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ARTICLE

Effects of Loquat (*Eriobotrya japonica* Lindl.) Leaf Extract with or without Ascorbic Acid on the Quality Characteristics of Semi-Dried Restructured Jerky during Storage

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Abstract Deterioration of jerky during storage is a major concern; this is usually combated with natural or synthetic antioxidants. This study aimed to evaluate the quality characteristics of semi-dried restructured jerky with and without loquat leaf extract (LE) powder and ascorbic acid (AA) during storage for 180 days. The jerkies were formulated with 0%, 0.15%, and 0.3% LE and/or 0.05% AA (Control, no antioxidant; AA, 0.05% AA; LE 0.15, 0.15% loquat LE; LE 0.15-AA, 0.15% loquat LE+0.05% AA; LE 0.3, 0.3% loquat LE; LE0.3-AA, 0.3% loquat LE+0.05% AA). LE is a phenolic compound, whose 1,1-diphenyl-2-picrylhydarzyl radical scavenging activity and metal chelating activity were found to be higher than AA. All antioxidant combinations having higher LE concentration and containing AA were effective in delaying protein and lipid oxidation compared to the control or AA. At the end of storage period, LE 0.15-AA and AA had higher CIE a* and lower shear force than the control. Therefore, the combination of 0.15% LE and 0.05% AA can result in reduced protein and lipid oxidation without any negative effect on the quality characteristics of semi-dried restructured jerky.

Keywords semi-dried, restructured jerky, loquat leaf, ascorbic acid, sulfhydryl concentration

Introduction

Jerky is a traditional dried meat product preserved by drying to reduce water activity; it is convenient, has a rich nutrient content, and is shelf-stable without refrigeration (Coradini et al., 2019). Jerky needs to be dried to a water activity value of ≤0.85 to achieve stability (Triyannanto and Lee, 2016). However, the drying process leads to a tough texture of jerky (An et al., 2010). While semi-dried or restructured jerky has a softer texture, it has high water activity resulting in protein and lipid oxidation (Yang et al., 2009), which is a major cause of deterioration of jerky quality during storage (Kim et al., 2022; Wongwiwat and Wattanachant, 2015).

Control or minimization of protein and lipid oxidation during storage of meat products can be accomplished using synthetic or natural antioxidants (Kim et al., 2014). However, the safety of synthetic antioxidants, such as 2,6-dibutyl-hydroxytoluene (BHT) and t-butyl-4-hydroxyanisole (BHA), is a concern, with respect to consumer health, due to their potential toxicological effects (Nassu et al., 2003). Therefore, demand for natural antioxidants has increased in the recent years (Xu et al., 2018). Use of natural antioxidants is considered safe and is readily accepted by consumers; moreover, the legislation does not require safety tests in case of "generally recognized as safe (GRAS)" (Nassu et al., 2003).

Ascorbic acid (AA) has been widely accepted to improve storage stability of meat products (Haak et al., 2009). It is approved as a GRAS substance and acts as a synergist when applied in combination with other antioxidants, promoting their antioxidant activity as well (Hwang et al., 2013). Li et al. (2013) had shown that plant polyphenols in combination with AA delayed lipid oxidation of dry-cured sausages, thereby maintaining their storage quality. Hwang et al. (2013) had reported that a combination of *ganghwayakssuk* extract and AA delayed lipid oxidation in raw chicken patties during storage, hence extending their shelf life.

Loquat (*Eriobotrya japonica* Lindl.) is a medicinal plant belonging to the Rosaceae family (Fu et al., 2019). It is commercially cultivated in Korea, India, Italy, and many other countries, and loquat leaf can be harvested regardless of the season (Dhiman et al., 2021). Pharmacological studies have shown that loquat leaf extract powders (LE) contain abundant polyphenols, including ellagic acid, chlorogenic acid, and neochlorogenic acid, which have anti-oxidant and anti-inflammatory effects (Kim et al., 2019). Till date, some studies applied loquat leaf to foods such as dumpling or fish cake (Park, 2012; Park, 2014). However, there has been no studies about meat product using loquat leaf, despite the high antioxidant capacity of loquat leaf. Accordingly, this study hypothesized that using loquat leaves has the potential to delay lipid oxidation values in meat products, and which could be further improved when combined with AA.

Thus, the objective of this study was to investigate the synergistic effects of loquat LE powder and AA on quality characteristics of semi-dried restructured jerky during storage at room temperature.

Materials and Methods

Preparation of loquat (*E. japonica* Lindl.) leaf extract powder

To prepare loquat LE powder, loquat leaf was purchased from Handsherb (Yeongcheon, Korea). The leaves were rinsed and dried in shade. The dried leaves were extracted with 80% ethyl alcohol at 60℃ for 3 h and the solvent was evaporated at 45℃ for 48 min in a vacuum extractor-concentrator (SSEE-1, SSE, Bucheon, Korea). The extract was then stored at –45℃ for 10 h at 2 mTorr and made into a powder using a freeze dryer (VTFD, Ilshin, Korea). The same procedure was repeated three times and the pH and color value of LE were as follows: pH=4.38±0.01, CIE L*=47.90±0.04, CIE a*=–4.86±0.02, and CIE b*=35.37±0.03.

Total phenolic content

The amount of total phenols in LE and AA was determined using the Folin-Ciocalteu spectrophotometric method (Singleton and Rossi, 1965). The LE and AA solutions were prepared at a concentration of 5 mg/mL in 80% ethanol and distilled water (DW), respectively. Gallic acid was used as a standard, and the total phenolic content of LE and AA was expressed as gallic acid equivalents (GAE; μg GAE/mL).

