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ARTICLE

Effects of Traditional Sauce Type and Storage Time on Quality Characteristics, Shelf-life and Flavor Compounds of Marinated Pork Cooked by Sous Vide Method

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Abstract The present study aimed at evaluating effects of traditional sauce type and storage time on shelf-life and flavor compounds of marinated pork cooked by Sous-Vide method. Five different traditional sauces (Meju soy sauce, Brewed soy sauce, Fish-soy sauce, Ishiru fish sauce and Anchovy fish sauce) purchased from Asian countries were used. After marination with the sauces, polyvinylchloride film bags containing the marinated pork samples were cooked using Sous-Vide method for 55°C for 5 h and 60°C for 30 min, and were then stored for 8 wk at 10°C. Results showed that the pork samples marinated with the sauces retarded the growth of total plate counts (TPC) during storage. At 8^{th} wk storage, TPC counts were significantly lower in all samples marinated with the sauces compared to control ($p<0.05$). Lipid oxidation level was significantly lower in the T2 (Meju soy sauce) and T6 (Anchovy fish sauce) compared to those of T3 (Brewed soy sauce) and T5 (Ishiru fish sauce) or control after 8 wk storage ($p>0.05$). Forty volatile flavor compounds were detected from the control and marinated samples at 4^{th} wk of storage. The pork marinated with Anchovy fish sauce presented significantly higher amounts of importantly pleasant flavor compounds such as; pyrazines and sulfur-containing compounds than those marinated with other remaining sauces and control. It is concluded that the marination with Anchovy fish sauce partly improved the shelf-life and increased amounts of pleasant flavor compounds of Sous-Vide cooked pork products during storage in comparison to the other remaining sauces.

Keywords traditional sauces, marination, Sous Vide, storage, quality, shelf-life

Introduction

Meat is known as the richest protein source and is widely consumed by consumers around the world. The meats are usually consumed after cooking and depending on each cuisine cultures the cooking methods may be differed such as; grilling, boiling, steaming etc. Currently, one of the widely applied cooking methods that is Sous Vide

(Antonella and Adriana, 2018; Baldwin, 2012). Because of increasing demands for minimally processed food products with their characteristics closer to that of the fresh products, which has resulted in the widespread utilization of the Sous Vide technique for shelf life extension and quality guarantee of fresh foods (Antonella and Adriana, 2018; Baldwin, 2012; Schellekens, 1996). The nature of this technology is a vacuum cooking by placing raw materials such as meats into heat-stable, air-impermeable pouches, vacuum packaging to remove air inside the bags, sealing, cooking under controlled conditions of temperature (50℃–65℃) and time (4–6 h), cooling, and then storing under chilling temperature foods (Antonella and Adriana, 2018; Baldwin, 2012; Schellekens, 1996). Till now, the Sous Vide technology has been used to prepare many dishes of vegetables and animal meats (e.g., pork, beef and lamb) (Roldan et al., 2015; Iborra-Bernad et al., 2014; Sanchez del Pulgar et al., 2012). This cooking method has been found to exerts the greatest advantage such as conservation of aroma flavor components, retaining favorable color, reducing heat damage to proteins and lipids, the loss of liquids and essential nutrients such as vitamins and antioxidants (Baldwin, 2012; Ghazala et al., 1995; Sanchez del Pulgar et al., 2012). Regarding the food stability point of view, the Sous Vide cooking offers advantages by improving shelf life of the products (the shelf life of Sous Vide cooked products ranges within 6–42 d) by controlling the microorganisms' growth and risk of microbial contamination as compared with conventional cook-chill techniques (Armstrong, 2000; Church and Parson, 1993; Jeong et al., 2018). More to the point, in many countries the demand for the ready-to-cook marinated meat has considerably increased in recent times (Sloan, 2010). In the food cultures of Asian countries (e.g., Korea, Japan and Vietnam etc.), soy, fish, fish-soy, and fish-soy-meat sauces are among the integral seasonings which are widely used in meat-based dishes making (Nam et al., 2010). Depending on the cuisine cultures in each country for example; in Korea, all foods require soy sauce as a main seasoning while, the fish sauce is the most important seasoning in Southeast Asian countries (Chung et al., 2016). Though the traditional sauces are daily used in many cuisines, however, limited scientific information (e.g., quality characteristics, shelf-life and flavor compounds) regarding their utilizations in the meat products marination is available. With the best of our knowledge, only few studies have reported about the meat marination with soy sauce (Kim et al., 2013; Yang et al., 2018) and these authors have shown an increased collagen solubility and proteolysis as well as palatable taste of the marinated products.

While, the Sous Vide technique has currently been serving as a good alternative for cooking of many meat dishes such as pork, beef and sheep meat (Jeong et al., 2018; Roldan et al., 2013; Sanchez del Pulgar et al., 2012), and the traditional sauces are widely used in many cuisine cultures for meat dishes cooking. However, there has been no published research evaluating the effects of traditional sauces utilization as marinades in marinated meat products cooked by this Sous Vide method. Thus, the objective of the present study was to examine the effects of traditional sauce types on the quality characteristics, shelf-life and flavor compounds of marinated pork cooked with the Sous Vide method during storage.

Materials and Methods

Materials

Pork meat (*longissimus dori* muscle) from female pigs (at 180 d old and fed with a commercial feed type) collected at 24 h after slaughter in a slaughterhouse (Nonghyup Mokwon Village, Gimje, Korea) were used in the present investigation. Meju soy sauce (original salt level 18.83%, total nitrogen 1.06% and pH 4.95) and Brewed soy sauce (original salt level 5.85%, total nitrogen 1.12% and pH 4.65) were purchased from local food marts in Jeonju, Korea (both kinds of these soy sauces are made using fermented soybean as the main ingredient, however, they are different from manufacturing methods in which the

Brewed soy sauce is made using the naturally brewed soybean and wheat while, the Meju soy sauce is made using the chemically fermented soybean). Fish-soy sauce (original salt level 8.08%, total nitrogen 0.95% and pH 4.98, made with a mixture of 50% (w/w) fermented anchovies and 20% (w/w) naturally brewed soybean) was purchased from local food company in Pu Yuan, China. Anchovy fish sauce (original salt 12.50%, total nitrogen 2.92% and pH 5.02, made mainly from naturally fermented anchovies with sea salt) was purchased from local food company in Phu Quoc, Vietnam. Ishiru fish sauce (original salt 13.27%, total nitrogen 1.93% and pH 5.05, made from fermented Japanese squid) was purchased from local food company in Isikawa, Japan. Before using, all the sauces were pasteurized by heating at 121℃ for 30 min in order to inactive the endogenic enzymes.

