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ARTICLE

Effects of Different Marination Conditions on Quality, Microbiological Properties, and Sensory Characteristics of Pork Ham Cooked by the Sous-vide Method

Kiyoung Jeong, Hyeonbin O, So Yeon Shin, and Young-Soon Kim*

Department of Food and Nutrition, Korea University, Seoul 02841, Korea

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***Corresponding author** : Young-Soon Kim
Department of Food and Nutrition,
Korea University, Seoul 02841, Korea
Tel: +82-2-3290-5638
Fax: +82-2-921-7207
E-mail: kteresa@korea.ac.kr

Abstract The aim of this study was to evaluate the effects of marinade under different conditions (temperature and vacuum) on pork ham cooked by the sous-vide method (61°C and 98.81% vacuum for 45 min). Control group was non-marinade pork ham. The samples were marinated under 1 of 4 conditions: 4°C, 98.81% vacuum (treatment group T_1); 4°C, atmospheric pressure (T_2); 20°C, 98.81% vacuum (T_3); and 20°C, atmospheric pressure (T_4). The pH value was higher in the control (6.02) than in the treatment groups (4.30–4.42, $p<0.001$). Shear force was the lowest in the control: 18.14 N. Lightness and redness values were higher in the control ($p<0.001$). The chroma value significantly decreased from 12.74 to 7.55 with marinade ($p<0.001$). Total viable and coliform counts of raw meat were 84.6 and 3.67 Log CFU/g, respectively. After the marinade, the total viable count decreased to 3.00–14.67 Log CFU/g ($p<0.001$). Coliforms were not detected. After sous-vide cooking, no viable microorganisms were detected in any group. Treatment groups generally showed high scores on consumer preference. The marinade and sous-vide cooking had a positive effect on sensory characteristics. They provided safe conditions for sanitary evaluation. As a result, it appears that marinade at refrigeration temperature is better than that at room temperature.

Keywords pork, marinade, sous-vide, microbiological safety

Introduction

Sous-vide cooking method at low temperature has a positive effect on tenderness of meat by weakens protein–protein association and gelation and increases water-holding capacity (Tornberg, 2005). However, when sous-vide-cooked meat is exposed to ambient air, myoglobin, which is not denatured at low temperatures, turns into oxymyoglobin and changes its color to red (Harold, 2010). Consumers misunderstand that if the cooked pork is pink, the meat is less cooked. Also, the sous-vide method prevents elimination of volatile substances, thereby leaving the boar odor in the meat. The red color and boar-odor have adverse effects on consumer preferences. We designed the experiment to improve the quality and the preference with marinade. We chose pork ham, which has a hard texture that is less preferred (Tokifuji et al., 2013).

Marinade is a process of treating meat with vinegar, salt, herbs, and oil before cooking. It improves quality and sensory properties, such as cooking yield, tenderness, water-holding capacity, and flavor (He et al., 2015). Moreover, it increases shelf life by reducing bacterial growth because of low pH and the presence of salts and herbs (Żochowska-Kujawska et al., 2012). The objective of this study was to evaluate the effects of marinade on quality and microbiological and sensory properties of pork ham cooked by the sous-vide method.

Materials and Methods

Experimental design

Pork ham was purchased from a livestock vendor (Cheongdam Livestock Industry Co., Ltd, Korea). The meat was cut into 2-cm-thick slices in 100 g portions and vacuum-sealed using a chamber vacuum sealer (Chamber Vacuum Sealer System 300 series, Polyscience Innovative Culinary Technology, USA). Marinade condition was set according to Lytou et al. (2017). The samples were marinated under 1 of 4 conditions: 4℃, 98.81% vacuum (treatment group T_1); 4℃, atmospheric pressure (T_2); 20℃, 98.81% vacuum (T_3); and 20℃, atmospheric pressure (T_4). Marinade composition employed in the study was as follows; lemon juice 70 mL, olive oil 30 mL, dried thyme 0.1 g, and salt 2.0 g. In addition, non-marinade pork ham was set as a control group. The groups of meat samples were cooked in a circulating thermostatic water bath (Sous-vide cooker, Fusionchef by Julabo, Germany). As the core temperature of pork ham reached 61℃, as evaluated using a food thermometer (PDT300, Comark, UK), sous-vide cooking was maintained at 61℃ and 98.81% vacuum for 45 min. The cooked pork ham was immediately cooled at 4℃ for 1 h.