1,1-Diphenyl-2-picrylhydarzyl (DPPH) radical scavenging activity

DPPH radical scavenging activity of LE and AA was determined based on the method described by Jung and Sim (2019). The activity was determined using linear regression of the concentration-response curve of the percentage of DPPH radical inhibition versus various sample concentrations. The IC_{50} value was expressed as the quantity of sample necessary to decrease the DPPH radical inhibition by 50%.

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

The ABTS radical scavenging capacity of LE and AA was determined according the method described by Omoba et al. (2015). The activity was determined using linear regression of the concentration-response curve of the percentage of ABTS radical inhibition versus various sample concentrations. The IC_{50} value was expressed as the quantity of sample necessary to decrease the ABTS radical inhibition by 50%.

Metal chelating activity

The chelation of ferrous ions by LE and AA was estimated according to the method described by Decker and Welch (1990). The activity was determined using linear regression of the concentration-response curve of the percentage of ferrous ion chelation versus various sample concentrations. The IC_{50} value was expressed as the quantity of sample required to chelate Fe^{2+} ions by 50%.

Preparation of semi-dried restructured jerky

Fresh pork ham (*Musculus semimembranosus, Musculus semitendinosus, Musculus biceps femoris*) was obtained from a local market (Jeonju, Korea) and ground (Φ-8 mm). Binding meat batter was prepared by mixing the ground meat (20%) with a gelatin solution (0.2% phosphate and 1% duck skin gelatin) for 1 min in a silent cutter (Nr-963009, Hermann Scharfen GmbH & Co., Postfach, Germany). Semi-dried restructured jerky batter was obtained by mixing the binding meat batter and ground meat (80%) with ice water (10%), soy source (3%), sugar (2.0%), salt (1.2%), carrageenan (0.3%), black pepper powder (0.15%), garlic powder (0.15%), onion powder (0.15%), and sodium nitrite (0.005%). The amount of each ingredient was calculated relative to the total ground meat weight. The binding meat batter, ground meat, and other ingredients were mixed in a silent cutter for 2 min. The resulting jerky batter was divided into six batches. Each batch of samples comprised of restructured jerkies with different levels of loquat LE powder (0%, 0.15%, and 0.3%) with or without AA (0% and 0.05%). The homogenized meat jerky batter was stuffed into a cellulose casing (Viskase Sale, Chicago, IL, USA: Φ-20 mm) and each jerky was prepared as 20-cm-long pieces. Samples dried at 55℃ for 90 min in a chamber (MAXi3501 chamber, Kerres, Postfach, Germany) were removed from the casing, and the drying process was carried out as follows: 55℃ (30 min) → 65℃ (180 min) → 80℃ (60 min) (Kim et al., 2020). The semi-dried restructured jerkies were vacuum-packed in polyethylene bags using a vacuum packager (HFV-500, Fujee, Hwaseong, Korea) and placed at room temperature for 180 days. The samples were taken on 1, 20, 45, 90, 135, and 180 day for different quality parameters. This study was independently repeated thrice (three batches).

Sulfhydryl concentration

The sulfhydryl concentration was measured by estimating protein oxidation using the method described by Berardo et al.

(2015). The sulfhydryl group was detected via its reaction with 5,5′-dithio-bis-2-nitrobenzoic acid to form 5-mercapto-2-nitrobenzioc acid, and the absorbance was measured at 412 nm using a microplate reader (SpectraMax Plus 384, Molecular Devices, CN, USA). Molar extinction coefficient of 14,000 mol/(L·cm) was used to calculate the sulfhydryl concentration in the jerkies, which was expressed as nmol/mg protein concentration.

Thiobarbituric acid-reactive substances (TBARS)

To analyze lipid oxidation, the TBARS value was measured according to the method of Tarladgis et al. (1960). Each restructured jerky sample (5 g) was homogenized with 50 mL of DW and 0.2 mL of 0.3% butylated hydroxytoluene (Sigma-Aldrich, St. Louis, MO, USA) in methanol (Daejung, Goryeong, Korea), and was transferred to a distillation flask. The homogenate was distilled with 47.5 mL of DW, 2.5 mL of 4 N HCl (Samchun, Seoul, Korea), and 1 mL of anti-foaming agent (KMK073, Shin-Etsu Silicone, Seoul, Korea), and 30 mL of the distillate was collected therefrom. Next, 5 mL of 0.02 M TBA (Sigma-Aldrich) in 90% acetic acid (Junsei Chemical, Tokyo, Japan) was added to each test tube containing 5 mL of the distillate and mixed. The tubes were capped and heated for 35 min at 100℃ and subsequently cooled under tap water (15℃). Absorbance of the supernatant was measured at 538 nm using a microplate reader (SpectraMax Plus 384, Molecular Devices). The amount of malondialdehyde (MDA) was calculated using a standard curve of 1,1,3,3-tetraethoxypoppane, and the TBARS value was reported as mg MDA per kg of sample.

pH

Approximately 5 g of the sample was homogenized for 1 min in 20 mL DW; pH of the homogenate was determined using a pH meter (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) calibrated with pH at 4, 7, and 10 standard solutions (Mettler-Toledo GmbH).

Color

The color of restructured jerkies during storage was measured as CIE (International Commission on Illumination) L*a*b* values by the colorimeter (CR-410, Minolta, Tokyo, Japan, D_{65} light source, 2° observer). The instrument was calibrated using a standard white plate (L*=+97.83, a*=–0.43, and b*=+1.98).

