Application of *Zataria multiflora* Boiss. and *Satureja hortensis* L. Essential Oils as Two Natural Antioxidants in Mayonnaise Formulated with Linseed Oil

Vahidyan H (M.Sc.)[1], Sahari MA (Ph.D.)[1]*, Barzegar M (Ph.D.)[1], Naghdi Badi H (Ph.D.)[2]

1- Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
2- Cultivation & Development Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
* Corresponding author: Department of Food Science and Technology, Tarbiat Modares University, P.O.Box: 14115-336, Tehran, Iran
Tel: + 98 – 21 – 48292323, Fax: + 98 – 21 – 48292200
Email: sahari@modares.ac.ir

Received: 13 Feb. 2012 **Accepted:** 8 Aug. 2012

Abstract

Background: **Mayonnaise is one of the food emulsions (O/W) that is used as a condiment in various foods.**

Objective: **Linseed oil (containing omega-3 fatty acids) was used at the replacement levels of 10, 15 and 20% of soybean oil in mayonnaise formulation and the sensory properties of samples were evaluated.**

Methods: **In the second part of this research, the *Zataria multiflora* Boiss. (ZMEO) and *Satureja hortensis* L. (SHEO) (500, 1000 and 1500) were added to oil of mayonnaise and their antioxidant effects, peroxide (PV) and conjucated diene (CD) values were compared with BHA syntethic antioxidant (100 and 200 ppm).**

Results: **15% of linseed oil used as replacer for soybean oil, showed no significant differences in the color, texture, taste, smell, mouth feel and overal acceptability compared with control samples, and this same ratio of 15 percent was used in subsequent tests. Statistical results of PV and CD indexes revealed that ZMEO (500) and the SHEO (1000 and 1500) had better antioxidant effect than BHA (100 and 200). In determination of secondary oxidation products, TBA value showed that ZMEO (500) was not significantly different than BHA (200) and that SHEO (1000) had better antioxidant activity than BHA (100 ppm).**

Conclusion: **The results of antioxidative effect and sensory properties (showed no significant difference in taste and smell score compared with control sample) of formulated mayonnaise showed that these natural antioxidants in mayonnaise formulation can ameliorate the quality of product as functional food due to advancing the public health.**

Keywords: ***Satureja hortensis* L., *Zataria multiflora* Boiss., Linseed oil, Mayonnaise, Natural antioxidant**

Introduction

Mayonnaise is a kind of semi-solid oil-in-water emulsion. It is traditionally prepared by carefully mixing a mixture of egg yolk, vinegar, oil, and spices (especially mustard) to maintain closely packed foam of oil droplets; it may also include salt, sugar or sweeteners, and other optional ingredients. The emulsion is formed by slowly blending oil with a pre-mix that consists of egg yolk, vinegar, and mustard because mixing the oil and aqueous phase at once will result in formation of a water-in-oil emulsion. It is probably one of the oldest and most widely used sauces in the world today [1].

Oxidation is one of the major causes of chemical spoilage, resulting in rancidity and/or deterioration of the nutritional quality, colour, flavour, texture and safety of foods. Lipid oxidation is a considerable problem in lipid-bearing foods, especially in food products containing lipids with highly polyunsaturated fatty acids (PUFA). Lipid oxidation of foods containing these lipids takes place almost instantly unless careful precautions are taken. Particular problems arise when the highly unsaturated oils are emulsified into various food systems [2].

There is at present increasing interest both in the industry and in scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants.