Preparation of marinated pork

In order to examine the effect of traditional sauce types on the quality attributes of the product, no seasoning or additives were used, the unique components used were the pork loin and sauces. The pork loins were cut into steaks (3 cm in thickness) with average weight of 300±10 g. Six different marination treatments of the fresh pork with: 1 with brine solution for control (T1) and 5 with different sauces (T2–T6) and were prepared in the present investigation, all the sauces were equalized to a same salt concentration (10%) using sea salt or drinking water. For the equalization, it was simply made by either adding more salt (if the salt level in the original sauces is lower than 10%) or by diluting the original sauces with drinking water (if the salt level in the original sauces is higher than 10%). A brine solution at the same concentration (10%, w/v) were used as the control marinade. The marination formulation was as follows: raw pork (66.7%), sauce (13.3%) and drinking water (20%). The marination was done by placing the pork steak each, the sauce and water as mentioned above into a heat-stable and air-impermeable pouches.

Sous Vide cooking

Prior to the Sous Vide cooking, the polyvinylchloride film bags containing the marinated meat were 80% vacuum-packaged, sealed and the placed into a cooking vessel (Korea). The cooking temperature and time were set at 55℃ for 5 h and then increasing to 60℃ for 30 min. When the cooking was completed, the samples from each treatment were divided into different storage groups: 0 d (the cooking day), 1, 2, 4, 6, and 8 wk at 10℃. For each storage group, 10 marinated and cooked steaks (approximately 3 kg) per treatment were used.

Microbiological measurement and identification

Total plate counts (TPC) on the samples were determined during storage. Approximately 10 g of each sample was aseptically taken and placed in sterile stomacher bag containing 90 mL of peptone water. After being homogenized for 1 min, serial dilutions were made using the peptone water. About 1 mL of the diluted sample was plated on Aerobic Agar Plate. The plates were incubated at 37℃ for 48 h in an incubator. Each sample was done in duplicates and TPC was expressed as log number of colony forming unit per gram sample (Log CFU/g). The diluted samples were also cultured on tryptic soy agar (Becton-Dickison Com. MD, USA). After incubating at 37℃ for 48 h, the representative single colonies were picked up from the plate and used for identification (Ba et al., 2018). Briefly, the colonies on the agar media were taken and applied for the 16S rRNA sequence analysis. Each colony was suspended in 60 μL of TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), boiled at 99℃ for 10 min and DNA template was obtained following centrifugation at 8,200×g for 10 min. The universal primer: 27f (5′-AGT TTG ATC CTG GCT CAG-3′) and 1490r (5′-GTT ACC TTG TTA CGA CTT C-3′) was used to amplify the 16S rDNA fragment. Following the PCR amplification, the DNA was sequenced using ABI-Prism Big Dye Determinator Cycle Sequencing Ready Reaction kit and ABI-Prism 377 Sequencer (Applied Biosystems Inc). Homology searches of the 16S rRNA sequences were performed at DDBJ (http://www.ddbj.nig.ac.jp) with Blast analysis program.

Warner-Bratzler shear force (WBSF)

The Warner-Bratzler shear force (WBSF) was determined using the method of Hwang et al. (2004). In each storage period, 3 samples were randomly taken from each the treatment. The sample (each) was made into six strips that had an average diameter of 0.5 inch and fiber parallel to the longest dimension of at least 2 cm. WBSF values of these 6 strips of each sample were measured using an Instron Universal Testing Machine (Model 3342; Instron Corporation, Norwood, MA, USA) and the shear force values expressed were expressed as kilograms of force (kgf).

Soluble collagen content

The total collagen content in the samples was determined by using the colorimetric method of Kolar (1990) with suitable modification. Particularly, approximately 2 g of sample was hydrolyzed for 16 h with 7N H_2SO_4 at 105℃. The hydrolysate was made up to 500 mL with distilled water and filtered. About 2 mL of the filtrate was taken and added with chloramine T solution in a tube and left for 20 min at room temperature. Thereafter, the 4-dimethylamino benzaldehyde solution was also added, and the mixture was heated at 60℃ for 15 min. The absorbance of samples and hydroxyproline standard was read at 558 nm using a spectrophotometer. A standard calibration curve was plotted for 4-hydroxyproline and regression line drawn. Collagen content was expressed in mg per 100 g sample, after converted hydroxyproline into collagen with a conversion factor of 7.14.

For insoluble (or heat stable) collagen content, the homogenized samples in Ringer's solution (Hill, 1966) were heated at 77℃ for 70 min, followed by centrifugation, residual fractions were hydrolyzed in 7N H_2SO_4 for 16 h at 105℃. The hydroxyproline content of the hydrolysate was determined after neutralization, according to the procedure of Kolar (1990). The soluble collagen content was calculated by the differences between the total and insoluble collagen contents.

Lipid oxidation

The TBARS content was determined to evaluate the lipid oxidation level in marinated pork samples cooked by Sous Vide method during storage using the protocol as described in our previous work Ba et al. (2016). The TBARS values were expressed as mg malonaldehyde/kg (MAD/kg) of sample. Three repetitions were applied for each sample in each treatment.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

In order to determine how the sauces affecting the proteolysis in the marinated products under Sous Vide cooking method, the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the method of Laemmli (1970). The protein concentration in the sample was adjusted to 2 mg mL^{-1} with SDS-PAGE sample buffer (2× treatment buffer, 2-mercaptoethanol, bromophenol blue; pH 6.8), and then heated to 95℃ for 5 min. The heated samples (15 μL each) were loaded into wells of 4% Stacking gel and a 12.5% Separating gel using a mini SDS PAGE electrophoresis system (Bio-Rad). A protein broad range marker (BIO-RAD) was also used.