Shear force

The sample was cut into 3 cm lengths dimension for shear force analysis, and shear force was determined using a texture analyzer (TA-XT plus, Stable Micro Systems, Surrey, UK), and technical replicates were nine per sample.

Moisture content and water activity

The moisture content of semi-dried restructured jerkies was measured using the AOAC (2000) method. The jerky was ground for the measurement of water activity, which was determined using a water activity meter (Novasina, Labmaster-aw, Lachen, Switzerland).

Microbial analysis

On days 1, 20, 45, 90, 135, and 180 of storage, 6 g of jerky sample was aseptically placed into a sterile stomacher bag with

225 mL of 0.1% peptone water and then homogenized for 3 min using the Stomacher Bag Mixer® 400 (Interscience, Osaka, Japan). These homogenates were serially diluted with sterile saline solution. For microbial analysis, 1 mL of the diluted sample was inoculated on 3M Petrifilm plates (3M Microbiology, Saint Paul, MN, USA) to determine aerobic plate counts, coliform/*Escherichia coli* count, and yeast and mold count. For total aerobic bacteria and coliform/*E. coli*, plates were incubated at 37℃ for 48 h, and for yeast and mold, they were incubated at 25℃ for 5 days in an incubation chamber (J070217, Jeio Tech, Korea). Each microbial count was recorded as Log colony-forming units per gram (Log CFU/g).

Statistical analysis

A t-test ($p<0.05$) was performed to compare total phenolic content and antioxidant activities between LE and AA, and LE and AA were considered as fixed terms and random terms were replicates. For the determination of jerky quality, LE concentration, addition of AA, and storage periods were considered as fixed terms and replicates were considered as random terms. A multifactorial analysis of variance, using the general linear model (GLM), was applied to determine the effects of LE concentration (0%, 0.15%, and 0.3%), with or without AA (0% and 0.05%), on storage period (1, 20, 45, 90, 135, and 180 days). The data were analyzed using a two-way analysis of variance with treatments and storage, using Duncan's multiple range test ($p<0.05$) with the SPSS statistical software program (SPSS Ver. 20.0, IBM, Chicago, IL, USA).

Results and Discussion

Total phenolic content and antioxidant activity of loquat leaf extract powder and ascorbic acid

Table 1 shows the total phenolic content and antioxidant activities (DPPH radical scavenging, ABTS radical scavenging, and metal chelating activities) of LE and AA. The total phenolic content of LE was 533.38 μg GAE/mL, whereas AA had no total phenolic content. The half maximal inhibitory concentration (IC_{50}) of metal chelating activity of LE was 5.71 mg/mL, whereas AA had no metal chelating activity. The IC_{50} of DPPH radical scavenging activity of LE was lower than that of AA ($p<0.05$). Moreover, the IC_{50} of ABTS radical scavenging activity of AA was lower than that of LE ($p<0.05$). Various analyses were used to evaluate the antioxidant activity of LE and AA, and the results showed the antioxidant activities to be comparable to that reported in previous studies (Kim et al., 2019; Lee and Kim, 2009). Phenolic compounds are secondary metabolites of plants and are associated with antioxidant activities (Hwang et al., 2010). Generally, the antioxidant activity of phenolic compounds is based on their metal ion chelation and hydrogen donating abilities (Wijekoon et al., 2011). Phenolic hydroxyl groups bind heavy metals that cause free radical formation, thereby inhibiting lipid peroxidation (Kısa et al., 2016).

Table 1. Total phenolic content and antioxidant activities of loquat leaf extract (LE) and ascorbic acid (AA)

Treatment	Loquat leaf extract	Ascorbic acid
Total phenolic content (μg GAE/mL)	533.38±5.49	NE
DPPH radical scavenging activity (IC_{50} value mg/mL)	0.06±0.00^{b}	0.10±0.00^{a}
ABTS radical scavenging activity (IC_{50} value mg/mL)	2.87±0.04^{a}	0.61±0.01^{b}
Metal chelating activity (IC_{50} value mg/mL)	5.71±0.03	NE

All values are mean±SE of three replicates.
^{a,b} Means within a row with different letters are significantly different.
GAE, gallic acid equivalents; NE, values were not estimated; DPPH, 1,1-diphenyl-2-picrylhydarzyl; ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

AA is a strong antioxidant, owing to its preventive effect on oxidation of other compounds following the donation of electrons (Kim et al., 2013a). In the present study, AA showed an excellent radical scavenging activity but no metal chelating activity. Since LE has metal chelating effect, a combination of LE and AA may be expected to have a synergistic effect as an oxidation inhibitor in semi-dried restructured jerky during storage.

