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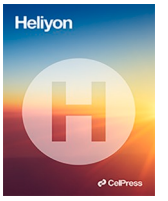

Research article

Enhancing hamburger shelf life and quality using gallic acid encapsulated in gelatin/tragacanth gum complex coacervate

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ABSTRACT

Considering mitigating oxidative degradation and inhibiting microbial proliferation in meat products, incorporating antioxidant and antimicrobial materials is critical to enhance shelf life, maintain quality, and ensure food safety. So, this study aimed to investigate the antimicrobial and antioxidant effects of encapsulated gallic acid on the quality of hamburgers during 30 days of storage. Gallic acid was microencapsulated in tragacanth gum/gelatin complex coacervate, and its encapsulation efficiency was optimized by the response surface method. The optimized encapsulation conditions were 1:4 polymer ratio (tragacanth to gelatin ratio); total polymer content, 0.9 %; pH, 3.5; and gallic acid content, 0.88 %, resulting in a 98 % encapsulation efficiency. The microcapsules were characterized using various techniques, including scanning electron microscopy, Fourier transform infrared spectroscopy, X-ray diffraction, thermogravimetric analysis, and differential scanning calorimetry. 400 ppm encapsulated gallic acid was added to the hamburger formulation, and various microbial properties, chemical analysis (peroxide value (POV) and thiobarbituric acid (TBA)), and sensory properties of the hamburgers were evaluated during storage. Results showed that gallic acid in the hamburgers decreased lipid oxidation from 0.126 to 0.103 mg MAD/kg in the TBA test and 12.73 to 11.03 meq/kg in the POV test during one month of storage. Also, phenolic compounds could prevent the growth and proliferation of spoilage microorganisms by damaging the microorganism cell walls and changing the metabolic processes. So, the amounts of total count and yeast and mold in the treated sample were lower than in the control sample. Significantly, adding encapsulated gallic acid did not negatively affect the flavor or overall evaluation of the samples. Overall, these findings suggested that encapsulated gallic acid is a suitable candidate to maintain chemical, microbial, and sensory characteristics of hamburgers over time.

1. Introduction

Improving foodstuff's shelf life and quality is essential for reducing food waste and economic losses. As the world faces population growth and climate change, extending shelf life becomes more important for creating resilient, sustainable, and efficient food distribution networks than before. Moreover, it aligns with consumer expectations for fresh, safe, and high-quality products. So,

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improving the shelf life and quality of foodstuff is becoming a key component of food systems.

Fat oxidation is one of the effective factors in reducing the shelf life of food products, which alters the flavor and taste of food (rancidity) and destroys vitamins and essential fatty acids in food, also causes problems among consumers by producing toxic substances [1]. Microbial growth is another factor influencing the shelf life and quality of foodstuff. The proliferation of bacteria, yeasts, and molds can instigate biochemical changes, accelerating spoilage processes and compromising the nutritional and sensory attributes of the food. In addition, pathogenic bacteria cause foodborne illnesses. Consequently, controlling fat oxidation and microbial growth is imperative for extending shelf life, maintaining quality, and ensuring the safety of food products [2].

So, natural antioxidant and antimicrobial agents are used in food formulations to control fat oxidation and microbial growth. Polyphenols are one of the most important bioactive components with antioxidant and antimicrobial activities that exist mainly in the human diet. Gallic acid is a natural phenolic compound found primarily in free or bound forms of tea leaves, berries, citrus fruits, and cereals [3]. Although gallic acid benefits human health, it is very unstable and susceptible to degradation under high temperatures and in the presence of light and oxygen [4]. Encapsulation is a valuable strategy for maintaining bioactive components by enveloping them within a protective matrix. This process involves the formation of a coating or capsule around the bioactive substance, shielding it from environmental factors such as light, heat, and oxygen. Encapsulating bioactive components enhances their stability, preventing degradation and preserving their functional properties. This protective barrier also enables controlled release, ensuring a gradual and sustained delivery of the bioactive compounds when incorporated into various foods [5].

In recent years, different encapsulation methods and wall materials have been used to encapsulate gallic acid. Briefly, Buljeta, Vukoja [6] added some gallic acid to pectin. Then, the precipitate obtained by centrifugation was frozen for 24 h at −18 °C, and then the encapsulated powder was obtained by freeze-drying [6]. In another study, pomegranate peel extract containing ellagic acid and gallic acid was encapsulated in alginate nanospheres using water in oil (w/o) emulsions [7]. Also, Choi and Chang encapsulated gallic acid in the pectic-polysaccharide complex of whey protein concentrate extracted from *Ulmus davidiana*. In this study, W/O/W multiple emulsion was formed and then turned into encapsulated powder using a spray drier [4].

One of the most used technologies for encapsulating natural compounds is complex coacervation, which involves electrostatic interaction between polymers of opposite charges and results in low water-soluble capsules with excellent controlled-release properties [8,9]. The charge density of the biopolymers, ionic strength, biopolymers ratio, accessibility of the interaction sites, and especially the pH of the biopolymers mixture are factors affecting the formation of complex coacervate [10].

Biopolymers such as gelatin have been widely used in previous studies to encapsulate vital and bioactive substances like tuna oil [11], sulforaphane [12], and olive leaf extract [10] by using a complex coacervation method. Gelatin is a denatured protein with a unique hydrophilic property, which causes it to be classified in the category of hydrocolloids [13]. Gelatin is a convenient option as a capsule wall due to its excellent emulsifying capacity, thickening ability, water-solubility, high stabilizing activity, and high cross-linking activity [14]. Tragacanth gum (TG), another material that can be used in encapsulation, is a heterogeneous complex and highly branched polysaccharide. TG is acid-resistant and slightly acidic with calcium, magnesium, and potassium cations [15].