Volatile flavor compounds analysis

Because of time and labor consuming, the volatile flavor compounds therefore were only assessed on the samples stored at 4th wk, not all the storage periods. All the procedures used for the analysis of the flavor compounds were followed our previously developed method (Ba et al., 2010) with minor modifications. Briefly, about 1 g of each sample was taken and placed into a 20-mL headspace vial and sealed with PTFE-faced silicone septum for extraction. The extraction of volatile flavor compounds was done using solid-phase micro-extraction (SPME). A SPME device containing carboxen–polydimethylsiloxane (75 μm) fibre (Supelco) was used and all the steps from extraction, absorption, desorption of the flavor compounds and fibre cleaning before/after each sample completion were done using a fully automated SPME sample preparation instrument (Model: AOC-5000 Plus) connected to Gas Chromatography (Model: 7890B GC, Agilent Technologies) with Mass Spectrophotometry (Model: 5977B MSD, Agilent Technologies). The SPME was carried out at 65℃ and agitated at 250 rpm for 60 min. The GC/MS conditions set were same as those mentioned in the above cited literature. The identifications of volatile compounds were performed by comparing their mass spectra with those already present in the Wiley registry of mass spectral data (Agilent Technologies) and/or by comparing their retention times with those of authentic external standards. Approximated quantities of the volatile compounds were quantified by comparison of their peak areas with that of the 2-methyl-3-heptanone internal standard obtained from the total ion chromatogram using a response factor of 1.

Statistical analysis

All the data were prepared on Excel and then analyzed using the Statistic Analysis System (SAS) package (SAS Institute, Cary, NC, USA, 2007). Means and standard errors were calculated for the variables. The data were analyzed by using the General Linear Model procedure considering sauce type and storage as the main effects. Means were compared using Duncan's Multiple Range Test. A three-factors model of Principle Component Analysis (PCA) was used to explore the correlations between the class of flavor compounds in the samples (variables) and marinating treatments with the five different sauces (observations).

Results and Discussion

Effect on total plate counts and bacterial species

The TPC is among the important microbial index reflecting the microbial quality of food products during storage as well as the practical handling in the production process. The TPC in the pork marinated with different sauces cooked with Sous Vide methods during storage are presented in Table 1. At 0 d, no statistical differences in the TPC occurred among the treatments, and the counts ranged from 1.84–2.27 Log CFU/g, however, the differences ($p<0.05$) were observed after 2, 4, 6, and 8 wk storage. For instance, at the 8th wk, the number of TPC was in the following order: T1 (control)>T3 (Brewed soy sauce)>T6 (Anchovy Fish sauce)>T2 (Meju soy sauce)>T5 (Ishiru fish sauce)>T4 (Fish Soy sauce), being the mean values of 5.15, 3.38, 3.15, 2.92, 2.74, and 1.90 CFU/g, respectively. The storage time also showed a significant effect on the TPC. However, depending on each sauce type used, the increasing rate also differed in which the T1 (control) showed the highest rate (increased by 2.96 Log) after 8 wk storage whereas most of the treatments with the traditional sauces showed a very low increasing rate for instance; the T2 only increased by 0.91 Log CFU/g after 8 wk storage. Whereas, the TPC in the T4 (marinade with fish soy sauce) was unchanged throughout the storage. The results indicating the stability of microbial quality

Table 1. Effect of marination with traditional sauces on total plate count of the marinated pork under Sous Vide cooking during storage (0–8 wk)

Treatment	Total plate count (Log CFU/g)					SEM
	0 d	2 wk	4 wk	6 wk	8 wk	
T1	2.19^{D}	2.12^{bD}	2.65^{bC}	3.68^{aB}	5.15^{aA}	0.23
T2	2.01^{B}	2.80^{aA}	2.68^{bA}	2.98^{bA}	2.92^{bA}	0.16
T3	2.27^{B}	3.19^{aB}	3.01^{aB}	3.42^{abA}	3.38^{bA}	0.51
T4	1.84	1.88^{c}	1.97^{c}	1.85^{c}	1.90^{b}	0.09
T5	1.89^{B}	1.95^{bcB}	2.51^{bcB}	3.23^{abA}	2.74^{bA}	0.21
T6	1.85^{B}	3.04^{aA}	3.25^{aA}	3.30^{abA}	3.15^{bA}	0.15

$^{a–c}$ Means within a column with different letters differ significantly p<0.05.
$^{A–C}$ Means within a row with different letters differ significantly p<0.05.
T1, marinated with brine water (control); T2, marinated with Meju soy sauce; T3, marinated with Brewed soy sauce; T4, marinated with Fish soy sauce; T5, marinated with Ishiru fish sauce; T6, marinated with Anchovy fish sauce.

of the treated samples in comparison to the control suggest that the sauces could inhibit the growth of TPC in the marinated pork product during storage. Regarding this, previous study has reported that fermented sauce is a potential source of antimicrobial substances (Kleekayai et al., 2015). In other meat types, Naveena et al. (2017) reported TPC of around 4 Log CFU/g breast chicken cooked with Sous Vide method after 60 d of storage. Hong et al. (2015) reported the TPC of 3.01 Log CFU/g for 14 d-stored chicken breasts cooked with Sous Vide method. Thus, when compared to the bacterial counts reported by these authors for the chicken breast samples cooked by Sous Vide, our results showed the lower counts in the pork samples marinated with the Ishiru fish sauce or Anchovy fish sauces.

The representative colonies appeared on the agar plates were taken and identified by the 16S rDNA sequencing. The results (Table 2) showed that some species/genus such as; *Enterococcus faecalis* was detected in the T1 (control) at 3.12 Log CFU/g, *Rhodococcus jialingiae* and *Nocardia coeliaca* were detected in the T2 at 2.16 and 2.42 Log CFU/g respectively, *Bacillus licheniformis* and *Bacillus paralicheniformis* were detected in the T3 at 3.15 and 3.22 Log CFU/g, respectively, *Kocuria marina* was detected in the T4 at 1.18 Log CFU/g, *Bacillus licheniformis* and *Bacillus paralicheniformis* both were detected in the T5 and T6. In general, these bacterial species are known as the non-pathogenic bacteria and most of them have also found in the meats cooked under the Sous Vide method in literature (Diaz et al., 2008).

Table 2. Bacterial species detected and identified from the marinated pork samples under Sous Vide cooking at 4 wk of storage

Treatment	Bacterial species
T1	*Enterococcus faecalis* (3.12 Log CFU/g)
T2	*Rhodococcus jialingiae* (2.16 Log CFU/g) and *Nocardia coeliaca* (2.42 Log CFU/g)
T3	*Bacillus licheniformis* (3.15 Log CFU g^{-1}) and *Bacillus paralicheniformis* (3.22 Log CFU/g)
T4	*Kocuria marina* (1.18 Log CFU/g)
T5	*Bacillus licheniformis* (3.31 Log CFU/g) and *Bacillus paralicheniformis* (2.62 Log CFU/g)
T6	*Bacillus licheniformis* (2.66 Log CFU/g) and *Bacillus paralicheniformis* (3.11 Log CFU/g)

T1, marinated with brine water (control); T2, marinated with Meju soy sauce; T3, marinated with Brewed soy sauce; T4, marinated with Fish soy sauce; T5, marinated with Ishiru fish sauce; T6, marinated with Anchovy fish sauce.