Sulfhydryl concentration and thiobarbituric acid-reactive substances (TBARS) activity

The sulfhydryl concentration and TBARS values of semi-dried restructured jerky prepared with LE and AA are shown in Table 2. When protein oxidation occurs, amino acid structures undergo some changes, such as formation of disulfide bridges, consequently leading to decreased sulfhydryl concentration (Turgut et al., 2017). The sulfhydryl concentration of jerky with LE and AA were higher ($p<0.05$) than that of the control at 1 day of storage. The sulfhydryl concentration of jerky samples decreased during storage ($p<0.001$) and significantly was high concentration related with an increase in LE concentration and

Table 2. Protein oxidation and lipid oxidation values in semi-dried restructured jerky prepared with loquat leaf extract (LE) and ascorbic acid (AA) during storage

Treatment	Storage (d)					
	1	20	45	90	135	180
Sulfhydryl concentration (nmol/mg protein)						
Control	48.27±0.13^{Fa}	38.61±0.15^{Fb}	33.80±0.09^{Fc}	28.99±0.06^{Fd}	24.33±0.04^{Fe}	18.91±0.06^{Ff}
AA	51.82±0.29^{Da}	45.76±0.31^{Eb}	43.84±0.06^{Dc}	38.88±0.13^{Dd}	32.52±0.21^{Ee}	26.14±0.09^{Ef}
LE 0.15	51.23±0.07^{Ea}	46.76±0.35^{Db}	39.41±0.12^{Ec}	35.81±0.15^{Ed}	33.08±0.09^{De}	29.37±0.04^{Df}
LE 0.15-AA	55.80±0.33^{Ca}	51.49±0.24^{Cb}	44.48±0.06^{Cc}	39.82±0.13^{Cd}	35.67±0.14^{Ce}	32.08±0.02^{Cf}
LE 0.3	56.79±0.03^{Ba}	53.55±0.25^{Bb}	46.73±0.20^{Bc}	44.48±0.06^{Bd}	39.73±0.10^{Be}	35.67±0.14^{Bf}
LE 0.3-AA	62.77±0.05^{Aa}	60.45±0.30^{Ab}	56.08±0.38^{Ac}	53.68±0.14^{Ad}	48.06±0.12^{Ae}	44.51±0.34^{Af}
TBARS (mg MDA/kg)						
Control	0.36±0.00^{Ae}	0.39±0.01^{Ad}	0.41±0.01^{Ac}	0.42±0.01^{Ab}	0.42±0.01^{Ab}	0.45±0.01^{Aa}
AA	0.35±0.00^{Ce}	0.36±0.00^{Bcd}	0.40±0.00^{BCc}	0.41±0.01^{Bb}	0.40±0.00^{CDb}	0.44±0.01^{Ba}
LE 0.15	0.35±0.00^{Be}	0.36±0.00^{Bd}	0.40±0.00^{Bc}	0.40±0.01^{BCc}	0.41±0.00^{Bb}	0.44±0.01^{BCa}
LE 0.15-AA	0.35±0.00^{Ce}	0.36±0.00^{BCd}	0.39±0.01^{Dc}	0.40±0.01^{CDbc}	0.40±0.00^{Db}	0.43±0.01^{DEa}
LE 0.3	0.35±0.00^{Ce}	0.36±0.00^{Cd}	0.39±0.00^{CDc}	0.40±0.01^{DEc}	0.41±0.01^{BCb}	0.43±0.01^{CDa}
LE 0.3-AA	0.35±0.00^{Cd}	0.35±0.00^{Dd}	0.39±0.00^{Dc}	0.39±0.01^{Ec}	0.40±0.01^{Db}	0.43±0.01^{Ea}

	Storage	LE	AA	Storage×LE	Storage×AA	LE×AA	Storage×LE×AA
Sulfhydryl concentration							
p-value	***	***	***	***	***	***	***
TBARS							
p-value	***	***	***	***	**	***	**

All values are mean±SE of three replicates.
Control, no antioxidant; AA, added 0.05% ascorbic acid; LE 0.15, added 0.15 loquat leaf extract; LE 0.15-AA, added 0.15% loquat leaf extract and 0.05% ascorbic acid; LE 0.3, added 0.3% loquat leaf extract; LE0.3-AA, added 0.3% loquat leaf extract and 0.05% ascorbic acid.
$^{A–F}$ Means sharing different letters in the same column are significantly different.
$^{a–f}$ Means sharing different letters in the same row are significantly different.
** $p<0.01$, *** $p<0.001$.
TBARS, thiobarbituric acid-reactive substances; MDA, malondialdehyde.

the addition of AA ($p<0.05$). Moreover, the combination (LE×AA) was found to be significantly effective for sulfhydryl concentration in jerky during storage ($p<0.001$); the highest sulfhydryl concentration was found in LE 0.3-AA, throughout the storage period ($p<0.05$). The inhibited reduction of sulfhydryl concentration could be explained by the phenolic compounds of LE and antioxidant activities of LE and AA (Table 1). In general, hydroxyl groups of phenolic compounds bind to proteins, forming complexes, which in turn inhibit protein oxidation (Hoffman et al., 2014). Similar results to this study were reported by Fourati et al. (2020). Minced beef without any addition showed the lowest sulfhydryl concentration when compared with the sample added with antioxidants at 0 storage day. It is difficult to explain exactly why there was a difference in sulfhydryl concentration from day 0 or 1 of storage. However, if oxidation during the manufacturing process of meat products was inhibited by antioxidants, the oxidation values (sulfhydryl concentration or TBARS value) could be different at the beginning of storage. Of course, additional research is needed for more detailed explanation. According to Fourati et al. (2020), sulfhydryl concentration of minced beef was also significantly decreased during storage. Xu et al. (2018) reported that the addition of mulberry polyphenol inhibited a decrease of sulfhydryl concentration in dried minced pork slices during the drying process and explained that phenolic compounds weaken protein oxidation in meat products. Moreover, metal chelation or DPPH and ABTS radical scavenging activity of LE and AA against free radicals was related to high sulfhydryl concentration of treatment compared to that in control.

