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ARTICLE

The Effectiveness of Calamansi (*Citrus microcarpa*) Extract in Enhancing the Shelf Life and Quality of Dakgalbi Made from Domestic Korean Chicken

Joko Sujiwo[1], Yousung Jung[1], Sangrok Lee[1], Dongwook Kim[1], Hee-Jeong Lee[1], Soomin Oh[1], Hyo-Joon Choo[2], and Aera Jang[1,*]

[1]Department of Applied Animal Science, Kangwon National University, Chuncheon 24341, Korea
[2]Poultry Research Institute, National Institute of Animal Science, Pyeongchang 25342, Korea

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***Corresponding author** : Aera Jang
Department of Applied Animal Science, Kangwon National University, Chuncheon 24341, Korea
Tel: +82-33-250-8643
Fax: +82-33-251-7719
E-mail: ajang@kangwon.ac.kr

***ORCID**
Joko Sujiwo
https://orcid.org/0000-0003-2078-3922
Yousung Jung
https://orcid.org/0000-0003-2095-2394
Sangrok Lee
https://orcid.org/0009-0001-6476-0127
Dongwook Kim
https://orcid.org/0000-0002-5496-1961
Hee-Jeong Lee
https://orcid.org/0000-0003-3806-482X
Soomin Oh
https://orcid.org/0009-0006-2403-4813
Hyo-Joon Choo
https://orcid.org/0000-0002-7747-5077
Aera Jang
https://orcid.org/0000-0003-1789-8956

Abstract This study investigated calamansi extract (CE) as a natural preservative for dakgalbi, a spicy Korean chicken dish. Chicken breast and thigh meat were treated with 0.14% or 0.18% CE and stored at 4℃ for 19 days. The antimicrobial and antioxidant properties of CE were evaluated before application. The total phenolic content of CE shows 15.22±0.39 mg GAE/g dry matter. The changes in proximate composition, pH, water holding capacity (WHC), instrumental color, microbial quality, thiobarbituric acid reactive substances (TBARS), and sensory properties of dakgalbi were assessed. The results showed that 0.18CE treatment of the breast meat significantly ($p<0.05$) enhanced WHC on day 19 of storage (56.47%) compared with that of the control (47.21%). Additionally, 0.18CE reduced total aerobic bacteria (5.48 Log CFU/g) and coliforms (3.29 Log CFU/g) compared to the control (6.27 and 3.75 Log CFU/g, respectively) on day 16 of storage. Moreover, CE application effectively retarded lipid oxidation, as demonstrated by the reduced TBARS values seen until the 19 days of storage, suggesting a potent antioxidative action. CE treatment effectively maintained the sensory quality without negatively affecting the sensory characteristics. The study concluded that the CE, particularly at 0.18%, significantly improved the preservation of dakgalbi, highlighting its potential as a natural preservative. This investigation underscores the importance of further research on the application of CE in various meat products and its mechanisms of action, aiming at enhancing food safety and sustainability within the food industry.

Keywords natural preservatives, chicken meat product, antimicrobial, antioxidant

Introduction

Dakgalbi, a popular South Korean dish, is made from spicy stir-fried chicken (made with boneless chicken pieces), rice cakes (*tteok*), and various vegetables. The chicken pieces are marinated in a sauce based on chili pepper paste (*gochujang*) and then grilled on a hot plate with sweet potatoes, scallions, cabbage, onions, and *tteok*. Dakgalbi sauce

is often made from garlic, ginger, soy sauce, red pepper paste, corn syrup, pepper, and sugar, which contribute to its flavor (Yoon et al., 2009).

Despite its popularity, dakgalbi, similar to other poultry dishes, has a limited shelf life and is susceptible to quality degradation over time. The limited shelf life of poultry meat is due to the rapid oxidation of unsaturated fatty acids and contamination by spoilage microorganisms (Kim et al., 2019). Current dakgalbi preservation methods, such as refrigeration, the use of cans (Khasanah et al., 2023) or retort packaging (Muhlisin et al., 2013), gamma ray radiation (Yoon et al., 2009), curing (Jeong et al., 2018) and the use of synthetic preservatives, can extend the shelf life but these methods may also affect the taste and nutritional value of the dish. While synthetic preservatives aid in preserving the quality, consistency, and shelf life of processed foods, they can affect taste by altering the original flavor and nutritional properties (Thakur et al., 2022). Moreover, consumers are increasingly seeking natural alternatives to synthetic preservatives due to health and environmental concerns (Naufalin, 2019). A study by Kim et al. (2011) identified that the addition of tomatoes has a positive effect on dakgalbi sauce. The study demonstrated that appropriate tomato addition positively impacts overall palatability. Incorporating tomatoes into dakgalbi sauce can enhance its appeal to foreign consumers and children who may be sensitive to spiciness.

To date, extensive investigations have been conducted on the application of natural compound extracts for the preservation of chicken meat during storage. These compounds are primarily used for their antimicrobial and antioxidant properties, which are largely attributed to their phenolic and flavonoid contents (Zhang et al., 2016). The proposed mechanism suggests that tiny fractures in polyphenols may penetrate microbial cells, subsequently disrupting their cellular homeostasis by interfering with nutrient absorption, electron transport, and nucleic and amino acid biosynthesis (Cho et al., 2023). Phenolic and flavonoid compounds can chelate metal ions, reducing their ability to generate free radicals and inhibit enzymes that produce free radicals, further contributing to their antioxidant activity (Kaurinovic and Vastag, 2019).

Exploring natural extracts with potent antioxidative and antimicrobial properties, such as calamansi (*Citrus microcarpa*), is crucial for enhancing the shelf life of poultry dishes, such as dakgalbi. Originating from Southeast Asia, calamansi, a potential Citrus genus member and a natural hybrid of the oval kumquat and mandarin (*Citrus japonica*×*Citrus reticulate*), has spread to various regions, including Hawaii, North America, Central India, and the West Indies (Cheong et al., 2012a). It is commonly used as a food seasoning, flavor enhancer, or food additive to boost iron absorption (Cheong et al., 2012b). In addition, numerous previous studies have shown that it is beneficial for preserving and enhancing the quality of various foods (Cho et al., 2023; Husni et al., 2021; Hussain et al., 2021; Jinap et al., 2018). Despite the promising potential of calamansi extract (CE), there is currently a gap in research regarding its application in improving the quality and prolonging the shelf life of poultry products, particularly dakgalbi, during storage. To elucidate the functional properties of CE relevant to dakgalbi preservation, this study will evaluate its total phenolic content (TPC) and antioxidant activity before the application on the dakgalbi. Previous research by Cho et al. (2023) reported a TPC of 12.11 mg GAE/g dry matter (DM) in CE. This high TPC suggests a rich presence of phenolic acids, like caffeic, p-coumaric, ferulic, and sinapic acids, as identified by Cheong et al. (2012a). These phenolic acids are believed to contribute to calamansi antioxidant properties. Therefore, this study has aimed to explore the effects of CE on the quality and shelf life of dakgalbi under refrigerated storage conditions.

Materials and Methods

Calamansi extraction and total phenolic content analysis

Frozen calamansi (*C. microcarpa*) were imported from Vietnam and purchased online through an online marketplace (Vmart

at coupang.com) from a Vietnamese local market. Before extraction, the calamansi was rinsed under running tap water and sterilized with 90% alcohol. The fruit was halved, and the peel and seeds were removed in order to obtain the calamansi pulp. Subsequently, the pulp was lyophilized, crushed, and sieved through a 20 mesh. The chosen fine calamansi powder was subjected to extraction by immersion in an ethanol solution with a concentration of 90% and a weight/volume ratio of 1:50. This process was carried out at a temperature of 25℃ and lasted for a period of 6 days. The CE, once ethanol-extracted, was subsequently concentrated at 45℃ using a rotary evaporator. This extraction method follows the procedure described by Cho et al. (2023). The condensed CE was freeze-dried and stored at –20℃ for analysis. The extraction yield was 55%.

