

Effects of Chitosan-based Coating Enriched with Peanut (*Arachis hypogaea*) Skin Extract on Physicochemical, Microbiological and Sensory Characteristics of Beef Burger During Cold Storage

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Abstract: The current study aimed to create a chitosan-based coating enriched with peanut skin extract (PSE) at a concentration of 0.5, 1 and 1.5%, and evaluate its impact on the physicochemical, microbiological and sensory characteristics of fresh beef burger stored at $4\pm 1^{\circ}\text{C}$ for 15 days. All coated burgers had significantly higher L^* , a^* and b^* values than the uncoated control during storage. Also, the results showed that application of chitosan coating enriched with 0.5, 1 and 1.5% PSE significantly increased total phenolic content (TPC) and antioxidant activity (AA), inhibited lipid oxidation as evaluated by thiobarbituric acid reactive substances (TBARS) and peroxide value (PV), retarded microbial growth and enhanced sensory characteristics of burger samples. Moreover, the positive effects of PSE on all investigated quality characteristics were proportional with the extract concentration. Therefore, using chitosan-based coating enriched with PSE enhances the stability of beef burger during cold storage and can be utilized in the meat industry.

Keywords: peanut skin extract, chitosan-based coating, total phenolic content, antioxidant activity, microbiological quality.

1. Introduction

Meat and meat products are usually considered as nutrient-rich foods and are also included in the food guide pyramid (Surendhiran *et al.*, 2020). Currently, meat and chicken products such as burgers, patties and sausages are widely consumed food products due to their appreciable sensory properties, nutritional quality, and low cost (Demirhan and Candoğan, 2017 and Feiner, 2006). Commonly noticed meat and meat products issues are microbiological spoilage and lipid oxidation, which impact their nutritional quality, sensory acceptability, and shelf life (Selani *et al.*, 2011). Consequently, nutritional value loss, sensory changes, and the generation of toxic substances are noted (Behbahani and Fooladi, 2018; Umaraw *et al.*, 2020). Therefore, meat processors should use effective technologies to extend the shelf-life of fresh and processed meats while keeping the nutritional qualities at a high level (Salimiraad *et al.*, 2022). To overcome these concerns, physical preservation methods such as refrigeration, fermentation, drying, smoking and canning as well as chemical antimicrobials and antioxidants can be utilized to extend the shelf-life, and minimize or prevent microbiological and chemical spoilage of meat and meat products (Umaraw *et al.*, 2020; Tyagi *et al.*, 2021). Traditional preservation methods have been replaced by new preservation methods such as coating, bio-preservative, and non-thermal methods (Zhou *et al.*, 2010).

Natural-based edible coatings, slime layers formed on the food surface, have been commonly utilized to preserve and maintain the quality of foodstuffs. These edible coatings exhibit sufficient protection against exterior factors, including water, gases, aroma, and microbes. Meat and meat products can be coated with polysaccharides (e.g., chitosan, alginate, starch, pectin, cellulose, and

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agar), proteins (e.g., wheat gluten, soy collagen, gelatin, casein, zein, egg albumin, and keratin), and lipid (e.g., fatty acids, waxes, and acyl-glycerol) based coatings to improve their physicochemical and quality characteristics, safety, and shelf-life during storage (Alizadeh-Sani *et al.*, 2019; Homayounpour *et al.*, 2020; Ludwicka *et al.*, 2020; Sheerzad *et al.*, 2024).

Chitosan, a natural polysaccharide polymer obtained from chitin (β -1,4-linked N-acetyl-D-glucosamine and D-glucosamine) deacetylation, is nature's second most abundant polysaccharide after cellulose. Chitosan is nontoxic biopolymer with excellent functional characteristics, including coating forming, antimicrobial, antifungal, and antioxidant characteristics (Dadvar *et al.*, 2021; Kumar *et al.*, 2020; Sun *et al.*, 2022). Based on its unique positive characteristics, it has recently been used to extend the shelf-life and enhance the quality of numerous foods, such as meat and meat products (Qiu *et al.*, 2021; Sheerzad *et al.*, 2024), fish and fish products (Zamani *et al.*, 2022), eggs (Ezazi *et al.*, 2021), and fruits and vegetables (Chettri *et al.*, 2023; Sharma *et al.*, 2024).

Recently, the agro-industrial field has grown significantly, resulting in an increase in waste and by-product generation. These products were generally sold at low prices, discarded or used as animal feed, and fertilizers. Moreover, wasting numerous natural antimicrobials and antioxidants (Adhikari *et al.*, 2019; Munekata *et al.*, 2016; Yu *et al.*, 2014). Several studies reported that peanut skin and its extract contained numerous bioactive components, antimicrobials and antioxidants, such as phenolic acids, flavonoids, bioflavonoids, isoflavones, flavanols, flavones, and stilbenes. Using peanut skin extract (PSE) in meat and chicken products exhibited a positive impact on lipid oxidation and microbial growth inhibition (Munekata *et al.*, 2015; Munekata *et al.*, 2016; O'Keefe and Wang, 2006; Serrano-León *et al.*, 2018). The main objective of the present study is to develop an edible coating system by incorporating PSE into chitosan solution, and evaluate its impact on the quality and shelf life of fresh beef burger during refrigerated storage at $4\pm1^\circ\text{C}$.

2. Materials and Methods

2.1. Materials

Beef brisket, flank, fat and spices were purchased from a local market in Ismailia, Egypt. Peanut skin was obtained as a gift from a Green Valley Company (Ismailia, Egypt). Chitosan from shrimp shells (deacetylation degree $> 95\%$ and viscosity $200 < \text{mpa.s}$), glycerol ($> 97\%$ purity) and acetic acid were purchased from Zhejiang Aoxing Biotech. Co. Ltd. (Zhejiang, China). 2-Thiobarbituric acid, Folin-Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals, reagents and solvents were of analytical grade or the highest grade available.

2.2. Methods

2.2.1. Preparation of peanut skin powder

Peanut kernel pieces were sieved and hand removed, and only clean peanut skin was used. The peanut skin was dried using a WT-binder dryer (type F115, Germany) at 45°C for 24 h. The dried peanut skin was finely pulverized using a Waring Commercial blender (HGB2WTS3, Torrington, Connecticut, USA), passed through a mesh 60 sieve shaker (Retsch, 5657 Haan, Germany), and then kept in a sealed polyethylene bag at $4\pm1^\circ\text{C}$ for 24 h for extraction.

2.2.2. Preparation of peanut skin extract

The peanut skin powder (25 g) was extracted twice with an absolute ethyl alcohol (250 mL) by a constant agitation using a shaker (Decoloring Table, TY-B, China) for 24 h at 100 rpm. The ethanolic solutions were collected and filtered, and the filtrates were evaporated using a Strike 300 rotary evaporation (Steroglass, Italy) at 40°C under vacuum to dryness. Dried PSE was kept at -18°C until use.

2.2.3. Preparation of coating solution

Chitosan-based coating solution was prepared according to the method described by Lekjing (2016). 2 g of chitosan were dissolved in 100 mL of 1% (w/v) glacial acetic acid with a constant agitation overnight at room temperature. After that, 0.5 mL of glycerol/g chitosan was added, then stirring for 30 min at room temperature. To prepare chitosan-based coating solutions enriched with PSE, 0.5, 1 and 1.5% of the extract were added to 100 mL of the prepared chitosan solution, and stirred for 6 h at room temperature.

