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Use of Encapsulated Garlic Oil in Low-Fat Salad Dressings: Physicochemical, Microbial and Sensory Properties

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Abstract

Background and Objective: Fresh garlic includes several preservative and medicinal characteristics; however, its major bioactive components react and convert to other chemical compounds as soon as its tissues are injured. Garlic oil is extracted from garlic and its use in food industries is limited due to its strong odor, taste, volatility and low solubility in aqueous solvents. The aim of the present study was to investigate physicochemical, microbial and sensory properties of low-fat salad dressings containing encapsulated garlic oil in ß-cyclodextrin (GO/ß-CD).

Material and Methods: Five types of salad dressing, including control with no garlic oil, a sample with free garlic oil and three samples containing 546, 818 and 1364 mg GO/ß-CD kg^{-1} salad dressing were prepared. Then, pH, color and antioxidant [(peroxide value and thiobarbituric acid reactive substances], antimicrobial (monitoring of four pathogens of *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Salmonella enterica*) and sensory (taste, color, odor and acceptability by 8-point hedonic test) properties of the samples were assessed during 41 days of storage at 4 °C.

Results and Conclusion: The minimum and the maximum color differences belonged to GO/ß-CD-3 sample containing 1364 mg GO/ß-CD kg^{-1} salad dressing and sample containing free GO, respectively. Yellowness of samples containing encapsulated GO decreased and direct relationships were seen between decreasing of the sample yellowness and quantity of GO/ß-CD sample. During the storage, pH and peroxide value PV slightly increased. *Staphylococcus aureus* was the most sensitive strain to free and encapsulated garlic oil. *Escherichia coli*, *Salmonella enterica* and *Bacillus cereus* showed lower sensitivities to the oil. Sensory results showed no significant differences between the tastes of control and free or encapsulated garlic oil and GO/ß-CD-2 samples containing 818 mg kg^{-1} encapsulated garlic oil, equal to 90 mg kg^{-1} free garlic oil. However, these two treatments included significant differences with samples containing free garlic oil. Results revealed that encapsulation of garlic oil with ß-CD included no adverse effects on taste of the salad dressings. Therefore, GO/ß-CD-2 (818 mg GO/ß-CD kg^{-1} salad dressing) can be suggested as a natural antimicrobial agent for the functional mayonnaise production.

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1. Introduction

In recent years, garlic has been one of the most used plants in food industries. Garlic is used in various forms such as dried, powder, puree, odorless tablet, garlic essential oil (GO), aged extract and encapsulated oil. Organosulfur compounds of GO include antioxidant, antimicrobial (Gram-negative and Grampositive bacteria) and anticarcinogenic

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characteristics [1-3]. The US Food and Drug Administration (US FDA) has approved garlic oil as a generally recognized as safe natural product. Because of its water insolubility, volatility, pungent odor and flavor and low stability, garlic use in food industries is usually limited [4,5]. Using encapsulation methods, GO solubility increases and is protected against light-induced reactions, oxidation and thermal processing as well as decreasing its undesired odors [6].

In one of the earliest studies on the use of GO in meat products, fresh garlic, garlic powder and GO were added to Chinese and chicken sausages, which showed that garlic and its derivatives included no significant antioxidant effects. However, fresh garlic included the highest antioxidant effects. It was concluded that organosulphur compounds were eliminated during garlic powder and GO production [7,8]. Using GO as a natural antimicrobial agent on fresh-cut tomatoes through a controlled release system, free GO included the highest antimicrobial activity. However, the food acceptability was very low. In contrast, samples containing the highest quantities of encapsulated GO showed the lowest microbial growth and the best sensory score [9]. A study on the antimicrobial activity of garlic extract on *Staphylococcus (S.) aureus* and *E. coli* O157:H7 in ready-to-cook chicken and *in-vitro* models showed significant antimicrobial effects of this extract as well as positive effects on the organoleptic properties of the garlic extract [10]. Mozaffari Nejad et al. showed good antibacterial characteristics of aqueous garlic extract on *S. aureus* growth on hamburgers at 4 ºC [11].

A few studies have been published on the use of encapsulated essential oils or extracts in salad dressings. Use of nanoliposomes containing pistachio green-hull phenolic compounds in mayonnaises was carried out by Rafiee et al. After four months of storage at 25 °C, the lowest peroxide value and thiobarbituric acid reactive substances (TBARS) and the best inhibitory effects on total viable counts of *Staphylococcus* (*S.*) *aureus*, molds and yeasts belonged to nanoliposomes containing 2% of lecithin and 1000 mg kg^{-1} phenolic compounds. Loaded nanoliposomes with pistachio green-hull included higher antimicrobial and antioxidant activities, compared to butylated hydroxy toluene (200 mg kg^{-1}) [12]. To fortify low-fat mayonnaises, Rahmani-Manglano et al. used microcapsules of fish oil (glucose syrup and maltodextrin

used as wall materials and whey protein hydrolysate as stabilizing agent). The authors monitored oxidative stabilities of the mayonnaises during storage at 4 and 25 °C. During storage, peroxide value increased; however, glucose syrup-based microcapsules included the best oxidative stabilities when loaded with whey protein hydrolysates to form film [13]. Formulation of functional mayonnaises by adding encapsulated brown algae extract (BAE) in nano-liposomes showed increased antibacterial efficiency of the extract against *Pseudomonas aeruginosa*, *E. coli* and *Bacillus* (*B.*) *cereus* [14]. At the end of storage, samples containing 200 mg kg^{-1} butylated hydroxytoluene loaded with nano-liposomes included significantly lower peroxide values and TBARS than those control and free BAE did. Addition of free BAE caused unpleasant changes in lightness, redness and yellowness as well as taste and acceptance, while nano-liposomes containing BAE and sodium benzoate (1000 mg kg^{-1}) decreased the total viable counts [14]. Mansouri *et al.* studied antibacterial effects of thyme essential oil nano-emulsion against *Salmonella Typhimurium*, *Escherichia* (*E.*) *coli* and *Listeria monocytogenes* in mayonnaises. The optimum nanoemulsion showed good antibacterial effects, the best sensory scores and long-term stabilities, which were linked to the slow release of the essential oils [15].

