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Effect of *Althaea rosea* flower gum loaded with *Thymbra spicata* (Zahter) essential oil coating on shelf life and quality of beef patties (Koefte) during cold storage

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Abstract. *The aim of this research is to determine the effect of Althaea rosea flower gum loaded with Thymbra spicata essential oils coating on packaged beef patties during cold storage. For this purpose, samples were evaluated in terms of pH, color, thiobarbituric acid reactive substances (TBARS), and microbiological properties. In addition, texture profile analysis (TPA) was performed to evaluate the textural properties of the beef patties. The essential oil treatment to the beef patties had a significant effect (p<0.05) on the pH values at the end of storage. The coating significantly affected the L* (lightness), a* (redness) and b* (yellowness) values (p<0.05). A similar situation was also found for lipid oxidation (1.00 µmol MDA (g). The coated samples with essential oil-treated had the lowest values of total aerobic bacteria (3.29 log CFU/g), yeast and mold (2.99 log CFU/g), lactic acid bacteria (2.23 log CFU/g), and total psychrophilic bacteria (2.58 log CFU/g). While the effect of the coating on the adhesiveness, gumminess, and chewiness values of the beef patties at the end of storage was significant (p<0.05), it did not affect other textural properties. Current research has shown that Althaea rosea flower gum can be used in edible coatings and, when fortified with Thymbra spicata essential oil, can be used in muscle foods for preservation and shelf-life extension.*

Keywords: *edible coating, beef patties, Thymbra spicata, Althaea rosea, shelf life*

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ФИЗИКО-ХИМИЧЕСКАЯ БИОЛОГИЯ

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Влияние покрытия из цветочной камеди *Althaea rosea*, обогащенной эфирным маслом *Thymbra spicata* (Zahter), на срок годности и качество говяжьих котлет при хранении в охлажденном виде

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Аннотация. *Целью данного исследования является определение влияния покрытия из цветочной камеди Althaea rosea, обогащенной эфирным маслом Thymbra spicata, на котлеты из говядины во время хранения в охлажденном виде. Для этого образцы оценивали по pH, цветности, содержанию веществ, реагирующих с тиобарбитуровой кислотой (TBARS), и по микробиологическим показателям. Кроме того, для оценки текстурных свойств котлет из говядины был проведен анализ профиля текстуры (TPA). Обработка говяжьих котлет эфирным маслом оказала большое влияние (p<0,05) на значения pH в конце хранения. Покрытие в большей степени воздействовало на значения L* (яркость), a* (покраснение) и b* (желтизна) (p<0,05). Аналогичная картина наблюдалась при исследовании окисления липидов (1,00 мкмоль МДА (г). Образцы с покрытием, обогащенным эфирным маслом, имели самые низкие значения общего количества аэробных бактерий (3,29 log КОЕ/г), дрожжей и плесени (2,99 log КОЕ/г), молочнокислых бактерий (2,23 log КОЕ/г) и общего количества психрофильных бактерий (2,58 log КОЕ/г). Хотя влияние покрытия на показатели клейкости, липкости и жевательности говяжьих котлет в конце* *хранения было значительным (p<0,05), оно не воздействовало на другие текстурные свойства. Текущие исследования показали, что цветочную камедь Алтея розового можно использовать в съедобных покрытиях, а при обогащении эфирным маслом Thymbra spicata ее можно применять в мясных продуктах для сохранения и продления срока годности.*

Ключевые слова: *съедобное покрытие, котлеты из говядины, Thymbra spicata, Althaea rosea, срок годности*

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INTRODUCTION

Meat and meat products have an important place in terms of human nutrition due to their nutritional value. In particular, the fact that it contains high protein, vitamins and minerals, as well as being rich and sufficient in essential amino acids, reflects the vital importance of this food product [1]. On the other hand, although the meat obtained from healthy animals under normal conditions is relatively sterile, its microbial load may increase due to animal residues/waste, contaminated tools/equipment, air, water and contamination from infected personnel during the production process. These microorganisms, which can be transmitted at various stages of the meat processing, may include pathogenic bacteria responsible for foodborne illness and spoilage bacteria that cause meat rot [2]. In addition to the negative bacterial effect, lipid oxidation that may occur due to the sensitivity of meat fat to oxidative changes is an important chemical reaction that reduces the nutritional value of meat and meat products. Natural or synthetic antioxidants can be used to prevent and/or minimize these effects [3].

