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Optimization of the Tenderization of Duck Breast Meat by Adenosine 5′-Monophosphate (AMP) using Response Surface Methodology

Daoying Wang[1], Shaoying Deng[1,2], Muhan Zhang[1], Zhiming Geng[1], Chong Sun[1], Huan Bian[1], Fang Liu[1], Yongzhi Zhu[1] and Weimin Xu[1]

[1] Institute of Agricultural Products Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, PR China
[2] Key Laboratory of Meat Processing and Quality Control, Ministry of Education, Nanjing Agricultural University, Nanjing 210095, PR China

This study aimed to characterize and optimize the tenderization condition of duck breast meat by adenosine 5′-monophosphate (AMP), with the aid of response surface methodology (RSM). The results showed that the optimal conditions for the tenderization of duck breast meat were at the NaCl concentration of 3.99 g/100 g, AMP concentration of 13.83 mmol/L, temperature of 15.32℃, and marinating time of 8 h. Compared with control duck breast meat, AMP combined with NaCl treatment demonstrated significant effects on improvement of meat tenderness and decrease of cooking loss. Such effects might be ascribed to the combination of a series of biochemical reactions, e.g. increase of muscle pH, dissociation of actomyosin and inhibition of meat shrinkage. Therefore, the mixture of AMP and NaCl could be regarded as an effective tenderization agent for duck breast meat.

Key words: adenosine 5′-monophosphate, duck, marinating, response surface methodology, tenderness

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Introduction

Adenosine 5′-monophosphate (AMP) is an endogenous purine nucleotide, which is a structural component of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and can be found in all living organisms involved in energy metabolism (Pascal, 2008). In food processing, AMP is often used in meat and poultry soups as a flavor enhancer (flavor modifier), or as food additives for specific nutritional purposes (Vinas *et al*., 2010).

Meat tenderness is an important indicator to assess meat quality and the major contributor to consumer acceptability of meat as well (Kong *et al*., 2008; Lee *et al*., 2008; Myers *et al*., 2009; Moeller *et al*., 2010). Tenderness can be determined by a trained panel or physical methods (Destefanis *et al*., 2008). The sensory evaluation of tenderness is linked to person and environment related factors such as the dining situation (Huffman *et al*., 1996), therefore the most widely laboratory method to measure meat tenderness is the Warner-Bratzler (WB) shear force determination (Combes *et al*., 2004; Tahergorabi *et al*., 2012).

Traditional Chinese duck meat products, such as water-boiled salted duck are well accepted by consumers in China and Southeast Asia due to their delicate flavor and tenderness (Xu *et al*., 2008). In Nanjing city alone, about thirty million ducks are consumed annually, and the consumption is still increasing (Wang *et al*., 2013; Wang *et al*., 2014b). How to control duck meat tenderness during storage and processing is extremely important since the hardening of the muscle during the processing of duck products severely affect their quality and palatability (Li *et al*., 2013).

AMP could dissociate actomyosin into myosin and actin (Okitani *et al*., 2008; Nakamura *et al*., 2012), which is closely related with meat tenderness (Taylor *et al*., 1995; Okitani *et al*., 2008; Wang *et al*., 2013). Our previous research found that tenderness was improved when duck breast muscle was treated with 10−40 mmol/L AMP (Dong *et al*., 2014). However, the effect of AMP was dependent on the treatment conditions. In the case of relatively high temperature, AMP had a rather weak impact on meat tenderness. We infer that AMP might be converted to IMP and ammonia by Adenosine deaminase (AMPD) (Shiraki *et al*., 1979). The activity of AMPD was influenced by marinating conditions such as salt, temperature etc. (Koch and Vallee, 1959), therefore under certain circumstance AMP is degraded before it permeates through the meat, hindering the effect on dissociating actomyosin and tenderizing meat. Thus it would be meaningful to regulate the marinating conditions and inhibit the activity of AMPD, thereby AMP

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Correspondence: W. Xu, Institute of Agricultural Products Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, PR China. (E-mail: weiminxu2002@aliyun.com)

could penetrate into the meat and exert the tenderization effect on meat. Our recent research has primarily confirmed that increasing NaCl content and decreasing the temperature could promote the effect of AMP (Wang *et al*., 2015). However, the optimized condition has not yet been developed.

The objectives of this study are: a) to optimize the treatment conditions (e.g. AMP content, NaCl content, and temperature, etc.) that bring meat the most tender using response surface methodology, and b) to assess duck meat quality (e.g. microstructure, actomyosin dissociation, pH, cooking loss, myofibril fragmentation index, etc.) under the optimum treatment conditions.

