



Effects of ethanolic extract from onion skin on the quality characteristics of beef patties during refrigerated storage

Cuntang WANG^{1,2*} , Xuanzhe AN¹, Zengming GAO¹, Ziyu LI¹, Shengxin TIAN¹, Yueyi LU¹

Abstract

In this study, the effects of ethanolic extract from onion skin (EEOS) on the storage quality of refrigerated beef patties were studied. EEOS concentrations of 0.00%, 0.01%, 0.05%, and 0.10% were added to beef patties. These beef patties were stored at 4 °C for 12 days and compared with 0.05 V_c treatment group. At the end of storage, compared to control, 0.1% EEOS effectively reduced the contents of metmyoglobin (MetMb) ($P < 0.05$) and thiobarbituric acid reactants (TBARS) ($P < 0.05$) in beef patties by 30.89% and 44.72%, respectively. On day 12, the carbonyl contents in 0.1% EEOS treatment increased only 3.3 times, lower than control and V_c treatment (4.5 and 4.1 times). Microbial contamination was significantly inhibited ($P < 0.05$). The antioxidant and antibacterial effects of EEOS were the main reasons for the improvement of pH and a* stability of beef patties. However, the addition of EEOS had no significant effect on cooking loss and texture properties except hardness and springiness. Overall, 0.1% EEOS had a positive effect on the sensory characteristics and storage quality of beef patties and could prolong the storage life of beef patties to 9 days. This showed that EEOS as a natural additive has great potential for beef storage and preservation.

Keywords: ethanolic extract of onion skin; beef patties; antioxidant; storage stability.

Practical Application: Onion skin is an important source of natural antioxidants as it is rich in flavonoids such as quercetin, kaempferol and anthocyanins. The pigment in onion skin especially affects the color of beef patty. Therefore, ethanolic extract from onion skin are added to beef patty to improve the color of beef. In addition, the microbial growth, lipid and protein oxidation in beef patty were inhibited during storage. This research could be useful for the food industry and consumers to know that ethanolic extract of onion skin is a more suitable natural coloring and antioxidant agent for beef patty. In this way, the quality and functional characteristics of beef patty shall be increased during processing and storage.

1 Introduction

Meat, as an important source of protein in the human diet, that demand is a daily on the increase (Alves et al., 2020; Hati et al., 2021). Among the meat products, the beef burger is one of the most popular, being especially consumed by young people (Alves et al., 2020). However, Oxidative reactions have always been the key technical problem restricting the transportation, sales, and storage of meat and meat products (Cui et al., 2021). Lipid and protein oxidation of meat products cause the loss of flavor, texture, and nutrients, thus affecting the acceptance of meat products and shortening their shelf-life (Ergezer & Serdaroğlu, 2018; Gómez et al., 2020). On the other hand, protein oxidation also leads to meat colour browning, because Fe²⁺ in the myoglobin heme group is oxidized to Fe³⁺, so that oxymyoglobin (OxyMb) in muscle changes into metmyoglobin (MetMb), and the colour of muscle turns brown (Gómez et al., 2020). Beef as red meat is prone to oxidative deterioration and colour browning due to higher myoglobin and iron oxidation levels, which will reduce consumers' purchase intention (Ergezer & Serdaroğlu, 2018; Jiao et al., 2020).

To solve these problems, synthetic antioxidants such as tert-butylhydroquinone (TBHQ), butyl hydroxytoluene (BHT), butyl hydroxyanisole (BHA) and propyl gallate (PG) are often used in

the production and processing of meat products to slow down the oxidation of lipid and protein (Cunha et al., 2018; Ergezer & Serdaroğlu, 2018). These antioxidants can effectively ameliorate oxidative stress of meat products and avoid the damage of macromolecules including the lipid and protein fractions, and they are favored by food manufacturers because of their low price (Ergezer & Serdaroğlu, 2018; Jiao et al., 2020). But synthetic antioxidants are strictly controlled by countries all over the world because of their possible adverse effects. For example, China strictly controls the dosage of BHT and BHA below 200 mg/kg, Brazil limits such synthetic antioxidants to 100 mg/kg, and the use of TBHQ has been banned in Europe and Canada (National Standard of the People's Republic of China, 2014; de Oliveira Ferreira et al., 2019). In addition to consumers' demands for healthy and safe food, natural antioxidants have gradually come into public view.

Natural antioxidants are a kind of polyphenol compounds with hydrogen donation ability, which mainly exist in natural plants such as herbs, spices, fruits and vegetables (Reddy et al., 2018). These plants show strong free radical scavenging ability and antibacterial activity due to the existence of phenolic acids, stilbenes and flavonoids in their extracts (Aquilani et al., 2018).

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¹ College of Food and Bioengineering, Qiqihar University, Qiqihar, China

² College of Food Science, Northeast Agricultural University, Harbin, China

*Corresponding author: wangcuntang1980@163.com

Therefore, researchers try to add these natural antioxidants to meat products to prevent lipid and protein oxidation. On the other hand, consumers expect these new meat products with natural antioxidants to taste, look and smell the same or better way as their traditionally formulated and processed (Paglarini et al., 2020). For the sensory evaluation of formula meat products, researchers often evaluate the quality of meat products by several sensory perception methodologies (Temporal Dominance of Sensations (TDS), Temporal Check- All-That-Apply (TCATP), and classification order, etc.), affective representation (ideal scale method, preference test, etc.), and conceptual representation (Q methodology) (Paglarini et al., 2020; Vidal et al., 2020). Studies have suggested that origanum vulgare extract significantly improves the oxidative stability of lamb burger, and effectively reduce thiobarbituric acid reactants (TBARS) by 80% when its concentration is 24 mL/kg (Fernandes et al., 2017). Beef patties added with clove extract showed lower lipid oxidation and higher redness value than those treated with V_C (Zahid et al., 2020). Red pitaya extracts not only did not affect the chemical composition and cooking loss of pork patties but also reduced the content of protein carbonyl and improved the colour stability during storage (Bellucci et al., 2021).

Onion skin, as a kind of recyclable waste in the food processing industry, has been proved to be rich in polyphenols (Celano et al., 2021). Flavonols and anthocyanins are the main characteristic metabolites of onion skin and present good antioxidant, antibacterial and antidiabetic potential (Benítez et al., 2017; Bedrniček et al., 2020; Celano et al., 2021). However, as a representative of flavonols in onion skin extract, quercetin extraction shows amphiphilic. It is poorly soluble in water, but is easily soluble in organic solvents such as ethanol and methanol (Gois Ruivo da Silva et al., 2020). The presence of water will improve the solubility of hydrophilic substances such as glycosides and enhance the surface area of solid-solvent contact (Gois Ruivo da Silva et al., 2020). At the same time, researchers generally choose the mixed solution of ethanol and water as the extraction agent of bioactive substances in onion skin for the sake of safety and avoiding environmental pollution. It is reported that 70% ethanol is the best solvent system for extracting bioactive substances from onion skin because the obtained quercetin has the highest content and purity (Gois Ruivo da Silva et al., 2020).

Based on the above theories, 70% ethanol was used as the extraction agent, and the living substances extracted from red onion skin were added to beef patties to evaluate the effects of ethanolic extract from onion skin (EEOS) on the physical and chemical properties, oxidative stability of lipid and protein, microbial pollution and sensory properties of beef patties, and compared with the treatment supplemented with antioxidant V_C .

2 Materials and methods

2.1 Materials

2-Thiobarbituric acid (2-TBA) was purchased from Coolaber Technology Co., Ltd. (Beijing, China). Guanidine hydrochloride was supplied by Aladdin Biochemical Technology Co., Ltd (Shanghai, China). 2,4-dinitrophenylhydrazine (DNPH) was

obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Anhydrous ethanol and trichloroacetic acid (TCA) were brought from Kemio Chemical Reagent Co., Ltd; (Tianjin, China). All other reagents were of analytical grade.