Color

The surface color value was measured using a colorimeter (CR-400, Konica Minolta, Japan). The values of lightness/darkness (CIE L*), redness/greenness (CIE a*), and yellowness/blueness (CIE b*) were obtained. The total color difference (*ΔE*) was calculated as described by Knispel (1991). The difference in *ΔE* was subdivided into six levels according to the color difference classification of the National Bureau of Standards (NBS) (Nimeroff, 1968). The difference in *ΔE* among the samples was classified as 0–0.5 (trace), 0.5–1.5 (slight), 1.5–3.0 (noticeable), 3.0–6.0 (appreciable), 6.0–12.0 (much), 12.0 or more (very much). The values of CIE L*, CIE a*, and CIE b* were used to calculate the total color difference (*ΔE*), NBS unit, and chroma according to the following equations:

$$\text{Total color difference } (\Delta E) = \sqrt{(\text{CIE L*}_{\text{samples}} - \text{CIE L*}_{\text{control}})^2 + (\text{CIE a*}_{\text{samples}} - \text{CIE a*}_{\text{control}})^2 + (\text{CIE b*}_{\text{samples}} - \text{CIE b*}_{\text{control}})^2}$$

$$\text{NBS unit} = \Delta E \times 0.92$$

$$\text{Chroma} = \sqrt{\text{CIE a*}^2 + \text{CIE b*}^2}$$

Marinade uptake

Marinade uptake was calculated from the weight before and after marinade (Yusop et al., 2010). Excess marinade was removed by applying a paper towel to the sample surfaces.

Cooking loss

Cooking loss was calculated by measuring the differences in weight before and after cooking. Each sample was blotted dry and weighed as per Honikel's method (Honikel, 1998).

pH

The pH value was measured by means of a pH meter (SP-701, Suntex Instruments Co., Ltd, Taiwan) in 10 g samples pulverized in 90 mL of distilled water for 60 s in a homogenizer (Unidrive 1000D, CAT M. Zipperer GmbH, Germany).

Shear force

Shear force was evaluated according to the American Meat Science Association guidelines (American Meat Science Association, 1995) and Honikel's method (Honikel, 1998). Some uniform portions of samples were cut into 1×1×1 cm cubes. The samples were sheared perpendicular to the fiber by means of a rheometer (Compac-100 II, Sun Scientific, Japan). The measurement conditions were as follows: probe 10, mode 1, crosshead speed of 250 mm/min, load cell of 50 N, and max load cell of 100 N.

Texture

Textural properties were analyzed following the method described by Honikel (1998) and Bourne (1978). Texture analysis was performed with a rheometer (Compac-100 II, Sun Scientific, Japan). Some uniform portions of samples were cut into 1×1×1 cm cubes. A two-bite compression test was conducted under the following conditions: probe 14, mode 20, crosshead speed of 250 mm/min, compressed to 50% of original height, and max load cell of 100 N.

Microbiological analysis

To determine the total viable and coliform counts in the samples, 25 g samples were homogenized in 225 mL of steriled 0.85% sodium chloride solution for 10 min using a stomacher (BagMixer® 400 W, Interscience, France). The samples were then subjected to a 10-fold serial dilution for the analysis. The total viable count was determined on a standard plate count agar (Difco, USA) after 45 h of incubation in an incubator (BI-600m, Jeio Tech, Korea) at 37°C. The coliform count was determined on petri film coliform count plates (3M, USA) after 24 h of incubation at 37°C. The analyses were carried out in triplicate as per the Ministry of Food and Drug Safety method (Ministry of Food and Drug Safety, 2013).

Survey of consumer preference

Survey of consumer preference was carried out with 20 panels. Samples were served on white porcelain trays. Each panelist used water and a piece of bread to clean their palate between samples. The samples were blind-coded with random 3-digit numbers. The panelists evaluated the samples for color, tenderness, flavor, sourness, juiciness, and overall acceptability on a 7-point scale.

Statistical analysis

Statistical significance of differences was verified by one-way analysis of variance (ANOVA) and Duncan's multiple-range test in the SPSS software (IBM SPSS Statistics 23, IBM Corporation, USA). In this experiment, each sample was tested in triplicate for statistical analysis.

