Journal of Medicinal Plants and By-products (2022) Special Issue: 67-75

Original Article

Antioxidant Efficacy of Biodegradable Starch Film Containing of *Bunium persicum* Essential Oil Nanoemulsion Fortified with Cinnamaldehyde

Running Title: Antioxidant Effect of Films Containing BPEO Nanoemulsion Fortified with CIN

Borzoo Taami[1], Kobra Rostamizadeh[2], Majid Aminzare[1] and Hassan Hassanzadazar[1*]

[1]*Department of Food Safety and Hygiene, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran*
[2]*Department of Medicinal Chemistry, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran*

Article History: Received: 06 June 2020/Accepted in revised form: 06 March 2021

Abstract

This study aimed to evaluate antioxidant effects of biodegradable starch film containing nanoemulsions of *Bunium persicum* (Boiss.) B.Fedtsch. essential oil (BPEO) fortified with Cinnamaldehyde (CIN). DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radicals scavenging assay and Folin-Ciocalteu methods were used to determine antioxidant potential of corn starch films containing nanoemulsions of *B. persicum* essential oil fortified with cinammaldehyde (NanoBP en cin). Prepared treatments were films containing BPEO, CIN, the combination of BPEO and CIN (BPEO+CIN), BPEO nanoemulsion and of BPEO nanoemulsion combined with CIN (BPEOne+CIN) and BPEO nanoemulsion fortified with CIN (NanoBP en cin) at 0.25-2.5–5-10 and 20 (mg/ml) concentrations in corn starch solution. Chemical analysis showed that the main constituents of the BPEO were cuminaldehyde (22.34%), carvacrol (15.70%), anisole (15.19%) and ortho-Cymene (12.04%), respectively. Droplet size and their polydispersity index (PDI) of BPEO nanoemulsion and NanoBP en cin were 131.1±7.2 nm (PDI=0.283) and 201± 24.57 nm (PDI=0.212), respectively. Phenolic compounds and antioxidant capacity of the treatments increased significantly with enhancing of the concentration of EO and CIN. ($P < 0.05$). The Films containing NanoBP at different concentrations had the more antioxidant effect than the other treatments. The lowest radical inhibition effect was for the films containing NanoBP en CIN. The results of this in vitro study showed that food packaging with corn starch films containing BPEO nanoemulsion is a good option for active packaging and have considerable antioxidant properties, but combining or fortification of nanoBPEO with other active ingredients like CIN are not recommended.

Keywords: Antioxidant, Starch film, Nanoemulsion, *Bunium persicum* essential oil, Cinnamaldehyde

Introduction

Organoleptic quality and safety of food can undergo undesirable changes due to the probably microbial and chemical spoilage such as lipid oxidation resulted decline in nutritional value, unpleasant flavor and food borne diseases [1]. Extending of food shelf life is one of the most important goals of food hygiene and the biggest challenge for the food industry [2]. In recent years, the food packaging industry has made great efforts to discover new ways to protect food products against environmental conditions, mechanical stresses, chemical and microbial corruption [3].

Active packaging is a new packaging system introduced to food industry using a variety of ingredients to increase shelf life and maintain or improve the food quality from production to consumption [2]. In this system of packaging, active ingredients are incorporated directly into packaging materials. Plastic packaging has to be minimized and eliminated in the food industry because of the serious environmental problems. Biopolymers are the best solution of this problem. Polysaccharide, protein or lipid based biopolymers are used as edible films or coating materials in active packaging [4]. Edible films and coatings are biodegradable can carry functional additives such as antioxidants, antimicrobials and tissue enhancers that have received considerable attention as a thin layer on the surface of food to control the physical, chemical, and microbial changes [4]. These additives are able to migrate from packaging to food and improve the food quality and safety, thereby prolonging the shelf life

*Corresponding Author: Department of Food Safety and Hygiene, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran
Email Address:Hassanzadazar_h@zums.ac.ir

of the food [5]. Chitin, chitosan, cellulose, pectin, starch derivatives, gums, alginate, carboxy methyl cellulose (CMC), galactomannans are main polysaccharides have been used to produce biodegradable films [4]. Starch as an available and renewable compound with very low oxygen permeability is widely used to produce edible films [6].

Antioxidants' adding to fatty foods lead to extending food shelf life. Their mechanism of action is to delay lipid peroxidation in food [7]. Since the beginning of the 20th century, different synthetic antioxidants such as butyl hydroxyisoenzole (BHA), butyl hydroxy toluene (BHT), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ) have been used in the food industry. But the use of these compounds is limited due to the possibility of their undesirable effects on human health. This has led to much research on natural antioxidants with plant origin in recent years. Essential oils (EOs) are more accepted by consumers as natural substitutes for chemical preservatives in food products [1]. They are aromatic and volatile oil compounds extracted from different parts of plants such as roots and leaves [3]. Many herbal essential oils are listed as the generally accepted as safe (GRAS) compounds have been approved by the FDA to use in food and beverage [8].

