The effect of thymol microcapsules prepared with xanthan and guar gums on the count of *staphylococcus aureus*, physicochemical and sensory properties of hamburger during frying

Parastoo Mesgaran Karimi[1],Mohammad Rabbani[2*], Afshin Akhondzadeh Basti[3] *Zahra BeigMohammadi[4]

1- PhD Student, Department of Food Science and Technology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran

2- Assistant Professor, Department of Food Science and Technology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran

3- Professors in Food Hygiene, Dept. of Food Hygiene, Faculty of Veterinary Mediciene, University of Tehran

4- Assistant Professor, Department of Food Science and Technology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran

Abstract

The preservative effect of the addition of thymol coated with xanthan gum and guar on meat quality parameters and sensory acceptability of fried hamburgers during a 21-day exposure was investigated. Eight treatments were studied: CON(burger without thymol); TYM0.5% - burger with 0.5% thymol, TYM1% - 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% guam gum. TCX1- burger with 1% thymol coated with 1% xanthan gum; TCG1- burger with 1% thymol coated with 1% guar gum and TCXG- burger with 1% thymol coated with 1% xanthan and guar gums. The control treatment had the highest energy level while the 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 group treated with thymol was lower than the control. The carbohydrate content of the samples increased with TCXG compared to the control treatment. Odour, 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 odour, 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. 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 level of bacteria.

Key words: Thymol, Xanthan, Guar, Physicochemical, Antioxidant, Antimicrobial, Frying hamburger

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) compounds is significantly affected by a number of factors, including temperature, time, muscle-fat ratio and salt content, when frying hamburger [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 hamburger.

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 oil of various plants as a white crystalline substance of 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* O_{157}:H_7, and *Clostridium perfringens*, thereby ensuring the maintenance of microbial quality in sausage products[13]. Furthermore, thymol enhances the quality of meat by promoting 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 reduce penetration force[17]. The incorporation of a blend of guar and xanthan gum into a traditional 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 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 the texture, inhibit the proliferation of pathogens, and improve the stability against oxidation 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. Material and method

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 spices 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 gr, 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 gr 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 gr 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 peroxide value (PV) was conducted in accordance with the methodology established by AOAC (2000). Initially, the extraction of fat from the meat was performed using the

Soxhlet method. Subsequently, 5 gr 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 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 peroxide value was determined through equation (3) and expressed as Milliequivalent peroxide per kg of sample.

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

S: the volume (ml)
N: normality of sodium thiosulfate solution
W: sample weight (kg)

2.3.2. Thiobarbituric acid value (TBARS)

The estimation of Thiobarbituric acid (TBARS)-value (mg malonaldehyde (mal)/kg) was conducted through the distillation technique, employing 2-thiobarbituric acid 0.02 M (Sigma Chemical Co. Ltd USA) as described by FAO (1986). In order to assess the quantity of malonaldehyde and secondary oxidation compounds. In brief, a 10 gr 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 milligram malonaldehyde equivalents per kilogram of sample (mg MAD/kg).

2.3.3. Measurement of volatile nitrogen substances (TVN)

For this purpose, 10 grams of the sample were combined with 2 grams of magnesium oxide and 300 milliliters 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 vapours 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 vapours inside the Erlenmeyer flask reached 150 mL. Subsequently, the solution resulting from the accumulation of distillation vapours was titrated with 0.1 normal sulfuric acid until the color reached that of onion skin. The quantity of nitrogen material present in the sample was determined in mg [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 observed the surface and morphology of microcapsules. To prepare microcapsules, they were located on a specimen stub and were coated via gold by 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 (FTIR and XRD)

Fourier Transform Infrared Spectroscopy (FTIR) profiles of the resulting nanoparticles were determined using a Bruker spectrometer, VERTEX 70, Germany). X-Ray Diffraction (XRD) measurement: Crystal structure of the samples was determined using Panalytical X-Ray Diffraction Spectrometer (Xpert Pro MPD, Nederland).

2.4. Enumeration of *staphylococcus aureus*

In a sterile environment, 10 gr of the sample separated. Then it was placed in a special sterile plastic bag and 90 ml of sterile Ringer's solution was added to it. The bag was transferred to Stomacher (Bagmixer, Interscience, France) device and homogenized for 3 minutes. Subsequently, the sample was diluted to a dilution of 10^5 mL. 1 mL was added to the tube containing 9 mL of peptone water (Merck, Germany), resulting in the preparation of the subsequent dilutions. After preparing serial dilutions, 100

microliters of samples were cultured in Baird Parker Agar for enumeration viable cells of *staphylococcus aureus* and the plates incubated at 37°C for 48 h.