TPC was analyzed using a slightly modified version of the Folin-Ciocalteu colorimetric method originally described by Singleton (1966). The 70% ethanol was used to dissolve the CE. The extract solution (2 mg/mL) was further diluted with methanol. A 0.5 mL sample of the diluted extract solution was mixed with 5 mL of distilled water and Folin-Ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, MO, USA). The mixture was then incubated for 3 min. Following this, 1 N Na_2CO_3 was added to the mixture which was then left to react for 90 min at 25℃ in a dark environment. After the reaction, the absorbance of the samples was measured at 760 nm using a Spectra Max M2 spectrophotometer (Molecular Devices, San Jose, CA, USA). A reference curve was established using gallic acid, and TPC was expressed in milligrams of gallic acid equivalent (GAE) for each gram of the sample.

Antioxidant activity of the calamansi extract

1,1-Diphenyl-2-pricrylhydrazyl

The method described previously by Blois (1958) was conducted with modifications to analyze 1,1-diphenyl-2-pricrylhydrazyl (DPPH) radical scavenging activity. A sample extract solution (1 mg/mL) was prepared. Then 100 μL of this solution was combined with 100 μL of a DPPH radicals solution (0.2 mM) dissolved in methanol, in a 96-well microplate. The reaction was then left for 30 min at 25℃ in a dark environment, allowing this mixture to react. The absorbance of the extract solution was gauged at 517 nm utilizing a SpectraMax M2 spectrophotometer (Molecular Devices). Trolox was used as a reference curve and DPPH measurements were expressed as mmol of trolox equivalent (TE) per gram of DM.

2,2’-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid

The method of Re et al. (1999) was used to ascertain the 2,2’-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity of freeze-dried CE. A 7 mM ABTS solution was combined with a 2.45 mM potassium persulfate solution and left at room temperature for 12 to 16 h to generate ABTS radicals. The reaction was conducted in the dark. The solution containing the radicals was diluted until the absorbance reached 0.700±0.02 at 735 nm. Subsequently, 50 μL of the sample was mixed with 950 μL of the ABTS radical solution and allowed to react in a dark environment at 30℃ for 30 min. The absorbance was measured at 735 nm. The results were assessed and are expressed as mmol TE per gram.

Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay was performed as previously described by Cho et al. (2023). The FRAP assay working solution was prepared using 10 mM 2,4,6-tripyridyl-S-triazine, 300 mM acetate buffer in 40 mM HCl, and a solution of 20 mM $FeCl_3 \cdot 6H_2O$. These components were combined in a 10:1:1 (v/v/v) ratio. A 25 μL sample of the extract (1 mg/mL) was combined with 175 μL of the FRAP working solution and reacted for 30 min at 37℃ in a dark environment. A SpectraMax M2 spectrophotometer (Molecular Devices) was used to measure the absorbance of the resulting

solution at 590 nm. FRAP activity was reported in terms of mmol TE per gram of DM.

Oxygen radical absorption capacity

The oxygen radical absorption capacity (ORAC) assay was conducted as previously described (Cho et al., 2023). The ORAC was measured using a mixture of a 25 μL extract sample (60 μg/mL) and 150 μL of 80 nM fluorescein, which was incubated for 15 min at a temperature of 37°C. After the incubation period, 25 μL of 150 mM 2,2'-azobis (2-amidinopropane) hydrochloride was introduced to produce peroxyl radicals. This led to a total volume of 200 μL in each well. The variation in absorbance of the sample extract that has undergone reaction was observed every minute, using 480 nm wavelength and an emission wavelength of 520 nm, at a temperature of 37°C. A SpectraMax M2 spectrophotometer (Molecular Devices) was used to conduct ORAC assays. A reference curve was established using Trolox, and the results are shown as mmol TE per gram.

Antimicrobial activity of calamansi extract

Strains of bacteria

The antibacterial properties of the ethanol extracts of calamansi were tested against four types of bacteria: *Listeria monocytogenes* (KCCM 40307), *Staphylococcus aureus* (KCCM 12256), *Escherichia coli* (KCCM 11234), and *Salmonella* Enteritidis (CCARM 8260). Three bacterial strains (*S.* Enteritidis, *S. aureus*, and *E. coli*) were cultured on Mueller-Hinton agar (MHA, MB Cell, Seoul, Korea) and subsequently incubated at 37°C for a period of 24 h. In contrast, *L. monocytogenes* was cultured on MHA and incubated at a reduced temperature of 30°C for an identical time span. After the incubation had been performed, a single colony from each bacterial type was transferred to 10 mL of sterile Mueller–Hinton broth (MHB, MB Cell). The samples were then subjected to their respective temperatures for further incubation. After three rounds of sub-culturing, the bacterial cells were analyzed using the paper-disc method.

Paper disc diffusion

The antibacterial properties of the ethanol extracts were evaluated using the paper disc diffusion method, which was slightly modified from that described previously by Ramos et al. (2006). Each extract was mixed with dimethyl sulfoxide (DMSO) to create solutions with specific densities of 1.25, 2.5, 5, and 10 mg/disc. These solutions were then sterilized using a filter with a 0.45 μm hydrophobic membrane, a product (Rephile Bioscience, Shanghai, China). A loopful of the culture was transferred into 10 mL of sterile MHB (MB Cell) to obtain the test organisms. This was then incubated at either 30°C or 37°C for 24 h. The cultures were then set at a concentration of 5–6 Log CFU/mL and inoculated onto MHA (MB Cell). Paper discs with diameters of 8 mm (Advantec, Tokyo, Japan) were placed on the MHA surface. Each extract was then immediately applied to the discs in 50 μL volumes. A negative control was set up by introducing 50 μL of DMSO into a sterile paper disc. A disc containing 0.01 mg of streptomycin was used for *L. monocytogenes*, *E. coli*, and *S. aureus*, and for *S.* Enteritidis, and another disc containing 0.20 mg of streptomycin was used to establish a positive control. Following this, the plates were incubated at either 30°C or 37°C for 24 h. After the incubation had been performed, the diameter of the inhibition zone was measured in millimeters using a digital caliper.

Preparation of dakgalbi

Domestic Korean chicken breast and thigh samples (*Woorimatdag* No. 1) were prepared separately. The skin was removed from the breast meat, and the thigh meat was prepared with intact skin. Both the breast and thigh meat samples were cut into

1 cm wide pieces. To prepare dakgalbi, each type of meat, including breast and thigh meat, was mixed separately with dakgalbi sauce. Table 1 shows that the dakgalbi sauce formulation incorporated with either 0.14% or 0.18% CE into the basic seasoning. The selection of 0.14% and 0.18% CE for the dakgalbi sauce was based on preliminary consumer trials to ensure these concentrations were acceptable without overpowering the flavor. Each sauce ingredient was blended individually, and the prepared sauce was mixed with meat at a ratio of 1:4 (sauce to meat). Adding the extract directly to the sauce ensures consistent distribution throughout and facilitates a more controlled evaluation of its effects compared to other application methods for the CE.

Packaging methods

The mixture was hermetically pressure-sealed utilizing a vacuum packer (Lovero SBV-400 TS, Sambo Tech, Gimpo, Korea) in commercial food-grade, bisphenol A-free vinyl plastic packaging (0.08 mm thick). The packed samples were stored in a refrigerator at 4℃ for a period of 19 days. Analyses were conducted on storage days 1, 8, 12, 16, and 19.

Proximate composition

The proximate composition was determined following procedures outlined by the Association of Official Agricultural Chemists (AOAC, 1995). The 105℃ oven was used to dry the dakgalbi sample for a duration of 12 h, to ascertain its moisture content based on the weight loss. The Kjeldahl method was used to measure the crude protein content. The crude fat content was determined by extraction using ether as the solvent. In a furnace at 550℃, the dakgalbi was incinerated to determine the crude ash content.

Measurement of pH value

Using a homogenizer (Polytron PT-2500 E, Kinematica, Malters, Switzerland), 90 mL of distilled water and 10 g of the sample were mixed for 30 s. An Orion 230 A pH meter (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure

Table 1. Formulation of dakgalbi sauce

Ingredients (%)	Treatment		
	Control	0.14CE	0.18CE
Soy sauce	40.00	40.00	40.00
Corn syrup	12.00	12.00	12.00
Sugar	12.00	12.00	12.00
Oyster sauce	5.00	5.00	5.00
Garlic	12.00	12.00	12.00
Ginger	2.00	2.00	2.00
Onion	8.00	8.00	8.00
Pepper	1.00	1.00	1.00
Cooking wine	6.00	6.00	6.00
Sesame oil	2.00	2.00	2.00
Calamansi extract	0.00	0.14	0.18

0.14CE, dakgalbi with the addition of 0.14% calamansi extract; 0.18CE, dakgalbi with the addition of 0.18% calamansi extract.

the pH of the homogenates directly.

