



Research Article

# Physicochemical characteristics of new breed white *Hypsizygus marmoreus* for cold storage after harvest

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**Abstract** Fresh mushrooms are vulnerable to browning and tissue changes after harvest. This study monitored the external appearance, physicochemical quality indicators, and nutritional and functional components of a white beech mushroom (*Hypsizygus marmoreus*) variety (EG2020) newly developed in Korea during cold storage for up to 42 days. Two existing varieties of white *H. marmoreus*, namely H6 from Korea and HKT from Japan, were used for comparison. The mechanical texture of EG2020 was superior to H6 and HKT due to the increasing hardness of the pileus with time. Browning, in terms of the total color difference during storage, was found to be the most severe in HKT. In terms of composition, EG2020 had the highest total free sugar content, a large amount of organic acids, and higher sugar content than H6 and HKT. EG2020 also contained the largest amount of  $\beta$ -glucan, and its amount increased during storage. In sensory evaluation, EG2020 received higher scores than HKT in flavor, taste, appearance, and texture. Therefore, the EG2020 variety is more stable than HKT during storage and distribution.

**Keywords** mushroom variety, post-harvest, cold storage, physicochemical characteristics, white *Hypsizygus marmoreus*



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## 1. Introduction

High stress, unbalanced nutrition, and physical inactivity contribute to many human diseases in today's fast-paced world. As a result, there is a growing interest in healthy foods (Chae et al., 2004). Edible mushrooms offer not only great flavor but also valuable nutrients and bioactive compounds that can serve medicinal purposes (Choi et al., 2016a; Lee et al., 2019). However, fresh mushrooms have a limited shelf life due to their high respiration rates and moisture content, leading to enzymatic browning and altered texture/flavor during storage. Unlike many fruits and vegetables, mushrooms lack an epidermis to protect them from physical damage and water loss. The degree of surface discoloration (such as browning), condition of the mushroom cap (pileus), and texture of the fruiting

body all serve as key quality indicators of mushrooms during distribution (Bae et al., 2010; Han et al., 1992; Mahajan et al., 2008). Therefore, it is crucial to develop measures to enhance the shelf life and maintain the freshness of mushrooms throughout their storage and distribution.

The beech mushroom (*Hypsizygos marmoreus*) from the Tricholomataceae family is commonly found in East Asia and Northern Europe. This mushroom is known for its clustering, dense texture and thick flesh, which gives it a relatively crisp texture (Bolormaa et al., 2011; Kim et al., 2018). It is rich in protein, low in fat, and contains a high level of glutamic acid which is well known as a rice refining component (Kim et al., 2016; Lim et al., 2010a). *H. marmoreus* can be divided into brown and white varieties based on the color of the pileus. The brown varieties are grown from tissue isolated from wild mushrooms, while the white varieties are produced through crossbreeding and taste less bitter than the brown ones (Bolormaa et al., 2012). Cultivation of this mushroom is time-consuming, taking approximately 100 days to reach maturity due to the extremely slow fungal growth. However, the slow growth rate also leads to a dense structure and a relatively long shelf life, making this mushroom suitable for domestic consumption and export (Lim et al., 2010b). Research is ongoing both in Korea and abroad to develop new varieties with excellent storage properties and improve the cultivation technology. Many applications have been filed to register new varieties. However, information is still lacking on how the active ingredients and quality characteristics of white *H. marmoreus* change during storage, especially a comparison between different cultivars (Lim et al., 2010c).

This study analyzed the appearance, physiochemical quality characteristics, nutritional profile, and

functional components of a white *H. marmoreus* variety newly developed in Korea called EG2020 (Evergreen, 2020). The changes of quality characteristics during storage were compared between EG2020 and two existing commercial cultivars, in order to assess the storage stability. Also, consumer palatability was examined using sensory evaluation.

## 2. Materials and methods

### 2.1. Materials and storage conditions

The EG2020 cultivar was developed in 2020 by Evergreen Farm in Cheongdo, North Gyeongsang Province, Korea (application number App-2020-400). EG2020 samples were obtained directly from the farm. A Korean variety (H6) and a Japanese variety (HKT) of white *H. marmoreus* were obtained at the market. All three varieties were cultivated under the same conditions. Only the edible part of the mushrooms was harvested from the medium bottle. After packing with oriented polypropylene (OPP) films using the modified atmosphere (MA) technique, the mushrooms were stored for 1-42 days in a refrigerator at 10°C and 55% relative humidity (QBR-1000s, GMS Co., Ltd., Yangju, Korea). Changes in quality characteristics during storage were analyzed and compared between different varieties. The pileus and stipe were cut off from a bottle badge to examine the morphological, physiochemical, nutritional, and antioxidant properties. Before chemical analysis, the sample was dried in a drying oven (FO-600M, Jeiotech, Daejeon, Korea) at 45°C, ground using a 40-mesh grinder, and stored in a deep freezer at -80°C.

### 2.2. Morphological characterization and measurement of weight loss rate

To examine the morphological characteristics, a

digital camera (Canon EOS 100D, Tokyo, Japan) was used to take pictures of the front and sides of the mushrooms. Also, the size of the pileus and length of the fruiting body for the three varieties were measured using a caliper. The weight loss rate was evaluated as a percentage on the 14<sup>th</sup>, 28<sup>th</sup>, and 42<sup>nd</sup> day as  $(W_t - W_{\text{storage day}}) / W_1 \times 100$ .

### 2.3. Determination of physicochemical and functional properties

#### 2.3.1. Texture

Changes in mushroom texture during storage were analyzed using a rheometer (texture analyzer, Compac-100, Sun Science Co., Tokyo, Japan). For measurement, the pileus and stipe of the mushroom were separated, pileus and stipe of equal size (height: 10 mm) and equal lengths (width: 15 mm) were prepared for each variety. The samples were measured at least 10 times, and the values were presented as mean  $\pm$  standard deviation (SD). Curves generated by the rheometer were used to determine the hardness, springiness, cohesiveness, and chewiness. The measurements were taken using a cylinder probe (No. 1, 20 mm diameter, circle) with a table speed of 120 mm/min.

#### 2.3.2. Color

To determine color changes in mushrooms during storage, the pileus and fruiting body were separated, and pilei of equal sizes and fruiting bodies of equal lengths were prepared for each variety. A colorimeter (JP/CR-300 series, Minolta, Osaka, Japan) was used to determine the lightness (L), redness (a), and yellowness (b) values for at least 10 samples from each variety, and the values were presented as mean  $\pm$  SD. The standard white plate had L=97.55, a=0.003, and b=1.63. The total color difference ( $\Delta E$ ) of mushroom samples during storage was calculated as

$$\Delta E = [(L_{\text{storage}} - L_1)^2 + (a_{\text{storage}} - a_1)^2 + (b_{\text{storage}} - b_1)^2]^{1/2}.$$

#### 2.3.3. pH and saccharinity

To monitor the pH change of mushrooms during storage, the sample was mixed with distilled water at pH 7.0 at a ratio of 1:9 (W/V) and homogenized at 25,000 rpm for 1 min (PT 1200 Polytron-Aggregate homogenizer, Kinematica Inc., NY, USA). The mixture was centrifuged at 4,000 rpm for 10 min (FLETA-40, Hanil Science Industrial, Gimpo, Korea), and the pH of the supernatant was measured three times using a pH meter (Orion 3-star, Thermo Scientific, Waltham, MA, USA). The soluble solid content was measured using the same supernatant with a digital refractometer (PAL-1, Atago, Tokyo, Japan). The measurements were taken at least three times, and the results were presented as mean  $\pm$  SD.

