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Effects of *Cissus quadrangularis L*. Powder Supplementation on Proximate Composition, Physicochemical and Textural Properties of Tteokgalbi

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Abstract We investigated *Cissus quadrangularis L.* powder (C) use as a natural additive to Tteokgalbi, a traditional Korean meat-based dish. Five distinct Tteokgalbi samples were treated: one without any additives (negative control; NC), one with 1.0% C (C1), 2.0% C (C2), 4.0% C (C3), and 0.10% ascorbic acid (positive control; PC). C addition resulted in changes in composition, quality, and sensory attributes. Moisture content decreased with higher C levels; crude protein varied among the groups, with C1 having the highest crude protein levels and C3 the lowest. Crude fat decreased with increasing C concentration, whereas the carbohydrate content increased. The water-holding capacity notably decreased in the C3 group, resulting in increased cooking loss with higher C concentrations. C treatment altered color and texture, reducing lightness (*L**) and increasing redness (*a**) before cooking and increasing *L** and *a** after cooking. Yellowness (*b**) decreased before cooking but increased thereafter. C-treated Tteokgalbi was less cohesive, chewy, and brittle compared to the NC. The C treatment increased the total phenolic and flavonoid contents and enhanced radical scavenging capacities. It also affects storage characteristics, lowers pH, and increases TBARS values. The microbial counts were lower in C2 and C3 after 11 days. These findings suggest the potential use of C as a natural meat additive.

Keywords: *Cissus quadrangularis*, Tteokgalbi, physicochemical properties, antioxidant capacities, sensory attributes

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Introduction

Tteokgalbi, a regional delicacy originating from Damyang, Haenam, and Gangjin in Jeollanamdo, Korea, is made from rib meat, giving it a resemblance to rice cakes. Despite its close connection to rib meat, which might pose challenges for individuals with dental sensitivities, Tteokgalbi is tender thanks to meticulous trimming, making it suitable for both youngsters and seniors. In the Joseon Dynasty, it was known as 'Hyogalbi' or 'Nogalbi,' serving as a rib dish designed for the elderly with dental concerns. The name 'Tteokgalbi' likely emerged in the 1960s due to its preparation method, which involves trimming the meat to resemble rice cakes.

Tteokgalbi is a Korean dish made by mixing minced or ground beef or pork with various ingredients and seasonings. These patties are either grilled or pan-fried. While specific nutritional details may vary depending on the recipe, a typical 100-gram serving of Tteokgalbi contains around 200 to 300 calories, with approximately 15 to 20 grams of protein, 10 to 15 grams of fat, and 10 to 15 grams of carbohydrates. Due to its specific recipe, Tteokgalbi falls under the category of energy-dense foods.

Energy-dense foods are those that provide a significant amount of energy in relation to their weight or volume. They often have a high concentration of fats and/or sugars, leading to their elevated energy density (Livingstone et al., 2022). Overconsumption of energy-dense foods can significantly contribute to weight gain and metabolic issues (Teo et al., 2021). To address these concerns, the food industry frequently explores the incorporation of functional ingredients into energy-dense foods, including meat-based products like Tteokgalbi. The addition of functional components to Tteokgalbi has the potential to enhance its nutritional profile, sensory qualities, shelf life, and health benefits.

Cissus, also known as *Cissus quadrangularis L.*, is a succulent plant traditionally recognized for its potential health advantages. Historically, *Cissus* has been used as a folk remedy for promoting bone health, addressing conditions like osteoarthritis (Lakshmanan et al., 2020; Lakshmanan et al., 2021), rheumatoid arthritis (Kumar et al. 2015], and fractures (Brahmkshatriya et al., 2010). It has also been employed as an anti-inflammatory agent for ailments such as asthma (Mishra et al., 2010) and specific gastrointestinal infections (Beserra et al., 2016), as well as for combating nutritional deficiencies like scurvy and anemia. More recently, *Cissus* has gained attention for its potential role in managing metabolic disorders such

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as obesity (Chatree et al., 2021) and diabetes (Kasar and Chidi, 2021). *Cissus* aids in obesity prevention by inhibiting intestinal fat absorption through the suppression of pancreatic lipase activity, reducing the formation of new fat cells in white adipose tissue, and enhancing β-oxidation through the induction of peroxisome proliferator activated receptor alpha (PPARα) (Lee et al., 2018). The notable biological functionality of *Cissus* is attributed to its phenolic compounds, including amyrin, sitosterol, friedelin, quercetin, genistein, and daidzein, which exhibit strong antioxidant properties (Nagani et al., 2011).

There are numerous beneficial effects of *Cissus*; however, the use of *Cissus* as a food additive in novel products has only just begun. In a previous study, the addition of *Cissus* significantly altered the physicochemical properties (pH, water holding capacity (WHC), and °Brix), colorimetric properties, and antioxidative properties of Yanggaeng (Jang et al., 2023). Therefore, we logically hypothesized that the addition of *Cissus* as a food additive in Tteokgalbi would also impact various characteristics of the novel food product. In this study, *Cissus* powder (C) was utilized as a natural additive in Tteokgalbi to investigate its effects on physicochemical properties and cold storage characteristics.

Materials and Methods

Preparation of *Cissus* powder

Cissus powder was generously provided by Nuon (Seongnam, South Korea). It was meticulously washed three times with deionized water (Biozoa Biological Supply, Seoul, Korea), and any remaining water was removed by spinning in a salad spinner (Windax, Seoul, Korea). The *Cissus* were processed into a powder using a previously described method (Kim et al., 2022) and stored in a deep freezer (Ilshin, Yangju, Korea) for subsequent analysis.

Recipe of Tteokgalbi treated with *Cissus* powder

The experimental Tteokgalbi was prepared according to established recipes (Lee et al., 2022). Fresh shoulder meat from pork was carefully selected from a local butcher shop (E-Mart, Chungju, Korea) to ensure that it was lean and boneless. The meat was then ground using a processor equipped with an 8 mm diameter grinder (Hankook Fujee Industries Co., Hwaseong, Korea).

