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Effects of Whey Protein Injection as a Curing Solution on Chicken Breast Meat

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Abstract The quality characteristics and storage stability of chicken breast meat (CBM) was investigated following the injection of whey protein (WP) as a curing ingredient. The moisture content of CBM decreased with increasing concentration of WP. The highest concentration of WP (7%) resulted in the lowest moisture and fat content and the highest protein content of CBM. Injection of WP elevated the pH and water holding capacity (WHC) of CBM. The cooking loss of CBM was significantly decreased with WP injections of 3% and higher. All WP injections increased the L* of the CBM but decreased the a* and b*. WP injection increased the springiness, cohesiveness, and chewiness and decreased the hardness of the CBM. WP injection increased 2-thiobarbituric acid reactive substances (TBARS) after 3 and 7 days of storage. The volatile basic nitrogen (VBN) content of the CBM increased with increased concentrations of WP. The total microbial count (TMC) of CBM injected with WP was higher initially and after 3 days of storage. Our results showed WP injection improved the WHC of CBM but decreased the storage stability by increasing TBARS, VBN and TMC.

Keywords chicken breast meat, whey protein, quality characteristics, storage stability

Introduction

Economic growth and an improved standard of living in Korea have contributed to an increased focus on personal health (Lee et al., 2018). Korean's diets, food preferences, and consumption patterns are highly dependent on nutritional information, which is a crucial factor in a healthy lifestyle. Koreans tend to select healthier processed meats when given the opportunity (Kim, 2011). Consumers recognize there are other high-quality protein sources in addition to meats and processed meats (Chung et al., 2018).

In Korea, the annual per capita consumption of chicken has increased from 5.98 kg in 1995 to 13.8 kg in 2016, and further increases are expected (Korea Meat Trade Association [KMTA], 2016). In 2017, chicken meat (13.3 kg) was the second most highly preferred and consumed meat in Korea after pork (24.5 kg) (Ministry of Agriculture Food and Rural Affairs, 2018). Recently there has been an increase in the consumption of portion cuts of meat, including breast, thigh, wing, and drumstick, as opposed to whole chickens. Korean consumers prefer the tender cuts of chicken, such as the legs and wings rather than the drier breasts. Preferences for particular cuts of chicken have considerable influence on imports. The number of chicken legs and wings imported (100,596 tons) was substantially higher than the number of chicken breasts imported (1,966 tons) (KMTA, 2016). Producers have attempted to physically and chemically modify chicken breast meat (CBM) to align with consumer preferences through tumbling, massaging, marinating, and curing by injection (Alvarado and McKee, 2007). CBM has generally been ground and used to make sausages or reconstructed ham. CBM consists of white muscle containing ~2% fat and ~20% protein, and fewer calories (109 kcal) than other meat and meat portion sources (Barbanti and Pasquini, 2005). CBM products are economically and industrially favorable. However, dryness and blandness have reduced consumer acceptance.

Whey protein (WP) is a nutritious soluble protein consisting of α -lactalbumin and β -lactoglobulin. It is a source of amino acids and performs a regulatory function as a biological reagent (Renner and Abd-EL-Salam, 1991; Moon and Jung, 2010). WP is used to increase the emulsification, solubility, bubble formation, water holding capacity (WHC), gelatinization, and viscosity of various foods, including meat products. Furthermore, WP can decrease the shear force and modify texture (Tungland and Meyer, 2002). WP has been added to processed meats (Cofrades et al, 2000), fermented foods, and functional foods (Kulmyrzaev et al., 2000; Moon, 2006) and is utilized to improve the overall quality of processed meats, reconstructed meats, coarse ground meats, comminuted meats, low-fat meats, and surimi (Youssef and Barbut, 2010). However, there are few studies on the effects of WP injection into meats as a curing solution, and its contribution to the overall quality and storage stability of CBM. This study was designed to investigate the quality and storage stability of CBM injected with WP. The data from this study will provide basic information for the development of CBM-based products that meet consumer needs.

