Research Article
APPLIED FOOD BIOTECHNOLOGY, 2023, 10 (2):141-154
Journal homepage: www.journals.sbmu.ac.ir/afb
pISSN: 2345-5357
eISSN: 2423-4214
OPEN ACCESS

Formulation of Sausage with Encapsulated GO: Physicochemical, Microbial and Sensory Properties

Khadijeh Khoshtinat[1,2], Mohsen Barzegar[1]*, Mohammad Ali Sahari[1], Zohreh Hamidi[1]

1- Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, P.O. Box 14155-336, Tehran, Iran.
2- Department of Food Technology Research, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, P.O.Box: 19395-4741, Tehran, Iran.

Abstract

Background and Objective: Consumers prefer not to use synthetic preservatives so, natural ones such as essential oils (in this research, garlic oil (GO) are mostly consumed in food products as an antimicrobial agents. Application of garlic oil in food industries is limited. Because of its water insolubility, volatility, pungent odour and flavor, and low stability. Encapsulation could eliminate its restrictions. In the present study, different concentrations of free and encapsulated garlic oil in ß-cyclodextrin (GO/ß-CD) were used for formulation of sausage

Material and Methods: Five types of sausage were produced, including control (without garlic oil), containing free garlic oil (GO), and three samples containing 546, 818, and 1364 mg of encapsulated garlic oil per kg. After that, changes of pH, color, oxidation indices (PV and TBARs), monitoring of *B. cereus*, *S. aureus*, *E. coli*, and *S. enterica*, and sensory (taste, color, odor, and acceptability by 8-point hedonic test) properties were studied during 29 days storage at 4ºC.

Results and Conclusion: By increasing the GO/ß-CD content of samples, the overall color changes decreased. The pH of all produced sausages remained constant. At the end of storage, PV was not significantly changed, and TBARs changing was very low. *S. aureus* was the most susceptible, followed by *S. enterica*, *E. coli*, and *B. cereus*. In sensory evaluation, the panelists did not distinguish between the control and the samples containing GO/ß-CD. Encapsulation of garlic oil with beta-cyclodextrin did not affect the taste of the produced sausages. Due to good antimicrobial properties of garlic oil, it can be used in meat products as a natural preservative.

Conflict of interest: The authors declare no conflict of interest.

Article Information

Article history:
- Received 7 Feb 2023
- Revised 22 Feb 2023
- Accepted 12 Mar 2023

Keywords:
- Antimicrobial properties
- Antioxidant effects
- ß-cyclodextrin
- Encapsulation
- Garlic oil
- Sausage

***Corresponding author:**

Mohsen Barzegar *
Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, P.O. Box 14155-336, Tehran, Iran.

Tel: +98(21)48292323
E-mail: mbb@modares.ac.ir

How to cite this article

Khoshtinat k, Barzegar M, Sahari MA, Hamidi Z. Formulation of Sausage with Encapsulated GO: Physicochemical, Microbial and Sensory Properties. Appl Food Biotechnol. 2023; 10 (2): 141-154. http://dx.doi.org/10.22037/afb.v10i2.41131

1. Introduction

As consumers prefer not to use synthetic preservatives, natural preservatives such as essential oils (EOs) are mostly used in food products as an antimicrobial agents. Although, garlic oil (GO) is approved as safe by Food and Drug Administration (FDA), use of this oil in food industries is limited. Because of its water insolubility, volatility, pungent odor and flavor and low stability [1, 2]. In addition, GO can be degraded by oxygen, light, heat and moisture. Therefore, encapsulation is one solution to improve its stabilization as well as solubility, antimicrobial characteristics and volatilization rate and decrease undesired odors. The FDA considered β-cyclodextrin safe for using as food additives [3]. Encapsulation of GO organosulfur compounds by β-CD, chitosan and soy protein isolate results in further solubility, higher stability and lower water activity as well as hygroscopicity [4]. In a previous report by the authors, the maximum release of GO from its inclusion complex (GO/β-CD) under *in-vitro* simulated intestinal fluid (SIF) was nearly 10.29% [5]. Results showed that free and encapsulated GO included significant effects on the pathogenic bacterial growth [6]. In another study, various products of garlic were used in production of sausages. The authors showed that garlic and its derivatives included no significant antioxidative effects. However, fresh garlic (FG) included the most effectiveness. They concluded that useful compounds were eliminated during production of garlic powder or EO [7,8]. In another study, garlic extract could control *Staphylococcus* (*S.*) *aureus* and *Escherichia* (*E.*) *coli* O157:H7 growth in ready-to-cook chickens. Results indicated significant antimicrobial effects of this extract and showed positive effects on organoleptic characteristics of the cooked chickens [9]. Mozaffari Nejad *et al.* studied antibacterial effects of aqueous garlic extract on *S. aureus* grows on hamburgers. Their results showed excellent antibacterial characteristics against the highlighted microorganisms [10]. Horita et al. prepared garlic extract using pressurized liquid extraction (PLE) and added this extract to frankfurters. The authors compared various characteristics of the samples containing PLE extract with those contained FG, commercial garlic powder and GO. The FG was a potent antioxidant and antimicrobial agent against spoilage bacteria [mesophilic aerobic bacteria, lactic acid bacteria (LAB), total and thermotolerant coliforms and psychrotrophic bacteria] during its shelf-life. Frankfurters containing commercial GO demonstrated the lowest overall acceptability, compared with the control sample [11].

Recently, researchers encapsulated GO using spray drying technique. They ued maltodextrin and Arabic gum as the wall materials. Microencapsulated GO (concentration of 20%) showed an intense antimicrobial activity, which increased the minced meat shelf-life [12,13]. Assessment of the lipid oxidation and sensory attributes of anchovy marinated with sunflower oil containing NaCl, alcohol vinegar, citric acid solution and various EOs (rosemary, coriander, laurel and GO) was carried out by the researchers. They showed retarding effects of GO on lipid oxidation, causing the most brightness after a 5-m cold storage [14]. Mahros et al. studied antimicrobial effects of FG and GO against the aerobic bacteria in beef meatballs during their cold storage at 4 °C. Moreover, FG showed a lower microbial count than that GO did. The FG (5%) and GO (600 mg/kg) included the best antimicrobial potentials [15]. Shrimp meat was covered by the edible film (chitosan containing GO) and stored in refrigerator. Coated samples showed lower pH, total volatile base nitrogen and oxidation rate after 11 d of storage. In addition, chitosan containing GO showed higher shelf-life (2 d), compared with samples coated by chitosan. Additionally, GO included no significant effects on total volatile base nitrogen and trimethyl amine nitrogen [16,17]. Use of GO/β-CD in low-fat salad dressing demonstrated good antimicrobial characteristics against four Gram-negative and Gram-positive bacteria with no adverse effects on its taste [18]. Although garlic and its EO include valuable characteristics due to the water insolubility, pungent odor and low stability, it cannot directly be used in production of sausages. In this study, encapsulated GO was used in sausage production at industrial scale for the 1st time. In addition, physicochemical, microbial and sensory characteristics of all samples were studied.

2. Materials and Methods

2.1. Materials

In this study, GO 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. 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), Tehran, Iran. The β -cyclodextrin (β-CD) and culture media [mannitol salt phenol red agar, trypticase soy agar, eosin methylene blue (EMB) agar and *Salmonella-shigella* (SS) agar] were purchased from Sigma-Aldrich, Missouri, USA, and Merck, Darmstadt, Germany, respectively. Bleached refined soybean oil without antioxidant was prepared by Oila, Tehran, Iran. Meat, powdered milk, salt, sodium caseinate, sodium polyphosphate, ascorbic acid, sodium nitrite, starch, wheat flour and condiments were purchased from the local markets. Other chemicals and solvents included analytical grade and used without further purifications (Sigma-Aldrich, Missouri, USA).

2.2. Methods

2.2.1. Encapsulation of garlic oil

In the present study, GO was encapsulated using coprecipitation method as reported in a previously published manuscript by the current study [5].

2.2.2. Formulation of sausages

Sausages were produced in Robat Meat Products Company, containing meat (55%), soybean oil (16% without antioxidant), powdered milk (2%), salt (1%), sodium caseinate (1%), sodium polyphosphate (0.4%), ascorbic acid (200 $mg.kg^{-1}$), sodium nitrite (120 $mg\ kg^{-1}$), starch (1%), wheat flour (1%), condiment (1.2%), ice and water mixture (up to 100%) and GO or GO/ß-CD. Five experimental treatments of sausages were produced, including control (without GO or GO/ß-CD), free GO (GO, 90 $mg\ kg^{-1}$), GO/ß-CD-1 (546 $mg\ kg^{-1}$ of loaded microcapsules, equivalent to 60 $mg\ kg^{-1}$ of free GO), GO/ß-CD-2 (818 $mg\ kg^{-1}$ of loaded microcapsules, equivalent to 90 $mg\ kg^{-1}$ of free GO) and GO/ß-CD-3 (1364 $mg\ kg^{-1}$ of the loaded microcapsules, equivalent to 150 $mg\ kg^{-1}$ of free GO). However, for sensory evaluation and ethics in the study, 120 $mg\ kg^{-1}$ of nitrite were added to some samples and soybean oil containing synthetic antioxidant was used. Formulated dough was manually filled, aerated and tied in factory-filled covers. Sausages were cooked for 1.5 h at 85 °C until the center point reached 75 °C. Then, these were stored in refrigerator for 24 h, cut into 20-g slices, wrapped in 5-layer polyethylene sheets under vacuum and restored in refrigerator until further use.

