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Improving tenderness of breast meat of spent-laying hens using marination in alkaline or acidic solutions

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Abstract

The current usage of spent-laying hen meat has been limited due to the toughness and non-juicy texture of its cooked meat. The objective of this research was to improve tenderness of spent-laying hen breast meat using alkaline or acidic solutions. Three hundred pieces of spent-laying hen breast meat were randomly arranged into 10 treatments including control (no marination), marinating with distilled water, sodium bicarbonate (SBC) (0.10, 0.15, 0.20, and 0.25 M), and lactic acid (LA) (0.05, 0.10, 0.15, and 0.20 M). SBC increased ($p < 0.05$) the pH of raw and cooked meats, whereas LA decreased ($p < 0.05$) the pH of those meats. High concentration of SBC and LA decreased ($p < 0.05$) lightness values on the surface of the cooked meat. A decrease in shear force values ($p < 0.05$) was found in the cooked meats with margination due to an increase in water-holding capacity (WHC). Microscopic images of cooked meat illustrated the porous structure in SBC marinated meat. In addition, the clear gaps between each muscle fiber were observed in cooked LA marinated meat. Muscle fiber diameter of the marinated samples was greater ($p < 0.05$) than those of the control group, indicating the swelling of muscle in the marinated samples. Therefore, marinations with SBC and LA increased tenderness of the cooked meat by either increasing WHC or changing muscle structure of the meat. Based on the current findings, solutions of 0.10 M LA and 0.15 M SBC can be potential marinades for improving tenderness of spent-laying hen breast meat.

Keywords: Spent-laying hen, Tenderness, Muscle protein, Marination

1. Introduction

In 2017, Thailand had a massive number of layer hens (approximately 58 million birds), and the number tends to increase every year [1]. These hens become spent-laying hens at the end of laying cycle (approximately 72 weeks of age). However, the utilization of spent-laying hen meat is still limited. The older age of spent-laying hens has a drastic effect on toughness and non-juiciness of the meat, resulting in the reduction of consumer acceptability. Therefore, the farmers are facing a problem in disposing their spent-laying hen meat, even when offered at a low price. Tenderness is considered to be the most important organoleptic characteristic of muscle food [2]. Theoretically, texture of spent-laying hen meat is very tough due to the tightly bound myofibrillar structure and the increasing cross-linkage in the connective tissue of the older animals [3]. One approach for improving tenderness of spent-laying hen meat can be achieved by using marination technology [4]. There are three methods for producing marinated products including immersion, injection, and tumbling. The tumbling method loosens the muscle structures, disrupts muscle cells and disintegrates the connection between the myofibers and the connective tissue to tenderize the meat [5]. Moreover, the tumbling method is employed for accelerating the extraction of meat proteins to the surface of meat pieces, enhancing water-holding capacity (WHC) of the meat [6]. The vacuum is applied using a tumbling process to enhance the penetration of marinade into the meat [7]. General ingredients in marinades are sodium chloride, phosphates and organic acids [8]. The functionality of the marinades directly depends on their ingredients. The most common and important ingredient of an alkaline marinade is phosphate [9]. Phosphate is usually used, in conjunction with sodium chloride, to increase WHC and reduce cooking loss in the meat process [10]. However, FAO/WHO organization has limited the use of phosphate in meat products due to its influence on consumer health [11]. Sodium bicarbonate is an alternative food ingredient option for alkaline marination. Yang and Petracci [12-13] reported that sodium bicarbonate could increase WHC and improve tenderness of pork and poultry meat. As for acidic marination, lactic acid, citric acid and acetic acid are usually used for improving meat qualities. Ke and Ergezer [14-15] reported that citric acid and acetic acid increased WHC and tenderness of beef. Aktas and coworkers [16] found that shear force values of lactic acid marinated beef samples were lower than those of the citric acid marinated beef samples. Moreover, Berge and coworkers [17] also reported that injection of 0.5 M of lactic acid into a collagen-rich beef muscle (*M. pectoralis profundus*) could reduce toughness of meat and increase the consumer acceptance score in tenderness. The overall goal of this project was to improve texture of spent-laying hen meat using marination. Regarding the composition and structure of spent-laying hen meat, it can be hypothesized that tenderization of the meat can be achieved by increasing WHC and changing the muscle structure of the meat using either alkaline or acidic marination. To test the hypothesis the effects of alkaline and acidic marinations on texture, WHC, and microstructure of spent-laying hen breast meat were investigated.

