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Fabrication and characterization of basil essential oil microcapsule-enriched mayonnaise and its antimicrobial properties against *Escherichia coli* and *Salmonella* Typhimurium

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ABSTRACT

Nowadays, as consumers tend to avoid foods containing synthetic preservatives, technologically processed plant extracts can be a good alternative to these preservatives. In this study, previously obtained basil essential oil microcapsules (BEOM) were added to mayonnaise in order to produce a microbiologically safe product with improved physicochemical properties. Mayonnaises were prepared with 0%, 0.3%, 0.6%, and 0.9% BEOM replacement of the total oil content, called Mayo-Control, Mayo-0.3% BEOM, Mayo-0.6% BEOM, and Mayo-0.9% BEOM, respectively. Additionally, Mayo-SP containing ethylene diamine tetra-acetic acid and potassium sorbate was prepared. The enriched mayonnaises displayed better antimicrobial activity against *Escherichia coli* than Mayo-SP and Mayo-Control. Mayo-SP showed the best antimicrobial activity against *Salmonella* Typhimurium, followed by Mayo-0.9% BEOM. At the end of storage, Mayo-0.9% BEOM had the highest apparent viscosity, G′, and G″ values due to its high content of gum molecules. Trans-2-heptanal, an oxidation product, was not identified in the enriched mayonnaises or Mayo-SP. Finally, BEOM were efficient in providing microbial safety of mayonnaise and also improved the product's oxidative stability, viscosity, and aroma.

1. Introduction

Basil (*Ocimum basilicum* L.) is used as a flavoring agent in culinary practices all over the world (Ghasemi Pirbalouti, Mahdad, & Craker, 2013). Functional compounds such as essential oils and extracts can be used to improve the color, aroma, and texture of food products and they can also be used as preservatives to increase the shelf life of the product. These compounds are generally not resistant to environmental factors and process conditions. At this point, microencapsulation technology is used to overcome this problem. Microencapsulation is the process of protecting the active ingredient (core material) within an encapsulating material (wall or shell material) (Paulo & Santos, 2017). One of the most common methods used in the encapsulation of essential oils and flavorings is spray drying. The active ingredient is dissolved or dispersed in a polymer solution containing aqueous or organic solvent, and then the solution is fed into the spray dryer and sprayed into the drying chamber. Finally, the microcapsules are obtained by removing water or solvent from the solution (Carvalho, Estevinho, & Santos, 2016).

Mayonnaise is one of the most commonly consumed sauces or condiments in the world today. The use of pasteurized egg yolk to produce mayonnaise minimizes the risk of contamination by bacteria (e.g., *Salmonella*). In addition, the aqueous phase with a pH below 4.1 ensures microbiological safety, and the ingredients such as salt, sugar, garlic, and mustard also contribute to its microbial safety. However, mixing mayonnaise with other ingredients for preparing salads can be dangerous to health because of increasing the pH of the environment. Additionally, salads are generally not heat-treated and are packed and stored at unsuitable temperatures. Therefore, salads prepared with mayonnaise increase the risk of *Salmonella* contamination. The application of natural antimicrobial agents to mayonnaise could be an

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effective multi-barrier technology. This technology can be defined as the application of combined protective measures to increase the microbiological stability, sensory quality, and economic and nutritional value of foods (Passos Lima da Silva & Dora Gombosy de Melo, 2012).

Natural antimicrobials have been applied to mayonnaise and mayonnaise-based salads to control microorganisms in previous studies. Monu, Techathuvanan, Wallis, Critzer, and Davidson (2016) tested the antimicrobial activity of clove, cinnamon, and thyme essential oils and the components of these oils, trans-cinnamaldehyde, cinnamic acid, eugenol, carvacrol, and thymol, against *Torulaspora delbruecki*, *Candida krusei*, *Schizosaccharomyces pombe*, and *Zygosaccharomyces bailii*. The most efficacious essential oils and components were applied to a mayonnaise-based salad dressing and trans-cinnamaldehyde showed good inhibitory properties against *S. pombe* and *Z. bailii* yeasts. Yolmeh, Habibi Najafi, Farhoosh, and Salehi (2014) investigated the use of annatto dye against *Escherichia coli* in mayonnaise; they found that the number of *E. coli* decreased when annatto dye was included in mayonnaise.

The incorporation of microcapsules in food systems is a challenging task and needs to be improved. Recently, the addition of microcapsules to mayonnaise to increase oxidative stability and nutritional value has been studied (Miguel et al., 2019; Rahmani-Manglano et al., 2020; Rojas et al., 2019). However, no study has been found in the literature on increasing the microbiological safety of mayonnaise by adding microcapsules. The hypothesis of this study is to provide a multi-barrier system for microbiologically safe mayonnaise by application of basil essential oil microcapsules (BEOM). Therefore, BEOM were added to mayonnaise in different ratios to determine their antimicrobial activity against *Escherichia coli* (biotype 1) and *Salmonella* Typhimurium (ATCC 14028). The BEOM-enriched mayonnaises were compared with mayonnaise containing synthetic antimicrobial and antioxidant agents. Changes in the rheological properties, aroma, and droplet size of the mayonnaises were determined.

2. Material and methods

2.1. Materials

Basil essential oil (BEO; *Ocimum basilicum* L., comoric type, refractive index: 1.512–1.590, d: 0.9560 g/cm^3) was obtained from Sigma-Aldrich (Steinheim, Germany). Standards (estragole, linalool, *trans*-cinnamaldehyde, limonene, 1,8-cineole, eugenol, and hexanal) were obtained from Sigma-Aldrich (Steinheim, Germany) and Fluka (Steinheim, Germany). Refined sunflower oil, pasteurized egg yolk, and cider vinegar were purchased from a local market in Ankara. All chemicals were of analytical grade.