2.2.3. Physicochemical analysis

During 29 days of storage at 4 ºC, physicochemical and microbial characteristics of the prepared sausages were assessed on days 1, 8, 15, 22 and 29 [18,7]. All experiments were carried out in triplicate and mean ±SD (standard deviation) was reported.

2.2.3.1. Assessment of color parameters

Hunterlab Instrument (Colorflex, Virginia, USA) was used to assess color of the samples. It was calibrated with a black and white tile (a* = -1.29, b* = 1.19 and L* = 92.23) at ambient temperature [11]. After opening the vacuum packages of sausage slices (0.5-cm thickness), samples were immediately transferred into clean glass plates and color attributes were assessed. Total color difference (ΔE), hue angle (H*), chroma (C*) and browning index (BI) were calculated using the equations of 1-4 [19,20]:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \quad [1]$$

$$H^* = \tan^{-1} [b^*/a^*] \quad [2]$$

$$C^* = [(a^*)^2 + (b^*)^2]^{0.5} \quad [3]$$

$$BI = \frac{100}{0.172}\left(\frac{a*+1.75L*}{5.647\,L*+a*-3.012\,b*} - 0.31\right) \quad [4]$$

2.2.3.2. Assessment of pH

In the present study, pH of the samples was assessed using pH meter (Metrohm, 827 pH Lab, the Switzerland) [21].

2.2.3.3. Assessment of peroxide value

Precisely, 1 g of sausages was weighed in a 15-ml centrifuge tubes. After adding a mixture of chloroform and methanol (1:2) to the sample, its oil was extracted using centrifuge (Sigma-Aldrich, Missouri, USA) at 18950 g for 2 min and filtered using Whatman filter papers no. 1 (Whatman, USA). Then, 7 ml of the filtrate were transferred to a fresh centrifuge tube and 2 ml of sodium chloride solution (0.5%) were added to the tube. The 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 (RT). Absorbance of the sample was measured at 500 nm [22]. This method was carried out for the control without the sample. Calibration curve was prepared using various concentrations of cumene hydroperoxide and development of the primary oxidation reaction was assessed using calibration equation ($y = 0.035x + 0.188$, $r = 0.9985$).

2.2.3.4. Thiobarbituric acid reactive substances assay

Thiobarbituric acid reactive substances (TBARS) was assessed using AOCS method [23]. After extraction of malondialdehyde and related additives and before spectrophotometric determination, TBARS reactants reacted directly with the meat sample. Malondialdehyde was extracted using the solvent or steam distillation, reacted with the TBA reagent and the adsorption rate of the resulting dye was read using spectrophotometer. As the distillation method did not include good accuracy and repeatability, direct reaction method with the reagent was used for the meat products. Use of other methods such as HPLC to assess TBARS in sausages and fish causes over estimation [24].

2.2.4. Microbial analysis of the produced samples

Four pathogens, including *B. cereus*, *S. aureus*, *E. coli* and *S. enterica*, were activated based on the provider's instructions. Each treatment was inoculated by 0.1 ml of 0.5 McFarland standard for each pathogen (*B. cereus,* 6.36 cfu ±0.32; *S. aureus,* 6.72 cfu ±0.22; *E. coli,* 6.00 cfu ±0.26; and *S. enterica,* 6.34 cfu ±0.35). In addition, 25 falcons (five treatments and five replications) were prepared containing 10 g of sausages inoculated by 0.1 ml of 0.5 McFarland standard for each pathogen [25]. On Days 1, 8, 15, 22 and 29 of the production, contents of each plate containing sausage samples were added to 90 ml of peptone water and mixed for 20 s at 200 rpm. These were transferred to sterile 250-ml Erlenmeyer flasks. Then, 100 µl of the content of each Erlenmeyer flask were transferred to a plate, containing similar culture media and cultured using surface culture. Plate count agar was cultured to precisely investigate bacterial colonies. Mannitol salt phenol red agar (MSA) was used for *S. aureus*, altering yellow color to red due to mannitol fermentation [26]. Trypticase soy agar (TSA) was used for *B. cereus* and the large colonies with jagged edges were counted [27]. Moreover, *E. coli* was cultured on EMB agar, which its colonies included metallic shine [28] and *S. shigella* (SS) agar was used to identify *S. enterica* with black colonies, resulting from sulfur consumption [29].

2.2.5. Sensory eavaluation of the produced sausages

Sensory evaluation was carried out by ten trained panelists. Five sausages were bached with a 3-digit code using random table. After removing samples from the vacuum packages, sausage slices were transferred on a tray for the panelists. Glasses of water and crispy breads were served to the penalists, which they were offered to consume between the two samples to remove the previous sample effects. Penalists scored the samples regarding their taste, odor, color and overall acceptability. Sensory analysis was carried out using 8-scale hedonic assay (1, highly undesirable; 8, highly desirable).

2.3. Statistical analysis

In this study, fully randomized design was used. Comparisons between the mean values were carried out using Tukey's test and Minitab software v.16 (Minitab, State College, Pennsylvania, USA). Sensorial results were analyzed and scores were transformed into grades and

reported as the mean of grades using IBM SPSS v.9.5.00 ed.21 (IBM Corporation, New York, USA). In this study, nonparametric Kruskal-Wallis test was used if asymptotic significances were reported ($p \leq 0.05$). Treatments were divided into two groups using Mann-Whitney test to reveal which treatments include significant differences.

3. Results and Discussion

3.1. Physicochemical analysis

Results of color, pH, PV and TBARS of the samples during storage are presented in Tables 1 and 2 and Figure 1.

3.1.1. Color

Results of redness (a*), yellowness (b*) and Luminosity (L*) of the samples are present in Table 1. Redness of all treatments was significantly different, up to the 4th sampling time. Therefore, no significant differences were seen between the GO and GO/ß-CD-1 treatments. On the last sampling day, differences between the GO and GO/ß-CD-2 treatments were not significant. The highest and the lowest redness values belonged to GO/ß-CD-3 and control samples, respectively. According to Yin and Cheng, adding garlic sulfur organic compounds significantly delays oxidation of oxymyoglobin pigments in red meats, resulting in changes in meat color from red to brown [30]. Therefore, the maximum a* in GO/ß-CD-3 treatment at the end of the storage might be due to the presence of the most GO quantity released in the product. In a study by Horita *et al.* a* of the samples containing free GO decreased after two weeks and then increased during refrigeration. At the end of storage, redness intensity of the samples decreased [11].

One of the instrumental color variables, a* of food products containing sugar increases when are caramelized [20]. It was shown that a* decreased after cooking process (pan frying) of combined burgers using a mixture of chicken meat and kilka fish [31]. Badawy et al. by studing antibacterial and antioxidant effects of chitosanmonoterpene nanoparticles and their uses in preserving minced meat showed that chitosan could affect red color retention for a much longer period, compared to other treatments and the controls [32]. They concluded that the protein myoglobin was actually responsible for creating red color of the raw meat, which is present in animal tissues and turns red when exposed to oxygen during processing. Furthermore, a* of emulsion-type sausages increased when sausage slices were converted into chips [33]. Skaljac et al. studied color characteristics of the vacuum-packed fermented sausages during storage [34]. During storage, a* significantly decreased in unpacked sausages, while vacuum-packed sausages included significantly lower a* on Days 30 and 60 of storage, which could be explained by the reaction of light-induced oxidation of the color pigments and formation of gray-brown metmyoglobin and likely decreased a* on the cutting surfaces of the sausages.

Yellowness of the control sample was significantly different from that of other treatments on Day 1. From the 3rd sampling time, b* included no significant differences between the treatments containing GO/ß-CD. In all treatments, yellowness decreased in Week 2 then increased until Week 4 and decreased again. In the 2nd, 3rd and 5th sampling dates, no significant differences were observed between GO and GO/ß-CD-2 treatments.