2.5. Sensory evaluation

Odour, color and overall acceptability of raw samples were evaluated by 8 trained panelists of the Food Hygiene and Control Department (with previous experience in burger processing and evaluation). The participants were asked about different qualitative characteristics. A sensory hedonic scheme ranging from 0 (very poor) to 8 (very good) was used, following the procedures of [3].

2.6. Statistical analyses

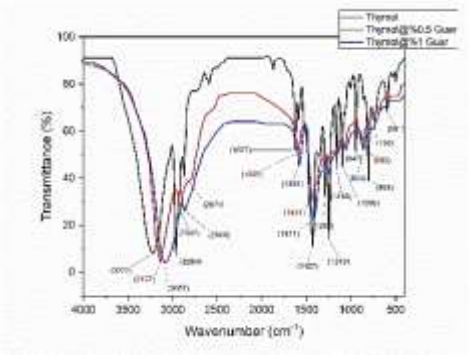
By performing various tests on samples, the data were recorded in Excel software. SPSS version 21 software was used for statistical analysis of the data, and P value less than 0.05 was considered statistically significant. Analysis of variance test was used to check the significant difference between treatment and control conditions. Duncan's statistical test was used to compare the difference between means at the 0.05 level. If the distribution of the data obtained from the research is normal, repeated measure ANOVA was used to investigate the changes in physicochemical, microbial and sensory characteristics in the treatments.

3. Results

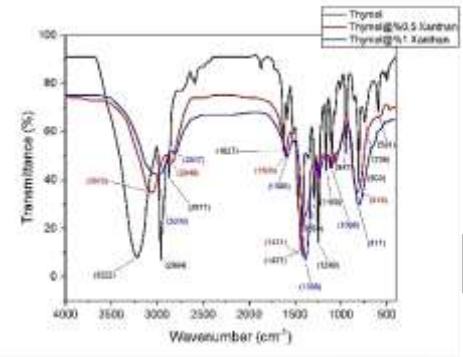
3.1. FTIR test analysis

The FT-IR test was used to check the chemical bonds in the samples. In FT-IR spectroscopy, the energies of the infrared rays coincide with the vibrational energies of molecules, and this matching causes the absorption of electromagnetic radiation energy by the sample. Therefore, by varying the frequency of the radiation in a specific range (infrared), a spectrum is obtained whose transmittance is reduced at some wavelengths, or in other words, it is absorbed by the molecules of the material. Therefore, by studying the absorption frequency of each spectrum, it is possible to understand the bonds in that material. Figure 1 shows the FT-IR 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.

A



B



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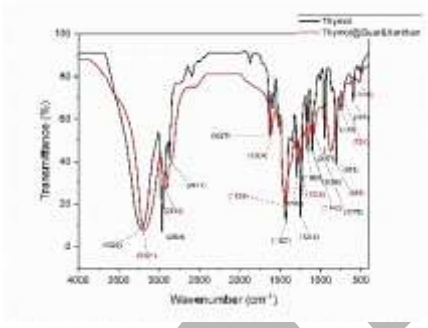


Figure 1. FT-IR spectra related to the samples of (a) thymol and thymol encapsulated in 0.5% and 1% guar, (b) thymol and thymol encapsulated in 0.5% and 1% xanthan and (c) thymol and thymol Encapsulated in guar and xanthan.

Table1. The position values of the peaks and functional groups and links related to each peak of the examined samples

Reference	Functional groups and links	Peak position (cm^{-1})	Sample
[1,2]	(stretching) -OH	3222	Thymol
[3]	(stretching) C-H	2964	
[3]	(stretching) C-H	2871	
[4]	(stretching) C=C/(bending) -OH	1627	
[5]	(bending) C-H	1427	
[5]	(bending) C-H	1289	
[6]	(stretching) C-OH	1249	
[6]	(stretching) OC-OH	1160	
[6]	(stretching) C-O-C	1098	
[7,8]	(dancer) C-H	947	
[7,8]	(cradle) C-H	804	
[7,8]	(transformation) C-H	738	
[7,8]	(off page) C-H	591	
[2,1]	(stretching) -OH	3137	Thymol@0.5%Guar

