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ARTICLE

The Effect of Hydrolysis Pre-Treatment by Flavourzyme on Meat Quality, Antioxidative Profiles, and Taste-Related Compounds in Samgyetang Breast Supplemented with Black Garlic

Farouq Heidar Barido[1,2], Hee Ju Kim[3], Sun Moon Kang[4], Aera Jang[1], Jae In Pak[1], and Sung Ki Lee[1,*]

[1]Department of Applied Animal Science, Kangwon National University, Chuncheon 24341, Korea
[2]Department of Animal Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta 57126, Indonesia
[3]Highlandinnovation Co., Ltd., Icheon 17392, Korea
[4]National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea

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***Corresponding author** : Sung Ki Lee
Department of Applied Animal Science, Kangwon National University, Chuncheon 24341, Korea
Tel: +82-33-250-8646
Fax: +82-33-259-5574
E-mail: skilee@kangwon.ac.kr

***ORCID**
Farouq Heidar Barido
https://orcid.org/0000-0002-3171-5426
Hee Ju Kim
https://orcid.org/0000-0003-4991-5001
Sun Moon Kang
https://orcid.org/0000-0003-3947-4337
Aera Jang
https://orcid.org/0000-0003-1789-8956
Jae In Pak
https://orcid.org/0000-0003-2563-0642
Sung Ki Lee
https://orcid.org/0000-0002-2989-4787

Abstract This study aimed to carefully investigate the effect of hydrolysis using Flavourzyme on meat quality, antioxidative status, and taste-related compounds in breast of Samgyetang that was supplemented with black garlic (BG). Four different treatment groups were compared: (1) conventional Samgyetang (control), (2) Samgyetang hydrolyzed with Flavourzyme (1%, v/w) (FS), (3) Samgyetang made with the BG extract without hydrolysis (NBG), and (4) BG samgyetang pre-treated with Flavourzyme (1%, v/w) in a water bath at 55°C for 2.5 h and hydrolyzed before being processed (HBG). All the treatment groups were cooked by retorting at conditions 121°C and 1.5 kg/cm^2 for 1 h. Improved umami profiles through the increase of umami-related nucleotides (5′-GMP, 5′-IMP) and free amino acids—aspartic acid and glumtamic acid, in Samgyetang breast was recorded following hydrolysis. The HBG group tended to impart stronger scavenging activity toward free radicals compared with the other two groups, while not differing with NBG group regarding suppressing malondialdehyde. Textural properties were improved through hydrolysis, wherein the shear force value decreased from 2.29 kgf in the control to 1.19 and 1.25 kgf in the FS and HBG group. Moisture percentages were highly retained, with the redness score increasing and the lightness color decreasing following hydrolysis. In conclusion, the results of this study can be a preliminary information of the effect of hydrolysis pre-treatment for BG samgyetang. Further experiments are required to compare various enzymes along with its organoleptic acceptances.

Keywords enzymatic hydrolysis, Flavourzyme, Samgyetang, black garlic, taste-properties

Introduction

Meat is among the most important protein sources that is rich in vitamins, minerals,

and fats, which cannot be found in any other food source to fulfill human nutritional needs (Lawrie and Ledward, 2006). Meat proteins are composed of essential amino acids at high concentrations, with various functions (Park et al., 2020). Carnosine, anserine, and methylhistidine are examples of short protein peptides found in meat that possess antioxidant properties that can scavenge both metal ions and reactive oxygen species (Kang et al., 2002). Pharmacologically, the inhibitory effect of angiotensin-I converting enzyme (ACE) on meat proteins may be associated with remedies for cardiovascular diseases caused by high blood pressure (Arihara et al., 2021). However, these functional properties are highly dependent on its digestible form available in the small intestine.

Enzymatic hydrolysis is the most frequently studied method for improving functional properties of meat (Gu et al., 2018; Zhang et al., 2010). It holds a low risk of carcinogen formation and low toxicity compared to chemical methods, and is known to develop certain amino acid sequences, that work as potent functional modulators, via structural alteration of the protein molecules with water (Mora et al., 2014). The resulting protein hydrolysate derived from chicken meat has been reported to contain a 3.2–14.0 μM IC50 value of ACE inhibitory peptides (Fujita et al., 2000). Another consequence of architectural changes in the molecular structure is the intensification of umami-related compounds [nucleotides and free amino acids (FAAs)] and increased protein solubility (Dong et al., 2020; Zeng et al., 2020).

