Food Science of Animal Resources

Food Sci. Anim. Resour. 2020 November 40(6):1033~1043 DOI https://doi.org/10.5851/kosfa.2020.e74

ARTICLE



Effects of Gelatin Hydrolysates Addition on Technological Properties and Lipid Oxidation of Cooked Sausage

Youn-Kyung Ham¹, Dong-Heon Song², Sin-Woo Noh², Tae-Wan Gu², Jae-Hyeok Lee², Tae-Kyung Kim³, Yun-Sang Choi⁴, and Hyun-Wook Kim^{2,*}

¹Department of Animal Resources Technology, Gyeongnam National University of Science and Technology, Jinju 52725, Korea

²Department of Animal Science & Biotechnology, Gyeongnam National University of Science and Technology, Jinju 52725, Korea

³Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Korea

⁴Food Processing Research Center, Korea Food Research Institute, Wanju 55365, Korea

Abstract This study investigated the impacts of gelatin hydrolysate addition on the technological properties and lipid oxidation stability of cooked sausage. Gelatin hydrolysate was prepared from pork and duck skin gelatin, through stepwise hydrolysis using collagenase and pepsin. The cooked sausages were formulated without gelatin (control) or with 1% pork skin gelatin, 1% duck skin gelatin, 1% pork skin gelatin hydrolysate, and 1% duck skin gelatin hydrolysate. The pH, color characteristics, protein solubility, cooking loss, and textural properties of cooked sausages were evaluated, and the 2-thiobarbituric acid reactive substances (TBARS) value was measured weekly to determine lipid oxidation stability during 4 wk of refrigerated storage. Enzymatic hydrolysis of gelatin decreased protein content and CIE L* but increased redness and yellowness (p<0.05). When 1% gelatin or gelatin hydrolysate was incorporated in cooked sausage, however, little to no impacts on pH value, moisture content, protein content, color characteristics, protein solubility, and cooking loss were found (p>0.05). The addition of 1% duck skin gelatin hydrolysate increased the cohesiveness and chewiness of cooked sausages. The inclusion of 1% duck skin gelatin accelerated lipid oxidation of cooked sausages during refrigerated storage (p<0.05), whereas duck skin gelatin hydrolysate caused a lower TBARS value in cooked sausage compared to duck skin gelatin. The results show comparable effects of gelatin and gelatin hydrolysate addition on the technological properties of cooked sausages; however, the oxidative stability of raw materials for gelatin extraction should be evaluated clearly in further studies.

Keywords antioxidant peptide, collagenase, duck skin, enzymatic hydrolysis, pepsin

Introduction

In the modern food industry, much effort to meet consumer demand for well-being

© Korean Society for Food Science of Animal Resources. This is an open access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licences/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

OPEN ACCESS

Received	July 31, 2020
Revised	August 18, 2020
Accepted	August 23, 2020

*Corresponding author : Hyun-Wook Kim Department of Animal Science & Biotechnology, Gyeongnam National University of Science and Technology, Jinju 52725, Korea Tel: +82-55-751-3261 Fax: +82-55-751-3267 E-mail: hwkim@gntech.ac.kr

*ORCID

Youn-Kyung Ham https://orcid.org/0000-0002-5659-5256 Dong-Heon Song https://orcid.org/0000-0002-4670-3321 Sin-Woo Noh https://orcid.org/0000-0001-5265-2607 Tae-Wan Gu https://orcid.org/0000-0003-1086-4817 Jae-Hyeok Lee https://orcid.org/0000-0002-7476-0796 Tae-Kyung Kim https://orcid.org/0000-0002-6349-4314 Yun-Sang Choi https://orcid.org/0000-0001-8060-6237 Hyun-Wook Kim

https://orcid.org/0000-0002-4397-9664

trends has been made by replacing artificial food additives with natural ingredients. This trend has now evolved into the concept of "clean label products" that are formulated with more natural ingredients and less processed (Aschemann-Witzel et al., 2019). To manufacture "clean label products", multifunctional food additives have been considered extensively to minimize the use of artificial food additives.