Water holding capacity

The water holding capacity (WHC) was determined according to the procedure outlined by Jung et al. (2022). A sample weighing 0.5 g, with the connective tissue discarded, was put into a water bath at 80℃ for a duration of 20 min. Subsequently, the solution was allowed to cool to room temperature for 10 min. The sample was then centrifuged at 2,000×g for 20 min at 4℃, after which the amount of water loss was calculated. The WHC, expressed as a percentage, was determined based on the water loss from centrifugation and the moisture content of the sample, calculated as follows:

$$\text{WHC (\%)} = (\text{Moisture content} - \text{Water loss}) / \text{Moisture content} \times 100 \quad (1)$$

$$\text{Water loss} = (\text{Weight before centrifugation} - \text{Weight after centrifugation}) / (\text{Sample weight} \times \text{Fat factor}) \times 100 \quad (2)$$

$$\text{Fat factor} = 1 - (\text{Crude fat} / 100). \quad (3)$$

Instrumental color

The color of the dakgalbi was measured using a CR-400 Minolta colorimeter (Minolta, Osaka, Japan), which featured an 8 mm aperture and utilized illuminant-C. The color parameters, namely, CIE L* for lightness, CIE a* for redness, and CIE b* for yellowness, were assessed 10 min after removing the vacuum packaging from the samples. After being removed from their packaging and placed on a plate, the samples underwent direct color measurement. This measurement encompassed the entire surface area of the meat pieces, including the cross-sectional area. These measurements were performed on day 1, 8, 12, 16, and 19 of storage.

Microbiological analysis

Petrifilm (Aerobic Plate Count and Coliform/*E. coli* Count Plates, 3M Company, St. Paul, MN, USA) was used to quantify total aerobic bacteria (TAB), coliforms, and *E. coli* counts. Each sample, weighing 3 g, was placed in a sterile bag containing 27 mL of sterile saline solution. A stomacher (BagMixer 400, Interscience, Saint-Nom la Bretèche, France) was used to homogenize the samples for 1 min. Following dilution with sterile saline solution, one milliliter of the homogenate was injected into a Petrifilm. The Petrifilm was then cultured at a temperature of 37℃ for a period of 48 h, after which the colony count was ascertained. The results were expressed as Log colony-forming units (CFU) per gram.

2-Thiobarbituric acid reactive substances

The 2-thiobarbituric acid reactive substances (TBARS) content was evaluated using the method outlined by Kim et al. (2019). A dakgalbi sample weighing 5 g was mixed with 50 μL of 7.2% tert-butyl-4-hydroxyanisole and 15 mL of distilled water. The mixture was then homogenized for 30 s using a Polytron PT-2500 E homogenizer (Kinematica). One milliliter of the homogenate was transferred to a test tube. Next, 2 mL of a solution containing thiobarbituric acid (TBA) and trichloroacetic acid (TCA; 20 mM TBA/15% TCA) was added to the tube. A blank sample consisting of 2 mL of each sample mixture was mixed with 2 mL of 15% TCA solution. The sample homogenate was then heated in a water bath at 90℃ for 15 min in order

to allow color development. After heating, the samples were chilled in cold water on ice for 10 min. Subsequently, they were centrifuged at 2,000×g at 4℃ for a duration of 15 min. The absorbance of the supernatant was measured at 531 nm using a SpectraMax M2 spectrophotometer (Molecular Devices). The malondialdehyde (MDA) content per kilogram of sample was expressed in terms of the TBARS value, calculated as follows:

$$\text{TBARS (mg MDA/kg)} = (\text{Absorbance of sample} - \text{Absorbance of the blank sample}) \times 5.88 \quad (4)$$

Sensory evaluation

The sensory attributes of dakgalbi were assessed by 15 assessors from the College of Animal Life Sciences at Kangwon National University. Dakgalbi was prepared by pan grilling. A 9-point hedonic scale was used to rate each attribute across the different test groups. The cooked samples were standardized to 1 cm in each dimension. The breast and thigh meat were provided for each experimental condition performed. Appearance, aroma, taste, flavor, and overall acceptability were evaluated on a scale where 1 point represented “extremely undesirable” and 9 points represented “extremely desirable.” Tenderness was rated on a scale where 1 point indicated “very tough” and 9 points indicated “very tender,” while juiciness was rated on a scale where 1 point meant “very dry” and 9 points meant “very juicy.” Off-flavor was rated on a scale where 1 point meant “very strong” and 9 points meant “very weak.” The study protocol was approved by the Institutional Review Board of Kangwon National University (KWNUIRB-2021-05-004-001). All the participants provided informed consent.

Statistical analysis

Analysis of variance (ANOVA) conducted within a general linear model was employed to examine the data using SAS software (version 9.2, SAS Institute, Cary, NC, USA). Tukey’s test was used to assess the significance of variations in mean values across samples. Statistically significant differences were defined as those with p-values less than 0.05. The experiment was conducted with three replications for each sample.

Result and Discussion

Antioxidant and antimicrobial activity of calamansi extract

The CE was extracted in 90% ethanol at a ratio of 1:50 for 6 days at 25℃ suggests a deliberate approach to maximizing the extraction efficiency of active compounds without compromising their quality or antioxidant activity. This prolonged extraction time allowed for better extraction of bioactive compounds including phenolic molecules and flavonoid contents which are known for their antioxidant properties (Antolak et al., 2018; Plaskova and Mlcek, 2023). This approach avoided heat, which can degrade these delicate compounds (Antony and Farid, 2022).

The CE antioxidant activity was measured using four different assays, DPPH, ABTS, FRAP, and ORAC, with values obtained of 44.27±6.63, 58.40±3.94, 49.35±0.56, and 284.45±13.65 mmol TE/g DM, respectively (Fig. 1). These results show that there is a higher antioxidant activity than those reported in a previous study by Cho et al. (2023), which recorded that the antioxidant activities of CE were 0.0114 mmol TE/g DM, 0.03 mmol TE/g DM, and 11.45 μmol TE/g DM for DPPH, FRAP, and ORAC, respectively. The observed differences between our study and the previous study (Cho et al., 2023) may be attributed to variations in the ethanol concentration and extraction duration. Jayaprakasha et al. (2001) highlighted that the choice and concentration of the extraction solvent play pivotal roles in the efficient extraction of antioxidant compounds from

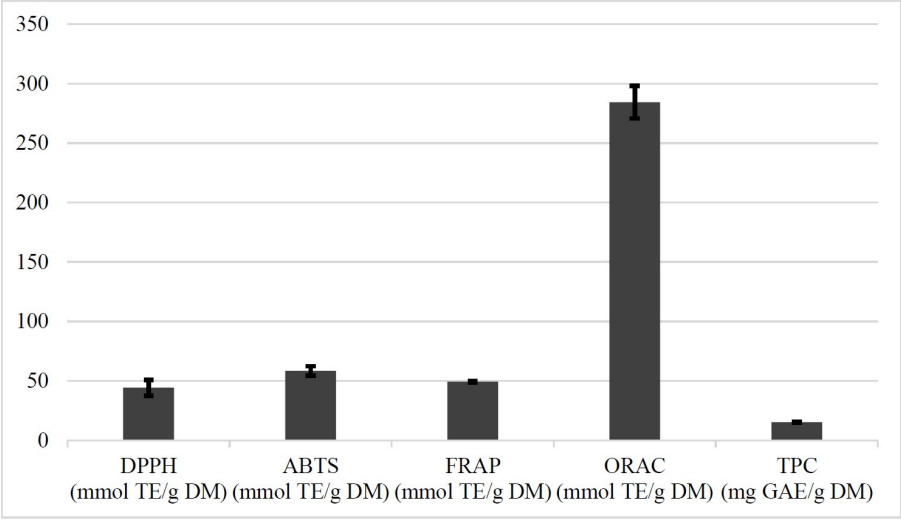

Fig. 1. Antioxidant activity and TPC of calamansi extract. DPPH, 1,1-diphenyl-2-pricrylhydrazyl; ABTS, 2,2′-azinobis-3-ethylbenzothiazoline- 6-sulfonic acid; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorption capacity; TPC, total phenolic content; TE, trolox equivalent; DM, dry matter; GAE, galic acid equivalent.

plant materials. Interestingly, this study found a TPC of 15.22±0.39 mg GAE/g DM, which is comparable to the 12.11 mg GAE/g DM reported by Cho et al. (2023). The presence of phenolic acids, such as caffeic, p-coumaric, ferulic, and sinapic acids, in calamansi is thought to contribute to its antioxidant properties, which are essential for maintaining the integrity of volatile chemical mixtures (Cheong et al., 2012a). The correlation between phenolic content and antioxidant capacity, as suggested by Barido et al. (2021), highlights the potential of CE to enhance the quality and shelf life of food products, such as dakgalbi, owing to its high antioxidant activity.