Black caraway (*Bunium perssicum*) (BP) is a perennial herb from the umbrella family that is native to Iran. The seeds of this plant are widely used as seasonings in traditional foods as well as in the food industry [9]. The main components in extracted EO of this plant which have been measured in various studies were γ-terpinene, couminaldehyde, limonene and ρ-cymene. There are numerous studies on the antioxidant properties of this plant that confirm the antioxidant properties of this plant [9-11]. Cinnamon also has good antioxidant properties due to its phenolic and other antioxidant compounds [7]. The most important component of cinnamon essential oil is cinnamaldehyde (CIN), which contains about 97.7% volatile oils of this essential oil and exhibits good bioactive properties. Today, the use of active ingredients in the EOs of various herbs, such as cinnamaldehyde, thymol, eugenol, carvacrol and limonene, has been accepted as essential preservatives that do not treat human health [8].

Direct use of EOs or their active ingredients in various dilutions is one of the most widely used methods to increase food safety and shelf life. However, the use of these compounds in food systems is largely limited due to their effects on sensory acceptance of food. In addition, due to the low solubility of the EOs in water, it has become relatively difficult to disperse these compounds in juicy food products [12]. These problems can be overcome by EO encapsulation. Currently, nanotechnology is increasingly becoming an important part of the food industry. By encapsulating antioxidant compounds in nanotechnology, the release of nanoparticles is controlled and accepted, resulting in minimized exposure to undesired reactions and adverse effects on the sensory properties of the food product. Nano scaling of EO increase the site of action per unit of mass and thus reducing the used amount of these compounds [13]. As the nanoparticles in the mass unit have a larger surface area, they are expected to be biologically more active. There are different types of delivery systems in nanotechnology that need to be formulated and processed properly. Nano-encapsulation, nanoemulsions, and nanoparticles appear to be effective for food applications [13]. Nanoemulsions are increasingly being used to encapsulate, protect and deliver lipophilic compounds in foods that are processed with minimal processing [12]. They can be added to an edible film ingredient. There are several reports about the beneficial effects of EO loaded nanoemulsions incorporated in edible films as food preservatives in food packaging [14-16]. But, based on our literature review, there are no studies have been conducted to evaluate antioxidant effects of the combination nanoemulsion of *B. persicum* (BPEOne) with CIN and fortified nanoemulsion of BPEO with CIN in edible films. Therefore, the purpose of this study was to evaluate antioxidant effects of biodegradable starch film containing nanoemulsions of *B. persicum* essential oil fortified with cinnamaldehyde.

Material and Methods

Materials

All used chemicals including corn starch, glycerol, Tween 80 and cinammaldehyde in this study were purchased from Merck (Merck, Darmstadt, Germany) and Sigma Aldrich, St. Louis, USA) Companies.

Essential Oil's Extraction

Dried seeds of black cumin (*B. persicum*) were purchased from a local market in Zanjan city, Iran. The taxonomic identification of plant materials was confirmed by the Iranian Institute of Medicinal Plants, Karaj, Iran with an IMPH-7036 voucher number. The hydro-distillation method was performed to extract of the EO. For this purpose, 100 g of plant seed was mixed with distilled water (900 cc) in 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 (Fig. 1). This procedure was performed about 15 times (using fresh materials each time) to obtain sufficient EO which dehydrated with sodium sulfate, filtered by 0.22 μm filters and stored in a dark tube at 4°C for further experiments.

GC/MS Analysis

GC/MS analysis of EO were performed using a Hewlett Packard 5890 (Agilent, USA) 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) [17].

Nanoemulsion Preparation of *B. persicum* Essential Oil

In this study, different formulations with different concentrations of emulsifier were set to determine the best mixture and most stable solution (Table 1). Then particle size was determined using a ZetaSizer (ZEN3600 Malvern, UK). The obtained nanoemulsions were stored in dark glass vials at 4 °C [15]. The droplet size of the nanoemulsions was measured frequently for 3 months with weekly interval and mixture with the smallest droplet size and stablity mixture was set as the main formula to continue our study. Based on these items, the 3th and 4th formula showed the best stability with acceptable droplet size. The best formula was determined based on their effect on prepared films. Briefly, to prepare the 4th nanoemulsion of BPEO, at first combination of 4.3% (w/w) of twin 80 with 5 ml of deionized water placed on a stirrer (RH basic 2, IKA, Germany) for 15 minutes to homogenize well then gradually 6% (w/w) of the BP essential oil was added to the solution and placed again on the stirrer for 15 minutes. The solution was homogenized using an ultrasound homogenizer (Silent Crusher M-Heidolph, Germany) for 15 minutes at 13500 rpm until BPEO nanoemulsions were obtained (Fig. 2).

To prepare of BPEO nanoemulsion fortified with CIN, a similar method was implemented to prepare BPEO nanoemulsions, except that cinnamaldehyde 6% (w/w) was added to the prepared solution in the previous step before homogenization.

Preparation of Starch Films Containing Different Concentrations of BPEO and Its Nanoemulsions

To prepare films, 3 grams of corn starch was dissolved in 100 mL distilled water plus with 1.8% glycerol as a plasticizer and heated at 90 °C on a magnetic stirrer for 10 min. After obtaining a uniform solution, the solution was cooled to about 40 °C and the antioxidant compound (BPEO and BPEO nanoemulsions) were added to the coating suspension at of 1.25 - 2.5 – 5- 10 and 20 (mg/mL) concentrations and homogenized for 2 minutes at 2000 rpm. 11 mL of this solution was poured in petri dish place at room temperature for 24 h to dry (18). Because of cracking prepared films by the 5th formula of BPEO nanoemulsion, the 4th formula was chosen as the best prepared nanoemulsion (Table 1). Prepared treatments were films containing BPEO, cinnamaldehyde, the combination of BPEO and CIN (BPEO+CIN), BPEO nanoemulsion and of BPEO nanoemulsion combined with cinammaldehyde (BPEOne+CIN) and BPEO nanoemulsion fortified with cinnamaldehyde (NanoBP en cin) in corn starch solution (Fig. 2).