Gelatin, which is obtained through the thermal hydrolysis of collagen in animal tissues, has recently been used to produce bioactive hydrolysates or peptides (Lafarga et al., 2017; Sarbon et al., 2018). Previous studies primarily used mammalian, poultry, and fish skin as gelatin sources, and several physiological benefits of gelatin hydrolysate, including antimicrobial, antioxidant, or antihypertensive activities have been found through *in vitro* and *in vivo* assays (Gómez-Guillén et al., 2011). Moreover, gelatin hydrolysates with antioxidative and/or antimicrobial activities could be used to extend the shelf stability of processed foods, resulting from the retardation of oxidative quality change and the inhibition of harmful microbial growth, respectively (Nikoo et al., 2015; Zhang et al., 2020).

In commercial restructured and/or emulsified meat products, gelatin (generally from 0.5 to 3.0 g/100 g) is used to improve water-holding capacity and textural properties (Lee and Chin, 2016). Moreover, it has been well documented that the positive functionality of gelatin in processed meat products results from mainly hydration properties and gel-forming ability of gelatin (Gómez-Guillén et al., 2011). Beyond the positive functionality, however, there is no available literature on the impact of gelatin on the oxidative stability of processed meat products during storage.

Commercial gelatin products used in the meat industry is mostly produced from pork skin and bovine hide (Karim and Bhat, 2009), but gelatin extraction materials have been diversified now into poultry and fishery sources due to the risk of infectious diseases and religious reasons (Tümerkan et al., 2019). In particular, poultry by-products such as feet and skins have been considered as promising materials for gelatin extraction (Noh et al., 2019; Park et al., 2013). However, although some previous studies have found the techno-functional advantages of duck skin gelatin in processed meat products (Kim et al., 2020), the efficiencies of pork and duck skin gelatin on the quality attributes of processed meat products have not been clearly compared.

In this preliminary study, the pork and duck skin gelatin hydrolysates were prepared through an enzymatic hydrolysis method that produced gelatin hydrolysates with antioxidant capacity (Lee et al., 2012), and the impacts of gelatin and its hydrolysate on technological properties and lipid oxidation stability of cooked sausage have been investigated.

Materials and Methods

Raw materials

Commercial pork skin gelatin (gel strength: 280 bloom, particle size: 5–15 mesh, Hangzhou Qunli Gelatin Chemical, Hangzhou, China) was purchased from a local market, and frozen duck skin used for gelatin extraction was also provided by a duck processing company (Farm Duck, Jeongeup, Korea). Pork ham and back fat were purchased from a local market after 48 h postmortem.

Gelatin extraction from duck skin

Duck skin gelatin was prepared according to a previously described extraction procedure (Kim et al., 2016; Tümerkan et al., 2019) with minor modifications. The frozen duck skin was thawed in a 4°C refrigerator for 24 h. The excessive subcutaneous fat was removed, and the duck skin was washed with tap water several times. The washed duck skin was

ground using a meat grinder (MN-22S, Hankook Fugee Industries, Hwaseong, Korea) equipped with an 8-mm plate. The ground duck skin was soaked in 1.5 volumes of 0.1 M NaOH for 16 h to remove non-collagenous proteins and neutralized with tap water (approximately pH 7.0). Acidic soaking of the duck skin was performed with 2 volumes of 0.5 M acetic acid for 12 h, and the swelled duck skin was neutralized again using tap water to pH 6.5–7.0. For hot-water extraction, the duck skin was blended with distilled water (1:1 ratio, w/w) and heated in a 65°C water bath for 3 h. After heating, the sample was filtrated through cheesecloth, and the gelatin solution was set in a 4°C refrigerator for 12 h. The upper fat layer was removed manually, and the gelatin layer was freeze-dried and pulverized for producing duck skin gelatin powder.

Enzymatic hydrolysis of gelatin

Gelatin hydrolysates were prepared through stepwise enzymatic hydrolysis using collagenase and pepsin, as described by Lee et al. (2012) with minor modifications. Six grams of pork or duck skin gelatin powder were dissolved in 540 mL of distilled water and gently homogenized, and a low concentration of gelatin solution was used to prevent gelation and coagulation. The gelatin solution was heated at 80°C for 10 min to inactivate any enzymes contained in the gelatin powders and cooled at room temperature for 2 h. The pH of the gelatin solution was adjusted to pH 7.0 using 1 M NaOH and finally diluted to 600 mL in a volumetric flask to form 1% gelatin concentration (w/v). The first enzymatic proteolysis of gelatin was performed with collagenase (EC3.4.24.3) at a 1:100 ratio (w/w), and the enzymatic reaction was placed in a 37°C incubator for 12 h with stirring at 250 rpm. The mixture was heated at 80°C for 10 min and cooled to inactivate the collagenase. The pH of the mixture was adjusted to pH 2.0 using 6 N HCl for the second enzymatic hydrolysis, and the mixture was treated with pepsin (EC3.4.23.1) at a 1:50 ratio (w/w). The second step was conducted under the same hydrolysis condition as above, and the product was heated at 80°C for 10 min and cooled to inactivate swas finally adjusted to pH 5.7 using 1 M NaOH and then centrifuged at 20,000×g for 10 min (4°C). The supernatant was freeze-dried and pulverized to obtain gelatin hydrolysate powder.