This study aimed to use encapsulated gallic acid by gelatin/tragacanth gum complex coacervate in hamburgers to extend its shelf life and quality. Our study addresses a gap in conventional preservation methods for hamburgers. Current approaches struggle with simultaneous challenges of oxidative degradation and microbial spoilage while maintaining sensory attributes. The novelty of our research lies in using gelatin/tragacanth gum complex coacervate for gallic acid encapsulation, offering a unique strategy for controlled release and targeted protection. This method provides a more effective and sustainable solution to enhance the shelf life and quality of hamburgers.

2. Material and methods

2.1. Material

Gelatin type B and thiobarbituric acid were procured from Sigma-Aldrich (Germany). Tragacanth gum (TG) (ribbon type, grade A) was obtained from a local pharmaceutical shop. Hydrochloric acid, gallic acid, chloroform, potassium iodide, phosphoric acid and trichloroacetic acid were purchased from Merck. All the chemicals were of analytical grade and were used without purification.

2.2. Encapsulation of gallic acid by using complex coacervation method

Gallic acid was encapsulated by the complex coacervation method according to the method introduced by García-Saldaña, Campas-Baypoli [12]with some modifications [12]. To prepare gelatin and TG dispersions, the known amounts of gelatin and TG powder were separately added to distilled water under magnetic stirring (300 rpm) at 45 ± 5 °C for 1h. The dispersion of TG stayed overnight at 4 °C for complete hydration of the macromolecules.

For the preparation of microcapsules, the gelatin solution was stirred by a mechanical stirrer (300 rpm) at 45 ± 5 °C for 15 min, and then gallic acid (0.3–1.1 g) was added to it. The mixture was stirred at 300 rpm for 30 min, and then TG solution was gradually added to it while the weight ratio of TG to gelatin (1:1, 1:2, 1:3, 1:4, and 1:5) and concentration of total biopolymer (0.1, 0.3, 0.5, 0.7, and 0.9) were maintained. Temperature was maintained at 45 °C. To produce microcapsules, the mixture was acidified by the dropwise addition of hydrochloric acid, and pH was reduced from 4.5 to 2.5 under magnetic stirring at 600 rpm, then mixed for 30 min. The mixture was kept in a refrigerator at 4 °C for 1h. The process of complex coacervation was continuously monitored by measuring the absorption at 600 nm using a UV–Vis spectrophotometer (Uniko 2100, USA). The coacervate phase deposited on the bottom of the solution was filtered using Whatman No.1 filter paper and then dried at 4 °C. The formation of microcapsules by complex coacervation

was confirmed in our previous study [16].

2.3. Experimental design

A systematic optimization, central composite designs (CCD), was used to estimate the reaction conditions for maximum encapsulation efficiency (% EE) of gallic acid in complex formation between gelatin and gum tragacanth using the RSM design. A set of 31 experiments were employed. Tragacanth to gelatin ratio (%W/V) (X_1), total polymer content (X_2), pH (X_3) and gallic acid content (% W/V) (X_4) were selected as the independent variables to maximize the response (encapsulation efficiency). The actual experimental runs 1–31 and % EE are shown in Table 1.

A second-order polynomial model was considered to correlate the relationship between the independent variables and the responses. Equation (1) indicates the effect of variables in terms of linear, quadratic, and cross-product terms.

$$Y = \beta 0 + \Sigma\, \beta i\, Xi + \Sigma\, \beta ii\, Xi^2 + \Sigma\, \beta ij\, Xi\, Xj \quad (1)$$

Where, Y is the response, Xi and Xj are the levels of variables, β_0 the constant term, βi the coefficient of the linear terms, βii the coefficient of the quadratic terms, and βij the coefficient of the cross-product terms. All the experimental data were statistically analyzed by Minitab software, version 17.0.

2.4. Encapsulation efficiency

The encapsulation efficiency (EE %) was determined according to equation (2) [10,17]:

$$EE\% = (W_t - W_s) / (W_t) \times 100 \quad (2)$$

Where, W_s is the surface gallic acid and W_t is the total gallic acid used in preparing microcapsules. For surface gallic acid measurement, about 0.1g of the microcapsule was dispersed in 1 ml solution of methanol: ethanol (1:1 v/v). Then, the sample was agitated in a vortex at ambient temperature and filtered by micro filter (Whatman No. 1). The amounts of the gallic acid were evaluated by recording the absorbance value in 271 nm.

The calibration curve was used to measure the gallic acid concentration. A known amount of gallic acid was dissolved in sufficient

Table 1
Responses of dependent variables for gallic acid encapsulation to independent variables.

Exp. No.	Variable levels				EE%
	X_1	X_2	X_3	X_4	
1	1:2	0.3	3	0.5	49.60
2	1:4	0.3	3	0.5	49.60
3	1:2	0.7	3	0.5	70.76
4	1:4	0.7	3	0.5	84.80
5	1:2	0.3	4	0.5	53.70
6	1:4	0.3	4	0.5	68.04
7	1:2	0.7	4	0.5	54.30
8	1:4	0.7	4	0.5	63.88
9	1:2	0.3	3	0.9	45.70
10	1:4	0.3	3	0.9	52.60
11	1:2	0.7	3	0.9	79.35
12	1:4	0.7	3	0.9	83.77
13	1:2	0.3	4	0.9	57.53
14	1:4	0.3	4	0.9	58.15
15	1:2	0.7	4	0.9	55.01
16	1:4	0.7	4	0.9	72.48
17	1:1	0.5	3.5	0.7	61.91
18	1:5	0.5	3.5	0.7	76.92
19	1:3	0.1	3.5	0.7	52.60
20	1:3	0.9	3.5	0.7	91.25
21	1:3	0.5	2.5	0.7	65.05
22	1:3	0.5	4.5	0.7	47.05
23	1:3	0.5	3.5	0.3	60.87
24	1:3	0.5	3.5	1.1	51.33
25	1:3	0.5	3.5	0.7	60.39
26	1:3	0.5	3.5	0.7	58.23
27	1:3	0.5	3.5	0.7	59.75
28	1:3	0.5	3.5	0.7	62.78
29	1:3	0.5	3.5	0.7	60.71
30	1:3	0.5	3.5	0.7	58.66
31	1:3	0.5	3.5	0.7	59.00