Shahsavari et al. investigated antioxidative activity of essential oil of *Bunium persicum* in soybean oil. The results showed that the *Bunium persicum* essential oil (BPEO) was able to reduce the oxidation rate of the soybean oil in the accelerated condition at 60 °C (oven test). The essential oil at 0.06% showed effect similar to BHA at 0.02%. Hence, BPEO could be used as an additive in food after screening [3]. Sahari et al. reported that tea seed oil has a natural antioxidant effect, and can enhance the shelf life of sunflower oil at 5% level [4]. Ayough et al. reported that the *Artemisia dracunculus* L. essential oil (ADEO) and *Matricaria chamomilla* L. essential oil (MCEO) are able to reduce the oxidation rate of soybean oil under accelerated conditions at 60 °C (oven test) [5]. Goli et al. reported that the pistachio hull extract (PHE) was effective in retarding oil deterioration at 60 °C, and in the concentration range of 0.02 - 0.06%, its activity increases [6]. Fazel et al. investigated the tea seed oil as natural antioxidants. They added tea seed oil at 5 and 10% levels to common kilka oil, and evaluated the peroxide (PV) and thiobarbitoric acid (TBA) values after 13 days at 60 °C for antioxidant effects. The antioxidant effects of tea seed oil in fish oil system was significantly different from the control for PV and TBA values and was preserved well during storage [7]. In another study, the antioxidant activity of *Zataria multiflora* L. essential oil in soybean oil at the concentration of 1000 ppm was comparable to that of BHA at concentration of 200 ppm [8]. Yasoubi et al. investigated the antioxidant activity of pomegranate (*Punica granatum* L.) peel extracts in soybean oil. The extract at concentration of 500 ppm was comparable to BHA and BHT at concentration of 200 ppm [9].

These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. that render spices and some herbs or their antioxidant components act as preservative agents in food [10].

Antioxidants are the compounds that, when added to food products, especially to lipids and lipid-containing foods, can increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons deterioration for food products during processing and storage. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity. Thus, a need for identifying alternative natural and safe sources of food antioxidant is created and the search for natural antioxidants, especially of plant origin, has notably increased in recent years [11].

Flaxseed (*Linum usitatissimum* L.) is economically important oilseed crop containing about 40% oil in the seed. Flaxseed (also known as linseed) is regaining its popularity from its traditional usage as a raw material in oil production because of the reported health benefits of n-3 fatty acids and its exceptionally high content of the n-3 fatty acid, alpha linolenic acid (ALA). According to, flaxseed oil usually contains greater than 50% of ALA. Flaxseed oil is extracted from the seed and can be consumed with food (as salad oil) or in nutraceutical forms [12].

The aim of this study was to evaluate antioxidant activity of *Zataria multiflora* Boiss. and *Satureja hortensis* L. essential oils on mayonnaise formulated with linseed oil (containing omega-3 fatty acids).

Material and Methods

Materials

Chloroform, ethanol, aceton, sodium thiosulfate, starch, acetic acid, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), butylated hydroxyanisole (BHA), potassium iodate, potassium sorbate were purchased from Merck (Darmstadt, Germany); cyclohexane from Riedel-dehaen Company, xantan from Provisco Company (Switzerland); carboxymethyl cellulose from Founders Room Company (Japan), potassium iodide from Applied Chemistry Company (Germany). All chemicals were analytical grade.

The essential oil of *Zataria multiflora* Boiss. (prepared by the method of vapor condensation) was purchased from Iranian Company Gyah Essence Gorgan (Golestan Province, Iran).

Satureja hortensis L. essential oil (SEO) was obtained from the Institute of Medicinal Plants and Natural Products Research, Karaj, Iran and kept at −4 °C until it was used.

The main material, flaxseed (*Linum usitatissimum* L.) was collected from the Plant and Seed Center of Jahad-e-Agriculture Ministry located in Karaj (Alborz Province, Iran). Soy oil non antioxidant was purchased from company Aliya Company (Golestan Province, Iran).

Extraction of linseed oil

The oil was extracted in laboratory by solvent method, that is, the seed were cleaned and ground and poured in some cartouches, then some cotton soaked in hexane was put upon them, the extraction was done at room temperature.

Production and storage of mayonnaise

The production of mayonnaise was carried out as described by [13] and comprised three successive stages: Blending of all ingredients except oil, addition of oil under controlled conditions during continuous blending.