Flavourzyme is a widely used enzyme that hydrolyzes a wide range of protein sources. It is prepared from non-genetically modified *Aspergillus oryzae* fungi through a specific fermentation processes and has both endo- and exopeptidase activities (Kristinsson and Rosco, 2000). These properties allow a wider range of substrates, thereby generating end products with a wide range of functions. Chae et al. (2003) have found altered inorganic substances and FAA contents of the soup prepared from Flavourzyme-hydrolyzed chicken meat, inferring the increased taste profiles from intensified FAAs and sodium content. Similarly, Gao et al. (2021) have observed a remarkable increase in the activity of small peptides and taste-related substances in Morel mushrooms following Flavourzyme-based hydrolysis, while possessing potent endo- and exopeptidase activities. Additionally, by using Flavourzyme as a hydrolyzing agent, the bovine collagen hydrolysate exhibited a greater inhibition of both gram-negative and gram- positive bacteria (Vidal et al., 2018).

With increased focus on healthy food habits, chicken meat has become a highly recommended meal (Kim et al., 2020). In Korea, the Korean ginseng chicken soup (Samgyetang), which is mainly prepared from chicken meat, stuffed with glutinous rice and certain medicinal plants, is commonly consumed during the summer season as it facilitates caloric restoration from strenuous activity (Jeong et al., 2020). Recently, in accordance to the sharp increase of samgyetang export overseas, Korean government through the Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry (IPET) have been pushing the development and innovation in Samgyetang including the utilization of boneless chicken to meet the foreign markets. To date, the study on Samgyetang involves selecting potential chicken breeds (Jeong et al., 2020; Lee et al., 2018), utilizing healthier salt ingredients (Barido and Lee, 2021a; Kim et al., 2019a), and including plants with bioactive compounds (Barido et al., 2020; Barido et al., 2021; Jeong et al., 2012; Jeong et al., 2013). However, the investigation on the effect of enzymatic hydrolysis pre-treatment is limited.

In our previous study (Barido et al., 2021), the inclusion of black garlic (BG), which contains abundant sulfur-containing compounds such as S-allyl cysteine, S-allyl mercapto cysteine, and diallyl sulfide (Ryu and Kang, 2017), and a high concentration of phenolic acids (Kimura et al., 2017), was proven to exhibit superior suppression of lipid oxidation products resulting from high temperature and pressure processing that adversely affect meat quality via upregulation of antioxidant activities. Hence, with above mentioned potential of the hydrolysis along with the antioxidative status of the BG, the purpose of this study was to investigate the effect of enzymatic hydrolysis pre-treatment on meat quality, antioxidant activity, and

taste-related compounds of retorted BG Samgyetang.

Materials and Methods

Preparation of BG extract

BG cloves used in this study were obtained from the Haena Food (20160506929-1, Seoul, Korea). The extraction of the BG was done according to the Kimura et al. (2017). Briefly, peeled BG was added to 10 volumes of deionized water (w/v) and grounded in a food blender (EBR9814W, Electrolux, Stockholm, Sweden) at 13,500×g for 1 min. The solution was placed in a water bath (BW-20G, Biotechnical Services, North Little Rock, AR, USA) at 80℃ for 1 h. The obtained BG extract was directly incubated at 4±2℃ for 1 h, followed by filtration using Whatman filter paper (No. 1). This solution containing the phenolic extract from BG with the pH value of 4.70 (data not shown) was added to Samgyetang at 5% (v/w) of the total weight.

Preparation of Samgyetang

Conventional Samgyetang was used as the control. It was prepared by placing fat-removed chicken breasts into a retort pouch, added with prepared broth (300 mL) that composed of *Astragalus membranaceus* root, mulberry branch, *Kalopanax septemlobus* branch, and licorice, Siberian ginseng with a salt concentration was set at 0.6% (w/w) according to Jeong et al. (2020). Flavourzyme samgyetang (FS) was prepared by hydrolyzing chicken breast prior to retorting with 1% of Flavourzyme (v/w). The hydrolysis was performed as described by Kong et al. (2017) in a water bath at 55℃ for 2.5 h. The hydrolyzed chicken samples were added with prepared broth similar to control into a retort pouch. Samgyetang supplemented with BG (NBG group) was prepared as described in our previous study (Barido et al., 2021) wherein, 200 mL of the prepared broth similar to the control was mixed with 100 mL of the prepared BG extract. To prepare hydrolyzed BG Samgyetang (HBG), deionized water was added to the chicken breast at 1:2 (w/v), followed by Flavourzyme at 1% of chicken breast (v/w). All prepared samples were directly subjected to high temperature and pressure of 121℃, 1.5 kg/cm^2 for 1 h with f_0 8 using an autoclave (AC-13, Jeio Tech, Daejeon, Korea). Subsequently, the breasts were separated from the broth by filtration using a mesh filter (600 μm) and assigned for further analysis of its quality, antioxidant activity, and taste-related compounds.

Proximate composition

The analysis of the proximate composition of the retorted chicken breast samples was done following the procedure by AOAC (2012). The percentage of sample's moisture was measured on a 1 g sample after oven drying at 105℃ for 24 h. Crude protein content was measured following the Kjeltec system procedure (2200 Kjeltec Auto Distillation Unit, Foss, Hillerød, Denmark). Crude fat was determined through the Soxhlet extraction method for 48 h, and the crude ash content was determined after burning in a muffle furnace (LEF-115S, Daihan Labtech, Namyangju, Korea) at 550℃. All analyses were performed in triplicate.

