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Comparison of Three Commercial Collagen Mixtures: Quality Characteristics of Marinated Pork Loin Ham

Running title: Effects of collagen mixtures on marinated loin ham

Abstract

Various commercial collagen mixtures aimed at improving the quality of meat products are available, but the optimal composition is unclear. This study aimed to compare the functional properties, including physicochemical characteristics and lipid oxidative stability, of loin ham marinated with three commercial collagen mixtures sold as food additives. The addition of collagen mixtures led to significant increases in the moisture content, water holding capacity, cooking yield, and instrumental tenderness, regardless of the type of collagen mixture. In particular, meat samples containing collagen mixture C showed the highest ($p<0.05$) water holding capacity and tenderness among all groups. Furthermore, collagen mixture B induced increases ($p<0.05$) in pH values in both raw and cooked samples. The a^* values of samples with collagen mixtures were lower ($p<0.05$) than those of samples without collagen mixtures. All collagen mixtures effectively improved oxidative stability during 7 days of storage at 4℃. The samples containing collagen mixture B had the lowest lipid oxidation ($p<0.05$) among groups. These results indicated that collagen mixture C could be used in injection brine to

28 enhance the quality characteristics of meat products, particularly the water holding capacity and
29 tenderness. Collagen mixture A could be used for meat products with high fat contents based
30 on its ability to improve lipid oxidative stability during long-term storage.
31 Keywords: collagen mixture, marinade ingredients, ham, quality characteristics
32
33

Introduction

The consumption of marinated meat has recently increased as consumer and retailer demand for ready-to-eat and convenience foods has increased. Marinade solutions commonly contain water, salt, and/or other functional ingredients with water-binding, tenderization, and flavor enhancement ability and/or antimicrobial or antioxidative activity (Alvarado and McKee, 2007). The quality characteristics of marinated meat can be affected by the type of solution, method, time, and temperature of marination. Injection and tumbling is a widely used approach to improve the quality properties of meat products; the marinade solution is uniformly dispersed into muscles for the effective extractability and solubility of myofibrillar proteins (Fidel Toldrá, 2010; Gamage et al., 2017; Kim et al., 2005). As a major ingredient in marinade solutions, salt increases the solubility of myofibrillar proteins and ionic strength of myofibrils (Wu and Smith, 1987), thereby improving the water retention or holding ability and tenderness of final meat products (Aktaş et al., 2003). Various additives, including inorganic salts, phosphates, and calcium chloride, are typically used in marination solutions (Lawrence et al., 2003). However, to meet consumer demand, ingredients derived from natural sources, such as kiwi, fig, pear, and ginger, have been used to improve the quality properties of marinated meat (Choe and Park, 1996; Park et al., 1999; Pawar et al., 2007).

Collagen contains approximately 30% protein and is widely used for the preparation of meat products owing to its functionality, including its effects on texture, water-binding ability,

adhesion, and cohesion (Gomez-Guillen et al., 2011). Previous studies have shown that collagen or collagen mixtures with other functional ingredients improve the water holding capacity (WHC) in cured ham, reduced fat sausage, and chicken nuggets (Choe and Kim, 2019; Kim et al., 2015; Schilling et al., 2003). For these reasons, commercial collagen mixtures from various sources, especially those derived from pork, are widely sold as food additives. However, to our knowledge, studies of the effects of commercial collagen mixtures composed of collagen, carrageenan, isolated soy protein, whey protein, and other components on injected/tumbled meat are lacking. In this study, we compare the functional effects of three commercial collagen mixtures on quality characteristics, including the proximate composition, pH, cooking yield, WHC, cooking loss, shear force, and color, of marinated pork loin using injection and tumbling. In addition, changes in lipid oxidation in the meat samples injected with three commercial pork collagen mixtures were examined at days 0 and 7 of refrigerated storage.