In this study, the paper disc diffusion method was used in order to assess the effectiveness of CE against various foodborne pathogens, including Gram-negative bacteria (*E. coli* and *S.* Enteritidis) and Gram-positive bacteria (*L. monocytogenes* and *S. aureus*). The assay measured the diameters of the inhibition zones formed by CE at different concentrations and when compared these findings with those of streptomycin, which served as a positive control. As detailed in Table 2, the CE demonstrated inhibition zones ranging from 10.63 to 11.35 mm against *E. coli*, with the most significant effect observed at a concentration of 10 mg/disc ($p<0.05$). Concentrations of CE below 2.5 mg/disc were ineffective in inhibiting the growth of all of the tested bacteria, showing no inhibition zones. The CE demonstrated an inhibitory effect against *S. aureus* with a zone of inhibition of 12.08 mm at a concentration of 10 mg/disc. This effect was on par with the inhibition observed for streptomycin, which produced a zone of 11.31 mm at a much lower concentration (0.01 mg/disc), although the difference was not statistically significant ($p>0.05$). The antimicrobial effect of CE began to manifest at a concentration of 5 mg/disc across all tested bacteria, with concentrations of 1.5 and 2.5 mg/disc having no impact. Furthermore, the results indicated that the CE had a more potent antimicrobial effect on Gram-negative bacteria than on Gram-positive bacteria, highlighting larger total inhibition zones. This observation is consistent with previous research by Mai-Prochnow et al. (2016) and may be attributed to the thinner cell walls of Gram-negative bacteria (1.5–10 nm), which are more susceptible to damage by phenolic acids than the thicker cell walls of Gram-positive bacteria (20–80 nm). In this study, the antimicrobial potency of CE was classified as strong, with inhibition zones of >10 mm (Vollmer et al., 2008) at a minimum effective concentration of 5 mg/disc. Cheong et al. (2012a) previously noted that the pronounced antimicrobial activity of calamansi stems from its rich content of phenolic acids, such as coumaric, sinapic, and caffeic acids. These compounds have been shown to exert significant stress on the main

Table 2. **The antimicrobial effect of calamansi extract, using the paper disc diffusion assay method (unit: mm)**

Compound	*Listeria monocytogenes*	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella* Enteritidis
PC	16.86^{A}	11.31^{A}	15.88^{A}	15.55^{A}
1.25 mg/disc	-	-	-	-
2.5 mg/disc	-	-	-	-
5 mg/disc	15.49^{A}	9.61^{B}	10.63^{C}	10.58^{B}
10 mg/disc	14.04^{A}	12.08^{A}	11.35^{B}	14.45^{A}
SEM	0.786	0.278	0.044	0.360

Values were expressed in millimeters (mm); an 8 mm diameter paper disc was included; PC positive control (streptomycin, 0.01 mg/disc for *E. coli*, *L. monocytogenes*, and *S. aureus*, 0.20 mg/disc for *S.* Enteritidis); - no clear zone detected.
$^{A–C}$ Means denoted by different superscripts within the same column indicate a significant difference ($p<0.05$).

component of the bacterial cell wall, peptidoglycan, which compromises cell integrity and leads to cell lysis. Additionally, these phenolic acids contribute to hyperacidification, which disrupts ATP synthesis and results in cell death (Cueva et al., 2010).

Proximate composition

Table 3 presents the proximate composition of dakgalbi treated with CE for both breast and thigh meat samples. The analysis revealed no significant differences ($p>0.05$) in the moisture, crude ash, crude protein, or crude fat content between the CE-treated and untreated samples of both meat types. This indicates that the application of CE does not affect the essential nutritional composition of meat, preserving vital nutritional qualities crucial for maintaining the quality and appeal of dakgalbi. Thus, CE can be regarded as an effective treatment for dakgalbi that does not compromise its nutritional integrity. These findings align with the results of a previous study (Muhlisin et al., 2013), which reported moisture, crude ash, crude protein, and crude fat contents of Chuncheon dakgalbi as 71.32%, 1.29%, 24.25%, and 2.71%, respectively. Similar results were obtained in this study. The moisture, crude protein, crude fat, and crude ash contents of the breast meat control group were 71.40%, 20.91%, 0.89%, and 1.91%, respectively. The 0.14CE of breast group treatment showed slight increases in all of the categories analyzed, with values of 71.46%, 22.54%, 0.91%, and 1.98%, respectively. The 0.18CE of breast

Table 3. **Effect of calamansi extract on proximate composition and of dakgalbi**

Meat cuts	Treatment	Proximate composition (%)			
		Moisture^{NS}	Crude protein^{NS}	Crude fat^{NS}	Crude ash^{NS}
Breast	Control	71.40	20.91	0.89	1.91
	0.14CE	71.46	22.54	0.91	1.98
	0.18CE	71.46	19.60	0.75	1.94
	SEM	0.240	1.116	0.049	0.063
Thigh	Control	70.87	17.54	4.76	2.88
	0.14CE	71.70	17.53	4.38	3.14
	0.18CE	72.52	17.37	4.71	3.00
	SEM	0.520	0.368	0.234	0.168

^{NS} Non-significant.
0.14CE, dakgalbi with 0.14% calamansi extract addition; 0.18CE, dakgalbi with 0.18% calamansi extract addition.

group treatment had a moisture content of 71.46%, crude protein content of 19.60%, crude fat content of 0.75%, and crude ash content of 1.94%. The moisture contents of the control group of thigh meat were 70.87%, crude protein of 17.54%, crude fat of 4.76%, and crude ash of 2.88%, respectively. The 0.14CE of the thigh treatment group had a moisture content of 71.70%, crude protein content of 17.53%, crude fat content of 4.38%, and crude ash content of 3.14%. The 0.18CE of the thigh treatment group had a moisture content of 72.52%, crude protein content of 17.37%, crude fat content of 4.71%, and crude ash content of 3.00%.

pH values of dakgalbi

Incorporating CE into dakgalbi sauce slightly reduced the pH value of both breast and thigh meat during the first 8 days of storage, as shown in Table 4. No significant differences ($p>0.05$) in pH were detected between the control and treated samples during this period, except for the addition of 0.14CE on the first day of storage. The pH trend showed a decrease for up to 19 d of storage for both meat types. This outcome aligns with the findings of Muhlisin et al. (2012), who noted a decline in the pH of dakgalbi treated with a curing mixture over 12 d of storage. Similarly, the pH of pork patty treated with CE decreased over the storage period (Cho et al., 2023). Despite these differences in the treatment compounds, the trend of decreasing pH over the storage period remained consistent across all previous studies. While a pH of 5.5–6.2 is generally considered safe for chicken consumption, with values below 5.5 or above 6.2 indicating spoilage (Sujiwo et al., 2018), all treatments in this study were no longer considered safe for consumption by the end of the 19-day storage period. This discrepancy may be due to the inclusion of sauce in the chicken mixture, potentially influencing the measured pH compared to plain meat. Typically, the pH value of meat has been found to decrease during the initial phase of storage, followed by an increase towards the end of the storage period. This increase in pH may have resulted from protein degradation caused by the activity of microorganisms or enzymes during storage (Kim et al., 2019). However, the expected increase in pH was not observed in the meat products in this study. The addition of sauces or marinades to raw meat can influence the microbial environment, thereby affecting the pH of meat products. The observed decrease in meat product pH over the storage period could be attributed to the presence of bacteria, such as lactic acid bacteria (Muhlisin et al., 2012).