Antioxidant Activity of Impregnated Films

The radical scavenging ability of prepared edible treatments was evaluated using ABTS and DPPH free radical scavenging assays and folin ciocaltue method described by Bitencourt CM *et al*. 2014, Moradi *et al*. 2012 and Salarbashi *et al*. 2014) respectively [19-21].

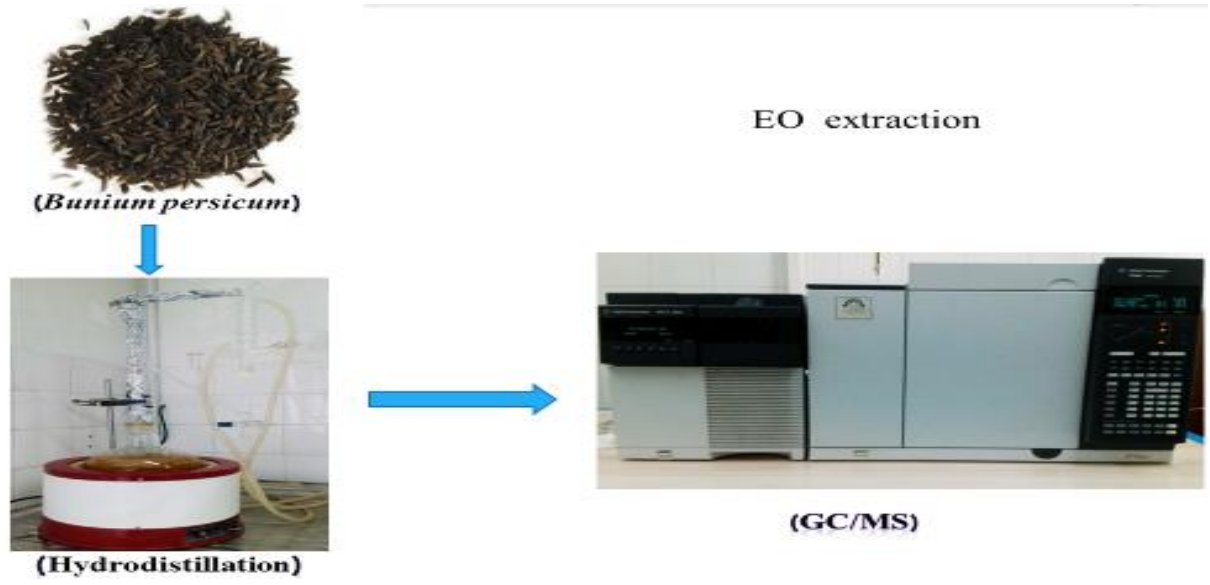

Fig. 1 Essential oil extraction and GC/MS

Table 1 Different formulation to choose best nanoemulsion solution based on PDI and droplet size

NO	BPEO (w/w%)	Tween 80 (w/w%)	Total volume	PDI±SD	Mean size±SD (nm)
1	6	1	80	0.351±0.08	126.7±2.39
2	6	2	80	0.330± 0.64	131.1±2.2.41
3	6	3	80	0.332±0.53	124.42±0.76
4	6	4	80	0.282±0.07	131.3±7.21
5	6	5	80	0.366±0.2	141.48±1.31
6	6	6	80	0.444±0.21	136.58±1.25
7	6	7	80	0.541±1.2	184.31±0.73
8	6	8	80	1.00±0.03	188.04±0.85
9	6	9	80	0.867±0.94	140.3±4.1
10	6	10	80	1.000±0.01	91.82±3.25

Nanoemulsion preparation of *B. persicum* essential oil fortified with cinnamaldehyde