Manufacture of cooked sausage

The excessive subcutaneous fat and connective tissues on the surface were removed. The pork ham and back fat were ground using a meat grinder with an 8 mm plate (MN-22S, Hankook Fugee Industries, Hwaseong, Korea). The ground pork, ground back fat, ice, and other ingredients were emulsified using a bowl cutter (Cutter C4W, Sirman, Marsango, Italy). All treatments were formulated with 60% (w/w) ground pork, 20% (w/w) ground pork back fat, and 20% (w/w) ice. Based on the total sample weight, 1.5% (w/w) NaCl, 0.3% (w/w) sodium tripolyphosphate, and 1% (w/w) gelatin or gelatin hydrolysates were added as follows: control (without gelatin), 1% (w/w) pork skin gelatin, 1% (w/w) pork skin gelatin hydrolysate, 1% (w/w) duck skin gelatin, and 1% (w/w) duck skin gelatin hydrolysate, respectively. The emulsified meat batter was stuffed into a collagen casing (diameter of 25 mm, #240, NIPPI, Tokyo, Japan), cooked in an 80°C water bath until the core temperature reached 75°C, and then, cooled in ice-water. The cooked sausages were placed at room temperature for 3 h to evaporate surface moisture. To determine lipid oxidation stability, the cooked sausages were vacuum-packaged and stored in a 4°C refrigerator for 4 wk. A total of three independent batches was prepared.

Physicochemical analysis

Chemical composition

The pH of the gelatin and hydrolysate powder (1% solution, w/v) was measured three times using an electronic pH-meter

(Orion Star[™] A211 pH Benchtop Meter, Thermo Scientific, USA). For meat batter and cooked sausage, the homogenate, which was prepared with 3 g of sample and 27 mL of distilled water, was used for pH analysis.

The moisture content of the cooked sausage was determined using the oven air-drying method (AOAC, 2007). The protein content of gelatin powder and cooked sausage was measured using a nitrogen protein analyzer (Rapid N Cube, Elementar, Langenselbold, Germany) and calculated using nitrogen-protein conversion factors of 5.55 and 6.25, respectively (Mariotti, 2008).

Color characteristics

The color characteristics of gelatin powder and cooked sausage were measured using a colorimeter (CR-400, Konica Minolta Sensing, Osaka, Japan) with an 8 mm aperture and 2° observer. The setting for the illuminant was a D₆₅ source. Calibration of the instrument was conducted with a calibration tile (CIE L*: +93.01, CIE a*: -0.25, CIE b*: +3.50), according to the manufacturer's manual. The CIE L*, a*, and b* values were taken five times on the cross section of each sample (internal color).

Protein solubility

The protein solubility of the meat batter was measured using the method described by Warner et al. (1997). Two grams of sample were homogenized with a buffer solution (1.1 M potassium iodide in 0.1 M potassium phosphate buffer, pH 7.2) using a homogenizer (HG-15A, Daihan Sci., Seoul, Korea) at 12,000 rpm for 2 min. The homogenate was stored in a 2°C refrigerator overnight and centrifuged at 1,500×g for 20 min (4°C). The supernatant was filtered through a filter paper (Whatman no. 1), and the protein concentration of the filtrate was quantified using the Biuret method (Gornall et al., 1949). Protein solubility was expressed in mg protein soluble fraction per gram of emulsified meat batter (mg/g).

Cooking loss

The cooking loss of cooked sausage was determined by the percent weight difference between the raw and cooked samples (Kim et al., 2015).