Table 4. Effect of calamansi extract on pH values of dakgalbi during storage at 4℃

Meat cuts	Treatment	Storage time (d)					SEM
		1	8	12	16	19	
Breast	Control	5.61^{Ab}	5.67^{Aa}	5.54^{Bc}	5.03^{Cd}	4.84^{Ce}	0.013
	0.14CE	5.59^{Bb}	5.64^{Aa}	5.63^{Aa}	5.39^{Bc}	5.13^{Bd}	0.009
	0.18CE	5.59^{Aba}	5.62^{Aa}	5.64^{Aa}	5.45^{Ab}	5.24^{Ac}	0.012
	SEM	0.005	0.014	0.015	0.013	0.009	
Thigh	Control	6.33^{Aa}	6.31^{Aa}	5.31^{Bb}	4.77^{Bc}	4.66^{Bd}	0.012
	0.14CE	6.29^{Aa}	6.27^{Aa}	5.61^{Ab}	4.87^{Ac}	4.75^{Ac}	0.026
	0.18CE	6.27^{Aa}	6.19^{Aa}	5.64^{Ab}	4.91^{Ac}	4.77^{Ad}	0.020
	SEM	0.018	0.029	0.026	0.012	0.010	

0.14CE, dakgalbi with 0.14% calamansi extract addition; 0.18CE, dakgalbi with 0.18% calamansi extract addition.
$^{A–C}$ Means denoted by different superscripts within the same column indicate a significant difference ($p<0.05$).
$^{a–e}$ Means denoted by different superscripts within the same row indicate a significant difference ($p<0.05$).

Water holding capacity of dakgalbi

The influence of the CE on the WHC of dakgalbi during storage at 4℃ was investigated in both breast and thigh meat cuts, as shown in Table 5. For the breast meat, the control group exhibited a significant decrease in the WHC from 57.75% on day 1 to 47.21% on day 19 ($p<0.05$). The 0.14CE WHC was 49.43% on day 1, which increased to 62.34% on day 8, followed by a slight decrease towards the end of the storage period, reaching 52.63% on day 19. The unexpected increase in WHC during storage could be attributed to protein degradation, as proteins break down, they may temporarily create new binding sites for water (Xu et al., 2023). The 0.18CE showed a relatively stable WHC, starting at 59.11% on day 1 with minor fluctuations and ending at 56.47% on day 19. In the thigh meat, the WHC of the control group decreased significantly from 53.29% on day 1 to 27.22% on day 19 ($p<0.05$). The 0.14CE treatment began with a WHC of 42.34% on day 1, peaking at 65.74% on day 8, and then decreasing to 31.58% by day 19. Treatment with 0.18CE resulted in a WHC of 45.55%, which decreased to 26.45% by the end of the storage period. The greater decrease in WHC observed in the 0.18CE treatment group may be attributed to changes in meat structure. Muscle breakdown during storage can create channels within the meat, facilitating water loss and consequently leading to a decrease in WHC. These results suggest that CE, particularly at 0.14% in breast meat and thigh meat, positively affects the WHC of dakgalbi during storage. CE treatment effectively maintained the WHC value throughout the storage period, whereas untreated dakgalbi exhibited a lower WHC by the end of the storage period. Higher WHC values are considered advantageous as they contribute to improved sensory characteristics like juiciness and tenderness. The WHC value in this study aligns with that of a previous study Muhlisin et al. (2012), which reported a WHC value of 48.24% for dakgalbi prepared from broiler meat. WHC serves as an indicator of the shelf life of meat or meat products during storage. Furthermore, the WHC is associated with meat pH value and color (Sujiwo et al., 2018). The WHC of meat is influenced by various factors such as the speed and degree of pH reduction, proteolysis, and protein oxidation (Li et al., 2018). In addition, protein denaturation affects the WHC. The process of denaturing proteins, which destroy water-binding sites, leading to increased hydrophobicity, along with key structural features such as myofibrillar lattice spacing, the expulsion of fluid to the extracellular space, and the formation of drip channels, plays a significant role regarding water loss in meat (Hughes et al., 2014).

Table 5. Effect of calamansi extract on the water holding capacity (%) of dakgalbi during storage at 4℃

Meat cuts	Treatment	Storage time (d)					SEM
		1	8	12	16	19	
Breast	Control	57.75^{Aa}	57.12^{Ba}	59.05^{Aa}	47.03^{Bb}	47.21^{Cb}	1.057
	0.14CE	49.43^{Bb}	62.34^{Aa}	58.99^{Aa}	50.01^{Ab}	52.63^{Bb}	0.833
	0.18CE	59.11^{Aa}	59.91^{Aa}	52.84^{Bbc}	50.32^{Ac}	56.47^{Aab}	0.792
	SEM	1.189	0.603	1.368	0.402	0.501	
Thigh	Control	53.29^{Aa}	58.43^{ABa}	34.32^{Bb}	30.37^{Abc}	27.22^{ABc}	1.233
	0.14CE	42.34^{Bb}	65.74^{Aa}	36.56^{Bbc}	27.39^{Ad}	31.58^{Adc}	1.622
	0.18CE	45.55^{Bab}	49.51^{Ba}	39.63^{Ab}	28.37^{Ac}	26.45^{Bc}	1.305
	SEM	0.969	2.229	0.550	1.561	1.053	

0.14CE, dakgalbi with 0.14% calamansi extract addition; 0.18CE, dakgalbi with 0.18% calamansi extract addition.
$^{A–C}$ Means denoted by different superscripts within the same column indicate a significant difference ($p<0.05$).
$^{a–d}$ Means denoted by different superscripts within the same row indicate a significant difference ($p<0.05$).

Instrumental color of dakgalbi

The effect of CE on the instrumental color of dakgalbi during storage at 4°C was evaluated, with findings presented in Table 6. This study assessed changes in color parameters (CIE L*, a*, and b*) for both breast and thigh meat treated with 0.14% and 0.18% CEs over storage periods of 1, 8, 12, 16, and 19 days. For the breast meat, the CIE L* values indicated a slight variation in lightness during the storage period. The control samples showed an increase in CIE L* towards the end of storage (47.23 at day 19), while the CE-treated samples demonstrated a decrease, particularly for the 0.14CE treatment group, which decreased to 43.00 by day 19. The CIE L* of the 0.18CE group was stable. Regarding the CIE a* values, which measure redness to greenness ratios, all treatments maintained relatively stable CIE a* levels throughout the storage period. In this study, the CIE a* values ranged from 3.55 to 10.37, which were higher compared to the typical color of chicken meat,

Table 6. Effect of calamansi extract on the instrumental color of dakgalbi during storage at 4°C

