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Full Length Research Paper

Application of *Lavandula officinalis* L. antioxidant of essential oils in shelf life of confectionary

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Chemically synthesized compounds such as butylated hydroxy-anisole (BHA) and butylated hydroxytoluene (BHT) are used as antioxidants in food products. The safety of some of these compounds have been a great concern. So, there is a great interest in finding antioxidants from natural sources for food. Lipids, known as *Lavandula officinalis* L. in this work, harvested in the north of Iran was distilled in a Clevenger apparatus to obtain essential oil, which was analyzed by gas chromatography (GC) and GC-mass spetrophotometry (MS). Antioxidant activity of essential oil was evaluated by peroxide value, iodine value and conjugated dienes. Also, the influence of this antioxidant on the shelf life of the confection's type was investigated. As a result, 1,8-cineole and borneol were the most used components in the chemical analysis of essential oils. Free fatty acid from the 1st to 9th week was increased significantly and as such, peroxide value increased directly with storage time. Variation of both synthetic and natural antioxidant in the aspect of iodine values was reduced slowly. However, conjugated dienes changes in oil sample containing essential oil and the BHA still remains the same.

Key words: Antioxidant, *Lavandula officinalis* L., GC-MS, peroxide value, iodine value.

INTRODUCTION

Essential oils contain a complex mixture of odorous and volatile compounds from secondary plant metabolism and are widely used in cosmetics as fragrance components, in food industry as flavoring additives and in a variety of household products as scenting agents (Mazzanti, 2002). Chemically synthesized compounds, such as butylated hydroxy-anisole (BHA) and butylated hydroxytoluene (BHT), are used as antioxidants in food products. However, some of these compounds have been troubled for their safety (Bran, 1975; Whysner et al., 1994). The use of BHA and BTH is proved to be carcinogenetic, while the use of synthetic antioxidants is restricted in several countries, (Meftahizade et al, 2010) because of their undesirable long-term toxicological effects, including carcinogenicity (Gazzini et al., 1998). As a result, there is a great interest in finding antioxidants from natural sources for food. Lipids containing polyunsaturated fatty acids are readily oxidized by molecular oxygen and such oxidation proceeds by a free radical chain mechanism (Aruoma 1998, Anwar et al, 2006). Therefore, there is traditionally, an increasing interest in the antioxidative activity of natural compounds (Amakura et al., 2002). Aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods (Hulin et al., 2002). Most of their properties are due to essential oils produced by their effective compounds and secondary metabolism (Adam et al., 1998). There are several methods for the evaluation of antioxidative action on fats and oils, such as assessment of unsaturated fatty acids, formation of free radicals

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Abbreviations: BHA, Butylated hydroxy-anisole; **BHT,** butylated hydroxytoluene; **POV,** peroxide value; **RBD,** refined, bleached and deodorized; **SFO,** sunflower oil samples; **FFA,** free fatty acid; **IV,** iodine values; **CD,** conjugated dienes.

and formation of primary oxidation products, that is, peroxide value (POV) (Becker et al., 2004; Branen, 1975).

Lavandula officinalis L. is indigenous to Southern Europe and is sometimes found growing wild in the Mediterranean area between the coast and the lower mountain slopes. It is cultivated throughout Europe as well as in different parts of Iran (Wichtl, 1994). Leaves and flower of lavandula has the highest amount of essential oils. Lavender oil, which is the essential oil obtained by the aerial part of *L. angustifolia* Mill, is predominantly used in aromatherapy as a relaxant, carminative and sedative agent (Cavanagh and Wilkinson, 2002; Sanz et al., 2004). It was traditionally used as an antiseptic agent in swabbing of wounds, for burns and insect bites and in veterinary prac-tice to kill lice and other animal parasites. There are various reports about combination of lavandula component in different regions of the world and much of them showed that phenol compounds are the main components. In this work, *L. officinalis L* harvested in the north of Iran was distilled in a Clevenger apparatus to obtain essential oil, which was analyzed by gas chromatography (GC) and GC-mass spectrophotometry (MS). The solid and liquid residues were solvent extracted to produce extracts, which were analyzed in terms of composition and antioxidant activity. The antioxidant activity was studied and some extracts were introduced into sunflower oil, being the oil samples analyzed by peroxide value and iodine value, to study the evolution of oil rancidity. Also, the influence of this antioxidant on the shelf life of local confection in Ilam, Iran was investigated and the comparison between natural and synthetic antioxidant (BHA) was done.