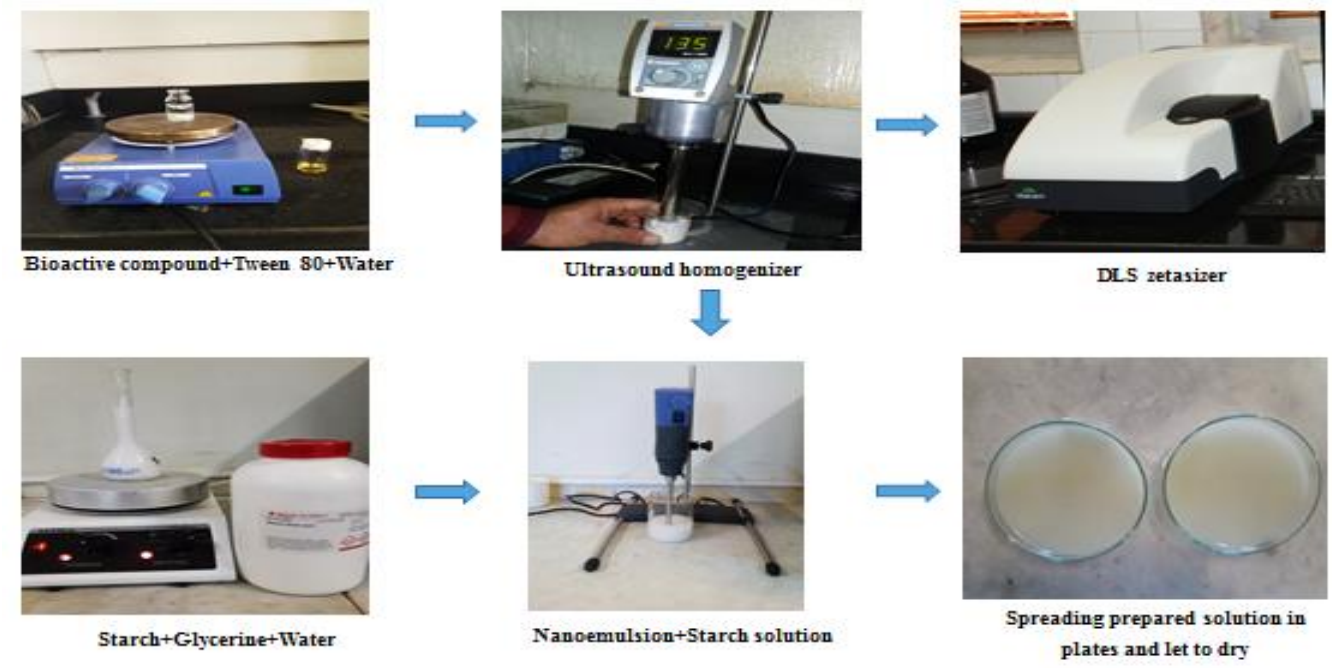

Fig. 2 Preparation of starch films containing different concentrations of BPEO and its nanoemulsions

Statistical Analysis

SPSS statistical software, Version 18.0 (SPSS Inc., IL, USA) was used for statistical analysis (ANOVA test) based on the three replications of each experiment. To analyze differences between the treatments, Tukey's post hoc test was used. Data were expressed as mean ±SD.

Results

GC/MS

As is shown in Table 2, twenty-nine compounds were identified, representing 99.35% of the BPEO. Chemical analysis showed that the main constituents of the EO were cuminaldehyde (22.34%), carvacrol (15.70%), anisole (15.19%) and ortho-Cymene (12.04%), respectively.

The droplet's size of BPEO nanoemulsions is illustrated in Figure 3. The droplet size and their polydispersity index (PDI) of BPEO nanoemulsion and fortified BPEO nanoemulsion with cinnamaldehyde were 131.1±7.2 nm (PDI=0.283) and 201±24.57 nm (PDI=0.212), respectively.

Total Phenolic Content

Table 3 shows the results of the loaded corn starch films using the Folin-Ciocalteu method. The results were stated in mg Gallic acid per gram. This method is based on the oxidation of phenolic groups by phosphomolybdate and phosphotungstenic acid. Subsequent oxidation and formation of green-blue complex was measured at 765 nm.

Antioxidant Activity of Impregnated Starch Films

In this study, the ability of different treatments to neutralize DPPH and ABTS free radicals was compared to BHT-containing films. According to the obtained results, in all prepared films, antioxidant power of the starch films was significantly increased by increasing concentration of the EO and CIN (In both free and nanoemulsion treatments) (Table 4) (P<0.05). The antioxidant activity of loaded treatments was attributed to the high content of phenolic compounds (Table 3 and Table 4). BHT was tested as a positive control to evaluate the accuracy of the DPPH and ABTS tests at a concentration of 1 mg/mL in prepared films with an antioxidant activity of 88.33% and 75.33% in DPPH and ABTS scavenging assays, respectively.

Discussion

Essential oils are very complex natural compounds that can contain about 20 to 60 compounds at different concentrations [9, 14]. It should be noted that the EO constituents of each studied plant are affected by several factors such as genetic diversity of plant and locality, climatic, seasonal and laboratory conditions (22). These factors may explain the difference between our study results and previous studies (Table 5).

Based on our literature review, there are no similar study conducted o BPEO nanoemulsions. Salvia-Trujillo *et al*., 2015 and Moghimi *et al*., 2015 conducted studies to evaluate antimicrobial effects of Thyme EO nanoemulsion. Mean droplets size of nanoemulsions was reported 86.12±0.25 and 143.2±6.2 nm, respectively [16].

Table 2 GC/MS analysis of *B. persicum* (Boiss.) B.Fedtsch. essential oil

NO	Compound name	Retention time	Area (%)	KI (Kovats Index)
1	α-Thujene	11.30	0.11	927
2	α-Pinene	11.69	0.32	934
3	β-Pinene	14.04	2.34	981
4	β-Myrcene	14.61	0.43	992
5	α-Phellandrene	15.55	1.28	1010
6	α-Terpinene	16.10	0.13	1021
7	Ortho-Cymene	16.64	12.04	1031
8	D-Limonene	16.78	2.14	1034
9	β-Phellandrene	16.89	0.39	1036
10	Gamma.-Terpinene	18.34	9.77	1064
11	Fenchone	20.07	0.40	1097
12	α-Terpinolene	20.52	0.16	1106
13	l-Menthone	23.53	0.18	1167
14	Menthol	24.59	0.22	1188
15	(-)-terpinen-4-ol	24.71	0.24	1190
16	α-Terpineol	25.52	0.59	1207
17	Estragole	2565	0.40	1210
18	Thymol methyl ether	26.97	0.17	1238
19	Carvacrol methyl ether	27.40	0.23	1247
20	Cuminaldehyde	28.06	22.34	1261
21	Trans-anethole	28.32	0.16	1266
22	Bornyl acetate	29.55	0.20	1293
23	Anisole	30.02	15.19	1303
24	Carvacrol	30.19	15.70	1307
25	α-Propylbenzyl alcohol	30.29	8.99	1309
26	Carvacrol	30.51	4.18	1314
27	α-Terpinyl acetate	32.35	0.45	1355
28	Caryophyllene	35.47	0.28	1427
29	Caryophyllene oxide	42.33	0.32	1596
	Total	-	99.35	-