One of the most important ways for meat and meat products to reach consumers safely is packaging. On the other hand, the amount of plastic used for food packaging on a global scale today is estimated to exceed one million tons per year, with an alarming annual growth. As a result, the disposal of plastic materials used in packaging into the environment has been seen as a serious waste problem and the concerns are increasing exponentially every year. Although there is no complete solution to this problem, the use of bio-based polymer packaging materials can be seen as the best alternative in the current circumstances [4]. In the last few years, researchers have demonstrated a wide variety of studies that the use of films and coatings made from biopolymer materials can be effective in extending the shelf life of foods. In this context, biopolymer materials could be synthesized from various organic materials such as carbohydrates, lipids and proteins [5, 6]. Polysaccharides, which are among these substances, are accepted and widely used for such applications due to their good film forming abilities. Being a rich source of polysaccharide materials and known as Hatmi in Turkey, *Althaea rosea* flowers (ARFs) are used in traditional medicine as a sedative, diuretic, antitussive and anti-inflammatory [7]. In particular, the gum content, which forms the polysaccharide structure of ARF, consists of rhamnose (Rha) and galactose (Gal), as well as uronic acids such as glucuronic acid (GlcA) and galacturonic acid (GalA) [8]. Moreover, studies have suggested that ARFs have immune-stimulatory [9], antimicrobial and antioxidant [10] effects. Yekta et al. [11] have obtained the gum (ARG) required for biofilm preparation by using the ARFs. They suggested the optimum condition of 1.5% ARG + 50% glycerol for biofilm preparation.

In the production of edible films and coatings, some natural antimicrobial and antioxidant compounds such as organic acids, nisin, plant extracts and essential oils can be used as additives instead of synthetic preservatives. In particular, the increasing interest of consumers in natural materials leads them to prefer fortified functional foods [12]. Toxic and carcinogenic effects that may be caused by synthetic preservatives overshadow the expected antimicrobial and antioxidant effects. In this context, essential oils (EOs) obtained from various aromatic and medicinal plants stand out as one of the most promising alternatives due to their strong antimicrobial and antioxidant effects [3]. One of these plants, *Thymbra spicata* (Lamiaceae) (*T. spicata*), also known as Zahter, is a plant with economic value used as a spice in foods and traditional medicine, widely found in Southeastern Anatolia and Mediterranean regions of Turkey [13]. In studies, antibacterial, antifungal, antimycobacterial and antioxidant activities of *T. spicata* essential oils (TSEO) containing carvacrol, γ terpinene and p-cymen components have been reported [14–19].

A new research topic that has not yet been explored is the addition of TSEO to the ARF gum-based coating material and the evaluation of related applications in the packaging of real foods (beef patties (koefte). Therefore, the present study was aimed to investigate the effects of ARG loaded with TSEO coatings on the physicochemical, microbiological and textural properties of beef patties stored at 4 °C for 6 days.

MATERIALS AND METHODS

Materials. Minced beef meat (25% fat) and NaCl used for beef patties mix were provided from local markets. The *Althaea rosea* flowers for extraction of gum and *Thymbra spicata* (Zahter) were collected from the provinces of Erzurum and Hatay, Turkey, respectively. Glycerol and Tween 80 were purchased by Merck (Darmstadt, Germany).

Gum extraction from Althaea rosea. ARG extraction was performed according to the method reported by Yekta et al. [11]. Briefly, the flowers purchased as dried were separated into smaller pieces with the help of a blender. Then, 100 g of flower powder was added to 1 L of ethanol and mixed for 1 h in a magnetic stirrer (Wisd MSH 20-D, Witeg, Germany). The mixture was incubated overnight at room temperature (25 °C). The mixture was then centrifuged at 8000 *g* for 10 min (Universal 320, Hettich, Germany) and the resulting pellets were treated with acetone. The resulting dry mass was then mixed with 2 L of distilled water and stirred for 2 h at 65 °C to remove the gum. Ethanol (99 %) was added to precipitate the polysaccharides and the mixture was kept at 4 °C overnight. After incubation, the precipitate was filtered through ordinary filter paper and then lyophilized in a freeze dryer (Free Zone, LabConco, USA).