MATERIALS AND METHODS

The aerial part of *L. officinalis* L. was collected in July 2009 in Mazandaran (north of Iran) and was air-dried. Before extraction, leaves were cut and the particle diameter after size reduction was estimated by the use of a sieving machine Retsch KS 1000 (Retsch, Haan, Germany), being the medium particle diameter calculated as 0.3 mm. Refined, bleached and deodorized (RBD) sunflower oil samples were used to investigate the antioxidant activity of *L. officinalis* essential oil. Sunflower oil was selected due to its high use in food as it is a rich source of linoleic acid and can easily undergo rancidity due to high degree of unsaturation (Shahidi et al., 1992). The essential oils of *L. officinalis* were obtained by using a Clevenger apparatus. About 100 g of dried plants (leaves) were used and the distillation was conducted for 4 h, while the amount of recovered oil was measured gravimetrically. Both the solid and liquid residues of Clevenger distillation were used for preparation of extracts with eventual antioxidant activity by using the method explained by Ribeiro et al. (2001). The ground solid residues were boiled with distilled water for about 2/5 h and were then filtered through a coffee filter paper. The collected filtrate was acidulated with a solution of 25% HCl to a pH of 2.5 and then followed by a filtration. The aqueous phase was mixed with disopropilether (10:3) and allowed to separate. The organic phases were combined, dried with MgSO4 filtered and evaporated using a rotary evaporator (Heidolph VV2000). Subsequently, the essential oils were analysed by gas chromatography on a Hewlett-Packard 5890 Series II chromatograph equipped with a FID detector and a DB5 column (5% phenyl and 95% dimethyl polysil oxane), 0.32 mm id 50 m and a film thickness of 0.17 m. The column temperature was programmed to hold at 65°C for 15 min, then heated to 180°C at a slope of 2°C/ min, with a final isothermal hold at 180°C for 30 min. The samples were injected using the split mode (split ratio 1:20), being the injection volume of 0.2 micro liter. The sample components were identified by comparing the retention times with those of chromatographic standard of the compounds (Sigma-Aldrich, Madrid, Spain). Peak areas were determined using a Hewlett-Packard 3396 Series II integrator. For quantitative analyses, the peak areas were converted to absolute values using response factors estimated from the standard compounds. Essential oils were also identified by GC-MS, using a gas chromatograph (shimadzu-9A) equipped with a DB-624 column (60 m x 0.32 mm id., film thickness 1.8 m) and linked to a MS detector (full scan), carrier gas and He that was adjusted to a linear velocity of 1.5 ml/min. However, the injection volume was 1 µl.

Determination of antioxidant activity in sunflower oils

Storage of samples

Seven refined, bleached and deodorized sunflower oil samples (SFO) were stored in triplicate in transparent polyethylene bottles of 250 ml capacity each. Out of a total of twenty one bottles, seven bottles contained 120 ml blank deodorized, refined and bleached SFO (control) and another seven bottles contained 200 ppm of BHA per 120 ml deodorized, refined and bleached sunflower oil samples.

Analysis of rancidity parameters in sunflower oil

Peroxide values (POV), free fatty acid (FFA) values and iodine values (IV) were determined by following the recommended methods of AOCS (AOCS, 1989), while conjugated dienes (CD) were determined according to the recommended methods of IUPAC (1987). For the determination of conjugated dienes, sunflower oil samples were diluted with iso-octane to bring the absorbance within the limits. The absorbance was measured at a wavelength of 232 and 268 nm for conjugated diene and triene values, respectively (Hitachi, U-o2001, Model 7400 spectrometer, Tokyo, Japan) (IUPAC, 1987; Gulcan and Bedia, 2007). The peroxide value was expressed as meq of oxygen/kg of fat and was determined by the iodine titration method. Extracted oil samples (2 g) were weighed into test tubes. The oxidation of the potassium iodide, in the acetic medium, by the active oxygen of the fat is followed by titration of the free iodine with sodium thiosulphate, using starch as indicator. Primary oxidation processes in oil mainly form hydro peroxides, which are measured by the POV. In general, the lower the POV, the better (time) the quality of the oil. The IV (iodine adsorption value) measures the number of reactive double bonds present in the oil. A higher IV number indicates more double bonds in the sample and therefore shows that greater care will be needed to slow down oxidation. This was done according to the methods of IUPAC (1987). Finally, an experiment was designed to evaluate the influence of synthetic (BHA and BHT) and natural antioxidant (*Lavandula* essential oil) on maintenance and taste of local confection during 9 weeks.