#### 2.3.4. Polyphenol oxidase (PPO) activity

PPO activity in mushrooms during storage was measured using a modified version of the method developed by Pizzocaro et al. (1993). After adding 20 mL of McIlvaine citric-phosphate buffer (pH 6.5) to 10 g of sample, the mixture was homogenized at 25,000 rpm for 1 min (PT 1200 Polytron-Aggregate homogenizer), followed by centrifugation at 4,000 rpm for 10 min (FLETA-40). After mixing 1 mL of the supernatant with 2 mL of 0.1 M catechol solution, the absorbance was measured at 420 nm for 3 min using a UV-visible spectrophotometer (Optizen 3220UV, Mecasys, Daejeon, Korea). The enzymatic activity (Unit/min/g) was presented as enzyme units, where a 0.001 increase of absorbance in 0.1 mL of enzymatic solution while 1 min was considered equivalent to one unit.

#### 2.3.5. $\beta$ -Glucan content

The  $\beta$ -glucan content of mushrooms during storage

was measured using a mushroom and yeast  $\beta$ -glucan kit (K-YBGL, Megazyme International Ireland Inc., Ireland) (Kwon, 2019). First, to measure the total glucan content, sulfuric acid (12 M, 2 mL) was added to 100 mg of dried sample and mixed in an ice water bath for 2 h on a shaker. Then, 10 mL of distilled water was added, and the mixture was incubated in a 100°C water bath for 2 h. After cooling the mixture, the acid was neutralized using 6 mL of 8 M NaOH, and the total volume was adjusted to 100 mL using 200 mM sodium acetate buffer (pH 4.5). A 1-mL aliquot of the solution was centrifuged at 13,000 rpm for 5 min. Next, 0.1 mL of the supernatant was mixed with 0.1 mL solution containing exo-1,3- $\beta$ -glucanase (20 U/mL) and  $\beta$ -glucosidase (4 U/mL). After keeping the mixture in 40°C water bath for 1 h, 3 mL of the glucose oxidase-peroxidase (GOPOD) reagent was added, followed by incubation in 40°C water bath for 20 min. The absorbance was measured at 510 nm using a UV-visible spectrophotometer (Optizen 3220UV). The total glucan content was computed using the  $\beta$ -glucan content calculator provided by Megazyme.

### 2.3.6. Free sugar and organic acid contents

Free sugars and organic acids produced during storage affect the saccharinity and flavor of white *H. marmoreus*. Their contents were analyzed using high-performance liquid chromatography (HPLC). The free sugar content was measured using an Alliance Waters Co. (Milford, MA, USA) HPLC system and Sugar-Pak I column ( $\emptyset$  6.5 mm $\times$ 300 mm, Waters Co.). To prepare the analyte, 1 g of dried mushroom was mixed with 10 mL of distilled water at room temperature on a shaking incubator at 200 rpm for 48 h. The extract was centrifuged at 4,000 rpm for 10 min (FLETA-40), and the supernatant was passed through a Whatman PVDF syringe filter

(13 mm, 0.2  $\mu$ m). HPLC conditions: 20  $\mu$ L of sample, 0.01 M Ca-EDTA (50 mg/1 L dH<sub>2</sub>O) as the mobile phase, flow rate of 0.5 mL/min, and column temperature of 90°C. A refractive index (RI) detector was used for the analysis.

The organic acid content was measured using a Prominence (Shimadzu Co.) HPLC system and PL Hi-Plex column ( $\emptyset$  7.7 mm $\times$ 300 mm, 8  $\mu$ M, Agilent Technologies, Germany). To prepare the analyte, 1 g of dried mushroom was mixed with 10 mL of 85% ethanol at room temperature on a shaking incubator at 200 rpm for 48 h. The extract was centrifuged at 4,000 rpm for 10 min (FLETA-40), and the supernatant was passed through a Whatman PVDF syringe filter (13 mm, 0.2  $\mu$ m). HPLC conditions: 20  $\mu$ L of sample, 0.005 M H<sub>2</sub>SO<sub>4</sub> in water as the mobile phase, flow rate of 0.5 mL/min, and column temperature of 65°C. An RI detector was used for the analysis.

### 2.3.7. Free amino acid content

The free amino acid content of mushroom samples during storage was measured using an automated amino acid analyzer (L-8900, Hitachi). First, 2 g of hot air-dried powder sample was mixed with 10 mL of distilled water and homogenized (PT 1200 Polytron-Aggregate homogenizer) at 25,000 rpm for 1 min. After centrifugation at 4,000 rpm for 10 min (FLETA-40), 1 mL of the supernatant was mixed with 1 mL of 5% trichloroacetic acid, centrifuged at 10,000 rpm for 10 min, diluted five times with 0.02 N HCl, and passed through a Whatman PVDF syringe filter (13 mm, 0.2  $\mu$ m). The filtrate was analyzed by HPLC under the following conditions: an injection volume of 20  $\mu$ L, PF-1,2,3,4,6, PF-RG, R-3, and C-1 as the mobile phase, and ninhydrin solution (Wako, Japan) as the developer. The amino acids were separated using an ion exchange column

(#2622SCPF) at a column temperature of 50°C and a reactor temperature of 135°C. A standard solution of free amino acids was prepared by mixing type ANII and Type B standard solutions (Wako).

#### 2.3.8. Total phenol content (TPC)

TPC of mushrooms during storage was measured using a scaled-down version of the Folin-Denis method (1912). The sample and distilled water were mixed at 1:9 W/V ratio and homogenized (PT 1200 Polytron-Aggregate homogenizer) at 25,000 rpm for 1 min, followed by centrifugation at 4,000 rpm for 10 min (FLETA-40). The supernatant (20  $\mu$ L) was added to a 96-well plate and mixed with 20  $\mu$ L of 95% ethanol and 60  $\mu$ L of distilled water. Then, 10  $\mu$ L of 1 N Folin-Ciocalteu reagent was added, and the mixture was left standing for 5 min. After adding 20  $\mu$ L of 5% Na<sub>2</sub>CO<sub>3</sub>, the mixture was left standing in the dark for 1 h. Absorbance was measured at 725 nm using a SPECTRO star Nano microplate reader (BMG LABTECH., Germany), and TPC was calculated using a standard gallic acid curve.

#### 2.3.9. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity of mushrooms during storage was measured using the Blois (1958) method. The sample and distilled water were mixed at 1:9 W/V and homogenized (PT 1200 Polytron-Aggregate homogenizer) at 25,000 rpm for 1 min, followed by centrifugation at 4,000 rpm for 10 min (FLETA-40). The supernatant (50  $\mu$ L) was added to a 96-well plate, mixed with 150  $\mu$ L of 60  $\mu$ M DPPH solution, and left standing in the dark for 15 min. Absorbance was measured at 517 nm using a SPECTRO star Nano microplate reader (BMG LABTECH., Germany), and DPPH radical scavenging activity (%) was calculated using the following formula:

$$(1 - \text{sample absorbance} / \text{control absorbance}) \times 100.$$

#### 2.4. Sensory evaluation of white *H. marmoreus* varieties

The sensory quality evaluation of EG2020 was compared with a Japanese variety (HKT). The samples were blanched in boiling water for 1 and 2 min and prepared as single servings. The evaluation involved 30 undergraduate and graduate Food Engineering students from Kyungpook University, who had more expert knowledge about foods and were trained for sensory evaluations. A 7-point scale (1=dislike extremely, 4=neither like nor dislike, 7=like extremely) was used to rate the overall acceptability, flavor, appearance, and texture. The sensory evaluation was approved by the Institutional Review Board (IRB) at Kyungpook National University (IRB No: KNU-2020-0128).

#### 2.5. Statistical analysis

All experiments were repeated at least three times, and the results are presented as mean  $\pm$  SD. The results were analyzed with one-way ANOVA and Duncan's multiple range test to determine significance using IBM SPSS Statistics 26 for Windows (IBM Corp., Armonk, NY, USA).  $p < 0.05$  was considered statistically significant.

## 3. Results and discussion

#### 3.1. Morphological changes and weight reduction during storage

First, we compared the frontal and side appearances of white *H. marmoreus* varieties after storing for different durations. According to Fig. 1, there was no obvious change in all three varieties (EG2020, HKT, and H6) in terms of the pileus condition and stem (stipe) texture, even on the final day of storage (day 42). The weight loss rate indicates the degree



Fig. 1. Appearance changes in white cultivar *H. marmoreus* during storage.