The MDA level, representing the formation of secondary lipid oxidation products and measured as TBARS, indicate the progression of lipid oxidation in meat and meat products (Turgut et al., 2017). On 1 day of storage, all treatments added LE and AA, either alone or in combination, possessed lower TBARS value than control ($p<0.05$). The TBARS values of all jerkies were increased during the storage period ($p<0.05$, Table 2). The jerky prepared with LE and AA, either alone or in combination, had lower TBARS values than the control during the storage period, thus indicating that LE, AA, and the combination (LE×AA) had a significant effect on TBARS values ($p<0.001$). Lipid oxidation can occur via three mechanisms: (i) free-radical chain reaction, (ii) metal- or enzyme- catalyzed oxidation, and (iii) photo-oxidation (Domínguez et al., 2019). The free-radical chain reaction is the most important mechanism of lipid oxidation in meat or meat products (Amaral et al., 2018). The iron present in myoglobin is a potent catalyst in various stages of lipid oxidation (Amaral et al., 2018; Domínguez et al., 2019). In the present study, as LE showed a strong metal chelating activity, its chelating activity with iron ions could possibly help retard lipid oxidation (Kong et al., 2010). Lee et al. (2011) had shown that increasing kimchi ethanolic extract concentration retarded the TBARS value of refrigerated cooked pork over 14 days of storage due to the strong metal-chelating activity. Our results indicated that the addition of LE and AA inhibited protein and lipid oxidation in semi-dried restructured jerky during storage, resulting in LE and AA being used as a radical scavenger and chelating agent in foodstuff. However, further study is needed to maximize the usability of LE to retard lipid oxidation value.

pH and color

The pH and color of the semi-dried restructured jerky, formulated with LE and AA, during storage are shown in Table 3. All treatments and control showed significantly decreased pH values during storage ($p<0.05$). The pH values of semi-dried restructured jerky significantly ($p<0.05$) decreased with an increase in LE concentration and addition of AA on day 1, and the combination (LE×AA) had a significant effect on the pH values. When we measured the pH of LE and AA in DW, the value was 4.38 and 2.02, respectively (data not shown). The pH of LE and AA might have been affected by the initial pH of the semi-dried restructured jerky. According to Bower et al. (2003), the pH of vacuum-packaged meat products decreases with storage.

Table 3. pH and color in semi-dried restructured jerky prepared with loquat leaf extract (LE) and ascorbic acid (AA) during storage

Treatment	Storage (d)					
	1	20	45	90	135	180
pH						
Control	6.15±0.01^{Aa}	6.12±0.01^{Ab}	6.12±0.01^{Ab}	6.11±0.01^{Abc}	6.11±0.01^{Ac}	6.10±0.01^{Ac}
AA	6.06±0.01^{Da}	6.06±0.01^{Ca}	6.06±0.01^{Ba}	6.06±0.01^{Ba}	6.06±0.01^{Ba}	6.01±0.01^{Db}
LE 0.15	6.09±0.01^{Ba}	6.07±0.01^{BCb}	6.07±0.01^{Bb}	6.06±0.01^{Bc}	6.06±0.01^{Bc}	6.05±0.01^{Bc}
LE 0.15-AA	6.05±0.01^{Ea}	6.04±0.01^{Db}	6.04±0.01^{Cb}	6.02±0.01^{Cc}	6.00±0.01^{Dd}	6.00±0.01^{Ee}
LE 0.3	6.08±0.01^{Ca}	6.08±0.01^{Ba}	6.06±0.01^{Bb}	6.06±0.01^{Bb}	6.05±0.01^{Bc}	6.03±0.01^{Cd}
LE 0.3-AA	6.04±0.01^{Fa}	6.03±0.01^{Db}	6.02±0.01^{Dc}	6.02±0.01^{Cc}	6.02±0.01^{Cc}	5.99±0.01^{Ed}
CIE L*						
Control	48.72±0.38^{ABe}	50.31±0.19^{Ad}	50.65±0.18^{Ad}	51.57±0.18^{Ac}	53.22±0.21^{Ab}	54.18±0.17^{Aa}
AA	49.07±0.19^{Ac}	49.13±0.19^{Bc}	50.38±0.08^{Ab}	50.58±0.30^{Bb}	50.93±0.13^{Bb}	51.68±0.38^{Ba}
LE 0.15	47.67±0.25^{CDd}	48.03±0.22^{Cd}	49.33±0.11^{Cc}	49.33±0.15^{Cc}	50.10±0.09^{Cb}	51.68±0.38^{Ba}
LE 0.15-AA	48.14±0.08^{BCc}	48.23±0.09^{Cc}	49.29±0.14^{Bb}	49.66±0.31^{Cb}	50.34±0.18^{Ca}	50.46±0.30^{Ca}
LE 0.3	46.43±0.19^{Ec}	46.82±0.13^{Dc}	46.84±0.15^{Dc}	47.96±0.20^{Db}	48.02±0.24^{Db}	48.90±0.16^{Da}
LE 0.3-AA	47.13±0.25^{DEb}	47.33±0.28^{Db}	47.56±0.12^{Eb}	47.69±0.22^{Db}	47.48±0.09^{Eb}	48.30±0.18^{Da}
CIE a*						
Control	14.63±0.12^{Ba}	14.35±0.18^{Aa}	13.80±0.07^{Bb}	12.92±0.22^{Bc}	12.48±0.15^{Bc}	11.65±0.27^{Cd}
AA	16.62±0.33^{Aa}	16.20±0.19^{Bab}	16.02±0.19^{Ab}	15.46±0.07^{Ac}	14.73±0.11^{Ad}	14.38±0.14^{Ad}
LE 0.15	11.91±0.33^{Ca}	11.56±0.32^{Dab}	11.31±0.18^{Dabc}	11.10±0.11^{Cbc}	10.74±0.07^{Cc}	9.52±0.05^{Dd}
LE 0.15-AA	14.25±0.14^{Ba}	13.33±0.25^{Cb}	13.33±0.13^{Cb}	12.79±0.01^{Bc}	12.75±0.12^{Bc}	12.56±0.17^{Bc}
LE 0.3	9.76±0.05^{Ea}	9.73±0.09^{Fa}	9.53±0.11^{Fa}	9.03±0.22^{Eab}	8.81±0.43^{Db}	8.73±0.27^{Eb}
LE 0.3-AA	10.55±0.12^{D}	10.54±0.04^{E}	10.43±0.10^{E}	10.40±0.10^{D}	10.21±0.05^{C}	10.04±0.38^{D}
CIE b*						
Control	12.09±0.03^{Fe}	14.56±0.24^{Ed}	15.28±0.24^{Ed}	16.17±0.32^{Ec}	17.36±0.32^{Db}	18.41±0.23^{Da}
AA	14.63±0.36^{Ec}	14.83±0.14^{Ec}	15.80±0.25^{Eb}	15.95±0.16^{Eb}	16.64±0.29^{Da}	17.05±0.12^{Ea}
LE 0.15	15.77±0.43^{Dd}	16.32±0.15^{Dcd}	16.99±0.05^{Dbc}	17.27±0.11^{Db}	18.66±0.42^{Ca}	19.09±0.09^{Ca}
LE 0.15-AA	17.57±0.15^{Cb}	17.79±0.19^{Cb}	18.00±0.19^{Cb}	18.22±0.23^{Cb}	19.31±0.40^{Ca}	19.62±0.14^{Ca}
LE 0.3	19.76±0.17^{Bc}	19.90±0.11^{Bc}	20.01±0.07^{Bc}	20.30±0.24^{Bbc}	20.84±0.33^{Bab}	20.95±0.16^{Ba}
LE 0.3-AA	21.83±0.22^{A}	21.97±0.18^{A}	22.10±0.18^{A}	22.23±0.36^{A}	22.63±0.35^{A}	22.67±0.30^{A}