X_1: Tragacanth to gelatin ratio (%W/V), X_2: total polymer content, X_3: pH and X_4: gallic acid content (%W/V).

deionized water. The absorbance values of gallic acid concentration (2–20 ppm) were recorded by using UV–Visible spectrophotometer in 271 nm.

2.5. Characterization of microcapsules

2.5.1. Chemical structure

The chemical structure of gallic acid, gelatin, tragacanth gum, blank microcapsules, and microcapsules containing gallic acid were analyzed by a Fourier transform infrared (FTIR) spectroscopy (Jasco 680-plus, Japan). FTIR analysis was performed in the range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1} at a scan speed of 2 mm/s.

2.5.2. Crystalline structure

X-ray diffraction (XRD) pattern was recorded by X'pert X-ray diffractometer (Philips, the Netherlands) with graphite monochromatized Cu Kα radiation ($\lambda = 1.54$ °A) and nickel filter in the range of $2\theta = 10$–100°. Blank microcapsules and gallic acid-loaded microcapsules were analyzed [18].

2.5.3. Thermal analysis

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) analysis of blank microcapsules and microcapsules containing gallic acid were performed by STA instruments (NETZSCH STA 449F3 Jupiter). The instrument was calibrated with an empty aluminum pan as a reference. Samples were weighed and analyzed in temperature scanning range from 25 to 550 °C with a heating rate of 10 °C/min. Nitrogen was used as the purge gas.

2.6. Preparation of hamburgers

The red beef meat (80 % lean beef and 20 % fat) was ground twice using a meat grinder. Then hamburger was prepared by mixing of minced meat (60 wt%) with other ingredients (40 wt %) including onion (28 %), vegetable oil (4 %), bread crumbs (3 %), non-fat dry milk (3 %), salt (1 %), spices (0.5 %), sodium polyphosphate (0.3 %), and spices juice (0.2 %). After mixing, the hamburger was formed using a steel mold with a weight of 100 ± 5 g, thickness of 0.5 cm and diameter of 9 cm. After, hamburger was placed between a polyvinyl chloride (PVC) film and the samples were kept at freezing temperature (−18 °C). To prepare hamburger containing encapsulated antioxidant, 400 mg/kg encapsulated gallic acid was added to 100 g hamburger. Encapsulation efficiency was used to calculate the amount of capsules containing 400 ppm of gallic acid. The other steps were similar to control sample (without encapsulated antioxidant). Both control and treated hamburgers were analyzed at 0, 15, and 30 days of storage at −18 °C.

2.7. Characterization of hamburger samples during storage

2.7.1. Peroxide value

Peroxide value (POV) was determined according to the AOAC (2000) methodology [19]. First, meat fat was extracted by Soxhlet method. Then, 5 g of the extracted oil was mixed with 30 ml acetic acid–chloroform with a ratio of 3:2 v/v to completely dissolve the fat. After, 5 ml saturated potassium iodide solution was added to the sample and the mixture was left in the dark for 1 min. Then, starch solution was added as an indicator. Finally, the sample was titrated with sodium thiosulfate solution (0.01 N). Peroxide value was calculated by equation (3) and expressed as milliequivalent peroxide per kg of sample.

$$\mathrm{POV}\ (\mathrm{meq}/\mathrm{kg}) = \frac{S \times N}{W} \times 1000 \tag{3}$$

Where S is the volume (ml) and N the normality of sodium thiosulfate solution and W is the sample weight (kg).

2.7.2. Thiobarbituric acid value (TBA)

Thiobarbituric acid (TBA) value was determined to evaluate the amount of malonaldehyde and secondary oxidation compounds according to the method of Tamsen et al. [20]. Briefly, 10 g sample was homogenized with 25 ml trichloroacetic acid 20 % in phosphoric acid (2 M) and then were mixed with 25 ml distilled water. After, the mixture was filtrated by Whatman paper (No. 41), then 5 ml of the extract was mixed with 5 ml TBA 0.01 M in 90 % acetic acid. The absorbance of the sample was measured at 532 nm by an UV–Visible spectrophotometer. TBA was expressed as milligram malonaldehyde equivalents per kg sample (mg MAD/kg).

2.7.3. Microbial analyses

10 g hamburger was homogenized in 90 ml sterile-buffered peptone water (BPW) solution, then 0.1 ml of serial dilutions were spread on plate count agar (PCA) and incubated at 37 ± 2 °C for 48–72 h or at 4 ± 1 °C for 10 days to determine mesophilic and psychrophilic bacteria, respectively. Also, to check the growth of mold and yeast, 0.1 ml of the primary suspension was spread on dichloran-rose bengal chloramphenicol agar (DRBC) and incubated at 25 ± 2 °C for 5 day [21]. Furthermore, 1 ml dilutions were inoculated on Lauryl Sulfate Broth (LSB) and Baird Parker Agar medium (BPA) to obtain *E. coli* and *S. aureus* counts, respectively, at 37 °C for 24 h.