Table 1. Changes in weight of white cultivar *H. marmoreus* during storage

Storage period (day)	Weight (g)			
	0	14	28	42
EG2020	184.54±5.80 <sup>1)a2)</sup>	184.5±4.17 <sup>a</sup>	180.21±2.60 <sup>a</sup>	180.65±3.52 <sup>a</sup>
H6	156.55±1.41 <sup>a</sup>	155.71±6.45 <sup>a</sup>	155.03±4.13 <sup>a</sup>	152.40±5.05 <sup>a</sup>
HKT	157.34±1.93 <sup>a</sup>	157.33±3.05 <sup>a</sup>	154.24±1.40 <sup>a</sup>	155.13±1.56 <sup>a</sup>

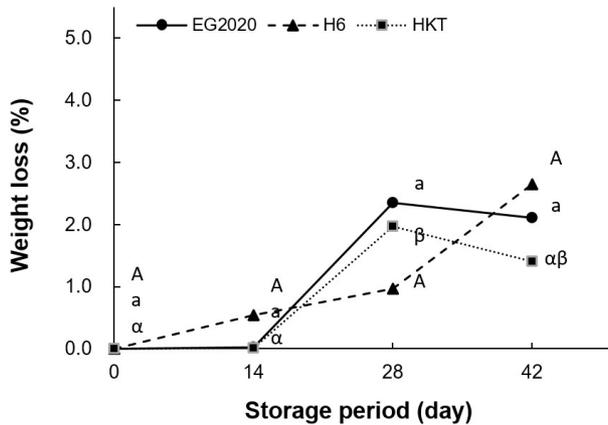
<sup>1)</sup>Mean±SD.

<sup>2)a-d</sup>Values with different superscripts were significantly different among groups at p<0.05 level by a Duncan's multiple range test.

of moisture loss during storage. As shown in Table 1 and Fig. 2, all mushroom samples continuously lost weight over the storage period. By day 42, the weight loss rate was 2.11% for EG2020, 2.65% for H6, and 1.40% for HKT. This is because the low temperature suppressed respiration and moisture

loss (Choi et al., 2016b).

To accurately quantify the changes in mushroom appearance, the size of pileus and length of stipe were measured using a caliper (Table 2). The cap size was ranked as EG2020 < H6 < HKT. For each variety, the cap size decreased from immediately



**Fig. 2.** Changes in weight loss of white cultivar *H. marmoreus* during on storage. Different superscripts indicate significant differences ( $p < 0.05$ ) by Duncan's multiple range test.

after harvest (day 0) to the end of storage (day 42): from  $18.53 \pm 3.93$  to  $15.80 \pm 2.07$  mm for EG2020, from  $20.80 \pm 3.00$  to  $17.13 \pm 3.16$  mm for H6, and from  $23.80 \pm 6.42$  to  $22.47 \pm 2.33$  mm for HKT. On the other hand, the stipe length of HKT was shorter than that of EG2020 and H6. The stipe length of EG2020 increased during storage, from  $72.80 \pm 11.89$  mm on day 0 to  $77.93 \pm 7.78$  mm on day 42. However, that of H6 changed very little during the same period (from  $81.20 \pm 6.61$  to  $80.40 \pm 11.00$  mm), while that of HKT decreased slightly (from  $72.67 \pm 6.42$  to  $66.87 \pm 6.62$  mm). Overall, the new variety EG2020 has smaller pilei than H6 and HKT but relatively more numerous pilei and stipes,

indicating a more compact form of growth and markedly greater weight compared to the other two varieties.

### 3.2. Texture changes during storage

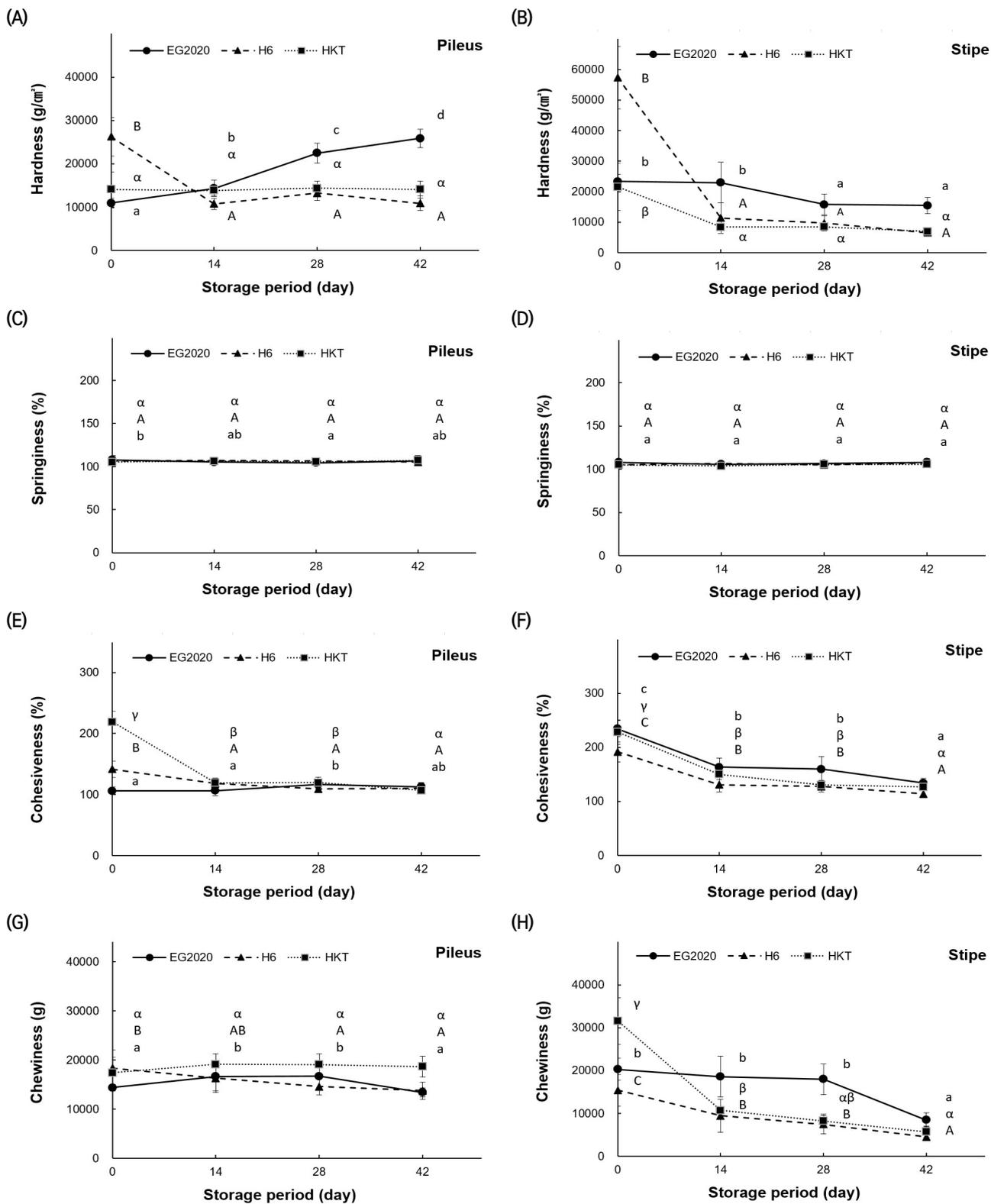
Fig. 3 shows texture changes of the three *H. marmoreus* varieties during storage. On day 0, H6 had the highest hardness in pileus and stipe ( $26,245.71 \pm 4,475.40$  and  $57,436.00 \pm 10,196.60$  g/cm<sup>2</sup>, respectively), but the hardness decreased markedly throughout storage, resulting in the lowest values among the three varieties by day 42. Therefore, H6 showed the greatest reduction in hardness over time. Remarkably, the pilei of EG2020 actually showed a higher hardness on day 42 than on day 0, from  $10,950.56 \pm 1,171.11$  to  $25,833.75 \pm 2,132.46$  g/cm<sup>2</sup>, while the stipe hardness decreased from  $23,383.75 \pm 2,222.82$  to  $15,468.75 \pm 2,645.74$  g/cm<sup>2</sup> during the same period. Cho et al. (2001a) reported that mushrooms are hard in the early phase of storage but become softer later due to respiration and transpiration. Here, EG2020 had the highest hardness after 42 days of storage, suggesting that this new variety is more robust after harvest than the existing varieties. The measured springiness was similar among the three varieties (103-107%), and there was little change over the storage period. The