Details of the synthesis and characterization of BEOM were given in our previous study (Ozdemir et al., 2021). The main compound of BEO was estragole (85.74%), followed by 1,8-cineole (3.30%), α-bergamotene (2.22%), linalool (1.57%), β-ocimene (0.82%), γ-cadinene (0.49%), and methyl eugenol (0.45%). BEO was encapsulated using a spray drying technique, and gum arabic (GA), maltodextrin (MD), and whey protein isolate (WPI) were used as wall materials (1 : 1 : 1, w/w). The wall materials (24%) were dispersed in distilled water (70%) and the solution was left standing overnight at room temperature. An emulsion was prepared by mixing the BEO (6%) and the wall material solution using a rotor–stator blender (Ultra-Turrax IKA T18 basic, Wilmington, USA) at 16,000 rpm. The microcapsules were obtained using a mini spray dryer (Model B-290; Büchi, Flawil, Switzerland) under the following conditions: inlet temperature of 150 °C, feed rate of 3 mL/min, drying air flow rate of 40 kg/h, and aspiration rate of 35 m^3/h. The powder recovery and encapsulation efficiency of the BEOM were 65.92% and 87.19%, respectively. The particle size value (D_{32}) of the BEOM was 3.16 μm and the microcapsules had irregular spherical shapes, dents, and shrunken surfaces. The glass transition temperature of BEOM was 69 ± 1 °C. *In vitro* release of estragole from the BEOM microcapsules in ethanol was 58.97% at the end of 48 h and the release rate depended simultaneously on the swelling and diffusion processes specific to a non-Fickian transport mechanism.

2.2. Antimicrobial activity of free BEO and BEOM

Minimum inhibitory concentrations (MICs) of free BEO and BEOM were determined using the broth dilution assay (Hill, Gomes, & Taylor, 2013) against *Escherichia coli* (biotype 1) and *Salmonella* Typhimurium (ATCC 14028) which were obtained from the Microbiology laboratory in the Department of Food Engineering at Ankara University.

Free BEO was solubilized in ethanol solution which was made of a mixture of dimethyl sulfoxide (DMSO, 1%), Tween 20 (5%), and ethanol (94%) (Moghaddam et al., 2014) while BEOM were solubilized in a water: ethanol solution (1:1, v/v). Dilutions of free BEO and BEOM were prepared in sterile TSB cuvettes (2 mL). The concentration of free BEO and BEOM added to the cuvettes varied from 350 to 1440 μg BEO/mL for *E. coli* and from 350 to 1200 μg BEO/mL for *S.* Typhimurium. Then, 0.1 mL of the inoculums (3 Log CFU/mL) was inoculated to the test cuvettes. For positive controls, the ethanol solution and water:ethanol solution were used at test concentrations. Next, the optical density at 630 nm (OD630) of the samples was recorded using a UV–vis spectrophotometer (Hitachi, U-2800A, Tokyo, Japan). The samples were incubated at 37 °C for 24 h and after the incubation the OD630 of the samples was measured again. The MIC was the lowest antimicrobial concentration that showed ≤0.05 change in OD630 after the incubation.

All cuvettes showing inhibition were tested for bactericidal capability by spreading 0.1 mL from the cuvettes onto PCA plates. The plates were incubated at 37 °C for 24 h and then the lowest concentration that showed no growth was considered the minimum bactericidal concentration (MBC) (Hill et al., 2013). Analyses were carried out in triplicate.

2.3. Preparation of mayonnaise

Five different mayonnaise formulations were prepared in this study. The recipe was based on the following formulation: sunflower oil (80%), egg yolk (9%), cider vinegar (7%), sugar (2%), water (1.5%), and salt (0.5%); this recipe was called Mayo-Control. The mayonnaises containing BEOM were prepared with 0.3%, 0.6%, and 0.9% BEOM replacement of the total oil content, which were called Mayo-0.3% BEOM, Mayo-0.6% BEOM, and Mayo-0.9% BEOM, respectively. Mayo-SP, in addition to the recipe of Mayo-Control, contained ethylene diamine tetra-acetic acid (EDTA, 0.075%) and potassium sorbate (PS, 0.1%).

Initially, egg yolk, water, sugar, and salt were mixed for 30 s at high speed in a stand mixer (Artisan, KitchenAid, Michigan, USA) (in Mayo-SP, EDTA and PS were added at this stage). Then, the oil was incorporated into the aqueous phase slowly (6 min 45 s) and the emulsion was mixed for 1 min. In the mayonnaises including BEOM, the microcapsules were dispersed homogenously in the oil.

2.4. Survival of E. coli and S. Typhimurium in mayonnaise samples

One hundred grams of each mayonnaise was weighed in sterile beakers after being freshly prepared; 0.1 mL of *E. coli* and *S.* Typhimurium cultures (3 Log CFU/mL) was inoculated to the mayonnaises and the samples were mixed homogenously using a stirrer. Then, 5 g of the inoculated mayonnaises was weighed in sterile plastic containers and kept at 25 °C. The samples were taken at certain time intervals (0, 3, 6, 9, 24, 48, and 72 h of storage) and diluted to appropriate concentrations with buffered saline solution (0.1%). Next, 0.1 mL of the diluted samples was spread on VRB and XLT4 plates. After incubation (37 °C, 24 h), the colonies were counted to determine the bacterial population in the samples. The analysis was performed in duplicate.

2.5. Storage of mayonnaise samples

Mayonnaise samples were put into 50 mL plastic containers and stored in an incubator at 25 °C for 6 weeks. Samples were taken at appropriate times for the analysis of pH, aroma, rheology, and droplet size distribution.

2.6. pH measurement

Ten grams of mayonnaise sample was weighed in a beaker and 90 mL of distilled water was added. The mixing was homogenized using a stirrer. The pH meter (Seven Compact pH-meter S220, Mettler Toledo AG, Switzerland) was calibrated using standard buffer solutions (pH 4, pH 7, and pH 10). The samples were analyzed at room temperature and the measurements were conducted in duplicate.