2.7.4. Sensory evaluation

The addition effect of microcapsules containing gallic acid on the taste, color, texture, and overall acceptance of hamburgers at the first day of production was investigated by ten semi-trained panelists. For this purpose, the pre-fried hamburgers (control sample and the treated sample separately) were deep-fried at 180 °C for 5 min in sunflower oil. A five-point hedonic scoring scale (Table S2) was employed as described by Kim, Kim [22].

3. Results and discussion

3.1. Analysis of the model

The content of EE obtained from the 31 experiments is listed in Table 1. Analysis of variance was performed to find the significant parameters affecting the EE. The analysis of variance (ANOVA) of response (Table S1) showed that the model is significant at $p < 0.05$. Corresponding p-values of variables suggest that among the test variables used in this study, X_1 (tragacanth to gelatin ratio), X_2 (total polymer content), X_3 (pH), $(X_1)^2$ (tragacanth to gelatin ratio × tragacanth to gelatin ratio), $(X_2)^2$ (total polymer content × total polymer content) and X_2X_3 (total polymer content × pH) are significant EE model terms with p-values of less than 0.05. Other terms are insignificant.

The coefficients of independent variables determined for the second-order polynomial model for EE is given below (equation (4)):

$$Y_{EE} = -71.3 - 18.38\ X_1 + 170.6\ X_2 + 56.1\ X_3 + 2.222 \times \overset{2}{1} + 71.2 \times \overset{2}{2} - 70.58\ X_2\ X_3\ (R^2 = 98.9\%) \quad (4)$$

3.2. Effect of tragacanth to gelatin ratio

The ratio of protein and polysaccharide is very important in controlling the charge balance and improving the interaction between these two polymers. To optimize the tragacanth to gelatin ratio, different ratios (1:1–1:5) were used. Contour plots (Fig. 1A and B) show the effect of tragacanth to gelatin ratio content and other reaction parameters on the EE. These plots show that the maximum EE was obtained at tragacanth to gelatin ratio of 1:5. In other words, EE increased by increasing the amount of protein in the studied

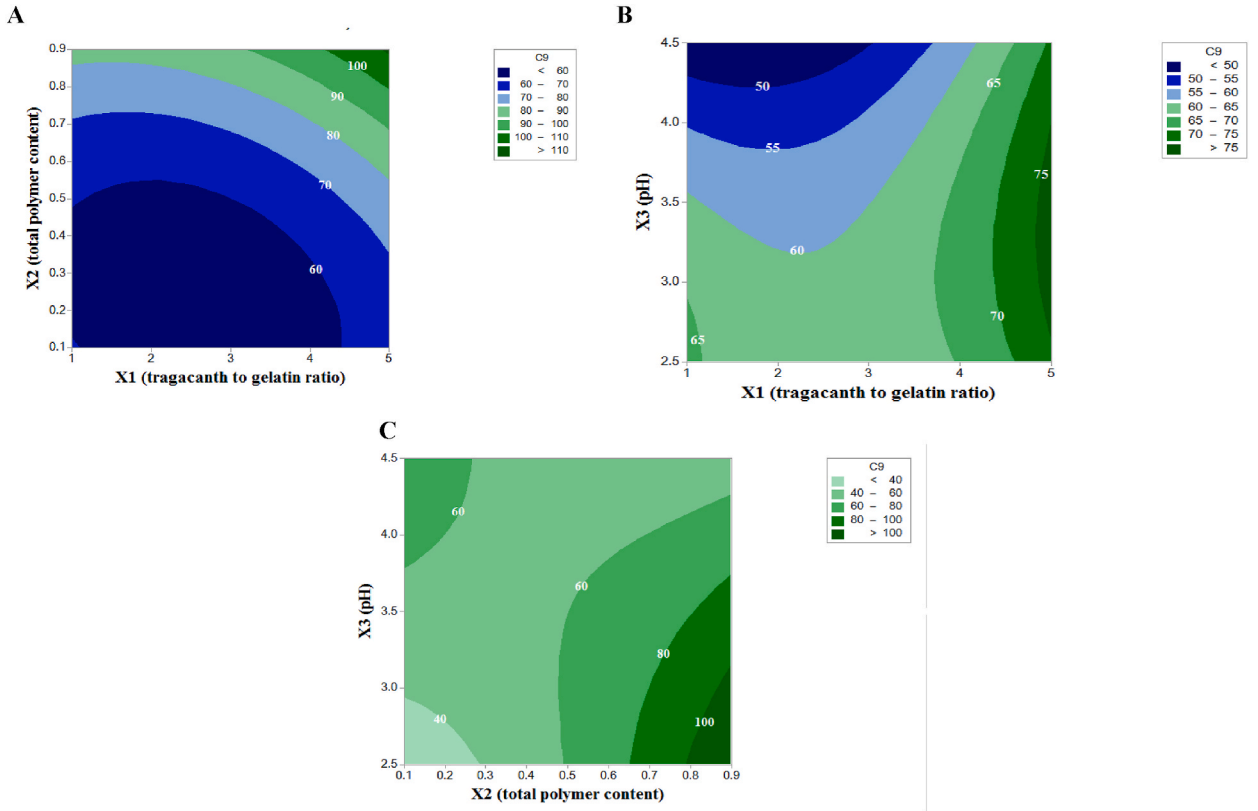

Fig. 1. Contour plots of mutual effect of tragacanth to gelatin ratio and total polymer content (A), tragacanth to gelatin ratio and pH (B), and total polymer content and pH (C) on the gallic acid encapsulation efficiency.