The ingredients in the mayonnaise were soybean oil 66%, full egg 13.5%, water 7.50%, vinegar 7.70%, sugar 3.85%, salt 1.50%, xantan 0.16%, potassium sorbate 0.1%, carboxymethyl cellulose 0.04% all of food grade.

Addition of essential oils in mayonnaise

The essential oils were added to oil of mayonnaise at the rates of 500, 1000 and 1500 ppm and BHA syntethic antioxidant at the rates of 100 and 200 ppm. Thereafter their antioxidant effects were evaluated by peroxide (PV), conjucated diene (CD) and thiobarbituric acid (TBA) values.

Samples of mayonnaise in Petri dishes with lids were stored in darkness for 8 weeks at 30ºC and three different methods for determination of oxidative changes in the mayonnaise were tested. The results were reported based on three replicates of the test.

Peroxide value (PV)

Peroxide value (PV) of samples was measured according to the AOCS method [14].

Conjugated diene hydroperoxides (CDH) assay

The oil sample of mayonnaise, 50 mg was mixed with 5 ml of cyclohexane and vortexed. CDH absorbance was recorded at 234 nm [15].

Thiobarbituric acid value (TBA)

1.00 g mayonnaise was dissolved in 3.5 ml cyclohexane, and 4.5 ml of 7.5% trichloroacetic acid (TCA) /0.34% thiobarbitoric acid (TBA) was subsequently added, and the resulting mixture was shaken for 5 min. After centrifuging for 15 min at 2870 g the TCA-TBA phase was removed and heated in a water bath at 100°C for10 min. The absorption at 532 nm was measured [14].

Separation oil of mayonnaise

Samples were first thoroughly homogenized then chloroform and methanol in ratio of 2:1, were added and shaken for 6 hr, then oil was separated by rotary evaporator [16].

Sensory evaluation

Early linseed oil (containing omega-3 fatty acids) was used at the replacement levels of 10, 15 and 20% of soybean oil in mayonnaise formulation and the sensory properties of these samples were evaluated. The attributes of color, texture, taste, smell, mouth feel and overal acceptability were assessed by panelists using Hedonic test.

Finally sensory evaluation and antioxidative effect of the essential oils treated with 500 ppm of *Zaturia moltiflora* B. (ZMEO) and 1000 ppm *Satureja hortensis* L. (SHEO) were evaluated. Mayonnaise samples were prepared by mixing mayonnaise oil with 500 ppm of ZMEO B. and 1000 ppm SHEO then the preparations were given to panelists to eat with salad. The panelists were asked to evaluate taste and smell of the mayonnaise samples on a scale from 5 to 1 indicating decreasing taste. A general taste score was calculated as the average of all grades. The data from the 15 independent panelists were pooled and the mean values and standard deviations were determined.

