The Effect of Microencapsulation of Phenolic Compounds from Lemon Waste by Persian and Basil Seed Gums on the Chemical and Microbiological Properties of Mayonnaise

Shima Shaygannia¹, Mohammad Reza Eshaghi¹, Mohammad Fazel², and Mahnaz Hashemiravan¹

¹Department of Food Science and Technology, Islamic Azad University of Varamin-Pishva Branch, Varamin, Tehran 33317-74895, Iran ²Department of Food Science and Technology, Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan 81595-158, Iran

ABSTRACT: Natural preservatives with high level of phenolic compounds, antioxidants and antimicrobial activities are used in mayonnaise to improve quality and safety due to their potential health benefits. Application of these compounds in production processes highlights many difficulties due to instability of their physical and chemical properties. Microencapsulation is used to address these restrictions. In this study, phenolic compounds from lemon waste were encapsulated with Persian gum (PG) and basil seed gum (BSG) as coating materials at different ratios (0:1, 1:0, and 1:1) at 15% (w/w) total biopolymer. We confirmed microencapsulation by scanning electron microscopy, and evaluate phenolic content, antioxidant activity, encapsulation efficiency, morphology, water solubility indexes, and water absorption indexes. Sample mayonnaise was prepared using microencapsulated polyphenols from lemon waste and extract (1,000 ppm of concentration), and control samples without extracts or microcapsules. All samples were subjected to chemical (measuring the peroxide, thiobarbituric acid, acidity, and color) and microbial (total count of microorganisms and *Escherichia coli*) analysis during 30 days of storage. BSG samples exhibited the highest capability to prevent oxidative deterioration during storage. Addition of microcapsules led to increases in parameter b* and decreases in parameters a* and L*. In general, PG/BSG microcapsules were considered optimal samples for production of mayonnaise, since they prevented mayonnaise deterioration and exhibited antioxidant and antimicrobial properties.

Keywords: basil seed gum, encapsulation, lemon waste, mayonnaise, Persian gum

INTRODUCTION

Mayonnaise is a widely consumed food product used as seasoning for different types of food (Liu et al., 2007). Due to its structure and composition, mayonnaise is highly susceptible to chemical and microbial deterioration. Chemical deterioration includes oxidation and hydrolysis of fat and oil types, which increases the content of compounds like peroxide, leading to sourness and changes to its color, taste, and texture (Frankel et al., 2002). Mayonnaise is susceptible to deterioration due to the structure of its lipid molecules, and their interactions with other molecules in their immediate vicinity, greatly influencing susceptibility to lipid oxidation (Coupland and McClements, 1996). Mayonnaise oxidation is intensified by free radicals generated from fat oxidation with other molecules like proteins, carbohydrates, and vitamins in the emulsion. In addition, undesirable changes in pH and acidity caused by microbial or chemical activity in mayonnaise leads to microbial deterioration (Shahidi and Zhong, 2005).

Therefore, synthetic antioxidants and chemical conservatives are added to mayonnaise to control oxidation and microbial activity, which has economic advantages despite the compounds being harmful to human health (Kwon et al., 2015). For example, the food conservative sodium benzoate inhibits activity of bacteria and fungi in acidic environments (e.g., in sauces and salad seasonings). However, sodium benzoate damages cell mitochondrial DNA, consequently inactivating the cell and leading to development of the Alzheimer's and Parkinson's diseases (Daescu et al., 1986).

Consumption of food prepared with natural sources of phenolic compounds with a wide range of antioxidant,

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Correspondence to Mohammad Reza Eshaghi, Tel: +989132129793, E-mail: mreshaghi@yahoo.com

Author information: Shima Shaygannia (Student), Mohammad Reza Eshaghi (Instructor), Mohammad Fazel (Instructor), Mahnaz Hashemiravan (Instructor)

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antimicrobial, and anti-mutagenic activities has recently increased (Kong et al., 2003). In Iran, 650,000 tons of citrus are produced annually, which accounts for 9% of the global production (Vand and Abdullah, 2012).

Lemon is a species of citrus. The large amounts of lemon waste (peels, seeds, and pulps) produced by lemon juice industries have attracted attention. Lemon exhibits antimicrobial and antioxidant activity as it contains phenolic compounds, including flavonoids, coumarin, and limonene (Altunkaya et al., 2013).

Polyphenols contain a large group of bioactive compounds, and are present in fruit, vegetables, seeds, and other parts of plants. For humans, diet is one of the most important sources of polyphenols, which exhibit antioxidant properties and roles in food preservation and human health (Shariatifar et al., 2014). These compounds are sensitive to heat, light, and oxidation due to unsaturated bands within their molecular structures (Halliwell, 2008).

