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Research Paper

The Effect of Thymol Microcapsules Prepared With Xanthan and Gu ar Gums on the Count of *Staphylococcus aureus*, and the Physicochemical and Sensory Properties of Hamburgers During Frying

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ABSTRACT

Introduction: The preservative effect of the addition of thymol coated with xanthan gum and guar gum on meat quality parameters and sensory acceptability of fried hamburgers during a 21-day storage period was investigated.

Materials & Methods: Eight treatments were studied: CON (burger without thymol); TYM 0.5% (burger with 0.5% thymol); TYM 1% (burger with 1% thymol); TCX 0.5% (burger with 0.5% thymol coated with 1% xanthan gum); TCG 0.5% (burger with 0.5% thymol coated with 1% guar gum); TCX 1 (burger with 1% thymol coated with 1% xanthan gum); TCG 1 (burger with 1% thymol coated with 1% guar gum); and TCXG (burger with 1% thymol coated with 1% xanthan and guar gums).

Results: The control treatment had the highest energy level, while TCG1 had the lowest. The use of thymol resulted in an increase ($P<0.05$) in the moisture content, particularly when the thymol was coated with xanthan and guar gums. The most significant ($P<0.05$) reduction in cooking loss and fat absorption was observed with TCXG treatment. The treatment groups showed an increase in ash levels compared to the control treatment. The protein content of the group treated with thymol was lower than the control. The carbohydrate content of the samples increased with TCXG compared to the control treatment. Odor, color, texture and general acceptability scores (P) were higher in the TCXG and TCG1 treatments and lowest in the CON treatment. From the beginning of the display (day 1) to day 21, there was a significant decrease in the scores for odor, color, texture, and overall acceptability. The results showed that coating thymol with xanthan and guar increased the b, a, and l indices compared to the control treatment.

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Conclusion: The study concluded that thymol can extend the shelf life of processed foods, making it a potential natural alternative to synthetic ingredients. The utilization of thymol, in both uncoated and coated forms, resulted in a significant reduction in the TVN index. The combination of thymol coated with xanthan and guar at a concentration of 1% proved to be the most effective in reducing the levels of bacteria.

1. Introduction

The cooking method exerts a significant influence on a range of meat quality attributes, including cooking rate, moisture content, protein and fat content, cooking yield, diameter changes, hardness, brittleness, and overall acceptability [1]. Additionally, frying gives rise to the formation of new compounds as a consequence of the exposure of the cooking oil or fat to elevated temperatures, air, and moisture. Hydrolysis occurs, resulting in the breaking of ester bonds and the release of free fatty acids, monoglycerides, and diglycerides [2]. These compounds possess a higher polarity and lower molecular weight than the original triglycerides [3]. The chemical nature of the substrate and the frying conditions influence the extent of oxidative reactions and hydrolysis during frying [4, 5]. The formation of carcinogenic heterocyclic aromatic amines (HAAs) is significantly affected by a number of factors, including temperature, time, muscle-fat ratio, and salt content, when frying hamburgers [6]. Additionally, the selection of frying oil can influence the formation of HAAs, with sunflower oil demonstrating the greatest yield of total HAAs in fried beef patties. Furthermore, the absorption of fat during frying can induce alterations in the fatty acid composition of the meat, which can have either advantageous or detrimental effects depending on the initial fatty acid makeup and the type of fat employed for frying [7, 8]. It is therefore crucial to take into account these factors when evaluating the nutritional quality of fried hamburgers.

The consumption of fried food has been positively associated with a number of adverse health outcomes, including cardiovascular events, overweight and obesity, type 2 diabetes mellitus (T2DM), hypertension, coronary heart disease, stroke, heart failure, and all-cause mortality [9]. The process of deep-frying can result in the formation of cytotoxic and genotoxic lipid oxidation products (LOPs), which may infiltrate fried foods and pose health risks when ingested [10, 11]. Therefore, it is crucial to consider the potential health risks associated with the consumption of fried hamburger and to adopt healthier cooking methods in order to minimize these risks [10, 11].

Thymol (2-isopropyl-5-methylphenol, IPMP), $C_{10}H_{14}O$, is a natural monoterpenoid, found in the oils of various plants as a white crystalline substance with a pleasant aromatic odor and strong antimicrobial properties [12]. The antioxidant and antimicrobial characteristics of thymol exert an influence on the quality of meat products. Thymol has been demonstrated to impede the oxidation of lipids and the alteration of color when stored in cold temperatures, thus preventing the rise in levels of malondialdehyde (MDA) and the adverse transformation of pigments [12]. Additionally, thymol functions as an agent that inhibits the growth of bacteria such as *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Clostridium perfringens*, thereby ensuring the maintenance of microbial quality in sausage products [13]. Furthermore, thymol enhances the quality of meat by promoting oxidative metabolism in muscle. Thymol has been demonstrated to enhance oxidative metabolism in muscle [14]. This leads to a reduction in drip loss and an improvement in meat quality in terms of tenderness and oxidative metabolism [15]. The antioxidant and antimicrobial properties of thymol assume a significant role in enhancing the quality and prolonging the shelf life of meat products [16]. Overall, thymol represents a natural and effective alternative for the preservation of meat products, offering additional health benefits.

The use of xanthan gum and guar gum has been demonstrated to exert a significant impact on the texture and visual appearance of meat products. The use of xanthan gum as a substitute for fat in low-fat meat emulsions, either alone or in combination with guar gum, has been shown to improve emulsion stability, cooking yield, juiciness, and to reduce penetration force [17]. The incorporation of xanthan gum into meat products resulted in an increase in protein and moisture content, as well as an improvement in oxidative quality and a notable enhancement in juiciness [18]. The substitution of xanthan gum and light salt for fat and common salt, respectively, in pork sausages led to a reduction in fat, energy, and sodium content, while simultaneously increasing moisture and potassium levels. Similarly, the incorporation of xanthan gum as a fat substitute in low-fat meat products resulted in an increase in protein and moisture content, a reduction in fat content, an improvement in oxidative quality, and the preservation of sensory characteristics similar to those of the high-fat versions [19].

The incorporation of xanthan and guar can facilitate the enrichment of meat products. These polysaccharides have been demonstrated to possess antibacterial and antioxidant properties, indicating their potential as natural preservatives for meat and meat products [20]. The incorporation of these polysaccharides has been shown to enhance texture, inhibit the proliferation of pathogens, and improve oxidative stability as well as the sensory properties of meat products [21, 22]. Furthermore, the utilization of a biodegradable film, which is based on bacterial exopolysaccharide (xanthan), has been proven to prolong the shelf life of chilled meat products while preserving their sensory, physicochemical, and microbiological attributes [18]. Therefore, the inclusion of xanthan and guar can improve both the nutritional value and quality of meat products. The objective of this study was to investigate the impact of thymol and thymol-coated xanthan gum, and guar gum on the physicochemical and sensory properties of fried hamburgers.