Materials and Methods

Preparation of loin samples injected with marination solution

Fresh pork loins were purchased from a local market (Seoul, Korea). After removing the subcutaneous and intramuscular fat and visible connective tissue, the loins were cut into 16 slices of equal weights (approximately 200 g) and sizes (height 15 cm). Three commercial

collagen mixtures (A, 20% pork collagen, 30% isolated soy protein, 30% konjac, and 12% carrageenan, and 8% guar gum; B, 20% pork collagen, 30% L-lysine monohydrochloride, 20% maltodextrin, 20% whey protein, 5% inulin, and 5% tapioca starch; C, 40% pork collagen, 30% L-lysine monohydrochloride, 20% whey protein, 8% maltodextrin, and 2% tapioca starch) were purchased from different companies (Gyeonggi-do, Korea). The composition (w/w) of the marinade solution was 93.6% water and 6.4% nitrite pickled salt (salt: nitrite = 99.4:0.6) for the control. For treatment groups, each commercial collagen mixture (A, B, or C; marinade solution : collagen mixture = 25 : 1) was completely dissolved in the marinade solution at 45℃ under mild stirring. The solution was injected into each slice of pork loin at a ratio of meat: solution of 10:2 (w/w) using an injector (PR8; RÜ HLE GmbH, Grafenhausen, Germany). The optimal amounts for injection were determined in our preliminary study. The injected pork slices were placed in plastic bags and intermittently tumbled for 90 min (45 min on, 15 min off) at 1 ± 1℃ in a tumbler (MKR150; RÜ HLE GmbH). After tumbling half of the raw sample was collected to determine the pH value, cooking yield, and color. The other part was dried at 60 ± 1℃ for 30 min, smoked at 65 ± 1℃ for 30 min, and cooked at 80 ± 1℃ for 30 min to reach an internal temperature of 72℃. For the lipid oxidation analysis, samples were stored at 3 ± 1℃ for 7 days.

Proximate composition

The proximate composition of each sample was analyzed as described by Lee et al. (2018) following standard AOAC (2012) methods.

pH values

The pH values were measured in a homogenate prepared with 4 g of meat sample and distilled water (16 mL) using a pH meter (Model S220; Mettler-Toledo, Greifensee, Switzerland). All determinations were performed in triplicate.

Water holding capacity (WHC)

The WHC of each sample was measured following the methods of Grau and Hamm (1953), with modifications. In brief, 300 mg of sample was placed on Whatman No. 2 filter paper and then pressed for 3 min with constant pressure using a binate plexiglass plate. Outer and inner sections were measured using a planimeter (Planix 7; Tamaya Technics Inc., Tokyo, Japan) to evaluate exuded moisture and meat, respectively. The ratio between the inner and outer section was defined as the WHC (%).

Cooking yield

Cooking yield was determined for individual samples by calculating the weight before and after cooking as follows:

Cooking yield (%) = [weight of cooked meat sample (g)/weight of raw meat sample (g)] × 100

Shear force measurement

For the shear force values of the cooked samples were determined using a Warner–Bratzler attachment on a texture analyzer (TA-XT2i; Stable Micro Systems Ltd., Godalming, UK). Test speeds were set to 2 mm/s. Data were collected and the shear force values (kg) were used to obtain the maximum force required to shear each sample.

Instrumental color

The colors of raw and cooked meat samples were determined using a colorimeter (CR-10; Minolta, Tokyo, Japan; illuminate C, calibrated with a white plate, CIE L^* = +97.83, CIE a^* = −0.43, CIE b^* = +1.98). Lightness (CIE L^* value), redness (CIE a^* value), and yellowness (CIE b^* value) values were recorded.

Determination of thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was assessed using the direct-distillation method as described by Tarladgis et al. (1960), with minor modifications. Samples were analyzed at days 0 and 7 of storage at 4℃ in triplicate. Each sample (10 g) was blended with 97 mL of distilled water prior to homogenization (AM-7; Nihon Seiki Kaisha Ltd., Tokyo, Japan) for 2 min and transferred to a distillation flask. Then, 2.5 mL of 4 N HCl and a few drops of an antifoaming agent, silicone o/w (KMK-73/ Shin-Etsu Silicone Co., Ltd., Seoul, Korea), were added. The mixture was distilled and 50 mL of distillate was collected. After filtration through Whatman No. 1 filter paper, 5 mL of extract was added to 5 mL of 0.005 mol L^{-1} 2-thiobarbituric acid and heated at 100℃ for 10 min. After cooling on ice, absorbance was measured at 532 nm and TBARS was calculated as mg of malonaldehyde per kg of sample.