Materials and Methods

Formulation and processing procedure

The five different CBM formulations used are provided in Table 1. Fifty 28-d-old Ross CBMs were purchased from Cheongam Food (Jeungpyeong, Korea) and trimmed to remove the skin, visible fat, and fascia. The CBMs were weighed and injected with a 30% curing solution (w/w) containing WP (Agri-mark Inc., Andover, ME, USA) using an injector (LJZ02-2, Leeseoph, Beijing, China). Curing solutions were prepared with various concentrations of WP [0% (control), 1% (WP1), 3% (WP3), 5% (WP5), and 7% (WP7); w/w; n=10]. Each group was analyzed for the experiments after vacuum-packing and storage at 4°C for 24 hours. All the data was reported as a mean with standard deviation.

Proximate composition

Moisture, protein, fat, and ash contents of the CBM were assessed according to standard method (AOAC, 2012; Bae et al., 2019).

Table 1. Chicken breast meat formulations by the injection of whey protein (WP)

	Treatments ¹⁾				
	C	WP1	WP3	WP5	WP7
Water	95.80	94.80	92.80	90.80	88.80
WP	-	1.00	3.00	5.00	7.00
Salt			3.33		
Sugar			0.87		
Total	100	100	100	100	100

¹⁾ Water, WP, salt, and sugar were added as percentage units of the sum of the major ingredients. C, no WP added control; WP1, WP3, WP5, and WP7, added 1%, 3%, 5%, and 7% of WP.

pH

Ten grams of each CBM sample was dissolved in 100 mL of distilled water and homogenized using a Stomacher (400 Lab blender, Seward, London, UK). The pH values were recorded using a standardized pH meter (WTW pH 720, Weilheim, Germany).

Meat color and water holding capacity (WHC)

The color and WHC of the CBM was assessed by a previously described methods (Bae et al., 2019).

Cooking and drip loss

Each CBM sample (approximately 4 cm cube) was vacuum-packed into a polypropylene bag and cooked in a water bath at 70°C for 40 min and cooled down to room temperature for 30 min to determine the cooking loss (%). The cooking loss (%) was determined by the following equation: [(initial CBM sample weight–CBM weight after cooking)/initial CBM sample weight]×100. To determine the drip loss (%), the vacuumed-packed CBM samples were refrigerated for 1 day. The drip loss (%) was determined by the following equation: [(initial CBM sample weight–CBM weight after storage)/initial CBM sample weight]×100.

Texture profile analysis (TPA)

For the TPA analysis, each CBM sample was cooked and cooled as described in cooking loss. TPA values were determined from the minced approximately 1 cm cubes using a rheometer (Sun Scientific Co., Tokyo, Japan, with Rheology data systems version 3.0 for Windows) using a constant table speed (60 mm/min) and 2 kg maximum load cell capacity.

Volatile basic nitrogen (VBN), 2-thiobarbituric acid reactive substances (TBARS) and total microbial count (TMB)

The VBN, TBARS and TMB values were measured using a set of standardized in-house experimental methods (Bae et al., 2019).

Statistical analysis

The data were tested by analysis of variance using the SAS program (2012) with the general linear model, and the

difference between samples were analyzed using Duncan's multiple range test.