Isolation and gas chromatography/mass spectrometry (GC/MS) analysis of the essential oil from T. spicata (Zahter). Plant material (50 g of *T. spicata*) was combined with 500 mL of water (plant material: water ratio of 1:10 w/v). Essential oil was obtained by conventional hydrodistillation using a Clevenger type apparatus for 3 h. The essential oils were dried by adding anhydrous sodium sulfate and stored in the dark at 4 °C until used in subsequent experiments. TSEO were qualitatively analyzed using a Shimadzu GCMS QP2010 instrument. The conditions were established as Gedikoğlu et al. [15]: the injector and detector temperatures were set at 250 °C. Temperature programming of the oven included an initial hold at 40 °C for 2 min and a rise to 240 °C at 4 °C/min and held for 53 min. Helium was the carrier gas, with a linear velocity of 43.4 cm/s. The samples were diluted with n-hexane (1:10, v/v), and a volume of 1.0 µl was injected into the GC with the injector in the split mode (split ratio: 1:25). The ionization voltage applied was 70 eV (electron volt), with a mass range m/z (mass number/ charge number) of 40–400 amu (atomic mass unit). Identification of volatile components was accomplished by comparison of using the total-ion chromatogram with the commercial libraries (NIST27 and WILEY7) of the GC–MS system. Peak area integration was used for the determination of percentage of combination.

Preparation of Beef patties (Koefte). Beef patties dough was obtained by adding 2 % NaCl to minced beef containing 25 % fat. After kneading by hand for 5 min, the dough was cut into 25 g pieces, rolled, then shaped and divided into 3 groups.

Preparation of Coating material. The coating material was prepared in a modified form of the method reported by Yekta et al. [11]. In summary, 3 g of ARG was added to 90 mL of distilled water and mixed at 65 °C until completely dissolved. Then glycerol (0.5 g/g ARG) was added as a plasticizer, and solutions were again warmed and stirred at 65 °C for 15 min. Tween 80 (Merck Chemicals Co., Darmstadt, Germany) at a final concentration of 0.4 % (w/v) was added to the solution as an emulsifier to help distribute and completely incorporate the TSEO in film-forming solutions. After 45 min of stirring, TSEO (1.5 % w/v) (determined as the best essential oil concentration for textural and sensorial properties of beef patties in preliminary studies) was added to ARG solution and homogenized at room temperature for 4 min at 13,000 rpm using an Ultra-Turrax homogenizer (Model T-25 Basic; IKA, Staufen, Germany).

Coating. Beef patties were immersed in coating solutions for 5 min and beef patties were removed from the solution and drained for 2 min. Then coated beef patties were dried in incubator at 30 °C for 1 h. Coating process was carried out in a sterile cabinet. The groups of beef patties were designed as no treatment control (C), ARG loaded with TSEO coating (ARG-TSEO) and ARG coating group (ARG). Prepared beef patties were placed on foam plates coated with stretch film and stored in the refrigerator at 4 °C for 6 days. pH, TBA and color values and microbiological characteristics of beef patties samples were determined during storage. Textural properties of beef patties samples were determined at the end of storage period (6th day).

Physiochemical analysis. The pH values of beef patties samples were measured with a pH 315i (WTW Xylem GmbH, Weilheim, Germany) after homogenization of 10 g sample with 100 ml deionized water for 1 min [20].

Lipid oxidation analysis. The determination of the oxidative rancidity of the samples was determined by 2-thiobarbiutric acid reactive substances (TBARS) analysis. For this purpose, 12 mL of trichloroacetic acid solution was added to 2 g sample and homogenized. The homogenate was filtered through a Whatman 1 filter followed by 3 mL of filtrate added to 3 mL of 0.02 M 2-thiobarbituric acid (TBA) solution. After the mixture was kept in a boiling water bath for 40 min, it was cooled for 5 min in cold water and then centrifuged at 2000 *g* for 5 min. After centrifugation, the supernatant was measured against blank in a UV–VIS spectrophotometer (DU730, Beckman Coulter Inc., USA) at 530 nm absorbance. The results of TBARS content were expressed as milligrams of malondialdehyde equivalents (MDA) per kilogram of beef patties sample [21].