In the previous reports by the current authors, garlic oil was successfully encapsulated with β-cyclodextrin using co-precipitation method. The maximum release of garlic oil from its inclusion complex (GO/β-CD) under *in-vitro* simulated intestinal fluid (SIF) was nearly 10.29 % [16]. Moreover, free and encapsulated GO showed significant effects on the pathogenic bacterial growth, including *B. cereus*, *S. aureus*, *E. coli* and *Salmonella* (*S.*) *enterica* [17]. However garlic and its essential oil include valuable characteristics, they cannot be used directly in production of low-fat salad dressings (LFSD) due to their water insolubility, pungent odor and low stability. In the present study, various concentrations of encapsulated garlic oil with β-CD were used for the formulation of LFSD. In addition, physicochemical, microbial and sensory properties of the samples were studied.

2. Materials and Methods

2.1. Materials

Garlic oil containing diallyl disulphide (41.33%), diallyl trisulphide (28.82%), methyl allyl sulphide (7.83%), allyl sulphide (6.65 %), diallyl tetrasulphide (3.93%) and 3-vinyl-1, 2-dithiocyclohex-5-ene (3.73%) was kindly provided by Magnolia Flavor and Fragrance (Saveh, Iran) and stored at 4 ºC until use. Microorganisms, including two Gram-positive strains of *B. cereus* PTCC 1015 and *S. aureus* PTCC 1431 and two Gram-negative strains of *E coli* PTCC 1330 and *S. enterica* PTCC 1709 were provided by the Iranian Research Organization for Science and Technology (IROST). The ß-cyclodextrin (β-CD) and culture media [mannitol salt phenol red agar, trypticase soy agar, eosin methylene blue agar and *S. shigella* agar] were purchased from Sigma-Aldrich, USA and Merck, Germany, respectively. Bleached and refined soybean oil without antioxidant was prepared from Oila, Iran. Vinegar, sugar, fresh egg and salt were purchased from local markets. Other chemicals and solvents included analytical grade and used with no further purification

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2.2. Methods

2.2.1. Encapsulation of GO

The complex of GO and β-CD was prepared using co-precipitation method [17]. First, β-CD was dissolved in ethanol: distilled water (1:2, v v^{-1}), mixed at 60 ºC and cooled down. Then, a mix of GO in ethanol (1:2, v v^{-1}) was prepared and mixed with the β-CD solution at 150 rpm for 24 h. This mixture was transferred to an ultrasonic bath (TECNO-GAZ, SPA, Italy) at 80 W for 4 h and then stored 4 °C overnight. The GO/ β-CD complex was filtered using membrane filters (Millipore 0.45-µm HATF filter, mixed cellulose acetate, Millipore, USA).

2.2.2. Formulation of low-fat salad dressings

Refined, bleached and deodorized soybean oil with no synthetic antioxidants was used (15% w w^{-1}) [18]. To prepare treatments of salad dressing, egg yolk (17% w w^{-1}), vinegar (4% w w^{-1}), sugar (5% w w^{-1}), salt (1.5% w w^{-1}) and carboxymethyl cellulose (0.1% w w^{-1}) were mixed together. After adding one-fourth of the needed water, mixture was homogenized using homogenizer (T18 Digital Ultra-Turrax IKA, Germany). Gradually, soybean oil and water were added to the homogen to produce homogenized LFSD. All the samples were stored at 4 °C until use.

To assess preservation activities of GO and GO/ß-CD capsules and sensory properties of the food products, the five treatments were produced as follows: control (with no GO and GO/ß-CD), free garlic oil (GO, 90 mg kg^{-1}), GO/ß-CD-1 (546 mg kg^{-1} of loaded microcapsulesw, equal to 60 mg kg^{-1} free GO), GO/ß-CD-2 (818 mg kg^{-1} loaded microcapsules, equal to 90 mg kg^{-1} free GO) and GO/ß-CD-3 (1364 mg kg^{-1} loaded microcapsules, equal to 150 mg kg^{-1} free GO) [8].

2.2.3. Chemical analysis

Physicochemical and microbial properties of the prepared LFSDs were analyzed during 41 days of storage at 4 ºC. Physicochemical properties such as color, pH, peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were analyzed. Moreover, LFSD properties were analyzed on Days 1, 11, 21, 31 and 41 of production [19,8]. All experiments were carried out in triplicate and results were reported as mean ±SD (standard deviation).

2.2.4. Assessment of color

Color of samples was assessed using Hunterlab Instrument (Colorflex, Virginia, USA). First, instrument was calibrated using black and white tile. Results were shown as a^* (redness), b^* (yellowness) and L^* (brightness) parameters. Total color difference (ΔE) was calculated using the following equation [20]:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \text{Eq.1}$$

2.2.5. Assessment of pH

The pH value of the LFSDs samples was assessed using pH meter (Metrohm 827 pH Lab, Switzerland) [21].

2.2.6. Assessment of PV

Precisely, 1 g of LFSD was weighted in a 15-ml centrifuge tube. After addition of a mixture of chloroform and methanol (1:2) to the tube, mixture was homogenized at 13500 rpm for 2 min using centrifuge (Sigma-Aldrich 3-30 k, USA) and filtered by Whatman filter papers no. 1. Then, 7 ml of the filterate were transferred to another centrifuge tube and mixed with 2 ml of sodium chloride solution (0.5%). Filtrated solution was centrifuged at 3000 rpm for 3 min to form two-phases, then 3 ml of the lower phase, 2 ml of a mixture of chloroform: methanol (1: 2) and 25 ml of ammonium thiocyanate (30%) were mixed and set at room temperature. Absorbances of the samples were measured at 500 nm [22]. This method was carried out for the control without addition of the sample. Calibration curve was prepared using various concentrations of cumene hydro-peroxide and development of the primary oxidation reaction was monitored using calibration curve equation (y = 0.035x + 0.188, r = 0.9985).

2.2.7. Assessment of TBARS

Thiobarbituric acid value is expressed as the malondia-ldehyde (mg) in one kg of the oil. Principle of the method is based on the colored reactions between thiobarbituric acid and secondary products of lipid oxidation. Absorbance of the pink solution of thiobarbituric acid and malondialdehyde addition reaction is read at 530-540 (532) nm. However, other components of alkenals, proteins, browning products and sugar decomposition products may react with thiobarbituric acid, forming colored compositions that can be confusing. To solve this problem, TBARS was measured using the reported method [23-25].