Meat cuts	Color	Treatment	Storage time (d)					SEM
			1	8	12	16	19	
Breast	CIE L*	Control	44.69^{Aab}	42.98^{Ab}	43.58^{Ab}	45.49^{Aab}	47.23^{Aa}	0.609
		0.14CE	44.00^{Aa}	42.64^{Aab}	41.62^{Bab}	41.04^{Bb}	43.00^{Bab}	0.587
		0.18CE	42.68^{Aab}	41.30^{Ab}	41.83^{Bab}	41.98^{Bab}	44.48^{ABa}	0.603
		SEM	0.744	0.688	0.367	0.401	0.689	
	CIE a*	Control	4.15^{Aa}	4.43^{Aa}	4.14^{Aa}	4.14^{Aa}	3.98^{Aa}	0.202
		0.14CE	3.78^{Aab}	4.19^{ABa}	4.30^{Aa}	3.86^{Aab}	3.55^{Ab}	0.131
		0.18CE	3.88^{Aabc}	3.82^{Bbc}	4.41^{Aa}	4.05^{Aab}	3.46^{Ac}	0.124
		SEM	0.192	0.115	0.157	0.165	0.144	
	CIE b*	Control	19.64^{Aa}	17.20^{Ab}	17.79^{Ab}	17.59^{Ab}	18.41^{Aab}	0.326
		0.14CE	18.65^{Aa}	17.14^{Aabc}	17.96^{Aab}	16.28^{Abc}	15.65^{Bc}	0.402
		0.18CE	19.54^{Aa}	16.49^{Ab}	17.08^{Ab}	16.69^{Ab}	16.87^{ABb}	0.395
		SEM	0.435	0.239	0.429	0.387	0.356	
Thigh	CIE L*	Control	41.57^{Ab}	42.61^{Ab}	42.70^{Ab}	47.02^{Aa}	50.42^{Aa}	0.754
		0.14CE	40.87^{Ad}	40.64^{Ad}	43.62^{Ac}	46.39^{Ab}	48.59^{Ba}	0.403
		0.18CE	41.12^{Ab}	39.82^{Ab}	41.97^{Ab}	45.85^{Aa}	47.74^{Ba}	0.655
		SEM	0.519	0.671	0.898	0.577	0.275	
	CIE a*	Control	10.29^{Aa}	9.58^{Aa}	10.37^{Aa}	9.61^{Aa}	8.14^{Aa}	0.525
		0.14CE	8.85^{Bab}	9.01^{Aab}	9.61^{Aa}	8.99^{Aab}	7.64^{Ab}	0.374
		0.18CE	9.00^{Bab}	9.50^{Aab}	9.89^{Aa}	9.20^{Aab}	8.03^{Ab}	0.372
		SEM	0.261	0.379	0.312	0.693	0.365	
	CIE b*	Control	16.02^{Aa}	14.60^{Aa}	15.20^{Aa}	14.66^{Aa}	16.67^{Aa}	0.486
		0.14CE	16.88^{Aa}	13.82^{Ab}	16.42^{Aa}	16.43^{Aa}	17.60^{Aa}	0.464
		0.18CE	16.67^{Aa}	14.96^{Aa}	16.23^{Aa}	16.77^{Aa}	16.98^{Aa}	0.475
		SEM	0.312	0.446	0.495	0.623	0.448	

0.14CE, dakgalbi with 0.14% calamansi extract addition; 0.18CE, dakgalbi with 0.18% calamansi extract addition.
^{A,B} Means denoted by different superscripts within the same column indicate a significant difference ($p<0.05$).
$^{a–d}$ Means denoted by different superscripts within the same row indicate a significant difference ($p<0.05$).

reported as 1.13 to 1.86 (Kim et al., 2019). In this experiment, chicken meat was combined with dakgalbi sauce, which has a natural red color. Consequently, the instrumental CIE a* of the meat was higher than that of typical chicken meat. The CIE b* values, indicative of yellowness, also varied with the storage time. The CIE b*, however, varied with storage duration; notably, the 0.14CE of the breast meat treatment group caused a significant decrease in CIE b* to 15.65 by day 19, showing significant difference when compared to the control group at the same storage point. Similar trends were observed in the thigh meat, with the general maintenance of color attributes throughout the storage period. However, there was a notable increase in CIE L* in the control group by day 19 (50.42), whereas CE-treated samples showed less pronounced changes. The CIE a* and CIE b* values for thigh meat also exhibited stability across treatments, with slight variations, indicating the protective effect of CE against color degradation over time. The application of antioxidants derived from natural sources can efficiently maintain color and flavor stability (Ribeiro et al., 2019), thus extending the shelf life of fresh, precooked, cooked, and processed meat. Regarding meat products, the oxidation of fat, which is present as unsaturated fat in membrane phospholipids, occurs during processing and storage. Oxidation reactions in meat not only diminish nutritional value by depleting essential fatty acids and vitamins, but also manifest as a decline in sensory quality, including changes in color, texture, and the emergence of rancid odors and flavors, thereby impacting consumer acceptance (Domínguez et al., 2019).

Microbiological changes

This study has assessed the impact of CE on the microbiological quality of raw dakgalbi stored at 4°C, focusing on TAB, coliforms, and the presence of *E. coli* in both breast and thigh meat over a storage period of 19 days. The results are summarized in Table 7. The TAB counts of breast meat indicated that the control samples experienced a progressive increase in bacterial load, reaching 7.07 Log CFU/g by day 19. In comparison, samples treated with 0.14CE and 0.18CE showed moderated growth, with final counts of 6.98 and 6.97 Log CFU/g, respectively, by the end of the storage period. Furthermore, the samples that underwent CE treatment exhibited significantly reduced values ($p<0.05$) when compared to the control samples on 12th and 16th days of storage. This suggests that the addition of CE effectively slowed the proliferation of TAB. The TAB in the untreated sample on day 12 of storage was almost unacceptable, based on the Korean meat microbiological guidelines published by the Ministry of Food and Drug Safety (MFDS, 2018), which state that the limit is 5×10^6 CFU/g, equivalent to 6.70 Log CFU/g. The TAB values of the control group were 6.21 Log CFU/g (breast meat) and 6.20 Log CFU/g (thigh meat) on day 12. In contrast, the CE-treated samples remained acceptable until day 16 of storage for the breast meat and until day 19 for the thigh meat. Coliform counts in the breast meat also revealed a general increase over time in the control group, peaking at 3.75 Log CFU/g by day 16 before slightly decreasing. The CE-treated samples exhibited a somewhat controlled increase, with the 0.18CE treatment notably maintaining lower coliform counts throughout the storage period, concluding with 2.55 Log CFU/g by day 19. The 0.18CE was also found to be significantly lower ($p<0.05$) than that of the control on day 16 of storage. *E. coli* was not detected in any of the samples throughout the study, indicating that all treatments maintained a level of microbial control that prevented the presence of this specific pathogen. Similar trends were observed for TAB in thigh meat. The control samples showed a gradual increase in the bacterial load, reaching 7.19 Log CFU/g on day 19. The application of 0.14CE and 0.18CE resulted in significantly lower ($p<0.05$) final bacterial counts of 6.67 and 6.10 Log CFU/g, respectively, demonstrating the antimicrobial effects of CE. Coliform counts in thigh meat demonstrated a variable pattern, with control samples maintaining higher counts than CE-treated samples. Notably, the 0.18CE treatment maintained consistently lower coliform levels ($p<0.05$), ending with 2.10 Log CFU/g by day 19, when compared to that of the control at 2.49 Log CFU/g. The results of this study align with those of a previous study (Cho et al.,

Table 7. Effect of calamansi extract on microbiological changes of dakgalbi during storage at 4°C (unit: Log CFU/g)

Meat cuts	Microorganism	Treatment	Storage time (d)					SEM
			1	8	12	16	19	
Breast	TAB	Control	3.92^{Ad}	4.74^{Ac}	6.21^{Ab}	6.27^{Ab}	7.07^{Aa}	0.081
		0.14CE	3.78^{Ad}	4.97^{Ac}	4.62^{Bc}	5.77^{Bb}	6.98^{Aa}	0.083
		0.18CE	3.79^{Ad}	4.81^{Ac}	4.64^{Bc}	5.48^{Bb}	6.97^{Aa}	0.063
		SEM	0.037	0.095	0.097	0.090	0.035	
	Coliforms	Control	2.86^{Ab}	2.98^{Ab}	3.12^{Ab}	3.75^{Aa}	2.82^{Ab}	0.094
		0.14CE	2.97^{Abc}	3.17^{Aab}	2.86^{Bbc}	3.43^{ABa}	2.62^{Ac}	0.095
		0.18CE	2.94^{Ab}	2.96^{Aab}	2.96^{ABab}	3.29^{Ba}	2.55^{Ac}	0.073
		SEM	0.043	0.063	0.051	0.078	0.155	
	Escherichia coli	Control	ND	ND	ND	ND	ND	-
		0.14CE	ND	ND	ND	ND	ND	-
		0.18CE	ND	ND	ND	ND	ND	-
		SEM	-	-	-	-	-	
Thigh	TAB	Control	4.00^{Ad}	5.46^{Ac}	6.20^{Ab}	6.27^{Ab}	7.19^{Aa}	0.065
		0.14CE	3.95^{Ad}	5.05^{Bc}	5.91^{Ab}	5.81^{Ab}	6.67^{Ba}	0.088
		0.18CE	3.97^{Ac}	5.06^{Bb}	4.94^{Bb}	5.44^{Aab}	6.10^{Ca}	0.155
		SEM	0.053	0.082	0.096	0.194	0.061	
	Coliforms	Control	2.34^{Ba}	2.10^{Ba}	2.59^{Aa}	2.49^{Aa}	2.49^{Aa}	0.135
		0.14CE	2.55^{Ab}	3.00^{Aa}	2.10^{Bc}	2.20^{Ac}	2.00^{Bc}	0.067
		0.18CE	2.63^{Aab}	3.07^{Aa}	2.00^{Bc}	2.30^{Ac}	2.10^{Bc}	0.099
		SEM	0.048	0.078	0.068	0.183	0.088	
	E. coli	Control	ND	ND	ND	ND	ND	-
		0.14CE	ND	ND	ND	ND	ND	-
		0.18CE	ND	ND	ND	ND	ND	-
		SEM	-	-	-	-	-	

0.14CE, dakgalbi with 0.14% calamansi extract addition; 0.18CE, dakgalbi with 0.18% calamansi extract addition.
^{A,B} Means denoted by different superscripts within the same column indicate a significant difference ($p<0.05$).
$^{a–c}$ Means denoted by different superscripts within the same row indicate a significant difference ($p<0.05$).
CFU, colony-forming units; ND, not detected; TAB, total aerobic bacteria.