2. Materials and Methods

2.1. Preparation of hamburgers

The beef, which consisted of 80% lean beef and 20% fat, underwent a double grinding process using a meat grinder. Subsequently, the minced meat, accounting for 60% by weight, was combined with other ingredients making up 40% by weight, including onion (28%), vegetable oil (4%), bread crumbs (3%), non-fat dry milk (3%), salt (1%), spices (0.5%), sodium polyphosphate (0.3%), and spice juice (0.2%), in order to prepare the hamburger. Once mixed, the hamburger was shaped using a steel mold with a weight of 100±5 g, a thickness of 0.5 cm, and a diameter of 9 cm. Following this, the hamburger was wrapped in a polyvinyl chloride (PVC) film and stored at a freezing temperature of -18 ˚C. In order to create a hamburger containing an encapsulated antioxidant, 400 mg/kg of encapsulated gallic acid was added to 100 g of hamburger. The encapsulation efficiency was then utilized to determine the quantity of capsules containing 400 ppm of gallic acid. The remaining steps were carried out in a similar manner to the control sample, which did not include an encapsulated antioxidant. Both the control and treated hamburgers were analyzed at intervals of 0, 15, and 30 days during storage at -18 ˚C.

2.2. Microencapsulation

The process of microencapsulation involved the incorporation of thymol (0.5% and 1%) with xanthan and guar. Initially, 0.01 g of thymol was dissolved in 1 mL of dimethyl sulfoxide (DMSO) solvent, followed by the addition of 1 mL of Tween 80. Subsequently, 100 mL of xanthan was added under controlled temperature conditions, and the mixture was homogenized for 10 min at 4000 rpm using a homogenizer. To preserve the integrity of the microcapsules and thymol, the resulting solution was then poured onto a plate and dried using a freeze dryer. The same method was employed for the preparation of microcapsules containing 1% guar and a combination of guar-xanthan (0.5% guar-0.5% xanthan).

2.3. Physicochemical characteristics

2.3.1. Peroxide value (PV)

The determination of PV was conducted in accordance with the methodology established by AOAC (2000) [23]. Initially, the extraction of fat from the meat was performed using the Soxhlet method. Subsequently, 5 g of the extracted oil was combined with 30 mL of acetic acid-chloroform in a 3:2 v/v ratio, enabling complete dissolution of the fat. Following this, 5 mL of a saturated potassium iodide solution was introduced to the sample and the resulting mixture was left in darkness for a duration of 1 min. To serve as an indicator, starch solution was then added. Lastly, the sample was titrated using a 0.01 N sodium thiosulfate solution. The PV was determined through Equation 1 and expressed as milliequivalents of peroxide per kg of sample.

$$1.\ \text{PO V (meq/kg)-}\frac{S\times N}{W}\times 1000$$

S: The volume (mL)

N: Normality of sodium thiosulfate solution

W: Sample weight (kg)

2.3.2. Thiobarbituric acid (TBARS) value

The estimation of TBARS value (mg MDA (mal)/kg) was conducted through the distillation technique, employing 2-TBARS 0.02 M (Sigma Chemical Co. Ltd, USA) as described by Food and Agriculture Organization (FAO) (1986) [23], in order to assess the quantity of MDA and secondary oxidation compounds. In brief, a 10 g sample was homogenized with 25 mL of 20% trichloroacetic acid in 2 M phosphoric acid and subsequently mixed with 25 mL of distilled water. The resulting mixture was filtered using Whatman paper (No. 41), and 5 mL of the extract was combined with 5 mL of 0.01 M TBARS in 90% acetic acid. The absorbance of the sample was then measured at 532 nm using a UV-Visible spectrophotometer. The TBARS value was reported as milligrams MDA equivalents per kilogram of sample (mg MDA/kg).

2.3.3. Measurement of volatile nitrogen substances (TVN)

For this purpose, 10 g of the sample were combined with 2 g of magnesium oxide and 300 mL of distilled water, with the addition of a few stones and a small quantity of anti-foam. The flask was heated for 15 min until the boiling point was reached. The vapors from the distillation flask were collected directly in the Erlenmeyer flask containing 25 mL of a 2% boric acid solution and a few drops of methyl red reagent, until the total volume of boric acid and condensed vapors inside the Erlenmeyer flask reached 150 mL. Subsequently, the solution resulting from the accumulation of distillation vapors was titrated with 0.1 normal sulfuric acid until the color reached that of onion skin. The quantity of nitrogen compounds present in the sample was determined in milligrams [3].

2.3.4. Characterization of microcapsules (SEM)

Scanning electron microscopy (SEM, LEO 440 I, England) and a light microscope (Leica Qwin 550) were applied to observe the surface and morphology of microcapsules. To prepare microcapsules, they were placed on a specimen stub and coated with gold using a sputter coater. After 10 min, the surface morphology of the prepared samples was examined by the SEM at an accelerating voltage of 10 kV

2.3.5. Characterization tests (fourier transform infrared spectroscopy (FTIR) and x-ray diffraction (XRD)

FTIR profiles of the resulting nanoparticles were determined using a Bruker spectrometer (VERTEX 70, Germany). XRD measurements of the crystal structure were performed using Panalytical X-Ray diffraction spectrometer (Xpert Pro MPD, Netheerlands).

2.4. Enumeration of *S. aureus*

In a sterile environment, 10 g of the sample was separated. Then it was placed in a special sterile plastic bag, and 90 mL of sterile Ringer's solution was added. The bag was transferred to a Stomacher (Bagmixer, Interscience, France) and homogenized for 3 minutes. Subsequently, the sample was diluted to a final volume of 10^5 CFU/mL. Then 1 mL was added to a tube containing 9 mL of peptone water (Merck, Germany), resulting in the preparation of the subsequent dilutions. After preparing serial dilutions, 100 µL of each sample was cultured in Baird-Parker agar for enumeration of viable *S. aureus* cells, and the plates were incubated at 37 ˚C for 48 h.

2.5. Sensory evaluation

Odor, color and overall acceptability of raw samples were evaluated by eight trained panelists from the Food Hygiene and Control Department (who had prior experience in burger processing and evaluation). The participants were asked to assess various qualitative characteristics. A sensory hedonic scale ranging from 0 (very poor) to 8 (very good) was used, following the procedures described in reference 3 [3].

2.6. Statistical analyses

By performing various tests on samples, the data were recorded in Excel software. SPSS software version 21 was used for statistical analysis, and $P|<0.05$ was considered statistically significant. Analysis of variance (ANOVA) was used to assess significant differences between treatment and control conditions. Duncan's multiple range test was used to compare the differences between means at the 0.05 level. If the distribution of the data was normal, repeated measures ANOVA was used to evaluate changes in physicochemical, microbial, and sensory characteristics across treatments.

3. Results

3.1. FTIR analysis

The FTIR test was used to check the chemical bonds in the samples. In FTIR spectroscopy, the energies of the infrared rays coincide with the vibrational energies of molecules, and this matching causes the absorption of electromagnetic radiation by the sample. Therefore, by varying the frequency of the radiation in a specific range (infrared), a spectrum is obtained in which transmittance is reduced at some wavelengths, or in other words, those wavelengths are absorbed by the molecules of the material. Therefore, by studying the absorption frequencies of each spectrum, it is possible to understand the bonds in that material. Figure 1 shows the FTIR spectra of the samples studied. The position of the peaks in each sample, together with the bonds and functional groups associated with each peak, are also given in Table 1.

