Effects of green tea leaf, lotus leaf, and kimchi powders on quality characteristics of chicken liver sausages

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Abstract
Liver sausage is flavorful and highly nutritious. However, liver has a relatively short shelf life due to acceleration of oxidation in the presence of endogenous enzymes and metals. Powders derived from natural sources, including plants or fruits, are applied to meat products for inhibiting oxidation without adverse effects on their quality. Hence, this study investigated the effects of natural powders derived from green tea leaf (GTL), lotus leaf (LL), and kimchi (KC) on the quality and change in lipid oxidation and freshness of chicken liver sausages during two weeks of storage. Chicken liver sausages were manufactured with chicken breast (70%) and liver (20%), pork back fat (5%), iced water (5%), various additives, and GTL, LL, and KC [0 (control) or 1%]. They were processed in three batches. For determination of the quality characteristics of chicken liver sausages with various plant powders, pH, color, and texture properties were assessed. In addition, lipid oxidation and freshness using thiobarbituric acid reactive substances (TBARS) and total volatile basic nitrogen (TVBN) were analyzed at day 0 and week 2 of refrigerated storage. Higher values were obtained for pH and cooking yield in sausage samples with LL and KC powders than in samples with the other treatments. For a* values, the sausage samples with KC showed similar (p > 0.05) values, whilst others had significantly lower values than the control. The addition of the three powders to sausage samples induced an increase (p < 0.05) in hardness, gumminess, and chewiness. The addition of plant powders did not influence TBARS and TVBN of sausage samples at the initial stage. However, after two weeks of storage, significantly lower TBARS and TVBN values were observed, and the sausage with KC (p < 0.05) showed the lowest values of both TBARS and TVBN. The results showed the potential ability of the three powders to improve the quality and inhibit lipid oxidation in liver sausages. Particularly, the addition of KC did not adversely affect the a* values of sausage samples. The effects on sensory properties and inhibition mechanisms of GTL, LL, and KC in meat products should be further studied.

Keywords: Antioxidant activity, Green tea leaf, Kimchi, Liver sausages, Lotus leaf, Quality characteristics

Introduction
Liver sausage is a conventional and commonly consumed sausage type in Europe due to its highly flavorful characteristics [1, 2]. Some studies have been conducted on its manufacturing process and the quality properties of meat products added with chicken

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liver including coarse sausage [2] and spreadable sausage [3, 4] as a main ingredient. Chicken liver contains 24.6% protein, 6% fat, and high amounts of vitamin A and essential elements, including Fe, Zn, Cu, Mn, Cd, Pb, Ni, and Cr [5]. However, liver has a relatively short shelf life and causes acceleration of oxidation due to the presence of endogenous enzymes and metals [6]. In addition, sausages made with chicken contain relatively high levels of unsaturated fatty acids. However, to the best of our knowledge, no studies have been reported on the inhibition of lipid oxidation in chicken liver sausages.

Plant sources, which contain polyphenols and flavonoids, have been applied to meat products as extracts or powders for inhibition or retardation of lipid oxidation during storage storage [7, 8]. Green tea has been generally consumed as a beverage and is a traditional remedial agent used in East Asia. Furthermore, the powerful antioxidant activity of green tea related to polyphenols including catechin has been reported [9]. Lotus leaf (LL), widely used in both food and medicine, is known to have antioxidant, free radical scavenging, antibacterial, and antianterivascular properties [10, 11]. Kimchi (KC), a traditional fermented food in Korea, possess antioxidant properties and contains carotenoids, vitamins, flavonoids, and other polyphenols [12]. Addition of powders containing high amounts of dietary fiber as natural antioxidants could affect the quality properties of meat products, such as color and/or texture [13, 14]. Hence, the aim of this study was to evaluate the effect of green tea leaf (GTL), LL, and KC powders on the quality characteristics of chicken liver sausages, including pH, color, texture properties, change in lipid oxidation, and freshness during two weeks of storage.