Results and Discussion

Proximate analysis

The proximate analysis results of the CBM according to the WP injection concentration are presented in Table 2. The moisture content of the CBM samples with WP injection was 77.54%–78.26%. The water content decreased with increasing concentration of WP injected (WP1, WP5 and WP7), with the lowest water content observed when 7% WP (WP7) was injected. This result is expected because the water content in formulation was decreased by increase of WP percentage. A previous study also showed that increased WP addition into meatballs reduced the moisture content (Serdaroglu, 2006). The protein content was significantly increased in the WP7-treated CBM. Hughes et al. (1998) also observed that WP addition increased the protein content in Frankfurter sausages. The fat content of the CBM samples decreased significantly when the WP injection exceeded 5% (WP5 and WP7). The ash content of the control and WP-treated groups ranged from 0.98 to 1.30, and there was no statistical difference between the control and treatment groups. Injection of curing solutions containing WP increased the amount of protein in CBM. WP consists of various proteins such as α -lactalbumin, β -lactoglobulin, serum albumin and immunoglobulin (Farrell et al., 2004). Although leaner meat is generally preferred, some countries such as Korea and Japan are not (Ngapo et al., 2007). Therefore, a sensory evaluation study may necessary to understand the acceptability, flavor, taste and other characteristics of WP-injected CBM containing higher protein.

Quality characteristics

The quality characteristics of CBM according to WP injection are summarized in Table 3. The injection of WP into CBM significantly increased the pH value regardless of the concentration of injected WP; WP3 and WP7 displayed the highest pH.

Table 2. Proximate analysis of chicken breast by the injection of whey protein (WP)

Item	C	WP1	WP3	WP5	WP7
Moisture	78.76±0.23 ^a	78.20±0.33 ^b	78.20±0.15 ^b	78.53±0.21 ^{ab}	77.54±0.13 ^c
Protein	18.56±0.50 ^b	19.20±0.41 ^b	18.73±0.07 ^b	19.17±0.16 ^b	20.27±0.15 ^a
Fat	1.59±0.25 ^{ab}	1.31±0.31 ^{bc}	1.85±0.03 ^a	0.95±0.14 ^c	0.98±0.18 ^c
Ash	0.98±0.38	1.27±0.13	1.21±0.05	1.30±0.05	1.20±0.09

^{a-c} Mean±SD with different superscript letters indicating significant differences ($p<0.05$).

C, no WP added control; WP1, WP3, WP5, and WP7, added 1%, 3%, 5%, and 7% of WP.

Table 3. pH, WHC, cooking loss, and drip loss of chicken breast meat by the injection of whey protein (WP)

Item	C	WP1	WP3	WP5	WP7
pH	6.15±0.01 ^c	6.21±0.03 ^b	6.28±0.01 ^a	6.22±0.01 ^b	6.26±0.01 ^a
WHC	58.52±1.90 ^b	61.19±2.30 ^{ab}	62.73±2.36 ^a	62.30±0.90 ^a	62.26±0.35 ^a
Cooking loss	16.71±0.70 ^a	14.72±0.73 ^{ab}	13.48±3.02 ^b	13.53±1.41 ^b	11.63±1.69 ^b
Drip loss	1.26±0.23	1.29±0.40	1.30±0.11	1.27±0.09	1.33±0.14

^{a-c} Mean±SD with different superscript letters indicating significant differences ($p<0.05$).

C, no WP added control; WP1, WP3, WP5, and WP7, added 1%, 3%, 5%, and 7% of WP; WHC, water holding capacity.

In a previous supportive study, the pH of turkey breast meat was also elevated upon the addition of different concentrations of WP (Sammel and Claus, 2003). WP is a surface-active globular protein that enhances WHC, gelatinization, and emulsification, and has the potential to stabilize lipid globules in food (Huffman, 1996; Sammel and Claus, 2003; Sun et al., 2007). The calculated WHC ranged from 58.52 (control) to 62.73 in CBM. The WHC was significantly increased when CBM contained over 3% WP (WP3, WP5, and WP7). Moreover, the injection of WP into CBM dramatically decreased the level of cooking loss when the concentration of WP injected was higher than 3% (WP3, WP5, and WP7). The drip loss in control and WP-injected CBM ranged from 1.26 to 1.33, which was not statistically different regardless of WP injection percentage. The pH level is an excellent indicator of the potential meat quality during meat processing. An increase in the pH in meat influences the texture profile and meat color by improving WHC. Consequently, the sensory evaluation of the meat and the meat quality may be potentially affected by characteristics such as lipid oxidation or protein degradation. The increase in pH and the gel-forming ability that occurs upon addition of WP (Lazidis et al., 2016) is considered to improve the WHC and, thus, possibly reduces cooking loss in CBM. In a previous study, the pH of turkey breast meat was elevated by the addition of different concentrations of WP (Sammel and Claus, 2003). In addition, Serdaroglu and Ozsumer (2003) also reported similar observations in a study using meatballs.