p-value	Storage	LE	AA	Storage×LE	Storage×AA	LE×AA	Storage×LE×AA
pH	***	***	***	***	***	***	***
CIE L*	***	***	***	***	***	***	*
CIE a*	***	***	***	***	**	***	NS
CIE b*	***	***	***	***	***	***	***

All values are mean±SE of three replicates.
Control, no antioxidant; AA, added 0.05% ascorbic acid; LE 0.15, added 0.15 loquat leaf extract; LE 0.15-AA, added 0.15% loquat leaf extract and 0.05% ascorbic acid; LE 0.3, added 0.3% loquat leaf extract; LE0.3-AA, added 0.3% loquat leaf extract and 0.05% ascorbic acid.
$^{A–F}$ Means sharing different letters in the same column are significantly different.
$^{a–e}$ Means sharing different letters in the same row are significantly different.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
NS, no significance.

As shown in Table 3, the control and treatments had significantly ($p<0.05$) increased CIE b* and decreased CIE a* during storage, except for LE 0.3-AA ($p>0.05$). During the initial days of storage, there was no significant difference in CIE a* between LE 0.15-AA and control ($p>0.05$). At the end of the storage period, LE 0.15-AA had a higher CIE a* than the control ($p<0.05$). The jerkies prepared with AA showed a higher CIE a* compared to those prepared with same amount of LE during storage ($p<0.05$), indicating that the addition of AA has a significant effect on the CIE a* ($p<0.001$). Lipid oxidation may catalyze pigment oxidation, and free radicals produced during oxidation may denature the myoglobin or oxidize the iron atoms, leading to a negative change in the color of products (Selani et al., 2011). The high antioxidant activity of LE is related to delayed or prevented oxidative reactions (Liu et al., 2016). AA generally improves color stability and leads to declined myoglobin oxidation in meat products (Hwang et al., 2013). Since jerkies prepared with LE and AA had low TBARS values compared to the control during storage (Table 2), the trend of inhibitory change of CIE a* may be due to inhibition of lipid oxidation. Additionally, the change of color values in the initial days of storage might be affected by the color of LE (CIE L*=47.90, CIE a*=–4.86, and CIE b*=35.37; data not shown).

Moisture content, water activity, and shear force

Moisture content and water activity in semi-dried restructured jerky, prepared with LE and AA, during storage are shown in Table 4. Both the parameters varied from 44.71% to 45.47% and from 0.882 to 0.890, respectively, on day 1, which matched the normal moisture content and water activity range of 20%–50% and 0.82–0.91, respectively, of semi-dried jerky (Chen et al., 2002). The moisture content of jerkies significantly decreased over storage days ($p<0.001$), except for samples from LE 0.3-AA (the reduction was not significant, $p>0.05$). At the end of the storage period, LE 0.3-AA, LE 0.15-AA, and AA showed higher ($p<0.05$) moisture content, compared to the same amount of LE, indicating that AA had a significant effect on moisture content ($p<0.01$). Similarly, the water activity of jerky samples significantly decreased during storage ($p<0.001$; Table 4). However, the increase in LE concentration had a significant effect on the inhibited water activity in the jerky during the storage period ($p<0.001$). Similar results were reported by Kim et al. (2012), who showed that the moisture content and water activity of pork jerky decreased during storage, leading to a tough texture of jerky during distribution and storage. Additionally, Choi et al. (2007) had reported that water is diffused and dehumidified from the products in packaging; therefore, moisture content and water activity of products may decrease during storage. Jerky requires a stable moisture content and water activity to avoid changes in quality properties during storage (Lim et al., 2013).