Texture profile analysis

Texture profile analysis of cooked sausage was conducted using a texture analyzer (CT3, Brookfield Engineering Laboratories, Middleboro, MA, USA). The sausages were equilibrated to room temperature (22°C) for 3 h, and four samples (2.5 cm height) were prepared from the middle portion of each sample. A twice compression cycle test (70% compression of the original sample height) was performed with a cylinder probe (diameter in 4 cm). Sample deformation curves were obtained with a 50 kg maximum load cell, and the analysis condition was as follows: pre-test speed 1.0 mm/s, post-test speed 5.0 mm/s, and head speed 2.0 mm/s. The values for hardness (kg), springiness (ratio), cohesiveness, gumminess (kg), and chewiness (kg) were presented (Bourne, 1978).

Lipid oxidation

Lipid oxidation of the cooked sausage was determined weekly using the 2-thiobarbituric acid reactive substance (TBARS) method of Buege and Aust (1978). The results were expressed as malondialdehyde mg/kg sample per kg of sample (mg MDA/kg sample).

Statistical analysis

The experimental design was a completely randomized block design with three independent batches. An analysis of variance (ANOVA) was performed on the measured variables using the one-way ANOVA procedure of the SPSS program (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to determine significant differences between means (p<0.05). For lipid oxidation, a two-way ANOVA was conducted, in which treatment and storage effect as the main effects, and their interaction were found.

Results and Discussion

Physicochemical properties of gelatins and gelatin hydrolysates

The protein content, pH, and color characteristics of pork and duck skin gelatin and their hydrolysates are shown in Table 1. The protein content of pork skin gelatin was 93.54 g/100 g, whereas duck skin gelatin showed a considerably lower protein content (78.71 g/100 g) (p<0.05). This result was likely that the commercial pork skin gelatin product was industrially purified through filtration and deionization processes. After stepwise enzymatic hydrolysis using collagenase and pepsin, the hydrolysates of pork and duck skin gelatins showed similar protein contents of 76.77 and 72.17 g/100 g, respectively (p>0.05). Previously, Kim et al. (2013) reported that the protein content of pork skin gelatin hydrolysate prepared through 0.3% flavourzyme treatment was 511.53 mg/g dry weight.

The pH value (pH 4.50) of pork skin gelatin was significantly lower than that of duck skin gelatin (pH 6.17); however, their hydrolysates showed similar pH values (p>0.05) since the final pH of gelatin hydrolysates were equally adjusted at the end of enzymatic hydrolysis process. According to a previous study, the final pH of gelatin is mainly affected by the acid/alkali treatment and neutralization in the gelatin extraction process (Kim et al., 2012), and the general pH after neutralization is about 5.0–7.0 (Noh et al., 2019). Thus, in this study, the pH difference between pork and duck skin gelatin could be associated with different manufacturing processes.

In terms of color characteristics, duck skin gelatin showed higher CIE L* but lower CIE b* (yellowness) than pork skin gelatin (p<0.05); there was no significant difference in redness between the two different gelatin sources. Stepwise enzymatic hydrolysis decreased lightness of both gelatin sources but increased redness (p<0.05). After enzymatic hydrolysis, the yellowness of duck skin gelatin significantly increased, but no change in yellowness was observed between pork skin gelatin and its hydrolysate. Between pork and duck skin gelatin hydrolysates, similar lightness and redness were observed, but duck

Table 1. Filysicochemical properties of pork and duck skin gelatins and their flydrolysates	Table 1. Physicochemical properties of pork and duck skin gelati	ins and their hydrolysates ¹⁾
---	--	--

Trait	Р	ork skin	D	ouck skin	SEM	
Tran	Gelatin	Gelatin hydrolysate	Gelatin	Gelatin hydrolysate	SEIVI	p-value
Protein content (g/100 g)	93.54ª	76.77 ^b	78.71 ^b	72.17 ^b	2.374	< 0.001
pH value	4.50 ^c	5.71 ^b	6.17 ^a	5.71 ^b	0.188	< 0.001
Color characteristics						
CIE L* (lightness)	89.91 ^b	84.60 ^c	95.69ª	79.16 ^c	1.866	< 0.001
CIE a* (redness)	-0.43 ^b	2.02 ^a	-0.99 ^b	2.02ª	0.513	0.002
CIE b* (yellowness)	13.99 ^b	14.08 ^b	5.99°	16.52ª	1.231	< 0.001

¹⁾Gelatin hydrolysates were prepared through a stepwise enzymatic hydrolysis using collagenase and pepsin.

^{a-c} Means sharing the same letters within a row are not significantly different (p>0.05).

skin gelatin hydrolysate showed a slightly higher yellowness than pork skin gelatin hydrolysate (p<0.05).