Color measurement

The instrumental color of the cooked breast samples was measured at five different points using a chromameter (CR-400, Konica Minolta Sensing, Osaka, Japan) that was previously calibrated using a white plate (2° observer, Illuminant C: Y=93.6, x=0.3134, y=0.3194). The lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*) were carefully recorded.

pH value

The pH value was measured in triplicate on each cooked breast slurry. After mixing 5 g sample with 45 mL of distilled water in a homogenizer (PH91, SMT, Tokyo, Japan), the pH value of the continuously stirred slurry samples was measured using a pH meter probe (Seven Easy pH, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) that previously been calibrated.

Shear force value

The cooked breast samples were made into a 1.5×1.5×1.5 cm^3 size and placed under the V blade of the TA-XT2*i* Plus (Stable Micro Systems, Surrey, UK) texture analyzer. Retorted samples were prepared at the uniform size of 1.5×1.5 cm. The measurement was performed on the interior of each samples parallel with the myofibrils orientation under V blade to avoid high variety of measurement due to the possible hardening that occurs toward the outside cooked edge of the sample. The analyses of shear force value for each samples were done with three replicates.

Water holding capacity (WHC) and cooking loss

The determination of the WHC was carried out according to the centrifugation method by Kristensen and Purslow (2001). The unit was expressed as a percentage and was calculated from the ratio of the total moisture content to the remaining water volume after being subjected to boiling in a water bath followed by centrifugation. While the percentage of cooking loss of the cooked breast samples was carefully by calculating the weight before and after cooking [$(W_1–W_2) –W_1$]. W1 denotes the weight of certain sample before retort processing, while W_2 denotes the weight of certain sample after experience retort processing.

Thiobarbituric acid reactive substances (TBARS) assay

The lipid oxidation rate was measured using 2-TBARS assays to quantify the malondialdehyde (MDA). In brief, cooked breast samples (0.5 g) were prepared in triplicate in a 25 mL TBARS test tube, added with an antioxidant mixture (0.1 mL), 1% TBA in 0.3% NaOH (3 mL), and assigned into a thorough vortex for 30 s. The 17 mL of 2.5% trichloroacetic acid in 36 mM HCl was added, sealed, and heated in a water bath (BW-20G, Biotechnical Services, North Little Rock, AR, USA) at 100℃ for 30 min. The tubes were immersed into ice water for 10 min once heating was completed. Each of 5 mL of the aqueous sample was taken into a new 15 mL conical tube, mixed with 3 mL of chloroform, and centrifuged at 2,400×g for 30 min at 4℃ (1248R, LaboGene, Lillerød, Denmark). The absorbance was recorded at 532 nm using a UV spectrophotometer (UV-mini 1240 PC, Shimadzu, Kyoto, Japan) and compared against a blank.

Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were performed to measure the antioxidant activity of the cooked breast samples based on the protocol by Islam et al. (2016). The extract solution (100 μL) was placed in 100 μL of methanolic solution containing DPPH radicals (0.2 mM). The mixture was allowed to react for 30 min at 25℃ in the dark. The absorbance of each extract solution was measured at 517 nm using a spectrophotometer (UV-mini 1240 PC, Shimadzu). The results were expressed as a scavenging percentage of free radicals.

Taste-related nucleotides

The concentration of taste-related nucleotides (5′-AMP, 5′-IMP, 5′-GMP, Inosine, and Hypoxanthine) was carried out following the method by Jayasena et al. (2014) with slight modifications via high-performance liquid chromatography (HPLC) (Agilent 1260 series, Agilent Technologies, Santa Clara, CA, USA) that equipped with a 4.6×150 mm C18 column (Agilent Technologies) and a diode array detector. Minced samples (5 g) were mixed with 25 mL 0.7 M perchloric acid and homogenized (Polytron R PT-2500 E, Kinematica, Malters, Switzerland). The homogenate was centrifuged at 2,000×g for 15 min at 0°C, filtered through filter paper (Whatman No. 4). The collected supernatant was adjusted to pH 6.5 with 5 N KOH. The supernatant was placed in a volumetric flask and adjusted to 100 mL with 0.7 M perchloric acid (pH 6.5, adjusted with 5 N KOH). After cooling for 30 min, the mixture was centrifuged at 1,000×g for 10 min (0°C), and the supernatant was filtered using 0.22 μm syringe filter and analyzed by HPLC (Agilent 1260 Infinity, Agilent Technologies). The wavelength was recorded at 254 nm, and the concentrations of 5′-nucleotide are expressed as μg/mg.