Droplet size measurement

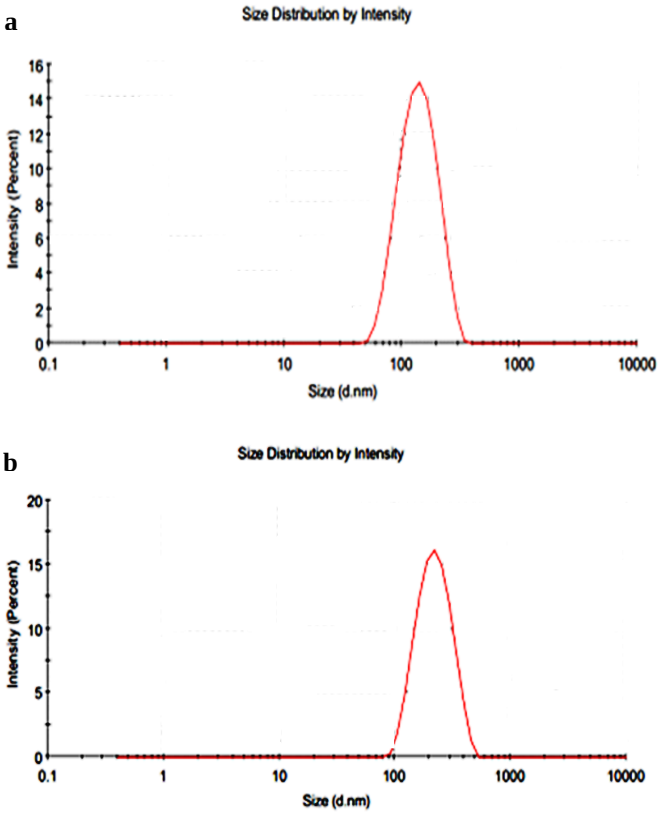

Fig. 3 Particle Size of BPEO nanoemulsion (a: Droplet size of BPEO nanoemulsion; b: Droplet size of BPEO nanoemulsion fortified with Cinnamaldehyde)

Several factors can impress on the final size of droplets in EO's nanoemulsions including type and concentration of EO and surfactant, preparing method and environmental condition (pH, presence of metallic ions and etc.) [29]. Emulsions are heterogeneous colloidal systems consisting of a mixture of two immiscible liquids.

These mixtures are thermodynamically unbalanced and may gradually lose their stability [30]. Many of the emulsion's properties such as stability, rheology, appearance, color, and texture depend on intermediate diameter and the size distribution of droplets. The smaller emulsion droplet size, the greater its stability against precipitation or creaming [30]. Therefore, precise control of droplet size in emulsions and the degree of non-uniformity of a size distribution of particles (PDI) prepared for various applications are of the utmost importance.

Results showed that phenolic compounds increased significantly with enhancing of the concentration of EO and CIN. ($P<0.05$). The highest amount of phenolic compounds was observed in the films containing nanoemulsion of BPEO in the range of 4.2 to 22.78 mg Gallic acid/g followed by BPEO treatments at concentrations of 1.25 to 20 mg/mL. Phenolic compounds in the BPEO+CIN treatment were lower than the films containing the BPEO alone. Among the combined treatments, the films containing nanoemulsions of BPEO and CIN (NanoBP+CIN) showed the highest phenolic compounds (20.3 mg gallic acid/g) at 20 mg/mL concentration. The lowest amount of phenolic compounds was also observed in the films containing nanoemulsions of BPEO fortified with CIN (NanoBP en CIN) (2.59 mg Gallic acid/g) (Table 3).

The phenolic compounds have an important role in enhancing the antioxidant properties of the EOs due to their high potential for inhibiting free radicals. Many factors can affect the amount of phenolic compounds in the plant's EO including geographical location, environmental and climatic conditions, growing season of plant, storage and processing conditions [3].

Films containing NanoBP at different concentrations had the more antioxidant effect than the other treatments and showed the closest antioxidant effect to the films containing BHT. The best inhibitory effects were seen at the 20 mg/mL concentration equal to 81.53% and 74.43% in DPPH and ABTS scavenging assays, respectively. This is probably due to the reduction in particle size and increasing the surface area to volume ratio at the nanoscale, which can enhance the scavenging effect of free radicals [3].