range. Further reactive anionic sites on the tragacanth chain were interacted with positive patches of protein at high protein to polysaccharide ratios. As a result, more complex coacervates were formed and gallic acid was encapsulated with higher efficiency. Also, EE increases with the increase of gelatin in the environment due to the higher hydrogen bonding between hydroxyl groups of gallic acid and carbonyl groups of gelatin [10]. It should be noticed that EE will decrease at higher amounts of tragacanth due to the steric hindrance between neighboring chains of tragacanth [23]. Devi and Maji [24], who encapsulated Neem seed oil in gelatin/sodium carboxymethyl cellulose using complex coacervation method reported similar result. Their results showed that EE was increased at higher ratios of gelatin to sodium carboxymethyl cellulose.

3.3. Effect of total polymer content

The effect of various content of total biopolymer (0.1–0.9 %) on EE has been shown in Fig. 1A and C. These contour plots show that EE increased with an increase in biopolymer concentration, which might be due to the fact that more positive patches of protein were accessible for binding with the anionic sites on the polysaccharide chain at higher biopolymer concentrations. So, more gallic acid was entrapped within complex coacervates [25]. On the other hand, the coating of gallic acid by the wall material will increase by increasing the concentration of polymer in the environment [4].

3.4. Effect of pH

Turbidity was measured to determine the appropriate pH range for complex coacervation, and its results are shown in Fig. S1. At pH 5–5.5, soluble complexes due to the interaction of polymers begin to form, and slowly, with the decrease of pH, insoluble complexes formed, while at pH 4-3.5, the most electrostatic association between TG and gelatin occurred, and as a result, the most amount of turbidity was observed. So, this pH range was the best range for the formation of coacervates. Also, at pH lower than 3.5, the turbidity decreased, which is due to the separation of the polymer complex [26].

The effect of pH variation (2.5–4.5) on EE is shown in Fig. 1B and C. These figures show that EE increased by pH decreasing. However, pH and total polymer content had significant cross-effects on EE. By decreasing pH (especially lower than 3.5), the total polymer content should be increased to obtain a higher EE of gallic acid. This implied that the highest level of tragacanth/gelatin complex was formed at a pH of around 3.5, which led to the better entrapment of the gallic acid into the complex. TG is an anionic

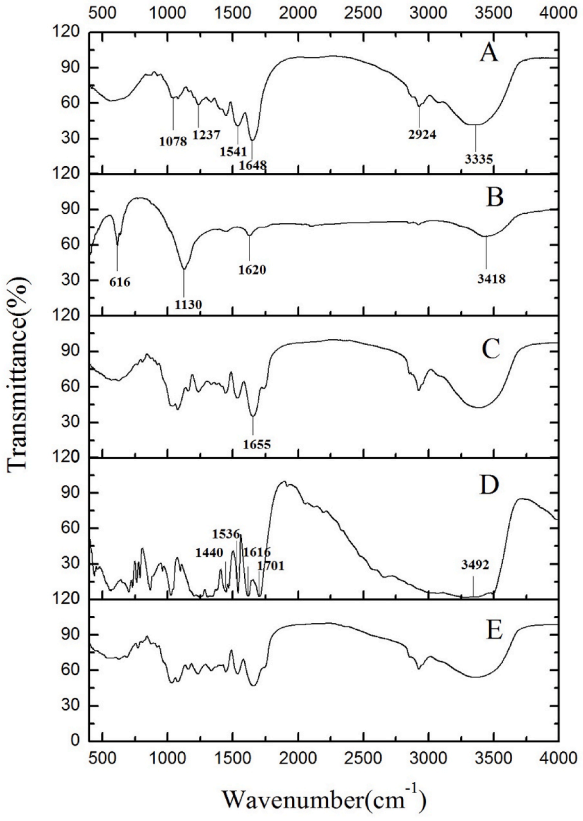

Fig. 2. FTIR spectra of gelatin (A), tragacanth gum (B), tragacanth/gelatin microcapsules (C), gallic acid (D), and tragacanth/gelatin microcapsules containing gallic acid (E).

polysaccharide, and gelatin in the studied pH range has a positive charge (the isoelectric point of type B gelatin is around 4.5–5), resulting in an electrostatic association between two biopolymers. 3.5–4 was the best pH for obtaining the highest complex coacervate. However, at pH lower than 3, the electrostatic association between the two polymers decreased due to the protonation of the carboxyl groups of TG, resulting in lower coacervate formation and lower EE [10]. Devi, Hazarika [27]observed a similar result, as 3.5 was the best pH for encapsulation of olive oil [27].

3.5. Effect of gallic acid content

To optimize the gallic acid content, different contents (0.3–1.1 %) were applied. The plots show the concentration of gallic acid did not affect the EE by considering other reaction conditions variations. Therefore, applying high concentrations of gallic acid did not increase EE. Jain, Thakur [25] showed similar results when microencapsulation of β-carotene by complex coacervation using casein and gum tragacanth [25].

3.6. Optimization of variables

Optimization of the gallic acid encapsulation in complex tragacanth/gelatin showed that all variables had their effect on the EE individually or in association with other variables except gallic acid concentration. The models predicted that tragacanth to gelatin ratio, 1:4; total polymer content, 0.9 %; pH, 3.5, and gallic acid content, 0.88 %; were the optimum conditions to achieve the maximum EE. The predicted optimized response was 98.9 %. Three experiments conducted according to predicted conditions, and the achieved EE was 98.7 ± 1.14, which showed the effectiveness of models to predict the response.

