Determination of Point of Sale and Consumption for Hanwoo Beef Based on Quality Grade and Aging Time

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Abstract This study aimed to determine the suitable point of sale and consumption of different quality grade (QG) Hanwoo short loin during aging period, based on physicochemical, sensory, and microbiological quality. Short loins obtained from the carcasses of 13 Hanwoo steers and 2 bulls with 5 different QGs (1++, 1+, 1, 2, and 3) were analyzed over 28 d. QG and aging time had significant effect on water holding capacity, color, shear force, total volatile basic nitrogen (TVBN) content, and sensory traits. Higher QG groups generally exhibited a lower shear force, nucleotide content, and water holding capacity, and higher L*, a*, and b* values. Acceptable tenderness (shear force <5.4 kg) in QG 1++, 1+, and intermediate QG (1 and 2) was achieved on days 7, 14, and 18, respectively, and QG 3 showed a shear force of 6.8 kg, even after 28 d. Regardless of QG, TVBN content below threshold levels (20–30 mg%) was observed throughout the 28 d aging period, while total plate counts above 7 Log CFU/g were seen at 21 d. In conclusion, it is recommended that Hanwoo beef with QG 1++, 1+, and intermediate QG (1 and 2) should be sold or consumed between 7 and 21, 14 and 21, 16 and 21 d, respectively. Beef with QG 3 should be sold or consumed within 21 d, based on microbial growth, even though it has not achieved desirable tenderness. For this reason, an additional tenderizing process is recommended before this beef is ready for consumption.

Keywords quality grade, Hanwoo, tenderness, microbial growth, point of sale or consumption

Introduction

Eating quality, including tenderness, flavor, and juiciness, in beef is an important...
characteristic that determines consumer palatability (Piao et al., 2018). In particular, tenderness is the most important criterion that determines consumer purchasing, followed by flavor (Lee et al., 2010). A higher amount of intramuscular fat (marbling) in beef increases the product’s tenderness and flavor, which in turn leads to greater consumer satisfaction (Lee et al., 2017). Hence, a number of attempts have been made to improve the tenderness and flavor of beef by controlling intrinsic and/or extrinsic factors (Choe et al., 2019; Lee et al., 2012).

The Korean carcass grading system has been implemented since 1992 and consists of five levels of quality grade [QG; 1++ (the highest grade), 1+, 1, 2, and 3 (the lowest grade)] and three levels (A, B, and C) of yield grade (Oh et al., 2017). The QG refers to marbling score, lean meat color, fat color, texture (firmness), and maturity. The yield grade refers to subcutaneous fat thickness, ribeye area, and carcass weight. In 2017, 10.1% and 33.3% of slaughtered Hanwoo cattle were graded QG 1++ and 1+, respectively, which increased consumer preference due to the large amount of marbling (KAPE, 2019). QG 1, 2, and 3 accounted for 28.7%, 20.3%, and 7.4% of slaughtered Hanwoo cattle, respectively, in 2017 (KAPE, 2019).

Postmortem aging is one of the methods to improve the tenderness and flavor of meat, which is commonly used in the meat industry. However, previous studies have shown that changes in meat flavor are dependent on the length of the aging period (Cho et al., 2016). The aging period that achieved the best palatability was shown to be dependent on the QG of Hanwoo beef (Yim et al., 2015). Similarly, Stetzer et al. (2008) reported that an extended aging time from 7 to 14 d resulted in a loss of positive flavor compounds. Therefore, it is worth investigating the effect of QG on the quality attributes of beef during postmortem aging.

Optimal aging time for different QG beef should be determined in order to define consumer meat preparation that will result in a desirable sensory quality. In addition, the optimal point of sale and consumption should be recommended to retailers. Therefore, the present study aimed to determine the relationship between QG of beef and aging time on physicochemical and sensory attributes, and to define the suitable point of sale and consumption for desirable sensory and hygienic quality.