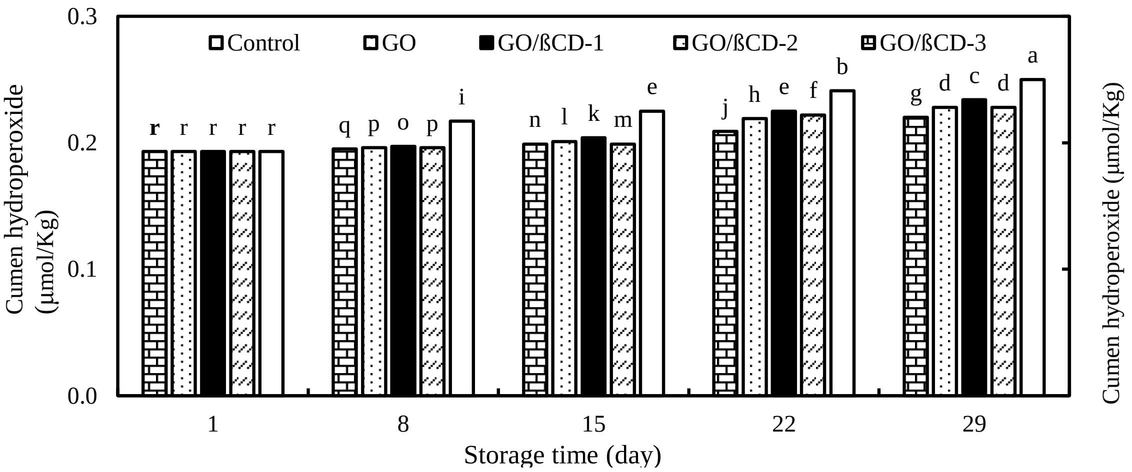

Figure 1. Changes in the peroxide values of the samples during 29 days of storage at 4 ºC. Control without garlic oil; GO (containing 90 mg kg^{-1} of free garlic oil); GO/ß-CD-1 (containing 546 mg kg^{-1} of GO/ß-CD equal to 60 mg kg^{-1} of garlic oil); GO/ß-CD-2 (containing 818 mg kg^{-1} of GO/ß-CD equal to 90 mg kg^{-1} of garlic oil); and GO/ß-CD-3 (containing 11364 mg kg^{-1} of GO/ß-CD equal to 150 mg kg^{-1} of garlic oil). Different lowercase letters in each column indicate significant differences between the treatments ($p \leq 0.05$).

Table 1. Changes in color parameters of the samples during 29 days of storage at 4 ºC*.

C	Treatments	Sampling days 1st	8th	15th	22nd	29th
a*	Control	9.11±0.02^{Ae}	7.81±0.04^{De}	8.05±0.04^{Ce}	8.34±0.03^{Bd}	7.34±0.08^{Ed}
	GO	12.57±0.09^{Ab}	10.84±0.05^{Dc}	11.05±0.03^{Cc}	11.52±0.04^{Bb}	10.62±0.06^{Eb}
	GO/ß-CD-1	12.84±0.03^{Aa}	12.12±0.01^{Ba}	12.94±0.03^{Aa}	11.54±0.08^{Cb}	10.26±0.12^{Dc}
	GO/ß-CD-2	11.53±0.03^{Bc}	11.13±0.13^{Cb}	11.60±0.03^{Bb}	11.91±0.01^{Aa}	10.58±0.07^{Db}
	GO/ß-CD-3	10.74±0.03^{Bd}	9.85±0.09^{Cd}	10.67±0.02^{Bd}	10.98±0.04^{Ac}	10.97±0.02^{Aa}
b*	Control	20.55±0.10^{Aa}	18.27±0.34^{Ba}	16.88±0.25^{Ca}	15.96±0.06^{Da}	14.27±0.22^{Ea}
	GO	16.34±0.04^{Ab}	14.13±0.18^{Cc}	15.02±0.02^{Bb}	15.29±0.05^{Bb}	13.76±0.04^{Ca}
	GO/ß-CD-1	16.48±0.03^{Ab}	15.94±0.05^{Bb}	15.22±0.05^{Cb}	15.07±0.04^{Cb}	14.06±0.02^{Da}
	GO/ß-CD-2	16.62±0.05^{Ab}	13.87±0.20^{Cc}	15.04±0.02^{Bb}	14.74±0.01^{Bb}	13.95±0.06^{Ca}
	GO/ß-CD-3	46.35±0.38^{Ad}	13.09±0.16^{Ed}	15.58±0.05^{Bb}	15.01±0.06^{Cb}	14.22±0.02^{Da}
L*	Control	68.51±0.04^{Ab}	66.84±0.03^{Bb}	65.04±0.02^{Cb}	64.34±0.06^{Db}	63.74±0.63^{Da}
	GO	69.91±0.06^{Aa}	66.44±0.12^{Bb}	64.52±0.14^{Cb}	63.54±0.12^{Db}	62.67±0.13^{Eb}
	GO/ß-CD-1	69.53±0.06^{Aa}	68.89±0.14^{Aa}	67.48±0.38^{Ba}	65.20±0.17^{Ca}	63.72±0.11^{Da}
	GO/ß-CD-2	67.81±0.01^{Ac}	66.59±0.49^{Bb}	65.06±0.40^{Cb}	64.06±0.02^{Db}	62.64±0.20^{Eb}
	GO/ß-CD-3	65.69±0.04^{Ad}	66.27±0.25^{Bc}	62.85±0.10^{Cc}	61.59±0.15^{Dc}	60.84±0.05^{Ec}

C	Treatments	Sampling days 1st	8th	15th	22nd	29th
C*	Control	22.48 ± 1.89^{Aa}	19.87 ± 1.95^{Aa}	18.70 ± 0.96^{Bb}	18.01 ± 0.05^{Cc}	16.05 ± 0.64^{Dc}
	GO	20.62 ± 0.08^{Ab}	17.81 ± 0.38^{Db}	18.65 ± 0.01^{Cb}	19.15 ± 0.04^{Bs}	17.38 ± 0.04^{Db}
	GO/ß-CD-1	20.89 ± 0.02^{Ab}	20.03 ±0.03^{Ba}	19.98 ± 0.04^{Ba}	18.98 ± 0.64^{Ca}	17.40 ± 0.09^{Db}
	GO/ß-CD-2	20.23 ± 0.04^{Ab}	17.78 ±0.55^{Db}	19.00 ±0.01^{Bb}	18.96 ± 0.00^{Ca}	17.51 ± 0.07^{Db}
	GO/ß-CD-3	19.56 ± 0.03^{Ac}	16.38± 0.32^{Dc}	18.89 ± 0.35^{Bb}	18.60 ± 0.05^{Bb}	17.96 ± 0.01^{Ca}
H*	Control	66.09 ± 0.01^{Ba}	66.85 ± 0.05^{Aa}	64.50 ± 0.00^{Ca}	62.41 ± 0.01^{Ea}	62.78 ± 0.04^{Da}
	GO	52.43 ± 0.01^{Db}	52.50 ± 0.02^{Cc}	53.66 ± 0.00^{Ac}	53.00 ± 0.01^{Bc}	52.34 ± 0.01^{Dc}
	GO/ß-CD-1	52.08 ± 0.00^{Dc}	52.75 ± 0.00^{Bc}	49.63 ± 0.00^{Ee}	52.56 ± 0.01^{Cd}	53.88 ± 0.02^{Ab}
	GO/ß-CD-2	55.25 ± 0.01^{Bb}	51.5 ± 0.02^{Cd}	52.36 ± 0.00^{Ad}	51.06 ± 0.00^{De}	42.82 ± 0.01^{Ed}
	GO/ß-CD-3	56.70 ± 0.01^{Ab}	53.04 ± 0.02^{Db}	55.60 ± 0.00^{Bb}	53.81 ± 0.01^{Cb}	52.35 ± 0.00^{Ec}
BI	Control	44.26 ± 0.02^{Aa}	39.45 ± 0.02^{Ba}	38.13 ± 0.02^{Cb}	37.05 ± 0.02^{D}	32.90 ± 0.02^{Ed}
	GO	38.78 ± 0.02^{Bc}	34.93 ± 0.02^{Ec}	38.06 ± 0.02^{Cc}	39.78 ± 0.02^{Aa}	36.24 ± 0.02^{Db}
	GO/ß-CD-1	39.55 ± 0.02^{Ab}	38.21 ± 0.02^{Cb}	38.58 ± 0.02^{Bb}	38.25 ± 0.02^{Cc}	35.78 ± 0.02^{Dc}
	GO/ß-CD-2	39.56 ± 0.02^{Ab}	34.66 ± 0.01^{Ec}	38.35 ± 0.02^{Cb}	38.75 ± 0.02^{Bb}	36.60 ± 0.02^{Db}
	GO/ß-CD-3	39.59 ± 0.02^{Bb}	32.02 ± 0.01^{Dd}	39.91 ± 0.02^{Aa}	39.97 ± 0.02^{Aa}	38.82 ± 0.02^{Ca}

Control without GO; GO (containing 90 mg/kg of free GO); GO/ß-CD-1 (containing 546 mg/kg of GO/ß-CD equal to the 60 mg/kg of free GO); GO/ß-CD-2 (containing 818 mg/kg of GO/ß-CD equal to the 90 mg/kg of free GO); and GO/ß-CD-3 (containing 11364 mg/kg of GO/ß-CD equal to the 150 mg/kg of free GO). BI: browning index; C: chroma; H*: hue angle; Different lowercase letters in each column indicate significant difference between treatments. Different uppercase letters in each row indicate a significant difference between treatments ($P \leq 0.05$).

Table 2. Changes in pH of the samples during 29 days storage at 4 ºC*.

Treatments	Sampling days 1st	8th	15th	22nd	29th
Control	6.66±0.01^{Ac}	6.89±0.08^{Bb}	7.00±0.03^{Bb}	7.00±0.07^{Bb}	7.86±0.02^{Bb}
GO	6.43±0.03^{Ab}	6.63±0.01^{Ba}	6.64±0.01^{Ba}	6.63±0.02^{Ba}	6.64±0.03^{Ba}
GO/ß-CD-1	6.41±0.01^{Aa}	6.61±0.01^{Ba}	6.62±0.01^{Ba}	6.63±0.01^{Ba}	6.54±0.02^{Ba}
GO/ß-CD-2	6.33±0.05^{Aa}	6.61±0.01^{Ba}	6.62±0.01^{Ba}	6.63±0.01^{Ba}	6.56±0.01^{Ba}
GO/ß-CD-3	6.38±0.03^{Aa}	6.61±0.01^{Ba}	6.62±0.01^{Ba}	6.63±0.01^{Ba}	6.61±0.03^{Ba}

* 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 ($P \leq 0.05$).