3.7. Characterization of gallic acid microcapsules

The FTIR spectra of gelatin, tragacanth, gallic acid, blank microcapsules and gallic acid-loaded microcapsules are shown in Fig. 2. The spectrum of gelatin (Fig. 2A) showed absorption bands at 3335 cm^{-1} (amino group), 2924 cm^{-1} (C–H stretching of alkenes), 1648 cm^{-1} (amid –I and CO and CN stretching), 1541 cm^{-1}(amide-II), 1237 cm^{1}(amide- III), and 1078 cm^{-1}(C–N stretching of amines) [28, 29].

FTIR spectrum of tragacanth gum (Fig. 2B) showed peaks at 3418 cm^{-1} (O–H stretching bonded absorption of carbohydrates),

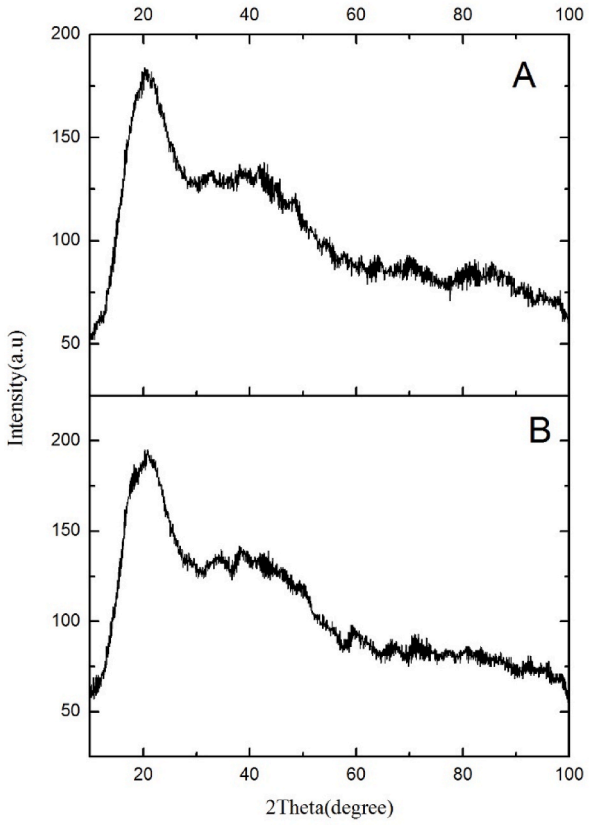

Fig. 3. XRD patterns of tragacanth/gelatin microcapsules (A) and microcapsules containing gallic acid (B).

1620 cm^{-1} (C═O of carboxylate acid), 1130 cm^{-1} (C–O of carboxylic acid), and 616 cm^{-1} (pyranose ring) [30].

The presence of the characteristic bands related to tragacanth gum and gelatin in tragacanth/gelatin complex spectra (Fig. 2C) revealed the existence of both polymers in the structure of microcapsules. In the spectrum of tragacanth/gelatin complex, there was a slight shift of the band of amide-I from 1648 cm^{-1} to 1655 cm^{-1}, which may be due to association of the negatively charged tragacanth gum with positively charged gelatin. Characteristic bands of gallic acid (Fig. 2D) appeared at 3492 cm^{-1} (O–H stretching), 1701 cm^{-1} (CO stretching of carboxylic acid), and 1616 cm^{-1} (C═C stretching). Moreover, in FTIR spectrum of gallic acid, the bands at 1536 and 1440 cm^{-1} were related to C–C aromatic ring stretching vibration [31,32]. The positions of these bands, especially stretching vibrations of the aromatic rings, were observed in gallic acid-loaded microcapsules (Fig. 2E). Based on the FTIR results, it was proven that the microcapsules contained the chemical ingredients and functional groups of the wall materials (tragacanth and gelatin) and the core active agent (gallic acid).

XRD analysis is used to detect crystalline and amorphous state of molecules. Fig. 3 shows XRD patterns of blank microcapsules and gallic acid-loaded microcapsules. XRD patterns of blank microcapsules (Fig. 3A) show a peak around 2θ value 20°, which reveals that tragacanth/gelatin complex is semi-crystalline. XRD pattern of microcapsules containing gallic acid (Fig. 3B) also shows similar structure of microcapsules. It indicates that the crystalline intensity of the complex did not change after gallic acid encapsulation. Alves, Mainardes [32] showed that gallic acid had a crystalline structure. However, it did not affect the crystalline structure after microencapsulation [32].

Fig. 4 shows the thermal analysis results of microcapsules with and without gallic acid. TGA analysis showed four steps for both samples. Also, three endothermic and three exothermic peaks were observed in the DSC thermogram of both samples. In the tragacanth/gelatin microcapsules containing gallic acid (Fig. 4A), the first reduction of the initial weight (7.9 %) occurred in the range of 80–180 °C. The first step of weight loss was related to moisture vaporization. The first weight reduction (9.78 %) also occurred in the control sample (Fig. 4B) in the temperature range of 80–180 °C due to water loss [25]. They are related to the first endothermic peak in the DSC thermogram of both samples (Fig. 4C and D). The second, third, and fourth weight losses in microcapsules containing gallic acid (Fig. 4A) occurred at 225–260 °C, 260–370 °C, and 370–550 °C, respectively, which are matched with the three exothermic peaks of the DSC test and are attributed to the depolymerization and polymer decomposition. There is about 64.52 % weight loss in this temperature range relative to the whole sample. These three loss steps were also observed for the control sample, with approximately 67.39 % weight loss relative to the entire sample. Aytac, Kusku [33] observed that microencapsulation did not affect the thermal resistance of gallic acid [33]. The second and third endothermic peaks in the DSC thermogram of both samples (Fig. 4C and D) at 227 °C and 444 °C were probably due to the conversion of the crystalline structures of the sample to amorphous forms [34].