Statistical analysis

The proximate composition, pH value, WHC, cooking yield, shear force, instrumental color, and TBARS were analyzed using two-way analysis of variance (ANOVA) and Duncan's multiple range test implemented in SAS (Release 8.01; SAS Institute Inc., Cary, NC, USA). The results were considered significant if $p < 0.05$ and values are expressed as means ± standard error. In addition, for pH values, instrumental color, and TBARS, the difference between raw and cooked samples or between the initial and final storage period within each

group was tested with the independent samples *t*-test.

Results and Discussion

Effects of three commercial collagen mixtures on proximate composition

The addition of collagen mixtures significantly increased the moisture content of marinated samples, regardless of the mixture type (Table 1). This result was consistent with previous results indicating that collagen immobilizes water during cooking, thereby increasing the moisture content in meat products (Daigle et al., 2005; Schilling et al., 2003). In addition, the ingredients of each collagen mixture including konjac, carrageenan, and tapioca starch could help enhancement in water retention ability of each sample as exhibiting gel formation (Chin et al., 2009; Desmond et al., 1998; Hinrichs et al., 2003). Crude protein, fat, and ash contents of marinated samples were not affected ($p>0.05$) by the addition and type of collagen mixtures.

Effects of three commercial collagen mixtures on pH values, water holding capacity, and cooking yield

The pH value of raw meat is an important determinant of the water retention/holding capacity. Raw meat with pH values of <5.7 yield final products with low WHC due to reduced electrostatic repulsion between proteins (Aktaş et al., 2003). In this study, raw meat supplemented with various collagen mixtures exhibited pH values of 5.73 to 5.91, which are

165 acceptable values for meat manufacturing (Table 2). The addition and type of collagen mixtures
166 did not influence ($p>0.05$) the pH values of both raw and cooked meat samples, except for the
167 sample with collagen mixture B. The addition of collagen mixture B led to the highest ($p<0.05$)
168 pH values for both raw and cooked meat samples among all groups. In this study, using collagen
169 mixture B (Table 2), the cooking yield and WHC were not substantially affected by the pH
170 value of raw pork. Regardless of the pH value of raw meat, the addition of the collagen mixture
171 significantly increased the cooking yield and WHC compared to those of samples without the
172 collagen mixture. The type of collagen mixture significantly influenced the WHC ($p < 0.05$); in
173 particular, the WHC was highest in meat samples containing collagen mixture C, which had an
174 intermediate pH value. This observation probably reflects the high level of pork collagen in
175 collagen mixture C, which can influence gel formation by the absorption of water during
176 thermal treatment (Osburn and Mandiso, 1998). According to Sosulski and McCurdy (1987),
177 protein enhances the gelation and swelling of muscle-based food products with high WHC. The
178 different components (non-meat ingredients) of the three commercial collagen mixtures may
179 affect the WHC. Previous studies have reported that collagen reduces syneresis in the final
180 product as non-meat protein, including isolated soy protein and transglutaminase, promote
181 water retention in interstitial spaces of the gel matrix (Pietrasik et al., 2006; Prestes et al., 2013).
182 In addition, the combination of collagen and non-meat ingredients enhance stability during heat
183 treatment and subsequent results in high cooking yields (Pietrasik et al., 2006).