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In the NC group, ground pork meat was combined with a mixture of soy sauce, NaCl, white sugar, honey, garlic, ginger, onion, scallion, rice wine, pepper, sesame oil, sesame seed, and flour. This formulation followed a method that involved peer review (Amelly et al., 2023). All ingredients except meat were procured from Cheiljedang, Seoul, Korea.

The PC group was established by adding ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA). The experimental groups were created by incorporating 1.00%, 2.00%, and 4.00% *Cissus* powder (Table 1). To ensure a consistent weight and shape of the experimental Tteokgalbi, we molded the mixture into a circular frame using a sterile Petri dish (SPL Life Sciences, Pocheon, Korea). The molds had a diameter of 10.0 cm and a thickness of 1.2 cm. These shaped Tteokgalbi samples were then refrigerated at 4 °C for durations of 1, 5, 7, or 11 days to maintain freshness during storage.

Proximate composition of Tteokgalbi treated with *Cissus* powder

To analyze the proximate composition of Tteokgalbi, a 10 g sample of Tteokgalbi was mixed with 100 mL of distilled water, and this process was repeated three times. The mixture was then homogenized using a Stomacher 400 Circulator (Seward, London, UK) for 2 min. Additionally, the moisture, crude protein, fat, and ash levels in the Tteokgalbi suspension were evaluated using peer-reviewed scientific methods, as previously described (Kim et al., 2022; Lee et al., 2022; Jang et al., 2023).

WHC and cooking loss of Tteokgalbi treated with *Cissus* powder

WHC and cooking-related moisture loss of Tteokgalbi were evaluated using well-established techniques and formulas documented in previous studies (Kim et al., 2022; Lee et al., 2022).

Hunter's colorimetric measurement of Tteokgalbi treated with *Cissus* powder before and after cooking

The L^* (lightness), a^* (redness), and b^* (yellowness) characteristics of uncooked and prepared Tteokgalbi were determined using a technique outlined in an earlier peer-reviewed investigation (Lee et al., 2023).

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Texture profile analysis (TPA) of Tteokgalbi treated with *Cissus* powder

The TPA values (springiness, cohesiveness, chewiness, and brittleness) of a 1 cm^3 minced Tteokgalbi cube were evaluated using a rheometer (Sun Scientific Co., Ltd., Tokyo, Japan, v 3.0) at a constant table speed of 60 mm/min following the methodology outlined in a previous study (Kim et al., 2022; Lee et al., 2022).

Antioxidative properties of Tteokgalbi treated with *Cissus* powder

The resultant Tteokgalbi homogenate is described in proximate composition of Tteokgalbi treated with *Cissus* powder. was then subjected to analysis for its total polyphenol content (TPC), total flavonoid content (TFC), as well as its ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radicals, using methods previously documented (Hwang and Kim, 2023; Jang et al., 2023).

Storage characteristics of Tteokgalbi

All Teokgalbi samples were prepared according to the methods outlined in described in proximate composition of Tteokgalbi treated with *Cissus* powder. pH levels were determined using a calibrated pH meter (WTW InoLab pH 720, Weilheim, Germany). The measurements for 2-thiobarbituric acid reactive substances (TBA), volatile basic nitrogen (VBN), and total microbial count (TMC) were conducted using established in-house procedures (Kim et al., 2022; Lee et al., 2022) at four time points: immediately after preparation (0), as well as after 5, 7, and 11 days of storage at 5°C.

Statistical analysis

Each experimental result was assessed three times to calculate mean values and standard deviations. One-way analysis of variance (ANOVA) was conducted using XLSTAT 2012 (Addinsoft, Inc., Paris, France). Tukey’s multiple-range test was used to assess the variations in outcomes following various treatments and the impact of storage differences (before and after cooking).

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Results

Proximate composition of Tteokgalbi

Table 2 shows the proximate composition of Tteokgalbi following treatment with varying concentrations of C. The NC group exhibited the highest moisture content in Tteokgalbi, whereas the application of C led to a significant and gradual reduction in the moisture content compared to the NC group. Furthermore, the crude protein content in Tteokgalbi ranged from 16.43 to 17.86, with the C1 group recording the highest crude protein content and the C3 group recording the lowest. The composition of crude fat gradually decreased with increasing additional C concentration; however, no statistical difference was observed. The crude ash content in Tteokgalbi spanned from 2.17 (NC) to 2.79 (C3), although no statistical difference was observed. In Tteokgalbi, the carbohydrate content steadily increased as the C concentration increased, reaching values of 5.74, 6.86, 8.15, and 9.74 for the NC, C1, C2, and C3 groups, respectively.

WHC and cooking loss of Tteokgalbi

Table 3 illustrates the water-holding capacity (WHC) and cooking loss characteristics of teokgalbi prepared using C. The PC (66.72%), C1 (64.74%), and C2 (62.91%) treatments maintained similar WHC levels as the NC group (63.54%). However, C3 had the lowest WHC (48.62%; $p<0.05$). The NC (9.65%) and PC (10.85%) groups displayed the lowest cooking loss, and as the quantity of C in Teokgalbi increased, cooking loss gradually increased ($p<0.05$). Therefore, the cooking loss rates in C1, C2, and C3 increased significantly to 16.45%, 16.54%, and 18.16%, respectively.

Chromaticity of Tteokgalbi before and after cooking

Figure 1 and Table 4 present the appearance and colorimetric characteristics of Tteokgalbi before and after cooking when treated with C. Concerning the color before cooking, the L^* value was notably high in the NC group (58.02), with PC treatment showing a similar L^* value (59.24). However, C treatment resulted in a decrease in the L^* value of Tteokgalbi, with the L^* values for the C1, C2, and C3 groups measuring 49.05, 45.56, and 43.98, respectively ($p<0.05$). The a^* value was the lowest in the NC (5.66) and PC (5.55) groups, but significantly increased as the amount

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of C treatment increased ($p<0.05$). As for the *b** value, the NC and PC groups had the highest values (19.79 and 18.69, respectively), which decreased in a dose-dependent manner with the addition of C ($p<0.05$).