Table 3. Changes in the microbial growth (log cfu ml^{-1}) of the samples during 29 days storage at 4 °C.

Microorganisms*	Treatments**	Sampling days 1st	8th	15th	22nd
B. cereus	Control	8.88±0.53 Ca	14.73±1.91 Ba	NC Aa	NC Aa
	GO	4.83±0.60 Bb	7.03±0.44 Bb	12.62±0.26 Ab	14.70±1.06 Ab
	GO/ß-CD-1	7.03±0.44 Ba	9.13±0.31 Bb	9.97±1.05 Bb	14.71±0.24 Ab
	GO/ß-CD-2	6.59±0.36 Bb	7.07±0.83 Bb	12.67±1.82 Ab	13.97±1.65 Ab
	GO/ß-CD-3	5.00±0.17 Bb	6.01±0.70 Bb	10.48±0.48 Ab	12.42±0.10 Ab
S. aureus	Control	8.87±0.51 Ca	10.18±0.20 Ba	NC Aa	NC Aa
	GO	4.17±0.38 Cc	1.92±0.40 Dc	3.30±0.00 Cc	5.69±0.36 Bc
	GO/ß-CD-1	5.88±0.89 Ab	3.58±0.36 Bb	4.98±0.03 Ab	6.63±0.55 Ab
	GO/ß-CD-2	4.70±0.00 Bc	-	-	-
	GO/ß-CD-3	-	-	-	-
E. coli	Control	8.20±0.17 Ba	9.53±1.20 Ba	NC Aa	NC Aa
	GO	5.48±0.86 Aa	5.99±0.27 Aa	8.60±0.00 Ab	9.92±0.95 Ab
	GO/ß-CD-1	6.92±0.93 Aa	7.03±0.44 Aa	8.97±0.35 Ab	10.70±1.54 Ab
	GO/ß-CD-2	6.75±0.74 Aa	6.87±0.51 Aa	7.79±0.61 Ab	9.97±1.23 Ab
	GO/ß-CD-3	6.00±0.00 Aa	5.48±0.48 Aa	5.96±1.03 Ab	10.20±1.01 Ab
S. entrica	Control	8.20±0.17 Ca	10.62±0.68 Ba	NC Aa	NC Aa
	GO	3.95±0.09 Bc	2.00±0.00 Cb	3.30±0.30 Bc	5.93±1.00 Ab
	GO/ß-CD-1	5.48±0.86 Ab	3.47±0.48 Bb	5.05±0.23 Ab	5.45±0.15 Ab
	GO/ß-CD-2	2.78±0.20 Cd	3.03±0.24 Cb	3.30±0.00 Cc	4.62±0.68 Bb
	GO/ß-CD-3	-	-	-	-

* 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. entrica,* 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 ≤0.05). NC; non-countable.

Horita et al. reported a downward, upward and re-downward trend in b* intensity of frankfurters containing GO [11]. On Day 1, L*of the treatments containing GO and GO/ß-CD-1 was not significantly different. In the 3rd and 4th sampling times, no significant differences were observed between the control and GO and GO/ß-CD-2. On the last day of refrigeration (Day 29), differences between luminosity of the treatments containing GO and GO/ß-CD-2 as well as the control. Moreover, GO/ß-CD-1 was not significant and the lowest L* value belonged to GO/ß-CD-3 treatment.

Magrinya et al. investigated effects of tocopherol and celery juice concentrate on qualitative and sensory characteristics of dried fermented sausages. Their results showed that L* and b * indices were significantly affected by the quantity of nitrate. Yellowness of the samples increased by increasing the quantity of celery juice concentrate in sausages. In contrast, redness index was not affected by nitrate content, tocopherol content and starter culture [35]. Moradi et al. showed that raw and cooked burgers with higher ratios of fish meat included darker color with lower yellowness (b*) index. However, b* index increased after cooking [31]. Researchers showed that b* index increased when sausage slices were converted into chips [33]. Results of color changes in unpacked and vacuum-packed fermented sausages during storage demonstrated that b* index significantly decreased in unpacked sausages. Changes of b* index during storage could be linked to the intensity of oxidation process and higher oxidation changes led to increased yellowness [34]. Horita et al. reported decreases in luminosity of frankfurters containing GO up to 20 d and then increases were seen [11]. They attributed this phenomenon to decomposition of similar pigments during storage. Aliyari et al. showed that color indices (b *, a *, L *) decreased after one month of refrigeration in sausages containing pomegranate peel extract, compared to the control sample [36].

A study reported that raw and cooked burgers included a lower ligthness (L*) index and L* index increased after pan frying. Ripoll et al. used dietary vitamin E and selenium (Se) to increase the shelf-life of modified atmosphere packaged light lamb meats and showed that lightness was affected by the storage time and dietary vitamin E content [37].

It is concluded that lightness increased with time. Naturally, lightness is unlinked to myoglobin chemical status changes; however, it is closely linked to muscle and protein structures, which affect water holding capacity. Meat oxidation decreases water reservation in myofibrils, increasing juice loss of the meats. Moreover, lipid oxidation could increase cell membrane permeability and induce juice loss. Uysal et al. showed that L* indices of sausage slices decreased when sausage slices were converted into chips [33].

During storage of the fermented sausages, L* index significantly decreased in unpacked sausages, while vacuum-packed sausages included significantly lower L* index on Days 30 and 60 of storage [34].

The overall color changes (ΔE) of the samples were calculated between Days 0 and 29 of storage. In general, ΔE of GO, GO/ß-CD-1, GO/ß-CD-2 and GO/ß-CD-3 treatments included 8.75, 11.47, 4.99 and 1.36, respectively. Compared to Day 1, the lowest (12.15 to 10.79) and the highest (15.76 to 4.29) changes belonged to GO/ß-CD-3 and GO/ß-CD-1, respectively. By increasing quantity of GO/ß-CD, ΔE of the samples decreased. In ΔE calculations, changes in a*, b* and L* indices were effective in the control treatment. Based on the results in Table 1, a* index seemed to include the greatest effect. Decreases in a* index of GO/ß-CD-1, GO/ß-CD-2 and GO/ß-CD-3 included 2.58, 0.95 and 0.31, respectively. Moarefian *et al.* showed that ΔE of sausages containing peppermint EO decreased by increasing the EO content. Treatments containing the highest quantity of EO included the mildest overall color changes between Day 2 and the last day of refrigeration [38]. Oxidation of myoglobin and oxyhemoglobin leads to the formation of brown methmyoglobin and decreases in redness of the meat. Moreover, decreasing the quantity of nitrite in sausages (up to 60%) includes significant effects on the color of the control treatment and subsequently ΔE. Redness of meat is linked to dereases of iron ions in myoglobin and antioxidants retain divalent iron ions and redness. Although GO is not a potent antioxidant, it showed a further power and GO/ß-CD-3 treatment included the lowest ΔE in other treatments at higher concentrations.

Chroma (C*) included no significant differences in control, free GO and GO/ß-CD-3 treatments during storage. Significant differences were shown between GO/ß-CD-1 and GO/ß-CD-2 in the 2nd and 3rd and the 3rd and 4th sampling dates, respectively. The average C* of the control decreased; however, it increased up to the 2nd time of sampling and then decreased for free and encapsulated GO. Skaljac et al. repoted that color of unpacked and vacuum-packed fermented sausages stored in refrigerator increased after 30 and 60 days [34]. As C* index is associated to the quantity of pigments and high values represent a further vivid color, meaning lack of greyness [37]. This decrease might be duo to decreases of pigments. Hue angle (H*) showed no significant differences during storage. The average H* of all samples decreased. Although, only GO/ß-CD-1 increased. Positive correlations were seen between H* and b* (as seen from eq. 2). Skaljac et al. repoted that H* of unpacked and vacuum-packed fermented sausages decreased after 60 days refrigeration. As H* includes correlations with the quantity of pigments [37], its decrease might be duo to decreases of pigments during storage. Browning index (BI) decreased during the storage with no significant differences, except for GO/ß-CD-1 in the 2nd and 4th times of sampling date and GO/ß-CD-3 in the 3rd and 4th times of sampling. Uysal et al. showed that BI increased after convertion of sausage slices to chips [33].

3.1.2. pH

The pH changes of the samples are present in Table 2. Based on the national standards of Iran, the maximum acceptable pH of all types of sausages must be in the range of 5.6-6.3 [39]. Thus, pH of all treatments was 0.18-0.46 U higher than that of the standard on Day 0. It is noteworthy that only two changes were made in formulation of the sausages. One change included use of refined soybean oil without synthetic antioxidants and the other one included use of free or encapsulated GO. The control sample (containing 120 mg kg^{-1} sodium nitrite) included the highest pH value on Day 0. At all times of sampling, pH differences between the control and other treatments were significant. The pH changes of all treatments were not significant during storage. Stability of pH in all the five treatments could be due to the use of sodium phosphate, including buffering effects. By preventing the pH changes, it controlled pH of the samples as stable. Yin and Cheng [30] showed that addition of sulfur-containing organic compounds of garlic to ground beef included no significant effects on pH. Sallama et al. [8] reported that samples containing FG included the highest initial pH, increased during storage. Horita et al. [11] demonstrated that pH of the sausages containing GO was lower than that of the control. This might be due to the presence of 2% sodium chloride in the control and use of various concentrations of calcium chloride in other samples.