184 **Effects of three commercial collagen mixtures on instrumental tenderness**

185 The instrumental tenderness of meat samples varied depending on the addition and type
186 of collagen mixtures (Fig. 1). In detail, the highest ($p<0.05$) and lowest ($p<0.05$) values were
187 observed in meat samples with collagen mixture C and without a collagen mixture, respectively.
188 Collagen mixture C enhanced the tenderness (24.7%) of meat samples compared to that of
189 collagen mixture-free samples. The improvement in tenderness might be explained by the
190 increase in the moisture content of the meat samples based on of the WHC results (Table 2).
191 Increased juiciness of meat products is associated with increased tenderness (Lee et al., 2018).

192

193 **Effects of three commercial collagen mixtures on instrumental color**

194 The color of meat and meat products is an important factor for consumer purchasing
195 decisions. In this study, the addition and type of collagen mixture did not influence ($p>0.05$)
196 L*, a*, and b* values of meat samples (Table 3). After cooking, L*, a*, and b* values increased
197 depending on the type of collagen mixture (Table 3). The addition of collagen mixtures led to
198 significant increases in the L* values of cooked samples, except in the group with collagen
199 mixture A. The addition of non-meat ingredients to meat products can lead to increases in
200 lightness (Choe and Kim, 2018). Control samples had higher ($p<0.05$) a* values than those of
201 samples with collagen mixtures. This observation may be explained by the intrinsic color of the
202 collagen mixture itself, as observed by Choe and Kim (2018). No significant difference in b*

values among cooked groups was observed, except in the group with collagen mixture B.

Effects of three commercial collagen mixtures on lipid oxidation during storage

TBARS levels indicate the amount of secondary lipid oxidation products, including aldehydes, and carbonyls, which cause the development of a rancid flavor (Choe et al., 2019). In this study, there were no significant differences in TBARS at the initial day of storage among groups, ranged from 0.049 to 0.058 mg MDA/kg meat samples (Fig 2). After 7 d of storage, TBARS levels increased ($p<0.05$) in all groups and the samples containing collagen mixtures showed the lower ($p<0.05$) lipid oxidation levels compared to sample with no collagen mixture. Especially, the samples injected with collagen mixture A had the lowest ($p<0.05$) values in TBARS. This result may be due to the presence of bioactive amino acids in collagen; this explanation is supported by previous results indicating that amino acids possessing antioxidant activity, including arginine, histidine, and methionine, are present in gelatin (collagen hydrolysate) (Alemán et al., 2011). Additionally, many researchers have attempted to retard lipid oxidation in injected meat products by adding natural ingredients. Jongberg et al. (2018) found that the incorporation of green tea and mate extracts into injection brine reduces lipid oxidation of injected chop samples during chilled storage for 7 days. Armentero et al. (2016) reported that cooked loin ham injected with a mixture of garlic, cinnamon, cloves, and rosemary shows a dramatic increase in stability against lipid oxidation. The difference in TBARS levels

among the groups was probably caused by antioxidant activity of various component of collagen mixtures.

Conclusion

In this study, we compared the functional effects of three commercial collagen mixtures on quality characteristics, including the proximate composition, pH, cooking yield, WHC, cooking loss, shear force, color, and lipid oxidative stability, of injected/tumbled loin ham. Our results indicated that collagen mixture C and collagen mixture A injected in brine could be used to improve quality characteristics and lipid oxidative stability, respectively, as possessing either greater WHC and tenderness or lipid oxidative stability. Further studies should evaluate the protein retardation effect of each commercial collagen mixture.

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Figure legends

Fig. 1. Shear force of pork loin injected with three different commercial collagen mixtures. Bars indicate standard error. [a-c]Values with different letters on the bar are significantly different ($p<0.05$). [1)]Control, samples injected with no collagen mixture; CM-A, samples injected with commercial collagen mixture A; CM-B, samples injected with commercial collagen mixture B; CM-C, samples injected with commercial collagen mixture C.

Fig. 2. Thiobarbituric acid reactive substances (TBARS) levels of pork loin injected with three different commercial collagen mixtures. Bars indicate standard error. [a-c]Values with different letters on the bar are significantly different ($p<0.05$). [1)]Control, samples injected with no collagen mixture; CM-A, samples injected with commercial collagen mixture A; CM-B, samples injected with commercial collagen mixture B; CM-C, samples injected with commercial collagen mixture C.