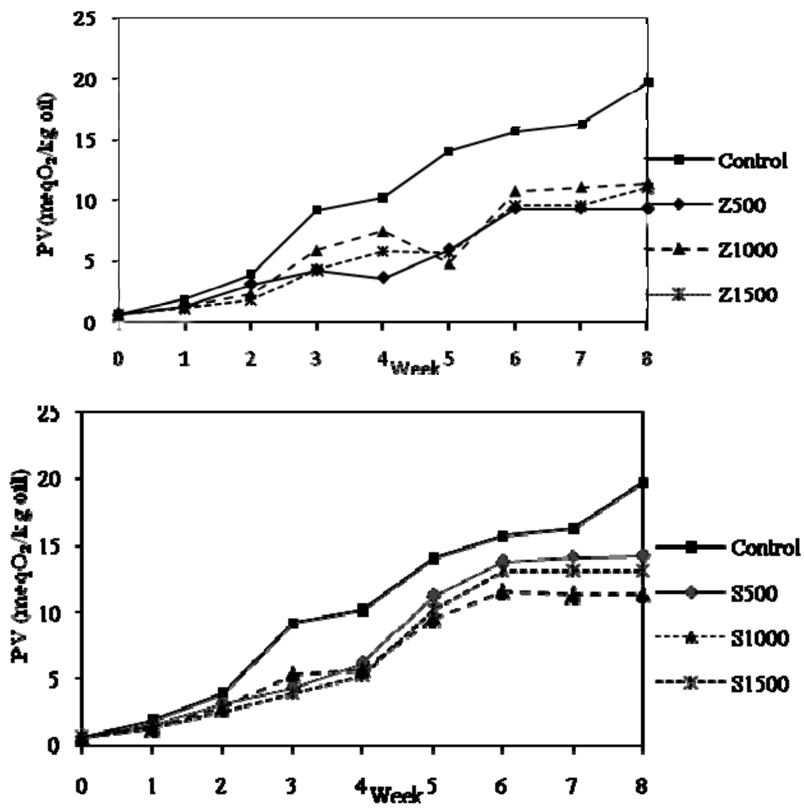
Statistical analysis

Experimental data was analysed using one-way analysis of variance (ANOVA) and significant differences among means from a triplicate analysis at ($p<0.05$) were determined by Duncan's multiple range test (DMRT) using the SPSS v.16.0 for Windows (SPSS Inc., Chicago) software.

Results

Peroxide value (PV)

Results of oxidative changes taking place in mayonnaise samples of different treatments (receiving different antioxidants) ZMEO and SHLO, synthetic antioxidant BHA and control after 8 week of storage at 30ºC are shown in Fig. 1.