Table 1. The peak positions and the functional groups and bonds associated with each peak of the examined samples

Ref.	Functional Groups and Links	Peak Position (cm^{-1})	Sample
[1, 2]	-OH (stretching)	3222	
[3]	C-H (stretching)	2964	
[3]	C-H (stretching)	2871	
[4]	-OH (bending)/C=C (stretching)	1627	
[5]	C-H (bending)	1427	
[5]	C-H (bending)	1289	
[6]	C-OH (stretching)	1249	Thymol
[6]	OC-OH (stretching)	1160	
[6]	C-O-C (stretching)	1098	
[7, 8]	C-H (dancer)	947	
[7, 8]	C-H (cradle)	804	
[7, 8]	C-H (transformation)	738	
[7, 8]	C-H (off page)	591	
[1, 2]	-OH (stretching)	3137	
[3]	C-H (stretching)	2942	
[4]	-OH (bending)/C=C (stretching)	1595	Thymol@0.5%Guar
[5]	C-H (bending)	1431	
[5]	C-H (bending)	1270	
[7, 8]	C-H (transformation)	882	
[1, 2]	-OH (stretching)	3087	
[3]	C-H (stretching)	2860	
[4]	-OH (bending)/C=C (stretching)	1581	Thymol@1%Guar
[5]	C-H (bending)	1421	
[5]	C-H (bending)	1241	
[7, 8]	C-H (transformation)	864	
[1, 2]	-OH (stretching)	3070	
[2]	C-H (stretching)	2848	
[4]	-OH (bending)/C=C (stretching)	1605	
[5]	C-H (bending)	1431	Thymol@0.5%Xanthan
[5]	C-H (bending)	1253	
[6]	C-O-C (stretching)	1099	
[7, 8]	C-H (transformation)	819	

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Ref.	Functional Groups and Links	Peak Position (cm^{-1})	Sample
[1, 2]	-OH (stretching)	3000	
[3]	C-H (stretching)	2817	
[4]	-OH (bending)/C=C (stretching)	1589	
[5]	C-H (bending)	1389	Thymol@1%Xanthan
[5]	C-H (bending)	1238	
[6]	C-O-C (stretching)	1098	
[7, 8]	C-H (transformation)	817	
[1, 2]	-OH (stretching)	3191	
[3]	C-H (stretching)	2942	
[4]	-OH (bending)/C=C (stretching)	1604	
[5]	C-H (bending)	1436	
[5]	C-H (bending)	1253	Thymol@Guar/Xanthan
[6]	OC-OH (stretching)	1142	
[7, 8]	C-H (cradle	880	
[7, 8]	C-H (transformation)	724	
[7, 8]	C-H (off page))	516	

In these FTIR spectra, the visible peak at about 3000-3400 cm^{-1} is related to the stretching vibrations of the O-H bond, caused by the presence of hydroxyl groups in these structures. It is clear that the position of this peak has shifted to lower wavenumbers in the guar-, xanthan-, and guar/xanthan-encapsulated samples compared to the pure thymol sample, due to the formation of hydrogen bonds between the hydroxyl groups in guar, xanthan, and thymol. In addition, an increase in the percentage of capsule materials (guar and xanthan) has caused more peak shifts, indicating an increase in hydrogen bonding between these structures. The lowest position of this peak corresponds to the sample of thymol encapsulated in 1% xanthan, followed by sample of thymol encapsulated in 0.5% xanthan, which shows that the hydroxyl groups in xanthan are more abundant than those in the structure of guar. This is also evident in the structure of this substance, and therefore xanthan is probably a more favorable structure for the encapsulation of thymol.

Moreover, the peaks situated within the wavenumber range of 2800 cm^{-1} to 2950 cm^{-1} are associated with the asymmetric and symmetric vibrations of the C-H bond present in both aliphatic and aromatic structures within the examined samples. Additionally, the shift of these peaks towards lower wavenumbers indicates an increase in the quantity of methyl and methylene (aliphatic) structures within the system. Thus, it is evident from the observable outcomes in the samples that the peaks reached their maximum values in the pure thymol sample, and upon encapsulation in guar and xanthan, these peaks exhibited a shift towards lower wavenumbers. Specifically, considering the chemical composition of thymol characterized by an aromatic ring, the incorporation of guar and xanthan structures resulted in an augmentation of aliphatic structures within the system, consequently shifting the peaks to towards lower wavenumbers. Furthermore, the disappearance or reduction in intensity of certain peaks following the encapsulation of thymol in guar and xanthan structures serves as evidence of the successful nature of this encapsulation process.

The bending vibration of the hydroxyl bonds is also located in the range of wavenumbers of about 1580 cm^{-1} to 1630 cm^{-1}, again clearly showing that the presence of guar and xanthan structures with higher percentages causes this peak to move towards lower wavenumbers. This shift is attributed to the formation of hydrogen bonds between the hydroxyl groups of these samples. The peak corresponding to the stretching vibration of the

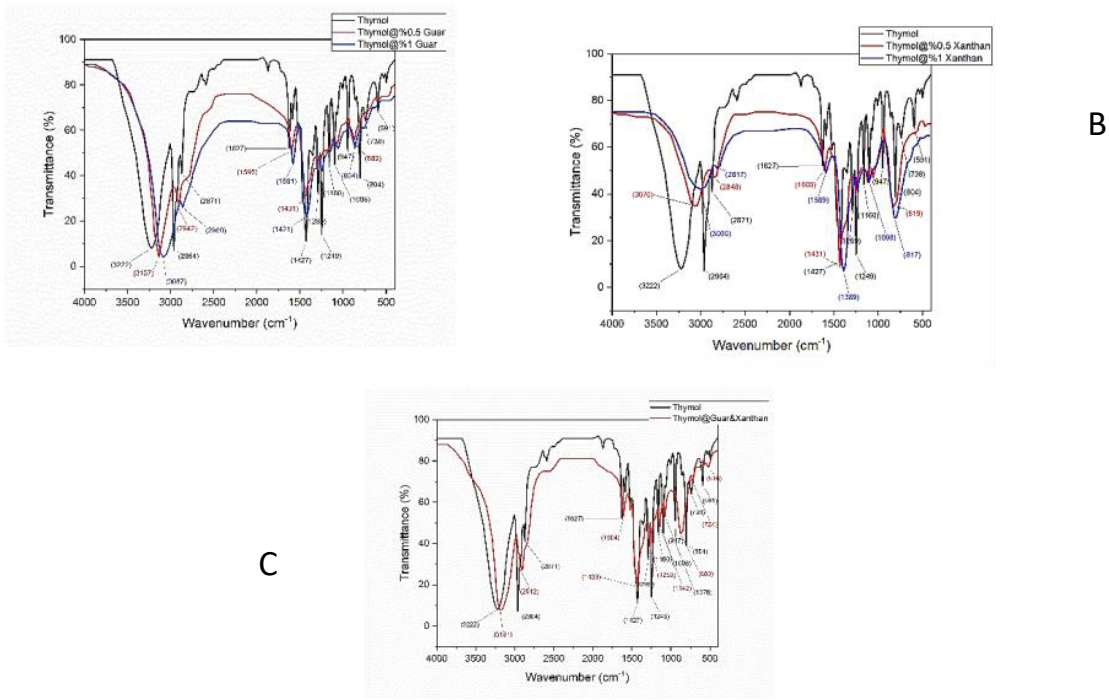

Figure 1. FTIR spectra of the samples

a) Thymol and thymol encapsulated in 0.5% and 1% guar; b) Thymol and thymol encapsulated in 0.5% and 1% xanthan; c) Thymol and thymol encapsulated in guar and xanthan

C=C bond in aromatic rings is also in the same range of wavenumbers. Peaks related to bending vibrations of C-H bonds in methyl and methylene structures have also produced multiple peaks in the wavenumber range of 1280 cm^{-1} to 1450 cm^{-1}.