[3]	(stretching) C-H	2942	
[4]	(stretching) C=C/(bending) -OH	1595	
[5]	(bending) C-H	1431	
[5]	(bending) C-H	1270	
[7,8]	(transformation) C-H	882	
[1,2]	(stretching) -OH	3087	Thymol@1%Guar
[3]	(stretching) C-H	2860	
[4]	(stretching) C=C/(bending) -OH	1581	
[5]	(bending) C-H	1421	
[5]	(bending) C-H	1241	
[7,8]	(transformation) C-H	864	
[1,2]	(stretching) -OH	3070	%XanthanThymol@0.5
[2]	(stretching) C-H	2848	
[4]	(stretching) C=C/(bending) -OH	1605	
[5]	(bending) C-H	1431	
[5]	(bending) C-H	1253	
[6]	(stretching) C-O-C	1099	
[7,8]	(transformation) C-H	819	
[1,2]	(stretching) -OH	3000	%XanthanThymol@1
[3]	(stretching) C-H	2817	
[4]	(stretching) C=C/(bending) -OH	1589	
[5]	(bending) C-H	1389	
[5]	(bending) C-H	1238	
[6]	(stretching) C-O-C	1098	
[7,8]	(transformation) C-H	817	
[1,2]	(stretching) -OH	3191	XanthanThymol@Guar/
[3]	(stretching) C-H	2942	
[4]	(stretching) C=C/(bending) -OH	1604	
[5]	(bending) C-H	1436	
[5]	(bending) C-H	1253	
[6]	(stretching) OC-OH	1142	
[7,8]	cradle) C-H	880	
[7,8]	(transformation) C-H	724	
[7,8]	((off page) C-H	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 intensity of this peak has shifted to lower wave numbers in the guar, xanthan and guar/xanthan encapsulated samples than in 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 and then the sample of thymol encapsulated in 0.5% xanthan, which shows that the hydroxyl groups in xanthan are more than these groups in the structure of guar, which 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 wave number 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 wave numbers indicates an escalation 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 wave numbers. 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 the right. Furthermore, the disappearance or reduction in intensity of certain peaks subsequent to 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 wave numbers 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 more towards the wave numbers of It has become less, as mentioned, due to the formation of hydrogen bonds between the hydroxyl groups of these samples. The peak corresponding to the stretching vibration of the C=C bond in aromatic rings is also in the same range of wave numbers. Peaks related to bending vibrations of C-H bonds in methyl and methylene structures have also produced multiple peaks in the wave number in the range of 1280 cm-1 to 1450 cm-1.

On the other hand, the multiple peaks located in the wave number 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. Also, in the presence of encapsulating phases, it is shifted 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, gualacil and aromatic ring structures have also shown absorption peaks at wave numbers 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 a proof 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, X-ray diffraction of the sample is used to check the properties of the sample. XRD can be used to determine the general parameters of the crystal structure. The X-ray diffraction pattern of the samples is shown in Figure 2.