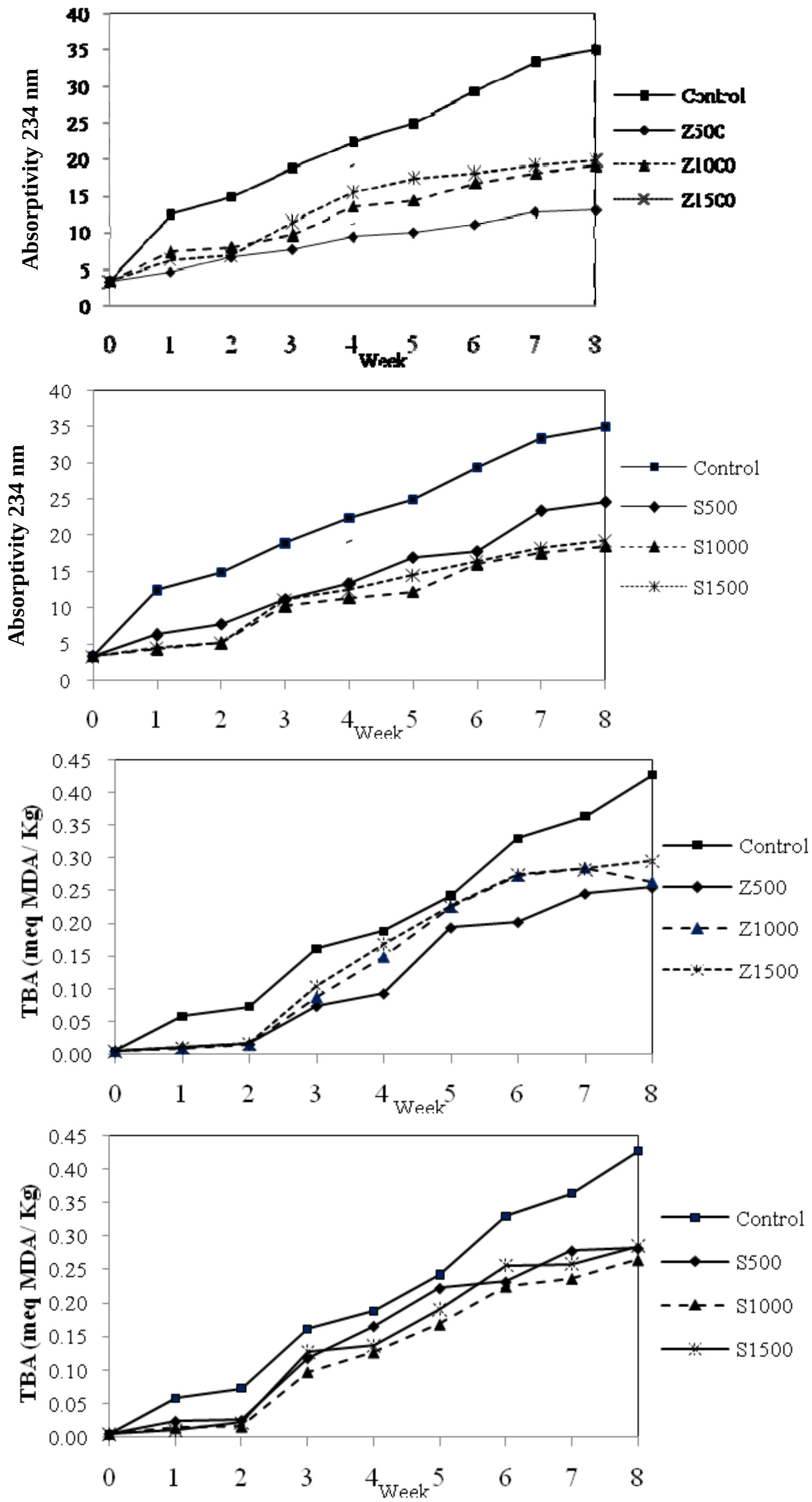


Fig. 1- Comparsion of the effects of different concentrations of ZMEO and SHLO on PV, CDH, TBA of mayonnaise containing linseed oil at weekly intervals

Results showed in different concentrations of ZMEO in comparision with blank sample (without any antioxidant), in last week of antioxidant activity, concentration of 1000 and 1500 ppm had no significant difference and concentration of 500 ppm was the best treatmeant and had significant difference in comparision with synthetic antioxidant of BHA. Also did not find any significant different between concentration of 1500 ppm of ZMEO and 200 ppm synthetic antioxidant of BHA. While in the case of SHLO at 1000 ppm showed significantly better antioxidant effect compared with BHA at 100 ppm (Fig. 2).

Conjugated diene hydroperoxides (CDH)

Results showed that between various concentrations of ZMEO in comparision with blank sample (without any antioxidant) in last week of antioxidant activity, concentration of 500 ppm was the best treatmeant and had significant difference in comparision with synthetic antioxidant of 100 and 200 ppm BHA.

In case of SHLO, however, results showed that concentration of 1000 ppm SHLO had significantly better antioxidant activity than that of 100 ppm BHA Fig. 2.

Thiobarbituric acid value (TBA)

Results showed that between various concentrations of ZMEO in comparision with blank sample (without any antioxidant), in last week of antioxidant activity, concentration of 500 ppm was the best treatmeant and had significant difference in comparision with synthetic antioxidant of 100 ppm BHA Fig. 2.

In short all concentrations of SHLO had an antioxidant activity similar to synthetic antioxidant of BHA at concentration of 100 ppm for inhibition of secondary products.

Also TBA values of 1000 ppm ZMEO and 1000 ppm SHLO have not shown significant differences Fig. 2.

Sensory evaluation

The results showed that 15% linseed oil used as replacer for soybean oil, caused no significant difference in the color, texture, taste, smell, mouth feel and overal acceptability in comparision with control samples, and this percent was used in subsequent tests. Also results of antioxidative efficacy and sensory properties (no significant difference in taste and smell score in comparision with contorol sample.