After cooking, the *L** values for both NC (64.17) and PC (61.79) groups remained statistically similar to those observed before cooking. In contrast, all C-treated Tteokgalbi groups exhibited higher *L** values after cooking (ranging from 45.58 to 53.16) compared to their respective values before cooking (ranging from 42.56 to 49.05). After cooking, the *a** values increased in the PC group (3.30) and all C-treated groups (ranging from 4.79 to 7.02) compared with the NC group (2.77). Notably, the *a** values for the PC (9.84) and C1 (11.37) groups were significantly elevated after cooking compared with their pre-cooking values. Furthermore, with the addition of relatively large amounts of C (C2 [6.45] and C3 [7.02]), a substantial increase in *a** value was observed after cooking. Interestingly, the *b** value displayed an opposite pattern to that of the C treatment before cooking. Surprisingly, all *b** values gradually decreased with the C treatment; however, after cooking, all *b** values dramatically increased in a dose-dependent manner.

Textural properties of Tteokgalbi

Table 5 provides a comprehensive overview of the textural attributes, including springiness, cohesiveness, chewiness, and brittleness, of Tteokgalbi prepared with C. Springiness ranged from 66.33 (C3) to 72.51 (NC), and there were no significant differences in springiness among all Tteokgalbi groups. However, Tteokgalbi prepared with C showed a significant reduction in cohesiveness, chewiness, and brittleness compared to NC Tteokgalbi. The cohesiveness of Tteokgalbi was the highest in the NC group (61.55%). However, in all other groups, including PC, C1, C2, and C3, cohesiveness ranged from 41.95 to 50.95, which was significantly lower than that in the NC group. The chewiness of Tteokgalbi was the highest in the NC group (2317.42 g). However, in all other groups, including PC, C1, C2, and C3, the chewiness ranged from 1019.99 to 1311.93 g, which was significantly lower than that in the NC group. Notably, Tteokgalbi prepared using C3 displayed the most pronounced reductions in chewiness and brittleness. The brittleness of Tteokgalbi was the highest in the NC group (171147.58 g), followed by the PC group (94292.52 g). The addition of C significantly reduced the brittleness of Tteokgalbi in C1 (77973.57 g), C2 (77564.81 g), and C3 (41203.77 g), compared to both the NC and PC groups.

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Antioxidative properties of Tteokgalbi

Table 6 presents an overview of the TPC and TFC values of Tteokgalbi samples treated with C. The TPC and TFC levels in Tteokgalbi increased proportionally as the amount of added C gradually increased. The highest TPC was observed in PC and C3, followed by C2, C1, and NC. With increasing quantities of C incorporated, both TPC and TFC values consistently increased in a gradual, dose-dependent manner. This trend persisted across different storage periods (0, 5, 7, and 11 days). However, after the 11-day storage period, the TPC and TFC values significantly decreased compared to their initial levels, regardless of the treatment applied.

Table 7 summarizes the DPPH and ABTS radical-scavenging capacities of C-treated Tteokgalbi during 11 days of cold storage at 4 °C. Throughout all time points of cold storage, the DPPH radical scavenging activities of the PC and all C groups were notably higher than those of the other treatment groups. After 11 days of storage, the DPPH radical-scavenging activity significantly declined in the NC, PC, and C1 groups. However, C2 and C3 maintained their DPPH radical scavenging activities at the same level as that on the initial storage day.

The ABTS radical-scavenging activities of the Tteokgalbi samples increased proportionally with increasing amounts of C and PC. The ABTS radical scavenging activity was highest in the C3 group, followed by the C2, PC, C1, and NC groups. Regardless of the storage period (ranging from 0 to 11 days), the ABTS radical-scavenging activity increased ($p<0.05$) in a dose-dependent manner as the amount of C treatment increased. Notably, the ABTS radical scavenging capacity did not change significantly in any treatment group during the storage period. In terms of FRAP, the Tteokgalbi samples from the PC and all C-treated groups displayed higher FRAP values. The FRAP values gradually declined with increasing cold storage time. On day 1 of storage, PC and all C treatments showed significantly higher FRAP values. However, after Day 5, C3 exhibited the highest FRAP values.

Storage characteristics of Tteokgalbi

Table 8 illustrates the variations in pH, TBARS, VBN, and TMC values in Tteokgalbi samples during 11 days of cold storage at 4 °C. Initially (day 0), the gradual increase in C resulted in a significant decrease in pH. After 11 days of cold storage, the pH values of all groups decreased

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significantly. Surprisingly, C3 initially exhibited the lowest pH, but the difference between days 11 and 0 was minimal (0.85) compared to the NC (1.26), PC (1.15), C1 (1.16), and C2 (0.85) groups. Therefore, we postulate that the additional C in Tteokgalbi may help prevent increases in the TBARS, VBN, and TMC values.

At the beginning of storage, all groups exhibited similar TBARS levels ranging from 0.01 to 0.03 mg malonaldehyde/kg. As the storage time increased, TBARS levels significantly increased across all treatments, which is consistent with previous findings in other meat products (Kim et al., 2022; Lee et al., 2022). In our study, the TBARS values were significantly and gradually elevated with increasing storage period. Overall, increasing C in Tteokgalbi significantly increased the TBARS values compared with NC. After day 5 of storage, the TBARS values for the NC and PC were the lowest at 0.41 and 0.38 mg malonaldehyde/kg. However, C1 (0.45 mg malonaldehyde/kg), C2 (0.59 mg malonaldehyde/kg), and C3 (0.71 mg malonaldehyde/kg) groups exhibited significantly elevated TBARS levels. After day 7 of storage, the TBARS values for the NC and PC were the lowest at 0.77 and 0.73 mg malonaldehyde/kg. However, C1 (0.60 mg malonaldehyde/kg), C2 (0.72 mg malonaldehyde/kg), and C3 (0.92 mg malonaldehyde/kg) groups showed significantly elevated TBARS levels. After day 11 of storage, the TBARS values for the NC and PC were the lowest at 1.61 and 1.44 mg malonaldehyde/kg. However, C1 (1.48 mg malonaldehyde/kg), C2 (1.57 mg malonaldehyde/kg), and C3 (1.81 mg malonaldehyde/kg) groups exhibited significantly elevated TBARS levels.