The strong antioxidant activity of phenolic compounds arises from their ability to scavenge free radicals. Indeed, radical scavenging activity is attributed to the presence of hydroxyl groups that replace aromatic rings. Phenolic compounds are present in many plant-based foods that have antioxidant activity (Lakshmanashetty et al., 2010).

Destruction of phenolic compounds can be prevented by microencapsulation, a process whereby bioactive compounds are trapped by biolipids, protecting them against the environment, thus increasing stability (Belščak-Cvitanović et al., 2011). Encapsulation can be achieved by biopolymers like starch compounds, agar, alginate, chitosan, and other gums (King, 1995). Arabic gum (AB) extracted from Acacia senegal trees is a type of polymer consisting of L-arabinose, D-galactose, and D-glucuronic acids and 2% pectin (Phillips and Williams, 2009) that is water soluble to produce a sticky solution. AB is effective for microencapsulation because it generates a stable emulsion with different types of oil in different pH ranges, and is resistant to acidic conditions. Consequently, AB has a high stability and appropriate functionality in acidic compounds (Anandaraman and Reineccius, 1986).

Persian gum (PG) is composed of galactose and arabinose and is applied as an emulsifier in acidic pH emulsion systems, high salt foods, and thermal processes. Similar to other gums, PG is water soluble and produces a sticky and viscous solution (Golkar et al., 2018).

Basil seed gum (BSG) exhibits a heterosaccharide structure that includes glucomannan, glucan, and xylan. BSG is an exclusive hydrocolloid categorized as an anionic gum and is added as an active ingredient in food formulation to increase consistency and gelation and to control microstructure, texture and aroma during storage (Dickinson, 2003; Hosseini-Parvar et al., 2010).

In the present study, we extracted phenolic compounds from lemon waste (peels and edible pulps) and microencapsulated them in gel matrixes of PG and BSG as substitutes to synthetic antioxidants and sodium benzoate in mayonnaise production. We assessed the effects of encapsulation on physical, chemical, sensory, and microbial properties of mayonnaise over one month. These phenolic compounds may represent new preservatives and flavorings for use in mayonnaise formulation.

MATERIALS AND METHODS

Materials

The primary ingredients consumed during mayonnaise preparation (oil without antioxidants, vinegar, mustard, pasteurized eggs, sugar, and salt) were purchased from a local supermarket in Isfahan, Iran. Lemons were obtained from a local farm in Shiraz. AB was provided from Sigma-Aldrich Co. (St. Louis, MO, USA). BSG and PG were purchased from a local market in Isfahan, Iran. Folin-Ciocalteau's phenol reagent and 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) were purchased from Merck KGaA (Darmstadt, Germany) and Sigma-Aldrich Co., respectively. Methanol, hexane, potassium chloride, trichloroacetic acid, and sodium bicarbonate were purchased from Sigma-Aldrich Co.. All other chemicals or solvents used were of analytical grade.

Extraction of polyphenols from lemon waste

Polyphenols were extracted from lemon by incubating 16 g lemon with 100 mg of 70% ethanol in an ultrasonic bath (60 Hz frequency, 60°C) for 2 h. The extracts were removed by a vacuum rotary evaporator machine at 40°C. The yield extract was filtered and then incubated in a freeze dryer for 48 h (Inoue et al., 2010).

Preparation the PG and BSG

Extraction of mucilage from Basil seeds was achieved following the method described by Hosseini-Parvar et al. (2010) with slight modifications. Extraction was performed using a water/seed ratio of 50:1 at 65°C, pH 7, for 20 min whilst stirring (500 rpm, RH basic 2, IKA, Staufen, Germany). A sterile extractor equipped with a rotating rough plate was used to separate the mucilage from the swollen seeds. The mucilage was then centrifuged and filtered to remove insoluble impurities. Finally, the filtrate was freeze-dried (Dena Vacuum, Tehran, Iran) at -40°C for 24 h and stored under dry, cool, and sterile conditions.

PG was prepared according to methods described in previous studies (Fadavi et al., 2014; Golkar et al., 2015a; Golkar et al., 2015b). Briefly, impurities were removed from lighter colored PG samples and the samples were milled (AQC 109, Agromatic AG, Zürich, Switzerland) and sieved (<500 μ m). PG powder was then dissolved

in distilled water with stirring (23°C, 500 rpm, RH basic 2, IKA) for 2 h and stored in a refrigerator for 24 h. The solution was centrifuged at 9,000 rpm for 15 min (PIT 320R, Hettichlab, Düsseldorf, Germany) to remove insoluble residues and the supernatant was collected and dried at -40 °C for 24 h.