Previously, Chuaychan et al. (2017) suggested that an increase in temperature during the drying process could increase the yellowness of gelatin hydrolysate powder, resulting from a Maillard reaction between reducing sugars and amino acids. In this study, since the duck skin gelatin hydrolysate powder was prepared through a lyophilization process, the increased yellowness was probably due to the heating process (80°C) for the inactivation of proteases during enzymatic proteolysis. In general, commercial gelatin generally presents a white color; however, the color characteristics of gelatin are not related to functional properties such as gel-forming ability, gel strength, and emulsifying capacity (Ockerman and Hansen, 1988). Therefore, the increased yellowness of gelatin hydrolysates may have little to no impact on the functional properties of gelatin hydrolysates.

Chemical composition and color of cooked sausages with pork skin gelatin, duck skin gelatin, and their hydrolysates

The chemical composition and color characteristics of cooked sausages prepared with gelatin or gelatin hydrolysates are shown in Table 2. The pH values of meat batter and cooked sausages were unaffected by the incorporation of gelatin or gelatin hydrolysate (p=0.174). Although there was significant difference in pH between pork and duck skin gelatin and gelatin hydrolysates (Table 1), it seemed that the inclusion level might be too small to change the pH of meat batter and cooked sausages. The addition of gelatin and gelatin hydrolysates had no significant effect on the moisture and protein content of the cooked sausage. The moisture content of the control and all treatments ranged from 61.19 to 62.41 g/100 g, and the protein content was from 16.36 to 19.89 g/100 g. As a similar result, it has been reported that the addition of 1% gelatin slightly increased the protein content of cooked sausage, whereas the moisture content was relatively decreased (Lee and Chin, 2016).

There was no change in the lightness and yellowness of the cooked sausages when 1% gelatin or gelatin hydrolysate were added (p>0.05). While the addition of gelatin and gelatin hydrolysate decreased the redness of the cooked sausage (p<0.05); however, the difference between the control and treatment was numerically too small. As a similar result, Lee and Chin

Trait Control ²⁾	(Courter 12)	Pork skin		Duck skin		SEM	
	Gelatin	Gelatin hydrolysate	Gelatin	Gelatin hydrolysate	SEM	p-value	
pH (meat batter)	5.91	5.85	5.96	5.95	5.91	0.016	0.312
pH (cooked sausage)	6.36	6.24	6.24	6.31	6.31	0.017	0.174
Moisture content (g/100 g)	62.41	61.49	61.19	61.87	62.22	0.222	0.429
Protein content (g/100 g)	16.36	18.12	19.89	18.76	19.23	0.821	0.896
Color characteristics							
CIE L* (lightness)	77.10	78.22	77.80	77.80	77.54	0.156	0.269
CIE a* (redness)	3.55 ^a	3.54 ^b	3.23 ^b	3.14 ^b	3.26 ^b	0.058	0.029
CIE b* (yellowness)	11.16	11.19	11.25	11.50	11.44	0.073	0.541

Table 2. Chemical composition and color characteristics of cooked sausages formulated with 1% pig and duck skin gelatins and their hydrolysates¹⁾

¹⁾Gelatin hydrolysates were prepared through a stepwise enzymatic hydrolysis using collagenase and pepsin.

²⁾Control was prepared without gelatin or gelatin hydrolysate, and other treatments were formulated with 1% gelatin or gelatin hydrolysate.

^{a,b} Means sharing the same letters within a row are not significantly different (p>0.05).

(2016) reported that the addition of 0.5%, 1.0%, and 1.5% pork skin gelatin had no impacts on lightness, redness, and yellowness of pork sausages. Our results indicate that the color changes of gelatin hydrolysate due to enzymatic hydrolysis may have no impacts on color characteristics of cooked sausages and suggest that this phenomenon could be affected by the inclusion level of gelatin and gelatin hydrolysate.

Protein solubility and cooking loss of cooked sausages with pork skin gelatin, duck skin gelatin, and their hydrolysates

The addition of 1% gelatin and gelatin hydrolysate caused an increasing trend in protein solubility of the meat batters (p=0.080; Table 3). This was probably related to the increased protein content with adding gelatin or gelatin hydrolysates. In terms of cooking loss, a decreasing trend was found due to the addition of 1% gelatin and gelatin hydrolysates (p=0.081). Previously, Lee and Chin (2016) reported that the addition of 1% pork skin gelatin had little effect on the water loss of cooked sausage. Recently, Noh et al. (2019) reported that the positive impacts of gelatin on the water-holding capacity of processed meat products could be associated with the functional properties rather than the interaction between myofibrillar proteins and gelatin added. Thus, the inclusion level of 1% gelatin or gelatin hydrolysate might be insufficient to form a gel matrix to entrap moisture in the meat batter.