Materials and Methods

Sample Preparation

One hundred and twenty three lean-type Cherry Valley ducks from a commercial feedlot were slaughtered by bleeding from a unilateral neck cut severing the left carotid artery and jugular vein (without stunning) in a commercial meat processing plant (Jiangsu furun Food Ltd, Xuzhou, China), each of which was about 2.0 kg. Two skinless, deboned breast fillets (*Pectoralis major*) muscles were removed from each carcass and subjected to 12 h aging in a refrigerator (4±1℃). A total of 246 duck fillets were trimmed off all visible fat and connective tissues, and cut to 7 cm ×5 cm×1.5 cm rectangular shaped samples with the average weight of 50±1 g. For single-factor analysis, 132 slices were subjected to different treatments at selected AMP concentration of 0−25 mmol/L, NaCl content of 1−6 g/100 g, temperature of 5−25℃ and kept for 4−20 h. Based on single-factor experiments, the response surface methodology was applied to estimate the effect of independent variables (NaCl, X_1; AMP, X_2; temperature, X_3) on the shear force (Y). A Box-Behnken design (BBD) was employed with 102 slices as shown in Table 1. The other 12 samples were used for comparison of the meat quality under the optimal AMP treatment conditions and the control that was treated with distilled water. All samples were placed into plastic bags individually and immersed in 50 mL AMP (adenosine 5′-monophosphate disodium salt, Sigma-Aldrich Chemical Co.) dissolved in various concentrations of salt solution. After a certain time treatment at corresponding temperature, samples were removed from solution and wiped with paper towel. A portion of samples was taken for the measurement of pH, myofibril fragmentation index (MFI), and actomyosin dissociation with 2 g of each test, which were stored at −40℃. The remainder of the portions was subjected to shear force, cooking loss and microstructure analysis immediately after the treatment. All experiments were undertaken in accordance with the guidelines of the regional Anima Ethics Committee and were approved by the Institutional Animal Care and Use Committee of Jiangsu Academy of Agricultural Sciences and conformed to the Declaration of Helsinki.

pH Value

Two grams of duck meat was homogenized at 5000 rpm with an Ultra Turrax homogenizer (T25, IKA, Labortechnik, Staufen, Germany) in 18 mL distilled water, and the pH of the homogenate was measured using a pH meter equipped with an electrode (Fernandez *et al*., 2002).

Myofibril Fragmentation Index (MFI)

MFI was determined by the method of Hopkins with slight

Table 1. **The Box-Behnken design and experimental results of response surface methodology**

Run	Independent variables[a]			Dependent variables[b]
	NaCl (g/100 g)	AMP (mmol/L)	Temperature (℃)	Shear force (g)
1	5	10	20	607.23
2	3	10	10	619.77
3	4	10	15	456.32
4	5	10	10	611.18
5	4	15	20	535.85
6	4	15	10	554.14
7	3	5	15	650.36
8	4	10	15	457.65
9	5	5	15	623.44
10	3	10	20	610.25
11	4	10	15	459.68
12	5	15	15	513.52
13	4	5	20	645.24
14	4	10	15	464.45
15	4	5	10	639.21
16	3	15	15	549.37
17	4	10	15	469.34

[a] Independent variables: X_1, NaCl; X_2, AMP; X_3, temperature.
[b] Dependent variables Y, shear force; data expressed as mean (n=6).

modifications (Zhang *et al*., 2013). Muscle tissue was pulverized in liquid nitrogen, and 0.5 g of powdered tissue was homogenized for 1 min in 30 mL 25 mM phosphate buffer (0.1 M Potassium Chloride, 1 mM EDTA, pH 7.0). The suspension was filtered to remove connective tissue, and residue was washed with 10 m*l* 25 mM phosphate buffer. Then filtrate was centrifuged at 1000×g for 15 min at 4℃, the precipitate was resuspended in 10 mL phosphate buffer and centrifuged again. This step was repeated twice more and the pellet was suspended in buffer solution. The protein concentration was diluted to 0.5 mg/mL and measured spectrophotometrically at 540 nm (UV 6100). MFI was calculated by multiplying readings with 150.