2.2.4. Beef burger preparation

Connective tissues and visible fat were trimmed from beef flank and brisket muscles to provide a lean meat, then the trimmed muscles were cut into pieces of 100 ± 10 g. Meat pieces and beef fat were separately minced through a 4 mm steel plate using a meat mincer (SAP Meat Mincer TC22. Italy). Beef burger was prepared by mixing 87.5% of minced meat, 12.5% of minced fat, 1.5% of sodium chloride, 0.022% of a spice mixture (clove, nutmeg and black pepper) and 0.03% of sodium tripolyphosphate for 5 min in a Kenwood meat mixer (Havant, UK). Burgers of 60 ± 1 g with 9 cm diameter were formed by hand press maker (Italman. Italy), and the following treatments were prepared: C: control without any coating, T1: samples coated with chitosan solution, T2: samples coated with chitosan solution+0.5% PSE, T3: samples coated with chitosan solution+1% PSE, T4: samples coated with chitosan solution+1.5% PSE. Burger samples were stored at $4 \pm 1^\circ\text{C}$ for 15 days and analyzed on days 0, 3, 6, 9, 12 and 15 of storage period.

2.2.5. Proximate analysis

The contents of moisture, protein, fat, and ash of burger treatments were performed according to the methods of the Association of Official Analytical Chemists (AOAC, 2012). Carbohydrate content was calculated by difference using the following equation:

$$\text{Carbohydrate \%} = 100 - (\text{Moisture \%} + \text{Protein \%} + \text{Fat \%} + \text{Ash \%})$$

2.2.6. pH determination

The values of pH of burger samples were determined according to the method of Tamsen *et al.* (2018) as follows: 5 g of sample were blended with 45 mL distilled water for 1 min, and then the pH of the homogenate was measured using a pH meter (Jenway 3510; Jenway Ltd., Essex, UK).

2.2.7. Color measurement

A Minolta CR-10 color reader (Osaka, Japan) was used to measure color parameters [where L^* measures lightness ($L^* = 0$ black, $L^* = 100$ white), a^* redness ($-50 = \text{green}$, $+50 = \text{red}$) and b^* yellowness ($-50 = \text{blue}$, $+50 = \text{yellow}$)] of meat samples. The surface color of burger samples was immediately measured after processing, and five readings were recorded for each sample.

2.2.8. Total phenolic content determination

Total phenolic content (TPC) was determined in the methanolic extract of burger samples using the Folin–Ciocalteu method of Osorio-Esquivel *et al.* (2011) with slight modifications. A 100 μL of methanolic extract were mixed with 900 μL of Folin–Ciocalteu phenol reagent, and was allowed to stand for 5 min at room temperature. After that, 0.75 μL of sodium bicarbonate solution (7%) was added to the mixture, vortexed for 30 sec, and allowed to stand for 90 min at room temperature. The absorbance was measured at 725 nm using a PG spectrophotometer and the results were given as mg of Gallic acid equivalents/g of burger sample.

2.2.9. Antioxidant activity determination

Antioxidant activity was estimated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical as per the procedure of Tamsen *et al.* (2018). 100 μL of the methanolic extract of burger samples were

mixed with 3.9 mL of methanol solution of DPPH, and the mixture was kept for 0.5 h at dark at room temperature. The absorbance was measured at 517 nm using a PG spectrophotometer. The antioxidant activity was estimated using the following equation:

$$\% \text{ Antioxidant activity} = (\text{Abs blank} - \text{Abs sample}) / \text{Abs blank} * 100$$

2.2.10. Lipid oxidation determination

2.2.10.1. Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) of burger samples were estimated according to the distillation procedure of Halvorsen and Kvernenes (2020) with some modifications. After the distillation of 10 g sample, 5 mL distillate were reacted with 5 mL TBA reagent (0.02 M) in a boiling water bath for 35 min. After cooling, the absorbance of the resultant pink color was measured at 532 nm using a PG spectrophotometer (Felsted, Dunmow, UK). The levels of TBARS were calculated by multiplying the absorbance by the factor 7.8, and were expressed as mg malonaldehyde (MD)/kg of burger.

2.2.10.2. Peroxide value

Peroxide value was determined based on the method described by Egan *et al.* (1997) as follows: 150 g of burger sample were blended with 250 mL of chloroform for 3 min in a blender (Matsushita ELEC. IND. Co. Ltd., Japan), and then the mixture was filtered. 25 mL of the filtrate were mixed with 37 mL of glacial acetic acid, and 1 mL of freshly prepared saturated potassium iodide solution. The titration was done with sodium thiosulphate (0.01 N) using 0.5 mL of starch indicator (1%). The results of peroxide value were expressed as meq O₂/kg of burger.

2.2.11. Microbiological analysis

Ten grams of the burger sample were homogenized with 90 mL of sterile peptone water (0.1%), and the required decimal dilutions up to 10⁻⁶ were prepared for the following microorganisms determinations: (1) total mesophilic count (TMC) and psychophilic count (PSC) were enumerated using plate count agar medium incubated at 37°C for 2 days and 7°C for 10 days, respectively, (2) yeast and mold count (YMC) was counted using plate count agar containing 100 µg/ml cidostane for 2 days of incubation at 25°C, and (3) coliform bacteria count (CFC) was enumerated on violet red bile agar for 24 h at 35-37°C. The microbial counts were expressed as log₁₀ CFU/g of burger sample.

2.2.12. Sensory evaluation

Burger samples were sensory evaluated according to the method of García *et al.* (2009). Burgers were grilled on a an electric grill (WA-BBQ 01, White Whale, China) at approximately 180°C for 3 min, then turned over and grilled for another 3 min. ten trained panelists of the staff members of the Food Technology Department, Faculty of Agriculture, Suez Canal University were asked to judge the color, taste, odor, texture, appearance, and overall acceptability using a 9-point hedonic descriptive scale, where 9 was given for like extremely to the sample and 1 for dislike extremely.

2.2.13. Statistical analysis

All measurements were conducted in triplicate except color measurement and sensory evaluation, and data are presented as mean ± SD. Two-way analysis of variance (ANOVA) accompanied with Duncan's multiple range test was used to estimate the significance at (p<0.05) level between the investigated treatments using SPSS software (version 17.0 for Windows, SPSS Inc., Chicago, USA).

3. Results and Discussion

3.1. Proximate analysis

The proximate analysis of coated and uncoated burger samples are presented in Table 1. From the results it could be noticed that application of chitosan-based coating and PSE had no significant (P>0.05) impact on moisture, protein, fat, ash and carbohydrate of burger samples. The results

showed that the uncoated control contained 59.01% moisture, 19.46% protein, 14.52% fat, 1.42% ash and 5.83% carbohydrate, while the coated burgers contained (58.49-58.79%) moisture, (19.22-19.37%) protein, (14.29-14.49%) fat, (1.50-1.67%) ash and (5.94-6.11%) carbohydrate. The obtained results are in accordance with those reported by Alirezalu *et al.* (2021), who found that there was no significant influence on the proximate analysis of fresh meat coated with ϵ -polylysine and stinging nettle extract. In addition, moisture, fat, ash and carbohydrate contents were lower and protein content was higher than those reported by Trujillo-Mayol *et al.*, (2021) for beef and soy burgers, and this may be due to the ingredients of burgers.