3.1.3. Peroxide value

As shown in Figure 1, PV of the samples increased slowly during storage. The PV changes of the control sample was higher than that of other treatments (0.057 µmol cumene hydroperoxide per kg of sample). On Day 29 of storage, PVs of GO and GO/ß-CD-2 samples were not significantly different. This might be attributed to the release of GO from GO/ß-CD. Quantitatively, PV of the GO/ß-CD-1 treatment was smaller than that of the control and GO/ß-CD-3 included the lowest PV after 29 days of storage. On Day 29, differences between the GO or GO/ß-CD and the control were statistically significant. Antioxidant characteristics of free and encapsulated GO were assessed using DPPH method [5]. It showed that free and encapsulated GO included weak antioxidant characteristics. This result was similar to that by Sun et al. [7]. By studying antioxidant activity of GO in shrimp meats, it was shown that coating decreased pH and accelerated lipid oxidation [17].

3.1.4. Thiobarbituric acid reactive substances

Quantity of thiobarbituric acid reactive substances was constant during storage. No significant differences were seen between the control and other samples during storage. Quantity of TBARS was less than the allowable level (data not shown). It is noteworthy that the vacuum packaging of sausage samples has caused less access to oxygen and hence lessened oil oxidation. Sallama et al. [8] showed that quantity of TBARS of chicken sausages containing GO was significantly lower than that of the control and more than that of the treatments containing FG, garlic powder and BHA. Effects of the packaging method (vacuum and modified atmosphere containing 20% of CO_2 and 80% of N_2) and storage time (210 days in refrigerator) on lipid oxidation and color stability of a type of fermented sausages studied by Rubio et al. [40]. Their results showed that TBARS increases of samples containing high linoleic acid were more than those containing high oleic acid and the two sample types were higher than that of the control. Treatments containing rosemary extract alone or with BHT and BHA included the lowest oxidation rates. After 45 days of storage, quantity of TBARS decreased, possibly due to the faster decomposition of hydroperoxides than their formation [41].

Feng et al. [42] reported that Chinese sausages containing higher fat content included the highest quantity of TBARS. During four weeks of storage in refrigerator, quantity of TBARS decreased after an initial increase, possibly due to the lower rate of fat oxidation and its conversion to malondialdehyde, compared to oxidation or oxidation and conversion to other compounds. Furthermore, they demonstrated that vacuum packaging significantly affected oxidation resistance of the samples stored in refrigerator. A study on effects of chitosan coatings incorporated with GO on the quality characteristics of shrimps stored at cold temperatures showed accelerating lipid oxidation. However, chitosan coating included antioxidative effects at earlier stages of the storage [16, 17]. Turan et al. revealed retarding effects of GO on lipid oxidation in marinated anchovys [14].

3.2. Microbial analysis

Changes in the microbial growth of the produced sausages are shown in Table 3. Based on ISIRI no. 2303, sausages must contain no *E. coli* or *S. aureus* in each gram of the product and *Salmonella* spp. must be negative for 25 g of the sample. [39]. In this study, GO/ß-CD-2 and GO/ß-CD-3 included no *S. aureus* after 15 days of storage, as well as GO/ß-CD-3 with no growth of *S. entrica* in the 2nd sampling time (8 d). No significant differences were seen between the samples containing GO and GO/ß-CD for *B. cereus* and *E. coli* at the end of storage. Encapsulated GO at 20% showed an intense antimicrobial activity and increased the minced meat shelf-life [12,13]. Mahros et al. revealed that FG could decrease aerobic bacterial counts of the beef meatballs better than that GO could during cold storage at 4 °C. Results of FG (5%) and GO (600 mg kg^{-1}) included the best antimicrobial potential and extended the shelf life during cold storage. Consumers did not accept it because of garlic strong flavor at high concentrations [15].

3.2.1. Staphylococcus aureus

Positive effects of free GO and GO/ß-CD on decreasing or stopping growth of the inoculated microorganisms (log 6.72 ±0.22 cfu ml^{-1}) was observed during the storage. In the control treatment (containing 120 mg kg^{-1} of sodium nitrite), number of *S. aureus* was uncountable in the 4th and 5th sampling times. In samples containing GO/ß-CD-2 and GO/ß- CD-3, S. *aureus* was not grown. These treatments prevented growth of these microorganisms. Moreover, 90 mg kg^{-1} GO included a better antimicrobial effect than that GO/ß-CD-1 did. Antimicrobial effects of GO on S. *aureus* growth were seen in free and encapsulated forms. Number of bacteria in the control sample was uncountable at Day 22 of storage, while that in samples containing GO/ß-CD-2 and GO/ß-CD-3 was negative on Days 15 and 8 of storage, respectively. Mozaffari Nejad et al. studied antibacterial effects of the aqueous garlic extract on *S. aureus* grown on a hamburger at 4 °C. They stated that garlic extract included good antibacterial characteristics against the highlighted microorganism [10]. Hussein et al. showed that improving safety of ready-to-eat sandwiches could derease the number of *S. aureus* using GO in tahini sauce [43].

3.2.2. Bacillus cereus

Number of *B. cereus* in the control smaple was uncountable (Day 22 of storage). On the last day of storage, GO/ß-CD-3 included the lowest number of *B. cereus*. From Day 15 to the end of the storage, differences were demonstrated between the numbers of *B. cereus* in GO and GO/ß-CD treatments with no significance, possibly due to the release of GO from the produced microcapsules during storage. Antimicrobial effects of free and encapsulated GO decreased from the 3rd time of sampling and hence number of *B. cereus* increased. Comparision of antimicrobial effects of free and encapsulated GO on *S. aureus* and *B. cereus* showed that *S. aureus* was more susceptible than *B. cereus*. In a previous study of the authors, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of 5^{-10} and 4^{-10} w v^{-1} for *S. aureus* and 3^{-10} and 3^{-10} w v^{-1} for *B. cereus* were reported, respectively. Growth of *S. aureus* was inhibited in presence of lower concentrations of free GO and GO/ß-CD, compared to *B. cereus*. Colonies were uncountable in the control sample on Day 29 of storage. Furthermore, GO, GO/ß-CD-1 and GO/ß-CD-3 colony numbers decreased during storage. This result was similar to that of a previous result by the authors; in which, *B. cereus* growth was inhibited by increased concentrations of free and encapsulated GO [13]. The *S. aureus* count significantly decreased in tahini sauce samples and ready-to-eat meat sandwiches [43].

3.2.3. Escherichia coli

All produced sausages included no *E. coli*. Number of *E. coli* in the control was uncountable from the 4th sampling time. At the end of the storage, no significant differences were reported between the other treatments regarding the number of *E. coli*. Antimicrobial effects of free and encapsulated GO decreased from the 3rd time of sampling. No significant differences were observed between the free and encapsulated GO treatments during storage. Additionally, *E. coli* was more susceptible to free and encapsulated GO, compared to that *B. cereus* was. This result was similar to that previously published by the present authors. The MIC and growth of *E. coli* were inhibited using lower concentrations of free and encapsulated GO, compared to those of *B. cereus* (10^{-4} instead of 10^{-3} w v^{-1}) [6]. Effects of garlic extract on *E. coli* O157:H7 in ready-to-cook chickens and *in-vitro* models resulted in significant antimicrobial effects [9]. Najjaa et al. verified inhibitory effects of microencapsulated GO against *E. coli* in minced meats [13].

3.2.4. Salmonella entrica

Produced sausages included no *S. enterica* in 25 g. Antimicrobial activity of sodium nitrite decreased in the control sample during the storage. In addition, number of *S. enterica* was uncountable from the 4th sampling time. From the 4th sampling time, antimicrobial effects of free and encapsulated GO decreased and number of *S. enterica* increased. Regarding GO/ß-CD-3 treatment, growth of *S. enterica* inhibited from the 2nd time of sampling. For other treatments, the lowest counted number belonged to GO/ß-CD-2. At the end of the storage, no significant differences were seen in antimicrobial effects of the treatment containing GO and GO/ß-CD. Comparison of the microbial counts of *E. coli* and *S. enterica* at the end of the storage demonstrated that *S. enterica* was more susceptible to free and encapsulated GO than that *E. coli* was. Although, results of MIC and MBC demonstrated similar susceptibilities [6]. It is noteworthy that antimicrobial activities of all chemical or natural compounds decrease over time. Hence by decreasing quantity of sodium nitrite, its antimicrobial effects decrease. As seen in control treatments, quantities of all four inoculated microorganisms were uncountable from Day 22.