Preparation of microcapsules

PG and BSG were encapsulated using polyphenol compounds as coating agents following the method described by Rutz et al. (2013) with some modifications. To prepare the microcapsules, mixtures of hydrocolloids at different PG/BSG ratios (1:0, 1:1, and 0:1) with 15% (w/w) total biopolymer were dissolved in water. Polyphenol extracts (containing 0.17 g of polyphenol) were then added so that the ratio of polyphenol extracts to wall material was 1:3. The mixture was stirred (500 rpm, RH basic 2, IKA) for 12 h at room temperature (23°C) and freeze dried (at -40°C for 24 h).

Evaluation the of microcapsule properties

Measurement of phenolic compounds: Phenolic compounds were measuring following the Folin-Ciocalteu method. First, 0.1 g of each sample was mixed with 20 mL methanol, and then rotated at 40°C to remove the methanol. Next, 0.5 mL diluted solution was added to 2.5 mL Folin reagent (10 g/100 mL), and 2 cc of 7.5% sodium carbonate solution was added. The obtained solution was then placed in the dark for 30 min before its absorption was measured at 760 nm (Ghasemi et al., 2009).

Measurement of the antioxidant activity: Antioxidant activity was determined following the method described by Brand-Williams et al. (1995), with modifications. First, 1 mg of microcapsules were diluted in 1 mL methanolethanol solution (50:50, v/v). The solution was incubated at 50°C for 20 min, centrifuged at 9,000 rpm, and then 0.1 mL was added to 3.9 mL DPPH solution in laboratory tubes. After incubation in dark for 30 min, the absorption was measured at 517 nm. The inhibition percentage was calculated according to equation 1:

Inhibition (%) =

Sample absorption – Control absorption Sample absorption ×100

Encapsulation efficiency: Encapsulation efficiency measured following the method described by Deladino et al. (2008) with slight modifications. To measure superficial phenolic compounds, 0.1 g of microcapsules were mixed with 5 mL methanol and centrifuged at 9,000 rpm for 10 min. To measure total phenol compounds, 0.1 g of microcapsules were mixed with 1 mL ethanol : acetic acid : water solution (42:8:50), incubated in an ultrasonic bath for 20 min and centrifuged at 9,000 rpm. The total phenol volume was measured following the Folin-Ciocalteu method and encapsulation efficiency were calculated according to equation 2:

Encapsulation efficiency (%) = Total phenolic compounds – Superfitial phenolic compounds ×100 Total phenolic compounds

Scanning electron microscopy (SEM): The microstructure of the microcapsules was examined by SEM (XL30, Philips, Guildford, UK). Samples were coated with gold/platinum at a thickness of about 15 nm and were analyzed by SEM (Zheng et al., 2011).

Water solubility index (WSI) and water absorption index (WAI): WSI and WAI were determined following the method described by Anderson (1982). Approximately 2.5 g of sample was suspended in 25 mL water, incubated in a water bath at 30°C for 30 min, and centrifuged at 4,350 g for 10 min. The supernatant was collected in a preweighted petri dish and dried in a vacuum oven at 105°C for 24 h. WSI was the amount of dry matter remaining following evaporation of the supernatant, expressed as a percentage of the dry matter in 2.5 g of sample. WAI was the weight of the solid pellet remaining after centrifugation divided by the amount of dry sample (Ahmed et al., 2011).

Preparation of mayonnaise

Mayonnaise was produced from the oil without antioxidant (65.2%), pasteurized egg (13.85%), water (8.2%), vinegar (7.7%), salt (1.5%), and mustard (0.3%) (all amounts are stated as % w/w). First, eggs and the powdered materials (salt, sugar, mustard powder, and citric acid) were homogenized, and then oil was added until a mayonnaise emulsion had been formed. Next, vinegar was added to the mayonnaise emulsion, followed by additives (PG, BSG, or PG/BSG extracts; 1,000 ppm in all samples) and distilled water whilst mixing.

Evaluation of the mayonnaise properties

Acid value measurements: Lipids were extracted from mayonnaise samples according to the method described by AOAC (2005). The acidity of the extracted lipids was determined and expressed as acid value percentages.