Microbiological analysis. Ten g of beef patties sample were taken aseptically and suspended in 90 mL sterile physiological saline solution (PSS) (0.85 % w/v) in sterile filter bag. Then the mixture homogenized in a stomacher (IUL Instruments, Barcelona, Spain) for 2 min [22]. A series of 10-fold dilutions for each sample were formed in sterile PSS, and suitable dilution of each sample was spread onto following appropriate selective medium; Tryptone Glucose Yeast Agar (Hi-Media Laboratories, Mumbai, India) for total viable and psychrophilic bacteria, incubated at 30 °C for 48 h and 7 °C for 10 days, respectively; Rose Bengal Chloramphenicol Agar (Hi-Media, India) for yeast and molds, incubated at 25 °C for 72 h, de Man Rogosa Sharpe Agar (Chemsolute, Germany) for Lactic acid bacteria (LAB), anaerobically incubated at 30 °C for 48 h. Colony were counted into log colony forming units per g of sample (log CFU/g).

Color analysis. The colorimetric parameters, including the L*, a*, and b* values, of the beef patties were measured using a colorimeter (CHN Spec, China). Five beef patties were used per treatment and per replicate.

Texture analysis. Texture profile analysis (TPA) was performed according to the procedure of Bourne [23] using a Texture Analyser (TA-XT Plus model, Stable Micro Systems, Godalming, Surrey, UK) with a trigger force of 5 g. The TPA tests were carried out using a cylindrical probe (SMS P/25; 25 mm diameter compression platen). The probe was 10 mm above the beef patties, and the test speed was 5 mm/sec. The beef patties were compressed twice at 50 % by probe. Hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience of the samples were determined by the Exponent Software.

Statistical analysis. Each experimental group composed of three biological replicates, and each biological replicate included at least two technical replicates to assess experimental accuracy and precision. Statistical analysis of the data obtained in the study was done using SPSS (SPSS Inc., Chicago, IL, USA) program. A one-way analysis of variance (ANOVA) with post hoc Tukey studentized range honestly significant difference (HSD) test (Dunnett T3 test for data not following normal distribution).

RESULTS AND DISCUSSION

Essential oil composition. A total of 40 compounds was identified from the GC/MS analysis and their peak area (%) and relative retention indices are shown in Tab. 1. Carvacrol (35.2 %), γ-terpinene (16.30 %), p-cymene (16.29 %), thymol (5.86 %), α-terpinene (5.02 %), and myrcene (4.07 %) were the main components of the essential oil of *T. spicata*. There are differences between the components detected in some studies where TSEO content was investigated and those detected in the current study. In particular, the number of components detected in the current study was relatively higher than in previous studies [17, 19, 24]. It was thought that this situation might have been caused by the extraction method of essential oil, genotype, soil conditions, humidity, temperature and season, harvest time, geographical region and intrinsic (cg/column, condition etc.) factors [25].

Physical parameters. The pH changes in the beef patties samples during the storage period are given in Tab. 2. As seen in the table, pH increased in all samples at the end of the storage period (p<0.05). At the end of the storage period, the pH of the beef patties was between 5.26 and 5.50. The increase observed in the control group beef patties was relatively higher than the coated beef patties. This increase observed in beef patties groups may be due to the accumulation of metabolites and the deamination of proteins due to the activities of bacteria in the meat [12]. On the other hand, it can be said that the relatively lower pH increase observed in the coated beef patties is due to the fact that the essential oils added to the edible coatings affect the gas permeability by decreasing or increasing the CO_2 solubility [26]. As a matter of fact, a similar result was reported by Şen and Kılıç [12] in cooked beef patties with edible coating containing acai powder and matcha extracts. Again, it was reported that the ones coated using rosemary extract remained at the pH level of 5.48 compared to the pH of 6.66 measured in uncoated chicken beef patties [27].

Significant visual changes in meat and meat products can be affected by many factors and ultimately affect consumers' perception of quality and freshness [28]. The changes in L*, a* and b* occurring in the beef patties samples during the storage period are given in Tab. 2. While L* value decreased until the 4th day of storage in all groups, it increased on the 6th day (p<0.05). On the other hand, at the end of the storage period, the L* value was found to be lower in the coated beef patties samples compared to the control group. This result was consistent with the study investigating the effect of soy-based edible film combined with thyme essential oils in beef patties [29]. Akcan et al. [30] reported that cooked beef patties treated with whey protein edible film combined with *Laurus nobilis* L. and Salvia officinalis had higher L* value at the end of the storage period. Redness, measured by the a* value of meat, is one of the most important components in terms of the most important color. Looking at the results in general, this value remained above 10 during the ripening period. On the other hand, a significant

Table 1. Composition determined by GC-MS analysis of the essential oils from *T. spicata* in this study
Таблица 1. Состав эфирных масел *T. spicata*, определенный с помощью анализа ГХ-МС в данном исследовании