**Table 2.** Changes in pileus and stipe diameter of white cultivar *H. marmoreus* during storage

Storage period (day)	Diameter (mm)					
	EG2020		H6		HKT	
	Pileus	Stipe	Pileus	Stipe	Pileus	Stipe
0	$18.53 \pm 3.93^{1) b2)}$	$72.80 \pm 11.89^a$	$20.80 \pm 3.00^b$	$81.20 \pm 6.61^a$	$23.80 \pm 6.42^a$	$72.67 \pm 6.42^b$
14	$17.80 \pm 3.19^{ab}$	$79.27 \pm 6.38^{ab}$	$18.20 \pm 3.35^{ab}$	$83.80 \pm 11.19^a$	$21.80 \pm 3.08^a$	$66.47 \pm 5.26^a$
28	$15.93 \pm 3.36^a$	$83.40 \pm 8.81^b$	$20.00 \pm 2.48^a$	$75.53 \pm 12.22^a$	$23.27 \pm 3.23^a$	$64.47 \pm 7.41^a$
42	$15.80 \pm 2.07^a$	$77.93 \pm 7.78^{ab}$	$17.13 \pm 3.16^a$	$80.40 \pm 11.00^a$	$22.47 \pm 2.33^a$	$66.87 \pm 6.62^a$

<sup>1)</sup> Mean  $\pm$  SD.

<sup>2)</sup> a-d Values with different superscripts were significantly different among groups at  $p < 0.05$  level by a Duncan's multiple range test.



**Fig. 3.** Changes in pileus (A, C, E, G) and stipe (B, D, F, H) texture of white cultivar *H. marmoreus* during storage. (A) Hardness of pileus, (B) Hardness of stipe, (C) Springiness of pileus, (D) Springiness of stipe, (E) Cohesiveness of pileus, (F) Cohesiveness of stipe, (G) Chewiness of pileus, (H) Chewiness of stipe. Different superscripts indicate significant differences ( $p < 0.05$ ) by Duncan's multiple range test.

measured cohesiveness differed among the varieties, with no similarities. Chewiness tended to decrease for both the pilei and stipes during storage, which is probably strongly influenced by changes in physical properties such as hardness.

### 3.3. Color changes during storage

The degree of browning in mushrooms during storage was quantified using a colorimeter, and the results are shown in Fig. 4. For each mushroom variety, the pileus and stipes were separated, and the color values of each part were measured. For the pileus of EG2020, the initial (L, a, b) values were  $(92.85 \pm 0.45, 0.12 \pm 0.09, \text{ and } 10.78 \pm 0.58)$  and did not change significantly during storage. For the stipe of EG2020, L  $(94.49 \pm 0.72)$  did not change significantly, while a decreased from  $0.07 \pm 0.06$  to  $-0.01 \pm 0.14$  and b increased slightly from  $6.77 \pm 0.91$  to  $7.41 \pm 1.09$  on day 42. For the pileus of H6, L  $(93.49 \pm 0.75)$  did not change significantly, while a decreased slightly from  $0.01 \pm 0.09$  to  $-0.04 \pm 0.10$  and b increased slightly from  $9.84 \pm 0.91$  to  $11.21 \pm 1.36$  on day 42. For the stipe of H6, L  $(93.71 \pm 1.24)$  did not change significantly, while a decreased slightly from  $0.06 \pm 0.07$  to  $-0.14 \pm 0.09$  and b increased from  $7.93 \pm 1.53$  to  $9.07 \pm 0.85$  on day 42. Finally, for the pileus of HKT, L decreased from  $92.32 \pm 1.11$  to  $88.18 \pm 2.41$ , a decreased slightly from  $0.30 \pm 0.15$  to  $-0.10 \pm 0.60$ , and b increased from  $12.08 \pm 1.30$  to  $15.63 \pm 1.23$  on day 42. For the stipe of HKT, L decreased from  $94.81 \pm 0.14$  to  $91.77 \pm 1.50$ , a decreased from  $0.14 \pm 0.09$  to  $-0.32 \pm 0.23$ , and b rose from  $7.79 \pm 0.44$  to  $11.49 \pm 0.82$  on day 42. HKT showed the greatest color change among the three varieties.

Color difference ( $\Delta E$ ) for the pileus over the storage period (from baseline to day 42) was the smallest for H6  $(1.76 \pm 1.15)$ , followed by EG2020

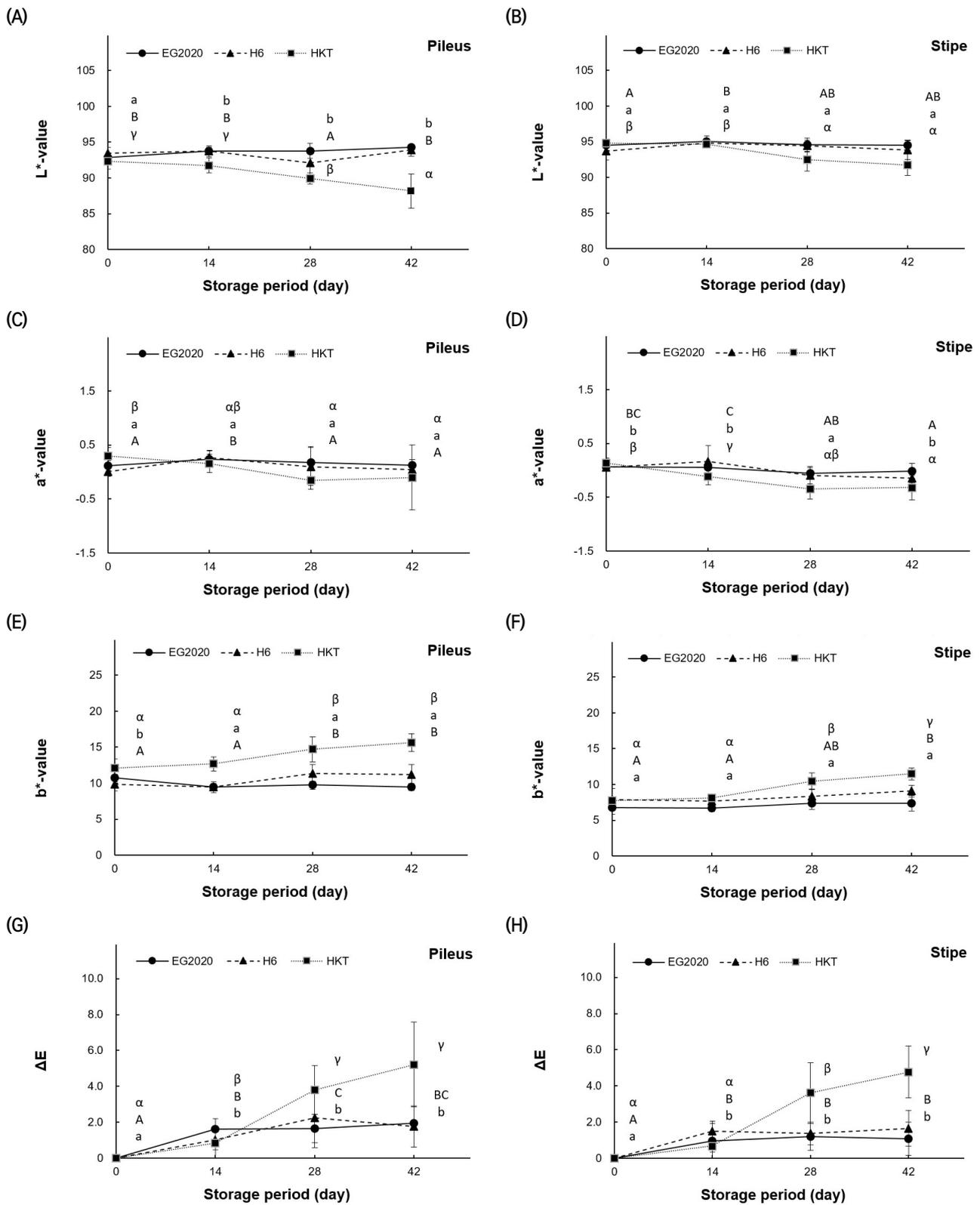
$(1.95 \pm 0.53)$  and HKT  $(5.22 \pm 2.35)$ .  $\Delta E$  of the stipe was the smallest for EG2020  $(1.08 \pm 0.91)$ , followed by H6  $(1.66 \pm 0.99)$  and HKT  $(4.77 \pm 1.44)$ . Hence, the  $\Delta E$  values also support that HKT shows the most browning among the three varieties.