Peroxide value (POV) measurements: POVs were determined according to the method described by Altunkaya et al. (2013), with slight modifications. First, the mayonnaise emulsion was broken and the oil was separated following the method described by Lagunes-Galvez et al. (2002). Here, the mayonnaise was frozen at -40° C for 24 h, allowed to thaw at 4°C for 24 h, and then centrifuged at 4,000 rpm for 20 min. Extracted oil (10 mg) was then dissolved in 2 mL methanol and butanol (1:1), 2 mL am-

monium iron(II) sulfate, 45 mM sulfuric acid, and 0.13 M ammonium thiocyanate. After 5 min, the absorption was measured at 510 nm using a ultraviolet-visible spectrophotometer.

Thiobarbituric acid (TBA) measurements: TBA content was determined according to the method described by Tananuwong and Tewaruth (2010), with slight modifications. Briefly, 50 mg of the oil phase of mayonnaise was mixed with butanol to 25 mL, and 5 mL of this compound was mixed with TBA reagent and placed in water bath at 95°C for 2 h. The absorption was measured at 530 nm using a spectrophotometer. TBA values was determined using equation 3:

TBA value=
$$50 \times \frac{(A_{sample} - A_{blank})}{m}$$

where A_{sample} is absorbance of sample, A_{blank} is absorbance of reagent blank, and m is the amount of oil sample amount (mg).

Color measurements: Color of the mayonnaise samples was measured using a ColorFlex $45^{\circ}/0^{\circ}$ spectrophotometers (Hunter Associates Laboratory, Inc., Reston, VA, USA). The samples were assessed by comparing L* (transparency), b* (yellowness), and a* (redness) (Worrasinchai et al., 2006).

Microbiological assessment: Total bacterial counts (TBC) and *Escherichia coli* content was determined according to the methods described by APHA (1992) and Fan et al. (2008). Briefly, 25 g of mayonnaise samples were mixed with 225 mL of 0.85% sterile salt solution and the solutions were diluted. TBC was determined by plate count agar after incubation at 35°C for 48 h (Fan et al., 2008). *E. coli* content was determined by culture environs of *E. coli* with tryptone water, *E. coli* broth, and oxidative reagents (APHA, 1992).

Statistical analysis

Statistical analysis was performed based on completely randomized factorial design and analysis of variance (ANOVA) using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA). Mean comparison tests were performed using least significant difference (LSD) methods at 5% significant level.

RESULTS AND DISCUSSION

Assessment of microcapsule properties

Total phenolic content: The total phenolic contents of the extract and the different lemon waste microcapsules (PG and BSG) are shown in Table 1. The total phenolic content of lemon extract was 30.5 ± 0.25 mg gallic acid/g, which is consistent with that described in a previous study (Esparza-Martínez et al., 2016) in which the total phenolic content in extracts subjected to different temperature treatments ranged from 15.67 to 36.81 mg gallic acid/g. The phenolic content of the lemon waste microcapsules depends on the compounds present in the core and wall materials. The highest and lowest total phenolic contents were observed for PG/BSG (17.91±0.36 mg gallic acid/g) and PG samples (13.92±0.33 mg gallic acid /g), respectively. PG is an ionic gum that creates mutual ion-dipole interactions with phenolic compounds, reducing phenolic content. Samples containing PG/BSG microcapsules may maintain higher amounts of phenolic compounds, while generating a particular structure with a high covering capability (Gharsallaoui et al., 2007). Single encapsulation of wall materials did not achieve all the required characteristics. However, the combination of different carbohydrates and proteins in multiple phenolic compounds may have acted to increase encapsulation efficiency.

Antioxidant activity: Hydroxyl radicals are the most reactive oxygen species, and can react effectively with biologic compounds including carbohydrates, DNA, polyunsaturated fatty acids, and proteins. Preventing these destructive reactions prolongs the permanence periods of the extracts (Youwei et al., 2008). The antioxidant activity of the phenolic extract and lemon waste microcapsules is shown in Table 1. Of the different microcapsules, BSG showed highest antioxidant activity ($61.19\pm0.34\%$),

Table 1. Total phenolic content, antioxidant activity, encapsulation efficiency, WSI and WAI of lemon waste extracts and microcapsules during storage

Samples	Total phenolic content	Antioxidant	Encapsulation	WSI (g/100 g	WAI (g/100 g
	(mg gallic acid/g)	activity (%)	efficiency (%)	dry solid)	dry solid)
BSG	16.87±0.25 ^b	61.19±0.34 ^b	70.72±0.76 ^a	11.53±0.69 ^a	24.76±0.65 ^c
PG/BSG	17.91±0.36 ^b	58.23±0.81 ^c	67.07±0.46 ^b	8.33±0.27 ^b	32.51±0.60 ^a
PG	13.92±0.33 ^c	53.95±0.38 ^d	65.06±0.40 ^b	13.28±0.59 ^a	27.11±0.03 ^b
Extract	30.50±0.25 ^a	73.21±0.23 ^a	—	—	–

The results were expressed as mean±standard deviation of three determinations.