Materials and Methods

Preparation of powders of GTL, LL, and KC
Green tea leaf, dried using a hot-air dryer, was purchased from Bosung in Chonnam, Korea. LL and Baechu KC (Chongga kimchi, Daesang FNF, Seoul, Korea) were purchased from a local market (Seoul, Korea). LL has been generally consumed as a beverage and is a traditional remedial agent used in East Asia. Furthermore, the powerful antioxidant activity of green tea related to polyphenols including catechin has been reported [9]. Lotus leaf (LL), widely used in both food and medicine, is known to have antioxidant, free radical scavenging, antibacterial, and antianterivascular properties [10, 11]. Kimchi (KC), a traditional fermented food in Korea, possess antioxidant properties and contains carotenoids, vitamins, flavonoids, and other polyphenols [12]. Addition of powders containing high amounts of dietary fiber as natural antioxidants could affect the quality properties of meat products, such as color and/or texture [13, 14]. Hence, the aim of this study was to evaluate the effect of green tea leaf (GTL), LL, and KC powders on the quality characteristics of chicken liver sausages, including pH, color, texture properties, change in lipid oxidation, and freshness during two weeks of storage.

Preparation of chicken liver sausages
The chicken liver sausages were produced in the following formulation: 70% chicken breast, 20% chicken liver, 5% pork back fat, 5% iced water, 2% isolated soy protein (ISP), 1.5% nitrite pickled salt [salt:nitrite = 99.4:0.6; nitrite pickled salt (NPS)], 0.25% sodium triphosphate, 0.5% sugar, 0.05% monosodium L-glutamate (MSG), 1.5% onion, 0.6% garlic, 0.2% ginger, and 0.03% black pepper. The ground chicken breast, pork back fat, and iced water were mixed in a silent cutter. Then, the additives (NPS, ISP, triphosphate, and spices) and 0% or 1% plant powders (GTL, LL, and KC) were added and homogenized. The ground chicken liver was added to the meat batter and mixed until the core temperature reached 9 °C ± 1 °C. The meat mixture was stuffed into collagen casings (#240, NIPPI Inc., Tokyo, Japan; approximately 25 mm diameter) using a stuffer (IS-8, Sirman, Marsango, Italy), and each sample was cooked at 75 °C ± 1 °C in a water bath for 30 min until the internal temperature reached 72 °C. The cooked samples were immediately cooled in an ice bath, vacuum packed, and stored at 4 °C until pH, color, texture properties, lipid oxidation, and total volatile basic nitrogen (TVBN) contents could be examined. The lipid oxidation and TVBN content were analyzed at week 0 and 2 of refrigerated storage (4 °C).

Determination of pH and cooking yield
The pH values for a mixture of sausage samples and distilled water (1:4) were determined using a pH-meter (Model 340, Mettler-Toledo GmbH Analytical, Schwerzenbach, Switzerland). The cooking yield in each treatment was determined by weighing meat batters before and after cooking and expressed in percentage.

Instrumental color evaluation
Color measurements were taken with a colorimeter (Chroma meter CR-210, Minolta, Japan; illuminant C, calibrated with a white standard plate L* = 97.83, a* = −0.43, and b* = +1.98), with an 8 mm diameter measuring area and a 50 mm diameter illumination area. Color values (CIE L*, a*, and b*) were measured on the surface of samples and results were taken in triplicate for each sample.

Texture profile analysis (TPA)
Samples were cut into sections with a height of 25 mm and ø 16 mm diameter. The textural properties for each sample were measured using a cylinder probe (ø 20 mm diameter), set attached to a Texture Analyzer (TA-XT2i, Stable Micro System
Application of plant powders in liver sausages

The test conditions were as follows: stroke, 2 kg; test speed, 2.0 mm/s; and distance, 8 mm. The texture profile analysis (TPA) parameters, namely hardness (kg·f), springiness, cohesiveness, gumminess, and chewiness (kg·f) were computed.

**Determination of thiobarbituric acid reactive substances (TBARS)**

Lipid oxidation was assessed using the direct-distillation method as described by Tarladgis et al. [15], with minor modifications. Each sample at day 0 and week 2 of refrigerated storage was analyzed in triplicate. Briefly, 10 g of the sample was blended with 50 mL of distilled water prior to homogenization (AM-7, Nihonseiki Kaisha Ltd., Japan) at 10,900 × g for 2 min and transferred to a distillation flask. The cup used for blending was washed with an additional 47.5 mL of distilled water, which was subsequently added to the same distillation flask containing 2.5 mL of 4 N HCl and a few drops of an antifoaming agent, silicone o/w (KMK-73, Shin-Etsu Silicone Co., Ltd., Seoul, Korea). Then, the mixture was distilled and 50 mL of distillate was collected. Five mL of 0.02 M 2-thiobarbituric acid in 90% acetic acid (TBA reagent) was added to a vial containing 5 mL of the distillate and mixed. The vials were capped and heated in a boiling water bath for 30 min to develop the chromogen and cooled to room temperature. The absorbance was measured at 538 nm (Libra S22, Biochrom Ltd., Cambridge, England) against a blank prepared with 5 mL distilled water and 5 mL TBA reagent. The K value was determined using 1,1,3,3-tetraethoxypropane (Sigma, USA) as the standard.