2.2.8. Assessment of antimicrobial activity

Four bacterial strains of *B. cereus*, *S. aureus*, *E. coli* and S. *enterica* were activated according to the provider's instructions. Bacteria were distributed in 1-ml conical tubes under sterilized condition and stored at -20 ºC until use. One day before the experiments, a tube was removed from the freezer and was added with 9 ml of triptone soy agar media. After storing at 37 ºC for 24 h, 0.5 McFarland standard (1.5 × 10^8 cell ml^{-1}) was prepared [26]. Each treatment was inoculated with 0.1 ml of 0.5 McFarland standard for each pathogen (*B. cereus*, 6.36 log cfu ±0.32; *S. aureus*, 6.72 ± log cfu 0.22; *E. coli*, 6.00 ± log cfu 0.26; and *S. enterica*, 6.34 log cfu ±0.35). Since these five treatments were prepared with five replications, 25 tubes were prepared with 10 ml of LFSDs that were inoculated with 0.1 ml of 0.5 McFarland standard for each pathogen [27].

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On Days 1, 11, 21, 31 and 41 of storage, 0.1 ml of the homogenized content was transferred to similar media and cultured using surface culture method. Plate count agar was used for the pathogens and their selective culture media to precisely investigate bacterial colonies. Mannitol salt phenol red agar was used for *S. aureus,* altering yellow to red color due to mannitol fermentation [28]. Tryptone soy agar was selected 3 for *B. cereus* and the large colonies with jagged edges were counted [29]. Moreover, *E. coli* was cultivated on eosin methylene blue (EMB) agar, which its colonies included metallic shiny appearance [30] and *S. shigella* (SS) agar was used to identify *S. enterica* with black colonies resulting from sulfur consumption [31].

2.2.9. Sensory eavaluation of the low-fat salad dressings containing free and encapsulated garlic essential oil

Sensory properties of the treatments were evaluated after 1, 11, 21, 31 and 41 days of storage at 4 ºC by ten trained faculty members, researchers and experts of Nutritional and Food Technology Research Institute, Shahid Beheshti University, Tehran, Iran. Five salad dressings were received a 3-digit code using random table.

After bringing samples out from the refrigerator and reaching the ambient temperature, samples were distributed into 5-ml disposal paper cups and tested by the panelists. A glass of water and crispy bread were served to the panelists to use between the two samples to remove the previous sample effects. Samples were analyzed based on their taste, odor, color and overall acceptability. Sensory analysis was carried out based on the eight scale (1, highly undesirable; 8, highly desirable).

2.3. Statistical analysis

Fully randomized design was used in this study. Comparison of the mean values was carried out using Tukey's test ($p \leq 95$ %) and Minitab Software v.16 (Minitab, USA). Using IBM SPSS v.9.5.00 ed.21 (IBM, USA), sensorial results were analyzed, which the scores were transformed to grades and reported as the mean of grades. In this study, nonparametric Kruskal-Wallis was carried out. If the asymptotic significance was seen (< 0.05), treatments were divided into two groups using Manwittney method to show which treatment included significant differences.

3. Results and Discussion

3.1. Chemical analysis

Tables 1 and 2 show results of color and pH of the samples during storage, respectively. In addition, Figures 1 and 2 represent PV and TBARS results, respectively

Table 1- The changes of color parameters of the samples during 41 days of storage at 4ºC[a].

Char	Treatments	Sampling days				
		1st	11th	21st	31st	41st
a*	Control	9.35±0.05 Aa	7.06±0.05 Bd	6.88±0.07 Cb	6.27±0.02 Db	5.16±0.05 Ea
	GO	8.90±0.06 Ab	7.94±0.06 Bb	5.93±0.02 Ce	5.57±0.03 Dd	2.40±0.02 Ec
	GO/ß-CD-1	9.24±0.03 Aa	8.23±0.07 Ba	7.80±0.08 Ca	7.40±0.02 Da	3.24±0.05 Eb
	GO/ß-CD-2	8.64±0.05 Ac	7.58±0.05 Bc	6.75±0.05 Cc	5.43±0.05 De	2.40±0.07 Ec
	GO/ß-CD-3	7.30±0.05 Ad	6.77±0.08 Be	6.42±0.08 Cd	5.91±0.04 Dc	2.52±0.04 Ec
b*	Control	51.53±0.38 Aa	41.74±0.32 Ba	40.76±0.11 Cc	40.08±0.34 Da	35.50±0.25 Ea
	GO	50.72±0.11 Ab	42.14±0.25 Ba	39.18±0.30 Ce	38.18±0.21 Dc	9.74±0.18 Ec
	GO/ß-CD-1	50.58±0.24 Ab	42.30±0.41 Ba	42.25±0.24 Ba	39.62±0.39 Cb	32.09±0.15 Db
	GO/ß-CD-2	48.97±0.26 Ac	45.03±0.38 Cb	41.41±0.36 Bb	38.34±0.22 Dc	30.08±0.02 Ec
	GO/ß-CD-3	46.35±0.38 Ad	41.89±0.03 Ba	39.66±0.27 Cd	37.52±0.16 Dd	29.42±0.07 Ec
L*	Control	76.86±0.07 Aa	69.66±0.15 Ba	68.85±0.14 Cb	66.11±0.07 Dd	64.71±0.18 Ed
	GO	75.37±0.08 Ad	69.05±0.12 Bb	68.07±0.10 Bb	67.95±0.04 Cb	65.71±0.13 Db
	GO/ß-CD-1	76.64±0.03 Ab	69.16±0.17 Cb	68.69±0.11 Ba	68.37±0.08 Da	66.14±0.10 Ea
	GO/ß-CD-2	76.16±0.07 Ac	69.07±0.02 Bb	69.14±0.04 Bb	67.57±0.12 Cc	65.39±0.12 Dc
	GO/ß-CD-3	75.52±0.05 Ad	68.84±0.03 Bc	68.04±0.03 Cc	66.06±0.09 Dd	64.63±0.06 Ed

[a] Control without garlic oil; GO (containing 90 mgkg^{-1} free GO); GO/ß-CD-1 (containing 546 mgkg^{-1} of GO/ß-CD equal to the 60 mgkg^{-1} free GO); GO/ß-CD-2 (containing 818 mgkg^{-1} of GO/ß-CD equal to the 90 mgkg^{-1} free GO); and GO/ß-CD-3 (containing 11364 mgkg^{-1} of GO/ß-CD equal to the 150 mgkg^{-1} free GO). Different lowercase letters in each column indicate significant difference between treatments.Different uppercase letters in each row indicate a significant difference between treatments ($p \leq 95\%$).