Textural properties of cooked sausages with pork skin gelatin, duck skin gelatin, and their hydrolysates

The addition of 1% gelatin and gelatin hydrolysate did not affect hardness, springiness, and gumminess of cooked sausage (p>0.05; Table 3). However, the addition of duck skin gelatin hydrolysate significantly increased the cohesiveness of cooked sausage. As a result, higher chewiness was observed for gelatin or gelatin hydrolysate treatments compared to the control (p<0.05). Previously, the impacts of gelatin on textural properties of cooked sausages have been inconsistent. Lee and Chin (2016) reported that the hardness, cohesiveness, gumminess, and chewiness of emulsion sausages decreased as the gelatin level increased. In addition, they reported that gelatin addition did not affect the springiness of emulsion sausage, since only a weak interaction between muscle protein and gelatin could occur (Lee and Chin, 2016). On the other hands, Jridi et al. (2015)

Trait	Control ²⁾ –	Pork skin		Duck skin		SEM	
Tran Control ^{-,}	Control ^{-/}	Gelatin	Gelatin hydrolysate	Gelatin	Gelatin hydrolysate	SEM	p-value
Protein solubility (mg/g)	74.77	93.68	80.81	90.52	83.90	2.681	0.080
Cooking loss (%)	2.37	1.82	1.63	1.99	1.90	0.089	0.081
Textural properties							
Hardness (kg)	7.61	8.82	8.21	9.15	8.12	0.303	0.596
Springiness (ratio)	0.68	0.68	0.73	0.79	0.77	0.009	0.051
Cohesiveness (unitless)	0.16 ^{bc}	0.16 ^c	0.21 ^{ab}	0.21 ^{ab}	0.22ª	0.019	0.045
Gumminess (kg)	1.20	1.37	1.68	1.90	1.90	0.090	0.195
Chewiness (kg)	0.14 ^c	0.17 ^{bc}	0.26 ^a	0.21 ^{ab}	0.24 ^{ab}	0.015	0.021

Table 3. Protein solubility, cooking loss, and textural properties of cooked sausages formulated with 1% pig and duck skin gelatins and
hydrolysates ¹⁾

¹⁾Gelatin hydrolysates were prepared through a stepwise enzymatic hydrolysis using collagenase and pepsin.

²⁾Control was prepared without gelatin or gelatin hydrolysate, and other treatments were formulated with 1% gelatin or gelatin hydrolysate.

^{a-c} Means sharing the same letters within a row are not significantly different (p>0.05).

indicated that fish gelatin addition (0%–1.5%) increased the hardness of turkey sausage but decreased cohesiveness. However, our results show that the addition of duck skin gelatin hydrolysate could increase the cohesiveness of cooked sausage. This is probably because the peptide groups in gelatin hydrolysate, which are available for the protein-protein interaction with muscle protein, could be exposed additionally. Moreover, since there are differences in amino acid composition and molecular size of gelatin, such positive effects of gelatin hydrolysate may be also different depending on gelatin extraction source.

Lipid oxidation of cooked sausages with gelatin hydrolysates

The effect of gelatin and gelatin hydrolysate addition on the lipid oxidation of cooked sausage during 4 wk of refrigerated storage is shown in Fig. 1. At the initial storage time (0 wk), the incorporation of duck skin gelatin or hydrolysate significantly increased the TBARS value of cooked sausages. Throughout the refrigerated storage period, the lipid oxidation of cooked sausages prepared with duck skin gelatin or hydrolysate accelerated consistently (p<0.05). As a result, the TBARS value of cooked sausage prepared with duck skin gelatin at 2 wk reached 1 mg MDA/kg sample, which is recognized as a limit of sensorial acceptance. For duck skin gelatin hydrolysate, the phenomenon of rapid lipid oxidation was alleviated slightly (0.88 mg MDA/kg at 4 wk). No changes in the TBARS value of the pork and duck skin gelatin hydrolysate treatment were observed during 4 wk of the refrigerated storage period (p>0.05). The pork skin gelatin hydrolysate treatment presented similar TBARS value to the control during overall storage period (p>0.05). According to Ch'ng et al. (2014), when