Table 1. Proximate analysis (% wet weight basis) of beef burgers coated with chitosan and peanut skin extract

Treatment	Constituent (%)				
	Moisture	Protein	Fat	Ash	Carbohydrate*
C	59.01±0.54 ^A	19.46±0.26 ^A	14.52±0.22 ^A	1.42±0.23 ^A	5.83±0.20 ^A
T1	58.79±0.33 ^A	19.22±0.22 ^A	14.42±0.20 ^A	1.67±0.27 ^A	5.94±0.24 ^A
T2	58.67±0.37 ^A	19.27±0.17 ^A	14.38±0.18 ^A	1.61±0.11 ^A	6.06±0.15 ^A
T3	58.54±0.47 ^A	19.25±0.13 ^A	14.29±0.29 ^A	1.50±0.25 ^A	6.11±0.21 ^A
T4	58.49±0.50 ^A	19.37±0.15 ^A	14.49±0.19 ^A	1.59±0.19 ^A	6.09±0.16 ^A

*Carbohydrate by difference

Data are mean values±SD of three replicates; ^ADifferent superscripts in each column replicate significant ($P<0.05$) differences between treatments; C: control without coating, T1: coated with chitosan, T2: coated with chitosan+0.5% PSE, T3: coated with chitosan+1% PSE, T4: coated with chitosan+1.5% PSE.

3.2. pH measurement

The pH value is an important indicator in the quality and shelf life assessing of fresh minced meat (Wang *et al.*, 2020). Changes in the pH values of control beef burger samples and those coated with chitosan and peanut skin extract during the 15 days of cold storage are presented in Table 2. Significant differences were observed among the uncoated control and coated burger samples (T1, T2, T3 and T4) at the beginning and throughout the whole storage period. The uncoated control had the highest initial pH value (6.21) compared with the coated burger samples (T1-T4; 6.07-6.15). Similar results were observed by Özvural *et al.* (2016) who reported that control hamburger patties had higher values of pH than coated samples. The lower values of pH in cumin essential oil loaded coated beef samples may be explained by the ability of coatings to reduce the permeability of CO₂ from the coatings and the accumulation of CO₂ may therefore cause pH decline in the samples (Behbahani *et al.*, 2020). The pH values of all burger samples decreased during the first 6 days of storage, whereas after the first 6 days of storage there was a gradual increase. The observed decrease of pH during the first days of storage might be due to lactic acid formation and the release of inorganic phosphates (Li *et al.*, 2020; Qiu *et al.*, 2014). The last increase of pH values is attributed to the accumulation of amines and other nitrogenous compounds (such as ammonia and trimethylamine) through the breakdown of proteins by endogenous enzymes and enzymes of spoilage bacteria (Biswas *et al.*, 2004; Nirmal *et al.*, 2011; Behbahani and Fooladi, 2018; Özvural *et al.* 2016).

Table 2. Effect of chitosan-based coatings incorporated with peanut skin extract on pH values of beef burgers during cold storage at 4±1°C

Treatment	Storage time (days)					
	0	3	6	9	12	15
C	6.21±0.01 ^{Aa}	6.13±0.01 ^{Ab}	5.78±0.01 ^{Ae}	5.80±0.02 ^{Ad}	5.82±0.02 ^{Ad}	5.89±0.02 ^{Ac}
T1	6.07±0.02 ^{Ca}	5.81±0.01 ^{Bb}	5.45±0.02 ^{Df}	5.48±0.01 ^{De}	5.65±0.02 ^{Bd}	5.71±0.01 ^{Bc}
T2	6.13±0.02 ^{Ba}	5.73±0.01 ^{Cb}	5.58±0.06 ^{Be}	5.63±0.02 ^{Bd}	5.65±0.01 ^{Bd}	5.68±0.01 ^{BCc}
T3	6.15±0.03 ^{Ba}	5.65±0.01 ^{Dc}	5.55±0.02 ^{BCd}	5.58±0.01 ^{Cd}	5.65±0.02 ^{Bc}	5.69±0.03 ^{Bb}
T4	6.12±0.02 ^{Ba}	5.53±0.02 ^{Ed}	5.54±0.03 ^{Cd}	5.58±0.03 ^{Cc}	5.60±0.01 ^{Cc}	5.66±0.02 ^{Cb}

Data are mean values±SD of three replicates; ^{A-D}Different superscripts in each column replicate significant ($P<0.05$) differences between treatments; ^{a-f}Different superscripts in each row replicate significant ($P<0.05$) differences between

storage days; C: control without coating, T1: coated with chitosan, T2: coated with chitosan+0.5% PSE, T3: coated with chitosan+1% PSE, T4: coated with chitosan+1.5% PSE.

3.3. Color measurement

The buying decision of meat and meat products can be affected by the myoglobin nature (Mancini and Hunt, 2005). The appearance of muscle foods can be influenced by the optical properties of edible coating materials. Changes in the color parameters (L^* , a^* and b^*) of control beef burger samples and those coated with chitosan and PSE during the 15 days of cold storage are presented in Table 3. All coated burgers with chitosan or chitosan and PSE had significantly ($p < 0.05$) higher L^* , a^* and b^* values than uncoated control during storage. Similar results were observed by Zhang *et al.*, (2020) for ready to cook pork chops coated with chitosan and bamboo vinegar, and Behbahani *et al.*, (2020) for beef coated with *shahri balangu* seed mucilage incorporated with cumin essential oil. The L^* , a^* and b^* values of all investigated burger samples decreased significantly during the cold storage could be attributed to the microbial activity and myoglobin and oxymyoglobin oxidation to metmyoglobin, producing muscles with an unattractive color (Cayré *et al.*, 2005; Ebadi *et al.*, 2019). Coated samples showed lower changes in all color parameters compared to control, and this might be due to the antioxidant and antimicrobial impact of chitosan and PSE. Moreover, burgers coated with chitosan and PSE (T2, T3 and T4) showed lower changes in all color parameters than those coated with chitosan (T1), and this is might be due to the antioxidant and antibacterial properties of bioactive polyphenols from PSE (Meng *et al.*, 2020).