Results and Discussion

Color

The results of the color assessment are displayed in Table 1. All the color values were affected by marinade. Meat color is associated with the quality of meat, and in particular, the higher the lightness, the better (Kim et al., 2012). The treated meat samples showed lower CIE L* (lightness) than the control (p<0.001). There was significant no difference among the treatment groups, at 52.89–54.72 (p<0.05). This observation is in line with the results obtained by Alahakoon et al. (2014), Ju (2011), and Yusop et al. (2010). CIE a* (redness) and chroma are related to the concentration of myoglobin as well as the degree of myoglobin denaturation (Vaudagna et al., 2008). The control samples had an intense red color (higher CIE a* and chroma) as compared with marinated samples (p<0.001). Similar observations were reported by Alahakoon et al. (2014). The value of ΔE (total color difference) was found to be the highest in group T_2 (8.74) and lowest in group T_4 (6.88, p<0.001). Classification of the values by NBS unit revealed a much difference between the control and treatment groups. Thus, the marinade exerted a strong effect on color attributes.

Marinade uptake and cooking loss

The results on the marinade uptake and cooking loss are presented in Table 2. Marinade uptake was significantly higher in the treatment groups than in the control (p<0.01). But, there were significant no difference among the treatment groups, at 0.84–1.59% (p<0.05). This observation is consistent with the data reported by Yusop et al. (2010), Lombard et al. (2011), and U-chupaj et al. (2017). The increase was due to water absorption during marinade. Lombard et al. (2011) reported that a marinade solution opens the muscle structure to accommodate liquid uptake. The cooking loss showed no significant difference among the groups of samples (p<0.05). This result is similar to that reported by He et al. (2015) and Yusop et al. (2010). It was demonstrated by Sánchez del Pulgar et al. (2012) that cooking loss is associated with textural properties.

pH

The results on pH are shown in Table 2. In general, pH has a strong influence on freshness, water retention, tenderness,

Table 1. Color values of marinated pork ham

Variables	CIE L*	CIE a*	CIE b*	ΔE	NBS unit	Chroma
Control	59.79±0.93[1)]^{a}	7.11±0.81^{a}	10.56±0.30^{a}	0.00±0.00^{c}	0.00±0.00^{c}	12.74±0.50^{a}
T_1	53.76±0.48^{b}	5.35±0.25^{b}	7.60±0.35^{b}	6.95±0.52^{b}	6.40±0.47^{b}	9.30±0.20^{b}
T_2	52.89±1.91^{b}	4.48±0.19^{cd}	6.07±0.54^{c}	8.74±1.19^{a}	8.04±1.09^{a}	7.55±0.51^{c}
T_3	54.72±1.06^{b}	4.21±0.32^{d}	6.77±0.73^{bc}	7.01±0.94^{b}	6.45±0.86^{b}	7.97±0.79^{c}
T_4	54.06±0.48^{b}	5.19±0.29^{bc}	7.33±0.47^{b}	6.88±0.18^{b}	6.33±0.16^{b}	8.98±0.54^{b}
F-value	18.450***	20.488***	35.329***	66.842***	66.842***	42.935***

[1)] Values are mean±standard deviation.
Data with different superscript letters in the same column are significantly different as analyzed by Duncan's multiple-range test (p<0.05).
*** p<0.001.
Control, non-marinated; T_1, 4°C for 1 h at 98.81% vacuum; T_2, 4°C for 1 h without vacuum; T_3, 20°C for 1 h at 98.81% vacuum; T_4, 20°C for 1 h without vacuum; NBS, National Bureau of Standards.