Actomyosin Dissociation

The method for actin extraction was performed according to Okitani *et al.* (2009), with slight modifications. Two grams minced duck meat was mixed with 20 mL of Weber-Edsall solution (0.6 M KCl/0.04 M $NaHCO_3$ /0.01 M Na_2CO_3; pH 7.2) and homogenized at 12000 rpm for 30 s twice at intervals of 10 s. The obtained homogenate was transferred to a beaker and shaken for 24 h at 4℃. One portion of this solution was collected as the actomyosin fraction. The other was mixed with distilled water to lower the KCl concentration to 0.2 M, and it was centrifuged at 12000 g for 20 min at 4℃. The protein concentration was measured with Bradford Protein Assay Kit. The supernatant and the extracted actomyosin fraction was mixed with the sample buffer (4% SDS, 10% 2-mercaptoethanol, 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris-HCl; pH 6.8) at a ratio of 2:1 (v/v) and boiled for 5 min. The samples with equal content of proteins (around 20 μg protein) loaded on each well were subjected to electrophoresis on 12 g/100 g SDS-polyacrylamide gels at a constant voltage of 200 V using a Mini PROTEAN Tetra cell (Bio-Rad Laboratories, Hercules, CA). Gels were transferred to 0.45 μm PVDF membrane (Millipore) using a Semi-Dry Electrophoretic Transfer Cell (Bio-Rad Laboratories, Hercules, CA). Proteins were detected with Polyclonal antibody to actin (Sigma Aldrich) at 1:1000 in 5 g/100 g skim milk powder solution. After overnight incubation at 4℃, the membrane was incubated with Horseradish peroxidase-labeled (HRP) anti-rabbit and anti-mouse secondary antibodies at 1:5000 dilutions. Images of DAB detection were captured by Gel Imager and then the intensities of bands in each lane were quantified using Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Cooking Loss

Each breast fillet was weighted accurately prior to cooking. Samples were cooked in plastic bags individually in a water bath kettle set at 80℃ until the internal meat temperature reached 75℃. After cooking, the breast fillets were cooled in tap water to the internal temperature of room temperature and wiped with paper to remove excess water and weighted immediately. Cooking loss was calculated as Cooking loss (%)=[(raw weight−cooked weight) /raw weight]×100 (Wang *et al*., 2014a).

Shear Force

Shear force was determined through the application of the Meullenet-Owens razor shear (MORS) test (Meullenet *et al*., 2004), using a texture analyzer (TVT-300XP, TexVol Instruments, Viken, Sweden) equipped with a razor blade. The crosshead speed was set at 2 mm/s, and the test was triggered by a 10 g contact force. The shear was perpendicular to the axis of muscle fibers. In each treatment, the MORS test value was determined in triplicates at predetermined locations on each of the fillets.

Microstructure

Scanning Electron Microscope (S-3000N, Hitachi High-Technologies Corporation, Tokyo, Japan) and Transmission Electron Microscope (H-7650, Hitachi, Japan) were employed for microstructure study on duck meat. After the shear force measurement, pieces (5 mm×5 mm×2 mm) for Scanning Electron Microscope (SEM) analysis were excised from samples and fixed in 2.5 mL/100 mL glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.3) at room temperature. The specimens were then rinsed with 0.1 mol/L phosphate buffer (pH 7.3) and dehydrated in 50, 70, 80, and 90 mL/100 mL ethanol, respectively for 15 min in each solution and 30 min in absolute ethanol three times. The specimens were freeze-dried and mounted on aluminum stubs and coated with gold for examination and photographing using a SEM. For Transmission Electron Microscope (TEM) analysis, pieces (1 cm×1 cm×2 mm) were fixed in 2.5 mL/100 mL glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.3) followed by a secondary fixation with 20 g/L osmium tetroxide at room temperature. The specimens were then dehydrated in 50, 70, 80, and 90 mL/100 mL ethanol, respectively for 15 min in each solution and 30 min in absolute ethanol three times. The samples were embedded in epoxy resin (Durcupan) and the resin was allowed to cure at 70℃ for 24−48 h. The cured resin blocks were cut in ultrathin section with ultramicrotome. The ultrathin sections were stained using a solution of 4 mL/100 mL uranyl acetate in ethanol for 10 min followed by an aqueous solution of Reynolds' lead (7 min) (Li *et al*., 2013).

