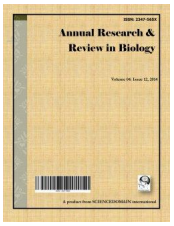

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Evaluation of *in vitro* Antioxidant Characteristics of Corn Starch Bioactive Films Impregnated with *Bunium persicum* and *Zataria multiflora* Essential Oils

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Authors' contributions

This work was carried out in collaboration between all authors. Author MA designed the study, performed the statistical analysis and wrote the protocol. Author MH wrote the first draft of the manuscript. Authors EA and ZA managed the analyses of the study. Author HH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Autoxidation is considered as one of the main factors responsible for deterioration which occurs during manufacturing, storage, distribution and final preparation of foods. Changes caused by lipid oxidation not only resulting to off-flavor, but also resulting to loss of color, decrease product's shelf life, nutritional loss and human diseases. Nowadays, edible films and coatings have attracted the considerable attention due to their potential to enhance food quality, food safety and their function as carrier for wide range of food additives specially antioxidants. In this study, the range of 1 to 20

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mg/ml concentrations from *Zataria multiflora* essential oil (ZMEO) and *Bunium persicum* essential oil (BPEO) incorporated in to corn starch film were used. Antioxidant activity of the bioactive films were determined by 2,2- diphenyl-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethyl benzo thiazoline-6-sulphonic acid, ABTS) and total phenolic content assays. Results showed that the films incorporated with EOs had better antioxidant properties compared with control group and this effect was significantly improved with increasing EOs concentration ($P < 0.05$). These finding pointed out that incorporation of ZMEO and BPEO as natural antioxidant agents into corn starch film could be considered as a potential factors for using in active packaging.

Keywords: Starch film; thyme; black cumin; DPPH; ABTS.

1. INTRODUCTION

For the past 50 years, Synthetic polymers commonly known as plastics have been widely used in a variety of packaging material. Plastics are most efficient, functional and cost-effective choice for many applications, but they have become a major source of waste-disposal problems due to their poor biodegradability [1]. Also, chemical compounds migration can occur from plastic films into food during processing and storage and once these compounds reach a certain level, the quality and safety of food may be jeopardized. To solve this problem, biodegradable films and coatings prepared from biopolymers, including proteins, polysaccharides and lipids or their combinations seems to be a good alternative to synthetic ones. They are defined as a thin layer applied on or between food components which can be consumed and provides a good barrier to gases, moisture and fats migration as well as mechanical protection. They can serve as a carrier for food additives like antioxidant and antimicrobial agents and reduce the deterioration and extending the shelf life without affecting the quality of the food [2,3].

Polysaccharides are able to form films, providing good barriers against gases like oxygen and carbon dioxide. Starch is a water-soluble polysaccharide which attracted much interest due to its biodegradability and mechanical properties. Furthermore, it is a relatively cost-effective choice compared with protein and lipid edible films and coatings. Starch is a mixture of two polymers amylose and amylopectin with different amounts of each component varying according to its plant source. Film-forming, mechanical and barrier properties appear to be influenced by amylose to amylopectin ratio. Generally, increasing amount of amylose has a direct relationship with abovementioned characteristics [4].

Oxidative rancidity is a major cause of food quality loss, leading to changes in appearance, texture, sensory properties, and nutritive value. Due to concern over the safety of synthetic antioxidant, extensive effort has been made to replace natural compounds instead of potentially toxic synthetic antioxidants. In this regard, use of natural preservatives such as plant essential oils with antioxidant activity into biodegradable films can improve shelf life, safety and quality of foods [5]. Plant essential oils are secondary metabolites that are generally recognized as safe (GRAS) as flavoring agents or preservatives for consumption by animals and human [6].

Zataria multiflora is one of the best-known spice and medicinal plant which has been widely used for treatment of respiratory tract infections and irritable bowel syndrome. This plant known as Avishan-e-Shirazi (in Iran) belongs to *Laminaceae* family and grows in Iran, Afghanistan and Pakistan [7]. *Zataria multiflora* essential oil (ZMEO) is extracted from flower shoots of this plant, having different effects such as pharmaceutical, antioxidant and antibacterial properties. Thymol, carvacrol, para-cymene, c-terpinene and b-caryophyllene are the main components of the essential oil [8,9].