Instrumental color and pigment determination

The color of WP-treated CBM are presented in Table 4. The lightness value (L^*) was higher (brighter) in all WP-injected groups than in the control. However, there was no difference in L^* among the different concentrations of WP. The a^* decreased in a dose-dependent manner with the injection of WP to CBM. The b^* decreased significantly when over 5% WP was added to CBM. Our experimental findings support an earlier study which demonstrated that WP addition to beef or pork Frankfurter sausage increased L^* but decreased a^* (Atughonu et al., 1998). Another study reported that WP addition to meat products did not affect the b^* (Serdaroglu et al., 2006). However, presently the b^* gradually decreased as the WP concentration in CBM increased. A previous study (Andres et al., 2006) compared the meat color in CBM sausage with addition of 0.64% and 1.94% WP. While previous studies demonstrated no significant differences in the brightness and the redness, 1.94% WP significantly increased the yellowness in comparison with 0.64% WP group. These results also support our experimental findings. Moreover, Youssef and Barbut (2010) showed that the addition of 1.5% WP significantly increased the brightness and decreased the redness of the meat while simultaneously increasing the hardness of meat (Atughonu et al., 1998).

Texture profile analysis (TPA)

TPA data (springiness, cohesiveness, chewiness, and hardness) after WP injection are presented in Table 5. The

Table 4. Color of chicken breast meat by the injection of whey protein (WP)

Item	C	WP1	WP3	WP5	WP7
L^*	55.23±2.16 ^b	61.92±1.48 ^a	62.96±2.74 ^a	62.83±1.61 ^a	62.32±1.36 ^a
a^*	3.47±0.98 ^a	1.27±0.13 ^b	0.99±0.16 ^{bc}	0.49±0.17 ^{bc}	0.38±0.20 ^c
b^*	6.54±1.84 ^a	6.63±0.67 ^a	5.61±0.79 ^{ab}	4.74±0.78 ^b	4.88±0.62 ^b

^{a-c} Mean±SD with different superscript letters indicating significant differences ($p < 0.05$). C, no WP added control; WP1, WP3, WP5, and WP7, added 1%, 3%, 5%, and 7% of WP.

Table 5. Texture profile analysis of chicken breast meat by the injection of whey protein (WP)

Item	C	WP1	WP3	WP5	WP7
Springiness (%)	40.10±2.37 ^b	55.22±2.38 ^a	52.05±3.97 ^a	54.85±3.16 ^a	54.13±2.38 ^a
Cohesiveness (%)	34.41±3.40 ^b	45.22±3.19 ^a	37.81±4.02 ^{ab}	44.07±7.45 ^a	37.56±7.03 ^{ab}
Chewiness (g)	183.7±29.08 ^b	269.48±33.52 ^a	168.73±14.70 ^b	280.2±27.83 ^a	275.09±22.20 ^a
Hardness (kg)	1.43±0.23 ^a	1.03±0.05 ^b	1.04±0.07 ^b	1.19±0.36 ^{ab}	1.20±0.16 ^{ab}

^{a,b} Mean±SD with different superscript letters indicating significant differences ($p<0.05$).