2.7. Volatile compound analysis

Changes in the volatile profiles of the mayonnaises were determined using headspace-solid phase microextraction (HS-SPME). Changes in the amount of estragole in the mayonnaises were also analyzed. Four grams of the mayonnaises was weighed accurately in headspace vials (20 mL) and the vials were sealed with polytetrafluoroethylene (PTFE)-coated silicone rubber septums (Agilent Technology Inc., Santa Clara, CA, USA). An 85 μm Carboxen/polydimethylsiloxane (CAR/PDMS) fiber was used to extract volatile compounds from the headspace above the mayonnaises. The samples were left at 50 °C in a thermoblock (Supelco, Bellefonte, PA, USA) for 15 min for their headspaces to equilibrate and then the fiber was exposed for 1 h to absorb the volatiles from the headspace. Afterwards, the fiber was desorbed into the GC/MS injection port for 10 min at 250 °C (split 1 : 10). The method was modified from that of Hartvigsen, Lund, Hansen, and Holmer (2000).

The volatile compounds in the mayonnaises were analyzed on an Agilent 7890A gas chromatograph (Agilent Technology Inc., Santa Clara, CA, USA) directly coupled to an Agilent 5975C mass selective detector (Agilent Technology Inc., Santa Clara, CA, USA), equipped with a DB-624 capillary column (30 m, 0.25 mm i.d. and 1.4 μm film thickness) (J&W Scientific, Folsom, CA, USA). The operating conditions were as follows: oven temperature was initially set at 40 °C for 5 min, then programmed to rise from 40 to 110 °C at 3 °C/min, then from 110 to 150 at 4 °C/min, then from 150 to 210 °C at 10 °C/min and kept at 210 °C for 12 min. The injector and detector temperatures were 250 °C. The carrier gas was helium at a linear flow rate of 1.0 mL/min; injection of the sample of diluted essential oil solution (1 : 50, v/v, in methanol) was done in split mode (1/100). Ion source temperature and energy ionization were 230 °C and 70 eV, respectively. Scanning (1 scan s^{-1}) was performed in the range of 40–400 *m/z* (Kiralan, Bayrak, Abdulaziz, & Ozbucak, 2012). The volatile components were identified using their Kováts retention indices (KI) by injecting *n*-alkane series (C5–C22) in the same conditions and the comparison of their mass spectra with those of Wiley 10 & NIST 14 library data of the GC/MS system. Additionally, pure standards of the major components of BEO (estragole, linalool, *trans*-cinnamaldehyde, limonene, 1,8-cineole, eugenol, and *trans*-2-heptanal) were used for identification. Measurements were performed in triplicate.

2.8. Rheological measurements

Rheological measurements of the mayonnaises were carried out at the end of the first day and end of the sixth week (day 43). Rheological analyses were evaluated using a Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria), equipped with a circulating water bath to control temperature. Analyses were performed at 25 °C in duplicate.

2.8.1. Steady shear viscosity

The cone and plate configuration was used to measure the steady shear viscosity of the mayonnaises (diameter 20 mm, cone angle 4°). The viscosity of the samples was measured in the shear rate ranging from 0.1 to 100 s^{-1} at 25 °C (Chang et al., 2017). The Herschel–Bulkley model was applied to determine the yield stress (τ_0), consistency (Pa.sn) (K), and the flow behavior (n) indices of the samples. The equation is as follows:

$$\tau = \tau_0 + K.\gamma^n$$

where τ: shear stress (Pa), τ_0: yield stress (Pa), K: consistency (Pa. s^n), γ: shear rate (s^{-1}), and n: flow behavior indices.

2.8.2. Dynamic viscoelastic measurement

The viscoelastic properties of the mayonnaises were evaluated using a parallel plate and gap distance of 1 mm at 25 °C. Initially, the amplitude sweep test was performed at a constant frequency of 1 Hz with an applied strain varying from 0.1% to 1% to determine the linear viscoelastic region (LVR) of the samples. Before the dynamic frequency sweep, samples were left to rest for 10 min to maintain temperature equilibrium and decrease stress. Next, the dynamic frequency sweep was performed in the range of 0.1–100 Hz at 25 °C to measure the viscoelastic parameters (Chang et al., 2017). The elastic modulus and the loss modulus were recorded (Rheoplus/32 V3.31 program).

2.9. Droplet size distribution

The droplet size distribution of the mayonnaises was measured by light scattering using laser diffraction in a Mastersizer 2000 (Hydro 2000 MU, Malvern Instruments, Malvern, UK). Analysis was performed at the end of the first day and end of the sixth week. Before the measurements, approximately 0.5 g of the sample was weighed and diluted with 100 mL of sodium dodecyl sulfate solution (0.2%) (Di Mattia, Balestra, Sacchetti, Neri, Mastrocola, & Pittia, 2015). The refractive indices of sunflower oil (1.51) and water (1.33) were used as particle and dispersant, respectively. The mayonnaise solution was diluted in recirculating water at 3000 rpm until an obscuration of 7% was achieved. Each sample was measured in duplicate.

2.10. Statistical analysis

Analysis of variance (ANOVA) was conducted using SAS Statistical software (version 9, SAS Institute, Cary, NC, USA) for Windows; and LSD comparisons were used to detect any significant differences ($p < 0.05$) between variables.

3. Results and discussion

3.1. Antimicrobial activity of free BEO and BEOM

The MICs and MBCs of free BEO and the BEOM against *E. coli* and *S.* Typhimurium are shown in Table 1. The MIC and MBC values for free BEO against *E. coli* were 400 and 450 μg/mL, respectively. The BEOM demonstrated significantly lower antimicrobial activity ($p < 0.05$) against *E. coli* than the free BEO. The MIC and MBC values of the BEOM

Table 1
Minimum inhibitory and minimum bactericidal concentrations (MIC, MBC) against *Escherichia coli* (biyotip 1) and *Salmonella* Typhimurium (ATCC 14028) for free BEO and BEOM.

