Advances in Bioresearch
Adv. Biores., Vol 7 (3) May 2016: 167-170
©2016 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV Value 8.21 [2014]

Advances in Bioresearch

ORIGINAL ARTICLE

Effect of *Anethum graveolens* L. extracted Oil on Oxidative Stability of Sunflower Oil during Storage

Nazila Eivazi Pargoo[a], Leila Pishkar[a]*, Mohammad Yari[b]
[a]Department of Biology, Islamshahr Branch, Islamic Azad University, Tehran,Iran
[b]Department of chemistry, Islamshahr Branch, Islamic Azad University, Tehran, Iran
Corresponding author address:leilapishkar@gmail.com

ABSTRACT
Oxidative degradation of lipids plays a crucial role in decreasing the shelf-life of food stuffs. The formation of lipid peroxidation is considerably accountable for the deterioration of lipid-containing food stuffs. Application of antioxidants during the manufacturing methods may induce a decreasing in the extent of lipid peroxidation. Spices and some herbs have received increased attention as sources of many effective antioxidants. The effect of plant extracted oils (Eos) from fennel; rosemary and ginger on the oxidative stability of sunflower oil during storage at room temperature have been studied. The primary question of this report was to characterize the EOs of A. graveolens and examine its antioxidant activity potential against sunflower oils peroxidation oil during storage at room temperature for 5 weeks. The GC/MS analysis of A. graveolens EO showed 29 compounds representing 78.23% of the total oil; α-phellandrene was the main constituent (27.87%), followed by limonene (21.33%), Dill apiole (20.41%) and carvone (11.34%). In general, the natural antioxidant (A. graveolens EO) utilized in this report showed a decrease of the rate of peroxide formation. Finally, it could be assumed that, A. graveolens EO are promising candidate as natural antioxidants.
Keywords: Anethum graveolens, Sunflower Oil, Oxidative stress

Received 01/01/2016 Accepted 23/03/2016

How to cite this article:
N E Pargoo, L Pishkar, M Yari. Effect of *Anethum graveolens* L. extracted Oil on Oxidative Stability of Sunflower Oil during Storage. Adv. Biores. Vol 7 [3] May 2016: 167-170.DOI: 10.15515/abr.0976-4585.7.3.167170

INTRODUCTION

Due to the high amount of unsaturated fatty acids in oils, the stabilization of these oils against oxidation has received a great attention. Oxidation of oils is one of the considerable modifications that happen during the processing and preparation of food material. These events not only results in the dropping of the food quality but also provide oxidized agents such as free radicals that drive decreased shelf – life of oils and participate in several diseases such as heart disease, cancer, intestine blockade and kidney stone. Therefore, stabilizing oils against oxidation is a crucial factor to prevent different diseases and go forward to a healthy diet [1,2]. The application of antioxidants is the most pivotal method of stabilizing oils, of stopping the oxidation of lipids, and of preventing oils from the damages induced by free radicals. It has been well documented that the oxidation of lipids is substantially prevented by adding antioxidants to oils and fats [3]. Man-made antioxidants play an important role as oil preservatives, but their utilization has been restricted in many countries due to reported side effects of their application [4]. For example, the supplement addition of these materials to the diets has induced lethal bleeding in the pleural space, pancreas, and changes in the thyroid gland and show carcinogenic effects [5]. Although man-made antioxidants are applied at very low concentrations, there is a crucial requirement for adding antioxidants without side effects. Since the conflicting results driving from from the long term use of these compounds in human cannot be failed to consider. Therefore, the exploration for substitutes of synthetic antioxidants has conducted to the nomination of numerous antioxidants found in plants [6]. There is an increased potential application by consumers in new sources of antioxidant products. Recently, probing of natural products for the designing of active materials with antioxidant effects from plant origin that can be used to the food industry has obtained interest [7]. Medicinal plants are known to yield certain bioactive compounds which react with other organisms in the environment and presenting