Bunium persicum is an economically important medicinal plant in the family *Apiaceae* which commonly called great pignut or black cumin. This plant grows in dry lands in Iran, used to be helpful in diarrhea and dyspepsia treatment and flavoring foods and beverages. The seed of *Bunium persicum* has essential oil (BPEO) and γ-terpinene, β-pinene and cuminaldehyde are major constituents represented on its oil [10].

2. MATERIALS AND METHODS

2.1 Materials

Materials were purchased from companies as follows: corn starch, BHT, ethanol, carbonate sodium, potassium persulfate, glycerol, Folin-Ciocalteu (FC) reagent and azinobis (3-

ethylbenzothiazoline-6-sulfonic acid) (ABTS˚) from Merck (Merck, Darmstadt, Germany), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH˚) and gallic acid from Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany).

2.2 Isolation of Essential Oils

The black cumin seeds and flower shoots of *Zataria multiflora* (aerial part) were purchased from a local market in Zanjan city, Iran. The taxonomic identification of plant materials was confirmed by Iranian Institute of Medicinal Plants, Karaj, Iran. The extraction of the EOs was performed using the hydro-distillation method. 100 g of each plant was separately ground and placed with water (900 cc) in distillation flask. The flask was coupled to a Clevenger type apparatus and heated at 100°C for 3 h and finally the upper liquid (EO) was isolated from the Clevenger apparatus. This procedure was performed about 15 times (using fresh materials each time) for each species to obtain sufficient EOs for further experiments, and the oils were dehydrated with sodium sulfate, filtered by 0.22 µm filters and were stored at 4°C.

2.3 Determination of Moisture Content and Yield

The seeds of black cumin and Arial parts of thyme (5 g) were placed with 80 ml cyclohexane in a 250 ml volumetric distillation flask. The flask was coupled to a condenser with a graduated volumetric collector and heated at boiling temperature for 2 h. After the distillation process, the volume of water in the collector was measured and expressed as the moisture content contained per 100 g sample. For the yield calculation, 350 g of dry spices were subjected to extraction by hydro distillation, and the essential oils obtained were quantified. In parallel to the moisture content measurement, the essential oil yield for dried plants were calculated to absolute dry weight (% v/w) [11].

2.4 GC-MS Analysis

GC/MS analysis of EOs were performed using a Hewlett Packard 5890 equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness). The Helium flow rate was 1 ml/min. The column temperature was initially 50°C and then gradually increased to 120°C at a 2°C/min rate, held for 3 min, and finally increased to 300°C. The MS procedure was operated through ionization energy of 70 eV. Thereafter, the compounds were identified by comparing their retention indices with those of authentic samples and the mass spectral data available in the library (Wiley-VCH 2001 data software, Weinheim, Germany) [11].

2.5 Preparation of Starch Film

Aqueous solutions of 3% (w/w) corn starch and 1.8% glycerol were prepared by heating at 90°C under agitation for 10 min to allow gelatinization. Afterwards the solution was cooled down to approx. 40°C and ZMEO and BPEO was added in concentration of 1 to 20 mg/ml (1, 2.5, 5, 10, 15 and 20 mg/ml) separately and homogenized with a ultra-turrax during 2 min at 2000 rpm till obtaining a homogeneous mixture. Aliquots of 25 mL of solutions were cast on Teflon petri dishes (ϕ = 10 cm) and dried at room temperature for 24 h [12].

2.6 Determination of Total Phenolic Content (TPC)

The TPC of the films was determined according to the Folin-Ciocalteu method. Twenty-five mg of each film sample was dissolved in 5 ml of distilled water, then the extract solution (0.1 ml), distilled water (7 ml), and Folin–Ciocalteu reagent (0.5 ml) were mixed and kept at room temperature for 8 min, after which 1.5 ml sodium carbonate (2%, w/v) and water were added to obtain a final volume of 10 ml. The mixture was stored in darkness and at room temperature for 2 h. The absorbance values were then measured at 765 nm using a spectrophotometer. The methanol solution was used as a blank. All assays were carried out in triplicate. A calibration curve was drawn using gallic acid in specific concentrations and the total phenolic content of the films was expressed as mg gallic acid equivalents (GAE) per gram of dried film according to the following equation [13]:

$$T = \frac{C.V}{M}$$

Where *T* is total content of phenolics compound (mg per gram dried film), *C* is the concentration of gallic acid obtained from the calibration curve (mg/ml), *V* is the volume of film extract (ml) and *M* is the weight of dried film (gram).