Sample	*Escherichia coli*		*Salmonella* Typhimurium	
	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)
BEO	400^b	450^b	700^b	800^b
BEOM	1000^a	1100^a	800^a	900^a

a–b: The values with different superscript letters (within columns) indicate significant differences ($p < 0.05$).

against *E. coli* were found to be 1000 and 1100 μg/mL, respectively. Similar studies have been found in the literature. Beatovic et al. (2015) evaluated the antimicrobial activity of the essential oils of twelve basil cultivars grown in Serbia. Siam Queen cultivar belonged to the "estragole chemotype" (83.6%) and the MIC and MBC values for the oil of 1.25 and 4.50 μg/mL, respectively, against both *E. coli* (ATCC 35210) and *S.* Typhimurium (ATCC 13311). The MIC and the MBC values given in our study were higher than those given by Beatovic et al. (2015). Additionally, they reported that the MIC and MBC values for the twelve BEO ranged from 0.28 to 21.83 μg/mL, respectively, against both E. coli and S. Typhimurium and emphasized the importance of chemical composition of the oils. Apart from the chemical composition of the essential oils, microorganism strains and experimental conditions and procedures may be the cause of difference between our results and those reported by Beatovic et al. (2015). Moghaddam et al. (2014) tested BEO (*Ocimum ciliatum*) against ten plant pathogens and reported MICs ranging from 1.1 to 6.1 mg/mL and MBCs ranging from 4.7 to 30 mg/mL. The main component of the BEO used by the authors was estragole (87.63%) just like in our study (85.74%).

Free BEO showed an MIC and MBC of 700 and 800 μg/mL, respectively, against *S.* Typhimurium. Free BEO possessed higher antimicrobial activity than the BEOM which presented an MIC and MBC of 800 and 900 μg/mL for *S.* Typhimurium, respectively. The antimicrobial activity of essential oils is strongly related to their composition. Rattanachaikunsopon and Phumkhachorn (2010) reported an MIC for BEO containing mainly linalool (64.35%) and 1,8-cineol (12.28%) of 40 μg/mL against *Salmonella* Enteritidis. The composition of the oil and the *Salmonella* strain used in our study were different from the oil and strain used by Rattanachaikunsopon and Phumkhachorn (2010), and so it is expected that the MIC values from these studies are different.

Free BEO showed higher antimicrobial activity than the BEOM against *E. coli* and *S.* Typhimurium ($p < 0.05$). This situation could have originated from the release properties of BEO from the microcapsules. Ngamakeue and Chitprasert (2016) encapsulated BEO inside gelatin using a simple coacervation method. In this study, the BEO–gelatin microcapsules generally possessed the same MIC and MBC values as free BEO, and some formulations of the microcapsules even demonstrated higher antimicrobial activity against *E. coli* (O157:H7), *S.* Typhimurium (TISTR 292), and *Staphylococcus aureus* (TISTR 029). On the other hand, the opposite of this situation has been observed. Ozdemir et al. (2018) encapsulated black pepper oleoresin using β-cyclodextrin by freeze drying and kneading methods. They stated that free black pepper oleoresin had significantly higher antimicrobial activity than its encapsulated forms against *E. coli* O157:H7 and *Listeria monocytogenes*.

3.2. pH measurement

The pH values of mayonnaise samples determined over a storage period of 6 weeks are shown in Table 2. The pH values of Mayo-SP were higher than those of the others during the storage period ($p < 0.05$), which could be due to the PS contained in the formulation of Mayo-SP. Arslan, Ilhan, Vardar, and Karabulut (2008) evaluated the antimicrobial activity of food additives against soil-borne pathogens and stated that the addition of PS to soil causes an increase in the pH of soil. Additionally, the mayonnaises enriched with BEOM had higher pH values than that of Mayo-Control. Our results are in accordance with data in the literature. Chatterjee and Bhattacharjee (2015) added clove (*Syzygium aromaticum* Linn.) extract and mustard to mayonnaise and investigated the pH values of mayonnaise samples over 6 months. In the first 4 months of storage, the mayonnaises enriched with clove extract and mustard exhibited higher pH values than that of the control.

3.3. Survival of E. coli and S. Typhimurium in mayonnaise samples

The *E. coli* count of the mayonnaises (Log CFU/g) is shown in Table 3. Mayo-Control possessed the highest decrease in viable count (1.37 Log CFU/g) at 24 h, followed by Mayo-0.9% BEOM (1.33 Log CFU/g). The difference between Mayo-Control and the mayonnaises enriched with BEOM was not significant ($p > 0.05$) and Mayo-SP had the highest count ($p < 0.05$). At the end of storage, the viable count in the mayonnaises enriched with BEOM was significantly lower than that in Mayo-Control and Mayo-SP ($p < 0.05$), which could be related to the release mechanisms of the microcapsules. BEOM may have needed time to release their contents, and therefore the appearance of antimicrobial activity of the volatile compounds against *E. coli* could have been delayed.

Table 2
pH of the mayonnaises.

Time (week)	Mayo –Control	Mayo – SP	Mayo – 0.3% BEOM	Mayo – 0.6% BEOM	Mayo – 0.9% BEOM
0	4.08 ± 0.01^{BCb}	4.23 ± 0.00^{Ac}	4.07 ± 0.00^{Cc}	4.08 ± 0.01^{BCb}	4.11 ± 0.02^{Bb}
1	4.03 ± 0.04^{BCc}	4.20 ± 0.01^{Ad}	3.99 ± 0.01^{Ce}	4.02 ± 0.01^{BCc}	4.05 ± 0.01^{Bd}
2	3.96 ± 0.00^{Ed}	4.18 ± 0.01^{Ae}	3.99 ± 0.00^{Ded}	4.01 ± 0.00^{Cc}	4.05 ± 0.00^{Bcd}
3	3.97 ± 0.03^{Dd}	4.17 ± 0.00^{Ae}	4.00 ± 0.00^{CDd}	4.03 ± 0.01^{Cc}	4.07 ± 0.00^{Bc}
4	4.12 ± 0.01^{Eab}	4.33 ± 0.01^{Ab}	4.15 ± 0.01^{Db}	4.17 ± 0.01^{Ca}	4.20 ± 0.00^{Ba}
5	4.11 ± 0.01^{Eab}	4.33 ± 0.01^{Ab}	4.14 ± 0.01^{Db}	4.18 ± 0.01^{Ca}	4.21 ± 0.01^{Ba}
6	4.15 ± 0.01^{Da}	4.34 ± 0.00^{Aa}	4.17 ± 0.01^{CDa}	4.19 ± 0.01^{Ca}	4.22 ± 0.01^{Ba}

A–E: The means with different uppercase letters among the samples (within rows) indicate significant differences ($p < 0.05$).
a–e: The means with different lowercase letters among storage days (within columns) indicate significant differences ($p < 0.05$).