### 3.4. PPO activity during storage

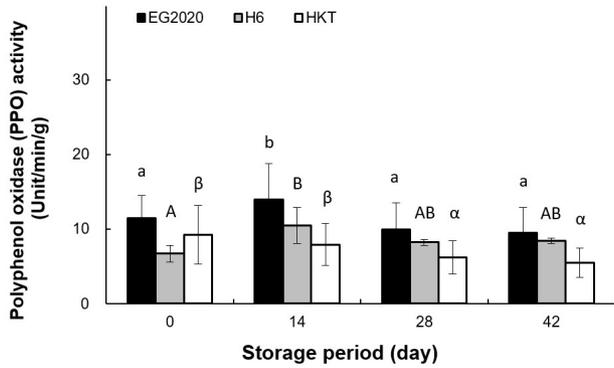
The browning of mushroom surface can be attributed to non-enzymatic browning caused by chemical changes as well as enzymatic browning caused by the PPO enzyme (Martinez and Whitaker, 1995). The PPO activity of three white *H. marmoreus* varieties was measured over the storage period, and the results are shown in Fig. 5. Among the three varieties, EG2020 had the highest initial PPO activity  $(11.43 \pm 3.10\%)$ , which increased to  $13.94 \pm 4.81\%$  on day 14, followed by a decrease after day 28. H6 showed a slight increase in PPO activity from  $6.72 \pm 1.12\%$  on day 0 to  $10.47 \pm 2.37\%$  on day 14, followed by a decrease after day 28. On the other hand, HKT showed a decreasing trend in PPO activity, from  $9.23 \pm 3.92\%$  on day 0 to  $5.47 \pm 1.93\%$  on day 42. The color change and PPO activity in white *H. marmoreus* varieties showed opposite trends. Burton et al. (1993) reported that enzymes and phenolic compounds exist in different cellular compartments, and that color change occurs when they react with each other after cell membranes collapse due to aging after harvesting. Murr and Morris (1974) also reported that discoloration progressed even when the PPO activity was suppressed. We speculated that the new variety EG2020 shows slower browning despite having a higher PPO activity than H6 and HKT due to its slower aging process.

### 3.5. Changes in pH and saccharinity during storage

pH changes in the mushroom varieties over the



**Fig. 4.** Changes in Hunter color values of white cultivar *H. marmoreus* during storage. (A) L-value of pileus, (B) L-value of stipe, (C) a-value of pileus, (D) a-value of stipe, (E) b-value of pileus, (F) b-value of stipe, (G)  $\Delta E$  of pileus, (H)  $\Delta E$  of stipe. Different superscripts indicate significant differences ( $p < 0.05$ ) by Duncan's multiple range test.



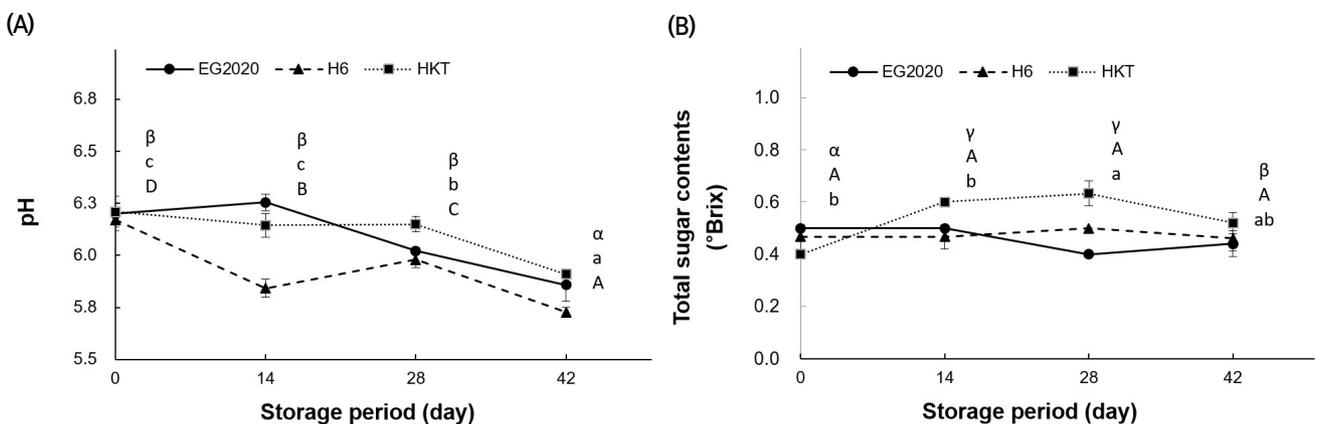
**Fig. 5.** Polyphenol oxidase (PPO) changes in white cultivar *H. marmoreus* during storage. Different superscripts indicate significant differences ( $p < 0.05$ ) by Duncan's multiple range test.

storage period are shown in Fig. 6(A). The three varieties had similar pH values on day 0 ( $6.20 \pm 0.02$  for EG2020,  $6.17 \pm 0.05$  for H6, and  $6.21 \pm 0.07$  for HKT). During storage, the pH decreased but the trend depended on the time and variety. In the case of EG2020, it changed very little from day 0 to day 14 but then decreased to  $6.02 \pm 0.02$  on day 28 and  $5.86 \pm 0.08$  on day 42. In the case of H6, the pH rapidly decreased from  $6.17 \pm 0.05$  on day 0 to  $5.84 \pm 0.04$  on day 14 and  $5.73 \pm 0.02$  on day 42. HKT showed very small pH changes from day 0 to day 14 ( $6.14 \pm 0.06$ ) and day 28 ( $6.15 \pm 0.04$ ), but dropped dramatically afterwards to reach  $5.91 \pm 0.02$  on day 42.

Changes in saccharinity of the three mushroom varieties over the storage period are shown in Fig. 6(B). Saccharinity on day 0 was the highest for EG2020 ( $0.5 \pm 0.00$  °Brix), followed by H6 ( $0.47 \pm 0.05$  °Brix) and HKT ( $0.40 \pm 0.00$  °Brix), indicating that the new variety EG2020 has the highest soluble solid content. For EG2020, saccharinity decreased to  $0.44 \pm 0.05$  °Brix on day 42. For H6, it slightly increased to  $0.50 \pm 0.00$  °Brix on day 28 but decreased again to  $0.46 \pm 0.05$  °Brix on day 42. For HKT, it increased to  $0.63 \pm 0.05$  °Brix on day 28 and decreased to  $0.52 \pm 0.04$  °Brix on day 42. These results show that throughout storage, the new variety EG2020 and existing Korean variety H6 maintained a consistent level of saccharinity, which is correlated to the umami taste.

### 3.6. Changes in free sugar content during storage

Free sugar content affects the sweetness and flavor of white *H. marmoreus*. Table 3 shows the changes in free sugar content over the storage period. On day 0, the total sugar content was the highest for EG2020 (4,779 ppm), followed by H6 (1,717 ppm) and HKT (1,688 ppm). Throughout storage, all three varieties showed a marked increase



**Fig. 6.** Changes in (A) pH and (B) total sugar contents of white cultivar *H. marmoreus* depending on storage. Different superscripts indicate significant differences ( $p < 0.05$ ) by Duncan's multiple range test.