2.7 DPPH˚ Free Radical Scavenging Assay

The efficacy of the films to scavenge DPPH radicals was determined using

spectrophotometry method on basis of bleaching of the bluish-red or purple color of DPPH solution as a reagent. Every sample (25 mg) of each film was dissolved in 3 mL of distilled water, and then a 2.8 mL of film extract solution were mixed with 0.2 mL of 1 mM methanolic solutions of DPPH. The absorbance at 517 nm was measured after the solution had been allowed to stand in the dark at ambient temperature for 30 min. BHT was used as the positive control. The percentage of DPPH radical-scavenging activity was calculated using following equation [14]:

$$\text{DPPH}^{\circ}\text{ scavenging effect}(\%) = \frac{Abs_{DPPH} - Abs_{sample}}{Abs_{DPPH}} * 100$$

Where Abs $_{DPPH}$ = the absorbance of the DPPH radical solution and Abs $_{sample}$ = the absorbance of the sample.

2.8 ABTS ˚ Free Radical Scavenging Assay

The antioxidant capacity of the films was measured by employing the ABTS˚ free radical scavenging method. Initially, a solution containing ABTS˚ radical (7 mM) and potassium persulfate (2.45 mM) (1:0.5) was kept in the dark for 18 h. An aliquot of this solution was diluted with ethanol to an absorbance of 0.70±0.03 at 734 nm to obtain the ABTS˚ working solution. A sample of the solubilized film (60 µl) was added to the ABTS˚ working solution (2940 µl) and the mixture was incubated at 37°C for 10 min in the dark and then centrifuged (5 min, 3000 rpm). BHT was used as the positive control. The antioxidant capacity was calculated by using the following equation [15]:

$$\text{ABTS}^{\circ}\text{ scavenging effect}(\%) = \frac{Abs_{\text{ABTS}} - Abs_{sample}}{Abs_{\text{ABTS}}} * 100$$

Where Abs_{ABTS} = the absorbance of the ABTS˚ solution and Abs_{sample} = the absorbance of the sample.

2.9 Statistical Analysis

Statistical analysis of data was performed using SPSS (Version 19.0 for Windows; SPSS Inc.). All the experiments were carried out in triplicate. Differences between the values were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with α=0.05.

3. RESULTS

3.1 Chemical Composition

The chemical compositions of BPEO and ZMEO are presented in Table 1. The average essential oil yields based on the dry weight of the black cumin seeds and flower shoots of thyme were determined as 0.8% and 1.2% (v/w) respectively. The main components of BPEO were cuminaldehyde (22.34%), carvacrol (19.88%) and anisole (15.19%), and the major compounds of ZMEO were carvacrol (36.62%), thymol (17.86%) and *p*-cymene (11.35%).

3.2 Total Phenolic Content

Foline-Ciocalteu phenol reagent is used to find a crude estimate of the amount of phenolic groups present in corn starch film incorporated essential oils. The results showed that total phenolic content in the corn starch films significantly was increased ($P \leq 0.05$) with increasing EOs concentration. The TPC content of ZMEO films was higher than those with BPEO (Fig. 1).

3.3 DPPH˚ Free Radical Scavenging Assay

DPPH assay is very convenient test for the quick screening of samples for determination of radical scavenging activity. This method is based on reduction of DPPH, a stable free radical, to be quenched and thereby decolorize in the presence of antioxidants [16]. The results showed that DPPH scavenging activity of the corn starch films significantly was increased with increasing EO concentration as shown in Fig. 2 ($P < 0.05$).

3.4 ABTS˚ Free Radical Scavenging Assay

ABTS is a de colorization assay, which radical is produced directly in a stable form prior to reaction with putative antioxidants, which involves the production of the blue/green ABTS chromophore through the reaction of ABTS with potassium persulfate [17]. The results showed that ABTS scavenging activity of the corn starch films significantly was increased with increasing EO concentration ($P < 0.05$) as shown in Fig. 3.