The *S.* Typhimurium count (Log CFU/g) of the mayonnaises is presented in Table 3. The number of *S.* Typhimurium organisms in Mayo-PS declined more quickly than in the others during storage (approximately 4 Log CFU/g). After Mayo-SP, the most effective sample against *S.* Typhimurium was Mayo-0.9% BEOM (3.34 Log CFU/g) at 24 h. The smallest decrease in viable count was found for Mayo-Control after 24 h ($p < 0.05$).

According to these results, it is shown that the BEOM demonstrated good antimicrobial activity against *E. coli* and *S.* Typhimurium in the mayonnaises. Passos Lima da Silva and Dora Gombosy de Melo (2012) obtained similar results with the use of oregano essential oil in mayonnaise salads against *S.* Enteritidis. The decline in viable counts of *S.* Enteritidis in the oil added to salads (0.2%) during storage (8 °C/24 h) was greater than in the control sample. In addition, Mayo-SP was found to be the most effective sample against *S.* Typhimurium while this situation was not observed against *E. coli*. The combination of PS and EDTA in Mayo-SP may have reacted differently against *E. coli* and *S.* Typhimurium. Similar results were obtained by Wan Norhana, Poole, Deeth, and Dykes (2012) who investigated the antimicrobial activity of nisin, EDTA, PS, sodium benzoate, and sodium diacetate against *L. monocytogenes* (V7) and *S.* Typhimurium (ATCC 14028) in shrimp samples. Use of the combination of nisin, EDTA, and PS resulted in antimicrobial activity against *L. monocytogenes* while a similar phenomenon was not observed against *S.* Typhimurium. Additionally, the decrease in *E. coli* and *S.* Typhimurium counts for all mayonnaises is related to their low pH.

3.4. Aroma analysis

At the beginning of storage, the principal component of Mayo-Control was acetic acid (98.62%) that arose from cider vinegar. The main compound of Mayo-SP was sorbic acid (54.07%), followed by acetic acid (46.15%), showing that PS changed to sorbic acid in Mayo-SP. The main compound of the mayonnaises enriched with BEOM was acetic acid (Mayo-0.3% BEOM: 58.28%, Mayo-0.6% BEOM: 51.68,

Table 3
Survival of the *Escherichia coli* and *Salmonella* Typhimurium in the mayonnaises during storage at 25°C.

	E. coli (log CFU/g)					*S.* Typhimurium (log CFU/g)				
Time (hour)	Mayo-Control	Mayo-SP	Mayo-0.3% BEOM	Mayo-0.6% BEOM	Mayo-0.9% BEOM	Mayo-Control	Mayo-SP	Mayo-0.3% BEOM	Mayo-0.6% BEOM	Mayo-0.9% BEOM
0	6.37 ± 0.08^{Aa}	6.34 ± 0.01^{Aa}	6.34 ± 0.05^{Aa}	6.35 ± 0.00^{Aa}	6.39 ± 0.06^{Aa}	6.06 ± 0.00^{Aa}	6.11 ± 0.05^{Aa}	6.07 ± 0.02^{Aa}	6.09 ± 0.00^{Aa}	6.33 ± 0.23^{Aa}
3	5.69 ± 0.14^{Bb}	6.13 ± 0.06^{Aa}	5.68 ± 0.04^{Bb}	5.74 ± 0.05^{ABb}	5.86 ± 0.33^{ABb}	5.80 ± 0.11^{Aa}	5.71 ± 0.13^{Aa}	5.50 ± 0.09^{Ab}	5.59 ± 0.15^{Ab}	5.69 ± 0.14^{Ab}
6	5.68 ± 0.27^{Ab}	5.76 ± 0.18^{Ab}	5.58 ± 0.04^{Abc}	5.48 ± 0.09^{Abc}	5.62 ± 0.14^{Ab}	5.03 ± 0.13^{Ab}	4.96 ± 0.31^{Ab}	5.14 ± 0.11^{Ab}	4.97 ± 0.05^{Ac}	5.07 ± 0.00^{Ac}
9	5.50 ± 0.08^{Abc}	5.44 ± 0.00^{Ac}	5.42 ± 0.03^{Ac}	5.48 ± 0.10^{Abc}	5.48 ± 0.06^{Abc}	4.91 ± 0.01^{Ab}	4.25 ± 0.10^{Bc}	4.68 ± 0.36^{ABc}	4.56 ± 0.18^{ABd}	4.73 ± 0.02^{Ac}
24	4.98 ± 0.17^{Bc}	5.49 ± 0.14^{Abc}	5.06 ± 0.02^{Bd}	5.20 ± 0.14^{Bc}	5.06 ± 0.12^{Bc}	4.48 ± 0.15^{Ac}	*	4.27 ± 0.01^{Ac}	3.80 ± 0.02^{Be}	3.34 ± 0.12^{Cd}
48	4.60 ± 0.23^{ABd}	5.04 ± 0.15^{Ad}	4.56 ± 0.22^{ABe}	4.67 ± 0.38^{ABd}	4.01 ± 0.38^{Bd}	*	*	*	*	*
72	2.98 ± 0.30^{Be}	4.54 ± 0.15^{Ae}	1.68 ± 0.13^{Cf}	1.65 ± 0.14^{Ce}	2.04 ± 0.03^{Ce}					

A–C: The means with different uppercase letters among the samples (for the same row) indicate significant differences ($p < 0.05$) for each microorganism.
a–f: The means with different lowercase letters among storage hours (for the same column) indicate significant differences ($p < 0.05$) for each microorganism.
*: Colony counts were below 2 log CFU/g.