On the other hand, the multiple peaks located in the wavenumber range of 1000 cm^{-1} to 1250 cm^{-1} are related to different carbon-oxygen bonds such as C-OH and C-O-C, which are known to be functional groups. In the presence of encapsulating phases, these peaks shift to lower wavenumbers compared to the pure thymol sample, which is further evidence for the existence of hydrogen bonds between the compounds in these samples. Dancing, rocking, deformation, and out-of-plane vibrations of C-H bonds in syringyl, guaiacil, and aromatic ring structures have also shown absorption peaks at wavenumbers below 1000 cm^{-1}. In addition, as mentioned for the peaks related to the stretching vibration of the C-H bonds, the disappearance or reduction in intensity of some of these peaks after the encapsulation process of thymol in guar and xanthan structures can be considered evidence of the success of this encapsulation process.

4.2. XRD analysis

XRD or x-ray diffraction, is an old and widely used technique for investigating the properties of crystals. In this method, the x-ray diffraction pattern of the sample is used to check its properties. XRD can be used to determine the general parameters of the crystal structure. The x-ray diffraction pattern of the sample is shown in Figure 2.

In the x-ray diffraction pattern of pure thymol, peaks located at 2θ values of approximately around 11.9, 14.7, 17.7, 19.2, 22.3, 24.1, 28.8, 29.6, 30.8, 31.8, and 36.3 degrees are observable. This particular diffraction pattern for thymol has been previously documented by Zhou et al. [25] and Trivedi et al. [26], illustrating the crystal structure of the aforementioned compound.

Scherrer's equation (Equation 2) is used to determine the size of crystals in this material.

2. $D=K\lambda/(FWHM)\times\cos(\theta)$

In this relationship, D is the size of the crystal, K is the shape factor, λ is the wavelength of the x-ray (1.54 Å), FWHM is the full width at half maximum, and θ is the position of the peak.

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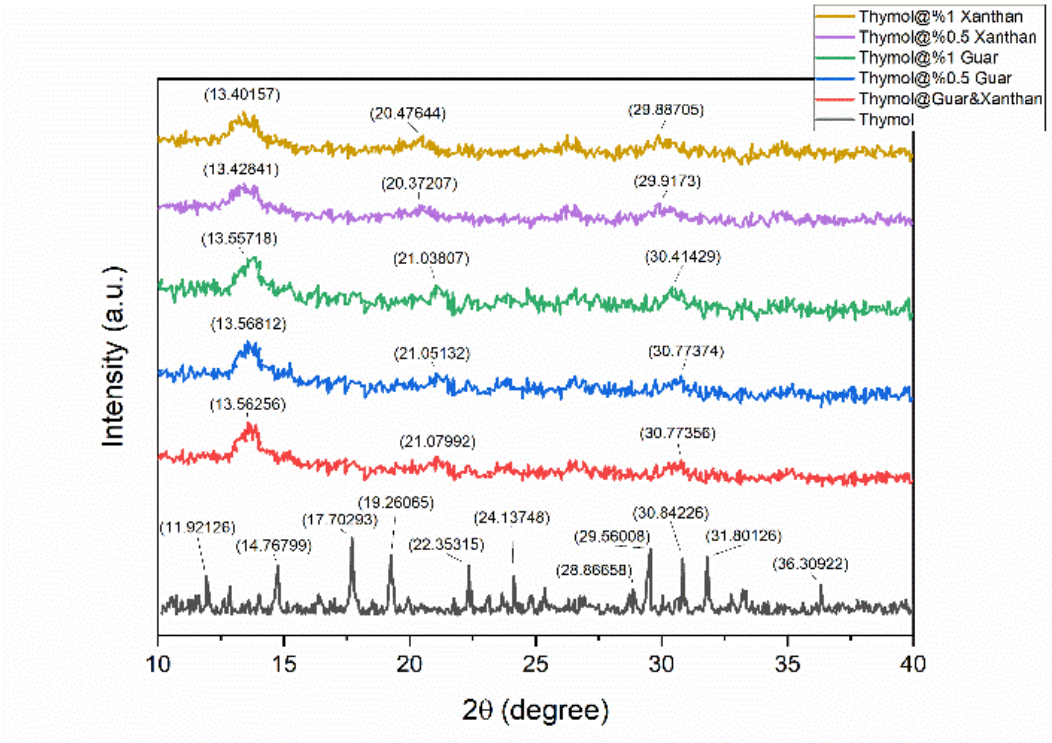

Figure 2. X-ray diffraction patterns of the samples

Given the value of cosθ and FWHM, and the fixed values of λ (1.54 Å) and k (0.9), the value of the crystal size is obtained from Scherrer's equation, which, given that λ is in angstroms, yields a result also in angstroms. The crystal size in nanometers was calculated by dividing the obtained value by 10. For the most intense peak (at angle of 2θ=17.7º), the value is 38.1 nm.

In the samples of thymol encapsulated with guar, xanthan, and guar/xanthan, there are no traces of the sharp peaks and crystalline structure of thymol. Instead, relatively broad peaks at angles of about 13.5, 21, and 30 degrees are observed, which are characteristic of the structure of amorphous encapsulating agents. Similar diffraction patterns have been observed for amorphous guar and xanthan in other similar articles. The broader diffraction peaks in the samples containing xanthan compared to those containing guar indicate that this structure is more amorphous than the structure of guar. Also, the slight shift of the diffraction peaks in the xanthan gum samples to lower angles than in the guar gum samples indicates the greater spacing of the crystal planes in these samples compared to the guar gum samples, which indicates the more amorphous structure of the xanthan gum samples. Therefore, the XRD test results, together with the FTIR test results, prove the successful encapsulation of thymol in guar and xanthan gum structures.

4.3. Analysis of SEM results

To investigate the microstructure of thymol samples encapsulated in different amounts of guar, xanthan, and guar/xanthan blends, an SEM test was performed, and the resulting micrographs are shown in Figure 3.