Table 3 Total phenolic content of loaded corn starch films by Folin-Ciocalteau method ($P<0.05$)

	TP concentration (mg Galic acid /g)				
Studied groups(mg/mL)	1.25 (mg/mL)	2.5 (mg/mL)	5 (mg/mL)	10 (mg/mL)	20 (mg/mL)
BPEO	3.72±0.21 ACEa	7.44±0.19 ADEb	11.44±0.93 ACEc	14.88±0.84 ACDEFd	20.8±0.25 ACEFe
CIN	5.25±0.35 Ba	6.16±0.41 BCDEb	7.37±0.25 BFc	7.87±0.04 Bd	12.54±0.69 Be
BP+CIN	3.23±0.32 CADEa	6.52±0.14 CBDEb	10.83±0.98 CAEc	14.5±0.62 CADEFd	20.13±0.39 CAEFe
Nano BP	4.2±0.58 DABEa	6.79±0.41 DABCEb	13.16±0.42 Dc	16±0.3 DACEFd	22.78±0.7 De
Nano BP+CIN	3.37±0.71 ECFa	6.79±0.19 EBCDb	10.96±0.11 EACc	16.17±0.05 EACDFd	20.3±0.32 EACFe
NanoBP en cin	2.59±0.3 FCEa	3.9±0.33 Fa	7.43±0.21 FBb	14.24±0.31 FACDEc	19.2±1.27 FACEd

Different uppercase letters show significant difference between treatments in each concentration (column)
Different lowercase letters show significant difference for each treatments in different concentrations (row)
BPEO: *Bunium percicum* essential oil; CIN: Cinamaldehyde; BP+CIN: Combination of BP essential oil and CIN; Nano BP: Nanoemulsion of BPEO; Nano BP+CIN: Combination of BP essential oil with CIN; NanoBP en cin: fortified NanoBP with CIN.

Table 4 Radical scavenging ability of stach films containg different concentrations of BPEO, CIN, BP nanoemulsions based on DPPH and ABTS scavenging assays (Mean±Standard Deviation)

Test	Studied groups	Concentrations (mg/ml)					
		1	1.25	2.5	5	10	20
DPPH	*B. persicum*	-	0.8 Aa±51.73	57.13±0.8 Ab	0.23 Ac±59.06	0.8 Ad±62.13	0.8 Ae±69.23
	Cinnamaldehyde	-	0.41 Ba±13.86	0.7 Bb±15.73	0.6 Bc±17.3	0.5 Bc±18.5	0.4 Bd±20.83
	BP + CIN	-	0.5 Ca±37.5	0.25 Cb±40.23	0.64 Cc±42.26	0.52 Cd±44.6	0.25 Ce±49.73
	Nano BP	-	0.9 Da±62.13	0.56 Db±67.46	0.35 Dc±70.33	0.55 Dd±72.96	0.5 De±81.53
	Nano BP+CIN	-	1.13 Ca±39.2	0.28 Eb±42.8	0.63 Ec±44.55	0.28 Ed±47.2	0.28 Ee±51.2
	NanoBP & cin	-	1.41 Fa±34	0.28 Fb±37.6	0.77 Fc±39.45	0.07 Fd±40.95	0.7 Fe±45.5
	BHT	88.33±0.57					
ABTS	*B. persicum*	-	28.76±0.68 Aa	41.43±0.51 Ab	44±1.0 Ac	47.23±0.68 Ad	62.3±0.36 Ae
	Cinnamaldehyde	-	10.93±0.11 Ba	11.93±0.2 Bb	13.7±0.36 Bc	14.76±0.25 Bc	19.06±0.4 Bd
	BP + CIN	-	24.53±0.5 Ca	27.23±0.32 Cb	27.26±0.57 Cb	30.66±0.55 Cc	41.23±0.77 Cd
	Nano BP	-	46.36±0.6 Da	50.46±0.86 Db	52.63±0.55 Dc	56.56±0.55 Dd	74.43±0.51 De
	Nano BP + CIN	-	27.53±0.5 Ea	30.06±0.11 Eb	30.8±0.81 Eb	33.6±0.95 Ec	44.36±0.55 Ed
	NanoBP & cin	-	23.11±0.85 Ca	25.06±0.6 Fb	26.93±0.11 Cc	29.7±0.65 Cd	39.43±0.51 Fe
	BHT	75.33%±0.57					

Different uppercase letters show significant difference between treatments in each concentration (column)
Different lowercase letters show significant difference for each treatments in different concentrations(row)
BPEO: *Bunium percicum* essential oil; CIN: Cinamaldehyde; BP+CIN: Combination of BP essential oil and CIN; Nano BP: Nanoemulsion of BPEO; Nano BP+CIN: Combination of BP essential oil with CIN; NanoBP en cin: fortified NanoBP with CIN.