328 **Table 1. Proximate composition of pork loin injected with three different commercial**
329 **collagen mixtures**

Traits (%)	Control[1)]	Type of commercial collagen mixture		
		A	B	C
Moisture	66.89±0.69[b]	69.32±0.24[a]	69.73±0.58[a]	70.45±0.47[a]
Protein	23.13±1.57	23.01±1.37	24.26±0.88	23.68±0.84
Fat	5.07±0.24	4.05±0.33	4.06±0.37	3.85±0.62
Ash	1.92±0.09	2.05±0.03	2.13±0.11	2.12±0.06

330 All values are mean ± standard error of four replicates.

331 [a-c]Values with different letters on the bar are significantly different ($p<0.05$). [1)]Control, samples injected
332 with no collagen mixture; CM-A, samples injected with commercial collagen mixture A; CM-B, samples
333 injected with commercial collagen mixture B; CM-C, samples injected with commercial collagen
334 mixture C.
335
336

Table 2. The pH values and water holding capacity, and cooking yield of pork loin injected with three different commercial collagen mixtures

Traits		Control[1)]	Type of commercial collagen mixture		
			A[1)]	B	C
pH	*Raw*	5.79±0.01^{by}	5.70±0.02^{by}	5.91±0.02^{ay}	5.73±0.01^{by}
	Cooked	5.92±0.02^{bx}	5.92±0.02^{bx}	6.13±0.01^{ax}	5.95±0.01^{bx}
Water holding capacity (%)		31.09±1.35^{c}	52.73±3.52^{b}	52.41±3.67^{b}	62.63±2.46^{a}
Cooking yield (%)		75.52±0.92^{a}	78.70±0.52^{b}	80.10±1.68^{b}	81.62±1.29^{b}

All values are mean ± standard error of four replicates.

$^{a\text{-}c}$Values with different superscript letters within the same row differ significantly ($p<0.05$).

^{x,y}Values with different superscript letters within the same column differ significantly ($p<0.05$).

[1)]Control, samples injected with no collagen mixture; A, samples injected with commercial collagen mixture A; B, samples injected with commercial collagen mixture B; C, samples injected with commercial collagen mixture C.

Table 3. Instrumental color profile of pork loin injected with three different commercial collagen mixtures

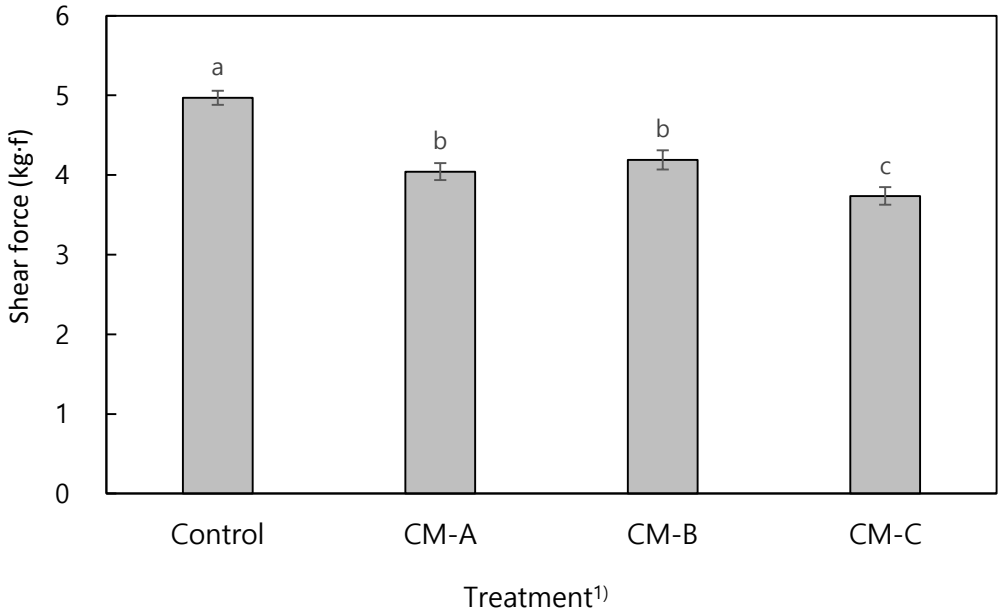
Traits		Control	Type of commercial collagen mixture		
			A	B	C
Raw	CIE L*	55.0±0.73^{x}	55.28±1.51^{x}	54.37±1.06^{x}	54.49±1.18^{x}
	CIE a*	6.85±0.49^{y}	7.12±0.36^{y}	7.24±0.24^{y}	7.16±0.27^{y}
	CIE b*	4.67±0.35^{y}	4.93±0.32^{y}	4.69±0.20^{y}	4.72±0.29^{y}
Cooked	CIE L*	47.05±0.77^{by}	47.47±0.78^{bx}	49.81±0.59^{ay}	51.06±0.62^{ay}
	CIE a*	17.45±0.37^{ax}	15.71±0.38^{bx}	16.10±0.27^{bx}	16.01±0.31^{bx}
	CIE b*	36.55±0.30^{ax}	35.60±0.28^{ax}	33.67±0.37^{bx}	35.46±0.34^{ax}