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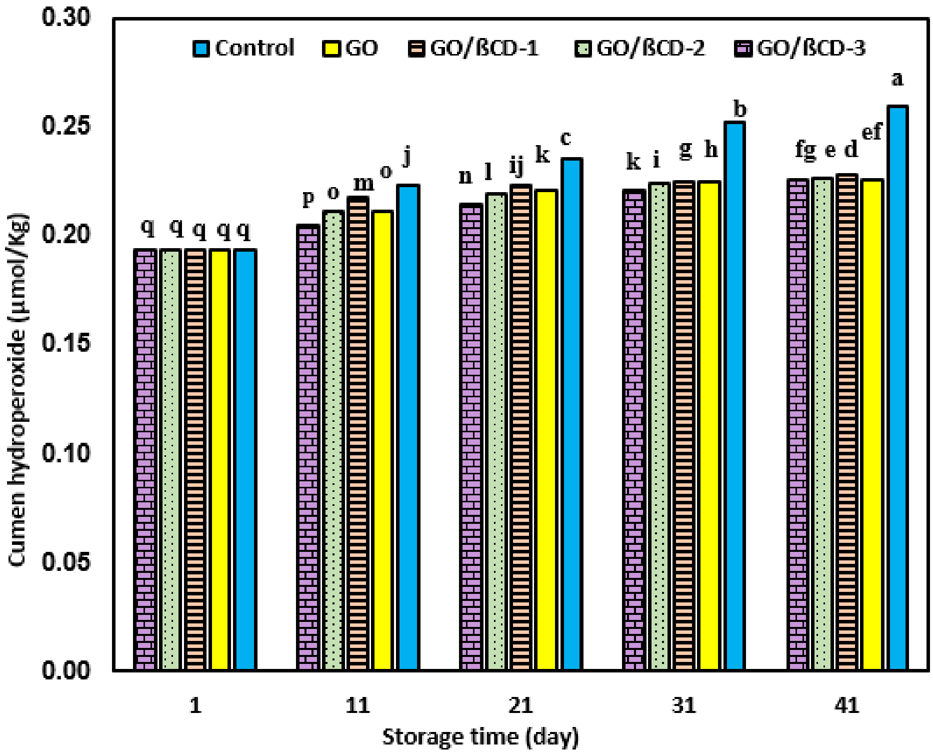

Fig. 1. The changes of peroxide value of samples during 41 days of storage at 4 ºC. Control without garlic oil; GO (containing 90 mg kg^{-1} free GO); GO/ß-CD-1 (containing 546 mg kg^{-1} of GO/ß-CD equal to the 60 mg kg^{-1} GO); GO/ß-CD-2 (containing 818 mg kg^{-1} of GO/ß-CD equal to the 90 mg kg^{-1} GO); and GO/ß-CD-3 (containing 11364 mg kg^{-1} of GO/ß-CD equal to the 150 mg kg^{-1} GO). Different lowercase letters in each column indicate significant difference between treatments (p ≤ 95%).

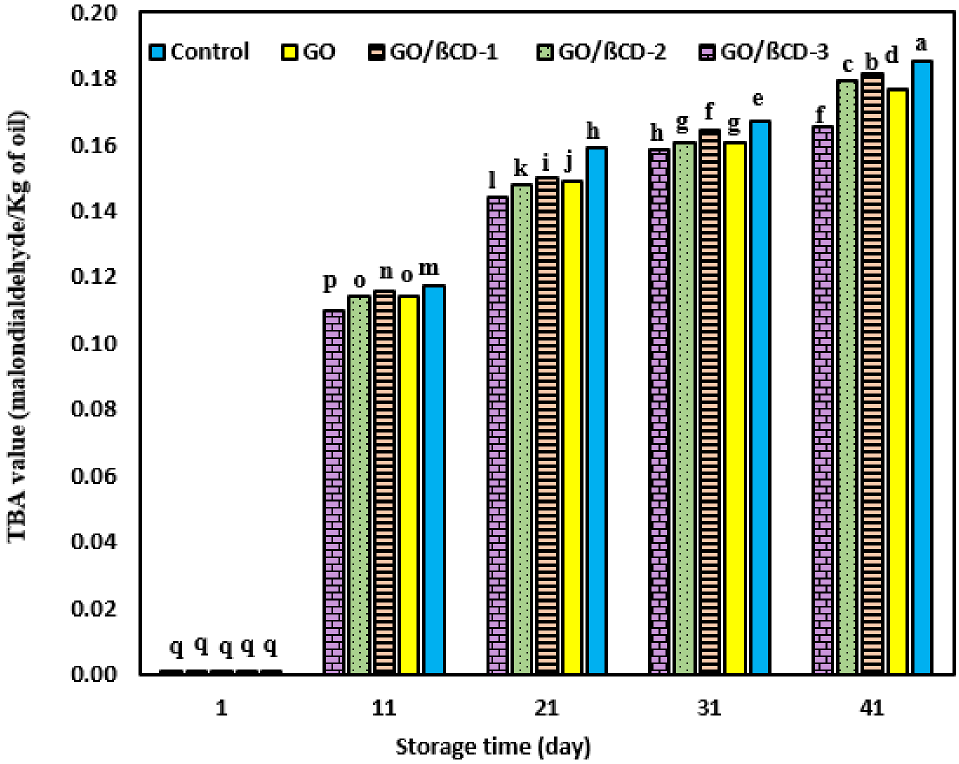

Fig. 2. The changes of TBARS values of samples during 41 days of storage at 4ºC. For abbreviations see

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3.2. Color

Results of redness (a*), yellowness (b*) and luminosity (L*) of various treatments are shown in Table 1. The a* value decreased in all treatments during storage. The highest decrease of a* belonged to the free GO treatment (6.5 U) and the lowest change belonged to the control sample (4.2 U). In all treatments, b* value decreased during the storage. At the end of storage, no significant differences were seen between b* of the samples containing free GO, GO/ß-CD-2 and GO/ß-CD-3, all included the lowest b* values. However, the control sample included the highest b* value during storage.

The L* values of all samples decreased during storage and the lowest values were linked to the control and GO/ß-CD-3, while GO/ß-CD-1 included the highest L* value. By increasing the quanty of GO/ß-CD microcapsules in the samples, the L* value decreased at first and at last days of the storage.

The overall color changes (ΔE) of the samples were monitored during storage. Furthermore, GO/ß-CD-3 and free GO treatments showed the lowest and the highest ΔE, respectively (on Days 1 and 41 of storage). It seemed that b* value included the most effects on ΔE.