Table 5 Comparison of main compounds of BPEO reported in different studies

Main components	Part of Plant	Origins of plant	References
Cuminaldehyde (22.34%), Carvacrol (15.7%), Anisole (15.19%), Ortho-Cymene (12.04%), Gamma.-Terpinene (9.77%)	Seed	Zanjan, Iran	Present study
γ –Terpinene (21.86%), Cuminaldehyde (17.28%), ρ -Cymene (6.21%), Acetylphenylcarbinol (5.83%), 1-Limonene (2.47%)	Seed	Yazd, Iran	[10]
Cuminaldehyde (11.4%), γ -Terpinene (11.37%), α -Pinene (11.27%), α –Terpinene (11.13%), S-3-Carene (5.74%)	Seed	Kerman,iran	[22]
ρ -Cymene (25.7%), γ –Terpinene (23.9%), Cuminaldehyde (22.6%), p-Mentha-l'3-dien-7-al and p-Mentha- 1,4-dien-7-al (21.9%), β –Pinene (0.5%)	Seed	India	[23]
Carvone (23.3%), Limonene (18.2%), Germacrene D (16.2%), trans-Dihydrocarvone (14.0%), Carvacrol (6.7%)	Fruit	Italy	[24]
Carvone (78.8%), Limonene (10.1%), Cis-Limonene oxide (1.8%), trans-Carveol(1.3%), Menthone(1.2%)	Fruit	Serbia	[25]
Limonene (48.4 %), Carvone (31.1 %), Apiole (12.3 %), Anethole (2.7%), cis-Dihydrocarvone (2%)	Seed	China	[26]
γ –Terpinene (44.2%), Cuminaldehyde (16.9%), ρ -Cymene (8%), Bornyl acetate (2.9%), 1,8-Cineole (2.9%)	Seed	Mashhad, Iran	[27]
γ-Terpinene (46.1%), Cuminaldehyde (15%), ρ-Cymene (6.7%), Limonene (5.9%), α- Pinene (2.7%)	Seed	Kerman, Iran	[28]

The highest inhibitory effect against DPPH and ABTS free radicals in films containing BPEO at a concentration of 20 mg/mL was 69.23% and 62.3%, respectively which were less active than BHT (P <0.05). Aminzare *et al*. 2017 reported that the highest antioxidant properties of starch films containing this EO at a concentration 20 mg/mL was 71% and 62% based on DPPH and ABTS scavenging assays, respectively which is consistent with the results of our study [3].

In both methods CIN containing films had lower inhibitory effects than other treatments (Table 4). It can be due to lower amounts of phenolic compounds (which are highly capable of absorbing free radicals) in films containing CIN (Table 3) (7). This was attributed to the weak synergistic ability of CIN in combination with other compounds as well as its volatility and low heat resistance [7].

Employing the DPPH and ABTS methods, the highest antioxidant activity in films containing combination of NanoBP+CIN at a concentration of 20 mg/mL were 51.2% and 44.36%, respectively, which was less than the antioxidant activity of the films containing NanoBP alone as well as the films containing BPEO (Table 4). Films

containing BPEO+CIN at higher concentration (20mg/mL) showed a 49.73% and 41.23% inhibition activity, respectively, which were lower than films containing BPEO at this concentration (Table 4). Combined treatments containing CIN did not have a significant effect on the antioxidant properties compared to treatments containing BPEO alone. It seems, bonding active sites of CIN and active ingredients of EO can be the main reason of reducing the antioxidant potency of the EO.

Among the combined treatments based on both DPPH and ABTS methods, the lowest radical inhibition effect was for the films containing NanoBP en CIN (45.5% and 39.43%) at a concentration of 20 mg/mL. In addition to bonding of CIN with EO, the possible reason for this reaction could be acting of CIN as a coating on the surface of the nanoparticles which does not allow the interaction between essential oils and free radicals. According to these results, it is possible to recommend loaded starch films with BPEO nanoemulsion as a potential edible food coating to prevent oxidative stress in food.

Conclusion

The antioxidant activity of loaded films increased significantly with increasing concentrations of BPEO and CIN (P <0.05). The results of this in vitro study show that food packaging with corn starch films containing BPEO nanoemulsion is a good option for active packaging and have considerable antioxidant properties, but combining or fortification of nanoBPEO with other active ingredients like CIN are not recommended.

Acknowledgments

The authors thank to Zanjan University of Medical Sciences for financial support under Grant number (A-12-940-1).