Table 2. Characteristics of marinated pork ham

Variables	Marinade uptake (%)	Cooking loss (%)	pH	Shear force (N)	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N·mm)
Control	0.00±0.00[b]	19.94±0.53[NS]	6.02±0.01[a]	18.14±0.85[1)b]	23.60±2.50[NS]	5.16±0.08[NS]	2.28±0.33[NS]	275.62±25.79[NS]
T_1	0.84±0.76[a]	20.84±7.12	4.41±0.01[b]	21.77±2.51[a]	27.43±0.99	5.19±0.05	1.90±0.26	269.87±41.62
T_2	1.10±0.09[a]	19.84±4.50	4.36±0.01[c]	22.54±2.26[a]	25.73±3.23	5.19±0.05	2.12±0.22	281.04±7.01
T_3	1.19±0.31[a]	22.13±2.43	4.42±0.02[b]	21.80±0.37[a]	27.69±2.33	5.13±0.09	2.06±0.14	291.56±22.38
T_4	1.59±0.43[a]	18.59±3.65	4.30±0.01[d]	22.46±0.87[a]	28.01±2.24	5.27±0.05	2.08±0.13	306.70±37.71
F-value	0.009**	0.287	16,281.933***	3.824*	1.808	1.734	1.101	0.727

[1)] Values are mean±standard deviation.
Data with different superscript letters in the same column are significantly different as analyzed by Duncan's multiple-range test ($p<0.05$).
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
Control, non-marinated; T_1, 4°C for 1 h at 98.81% vacuum; T_2, 4°C for 1 h without vacuum; T_3, 20°C for 1 h at 98.81% vacuum; T_4, 20°C for 1 h without vacuum; NS, not significant.

color, textural properties, and storage stability of meat (Kim et al., 2008). The pH level was the highest in the control and the lowest in group T_4 ($p<0.001$). The treated samples showed lower pH (4.30–4.42) than the control did (6.02, $p<0.001$). This finding is similar to those reported by Burke et al. (2003), Jin et al. (2016), Seong et al. (2012), and Topuz et al. (2014). It was assumed that low pH of lemon juice had an effect on pork ham here.

Shear force and texture

The results of the shear force analysis are given in Table 2. Shear force is a standard method of measuring the degree of softness of meat. It measures the amount of force required to cut meat while shearing it with a blunt blade (Jeon et al., 2013). The shear force is highly correlated with the hardness and tenderness. The higher the shear force, the tougher the meat is, and the lower the shear force, the tougher the meat is (Kim et al., 2015). It is related to consumer preference and may be one of the most important attributes of meat products (Wheeler et al., 1990). Meat with low shear force values is desirable (Jouki et al., 2011). Gault (1984) reported that the degree of tenderness varies with marinade methods. Gao et al. (2015) and Kim et al. (2015) found that marinade has a positive effect on texture of meat. Studies on the positive effects of marinade containing acid on texture of meat and the mechanism of action are still in progress (Seong et al., 2012). Groups T_1 (21.77 N), T_2 (22.54 N), T_3 (21.80 N), and T_4 (22.46 N) showed significantly higher shear force as compared to the control (18.14 N, $p<0.001$). It is likely that this result means that protein is solidified by acid and salt, and shear force increases accordingly. The results of the analysis of textural properties are presented in Table 2. Although shear force showed a slight difference between the control and treated samples, texture analysis did not show any significant differences among the samples. In comparison with the control, marinated samples showed lower hardness, but the difference was not significant ($p<0.05$). Furthermore, the meat samples did not significantly differ in springiness, cohesiveness, and chewiness ($p<0.05$). The textural properties were not affected by different types of marinade.

Microbiological analysis

The total viable and coliform counts are displayed in Table 3. There are two major hurdles for microbes in this study. The first is sous-vide cooking. Wang et al. (2004) reported that sous-vide–processed products show high resistance to microbial growth during storage. Creed (1998) reported that vacuum sealing reduces bacterial growth. The second factor is marinade. Lemon juice, salt, thyme, and olive oil used in the marinade inhibit bacterial growth. Lemon juice causes denaturation of

Table 3. **The total viable and coliform counts in marinated pork ham**

Variables		Total viable count (CFU/g)	Coliform count (CFU/g)
Marinade	Control	84.67±2.08[1)a]	3.67±1.15[a]
	T_1	3.00±1.00[e]	ND
	T_2	12.00±2.00[c]	ND
	T_3	6.00±1.00[d]	ND
	T_4	14.67±1.53[b]	ND
Cooked	Control	ND	ND
	T_1	ND	ND
	T_2	ND	ND
	T_3	ND	ND
	T_4	ND	ND
F-value		1,384.500***	30.250***