**Table 3.** Changes in free sugar of white cultivar *Hypsizygus marmoreus* during storage

Sample	Free sugar content (ppm)	Storage period (day)			
		0	14	28	42
EG2020	Total free sugar	4,779	7,226	11,274	5,257
	Sucrose	2,990	5,886	9,922	3,334
	Lactose	ND <sup>1)</sup>	ND	ND	ND
	Glucose	599	58	ND	697
	Fructose	1,187	1,279	1,349	1,225
H6	Total free sugar	1,717	8,348	7,865	6,724
	Sucrose	ND	6,766	6,140	4,908
	Lactose	ND	ND	ND	ND
	Glucose	152	ND	ND	ND
	Fructose	1,564	1,581	1,724	1,815
HKT	Total free sugar	1,688	6,693	6,809	3,408
	Sucrose	ND	5,358	5,455	2,213
	Lactose	ND	ND	ND	ND
	Glucose	101	ND	12	ND
	Fructose	1,585	1,333	1,340	1,193

<sup>1)</sup>Not detected.

in free sugar content. EG2020 reached the highest value on day 28 (11,274 ppm), which was nearly halved by day 42 (5,257 ppm). In the other two varieties, the initial free sugar content was very low. However, the sucrose content changed substantially during storage, leading to an increased free sugar content. In the case of H6, the free sugar content rapidly rose from 1,717 ppm on day 0 to a peak value of 8,348 ppm on day 14, and then slowly decreased to 7,865 ppm on day 28 and 6,724 ppm on day 42. Similarly, HKT showed a rapid increase in free sugar content from 1,688 ppm on day 0 to 6,693 ppm on day 14, peaking at 6,809 ppm on day 28, and then decreasing rapidly to 3,408 ppm on day 42. The composition of free sugars was similar among the three varieties. The sucrose content was the highest, followed by fructose. Given that the new variety EG2020 displayed the highest free sugar

content throughout the storage period, it should display the strongest sweet.

### 3.7. Changes in organic acid content during storage

The flavor of mushrooms is also affected by the organic acids. Here, we monitored the organic acid content over the storage period, and the results are shown in Table 4. H6 had the highest total organic acid content (1,445–3,592 ppm), while its level was similar between EG2020 (1,566–2,784 ppm) and HKT (1,590–2,494 ppm). During the storage period, the total organic acid content generally decreased with time. The composition of free amino acids varied among the three mushroom varieties. In EG2020, the most abundant organic acid was malic acid, which gives a smooth and refined acidic taste, and the percentage of succinic acid, which produces umami, was also high. In H6 and HKT, malic acid

**Table 4.** Changes in organic acid of white cultivar *Hypsizygus marmoreus* during storage

Sample	Organic acid content (ppm)	Storage period (day)			
		0	14	28	42
EG2020	Total organic acid	2,784	1,799	1,857	1,566
	Citric acid	ND <sup>1)</sup>	ND	ND	ND
	Malic acid	2,075	1,405	1,602	1,355
	Succinic acid	446	236	105	103
	Lactic acid	104	61	49	26
	Acetic acid	138	96	107	81
H6	Total organic acid	3,592	2,036	1,478	1,445
	Citric acid	ND	ND	ND	ND
	Malic acid	1,578	1,674	1,354	1,187
	Succinic acid	716	192	57	107
	Lactic acid	188	123	25	47
	Acetic acid	1,110	48	42	103
HKT	Total organic acid	2,494	1,590	1,832	2,156
	Citric acid	ND	ND	ND	ND
	Malic acid	946	1,265	1,536	1,533
	Succinic acid	118	170	214	463
	Lactic acid	88	76	49	66
	Acetic acid	1,343	80	33	95

<sup>1)</sup>Not detected.

was also the most abundant organic acid, but acetic acid, which produces a tangy sour taste, became the second-most abundant one soon after harvest. These results suggest that consumers are likely to favor the new EG2020 variety due to its sweet, refined sour taste and umami flavor.

### 3.8. Changes in free amino acid content during storage

It is generally known that mushrooms with a higher free amino acid content also taste better (Xu et al., 2007). In this study, we compared the changes of free amino acid content in the three varieties of white *H. marmoreus* over the storage period. As shown in Table 5, HKT had the highest total amino acid content on day 0 (23.37 mg/mL), but it decreased

over time to reach 19.49 mg/mL on day 42. H6 also showed a decrease in total amino acid content from 18.35 mg/mL on day 0 to 17.07 mg/mL on day 42. In contrast, after a decrease from 18.15 mg/mL on day 0, EG2020 showed a higher total amino acid content on day 42 (18.49 mg/mL). Furthermore, all three varieties contained all the essential amino acids except for tryptophan.

We also analyzed the contents of glutamic acid and aspartic acid that produce the umami flavor. On day 0, HKT had the highest glutamic acid content (4.93 mg/mL) and aspartic acid content (1.72 mg/mL). However, the glutamic acid content decreased markedly over the storage period. H6 and EG2020 also showed a similar pattern of decline in

**Table 5.** Changes in free amino acid concentrations of white cultivar *Hypsizygus marmoreus* during storage

(1) Free amino acid (mg/mL)		Storage period (day)											
		EG2020				H6				HKT			
		0	14	28	42	0	14	28	42	0	14	28	42
Total free amino acid		18.15	15.813	17.500	18.491	18.35	15.032	15.920	17.065	23.37	16.516	20.621	19.491
Essential amino acid	Lysine	1.466	1.587	1.558	1.861	0.870	1.426	1.812	1.612	1.273	1.477	1.731	1.756
	Methionine	0.179	0.221	0.231	0.259	0.174	0.223	0.246	0.254	0.133	0.237	0.229	0.249
	Leucine	1.056	1.155	1.207	1.314	0.972	1.145	1.357	1.298	1.257	1.227	1.243	1.316
	Phenylalanine	0.677	0.686	0.717	0.804	0.556	0.677	0.768	0.762	0.642	0.722	0.744	0.765
	Valine	0.768	0.729	0.845	0.845	0.894	0.722	0.989	0.820	1.048	0.775	0.901	0.895
	Histidine	0.339	0.377	0.371	0.468	0.258	0.332	0.422	0.389	0.307	0.337	0.436	0.413
	Threonine	0.640	0.579	0.638	0.734	0.578	0.540	0.766	0.664	0.719	0.589	0.712	0.732
	Isoleucine	0.549	0.566	0.651	0.636	0.576	0.570	0.746	0.627	0.709	0.615	0.672	0.668
Non-essential amino acid	Glycine	0.517	0.456	0.533	0.583	0.527	0.446	0.611	0.550	0.611	0.479	0.593	0.562
	Cystine	0.237	ND <sup>1)</sup>	ND	0.165	0.449	0.070	ND	0.158	0.497	0.091	ND	0.206
	Arginine	1.313	1.491	1.467	1.761	0.856	1.448	2.110	1.628	0.887	1.650	1.527	2.248
(2) Free amino acid (mg/mL)		Storage period (day)											
		EG2020				H6				HKT			
		0	14	28	42	0	14	28	42	0	14	28	42
Tyrosine		0.618	0.652	0.663	0.799	0.493	0.610	0.757	0.725	0.585	0.661	0.730	0.768
Proline		0.519	0.417	0.413	0.436	0.513	0.354	0.410	0.394	0.733	0.318	0.554	0.336
Glutamic acid		2.133	1.756	1.709	1.773	4.799	1.765	2.110	1.663	4.929	1.833	1.803	1.914
Aspartic acid		1.024	0.853	0.968	0.893	1.140	0.908	1.601	0.926	1.722	1.222	0.852	1.626
Serine		0.846	0.769	0.872	0.998	0.538	0.753	1.014	0.940	0.721	0.805	0.931	1.030
Alanine		1.628	1.574	1.524	1.850	1.740	1.438	1.878	1.718	2.031	1.614	1.696	2.007
Phosphoserine		0.102	0.064	0.072	0.068	0.155	0.060	0.099	0.068	0.173	0.079	0.053	0.128
Taurine		0.060	0.083	0.049	0.066	0.068	0.061	0.106	0.064	0.105	0.110	0.041	ND
Phosphoethanol amine		0.097	0.062	0.072	ND	0.095	0.074	0.109	ND	0.138	0.089	0.068	ND
Urea		1.738	ND	ND	ND	1.070	ND	0.955	ND	1.836	ND	0.249	ND
Sarcosine		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
$\alpha$ -Amino adipic acid		0.051	0.106	0.084	0.154	0.085	0.084	0.085	0.129	0.065	0.081	0.095	0.124
Citrulline		ND	ND	ND	ND	ND	ND	ND	ND	0.592	ND	ND	ND
$\alpha$ -Amino-n-butyric acid		ND	0.025	0.023	ND	ND	0.034	ND	ND	ND	0.029	0.020	ND
Cystathionine		0.076	0.073	0.094	0.096	0.055	0.081	0.126	0.101	0.112	0.092	0.085	0.137