Initially, the VBN content in the Tteokgalbi samples ranged from 8.84 to 10.20 mg/100 g. By day 5, both the C2 (10.38 mg/100 g) and C3 groups (10.03 mg/100 g) groups exhibited significantly lower VBN contents than the NC group (13.79 mg/100 g). However, after day 7, the VBN values ranged from 13.94 to 16.40 mg/100 g, and there were no statistically significant differences noted between the C-added groups and the NC group. The overall VBN values were higher than the VBN values observed initially or on day 5. After day 11 of storage, all VBN values were higher than the initial, day 5, and day 7 periods. Furthermore, after day 11 of storage, only the PC group exhibited the lowest VBN value, whereas that of the other ranged from 26.06 26.24. Therefore, treatment C did not significantly prevent the increase in VBN content in Tteokgalbi until the 5-day storage period.

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The total microbial count (TMC) ranged from 4.14 to 4.42 log colony-forming units (CFUs)/g at the beginning of cold storage and gradually increased to 4.27–4.56, 5.91–6.59, and 8.36–8.59 log CFUs/g after 5, 7, and 11 days of cold storage, respectively. Initially, TMC was recorded in the NC group at 4.36 log CFUs/g, and PC and all C treatments did not significantly alter the TMC values in the Tteokgalbi samples. After 5 days of cold storage, the total number of microorganisms in the Tteokgalbi samples significantly increased ($p<0.05$) compared with that in the initial storage. After day 5, PC exhibited lower TMC values than the other treatments. The highest TMC values after 7 days were observed in the NC and C1 group (6.41 and 6.59 log CFUs/g, respectively), whereas C2 and C3 resulted in lower TMC values than the other groups. Furthermore, relatively lower TMC levels in C2 and C3 were maintained on day 11 of cold storage; therefore, C2 and C3 exhibited TMC values of 8.32 and 8.38 log CFUs/g, respectively, which were lower than that of the NC (8.59 log CFUs/g).

Discussion

Cissus, native to Africa and Asia, has a long history in traditional medicine for treating conditions such as bone fractures (Azam et al., 2023) and joint pain (Lakshmanan et al., 2021). Scientific research supports its effectiveness in reducing pain and improving joint function in osteoarthritis (Bloomer et al., 2013), as well as in diminishing inflammation and oxidative stress in metabolic syndrome (Lekshmi et al., 2015). Furthermore, Cissus may help prevent weight gain by reducing waist circumference and affecting adiponectin levels (Toor et al., 2020; Chatree et al., 2021). Although Cissus is available in various forms (Brahmkshatriya et al., 2015), its use as a supplement has not been extensively studied, particularly regarding its potential as a common food ingredient. Therefore, this study aimed to investigate the feasibility of using Cissus, recognized for its distinctive components and functional properties, in the food industry. Our hypothesis was that incorporating Cissus as a food additive in Tteokgalbi would influence its physicochemical, textural, and antioxidative properties.

The addition of *Cissus* significantly altered the physicochemical properties of Teokgalbi. The addition of C significantly decreased the WHC and increased cooking loss. WHC is a crucial factor in evaluating meat quality in terms of juiciness and tenderness (Geletu et al., 2021). Postmortem cellular glycolysis produces lactic acid, which acidifies meat products (Stajkovic et

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al., 2019). Consequently, this acidification lowers the pH, which has the potential to diminish protein WHC through charge shielding, ultimately resulting in reduced water retention (Jankowiak et al., 2021). Therefore, the reduction in WHC observed in the C3 group may be closely linked to the decline in the pH of Tteokgalbi. Similarly, Hanwoo Tteokgalbi patties treated with either onion skin or blackcurrant powder also exhibited increased cooking loss compared with control patties (Chung et al., 2018). The addition of natural components has a dual effect on cooking loss. Natural substances with higher fiber content enhance WHC, leading to a reduction in cooking loss (Jeon and Choi, 2012). However, if a natural additive has a low pH, it may decrease cooking loss, thereby triggering an increase in it (Chung et al., 2018). Water loss from meat products occurs when the bond between water molecules and proteins weakens and is influenced by the pH of the meat product (Feng et al., 2015).

The addition of C significantly altered the colorimetric characteristics of Tteokgalbi. The meat hue is influenced by the oxidation level of myoglobin, which is determined by the ratio of oxymyoglobin to metmyoglobin. For example, when there is an increase in metmyoglobin formation in meat, the meat tends to appear brighter appearance (Lakshmanan et al., 2020; Cheng et al., 2021). In our experimental context, the L^* value of Tteokgalbi with the addition of C might indicate a browning reaction triggered by amino acids and reducing sugars. Conversely, the change in the a^* value might be closely associated with the inherent color components in C, which have the potential to alter the pigmentation of Tteokgalbi, especially because the additional C treatment changed the color from green to magenta. The a^* value has been found to be positively correlated with various factors, including the total pigment content from additives, myoglobin levels, and ion concentration (Asami et al., 2019; Krycki et al., 2021). The most intriguing colorimetric change was observed in the b^* values, as they responded in opposite directions before and after cooking Tteokgalbi. The increase in b^* value after cooking may be linked to the emergence of yellowish components in group C after heating. Upon evaluating the color of Tteokgalbi before and after cooking, we concluded that the color change was intricately linked to the extent of the browning reaction occurring during heating and cooking, as well as the presence of variable inherent components influenced by heating from the natural additives present in C.