antioxidant behavior [8]. Plant secondary metabolism is considerably linked to defense mechanisms and is the source of numerous chemical compounds [9]. Among plant extracts, essential oils (EOs) are obtaining enhancing potential interest in the food industries because of their comparatively safe properties, their wide usage by consumers, and their advantageous for potential multi-purpose functional application [10]. These oils are thought to play a pivotal role in plant defense mechanisms against free radicals [11]). *Anethum graveolens* L. (dill) is known as a one of the most pivotal culinary herbs in the world. The leaves can be added in diet such as salads and soups; the seeds can be consumed in bread and soups. Different pharmacological effects of *A. graveolens* such as antimicrobial, anticancer, antispasmodic, antidiabetic, and anti-inflammatory have been documented [12]. In addition, some studies have demonstrated that *A. graveolens* seed oil prevents some spoilage fungi [13]. Safflower (*Carthamus tinctorius* L.) provides particularly branched, herbaceous, thistle-like annual plant. It is widely used for vegetable oil extracted from the seeds. Plants possess 30 to 150 cm tall with having globular flower heads and providing yellow, orange, or red flowers. Each branch will usually possess from one to five flower heads demonstrating 15 to 20 seeds per head. There are two types of safflower that are used to extract different kinds of oil: one has high monounsaturated fatty acid like oleic acid and the other has high polyunsaturated fatty acid like linoleic acid. Commercially the uppermost edible oil is the former one, which has lower saturates fatty acids than olive oil. The primary objective of this study was to characterize the EOs of *A. graveolens* and study its antioxidant activity potential against sunflower oils peroxidation.

MATERIAL AND METHODS

Plant material and extraction of *A. graveolens* oil

The aerial parts of *A. graveolens* were collected during June and July in 2014 and 2015. EO was isolated by hydrodistillation for 4 h using a Clevenger-type apparatus, according to the procedure accepted in the Council of Europe (14). The extracted EO was dried using sodium solphate and stored in dark at 4°C.

Extraction of *Carthamus tinctorius* L. oil

The seeds are first washed to remove any additional stuff that might interfere with the Nasban Cold Press Oil Extractor Machine. In the pressing process, two cylindrical rolls press the seeds so that cells are cracked and oil can be release out of the flakes without passing through a cell wall.

Essential oil analysis

Composition of the *A. graveolens* EO was assessed by gas chromatography (GC) and mass spectrophotometry (GC/MS). The GC analysis was done using an Agilent Technologies 6890 with a silica capillary HP-5 column (30 m × 0.25 mm i.d.; film thickness 0.25 μm). The injector and detector temperatures were kept at 250 °C and 280°C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml/min; oven temperature program was 40–210 °C at the rate of 3 °C/min and then programmed to 240 °C at the rate of 15 °C/min and finally held isothermally for 5 min; the split ratio was 1:50. GC/MS analysis was done by application of an Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30 m × 0.25 mm i.d.; film thickness 0.25 μm) coupled with 5975-C mass spectrometer. Ion source and interface temperatures were set at 230 °C and 280 °C, respectively. The constituents of the EO were identified for ***n***-alkanes (C_6–C_{25}). Identification of individual materials was carried out by comparison of their mass spectra with those of the internal mass spectra library.

Peroxide Value (PV) assay:

The peroxide value was determined by AOCS official method (15). A certified amount of filtered oil was accurately weighed to 30 mg in a 50 mL flask. 30 mL of 3:2 acetic acid-chloroform was added and mix gently and 0.5 mL of saturated potassium iodide solution was added incubated for 1 min at room temperature and finally added to the oil samples. The samples were titrated against 0.1 N sodium thiosulfate. This titration continues until the yellow iodine color vanished. Starch indicator was also added and the titration was continued with 0.1 N sodium thiosulfate until the blue color of sample vanished. The blank titration value was calculated by using Equation 1.

Peroxide value = (S-B) ×N/W

Where: S is the volume of titrated sample (mL), B is the volume of titrated blank (mL), N is the normality of sodium thiosulfate solution and W is the weight of oil (g).

DATA ANALYSIS

All the experiments were performed in triplicate, with the results being expressed as mean ± SEM of three independent experiments. The data were compared using one-way ANOVA, with a Dunnett's multiple comparison tests. The differences between the data were regarded significant for values of $\boldsymbol{p} < 0.05$.

RESULTS AND DISCUSSION

Results obtained by the GC/MS chemical analysis of *A. graveolens* EO is summarized in Table 1. In total, 29 compounds were identified. The GC/MS analysis of *A. graveolens* EO showed 29 compounds representing 78.23% of the total oil; α-phellandrene was the main constituent (27.87%), followed by limonene (21.33%), Dill apiole (20.41%) and carvone (11.34%).