2. Materials and methods

2.1 Sample collection

Breast meat samples in this study were kindly obtained from a commercial farm (Sun Food International Co. Ltd., Saraburi, Thailand). The spent-laying hens (70-75 weeks old Lohmann Brown) were slaughtered and conventionally processed at a local slaughter-house. The breast meat (approximately 2 kg) was individually vacuum-packed in a polyethylene bag and stored at −18 ˚C until use for the study. Before any experiment, the meat was thawed overnight at 10 ˚C. The sodium bicarbonate ($NaHCO_3$; SBC) was purchased from Solvay (Brussels, Belgium) and the lactic acid ($C_3H_6O_3$; LA) was obtained from Purac (Gorinchem, The Netherlands).

2.2 Sample treatment

After thawing overnight at 10 ˚C, 300 pieces of spent-laying hen breast meat (70-90 g) were randomly arranged into 10 treatments, including control (not marinated), marinating with distilled water (DW), sodium bicarbonate (SBC) (0.10, 0.15, 0.20, and 0.25 M), and lactic acid (LA) (0.05, 0.10, 0.15, and 0.20 M). The meat was marinated with the solutions using the ratio of meat (g) and liquid (ml) of 1:1. A vacuum tumbler machine (MGH-20, Vicchi Engineering, Thailand) was used for marinating meat. Tumbling was applied using a vacuum of 0.4-0.5 bars at 20 rpm and 10 ˚C for 1 h. Of 30 samples of each treatment, 10 breasts were used for evaluating the pH, marinade uptake, drip loss, muscle fiber diameter, and microstructure of raw meat. The remaining meat samples were cooked using a water immersion method at 95 ˚C until the core temperature reached 80 ˚C. Ten pieces of the cooked breast meat were used for evaluating the pH, cooking loss, lightness, moisture content, muscle fiber diameter, and microstructure. The others (10 samples) were used for determining the Warner-Bratzler shear force of cooked meat.

2.3 Sample analysis

2.3.1 pH

The pH of raw and cooked spent-laying hen meat was measured using a pH meter (AD8000, Better Syndicate Co., Ltd, Thailand) combined with glass electrode according to Qiao and coworkers [18].

2.3.2 Color

The lightness (L^*) of the meat was determined on the surface of the sample using a Spectrophotometer UltraScan PRO (D65, Hunter Associates Laboratory Inc., USA) and reported in CIE system color profile.

2.3.3 Marinade uptake

According to Wongwiwat and coworkers [4], marinade uptake was calculated as the ratio of meat weight after tumbling process (W_1) to meat weight before tumbling process (W_0) using the following equation:

$$Marinade\ uptake(\%) = 100 \times \frac{W_1 - W_0}{W_0} \quad (1)$$

2.3.4 Drip loss

After the tumbling process, the meat was weighed (W_1), packed in a plastic bag and hung at 10 ˚C for 24 h. The meat was then removed from the bag and re-weighed (W_2). Drip loss was calculated as described by Yang and coworkers [12].

$$Drip\ loss\ (\%) = 100 \times \frac{W_1 - W_2}{W_1} \quad (2)$$

2.3.5 Cooking loss

After the tumbling process, the meat was weighed (W_1) and packed in a plastic bag. The meat was then cooked at 95 ˚C using water immersion until the core temperature of the meat reached 80 ˚C. The cooked meat was immediately cooled in ice-cold water until the core temperature reached 10 ˚C. The cooked meat then rested for 1 h at room temperature until the core temperature reached 25 ˚C. The cooked meat was removed from the bag and re-weighed (W_3). Cooking loss was calculated as described by Yang and coworkers [12].

$$Cooking\ loss\ (\%) = 100 \times \frac{W_1 - W_3}{W_1} \quad (3)$$

2.3.6 Moisture content

According to AOAC [19], the cooked meat was ground in a blender for 1 min. Two to five grams of ground meat samples ($W_{initial}$) were placed in a moisture dish and dried in a conventional oven (air drying) at 100-105 ˚C for 16-18 h. After drying, the samples were re-weighed (W_{dried}). Moisture content was determined as stated in the following equation:

$$Moisture\ (\%) = 100 \times \frac{W_{initial} - W_{dried}}{W_{initial}} \quad (4)$$