3.3. PH

Changes in pH of the samples are presented in Table 2. According to the national standards of Iran, the maximum acceptable pH for mayonnaise and salad dressing is 4.10 [32]. Therefore, pH of the produced samples was slightly greater than this limit (maximum 4.70). As fresh egg (17%), salt (1.5%), sugar (5%) and vinegar (4%) were used for the preparation of salad dressings, it seemed that vinegar was the most affecting factor on pH of these products. The pH difference of the samples on the production day was 0.07 with no significant differences between the samples containing free and encapsulated GO (GO/ß-CD). At the end of the storage, pH differences of the samples were very low (~ 0.02) with no significant differences.

3.4. PV

In this study, PV of the samples increased slowly. However, PV of the control was greater than that of other treatments (Fig. 1). At the end of the storage, no significant differences were seen between the PV of samples containing free GO and GO/ß-CD-2 (both included 90 mg of free GO) and the control sample included the highest PV with no significance, compared to that of other samples (~ 0.02 µmol cuman hydroperoxide kg^{-1} oil). It could be concluded that free GO or its encapsulated form included weak antioxidant activity.

3.5. TBARS

During storage, TBARS content increased and the rate of enhancement was higher in the control sample (Fig. 2). After 41 days of storage, the highest and the lowest TBARS values belonged to the control and GO/ß-CD-3 treatment, respectively.

3.6. Microbial analysis

Changes in microbial growth of the produced LFSDs are shown in Table 3. In general, *S. aureus*, *B. cereus*, *E. coli* and *S. enterica* were inoculated into samples containing no microorganisms. According to the ISIRI No. 2965, salad dressing must include no *E. coli* and *S. aureus* in each gram of the product and *Salmonella* result must be negative in 25 g of the sample. Moreover, samples must not necessarly include *S. aureus* (for the products which pH is greater than 4.1) [33]. In this study, samples included no *B. cereus* and *E. coli;* whowever, *S. entirca* and non-coagulative *S. aureus* were reported after Day 21 of storage. Contamination source of *Salmonella* and S. *aureus* were possibly contaminated raw egg yolks and eggshells, respectively.

The antimicrobial effects of GO on S. *aureus* growth was recorded in free and encapsulated forms. The control sample was non-countable at Day 41 of storage, while samples containing GO, GO/ß-CD-2 and GO/ß-CD-3 were negative from Day 31 as well as GO/ß-CD-1 sample on Day 41 of storage.

Table 2- The changes of pH of the samples during 41 days storage at 4°C*.

Treatments	Sampling days				
	1st	11th	21st	31st	41st
Control	4.67±0.03 Ca	4.74±0.02 Ba	4.84±0.01 Aa	4.85±0.01 Aa	4.86±0.02 Aa
GO	4.71±0.02 Da	4.70±0.01 Ca	4.75±0.00 Bb	4.82±0.01 Aa	4.84±0.01 Aa
GO/ß-CD-1	4.69±0.01 Ca	4.73±0.02 Ba	4.75±0.02 Bb	4.84±0.01 Aa	4.84±0.00 Aa
GO/ß-CD-2	4.64±0.01 Da	4,68±0.01 Ca	4.74±0.02 Bb	4.78±0.01 Aa	4.80±0.01 Aa
GO/ß-CD-3	4.64±0.01 Ca	4.66±0.02 Ca	4.71±0.02 Bb	4.81±0.00 Aa	4.83±0.01 Aa

* For abbreviation see Table 1 footnote. Different lowercase letters in each column indicate significant difference between treatments.Different uppercase letters in each row indicate a significant difference between treatments (p ≤ 95%).

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Table 3- The changes of the microbial growth (logcfu ml^{-1}) of the samples during 41 days storage at 4ºC.

Microorganisms*	Treatments**	Sampling days			
		11th	21st	31st	41st
B. cereus	Control	6.85±0.84 Ca	9.05±0.51 Ba	NC Aa	NC Aa
	GO	3.72±0.22 Bc	3.00±0.00 Bb	3.52±0.53 Bb	4.32±0.59 Bb
	GO/ß-CD-1	5.30±0.61 Ab	4.87±0.23 Ab	5.03±0.26 Ab	5.14±0.29 Ab
	GO/ß-CD-2	3.70±0.69 Bc	3.82±0.99 Bb	3.77±0.68 Bb	4.03±0.51 Bb
	GO/ß-CD-3	2.98±0.30 Bc	2.00±0.00 Bb	3.30±0.61 Bb	3.46±0.41 Bb
S. aureus	Control	10.97±0.03 Ca	12.00±0.70 Ba	NC Aa	NC Aa
	GO	3.00±0.00 Bb	-	-	-
	GO/ß-CD-1	3.60±0.36 Bb	1.48±0.00 Cb	-	-
	GO/ß-CD-2	2.70±0.00 Bb	-	-	-
	GO/ß-CD-3	2.30±0.30 Bb	-	-	-
E. coli	Control	10.48±0.32 Ca	12.59±0.36 Ba	NC Aa	NC Aa
	GO	5.43±0.51 Ab	3.48±0.32 Bb	3.00±0.30 Bb	2.00±0.00 Cb
	GO/ß-CD-1	5.89±0.94 Ab	3.60±0.30 Bb	3.01±0.27 Bb	2.00±0.00 Cb
	GO/ß-CD-2	5.69±0.09 Ab	-	-	-
	GO/ß-CD-3	4.00±0.00 Bb	-	-	-
S. enterica	Control	11.65±1.52 Ca	13.60±0.52 Ba	NC Aa	NC Aa
	GO	2.00±0.00 Bc	2.30±0.35 Bb	2.30±0.00 Bb	2.75±0.39 Bb
	GO/ß-CD-1	4.48±0.49 Bb	3.40±0.78 Bb	2.84±0.06 Bb	1.95±0.05 Cc
	GO/ß-CD-2	2.78±0.12 Bc	2.70±0.20 Bb	2.30±0.00 Bb	1.90±0.00 Cc
	GO/ß-CD-3	2.70±0.40 Bc	-	-	-

* Each treatment was inoculated by 0.1 ml of 0.5 McFarland standard for each pathogens (*B.cereus*, 6.36 ±0.32; *S. aureus*, 6.72 ±0.22; *E. coli*, 6.00±0.26; and *S. enterica* , 6.34±0.35 cfu). ** For abbreviation see Table 1 footnote. Different lowercase letters in each column indicate significant difference between treatments.Different uppercase letters in each row indicate a significant difference between treatments ($p \leq 95\%$). NC; non-countable.