References

1. Mazidi S, Rezaei K, Golmakani M, Sharifan A, Rezazadeh S. Antioxidant activity of essential oil from Black zira (*Bunium persicum* Boiss.) obtained by microwave-assisted hydrodistillation. J Agric Sci Technol. 2012;14:1013-1022.
2. Gómez-Estaca J, López-de-Dicastillo C, Hernández-Muñoz P, Catalá R, Gavara R. Advances in antioxidant active food packaging. Trends Food Sci Technol. 2014;35:42-51.
3. Aminzare M, Amiri E, Abbasi Z, Hassanzadazar H, Hashemi M. Evaluation of in vitro antioxidant characteristics of corn starch bioactive films impregnated with *Bunium persicum* and *Zataria multiflora* essential oils. Annu Res Rev Biol. 2017:1-9.
4. Ferreira A, Alves V, Coelhoso I. Polysaccharide-based membranes in food packaging applications. Membranes. 2016;6:22.
5. Piñeros-Hernandez D, Medina-Jaramillo C, López-Córdoba A, Goyanes S. Edible cassava starch films carrying rosemary antioxidant extracts for potential use as active food packaging. Food Hydrocoll. 2017;63:488-495.
6. Acosta S, Chiralt A, Santamarina P, Rosello J, González-Martínez C, Cháfer M. Antifungal films based on starch-gelatin blend, containing essential oils. Food Hydrocoll. 2016;61:233-240.
7. İnanç Horuz T, Maskan M. Effect of Cinnamaldehyde on oxidative stability of several fats and oils at elevated temperatures. Cogent Food Agric. 2015;1:1071725.
8. Prakash B, Singh P, Kedia A, Dubey N. Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant activities and in vivo efficacy in food system. Food Res Int. 2012;49:201-208.
9. Foroumadi A, Asadipour A, Arabpour F, Amanzadeh Y. Composition of the essential Oil of *Bunium persicum* (Boiss.) B. Fedtsch. from Iran. J Essent Oil Res. 2002;14:161-162.
10. Haghirogsadat F, Kalantar S, Sheykhha M, Hokmolahi F, Azimzade M. Survey of effective compounds and antioxidant properties of essential oil of black cumin in Yazd province(In Persian). J Shaheed Sadoughi Univ Med Sci. 2015;22:1592-1603.
11. Oroojalian F, Kasra-Kermanshahi R, Azizi M, Bassami MR. Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. Food Chem. 2010;120:765-770.
12. Acevedo-Fani A, Salvia-Trujillo L, Rojas-Graü M A, Martín-Belloso O. Edible films from essential-oil-loaded nanoemulsions: Physicochemical characterization and antimicrobial properties. Food Hydrocoll. 2015;47:168-177.
13. Bhuyan D, Greene GW, Das RK. Prospects and application of nanobiotechnology in food preservation: molecular perspectives. Crit Rev Biotechnol. 2019;39:759-778.
14. Noori S, Zeynali F, Almasi H. Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (Zingiber officinale) essential oil and its effect on safety and quality attributes of chicken breast fillets. Food Control. 2018;84:312-320.
15. Gahruie HH, Ziaee E, Eskandari MH, Hosseini SMH. Characterization of basil seed gum-based edible films incorporated with *Zataria multiflora* essential oil nanoemulsion. Carbohydr Polym. 2017;166:93-103.
16. Salvia-Trujillo L, Rojas-Graü A, Soliva-Fortuny R, Martín-Belloso O. Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils. Food Hydrocoll. 2015;43:547-556.
17. Sari T, Mann B, Kumar R, Singh R, Sharma R, Bhardwaj M. Preparation and characterization of nanoemulsion encapsulating curcumin. Food Hydrocoll. 2015;43:540-546.
18. Corrales M, Han J H, Tauscher B. Antimicrobial properties of grape seed extracts and their effectiveness after incorporation into pea starch films. Int J Food Sci Technol. 2009;44:425-433.
19. Bitencourt CM, Fávaro-Trindade CS, Sobral PJdA, Carvalho Rad. Gelatin-based films additivated with curcuma ethanol extract: Antioxidant activity and physical properties of films. Food Hydrocoll. 2014;40:145-152.
20. Moradi M, Tajik H, Rohani SMR, Oromiehie AR, Malekinejad H, Aliakbarlu J, Hadian M. Characterization of antioxidant chitosan film incorporated with *Zataria multiflora* Boiss essential oil and grape seed extract. Food Sci Technol. 2012;46:477-484.

21. Salarbashi D, Tajik S, Shojaee-Aliabadi S, Ghasemlou M, Moayyed H, Khaksar R, Noghabi MS. Development of new active packaging film made from a soluble soybean polysaccharide incorporated *Zataria multiflora* Boiss and *Mentha pulegium* essential oils. Food Chem. 2014;146:614-622.
22. Ehsani A, Hashemi M, Naghibi SS, Mohammadi S, Khalili Sadaghiani S. Properties of *Bunium Persicum* Essential Oil and its Application in Iranian White Cheese Against *Listeria Monocytogenes* and *Escherichia Coli* O157: H7. J Food Saf. 2016;36:563-570.
23. Thappa R, Ghosh S, Agarwal S, Raina A K, Jamwal P. Comparative studies on the major volatiles of Kalazira (*Bunium persicum* seed) of wild and cultivated sources. Food Chem. 1991;41:129-134.
24. Iacobellis NS, Cantore P, Capasso F, Senatore F. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J Agric Food Chem. 2005; 53:57-61.
25. Samojlik I, Lakić N, Mimica-Dukić N, Đaković-Švajcer K, Božin B. Antioxidant and hepatoprotective potential of essential oils of coriander (*Coriandrum sativum* L.) and caraway (*Carum carvi* L.)(Apiaceae). J Agric Food Chem. 2010;58:8848-8853.
26. Jiang Z-T, Sun M-L, Li R, Wang Y. Essential oil composition of Chinese caraway (*Carum carvi* L.). J Essent Oil-Bear Plants. 2011;14:379-382.
27. Jamshidi A, Khanzadi S, Azizi M, Azizzadeh M, Hashemi M. Modeling the growth of *Staphylococcus aureus* as affected by black zira (*Bunium persicum*) essential oil, temperature, pH and inoculum levels. Vet Res Forum. 2014;5:107-114.
28. Kareshk AT, Keyhani A, Mahmoudvand H, Oliaei RT, Asadi A, Andishmand M, Azzizian H, Babaei Z, Zia-Ali N. Efficacy of the *Bunium persicum* (Boiss) essential oil against acute toxoplasmosis in mice model. Iran J Parasitol. 2015;10:625.
29. Villamiel M, García-Pérez JV, Montilla A, Carcel JA, Benedito J. Ultrasound in food processing: Recent advances. John Wiley & Sons. 2017.
30. McClements DJ. Food emulsions: principles, practices, and techniques. CRC press. 2015.