Table 1. Chemical composition of *Bunium persicum* and *Zataria multiflora* essential oils

	***Bunium persicum* essential oil**			***Zataria multiflora* essential oil**		
No.	**Compound name**	**Area (%)**	**KI***	**Compound name**	**Area (%)**	**KI***
1	α-Thujene	0.11	927	α-Thujene	0.15	927
2	α-Pinene	0.32	934	α-Pinene	1.83	934
3	β-Pinene	2.34	981	β-Pinene	0.45	981
4	β-Myrcene	0.43	992	β-Myrcene	0.87	992
5	α-Phellandrene	1.28	1010	α-Phellandrene	0.39	1010
6	α-Terpinene	0.13	1021	α-Terpinene	0.48	1021
7	*o*-Cymene	12.04	1031	*p*-Cymene	11.35	1032
8	D-Limonene	2.14	1034	D-Limonene	0.88	1034
9	β-Phellandrene	0.39	1036	1,8-Cineole	0.75	1036
10	γ – Terpinene	9.77	1064	γ -Terpinene	4.74	1064
11	Fenchone	0.40	1097	Linalool	7.98	1108
12	α-Terpinolene	0.16	1106	Terpinen-4-ol	0.65	1190
13	l-Menthone	0.18	1167	α-Terpineol	0.80	1207
14	Menthol	0.22	1188	Thymol methyl ether	0.44	1238
15	(-)-terpinen-4-ol	0.24	1190	Carvacrol methyl ether	2.02	1247
16	α-Terpineol	0.59	1207	2-Methyl-3-phenyl-propanal	0.69	1258
17	Estragole	0.40	1210	Carvone	0.50	1263
18	Thymol methyl ether	0.17	1238	Thymol	17.86	1307
19	Carvacrol methyl ether	0.23	1247	Carvacrol	36.62	1319
20	Cuminaldehyde	22.34	1261	Thymol acetate	0.60	1356
21	*trans*-Anethole	0.16	1266	Carvacrol acetate	2.01	1375
22	Bornyl acetate	0.20	1293	β-Caryophyllene	0.81	1427
23	Anisole	15.19	1303	(+)-Aromadendrene	0.77	1447
24	Carvacrol	19.88	1307	β-Spathulenol	0.88	1592
25	α-Propylbenzyl alcohol	8.99	1309	Caryophyllene oxide	1.37	1596
26	α-Terpinyl acetate	0.45	1355	-	-	-
27	Caryophyllene	0.28	1427	-	-	-
28	Caryophyllene oxide	0.32	1596	-	-	-
	Total		**99.35**	**Total**		**95.89**

** Kovats retention indices*

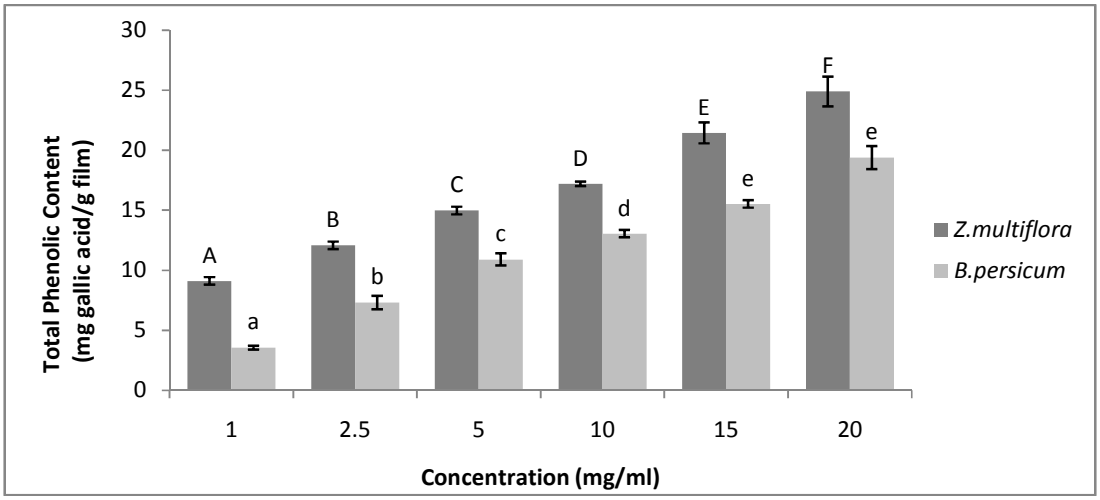

Fig. 1. Total phenolic content of corn starch films incorporated with *Zataria multiflora* and *Bunium persicum* EOs. Different letters in each essential oil indicate significant differences between doses ($P < 0.05$)