Free amino acid (FAA) contents

The concentration of FAA in cooked breast samples were determined in triplicate according to the method by Rahman et al. (2018) with slight modifications. Briefly, finely ground sample (500 mg) and 6 N HCl (20 mL) were placed into a 25 mL test tube. After being flushed with N_2 gas for 30 s, a sealed tube was subjected to hydrolysis at 110°C for 16 h. Then, 100 μL of the hydrolyzed solution was evaporated under N_2 gas for another 30 s, dissolved with 1 mL of Milli-Q water, vortexed, and the mixtures were filtered through a 0.45 μm PTFE filter. The FAA was then quantified using HPLC (Agilent 1260 series, Agilent Technologies) equipped with a C18 column size 4.6×150 mm, with 5-μm particle size (Agilent Technologies), and a 338 nm detection wavelength at 40°C. Mobile phase A was 40 mM NaH_2PO_4, pH 7.8, and mobile phase B was composed of 45% acetonitrile, 45% methanol, and 10% Milli-Q water.

Statistical analysis

One-way analysis of variance (ANOVA) using R-version 3.6.1 (The R-foundation for Statistical Computing, Vienna, Austria) was used to analyze the differences in proximate composition, instrumental color, meat quality, antioxidant activity, taste-related nucleotides, and FAA contents among treatments. Each samples in six replications were statistically calculated and the significance values with p-values lower than 0.05 were assigned to Duncan's multiple range test to obtain superscript.

Results and Discussion

Proximate composition

The proximate composition, including moisture, crude fat, crude protein, and ash percentage, was compared among the treatment groups and is displayed in Table 1. As shown, the highest moisture content was observed in the HBG (73.98%) and Samgyetang that was hydrolyzed with flavorzyme before retorting (FS) group (70.62%), in comparison to the control (66.98%) and NBG (67.71%) groups ($p>0.05$). These moisture percentages were within the normal range for retorted breast meat from commercial broiler chickens, as per Jeong et al. (2020). Regardless of the BG addition, hydrolysis pre-treatment in this study did significant effect on moisture content. It is possibly attributed to the changes of hydrophilic ability of the meat proteins from enzymolysis that causing more interaction between water molecules and free peptides (Kong et al., 2017). In contrast, the proteins degraded dramatically after hydrolysis unlike in the control and NBG groups ($p<0.05$). Such

Table 1. **Proximate composition of Samgyetang breast influenced by enzymatic hydrolysis pre-treatment**

Variables	Treatments				SEM	p-value
	Control	FS	NBG	HBG		
Moisture (%)	66.98[d]	70.62[b]	67.71[c]	73.98[a]	0.61	<0.05
Crude fat (%)	1.71	1.90	1.86	1.56	0.06	1.09
Crude protein (%)	30.50[a]	24.41[b]	29.62[a]	23.65[b]	0.59	<0.05
Crude ash (%)	0.81	0.80	0.82	0.81	0.00	1.01

[a–c] Mean values within the same rows with the different superscripts are significantly different among treatments ($p<0.05$).
Control, conventional Samgyetang made without addition of black garlic extract and receiving no hydrolyzation; FS, Samgyetang that was hydrolyzed with flavorzyme before retorting; NBG, Samgyetang made with the addition of black garlic extract without hydrolyzation; HBG, Enzymatically hydrolyzed black garlic Samgyetang.

macromolecular degradation during hydrolysis may result in altered protein structures, as well as other properties like hydrophilicity of the protein that may increase and consequently retain more water within the inner muscle (Ha et al., 2013; Naveena et al., 2004; Sharma and Vaidya, 2018). However, another outcome from the cleavage of protein macromolecule during hydrolyzation is the formation of shorter chain and lower molecular weight protein called free peptides that easily dissolved into water, causing lower protein content (Dong et al., 2020; Giménez et al., 2009) that also observed in this study. No noticeable differences were observed in crude fat and ash percentages among the treatment groups.

Color measurement

The surface color profile of the cooked breast samples following hydrolyzation were shown in Table 2. The addition of BG extract on Samgyetang was observed to intensify the redness color in our previous study when compared to the conventional one (Barido et al., 2021). This study confirmed the previous finding, wherein the conventional Samgyetang had the lowest redness score among treatments. Moreover, as also seen in this study, the hydrolyzation produced breast samples with the highest redness score among control and BG treatment groups ($p<0.05$). However, the light color was observed at the lowest score in the hydrolyzed group among treatments. The order of lightness values from the highest to the lowest was control, FS, BG treatment, and hydrolyzed group respectively ($p<0.05$). The result of this study implied the possible darkening effect from the hydrolyzation process on the color profile of the chicken breast. No statistical differences were observed in terms of the yellow color of the chicken breast following treatments. The basic color of the extract solution may hold the important factor to attribute the red color intensification on the BG-treated Samgyetang, as they could largely alter the surface color through permeation into the muscle environment (Jin et al., 2015). While the darkening effect after hydrolyzation might be

Table 2. **Color measurement of Samgyetang breast influenced by enzymatic hydrolysis pre-treatment**