Parameters	Treatment	Storage time (days)					
		0	3	6	9	12	15
L^*	C	35.17±0.03 ^{Da}	34.69±0.02 ^{Eb}	32.54±0.51 ^{Cc}	29.13±0.04 ^{Dd}	27.28±0.03 ^{Ce}	25.24±0.04 ^{Cf}
	T1	35.52±0.04 ^{Ca}	34.89±0.10 ^{Db}	33.29±0.03 ^{Bc}	29.22±0.03 ^{Cd}	27.34±0.04 ^{Ce}	25.31±0.02 ^{Cf}
	T2	35.65±0.05 ^{BCa}	35.20±0.02 ^{Cb}	33.30±0.03 ^{Bc}	29.28±0.03 ^{Cd}	27.85±0.40 ^{Be}	25.37±0.02 ^{BCf}
	T3	35.78±0.03 ^{Ba}	35.34±0.02 ^{Bb}	33.66±0.05 ^{ABc}	29.38±0.02 ^{Bd}	28.18±0.03 ^{ABe}	25.64±0.36 ^{Bf}
	T4	36.23±0.04 ^{Aa}	35.58±0.03 ^{Ab}	33.99±0.06 ^{Ac}	30.07±0.07 ^{Ad}	28.24±0.03 ^{Ae}	26.11±0.03 ^{Af}
a^*	C	10.34±0.01 ^{Da}	8.67±0.02 ^{Cb}	7.39±0.02 ^{Bc}	6.12±0.02 ^{Bd}	5.68±0.02 ^{De}	5.13±0.02 ^{Df}
	T1	10.15±0.03 ^{Ea}	9.30±0.28 ^{Bb}	9.19±0.28 ^{Ab}	9.01±0.40 ^{Ab}	8.15±0.02 ^{Cc}	7.97±0.03 ^{Cc}
	T2	10.73±0.02 ^{Aa}	9.56±0.34 ^{ABb}	9.42±0.30 ^{Ab}	9.16±0.30 ^{Abc}	8.80±0.02 ^{AcD}	8.45±0.01 ^{Ad}
	T3	10.44±0.02 ^{Ca}	9.43±0.02 ^{ABb}	9.22±0.02 ^{Ac}	9.17±0.02 ^{Ad}	8.56±0.03 ^{Be}	8.13±0.02 ^{Bf}
	T4	10.54±0.02 ^{Ba}	9.74±0.02 ^{Ab}	9.47±0.03 ^{Ac}	9.33±0.02 ^{Ad}	8.57±0.02 ^{Be}	8.44±0.02 ^{Af}
b^*	C	9.23±0.01 ^{Ea}	9.08±0.02 ^{Db}	8.74±0.03 ^{Ec}	8.18±0.02 ^{Ed}	8.15±0.02 ^{Ed}	8.08±0.04 ^{Ce}
	T1	9.54±0.02 ^{Da}	9.14±0.02 ^{Dcd}	9.10±0.03 ^{De}	9.25±0.02 ^{Cb}	9.13±0.20 ^{Cde}	9.17±0.02 ^{Bc}
	T2	9.95±0.04 ^{Ca}	9.64±0.02 ^{Cb}	9.34±0.03 ^{Cc}	9.17±0.03 ^{Dd}	9.08±0.03 ^{De}	9.19±0.02 ^{Bd}
	T3	10.05±0.03 ^{Ba}	9.73±0.02 ^{Bb}	9.66±0.02 ^{Bc}	9.66±0.03 ^{Bc}	9.34±0.02 ^{Ad}	9.25±0.03 ^{Ae}
	T4	10.24±0.02 ^{Aa}	10.01±0.06 ^{Ab}	9.75±0.03 ^{Ac}	9.75±0.04 ^{Ac}	9.25±0.03 ^{Bd}	9.25±0.02 ^{Ad}

Table 3. Effect of chitosan-based coatings incorporated with peanut skin extract on L^* , a^* and b^* values of beef burgers during cold storage at 4±1°C

Data are mean values±SD of five replicates; ^{A-E}Different superscripts in each column replicate significant ($P < 0.05$) differences between treatments; ^{a-f}Different superscripts in each row replicate significant ($P < 0.05$) differences between storage days; C: control without coating, T1: coated with chitosan, T2: coated with chitosan+0.5% PSE, T3: coated with chitosan+1% PSE, T4: coated with chitosan+1.5% PSE.

3.4. Total phenolic content, antioxidant activity, thiobarbituric acid reactive substances and peroxide value

Phenolic components, secondary metabolites presented in fruits, vegetables, cereals, and legumes, in plant extracts are known as functional ingredients with antioxidant, antimicrobial, and healthy properties (Alirezalu *et al.*, 2020; Munekata *et al.*, 2020). Changes in the levels of total phenolic content (TPC), antioxidant activity (AA), thiobarbituric acid reactive substances (TBARS) and peroxide value (PV) of control beef burger samples and those coated with chitosan and PSE during the 15 days of cold storage are presented in Table 4. Results showed that, adding PSE to chitosan-based coatings significantly ($p < 0.05$) increased the TPC of treated burger samples, and this might be attributed to the higher content of phenolic components of PSE (Munekata *et al.*, 2016; Yu *et al.*, 2005; Rusak *et al.*, 2008). Furthermore, the levels of TPC in burger samples were directly proportional to the PSE level, thus T4 exhibited the greatest levels of TPC. In all treated burgers, TPC progressively decreased ($P < 0.05$) during cold storage. The TPC reduction might be due to the oxidation reactions during cold storage (Daskalaki *et al.*, 2009). Among all investigated samples, the highest loss in TPC was observed in the control (C; 13.49%), while the lowest loss was noted in T3 with 7.24%. Similar decreasing trend during refrigerated storage for beef coated with polylysine and stinging nettle extract were observed by (Rusak *et al.*, 2008).

A significant ($p < 0.05$) increase of AA was noticed in all coated burgers (20.58-34.08%) as compared to control (17.34%). Additionally, the gradient rise in the concentration of the added PSE caused a proportional rise in the AA. Therefore, T4 had the highest AA throughout the whole storage period, and this might be attributed to its high TPC content (Table 4). A positive correlation between TPC and AA has been documented, and numerous phenolic components with chelating and antioxidant properties were observed in peanut skin indicating that they may be applied to meat products (Munekata *et al.*, 2015; Tagrida and Benjakul, 2021). Moreover, coating contain chitosan could preserve the higher levels of phenolic compounds and AA in coated herring, and this is might be due to their oxygen barrier capacity. According to Meng *et al.* (2020), the higher PSE level in starch-chitosan film was associated with a rise in reducing power. The AA of all samples gradually declined during the 15 days of storage. The decrease in AA might be due to the decomposition of TPC during storage.

TBARS and PV are main indicators of the lipid oxidation degree, which are induced by the formation of secondary oxidation products such as malondialdehyde and primary oxidation products such as hydroperoxides, respectively (Nogueira *et al.*, 2019; Saengsorn and Jimtaisong, 2017). The initial TBARS values of control and coated burger samples, C, T1, T2, T3 and T4, were 0.38, 0.35, 0.34, 0.33 and 0.32 mg MDA/kg, respectively which was consistent with the 0.27-0.33 mg MDA/kg by Amjadi *et al.* (2020) for coated and uncoated fresh beef samples. As presented in Table 4, the TBARS values of all coated and uncoated burger samples increased significantly throughout the storage time. Furthermore, all TBARS values measured for coated burger samples were significantly ($p < 0.05$) lower than those of uncoated control. T4 exhibited strong inhibition impact on lipid oxidation, recorded the lowest TBARS value of 0.44 MDA mg/kg burger at the 15th day of cold storage. An increase in TBARS values was linked to unsaturated fatty acids oxidation and partial dehydration (Nowzari *et al.*, 2013). It has been stated that the permitted level for TBARS in meat and meat products is 1 mg MDA/kg (Behbahani and Fooladi, 2018). Therefore, uncoated control was rejected from the 12 day of storage. The lipid oxidation protection in coated burger samples may be attributed to the synergism between antioxidant effect and oxygen barrier properties of chitosan (Ojagh *et al.*, 2010; Antoniewski *et al.*, 2007; Yen *et al.*, 2008). Phenolic compounds have the ability to prevent lipid oxidation even at low concentration by capturing the free radicles that starts the oxidation reactions (Sikorski, 2016) Adding PSE to chitosan-coated burgers can significantly increase the antioxidant ability of chitosan.