Mayo-0.9% BEOM: 48.82%), followed by estragole. The amount of estragole increased with the ratio of BEOM for the mayonnaises enriched with BEOM: 38.78%, 44.77%, and 47.11% in Mayo-0.3% BEOM, Mayo-0.6% BEOM, and Mayo-0.9% BEOM, respectively. As well as estragole, anethole and *trans*-anethole were detected in the mayonnaises enriched with BEOM.

After 2 weeks of storage, *trans*-2-heptanal was identified in Mayo-Control (0.97%) and its ratio continued to increase during storage. Trans-2-heptanal is an aldehyde and its existence generally arises from oxidation of oils, especially linoleic and linolenic acids (Choe & Min, 2007). Jacobsen et al. (2000) reported that *trans*-2-heptanal was identified in mayonnaises containing fish oil because of oxidation. Trans-2-heptanal was not detected in Mayo-SP or the mayonnaises enriched with BEOM, which indicates that EDTA and BEOM prevented the oxidation of these mayonnaises.

Benzaldehyde was detected in Mayo-Control at 4 and 6 weeks of storage (0.78% and 0.92%, respectively). Benzaldehyde can be found naturally in some plants and also it can form as a result of the oxidation of benzyl alcohol. Hartvigsen et al. (2000) identified benzaldehyde as an oxidation product in mayonnaise enriched with fish oil. The determination of benzaldehyde and *trans*-2-heptanal in Mayo-Control indicates that oxidation in this sample was more advanced compared to the others. Phenolic components in BEO may have prevented the formation of oxidation products in the mayonnaises enriched with BEOM, acting like an antioxidant (Alves-Silva et al., 2013).

3.5. Rheological measurements

3.5.1. Steady shear viscosity

The viscosity of the mayonnaise samples was measured as a function of the shear rate (1–100 s^{-1}). As shown in Fig. 1, all samples showed shear thinning behavior (pseudoplastic), as the apparent viscosity decreased with the increase in shear rate. This phenomenon originates from structural deformation of the network structure of oil or protein droplets. When the droplets become close to each other in solid-like emulsions, a three-dimensional network of droplets forms. As the shear rate increases, the droplets become deformed and deteriorate, which may lead to a decrement in viscosity (Chang et al., 2017). In addition, the hydrodynamic forces generated could be the reason for the structural collapse of the molecules (Izidoro, Scheer, Sierakowski, & Haminiuk, 2008).

The measured shear stress and shear rate data of the mayonnaises were fitted to the Herschel–Bulkley model at 25 °C (R^2, 0.9989–0.9997). Use of the Herschel–Bulkley model to describe the flow properties of

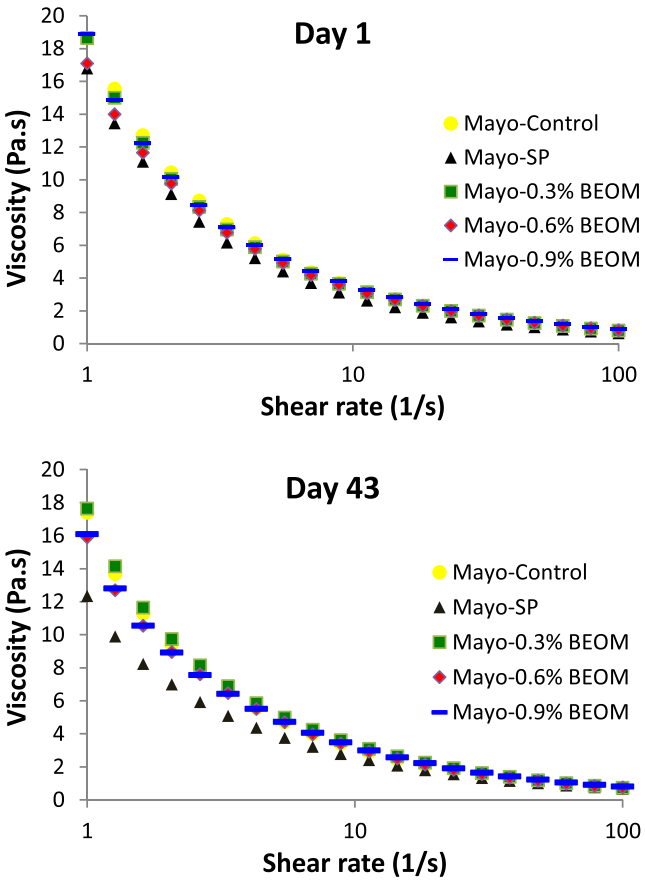

Fig. 1. Viscosity of the mayonnaises as a function of shear rate.

mayonnaise has been reported widely in the literature (Chivero, Gohtani, Yoshii, & Nakamura, 2016; Izidoro et al., 2008; Mun et al., 2009). The rheological parameters from the Herschel–Bulkley model for the mayonnaises are shown in Table 4. The flow behavior index (n) values of the mayonnaises changed from 0.43 to 0.48 and from 0.39 to 0.46 after 1 and 43 days of storage, respectively. These values prove that the mayonnaises show shear thinning behavior ($n < 1$). The consistency coefficient (K) measures the viscous nature of emulsions and high K values indicate a strong emulsion structure (Ng, Lai, Abas, Lim, & Tan, 2014).

Table 4
Herschel-Bulkley model parameters, apparent viscosity (5 s^{-1}), and droplet size of the mayonnaises.