Effect on WBSF

WBSF is the most commonly used measurement to determine whether a meat that is tender or tough. The results of WBSF measurement on the samples marinated with different sauces cooked with Sous Vide during storage are presented in Table 3. Our results showed that the type of sauces affected the WBFS values of the samples. At the initial measurement (d 0), the T1, T2, and T3 showed higher WBSF values (2.21, 2.23, and 2.26 kgf, respectively) than those of the T4 (1.52 kgf), T5 (1.95 kgf) ($p<0.05$). However, after 8 wk of storage, the highest value (4.07 kgf) was found in the T3 (marinated with Brewed soy sauce), followed by T6 (Anchovy fish sauce), T4 (Fish soy sauce) and T1 (control) ($p<0.05$). The storage time seemed to have a minor effect on the WBSF, for instance the T3 and T6 increased in shear force values (by 1.81 and 114 kgf, respectively), while the T1 (control), T2, T4, and T5 maintained their WBSF values unchanged after 8 wk of storage ($p>0.05$). These results may suggest that the sauce types, particularly the ingredients and/or methods used for manufacturing the sauces showed a greater effect on the WBSF values of the products rather than the storage time when the Sous Vide cooking method was applied. Regarding this, previous studies demonstrated that the Sous Vide cooking method could retain the consistent textural characteristics of meat products during storage (Keller et al., 2008; Schafheitle, 1990).

Effect on lipid oxidation

The TBARS content has been measured and used to reflect the lipid oxidation level in meats and meat products (Ba et al., 2016; Grun et al., 2006). Effect of marination with sauce types on the concentrations of TBARS of the Sous Vide-cooked samples during storage are presented in Table 4. At 0 d of storage, the T2 and T4 presented the significantly ($p<0.05$) higher TBARS values (0.80 and 0.96 mg MDA/kg, respectively) than the other remaining treatments, while the values in the T3, T5, and T6 were similar to that of the control ($p>0.05$). After 8 wk of storage, the TBARS values were significantly higher in the T1 (control, 1.11 MDA/kg), T3 (Brewed soy sauce, 1.33 MDA/kg) and T5 (Ishiru fish sauce, 1.15 MDA/kg) as compared with those of the T2 (Meju soy sauce, 0.82 MDA/kg) and T6 (Anchovy fish sauce, 0.87 MDA/kg) ($p>0.05$). These results indicate that the marination with Anchovy fish sauce and Meju soy sauce produced the lowest TBARS values in the products as compared to the ones marinated with Brewed soy sauce and Ishiru fish sauce or brine solution (control). According the storage effect, significant ($p<0.05$) increases in TBARS only were observed in the T1 (control), T3 (Brewed soy sauce), and T5 (Ishiru fish sauce), with the increasing rate by 0.46, 0.71, and 0.47 mg MDA/kg, respectively after 8 wk of storage.

Table 3. Effect of marination with traditional sauces on WBSF (kgf) in the marinated pork under Sous Vide cooking during storage (0–8 wk)

Treatment	WBSF (kgf)					SEM
	0 d	2 wk	4 wk	6 wk	8 wk	
T1	2.21^{a}	2.31^{ab}	2.36^{ab}	2.04^{b}	2.32^{bc}	0.13
T2	2.23^{a}	1.54^{c}	2.65^{a}	2.47^{b}	1.97^{c}	0.44
T3	2.26^{aB}	3.06^{aA}	3.03^{aA}	4.08^{aA}	4.07^{aA}	0.78
T4	1.52^{b}	1.96^{ab}	2.33^{ab}	2.27^{b}	2.59^{b}	0.41
T5	1.95^{ab}	1.65^{c}	1.65^{b}	1.41^{c}	2.02^{bc}	0.25
T6	1.63^{bB}	1.88^{abcB}	1.77^{bB}	2.94^{bA}	2.77^{bA}	0.61

$^{a–c}$ Means within a column with different letters differ significantly $p<0.05$.
$^{A–C}$ Means within a row with different letters differ significantly $p<0.05$.
WBSF, Warner-Bratzler shear force; T1, marinated with brine water (control); T2, marinated with Meju soy sauce; T3, marinated with Brewed soy sauce; T4, marinated with Fish soy sauce; T5, marinated with Ishiru fish sauce; T6, marinated with Anchovy fish sauce.

Table 4. Effect of marination with traditional sauces on TBARS content of the marinated pork under Sous Vide cooking during storage (0–8 wk)

Treatment	TBARS (mg MDA/kg sample)					SEM
	0 d	2 wk	4 wk	6 wk	8 wk	
T1	0.65^{bB}	0.76^{bB}	0.76^{cB}	0.89^{cB}	1.11^{aA}	0.17
T2	0.80^{a}	0.71^{b}	1.32^{ab}	1.03^{ab}	0.82^{b}	0.25
T3	0.62^{bB}	1.33^{aA}	1.56^{aA}	1.34^{aA}	1.33^{aA}	0.36
T4	0.96^{a}	1.19^{a}	1.09^{b}	1.18^{b}	1.06^{ab}	0.10
T5	0.68^{bB}	0.90^{abB}	1.39^{abA}	1.07^{cA}	1.15^{aA}	0.28
T6	0.63^{b}	0.68^{b}	0.84^{bc}	1.26^{a}	0.87^{b}	0.25

[a–c] Means within a column with different letters differ significantly p<0.05.
[A–C] Means within a row with different letters differ significantly p<0.05.
T1, marinated with brine water (control); T2, marinated with Meju soy sauce; T3, marinated with Brewed soy sauce; T4, marinated with Fish soy sauce; T5, marinated with Ishiru fish sauce; T6, marinated with Anchovy fish sauce.

Whereas, no changes were found for the other remaining treatments after 8 wk of storage (p>0.05). In comparison with our results, previous studies such as Naveena et al. (2017) have showed a lower TBARS (0.2–0.3 mg MDA/kg) for sausages cooked by Sous Vide method at 0-d storage. While, Sun et al. (2017) reported much higher values (2–3 mg MDA/kg) for the beef steaks cooked by Sous Vide method.