Different letters (a-d) in the column indicate significant differences (P < 0.05).

BSG, basil seed gum; PG, Persian gum; Extract, lemon extract free encapsulated; WSI, water solubility index; WAI, water absorption index.

whereas PG showed had the lowest antioxidant activity $(53.95\pm0.38\%)$. In general, samples containing BSG had high antioxidant activity, which may be attributed to the high content of phenolic compounds and presence of aromatic compounds in basil (Zheng et al., 2011). Similar observations were reported in a study by Ahmed et al. (2010) for AB and maltodextrin as wall materials for purple sweet potatoes. The authors suggested that antioxidant activity depends on amounts of wall material and bioactive compounds, and that some biopolymers have antioxidant properties.

Many studies have shown a direct correlation between phenolic content and antioxidant properties, whereby PG /BSG samples with the highest phenolic compound contents have lower antioxidant properties compared with BSG samples. The antioxidant properties of polyphenols arise from their redox properties, which allow them to act as reducing agents, hydrogen donators, metal chelators, and single oxygen quenchers (Piluzza and Bullitta, 2011).

The antioxidant activity of lemon extracts was higher than that of the encapsulated samples. This may be attributed to deficient extraction of the phenolic compounds in microcapsules and extract reaction with the capsule membrane, leading to reduction in antioxidant activity. da Rosa et al. (2014) obtained similar results, indicating extract and microcapsule inhibition of 93.07% and 80.38 \sim 90.75%, respectively.

Encapsulation efficiency: Encapsulation efficiency indicates capability of the wall material in preserving core materials and is one of important factors for determining sustainability of capsulated compounds (Hogan et al., 2001). Encapsulation efficiency of the lemon waste extracts ranged from 65.06% to 70.72% (Table 1). Higher efficiencies were observed for BSG samples compared with the PG/BSG samples, attributed to its capability for film formation, biodegradability, and tendency to form a concentrated network in drying processes. This can be explained by the low functionality of PG and reduction of phenol compounds (Golkar et al., 2018). In addition, encapsulation efficiency depended on the type of encapsulated compounds, with the combination of extracts with different

physical and chemical properties promoting different microcapsule behaviors. In a similar study, da Rosa et al. (2014) assessed the phenol compounds of blackberry with β -cyclodextrin gum, chitosan, and a mixture of both. The authors showed that encapsulation efficiency is influenced by the phenol compounds and the type of materials applied in the wall.

SEM: Fig. 1 shows the external structure and morphology of the microcapsules. The microcapsules coated with PG and PG/BSG showed irregular shapes, while the microcapsules coated with BSG showed smooth surfaces and uniform appearance, attributed to the chemical structures of the gum. Moreover, deep grooves were observed on the samples coated in BSG or PG/BSG, which might be due to the hydrophilic groups and enhancement of hydrogen bonds. The presence of deep and large grooves in the samples increased the sensitivity of the samples to oxidation. In general, the differences in the sample shapes could have arisen from differences in conductivity, concentration of the solution and the capsule contents.

In general, freeze-dried encapsulated powder had an irregular shape and flaky structure. Furthermore, micro grooves may be formed due to ice sublimation during freeze-drying. In a previous study, Pasrija et al. (2015) reported similar morphologies for freeze-dried microcapsules containing green tea polyphenols. SEM images of BSG showed that BSG has a fibril structure and contains a globular structure similar to that of the scattered cotton (Naji-Tabasi and Razavi, 2017).

WSI and WAI: In addition, release of the encapsulated compounds is influenced by solubility of the wall materials and absorption of solvent by the capsules. WSI and WSI of the microencapsulated lemon waste extracts are shown in Table 1. Samples containing PG demonstrated significantly higher (P<0.05) water solubility (13.28±0.59%), meaning that it can be easily and effectively dissolved and reconstituted in water for use with bioactive compounds in aqueous systems. Whereas PG/BSG sample showed the lowest water solubility, possibly due to cross-linking of the carbohydrates with other compounds, which reduces solubility and swelling power (Uslu and



Fig. 1. SEM micrographs of encapsulated lemon waste extracts with PG (A), BSG (B), and PG/BSG (C). BSG, basil seed gum; PG, Persian gum.

Polat, 2012). Tan et al. (2015) showed that WSI and WAI directly depend on the properties of the coating materials. In general, a high water solubility is important for application of powders in the food and pharmaceutical industries as it allows them to be easily incorporated into evenly distributed products.