Component	Area (%)	RI*
Methyl isovalerate	0.10	773
2,5-diethyltetrahydrofuran	0.02	896
3-methylapopinene	0.02	927
α-thujone	2.30	927
α-pinene	1.96	933
Vinyl amyl carbinol	1.36	978
2,2-Dimethyl-5-methylene norbornane	0.32	954
Sabinene	0.04	972
Beta-pinene	0.52	978
5-Methyl-3-heptanone	0.04	938
Myrcene	4.07	991
3-Octanol	0.60	999
α-phellandrene	0.78	1007
3-Carene	0.37	1009
α-terpinene	5.02	1018
p-cymene	16.29	1025
Beta-phellandrene	1.74	1031
(E)-β-ocimene	0.17	1046
γ-terpinene	16.3	1058
Trans-4-thujanol	0.19	1069
Terpinolene	0.67	1086
Linalool	0.51	1101
cis-4-(Isopropyl)-1-methylcyclohex-2-en-1-ol	0.11	1124
Borneol	0.67	1173
4-Carvomenthenol	2.47	1184
p-cymen-8-ol	0.16	1189
α-terpineol	0.50	1195
cis-Dihydrocarvone	0.27	1198
d-Dihydrocarvone	0.14	1204
cis-3-Hexenyl pentanoate	0.06	1282
Thymol methyl ether	0.11	1239
Carvone	0.09	1246
Thymol	5.86	1293
Carvacrol	35.2	1300
Caryophyllene	3.79	1449
9-epi-(E)-Caryophyllene	0.20	1464
α-humulene	0.19	1454
γ-gurjunene	0.11	1476
Caryophyllene oxide	0.38	1587
Total	100%	

Note. *RI – Retention index.

Table 2. pH and color changes of control and coated beef patties during the storage period (6 day)
Таблица 2. Изменения pH и цвета контрольных и покрытых оболочкой говяжьих котлет в течение периода хранения (6 дней)

Parameter	Group	Day 0	Day 2	Day 4	Day 6
pH	C	5.34±0.02^{Aa}	5.09±0.02^{b}	5.42±0.05^{Aac}	5.50±0.03^{Ac}
	ARG	5.11±0.03^{Ba}	5.11±0.05^{a}	5.32±0.02^{Bb}	5.34±0.03^{Bb}
	ARG-TSEO	5.21±0.03^{Cab}	5.17±0.03^{a}	5.25±0.02^{Bb}	5.26±0.02^{Cb}
L*	C	47.47±0.42^{Aa}	44.63±1.70^{Ab}	37.33±0.83^{ABc}	43.52±0.52^{Ab}
	ARG	44.83±0.15^{Ba}	42.74±0.44^{ABab}	39.99±1.31^{Ac}	40.66±1.13^{ABbc}
	ARG-TSEO	43.20±0.20^{Ca}	41.69±0.72^{Bab}	35.35±1.40^{Bb}	38.92±1.57^{Bc}
a*	C	19.13±0.11^{Aa}	20.40±0.38^{ab}	18.71±0.52^{Aa}	21.48±1.17^{Ab}
	ARG	19.69±0.13^{Ba}	21.65±1.25^{b}	17.27±0.13^{Bc}	13.93±0.41^{Bd}
	ARG-TSEO	19.41±0.22^{ABa}	21.67±1.59^{b}	16.73±0.16^{Bc}	13.53±0.29^{Bd}
b*	C	10.11±0.11	9.59±0.30^{A}	9.62±0.32	10.04±0.12^{A}
	ARG	10.12±0.09^{a}	9.54±0.17^{Ab}	9.59±0.13^{b}	9.17±0.10^{Bc}
	ARG-TSEO	10.12±0.13^{ab}	10.63±0.29^{Ba}	9.55±0.31^{b}	8.47±0.30^{Cc}

Note. *Values are given as mean ± standard deviation. N = 3 biological replicates. Different uppercase letters in the same column indicate significant difference during storage while different lowercase letters in the same row indicate significant difference among the samples separately for each parameter ($p<0.05$). C: Beef patties without coating, ARG: Beef patties with *A. rosea* gum coating, ARG-TSEO: Beef patties with *A. rosea* gum coating loaded with *T. spicata* essential oil.

overall decrease in the a* value of the patties treated with TSEO-containing films was observed during the storage period ($p< 0.05$). On the last day of storage, beef patties coated with and without TSEO had lower a* values than the control group. This change observed at the end of the storage period has also been reported in edible coated beef patties and meats in different studies [30, 31, 29]. The yellowness (b* value) affected by properties such as pH, water activity and oxidation were generally decreased in all groups ($p<0.05$). On the other hand, this decrease was more evident in the coated beef patties samples compared to the control group. Kodal Coşkun et al. [29] reported lower b* values in beef patties coated with soy-based edible film combined with thyme essential oils after 12 days of storage compared to the control group.