2.2 Preparation of EEOS

Red onion skins were collected from the wholesale market in Qiqihar, China. Onion skins were thoroughly washed with distilled water to remove sediment and dried at a constant temperature of 50 °C in an electric blast drying oven (GZX-9146 MBE, Shanghai Boxun Industrial Co., Ltd, Shanghai, China). After that, the onion skins were ground with a multifunctional pulverizer (Sufeng Industry and Trade Co., Ltd, Zhejiang, China) and sieved with a 100-mesh sieve to obtain onion skin powder. Approximately 100 g of onion skin powder was maintained for 12 h in 2000 mL of 70% ethanol solution at room temperature. The extract solution was centrifuged (6000 rpm) for 10 min to remove the residue powder, which was extracted again by the above method. The two supernatants were combined and evaporated under reduced pressure in a rotating evaporator at 50 °C and then transferred to a vacuum freeze dryer (2.5 L freeze dry system, Labconco Co., Kansas, USA) for vacuum drying at - 40°C for 48 h. The lyophilized powder was sealed and stored at - 20 °C.

2.3 Manufacture of beef patties

The fresh beef loin was purchased from a local market in Qiqihar, China and transported to the laboratory in a fresh-preserved box. The beef loin with excess fat and connective tissue removed was immediately ground by a meat grinder (JR-32L, Zhucheng Huagang Machinery Co., Ltd, Shandong, China) and pass through a plate having 6 mm holes. The recipe for beef patties contained the following ingredients: 1080 g beef, 120 g ice water, 1.5% (w/w) salt and different proportions of EEOS (0% w/w, 0.01% w/w, 0.05% w/w and 0.1% w/w) according to Abdelhakam et al. (2019). Beef patties with 0.05% V_C were used as the positive control. All minced beef stirred evenly was made into meat patties weighing 80 g, 10 cm in diameter and 1 cm thick with a hand-held meat patty mold. Beef patties were packaged with a family vacuum packaging machine (DS3600, Shenzhen Dingsheng Electric Appliance Co., Ltd., Guangdong, China), refrigerated at 4 °C and analyzed at 0, 3, 6, 9 and 12 days.

2.4 Analysis of pH and colour of beef patties

The analysis of pH referred to the method of Biswas et al. (2012). Homogenizing 10 g of sample ($n = 3$) with a mortar in 50 mL of distilled water. After, the pH value was measured with a digital pH meter (Five Easy Plus, METTLER TOLEDO instrument (Shanghai) Co., Ltd., Shanghai, China).

A portable colorimeter (CR-10 Plus, Konica Minolta optics Co., Ltd, Shanghai, China) was used to determine the colour parameters of the samples ($n = 3$). The colorimeter adopts a D65 observation light source, 8 mm measurement aperture and 10 ° observation angle. Five different sites were measured for each sample. The total colour difference (ΔE^*) was calculated according to the methodology of Bellucci et al. (2021) Equation 1:

$$\Delta E^* = \sqrt{(L_{3-12}^* - L_0^*)^2 + (a_{3-12}^* - a_0^*)^2 + (b_{3-12}^* - b_0^*)^2} \quad (1)$$

where L_0^* , a_0^* and b_0^* are the lightness (L^*) value, redness (a^*) value and yellowness (b^*) value of day 0; L_{3-12}^* , a_{3-12}^* , b_{3-12}^* are the L^* value, a^* value and b^* value of day 3-12.

2.5 Cooking loss and texture profile of beef patties

The determination of cooking loss of beef patties was based on the method of Bellucci et al. (2021). The cooking loss of beef patties before and after cooking was calculated by the following Equation 2:

$$\text{Cooking loss (\%)} = \frac{\text{Raw weight} - \text{Cooking weight}}{\text{Raw weight}} \times 100 \quad (2)$$

The texture profile of beef patties was analyzed regarding the method of Bahmanyar et al. (2021) and slightly modified. Beef pieces (2 cm × 2 cm × 1 cm) were placed on the stage of a texture analyzer (TMS PRO, FTC Co., USA) to analyze the hardness, cohesiveness, springiness, gumminess and chewiness of the samples. Before analysis, it was determined that the probe for texture profile analysis is a 38.1 mm cylindrical plastic probe and the pickup height of the probe is 20 mm. The samples were compressed by 50% at a decent speed of 2 mm/s.

2.6 Determination of MetMb content of beef patties

MetMb content of beef patties was determined according to Chauhan et al. (2019) and Cui et al. (2021). A 3 g sample was homogenized with pestle and mortar after adding 30 mL 0.04 M phosphate buffer solution (pH 6.8, 4°C), and then refrigerate for 1 h at 4 °C. The samples were centrifuged with a high-speed freezing centrifuge (TG-16G, Changzhou Longhe Instrument Manufacturing Co., Ltd., Jiangsu, China) for 10 min (8000 rpm, 4°C) after incubation. The obtained supernatant was filtered with Whatman No. 1 filter paper (1001-150, Shanghai Weixi Biotechnology Co., Ltd., Shanghai, China). The absorbance of the filtrate at 525, 572 and 700 nm were measured by a UV-vis spectrophotometer (UV-5100, Metash Instruments Co., Ltd, Shanghai, Chain). The calculation formula of MetMb content was as follows Equation 3:

$$\text{MetMb (\%)} = \left(1.395 - \frac{\text{Abs}_{572} - \text{Abs}_{700}}{\text{Abs}_{525} - \text{Abs}_{700}} \right) \times 100 \quad (3)$$

2.7 Determination of TBARS of beef patties

TBARS values were determined according to the method of Witte et al. (1970) with suitable modification. About 10 g of sample was homogenized in 25 mL of 20% TCA solution (4 °C), using a mortar. Then rinsing the mortar with 25 mL distilled water (4 °C) and mixing it into a 100 mL beaker. The mixture was filtered through Whatman No. 1 filter paper (1001-150, Shanghai Weixi Biotechnology Co., Ltd., Shanghai, China). 3 mL of 5 mM 2-TBA reagent was added into a centrifuge tube

containing 3 mL of filtrate and place in a digital display constant temperature water bath pot (SYJ-4, Shanghai Bosun Industrial Co., Ltd., Shanghai, China) for 30 min (95 °C). At the end of the time, the reaction was terminated by chilled water. The reagent blank test was to use 3 mL of 10% TCA instead of sample filtrate. The absorbance at 532 nm of the sample was measured with a UV-vis spectrophotometer (UV-5100, Metash Instruments Co., Ltd, Shanghai, Chain). The absorbance multiplied by the coefficient 5.2 was used as the TBARS values of the sample and the results were expressed as mg malonaldehyde (MDA)/kg of sample.

2.8 Determination of protein carbonyl content of beef patties

The carbonyl content was measured as described by Vuorela et al. (2005). A 10 g of sample was triturated with pestle and mortar in 10 mL KCl (0.15 M, 4 °C). Two aliquots of 0.2 mL homogenate respectively added 1 mL of 10% TCA (4 °C). Then they were centrifuged at 5000 rpm for 5 min. One precipitation was added to 1 mL of 2 M HCl (4 °C) for protein quantification and another was added to 0.2% DNPH (4 °C) for carbonyl quantification. After incubation at room temperature for 1 h, they were added to 1 mL of 10% TCA and centrifuged at 5000 rpm for 5 min. The resulting precipitate was washed three times with 1 mL ethanol/ethyl acetate (1:1) to remove excess DNPH. The remaining small particles were dissolved in 1.5 mL of 6 M guanidine hydrochloride solution and centrifuged for 2 min (5000 rpm). The concentration of carbonyl and protein was determined by spectrophotometry at 370 nm and 280 nm. The results were expressed using bovine serum albumin as standard: nmol carbonyl/mg protein, Equation 4.

$$\text{Carbonyls} = \frac{\text{Abs}_{370}}{21.0 \text{ mM}^{-1} \text{ cm}^{-1}} \times 1000 \quad (4)$$

where 21.0 mM⁻¹ cm⁻¹ is the molar extinction coefficient of carbonyls.