2023), which reported that treatment of pork patties with calamansi pulp extract significantly reduced the number of bacteria, including TAB, lactic acid bacteria, Enterobacteriaceae, and *Pseudomonas* spp. The presence of phenolic acids in the CE could potentially account for its antimicrobial effects, leading to a reduction in the microbial population of dakgalbi compared to the untreated sample. A possible mechanism underlying the antimicrobial properties of phenolic acids against pathogens is hyperacidification at the plasma membrane interface caused by the dissociation of the phenolic acids (Cueva et al., 2010). Hyperacidification alters cell membrane potential, thus making it more permeable. This change also affects the sodium-potassium ATPase pump, which plays a crucial role in ATP synthesis (Vattem et al., 2005). In addition, the findings of this study align with those of a previous study, by Hussain et al. (2021) which demonstrated that the synergistic interaction of ferulic, p-coumaric, and sinapic acids in calamansi inhibited the growth of bacteria and preserved the physical quality of

chicken fillets marinated in a blend of calamansi and chili paste during storage.

The 2-thiobarbituric acid reactive substances values of dakgalbi

This study assessed the effects of CE on the TBARS values, indicative of lipid oxidation, in dakgalbi stored at 4℃, as presented in Table 8. Initially, the TBARS values were relatively low across all breast meat groups. As time progressed, the TBARS values increased in both the treated and control samples, reflecting the natural progression of lipid oxidation. Towards the end of the storage period, a slight decrease in TBARS values was observed in the samples treated with 0.14CE and 0.18CE. However, as the control group also did not show a significant difference in TBARS values towards the end of the storage period, it seems that there is no significant effect of CE on the breast meat group. A similar trend was observed for the thigh meat. The TBARS values of the control, 0.14CE, and 0.18CE group increased until day 16, indicating the vulnerability of meat lipids to oxidative deterioration over time. However, by day 19, a reduction in the TBARS values was observed in all treatment group. It is noteworthy that on day 8 of storage, the TBARS value of the 0.14CE treatment was significantly lower than that of the control in both breast and thigh meat, highlighting the early antioxidant effect of the CE. This aligns with the findings of Kim et al. (2018), who reported that lemons, a member of the citrus family, could significantly reduce lipid oxidation in chicken meat during storage. Lipid oxidation has been shown to lead to a decline in the sensory qualities of meat, which can be explained by aldehydes reacting with compounds to produce substances unreactive to TBA, or by the TBARS values following an 'induction-propagation-termination' cycle, resulting in fluctuating patterns of increase and decrease (Lee et al., 2021).

Sensory evaluation of dakgalbi

The sensory properties of chicken breast dakgalbi treated with CE and stored at 4℃ were assessed over a 12-day period, focusing on appearance, aroma, taste, flavor, off-flavor, juiciness, tenderness, and overall acceptability (Table 9). Throughout the 12-day storage period, the sensory evaluation of chicken breast dakgalbi treated with CE and the control group revealed no significant differences in most traits, including appearance, aroma, off-flavor, juiciness, tenderness, or overall acceptability. This

Table 8. Effects of calamansi extract on the 2-thiobarbituric acid reactive substances (TBARS) values of dakgalbi during storage at 4℃ (unit: mg MDA/kg)

Meat cuts	Treatment	Storage time (d)					SEM
		1	8	12	16	19	
Breast	Control	0.980^{Aa}	1.135^{Aa}	1.169^{Aa}	1.100^{Aa}	0.997^{Aa}	0.055
	0.14CE	0.931^{Ac}	1.041^{Bab}	1.024^{Aac}	1.091^{Aa}	0.960^{Abc}	0.022
	0.18CE	0.966^{Ac}	1.132^{Aa}	1.064^{Aab}	1.087^{Aa}	0.989^{Abc}	0.017
	SEM	0.033	0.019	0.063	0.022	0.021	
Thigh	Control	1.387^{Ab}	1.518^{Aab}	1.622^{Aa}	1.656^{ABa}	1.369^{Ab}	0.038
	0.14CE	1.213^{Bc}	1.302^{Bc}	1.575^{Aab}	1.615^{Ba}	1.403^{Abc}	0.042
	0.18CE	1.220^{Bc}	1.531^{Aab}	1.574^{Aab}	1.687^{Aa}	1.472^{Ab}	0.035
	SEM	0.023	0.042	0.063	0.013	0.031	

0.14CE, dakgalbi with 0.14% calamansi extract addition; 0.18CE, dakgalbi with 0.18% calamansi extract addition.
^{A,B} Means denoted by different superscripts within the same column indicate a significant difference ($p<0.05$).
$^{a–c}$ Means denoted by different superscripts within the same row indicate a significant difference ($p<0.05$).
MDA, malondialdehyde.

Table 9. Effect of calamansi extract on sensory properties of breast dakgalbi during storage at 4℃

Trait	Treatment	Storage time (d)			SEM
		1	8	12	
Appearance	Control	7.66^{Aa}	7.60^{Aa}	7.33^{Aa}	0.219
	0.14CE	8.13^{Aa}	7.07^{Ab}	7.47^{Aab}	0.293
	0.18CE	8.13^{Aa}	7.33^{Aab}	7.07^{Ab}	0.219
	SEM	0.222	0.263	0.251	
Aroma	Control	7.13^{Aa}	7.60^{Aa}	6.80^{Aa}	0.238
	0.14CE	7.27^{Aa}	6.87^{Aa}	7.13^{Aa}	0.282
	0.18CE	7.27^{Aa}	6.60^{Aa}	6.80^{Aa}	0.388
	SEM	0.237	0.392	0.277	
Taste	Control	7.00^{Aab}	7.60^{Aa}	6.60^{Ab}	0.273
	0.14CE	7.20^{Aa}	6.33^{Aa}	7.13^{Aa}	0.368
	0.18CE	7.33^{Aa}	6.20^{Aa}	6.60^{Aa}	0.363
	SEM	0.257	0.424	0.310	
Flavor	Control	7.07^{Aab}	7.47^{Aa}	6.27^{Ab}	0.237
	0.14CE	6.93^{Aa}	6.67^{ABa}	6.80^{Aa}	0.325
	0.18CE	7.00^{Aa}	6.13^{Ba}	6.53^{Aa}	0.385
	SEM	0.257	0.371	0.326	
Off-flavor	Control	7.27^{Aab}	7.67^{Aa}	6.80^{Ab}	0.247
	0.14CE	7.20^{Aa}	6.40^{Aa}	7.13^{Aa}	0.360
	0.18CE	7.07^{Aa}	6.27^{Aa}	6.87^{Aa}	0.389
	SEM	0.233	0.439	0.308	
Juiciness	Control	5.53^{Aa}	6.00^{Aa}	5.80^{Aa}	0.314
	0.14CE	5.27^{Aa}	5.73^{Aa}	5.87^{Aa}	0.302
	0.18CE	5.60^{Aa}	5.67^{Aa}	6.00^{Aa}	0.415
	SEM	0.324	0.422	0.282	
Tenderness	Control	6.27^{Aa}	6.07^{Aa}	6.07^{Aa}	0.325
	0.14CE	6.33^{Aa}	5.87^{Aa}	6.33^{Aa}	0.357
	0.18CE	6.47^{Aa}	6.00^{Aa}	6.47^{Aa}	0.368
	SEM	0.276	0.444	0.308	
Overall acceptability	Control	6.93^{Aa}	7.20^{Aa}	6.47^{Aa}	0.255
	0.14CE	7.00^{Aa}	6.40^{Aa}	6.80^{Aa}	0.360
	0.18CE	6.80^{Aa}	6.33^{Aa}	6.40^{Aa}	0.389
	SEM	0.274	0.420	0.307	