Only one study has been published on the antimicrobial effects of encapsulated GO, showing inhibitory effects on coagulase-positive staphylococci [13]. Antimicrobial effects of GO in sausages were studied in chicken sausages by counting aerobic microorganisms; in which, effects of FG were better than those in garlic powder and GO [8]. Magrinya et al. [35] showed that number of lactobacilli and staphylococci increased after 45 d of storage. It was concluded that low storage temperature, nitrate concentration and vacuum packaging could decrease microbial population. In another study, Horita et al. [11] counted mesophilous aerobic bacteria, LAB, heat-tolerant coliforms and cold-tolerant bacteria in low-sodium sausages containing garlic and its derivatives. They demonstrated that by appropriate thermal process and presence of sodium nitrate, number of primary microorganisms of the produced treatments was less than ten $cfucm^2$ after 60 days of storage. Due to the lack of bioactive compounds, such as allicin, treatments containing garlic extract and garlic powder included the highest growth rates of microorganisms; however, FG showed excellent antimicrobial characteristics. Using aqueous extracts of pistachio skin in sausages, a total count of microorganisms was not significantly different until Day 2 and significant differences were observed between the control and treatments containing 1250 mg kg^{-1} of the extract only on the last day of storage. No growth of *C. perfringens*, coliforms, molds and yeasts were reported in all treatments [36]. During 30 days of storing sausages containing aqueous extract of the pomegranate peel in refrigerator, the total microbial count increased. However, no traces of molds and yeasts were recorded as well as coliforms and *C. perfringens* [36].

3.3. Sensory evaluation of samples containing free and encapsulated garlic oil

Table 4 presents sensory scores of the samples during storage.

3.3.1. Taste

Significant differences were not reported between the average taste scores of the sausages until the 4th time of evaluation. Taste scores of the control decreased up to Day 29. Taste scores of the treatment containing free GO decreased until the 4th sampling time and increased at the last sampling time. In treatment containing GO/ß-CD-1, the average taste scores of the 3rd and 4th times of sampling were not significantly different and decreased during the storage. Contrary to the control treatment, the average scores of the GO/ß-CD-3 increased during storage. Differences in the average taste score of the treatments containing free and encapsulated GO in the 4th and 5th times of the evaluation were not significant. On Day 29, the highest mean taste score belonged to GO/ß-CD-1 and GO/ß-CD-2 and the lowest mean taste score to GO treatment.

3.3.2. Odor

No significant differences were observed between the treatments during storage. On Day 29 of storage, the highest and the lowest average odor scores belonged to the control and GO/ß-CD-3, respectively. Mahros *et al.* reported that strong garlic flavor, particularly at high concentrations, decreased the consumers` acceptability of the samples [15].

Table 4. Changes in sensory characteristics of the samples during 29 days storage at 4 °C.

Characteristic	Treatments*	Sampling days									
		1st		8th		15th		22nd		29th	
		Rank mean	Arithmetic mean	Rank mean	Arithmetic mean	Rank mean	Arithmetic mean	Rank mean	Arithmetic mean	Rank mean	Arithmetic mean
Taste	Control	31.2 ^{Aa}	6.30±0.95	32.9 ^{Ba}	5.00±1.33	27.3 ^{Bc}	6.40±1.07	25.0 ^{Ca}	5.60±1.43	24.0 ^{Ca}	5.50±1.18
	GO	17.6 ^{Aa}	6.20±1.13	17.50 ^{Aa}	5.00±0.82	13.7 ^{Bb}	6.00±0.94	13.3 ^{Ba}	5.40±1.26	15.0 ^{Bac}	5.40±0.84
	GO/ß-CD-1	33.1 ^{Aa}	5.80±1.13	32.6 ^{Ca}	4.50±1.18	31.9 ^{Cb}	6.00±0.82	31.3 ^{Db}	5.90±0.99	31.0 ^{Da}	5.60±0.70
	GO/ß-CD-2	23.7 ^{Aa}	5.70±1.16	23.9 ^{Aa}	4.50±1.18	30.0 ^{Cb}	6.10±1.10	31.7 ^{Db}	6.10±0.74	31.2 ^{Da}	5.70±0.82
	GO/ß-CD-3	22.0 ^{Aa}	5.60±1.58	22.4 ^{Aa}	4.50±1.35	24.7 ^{Da}	6.20±1.23	26.3 ^{Aa}	6.20±0.92	26.5 ^{Aa}	5.70±1.06
Odour	Control	30.6 ^{Aa}	5.80±1.32	28.9 ^{Ba}	4.50±1.65	35.0 ^{Ca}	6.00±1.05	34.5 ^{Ca}	5.00±1.25	30.8 ^{Aa}	5.10±0.74
	GO	17.9 ^{Ca}	5.40±1.43	21.3 ^{Aa}	4.60±1.35	19.4 ^{Aa}	5.70±1.16	18.6 ^{Aa}	4.90±1.66	24.1 ^{Da}	4.50±1.27
	GO/ß-CD-1	33.6 ^{Ba}	6.20±1.47	32.6 ^{Ca}	4.70±1.34	30.3 ^{Ba}	5.70±1.06	27.3 ^{Ba}	4.80±1.03	27.2 ^{Ba}	5.00±1.49
	GO/ß-CD-2	22.5 ^{Da}	6.40±0.97	24.9 ^{Da}	4.90±1.29	20.1 ^{Aa}	5.70±1.49	22.4 ^{Da}	5.30±1.16	23.1 ^{Da}	5.50±0.97
	GO/ß-CD-3	23.1 ^{Da}	6.10±1.20	20.0 ^{Aa}	5.50±1.43	22.9 ^{Da}	5.80±1.32	24.8 ^{Da}	5.50±1.27	22.4 ^{Ca}	5.30±1.16
Color	Control	32.1 ^{Aa}	6.40±0.84	29.4 ^{Ba}	6.10±0.74	28.7 ^{Ba}	6.00±0.67	27.1 ^{Ba}	5.70±0.82	24.2 ^{Da}	5.60±0.84
	GO	27.9 ^{Ba}	5.80±1.12	26.0 ^{Ca}	5.60±0.84	22.7 ^{Da}	5.60±0.70	25.7 ^{Ca}	5.60±0.52	24.6 ^{Da}	5.10±1.10
	GO/ß-CD-1	26.0 ^{Ca}	5.80±1.13	26.5 ^{Ca}	5.30±0.95	28.8 ^{Ba}	5.70±0.82	26.7 ^{Ca}	5.40±1.26	24.6 ^{Da}	5.10±1.20
	GO/ß-CD-2	21.7 ^{Da}	5.80±1.47	25.3 ^{Ca}	5.80±0.79	26.3 ^{Ca}	5.70±1.25	24.3 ^{Da}	5.80±0.63	29.2 ^{Ba}	5.70±0.67
	GO/ß-CD-3	19.9 ^{Ea}	5.70±1.06	20.4 ^{Ea}	5.50±1.18	21.2 ^{Da}	5.50±1.18	23.8 ^{Da}	5.90±0.88	25.0 ^{Da}	5.60±0.97
Acceptability	Control	28.8 ^{Da}	6.00±1.15	29.7 ^{Da}	5.20±1.13	30.0 ^{Da}	6.00±1.05	31.7 ^{Da}	5.50±1.18	31.6 ^{Da}	5.80±1.23
	GO	19.7 ^{Aa}	5.90±1.45	14.8 ^{Ea}	4.60±1.17	15.9 ^{Ea}	6.10±1.00	14.7 ^{Eb}	5.50±0.85	19.9 ^{Aa}	5.50±1.51
	GO/ß-CD-1	29.6 ^{Da}	5.90±1.37	32.7 ^{Ea}	4.80±1.03	32.3 ^{Da}	5.90±0.74	31.6 ^{Da}	5.50±0.85	28.9 ^{Da}	5.40±0.84
	GO/ß-CD-2	23.4 ^{Ba}	6.10±0.99	25.1 ^{Ba}	4.70±1.06	25.5 ^{Ba}	6.00±0.94	26.2 ^{Ba}	5.60±1.07	24.4 ^{Ba}	5.40±0.97
	GO/ß-CD-3	26.1 ^{Ca}	6.30±1.49	25.3 ^{Ba}	5.10±1.73	23.8 ^{Ba}	6.10±1.45	23.4 ^{Ba}	5.60±1.07	23.7 ^{Ba}	5.60±1.17

* For abbreviation see Table 1 footnote. Different lowercase letters in each column indicate significant difference between treatments. Different uppercas5.60±e letters in each row indicate a significant difference between treatments ($P \leq 0.05$).

3.3.3. Color

No significant differences were seen between the treatments regarding the average color scores during the storage. At the end of the evaluation, the highest average color score belonged to the GO/ß-CD-2.