2.9 Total volatile base nitrogen (TVB-N) content of beef patties

According to the method of GB 5009.228-2016 (National Standard of the People's Republic of China, 2016b), a 10 g sample was homogenized with 100 mL distilled water using mortar and pestle. After incubation at room temperature for 30 min, the homogenate was filtered. The obtained filtrate was distilled by the Kjeldahl distillation unit, and the timing starts when the first drop of condensate drops. The distillate was introduced into a beaker containing 10 mL of 2% boric acid solution and 5-6 drops of mixed indicator (0.2% methyl red solution and 0.1% methylene blue solution are mixed equally when used.). After 5 min, the collected solution was immediately titrated to blue-violet color with 0.01 M HCl with the removal of the catheter. At the same time, distilled water was used to replace sample solution with a reagent blank experiment. TVB-N values were expressed in mg of TVB-N per 100 g of beef, and the calculation formula was as follows Equation 5:

$$X(\text{mg}/100\text{g}) = \frac{(V_1 - V_2) \times M \times 14}{m \times 5/100} \quad (5)$$

where V_1 is the volume of HCl standard solution consumed by the sample solution for determination (mL); V_2 is the volume of HCl standard solution consumed by reagent blank (mL); M is the molar concentration of HCl standard solution; 14 is the milligrams of nitrogen equivalent to 1 mL of 1 M HCl standard solution; m is the mass of the sample (g).

2.10 Total plate count of beef patties

The total plate count of beef patties during storage was measured by the plate pouring method (National Standard of the People's Republic of China, 2016a). A 10 g of sample was homogenized in 90 mL of 0.85% sterile normal saline for 30 min. Serial decimal dilutions were prepared with sterile normal saline. 1 mL of diluent was poured into a plate and covered with sterile plate count agar, and cultured at 37 °C for 48 h. Following culture, the number of colonies was recorded and expressed as \log_{10} CFU/g.

2.11 Sensory analysis of beef patties

Beef patties were evaluated by descriptive analysis and performed hedonic tests of sensory evaluators within the allowable range (Alejandro et al., 2019). Fifty panelists who were trained in advance by a meeting participated in this study. The raw meat patties including the control and V_C treatment group were scored by each panelist on a 9-point scale. The score was 1-9, 1 was disliked extremely, and 9 was like extremely. The descriptors used were colour, odor, juiciness, elasticity and overall acceptance of the raw meat patties. All analyses were performed in separate compartments and randomly cod were delivered to each panelist in a white tray.

2.12 Statistical analysis

All experiments were done in duplicate. Data statistics software (SPSS 23, IMB, USA) was used to analyze the results obtained by analysis of variance (ANOVA). Duncan's test ($p < 0.05$) was used to compare the significant differences between the data means.

3 Results and discussion

3.1 pH of beef patties

As revealed in Table 1, the pH values of beef patties were significantly affected both by storage time and additives ($P < 0.05$). The addition of EEOS maintained the pH stability of beef patties during storage. At day 0, the pH values of all treatment groups were close to the isoelectric point of myofibrillar protein 5.3-5.5. At the end of storage, the pH value of each treatment group all showed an increasing trend, and the pH value of control increased from 5.57 to 5.79. In comparison with the control, the addition of 0.05% and 0.1% EEOS could significantly reduce the pH values and improve the pH stability of beef patties, which also effected the stabilization of the a^* values of beef patties (Aksu et al., 2020). The increase of pH values during the storage period might

be related to the breeding of spoilage bacteria, which would decompose proteins to produce NH_3 and aminates compounds (Hajlaoui et al., 2019). On the other hand, fatty acids and their oxidation products of meat would also increase pH value and affect the storage quality of beef patties (Hajlaoui et al., 2019). 0.1% EEOS was the most effective against retaining the pH stability of beef patties. This was attributed to the higher concentration and the rich polyphenols (quercetin, kaempferol and myricetin) in EEOS. A similar study was reported that ethanolic extract of *Morus alba* L. leaves can significantly delay the increase of pH values of chilled pork and keep the pH at the level of fresh meat (Cui et al., 2021). It might be seen that natural plant extracts have great potential for improving the storage stability of meat.

3.2 Colour of beef patties

Colour is an important parameter to evaluate the freshness of beef. It is the most commonly used standard for consumers to judge the shelf-life and acceptability of fresh meat, and the main appearance factor that determines consumers' purchasing desire (Chauhan et al., 2019). The effects of EEOS treatment for the colour of beef patties were shown in Table 1, that L^* , a^* and b^* values of beef patties decreased during storage, and the colour stability of beef patties could be significantly improved by adding EEOS. In the initial stage of storage, the L^* value of beef patties increased slightly, but there was no significant difference in other treatments except the control. After the 6th day, the L^* value of beef patties began to decline. In addition, EEOS significantly reduced the L^* value of beef patties compared with the control and V_C treatment groups. The storage time had no significant effect on the b^* value of beef patty ($P > 0.05$). During initial storage, b^* values of beef patties were significantly reduced ($P < 0.05$) in 0.05% and 0.1% EEOS treatment groups. After the 6th day, there was no significant difference in all treatment groups. Gómez et al. (2020) also reported the L^* , b^* and a^* values of beef wrapped in overwrap decreased gradually during storage.

The bright red color of fresh beef mainly comes from the oxidation of myoglobin to OxyMb (Fernandes et al., 2017). But, the instability of OxyMb is the main reason for the loss of beef red. During storage, the a^* value of beef patties was significantly correlated with storage time and EEOS dosage ($P < 0.05$). On day 12, 0.1% EEOS treatment had the highest a^* value of 10.1, which was higher than 8.9 in V_C treatment and 6.8 in control. It could also be observed from Table 1 that EEOS treatments showed lower ΔE^* , which indicated that they have higher oxidation stability than the control sample. These results were consistent with the studies of Bellucci et al. (2021) and Cando et al. (2014) that red peel pitaya and willowherb extracts could reduce pork or beef patties ΔE^* , and improve the colour stability of meat. This is because polyphenols in natural plant extracts act as antioxidants to effectively slow down the conversion from OxyMb to MetMb (brown). Moreover, the anthocyanins in plant extracts also attracted the attention of researchers. The anthocyanins rich in black rice extract was the reason for the decrease of b^* value, but it was vital to reduce the red loss of beef patties. Otherwise, the colour stability of beef patties was enhanced by anthocyanins in EEOS penetrate surface muscle cells as natural pigments.

Table 1. pH values and colour parameters of beef patties during refrigerated storage.