Colonies were non-countable in the control sample at the end of storage while colony numbers of GO, GO/ß-CD-1 and GO/ß-CD-3 treatments decreased during storage. By comparing the growth rates of *S. aureus* and *B. cereus*, it was seen that *S. aureus* was further sensitive to free and encapsulated GO treatments. This finding was similar to the authors' previous finding; in which, *B. cereus* growth was inhibited by increased concentrations of free GO and GO/ß-CD [17].

The current results revealed that colonies were non-countable in the control sample at Day 41 of storage. Furthermore, *E. coli* growth in GO/ß-CD-2 and GO/ß-CD-3 treatments was negative since Day 31. Antimicrobial activity was enhanced by increasing the concentration of encaps-ulated GO. For example, *E. coli* colonies in GO/ß-CD-3 were fewer than those in GO/ß-CD-2 on Day 11. No significant differences were seen between the free GO and GO/ß-CD-1 treatments in all sampling dates.

From Day 21 of storage, no *S. enterica* strain was observed in GO/ß-CD-3 sample. No significant differences were seen between GO, GO/ß-CD-1 and GO/ß-CD-2 since Day 31of storage. In fact, *E. coli* was more sensitive to GO and GO/ß-CD than that *S. enterica* was, possibly because *E. coli* included no growth after 21 days of storage. However, *S. enterica* grew in GO/ß-CD-2 treatment up to the end of storage.

3.7. Sensory evaluation of samples containing free and encapsulated GO

Table 4 shows sensory scores of the samples during 41 days of storage at 4 ºC. The panelist scores were changed to grades by the software.

The average taste scores showed no significant differe-nces within the treatments up to the end of storage. The control sample showed increases up to Day 41.

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Table 4- The changes of sensory characteristics of the samples during 41 days storage at 4°C.

Characteristics	Treatments*	Sampling days				
		1st	11th	21st	31st	41st
Taste	Control	28.50^{Dc}	27.95^{Da}	32.20^{Db}	37.40^{Ad}	26.80^{Cc}
	GO	23.15^{Ac}	19.10^{Ea}	19.50^{Eb}	19.80^{Ea}	23.70^{Dc}
	GO/ß-CD-1	26.10^{Cc}	26.80^{Ca}	26.90^{Cb}	28.15^{Cd}	28.90^{Ac}
	GO/ß-CD-2	25.20^{Bc}	24.80^{Ba}	24.15^{Bb}	17.10^{Ab}	28.20^{Ac}
	GO/ß-CD-3	24.55^{Bc}	28.85^{Da}	23.75^{Bb}	25.05^{Bd}	19.90^{Ec}
Odor	Control	29.25^{Dc}	30.85^{Ed}	32.05^{Ae}	31.20^{Bb}	32.70^{Db}
	GO	24.20^{Bc}	20.10^{Ad}	21.20^{De}	26.20^{Cb}	24.85^{Cb}
	GO/ß-CD-1	29.95^{Dc}	28.00^{Dd}	28.35^{Be}	25.65^{Cb}	25.70^{Cb}
	GO/ß-CD-2	23.15^{Bc}	21.95^{Cd}	18.90^{Ee}	23.50^{Db}	24.00^{Eb}
	GO/ß-CD-3	20.95^{Ac}	26.60^{Bd}	27.00^{Ce}	20.95^{Ad}	20.25^{Ab}
Color	Control	23.40^{Dd}	22.15^{De}	23.10^{Db}	29.70^{Bd}	24.55^{Ec}
	GO	29.00^{Ad}	24.10^{Ce}	22.10^{Db}	23.60^{Cd}	24.55^{Ec}
	GO/ß-CD-1	21.50^{Ed}	24.10^{Ce}	24.40^{Cb}	23.50^{Cd}	23.30^{Dc}
	GO/ß-CD-2	27.85^{Bd}	29.55^{Ae}	30.10^{Ab}	21.50^{Dd}	27.90^{Bc}
	GO/ß-CD-3	25.75^{Cd}	27.60^{Be}	27.80^{Bb}	29.20^{Bd}	27.20^{Bc}
Acceptability	Control	27.75^{Da}	27.70^{Ed}	32.75^{Ab}	34.60^{Bc}	27.75^{De}
	GO	24.65^{Ba}	22.05^{Cd}	22.20^{Cb}	18.10^{Ae}	23.55^{Ce}
	GO/ß-CD-1	29.05^{Ea}	27.45^{Ed}	25.75^{Eb}	27.25^{Gb}	26.70^{Ee}
	GO/ß-CD-2	24.65^{Ca}	24.95^{Bd}	22.85^{Cb}	20.95^{Dc}	24.35^{Ae}
	GO/ß-CD-3	21.40^{Aa}	25.35^{Bd}	23.95^{Bb}	26.60^{Ec}	25.15^{Be}

* For abbreviation see Table 1 footnote. Different lowercase letters in each column indicate significant difference between treatments.Different uppercase letters in each row indicate a significant difference between treatments ($p \leq 95\%$).

Taste score of the sample containing free GO decreased on Day 11 and then increased until the end of the experiments. Furthermore, GO/ß-CD-1 increased in all sampling dates, whereas taste of GO/ß-CD-2 treatment decreased up to Day 31e of storage and then increased significantly at the end of storage. Fluctuating results were observed for GO/ß-CD-3.

No significant differences were seen between the samples during storage. The control sample increased, except for the final stage. Moreover, GO and GO/ß-CD-1 included similar patterns up to Day 31 of storage. Odor scores of GO/ß-CD-1 were constant at Day 31 and 41, while GO odor score decreased after 41 days of storage, compared with the score of Day 31. Odor scores of GO/ß-CD-2 enhanced after 21 day of storage while odor scores of GO/ß-CD-3 decreased.

Statistical analysis of color scores showed no significant differences between the samples. For the control treatment, an increase was seen up to Day 31, except after Day 11. Color scores of GO treatment increased at Days 31 and 41 of storage. Two treatments of GO/ß-CD-2 and GO/ß-CD-3 achieved identical scores up to the end of the experiments. Sample scores for overall acceptability were not statistically different up to Day 31 of storage. Sensory results revealed slight differences in sensory attributes; however, tastes included significant differences within the treatments on Day 31. Furthermore, GO and GO/ß-CD-2 treatments included several differences with the control. However, no significances were observed between the GO/ß-CD-1 and GO/ß-CD-3 and the control. Therefore, it might be concluded that encapsulation of GO with ß-cyclodextrin included no adverse effects on garlic salad dressing taste.