C, no WP added control; WP1, WP3, WP5, and WP7, added 1%, 3%, 5%, and 7% of WP.

springiness was markedly elevated in all the CBM groups injected with WP (52.05–55.22) compared to the control (44.10). Cohesiveness and chewiness were enhanced in WP1 and WP5 compared to the control, but that of the WP3 and WP7 was similar to the control. We observed a trend of reduction in hardness with an increase in the concentration of the WP injected into CBM (1.03–1.20) compared to the control (1.43). Up to 3% WP (WP1 and WP3) injected into CBM considerably decreased the hardness compared to the control. WP is widely accepted as a texture-enhancing additive (Andres et al., 2006; Holland, 1984; Shie, 2004), and it has been reported that WP addition attenuates the hardness and chewiness but elevates the cohesiveness in sausages. Taken together, the introduction of WP into meat contributes to the formation of solid texture of the meat emulsion due to the alteration of gel-forming ability. However, the hardness of CBM significantly decreased after WP injection, which may imply that WP is retained in the moisture in CBM and can enhance the tenderness of the meat.

Storage stability

The storage characteristics of CBM based on the concentration of WP injection are shown in Table 6. The TBARS value on day 0 ranged from 0.11 to 0.15 mg malondialdehyde/kg meat protein with the WP3 group showing the highest value and WP1 showing the lowest (WP3>WP7>C=WP5>WP1). The TBARS value on day 3 was higher than that on day 0, with WP7 (0.46 mg malondialdehyde/kg meat protein) and WP5 (0.33 mg malondialdehyde/kg meat protein) showing higher TBARS values than the control (0.20 mg malondialdehyde/kg meat protein). The TBARS value on day 7 was also increased, and all

Table 6. Storage stability of the chicken breast meat by the injection of whey protein (WP)

Item	Storage (day)	C	WP1	WP3	WP5	WP7
TBARS (mg malondialdehyde /kg meat)	0	0.14±0.01 ^c	0.11±0.01 ^a	0.18±0.00 ^a	0.14±0.01 ^c	0.15±0.00 ^b
	3	0.20±0.01 ^c	0.26±0.03 ^{bc}	0.23±0.01 ^{bc}	0.33±0.01 ^b	0.46±0.21 ^a
	7	0.25±0.01 ^d	0.28±0.01 ^c	0.45±0.01 ^a	0.42±0.01 ^b	0.46±0.01 ^a
VBN (mg/100 g)	0	10.56±0.54 ^{ab}	9.79±0.31 ^b	10.88±0.41 ^a	10.98±0.27 ^a	10.24±0.57 ^{ab}
	3	17.11±0.15 ^e	23.28±0.96 ^a	18.62±0.63 ^d	22.19±0.31 ^b	19.99±0.31 ^c
	7	14.55±0.54 ^d	20.63±0.69 ^c	26.95±1.10 ^a	25.57±0.31 ^b	26.49±0.27 ^{ab}
TMC (CFU/g)	0	3.61±0.21 ^c	5.46±0.02 ^b	5.76±0.05 ^a	5.55±0.02 ^{ab}	5.48±0.01 ^b
	3	4.45±0.21 ^d	7.49±0.10 ^a	7.36±0.02 ^{ab}	6.94±0.04 ^c	7.09±0.05 ^{bc}
	7	7.15±0.10 ^{ab}	7.13±0.13 ^{ab}	7.51±0.03 ^a	6.80±0.28 ^b	7.23±0.05 ^a

^{a-d} Mean±SD with different superscript letters indicating significant differences ($p<0.05$).

C, no WP added control; WP1, WP3, WP5, and WP7, added 1%, 3%, 5%, and 7% of WP; TBARS, 2-thiobarbituric acid reactive substances; VBN, volatile basic nitrogen; TMC, total microbial count.