Parameters	Storage time (days)	Treatments				
		Control	0.01% EEOS	0.05% EEOS	0.1% EEOS	V _C
pH	0	5.57 ± 0.03 ^{Ca}	5.53 ± 0.01 ^{Cb}	5.38 ± 0.01 ^{Cd}	5.45 ± 0.02 ^{Cc}	5.40 ± 0.02 ^{Dd}
	3	5.55 ± 0.04 ^{Ca}	5.56 ± 0.02 ^{Ca}	5.48 ± 0.01 ^{BCb}	5.45 ± 0.02 ^{Cb}	5.47 ± 0.02 ^{CDB}
	6	5.62 ± 0.04 ^{Ca}	5.62 ± 0.09 ^{BCa}	5.50 ± 0.11 ^{BCb}	5.46 ± 0.06 ^{Cc}	5.51 ± 0.09 ^{Cb}
	9	5.71 ± 0.03 ^{Ba}	5.68 ± 0.06 ^{ABab}	5.58 ± 0.11 ^{ABbc}	5.55 ± 0.05 ^{Bc}	5.60 ± 0.04 ^{Bbc}
	12	5.79 ± 0.05 ^{Aa}	5.74 ± 0.04 ^{Aab}	5.69 ± 0.06 ^{Ab}	5.65 ± 0.06 ^{Ab}	5.70 ± 0.02 ^{Ab}
L*	0	31.9 ± 0.64 ^{Ba}	31.9 ± 0.42 ^{ABa}	30.9 ± 0.68 ^{ABa}	31.7 ± 0.58 ^{Aa}	32.5 ± 0.65 ^{ABa}
	3	34.1 ± 0.58 ^{Aa}	33.1 ± 0.25 ^{Aa}	31.5 ± 0.45 ^{Ab}	31.1 ± 0.67 ^{Ab}	33.4 ± 1.02 ^{Aa}
	6	33.5 ± 0.93 ^{Aa}	33.2 ± 1.40 ^{Aab}	31.8 ± 0.43 ^{Bbc}	31.5 ± 0.22 ^{Ac}	33.6 ± 0.45 ^{Aa}
	9	32.0 ± 0.95 ^{Ba}	30.7 ± 1.33 ^{Bbc}	29.1 ± 1.19 ^{Bc}	30.1 ± 0.26 ^{Abc}	31.5 ± 0.42 ^{Bab}
	12	31.6 ± 0.19 ^{Bab}	30.8 ± 0.58 ^{Bbc}	30.4 ± 1.22 ^{ABc}	29.1 ± 0.45 ^{Ad}	32.5 ± 0.05 ^{ABa}
a*	0	11.8 ± 0.49 ^{Aab}	11.9 ± 1.15 ^{Aab}	11.2 ± 0.04 ^{Aab}	10.7 ± 0.93 ^{Ab}	12.3 ± 0.60 ^{Aa}
	3	11.0 ± 0.13 ^{Bb}	11.1 ± 0.05 ^{Ab}	11.1 ± 0.05 ^{Ab}	10.9 ± 0.25 ^{Ab}	11.6 ± 0.20 ^{ABa}
	6	10.4 ± 0.28 ^{Ba}	10.7 ± 0.15 ^{Aa}	10.8 ± 0.63 ^{Aa}	10.7 ± 0.63 ^{Aa}	10.7 ± 1.05 ^{Ba}
	9	8.7 ± 0.47 ^{Cb}	9.2 ± 0.92 ^{Bab}	9.8 ± 0.34 ^{Ba}	10.2 ± 0.51 ^{Aa}	9.5 ± 0.24 ^{Cab}
	12	6.8 ± 0.43 ^{Dd}	7.3 ± 0.37 ^{Ccd}	8.9 ± 0.90 ^{Bb}	10.1 ± 0.31 ^{Aa}	8.0 ± 0.41 ^{Dc}
b*	0	11.2 ± 0.07 ^{Aa}	11.4 ± 0.12 ^{Aa}	10.6 ± 0.12 ^{Ab}	9.5 ± 0.08 ^{Ac}	11.1 ± 0.44 ^{Ab}
	3	11.1 ± 0.55 ^{Aab}	11.1 ± 0.07 ^{Aab}	10.3 ± 0.46 ^{Ab}	9.2 ± 0.27 ^{Ac}	11.4 ± 0.36 ^{Aa}
	6	10.9 ± 0.79 ^{Aab}	11.5 ± 0.54 ^{Aa}	9.4 ± 1.42 ^{Abc}	8.9 ± 1.22 ^{Ac}	11.1 ± 0.71 ^{Ab}
	9	10.9 ± 0.17 ^{Aa}	9.7 ± 1.90 ^{Aa}	9.7 ± 0.87 ^{Aa}	9.9 ± 0.66 ^{Aa}	10.2 ± 0.27 ^{Ba}
	12	10.4 ± 0.67 ^{Aa}	10.1 ± 0.82 ^{Aa}	10.2 ± 0.80 ^{Aa}	9.1 ± 0.16 ^{Aa}	9.8 ± 0.33 ^{Ba}
ΔE*	0-3	2.7 ± 0.41 ^{Ba}	2.1 ± 0.21 ^{Ca}	2.8 ± 0.14 ^{Aa}	2.8 ± 0.22 ^{Aa}	2.5 ± 0.41 ^{Ba}
	0-6	2.9 ± 0.43 ^{Ba}	2.8 ± 0.73 ^{B^Ca}	2.9 ± 0.72 ^{Aa}	2.8 ± 0.48 ^{Aa}	2.7 ± 0.70 ^{Ba}
	0-9	3.4 ± 0.64 ^{Ba}	4.2 ± 0.95 ^{ABa}	3.9 ± 0.21 ^{Aa}	3.5 ± 0.38 ^{Aa}	2.9 ± 0.87 ^{ABa}
	0-12	5.4 ± 0.77 ^{Aa}	5.2 ± 0.15 ^{Aa}	2.9 ± 0.91 ^{Ab}	3.5 ± 0.59 ^{Aab}	4.9 ± 0.41 ^{Aab}

^{A-D}Mean values in the same column (same treatment in different days) with different letters indicate significant difference ($P < 0.05$); ^{a-d}Mean values in the same row (different treatment in the same day) with different letters indicate significant difference ($P < 0.05$); Treatments: Control: patties prepared without antioxidant; 0.01% EEOS: patties prepared with ethanolic extract of onion skin at 100 mg·kg⁻¹; 0.05% EEOS: patties prepared with ethanolic extract of onion skin at 500 mg·kg⁻¹; 0.1% EEOS: patties prepared with ethanolic extract of onion skin at 1000 mg·kg⁻¹; V_C: patties prepared with V_C at 500 mg·kg⁻¹.

3.3 Cooking loss of beef patties

Cooking loss is mainly to research the loss of water in meat, which is of great significance for the meat industry to seek methods to improve the water retention of meat. As presented in Figure 1A, the cooking loss of beef patties reduced gradually during storage, and the addition of EEOS had no significant effect on the cooking loss ($P > 0.05$). On day 0, both treatment groups with 0.05% and 0.1% EEOS showed a higher cooking loss, which may be attributed to the presence of hydrophobic substances in EEOS increased the water exudation in the meat patties. After day 0, the water holding capacity of beef patties decreased, resulting in a large amount of water seepage. This phenomenon was also observed in the samples on day 3, and there was a large amount of juice in the packaging bag. These indicated that the addition of EEOS and V_C could not enhance the water holding capacity of beef patties, which was consistent with some research results of Das et al. (2016) and Hajrawati et al. (2019). Litchi peel extracts and cembra leaf extract did not affect

the cooking loss of meat (Das et al., 2016; Hajrawati et al., 2019). In contrast, grape pomace powder and potatoes extracts reduced the cooking loss of beef patties (Abdelhakam et al., 2019; Colle et al., 2019). They attributed this to the presence of polysaccharides. For example, the cellulose in grape pomace and the starch in potato both could enhance the water holding capacity of meat patties. However, onion skins extracted by 70% ethanol were mainly polyphenols, so it did not affect on the cooking loss of beef patties.

3.4 Texture profile beef patties

The analysis results of texture properties of beef patties during storage were showed in Table 2. There was no significant difference in cohesiveness, gumminess, and chewiness of beef patties between different treatments and storage time ($P > 0.05$). But, the incorporation of EEOS significantly reduced the hardness of beef patties. It was reported that the textural properties of meat are related to myofibrillar proteins because the presence of

Table 2. Effect of EEOS on textural properties of beef patties.