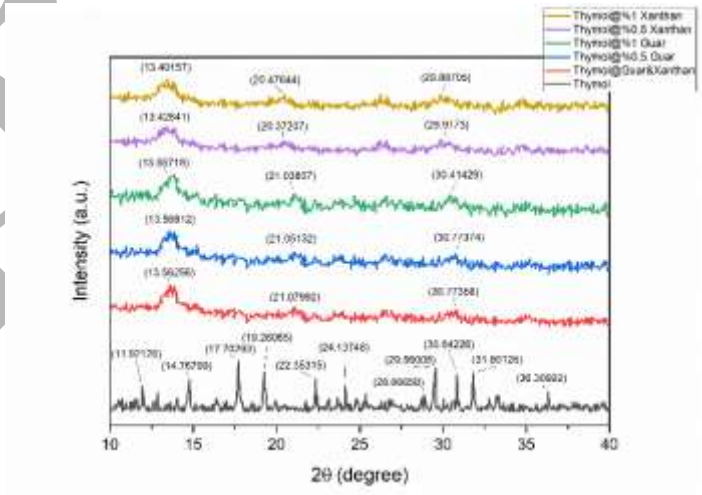


Figure 2. X-ray diffraction patterns of the samples

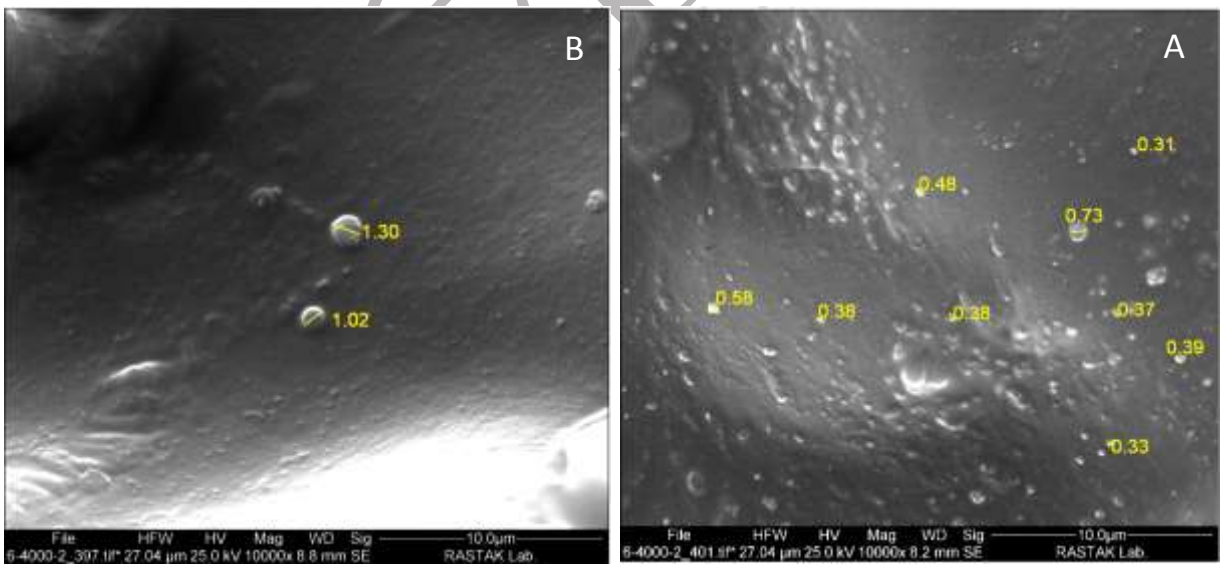
In the X-ray diffraction pattern of pure thymol, peaks located at 2θ values approximately around 11.9, 14.7, 17.7, 19.2, 22.3, 24.1, 28.8, 29.6, 30.8, 31.8, and 36.3 degrees are observable. This particular diffraction pattern for thymol has been previously documented by Zhou et al and Trivedi et al [10], illustrating the crystal structure of the aforementioned compound.
Scherer's relation (relation (1)) is used to determine the size of crystals in this material.

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

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 Angstroms), FWHM is the full width at half maximum and θ is the position of the peak.
Given the value of cos (θ) and FWHM, and the fixed values of λ (1.54 Angstroms) and k (0.9), the value of the crystal size is obtained from Scherer's relation, which, given that λ was in Angstroms, is also in Angstroms. The crystal size in nanometers is calculated by dividing the value obtained by 10. By calculating this parameter, the most intense peak (the peak at an angle of 17.7^0) is equal to 38.1 nm.
In the samples of thymol encapsulated with guar and xanthan and guar/xanthan there are no traces of the sharp peaks and crystalline structure of thymol and instead relatively broad peaks at angles of about 13.5, 21 and 30 degrees are observed, which is characteristic of the structure of crystalline (amorphous) encapsulating agents and 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 non-crystalline structure of the xanthan gum samples. Proves 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, an SEM test was performed and the resulting micrographs are shown in Figure 3.