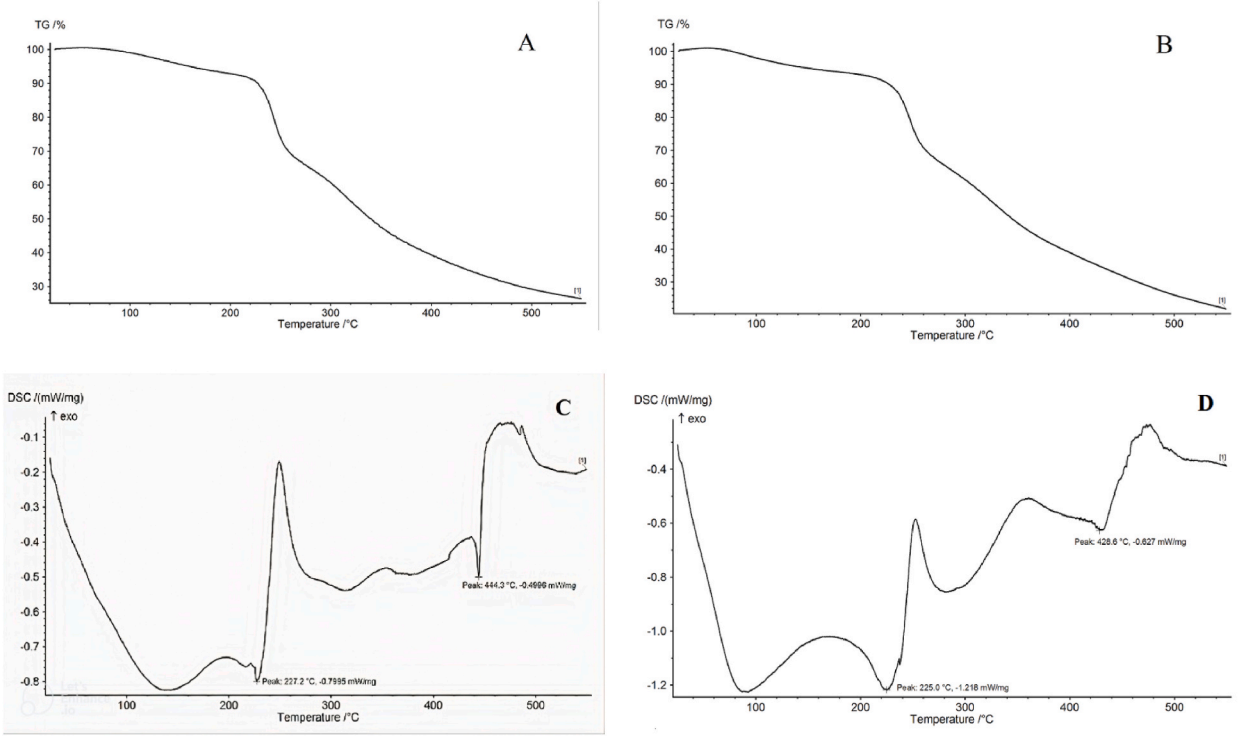

Fig. 4. (A) TGA thermogram of microcapsules containing gallic acid, (B) TGA thermogram of control sample, (C) DSC thermogram of microcapsules containing gallic acid, (D) DSC thermogram of control sample.

3.8. Investigation of chemical and microbial properties of hamburger during storage

The peroxide value (Table 2) shows the progress of oxidation in both samples (the control sample and the sample containing microcapsules) during 30 days of storage. The decomposition of unstable hydroperoxides leads to the formation of secondary oxidation products, such as aldehydes, affecting sensory characteristics and food quality. Table 2 also shows significant changes ($p < 0.05$) between the two samples after 15 and 30 days. After 30 days, the control sample had a 76.2 % increase in peroxide value, while the sample containing encapsulated gallic acid had a 72.8 % increase. Due to phenolic compounds, the hamburgers containing gallic acid microcapsules reduced the oxidation process and POV. This reduction in POV in the sample containing microcapsules can be attributed to the antioxidant property of gallic acid, which can decompose the hydroperoxides created due to lipid oxidation. Phenolic compounds have efficient free radical scavengers or chain-breaking characteristics, which work by giving a hydrogen atom from their –OH groups. The resultant phenolic radicals are thermodynamically stable due to the delocalization of radicals through the phenolic ring structure [35]. The maximum permissible amount of peroxide for human consumption is about 15 meq/kg lipids [36]. The value of the two samples did not exceed this value after 30 days.

Both control and treated hamburgers were analyzed at 0, 15, and 30 of storage at −18 °C.

Table 2 also shows the TBA changes of both control and treated hamburgers during the storage at −18 °C for one month. The TBA shows the amounts of secondary products from lipid oxidation, especially aldehydes. According to Table 2, the amount of TBA in the two samples increased significantly after 30 days due to the conversion of hydroperoxides into aldehyde and other secondary oxidation products. However, on the 30th day, the amounts of secondary products resulting from lipid oxidation were significantly lower in the hamburgers containing phenolic compounds (gallic acid) than in the control sample due to the effect of phenolic compounds on lipid oxidation. Phenolic compounds are responsible for absorbing and neutralizing free radicals, quenching singlet oxygen or decomposing peroxides, and donating hydrogen, producing stable and low-energy radicals with very low reactivity [20]. de Paiva, Trindade [37] observed that adding 500 mg/kg of natural licorice extract to caiman meat nuggets caused a significant reduction of TBA [37]. Moreover, Das, Rajkumar [38]found that 0.1 % Moringa oleiferia leaves extract retarded lipid oxidation in cooked goat meat patties [38].