Discussion

Differences in chemical compositions of the extracts obtained from different parts of the world could be due to use of various species; and different climates, cultivation, and storage conditions. Differences in the composition of the oils and extracts affect their antioxidant properties [17]. The antioxidant activity of natural antioxidants is higher than those reported by other researches. Shahidi et al. reported that the antioxidant effect of aromatic plants is due to the presence of hydroxyl groups in their phenolic compounds. Previous studies by Pokorny et al. showed that sage extract has a strong antioxidant effect in sunflower oil [18, 19]. The antioxidative

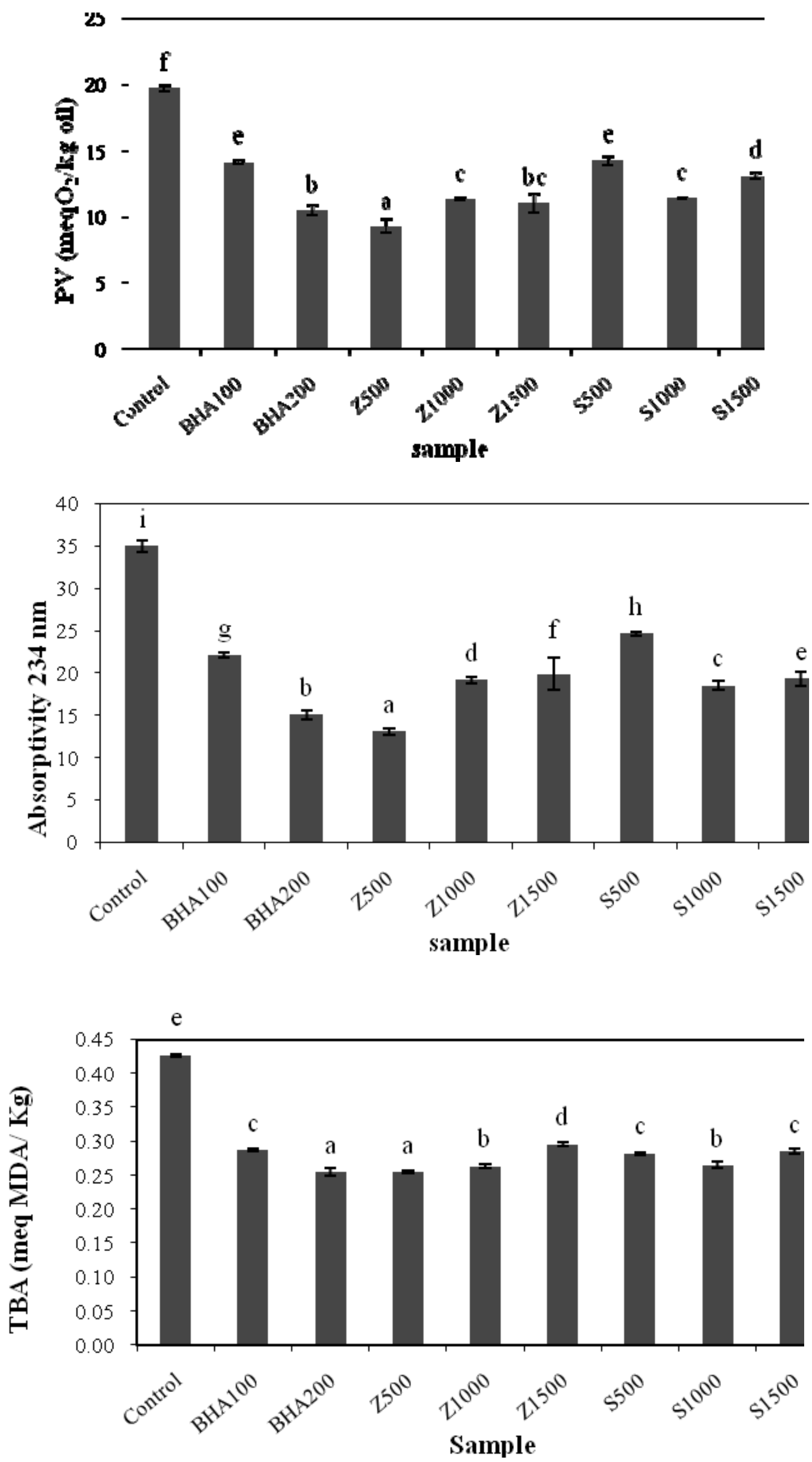


Fig. 2- Shelf-life comparsion of (as for PV, CDH and TBA) of mayonnaise containing 15% linseed oil and ZMEO, SHEO and BHA in the last week.