RESULTS AND DISCUSSION

1,8-Cineole and borneol were the main components in chemical analysis of essential oils from *L. officinalis* L. harvested from Mazandaran, North of Iran (Table 1). In

Table 1. Some components of essential oils obtained from *Lavandula officinalis* leaves and flowers.

Component	Retention time	Mass percentage
Linalool oxide	1211	0.98
Alpha-pinene	1231	3.22
Alpha- terpinene	1237	5.7
Borneol	1259	11.32
Camphor	1289	2.23
Menthol	1298	3.4
Eucarvone	1315	0.53
Dihydrocarveol acetate	1347	0.73
Terpinolene	1389	0.35
Eugenol	1402	1.07
Beta- caryophyllene	1412	2.23
Viridiflorol	1424	5.7
1,8-Cineole	1432	25.7

Table 2. Free fatty acid values in various RBD sunflower oil samples during 9 weeks.

No of weeks	Control sample	Essential oil sample	BHA sample
1st	0.057± 0.002	0.052±0.008	0.048±0.002
2nd	0.069±0.003	0.060±0.005	0.054±0.006
3rd	0.078±0.002	0.065±0.006	0.057±0.001
4th	0.94±0.001	0.067±0.004	0.059±0.002
5th	0.128±0.005	0.071±0.001	0.064±0.002
6th	0.152±0.003	0.076±0.006	0.076±0.004
7th	0.232±0.004	0.082±0.002	0.099±0.004
8th	0.273±0.008	0.091±0.002	0.101±0.004
9th	0.295±0.007	0.1±0.012	0.115±0.007

Original value of control sample was 0.042 ± 0.002.

view of the dissimilar compounds or different amount of the same compounds being present in different part of the plant body, the antioxidant potential of these parts of the plant could also be different (Sithisarm et al., 2005; Skerget et al, 2005). Afsharpor and Azarbayejani (2006) reported that dead *L. officinalis*, harvested from Isfahan, Iran, produce 1 to 3% essential oil, containing mainly monoterpenes (the most important component) of which linalyl acetate (30 - 55%), linalool (20 to 35%), ocimene, cineole (1,8-cineole) and camphor are part. Verma et al. (2009) reported that the essential oil content in the inflorescence of different accessions of lavender grown in temperate parts of Kashmir was only 0.80 to 1.3%. These variations could either be due to difference of the plant genotype or to the altitude and microclimate of the cultivation area. Also, it may be attributed to the different soils and especially, the climatic conditions (Evans, 1996).

As seen in Table 2, the changes in free fatty acid values show that free fatty acid from 1st to 9th week were enhanced significantly in comparison with control samples (free from BHA or essential oil), but this increase is low both in BHA and essential oil. Also, changes in oil contain BHA and the essential oil was the same approximately. This confirms the antioxidant ability of *L. officianlis* essential oils. Gradually, due to hydrolysis of triglycerides, fatty acid will be released and this phenomenon will be accelerated by a reaction of oil with moisture (Freja et al., 1999; Farag et al, 1989).

Peroxide values changes have been shown in Table 3 and peroxide value is one of the most widely used tests for oxidative rancidity in oils and fats. For this, oxidation degree on sunflower oil samples was determined by measuring POV in the absence and presence of anti-oxidants for 9 weeks. Results showed that peroxide value increased linearly with storage time. POV was 1.28 ± 0.02 in the 1st week and immediately jumped to 8.24 ± 0.04 in the 8th week. These changes were significant, thereby indicating the noticeable phenomenon of lipid oxidation, but in the sample containing BHA/essential oil, this procedure was increased gradually, till it was 1.15 ± 0.04 and 1.54 ± 0.07 in BHA and essential oil samples,

Table 3. Peroxide values (meqkg^{-1}) of various RBD sunflower oil samples.