The shear force values of semi-dried restructured jerky increased with increasing LE concentration ($p<0.001$, Table 4). The shear force value in the control and treatment groups significantly ($p<0.05$) increased during storage, and the sharpest incline occurred in the control. At the end of the storage period, LE 0.15-AA, LE 0.15, and AA had lower shear force than the control ($p<0.05$). The increased shear force could be explained by increased protein oxidation and moisture content during storage. Disulfide bond formation caused by protein oxidation might weaken protein solubility, resulting in protein aggregation and complex formation (Xu et al., 2018). In this study, the addition of LE and AA inhibited protein and lipid oxidation; thus these might have also inhibited the increase in shear force in semi-dried restructured jerky during storage. Additionally, Li et al. (2014) had reported that the moisture content was extremely significantly correlated with shear force, and shear force value can represent the parameters of both moisture content and texture quality. The high moisture content of LE 0.15-AA, LE 0.15, and AA compared to that in the control seemed to be related to their low shear force at the end of the storage period (Table 4). All the results together suggested that a combination of LE and AA could minimize the texture deterioration of semi-dried restructured jerky caused by protein and lipid oxidation.

Table 4. Moisture content, water activity, and shear force in semi-dried restructured jerky prepared with loquat leaf extract (LE) and ascorbic acid (AA) during storage

Treatment	Storage (d)					
	1	20	45	90	135	180
Moisture content (%)						
Control	45.47±0.33^{a}	44.70±0.19^{ab}	44.20±0.40^{bc}	43.72±0.24^{Cc}	43.67±0.17^{c}	42.53±0.20^{Dd}
AA	45.29±0.16^{a}	44.97±0.28^{ab}	44.97±0.09^{ab}	44.54±0.13^{Abc}	44.23±0.12^{c}	43.70±0.14^{ABd}
LE 0.15	45.11±0.16^{a}	44.93±0.16^{ab}	44.81±0.55^{abc}	44.21±0.07^{ABbc}	44.06±0.05^{c}	43.13±0.02^{Cd}
LE 0.15-AA	44.81±0.28^{a}	44.61±0.14^{ab}	44.49±0.12^{ab}	44.33±0.10^{Aabc}	44.24±0.11^{bc}	43.90±0.17^{Ac}
LE 0.3	44.99±0.30^{a}	44.67±0.15^{a}	44.57±0.22^{a}	43.87±0.09^{BCb}	43.55±0.30^{b}	43.35±0.20^{BCb}
LE 0.3-AA	44.71±0.05	44.63±0.28	44.24±0.47	44.09±0.14^{ABC}	44.00±0.26	44.16±0.06^{A}
Water activity						
Control	0.890±0.001^{a}	0.888±0.003^{Aa}	0.886±0.001^{a}	0.886±0.002^{Aa}	0.869±0.002^{b}	0.860±0.001^{Ec}
AA	0.884±0.001^{a}	0.887±0.001^{Aa}	0.885±0.001^{a}	0.874±0.002^{Bb}	0.872±0.001^{b}	0.862±0.000^{Dc}
LE 0.15	0.882±0.003^{a}	0.880±0.000^{BCa}	0.883±0.002^{a}	0.874±0.002^{Bb}	0.872±0.000^{b}	0.864±0.000^{Cc}
LE 0.15-AA	0.882±0.002^{a}	0.877±0.001^{Cab}	0.877±0.003^{ab}	0.872±0.001^{Bbc}	0.869±0.002^{bc}	0.866±0.000^{Bc}
LE 0.3	0.886±0.000^{a}	0.883±0.001^{ABa}	0.885±0.003^{a}	0.874±0.003^{Bb}	0.874±0.002^{b}	0.868±0.000^{Ab}
LE 0.3-AA	0.884±0.002^{a}	0.882±0.006^{ABa}	0.884±0.002^{a}	0.877±0.002^{Bab}	0.870±0.001^{bc}	0.868±0.000^{ABc}
Shear force (N)						
Control	144.67±0.36^{De}	150.49±0.34^{Dd}	151.37±0.26^{Cd}	154.31±0.39^{Ec}	159.15±0.23^{Cb}	165.30±0.43^{Ca}
AA	145.33±0.36^{Df}	147.14±0.42^{Ee}	148.08±0.29^{Dd}	151.01±0.25^{Fc}	153.79±0.22^{Db}	157.00±0.31^{Fa}
LE 0.15	153.59±0.09^{Cf}	154.47±0.10^{Ce}	156.20±0.01^{Bd}	158.62±0.35^{Cc}	160.43±0.46^{Bb}	161.73±0.32^{Da}
LE 0.15-AA	154.97±0.33^{Bd}	155.72±0.29^{Bcd}	156.12±0.16^{Bc}	157.14±0.20^{Db}	158.80±0.31^{Ca}	159.37±0.41^{Ea}
LE 0.3	166.90±0.27^{Ae}	167.04±0.12^{Ae}	167.91±0.18^{Ad}	169.18±0.07^{Bc}	171.11±0.31^{Ab}	172.60±0.30^{Aa}
LE 0.3-AA	167.52±0.16^{Ab}	167.56±0.24^{Ab}	168.23±0.32^{Ab}	170.77±0.46^{Aa}	170.77±0.41^{Aa}	171.15±0.44^{Ba}

p-value	Storage	LE	AA	Storage×LE	Storage×AA	LE×AA	Storage×LE×AA
Moisture content	***	NS	**	NS	***	*	NS
Water activity	***	***	*	***	NS	NS	*
Shear force	***	***	***	***	***	***	***