Statistical Analysis

For single-factor analysis, the differences between each group was evaluated by one-way analysis of variance (ANOVA) using the SPSS 18.0 (Argyrous, 2011). Differences were regarded as significant at $P<0.05$. The experimental data of RSM were statistically analyzed, using the Design-Expert 8.0.6 software (State-Ease, Inc., Minneapolis MN, USA). The quadratic response surface analysis was based on multiple linear regression analysis, which took into account the main, the quadratic, and the interaction effects according to the following equation:

$$Y=b_0+\sum_{i=1}^{3} b_i X_i+\sum_{i=1}^{3} b_{ii} X_i^2+\sum_{i<j=1}^{3} b_{ij} X_i X_j \quad (1)$$

where Y is the dependent or response variable, b_0, b_i, b_{ii}, and b_{ij} are the intercept, linear, quadratic, and interaction coefficients, respectively, and X_i and X_j are independent variables. The results were analyzed using ANOVA. The optimal ultrasonic treatment conditions were estimated

through regression analysis and three-dimensional response surface plots of the independent variables and each dependent variable.

Results and Discussion

Effects of AMP Concentration, NaCl Concentration, and Temperature on Shear Force of Duck Breast Meat

In order to develop a model for optimization of AMP tenderization of duck breast meat, effects of AMP concentration, NaCl concentration, and temperature on tenderness of duck breast meat were investigated respectively. Variations in meat tenderness, in terms of shear force, were observed under every single-factor test, and the results were presented in Fig. 1.

In the single-factor test for the effect of AMP concentration on meat tenderness, AMP concentration varied from 0 to 25 mmol/L, and marination was carried out at 5℃ for 8 h with NaCl concentration of 0.9 g/100 g. The shear force value decreased from 1004.43 g to 773.04 g with increased AMP concentration from 0 to 25 mmol/L (Fig. 1a). In the range of 0−10 mmol/L, every change of 5 mmol/L in AMP concentration resulted in a significant variation of shear force ($P<0.05$). However, such a significant variation could not be observed for every change of 5 mmol/L in AMP concentration in the range of 10−25 mmol/L, although shear force still decreased with the increasing of AMP concentration. Therefore, the optimum AMP concentration was selected at 10 mmol/L with respect to the future application of AMP in meat.

In the single-factor test for the effect of NaCl concentration on meat tenderness, NaCl concentration varied in the range of 1−6 g/100 g, and marination was carried out at 5℃ for 8 h with 10 mmol/L AMP concentration. The shear force decreased significantly ($P<0.05$) with increased NaCl concentration from 1 to 4 g/100 g, followed by a significant increase ($P<0.05$) when the concentration was elevated to 5 and 6 g/100 g, and unchangeable afterwards ($P>0.05$) (Fig. 1b). NaCl exerts an inhibition on the activity of AMPD (Zhu *et al*., 2013), which may result in the less degradation of AMP and consequently the decrease of shear force. However, higher NaCl concentration resulted in higher hardness due to the compaction of myofibrillar structure and an inhibitory effect on calpains activity (Shomer *et al*., 1987; Geesink and Koohmaraie, 2000). Therefore, the NaCl concentration of 4 g/100 g was selected as a central point for RSM.

The effect of temperature on meat tenderness was studied within the range of 5−25℃, and marination was carried out for 8 h with NaCl concentration of 4 g/100 g and AMP concentration of 10 mmol/L. The shear force decreased significantly ($P<0.05$) with increasing of temperature from 5 to 15℃, while it almost varied in the opposite direction with temperature ranging from 15 to 25℃ (Fig. 1c). The increasing of temperature favors permeating of AMP into duck meat and improvement of meat tenderness, however, further increasing of temperature results in activating of AMPD which may convert AMP to IMP (Zhu *et al*., 2013) and impair the effect of AMP on meat tenderness. Therefore, optimum temperature was set at 15℃ for development of the model.

The effect of time on meat tenderness was also investigated within the range of 4−20 h, and marination was carried out at 15℃ while NaCl concentration was 4 g/100 g and AMP concentration was 10 mmol/L (Fig. 1d). The shear