**Determination of total volatile basic nitrogen (TVBN)**

TVBN of the meat samples (at day 0 and week 2 of storage) was determined by the Conway microdiffusion method [8] and the results were expressed in mg N/100 g of sample.

**Statistical analysis**

Statistical analysis was performed by one-way analysis of variance (ANOVA), and significant differences (p < 0.05) were detected by Duncan’s multiple range test using SAS software (SAS, Release 8.01, SAS Institute Inc., USA). The values are expressed as means ± standard deviation.

**Results and Discussion**

**pH and cooking yield**

The pH values of chicken liver sausages were affected by the type of powder (Table 1). The addition of GTL and LL powders to the sausages did not induce significant change in pH values compared to the control. However, sausage samples with KC powder had the lowest (p < 0.05) pH values among the treatments. This result might be due to the presence of organic acids in KC. Similarly, Choe et al. [1] found that the pH of spreadable liver sausages increased with an increase in KC powder.

The cooking yield of meat and meat products is closely associated with fat and water retention [16]. In this study, the cooking yield of sausages added with GTL and LL powders did not differ from (p > 0.05) that of the control. On the other hand, the addition of KC in sausage samples resulted in a significant increase in cooking yield. A similar trend was found in spreadable liver sausages containing KC powder with addition levels of 1%, 2%, and 3% [1]. This result is supported by many authors who have documented an enhancement on water binding or holding ability by the addition of powders derived from plant and fruit containing dietary fiber in meat products [17, 18].

**Color**

The addition of non-meat ingredients in meat products could induce undesirable change in color, depending on its intrinsic color and addition level [19, 20]. In this study, the addition of the powders (GTL, LL, and KC) to sausage samples significantly influenced the change in color (Table 1). The sausage samples containing GTL, LL, and KC powders showed lower (p < 0.05) values of L* than the control. The three different powders led to a decrease in L* values. There was no difference (p > 0.05) in a*

### Table 1. Effect of different plant powders on pH, cooking yield, and color of chicken liver sausages

| Traits                  | Control         | GTL            | LL            | KC            |
|-------------------------|-----------------|----------------|---------------|---------------|
| pH                      | 6.39 ± 0.02     | 6.38 ± 0.03    | 6.37 ± 0.02   | 6.32 ± 0.02   |
| Cooking yield           | 95.38 ± 0.61    | 95.12 ± 0.89   | 94.92 ± 0.60  | 97.18 ± 0.53  |
| Color                   |                 |                |               |               |
| L*                      | 65.52 ± 0.95    | 62.81 ± 0.85   | 59.63 ± 0.72  | 63.80 ± 0.47  |
| a*                      | 12.38 ± 0.41    | 5.54 ± 0.22    | 5.89 ± 0.20   | 12.47 ± 0.21  |
| b*                      | 19.91 ± 0.63    | 21.78 ± 0.45   | 18.75 ± 0.33  | 21.84 ± 0.41  |

Means±SD with different superscript in the same row differ significantly (p < 0.05).

| Traits                  | Control         | GTL            | LL            | KC            |
|-------------------------|-----------------|----------------|---------------|---------------|
| pH                      | 6.39 ± 0.02     | 6.38 ± 0.03    | 6.37 ± 0.02   | 6.32 ± 0.02   |
| Cooking yield           | 95.38 ± 0.61    | 95.12 ± 0.89   | 94.92 ± 0.60  | 97.18 ± 0.53  |
| Color                   |                 |                |               |               |
| L*                      | 65.52 ± 0.95    | 62.81 ± 0.85   | 59.63 ± 0.72  | 63.80 ± 0.47  |
| a*                      | 12.38 ± 0.41    | 5.54 ± 0.22    | 5.89 ± 0.20   | 12.47 ± 0.21  |
| b*                      | 19.91 ± 0.63    | 21.78 ± 0.45   | 18.75 ± 0.33  | 21.84 ± 0.41  |

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values between the control and sausage samples with KC powder. This result might be due to the intrinsic color of KC powder. The sausage samples with GTL and LL powders showed significantly lower a* values than those of the control and samples treated with KC powder. Some non-meat substance showing greenish color could induce higher b* values in meat products. In this study, the sausage samples with GTL and KC powders showed a significant increase in b* values compared to the control and samples treated with LL powder. The sausage samples with LL had the lowest (p < 0.05) b* values among the treatments.