**Materials and Methods**

**Animals and sample preparation**

Short loins (M. longissimus lumborum) from 1st to 6th lumbar vertebrae from the both sides of the carcasses of 13 Hanwoo steers and 2 bulls (the range of carcass weights 403–485 kg) from five different QG groups (1++, 1+, 1, 2, 3; n=3/group) were used in this study (Table 1). The 2 bulls were used inevitably due to the short of supply of QG 3 beef from steer. All the samples (30 short loins, a pair from 13 steers and 2 bulls) were purchased from a local auction market of beef cuts where their carcass characteristics including grading scores of Korean carcass quality grading standard (MAFRA, 2017) were open to auction purchasers prior to auctioning. All the beef cuts were fabricated and vacuum packaged on the day of grading. Next day (day 3 postmortem) the beef cuts were listed and purchased on the auction market. After grading, the short loins were immediately vacuum packaged and transferred to a laboratory of Korean Institute for Animal Product Quality Evaluation. Short loins from left sides of beef carcasses were designated to sensory evaluation and the ones from right sides to physicochemical and microbial analyses. Each short loins were cut into eleven slices of even thickness (2.5 cm) and then vacuum-packaged. The eleven slices of each short loins were randomly assigned to one of 11 sections based on aging period (4, 7, 9, 11, 14, 16, 18, 21, 23, 25, and 28 d). The aging of meat samples was conducted at 2±1°C and the physicochemical and microbial analysis and sensory evaluation were examined at each aging time.
Effect of Aging on Beef with Different Quality Grades

The pH of meat samples was measured in triplicate using a pH meter (PHM 201, Radiometer, Villeurbanne, France). The homogenate was prepared by blending a 10 g meat sample with 90 mL distilled water for 1 min with a homogenizer (Ultra-turrax T25-S1, IKA, Staufen, Germany).

The proximate composition was obtained with a slightly modified method of AOAC (AOAC, 2000). Briefly, the moisture content was obtained by drying each sample (3 g) placed in an aluminum dish at 104℃ for 15 h. The crude protein contents were measured by the Kjeldahl method (APO45, Gerhardt Ltd., Idar-Oberstein, Germany). The crude fat contents were measured using the Soxhlet extraction system (TT 12/A, Gerhardt Ltd., Idar-Oberstein, Germany). The crude ash content was measured by incinerating of (2 g) each sample in a furnace at 600℃ overnight.

Instrumental surface color (CIE L*, a*, and b*) was measured in three random places on each meat sample using a Minolta Color Meter (Model CR-400, Minolta Co. Ltd., Osaka, Japan) calibrated with a standard white tile (L*=94.4, a*=0.313, b*=0.319) after 30 min blooming at room temperature.

Shear force of the sample (2.5 cm thick) was measured according to the method of Wheeler et al. (2001). The steak samples were flipped over when the internal temperature reached 35℃ and cooking was done when the samples had reached a core temperature of 70℃ using an electric pan (TG-60051, Tefal, Rumilly, France). Six cores of 1.27 cm diameter were made for each sample and peak force was determined using a V-shaped shear blade with a cross-head speed of 400 mm/min (TA-XT2i, Texture technologies Corp., Scarsdale, USA) and expressed as kgF.

TVBN was determined by the Conway micro diffusion method (PSQ, 1980) with slight modification and the results were

| Quality grade | Marbling score | Lean meat color | Fat color | Texture | Bone maturity | Carcass weight (kg) | Back fat thickness (mm) | Ribeye area (cm²) | Yield index |
|---------------|----------------|----------------|-----------|---------|---------------|---------------------|------------------------|-------------------|------------|
| 1++           | 9              | 4.3±0.33       | 2.7±0.33  | 11.3±0.33 | 2.0±0.00      | 407.7±21.36         | 16.7±0.88              | 92.3±7.22        | 63.3±0.88   |
| 1+            | 7              | 5.0±0.00       | 3.0±0.00  | 12.0±0.00 | 2.3±0.33      | 485.0±24.56         | 17.7±3.18              | 95.7±3.53        | 61.0±2.31   |
| 1             | 5              | 5.0±0.00       | 3.0±0.00  | 15.7±2.67 | 2.7±0.33      | 454.7±13.35         | 13.7±0.67              | 93.0±5.51        | 64.0±1.00   |
| 2             | 3              | 4.7±0.33       | 2.7±0.33  | 21.3±0.33 | 2.0±0.00      | 403.3±28.95         | 13.0±1.53              | 82.3±6.67        | 64.3±0.88   |
| 3             | 1              | 5.0±0.00       | 3.0±0.00  | 21.0±0.00 | 3.0±1.00      | 443.7±28.69         | 6.7±1.45               | 93.7±2.67        | 69.0±1.00   |

1) Lean meat color: 1, bright red; 7, dark red.
2) Fat color: 1, white; 7, yellowish.
3) Texture: 1, very fine; 3, very coarse.
4) Skeletal maturity: 1, youthful; 9, mature.
5) Mean±SEM.