Variables	Treatments				SEM	p-value
	Control	FS	NBG	HBG		
L*	76.77[a]	77.28[a]	64.71[b]	57.42[c]	1.61	<0.05
a*	5.46[c]	4.99[c]	8.99[b]	11.35[a]	0.42	<0.05
b*	16.93	15.73	14.25	15.48	0.33	0.12

[a–c] Mean values within the same rows with the different superscripts are significantly different among treatments ($p<0.05$).
Control, conventional Samgyetang made without addition of black garlic extract and receiving no hydrolyzation; FS, Samgyetang that was hydrolyzed with flavorzyme before retorting; NBG, Samgyetang made with the addition of black garlic extract without hydrolyzation; HBG, Enzymatically hydrolyzed black garlic Samgyetang.

due to the dissolution effect of some colored pigments during browning reaction under Maillard reaction, which confirms the study of Gao et al. (2021). These color alterations highly influence consumer preferences toward meat products (Utama et al., 2020).

pH and cooking loss

Table 3 shows the pH values of the cooked breast samples for each treatment. Hydrolysis significantly increased the pH value compared to that in both the control and BG treatments groups ($p<0.05$). However, this study did not find any further differences in cooking loss percentage between the control and the BG treatment groups ($p>0.05$). Both BG treatment and hydrolysis were shown to not significantly affect the cooking loss of the Samgyetang. Accordingly, our afore performed studies, cooking loss was tended to be more influenced by the cooking method that the BG extract additions (Barido et al., 2021; Barido et al., 2022). The pH of the breast meat sample in the control, after retorting, was 6.19, slightly lower but within the normal range compared to the previous study on Samgyetang made from commercial broilers that were processed via retort processing (Jeong et al., 2020; Kim et al., 2019b). The increase in pH following hydrolysis with petidases might be attributed to the hydrophilic nature of cleaved protein molecules that influence the water potential or its tendency to pair with free peptides, thus results in increased water retention (Shao et al., 2016), hence supporting the previous report by Ang and Ismail-Fitry (2019). In addition, although biochemical alterations may have a negative correlation with one of the important economic traits such as cooking loss (Barido and Lee, 2021b), hydrolysis did not seem to contribute to it, as the percentage of cooking loss did not differ among treatment groups ($p>0.05$).

Shear force and water holding capacity (WHC)

Enzymatic hydrolysis positively affected the textural properties of the cooked breast samples. As seen in Table 3, FS and HBG chicken breast had the lowest shear force value with 1.19 and 1.25 kgf among all treatment groups ($p<0.05$) with the control and NBG groups having 2.29 and 1.92 kgf, respectively ($p>0.05$). Enzymatic hydrolysis pre-treatment is one of the methods used to enhance the flavor and texture properties of protein sources. Dong et al. (2020) reasoned the possible improvement in physicochemical quality, including texture, upon hydrolyzation to be a consequence of increased protein solubility and protein degradation, resulting in notable enhancement of short-chain peptides with less effect on organoleptic profiles. Another possible mechanism may be the higher retention of water that allows the heightened juiciness perception toward meat products (Barido and Lee, 2021c). Miller et al. (1995) mentioned that the difference in shear force value of even

Table 3. Meat quality traits of Samgyetang breast influenced by enzymatic hydrolysis pre-treatment

Variables	Treatments				SEM	p-value
	Control	FS	NBG	HBG		
pH	6.19[b]	6.44[a]	6.13[b]	6.41[a]	0.04	<0.05
Shear force (kgf)	2.29[a]	1.19[b]	1.92[a]	1.25[b]	0.07	<0.05
WHC (%)	69.65	68.22	70.84	69.19	0.11	0.15
Cooking loss (%)	38.15	38.09	37.91	38.45	0.34	1.12

[a,b] Mean values within the same rows with the different superscripts are significantly different among treatments ($p<0.05$).
Control, conventional Samgyetang made without addition of black garlic extract and receiving no hydrolyzation; FS, Samgyetang that was hydrolyzed with flavorzyme before retorting; NBG, Samgyetang made with the addition of black garlic extract without hydrolyzation; HBG, enzymatically hydrolyzed black garlic Samgyetang; WHC, water holding capacity.

0.5 kgf is sufficient to be detectable by the consumer. In accordance, Huffman et al. (1996) also mentioned that a variation of 1 kgf is sufficient for consumers to notice the distinct differences among meat textures. This implied the efficacy of enzymolysis pre-treatment in improving meat texture profiles. Concerning the WHC, the percentage was in the previous reported range by Jeong et al. (2020), with minor variations ($p>0.05$). Together with cooking loss, WHC is essential economic traits in poultry processing industries due to its high correlation with the yield loss after cooking (Kristensen and Purslow, 2001). In this study, both hydrolyzation and BG extract addition were shown to not detrimentally affect the WHC percentage of the samgyetang samples.