**Lipid oxidation**

Lipid oxidation is commonly determined by measuring thiobarbituric acid reactive substances (TBARS), which are secondary lipid reaction products including aldehydes, carbonyls, thiobarbituric acid reactive substances (TBARS), which are generally used as an indicator of freshness of meat and meat products. The TBARS values in sausage samples increased with an increase in storage time, showing 0.19–0.28 mg MDA/kg meat. The result is probably due to the oxidation of unsaturated fatty acids of sausage samples. However, in this study, TBARS values during two weeks of storage were below the threshold value (0.5 mg MDA/kg meat) [24] in meat products with a rancid taste. The sausage samples containing plant powders generally showed lower (p < 0.05) values in TBARS compared to that of the control after two weeks of storage. This result indicates that lipid oxidation was effectively suppressed by GTL, LL, and KC powders, having high levels of phenolic compounds, compared to the control during storage. Many authors have reported on the antioxidant activities of GTL, LL, and KC [19, 25, 26]. The antioxidant activities of phenolic compounds are closely associated with the hydroxyl group attached to the aromatic ring, which is able to donate electrons with hydrogen atoms and neutralize free radicals. This mechanism blocks further degradation of more active oxidant forms, such as malondialdehyde [27].

**TVBN**

During storage, the action of microorganisms or enzymes can lead to decomposition of protein in meat and meat products and then ammonia, trimethylamine, and dimethylamine are produced, which can be measured as the TVBN content [8]. For this reason, TVBN is generally used as an indicator of freshness of meat and meat products. The TVBN values in sausage samples showed a similar trend to TBARS (Fig. 2). There was no significant difference in TVBN values among the treatments at day 0 of storage. However, at week 2 of storage, the control showed the highest (p < 0.05) values in TVBN of sausage samples relatively and the TVBN values depended on the powder type. Thus, at the final storage, the TVBN values of sausage samples were in the following order: control (13.11 mg%) > GTL (10.83 mg%) > LL (9.24 mg%) > KC (7.96 mg%).

### Table 2. Effect of different plant powders on texture properties of chicken liver sausages

| Traits         | Control       | GTL            | LL            | KC            |
|----------------|---------------|----------------|---------------|---------------|
| Hardness (kg)  | 0.43 ± 0.01e  | 0.49 ± 0.01e   | 0.53 ± 0.01e  | 0.48 ± 0.006  |
| Springiness    | 0.96 ± 0.03   | 0.94 ± 0.03    | 0.93 ± 0.05   | 0.97 ± 0.04   |
| Cohesiveness   | 0.50 ± 0.03   | 0.51 ± 0.01    | 0.51 ± 0.02   | 0.53 ± 0.04   |
| Gumminess (kg) | 0.22 ± 0.01f  | 0.25 ± 0.01f   | 0.27 ± 0.01f  | 0.25 ± 0.02fe |
| Chewiness (kg) | 0.21 ± 0.02f  | 0.24 ± 0.01f   | 0.25 ± 0.02f  | 0.24 ± 0.02f  |

Means±SD with different superscript in the same row differ significantly (p < 0.05).

Control, chicken liver sausages without plant powders; GTL, chicken liver sausages with 1% green tea leaf powder; LL, chicken liver sausages with 1% lotus leaf powder; KC, chicken liver sausages with 1% kimchi powder.
KC (8.49 mg%). This result was probably due to the antimicrobial effect of GTL, LL, and KC, which was supported by previous studies [11, 28].

**Conclusion**

Based on the results of this study, GTL, LL, and KC powders effectively retard lipid oxidation and maintain freshness in chicken liver sausages. In particular, the addition of KC powder showed great improvement in the quality characteristics like cooking yield and similar a* values to control. Further studies based on the inhibition mechanisms of GTL, LL, and KC powders during storage against lipid and protein oxidation of meat products and their sensory properties should be performed.

**Competing interests**

No potential conflict of interest relevant to this article was reported.

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Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

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Software: Kim GW.
Investigation: Choe J, Kim GW.
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Ethics approval and consent to participate

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

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