Effect on soluble collagen content

In order to determine whether the marination with different sauces affects the collagen degradation during the storage, the soluble collagen content was measured. The soluble collagen content in the marinated pork samples during storage is present in Table 5. At 0 d, the soluble collagen content among the treatments ranged from 0.04–0.08 g/100 g, in which the T4 showed the significantly (p<0.05) higher level (0.08 g/100 g). After 8 wk of storage, all the samples marinated with the sauces (T2–T6) had significantly (p<0.05) higher soluble collagen contents than the control (T1). Noticeably, all the samples marinated with the traditional sauces showed significantly (p<0.05) higher the soluble collagen content than the control samples on all

Table 5. Effect of marination with traditional sauces on soluble collagen content of the marinated pork under Sous Vide cooking during storage (0–8 wk)

Treatment	Soluble collagen content (g/100 g)					SEM
	0 d	2 wk	4 wk	6 wk	8 wk	
T1	0.04^{b}	0.06^{b}	0.05^{b}	0.06^{b}	0.05^{b}	0.01
T2	0.05^{bB}	0.06^{bB}	0.10^{abA}	0.10^{abA}	0.09^{aA}	0.03
T3	0.04^{bB}	0.05^{bB}	0.11^{aA}	0.11^{aA}	0.09^{aA}	0.04
T4	0.08^{aB}	0.08^{abB}	0.12^{aA}	0.12^{aA}	0.09^{aAB}	0.02
T5	0.05^{bB}	0.12^{aA}	0.10^{abA}	0.09^{abA}	0.09^{aA}	0.03
T6	0.07^{bB}	0.10^{aA}	0.11^{bA}	0.11^{bA}	0.10^{aA}	0.02

[a,b] Means within a column with different letters differ significantly p<0.05.
[A,B] Means within a row with different letters differ significantly p<0.05.
SEM, standard error of the means; T1, marinated with brine water (control); T2, marinated with Meju soy sauce; T3, marinated with Brewed soy sauce; T4, marinated with Fish soy sauce; T5, marinated with Ishiru fish sauce; T6, marinated with Anchovy fish sauce.

the wk examined. Regarding the storage effect, we observed that increasing storage time significantly ($p<0.05$) increased the soluble collagen contents in all the treated samples, excepts the control samples. Currently, we cannot offer an exact explanation why soluble collagen contents in the sauces-marinated samples increased with the storage time, however, this could be related to the increased collagen degradation levels caused by the added sauces or these sauces favored the degradation process in the samples during storage. These obtained results are in agreement with those reported for beef by Kim et al. (2018).

Effect on protein degradation

In order to elucidate how the proteolytic activity differed among the treatments marinated with the traditional sauces under the Sous Vide cooking treatment, the SDS-PAGE analysis was done and the results are shown in Fig. 1. The SDS-PAGE of the proteins extracted from the treatments at the storage of 0 d and 2 wk were compared with each other. Results show that the bands with larger molecular weights (25 and 40 kDa) were denser in all the samples at 0 d of storage. After 2 wk, we observed that the disappearance of protein bands with molecular weight of 20 kDa in the T3 to T6 whereas, it appeared at light density in the T1 (control) and T2, indicating the significant differences in breaking down of myofibrillar proteins among the treatments. While, bands with molecular weight of about 23 kDa showed lesser density in the samples marinated with Brewed soy sauce (T3) and Fish soy sauce (T4) as compared with other treatments. Our obtained results are in agreement with those reported for chicken sausages cooked with Sous Vide in literature (Naveena et al., 2017). The variations in the densities of the particular protein bands among the treatments could be related to the effects of type of sauces which caused different proteins degradation patterns, and these proteins degradation are expected to improve the taste characteristics of the Sous Vide cooked marinated pork.

Volatile flavor compounds

A total of forty volatile flavor compounds were detected in the pork samples marinated with different sauces at 4th wk of

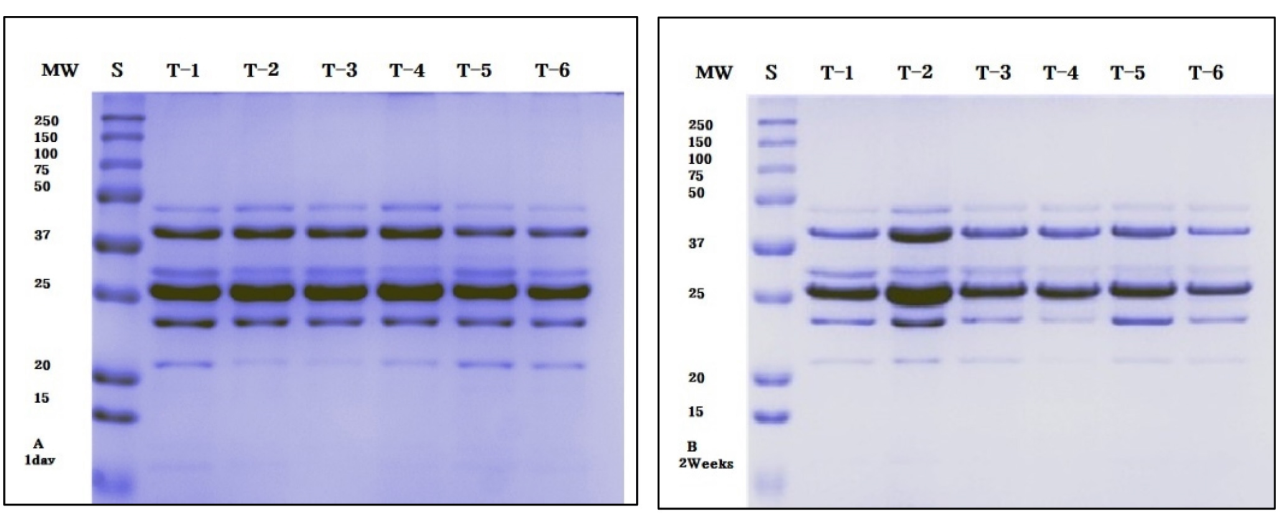

Fig. 1. The SDS-PAGE results of marinated pork cooked with Sous Vide method at 0 d and 2 wk storage. MW, standard markers molecular weight; T-1, marinated with salt water sauce; T-2, marinated with Meju soy sauce; T-3, marinated with Brewed soy sauce; T-4, marinated with Fish soy sauce; T-5, marinated with Ishiru fish sauce; T-6, marinated with Anchovy fish sauce; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