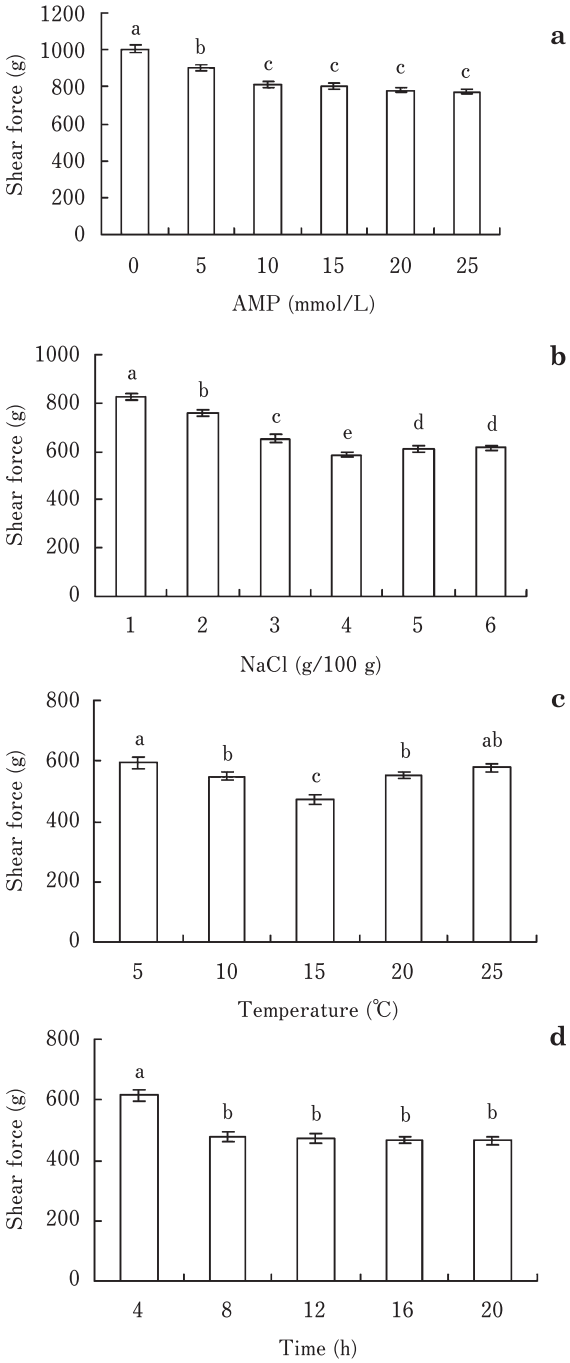

Fig. 1. **Effects of AMP (a), NaCl (b), temperature (c) and time (d) on the shear force of duck.**

force decreased significantly with time ($P<0.05$) in the first 8 h, and it was almost unchanged in the following 12 h. This result indicated 8 h was sufficient for AMP to permeate into duck meat.

Analysis of the Model

Response surface methodology (RSM), a statistical approach for modeling and optimizing a response that is affected by one or more factors, has been widely used in food research in recent years (Wang *et al*., 2007; Kim *et al*., 2013; Hu *et al*., 2014). In the present study, RSM was employed to develop a model for optimization of AMP and NaCl tenderization of meat. Based on the single factor experiment, the salt concentration, AMP concentration, and temperature were set in the range of 3−5 g/100 g, 5−15 mmol/L and 10−20℃ respectively in RSM. The results of 17 runs, using BBD, are provided in Table 1. By applying multiple regression analysis on the experimental data, the model for the response variable could be expressed by the following quadratic polynomial equation in the form of coded values:

$$Y=2630.12575-573.738X_1-46.37505X_2 -95.35945X_3-0.4465X_1X_2+0.2785X_1X_3 -0.2432X_2X_3+70.591X_1^2+2.08374X_2^2+3.20114X_3^2 \quad (2)$$

Analysis of variance (ANOVA) for the model is shown in Table 2. The determination coefficient ($r^2=0.9946$) indicated that the model was highly significant. The value of lack of fit test (0.1003) was higher than 0.05, which was not significant relative to the pure error, indicating that the fitting model was adequate to describe the experimental data (Yin *et al*., 2010; Hu *et al*., 2014; Liu *et al*., 2014).

In analysis of variance, p-value and F-value are used to reflect the significance of corresponding variables. As given in Table 2, Comparing the linear, interaction, and quadratic terms, it can be concluded that AMP (X_2), NaCl concentration (X_1^2), AMP concentration (X_2^2), and temperature (X_3^2) were highly significant to shear force with the p-values lower than 0.01 and NaCl concentration (X_1) was the significant term with the p-values lower than 0.05. The order of the linear term effect on shear force can be arranged as follows: AMP concentration (X_2)＞NaCl concentration (X_1)＞temperature (X_3). However, no significant interaction effects could be found between NaCl concentration and AMP concentration (X_1X_2), NaCl concentration and temperature (X_1X_3), and AMP concentration and temperature (X_2X_3). Therefore, these interaction effects were insignificant or negligible terms.