[1)] Values are mean±standard deviation.
Data with different superscript letters in the same column are significantly different, as analyzed by Duncan's multiple-range test ($p<0.05$).
*** $p<0.001$.
CFU, colony-forming unit, the units used to estimate the number of viable bacteria or fungal cells in a sample; Control, non-marinated; T_1, 4℃ for 1 h at 98.81% vacuum; T_2, 4℃ for 1 h without vacuum; T_3, 20℃ for 1 h at 98.81% vacuum; T_4, 20℃ for 1 h without vacuum; ND, not detected.

microorganisms and affects water-holding capacity. Salt increases moisture retention to prevent its use by microorganisms. Thyme has excellent antiseptic, antioxidant, and insecticidal properties and a food preservation effect (Goli et al., 2012; Khan et al., 2016; Kim et al., 2006). In this study, the total viable and coliform counts of raw meat were 84.6 and 3.67 CFU/g, respectively. After the marinade, the total viable counts decreased to 3.00–14.67 CFU/g ($p<0.001$). In a comparison of the control with the treatment groups T_1 (3.00 CFU/g), T_2 (12.00 CFU/g), T_3 (6.00 CFU/g), and T_4 (14.67 CFU/g), there were significant differences ($p<0.001$). This result indicated that vacuum and low temperature during marinade inhibited microbial growth. Coliforms were not detected. After sous-vide cooking, no viable microorganisms were detected in any groups.

Survey of consumer preference

The results of the survey of consumer preference are detailed in Table 4. Although color, tenderness, and overall acceptability showed significant differences among the samples, flavor, sourness, and juiciness did not. Color levels were the lowest in the control (3.59, $p<0.001$). This finding is similar to the data reported by Alahakoon et al. (2014) and Gao et al. (2015). It is assumed that the color of marinated meat has a positive effect on consumer preferences. Tenderness was the

Table 4. **Survey of consumer preference of marinated pork ham**

Variables	Color	Tenderness	Flavor	Sourness	Juiciness	Overall preference
Control	3.59±1.66[1)b]	6.47±1.74[a]	4.65±1.69[NS]	5.94±2.01[NS]	5.88±1.36[NS]	5.18±1.59[b]
T_1	5.76±1.20[a]	7.24±1.03[a]	6.18±1.67	5.94±1.78	6.41±1.62	6.06±1.52[ab]
T_2	6.19±1.22[a]	6.56±1.86[a]	5.81±2.04	5.94±1.95	6.81±1.56	6.76±1.64[a]
T_3	5.82±1.07[a]	6.18±2.04[ab]	5.41±1.00	5.47±1.42	6.00±1.54	5.76±1.39[ab]
T_4	6.00±1.27[a]	5.29±0.85[b]	5.24±1.25	5.29±1.57	6.12±1.41	5.59±1.28[b]
F-value	11.658***	3.501*	2.446	0.580	1.335	2.694*

[1)] Values are mean±standard deviation.
Data with different superscript letters in the same column are significantly different, as analyzed by Duncan's multiple-range test ($p<0.05$).
* $p<0.05$, *** $p<0.001$.
Control, non-marinated; T_1, 4℃ for 1 h at 98.81% vacuum; T_2, 4℃ for 1 h without vacuum; T_3, 20℃ for 1 h at 98.81% vacuum; T_4, 20℃ for 1 h without vacuum; NS, not significant.

highest in group T_1 (7.24, $p<0.05$). This result is consistent with the study published by Gao et al. (2015). Tenderness of meat is the main quality criterion and is associated with heat-induced deformation of muscle tissue (Christensen et al., 2011). Overall acceptability was higher in the treated samples of meat (5.59–6.76) than in the control (5.18, $p<0.05$). This observation is in line with the findings of Gao et al. (2015), Ju (2011), and Topuz et al. (2014).

Conclusion

The purpose of this study was to improve the texture and microbiological and sensory properties of pork ham by sous-vide cooking and marinade. The red color and boar odor, which are noticeable after sous-vide cooking, have adverse effects on consumer preferences. To solve this problem, we tried to find optimal marinade conditions (vacuum and temperature). The marinade and sous-vide cooking not only had a positive effect on sensory characteristics but also provided safe conditions for sanitary evaluation. T_2 received especially high scores on consumer preference. It appears that marinade at refrigeration temperature is better than that at room temperature.

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