3.3.4. Overall acceptability

Differences between the overall acceptabilities of all samples were not significant during storage. In the last evaluation, control and free GO treatments included the highest and the lowest average acceptability scores, respectively. In quantitative changes in the average scores of indicators in five times of sensory evaluation, sausages included quite differences only in the 3rd time of evaluation for the taste index. Mann-Whitney test showed that free GO and GO/ß-CD-1 were quite different; however, control treatment was not significantly different from other treatments. It can be concluded that until the 3rd time of evaluation, the panelists did not differentiate between the control and other treatments regarding the four indicators of taste, smell, color and acceptability. By comparing sensory scores of the free and encapsulated GO, it is significant that differnces between GO, GO/ß-CD-2 and GO/ß-CD-3 are not statistically significant. In the 4th time of evaluation, treatments included significant differences in terms of taste and acceptability. Mann-Whitney test showed significant differences between GO with the GO/ß-CD-1 and GO/ß-CD-2 as well as the control and GO on taste and acceptability, respectively. The panelists did not recognize significant differences between the control and sausage treatments containing GO/ß-CD. Thus, it might be concluded that encapsulation of GO with β cyclodextrin included no adverse effects on garlic sausage taste. There are a few studies on sensory evaluation of the encapsulated GO. Ranjbar *et al.* indicated that garlic extract positively affected organoleptic characteristics of the cooked chickens at its optimum concentration [10].

4. Conclusion

In this study, free and encapsulated GO were used in sausage formulation. In general, ΔE decreased during storage at 4 °C. It seemed that a* (redness) was the most affecting factor. Moreover, pH of all samples was slightly more than the standard limit with no significanct differences. In this study, PV assay showed that free and encapsulated GO included poor antioxidant activities. In addition, TBARS results indicated weak antioxidant activities of free and encapsulated GO. Microbial experiments showed that *S. aureus* followed by *S. enterica*, *E.coli* and *B. cereus* were susceptible to encapsulated GO. Sensory results demonstrated no differences between the control and GO/ß-CD samples after 22 days of storage. It can be concluded that encapsulation of GO with β-cyclodextrin do not change the taste of produced sausages. Furthermore, GO/ß-CD can be recommended as a natural preservative due to its good antimicrobial characteristics.

5. Acknowledgements

This study was supported by a grant from Tarbiat Modares University and National Nutrition and Food Technology Research Institute (NNFTRI), Shahid Beheshti University of Medical Sciences. The authors thank Robat Meat Products for sausage production. Moreover, the authors thank Magnolia Flavor and Fragrance for providing GO.

6. Conflict of Interest

The authors report no conflicts of interest.

7. Authors Contributions

Khoshtinat K. “investigation and writing—original draft preparation”; Barzegar M. “conceptualization, writing—review, editing and supervision”; Sahari M.A. “advisor”; Hamidi Z. “advisor”.

References

1- Santhosha SG, Jamuna P, Prabhavathi SN. Bioactive components of garlic and their physiological role in health maintenance: A review. Food Biosci. 2013; 3:59-74. http://dx.doi.org/10.1016/j.fbio.2013.07.001

2- Piletti R, Zanetti M, Jung G, de Mello JMM, Dalcanton F, Soares C, Riella HG, Fiori MA. Microencapsulation of GO by β-cyclodextrin as a thermal protection method for antibacterial action. Mater Sci Eng C. 2019; 94: 139-149. https://doi:org/10.1016/j.msec.2018.09.037

3- FDA. http://www.fda.gov/Food/Ingredients Packaging Labeling/GRAS/ucm154182.htm

4- Tavares L, Santos L, Zapata Norena CP. Microencapsulation of organosulfur compounds from GO using β-cyclodextrin and complex of soy protein isolate and chitosan as wall materials: A comparative study. Powder Technol. 2021; 390:103-111. https://doi.10.1016/j.powtec.2021.05.080

5- Khoshtinat Kh, Barzegar M, Sahari MA, Hamidi Z. Encapsulation of Iranian GO with β-cyclodextrin: Optimization and its characterization. J Agric Sci Technol. 2017; 19(1): 97-111 http://jast.modares.ac.ir/article-23-6097-en.html

6- Khoshtinat Kh, Barzegar M, Sahari MA, Hamidi Z. Comparison of antioxidant and antibacterial activities of free and encapsulated GO with beta-cyclodextrin. Appl Food Biotechnol. 2016; 3(4) 254-268. https://doi.org/10.22037/afb.v3i4.12631

7- Sun YM, Ockerman HW, Marriott NG. Garlic in Chinese sausage. J Muscle Foods. 2000; 11, 35- 43.

8- Sallama KI, Ishioroshi M, Samejima K. Antioxidant and antimicrobial effects of garlic in chicken sausage. LWT. 2004; 37: 849- 855. https://doi:10.1016/j.lwt.2004.04.001

9- Ranjbar M, Sharifan A, Shabani S, Aminafshar M. The antimicrobial effect of garlic extracts on *Staphylococcus aureus* and *Escherichia coli* 0157:H7 in ready to cook chicken. J Food Technol Nutr. 2014; 11: 57- 66 (in persain).

10- Mozaffari Nejad AS, Shabani S, Bayat M, Hosseini SE. Antibacterial effect of garlic aqueous extract on *Staphylococcus aureus* in hamburger. Jundishapur J Microbiol. 2014; 7(11): e13134.
https://doi:10.5812/jjm.13134

11- Horita CN, Far ıas-Campomanes AM, Barbosa TS, Esmerino EA, Gomes da Cruz A, Bolini, HMA, Meireles MAA, Pollonio MAR. The antimicrobial, antioxidant and sensory properties of garlic and its derivatives in Brazilian low-sodium frankfurters along shelf-life. Food Res Inter. 2016; 84: 1-8.
https://doi: 10.1016/j.foodres.2016.02.006

12- Mehta N, Kumar P, Verma AK, Umaraw P, Kumar Y, Malav OP, Qurni Sazili A, Dominguez R, Lorenzo JM. Microencapsulation as a noble technique for the application of bioactive compounds in the food industry: A comprehensive review. Appl Sci. 2022; 12: 1424.
https://doi.org/10.3390/app12031424

13- Najjaa H, Chekki R, Elfalleh W, Tlili H, Jaballah S, Bouzouita N. Freeze-dried, oven-dried and microencapsulation of essential oil from *Allium sativum* as potential preservative agents of minced meat. Food Sci Nutr. 2020; 8, 1995-2003.
https://doi.org/10.1002/fsn3.1487

14- Turan H, Kocatepe D, Keskin I, Altan CO, Bayram Kostekli B, Candan C, Ceylan A. Interaction between rancidity and organoleptic parameters of anchovy marinade (*Engraulis encrasicolus* L. 1758) include essential oils. J Food Sci Technol. 2017; 54(10):3036-3043.
https://doi.10.1007/s13197-017-2605-9

15- Mahros MA, Eltanahy AR, Abd-Elghany SM Sallam KI. The antimicrobial effect of fresh garlic and GO supplemented with ground beef. Mansoura Vet Med J. 2021; 22(2): 48-51.
https://doi.10.35943/mvmj.2021.67329.1045

16- Hashemi M, Hashemi M, Daneshamooz Sh, Raeisi M, Jannat B, Taheri Sh, Noori SMA. An overview on antioxidants activity of polysaccharide edible films and coatings contains essential oils and herb extracts in meat and meat products. Adv Anim Vet Sci. 2020; 8 (2): 198-207.
https://doi.10.17582/journal.aavs/2020/8.2.198.207

17- Asik E, Candogan K. Effects of chitosan coatings incorporated with GO on quality characteristics of shrimp J Food Qual. 2014; 37(4): 237- 246.
https://doi.org/10.1111/jfq.12088

18- Khoshtinat Kh, Barzegar M, Sahari MA, Hamidi Z. Application of encapsulated GO in low-fat salad dressings: Physicochemical, microbial and sensory properties. Appl Food Biotechnol. 2022, 9 (2):113-125.

https://dx.doi.org/afb.v9i2.37619

19- Laca A, Senz MC, Paredes B, Diaz M. Rheological properties, stability and sensory evaluation of low- cholesterol mayonnaises prepared using egg yolk granules as emulsifying agent. J Food Eng. 2010; 97: 243- 252.
https://doi.org/10.1016/j.jfoodeng.2009.10.017

20- Azab DEH, Almoselhy RIM, Mahmoud MH. Improving the quality characteristics of low fat toffee by using mango kernel fat, pectin and high-speed homogenizer. J Food Process Preserv. 2022; 46: 12, e17235.
https://doi.org/10.1111/jfpp.17235

21- ISO 2917:1999- Meat and meat products- Measurement of pH-Reference method.

22- Maqsood S, Benjakul S. Synergistic effect of tannic acid and modified atmospheric packaging on the prevention of lipid oxidation and quality losses of refrigerated striped catfish slices. Food Chem. 2010; 121: 29- 38.
https://doi:10.1016/j.foodchem.2009.11.086

23- Firestone D. Ed. AOCS recommended practice Cd, 19-90. Animal and vegetable fats and oils, determination of 2-Thiobarbituric acid value direct method. Urbana, IL: American Oil Chemists Society; 2014.

24- ISO. 2005. Meat and meat products - Determination of thiobarbituric acid value in meat- Test method (13401).

25- ISO 6887-2:2017, Microbiology of the food chain-Preparation of test samples, initial suspension and decimal dilutions for microbiological examination- Part2: Specific rules for the preparation of meat and meat products.

26- ISO 6888-1: 2021. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase-positive *Staphylococci* (*Staphylococcus aureus* and other species)- Part 1 :Technique using Baird-Parker agar medium Amendment 1: Inclusion of precision data.

27- ISO 21871:2006. Microbiology of food and animal feeding stuffs horizontal method for the determination of low numbers of presumptive *Bacillus cereus*- Most probable number technique and detection method.