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Furthermore, the addition of carbon altered the textural properties of Tteokgalbi. Typically, increasing the WHC through the addition of natural food additives such as watermelon radish powder (Kim et al., 202) and seaweed (Gullón et al., 2020; Munsu et al., 2021) to meat products enhances their hardness and chewiness, possibly because of their higher fiber content. However, in this study, as previously reported, the addition of C resulted in a decrease in the WHC of Tteokgalbi. Consequently, the reduction in WHC due to the addition of C may lead to increased cooking loss, but decreased chewiness and brittleness.

Surprisingly, the addition of C had a significant impact on the storage characteristics of Tteokgalbi. Preserving meat products requires greater antioxidant capabilities owing to the temporary increase in microbial growth and oxidation of fatty acids. However, incorporation of specific natural components such as watermelon radish (Kim et al., 2022) and *Boesenbergia pandurata* (Roxb.) (Lee et al., 2022), onions (Chung et al., 2018), and avocado puree (Wang et al., 2022) in meat products can help maintain elevated radical-scavenging capabilities compared to control products, as these ingredients typically contain higher levels of TPC and/or TFC. In this study, we also observed increased TPC, TFC, and various radical scavenging activities in response to the C treatments. Therefore, the enhanced antioxidant activity resulting from C addition may positively affect microbial growth regulation in meat products treated with C.

The increase in TBARS is perplexing, considering that the addition of C is expected to enhance antioxidative capacity. Typically, the antioxidant capacity and TBARS are inversely related. This puzzling result may be attributed to the nature of C, which is known for its ability to inhibit fat absorption in humans due to the presence of isorhamnetin and quercetin (Jiang et al., 2019). Tteokgalbi is rich in fat, and if the microbial utilization of fatty acids is hindered, the undigested fatty acids could be more prone to oxidation. To date, the metabolism of fatty acids by microbes in the presence of C has not been comprehensively studied. Therefore, our data suggest that unabsorbed fatty acids metabolized by microbes may lead to increased TBARS values in carbon supplemented Tteokgalbi.

VBN content is a vital indicator used to assess the freshness of animal meat products by quantifying nitrogen containing compounds, such as dimethyl amine, trimethyl amine, and ammonia. Normally, fresh meat products have a VBN content in the range of 5 to 10 mg/100 g. However, during the initial stages of spoilage, the VBN content can increase to 30–40 mg/100 g

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(Ha et al., 2019). According to the Korean Food Sanitation Act, VBN levels in packaged and raw meat must not exceed 20 mg/100 g to ensure compliance (Ministry of Food and Drug Safety, 2014). Our experimental Tteokgalbi recipe had an initial VBN content of ~ 10 mg/100 g. However, with increasing storage period, the overall VBN values gradually and significantly increased. Interestingly, on day 5 of storage, the addition of C to Tteokgalbi (C2 and C3) significantly reduced VBN content compared to both NC and PC. On day 7 of storage, the addition of C to Tteokgalbi (C1 and C2) also significantly decreased VBN content compared to both NC and PC. This reduction in VBN content in C2 and C3 on day 5 may be associated with the induction of antioxidant capacity in Tteokgalbi. However, the lack of reduction in VBN content in C3 on storage day seven, despite the significant reduction in TBN content observed on day five, may be attributed to the inherent functional properties of C. As mentioned previously, C addition may promote the growth of unabsorbed microbes in Tteokgalbi.

The International Commission on Microbiological Specifications for Foods provides guidelines that state that the total bacterial count should not exceed 7 log CFU/g (Swanson, 2011). In South Korea, the Livestock Products Processing Act defines freshness requirements for meat products, specifying that the bacterial count should not exceed 5 log CFUs/cm^3 (Ministry of Food and Drug Safety 2014). Hence, even without utilizing a specific packaging system, our experimental Tteokgalbi met the criteria set by the Livestock Products Processing Act of South Korea on day of cold storage. However, no significant change in TMC was observed with the addition of C.

ConclusionIn summary, incorporating *Cissus* as a natural ingredient into Tteokgalbi significantly influenced its composition, quality, and sensory attributes. Notable changes were observed in proximate composition, WHC, and cooking loss, including decreased moisture content and variations in crude protein and fat levels. The carbohydrate content increased with higher *Cissus* concentrations. WHC decreased in the C3 group, potentially due to a decrease in pH, and cooking loss increased with higher *Cissus* concentrations, likely because of changes in WHC. Furthermore, *Cissus* altered colorimetric and textural characteristics, affecting the L^*, a^*, and b^* values both before and after cooking. Interestingly, *Cissus*-treated Tteokgalbi exhibited reduced cohesiveness, chewiness, and brittleness compared to the non-*Cissus* (NC) treated

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group. Additionally, *Cissus* treatment enhanced radical scavenging capacities, increasing the total phenolic and flavonoid content in a dose-dependent manner. DPPH and ABTS radical-scavenging capacities were notably higher in *Cissus*-treated groups, indicating enhanced antioxidative activity. *Cissus* treatment also influenced storage characteristics, with lower pH and increased TBARS values during storage, indicative of lipid oxidation. TMC was lower in the C2 and C3 groups after 11 days of cold storage. These findings indicate the potential of using *Cissus* as a natural additive in meat products, but further research is necessary to fully comprehend its effects on meat quality and storage stability. The C3 modified the proximate composition, physicochemical, and textural properties of Tteokgalbi significantly, and also improved its antioxidant capacity. However, additional research is needed to address the shortcomings in the storage characteristics of the C3. As a subsequent step, conducting a consumer preference survey would be beneficial to determine the optimal ratio of *Cissus* powder in Tteokgalbi.

Conflicts of Interest

The authors declare no potential conflict of interest.