Lipid oxidation. Lipid oxidation, which is considered as one of the main factors limiting the shelf life of muscle foods, is one of the important quality criteria [27]. In the current study, the lipid oxidation level was measured in terms of thiobarbituric acid (TBA) and is presented in Tab. 3. As seen in the table, TBA values were generally significantly lower in all groups during storage ($p<0.05$). At the end of storage, the lowest TBA value of 1.00 was observed in ARG-coated beef patties loaded with TSEO. Although this difference was statistically significant, it was found to be lower in ARG coated beef patties without TSEO when compared to the control group ($p<0.05$). This indicates that ARG films delay oxidation by forming a possible oxygen barrier mechanism. On the other hand, active ingredients such as carvacrol and thymol, which both ARG and TSEO contain, highlight the potential role of edible coating as antioxidative [29]. Similar findings have been reported in different studies in which edible beef patties coating was applied [30, 29]. It has been suggested that the addition of 0.05, 0.10 and 0.15 % sage to Chinese sausage has lower TBA values compared to controls [32]. Another study [33] reported that the addition of thyme oil (3 %) to raw and cooked pork and beef mince samples significantly reduced TBA values after 12 days at 4 °C. These results are in agreement with our results in the present study.

Microbiological characteristics. The effect of ARG loaded with *T. spicata* essential oils edible coating on the shelf life of packaged beef patties is shown in Fig. The effect of ARG and thyme oil-loaded ARGs on the total aerobic bacteria (TAB) count both during and at the end of the storage period had a significant effect ($p<0.05$) (Fig. a). The edible coating with ARG showed

Table 3. TBA values of beef patties samples during the storage period
Таблица 3. Содержание тиобарбитуровой кислоты в образцах говяжьих котлет в течение срока хранения

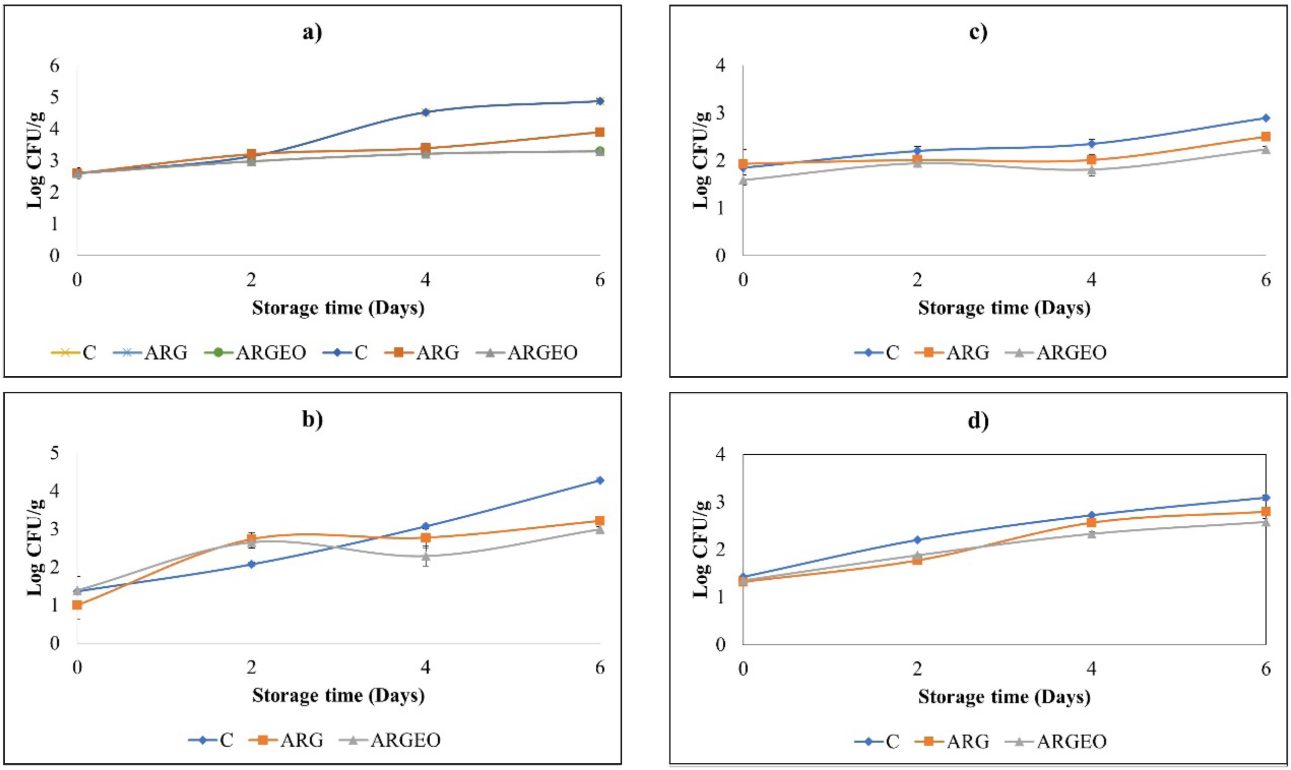
Storage day	Groups		
	C	ARG	ARG-TSEO
0	1.08±0.04^{A}	1.08±0.03^{A}	1.10±0.05^{A}
2	1.18±0.02^{Ba}	1.13±0.04^{ABa}	0.90±0.04^{Bb}
4	1.36±0.01^{Ca}	1.21±0.06^{Bb}	0.92±0.03^{Bc}
6	1.49±0.03^{Da}	1.33±0.02^{Cb}	1.00±0.04^{ABc}