Properties	Storage time(days)	Treatments				
		Control	0.01% EEOS	0.05% EEOS	0.1% EEOS	V _C
Hardness(g)	0	22.40 ± 3.27 ^{Ba}	19.49 ± 1.84 ^{Aa}	19.82 ± 1.20 ^{Aa}	21.89 ± 0.91 ^{Aa}	21.10 ± 1.15 ^{Ba}
	3	22.43 ± 1.54 ^{Bab}	19.72 ± 1.34 ^{Abc}	21.26 ± 1.46 ^{Ac}	24.11 ± 0.71 ^{Aa}	22.43 ± 0.95 ^{Bab}
	6	28.83 ± 1.03 ^{Aa}	20.78 ± 1.83 ^{Ab}	19.18 ± 1.01 ^{Ab}	20.53 ± 2.69 ^{Ab}	28.68 ± 1.14 ^{Aa}
	9	21.74 ± 1.38 ^{Bab}	17.65 ± 2.03 ^{Ab}	20.23 ± 3.17 ^{Aab}	21.68 ± 2.55 ^{Aab}	22.92 ± 2.45 ^{Ba}
	12	24.87 ± 1.84 ^{Ba}	18.29 ± 0.54 ^{Ab}	19.84 ± 2.12 ^{Ab}	23.64 ± 1.48 ^{Aa}	20.17 ± 1.31 ^{Bb}
Cohesiveness	0	0.22 ± 0.02 ^{Aa}	0.23 ± 0.01 ^{Aa}	0.24 ± 0.02 ^{Aa}	0.24 ± 0.01 ^{Aa}	0.24 ± 0.01 ^{Aa}
	3	0.24 ± 0.01 ^{Aa}	0.25 ± 0.02 ^{Aa}	0.24 ± 0.02 ^{Aa}	0.24 ± 0.01 ^{Aa}	0.26 ± 0.01 ^{Aa}
	6	0.22 ± 0.01 ^{Aab}	0.22 ± 0.02 ^{Aab}	0.23 ± 0.01 ^{Aab}	0.24 ± 0.01 ^{Aa}	0.20 ± 0.04 ^{Ab}
	9	0.23 ± 0.01 ^{Aa}	0.24 ± 0.01 ^{Aa}	0.26 ± 0.03 ^{Aa}	0.26 ± 0.02 ^{Aa}	0.23 ± 0.01 ^{Aa}
	12	0.24 ± 0.02 ^{Aa}	0.24 ± 0.02 ^{Aa}	0.24 ± 0.01 ^{Aa}	0.24 ± 0.01 ^{Aa}	0.24 ± 0.01 ^{Aa}
Springiness (mm)	0	0.95 ± 0.12 ^{ABab}	0.86 ± 0.02 ^{ABb}	0.88 ± 0.05 ^{ABb}	1.08 ± 0.13 ^{Aa}	0.83 ± 0.03 ^{Cb}
	3	0.92 ± 0.08 ^{ABa}	0.83 ± 0.04 ^{ABa}	0.93 ± 0.13 ^{ABa}	0.96 ± 0.12 ^{Aa}	0.90 ± 0.03 ^{BCa}
	6	1.00 ± 0.15 ^{Aa}	1.02 ± 0.16 ^{Aa}	1.02 ± 0.03 ^{Aa}	0.92 ± 0.03 ^{Aa}	1.00 ± 0.09 ^{BCa}
	9	0.79 ± 0.03 ^{Ba}	0.72 ± 0.11 ^{Ba}	0.78 ± 0.04 ^{Ba}	0.88 ± 0.14 ^{Aa}	0.88 ± 0.09 ^{ABa}
	12	0.88 ± 0.08 ^{ABab}	0.75 ± 0.07 ^{Bb}	0.81 ± 0.19 ^{ABb}	0.91 ± 0.14 ^{Aab}	1.09 ± 0.11 ^{Aa}
Gumminess (g)	0	5.34 ± 1.16 ^{ABa}	4.44 ± 0.35 ^{Aa}	4.79 ± 0.56 ^{Aa}	5.15 ± 0.11 ^{Aa}	5.93 ± 0.30 ^{Aa}
	3	5.48 ± 0.57 ^{ABa}	4.84 ± 0.01 ^{Aa}	5.08 ± 0.74 ^{Aa}	5.71 ± 0.36 ^{Aa}	5.74 ± 0.15 ^{Aa}
	6	6.51 ± 0.32 ^{Aa}	4.48 ± 0.28 ^{Ac}	4.51 ± 0.21 ^{Ac}	4.82 ± 0.70 ^{Ac}	5.63 ± 0.18 ^{Ab}
	9	4.92 ± 0.19 ^{Ba}	4.16 ± 0.60 ^{Aa}	5.34 ± 1.55 ^{Aa}	5.54 ± 0.99 ^{Aa}	5.34 ± 0.66 ^{Aa}
	12	6.03 ± 0.61 ^{ABa}	4.47 ± 0.44 ^{Ac}	4.67 ± 0.49 ^{Ac}	5.75 ± 0.28 ^{Aab}	4.92 ± 0.62 ^{Abc}
Chewiness (g·m)	0	4.87 ± 0.48 ^{ABa}	4.90 ± 0.95 ^{Aa}	4.07 ± 0.52 ^{Aa}	5.27 ± 0.38 ^{Aa}	4.24 ± 0.24 ^{Aa}
	3	5.03 ± 0.84 ^{ABab}	3.99 ± 0.20 ^{Ab}	4.79 ± 0.37 ^{Aab}	5.68 ± 0.31 ^{Aa}	5.17 ± 0.10 ^{Aab}
	6	6.52 ± 1.07 ^{Aa}	4.49 ± 0.46 ^{Aa}	4.62 ± 0.37 ^{Aa}	5.27 ± 0.70 ^{Aa}	5.61 ± 0.69 ^{Aa}
	9	3.92 ± 0.31 ^{Ba}	3.05 ± 0.87 ^{Aa}	4.20 ± 0.46 ^{Aa}	4.87 ± 0.93 ^{Aa}	4.74 ± 1.03 ^{Aa}
	12	5.29 ± 0.03 ^{ABa}	3.32 ± 0.70 ^{Aa}	4.75 ± 0.75 ^{Aa}	5.25 ± 0.86 ^{Aa}	5.42 ± 1.06 ^{Aa}

^{A-C} Mean values in the same column (same treatment in different days) with different letters indicate significant difference ($P < 0.05$); ^{a-c} Mean values in the same row (different treatment in the same day) with different letters indicate significant difference ($P < 0.05$); Treatments: Control: patties prepared without antioxidant; 0.01% EEOS: patties prepared with ethanolic extract of onion skin at 100 mg·kg⁻¹; 0.05% EEOS: patties prepared with ethanolic extract of onion skin at 500 mg·kg⁻¹; 0.1% EEOS: patties prepared with ethanolic extract of onion skin at 1000 mg·kg⁻¹; V_C: patties prepared with V_C at 500 mg·kg⁻¹.

free radicals produced by lipid oxidation and microbial pollution would accelerate the degradation of myofibrillar proteins (Jiao et al., 2020; Patinho et al., 2021). But, myofibrillar proteins might be inhibited by the flavonoids in onion skin due to the effects of antioxidation and bacteriostasis. This also explained why storage time has no significant effect on the springiness of beef patties treated with 0.1% EEOS. And at the end of storage, the springiness and hardness of 0.1% EEOS treatment were better than those of V_C treatment. Jiao et al. (2020) believed that the free radicals produced by lipid oxidation would induce the cross-links between protein and protein or lipid, but the antioxidant and antibacterial effects of polyphenols could inhibit the degradation of myofibrillar proteins, to stabilize the protein cross-links network. Bellucci et al. (2021) found that pitaya extract had little effect on texture parameters of pork patties except for cohesiveness. Pérez-Báez et al. (2020) obtained different results that roselle extract and potato peel flour significantly reduced

the hardness, cohesivity, and springiness of beef patties. Such different effects for springiness, hardness, cohesiveness, and chewiness were also noted by Öztürk & Turhan. (2020) in beef meatballs added with pumpkin seed kernel flour. He attributed these discrepancies of texture parameters to the differences in the composition, quantity and source of natural substances in different formula meat products (Öztürk & Turhan, 2020).