The amounts of *E. coli* and *S. aureus* in the control sample and the sample containing gallic acid microcapsules were 0 and less than 10^2 CFU per gram, respectively, during one month (Table 3). However, according to Table 3, the TC of the hamburger containing microcapsules and the control sample was significantly different after 30 days of storage. So, the TC of the hamburger containing microcapsules was much lower than the control sample due to the presence of phenolic compounds. Also, the amounts of mold and yeast were significantly lower in the sample containing microcapsules than in the control sample. Phenolic products exhibit antimicrobial activity through multiple mechanisms, including destabilizing and permeabilizing cytoplasmic membranes, inhibiting enzymes through oxidation, and forming reactive quinones that can react with amino acids and proteins. These actions may involve specific interactions with sulfhydryl groups or more nonspecific interactions with proteins. Phenols can effectively hinder the production of nucleic acids in both Gram-negative and Gram-positive bacteria [39]. It was confirmed that phenolic acids, especially gallic acid, alter the hydrophobicity of bacterial cells by affecting their physicochemical surface properties [40]. Over time, the inhibitory effect of gallic acid on microbial growth may diminish as the compound is metabolized or degraded by the microorganisms. However, the duration of this effect can vary depending on the specific microorganism and the concentration of gallic acid [41]. Lattanzio, Lattanzio [42] reported that gallic acid prevents sporulation and germination of mold by chelating some metal ions, which is required for the growth of microorganisms. The present study confirms the antioxidant and antimicrobial activity of microcapsules containing gallic acid added to hamburgers, which maintains the quality of the meat products.

Sensory scores assessment for color, taste, texture, and overall acceptance of hamburgers treated with and without microcapsules containing phenolic compounds showed no significant difference between two samples (Table S3). So, adding encapsulated gallic acid to the hamburger had no effect on overall consumer acceptance. Pourashouri, Shabanpour [43] also did not observe any significant difference between nugget samples containing different percentages of encapsulated fish oil.

4. Conclusion

This study demonstrates the efficient encapsulation of gallic acid using gelatin and tragacanth gum through complex coacervation, achieving a maximum encapsulation efficiency of 98.7 % at tragacanth to gelatin ratio, 1:4; total polymer content, 0.9 %; pH, 3.5; and gallic acid content, 0.88 %. The application of these microcapsules in hamburgers reveals the ability of gallic acid to mitigate oxidative

Table 2
TBA and POV of hamburgers during one month of storage.

Test	Sample	Storage time (day)		
		0	15	30
TBA (mg MAD/kg)	Control	0.087 ± 0.008^{cA}	0.114 ± 0.010^{bA}	0.126 ± 0.061^{aA}
	Microcapsules contained	0.079 ± 0.009^{bA}	0.083 ± 0.008^{bB}	0.103 ± 0.004^{aB}
POV (meq/kg.)	Control	3.03 ± 0.20^{cA}	9.93 ± 0.45^{bA}	12.73 ± 0.15^{aA}
	Microcapsules contained	3.00 ± 0.10^{cA}	8.43 ± 0.40^{bB}	11.03 ± 0.15^{aB}

Different small letters in each row indicate a significant difference between the samples ($p < 0.05$).
Different capital letters in each column indicate a significant difference between the samples ($p < 0.05$).

Table 3
Microbial evaluation of hamburgers during one month of storage.

Test	Sample	Storage time (day)		
		0	15	30
Mold and Yeast	Control	40 ± 0^{cB}	72 ± 2.82^{bA}	82.5 ± 3.53^{aA}
	Microcapsules contained	50 ± 0^{bA}	53.5 ± 4.94^{bB}	67 ± 9.89^{aB}
Total count	Control	22500 ± 707^{bA}	24500 ± 707^{bA}	32500 ± 3535^{aA}
	Microcapsules contained	19500 ± 707^{aA}	24500 ± 2121^{aA}	20500 ± 707^{aB}
E. coli	Control	0 ± 0	0 ± 0	0 ± 0
	Microcapsules contained	0 ± 0	0 ± 0	0 ± 0
S. aureus	Control	$>10^2\pm0$	$>10^2\pm0$	$>10^2\pm0$
	Microcapsules contained	$>10^2\pm0$	$>10^2\pm0$	$>10^2\pm0$

Different small letters in each row indicate a significant difference between the samples ($p < 0.05$).
Different capital letters in each column indicate a significant difference between the samples ($p < 0.05$).

reactions and inhibit spoilage microorganisms, particularly molds and yeast, during one month of storage. Importantly, sensory evaluation indicates no significant difference between treated and control hamburgers. The results underscore the high potential of gallic acid coated with gelatin and tragacanth gum as a natural and effective solution for enhancing the shelf life of meat products, offering a viable alternative to artificial antibacterial and antioxidant agents.

Ethics statement

The authors state that sensory evaluations of the type we conducted do not require formal ethical approval in Iran. However, we adhered to general ethical principles to ensure the safety and comfort of our participants. Before participating in the sensory evaluation, all participants were informed about the nature and purpose of the study, and their written informed consent was obtained.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Elham Asghari-Varzaneh: Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Safourasadat Sharifian-Mobarakeh:** Methodology, Formal analysis, Data curation. **Hajar Shekarchizadeh:** Writing – review & editing, Validation, Project administration, Investigation, Conceptualization.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24917.

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