Antioxidant activity

The antioxidant activity of the cooked breast samples was measured using the TBARS assay and DPPH radical scavenging activity. With respect to the lipid oxidation rate, as seen in Fig. 1, both the NBG and HBG groups shared a similar suppression effect on MDA, compared to the control and FS ($p<0.05$). The lipid oxidation product formation was lowest in the HBG group (0.27 mg MDA/kg), intermediate in the NBG treatment (0.33 mg MDA/kg), and the highest in the FS (0.39 mg MDA/kg) and control group (0.42 mg MDA/kg). As expected, the addition of BG extract to Samgyetang resulted in a remarkable improvement in the antioxidative status of chicken meat, as also observed in our previous report (Barido et al., 2021). This was also in agreement with a previous report by Lee et al. (2018) on pork patties, which implied that BG extracts possessed potent antioxidant compounds. Further consolidation was obtained from the DPPH radical scavenging activity (Fig. 2), which was found to be the highest in the HBG group (50.22%), followed by that in the NBG treatment (46.57%), FS (34.17%) and control (31.20%).

Based on the DPPH assay obtained by this study, interestingly, the hydrolyzed group tended to exhibit stronger scavenging activity toward free radical when compared to the BG Samgyetang receiving no enzymatic hydrolyzation. However, both hydrolyzed group and BG treatment shared a significantly higher scavenging percentage than that of the control. The proper

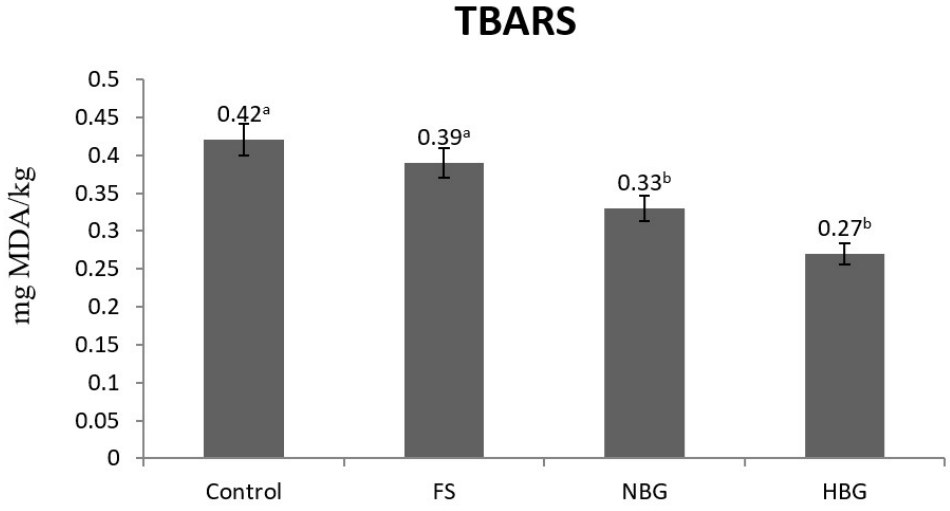

Fig. 1. Lipid oxidation rate measured of Samgyetang breast influenced by enzymatic hydrolysis pre-treatment. [a,b] Mean values with the different superscripts are significantly different among treatments ($p<0.05$). TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; Control, conventional Samgyetang made without addition of black garlic extract and receiving no hydrolyzation; FS, Samgyetang that was hydrolyzed with flavorzyme before retorting; NBG, Samgyetang made with the addition of black garlic extract without hydrolyzation; HBG, enzymatically hydrolyzed black garlic Samgyetang.