storage (Table 6). Based on the chemical groups, these are classified into: aldehydes (18), furans (3), pyrazines (8), sulfur containing compounds (4), alcohols (4) and ketones (3). It is well known that the volatile compounds formed during cooking are mainly responsible for the development of flavor characteristics of cooked meat (Ba et al., 2012; Ba et al., 2013; Mottram, 1994). The statistical analysis results showed that the type of sauces used significantly affected the concentrations of most of the detected compounds. Amongst, aldehydes are among the most predominant class in term of both quality and quantity. It is well known that most aldehydes are formed by the oxidation/degradation of unsaturated fatty acids (e.g., mono and polyunsaturated fatty acids) in meat during cooking/heating (Ba et al., 2012; Mottram, 1995). In the present study, the control samples presented higher amounts for some of these aldehydes (e.g., hexanal, heptanal, nonanal and E-2-decenal) as compared with the treated samples (T2–T6) ($p<0.05$). Among the aldehydes, octanal with its pleasant fatty-oily flavors, is formed from the oxidation of oleic acid (Bading, 1970), the level of this compound was significantly ($p<0.05$) higher in the treated samples (T2–T6) than the control (T1). Similar to our finding, most of the aldehydes detected in the samples in the present study have also been detected in lamb meat cooked with Sous Vide (Rhodehamel, 1992).

Table 6. Effect of marination with traditional sauce types on the concentration (μg/g) of volatile flavor compounds of the marinated pork under Sous Vide cooking at 4th storage wk

	RT (min)	T1	T2	T3	T4	T5	T6	SEM	IM[1)]
Aldehydes									
Propanal	1.69	0.01	0.03	0.03	0.04	0.03	0.02	0.004	MS+STD
3-Methyl butanal	2.69	0.02^{d}	0.21^{c}	0.34^{a}	0.25^{b}	0.27^{ab}	0.06	0.006	MS+STD
2-Methyl butanal	2.80	0.03^{d}	0.24^{c}	0.45^{a}	0.32^{b}	0.37^{b}	0.11	0.007	MS+STD
Hexanal	6.10	0.76^{a}	0.27^{c}	0.15^{d}	0.35^{bc}	0.25^{cd}	0.37^{b}	0.186	MS+STD
Furfural	7.07	0.07^{b}	0.20^{a}	0.11^{b}	0.15^{ab}	0.11^{b}	0.19^{a}	0.016	MS+STD
Heptanal	9.26	0.40^{a}	0.13^{b}	0.10^{b}	0.18^{b}	0.13^{b}	0.08^{b}	0.083	MS+STD
Benzaldehyde	10.87	0.81^{c}	0.86^{bc}	1.47^{ab}	1.66^{a}	1.07^{b}	1.85^{a}	0.160	MS+STD
Octanal	11.91	0.10^{d}	0.26^{cb}	0.20^{c}	0.41^{a}	0.31^{ab}	0.30^{b}	0.000	MS+STD
E-2-Octenal	13.18	0.03^{b}	0.02^{b}	0.001^{b}	0.001^{b}	0.03^{b}	0.20^{a}	0.011	MS+STD
2-Nonenal	13.36	0.02	0.02	0.03	0.03	0.02	0.06	0.003	MS+STD
Nonanal	14.20	1.36^{a}	0.91^{b}	0.86^{b}	0.89^{b}	0.90^{b}	0.84^{b}	0.281	MS+STD
E-2-Nonenal	15.31	0.04	0.02	0.02	0.02	0.02	0.03	0.007	MS+STD
Decanal	16.21	0.04^{b}	0.02^{b}	0.02^{b}	0.04^{b}	0.05^{ab}	0.06^{a}	0.010	MS+STD
E-2-Decenal	17.26	0.11^{a}	0.04^{b}	0.04^{b}	0.05^{b}	0.05^{b}	0.07^{b}	0.025	MS+STD
Benzenacetaldehyde	17.40	0.01^{b}	0.01^{b}	0.06^{ab}	0.04^{b}	0.04^{b}	0.08^{a}	0.003	MS+STD
EE-2,4-Decadienal	18.28	0.02^{b}	0.01^{b}	0.01^{b}	0.01^{b}	0.01^{b}	0.05^{a}	0.004	MS+STD
E-2-Dodecenal	19.06	0.07	0.02	0.02	0.05	0.04	0.05	0.019	MS+STD
Tetradecanal	22.97	0.01	0.01	0.001	0.01	0.01	0.02	0.002	MS+STD
∑ *Aldehydes*		3.80^{b}	3.28^{c}	3.91^{ab}	4.48^{a}	3.71^{bc}	4.43^{a}	0.69	
Furan									
3-Methyl furan	2.25	0.01^{b}	0.04^{b}	0.02^{b}	0.01^{b}	0.01^{b}	0.11^{a}	0.02	MS+STD
2-Pentyl furan	11.58	1.12^{a}	0.42^{c}	0.31^{d}	0.83^{b}	0.63^{bc}	0.38^{cd}	0.06	MS+STD

Table 6. **Effect of marination with traditional sauce types on the concentration (μg/g) of volatile flavor compounds of the marinated pork under Sous Vide cooking at 4th storage wk (continued)**