2.3.7 Warner-Bratzler shear force

The texture of the cooked samples was evaluated using Warner-Bratzler shear test following the protocol described by Liu and coworkers [20]. Cooked meat was cut parallel to muscle fiber orientation into meat cubes (1 cm × 2 cm × 1 cm). Shear force was analyzed using a TA-XT2i texture analyzer (Stable Micro System, Godalming, UK) with a 3-mm-thick Warner-Bratzler blade. The operating parameters consisted of a pre-test speed of 2.0 mm/s, test speed of 1.0 mm/s, post-test speed of 2.0 mm/s, distance of 25 mm and 25 kg of load cell. The force required to shear the samples was expressed as a Newton (N). Maximum force recorded during the test was reported as hardness of the samples.

2.3.8 Microstructure of muscle

Only the control and the samples treated with DW, 0.25 M of SBC and 0.20 M of LA were used for evaluating the microstructure of muscle. The microstructure of the samples was determined using a scanning electron microscope (SEM) (Quanta 450, FEI, Czech Republic) according to Wattanachant and coworkers [21]. Raw and cooked meats were cut into 1.0 x 1.0 x 0.5 cm cubes and fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer, pH 7.3, for 2 h at room temperature. Then, the specimens were rinsed with 0.1 M phosphate buffer, pH 7.3 and dehydrated in a series of 25%, 50%, 70%, 95%, and absolute ethanol (twice), for 1 h in each solution, respectively. The specimens were cut with liquid nitrogen and mounted on aluminum stubs and coated with gold. The specimens were photographed under the SEM using an accelerating voltage of 5 kV. The videoprints were taken at magnification of 500x. Muscle fiber diameter was measured on videoprints using ImageJ program.

2.4 Statistical analysis

All statistical analyses were performed using an SPSS 12.0 program. The effects of marinade treatments were evaluated based on a completely randomized design using analysis of variance. Significant differences at 95% confidence level between treatment means were analyzed by Duncan's multiple range test. In addition, the differences in means between raw and cooked samples within the same level of marination were examined using Student's t-test.

3. Results and discussion

3.1 Marinade and meat pH

Marinade pH ranged from 8.33 to 8.47 for SBC solution and from 2.06 to 2.41 for LA solution as shown in Table 1. The pH values of raw and cooked SBC-marinated meats were significantly ($p < 0.05$) increased as compared to that of control, whereas those pH values were significantly decreased with increasing LA ($p < 0.05$). Similar results in meat pH were reported for SBC and LA [12,16]. For all treatments, the pH of raw meat was lower than that of cooked meat. The increased pH of the cooked meat was probably due to the exposure of imidazolium, the residue group of basic amino acids such as histidine, during cooking [22]. Kim and coworkers [23] reported similar results that the pH of cooked chicken breast meat was greater than uncooked meat.

Table 1 pH of marinades and effects of marination on pH of raw and cooked spent-laying hen breast meats[1].

Treatment	pH		
	Marinade	Raw meat	Cooked meat
Control	-	$5.70^{f}\pm0.02$	$5.97^{e*}\pm0.02$
DW	$6.86^{d}\pm0.01$	$5.76^{e}\pm0.02$	$5.96^{e*}\pm0.02$
SBC 0.10 M	$8.33^{c}\pm0.01$	$7.26^{d}\pm0.02$	$7.41^{d*}\pm0.02$
SBC 0.15 M	$8.35^{bc}\pm0.03$	$7.48^{c}\pm0.03$	$7.79^{c*}\pm0.02$
SBC 0.20 M	$8.41^{b}\pm0.02$	$7.65^{b}\pm0.01$	$7.86^{b*}\pm0.03$
SBC 0.25 M	$8.47^{a}\pm0.02$	$7.80^{a}\pm0.02$	$7.99^{a*}\pm0.03$
LA 0.05 M	$2.41^{e}\pm0.02$	$5.39^{g}\pm0.02$	$5.67^{f*}\pm0.02$
LA 0.10 M	$2.23^{f}\pm0.04$	$4.92^{h}\pm0.02$	$5.26^{g*}\pm0.02$
LA 0.15 M	$2.14^{g}\pm0.03$	$4.77^{i}\pm0.02$	$4.93^{h*}\pm0.02$
LA 0.20 M	$2.06^{h}\pm0.04$	$4.57^{j}\pm0.02$	$4.63^{i*}\pm0.02$

[1] Data are expressed as mean ± standard deviation.
Values in the same column with different letters are different ($p < 0.05$).
[*]Significant differences ($p < 0.05$) between raw and cooked samples within the same treatment were analyzed by Student's t-test.