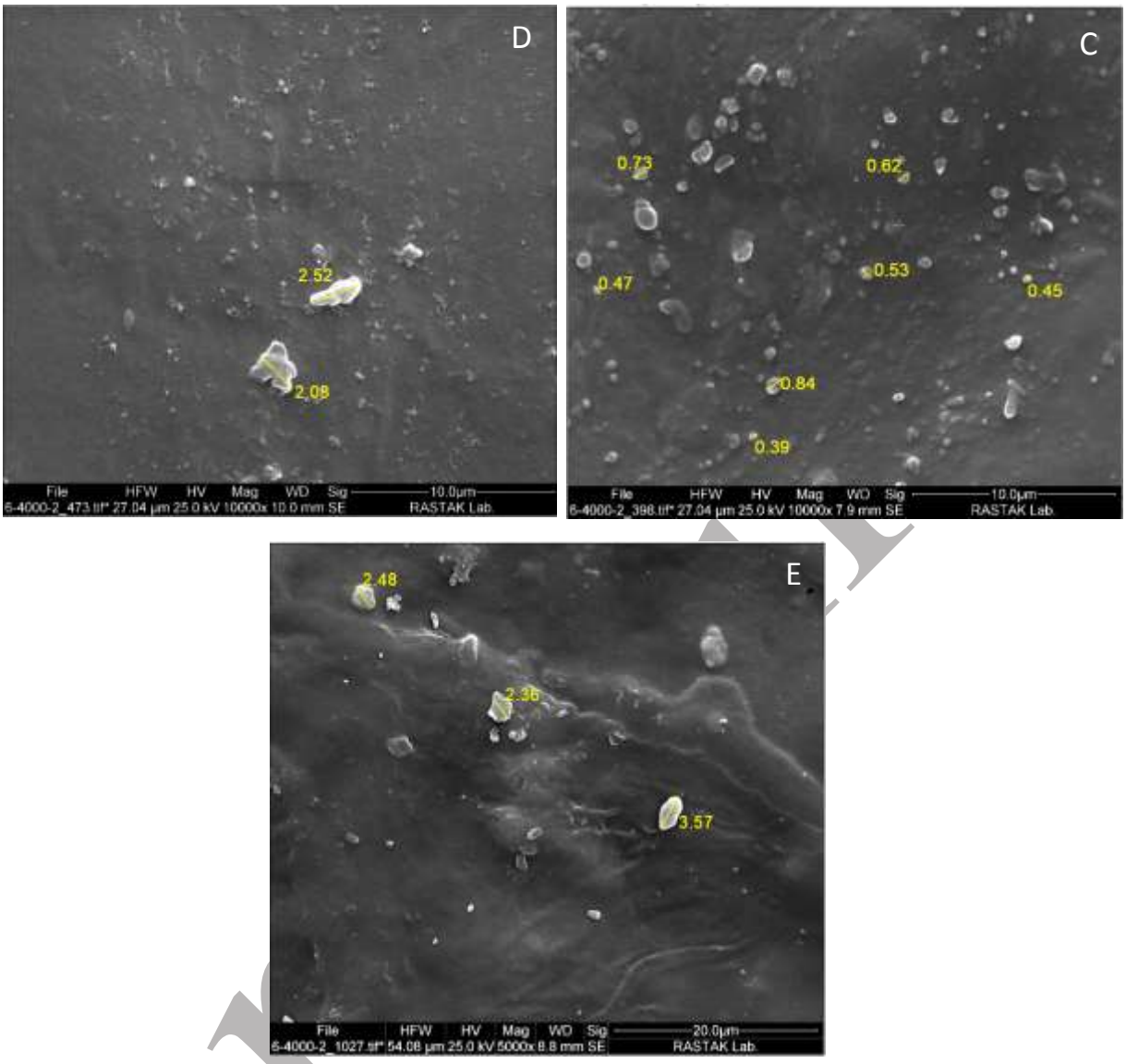


Figure 3. SEM micrographs of thymol samples encapsulated in (a) 0.5% guar, (b) 1% guar, (c) 0.5% xanthan, (d) 1% xanthan and (e) guar-xanthan mixture.

It is clear that in all the micrographs there are capsules on 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 has increased and their dispersion has decreased. According to Figure 1(a), the average size of the microcapsules prepared with 0.5% guar is about 0.44 µm, while according to Figure 1(b), this size is 1.2 µm for the microcapsules prepared with 1% guar. According to Figure 1 (c), the average size of the microcapsules prepared with 0.5% xanthan is about 0.57 µm, while according to Figure 1 (d) this size is 2.3 µm for the microcapsules prepared with 1% xanthan. It is therefore clear that increasing the percentages of guar and xanthan significantly increased 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 was larger than in the samples encapsulated with guar. In addition, in the sample encapsulated in the guar/xanthan mixture (Figure 1(c)), the microcapsules with an average particle size of 2.8 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 the 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 non-coated thymol and control. The TCX1, TCG1, and TCXG 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 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 the TCXG.