the WP-treated CBM had higher TBARS values (0.28–0.46 mg malondialdehyde/kg meat protein) than the control (0.25 mg malondialdehyde/kg meat protein). The initial storage VBN value on day 0 was higher in WP5 (10.98) than WP1 (9.79). The VBN values, expressed as mg/100 g, were significantly higher for all WP-injected CBM than the control on day 3 and 7 after storage. In the control group, the VBN values were 17.11 and 14.55 mg/100 g on day 3 and 7 after storage, respectively. In the WP-injected groups, the VBN values ranged between 18.62–23.28 mg/100 g (on day 3 of storage), and between 20.63–26.95 mg/100 g (on day 7 of storage), respectively. The guidelines from the Ministry of Food and Drug Safety (2002) state that the VBN content should be maintained below 20 mg/100 g, but the WP injection exceeded this limit. Cresopo et al. (1978) reported that, during storage, food proteins were degraded into smaller amino acids and produced inorganic nitrogen. In general, the VBN in fresh meat should be under 10–20 mg/100 g, and it is thought that early spoilage can change these values to 30–40 mg/100 g. Total microbial count (TMC) was higher in the WP-injected CBM than in the control initially and 3 days after storage. The TMC values for the control were 3.61 and 4.45 log colony forming units (CFU)/g on day 0 and day 3 after storage, respectively. In the WP-injected groups, the TMC values ranged between 5.46–5.76 Log CFU/g at initial storage and 6.94–7.49 Log CFU/g at 3 days after storage, respectively. The TMC values on day 7 after storage ranged between 6.8 Log CFU/g (WP5) and 7.23 Log CFU/g (WP7); the TMC value was significantly lower in WP5 than in WP3 and WP7. Lamkey et al. (1991), who reported that TMC values higher than 8 Log CFU/g indicated that the meat was in the spoilage stage caused by microbial activities.

Rajesh Kumar et al. (2007) reported that the addition of 20% WP into pork nuggets instead of water resulted in very low values of TBARS during a 28 day storage period. This observation reflected that the protein and lactose content of WP that can lead to the formation of antioxidants due the Maillard reaction upon heating (Pena-Ramos and Xiong, 2001). In addition, Pena-Ramos and Xiong (2003) reported that the addition of 2% WP into pork patty resulted in higher antioxidative activity compared to the control. However, WP injection in our study did not alter the antioxidative activity since WP was an extracted protein product.

Meat protein decomposes during storage due to microorganism- or enzyme-dependent breakdown. The VBN content is an important indicator to evaluate the freshness of meat or meat products. There was no difference in the VBN content between the control and WP-injected groups after the initial storage. However, after refrigeration, the VBN content increased markedly compared to control group on day 3 and 7. Hence, WP may play a role in protein decomposition of CBM, since WP-injected CBM had high concentrations of proteins such as α -lactalbumin, β -lactoglobulin, serum albumin, and immunoglobulin. In addition, the values of the total TMC were higher in WP-injected CBM than in the control CBM due to the proliferation of microorganisms-produced metabolites during storage. On the other hand, WP injected-groups showed higher level of the initial TMC, because WP may possess probiotics or other beneficial microorganisms.

Conclusion

The collective results indicate that the injection of WP-containing curing solution into CBM enhances WHC and textural quality. However, it may have negative effects on the storage properties. Further studies are needed to overcome the limitation of WP in storage stability, which is important for its industrial usage. Natural components, such as spices and herbs, have strong antimicrobial activity (Bae et al., 2019; Holley and Patel, 2005). Their addition to the WP solution may help alleviate the storage issue in WP-injected CBM.

Conflicts of Interest

The authors declare no potential conflict of interest.

Author Contributions

Conceptualization: Ha JH, Choi YI, Lee HJ. Data curation: Ha JH, Lee JH, Lee JJ, Choi YI, Lee HJ. Editing: Ha JH, Lee JH, Lee JJ, Choi YI, Lee HJ. Software: Lee JH, Lee HJ. Investigation: Ha JH, Lee JH, Choi YI, Lee HJ. Writing - original draft: Ha JH, Lee JH, Lee JJ, Choi YI, Lee HJ. Writing - review & editing: Ha JH, Lee JH, Lee JJ, Choi YI, Lee HJ.

Ethics Approval

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

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