(continued)

(3) Free amino acid (mg/mL)	Storage period (day)											
	EG2020				H6				HKT			
	0	14	28	42	0	14	28	42	0	14	28	42
$\beta$ -Alanine	0.045	0.039	0.037	0.060	0.056	0.044	0.043	0.087	0.061	0.049	0.040	0.069
$\beta$ -Amino isobutyric acid	0.070	0.065	0.051	0.124	0.070	0.044	0.038	0.071	0.084	0.071	0.027	0.080
$\gamma$ -Amino-n-butyric acid	0.028	0.026	0.016	0.033	0.033	0.014	0.021	0.031	0.039	0.022	0.022	0.028
Ethanol amine	0.072	0.090	0.105	0.132	0.122	0.103	0.111	0.129	0.059	0.094	0.128	0.127
Ammonia	0.040	0.025	0.042	0.053	0.111	0.026	0.063	0.036	0.135	0.036	0.047	0.052
Hydroxylysine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ornithine	1.301	1.308	0.950	1.564	0.607	1.001	1.332	1.216	1.306	1.138	1.317	1.747
1-Methylhistidine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-Methylhistidine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Anserine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carnosine	ND	0.004	ND	0.018	ND	0.006	ND	0.040	ND	0.010	ND	0.037
Hydroxyproline	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

<sup>1)</sup>Not detected.

these two amino acids associated with umami.

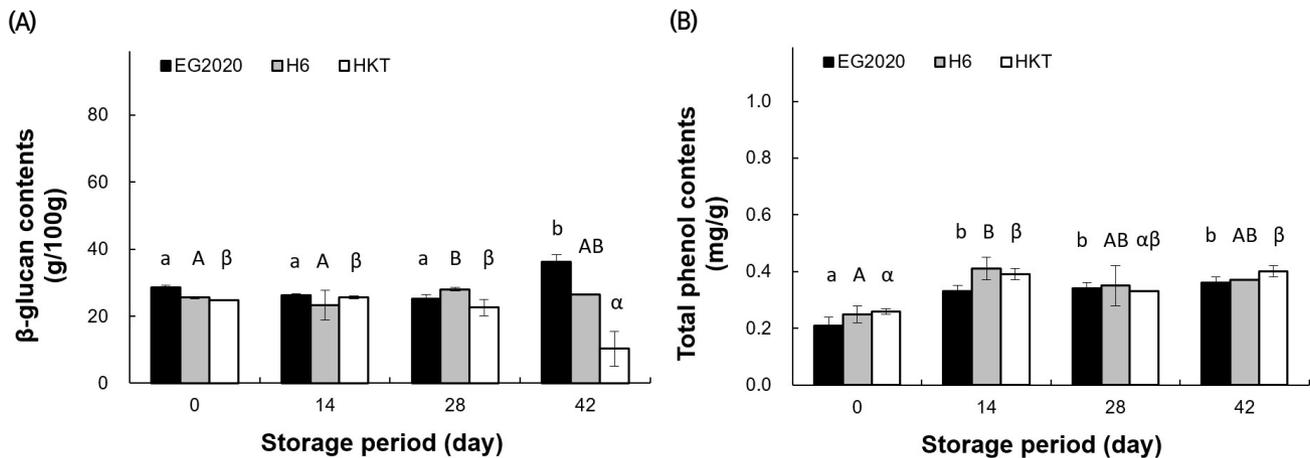
Next, we compared the changes in the contents of amino acids that produce a bitter taste. EG2020 had the highest arginine content both on day 0 (1.31 mg/mL) and day 42 (1.76 mg/mL). The relative abundance of these amino acids in EG2020 was ranked as valine > leucine > phenylalanine > tyrosine > isoleucine > histidine > methionine. Most bitter-tasting amino acids tended to become enriched during storage in all three varieties. Park et al. (2011) reported that *H. marmoreus* contains a total of 17 amino acids. Particularly, they found relatively high percentages of glutamic acid, serine, arginine, and leucine, in agreement with our study.

### 3.9. changes in $\beta$ -glucan content and TPC during storage

$\beta$ -Glucan is the classic useful ingredient of mushrooms. Fig. 7(A) compares the  $\beta$ -glucan content in the three white *H. marmoreus* varieties during storage. The new variety EG2020 had the highest  $\beta$ -

glucan content, being  $28.53 \pm 0.68$  g/100 g on day 0 and  $6.28 \pm 2.07$  g/100 g on day 42. The  $\beta$ -glucan content of H6 was  $25.53 \pm 0.27$  g/100 g on day 0 and  $26.43 \pm 0.19$  g/100 g on day 42, while that of HKT was  $24.8 \pm 0.02$  g/100 g on day 0 and  $10.31 \pm 5.19$  g/100 g on day 42. The trend of  $\beta$ -glucan content with time differed among mushroom varieties, increasing in EG2020 and decreasing markedly in HKT.

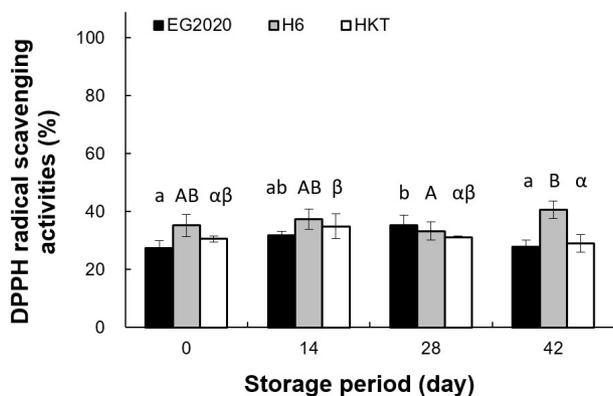
The TPC values of three mushroom varieties during storage are shown in Fig. 7(B). On day 0, TPC was ranked as HKT > H6 > EG2020. Moreover, it increased over the whole storage period: from  $0.26 \pm 0.01$  mg/g on day 0 to  $0.40 \pm 0.02$  mg/g on day 42 in HKT, from  $0.25 \pm 0.03$  to  $0.37 \pm 0.00$  mg/g in H6, and from  $0.21 \pm 0.03$  to  $0.36 \pm 0.02$  mg/g in EG2020. The TPC values on day 0 were lower than those of wild mushrooms (0.391–1.725 mg/g) reported by Ferreira et al. (2009). However, the trends of change over the storage period were similar across the varieties.



**Fig. 7.** Changes in (A)  $\beta$ -glucan and (B) total phenolics contents of white cultivar *H. marmoreus* during storage. Different superscripts indicate significant differences ( $p < 0.05$ ) by Duncan's multiple range test.