All values are mean±SE of three replicates.
Control, no antioxidant; AA, added 0.05% ascorbic acid; LE 0.15, added 0.15 loquat leaf extract; LE 0.15-AA, added 0.15% loquat leaf extract and 0.05% ascorbic acid; LE 0.3, added 0.3% loquat leaf extract; LE0.3-AA, added 0.3% loquat leaf extract and 0.05% ascorbic acid.
$^{A–F}$ Means sharing different letters in the same column are significantly different.
$^{a–f}$ Means sharing different letters in the same row are significantly different.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
NS, no significance.

Microbial analysis

Results obtained from the microbial analyses of semi-dried restructured jerky prepared with LE and AA during storage are shown in Table 5. The total aerobic bacteria count of all jerkies after storage of 45 days was detected, and the total aerobic bacteria count of all jerkies increased during storage, regardless of control and treatments ($p<0.05$). Coliform/*E. coli*, yeast, and molds were not detected in all jerkies during storage (date not shown). At present, the established limit for total aerobic

Table 5. Microbial analysis in semi-dried restructured jerky prepared with loquat leaf extract (LE) and ascorbic acid (AA) during storage

Treatment	Storage (d)					
	1	20	45	90	135	180
Total aerobic bacteria (Log CFU/g)						
Control	ND[d1)]	ND[d]	ND[d]	3.49±0.06[c]	4.07±0.04[b]	5.39±0.06[a]
AA	ND[d]	ND[d]	ND[d]	3.47±0.06[c]	4.08±0.04[b]	5.40±0.07[a]
LE 0.15	ND[d]	ND[d]	ND[d]	3.52±0.06[c]	4.10±0.04[b]	5.38±0.07[a]
LE 0.15-AA	ND[d]	ND[d]	ND[d]	3.50±0.05[c]	4.07±0.05[b]	5.41±0.07[a]
LE 0.3	ND[d]	ND[d]	ND[d]	3.47±0.05[c]	4.09±0.06[b]	5.42±0.07[a]
LE 0.3-AA	ND[d]	ND[d]	ND[d]	3.54±0.04[c]	4.08±0.03[b]	5.41±0.07[a]

p-value	Storage	LE	AA	Storage×LE	Storage×AA	LE×AA	Storage×LE×AA
Total aerobic bacteria	***	NS	NS	NS	NS	NS	NS

All values are mean±SE of three replicates.
Control, no antioxidant; AA, added 0.05% ascorbic acid; LE 0.15, added 0.15 loquat leaf extract; LE 0.15-AA, added 0.15% loquat leaf extract and 0.05% ascorbic acid; LE 0.3, added 0.3% loquat leaf extract; LE0.3-AA, added 0.3% loquat leaf extract and 0.05% ascorbic acid.
[1)] ND, not detected with the detection limit of $<10^1$ CFU/g.
[a–d] Means sharing different letters in the same row are significantly different.
*** $p<0.001$.
NS, no significance.

bacteria counts in protective dried meat, including jerky, is below 5.0 Log CFU/g (Gaikwad et al., 2020); in this study, after a 135 day storage period, the jerky was still in agreement with these criteria (Table 5). Kim et al. (2013b) had reported similar results of increased total aerobic bacterial count, in addition to no detected mold and coliform bacterial populations in pork jerky during a 2 month storage period, regardless of the control (without leek extract); the jerky was prepared with leek extract. Lim et al. (2012) had also reported that the total plate count of sun-dried beef jerky increased regardless of the addition of *Citrus junos seib* and *Prunus mume* extracts during storage. Several studies have noted that the fermented loquat leaf ethanol extract possesses antimicrobial activity and that the differences in the quantity of loquat LE can result in differences in their antimicrobial activity, which can consequently affect microbial growth on meat products with loquat LE (Dhiman et al., 2021; Fu et al., 2019; Liu et al., 2016). Further studies would be required to test the changes in microbial growth in semi-dry structured jerky supplemented with various concentrations of LE during storage.

Conclusion

This study reported that LE and AA have high antioxidant activities, as shown by their metal chelating, as well as DPPH and ABTS radical scavenging activities. The combination of LE and AA retarded protein and lipid oxidation (as per sulfhydryl concentration and TBARS value) in semi-dried restructured jerky, during storage. The combination of 0.15% loquat LE powder and 0.05% AA was determined to be applicable as a natural antioxidant for preserving the quality of semi-dried restructured jerky, and it has a potential application in preserving meat and meat products.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Kim SM, Choi YS. Data curation: Kim SM, Kim TK, Kang MC, Choi YS. Formal analysis: Kim SM, Kim TK, Cha JY. Methodology: Kim SM, Kim TK, Yong HI. Software: Yong HI. Validation: Kang MC, Choi YS. Investigation: Choi YS. Writing - original draft: Kim SM, Kang MC, Cha JY, Choi YS. Writing - review & editing: Kim SM, Kim TK, Kang MC, Cha JY, Yong HI, Choi YS.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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