It is clear that in all the micrographs, there are capsules visible in the images, which are guar, xanthan, and guar/xanthan microcapsules containing thymol. Comparing the micrographs of thymol encapsulated in different amounts of guar and xanthan, it is clear that by increasing the concentration of these two encapsulating agents from 0.5% to 1%, the size of the microcapsules increases and their dispersion decreases. According to Figure 1a, the average size of the microcapsules prepared with 0.5% guar is about 0.44 µm, while according to Figure 1b, this size is 1.2 µm for the microcapsules prepared with 1% guar. According to Figure 1c, the average size of the microcapsules prepared with 0.5% xanthan is about 0.57 µm, while according to Figure 1d this size is 2.3 µm for the microcapsules prepared with 1% xanthan. It is therefore clear that increasing the concentration of guar and xanthan significantly increases the size of the microcapsules. It is also clear that the size of the microcapsules in the samples encapsulated with xanthan at both 0.5% and 1% concentration is larger than in the samples encapsulated with guar. In addition, in the sample encapsulated in the

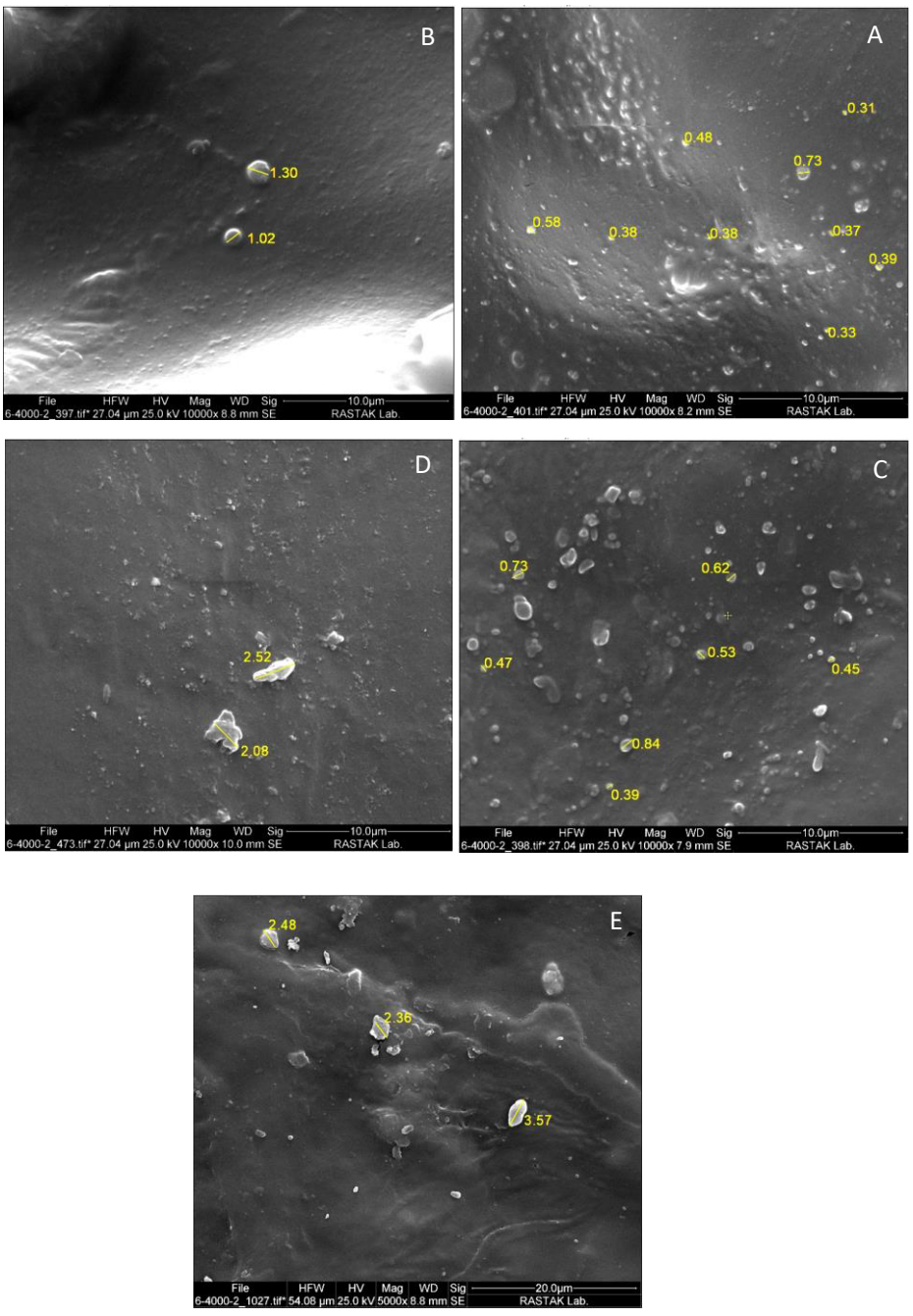

Figure 3. SEM micrographs of thymol samples encapsulated in (A) 0.5% guar, (B) 1% guar, (C) 0.5% xanthan, (D) 1% xanthan, and (E) guar-xanthan mixture

guar/xanthan mixture (Figure 1c), the microcapsules, with an average particle size of 2.8 µm, have the largest size among the other samples studied.

4.4. Physicochemical analyses

The impact of the experimental treatments on these factors was determined to be statistically significant ($P<0.05$) (Table 1). Specifically, the energy level demonstrated a noteworthy decline in hamburger samples subjected to TCX1, TCG1, and TCXG treatments, when compared to the control treatment. It is worth noting that the control treatment exhibited the highest energy level, whereas TCG1 resulted in the lowest energy level. The use of thymol resulted in an increase ($P<0.05$) in moisture content of burgers, particularly when the thymol was coated with xanthan and guar gums. The coated thymol displayed a significantly higher moisture percentage compared to the

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Table 1. Chemical composition, cooking loss, and fat attraction of hamburgers treated with thymol or thymol coated with xanthan and guar gums

Treatment	Mean±SD			
	Energy	Moisture	Cooking Loss	Fat Attraction
CON	551.03±0.37^{a}	56.21±0.12^{d}	11.06±0.25^{a}	9.82±0.06^{a}
TYM0.5	550.24±1.07^{a}	56.88±0.12^{c}	10.7±0.46^{a}	9.01±0.54^{b}
TYM1	549.3±0.32^{a}	57.26±0.02^{b}	9.98±0.05^{b}	8.41±0.52^{b}
TCX0.5	549.36±0.47^{a}	58.06±0.12^{a}	9.69±0.04^{c}	8.54±0.11^{b}
TCG0.5	549.12±0.6^{a}	58.08±0.23^{a}	9.62±0.08^{c}	8.21±0.63^{b}
TCX1	548.14±1.02^{a}	58.47±0.15^{a}	9.35±0.06^{d}	7.26±0.09^{c}
TCG1	547.74±0.36^{a}	58.15±0.16^{a}	9.33±0.08^{d}	6.98±0.23^{c}
TCXG	548.39±0.72^{a}	58.22±0.18^{a}	9.3±0.08^{d}	6.75±0.19^{c}

Note: Different letters indicate significant differences between treatments (P<0.05). CON: Beef burger without thymol; TYM0.5: Beef burger containing 0.5% thymol; TYM1%: Beef burger with 1% thymol; TCX0.5: Burger with 0.5% thymol coated with 1% xanthan gum; TCG0.5: Burger with 0.5% thymol coated with 1% guar gum; TCX1: Burger with 1% thymol coated with 1% xanthan gum; TCG1: Burger with 1% thymol coated with 1% guar gum; TCXG: Burger with 1% thymol coated with 1% xanthan and guar gum.

non-coated thymol and control. The TCX1, TCG1, and TCXG treatments exhibited the highest moisture content. Furthermore, cooking loss in the hamburger samples was reduced when thymol and coated thymol treatments were applied. The most significant (P<0.05) decrease in cooking loss was observed in the TCXG treatment. Additionally, the percentage of fat absorption in the hamburger experienced a significant decrease upon the application of thymol. The most substantial decrease in fat absorption was observed in TCXG.