effects of sage, thyme, and oregano in rapeseed oil during storage at 60 °C were confirmed by Takacsova et al. [20]. Yanishlieva and Marinova reported that the extract of *Melissa offcinalis* L. (lemon balm) showed antioxidative action [21]. It is difficult to evaluate natural antioxidants in oils and food emulsions in view of the complex interfacial affinities between air-oil and oil-water interfaces involved. Frankel et al. and Huang et al. showed that the relative effectiveness of lipophilic and hydrophilic antioxidants was dependent on the lipid substrate, physical state (bulk oil, emulsion), antioxidant concentration, oxidation time and temperature, and the analytical method used to determine the extent and end point of oxidation. Moreover, the hydrophilic antioxidants become diluted in water phase and cannot adequately protect the oil in the oil water interface [22, 23].

Madsen et al. investigated the antioxidant activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus oficinalis* L.) in French dressing during dark and light storage at 19 °C for up to 24 weeks. They found that for oil-in-water emulsion as dressing, addition of 0.15% produces a significantly better antioxidative protection than addition of 80 ppm propyl gallate. Addition of freeze-dried methanol extract of the two spices in an equivalent concentration had lower antioxidative effect; but the effect was comparable to the effect of propyl gallate [16]. Abdalla and Roozen evaluated the antioxidative activities of plant extracts in the sunflower oil and its 20% oil-in-water emulsion at 60 °C in the dark. They concluded that thyme and lemon balm extracts inhibited hexanal generation more than formation of conjugated dienes in both oil and emulsion. Oregano extract was more active in oil than in emulsion. Catnip and hyssop extracts (600 ppm) showed prooxidative action to sunflower oil at 60 °C. In emulsions, during additional incubation, catnip extract (600 ppm) was active and significantly inhibited the formation of conjugated dienes compared with BHT (300 ppm) [24].

Conclusion

The results obtained in this study showed that ZSO and SEO can be used as a natural antioxidant ingredients in the mayonnaise linseed oil (containing omega-3 fatty acids) as a functional food due to advancing the public health.

Acknowledgement

The authors gratefully acknowledge the Research Council of Tarbiat Modares University and Center of Excellency for Recycling and Losses of Strategic Agricultural Products for their financial support and Kalleh Dairy Company for providing raw material.

References

1. Depree JA, Savage GP. Physical and flavour stability of mayonnaise. *Trends Food Sci. Technol.* 2001; 112: 157 - 63.

2. Frankel EN, Satue-Gracia T, Meyer AS, German JB. Oxidative stability of fish and algae oils containing long-chain polyunsaturated fatty acids in bulk and in oil-in-water emulsions. *J. Agric. Food Chem.* 2002; 50: 2094 - 9.

3. Sahsavari N, Barzegar M, Sahari MA, Naghdiabadi M. Antioxidant activity and chemical characterization of essentional oil of *Bunium persicum*. *Plant Food Hum. Nutr.* 2008; 63: 183 - 8.

4. Sahari MA, Ataii D, Hamedi M. Characteristics of tea seed oil in comparison with sunflower and olive oils and its effect as a natural antioxidant. *J. Amer. Oil Chem. Soci.* 2004; 81: 585 - 8.

5. Ayoughi F, Barzegar M, Sahari MA, Naghdiabadi M. Chemical compositions of essential oils of *Aretemisia dracunculus* L. and *Matricaria chamomilla* L. and evalution of their antioxidative effect. *J. Agric. Sci. Technol.* 2011; 13: 70 - 88.