**Table 1. Carcass traits of Hanwoo beef with different quality grade (n=15)**
expressed as mg%. The amount of TVBN was calculated using the following equation:

$$TVBN \text{ (mg%) } = \left( \frac{T - B}{F \times 28/S} \right) \times 100$$

where T is the titration volume for the extracted sample (mL), B is the titration volume of blank (mL), F is the factor value of 0.02 N H$_2$SO$_4$, and S is the weight of meat sample (g).

**Microbial analysis**

For total aerobic plate count (TPC) analysis, 10 g of each meat sample was homogenized with 90 mL distilled water using a stomacher (BA 7021, Seward Medical Ltd., London, England) for 90 s. TPCs were analyzed according to the Standards for Processing and Ingredients Specifications of Livestock Products, Animal, Plant and Fisheries Quarantine and Inspection Agency Notification (QIA, 2014). The meat homogenate was serially diluted with distilled water by 10-folds. Portions of the samples (0.1 mL) were plated separately on each plate and spread thoroughly. TPCs were enumerated on plate count agar (Difco™, Laboratories, Detroit, USA) and colonies were counted after incubation at 35±1℃ for 48 h. All analyses were performed in triplicate, and results expressed as logarithm colony-forming units per gram of samples (Log CFU/g).

**Nucleotide content**

The nucleotide compounds [adenosine 5’-monophosphate (AMP), inosine 5’-monophosphate (IMP), inosine, and hypoxanthine] were extracted from the meat samples according to the method of Nakatani et al. (1986). The extract was filtered through a syringe filter (0.45 μm) into a glass vial and injected into a high-performance liquid chromatography (HPLC; LC-10AD, Shimadzu Ltd., Kyoto, Japan). The analytical conditions were as follows: injection volume, 3 mL; mobile phase, 1% trimethylamine/phosphoric acid (pH 6.5); flow rate, 1.0 mL/min; column and running temperature, Zorbax Eclipse (150×4.6 mm$^2$, 4 μm particles; Agilent Technologies, Palo Alto, USA) at 40℃; and detector, UV/Vis detector at 254 nm. The peak area was calculated from a standard curve obtained using a standard AMP (Sigma-Aldrich, St. Louis, USA), IMP (Sigma-Aldrich), inosine (Sigma-Aldrich), hypoxanthine (Sigma-Aldrich).

**Sensory evaluation**

Sensory evaluation was performed for seven traits of which 6 traits of juiciness, muscle fiber tenderness, overall tenderness, connective tissue amount, flavor intensity and overall palatability in 8-hedonic scale (1=extremely undesirable, 8=extremely desirable) were referenced to the guidelines for cookery and sensory evaluation of meat (AMSA, 2015) and of which one trait of “fatty” (which is “gireumgi” in Korean) was referred to the method of Choi (2010). All panel members were selected screening test according to the method of Kim (1997) using ranking and triangle test. Each panelist evaluated fifteen different beef samples at 4, 7, 9, 11, 14, 16, 18, 21, 23, 25, and 28 d of aging.

For sensory evaluation, each sample was cooked on pre-heated electric pan (TG-60051, Tefal, Rumilly, France). The sample was flipped over when internal temperature reached 35℃ and removed when that reached 70℃, monitored with a digital thermometer (Testo-925, Testo GmbH, Lenzkirch, Germany). The samples were wrapped in aluminum foil and placed in a preheated oven (65℃) until served to panelists. The samples were cooled for 2 min and were cut into 10 mm length×10 mm width×20 mm height. Then, samples were placed on white plastic plate with 3-digit random code and served to the panelists and the panelists cleanse their palate with water between samples.
Statistical analysis

For pH and proximate composition, statistical analysis was performed by one-way Analysis of Variance (ANOVA), and significant differences at level of p<0.05 were detected by Duncan's multiple range test using SAS software (SAS Institute Inc., USA). The values are expressed as means±standard error. For physicochemical, microbial and sensory analyses, QG (1++, 1+, 1, 2, and 3) and aging time (day of 4, 7, 9, 11, 14, 16, 18, 21, 23, 25, and 28) effects and their interactions were examined as a repeated measure using General Linear Model (GLM) procedure of SAS. All means were separated using the least square means (LSMeans) function with PDIF option. The values are expressed as LSMeans±standard error.