The influence of interventions on the percentage of ash, protein, and carbohydrates in the hamburger samples was found to be statistically significant (P<0.05), while it was

Table 2. Chemical composition of hamburgers treated with thymol or thymol coated with xanthan and guar gums

Treatment	Mean±SD			
	Ash	Protein	Fat	Carbohydrate
CON	2.65±0.08^{b}	54.83±0.2^{a}	32.33±0.14^{a}	10.19±0.07^{d}
TYM0.5	2.8±0.02^{a}	54.37±0.2^{b}	32.28±0.2^{a}	10.55±0.01^{c}
TYM1	2.86±0.03^{a}	54.25±0.35^{b}	32.15±0.05^{a}	10.74±0.03^{b}
TCX0.5	2.87±0.05^{a}	53.78±0.21^{b}	32.17±0.09^{a}	11.19±0.07^{a}
TCG0.5	2.98±0.07^{a}	53.78±0.2^{b}	32.21±0.07^{a}	11.03±0.14^{a}
TCX1	3.09±0.15^{a}	53.55±0.05^{c}	32.1±0.07^{a}	11.27±0.28^{a}
TCG1	3.09±0.14^{a}	53.55±0^{c}	32.02±0.18^{a}	11.34±0.33^{a}
TCXG	3.04±0.06^{a}	53.55±0.05^{c}	32.11±0.13^{a}	11.29±0.38^{a}

Note: Different letters indicate significant differences between treatments (P<0.05). CON: Beef burger without thymol; TYM0.5: Beef burger containing 0.5% thymol; TYM1%: Beef burger with 1% thymol; TCX0.5: Burger with 0.5% thymol coated with 1% xanthan gum; TCG0.5: Burger with 0.5% thymol coated with 1% guar gum; TCX1: Burger with 1% thymol coated with 1% xanthan gum; TCG1: Burger with 1% thymol coated with 1% guar gum; TCXG: Burger with 1% thymol coated with 1% xanthan and guar gum.

Table 3. Sensory evaluation of hamburgers treated with thymol or thymol coated with xanthan and guar gums

Treatment	Mean±SD			
	Odor	Color	Texture	Acceptability
CON	2.5±0.03^{e}	2.92±0.05^{d}	3.08±0.06^{c}	2.58±0.02^{e}
TYM0.5	2.58±0.08^{e}	3.08±0.02^{c}	3.33±0.01^{b}	3.08±0.09^{d}
TYM1	2.92±0.11^{d}	3.08±0.04^{c}	3.33±0.04^{b}	3.17±0.06^{d}
TCX0.5	3.33±0.09^{c}	3.5±0.05^{b}	3.42±0.07^{b}	3.5±0.05^{c}
TCG0.5	3.67±0.08^{b}	2.58±0.01^{e}	3.5±0.02^{b}	3.75±0.03^{b}
TCX1	3.83±0.04^{b}	3.67±0.03^{a}	3.58±0.03^{a}	3.75±0.01^{b}
TCG1	3.92±0.02^{a}	3.75±0.08^{a}	3.68±0.11^{a}	3.91±0.13^{a}
TCXG	4±0.06^{a}	3.92±0.1^{a}	3.75±0.06^{a}	4.08±0.09^{a}

Note: Different letters indicate significant differences between treatments (P<0.05). CON: Beef burger without thymol; TYM0.5: Beef burger containing 0.5% thymol; TYM1%: Beef burger with 1% thymol; TCX0.5: Burger with 0.5% thymol coated with 1% xanthan gum; TCG0.5: Burger with 0.5% thymol coated with 1% guar gum; TCX1: Burger with 1% thymol coated with 1% xanthan gum; TCG1: Burger with 1% thymol coated with 1% guar gum; TCXG: Burger with 1% thymol coated with 1% xanthan and guar gum.

not significant for fat levels. The utilization of thymol and thymol coated with xanthan and guar resulted in increased levels of ash compared to the control treatment. Consequently, TCX1 and TCG1 exhibited the highest percentages of ash. Conversely, the protein levels in burgers treated with thymol were lower compared to the control. The control demonstrated the highest protein percentage, whereas the lowest was observed in TCXG. Furthermore, the carbohydrate percentage in the samples increased with TCXG treatment in comparison to the control treatment. The treatments TCX1, TCG1, and TCXG displayed the highest amounts of carbohydrates (Table 2).

Table 4. Evolution of CIE color parameters (L*, a*, b*) in hamburgers treated with thymol or thymol coated with xanthan and guar gums

Treatment	Mean±SD		
	l*	a*	b*
CON	54.75±0.07^{d}	15.84±0.05^{e}	19.59±0.08^{c}
TYM0.5	54.9±0.1^{d}	15.98±0.05^{d}	20.25±0.11^{d}
TYM1	55.09±0.19^{d}	16.21±0.02^{c}	22.06±0.19^{a}
TCX0.5	55.35±0.08^{c}	16.6±0.09^{b}	21.34±0.13^{b}
TCG0.5	55.49±0.01^{b}	16.55±0.03^{b}	21.35±0.16^{b}
TCX1	55.86±0.18^{a}	17.11±0.25^{a}	21.3±0.12^{b}
TCG1	56.01±0.14^{a}	16.89±0.13^{a}	20.84±0.22^{c}
TCXG	56.03±0.12^{a}	16.52±0.11^{b}	20.75±0.15^{c}

Note: Different letters indicate significant differences between treatments (P<0.05). CON: Beef burger without thymol; TYM0.5: Beef burger containing 0.5% thymol; TYM1%: Beef burger with 1% thymol; TCX0.5: Burger with 0.5% thymol coated with 1% xanthan gum; TCG0.5: Burger with 0.5% thymol coated with 1% guar gum; TCX1: Burger with 1% thymol coated with 1% xanthan gum; TCG1: Burger with 1% thymol coated with 1% guar gum; TCXG: Burger with 1% thymol coated with 1% xanthan and guar gum.