3.5 MetMb content of beef patties

The colour of meat mainly comes from pigment, such as myoglobin. During the cutting process of fresh meat, once myoglobin is exposed to oxygen, it will quickly oxidize to OxyMb (bright red), which is a colour that consumers are willing to wear (Hernández Salueña et al., 2019). With the prolonged storage time, MetMb accumulated continuously, and the meat showed disappointing brown. However, the content of MetMb

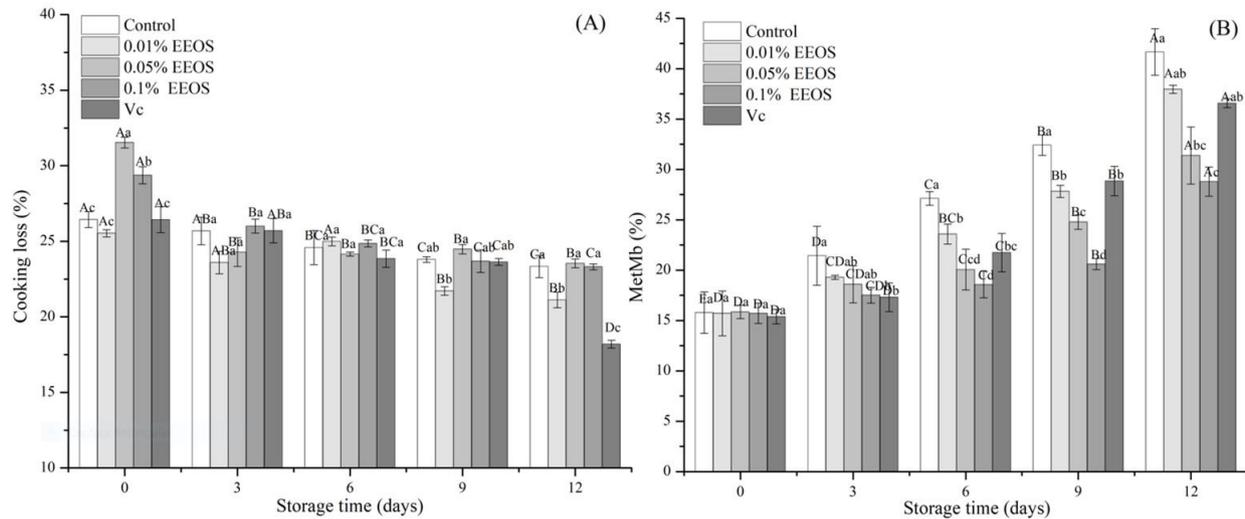


Figure 1. Cooking loss and MetMb content of beef patties during refrigerated storage. ^{A-E} Mean values in same treatment in different days indicate significant difference ($P < 0.05$); ^{a-d} Mean values in different treatment in the same day indicate significant difference ($P < 0.05$); Treatments: Control: patties prepared without antioxidant; 0.01% EEOS: patties prepared with ethanolic extract of onion skin at $100 \text{ mg}\cdot\text{kg}^{-1}$; 0.05% EEOS: patties prepared with ethanolic extract of onion skin at $500 \text{ mg}\cdot\text{kg}^{-1}$; 0.1% EEOS: patties prepared with ethanolic extract of onion skin at $1000 \text{ mg}\cdot\text{kg}^{-1}$; V_c : patties prepared with V_c at $500 \text{ mg}\cdot\text{kg}^{-1}$.

was significantly reduced ($P < 0.05$) by the addition of EEOS and presented a dose-dependent effect (Figure 1B). On day 0, there was no significant difference among the treatment groups ($P > 0.05$). The content of MetMb in beef patties increased with the extension of storage time. At the end of storage, the content of MetMb in the control was 41.66%. Compared with that, the content of MetMb in EEOS treatments (0.01%, 0.05% and 0.1%) decreased by 8.91%, 24.68% and 30.89%, respectively. The effect of inhibiting MetMb accumulation was better than V_c when the dosage of EEOS was greater than 0.05%. This was due to the presence of polyphenols in EEOS. Papuc et al. (2018) thought that quercetin, kaempferol, and myricetin are strong reducers, which could reduce MetMb to Oxymb. In addition, Chauhan et al. (2019) also found that the inhibition of *Terminalia arjuna* fruit extracts on lipid and OxyMb was the reason for the decrease of MetMb content in pork. The primary and secondary products of lipid oxidation would accelerate the accumulation of MetMb. The accumulation of MetMb was reduced by the inhibitory effect of EEOS on lipid oxidation and OxyMb loss. Similar conclusions were also obtained in both experiments of pork with ethanolic extracts from *Morus alba L.* leaves or hawthorn berry (Cui et al., 2021; Papuc et al., 2018).

3.6 TBARS value of beef patties

As an important component of meat, lipid can improve meat flavor and water retention. However, the lipid is easily oxidized and deteriorated in the presence of light, radiation, pro-oxidants or reactive oxygen species (Cunha et al., 2018). Lipid oxidation is a complex chain reaction process, which promotes the oxidative decomposition of fatty acids by generating free radicals and produces secondary oxidation products such as aldehydes, ketones, and alkanes that affect the sensory and flavor of meat (Cunha et al., 2018; Reddy et al., 2018).

As showed in Figure 2A, TBARS value increased with the extension of storage time, and the addition of EEOS significantly reduced the TBARS value of beef patties ($P < 0.05$). The TBARS value of each treatment group had no significant difference at the initial of storage but increased sharply from day 6. At the end of the storage, the TBARS value of control increased from 0.07 to 1.23 mg MDA/kg . Compared with the control, adding 0.1% EEOS could effectively reduce TBARS by 44.72%. Furthermore, the TBARS value of 0.5% EEOS treatment was close to that of V_c treatment, which indicated that EEOS possessed a stronger ability to inhibit the formation of secondary oxidation products of lipid. The chain initiation of lipid oxidation could be inhibited by scavenging free radicals by polyphenols in onion skins (Reddy et al., 2018). Meanwhile, polyphenols could also chelate metal ions (such as Fe^{2+} , Fe^{3+}) to reduce the generation of oxidants, to inhibit lipid oxidation (Reddy et al., 2018).

Traditionally, meat with a TBARS value of less than 6.6 mg MDA/kg was defined as fresh meat (Jiao et al., 2020). TBARS values of beef patties treated with EEOS (0.05% and 0.1%) and V_c were still lower than 0.66 mg MDA/kg on day 9. On day 12, only the TBARS value of 0.68 mg MDA/kg in the 0.1% EEOS treatment was close to the fresh meat standard. In general, the addition of 0.1% EEOS could prolong the shelf-life of the beef patty to the 9th day. A low TBARS value of beef patties was maintained by thinned young kiwifruit extract on the 7th day of storage (Jiao et al., 2020). It is reported that the ethanolic extract from *Morus alba L.* leaves could significantly inhibit pork lipid oxidation (Cui et al., 2021). Chauhan et al. (2019) also proved that 60% ethanol extract of *Terminalia arjuna* fruit significantly reduced TBARS of pork by 60.25%. All these studies showed that natural plants extract can effectively inhibit meat lipid oxidation, and have great potential for the research and application of natural antioxidants.

3.7 Carbonyl content of beef patties

Protein in meat is oxidized directly induced by reactive oxygen species or indirectly induced by lipid oxidation products, myoglobin or metal catalysts, bringing on the formation of protein carbonyl (Cunha et al., 2018). The formation of carbonyl can lead to protein degradation, fracture or cross-linking, resulting in changes in meat colour and texture and loss of nutritional quality (Chauhan et al., 2019). Therefore, carbon content becomes an important index to evaluate meat protein oxidation. The effect of EEOS on carbonyl contents of beef patties during storage as illustrated in Figure 2B. During the storage, the carbonyl content increased significantly ($P < 0.05$) due to oxidation of proteins, and at the end of storage, the carbonyl content of the control increased from 4.05 nmol/mg protein to 18.41 nmol/mg protein. In general, carbonyl contents in the control increased by 4.5 times, and carbonyl content in the treatments added with 0.01%, 0.05%, 0.1% EEOS, and V_C increased by 4.4, 3.8, 3.3 and 4.1 times respectively. This indicated that EEOS was superior to V_C in inhibiting protein oxidation, which is similar to the inhibition of EEOS on lipid oxidation. Chauhan et al. (2019) thought that the formation of carbonyl was mainly related to the interaction of aldehydes produced by lipid oxidation and protein.

The incorporation of 0.05% and 0.1% EEOS significantly ($P < 0.05$) reduced the level of protein carbonyl and presented a dose-dependent effect. Similar studies found that compared with control, the protein oxidation inhibition rate of pork patties treated with different concentrations of pitaya extract was 24%-30% (Bellucci et al., 2021).