Samples coated with PG/BSG showed the highest WAI ($32.51\pm0.60\%$), whereas lowest WAI were observed for those coated with PG ($24.76\pm0.65\%$). Therefore, samples coated with PG is less likely to be influenced by humidity and is more stable under storage conditions (Tan et al., 2015). These results suggest that the percentage of water absorption decreases with increased water solubility of the microcapsules. In a previous study, Moreira et al. (2009) obtained similar results for acerola pomace extracts. Furthermore, according to Ahmed et al. (2010) agglomeration of the encapsulated particles could contribute to a smaller WAI and, subsequently, increase WSI.

Assessing the mayonnaise properties

Acidity values: Acidity is one of important chemical features of salad sauces/dressings like mayonnaise. There is a direct relationship between storage duration and acidity. However, an increase in acidity leaves an unpleasant taste in the sauce. The acid values of the coated microencapsulated mayonnaise were gradually but significantly (P < 0.05) increased with the storage period, reaching the maximum level after 30 days (Table 2). The lowest acidity values during storage were observed for BSG samples, while the highest acidity values were observed for the control samples and the samples containing lemon waste extracts. This increase in acidity is attributed to the low pH of mayonnaise, which causes iron bridges between iron and phosvitin to break and release iron. Iron can activate oxidative and hydrolytic enzymes present in eggs (Stefanow, 1989; Kishk and Elsheshetawy, 2013). According to Kishk (1997), free fatty acids may be produced by oxidation of the double bonds of unsaturated fatty acid esters. Also, growth of acid-tolerant non-pathogenic bacteria, such as Lactobacillus, may be effective in this respect due to the water content of mayonnaise (Karas et al., 2002). A high moisture content in mayonnaise during storage induces lipolysis followed by fat hydrolysis and accumulation of free fatty acids (Koczoñ et al., 2008). In a previous study, El-Bostany et al. (2011) assessed pH and acidity percentage of low-fat mayonnaise produced from potato powder. Over 60 days of storage, there was a reduction in pH and percentage increases in acidity.

POVs: POVs are qualitative parameters and indexes related to chemical deterioration of oils, and are considered a criterion for measuring hydroperoxide generated in products during the initial rate of oxidation (Alobo, 2001). Mayonnaise is an oil-in-water emulsion. The oil phase is susceptible to oxidative deterioration in connection with a vast surface of water, whereas the water phase contains high volumes of oxygen, which lead to increased oxidation (Gorji et al., 2016). In our study, POVs in the prepared mayonnaise samples were significantly (P<0.05) increased throughout 30 days of storage.

After 30 days of storage, POV peaked at 27.32±0.1 meqO₂/kg oil in the control samples, with significant differences (P < 0.05) observed compared with all other samples (Fig. 2). Samples containing PG/BSG exhibited lowest POVs (10.40±0.11 meqO₂/kg oil) within 30 days, followed by samples containing BSG (12.40±0.31 meqO₂/ kg oil). This can be related to the oxygen barrier characteristics of PG- and BSG-based encapsulation. Formation of hydroperoxides is further decreased in BSG samples due to antioxidant properties of BSG with phenolic and aromatic compounds, which reduce the POV (Milani and Golkar, 2019). In a previous study, Bagheri et al. (2015) revealed that fennel extracts prevent oil fat oxidation due to a high content of phenolic compounds. These results were determined by the antioxidant activity of the extracts and encapsulated extracts in preventing kilka fish oxidation, whereby the antioxidant effect was highest for the encapsulated extracts. Our results are consistent with the previous studies, indicating encapsulation has potential to improve antioxidant activity of the lemon extracts in mayonnaise by prolonging its availability.

Evidence of encapsulation improving bioactivity and bi-

 Table 2. Acid values of mayonnaise samples prepared using extracts and microcapsules of lemon waste during storage (unit: mg KOH/g)

Samples	0	10	20	30
Control BSG PG/BSG PG Extract	$\begin{array}{c} 0.84 \pm 0.016^{abD} \\ 0.83 \pm 0.003^{abB} \\ 0.85 \pm 0.004^{aC} \\ 0.82 \pm 0.006^{bD} \\ 0.84 \pm 0.016^{abD} \end{array}$	$\begin{array}{c} 0.87 {\pm} 0.024^{\rm aC} \\ 0.81 {\pm} 0.006^{\rm bC} \\ 0.89 {\pm} 0.003^{\rm aB} \\ 0.90 {\pm} 0.006^{\rm aB} \\ 0.87 {\pm} 0.024^{\rm aC} \end{array}$	0.93 ± 0.004^{aB} 0.83 ± 0.003^{dB} 0.90 ± 0.005^{bA} 0.89 ± 0.001^{cC} 0.90 ± 0.004^{bB}	$\begin{array}{c} 1.04 \pm 0.005^{aA} \\ 0.87 \pm 0.003^{eA} \\ 0.90 \pm 0.001^{dA} \\ 0.91 \pm 0.003^{cA} \\ 0.95 \pm 0.003^{bA} \end{array}$

The results were expressed as mean±standard deviation of three determinations.