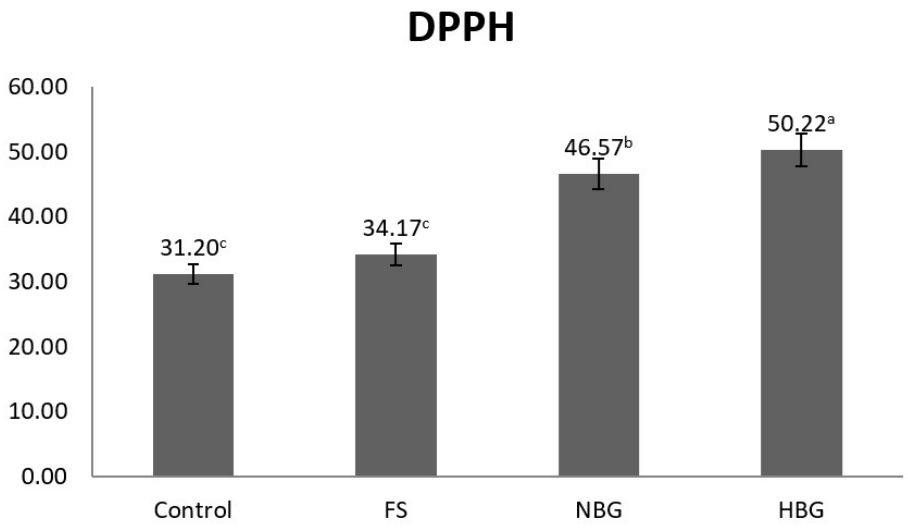

Fig. 2. DPPH radical scavenging activity of Samgyetang breast influenced by enzymatic hydrolysis pre-treatment. [a–c] Mean values with the different superscripts are significantly different among treatments ($p<0.05$). DPPH, 2,2-diphenyl-1-picrylhydrazyl; Control, conventional Samgyetang made without addition of black garlic extract and receiving no hydrolyzation; FS, Samgyetang that was hydrolyzed with flavorzyme before retorting; NBG, Samgyetang made with the addition of black garlic extract without hydrolyzation; HBG, enzymatically hydrolyzed black garlic Samgyetang.

method used for hydrolyzation is critical for generating end products with improved functionalities. Using Flavourzyme as a the hydrolyzing agent, can allow a broad range of substrate specificities due ability to modulate both endo- and exopeptidase activities, resulting in the enhancement of amino acids with spacious properties (Giménez et al., 2009). The negatively charged amino acids are strong antioxidants that serve to donors the exaggerated electrons thereby scavenging the free radicals (Onuh et al., 2014).

Taste-related nucleotides

Taste-related nucleotides, like 5′-IMP, 5′-GMP, 5′-AMP, inosine, and hypoxanthine, were analyzed by HPLC, and the results are presented in Table 4. Among the nucleotides recorded in the cooked breast samples, inosine and 5′-IMP were predominantly found across all the treatments. The strong umami nucleotides, 5′-IMP, 5′-GMP were dramatically upregulated following hydrolyzation of BG Samgyetang, with the 5′-IMP concentration being 146.68 μg/mg, 130.72 μg/mg, 123.09 μg/mg, and 127.36 μg/mg for HBG, FS, NBG and control group, respectively. Meanwhile, the concentration observed for 5′-GMP were 15.13 μg/mg, 12.03 μg/mg, 10.62 μg/mg, and 5.78 μg/mg, for HBG, FS, BG, and control group respectively. The results of this study implied the potential flavouring properties from the addition of BG as well as confirmed the efficacy of hydrolysis in enhancing the umami taste properties of meat proteins (Kong et al., 2017).

Two main nucleotide compounds may impart the umami taste in meat: Purine ribonucleotides with a phosphate ester on the 5′-carbon of the ribose section and a hydroxyl group on the 6′-carbon of the purine ring. However, the strength of the umami properties of these nucleotide compounds is highly dependent on their structure. The 5′-GMP, 5′-IMP, and 5′-AMP are known to be strong umami compounds, while the purine nucleotide with 6′-carbon, such as inosine, may also give the umami taste, but is weaker than the ribonucleotide with phosphate ester at the C-5 of the ribose section (Smith and Hong-Sum, 2011). In addition, the purine ribonucleotides phosphorylated at C-2′ or C-3′ of the ribose section can be classified as tasteless or potential umami compounds upon reaction with certain amino acids (Dermiki et al., 2013).

Table 4. Taste-related nucleotides of Samgyetang breast influenced by enzymatic hydrolysis pre-treatment

Variables[1)]	Treatments				SEM	p-value
	Control	FS	NBG	HBG		
5′-GMP	5.78[d]	12.03[b]	10.62[c]	15.13[a]	0.81	<0.05
5′-IMP	127.36[c]	130.72[b]	123.09[c]	146.68[a]	3.70	<0.05
5′-AMP	ND	ND	ND	ND	ND	ND
Inosine	214.42[b]	177.39	226.18[a]	172.96[c]	6.42	<0.05
Hypoxanthine	ND	ND	ND	ND	ND	ND

[1)] 5′-GMP, guanosine monophosphate; 5′-IMP, inosine monophosphate; 5′-AMP, adenosine monophosphate.
[a–c] Mean values within the same rows with the different superscripts are significantly different among treatments ($p<0.05$).
Control, conventional Samgyetang made without addition of black garlic extract and receiving no hydrolyzation; FS, Samgyetang that was hydrolyzed with flavorzyme before retorting; NBG, Samgyetang made with the addition of black garlic extract without hydrolyzation; HBG, enzymatically hydrolyzed black garlic Samgyetang; ND, not detected.