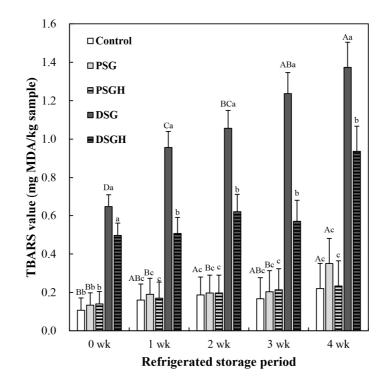


Fig. 1. Changes in 2-thiobarbituric acid reactive substances (TBARS) of cooked sausages formulated with 1% pork and duck skin gelatins and their hydrolysates. Gelatin hydrolysates were prepared through a stepwise enzymatic procedure using collagen and pepsin. ^{A–D} Means with the same letter within each treatment are not significantly different (p>0.05). ^{a–c} Means with the same letter within each storage period are not significantly different (p>0.05). Control, without gelatin or gelatin hydrolysate, PSG, 1% pork skin gelatin; PSGH, 1% pork skin gelatin hydrolysate; DSG, 1% duck skin gelatin; DSGH, 1% duck skin gelatin hydrolysate. Error bars represent standard error of the means.

0.5% of commercial gelatin, cold water fish skin gelatin, and bovine gelatin was added to chicken sausages, rapid lipid oxidation occurred during 3 wk of refrigerated storage. Thus, fatty acids and/or other pro-oxidants contained in raw skin materials for gelatin extraction could be incorporated into gelatin powder, which might be associated with the accelerated lipid oxidation of meat products formulated with gelatin.

Conclusion

In conclusion, this study shows comparable effects of gelatin and gelatin hydrolysate addition on the technological properties of cooked sausages. In particular, the addition of 1% gelatin hydrolysate could increase the cohesiveness and chewiness of cooked sausages. However, duck skin gelatin considerably accelerated the lipid oxidation of cooked sausages during 4 wk of the refrigerated storage period, although duck skin gelatin hydrolysate prepared through the stepwise enzymatic hydrolysis using collagenase and pepsin could alleviate the accelerated lipid oxidation. In further studies, an extraction process that can minimize the incorporation of lipid and/or pro-oxidant compounds should be considered for developing a multifunctional gelatin hydrolysate that provides antioxidant capacity as well as technological benefits.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgements

This research was supported High Value-added Food Technology Development Program (2018-118011) and Research Program (119015-03-2-HD060) by the Ministry of Agriculture, Food and Rural Affairs (Korea).

Author Contributions

Conceptualization: Ham YK, Song DH, Choi YS, Kim HW. Data curation: Ham YK, Noh SW, Gu TW, Lee JH, Kim TK. Formal analysis: Ham YK, Noh SW, Gu TW, Lee JH, Kim TK. Software: Ham YK. Validation: Song DH. Investigation: Choi YS, Kim HW. Writing -original draft: Ham YK. Writing -review & editing: Ham YK, Song DH, Noh SW, Gu TW, Lee JH, Kim TK, Choi YS, Kim HW.

Ethics Approval

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

References

AOAC. 2007. Official methods of analysis of AOAC International. 18th ed. AOAC International, Gaithersburg, MD, USA.
Aschemann-Witzel J, Varela P, Peschel AO. 2019. Consumers' categorization of food ingredients: Do consumers perceive them as 'clean label' producers expect? An exploration with projective mapping. Food Qual Prefer 71:117-128.

Bourne MC. 1978. Texture profile analysis. Food Technol 32:62-72.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. Methods Enzymol 52:302-310.