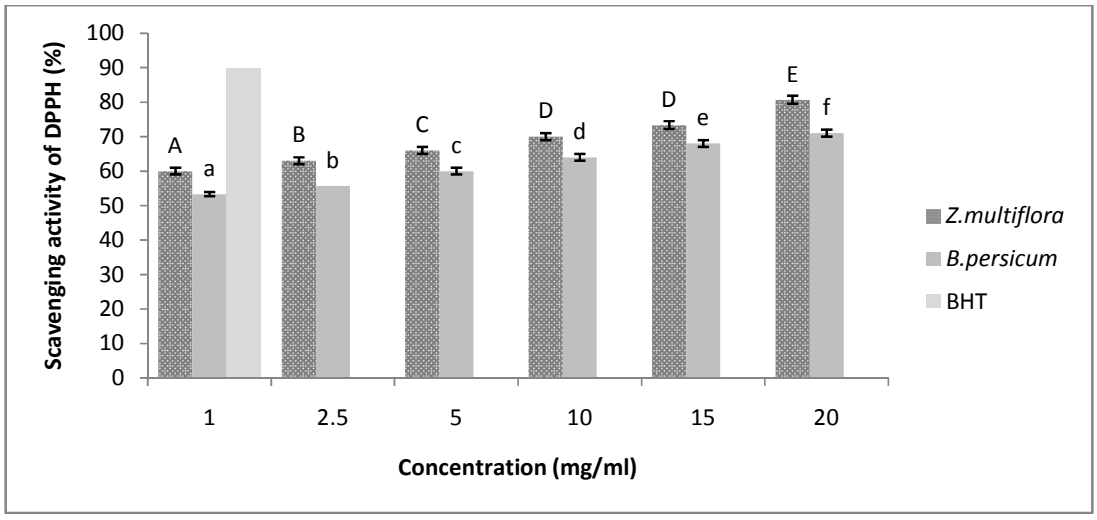

Fig. 2. DPPH scavenging activity of corn starch films incorporated with *Zataria multiflora* and *Bunium persicum* EOs. Different letters in each essential oil indicate significant differences between doses ($P < 0.05$). The BHT (1 mg/ml) was used as positive control

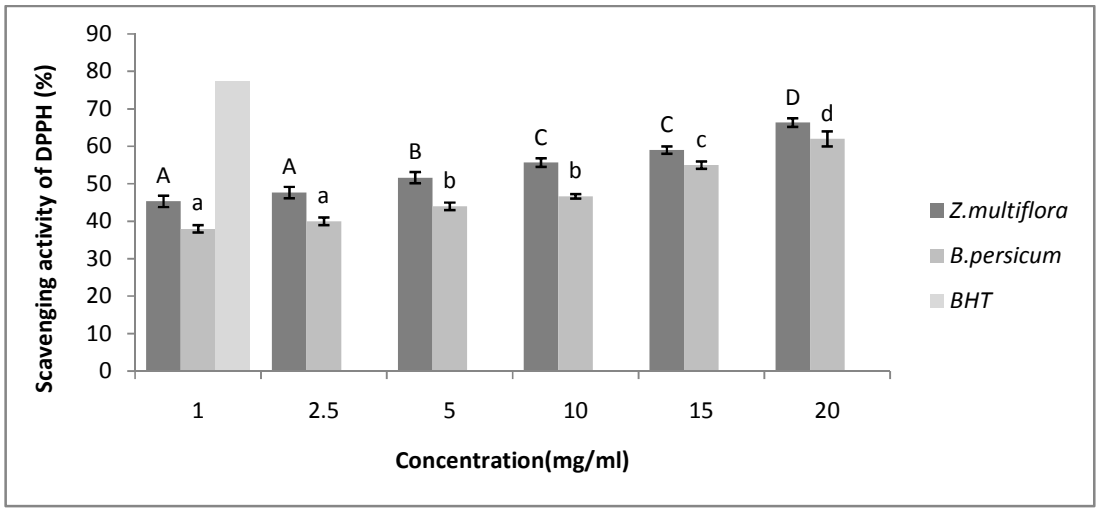

Fig. 3. ABTS scavenging of corn starch films incorporated with *Zataria multiflora* and *Bunium persicum* EOs. Different letters in each essential oil indicate significant differences between doses ($P < 0.05$). The BHT (1 mg/ml) was used as positive control

4. DISCUSSION

4.1 GC-MS

Chemical composition of the EO from *Bunium persicum* harvested in Zanjan city, Iran, was dominated by Cuminaldehyde, Carvacrol, and Anisole respectively. The results also showed that the EO of *Zataria multiflora* was characterized by the presence of three dominating components: Carvacrol (36.62%), thymol (17.86%) and p-Cymene respectively. These compositions were qualitatively the same as the oils from former studies [18,19].