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Author Contributions

Conceptualization: Lee J-J. Data curation: Lee J-J, Choi J-S, Ha J-H. Formal analysis: Lee J-J, Lee J. Methodology: Choi J-S, Ha J-H. Software: Lee J-J, Choi J-S, Ha J-H. Validation: Lee J-J, Lee J, Choi J-S, Ha J-H. Investigation: Lee J-J, Lee J, Choi J-S, Ha J-H. Writing - original draft: Lee J-J, Ha J-H. Writing - review & editing: Lee J-J, Lee J, Choi J-S, Ha J-H.

Ethics Approval

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

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Table 1. Formula of Tteokgalbi prepared with different levels of *Cissus* powder

Ingredients	Treatments[1]				
	NC	PC	C1	C2	C3
Pork	80.00	79.90	79.00	78.00	76.00
Cissus quadrangularis powder (C)	-	-	1.00	2.00	4.00
Ascorbic acid	-	0.10	-	-	-
Soy sauce	4.00	4.00	4.00	4.00	4.00
NaCl	1.40	1.40	1.40	1.40	1.40
White sugar	3.60	3.60	3.60	3.60	3.60
Honey	0.50	0.50	0.50	0.50	0.50
Garlic	1.30	1.30	1.30	1.30	1.30
Ginger	0.40	0.40	0.40	0.40	0.40
Onion	3.70	3.70	3.70	3.70	3.70
Scallion	1.10	1.10	1.10	1.10	1.10
Rice wine	0.50	0.50	0.50	0.50	0.50
Pepper	0.20	0.20	0.20	0.20	0.20
Sesame oil	2.00	2.00	2.00	2.00	2.00
Sesame seed	0.30	0.30	0.30	0.30	0.30
Flour	1.00	1.00	1.00	1.00	1.00

[1]NC, Tteokgalbi treated with 0.00% *Cissus quadrangularis* powder (C); PC, Tteokgalbi treated with 0.10% ascorbic acid as a positive reference; C1, Tteokgalbi treated with 1.00% C; C2, Tteokgalbi treated with 2.00% C; C3, Tteokgalbi treated with 4.00% C.

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Table 2. Proximate compositions of Tteokgalbi prepared with different levels of *Cissus* powder

Proximate compositions (%)	Treatments[1]				
	NC	PC	C1	C2	C3
Moisture	69.03±0.30[a]	68.00±0.42[b]	67.22±0.39[b]	66.01±0.30[c]	65.73±0.48[c]
Crude protein	17.52±0.64[ab]	17.40±0.10[ab]	17.86±0.69[a]	17.01±0.48[ab]	16.43±0.36[b]
Crude fat	5.53±0.35	5.67±0.35	5.40±0.14	5.37±0.12	5.30±0.31
Crude ash	2.17±0.57	2.22±0.29	2.65±0.12	2.73±0.02	2.79±0.11
Carbohydrate	5.74±0.16[c]	6.71±0.34[bc]	6.86±0.72[bc]	8.15±0.18[b]	9.74±0.92[a]

[1]Treatments are shown in Table 1. Values are expressed as means ± standard deviation (n=3). [a-c]Values with different superscripted small letters within the same row differ significantly by tukey's multiple range test ($p<0.05$).

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Table 3. Water-holding capacity (WHC) and cooking loss of Tteokgalbi prepared with different levels of *Cissus* powder

	Treatments[1]				
	NC	PC	C1	C2	C3
WHC (%)	63.54±5.14^{a}	66.72±10.43^{a}	64.74±8.67^{a}	62.91±9.36^{a}	48.62±7.73^{b}
Cooking loss (%)	9.65±0.15^{b}	10.85±0.07^{b}	16.45±1.70^{a}	16.54±0.22^{a}	18.16±4.01^{a}

[1]Treatments are shown in Table 1. Values are expressed as means ± standard deviation (n=3). $^{a\text{-}b}$Values with different superscripted small letters within the same row differ significantly by tukey's multiple range test (p<0.05).

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Table 4. Hunter color properties of Tteokgalbi prepared with different levels of *Cissus* powder

Color		Treatments[1]				
		NC	PC	C1	C2	C3
Color before cooking	*L**	58.02±1.13^{a}	59.24±0.70^{a}	49.05±2.54^{b}	42.56±0.63^{c}	43.98±2.92^{c}
	*a**	5.66±0.96^{ab}	5.55±0.23^{b}	6.55±1.19^{ab}	7.10±1.49^{ab}	8.14±0.22^{a}
	*b**	19.79±0.43^{a}	18.69±0.02^{a}	17.71±1.08^{a}	16.77±1.20^{ab}	13.17±2.99^{b}
Color after cooking	*L**	64.17±1.03^{a}	61.79±1.10^{a}	53.16±2.29^{b}	48.32±1.03^{c}	45.58±0.06^{c}
	*a**	2.77±0.54^{c}	3.30±0.70^{bc}	4.79±0.78^{b}	6.45±0.20^{a}	7.02±0.38^{a}
	*b**	16.49±0.11^{c}	17.79±1.00^{c}	19.68±0.52^{b}	20.70±0.68^{ab}	21.48±0.51^{a}

[1]Treatments are shown in Table 1. Values are expressed as means ± standard deviation (n=3). [a-c]Values with different superscripted small letters within the same row differ significantly by tukey's multiple range test ($p<0.05$).

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Table 5. Textural properties of Tteokgalbi prepared with different levels of *Cissus* powder

Items	Treatments[1]				
	NC	PC	C1	C2	C3
Springiness (%)	72.51±2.52	71.96±1.51	69.88±3.13	69.99±3.13	66.33±9.62
Cohesiveness (%)	61.55±6.26[a]	41.95±4.30[b]	50.95±1.69[b]	47.10±0.94[b]	50.56±2.50[b]
Chewiness (g)	2317.42±80.74[a]	1311.93±96.66[b]	1153.33±92.37[bc]	1149.97±65.89[bc]	1019.99±89.05[c]
Brittleness(g)	171147.58±1119.04[a]	94292.52±1334.22[b]	77973.57±571.26[c]	77564.81±864.57[c]	41203.77±1090.31[d]

[1]Treatments are shown in Table 1. Values are expressed as means ± standard deviation (n=3). [a-d]Values with different superscripted small letters within the same row differ significantly by tukey's multiple range test ($p<0.05$).