At day 10 of storage, the carbonyl content of the treatment added clove extract and V_C decreased significantly and was lower than that of the control and BHT (Zahid et al., 2020). Natural

polyphenols might act on protein free radical chain reaction, thereby blocking the protein oxidation pathway. Cunha et al. (2018) thought that when reactive oxygen species remove hydrogen atoms from proteins, they will generate carbon-centered free radicals ($P\bullet$), which were converted into peroxy radicals ($POO\bullet$) in the presence of oxygen, and then convert alkyl peroxide ($POOH$) by obtaining hydrogen atoms from another molecule. EEOS could inhibit the free radical chain reaction of proteins by preventing the formation of free radicals and the reproduction of reactive oxygen species (Reddy et al., 2018). In addition, $POOH$ may continue to react with Fe^{2+} or free peroxy radical ($HO_2\cdot$) to form alkoxy groups (Cunha et al., 2018).

Flavonoids in EEOS might also act on this process, chelating metal ions and removing them freely (Reddy et al., 2018).

3.8 TVB-N content of beef patties

TVB-N is a class of volatile substances containing ammonia and amines, which were produced by endogenous enzymes and spoilage bacteria decomposing proteins and other nitrogen (Jiao et al., 2020). Storage time and exogenous substances had significant ($P < 0.05$) effects on the TVB-N values of beef patties (Figure 3A). The TVB-N values gradually increased with the extension of storage time, which was consistent with the study of Fan et al. (2019). During the storage process, the spoilage flora continued to increase, and the TVB-N value of the control (15.63 mg/100 g) exceeded the first-class fresh meat standard of 15 mg/100 g on day 6 (Cui et al., 2021). The treatments supplemented with EEOS and V_C remained at the fresh meat level. On day 9, the TVB-N values of the control and 0.01% EEOS treatment both transcended the standard of second-class fresh meat by 20 mg/100 g (Cui et al., 2021). At the end of storage,

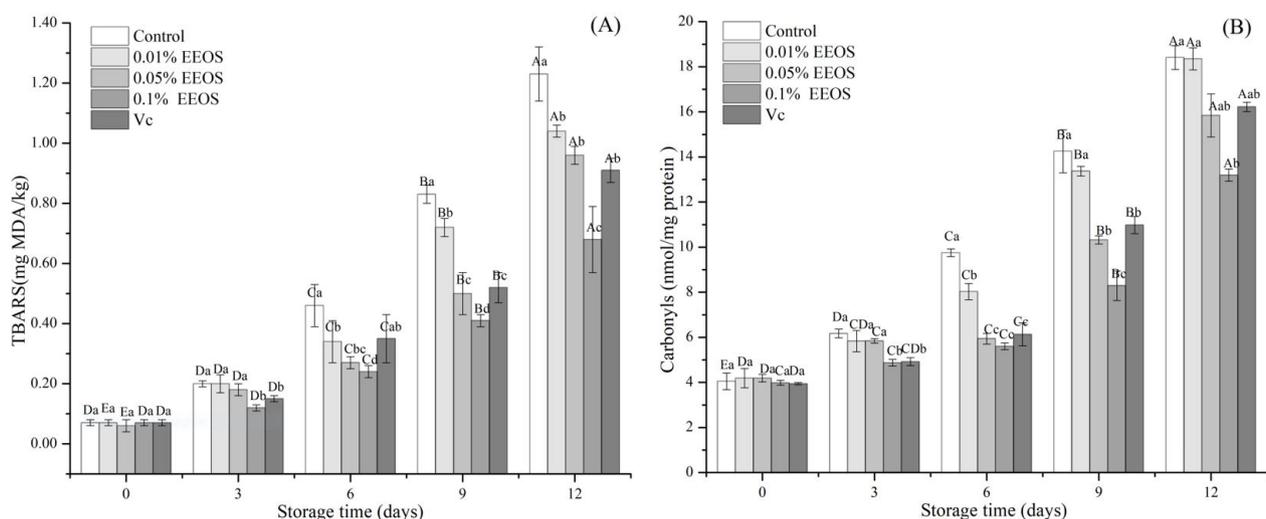


Figure 2. Evolution of TBARS values (A) and total carbonyl content (B) in Beef patties during refrigerated storage. ^{A-E} Mean values in same treatment in different days indicate significant difference ($P < 0.05$); ^{a-d} Mean values in different treatment in the same day indicate significant difference ($P < 0.05$); Treatments: Control: patties prepared without antioxidant; 0.01% EEOS: patties prepared with ethanolic extract of onion skin at 100 mg·kg⁻¹; 0.05% EEOS: patties prepared with ethanolic extract of onion skin at 500 mg·kg⁻¹; 0.1% EEOS: patties prepared with ethanolic extract of onion skin at 1000 mg·kg⁻¹; V_C : patties prepared with V_C at 500 mg·kg⁻¹.

only the TVB-N value (20.72 mg/100 g) of 0.1% EEOS treatment was close to the second-class fresh meat standard. This showed that 0.1% EEOS can extend the shelf-life of beef patties to the 9th day. This was similar to the report of Cui et al. (2021), the ethanolic extract from *Morus alba* L. leaves still maintained the chilled pork at the fresh meat level on day 9. Jiao et al. (2020) found that thinned young kiwifruit extract was better than epicatechin and potassium sorbate in prolonging the shelf-life of beef. These studies showed that natural antioxidants have a significant effect on prolonging the shelf life of meat. According to a previous report, TVB-N was not only related to microbial growth but also related to meat oxidation (Jiao et al., 2020). The existence of EEOS in beef patties inhibited the oxidation of lipid and protein, on the other hand, it might also inhibit the growth of microorganisms.

3.9 Total plate count

The reproduction of microorganisms will accelerate the oxidative decomposition of fat and protein and greatly shorten the shelf-life of fresh meat. Figure 3B exhibited the effects of storage time and antioxidants on the microbial flora of beef patties. The addition of EEOS significantly reduced the total plate count of microbial ($P < 0.05$). On day 0, the number of microorganisms in each treatment was 3.18-3.28 \log_{10} CFU/g, and there was no significant difference ($P > 0.05$). After day 6, the total plate count of microbial in control increased sharply, which might be due to the microbial growth is in the logarithmic growth stage. The beef patties with EEOS and V_c could still maintain a low total plate count of microbial.

The total plate count of microbial reached 7 \log_{10} CFU/g and was identified as rotten meat (Gómez et al., 2020). On day 9, only

the total plate count of microbial in the control (7.26 \log_{10} CFU/g) exceeded 7 \log_{10} CFU/g. At the end of storage, all treatments reached the standard of rotten meat, but the microbial pollution degree of beef patties added with 0.1% EEOS was much lower than that of V_c treatment. Ham et al. (2019) reported that V_c had no significant effect on the total plate count of chicken breast. Some scholars had found that natural polyphenols can interact with various components on bacterial cell walls or cell membranes to change their structure and function so that bacteria lose structural integrity (Prommachart et al., 2020). It was reported that red grapes pomace powder significantly reduced the total bacterial count in hamburgers, and ethanolic extract from *Morus alba* L. leaves could inhibit the microbial growth of chilled pork (Abdelhakam et al., 2019; Cui et al., 2021).

3.10 Sensory analysis

As an important means to evaluate the new formulation of beef patties, sensory characteristics are also a parameter to predict consumer acceptability (Chauhan et al., 2019). As shown in Figure 4A, the addition of EEOS improved the colour score of beef patties. At the beginning of storage, there was no significant difference among the treatments ($P > 0.05$). On day 12, the average score of each group added EEOS was 4.28, much higher than that of the control 2.83. The researchers believed that the loss of beef red is related to the accumulation of MetMb, and OxyMb is constantly transformed into MetMb during storage (Chauhan et al., 2019). This might be the reason for the continuous decline of colour score, but the addition of EEOS and V_c could significantly inhibit the generation of MetMb. Bellucci et al. (2021) reported that the higher a^* values were obtained in pork patties containing pitaya extract.