4.2 Total Phenolic Content

The total phenolic content of essential oils was determined by using spectrophotometric method and the reagent Folin-Ciocalteu and gallic acid was used as standard. In our study, the phenol content of films incorporated essential oils of *Zataria multiflora* and *Bunium persicum* were in the range of 10-24 and 3-20 mg gallic acid/g film,

respectively. On the basis of FC results showed in Fig. 1, total phenolic content in the corn starch films significantly was increased with increasing EOs concentration ($P < 0.05$). The highest TP content was observed in film incorporating 20 mg/ml of ZMEO. According to Dashipour et al. [20] study on carboxymethyl cellulose films containing *Zataria multiflora* essential oil, total phenolic content was 20 mg GA/g film in concentration of 3% of essential oil, this result is an agreement with our study. It was determined that positive correlation was between antioxidant activity and amount of total phenolic content in BPEO.

4.3 DPPH Scavenging Assay

This method was used to indicate antioxidant activity of the film. Starch films showed radical scavenging activity of 80 and 71% in highest concentration for ZMEO and BPEO, respectively. As the concentration of EOs increased, DPPH scavenging activity of the films increased significantly ($P < 0.05$) as shown in Fig. 2 but it was lower than synthetic antioxidant agent BHT (89%). It seems that this activity is mostly related to the presence of the phenolic compounds in the polar fraction [21,22]. Similarly, in a former study, antioxidant effect of carvacrol and thymol were compared with BHT using DPPH method and results showed that thymol antioxidant activity was superior to that of carvacrol, possibly due to greater steric hindrace of the thymol phenolic group; but BHT with a hydroxyl group sterically hindered had higher antioxidative activity, when compared to both carvacrol and thymol [23].

ZMEO exhibited a higher level of radical scavenging activity than BPEO films. Similar result was found in a study for active film formulated with *Zataria multiflora Boiss* and *Mentha pulegium* essential oils [14] as well as a nonocomposit film containing rosemary essential oil [24]. In another study soybean film incorporated with 1% and 2% *Zataria multiflora Boiss* EO exhibited 59% and 69% DPPH scavenging activity [13] and show a bit lower than our study. This difference possibly due to different reaction time with DPPH˚ and another reason is the concentration of the antioxidant versus DPPH˚ [25].

4.4 ABTS Scavenging Assay

According to the results obtained, starch films containing different *Zataria multiflora* and *Bunium persicum* concentrations decolorize ABTS dose-dependently. As it is obvious, the following Fig. 3 by increasing in the EOs concentrations results more intensive increasing in antioxidant activity ($P < 0.05$) and they were 66 and 62% in the highest concentration for films incorporated ZMEO and BPEO, respectively but they showed lower antioxidant activity than BHT (77%). In addition ZMEO shows better antioxidant activity than BPEO. Zangiabadi et al. [26] reported that the scavenging capability of ZMEO and BPEO were 60 and 70% respectively.

Generally, direct use of essential oils is often limited in final product, due to their strong stimulation of human gustatory and olfactory senses. So, using active packaging with no contact between the essential oil and the food has solved this issue and many studies have shown that addition of essential oils and extracts into films not only has no negative effects on sensory attributes but also, can prevent taste transfer, reduce organoleptic changes and distribution of the active compounds in the headspace [27,28].

The result obtained in this work indicated that starch is a natural polymer has great potential for usage in bio-based packaging materials. Also, ZMEO and BPEO are good sources of natural antioxidants however antioxidant activity of ZMEO was higher than BPEO. Thymol and carvacrol are the main compounds in *Zataria multiflora* essential oil and γ-terpinene, β-pinene and cuminaldehyde are major constituents *in Bunium persicum* essential oil. High antioxidant effect of films incorporated these two essential oils could be related the presence of these phenolic compounds on their essential oils [29, 30]. Phenolic compounds are able to act as antioxidants in different ways. They can scavenge some active species, containing hydroxyl, peroxyl and superoxide radicals or bind pro-oxidant metals, like iron and copper, avoiding the formation of free radicals [31].

Moreover, the antioxidant effect of ZMEO and BPEO enriched films should be determined on an entire model food.

5. CONCLUSION

The present study revealed significant antioxidant effect of *B. persicum* essential oil and *Z. multiflora* essential oils for scavenging free radicals; although *Z. multiflora* EO had better antioxidant properties than *B. persicum* EO. Based on the results, a remarkable potency was identified in antioxidant activity of both EOs using

different assays; therefore they can be used as natural preservatives in food industries.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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