6. Goli AH, Barzegar M, Sahari MA. Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. *Food Chem.* 2005; 92: 521 - 5.

7. Fazel M, Sahari MA, Barzegar M. Determination of main tea seed oil antioxidant and their effects on common kilka oil. *Inter. Food Rese. J.* 2008; 15: 209 - 17.

8. Zhang H, Feng C, Wang X. Evaluation of antioxidant activity of parsley (*Petroselinum crispum*) essential oil and identification of its antioxidant constituents. *Food Chem.* 2006; 39: 833 - 9.

9. Yasoubi P, Barzegar M, Azizi MA. Total phenolic contenta and antioxidant activity of pomegranate (*Punica granatum*) peel extract. *J. Agric. Sci. and Techno.* 2007; 9: 35 - 42.

10. Calucci L, Pinzono C, Zandomeneghi M, Capocchi A. Effects of gamma-irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. *J. Agric. Food Chem.* 2003; 51: 927 - 34.

11. Zainol MK, Abd-Hamid A, Yusof S, Muse R. Antioxidant activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* L. urban. *Food Chem.* 2003; 81: 575-581.

12. Hassan-zadeh A, Sahari MA, Barzegar B. Optimization of the ω-3 extraction as a functional food from flaxseed. *Inter. J. Food Sci. Nutr.* 2008; 59: 526 - 34.

13. Leuschner RG, Boughtflower MP. Standardized laboratory-scale preparation of mayonnaise containing low levels of it *Salmonella enterica* serovar Enteritidis. *J. Food Protec.* 2001; 64: 623 - 9.

14. AOCS. In: D Firestone, (Ed.), Official Methods and Recommendation Practices of the American Oil Chemists' Society (4^{th} ed.). Champaign: AOCS. 1989.

15. Karawita R, Siriwardhana N, Lee KW, Heo MS, Yeo IK, Lee YD. Jeon YJ. Reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition properties of diferent solvent fractions from *Hizikia usiformis*. *Euro. Food Resea. Tech.* 2005; 220: 363 - 71.

16. Chen IS, Shen CSJ, Sheppard AJ. Comparison of methylen chloride and chloroform for the extraction of fats from food products. Division of Nutrition, Food and Drug Administration, 1981, pp: 599 - 661.

17. Najafpour-navayi M, Mirza M. Comparision of chemical components of *Hyssopus officinalis* L. esential oil *in vitro* and in natural habitat. *Iranian J. Medic. Arom. Plants* 2003; 18: 41 - 53 (In Persian).

18. Shahidi F, Janitha PK, Wanasundara PD. *Crit. Rev. Food Sci. Nutr.* 1992; 32: 67 - 103.

19. Pokorny J, Nguyen H, Korczak J. Antioxidant activities of rosemary and sage extracts in sunfower oil. Nahrung. 1997; 14: 176 - 7.

20. Takacsova M, Pribela A, Faktorova M. Study of the antioxidative effect of thyme, sage, juniper and oregano. *Die Nahrung* 1995; 39: 241 - 3.

21. Yanishlieva N, Marinova E. Antioxidant activity of selected species of the family *Lamiaceae* grown in Bulgaria. *Die Nahrung* 1995; 39: 458 - 63.

22. Frankel E, Huang S, Prior E, Aeschbach R. Evaluation of antioxidant activity of rosemary extracts, carnosol and carnosic acid in bulk vegetable oils and fish oil and their emulsions. *J. Sci Food Agric.* 1996; 72: 201 - 8.

23. Madsen HL, Serensen B, Skibsted LH, Bertelsen G. The antioxidative activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus oflcinalis* L.) in dressing stored exposed to light or in darkness. *Food Chem.* 1998; 63: 173 - 80.

24. Abdalla AE, Roozen JP. Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. *Food Chem.* 1999; 64: 323 - 9.