Results and Discussion

pH and proximate composition

In Hanwoo beef, pH value was not affected by QG (p>0.05), which ranged from 5.2 to 5.5, while chemical composition was dependent on QG (Table 2). Moisture, crude protein, and crude ash contents of Hanwoo beef decreased as the QG increased (p<0.05). A previous study reported a negative relationship between moisture content and QG in Hanwoo beef (Yim et al., 2015). In contrast, the crude fat content in Hanwoo beef showed a positive correlation with QG, which is likely due to the fact that QG is largely determined by intramuscular fat (KAPE, 2019).

Physicochemical properties

A correlation between QG and aging time was observed for WHC (p=0.001) (Fig. 1). In general, the lowest QG group (3) showed the highest WHC for all groups during the entire aging period, except for 14 and 23 d. A similar result was observed by Yim et al. (2015), who reported a negative relationship between QG and WHC in Hanwoo beef over 25 d of storage (p<0.05). However, Piao et al. (2015) reported that Hanwoo loin with QG 2 showed a higher cooking water loss than those with QG 1++, 1+, and 1, while no difference was found among QGs in rump meat. This water loss during cooking was negatively related to tenderness and juiciness, with R²=–0.69 and –0.67, respectively. The WHC for other groups fluctuated during storage, and exhibited lower values than the QG 3 group.

For L*, a*, and b* values, a significant correlation between QG and aging time was identified (p=0.006, p<0.0001, and p=0.0003, respectively) (data not shown). The L* and b* values were affected equally by both effects (p<0.0001), while the effect of QG was greater than that of aging time (F values 86.94 and 8.34 for L* and 115.12 and 12.72 for b*, respectively). For L* and b* values, a higher QG generally induced higher values, while the QG 1++ and 3 groups demonstrated the highest

*–e Different letters within the same column differ significantly (p<0.05).
1) Means±SE.

Table 2. pH values and proximate composition of Hanwoo beef with different quality grades

| Quality grade | pH     | Moisture (%) | Protein (%) | Fat (%) | Ash (%) |
|---------------|--------|--------------|-------------|---------|---------|
| 1++           | 5.3±0.081) | 55.0±2.42d  | 17.7±0.28d  | 26.0±2.41a | 0.76±0.05c |
| 1+            | 5.3±0.04  | 60.6±1.52c  | 19.5±0.27c  | 19.0±2.15b | 0.85±0.05b |
| 1             | 5.3±0.10  | 63.4±0.37b  | 19.3±0.22c  | 15.3±0.75c | 0.87±0.03b |
| 2             | 5.4±0.07  | 65.4±0.91b  | 20.5±0.37b  | 11.5±2.11d | 0.98±0.03a |
| 3             | 5.4±0.04  | 71.3±0.91a  | 21.3±0.36a  | 6.05±0.68e  | 0.99±0.01a |

1) Means±SE.
and lowest values in Hanwoo beef over the entire aging period, respectively (p<0.05). This result could be due to the fact that the intramuscular fat content of meat is a major determination factor of QG according to the Korean meat grading system (Jo et al., 2012). In this study, the effect of aging time was greater for $a^*$ values than for QG (F values 29.68 and 8.83). The $a^*$ values of all QG groups significantly decreased at day 16 and increased thereafter, except for groups 1+ and 3. However, a clear trend was not observed for $a^*$ values.