28- ISO 7251:2005. Microbiology of food and animal feeding stuffs - Detection and enumeration of presumptive *Escherichia coli* - Most probable number technique

29- ISO 6579-1:2017. Microbiology of the food chain- Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp.

30- Yin MC, Cheng WS. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. Meat Sci. 2003; 63:23- 28.
https://doi:10.1016/S0309-1740(02)00047-5

31. Moradi Y, Motallebi AA, Mosaddegh M, Shahrokhi S, Rafipour F, Zareh Gashti G, Safavi E, Babaei M. Investigating the possibility of producing a combined burger using a mixture of chicken meat and kilka fish. Project report No.44642. 2011. Iranian Fisheries Research Institute.

32. Badawy MEI, Lotfy TMR, Shawir SMS. Facile synthesis and characterizations of antibacterial and antioxidant of chitosanmonoterpene nanoparticles and their applications in preserving minced meat. Int J Biol Macromol. 2020 (156):127-136.
https://doi.org/10.1016/j.ijbiomac.2020.04.044

33. Uysal PC, Eniste I, Cifcci M, Simsek A, Kılıc B. Effects of different packaging methods and storage temperatures on physicochemical, microbiological, textural and sensorial properties of emulsion-type sausage chips. J Stored Prod Res. 2022 (98):102002.
https://doi.org/10.1016/j.jspr.2022.102002

34. Skaljac S, Jokanovic M, Tomovic V, Ivic M, Sojic B, Ikonic P, Peulic T. Colour characteristics of vacuum packed fermented sausage during storage. The 60th International Meat Industry Conference Meatcon 2019 IOP Conf. Series: Earth Environ Sci. 2019; 333: 012101
https://doi.org/10.1088/1755-1315/333/1/012101

35- Magrinya N, Bou R, Rius N, Codony R, Guardiola F. Effect of tocopherol extract, *Staphylococcus carnosus* culture and celery concentrate addition on quality parameters of organic and conventional dry- cured sausages. J Agric Food Chem. 2009;57(19): 8963- 8972.
https://doi.org/10.1021/jf901104h

36- Aliyari P, Bakhshi Kazaj F, Barzegar M, Ahmadi Gavlighi H. Production of functional sausage using pomegranate peel and pistachio green hull extracts as natural preservatives. J Agric Sci Technol. 2020; 22 (1):159- 172. http://jast.modares.ac.ir/article-23-20582-en.html

37. Ripoll GM, Joy M, Munoz F .Use of dietary vitamin E and selenium (Se) to increase the shelf life of modified atmosphere packaged light lamb meat. Meat Sci. 2011; 87(1): 88-93. https://doi.org/10.1016/j.meatsci.2010.09.008

38- Moarefian M, Barzegar M, Sattari M. *Cinnamomum zinnzmomu*m essential oil as a natural antioxidant and antibacterial in cooked sausage. J Food Biochem. 2013; 37: 62- 69. https://doi.org/10.1111/j.1745-4514.2011.00600.x

39- Iranian National Standardization Organization (INSO). Sausages- Specifications and test methods. 2303, 4th Revision. 2021; ICS: 67.120.10.

40- Rubio B, Martinez B, Garcia-Cachan MD, Rovira J, Jaime I. Effect of the packaging method and the storage time on lipid oxidation and colour stability on dry fermented sausage salchichòn manufactured with raw material with a high level of mono and polyunsaturated fatty acids. Meat Sci. 2008; 80:1182-1187. https://doi.org/10.1016/j.meatsci.2008.05.012

41- Trindade RA, Mancini-Filho J, Villavicencio ALCH. Natural antioxidants protecting irradiated beef burgers from lipid oxidation. LWT. 2010; 43: 98- 104. https://doi.org/10.1016/j.lwt.2009.06.013

42- Feng T, Ye R, Zhuang H, Rong Z, Fang Z, Wang Y, Gu Z, Jin Z. Physicochemical properties and sensory evaluation of Mesona Blumes gum/rice starch mixed gels as fat-substitutes in Chinese cantonese-style sausage. Food Res Int. 2013; 50:85-93.

43- Hussein MA, Mohamed SH, Tharwat AE, Hafez AEE, Gomaa RA. Effect of ingredients on the bacteriological profile of ready to eat meat sandwiches. J Anim Health Prod. 2021; 9:69- 75. http://doi.org/10.17582/journal.jahp/2021/9.s1.69.75

Research Article
pISSN: 2345-5357
eISSN: 2423-4214
APPLIED FOOD BIOTECHNOLOGY, 2023, 10 (2): 141-154
Journal homepage: www.journals.sbmu.ac.ir/afb

فرمولاسیون سوسیس با روغن سیر ریزپوشانی شده: خواص فیزیکوشیمیایی، میکروبی و حسی سوسیس

خدیجه خوش طینت[۱و۲]، محسن برزگر[۱*]، محمد علی سحری[۱]، زهره حمیدی[۱]

۱- گروه علوم و مهندسی صنایع غذایی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران.

۲- گروه تحقیقات صنایع غذایی، دانشکده علوم تغذیه و صنایع غذایی، انستیتو تحقیقات تغذیه ای و صنایع غذایی کشور، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران.

چکیده

سابقه و هدف: - مصرف‌کنندگان ترجیح می‌دهند نگهدارنده‌های مصنوعی مصرف نکنند، بنابراین، انواع طبیعی مانند اسانس ها (در این تحقیق روغن سیر) در بسیاری از فرآورده‌های غذایی به‌عنوان ترکیبات ضدمیکروبی به‌طور گسترده مصرف می شود. اگرچه، روغن سیر به تایید سازمان غذا و دارو آمریکا رسیده است، کاربرد آن در صنایع غذایی به‌علت حلالیت کم در آب، فراریت، طعم و بوی تند و پایداری کم محدود است. از آن جا که روغن سیر می‌تواند توسط اکسیژن، نور، حرارت و رطوبت تجزیه شود: ریزپوشانی آن با ترکیبات تجزیه‌پذیر، غیرسمی و مقاوم به حرارت، مانند بتاسیکلودکسترین، می‌تواند‌این محدودیت‌ها را ازبین ببرد. در مطالعه حاضر، درصدهای مختلفی از روغن سیر ریزپوشانی شده در فرمولاسیون سوسیس به کار برده شد و خواص فیزیکوشیمیایی، میکروبی، و حسی آن‌ها بررسی شد.

مواد و روش ها: پنج نوع سوسیس (شاهد (بدون روغن سیر)، حاوی روغن سیر آزاد (GO)، و سه تیمار حاوی ۵۴۶، ۸۱۸ و ۱۳۶۴ میلی‌گرم از روغن سیر ریزپوشانی شده (GO/ß-CD) در هر کیلوگرم تولید شد. سپس، تغییرات pH، رنگ، شاخص های اکسایش (عدد پراکسید و TBARS)، ضدمیکروبی (پایش ۴ ریززنده بیماری‌زا؛ *باسیلوس سرئوس*، *استافیلوکوکوس اورئوس*، *اشرشیا کلی*، و *سالمونلا انتریکا*)، و حسی (طعم، رنگ، بو و قابلیت پذیرش توسط آزمون هدونیک ۸-امتیازی) در مدت ۲۹ روز نگهداری در ۴ درجه سانتی گراد ارزیابی شد.

یافته‌ها و نتیجه‌گیری: در تیمارهای سوسیس، با افزایش میزان سیر ریزپوشانی شده تغییرات کلی رنگ کاهش یافت. میزان pH در تمام تیمارها ثابت بود. در پایان دوره نگهداری، عدد پراکسید تغییر معنی‌داری نداشت و تغییرات TBARs بسیار ناچیز بود که می‌تواند به حضور روغن سیر و تاثیر بسته‌بندی تحت خلا برش‌های سوسیس مربوط باشد. *استافیلوکوکوس اورئوس* حساس ترین ریززنده بیماری‌زا بود و پس از آن به‌ترتیب *سالمونلا انتریکا*، *اشرشیا کلی و باسیلوس سرئوس* قرار داشتند. در ارزیابی حسی، ارزیاب‌ها نتوانستند تفاوتی بین نمونه شاهد و نمونه‌های حاوی روغن سیر ریزپوشانی شده بیابند. به‌طور کلی، ریزپوشانی روغن سیر با بتاسیکلودکسترین بوی تند آن را مهار و تاثیر سوئی بر سوسیس نداشت. در نهایت، به دلیل خواص ضدمیکروبی خوب آن می‌تواند به‌عنوان یک نگهدارنده طبیعی در فرآورده‌های گوشتی استفاده شود.

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

تاریخچه مقاله

دریافت ۷ فوریه ۲۰۲۳
داوری ۲۲ فوریه ۲۰۲۳
پذیرش ۱۲ مارس ۲۰۲۲

واژگان کلیدی

- روغن سیر
- بتا سیکلودکسترین
- ریزپوشانی
- سوسیس، خواص فیزیکوشیمیایی
- خواص ضدمیکروبی

***نویسنده مسئول**

محسن برزگر

گروه علوم و مهندسی صنایع غذایی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران

تلفن: ۹۸(۲۱)۴۸۲۹۲۳۲۳+

پست الکترونیک:
mbb@modares.ac.ir