Analysis of Response Surfaces

Three-dimensional (3D) response surface plots and contour plots could intuitively show the effect of each variable on the dependent variable. From Fig. 2, It can be observed that both NaCl concentration (X_1) and AMP concentration (X_2) had significant effect on shear force. As shown in Fig. 2a, at the fixed temperature of 15℃, increases in both NaCl concentration and AMP concentration would result in a decrease in shear force. However, further increase in NaCl concentration to above 4 g/100 g would lead to the increase in shear force. The elliptical contour plot in Fig. 2a1 well matched with the results in Table 2.

As shown in Fig. 2b, at the fixed NaCl concentration of 4 g/100 g, an increase in AMP concentration resulted in a persistent decrease in shear force. Shear force decreased initially followed by a sharp increase with the increasing of the temperature and the lowest shear force of 482.75 g was achieved when AMP was at the optimum concentration. The mutual interaction between AMP concentration (X_2) and temperature (X_3) was not found (Fig. 2b1), which was also consistent with the results in Table 2.

The effect of NaCl concentration (X_2) and temperature (X_3) on the shear force is shown in Fig. 2c. At the fixed AMP concentration of 10 mmol/L, shear force decreased initially and then increased considerably with the increase of NaCl and temperature, and under the optimum condition, the lowest shear force was 476.42 g. Contour plot in Fig. 2c1

Table 2. **ANOVA for response surface quadratic model: estimated regression model of relationship between dependent variables and independent variables**

Source	Sum of squares	DF	Mean square	F-value	*p*-value
Model	87564.83	9	9729.43	142.99	＜0.0001
X_1-NaCl	691.55	1	691.55	10.16	0.0153
X_2-AMP	20540.60	1	20540.60	301.87	＜0.0001
X_3-Temperature	82.75	1	82.75	1.22	0.3066
X_1X_2	19.94	1	19.94	0.29	0.6051
X_1X_3	7.76	1	7.76	0.11	0.7455
X_2X_3	147.87	1	147.87	2.17	0.1839
X_1^2	20981.43	1	20981.43	308.35	＜0.0001
X_2^2	11426.24	1	11426.24	167.92	＜0.0001
X_3^2	26966.57	1	26966.57	396.31	＜0.0001
Residual	476.31	7	68.04		
Lack of fit	361.18	3	120.39	4.18	0.1003
Pure error	115.13	4	28.78		
Cor total	88041.15	16			
Coefficient of determination (r^2)	0.9946				