For shear force, there was a significant interaction between QG and aging time (p=0.025) (Fig. 2). On the first day of the aging period (4 d), Hanwoo sirloin short loin with QG 1++ and 1+ recorded 21.8 and 22.2 kgF in shear force values,
respectively which were lower than QG 3 (29.5 kgF; p<0.05). The shear values of QG 1 and 2 were in between those of QG 1++, 1+, and QG 3. The shear values for QG1++ exhibiting 21.8 kgF were the lowest among all the QGs. However, this value of 21.8 kgF would be too much higher compared to those (4–9 kgF) reported so far in the previous reports (Shackelford et al., 1991). Possible explanations would be the differences in sample preparations that in most studies steak samples frozen for a certain period and then thawed before sensory analysis were evaluated for sensory evaluations while in this study loin samples had never been frozen but only refrigerated by the designated time (4 d postmortem) of analysis. Thus, loin steaks at 4 d of postmortem could be still tough for consumptions. In this study, the loin steaks after 7 d postmortem showed the shear force values under 10 kgF likewise to other research reports. The other possible explanation would be the use of different type of shear that Kramer-type shear was used in this study while the Warner-Bratzler type of shear had been used in many other studies. No difference was found between QG 1 and 2. As numerous studies have shown, meat tenderness is closely affected by the degree of marbling (Jo et al., 2012; Oh et al., 2017). However, regardless of QG, the shear force decreased rapidly (average 78.0%) between days 4 and 7 of aging, even though the order of the shear force value of the sample among QGs was not changed. In general, shear force values did not change significantly after 7 d in Hanwoo sirloin for all QGs ranging from 4.6–6.7 kg, except for QG 1++ and 3. The accelerated tenderization rate between days 4 and 7 could be linked to the fact that degradation of myofibrils by intramuscular proteases is most active within 3 d postmortem (Rhee et al., 2000). An acceptable degree of tenderness (shear force <5.4 kg; Destefanis et al., 2008) in QGs 1++, 1+, 1, and 2 was detected at days 7, 14, 16, and 18, respectively. The QG 3 group cannot be recommended for consumption even at the end of the aging period (28 d) on the basis of tenderness because its shear force value of 6.8 kg is not acceptable according to Destefanis et al. (2008). For this reason, an additional tenderization process should be performed before consumption of QG 3 meat.

During an extended storage time, the protein in meat can be decomposed by microorganisms or endogenous enzymes, and TVBN compounds, such as amines and ammonia, are produced (Choe et al., 2017). Thus, TVBN content is used as a freshness indicator in muscle-based food. A significant (p<0.0001) interaction was observed for TVBN content between QG and aging time (data not shown). QG 1++ showed the lowest (p<0.05) TVBN content over the entire storage period, except for day 18. TVBN content was significantly higher (from 6.9 to 8.3 mg%) in lower QG when comparing grade 1++ with 3, until day 14 of aging. It could be inferred that the lower QG had higher amine production due to its higher relative protein composition (Table 2). TVBN content for all QG groups fluctuated slightly from days 16 to 23 and decreased in the lower QG groups (1, 2, and 3) at day 25. Values in all groups reached the highest levels at day 28 of aging, ranging from 11.8 to 13.3 mg%. However, this range of TVBN values is below the threshold value (20–30 mg%) for fresh meat.

**Microbial analysis**

It is well known that microbial growth in meat is closely associated with deterioration of freshness, and sensory properties that produce undesirable volatile metabolites during storage. In the present study, no significant correlation (p=0.84) was observed in TPC between QG and aging period. The two main factors (QG and aging period) had a significant influence on TPC (p<0.01 and p<0.0001, respectively) (Fig. 3A and B). The QG 1 group showed a higher TPC (5.8 Log CFU/g) than the other groups (Fig. 3A), and there was no significant difference among the other groups. The reason for a higher TPC in QG 1 samples is not clear, but the difference is negligible when the growth rate of microorganisms is considered. In addition, the QG, mainly intramuscular fat levels, was not closely related to microbial growth (Jennings et al., 1978). After an extended aging time, the TPC of beef samples significantly increased, reaching 7.04 Log CFU/g at day 21 of aging (Fig. 3B). Based on
this result, it is suggested that Hanwoo beef should be consumed before 21 d of aging regardless of QG, because a TPC above 7 Log CFU/g in TPC indicates meat spoilage or an unacceptable quality with an off-odor (MFDS, 2014).

**Nucleotides content**

Fresh meat contains essential flavor precursors including metabolic products of nucleotides (non-volatile compounds), which contribute to the flavor of cooked meat (Tikk et al., 2006). Metabolic products of nucleotides such as inosine 5'-monophosphate (IMP), inosine, and hypoxanthine possess umami taste characteristics (Mateo et al., 1996).