Different small letters (a-e) in the same column and capital letters (A-D) in the same row indicate significant (P<0.05) differences during the 30-day storage.

Control, non-encapsulated samples; BSG, basil seed gum; PG, Persian gum; Extract, lemon extract free encapsulated.



Fig. 2. Peroxide values (meqO₂/kg) measured as peroxide and hydroperoxide concentrations in lipid extracts of mayonnaise samples prepared using extracts and microcapsules of lemon waste with PG and BSG during storage. Different letters (a-l) indicate significant (P<0.05) differences between results. Values are mean±standard deviation of three experimental repeats. Control, non-encapsulated samples; BSG, basil seed gum; PG, Persian gum; Extract, lemon extract free encapsulated.

oavailability of polyphenols has been reported in previous studies (Fang and Bhandari, 2010). However, it has also been reported that higher concentrations of extracts may act as better pro-oxidants (Sarah et al., 2010). Rasmy et al. (2012) assessed the antioxidant effect of rosemary alcoholic extracts on mayonnaise formulation and found that applying 400 μ g of extract (concentration in 1 g of mayonnaise) reduced POV compared with the control group. Moreover, similar to the increase in POVs after 4 months, mayonnaise-containing rosemary followed a slower increase compared to butylated hydroxyanisole antioxidants.



Fig. 3. Thiobarbituric acid (TBA) [mg malondialdehyde (MA)/kg] reactive substance value of lipid extracts of mayonnaise samples prepared using extracts and microcapsules of lemon waste with PG and BSG during storage. Different letters (a-j) indicate significant (P<0.05) differences between results. Values are mean±standard deviation of three experimental repeats. Control, non-encapsulated samples; BSG, basil seed gum; PG, Persian gum; Extract, lemon extract free encapsulated.

TBA values: Primary oxidation products are highly instable, and therefore decompose rapidly into secondary oxidation compounds (Whitaker, 2001). TBA values were significantly (P<0.05) increased in the prepared mayonnaise samples, with highest values observed after 30 days of storage. At the beginning of storage, TBA values are trivial because this index is formed due to hydroperoxide decomposition. Whereas during the storage period, hydroperoxide is converted into ketones and aldehydes (Daoud et al., 2019). Samples containing PG/BSG had the lowest TBA values [0.28±0.04 mg malondialdehyde (MA)/kg oil], and control samples had the highest TBA values (0.52±0.01 mg MA/kg oil, Fig. 3) after 30 days of storage. The results emphasized that encapsulation of mayonnaise with PG and BSG coated lemon waste extracts decreases oxidation and increases the shelf-life. This can be attributed to the protective effects of encapsulation against oxygen and the antioxidant activity of phenolic compounds present in lemon waste extracts. In addition to encapsulation providing effective barriers against oxygen, BSG and PG significantly (P < 0.05) enhanced the antioxidative properties of encapsulation. The reduction in TBA values may therefore be attributed to phenolic compounds such as tannin, and antioxidative properties of Persian gums, and the antimicrobial effect of basil on fat oxidation-related bacterial-enzyme reactions (Buonocore et al., 2002). The lemon waste extracts had higher TBA value compared with the microcapsule samples, consistent with results from previous studies. For example, Gortzi et al. (2006) demonstrated that antioxidant activity was higher in thymus encapsulated with liposomes than that of non-encapsulated extracts.

Color evaluation: The results of color parameter (L*, a*, and b*) evaluation of the extracts and microencapsulated lemon waste polyphenols with different coating materials are shown in Table 3.

There were no significantly differences in the L* values of the control samples and extract samples. The lowest L* values were observed for BSG sample (85.30 ± 0.10).