Table 5. The impact of thymol and thymol coated with xanthan and guar on TVN, PV, and TBARS levels in hamburgers during storage

Level	Treatment	Mean±SD			
		Storage (d)			
		1	7	14	21
TBARS	CON	0.14±0.01^{a}	0.21±0.02^{a}	0.29±0^{a}	0.35±0.02^{a}
	TYM0.5	0.12±0.02^{a}	0.16±0.03^{a}	0.24±0.02^{b}	0.3±0.01^{b}
	TYM1	0.09±0.03^{a}	0.14±0^{a}	0.23±0.02^{b}	0.27±0.01^{c}
	TCX0.5	0.1±0.04^{a}	0.14±0.02^{a}	0.21±0.01^{b}	0.27±0^{c}
	TCG0.5	0.11±0.02^{c}	0.15±0.04^{a}	0.22±0.01^{c}	0.28±0^{c}
	TCX1	0.1±0.01^{a}	0.12±0.03^{a}	0.18±0^{b}	0.25±0^{d}
	TCG1	0.11±0.03^{a}	0.15±0.02^{a}	0.23±0.03^{b}	0.26±0^{c}
	TCXG	0.08±0.01^{a}	0.11±0.03^{a}	0.16±0.02^{c}	0.24±0.01^{d}
PV	CON	0.41±0.08^{a}	1.03±0.08^{a}	1.2±0.04^{a}	1.36±0.08^{a}
	TYM0.5	0.43±0.03^{a}	0.89±0.03^{b}	1.01±0.03^{b}	1.19±0.03^{b}
	TYM1	0.4±0.01^{a}	0.78±0.01^{c}	0.93±0.03^{c}	1.02±0.01^{c}
	TCX0.5	0.38±0.01^{a}	0.79±0.01^{c}	0.9±0.01^{c}	0.99±0.01^{d}
	TCG0.5	0.41±0.02^{a}	0.74±0.02^{d}	0.89±0.02^{d}	0.98±0.02^{d}
	TCX1	0.42±0.01^{a}	0.69±0.01^{e}	0.83±0.02^{e}	0.94±0.01^{e}
	TCG1	0.37±0.03^{a}	0.66±0.03^{e}	0.8±0.02^{e}	0.88±0.03^{f}
	TCXG	0.34±0^{a}	0.68±0^{e}	0.79±0.01^{e}	0.84±0^{g}
TVN	CON	9.45±0.14^{a}	11.02±0.11^{a}	12.48±0.13^{a}	15.29±0.21^{a}
	TYM0.5	9.36±0.15^{a}	10.77±0.1^{b}	11.29±0.1^{b}	13.59±0.16^{b}
	TYM1	9.84±0.08^{a}	10.21±0.03^{c}	10.99±0.07^{c}	11.84±0.03^{c}
	TCX0.5	9.76±0.03^{a}	10.14±0.04^{c}	10.85±0.05^{c}	11.76±0.04^{d}
	TCG0.5	9.94±0.06^{a}	10.13±0.1^{c}	10.91±0.12^{c}	11.94±0.1^{c}
	TCX1	9.35±0.07^{a}	10.09±0.06^{c}	10.52±0.01^{e}	11.35±0.06^{f}
	TCG1	9.55±0.05^{a}	10.16±0.09^{c}	10.64±0.02^{d}	11.55±0.09^{e}
	TCXG	9.99±0.09^{a}	09.79±0.09^{d}	10.23±0.11^{f}	10.99±0.14^{g}

Note: Different letters indicate significant differences between treatments ($P<0.05$). CON: Beef burger without thymol; TYM0.5: Beef burger containing 0.5% thymol; TYM1%: Beef burger with 1% thymol; TCX0.5: Burger with 0.5% thymol coated with 1% xanthan gum; TCG0.5: Burger with 0.5% thymol coated with 1% guar gum; TCX1: Burger with 1% thymol coated with 1% xanthan gum; TCG1: Burger with 1% thymol coated with 1% guar gum; TCXG: Burger with 1% thymol coated with 1% xanthan and guar gum.

Table 6. The impact of thymol and thymol coated with xanthan and guar on the enumeration of *S. aureus* in hamburgers during storage

Bacteria	Treatment	Mean±SD			
		Storage (d)			
		1	7	14	21
S. aureus	CON	2.08±0.03^{a}	3.16±0.1^{a}	3.91±0.01^{a}	4.53±0.11^{a}
	TYM0.5	1.71±0.16^{b}	2.75±0.03^{b}	3.5±0.1^{b}	4.08±0.13^{b}
	TYM1	1.63±0.08^{b}	2.35±0.05^{d}	3.1±0.02^{d}	3.72±0.06^{c}
	TCX0.5	1.43±0.13^{b}	2.25±0.09^{d}	3±0.08^{d}	3.62±0.03^{d}
	TCG0.5	1.51±0.03^{b}	2.38±0.04^{d}	3.13±0.11^{d}	3.75±0.05^{c}
	TCX1	1.56±0.09^{b}	2.17±0.06^{e}	2.92±0.12^{d}	3.54±0.02^{e}
	TCG1	1.62±0.11^{b}	2.27±0.07^{d}	3.02±0.05^{d}	3.64±0.01^{d}
	TCXG	1.77±0.14^{b}	2.59±0^{c}	3.34±0.03^{c}	3.96±0.04^{b}

Note: Different letters indicate significant differences between treatments (P<0.05). CON: Beef burger without thymol; TYM0.5: Beef burger containing 0.5% thymol; TYM1%: Beef burger with 1% thymol; TCX0.5: Burger with 0.5% thymol coated with 1% xanthan gum; TCG0.5: Burger with 0.5% thymol coated with 1% guar gum; TCX1: Burger with 1% thymol coated with 1% xanthan gum; TCG1: Burger with 1% thymol coated with 1% guar gum; TCXG: Burger with 1% thymol coated with 1% xanthan and guar gum.

Different uppercase letters indicate significant differences between treatments on the same day of display (P<0.05). Different lowercase letters indicate significant differences within the same treatment across the display period (P<0.05).

4.5. Sensory evaluation

For all parameters evaluated in sensory acceptability (odor, color, texture, and overall acceptability), differences (P<0.05) were observed between treatments (Table 3). Higher odor and color scores (P<0.05) were found in the TCXG and TCG1 treatments, and lowest scores were observed in the CON treatment. The TCG1 and TCXG treatments had the highest texture and overall acceptability scores, while the control treatment had the lowest scores. From the beginning of the display (day 1) to day 21, there was a significant decrease in the scores for odor, color, texture, and overall acceptability.

Different lowercase letters indicate significant differences within the same treatment across the display period (P<0.05).

4.6. Color parameters

The results of the mean comparison of color characteristics of hamburger samples containing thymol and thymol coated with xanthan and guar showed that coating thymol with xanthan and guar increased the b*, a*, and l* indices compared to the control treatment. From the beginning of the display (day 1) to day 21, the a* and b* indices increased, while the l index decreased significantly (Table 4).

Different lowercase letters indicate significant differences within the same treatment across the display period (P<0.05).