4. Discussion

4.1. Color

Laca et al. [20] reported that all color parameters significantly changed during storage in low-cholesterol mayonnaises prepared with egg yolk micropigments as well as the control sample. After Week 2, samples containing egg yolk micro-pigments included lower redness than that the control sample did, possibly due to the use of turmeric in industrial mayonnaises.

During the oxidative stabilization of mayonnaises with grape seed extracts, redness increased after 30 and decreased after 60 days of storage [34]. Ghazaei et al. [35] who replaced

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a part of egg yolk with modified starch to produce low-fat mayonnaise, reported that increases in modified starch content decreased a^* value with no significant differences between the treatments.

Decreases in yellowness were reported by Laca et al. [20] in low-cholesterol mayonnaise within the storage time, which was the least in the control sample (industrial mayonnaises) started from the second week of storage. These decreases were seen in treatments containing fresh egg yolk and egg yolk micro-pigments, except for Week 3. The value of b^* decreased by increasing the storage time in all mayonnaise samples containing grape seed oil [34]. Ghazaei et al. [35] showed that by increasing the replacement of egg yolk with a modified starch in low-fat mayonnaise, the b^* value decreased. However, no significant differences were seen between the samples.

Laca et al., showed that L^* values were more stable, compared to redness and yellowness in low-cholesterol mayonnaises [20]. In fact, L^* values of all treatments were stable untill Week 2, then slightly increased after Week 3 (control sample) and remained constant up to the end of storage. In mayonnaises containing egg yolk, L^* values were constant up to Week 2, increased after Week 3 and then were constant until the end of storage. The L^* values of the samples containing micro-pigments of egg yolk decreased during storage. In addition, L^* values decreased in mayonnaise samples containing grape seed oil during storage [34].

Increasing of ΔE in all samples after 11 days of storage was similar to that reported by Laca et al. [20], who reported that ΔE increased and was constant after one-month storage of the control sample (industrial sample). In production of mayonnaises with grape extract, it was explained that these changes might be due to the proanthocyanin oxidative decomposition and the resulting undesirable brown color [34]. Another report showed that addition of free brown algae extra caused unpleasant changes in lightness, redness, yellowness, taste and acceptance of mayonnaises. In contrast, nano-liposomes loaded with this extract included good color and acceptability [14].

4.2. pH

Changes in pH of the current study were very low, which was similar to the changes in pH of a study on the release of sauce flavor containing various quantities of garlic oil and fat. It was reported that by increasing the garlic oil content, pH change was negligible and the flavor release was not correlated with the fat content [33]. Ghazaei et al. investigated that pH of the control sample (with no modified starch) was higher than that of other treatments. By increasing the modified starch content, pH significantly decreased [35].

4.3. PV

Finding of this study was similar to the finding reported by Vahidyan et al. [36], who investigated antioxidant effects of thyme and savory extracts on mayonnaises through assessment of PV. They reported no significant differences in PVs of samples containing 500, 1000 and 1500 mg kg^{-1} thyme extract after eight weeks of storage at 30 ºC. In contrast, mayonnaise samples containing grape seed extract included lower PVs than that the control (samples with no essential oil) did [36]. However, Rafiee et al. showed that mayonnaises containing nanoliposomes loaded with pistachio green hull phenolic compounds included the lowest PV and TBARS after four months of storage at room temperature. Generally, plant extracts show better antioxidant activities than those the essential oils do, recommended as natural antioxidants [12]. Oxidative stability of the low-fat mayonnaises containing glucose syrup-based microcapsules of fish oil was studied during storage at cold and room temperatures. The PV of the samples increased during storage; however, mayonnaises loaded with whey protein hydrolysates included the best oxidative stability [13].

Loading of brown algae extract (BAE) in nanoliposomes and its uses in functional mayonnaises showed significantly a lower PV than that the control and samples containing free BAE did [14]. Generally, lipid oxidation reactions of mayonnaises start at the interfacial region. Hydroperoxides located at the droplet surface can quickly contact with pro-oxidants such as transition metals from aqueous media. Transition metals such as iron can accelerate oxidation reactions by promoting lipid hydroperoxide degradation into extremely reactive peroxyl and alkoxyl radicals, which further propagate oxidation [37]. Egg yolk as an emulsifier ingredient of mayonnaises includes a high iron content. Mayonnaises include an acidic pH, which catalyses cleavage of iron from egg yolk proteins and therefore enhances availability of iron as an oxidation initiator. High polyunsaturated fatty acid content of soybean oil makes it less stable against oxidation reactions. Similar to most bioactive compounds, natural antioxidants such as garlic oil contain chemically reactive species which may interact with food matrix ingredients and thus decrease their antioxidant activities.

4.4. TBARS

Compared to the control, higher TBARS quantities of the treatments were not similar to those reported by Vahidyan et al. [36]. They showed that mayonnaises containing linseed oil and 500 mg kg^{-1} thyme and savory extracts included the highest antioxidant activities at the end of storage. Thyme extract at all concentrations showed antioxidant activities of 100 mg kg^{-1} BHA. In another study, TBA value decreased significantly by rising the extract concentration in mayonnaises containing grape seed extracts. However, rate

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of increases within two first weeks was higher than that within the six subsequent weeks of storage [34]. Previous reports showed that encapsulated natural extracts included higher antioxidant activities than those capsules loaded with essential oils did [12,14].

4.5. Microbial analysis

The present study showed that *S. aureus* was further sensitive to free and capsulated GO. This finding was similar to that of previous studies; in which, *B. cereus* growth was inhibited by increasing the quantities of free GO and GO/ß-CD [17]. Results showed that the antibacterial effects of free GO and GO/ß-CD on Gram-positive bacteria (*S. aureus* and *B. cereus*) were higher than those on Gram-negative bacteria (*E. coli* and *S. enterica*). Essential oils, including garlic oil, lead to cellular leakage, cytoplasm coagulation and cellular death via cell wall, cytoplasmic membrane and membrane protein destructions [38]. These hydrophobic compositions include high tendencies to bind to bacterial cell membrane lipids. Based on Burt`s theory [39], Gram-positive bacteria are more sensitive to essential oils than Gram-negative bacteria. This may be due to the outer layer of the cell wall in Gram-negative bacteria, which acts as an impenetrable barrier and hence restricts hydrophobic composition accessibility to cellular contents [40].