Note. *Values are given as mean ± standard deviation. N = 3 biological replicates. Different uppercase letters in the same column indicate significant difference during storage while different lowercase letters in the same row indicate significant difference among the samples separately for each parameter ($p<0.05$). C: Beef patties without coating, ARG: Beef patties with *A. rosea* gum coating, ARG-TSEO: Beef patties with *A. rosea* gum coating loaded with *T. spicata* essential oil.

an antimicrobial effect against TAB. On the other hand, the antagonistic effect of the essential oil added into the coating on the growth of TAB was found to be higher. The antimicrobial activity of pectin edible coatings prepared by adding *T. spicata* essential oil against TAB on sliced bolognas was reported by Gedikoğlu [34]. Consistent with our present study, Antoniadou et al. [35] reported that the number of TAB decreased significantly in beef patties coated edible with chitosan and this effect existed until the end of the 14-day storage period. It is generally known that when the microbial count in food reaches 10^6 CFU/g, food becomes unacceptable to eat [34]. Based on this information, this value was not reached at the end of the storage period in all groups. However, if the storage period is extended, it can be predicted that especially the control group may be at a level close to this value. This indicates that the edible coating improves the shelf life of the beef patties.

Yeast and mold growth results in the groups are shown in Fig. b. Overall, ARG edible coating loaded with TSEO had a significant ($p<0.05$) effect on yeast and mold growth compared to the control group. Especially at the end of the storage period, this difference became more pronounced. Again, the use of essential oils in the coating caused an antagonistic effect on the current parameter. At the end of the storage period, the C treatment had the highest yeast and mold counts at 4.28 log CFU/g, while the ARG and ARGEO treatment had the lower count at 3.21 and 2.99 log CFU/g, respectively. Gedikoğlu [34] reported that, contrary to our results, there was no difference between the number of yeast and mold in the storage period, but at the end of the storage period (25th day), the number of yeast and molds decreased significantly in sliced bolognas coated with pectin fortified with TSEO.

Lactic acid bacteria count of control samples and ARG-coated samples did not differ at day 0 and 2 (Fig. d). However, there was a logarithmic difference of approximately 0.5 in all groups after day 4. This difference was found to be statistically significant at the end of the storage period in the control samples and the samples treated with ARG and ARGEO ($p<0.05$). Antoniadou et al. [35] reported that LAB counts were lower in chitosan-coated beef patties during the storage period compared to the control group, but there was no difference at the end of the storage period. On the other hand, Gedikoğlu [34] reported that the LAB number (1.72 log CFU/g) of sliced bolognas coated with TSEO loaded pectin was lower than those in the normal coating and control group. In another study [27], it was suggested that the number of LAB obtained from chicken beef patties coated with rosemary essential oil was lower than that of the control group.