	Day 0					Day 43				
	Mayo-Control	Mayo-SP	Mayo-0.3% BEOM	Mayo-0.6% BEOM	Mayo-0.9% BEOM	Mayo-Control	Mayo-SP	Mayo-0.3% BEOM	Mayo-0.6% BEOM	Mayo-0.9% BEOM
τ_o	9.21 ± 3.59^{a}	10.62 ± 0.90^{a}	8.27 ± 0.26^{a}	6.56 ± 2.94^{a}	8.51 ± 2.13^{a}	6.30 ± 1.31^{a}	4.30 ± 0.76^{b}	4.92 ± 1.57^{a}	5.62 ± 0.06^{a}	6.26 ± 0.92^{a}
K	9.28 ± 0.96^{a}	5.87 ± 0.34^{b}	9.21 ± 0.02^{a}	9.81 ± 1.33^{a}	8.99 ± 3.25^{a}	10.05 ± 2.92^{a}	7.42 ± 0.03^{a}	11.70 ± 2.14^{a}	9.69 ± 1.00^{a}	9.02 ± 1.30^{a}
n	0.43 ± 0.01^{a}	0.48 ± 0.01^{a}	0.45 ± 0.00^{a}	0.44 ± 0.01^{a}	0.48 ± 0.08^{a}	0.41 ± 0.05^{a}	0.46 ± 0.00^{a}	0.39 ± 0.02^{a}	0.43 ± 0.02^{a}	0.46 ± 0.04^{a}
R^2	0.9989	0.9997	0.9992	0.9996	0.9996	0.9995	0.9997	0.9992	0.9992	0.9991
Viscosity (Pa.s)	5.11 ± 1.05^{Aa}	4.41 ± 0.01^{Aa}	5.00 ± 0.10^{Aa}	4.91 ± 0.14^{Aa}	5.15 ± 0.40^{Aa}	4.72 ± 0.54^{Aa}	3.76 ± 0.15^{Bb}	4.98 ± 0.34^{Aa}	4.70 ± 0.27^{Aa}	4.73 ± 0.08^{Aa}
Droplet size (D_{32}, μm)	10.56 ± 0.19^{Bb}	8.19 ± 0.39^{Db}	9.56 ± 0.02^{Ca}	11.54 ± 0.19^{Aa}	10.56 ± 0.25^{Ba}	15.02 ± 0.63^{Aa}	14.73 ± 1.10^{Aa}	10.33 ± 0.75^{Ba}	9.56 ± 0.12^{Bb}	10.05 ± 1.05^{Ba}

A–D: The means with different uppercase letters among the samples indicate significant differences ($p < 0.05$) at same day.
a–b: The means with different lowercase letters among storage days indicate significant differences ($p < 0.05$) in same group.

The K values of Mayo-Control and the mayonnaises enriched with BEOM were higher than that of Mayo-SP at the beginning and end of storage. However, these differences were not statistically important ($p > 0.05$). Yield stress (τ_o) values of the mayonnaise samples were in accordance with the literature (Liu, Xu, & Guo, 2007).

The apparent viscosity of the mayonnaise samples is given in Table 4. Mayo-0.9% BEOM had the highest viscosity while Mayo-SP had the lowest viscosity at the beginning and end of storage. The high amount of GA in Mayo-0.9% BEOM could have generated the highest apparent viscosity of the emulsion due to its semi-rigid conformation, high molecular weight, and a branched structure with a long chain (Carneiro, Tonon, Grosso, & Hubinger, 2013). However, only the difference between the apparent viscosity of the mayonnaise samples at the end of storage is statistically significant ($p < 0.05$). Our findings were supported by those of Miguel et al. (2019) who added fish oil-loaded zein capsules to mayonnaise and observed an increase in the viscosity of mayonnaise. On the other hand, it was observed that the viscosity of Mayo-SP decreased during storage. Similar results were reported by Verma and Razdan (2002) who investigated the effect of different preservatives (cetrimide, sorbic acid, benzoic acid, and methyl paraben) on the apparent viscosity of leucaena mucilage. The apparent viscosity of the mucilages decreased during storage and at the end of storage (day 32); there was a significant difference in the apparent viscosity of the mucilages.

3.5.2. Dynamic viscoelastic measurement

The frequency sweep test was performed to characterize the viscoelastic properties of the mayonnaises as a function of frequency (0.1–100 Hz) at 25 °C. The storage modulus (G′) and loss modulus (G″) of the mayonnaises at the beginning and end of storage are presented in Fig. 2.

G′ values were higher than G″ values in all mayonnaises both at the beginning and end of storage, which is a characteristic behavior for concentrated emulsions. The increase in G′ values with increasing