	RT (min)	T1	T2	T3	T4	T5	T6	SEM	IM[1)]
2-Heptylfuran	15.93	0.05	0.04	0.02	0.04	0.04	0.06	0.01	MS+STD
∑ *Furans*		1.26^{a}	0.52^{cb}	0.36^{c}	0.95^{ab}	0.76^{b}	0.56^{bc}	0.33	
Pyrazines									
Pyrazine	4.06	0.02^{b}	0.03^{b}	0.05^{b}	0.04^{b}	0.05^{b}	0.15^{a}	0.05	MS+STD
Methylpyrazine	6.82	0.09^{c}	0.55^{b}	0.30^{b}	0.23^{b}	0.26^{b}	1.20^{a}	0.38	MS+STD
2,5-Dimethyl pyrazine	9.50	0.05^{c}	0.50^{b}	0.27^{b}	0.22^{b}	0.21^{b}	1.48^{a}	0.50	MS+STD
2-Ethyl-6-methylpyrazine	11.74	ND	0.07^{b}	0.08^{b}	0.10^{b}	0.08^{b}	0.27^{a}	0.09	MS
Trimethyl pyrazine	11.83	ND	0.06^{b}	0.09^{b}	0.12^{b}	0.10^{b}	0.26^{a}	0.08	MS
3-Ethyl-6-methylpyrazine	12.20	ND	ND	0.09^{b}	0.06^{b}	0.08^{b}	0.27^{a}	0.09	MS
Acetyl pyrazine	12.33	ND	0.05^{b}	0.05^{b}	0.05^{b}	0.05^{b}	0.18^{a}	0.06	MS
2-Ethyl-3,5-dimethylpyrazine	13.55	0.03^{b}	0.04^{b}	0.08^{b}	0.06^{b}	0.06^{b}	0.24^{a}	0.07	MS
∑ *Pyrazines*		0.19^{c}	1.31^{b}	1.01^{b}	0.87^{b}	0.88^{b}	4.05^{a}	1.29	
Sulfure-containing compounds									
Carbon disulfide	1.86	0.01	0.01	ND	ND	ND	ND	ND	MS+STD
Dimethyl disulfide	4.26	0.02^{b}	0.04^{b}	0.05^{b}	0.07^{b}	0.11^{b}	2.10^{a}	0.58	MS+STD
2-Methylpyriridine	6.66	ND	ND	0.01	0.01	0.01	0.01	0.01	MS+STD
4-Pyridinol	13.68	0.02	ND	ND	0.02	0.02	0.02	0.01	MS
∑ *Sulfur-compounds*		0.05^{c}	0.05^{c}	0.06^{c}	0.10^{b}	0.14^{b}	2.13^{a}	0.16	
Alcohols									
1-Pentanol	4.99	0.05^{b}	0.03^{b}	ND	0.11^{a}	0.03^{b}	0.03^{b}	0.04	MS+STD
2-Furanmethanol	7.78	0.07d	0.42^{b}	0.31^{c}	0.26^{c}	0.25^{c}	0.75^{a}	0.22	MS+STD
Hexanol	8.29	0.04^{c}	0.15^{a}	0.01^{c}	0.01^{c}	0.01^{c}	0.12^{ab}	0.06	MS+STD
1-Octanol	13.44	0.12^{a}	0.04^{b}	0.05^{b}	0.08^{b}	0.07^{b}	0.11^{a}	0.03	MS+STD
∑ *Alcohol*		0.29^{b}	0.64^{b}	0.38^{b}	0.46^{b}	0.36^{b}	1.01^{a}	0.26	
Ketones									
2-Heptanone	8.88	0.08^{a}	0.02^{b}	0.03^{b}	0.05^{b}	0.04^{b}	0.02^{b}	0.02	MS+STD
3,5-Heptadien-2-one	15.75	0.02^{b}	0.05^{b}	0.04^{b}	0.05^{b}	0.04^{b}	0.10^{a}	0.03	MS+STD
2,3-Dihydro-3,5-dihydroxyl-6-methyl-4H-pyran-4-one	14.94	0.01^{b}	0.06^{b}	0.13^{a}	0.08^{b}	0.08^{b}	0.11^{a}	0.04	MS
∑ *Ketones*		0.11^{b}	0.14^{b}	0.20^{ab}	0.18^{b}	0.15^{b}	0.23^{a}	0.05	

[a–d] Means within a row with different letters differ significantly $p<0.05$.
[1)] IM, identification method; The compounds were identified by either mass spectra (MS) from library or authentic standards (STD).
RT, retention time; ND, not detected; T1, marinated with brine water (control); T2, marinated with Meju soy sauce; T3, marinated with Brewed soy sauce; T4, marinated with Fish soy sauce; T5, marinated with Ishiru fish sauce; T6, marinated with Anchovy fish sauce.

Furans have generally high odor-detection thresholds thus they seem to have limit contribution to the development of flavor characteristics of cooked meat, and they are known to formed from the oxidation of fatty acids (Ba et al., 2013). All of these furans have also been found in cooked meat and meat cooked by Sous Vide method (Ba et al., 2010; Rhodehamel, 1992). The 2-pentyl furan is formed from the oxidation of linoleic acid (Mottram, 1994), its levels were higher in the control

(T1) than the treated samples (T2–T6) ($p<0.05$).

Pyrazines are known to have low odor-detection thresholds, are among the most important compounds contributing to the roasty flavor of cooked meat products (Mottram, 1994). In the present study, 8 pyrazines were detected on the samples. Our results showed that the type of sauces used for marination significantly affected the amounts of all the pyrazines. Noticeably, the four compounds such as 2-ethyl-6-methylpyrazine, trimethyl pyrazine, 3-ethyl-6-methylpyrazine and acetyl pyrazine were not found on the control samples. While, the compound named as 3-ethyl-6-methylpyrazine was not found in the samples marinated with Meju soy sauce. All the samples marinated with the sauces such as Meju soy sauce, Brewed soy sauce, Fish soy sauce or Anchovy fish sauce had the significantly higher amounts of all the pyrazines as compared to the control ($p<0.05$). Interestingly, the samples marinated with Anchovy fish sauce exhibited significantly higher the total pyrazines content compared to those marinated with the other remaining sauces ($p<0.05$). Pyrazines are usually produced from the Mallard reaction between nitrogen source (e.g., amino acids) and ribose during cooking (Ba et al., 2013; Mottram, 1994). Thus, the higher amount of total pyrazines in the treatment with Anchovy fish sauce (T6) could be attributed to the higher total nitrogen content (2.92%) in this sauce type, which provided more nitrogen sources (e.g., free amino acids) for the Mallard reaction during cooking (Mottram, 1994).

The sulfur-containing flavor compounds are important for the deployment of cooked meat flavors (Ba et al., 2012; Mottram, 1994), and they are generally formed from the Mallard reaction between the sulfur-containing amino acids and a sugar (Ba et al., 2012). Among the 4 sulfur-containing compounds, dimethyl disulfide was detected on all the samples while, the carbon disulfide was only found in the control (T1) and those marinated with Meju soy sauce (T2) whereas, the 2-methylpyriridine was not found in these two treatments. In general, the samples marinated with Anchovy fish sauce (T6) presented the significantly ($p<0.05$) higher amount of the sulfur-containing compounds such as dimethyl disulfide. Similar to our results, Rhodehamel (1992) have also reported these compounds in Sous Vide cooked meat. The results indicating the variations in the sulfur-containing compounds could be related to the differences in the level of sulfur-containing amino acids among the treatments.