### 3.10. Changes in antioxidant activity during storage

DPPH radical scavenging activities of the three white *H. marmoreus* varieties over the storage period are shown in Fig. 8. On day 0, H6 had the highest activity ( $35.19 \pm 3.90\%$ ), followed by HKT ( $30.56 \pm 1.00\%$ ) and EG2020 ( $27.29 \pm 2.76\%$ ). The changes of DPPH radical scavenging activity during storage depended on the variety. For EG2020, it increased slightly until day 28 but decreased by day 42. For H6, it increased continuously during the storage period. For HKT, it increased until day 14



**Fig. 8.** Changes in DPPH radical scavenging activities of white cultivar *H. marmoreus* during storage. Different superscripts indicate significant differences ( $p < 0.05$ ) by Duncan's multiple range test.

but slowly decreased through day 28 and 42. Choi et al. (2017) reported that the DPPH radical scavenging activities of extracts from two other edible mushroom species, *Lentinula edodes* and *Agaricus Bosporus*, declined continuously during low-temperature storage. These results suggest that the antioxidant properties of white *H. marmoreus* can be preserved by controlling the duration of low-temperature storage.

### 3.11. Palatability of EG2020 and HKT varieties

Palatability evaluation was performed to compare the sensory qualities of EG2020 with a Japanese variety currently marketed in Korea (HKT). As shown in Table 6, EG2020 has a markedly higher overall palatability than HKT ( $4.23 \pm 1.21$  vs.  $3.76 \pm 1.68$ ) as well as a higher flavor rating ( $4.42 \pm 1.04$  vs.  $4.10 \pm 0.98$ ). However, EG2020 also has a higher off-flavor rating, suggesting that the volatile aromatic ingredients in this new variety are sensed more strongly. In terms of taste, EG2020 is rated higher in terms of sweetness than HKT ( $3.97 \pm 1.62$  vs.  $3.45 \pm 1.56$ ). Aftertaste, the taste that remains in the mouth after swallowing, is rated slightly higher for EG2020 than HKT ( $4.32 \pm 1.51$  vs.  $3.61 \pm 1.68$ ). In terms of appearance, EG2020

**Table 6.** Sensory scores of white cultivar *Hypsizygus marmoreus* during storage

Sensory parameter		Sensory score <sup>1)</sup>			
		EG2020		HKT	
		1 min	2 min	1 min	2 min
Overall acceptability		4.23±1.21 <sup>2)a3)</sup>	4.16±1.46 <sup>a</sup>	3.76±1.68 <sup>a</sup>	3.87±1.58 <sup>a</sup>
Flavor	Aroma	4.42±1.04 <sup>a</sup>	4.23±1.01 <sup>a</sup>	4.10±0.98 <sup>a</sup>	4.10±1.23 <sup>a</sup>
	Odor	3.29±1.44 <sup>a</sup>	3.29±1.53 <sup>a</sup>	3.13±1.43 <sup>a</sup>	3.45±1.60 <sup>a</sup>
Taste	Sourness	3.32±1.87 <sup>a</sup>	2.90±1.59 <sup>a</sup>	3.45±1.62 <sup>a</sup>	3.06±1.87 <sup>a</sup>
	Bitters	3.58±2.11 <sup>a</sup>	3.45±1.76 <sup>a</sup>	3.48±1.95 <sup>a</sup>	3.48±2.18 <sup>a</sup>
	Sweetness	3.97±1.62 <sup>a</sup>	3.52±1.72 <sup>a</sup>	3.45±1.56 <sup>a</sup>	3.58±1.56 <sup>a</sup>
	After taste	4.32±1.51 <sup>a</sup>	3.84±1.80 <sup>a</sup>	3.61±1.68 <sup>a</sup>	3.77±1.64 <sup>a</sup>
Appearance	Color	5.42±1.36 <sup>ab</sup>	4.58±1.56 <sup>b</sup>	4.97±1.40 <sup>a</sup>	4.65±1.43 <sup>ab</sup>
	Shape	4.87±1.56 <sup>a</sup>	4.84±1.59 <sup>a</sup>	4.68±1.38 <sup>a</sup>	4.77±1.66 <sup>a</sup>
Texture	Hardness	3.26±1.48 <sup>a</sup>	3.65±1.56 <sup>a</sup>	3.55±1.19 <sup>a</sup>	3.90±1.38 <sup>a</sup>
	Chewiness	3.90±1.78 <sup>a</sup>	4.16±1.67 <sup>a</sup>	3.84±1.32 <sup>a</sup>	4.32±1.38 <sup>a</sup>

<sup>1)</sup>Sensory evaluation was performed using 7-hedonic scale as very good (7 point), slightly good (5 point), normal (4 point), slightly bad (3 point), very bad (1 point).

<sup>2)</sup>Mean±SD.

<sup>3)a-c</sup>Values with different alphabets in the column were significantly different among groups at p<0.05 level by a Duncan's multiple range test.

is rated very favorably in color compared to HKT ( $5.42 \pm 1.36$  vs.  $4.97 \pm 1.40$ ) and also more favorably in shape. In terms of texture, EG2020 was perceived as slightly softer than HKT ( $3.26 \pm 1.48$  vs.  $3.55 \pm 1.19$ ) but with a slightly higher chewiness ( $3.90 \pm 1.78$  vs.  $3.84 \pm 1.32$ ). The sensory evaluation results show that the new EG2020 variety has superior palatability compared to HKT in terms of flavor, taste, appearance, and texture. Cho et al. (2001b) reported that while the marketability of mushrooms is affected by nutritional and physiochemical properties, consumers rate mushrooms based on their sensory palatability. Hence, we expect consumers to welcome EG2020 once it becomes available on the market.

## 4. Summary

The storage and distribution of fresh mushrooms

are impaired by their vulnerability to browning and undesirable texture changes over time. In the present study, we compared a new variety of white *H. marmoreus* (EG2020) with two commercially available varieties (H6 from Korea and HKT from Japan). The appearance, physiochemical quality indicators, nutritional components, and functional ingredients were monitored during cold storage for up to 42 days. Our results revealed no marked changes in pileus appearance and stipe texture in all three varieties even after 42 days. Although the new variety EG2020 had slightly smaller pileus sizes compared to H6 and HKT, the clusters contained substantially more pilei and stipes, leading to a more compact growth form as well as a higher overall weight. In terms of mechanical texture, the pilei of EG2020 tended to increase in hardness during storage compared to the other two varieties.

Hence, EG2020 is expected to have a better ability to survive storage and transportation. Color change (indicated by  $\Delta E$ ) of the pileus over time was ranked as H6 ( $1.76 \pm 1.15$ ) < EG2020 ( $1.95 \pm 0.53$ ) < HKT ( $5.22 \pm 2.35$ ), while that of the stipes was ranked as EG2020 ( $1.08 \pm 0.91$ ) < H6 ( $1.66 \pm 0.99$ ) < HKT ( $4.77 \pm 1.44$ ). Overall, HKT showed the most serious browning among the three varieties. All three varieties showed a declining pH value and consistent saccharinity over the storage period. Free sugar contents affect the sweetness and flavor of foods, and the new variety EG2020 had the highest total free sugar content. In addition to its high saccharinity, EG2020 was also found to contain abundant organic acids that produce a refined sour taste and umami. These results suggest that the new variety EG2020 should be well accepted by consumers due to its improved flavor. EG2020 also had the highest  $\beta$ -glucan content that increased over the storage period. Finally, a sensory evaluation was performed to compare the palatability of EG2020 and HKT. EG2020 was given the highest rating in all evaluated aspects, namely the flavor, taste, appearance, and texture, further supporting that this new mushroom variety should have good overall acceptability.

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### Conflict of interests

The authors declare no potential conflicts of interest.

### Author contributions

Conceptualization: Park JS, Park HJ, Jung HY, Cho YJ.  
Methodology: Park JS, Park HJ, Kim JS, Jeong DE, Han

CW. Formal analysis: Park HJ, Lee SY, Jung HY, Cho YJ. Validation: Park JS, Kim JS, Jeong DE, Han CW. Writing - original draft: Park JS, Park HJ. Writing - review & editing: Kim JS, Jung HY, Cho YJ.

### Ethics approval

This research was approved by IRB from the Kyungpook National University (approval no. KNU-2020-0128).

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