Statistical analysis revealed that no significant ($p > 0.05$) differences were observed between the uncoated control and coated burger samples at the day of processing. The initial peroxide values ranged from 1.65 meq oxygen/kg in T4 to 1.67 meq oxygen/kg in control. The obtained PV were

close to those reported by Nagarajan *et al.* (2021). Also, the treatments coated with chitosan and PSE (T2, T3 and T4) exhibited the lowest ($p<0.05$) PV levels, while the uncoated control sample (C) had the highest ($p<0.05$) values at any sampling time of storage. In agreement with our results, the positive impacts of coating with chitosan and/or plant extracts on the protection against lipid oxidation are well documented (Nagarajan *et al.*, 2021; Wang *et al.*, 2020). These results are possibly attributed to lower oxygen permeability of chitosan and the antioxidant capacity of PSE due to its high polyphenol and flavonoid contents (Meng *et al.*, 2020). During storage, the PV of all burger samples was significantly ($p<0.05$) rose, as expected, due to the formation of primary product as a result of lipid oxidation. Significant increase of PV was observed in uncoated control compared with coated samples. Similar trend was noticed by Balti *et al.* (2020) for coated shrimp. Behbahani *et al.* (2017) reported that the allowable limit of PV for meat was 7.0 meq oxygen/kg. From the obtained results, the uncoated control topped the permitted level on the 15th day of storage and therefore the control's shelf life was equivalent to 12 days.

Table 4. Effect of chitosan-based coatings incorporated with peanut skin extract on TPC, AA* TBARS and PV of beef burgers during cold storage at $4\pm1^{\circ}\text{C}$

Parameters	Treatment	Storage time (days)					
		0	3	6	9	12	15
TPC	C	43.00 \pm 0.74 ^{Ca}	41.78 \pm 0.38 ^{Eb}	41.02 \pm 0.23 ^{Eb}	39.90 \pm 0.38 ^{Ec}	38.78 \pm 0.46 ^{Ed}	37.20 \pm 0.38 ^{De}
	T1	44.14 \pm 0.96 ^{Ca}	44.53 \pm 0.58 ^{Da}	43.46 \pm 0.54 ^{Dab}	42.09 \pm 0.32 ^{Dab}	41.32 \pm 0.23 ^{Db}	41.44 \pm 0.22 ^{Cb}
	T2	66.97 \pm 0.49 ^{Ba}	66.41 \pm 0.67 ^{Ca}	64.68 \pm 0.62 ^{Cb}	63.36 \pm 0.46 ^{Cc}	62.39 \pm 0.23 ^{Cd}	60.51 \pm 0.62 ^{Be}
	T3	70.28 \pm 0.38 ^{Aa}	69.62 \pm 0.55 ^{Ba}	68.50 \pm 0.23 ^{Bab}	67.63 \pm 0.40 ^{Babc}	66.62 \pm 0.32 ^{Bbc}	65.19 \pm 0.31 ^{Ac}
	T4	72.16 \pm 0.32 ^{Aa}	71.70 \pm 0.23 ^{Aa}	70.64 \pm 0.47 ^{Ab}	69.41 \pm 0.38 ^{Ac}	67.94 \pm 0.46 ^{Ad}	66.31 \pm 0.54 ^{Ae}
AA*	C	17.34 \pm 0.42 ^{Ea}	14.88 \pm 0.24 ^{Eb}	11.95 \pm 0.19 ^{Ec}	11.52 \pm 0.25 ^{Ecd}	11.20 \pm 0.28 ^{Ed}	9.09 \pm 0.51 ^{Ee}
	T1	20.58 \pm 0.30 ^{Da}	20.37 \pm 0.32 ^{Da}	17.85 \pm 0.04 ^{Db}	16.99 \pm 0.26 ^{Dc}	16.74 \pm 0.20 ^{Dc}	14.45 \pm 0.02 ^{Dd}
	T2	29.38 \pm 0.30 ^{Ca}	27.71 \pm 0.24 ^{Cb}	24.64 \pm 0.31 ^{Cc}	24.36 \pm 0.25 ^{Ccd}	23.87 \pm 0.34 ^{Cd}	22.80 \pm 0.58 ^{Ce}
	T3	31.79 \pm 0.24 ^{Ba}	29.64 \pm 0.12 ^{Bb}	26.25 \pm 0.50 ^{Bc}	25.83 \pm 0.56 ^{Bcd}	25.35 \pm 0.61 ^{Bde}	24.58 \pm 0.36 ^{Be}
	T4	34.08 \pm 0.30 ^{Aa}	32.41 \pm 0.24 ^{Ab}	29.32 \pm 0.31 ^{Ac}	28.76 \pm 0.25 ^{Ac}	28.04 \pm 0.21 ^{Ad}	27.22 \pm 0.60 ^{Ae}
TBARS	C	0.38 \pm 0.01 ^{Af}	0.73 \pm 0.02 ^{Ae}	0.87 \pm 0.02 ^{Ad}	0.97 \pm 0.03 ^{Ac}	1.07 \pm 0.02 ^{Ab}	1.24 \pm 0.04 ^{Aa}
	T1	0.35 \pm 0.01 ^{Bf}	0.49 \pm 0.02 ^{Be}	0.58 \pm 0.01 ^{Bd}	0.62 \pm 0.01 ^{Bc}	0.66 \pm 0.02 ^{Bb}	0.76 \pm 0.03 ^{Ba}
	T2	0.34 \pm 0.01 ^{BCf}	0.39 \pm 0.02 ^{Ce}	0.44 \pm 0.01 ^{Cd}	0.48 \pm 0.01 ^{Cc}	0.53 \pm 0.02 ^{Cb}	0.57 \pm 0.01 ^{Ca}
	T3	0.33 \pm 0.01 ^{CDf}	0.37 \pm 0.01 ^{Ce}	0.42 \pm 0.02 ^{Dd}	0.47 \pm 0.02 ^{CDc}	0.50 \pm 0.01 ^{Db}	0.54 \pm 0.01 ^{CDa}
	T4	0.32 \pm 0.01 ^{Dd}	0.34 \pm 0.01 ^{Dd}	0.41 \pm 0.01 ^{Dc}	0.45 \pm 0.01 ^{Db}	0.49 \pm 0.01 ^{Da}	0.50 \pm 0.01 ^{Da}
PV	C	1.67 \pm 0.10 ^{Af}	3.35 \pm 0.05 ^{Ae}	4.20 \pm 0.10 ^{Ad}	5.93 \pm 0.03 ^{Ac}	6.87 \pm 0.03 ^{Ab}	7.93 \pm 0.03 ^{Aa}
	T1	1.67 \pm 0.10 ^{Af}	2.20 \pm 0.10 ^{Be}	2.85 \pm 0.05 ^{Bd}	3.75 \pm 0.05 ^{Bc}	4.55 \pm 0.05 ^{Bb}	5.43 \pm 0.04 ^{Ba}
	T2	1.68 \pm 0.05 ^{Af}	2.10 \pm 0.10 ^{Be}	2.73 \pm 0.08 ^{Bd}	3.43 \pm 0.03 ^{Cc}	4.35 \pm 0.05 ^{Cb}	5.35 \pm 0.05 ^{Ba}
	T3	1.65 \pm 0.05 ^{Af}	2.02 \pm 0.25 ^{Be}	2.46 \pm 0.05 ^{Cd}	3.30 \pm 0.10 ^{Dc}	4.15 \pm 0.05 ^{Db}	5.15 \pm 0.05 ^{Ca}
	T4	1.65 \pm 0.05 ^{Ae}	1.65 \pm 0.05 ^{Ce}	2.38 \pm 0.08 ^{Cd}	3.15 \pm 0.05 ^{Ec}	4.10 \pm 0.10 ^{Db}	5.10 \pm 0.10 ^{Ca}