3.2 WHC of the meat

The effects of SBC and LA marination on marinade uptake, drip loss, cooking loss and moisture content of spent-laying hen meat are shown in Table 2. Marinade uptake of SBC and LA marinated samples was significantly greater ($p < 0.05$) than that of the DW sample, except for 0.05 M of LA marinated sample. Drip loss and cooking loss were significantly increased ($p < 0.05$) by SBC and LA marination as compared to those of the control, indicating excessive marinade solutions in the meat. However, as marinade concentration increased, the SBC and LA marinated samples showed significant decreases in drip loss and cooking loss ($p < 0.05$). Even though drip loss and cooking loss of the marinated samples increased, the moisture content of the marinated samples was still greater ($p < 0.05$) than that of the control. An increase in moisture content was due to an increased ability of the meat proteins to retain water. Because SBC and LA could alter the pH of the meat, the meat proteins were increased in negative charges (by SBC) or positive charges (by LA). Increasing negative or positive charges enhanced the interaction between meat proteins and water molecules, thus increasing the WHC of the marinated meat [15-16,24].

Table 2 Marinade uptake, drip loss, cooking loss, moisture content, lightness (L*), and shear force of spent-laying hen meat[1].

Treatment	Marinade uptake (%)	Drip loss (%)	Cooking loss (%)	Moisture content (%)	Lightness (L*)	Shear force (N)
Control	nd	2.13^{h}±0.33	22.71^{g}±2.22	67.19^{h}±0.46	73.31^{a}±0.59	58.99^{a}±2.86
DW	32.32^{d}±1.92	8.21^{c}±0.96	39.94^{a}±0.70	65.85^{i}±0.33	73.21^{a}±0.56	49.36^{b}±1.46
SBC 0.10 M	46.09^{c}±2.71	9.79^{b}±0.31	32.24^{cd}±2.08	74.59^{e}±0.03	73.30^{a}±0.86	37.67^{d}±1.80
SBC 0.15 M	58.28^{ab}±4.89	6.73^{de}±0.70	30.60^{cde}±2.19	77.19^{cd}±0.22	72.17^{b}±0.74	27.42^{ef}±2.72
SBC 0.20 M	62.34^{a}±4.35	4.79^{fg}±0.48	29.72^{def}±1.87	77.72^{b}±0.15	71.79^{b}±0.56	25.58^{f}±2.75
SBC 0.25 M	65.57^{a}±2.58	3.68^{g}±0.73	27.61^{f}±2.18	78.86^{a}±0.16	71.69^{b}±0.86	22.12^{g}±2.14
LA 0.05 M	33.78^{d}±1.99	17.73^{a}±0.84	39.48^{a}±1.47	69.42^{g}±0.17	73.68^{a}±0.69	43.85^{c}±2.06
LA 0.10 M	43.45^{c}±3.61	10.55^{b}±0.84	35.61^{b}±1.69	73.10^{f}±0.07	70.84^{c}±0.44	38.17^{d}±1.94
LA 0.15 M	53.95^{b}±1.71	7.52^{cd}±0.50	32.69^{c}±1.74	76.88^{d}±0.10	67.44^{d}±0.83	29.84^{e}±1.46
LA 0.20 M	61.12^{a}±1.74	5.83^{ef}±0.47	28.27^{ef}±1.56	77.36^{c}±0.11	62.60^{e}±0.93	25.11^{f}±1.87

[1] Data are expressed as mean ± standard deviation.
Values in the same column with different letters are different ($p < 0.05$).
nd means not determined.