4.7. Total volatile nitrogen (TVN), Lipid peroxidation (TBARS and PV)

The investigation of lipid peroxidation indices in fried hamburgers showed that the use of thymol and coated thymol caused a significant decrease in the TBARS value and peroxide number. The lowest values of peroxide number and TBARS index were observed in the TCXG treatment, and the highest values were found in the control treatment. The lipid peroxidation indices also increased significantly over time (Table 5). The investigation into TVN levels in fried hamburgers revealed that the use of thymol and coated thymol resulted in a notable reduction in TVN. The TCXG treatment demonstrated the lowest TVN levels, while the control treatment exhibited the highest. Furthermore, the TVN levels increased significantly over time (Table 5).

Different lowercase letters indicate significant differences within the same treatment across the display period (P<0.05).

4.8. Enumeration of *S. aureus*

The count of *S. aureus* in the hamburger samples increased significantly over time, particularly with prolonged storage. Furthermore, the use of thymol and thymol coated with xanthan and guar gums resulted in a significant reduction in the count of *S. aureus* compared to the control treatment. It is worth noting that the treatments with thymol coated with xanthan and guar at a concentration of 1%, thymol coated with xanthan at a concentration of 1%, and thymol coated with guar at 1% showed the most significant reduction in bacteria levels in the hamburger samples (Table 6).

Different lowercase letters indicate significant differences within the same treatment across the display period (P<0.05).

5. Discussion

There is a growing preference among consumers for the use of medicinal plant essential oils as natural additives to improve food safety. This shift is driven by concerns about the negative health impacts of chemical preservatives [27]. Essential oils are rich in beneficial natural compounds that contribute to human health. They serve as valuable additives in diverse industries such as food, pharmaceuticals, and cosmetics [5]. Despite their benefits, essential oils face limitations as food preservatives due to their susceptibility to oxiation, light, and high temperatures, as well as degradation during production and storage. The encapsulation of essential oils in suitable wall materials is of great importance for overcoming the aforementioned challenges. This process aims to enhance oxidative stability, offer controlled release, and extend the shelf life of the product [28]. It is thought that encapsulation may prove to be a successful technique for preserving the fragrance of essential oils by preventing degradation and evaporation.

The acceptance of food products by consumers is a key determinant of the future direction of the market and a significant influence on the application of new technologies in the food processing industry [29, 30]. Consumer behavior can be related to the personal and culinary experiences of consumers [31]. The aroma of thymol had a positive effect on the smell of the samples. However, this resulted in a negative effect on the taste. The concentration of thymol may act as a limiting factor in consumer acceptability, as evidenced by the high level of acceptability observed when thymol was added at a lower concentration and mixed with thymol essential oil. A comparable phenomenon was documented, identifying discrepancies in the sensory assessment of minced meat treated with Chinese cinnamon and cinnamon bark essential oils. They observed that the lowest acceptability ratings for odor and taste were associated with higher oil concentrations. Overall acceptability is often strongly correlated with other sensory attributes, such as taste and tenderness [32, 33].

As anticipated, the TBARS values increased in accordance with the autocatalytic nature of the lipid oxidation reaction. The rate of oxidation increased as the reaction proceeded. Despite the overall increase, the TBARS values on day 21 of storage were within the acceptable range for the oxidized hamburger samples and consistent with the findings of Campo et al. (2006) [28]. Other authors' similar outcomes have been documented when sage was incorporated into beef burgers during prolonged cold storage, as well as when chestnut extract was employed to extend beef shelf life. Additionally, basil (*Ocimum basilicum* L.) essential oil has been utilized in beef burgers, and copaiba essential oil has been applied to extend the shelf life of hamburgers. Sheep meat has also been observed in these studies.

The xanthan and guar-coated burgers exhibited greater tenderness than the control sample, with a subsequent decline in tenderness observed after 14 days. The coating also enhanced the sensory qualities of the product. The study suggests that thymol represents a natural alternative to synthetic preservatives, particularly in the form of xanthan and guar thymol coating, which enhances both sensory properties and shelf life at low concentrations, as evaluated by consumers.

TVNs are volatile nitrogen compounds that form when protein-rich food begins to spoil. Microbes in meat affect the production of TVNs. Table 5 shows the TVN results for different burgers stored over time. The amount of TVN in all samples increased over time. There was a significant difference between samples, caused by bacterial breakdown of amino acids, producing ammonia, ethylamines, and other volatile bases. This result is similar to those reported by Hematian et al. (2022) [34], who monitored the freshness of rainbow trout fillets in a gelatin film containing *Coleus scutellarioides*. They found that the TVN rate increased over 16 hours at 25 ˚C. Fewer TVNs were found in burgers with thymol, coated with xanthan and nanoencapsulated guar. This is similar to what was reported by Hosseini [35] in fish fillets. TVN

values decreased during storage. This is because thymol is a natural antioxidant in beef. This finding agrees with the observations of Jong et al. (2005) and follows the rules of Standard No. 2688 of 1987, which says that TVN values should not exceed 14 mg/100 g of meat [3].

The main harmful bacteria found in meat are: Salmonella spp., *S. aureus*, *Listeria monocytogenes*, *C. perfringens*, *Clostridium botulinum*, *E. coli* O157:H7, Pseudomonas, Acinetobacter, Enterobacter, *Lactobacillus* sp., and *Proteus* spp. [36].

Essential oils are a rich source of natural antioxidants, tannins and phenolic acids. The concentration of TBARS is used to indicate oxidation in meat products. Essential oils have many antibacterial properties, including geraniol, menthol, cinnamyl alcohol, linalool, citronellol, carvacrol, cinnamaldehyde, eugenol, thymol, estragole, and chavicol [37]. These compounds can kill bacteria and stop them from causing disease.

Thyme contains antimicrobial compounds that inhibit the growth of bacteria. Thyme oil is also an excellent antioxidant when added to minced meat. It reduces TVN and TBARS.

The present study demonstrated that the use of thymol, thymol coated with xanthan, thymol coated with guar, and thymol coated with xanthan and guar significantly reduced the enumeration of *S. aureus* in hamburger samples. The present study's findings agree with those of previous research [33, 37-40].

5. Conclusion

Adding and coating thymol oil increased antioxidant activity and reduced lipid oxidation compared to the control. These effects were seen for up to 21 days. Thymol also made meat look more attractive for a longer period. Hamburgers coated with a mixture of xanthan and guar gum-thymol had lower cooking loss than other treatments. This was especially noticeable in the final screening periods (days 14 and 21).

The results showed that the thymol coating with xanthan and guar had the biggest impact compared to the other samples. It inhibited bacterial from growing and improved the meat in other ways, excluding color. This study shows that thymol and its nanoencapsulated coating can kill bacteria and protect meat from spoilage. Further studies are needed to determine whether this approach is effective with other types of meat. It is also important to use packaging or other forms of coverage to store this product for long-term use.

Ethical Considerations

Compliance with ethical guidelines

The authors of this study declare that all steps were carried out in accordance with the principles of ethics.

Data availability

The data used to support the findings of this study is available from the corresponding author upon reasonable request.

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Authors' contributions

Samples collection and experiments: Parastoo Mesgaran Karimi; data analysis and writing: Mohammad Rabbani, Afshin Akhondzadeh Basti, Zahra BeigMohammadi.

Conflict of interest

The authors declared no conflict of interest

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