Data are mean values \pm SD of three replicates; ^{A-E}Different superscripts in each column replicate significant ($P<0.05$) differences between treatments; ^{a-f}Different superscripts in each row replicate significant ($P<0.05$) differences between storage days; C: control without coating, T1: coated with chitosan, T2: coated with chitosan+0.5% PSE, T3: coated with chitosan+1% PSE, T4: coated with chitosan+1.5% PSE.

3.5. Microbiological analysis

Changes in total mesophilic count (TMC), psychophilic count (SPC), yeast and mold count (YMC), and coliform count (CFC) of control beef burger samples and those coated with chitosan and PSE during the 15 days of cold storage are presented in Table 5. The initial TMC in all burger samples varied from 2.44 to 2.52 log CFU/g, indicating a high quality of meat and meat products. The coated burgers showed significantly lower TMC than uncoated control at every observation time throughout the whole storage period. The TMC in control burgers increased to 7.09 log CFU/g at 15 days of storage. The International Commission of Microbiological Specification for Foods (ICMSF, 1986) reported that the maximum allowable TMC level for fresh beef is 7 log CFU/g. From the obtained results it could be concluded that the control burgers were acceptable in the term of TMC

(5.84 log CFU/g) on the twelve day of storage. TMC of all uncoated and coated burgers rose significantly ($p<0.05$) as the time of storage increased. This may be due to the presence of many phenolic compounds in peanut skin such as gallic, ferulic, caffeic, chlorogenic, 4-hydroxybenzoic acid, p-coumaric, salicylic and vanillic acids which exhibit many biological characteristics such as antibacterial, anti-algal, antioxidant and anti-inflammatory activities (Bodoira *et al.*, 2022; Bodoira and Maestri, 2020; Kumar *et al.*, 2021; Sun *et al.*, 2023). This finding are similar to those observed by Ebadi *et al.* (2019) for *Nemipterus japonicus* fillet coated with chitosan and/or propolis extract.

On the first day of storage, the SPC was significantly ($p<0.05$) higher in the uncoated control (1.76 log CFU/g; C) compared to the other coated samples (1.45-1.79 log CFU/g; T2, T3 and T4). The SPC of all burgers increased gradually as a function of storage period ($p<0.05$). The rate of increasing of SPC in the control was higher than that of the coated burgers. The lower SPC of coated burgers compared with control burgers might be attributed to the antimicrobial potential of chitosan Serrano-León *et al.* (2018) reported that chitosan film enriched with PSE significantly reduced psychrotrophic count of restructured chicken products compared to control.

At day 0, the YMC in all burger samples varied from 1.50 to 1.52 log CFU/g. Significant ($p<0.05$) differences were detected in YMC among the control and coated burgers. During cold storage, YMC gradually increased in all investigated samples, whilst the uncoated control showed a higher rate of increase than the coated samples. At day 15, YMC was significantly ($p<0.05$) lower in chitosan or chitosan incorporated with PSE coated samples compared to control, and T4 had the lowest YMC. Since mold species are aerobic organisms that mostly grow on the meat's surface, the edible coatings served as a barrier of oxygen and inhibited the growth of aerobic microorganisms on the meat slices (Behbahani *et al.*, 2020).

Parameters	Treatment	Storage time (days)					
		0	3	6	9	12	15
Total mesophilic count	C	2.52±0.06 ^{Af}	3.86±0.02 ^{Ae}	4.99±0.02 ^{Ad}	5.53±0.02 ^{Ac}	5.84±0.02 ^{Ab}	7.09±0.01 ^{Aa}
	T1	2.49±0.01 ^{ABf}	3.64±0.01 ^{Be}	3.76±0.01 ^{Bd}	4.47±0.01 ^{Bc}	5.30±0.01 ^{Bb}	5.54±0.01 ^{Ba}

Table 5. Effect of chitosan-based coatings incorporated with peanut skin extract on total mesophilic, psychrophilic, yeast and mold, and Coliform count (log₁₀ CFU/g) of beef burgers during cold storage at 4±1°C