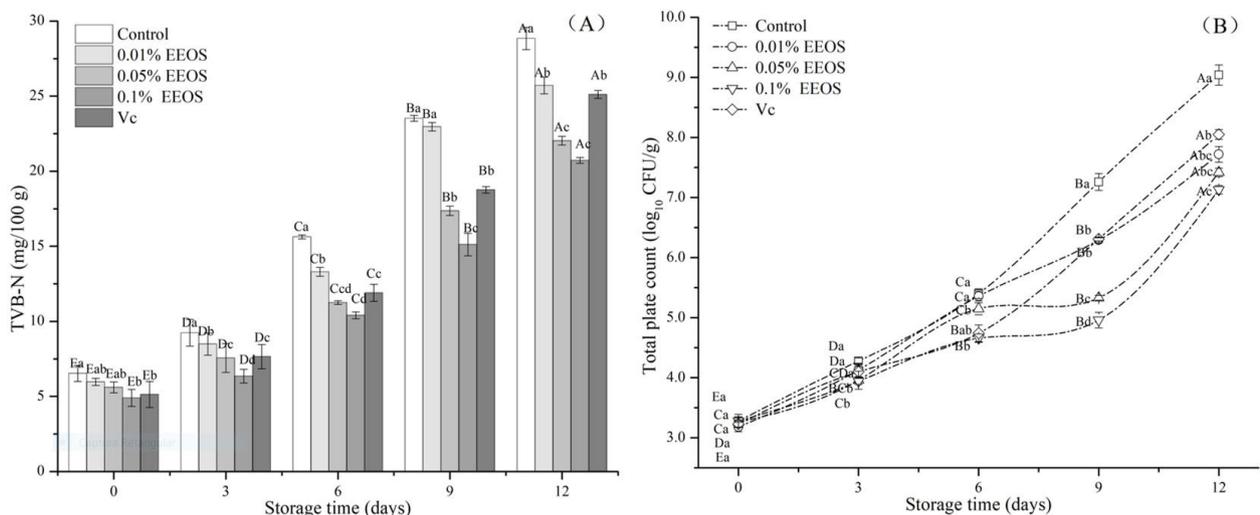


Figure 3. TVB-N content (A) and total plate count (B) of beef patties during refrigerated storage. ^{A-E}Mean values in same treatment in different days indicate significant difference ($P < 0.05$); ^{a-d}Mean values in different treatment in the same day indicate significant difference ($P < 0.05$); Treatments: Control: patties prepared without antioxidant; 0.01% EEOS: patties prepared with ethanolic extract of onion skin at 100 $\text{mg}\cdot\text{kg}^{-1}$; 0.05% EEOS: patties prepared with ethanolic extract of onion skin at 500 $\text{mg}\cdot\text{kg}^{-1}$; 0.1% EEOS: patties prepared with ethanolic extract of onion skin at 1000 $\text{mg}\cdot\text{kg}^{-1}$; V_c : patties prepared with V_c at 500 $\text{mg}\cdot\text{kg}^{-1}$.

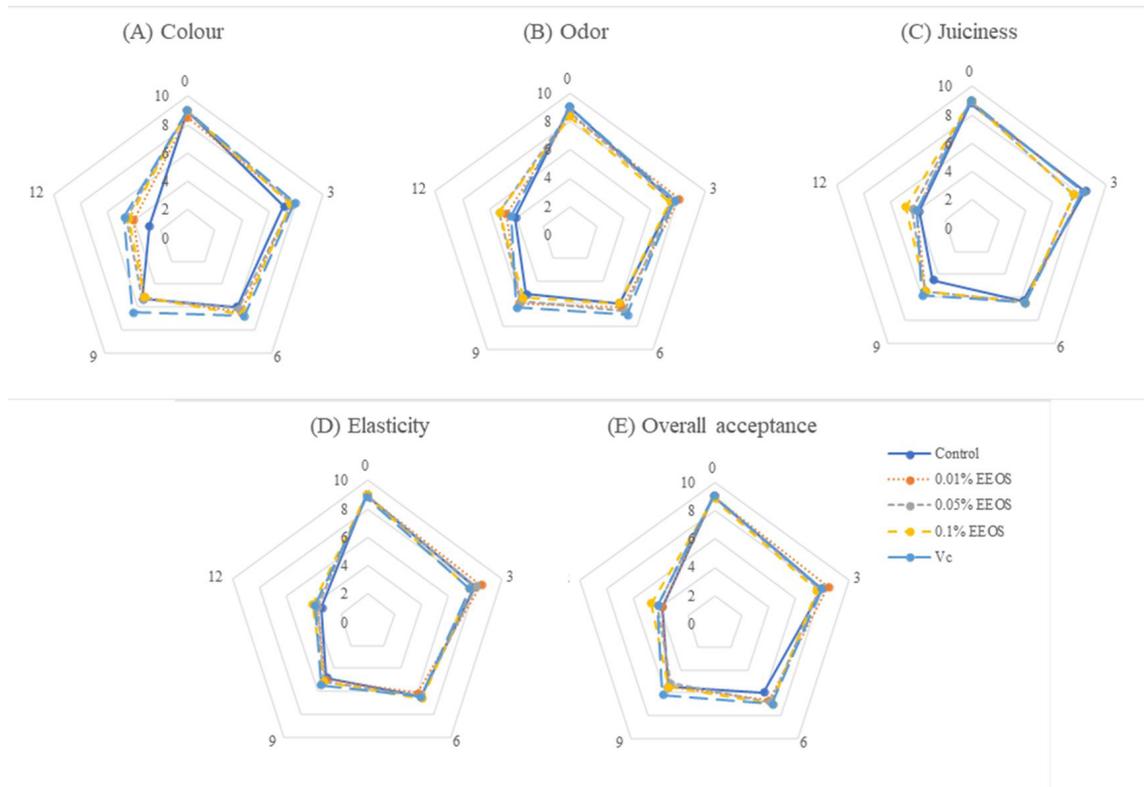


Figure 4. Sensory analysis of beef patties with the addition of EEOS. Treatments: Control: patties prepared without antioxidant; 0.01% EEOS: patties prepared with ethanolic extract of onion skin at $100 \text{ mg}\cdot\text{kg}^{-1}$; 0.05% EEOS: patties prepared with ethanolic extract of onion skin at $500 \text{ mg}\cdot\text{kg}^{-1}$; 0.1% EEOS: patties prepared with ethanolic extract of onion skin at $1000 \text{ mg}\cdot\text{kg}^{-1}$; V_c : patties prepared with V_c at $500 \text{ mg}\cdot\text{kg}^{-1}$.

In addition, EEOS had no significant influence on the juiciness and elasticity of beef patties (Figure 4C and D). But, EEOS produced a positive effect on the odor of beef patties because it contains onion specific flavor precursors sulfur and alk(en)yl cysteine sulphoxides (Bedrniček et al., 2019) (Figure 4B). It was reported that onion peel powder also has a positive effect on the pleasantness of the odor of fish sausages (Bedrniček et al., 2020). Overall, the beef patty with 0.1% EEOS received the best evaluation and was more popular with panelists than the V_c treatment. This conclusion could also be drawn from the overall acceptability of beef patties (Figure 4E). Fan et al. (2019) found that 1.0% *Portulaca oleracea L.* extract can improve the odor, texture, taste, and overall acceptability of cooked pork slices. Jiao et al. (2020) even concluded that thinned young kiwifruit extract would not adversely affect the sensory characteristics of beef and could be incorporated into the meat as a natural antioxidant.

4 Conclusions

In this study, onion skins extract, as an important source of quercetin, exhibited great potential in improving the storage stability of beef patties. The pH and colour stability of beef patties were improved by EEOS. The contents of MetMb, TBARS, and carbonyl reduced with the increase of EEOS. This dose-dependent effect was due to the abundance of polyphenols in EEOS. At the end of storage, the incorporation of EEOS significantly inhibited microbial growth and reduced TVB-N values. 0.1% EEOS not only

prolonged the freshness of beef patty to the 9th day but also had a positive impact on sensory characteristics, which was superior to the recognized antioxidant V_c . This shows that the recycling of onion skins can realize waste resources, and its application in meat preservation can improve meat storage stability and extend shelf-life.

Notes

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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