All values are mean ± standard error of four replicates.

^{a,b}Values with different superscript letters within the same row differ significantly ($p<0.05$).

^{x,y}Values with different superscript letters within the same column differ significantly ($p<0.05$).

$^{1)}$Control, samples injected with no collagen mixture; A, samples injected with commercial collagen mixture A; B, samples injected with commercial collagen mixture B; C, samples injected with commercial collagen mixture C.



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358 **Fig. 1. Shear force of pork loin injected with three different commercial collagen mixtures.**

359 Bars indicate standard error. [a-c]Values with different letters on the bar are significantly different ($p<0.05$).

360 [1)]Control, samples injected with no collagen mixture; CM-A, samples injected with commercial collagen

361 mixture A; CM-B, samples injected with commercial collagen mixture B; CM-C, samples injected with

362 commercial collagen mixture C.

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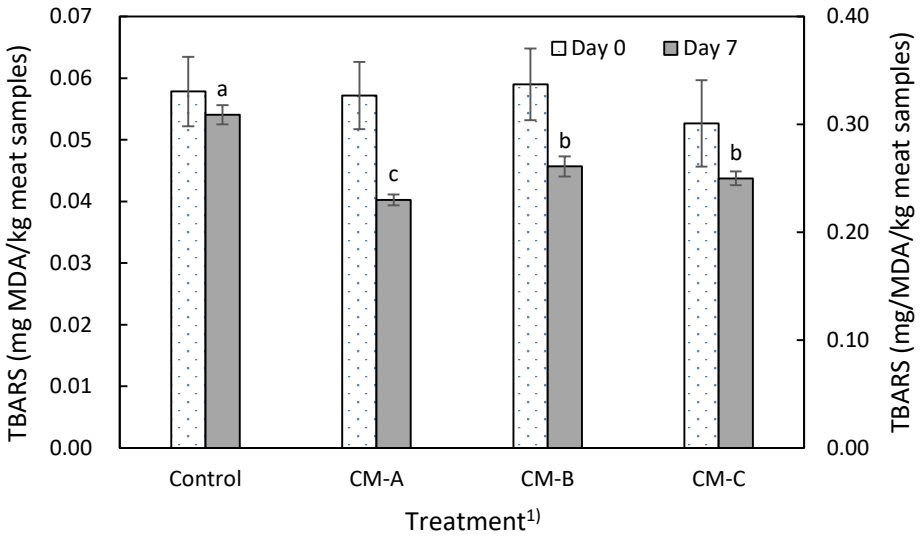

364

365 **Fig. 2. Thiobarbituric acid reactive substances (TBARS) levels of pork loin injected with**

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