Free amino acid (FAA) contents

A total of 17 FAAs were recorded in and compared among the groups, in this study. As seen in Table 5, the hydrolysis resulted in the highest total FAA content in HBG group compared with those of the FS, NBG and control groups ($p<0.05$). Furthermore, the umami-related FAA (Asp and Glu) were remarkably intensified, wherein the concentration of Asp in the HBG group (18.31 μg/mg) was the highest followed by FS (16.35 μg/mg), NBG (16.07 μg/mg) and control group (15.21 μg/mg; $p<0.05$). Similarly, the Glu concentration in the HBG group (31.08 μg/mg) was higher when compared to FS (26.02 μg/mg), NBG treatment (22.31 μg/mg), and the control group (20.89 μg/mg). Additionally, the HBG and NBG treatment groups shared similar contents of the sweet FAA, alanine (Ala), which was higher than that in the control group ($p<0.05$). However, the hydrolysis of BG Samgyetang with Flavourzyme may enhance the exposure of hydrophobic amino acids like methionine (Met), possibly imparting a bitter sensation. Apart from its bitterness, Met is a sulfur-containing FAA with antioxidant properties (Smith and Hong-Shum, 2011). The endo- and exopeptidase properties possessed by Flavourzyme may cleave protein molecules both at the amino- and carboxyl-terminal, as well as at the internal cleavage sites (Ang and Ismail-Fitry, 2019; Gao et al., 2021; Kong et al., 2017), resulting in more varied FAAs.

Conclusion

This study aimed to investigate the effects of enzymatic hydrolysispre-treatment on the meat quality and functional properties of BG supplemented Samgyetang. The intensification of two umami-related nucleotides (5′-GMP and 5′-IMP) and FAAs (Asp and Glu) were the results of enzymatic hydrolyzation on Samgyetang, irrespective of the BG addition. The HBG exhibited a stronger scavenging activity toward free radicals when compared to FS, NBG and control groups, indicating the antioxidant potential of the BG addition. The texture properties were dramatically improved upon hydrolysis, from 2.29 kgf in control and 1.19 and 1.25 kgf in the FS and HBG group. Moisture was highly retained, while redness increased, and lightness color decreased following hydrolysis of cooked breast meat. No notable adverse effects of the hydrolysis on the quality, taste-related compounds, and functional properties of BG Samgyetang were observed in this study. The results of this study can be a preliminary information of the effect of hydrolysis pre-treatment for retorted BG samgyetang. Further experiments are required to compare various enzymes along with its organoleptic acceptances.

Table 5. **Free amino acid contents of Samgyetang breast influenced by enzymatic hydrolysis pre-treatment**

Variables	Treatments				SEM	p-value
	Control	FS	NBG	HBG		
Aspartic acid (Asp)	15.21^{c}	16.35^{b}	16.07^{b}	18.31^{a}	0.27	<0.05
Threonine (Thr)	6.37	6.09	6.44	6.24	0.11	1.01
Serine (Ser)	4.87	4.93	5.02	5.14	0.05	0.10
Glutamic acid (Glu)	20.89^{c}	26.02^{b}	22.31^{c}	31.08^{a}	2.71	<0.05
Glycine (Gly)	3.04	3.06	3.11	3.01	0.04	0.11
Alanine (Ala)	15.17^{b}	16.18^{b}	19.22^{a}	20.08^{a}	1.13	<0.05
Cysteine (Cys)	ND	ND	ND	ND	ND	ND
Valine (Val)	3.09	3.14	3.11	3.07	0.04	0.41
Methionine (Met)	1.91^{b}	1.99^{b}	1.90^{b}	4.47^{a}	1.07	<0.05
Isoleucine (Ile)	2.31	2.30	2.28	2.35	0.09	0.68
Leucine (Leu)	5.12	5.03	5.09	4.99	0.23	0.17
Tyrosine (Tyr)	1.57	1.47	1.48	1.54	0.19	0.13
Phenylalanine (Phe)	2.15	2.31	2.22	2.18	0.14	0.35
Lysine (Lys)	10.22	10.58	11.21	11.72	0.03	0.22
Arginine (Arg)	8.13	8.00	7.98	8.05	0.55	1.11
Proline (Pro)	3.77	3.69	3.61	3.49	0.17	1.27
Total FAA	103.82^{c}	111.34^{b}	111.05^{b}	125.72^{a}	4.09	<0.05

$^{a–c}$ Mean values within the same rows with the different superscripts are significantly different among treatments ($p<0.05$).
Control, conventional Samgyetang made without addition of black garlic extract and receiving no hydrolyzation; FS, Samgyetang that was hydrolyzed with flavorzyme before retorting; NBG, Samgyetang made with the addition of black garlic extract without hydrolyzation; HBG, enzymatically hydrolyzed black garlic Samgyetang; ND, not detected; FAA, free amino acid.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Barido FH, Kim HJ, Kang SM, Jang A, Pak JI, Lee SK. Data curation: Barido FH. Formal analysis: Barido FH. Methodology: Barido FH, Kang SM. Software: Barido FH. Validation: Barido FH, Kim HJ, Kang SM, Jang A, Pak JI, Lee SK. Investigation: Barido FH. Writing - original draft: Barido FH. Writing - review & editing: Barido FH, Kim HJ, Kang SM, Jang A, Pak JI, Lee SK.

Ethics Approval

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

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