Investigation of low-cholesterol mayonnaises with egg yolk granules showed that the microbial growth was accelerated by increasing the fresh egg yolk. It was concluded that use of dried granules was better, which lowered the total count of microorganisms and hence increased the storage ability of mayonnaises. The fresh egg treatment (commercial mayonnaises) showed less microbial loads as the fresh egg included physical and chemical barriers against microorganisms with less microbial loads [20].

Nanoliposomes containing pistachio green hull phenolic compounds included the best inhibitory effects on *S. aureus*, molds and yeasts in functional mayonnaises [12]. Antibacterial efficiency of the brown algae extract against *P. aeruginosa*, *E. coli* and *B. cereus* increased when the extract was loaded in nano-liposomes [14]. Mansouri et al. demonstrated the antibacterial effects of thyme essential oil nanoemulsion against *S. Typhimurium*, *E. coli* and *L. monocytogenes* in mayonnaises [15].

4.6. Sensory evaluation of treatments containing free and encapsulated GO

As significant differences were seen between the free GO and GO/ß-CD-2 and the control while no differences were observed between the GO/ß-CD-1 and GO/ß-CD-3 and the control, it seemed that encapsulation of GO with ß-cyclodextrin included no adverse effects on the taste of garlic salad dressings, especially, GO/ß-CD-2.

Laca et al. [20] reported that panelists gave better scores to egg yolk granule-containing samples, compared to raw egg yolk. Vahidyan et al. [36] showed that a 15% replacement of soybean oil by linseed oil did not led to significant changes in color, texture, taste, odor, mouthfeel and overall acceptability of salad dressings containing thyme and savory essential oils. Sensory analysis of mayonnaises containing various concentrations of grape seed extract revealed that the control sample (with no grape seed extracts) included the highest score. The maximum concentration of the grape seed extract received higher scores for taste, odor and texture than those the control sample did. However, overall acceptability and color were better in the control [34]. Mansouri et al. reported that nanoemulsion of thyme essential oil received the best sensory scores, possibly due to the slow release of essential oils [15].

5. Conclusion

During the storage of produced LFSD at 4 ºC, the overall color changes (ΔE) of the GO/ß-CD-3 treatment was lowest and it seems that b^* has the most influence on the ΔE parameter. The pH changes of samples were low and its changes was over the range of 4.64- 4.84. PVs of all samples slowly increased during storage. At the end of storage, there was no significant difference between PV of samples containing free GO and GO/ß-CD-2. In addition, TBARS values of all samples increased during storage, but control had the highest value. *S. aureus* was the most sensitive pathogen to GO/ß-CD, followed by *E. coli*, *S. enterica* and *B. cereus*. Sensory results indicated no significant difference between the control and sample containing microcapsules of garlic oil (GO/ß-CD-2). GO treatment got the lowest sensory score. So, encapsulation of garlic oil with ß-CD had no side effect on sensory properties of produced LFSD and considering its good antimicrobial properties, it can be suggested as a natural antimicrobial agent for the production of functional low-fat salad dressing.

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7. Conflict of Interest

The authors report no conflicts of interest.

8. Authors Contributions

Khoshtinat K. “Investigation and writing- original draft preparation”; Barzegar M. “Conceptualization, writing-review and editing and supervision”; Sahari M.A. “Advisor”; Hamidi Z. “Advisor”.

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چکیده

سابقه و هدف: سیر تازه خواص دارویی و نگهدارندگی دارد اما به محض آسیب دیدن بافت آن، ترکیبات زیست‌فعال مهم آن به سایر ترکیبات شیمیایی تبدیل می‌شوند. روغن سیر (GO) از سیر استخراج می‌شود، که به علت طعم و بوی شدید، فراریت، حلالیت کم در حلال‌های آبی، کاربرد بسیار محدودی در صنعت غذا دارد. هدف مطالعه حاضر بررسی خواص فیزیکوشیمیایی، میکروبی و حسی سس سالاد کم چرب حاوی روغن سیر ریزپوشانی شده با بتاسیکلودکسترین است (GO/ß-CD).

مواد و روش ها: پنج نمونه سس سالاد شامل شاهد (فاقد روغن سیر)، حاوی روغن سیر آزاد، و سه نمونه حاوی غلظت های مختلف GO/ß-CD (۵۴۸، ۸۱۸، و ۱۳۶۴ mg.Kg^{-1}) تولید شد. سپس، تغییرات pH، رنگ، فعالیت ضداکسایشی (عدد پراکسید و تیوباربیتوریک اسید)، ضدمیکروبی (پایش ۴ ریزاندامگان بیماری‌زا شامل *باسیلوس سرئوس*، *استافیلوکوکوس اورئوس*، *اشرشیا کلی* و *سالمونلا انتریکا*) و حسی (طعم، رنگ، بو و قابلیت پذیرش با آزمون هدونیک ۸ امتیازی) آن ها در مدت ۴۱ روز نگهداری در۴°C بررسی شد.

یافته‌ها و نتیجه‌گیری: در تیمارهای سس سالاد، کمینه و بیشینه تغییر رنگ به‌ترتیب به تیمارهای GO/ß-CD-3 (حاوی بیشترین میزان روغن سیر ریزپوشانی شده) و نمونه حاوی روغن سیر آزاد تعلق داشت. زردی نمونه‌های حاوی روغن سیر ریزپوشانی شده کاهش یافت و ارتباط مستقیمی بین کاهش زردی نمونه‌ها با میزان GO/ß-CD وجود داشت. درمدت نگهداری، pH و PV به آهستگی افزایش یافتند، *استافیلوکوکوس اورئوس* حساس‌ترین ریززنده بیماری زا به روغن سیر آزاد و GO/ß-CD بود و *اشرشیا کلی*، *سالمونلا انتریکا* و *باسیلوس سرئوس* به‌ترتیب در مرتبه‌های بعدی قرار داشتند. نتایج ارزیابی حسی نشان داد تفاوت معنی‌داری بین طعم نمونه شاهد و GO/ß-CD-2 (حاوی ۸۱۸ mg.Kg^{-1} معادل ۹۰ mg.Kg^{-1} روغن سیر آزاد) وجود ندارد، اما این دوتیمار تفادت معنی داری با نمونه‌های حاوی روغن سیر آزاد داشتند. نتایج دلالت بر این دارد که ریزپوشانی روغن سیر با بتا-سیکلودکسترین تاثیر منفی بر طعم سس سالاد نداشت و به لحاظ خواص ضدمیکروبی خوبی که دارد، می‌تواند به‌عنوان یک عامل ضدمیکروبی طبیعی پیشنهاد شود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- سس سالاد کم چرب

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