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

	ENERGY	MOISTURE	COOKING LOSS	FAT ATTRACTION
Treatment*				
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}	10.70±0.46^{a}	9.01±0.54^{b}
TYM1	549.30±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±0.60^{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}	9.30±0.08^{d}	6.75±0.19^{c}

Different letters indicate significant differences between treatments (P< 0.05). *CON–beef burger, without thymol; TYM0.5— beef burger containing 0.5 % of thymol, TYM1%- beef burger with 1% of thymol; TCX0.5- burger with 0.5% of thymol coated with 1% xanthan gum; TCG0.5 - burger with 0.5% of thymol coated with 1% guar gum, TCX1- burger with 1% of thymol coated with 1% xanthan gum; TCG1- burger with 1% of thymol coated with 1% guar gum and TCXG- burger with 1% of thymol coated with 1% xanthan and guar gum.

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 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 the TCXG. Furthermore, the carbohydrate percentage in the samples increased with TCXG in comparison to the control treatment. The treatments of TCX1, TCG1, and TCXG displayed the highest amounts of carbohydrates (Table 2).

Table 2. Chemical composition of hamburgers treated with thymol or thymol coated with xanthan and guar gums (Mean±sd).

	ASH	PROTEIN	FAT	CARBOHYDRATE
Treatment				
CON	2.65±0.08^{b}	54.83±0.20^{a}	32.33±0.14^{a}	10.19±0.07^{d}
TYM0.5	2.80±0.02^{a}	54.37±0.20^{b}	32.28±0.20^{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±0.20^{b}	32.21±0.07^{a}	11.03±0.14^{a}
TCX1	3.09±0.15^{a}	53.55±0.05^{c}	32.10±0.07^{a}	11.27±0.28^{a}
TCG1	3.09±0.14^{a}	53.55±0.00^{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}

Different uppercase letters indicate significant differences between treatments in the same day of display (P< 0.05). Different lowercase letters indicate significant differences in the same treatment along display (P< 0.05).

4.5. Sensory evaluation

For all parameters evaluated in sensory acceptability (odor, color, texture, and overall acceptability), differences (P) were observed between treatments (Table 3). The odor and color obtained a higher score (P) were found in the TCXG and TCG1 treatment and lowest in the CON treatment. The TCG1 and TCXG treatments had the highest texture and general 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 odour, color, texture and overall acceptability.

Table 3. Sensory evaluation of hamburgers treated with thymol or thymol coated with xanthan and guar gums (Mean±sd).

Treatment	ODOR	COLOR	TEXTURE	ACCEPTABILITY
CON	2.50±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}	3.50±0.05^{b}	3.42±0.07^{b}	3.50±0.05^{c}
TCG0.5	3.67±0.08^{b}	2.58±0.01^{e}	3.50±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	4.00±0.06^{a}	3.92±0.10^{a}	3.75±0.06^{a}	4.08±0.09^{a}

Different lowercase letters indicate significant differences in the same treatment along display (P< 0.05).

4.6. Color parameters

The results of 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 indices a* and b* increased and the l index decreased significantly (Table 4).

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

Treatment	l*	a*	b*
CON	54.75±0.07^{d}	15.84±0.05^{e}	19.59±0.08^{c}
TYM0.5	54.90±0.10^{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}	16.60±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}	21.30±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}

Different lowercase letters indicate significant differences in the same treatment along display (P< 0.05).

4.7. Total volatile nitrogen (TVN), Lipid oxidation (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 TBARS index and peroxide number. The lowest values of peroxide number and TBARS index were observed in the TCXG treatment and the highest values in the control treatment. The lipid peroxidation indices also increased significantly over time (Table 5). The investigation into total volatile nitrogen (TVN) indices 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 index demonstrated a significant increase over time (Table 5).

Table 5. The impact of thymol and thymol-coated with xanthan and guar on the TVN, PV and TBARS of hamburgers during storage (Mean±sd).