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Table 6. Changes in total polyphenols content and total flavonoids content of Tteokgalbi prepared with different levels of *Cissus* powder during storage at 4℃ for 11 days

	Storage days	Treatments[1]				
		NC	PC	C1	C2	C3
TPC[2] (mg GAE[3]/g)	1	49.47±5.20^{cA}	88.43±3.30^{aA}	72.10±4.75^{bA}	75.67±11.16^{bA}	98.84±9.68^{aA}
	5	45.70±4.05^{cB}	63.50±4.60^{aB}	67.54±2.43 ^{abAB}	74.90±3.03^{abA}	73.57±15.69^{aB}
	7	35.10±3.37^{dC}	60.92±4.48^{cB}	59.84±2.96^{bB}	63.42±5.06^{aAB}	76.77±2.55^{aB}
	11	33.10±10.89^{bC}	47.48±3.54^{aC}	51.61±1.17^{aB}	50.94±11.94^{aB}	67.96±1.48^{aB}
TFC[4] (mg QE[5]/g)	1	<LLOQ^{6d}	6.93±0.96^{dA}	17.92±3.55^{cA}	37.81±1.18^{bA}	56.36±4.77^{aA}
	5	<LLOQ ^{D}	4.40±2.60^{dB}	15.22±0.53^{cAB}	30.68±1.43^{bB}	52.07±3.51^{aA}
	7	<LLOQ ^{d}	3.09±0.58^{dC}	13.68±1.78^{cAB}	24.00±0.80^{bC}	36.77±2.33^{aB}
	11	<LLOQ ^{d}	1.70±0.44^{dD}	12.07±1.62^{cB}	20.94±0.41^{bD}	34.88±1.83^{aB}

[1]Treatments are shown in Table 1. Values are expressed as means ± standard deviation (n=3). $^{a\text{-}d}$Values with different superscripted small letters within the same row differ significantly by tukey's multiple range test ($p<0.05$). $^{A\text{-}D}$Values with different superscripted capital letters within the same column differ significantly by tukey's multiple range test ($p<0.05$). [2]TPC; Total polyphenol content, [3]GAE; Gallic acid equivalent, [4]TFC; Total flavonoid content, [5]QE; Quercetin equivalent, [6]LLOQ; Lower limit of quantification.

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Table 7. Changes in DPPH radical scavenging capacity, ABTS radical scavenging capacity and Ferric reducing antioxidant power (FRAP) of Tteokgalbi prepared with different levels of *Cissus* powder during storage at 4℃ for 11 days

	Storage days	Treatments[1]				
		NC	PC	C1	C2	C3
DPPH radical scavenging activities (Inhibition %)	1	15.86±5.73^{dA}	44.34±7.34^{bA}	34.50±2.44^{cAB}	58.46±5.01^{b}	72.94±2.84^{a}
	5	14.65±0.95^{eAB}	43.34±4.05^{cA}	33.13±3.25^{dAB}	53.03±1.35^{b}	69.56±2.79^{a}
	7	13.42±1.75^{eAB}	43.05±4.90^{cA}	30.80±0.63^{dA}	51.04±3.65^{b}	64.45±2.54^{a}
	11	11.09±3.41^{eB}	34.34±2.73^{cB}	26.19±0.91^{dB}	46.73±4.17^{b}	62.31±1.86^{a}
ABTS radical scavenging activities (Inhibition %)	1	22.95±0.33^{e}	31.63±1.37^{c}	28.52±1.15^{d}	33.92±0.76^{b}	40.18±2.55^{a}
	5	22.25±6.58^{e}	31.98±1.33^{c}	29.50±0.79^{d}	34.49±0.56^{b}	39.29±2.18^{a}
	7	22.24±1.45^{d}	27.77±2.25^{c}	28.64±1.52^{bc}	32.05±2.08^{b}	36.39±2.21^{a}
	11	23.09±0.37^{d}	29.11±2.88^{c}	28.36±0.30^{c}	33.49±1.08^{b}	40.66±2.05^{a}
FRAP[5] (mM $FeSO_4$/g)	1	69.79±31.43^{aB}	475.44±52.41^{aB}	340.50±10.68^{aB}	552.92±8.81^{aA}	875.38±23.08^{aA}
	5	47.71±12.50^{eAB}	425.08±66.54^{cA}	281.92±11.90^{dA}	534.58±8.92^{bA}	720.75±12.84^{aB}
	7	29.25±5.11^{eA}	414.04±24.48^{bAB}	234.88±10.05^{dC}	442.63±3.06^{cB}	642.56±10.83^{aC}
	11	20.06±5.24^{dB}	389.63±24.46^{bB}	224.33±11.13^{cB}	404.19±7.73^{bC}	525.75±9.97^{aD}

[1]Treatments are shown in Table 1. Values are expressed as means ± standard deviation (n=3). $^{a-d}$Values with different superscripted small letters within the same row differ significantly by tukey's multiple range test ($p<0.05$). $^{A-D}$Values with different superscripted capital letters within the same column differ significantly by tukey's multiple range test ($p<0.05$).