Psychrophilic count	T2	2.46±0.01 ^{BCf}	3.59±0.01 ^{Ce}	3.68±0.01 ^{Cd}	4.41±0.01 ^{Cc}	5.08±0.01 ^{Cb}	5.51±0.01 ^{Ca}
	T3	2.44±0.01 ^{Cf}	3.56±0.01 ^{De}	3.64±0.01 ^{Dd}	4.36±0.01 ^{Dc}	5.06±0.01 ^{Cb}	5.49±0.01 ^{Da}
	T4	2.50±0.03 ^{ABf}	3.52±0.01 ^{Ee}	3.61±0.01 ^{Ed}	4.34±0.01 ^{Dc}	5.03±0.01 ^{Db}	5.45±0.01 ^{Ea}
	C	1.76±0.02 ^{Bf}	2.28±0.02 ^{Ad}	3.88±0.02 ^{Ac}	3.91±0.02 ^{Ac}	4.45±0.03 ^{Ab}	4.92±0.02 ^{Aa}
Yeast and mold count	T1	1.79±0.02 ^{Ae}	1.94±0.02 ^{Be}	2.84±0.01 ^{Bd}	3.37±0.02 ^{Bc}	3.87±0.02 ^{Bb}	4.07±0.03 ^{Ba}
	T2	1.68±0.01 ^{Cf}	1.83±0.01 ^{Ce}	2.84±0.02 ^{Bd}	3.05±0.02 ^{Cc}	3.26±0.02 ^{Cb}	3.87±0.02 ^{Ca}
	T3	1.64±0.01 ^{Df}	1.67±0.01 ^{De}	2.69±0.01 ^{Cd}	2.90±0.01 ^{Dc}	3.11±0.01 ^{Db}	3.72±0.01 ^{Da}
	T4	1.45±0.02 ^{Ef}	1.64±0.01 ^{Ee}	2.65±0.02 ^{Dd}	2.87±0.02 ^{Dc}	3.10±0.01 ^{Db}	3.70±0.01 ^{Da}
Coliform count	C	1.51±0.03 ^{Af}	1.79±0.01 ^{Ae}	2.84±0.01 ^{Ad}	2.86±0.02 ^{Ac}	2.89±0.01 ^{Ab}	3.91±0.01 ^{Aa}
	T1	1.52±0.03 ^{Af}	1.63±0.02 ^{Be}	2.79±0.01 ^{Bd}	2.81±0.01 ^{Bc}	2.84±0.01 ^{Bb}	3.26±0.01 ^{Ba}
	T2	1.50±0.05 ^{Af}	1.55±0.01 ^{Ce}	1.85±0.01 ^{Cd}	2.08±0.01 ^{Cc}	2.30±0.01 ^{Cb}	2.83±0.02 ^{Ca}
	T3	1.51±0.05 ^{Ae}	1.52±0.01 ^{De}	1.82±0.01 ^{Dd}	2.04±0.02 ^{Dc}	2.28±0.01 ^{Db}	2.78±0.01 ^{Da}
Coliform count	T4	1.50±0.04 ^{Ae}	1.50±0.01 ^{De}	1.80±0.01 ^{Ed}	2.02±0.02 ^{Dc}	2.26±0.01 ^{Eb}	2.76±0.02 ^{Da}
	C	1.64±0.05 ^{Ae}	1.90±0.01 ^{Ad}	2.83±0.02 ^{Ac}	2.87±0.01 ^{Ac}	2.96±0.01 ^{Ab}	3.50±0.02 ^{Aa}
	T1	1.66±0.01 ^{Ad}	1.67±0.01 ^{Bd}	2.71±0.02 ^{Bc}	2.78±0.02 ^{Bb}	2.83±0.02 ^{Bb}	2.76±0.01 ^{Ba}
	T2	1.62±0.01 ^{Ae}	1.64±0.02 ^{Bd}	1.65±0.02 ^{Dd}	1.71±0.01 ^{Dc}	1.90±0.01 ^{Cb}	2.27±0.01 ^{Ca}
Coliform count	T3	1.46±0.01 ^{Be}	1.50±0.02 ^{Cd}	1.50±0.01 ^{Ed}	1.56±0.02 ^{Ec}	1.76±0.01 ^{Db}	2.24±0.01 ^{Da}
	T4	1.46±0.05 ^{Bf}	1.65±0.03 ^{Be}	1.69±0.01 ^{Cd}	1.77±0.02 ^{Cc}	1.87±0.03 ^{Cb}	2.20±0.01 ^{Ea}

Data are mean values±SD of three replicates; ^{A-E}Different superscripts in each column replicate significant ($P<0.05$) differences between treatments; ^{a-f}Different superscripts in each row replicate significant ($P<0.05$) differences between storage days; C: control without coating, T1: coated with chitosan, T2: coated with chitosan+0.5% PSE, T3: coated with chitosan+1% PSE, T4: coated with chitosan+1.5% PSE.

The initial counts of Coliform (CFC) in all burger samples varied from 1.46 to 1.66 log CFU/g, and T1 had the highest count. During the storage period, the CFC of all burger samples under investigation increased; and T2 had the lowest rate increase of increase (40.12%). Similar results were observed by Behbahani *et al.* (2020) who reported that coated beef slices had lower coliform count than uncoated control at any observed day of storage. The noted results could be attributed to the antibacterial effect of PSE-incorporated edible coating (T2, T3 and T4). Microbial-suppressing potential of edible coatings enriched with numerous plant extracts in meat products has been reported (Mehdizadeh *et al.*, 2020, Keykhosravi *et al.*, 2020).

3.6. Sensory evaluation

Visual appearance has a significant impact on the quality of meat and meat products, and especially the color influences consumers' assessments of these products (Adamsen *et al.*, 2006). The sensory properties of uncoated and coated burger samples were assessed and statistically analyzed for color, taste, odor, texture, appearance and overall acceptability as presented in Table 6. The data indicated that burgers coated with chitosan and 1.5% PSE (T4) had the highest score values of all investigated sensorial properties during the cold storage period, followed by burgers coated with chitosan and 1% PSE (T3), burgers coated with chitosan and 0.5% PSE (T2) and burgers coated with chitosan (T1). While, uncoated control burgers (C) had the lowest score values. These results are in agreement with those reported by Ebadi *et al.* (2019) who studied the effect of chitosan and/or propolis extract on color, odor and overall preference attributes of *Nemipterus japonicus* fillet, and found that fillet coated with chitosan and PE had higher scores than uncoated control and chitosan-based coated samples during storage. Moreover, Siripatravan and Noipha (2012) reported that odor, color and overall acceptability significantly decreased with increasing storage time. Songsaeng (2014) reported that the appearance scores significantly increased with the clove oil concentration. In contrast, the scores of odor decreased with increasing the clove oil concentration.

In addition, the results of sensory analysis showed that color, taste, odor, texture, appearance and overall acceptability scores decreased with increasing storage time. Discoloration and off-odor could

be attributed to microbial growth, lipid oxidation and slime formation during storage (Siripatrawan and Noipha, 2012). The deterioration of sensory characteristics was faster in the uncoated control compared to the coated burgers. Similarly, Mehdizadeha *et al.*, (2020) found that beef samples coated with chitosan-starch film enriched with pomegranate peel extract and thyme essential oil had the lowest rate of reduction in color, odor and overall acceptability properties, and coated beef samples showed higher acceptability at the end of storage. This could be due to the inhibition of microbial spoilage and lipid oxidation. Additionally, Munekata *et al.* (2016) reported that adding PSE decreased the loss of sensory characteristics of sheep patties.

Table 6. Effect of chitosan-based coatings incorporated with peanut skin extract on the sensory evaluation of beef burgers during cold storage at 4±1°C