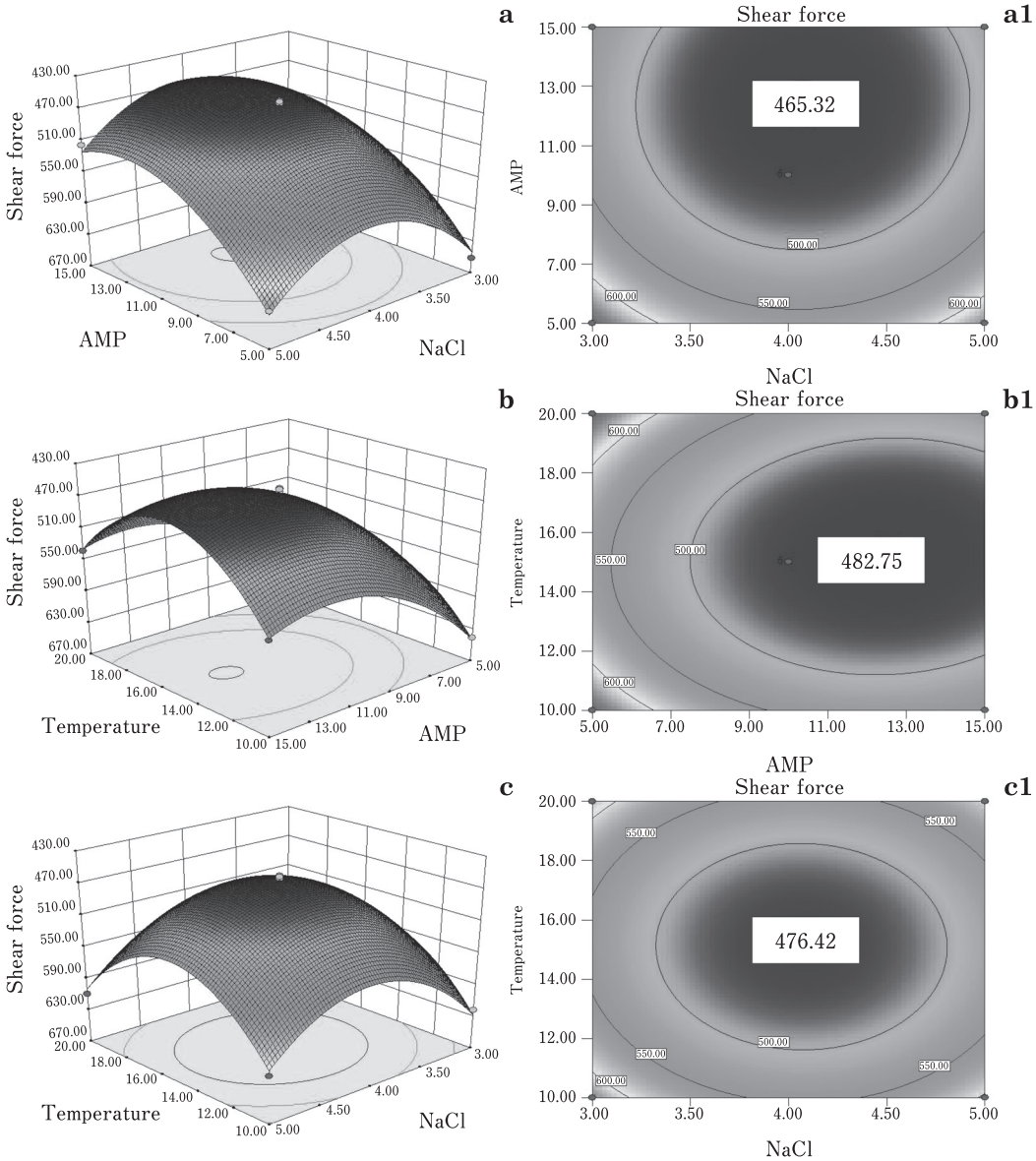

Fig. 2. **Response surface plots and contour plots of the shear force in response to changes in AMP (mmol/L), NaCl (g/100 g) and temperature (℃).** a, a1: AMP and NaCl; b, b1: AMP and temperature; c, c1: NaCl and temperature.

shows that NaCl concentration does not affect shear force significantly when compared with temperature.

Based on the above results, the verification was performed to evaluate the optimal processing conditions on shear force. The optimal conditions were found to be at NaCl concentration of 3.99 g/100 g, AMP concentration of 13.83 mmol/L, and temperature of 15.32℃, under which the predicted shear force is 453.13 g. However, considering the operability in practical environment, the optimal conditions were modified at NaCl concentration (X_1) of 3.99 g/100 g, AMP concentration (X_2) of 13.83 mmol/L, and temperature (X_3) of 15.3℃, under which the experimental shear force is 453.82 g (Table 3). Only small deviations were found between the actual shear force and the predicted ones. Thus, the RSM model was satisfactory and accurate.

Comparison of AMP Treated Duck Breast Meat with Control Duck Breast Meat

As shown in Table 3, AMP treatment could notably decrease the shear force and cooking loss while increase meat pH and MFI as compared to control. As we expected, a larger amount of actin was liberated from actomyosin after AMP and NaCl treatment (Fig. 3), which was consistent with

the previous studies that AMP could dissociate actin from myosin and contribute to improved meat tenderness (Wang *et al*., 2015 and Okitani *et al*., 2008). The actin amounts were equal between control and AMP treated samples in actomyosin fraction which demonstrated the actomyosin extracted were equivalent between these samples (Fig. 3b). Myofibrillar structures of AMP treated and control duck breast meats are shown in Fig. 4. On the transverse sections of control meat, gaps between muscle fibers were plainly visible (Fig. 4a1), which was similar to those reported by Wattanachant *et al*., (2005) who examined the microstructure of chicken meat. Contrarily, the gaps between muscle fibers became smaller when treated with AMP and NaCl, which may attributed to the penetration of the curing agents and the strong solubilizing of the proteins that made the solutes accumulated in the intercellular spaces. (Fig. 4b1) (Larrea *et al*., 2007). The TEM micrograph showed that in control samples, myofibrils were closely packed, with distinctive I band and A band, and plainly visible Z-line and M-line (Fig. 4a2). While in AMP and NaCl treated samples, remarkable structural changes were observed, and solubiliza-

Table 3. **Effect of AMP on shear force (g), pH, cooking loss (%) and MFI of duck breast meat**

	Contol	AMP treatment
Shear force	1038.54±16[a]	453.82±4.97[b]
pH	6.02±0.01[b]	6.30±0.02[a]
Cooking loss	29.35±0.87[a]	14.82±0.70[b]
MFI	163.37±3.56[b]	276.33±4.99[a]

[a, b] Means in the same row with different letters differ significantly ($P<0.05$); data expressed as mean±SD ($n=6$).