There was no significant relationship between QG and aging period for nucleotide content. Significant QG and aging period effects were seen on the content of most nucleotides (IMP, inosine, and hypoxanthine), and AMP content was significantly affected by the aging period (Fig. 4A and B). The 1++ group with the highest fat content showed the lowest values of IMP, inosine, and hypoxanthine content among all QG groups (p<0.05). This result could be due to the fact that meat with a higher fat content generates lower amounts of nucleotide products compared to leaner meat, which usually comes from locomotor muscles (Xiong et al., 1999) and lower QG groups according to the present meat grading system. Even though there is a lower amount of nucleotide derivatives, the highest QG beef is preferred by consumers because it contains...
large amounts of fat-soluble materials, including aromatic compounds and short-chain fatty acids (Xiong et al., 1999). Piao et al. (2017) also reported that Hanwoo loin of higher QGs contained more oleic acid and volatile hydrocarbons generated from lipid oxidation in beef, including n-pentane, n-hexane, and 2-butene, which show a positive correlation with QG and crude fat content. With an extended aging time, AMP and IMP content significantly decreased, and inosine content significantly increased. The increase in inosine content can be explained by the conversation of IMP during aging. Dashmaa et al. (2013) reported a negative relationship between IMP and inosine and hypoxanthine in Hanwoo muscle, both of which are produced by endogenous enzymes or microorganisms from IMP during aging.

**Sensory properties**

For sensory attributes, QG×aging period showed a significant interaction (p=0.0003, p=0.0008, p=0.0167, and p<0.0001, for juiciness, tenderness, flavor, and overall acceptability, respectively; data not shown). There was no statistically significant difference in juiciness among the QG groups (4.9–5.6), except for QG 3, which exhibited a significantly lower juiciness value (3.8) at the initial aging time. This difference between the QG 3 group and other QG groups continued until the end of the aging period. For the 1++ group, juiciness increased slightly until day 11 and was maintained until day 18, then decreased thereafter to levels similar to those on the initial day. For 1+, 1, 2 groups, no noticeable change in juiciness was observed during the aging period, other than a slight increase at day 11. The panel detected a significant difference in tenderness (2.9 vs 4.7–5.3), flavor (3.8 vs 5.0–5.5), and overall acceptability (3.0 vs 4.7–5.2) between QG 3 and the other groups (QG 1++, 1+, 1, and 2) at the initial aging time. Significantly higher scores in QG 1++, 1+, 1, and 2 groups were observed for tenderness at day 7, 9, 11, and 11, respectively, compared to the initial aging time. The QG 1 and 2 groups scored a lower value in tenderness even at day 25 of aging. Otherwise, a significant increase in tenderness was observed in QG 2 group at day 23 of aging, compared to the initial aging time. Regarding flavor, there was no significant effect of aging in QGs 1++, 1+, and 1 until day 21, while lower scores (p<0.05) were observed from 23 days of aging regardless of QG. Furthermore, the QG 3 group exhibited a lower score (p<0.05) in flavor from 21 d of aging, while flavor in the QG 1 group was not affected (p>0.05) by the aging period, except at day 16. The sensory panel found an increase (p<0.05) in overall acceptability at day 7 and 11 for QG 1++ and other groups (1+, 1, and 2), respectively, and a decrease (p<0.05) at day 23 of aging for all QGs. No significant difference in overall acceptability was observed for the QG 3 group, ranging from 2.8 to 3.9 over the entire aging period.

**Conclusion**

The results of this study showed that the physicochemical and sensory properties of Hanwoo beef are significantly dependent on QG and aging time. A higher QG in beef generally leads to an acceleration in tenderness and loss of water retaining ability during aging, compared to lower QG counterparts. Based on microbial growth, all QG groups should be sold or consumed with 21 d of aging. Taken together, it is recommended that Hanwoo beef with the highest (1++), high (1+), and intermediate QG (1 and 2) should be sold or consumed after 7–21, 14–21, and 16–21 d of aging, respectively. Finally, Hanwoo beef with QG 3 requires an additional tenderization process, such as margination or injection, in order to reach desirable tenderness within the 21 d aging period in order to meet microbiological quality.

**Conflicts of Interest**

The authors declare no potential conflict of interest.
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Author’s Contributions

Conceptualization: Koh KC, Chung KY. Data curation: Kim HS, Kang SJ. Formal analysis: Chung KY, Choi CB. Software: Koh KC. Validation: Choe J, Jo C. Writing - original draft: Choe J. Writing - review & editing: Koh KC, Chung KY, Kim HS, Kang SJ, Choi CB, Jo C, Choe J.

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