 Table 3. Color measurements of mayonnaise samples prepared using extracts and microcapsules of lemon waste during storage

Samples	L*	a*	b*
Control BSG PG/BSG PG Extract	91.77±0.24 ^a 85.30±0.10 ^d 87.02±0.29 ^c 89.31±0.13 ^b 91.63±0.20 ^a	$\begin{array}{c} -1.31 {\pm} 0.03^{a} \\ -1.73 {\pm} 0.02^{c} \\ -1.68 {\pm} 0.06^{bc} \\ -1.58 {\pm} 0.03^{b} \\ -1.51 {\pm} 0.01^{b} \end{array}$	$\begin{array}{c} 8.48 \pm 0.07^{b} \\ 8.51 \pm 0.13^{b} \\ 9.39 \pm 0.15^{ab} \\ 10.21 \pm 0.06^{a} \\ 10.35 \pm 0.10^{a} \end{array}$

The results were expressed as mean \pm standard deviation of three determinations.

Different letters (a-d) in the column indicate significant differences (P<0.05).

Control, non-encapsulated samples; BSG, basil seed gum; PG, Persian gum; Extract, lemon extract free encapsulated.

Furthermore, the samples containing microcapsules had lower transparency compared with the control samples. The highest a* values (red-green) were determined for the control samples (-1.31 ± 0.03) , with the lowest values recorded for the BSG samples (-1.73 ± 0.2) . In addition, the lowest b* (green-blue) value was observed for the control sample.

Adding microcapsules to prepared mayonnaise samples decreased the L* and a* indexes, and increased the b* indexes. The reduction in the L* index is due to increases in particle diameter, which reduces light differentiation and product lightness. In a previous study, McClements and Demetriades (1998) reported that mayonnaise transparency decreases as the diameters of particles diameter in the emulsion increase.

Negative a* values were reported in the mayonnaise samples. This change in color toward greenness may be due to formation of phenolic polymer compounds. Adding PG increased yellowness (b*), whereas basil mucilage induced the opposite effect due to the color pigments in the extracts and gums. Our results are in line with those of a study by Amiri Aghdaei et al. (2014) which showed decreases in transparency (L*) and yellowness (b*) by addition of *Plantago psyllium* seed mucilage as an alternative to fat in low-fat mayonnaise.

Microbiological analysis: The total microbial count is presented in Fig. 4. Highest microorganism growth was observed in control samples, whereas samples containing BSG/PG and BSG had lowest microorganism growth during storage, indicative that encapsulation was effective in preventing microorganism growth. This antimicrobial



Fig. 4. Total bacterial counts of mayonnaise samples prepared using extracts and microcapsules of lemon waste with PG and BSG during storage. Different letters (a-k) indicate significant (*P*<0.05) differences between results. Values are mean±standard deviation of three experimental repeats. Control, non-encapsulated samples; BSG, basil seed gum; PG, Persian gum; Extract, lemon extract free encapsulated; CFU, colony-forming unit.

property is attributed to the phenolic compounds present in lemon extracts, PG and BSG, which inhibited microbial growth. Therefore, phenol compounds in lemon extracts, PG and BSG have antimicrobial activity against a wide spectrum of microorganisms (Buonocore et al., 2002). Indeed, BSG is a source of flavoring and antioxidants and contains essential oils, mainly 1,8-cineole, estragole and eugenol, flavonoids, and anthocyanin. BSG significantly increased antioxidant and antimicrobial properties of extracts compared with PG (da Costa et al., 2015).

In all samples, the total microorganism count was $<10^3$, resulting from organic acids in the mayonnaise reducing pH and acting as an antimicrobial material (Ma and Boye, 2013). All the samples indicated negative responses to *Escherichia* tests. When garden thyme extracts were added to mayonnaise, growth and activity of *Salmonella enteritidis* was inhibited in the presence of 0.4% extract. In a previous study, Juri et al. (2015) showed oregano essence inhibited growth of *S. enteritidis* in mayonnaise.

In the current study, microencapsulation of phenolic compounds extracted from the lemon waste was performed by PG, BSG, and BSG/PG. Highest EE and antioxidant activity were observed for sample coated with BSG (70.72% and 61.19%, respectively). Furthermore, we observed increased chemical stability of microencapsulated phenolic compounds. Samples containing microcapsules reduced peroxide production, stabilized free fatty acids, and prevented increases in acidity by eliminating and preventing formation of free radicals in the mayonnaise. Mayonnaise samples prepared using PG/BSG had significantly (P<0.05) lowest peroxide and TBA values during the storage period compared with control samples. Moreover, microencapsulation decreased L* and a* indexes, and increased b* indexes and were effective in reducing microbial growth and inhibiting Escherichia growth. Overall, polyphenol microcapsules from lemon waste have antioxidant and antimicrobial properties, and can be consumed as natural preservatives in mayonnaise.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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