Four alcohols such as 1-pentanol, 2-furanmethanol, hexanol and 1-octanol were detected on all the samples excepts the 1-pentanol which was not found in the samples marinated with Brewed soy sauce (T3). The type of sauces significantly affected the concentrations of alcohols for instance; the 2-furanmethanol was higher in all the treated samples as compared to the control. In general, the alcohols with their low odor-detection thresholds, are important compounds contributing the flavor of cooked meats (Mottram, 1994).

Regarding the total concentrations of the flavor classes, it was observed that significant differences occurred among the treatments, except the ketone class. The total aldehydes were in the following order: T4>T6>T3>T1>T5>T2, being the mean values of 4.48, 4.43, 3.91, 3.80, 3.71, and 3.28 μg/g, respectively. Importantly, the total pyrazines with pleasant flavors were significantly higher in all the treated samples (T2–T6) as compared to the control (T1). Similarly, total alcohols and ketones also were significantly higher in all the treated samples (T2–T6) as compared to the control (T1). Overall, the pork marinated with the traditional sauces and cooked by Sous Vide method produced higher amounts of importantly pleasant flavor compounds than the control marinated the salt solution only.

Additionally, the total concentrations of flavor classes were also analyzed by principle components (PC) and the results of first three PC are plotted in Fig. 2. The results showed that about 58.02% and 21.07%, and 11.90% of the variability were explained by the first two PCs (PC1 and PC2) and third PC (PC3), respectively. The PC1 was positively related with furan, aldehyde, sulfur-containing compound and ketone classes whereas, it was inversely related to pyrazine and alcohol classes.

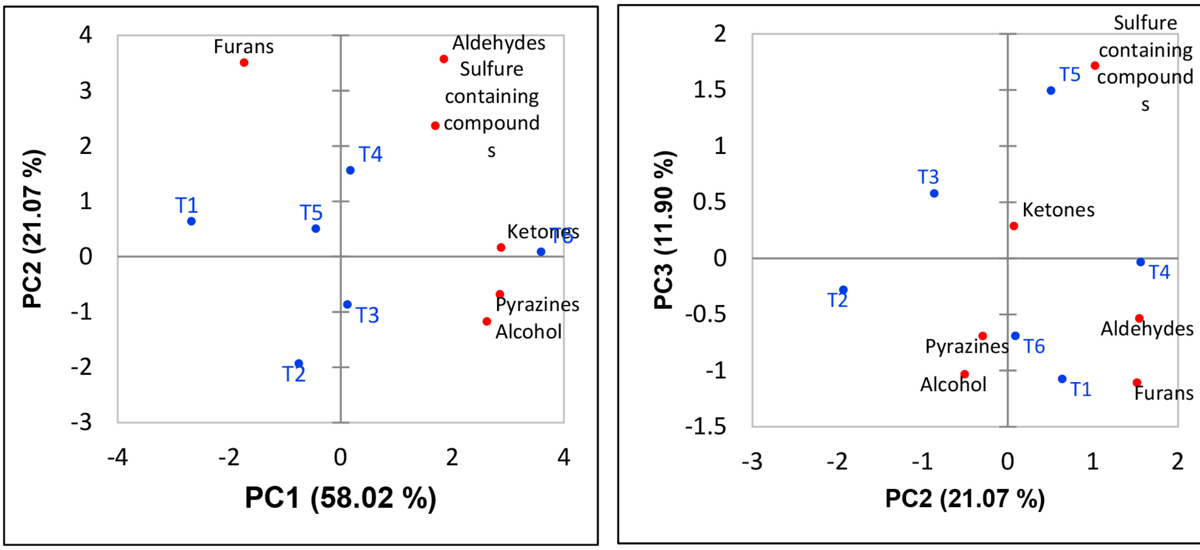

Fig. 2. Principle component analysis for the total amounts of flavor classes in different treatments cooked by Sous Vide method. T1, marinated with salt water sauce; T2, marinated with Meju soy sauce; T3, marinated with Brewed soy sauce; T4, marinated with Fish soy sauce; T5, marinated with Ishiru fish sauce; T6, marinated with Anchovy fish sauce.

The PC3 was positively related with sulfur-containing compound and ketone classes whereas, it was inversely related to aldehyde, alcohol, furan and pyrazine classes. The PC1 shows a clear difference between the T1 with the other treatments as it obtained higher score for the furan class, and it also indicates a clear difference between the T4 and T6 with the other treatments as it obtained higher scores for the aldehyde, pyrazine and sulfur-containing compound classes. The PC2 allows the separation of treatments according to the pyrazine and alcohol scores, discriminating the T6 from the other remaining treatments. While the PC3 also allows the separation of treatments according to the ketones score, discriminating the T3 and T6 from the other remaining treatments. These differences were also observed in the analytic results of volatile flavor compounds as presented in Table 6.

Conclusion

The present study for the first time, the pork loin was marinated with 5 different traditional sauces and were cooked by Sous Vide method. The pork samples marinated with Meju soy sauce or Anchovy fish sauce following by Sous Vide cooking generally improved the shelf-life stability as indicating by significant lower TPC and lipid oxidation level than those marinated with the other remaining sauces as well as control during storage. Six different bacterial species such as *Enterococcus faecalis*, *Rhodococcus jialingiae*, *Nocardia coeliaca, Bacillus licheniformis*, *Bacillus paralicheniformis*, *Kocuria marina,* were identified from the marinated samples using the 16S rDNA sequencing. Forty flavor compounds were detected from the marinated pork samples at 4th wk of storage. The pork samples marinated with Anchovy fish sauce exhibited significantly higher amounts of importantly pleasant flavor compounds such as; pyrazines and sulfur-containing compounds than those marinated with other remaining sauces and control. Thus, it may be concluded that the marination with Anchovy fish sauce produced more beneficial effects on the shelf-life stability and flavor characteristics improvement of the Sous Vide cooked pork products during storage in comparison with the other remaining sauces (e.g., Brewed soy sauce and Ishiru Fish sauce). Further study is needed to evaluate the effects of sauce type on the sensory quality as well as taste active compounds in the marinated products during storage.

Conflicts of Interest

The authors declare no potential conflict of interest.

Author Contributions

Conceptualization: Kim YA, Hwang IH. Data curation: Kim YA. Formal analysis: Kim YA. Methodology: Kim YA, Hoa VB. Software: Kim YA. Validation: Kim YA. Investigation: Kim YA. Writing - original draft: Kim YA. Writing - review & editing: Kim YA, Hoa VB, Hwang IH.

Ethics Approval

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

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