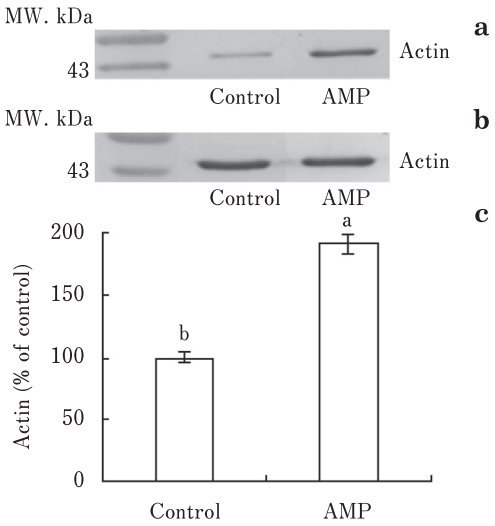

Fig. 3. **Actomyosin dissociation in duck breast meat treated by water alone (control) and by AMP treatment under optimized condition (AMP) as shown by western-blot (a) along with relative values (c) and total actin in extracted actomyosin fraction (b).**

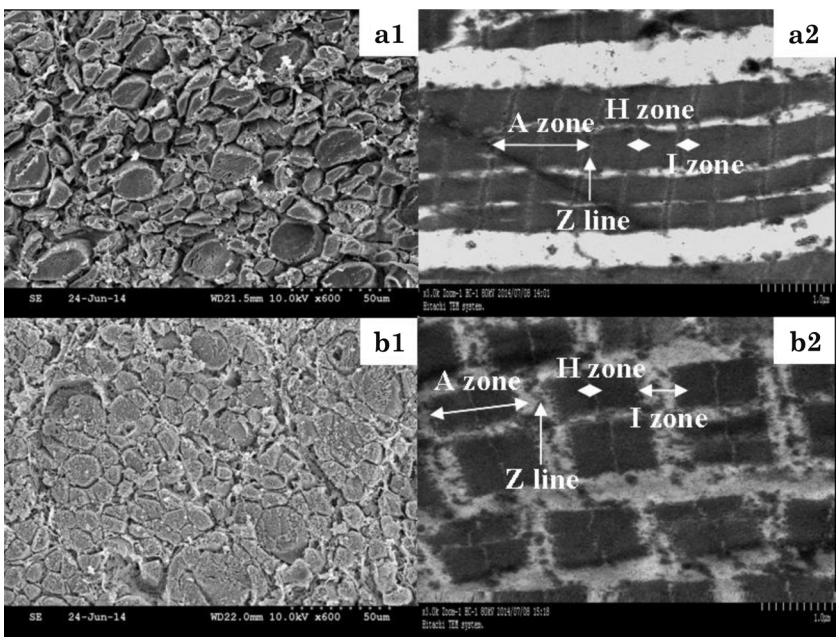

Fig. 4. **SEM (transverse section, 600×) and TEM (longitudinal section, 3000×) micrographs of cooked duck breast meat after AMP treatment.** a1, b1: Transverse section of control, AMP treated duck meat. a2, b2: Longitudinal section of control, AMP treated duck meat.

tion of myofibril proteins and loss of Z-disks occurred (Fig. 4b2). AMP probably could lead to the weakening of the longitudinal structure of myofibril and integrity of muscle, and it has been reported that the degradation of proteins such as nebulin and troponin-T could mediate the interaction of actin and myosin (Root and Wang, 1994; Lehman *et al*., 2001). These results indicated AMP together with NaCl treatment could damage the tissue structure and restrain the shrinkage of muscle fiber transversally and longitudinally, and ultimately could make the meat tender. AMP could be converted to IMP and ammonia by AMPD (Shiraki *et al.* 1979). The activity of AMPD is influenced by marinating conditions such as salt and temperature, therefore we infer AMPD activity was inhibited under the optimized condition, which allowed AMP to penetrate into the internal of meat and exert the tenderization effect on meat. Taken together, AMP and NaCl treatment under the optimum condition is very effective for tenderization of meat and improvement of overall meat quality.

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