3.3 Shear force values of the meat

As shown in Table 2, marination significantly reduced the shear force of the cooked samples ($p < 0.05$). Considering SBC, shear force values of the samples marinated with 0.10 M and 0.15 M SBC decreased by 36% and 54% from the value of control, respectively. However, a slight decrease in shear force values was observed when the SBC concentration increased to 0.20 M and 0.25 M. A similar trend was observed in the LA samples. The findings herein were consistent with previous reports [16,24]. Sheard and Tali [24] reported that the reduction in shear force can be attributed to the increased water content of the SBC marinated samples and a weakening of the myofibrillar structure. Moreover, SBC can produce carbon dioxide gas during the cooking process. The carbon dioxide gas possibly generated a porous structure in the meat, thus reducing the shear force values [25]. For LA marination, Aktas and coworkers [16] reported that 1.5% (0.2 M) of LA could reduce shear force values due to its ability to increase water content and solubilize the collagenous tissue in beef samples.

3.4 Meat color

The SBC and LA marinated samples had significantly ($p < 0.05$) lower L^* values (lightness) as compared to that of control, except for the 0.10 M of SBC and 0.05 M of LA marinated samples (Table 2). Similar results have been reported by others [26-27]. Petracci and Hosseini [13,27] reported that the SBC and LA decreased L^* values of cooked meat through the modification of pH. Within those pH ranges, WHC of the meat was increased, resulting in less free water to reflect light on the meat surface. In this regard, there was less light scattering on the surface of the marinated sample, reducing lightness of the meat surface.

3.5 Changes in muscle structure

On the transverse sections of the meat samples (Figure 1), the swelling of muscle fibers was visible in the raw marinated samples (Figure 1C, 1E, 1G). The swelling of the marinated samples is related to an increase in WHC of the meat. The air-filled pockets were observed in the cooked SBC marinated samples (Figure 1F), supporting the hypothesis of carbon dioxide production during cooking [24]. These air-filled pockets could disrupt muscle fiber structure, thus reducing the shear force value [25]. For the cooked LA marinated samples (Figure 1H), clear gaps between the muscle fibers were observed. This might be due to the solubilization of the endomysial collagen [17]. The muscle fiber diameters of raw and cooked spent-laying hen meat are shown in Table 3. SBC and LA significantly ($p < 0.05$) increased muscle fiber diameter of raw and cooked meat. An increase in muscle fiber diameter of the marinated samples is due to the swelling of muscle fibers. The changes in muscle fibers observed in this study agreed with Wongwiwat and coworkers [4] who studied the effects of phosphate and citric acid marination on spent-laying hen muscles. For all treatments, the muscle fiber diameter of cooked meat was lower than that of all raw meats. A decrease in the muscle fiber diameter of cooked meat indicated the shrinkage of muscle fiber during the cooking process [21].

Table 3 Effects of sodium bicarbonate and lactic acid marination on muscle fiber diameter of raw and cooked breast meat collected from spent-laying hens[1].

Treatment	Raw meat (μm)	Cooked meat (μm)
Control	$35.60^{c}\pm1.07$	$35.10^{b*}\pm1.37$
DW	$37.80^{b}\pm1.14$	$35.50^{b*}\pm1.18$
SBC 0.25 M	$39.30^{a}\pm0.95$	$37.20^{a*}\pm1.14$
LA 0.20 M	$39.10^{a}\pm0.99$	$37.60^{a*}\pm1.43$

[1] Data are expressed as mean ± standard deviation.
Values in the same column with different letters are different ($p < 0.05$).
*Significant differences ($p < 0.05$) between raw and cooked samples within the same treatment were analyzed by Student's t-test.