Table 1. Results obtained by the GC/MS chemical analysis of ***A. graveolens*** EO

Peak number	KI	Area (%)	Identified compound
1	867	0.06	hexanal
2	931	0.33	α thujene
3	939	1.73	α-pinene
4	976	0.25	sabinene
5	991	0.72	β-myrcene
6	1005	27.87	α-phellandrene
7	1018	0.29	α-terpinene
8	1031	21.33	Limonene
9	1062	0.06	Gamma-terpinene
10	1089	0.24	p-cymenene
11	1099	0.16	undecane
12	1119	0.06	Menth-2-en-1-ol
13	1155	0.16	Iso borneol
14	1184	5.46	Dill ether
15	1193	0.18	Dihydro carvone
16	1200	1.11	Trans carvone
17	1217	0.16	Trans carveol
18	1242	11.34	carvone
19	1290	0.17	Thymol
20	1298	0.40	Carvacrol
21	1308	0.12	9-undecenal
22	1489	0.44	Germacrene-D
23	1520	2.91	Myristicin
24	1554	0.18	Elemicin
25	1622	20.41	Dill apiole
26	1927	0.53	Hexa decanoic acid
27	1949	0.11	phytol
28	2092	0.26	9-12-octa decanoic acid
29	2500	0.11	pentacosane

Our result on chemical compounds of the *A. graveolens* EO shows a good agreement with some other reports (16, 17). The small differences in the chemical compounds of oil might arise from environmental differences and genetic changes of the plants.

Detection of peroxide number

Detection of peroxide number is the most widely used value which introduces the pivotal evidence of unsaturated fats in oil. It gives a value, to which an oil sample has experienced primary oxidation. The double bonds presented in oils play a considerable action in autoxidation. Oils which have a high content of unsaturation are most sensitive to autoxidation. All sunflower oil samples were measured for 5 consecutive weeks. The incubated oils were sampled every week after storing at room temperature for analytical experiments.

Table 2. Peroxide number of blank and presence of *A. graveolens* EO per weeks.

No	Sample	Week 1	Week 2	Week 3	Week 4	Week 5
1	Blank	6.35	6.89	7.41	8.00	8.59
2	0.1 mg/mL EO	5.91	6.49	6.91	7.45	7.99
3	0.2 mg/mL EO	5.42	5.90	6.45	6.93	7.48
4	0.3 mg/mL EO	4.91	5.30	5.79	6.18	6.49
5	0.4 mg/mL EO	3.45	3.80	4.28	4.69	5.19
6	0.5 mg/mL EO	3.12	3.44	3.72	4.15	4.49
7	0.6 mg/mL EO	2.75	2.80	2.00	2.90	3.22

It is well documented that deterioration process of edible oils could be prevented when the oil is rich with appreciable contents of plant phenolic compounds which act as antioxidants. This study was planned to examine the possible decrease in the rate of oxidation following addition of *A. graveolens* EO as natural source of antioxidants. The impacts of storage periods at room temperatures on the oxidative stability of sunflower oil incubated with *A. graveolens* EO are given in Table 2. Considerable increase was seen in the

peroxide number of sunflower oil during storage at room temperatures. As shown in Table 1, the peroxide value enhanced after 5 weeks of storage for control oil and (sunflower oil + without *A. graveolens* EO). The increase was greatly significant in sunflower oil without antioxidant as compared to sunflower oil sample in which *A. graveolens* EO were added. In general, the natural antioxidant (*A. graveolens* EO) utilized in this report showed a decrease of the rate of peroxide formation, because peroxide value of sunflower oil samples which incubated with *A. graveolens* EO were lower than that of control one. The decrement which showed in the peroxide value after five weeks of storage could be attributed to the rate of hydrolysis of peroxidic compounds was higher than the rate of peroxide formation which is in good agreement with literature [18].

CONCLUSION

The abovementioned outcomes proposed that extracted oil from *A. graveolens* EO possesses natural antioxidants and may be considered as a complement or alternative for food stuff, and maybe not single compound play a considerable role for this stability. A combination of a number of plant oils could provide a synergistic effect in increasing the oxidation stability of food stuff. It could be suggested that, essential oils of candidate aromatic plants yield a promising potential as natural antioxidants.

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