No of weeks	Control sample	Essential oil sample	BHA sample
1st	1.28± 0.02	0.48±0.02	0.50±0.04
2nd	2.45±0.03	0.53±0.01	0.55±0.01
3rd	2.68±0.04	0.62±0.01	0.59±0.05
4th	3.21±0.03	0.67±0.04	0.64±0.04
5th	3.82±0.05	0.75±0.04	0.76±0.04
6th	5.64±0.04	0.85±0.05	0.89±0.05
7th	6.22±0.05	0.90±0.07	0.91±0.01
8th	7.45±0.03	0.92±0.04	0.95±0.01
9 th	8.24±0.04	1.54±0.07	1.15±0.04

Original value of control sample was 0.88 ± 0.004.

Table 4. Iodine values of various RBD sunflower oil samples.

No of weeks	Control sample	Essential oil sample	BHA sample
1st	131±2.21	155±2.25	153±3.24
2nd	128±1.52	152±3.15	151±2.35
3rd	127±2.51	150±3.24	149±3.36
4th	118±1.24	147±2.51	148±3.24
5th	110±2.36	146±1.25	146±2.35
6th	108±1.21	145±2.13	145±2.35
7th	107±1.45	142±2.34	143±2.35
8th	106±1.27	140±2.36	140±3.24
9 th	110±1.15	137±1.24	130±2.15

Original value of control sample was 135 ± 1.28.

Table 5. Conjugated dienes in terms of molar extinction co-efficient.

No of weeks	Control sample	Essential oil sample	BHA sample
1st	0.25±0.02	0.15±0.02	0.17±0.02
2nd	0.27±0.03	0.17±0.01	0.21±0.01
3rd	0.35±0.04	0.19±0.02	0.25±0.03
4th	0.48±0.04	0.22±0.01	0.27±0.03
5th	0.57±0.07	0. 35±0.01	0.32±0.01
6th	0.65±0.03	0.42±0.02	0.44±0.01
7th	0.75±0.04	0. 47±0.01	0.49±0.02
8th	0.87±0.04	0.58±0.03	0.59±0.04

Original value of control sample was 0.18 ± 0.02.

respectively.

Table 4 shows the variation of both synthetic and natural antioxidant in the point of view of iodine values, which in oil samples contain essential oil, after 9 weeks of its arrival to 137 ± 1.24. Also, conjugated dienes is the main measure in oil oxidation, and investigation of this variation \is a valid factor, which changes in oil sample containing essential oil and BHA that are the same approximately (Table 5).

Essential oils due to antioxidant activity compounds have a main role in the long shelf life of foods and confectionary (Table 6). Increase in lipid oxidation parameters for control samples was statistically significant, while these variations in local confection containing BHA sample were not significant. This measure can have direct health benefits by decreasing the formation of reactive oxygen species in confectionary. However, the above-mentioned rancidity parameters are the main

Table 6. Influence of essential oil on storage time of confectionary in room temperature.

Storage time of confection in room temperature (week)	Control sample (free essential oils)		Samples containing essential oils	
	pH	Taste variation	pH	Taste variation
1st	6.3	-	6.0	-
2nd	6.1	-	6.1	-
3rd	5.7	-	5.9	-
4th	5.5	Little	5.8	-
5th	5.4	Considerably sour	5.8	-
6th	5.2	Sour	5.7	-
7th	3.6	Sour and acidous	5.6	Slightly sour
8th	3.5	Sour and acidous	5.5	Slightly sour
9th	3.2	Sour and acidous	5.5	Slightly sour

indicators of deterioration of fats.

Conclusion

L. officinalis L. essential oils are a good potential for anti-oxidant activity and can be used in preserving foods. Antioxidant activity is related to phenolic compounds like1,8-cineole and beta-caryophyllene. This applied research also shows that utilization of *L.* essential oils in delay decay of local confectionary is useful and this fact can be added to other confection around the world.

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