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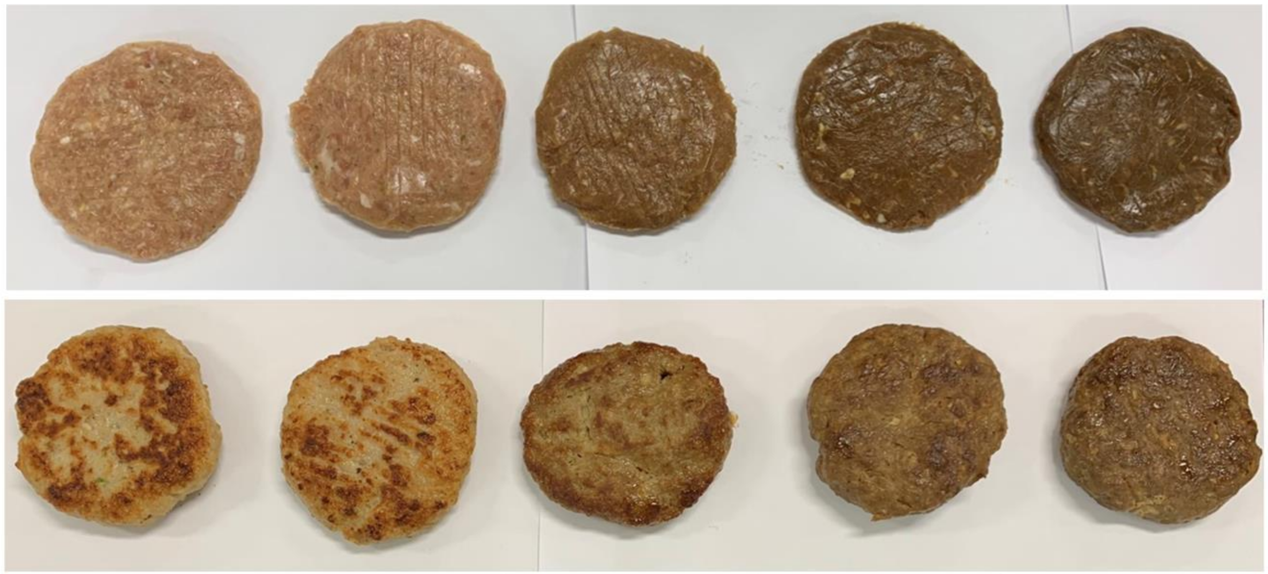
Table 8. Changes in pH, 2-thiobarbituric acid (TBARS), volatile basic nitrogen (VBN), and total microbial counts (TMC) of Tteokgalbi prepared with different levels of *Cissus* powder during storage at 4°C for 11 days

	Storage days	Treatments[1]				
		NC	PC	C1	C2	C3
pH	0	6.13±0.02^{aA}	5.98±0.04^{dA}	6.03±0.02^{bcA}	6.03±0.01^{bA}	5.99±0.01^{cdA}
	5	6.04±0.04^{aB}	5.89±0.01^{dB}	5.99±0.01^{bB}	5.97±0.01^{bcB}	5.95±0.01^{cB}
	7	5.25±0.01^{bC}	5.08±0.01^{cC}	5.10±0.01^{cC}	5.24±0.01^{aC}	5.65±0.02^{aC}
	11	4.87±0.02^{cD}	4.83±0.01^{dD}	4.87±0.02^{cD}	4.96±0.00^{bD}	5.14±0.01^{aD}
TBARS [2] (mg malonaldehyde/kg)	0	0.03±0.02^{D}	0.02±0.03^{D}	0.02±0.02^{D}	0.01±0.03^{D}	0.02±0.04^{D}
	5	0.41±0.03^{dC}	0.38±0.04^{dC}	0.45±0.00^{cC}	0.59±0.01^{bC}	0.71±0.02^{aC}
	7	0.77±0.02^{dB}	0.73±0.05^{dB}	0.60±0.01^{cB}	0.72±0.01^{bB}	0.92±0.02^{aB}
	11	1.61±0.11^{cA}	1.44±0.07^{cA}	1.48±0.03^{bcA}	1.57±0.10^{bA}	1.81±0.09^{aA}
VBN [3] (mg/100 g)	0	10.20±0.32^{D}	10.09±0.65^{D}	9.99±1.80^{C}	8.84±2.27^{C}	9.39±1.41^{C}
	5	13.79±0.84^{aC}	14.34±1.12^{aBC}	14.71±0.96^{aB}	10.38±0.65^{bC}	10.03±0.32^{bC}
	7	16.40±1.62^{B}	15.70±0.65^{B}	14.50±1.62^{B}	14.71±1.61^{B}	13.94±0.56^{B}
	11	26.24±0.97^{aA}	24.15±1.40^{bA}	26.06±0.64^{aA}	26.37±0.32^{aA}	26.24±0.56^{aA}
TMC [4] (log CFU/mL)	0	4.36±0.20^{C}	4.31±0.18^{CB}	4.14±0.13^{DB}	4.42±0.24^{C}	4.24±0.12^{D}
	5	4.53±0.07^{aC}	4.27±0.06^{cC}	4.56±0.11^{aC}	4.46±0.13^{abC}	4.45±0.07^{abC}
	7	6.41±0.03^{aB}	5.91±0.03^{cB}	6.59±0.15^{aB}	5.99±0.06^{cB}	6.01±0.08^{bB}
	11	8.59±0.24^{aA}	8.47±0.21^{aA}	8.43±0.23^{aA}	8.32±0.22^{bA}	8.38±0.12^{bA}

[1]Treatments are shown in Table 1. Values are expressed as means ± standard deviation (n=3). $^{a\text{-}d}$Values with different superscripted small letters within the same row differ significantly by tukey's multiple range test ($p<0.05$). $^{A\text{-}D}$Values with different superscripted capital letters within the same column differ significantly by tukey's multiple range test ($p<0.05$). [2]TBARS, 2- thiobarbituric acid; [3]VBN, volatile basic nitrogen; [4]TMC, total microbial counts.

ACCEPTED



NC	PC	C1	C2	C3

Fig. 1. Appreance of Tteokgalbi prepared with different levels of *Cissus* powder. NC, Tteokgalbi treated with 0.00% Cissus quadrangularis powder (C); PC, Tteokgalbi treated with 0.10% ascorbic acid as a positive control; C1, Tteokgalbi treated with 1.00% C; C2, Tteokgalbi treated with 2.00% C; C3, Tteokgalbi treated with 4.00% C.