Effect of edible coating on microbiological characteristics of beef patties samples: a – total aerobic bacteria; b – yeast and mold; c – psychrophilic bacteria; d – lactic acid bacteria. C: Beef patties without coating; ARG: Beef patties with *A. rosea* gum coating; ARGEO: Beef patties with *A. rosea* gum coating loaded with *T. spicata* essential oil

Влияние съедобного покрытия на микробиологические характеристики образцов котлет из говядины: а – суммарные аэробные бактерии; b – дрожжевые и плесневые бактерии; c – психрофильные бактерии; d – молочнокислые бактерии. С: котлеты из говядины без покрытия; ARG: котлеты из говядины с покрытием из камеди *A. rosea*; ARGEO: котлеты из говядины с покрытием из камеди *A. rosea* с добавлением эфирного масла *T. spicata*

Table 4. Texture profile analysis of beef patties
Таблица 4. Анализ текстурного профиля говяжьих котлет

Parameters	Groups		
	C	ARG	ARG-TSEO
Adhesiveness (Ns)	-81.79±30.77[A]	-28.6±14.49[B]	-26.11±8.06[B]
Springiness (mm)	0.96±0.03	0.91±0.06	0.92±0.03
Cohesiveness (Ratio)	0.38±0.03	0.37±0.06	0.38±0.02
Gumminess (N/mm^2)	155.18±25.51[A]	102.04±28.15[B]	55.46±32.47[B]
Chewiness (N.mm)	148.75±28.94[A]	92.98±28.7[B]	51.35±30.33[B]
Resilience (N/mm)	0.06±0.05	0.07±0.01	0.07±0.01

Note. *Values are given as mean ± standard deviation. N = 3 biological replicates. Means in the same row not sharing a common superscript are different ($p<0.05$). C: Beef patties without coating, ARG: Beef patties with *A. rosea* gum coating, ARG-TSEO: Beef patties with *A. rosea* gum coating loaded with *T. spicata* essential oil.

The total number of psychrophilic bacteria, which is accepted as an indicator of food spoilage in cold storage, was found to be between 1.32 and 1.45 in all groups at day 0. At day 6, this value was 3.09 log CFU/g for the control group and 2.79 and 2.58 log CFU/g for the ARG and ARGEO groups, respectively (Fig. d). The values remained below the acceptable limit at the end of the storage period. When the total psychrophilic bacteria numbers were compared between the groups, it was seen that the difference between the control and ARG and ARGEO groups was significant at the end of the storage period ($p<0.05$). Similar results were also reported by Can and Şahin [27].

Textural features. The texture analysis results of the beef patties samples at the end of the storage period are given in Tab. 4. There was no difference between the groups in terms of springiness, cohesiveness and resilience parameters. The results obtained showed that the coatings had a softening effect on the beef patties. These differences were found to be statistically significant ($p<0.05$), adhesiveness was highest in the control and lowest in the ARG and ARGEO groups. It was determined that the highest gumminess and chewiness values were in the control sample on the 6th day. In the control group, these values were determined as 155.18 and 148.75 (N/mm^2), respectively. Torusdağ et al. [36] reported that gelatin-based rosemary extract containing edible coatings reached lower chewing and stickiness values in beef patties compared to the control group after 10 days of storage, just like our result. In another study [37], the application of an edible coating enriched with antioxidant and antimicrobial substances to fish fillets at the end of storage, unlike our study, was determined to have the highest springiness.

CONCLUSIONS

The essential oils of *Thymbra spicata* were evaluated for its antioxidant, color, antimicrobial and textural properties in *Althaea rosea* flower gum-based edible coatings for packaged beef patties during cold storage. The application of *T. spicata* essential oil with ARG coating led to some changes in the appearance of beef patties by slight color differences but decreased the pH and lipid oxidation compared with beef patties without coating. Similar observations were detected ARG coated beef patties. On the other hand, when the beef patties coated with ARG loaded with *T. spicata* essential oil were examined in terms of total aerobic bacteria, psychrophilic bacteria, lactic acid bacteria and yeast/mold, it was determined that they had lower numbers. Moreover, the gumminess and chewiness values were higher in both groups of ARG and ARG loaded with *T. spicata* essential oil coating compared to the control group. This study revealed that ARG and ARG loaded with *T. spicata* (Zahter) essential oil coating could be a useful hurdle technology.

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The author performed the research, made a generalization on the basis of the results obtained and prepared the copyright for publication.

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