TBARS	**Storage (day)**			
	1	**7**	**14**	**21**
CON	0.14±0.01^{a}	0.21±0.02^{a}	0.29±0.00^{a}	0.35±0.02^{a}
TYM0.5	0.12±0.02^{a}	0.16±0.03^{a}	0.24±0.02^{b}	0.30±0.01^{b}
TYM1	0.09±0.03^{a}	0.14±0.00^{a}	0.23±0.02^{b}	0.27±0.01^{c}
TCX0.5	0.10±0.04^{a}	0.14±0.02^{a}	0.21±0.01^{b}	0.27±0.00^{c}
TCG0.5	0.11±0.02^{c}	0.15±0.04^{a}	0.22±0.01^{c}	0.28±0.00^{c}
TCX1	0.10±0.01^{a}	0.12±0.03^{a}	0.18±0.00^{b}	0.25±0.00^{d}
TCG1	0.11±0.03^{a}	0.15±0.02^{a}	0.23±0.03^{b}	0.26±0.00^{c}
TCXG	0.08±0.01^{a}	0.11±0.03^{a}	0.16±0.02^{c}	0.24±0.01^{d}
Peroxide -V				
CON	0.41±0.08^{a}	1.03±0.08^{a}	1.20±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	0.40±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}	0.90±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}	0.80±0.02^{e}	0.88±0.03^{f}
TCXG	0.34±0.00^{a}	0.68±0.00^{e}	0.79±0.01^{e}	0.84±0.00^{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±0.10^{b}	11.29±0.10^{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±0.10^{c}	10.91±0.12^{c}	11.94±0.10^{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}

Different lowercase letters indicate significant differences in the same treatment along display (P< 0.05).

4.8. Enumeration of *staphylococcus aureus*

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

Table 6. The impact of thymol and thymol-coated with xanthan and guar on the Enumeration of *Staphylococcus aureus* in hamburgers during storage (Mean±sd).

S. aureus	**Storage (day)**			
	1	**7**	**14**	**21**
CON	2.08±0.03^{a}	3.16±0.10^{a}	3.91±0.01^{a}	4.53±0.11^{a}
TYM0.5	1.71±0.16^{b}	2.75±0.03^{b}	3.50±0.10^{b}	4.08±0.13^{b}
TYM1	1.63±0.08^{b}	2.35±0.05^{d}	3.10±0.02^{d}	3.72±0.06^{c}
TCX0.5	1.43±0.13^{b}	2.25±0.09^{d}	3.00±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±0.00^{c}	3.34±0.03^{c}	3.96±0.04^{b}

Different lowercase letters indicate significant differences in the same treatment along display (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 [23]. 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 oxygen, 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 in order to overcome the aforementioned challenges. This process has the objective of enhancing oxidative stability, offering controlled release and extending the shelf life of the product[24]. 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[25, 26]. Consumer behaviour can be related to the personal and culinary experiences of consumers[27]. The odour 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; who identified 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[28].

As anticipated, the TBARS values increased in accordance with the autocatalytic nature of the lipid oxidation reaction. The rate of oxidation increases as the reaction proceeds. Despite the overall increase, the TBARS values on day 21 of screening were within the acceptable range for the oxidised hamburger sample and consistent with the findings of Campo et al. (2006) [24]. Other author's similar outcomes have been documented when sage has been incorporated into beef burgers during prolonged cold storage, as well as when chestnut extract has been 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 have 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 language is formal, neutral, and grammatically correct, with technical terms explained. Passive voice and impersonal construction are used, with a consistent citation style. 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 consumer

TVNs are amino acids that form when food with a lot of protein goes bad. 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, which is caused by bacteria breaking down amino acids, creating ammonia, ethylamines, and other volatile bases. This result is similar to those reported [30]), 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 [35] found in fish fillets. TVN values fell 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*., *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Clostridium botulinum*, *Escherichia coli* O_{157}:H_7, Pseudomonas, Acinetobacter, Enterobacter, Lactobacillus sp. and Proteus spp. [32].

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, and eugenol. Thymol, estragole, caran and chavicol[31].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 *Staphylococcus aureus* in hamburger samples. The present study's findings agree with those of previous research[29, 33, 34,36].

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 longer. Hamburgers coated with a mixture of xanthan and guar thymol had less 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 stopped bacteria from growing and made the meat better in other ways, apart from the color. This study shows that thymol and its nanoencapsulated coating can kill bacteria and protect meat from going bad. Further studies are needed to see if this works with other types of meat. It is also important to use packaging or other forms of coverage to store this product long-term.

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Ethics

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

Conflicts of Interest

The authors declare that they have no conflicts of interest

Data Availability

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

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