- Ch'ng SE, Ng MD, Pindi W, Kang OL, Abdullah A, Babji AS. 2014. Chicken sausages formulated with gelatin from different sources: A comparison of sensory acceptability and storage stability. World Appl Sci J 31:2062-2067.
- Chuaychan S, Benjakul S, Sae-Leaw T. 2017. Gelatin hydrolysate powder from the scales of spotted golden goatfish: Effect of drying conditions and juice fortification. Dry Technol 35:1195-1203.
- Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP. 2011. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocoll 25:1813-1827.
- Gornall AG, Bardawill CJ, David MM. 1949. Determination of serum proteins by means of the biuret reaction. J Biol Chem 177:751-766.
- Jridi M, Abdelhedi O, Souissi N, Kammoun M, Nasri M, Ayadi MA. 2015. Improvement of the physicochemical, textural and sensory properties of meat sausage by edible cuttlefish gelatin addition. Food Biosci 12:67-72.
- Karim AA, Bhat R. 2009. Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocoll 23:563-576.
- Kim DW, Park K, Ha G, Jung JR, Chang O, Ham JS, Jeong SG, Park BY, Song J, Jang A. 2013. Anti-oxidative and neuroprotective activities of pig skin gelatin hydrolysates. Korean J Food Sci Anim Resour 33:258-267.
- Kim HW, Lee YJ, Kim YHB. 2015. Efficacy of pectin and insoluble fiber extracted from soy hulls as a functional non-meat ingredient. LWT-Food Sci Technol 64:1071-1077.
- Kim HW, Song DH, Choi YS, Kim HY, Hwang KE, Park JH, Kim YJ, Choi JH, Kim CJ. 2012. Effects of soaking pH and extracting temperature on the physicochemical properties of chicken skin gelatin. Korean J Food Sci Anim Resour 32:316-322.
- Kim HW, Yeo IJ, Hwang KE, Song DH, Kim YJ, Ham YK, Jeong TJ, Choi YS, Kim CJ. 2016. Isolation and characterization of pepsin-soluble collagens from bones, skins, and tendons in duck feet. Korean J Food Sci Anim Resour 36:665-670.
- Kim SM, Kim TK, Ku SK, Kim MJ, Jung S, Yong HI, Choi YS. 2020. Quality characteristics of semi-dried restructured jerky: Combined effects of duck skin gelatin and carrageenan. J Anim Sci Technol 62:553-564.
- Lafarga T, Álvarez C, Hayes M. 2017. Bioactive peptides derived from bovine and porcine co-products: A review. J Food Biochem 41:e12418.
- Lee CH, Chin KB. 2016. Effects of pork gelatin levels on the physicochemical and textural properties of model sausages at different fat levels. LWT-Food Sci Technol 74:325-330.
- Lee SJ, Kim KH, Kim YS, Kim EK, Hwang JW, Lim BO, Moon SH, Jeon BT, Jeon YJ, Ahn CB, Park PJ. 2012. Biological activity from the gelatin hydrolysates of duck skin by-products. Process Biochem 47:1150-1154.
- Mariotti F, Tomé D, Mirand PP. 2008. Converting nitrogen into protein-Beyond 6.25 and Jones' factors. Crit Rev Food Sci Nutr 48:177-184.
- Nikoo M, Benjakul S, Xu X. 2015. Antioxidant and cryoprotective effects of Amur sturgeon skin gelatin hydrolysate in unwashed fish mince. Food Chem 181:295-303.
- Noh SW, Song DH, Ham YK, Kim TK, Choi YS, Kim HW. 2019. Interaction of porcine myofibrillar proteins and various gelatins: Impacts on gel properties. Food Sci Anim Resour 39:229-239.
- Ockerman HW, Hansen CL. 1988. Glue and gelatin. In Animal by-product processing. Ellis Horwood, New York, NY, USA. pp 132-157.
- Park JH, Choe JH, Kim HW, Hwang KE, Song DH, Yeo EJ, Kim HY, Choi YS, Lee SH, Kim CJ. 2013. Effects of various

extraction methods on quality characteristics of duck feet gelatin. Korean J Food Sci Anim Resour 33:162-169.

- Sarbon NM, Badii F, Howell NK. 2018. Purification and characterization of antioxidative peptides derived from chicken skin gelatin hydrolysate. Food Hydrocoll 85:311-320.
- Tümerkan ETA, Cansu Ü, Boran G, Mac Regenstein J, Özoğul F. 2019. Physiochemical and functional properties of gelatin obtained from tuna, frog and chicken skins. Food Chem 287:273-279.
- Warner RD, Kauffman RG, Greaser ML. 1997. Muscle protein changes post mortem in relation to pork quality traits. Meat Sci 45:339-352.
- Zhang L, Shan Y, Hong H, Luo Y, Hong X, Ye W. 2020. Prevention of protein and lipid oxidation in freeze-thawed bighead carp (*Hypophthalmichthys nobilis*) fillets using silver carp (*Hypophthalmichthys molitrix*) fin hydrolysates. LWT-Food Sci Technol 123:109050.