Parameters	Treatment	Storage time (days)					
		0	3	6	9	12	15
Color	C	8.63±0.15 ^{Ba}	8.30±0.30 ^{Bab}	7.90±0.46 ^{Babc}	7.57±0.51 ^{Bbcd}	7.37±0.51 ^{Bcd}	7.07±0.51 ^{Bd}
	T1	8.70±0.10 ^{Ba}	8.43±0.21 ^{Bab}	8.23±0.21 ^{Bbc}	8.03±0.15 ^{Bcd}	7.77±0.25 ^{Bde}	7.53±0.25 ^{Be}
	T2	9.57±0.15 ^{Aa}	9.23±0.21 ^{Aab}	8.93±0.21 ^{Abc}	8.80±0.20 ^{Ac}	8.60±0.20 ^{Acd}	8.30±0.30 ^{Ad}
	T3	9.70±0.10 ^{Aa}	9.60±0.10 ^{Aab}	9.27±0.25 ^{Abc}	8.93±0.21 ^{Acd}	8.73±0.21 ^{Ade}	8.50±0.27 ^{Ae}
Taste	T4	9.33±0.42 ^{Aa}	9.17±0.38 ^{Aab}	8.90±0.27 ^{Aabc}	8.67±0.21 ^{Abc}	8.80±0.10 ^{Abc}	8.50±0.10 ^{Ac}
	C	8.60±0.10 ^{Ba}	8.40±0.10 ^{Ca}	7.70±0.20 ^{Cb}	7.43±0.15 ^{Dbc}	7.20±0.20 ^{Bcd}	7.00±0.20 ^{Bd}
	T1	8.67±0.15 ^{Ba}	8.53±0.15 ^{Ca}	8.27±0.25 ^{Bab}	8.00±0.20 ^{Cb}	7.60±0.30 ^{Bc}	7.23±0.25 ^{Bc}
	T2	9.17±0.15 ^{Aa}	8.90±0.10 ^{Bab}	8.67±0.15 ^{Abc}	8.47±0.15 ^{Bcd}	8.23±0.25 ^{Ade}	7.93±0.25 ^{Ae}
Odor	T3	9.37±0.15 ^{Aa}	9.17±0.15 ^{Aab}	8.87±0.15 ^{Abc}	8.67±0.15 ^{ABcd}	8.37±0.25 ^{Ade}	8.07±0.25 ^{Ae}
	T4	9.40±0.10 ^{Aa}	9.27±0.15 ^{Aa}	9.00±0.20 ^{Aab}	8.83±0.15 ^{Abc}	8.53±0.31 ^{Accd}	8.27±0.35 ^{Ad}
	C	8.60±0.10 ^{Ba}	8.43±0.21 ^{Ba}	8.13±0.12 ^{Cb}	7.90±0.10 ^{Db}	7.57±0.15 ^{Cc}	7.20±0.10 ^{Cd}
	T1	8.77±0.15 ^{Ba}	8.57±0.15 ^{Bab}	8.40±0.20 ^{Bbc}	8.20±0.20 ^{Ccd}	8.00±0.10 ^{Bde}	7.80±0.20 ^{Be}
Texture	T2	9.30±0.10 ^{Aa}	9.10±0.10 ^{Aab}	8.90±0.10 ^{Ab}	8.63±0.15 ^{Bc}	8.40±0.20 ^{Acd}	8.30±0.10 ^{Ad}
	T3	9.47±0.15 ^{Aa}	9.30±0.10 ^{Aab}	9.10±0.10 ^{Abc}	8.87±0.15 ^{ABc}	8.53±0.25 ^{Ad}	8.57±0.15 ^{Ad}
	T4	9.33±0.15 ^{Aa}	9.20±0.10 ^{Aab}	9.10±0.10 ^{Aab}	8.97±0.15 ^{Ab}	8.67±0.25 ^{Ac}	8.57±0.15 ^{Ac}
	C	8.40±0.06 ^{BCa}	8.17±0.06 ^{Bb}	8.00±0.10 ^{Bc}	7.97±0.06 ^{Bc}	7.90±0.10 ^{Bc}	7.90±0.10 ^{Bc}
Appearance	T1	8.30±0.20 ^{Ca}	8.13±0.15 ^{Bab}	8.03±0.15 ^{Bab}	7.93±0.15 ^{Bb}	7.93±0.12 ^{Bb}	7.90±0.10 ^{Bb}
	T2	8.40±0.10 ^{BCab}	8.47±0.21 ^{Aa}	8.33±0.15 ^{Aabc}	8.20±0.10 ^{ABcd}	8.10±0.10 ^{ABcd}	8.00±0.10 ^{ABd}
	T3	8.60±0.10 ^{ABa}	8.50±0.10 ^{Aa}	8.40±0.10 ^{Aab}	8.40±0.10 ^{Aab}	8.23±0.15 ^{Abc}	8.10±0.10 ^{ABc}
	T4	8.80±0.10 ^{Aa}	8.67±0.15 ^{Aab}	8.40±0.10 ^{Ab}	8.40±0.10 ^{Ab}	8.27±0.15 ^{Ab}	8.17±0.21 ^{Ab}
Overall acceptability	C	7.93±0.15 ^{Ba}	7.83±0.15 ^{Bab}	7.60±0.17 ^{Bbc}	7.47±0.06 ^{Bcd}	7.30±0.10 ^{Bd}	6.73±0.21 ^{Ce}
	T1	8.17±0.15 ^{Ba}	7.97±0.15 ^{Bab}	7.80±0.27 ^{Bbc}	7.63±0.15 ^{Bbc}	7.47±0.21 ^{Bcd}	7.27±0.21 ^{Bd}
	T2	8.90±0.20 ^{Aa}	8.67±0.21 ^{Aab}	8.43±0.25 ^{Abc}	8.20±0.20 ^{Acd}	7.97±0.15 ^{Ade}	7.77±0.15 ^{Ae}
	T3	8.93±0.31 ^{Aa}	8.77±0.25 ^{Aa}	8.47±0.35 ^{Aab}	8.23±0.31 ^{Abc}	7.97±0.25 ^{Abc}	7.73±0.21 ^{Ac}
	T4	8.87±0.15 ^{Aa}	8.83±0.15 ^{Aa}	8.30±0.10 ^{Ab}	8.20±0.10 ^{Ab}	8.10±0.10 ^{Ab}	7.83±0.06 ^{Ac}
	C	8.80±0.10 ^{Ca}	8.40±0.20 ^{Bb}	8.10±0.10 ^{Cc}	7.60±0.10 ^{Cd}	7.00±0.10 ^{Ce}	6.70±0.10 ^{Cf}
	T1	8.70±0.10 ^{Ca}	8.60±0.10 ^{Ba}	8.47±0.12 ^{Bab}	8.27±0.21 ^{Bbc}	8.13±0.15 ^{Bcd}	7.93±0.15 ^{Bd}
	T2	9.10±0.10 ^{Ba}	8.97±0.15 ^{Aab}	8.87±0.15 ^{Abc}	8.70±0.10 ^{Acd}	8.50±0.10 ^{Ade}	8.30±0.10 ^{Ae}
	T3	9.10±0.10 ^{Ba}	8.97±0.15 ^{Aab}	8.83±0.15 ^{Abc}	8.63±0.15 ^{Acd}	8.50±0.10 ^{Ade}	8.30±0.10 ^{Ae}
	T4	9.30±0.10 ^{Aa}	9.10±0.10 ^{Aab}	8.90±0.10 ^{Abc}	8.73±0.06 ^{Acd}	8.53±0.15 ^{Ade}	8.33±0.15 ^{Ae}

Data are mean values±SD of ten replicates; ^{A-D}Different superscripts in each column replicate significant ($P<0.05$) differences between treatments; ^{a-f}Different superscripts in each row replicate significant ($P<0.05$) differences between storage days; C: control without coating, T1: coated with chitosan, T2: coated with chitosan+0.5% PSE, T3: coated with chitosan+1% PSE, T4: coated with chitosan+1.5% PSE.

4. Conclusion

In conclusion, the results indicated that application of chitosan-based coating enriched with PSE had positive impacts on physicochemical, microbiological and sensorial characteristics of beef burger during cold storage for 15 days. Chitosan and PSE coating on the beef burger surface significantly increased TPC and AA and reduced levels of TBARS and PV. Moreover, burgers coating with chitosan and PSE effectively inhibited TMC, PSC, YMC and CFC, and maintained all sensory characteristics. Therefore, chitosan-based coating enriched with PSE can be utilized to retard microbiological and oxidative deterioration, and extend the shelf-life of beef burger during cold storage.

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Conflict of interest

The authors declared that they have no conflict of interest.

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