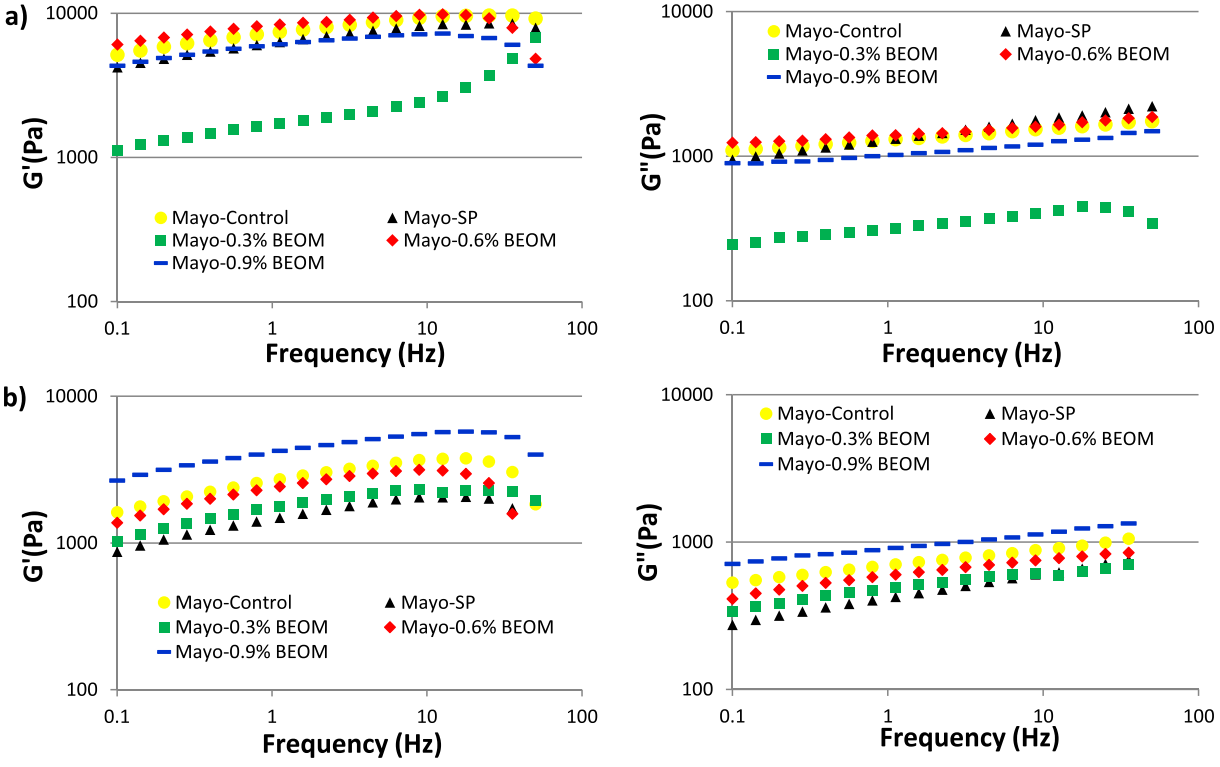

Fig. 2. Storage modulus (G′) and loss modulus (G″) of the mayonnaises at 1st day (a) and 43rd day (b) of the storage.

frequency could be associated with the presence of strong interactions between the droplets that contribute to the elastic modulus and need more time to relax (Di Mattia et al., 2015). At the beginning of storage, it was observed that G′ and G″ values of all samples were close to each other except for that of Mayo-0.3% BEOM which had lower values. The lower G′ values could be related to its lower dispersion level and so a lesser degree of interactions between the droplets. Lower G″ values indicate that an emulsion needs low stresses to flow and exhibits a more liquid-like behavior (Di Mattia et al., 2015). At the end of storage, Mayo-0.9% BEOM had higher G′ and G″ values than the others. This could be connected to the large amount of gum molecules in the medium. Gum molecules cause osmotic pressure and force droplets to have a strong gel structure (Hashemi et al., 2018). Mayo-SP had the lowest G′ and G″ values at the end of storage, indicating a more liquid-like behavior and a decrease in elastic properties. This situation could be related to the EDTA and PS content of the sample, as with the apparent viscosity results.

Consequently, enrichment of mayonnaise with 0.9% BEOM contributed to the viscosity of mayonnaise, increased its elasticity by acting as a plasticizer, and thus might lead to higher G′ and G″ values. The amount of thickeners and emulsifiers can make significant changes to the rheological properties of emulsions, which was supported by Mun et al. (2009). They added 3.8% and 5.6% 4αGTase-treated starch to mayonnaise samples with and without xanthan gum. In the samples without xanthan gum, there was a significant increase in G′ values with an increase in starch ratio. While the G″ values were greater than the G′ values in the sample containing 3.8% starch, the opposite was the case in the sample containing 5.4% starch.

3.6. Droplet size distribution

The change in droplet size of the mayonnaises during storage is shown in Table 4. The droplet size of Mayo-Control and Mayo-SP was 10.56 ± 0.19 and 8.19 ± 0.39 μm, respectively, at the beginning of storage while it increased to 15.02 ± 0.63 and 14.73 ± 1.10 μm ($p < 0.05$), respectively, at the end of storage. The increase in droplet size of the emulsions could be related to coalescence phenomena in which two or more droplets combine to generate a single larger droplet. This situation results in the formation of an oil layer, which is known as oiling-off (Ng et al., 2014). Our results show that the Mayo-Control and Mayo-SP emulsions were not stable and their droplets tended to agglomerate. Similar results were reported by Ng et al. (2014) who prepared model emulsions using different amounts of palm olein-based diacylglycerol. They reported that the droplet size of the emulsions increased during 4 weeks of storage. The increase in droplet size of Mayo-0.3% BEOM was quite small in comparison with those of Mayo-Control and Mayo-SP samples and was not statistically significant ($p > 0.05$). The addition of 0.3% BEOM to mayonnaise could delay the coalescence of oil droplets by increasing the viscosity of the continuous phase and the formation of a gel network (Santipanichwong & Suphantharika, 2007). The change in apparent viscosity of Mayo-0.3% BEOM during storage (Table 4) supports this relation. When the change in droplet size of Mayo-0.6% BEOM and Mayo-0.9% BEOM samples is considered, it is seen that these values decreased at the end of storage. However, the decrease in droplet size of Mayo-0.9% BEOM was not statistically significant ($p > 0.05$). The decrease in droplet size of emulsions over time is often associated with solubilization. Apolar molecules can be solubilized in an aqueous surfactant solution by incorporating them into micelles or colloids (Santipanichwong & Suphantharika, 2007). By adding 0.6% and 0.9% BEOM to the mayonnaises, the amount of WPI in the mayonnaises increased and WPI may have served as a surfactant. Similar results were reported by Santipanichwong and Suphantharika (2007) who investigated the change in droplet size of mayonnaise samples containing different amounts of lutein. While the droplet size of the samples containing a low amount of lutein (25 and 50 mg/kg) increased at the end of storage, that of the samples containing a high amount of lutein (75 mg/kg) decreased.

4. Conclusions

BEOM demonstrated significant antimicrobial activity against *E. coli* and *S.* Typhimurium in the mayonnaises. Moreover, BEOM showed even better antimicrobial activity than PS against *E. coli* in mayonnaise. The addition of 0.9% BEOM to the mayonnaise resulted in higher viscosity, G′, and G″ values than in the others at the end of storage because of the high amount of gum molecules in the medium. Trans-2-heptanal was not identified in Mayo-SP or the mayonnaises enriched with BEOM, which indicates that BEOM performed as an antioxidant agent due to their phenolic components, just like EDTA. The addition of 0.3% BEOM to the mayonnaise resulted in the formation of an emulsion with stable droplet sizes during storage. These results indicate that the addition of BEOM can be considered as a hurdle technology to improve microbial safety and extend the shelf life of uncooked food products. Additionally, BEOM have the potential to be used as a natural antimicrobial and antioxidant agent, but this issue needs to be investigated in depth in future research.

CRediT authorship contribution statement

Necla Ozdemir: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing. **Ali Bayrak:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing. **Tuba Tat:** Data curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Zühre Nur Yanık:** Data curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **A. Kadir Halkman:** Data curation, Formal analysis, Investigation, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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