0.14CE, dakgalbi with 0.14% calamansi extract addition; 0.18CE, dakgalbi with 0.18% calamansi extract addition.
^{A,B} Means denoted by different superscripts within the same column indicate a significant difference ($p<0.05$).
^{a,b} Means denoted by different superscripts within the same row indicate a significant difference ($p<0.05$).

consistency suggests that the addition of 0.14% and 0.18% CE did not adversely affect the sensory characteristics of dakgalbi, therefore maintaining its acceptability among the panelists. The only exception was observed in the flavor trait on day 8 of

Table 10. Effect of calamansi extract on sensory properties of thigh dakgalbi during storage at 4℃

Trait	Treatment	Storage time (d)			SEM
		1	8	12	
Appearance	Control	7.73^{Aa}	7.53^{Aa}	7.40^{Aa}	0.179
	0.14CE	7.60^{Aa}	7.60^{Aa}	7.47^{Aa}	0.190
	0.18CE	8.00^{Aa}	7.80^{Aab}	7.20^{Ab}	0.181
	SEM	0.161	0.194	0.194	
Aroma	Control	7.20^{Aab}	7.80^{Aa}	6.80^{Ab}	0.255
	0.14CE	7.53^{Aa}	7.20^{Aa}	7.27^{Aa}	0.249
	0.18CE	7.60^{Aa}	7.40^{Aa}	7.27^{Aa}	0.239
	SEM	0.269	0.204	0.265	
Taste	Control	6.67^{Aa}	7.40^{Aa}	6.33^{Ba}	0.314
	0.14CE	7.47^{Aa}	7.20^{Aa}	7.27^{Aa}	0.261
	0.18CE	7.40^{Aa}	7.20^{Aa}	6.93^{ABa}	0.248
	SEM	0.310	0.253	0.261	
Flavor	Control	6.67^{Aa}	7.13^{Aa}	6.67^{Aa}	0.324
	0.14CE	7.47^{Aa}	6.93^{Aa}	7.07^{Aa}	0.263
	0.18CE	7.47^{Aa}	7.27^{Aa}	6.60^{Aa}	0.293
	SEM	0.325	0.256	0.297	
Off-flavor	Control	7.40^{Aa}	7.27^{Aa}	6.53^{Aa}	0.276
	0.14CE	7.40^{Aa}	7.27^{Aa}	7.20^{Aa}	0.261
	0.18CE	7.60^{Aa}	7.13^{Aa}	7.00^{Aa}	0.262
	SEM	0.260	0.250	0.287	
Juiciness	Control	7.06^{Aab}	7.60^{Aa}	6.53^{Ab}	0.226
	0.14CE	7.40^{Aa}	6.93^{Aa}	6.80^{Aa}	0.310
	0.18CE	7.47^{Aa}	7.13^{Aab}	6.60^{Ab}	0.249
	SEM	0.270	0.265	0.257	
Tenderness	Control	7.47^{Aa}	7.47^{Aa}	6.73^{Aa}	0.246
	0.14CE	7.40^{Aa}	6.87^{Aa}	6.93^{Aa}	0.356
	0.18CE	7.27^{Aa}	7.13^{Aa}	6.60^{Aa}	0.304
	SEM	0.338	0.267	0.307	
Overall acceptability	Control	7.00^{Aab}	7.33^{Aa}	6.47^{Bb}	0.229
	0.14CE	7.33^{Aa}	6.67^{Aa}	7.33^{Aa}	0.303
	0.18CE	7.40^{Aa}	7.13^{Aa}	6.80^{ABa}	0.257
	SEM	0.267	0.293	0.230	

0.14CE, dakgalbi with 0.14% calamansi extract addition; 0.18CE, dakgalbi with 0.18% calamansi extract addition.
^{A,B} Means denoted by different superscripts within the same column indicate a significant difference ($p<0.05$).
^{a,b} Means denoted by different superscripts within the same row indicate a significant difference ($p<0.05$).

storage, where a significant difference ($p<0.05$) indicated a variation in flavor perception, particularly in the 0.18% CE treatment. During the storage period, a significant decrease ($p<0.05$) in taste and flavor was recorded in the control group,

indicating a deterioration of these sensory attributes over time. In contrast, CE treatment on dakgalbi prepared from breast meat effectively maintained sensory traits, with no significant decrease in taste or flavor. Similarly, dakgalbi prepared fromthigh meat revealed that CE treatment effectively maintained the sensory quality without negatively affecting the sensory characteristics after 12 days of storage (Table 10). Interestingly, in the case of the thigh meat, CE treatment not only preserved the sensory traits, but also showed a notable improvement in taste and overall acceptability by day 12 of storage, particularly in samples treated with 0.14% CE compared to the control. This indicates that CE, beyond its role in preservation, may also contribute positively to the sensory profile of dakgalbi over time. In line with these findings Budiarto et al. (2024), the hedonic quality of chicken meat significantly increased after storage when treated with citrus-based additives. The preservation of sensory qualities in CE-treated dakgalbi underscores the efficacy of CE in maintaining the desirable sensory attributes of the product over time. The absence of any significant differences between the control and CE-treated groups for almost all of the sensory traits throughout the storage period highlights the potential of CE as a natural preservative. It effectively maintained the sensory quality of dakgalbi without compromising its acceptability. The results of the sensory properties in this study are in accordance with the findings of Cho et al. (2023), who reported that the addition of CE to pork patties preserves and improves their quality, including sensory properties. Assessing sensory properties after 12 days of storage could be beneficial for developing future meal products, like home meal replacements. Notably, Muhlisin et al. (2013) also evaluated the chemical changes of dakgalbi after 4 weeks in storage.

Conclusion

Incorporating CE into dakgalbi shows potential to improve the quality and shelf life of both chicken breast and thigh meat. This is likely due to the antioxidant properties, as evidenced by the TBARS evaluation, and its antimicrobial effects. This study demonstrated that the addition of CE effectively preserved the WHC, microbiological quality, and TBARS value during storage at 4°C. The results demonstrate that CE, particularly at concentrations of 0.14% (0.14CE) of thigh meat considered more desirable compared to the other group. Furthermore, CE treatment effectively maintained the sensory quality without negatively affecting the sensory characteristics. Overall, our study underscores the potential of CE as a natural preservative for dakgalbi, offering a sustainable approach to prolong its shelf life and ensure consumer satisfaction. However, this investigation has certain limitations that warrant further research. Future studies should aim to explore a wider range of microorganisms, extend the study to other meat types or meat products, and assess different concentrations and combinations of CEs. Additionally evaluating the stability and efficacy of the antioxidant properties post-heating to ensure the practical application of CE in cooked food products. Addressing these aspects will not only confirm the efficacy of CE, but also broaden its practical applications in the food industry, contributing to the development of safer and more sustainable food preservation techniques.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Kim D, Jang A. Data curation: Jung Y, Lee S, Lee HJ, Oh S. Formal analysis: Jung S, Kim D, Lee HJ, Oh S. Methodology: Kim D, Choo HJ, Jang A. Software: Sujiwo J, Jung Y, Lee S, Lee HJ, Oh S. Validation: Kim D, Choo HJ, Jang A. Investigation: Kim D, Choo HJ, Jang A. Writing - original draft: Sujiwo J, Jung Y, Kim D, Choo HJ, Jang A. Writing - review & editing: Sujiwo J, Jung Y, Lee S, Kim D, Lee HJ, Oh S, Choo HJ, Jang A.

Ethics Approval

The sensory evaluation received ethical approval from the Institutional Review Board of Kangwon National University (KWNUIRB-2021-05-004-001).

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