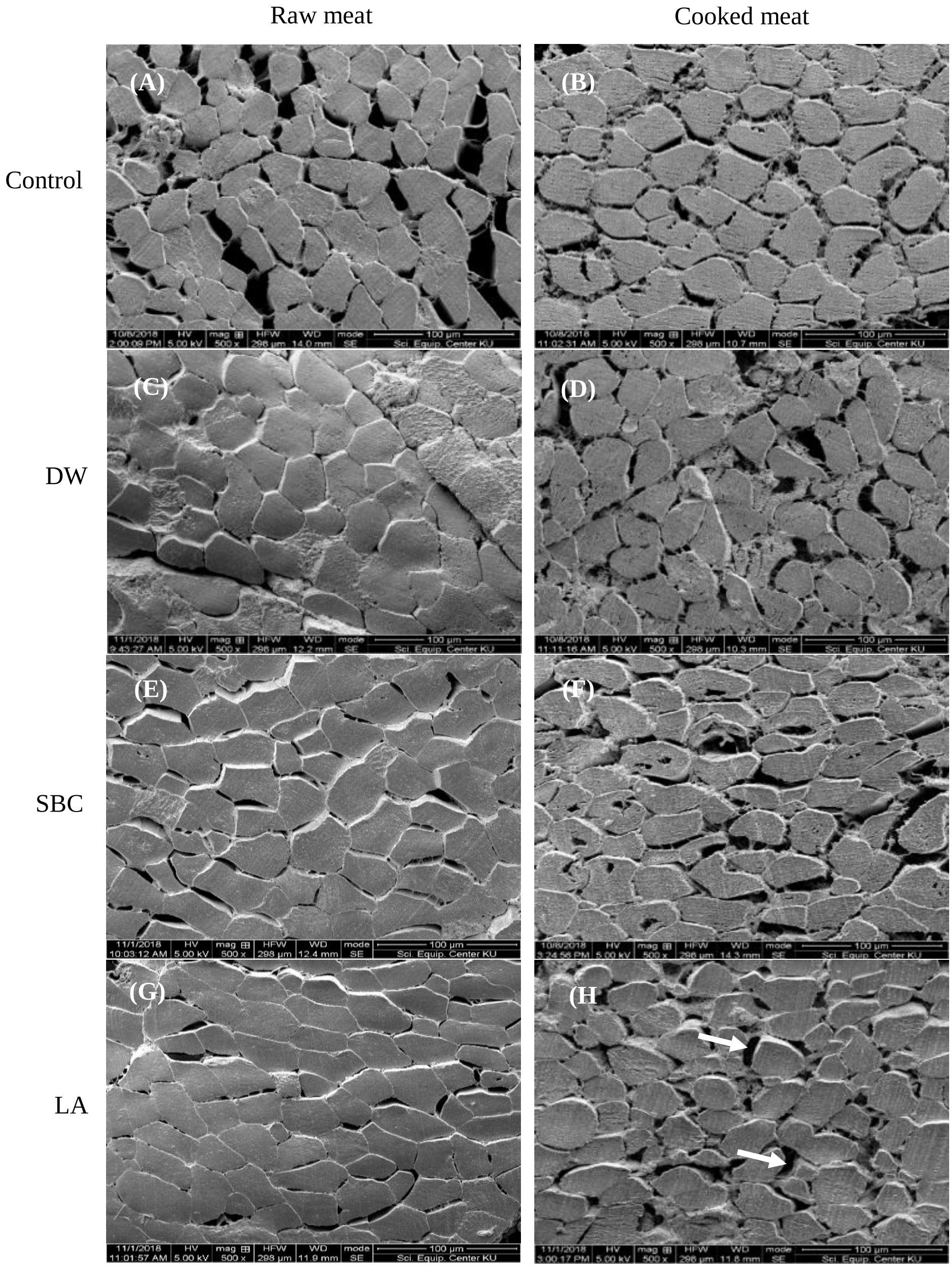

Figure 1 SEM micrographs of transverse sections of raw and cooked spent-laying hen meats; (A-B) control, (C-D) DW marinated meat, (E-F) 0.25 M SBC marinated meat, (G-H) 0.20 M LA marinated meat. The arrows indicate clear gaps between muscle fibers.

4. Conclusions

The results of this study indicated the effectiveness of SBC and LA for marination in improving tenderness of cooked breast meat obtained from spent-laying hens. It was clearly observed that the pH, lightness, WHC, shear force, and muscle microstructure of spent-laying hen meat were directly affected by the SBC and LA solutions. Based on the current results, 0.10 M of LA solution and 0.15 M of SBC solution are recommended, as at this concentration the marinades effectively decreased shear force values of the cooked spent-laying hen breast meat by 36% and 54%, respectively. Although marinated meats with these concentrations had statistically lower lightness based on an instrumental determination, the slightly reduced lightness of meat surface might not be detected by consumers. To provide more comprehension, further study regarding consumer acceptance is under investigation.

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