PAPER

# EFFECT OF SOME AMINO ACIDS ON YIELD AND CHARACTERISTICS OF PACIFIC WHITE SHRIMP TREATED WITH ALKALINE SOAKING SOLUTION

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## ABSTRACT

Effects of alkaline soaking solution (ASS), 0.75% NaOH containing 2.5% NaCl, pH 11.5, containing different amino acids at various concentrations on yield and characteristics of Pacific white shrimp *(Litopenaeus vannamei)* were studied. The lowest cooking loss with the highest cooking yield was obtained for the sample treated with ASS containing 3% glutamic acid ($P<0.05$). When shrimp were treated with ASS having 3% monosodium glutamate (MSG) with the same mole equivalent to glutamic acid, the higher weight gain but slightly lower cooking yield were obtained ($P<0.05$). ASS containing 3% MSG had no effect on color and shear force of cooked shrimp.

*Keywords*: glutamic acid, monosodium glutamate, amino acids, alkaline, treatment, Pacific white shrimp

## 1. INTRODUCTION

Nowadays, Pacific white shrimp *(Litopenaeus vannamei)* is one of important species having a high market demand worldwide due to its appealed appearance, taste, flavor and texture (MANHEEM, 2013). Thailand is well known as the world largest shrimp producer, manufacturer and exporter. In 2013, shrimp was exported from Thailand with the value of 28,617 million bahts (THE CUSTOMS DEPARTMENT, 2013). Shrimp processing, especially freezing, can lead to the denaturation or aggregation of proteins (CARNETRO *et al*., 2013). These changes can result in drip loss and sensorial changes in the product (GONÇALVES and RIBEIRO, 2009). Furthermore, cooking also causes the changes in quality attributes, mainly by affecting textural and physicochemical properties and inducing weight loss (CARNETRO *et al*., 2013). The addition of water binding agent is therefore required in order to retain the quality of shrimp during processing. Phosphate and bicarbonate have been widely used as water binding agents, which can increase water uptake and lower cooking loss (RATTANASATHERIN *et al*., 2008; CARNETRO *et al*., 2013 and CHATARASUWAN *et al*., 2011). Bicarbonate treatment could increase the weight gain of shrimp, mainly due to the repulsion between protein molecules mediated by its alkaline pH (CHATARASUWAN *et al*., 2011). Due to the strict regulation of the uses of phosphate and bicarbonate in shrimp and shrimp products, an alternative additive with the property equivalent to both phosphate and bicarbonate are currently gaining attention for shrimp processing industry.

Some amino acids were reported to retard protein denaturation and retain protein functionality of frozen fish muscle (CAMPO-DEANO *et al*., 2009; ZHOU *et al*., 2006). Owing to the hydrophilic nature of some amino acids, their uses along with alkaline treatment could show the synergism in water uptake or water binding of shrimp muscle. Amino acid with different side chains, pI and molecular properties might exhibit varying efficacy as processing aid for shrimp processing. Monosodium glutamate (MSG) is the sodium salt of glutamic acid. It has been used widely as a flavor enhancer with umami taste. Since it is readily soluble in water, it can be used with ease for shrimp treatment and yield the product with improved properties. However, no information regarding the uses of amino acids and their salts as the processing aid for quality improvement of shrimp exists. The objective of this study was to investigate the impact of amino acids in conjunction with alkaline treatment on yield and characteristics of Pacific white shrimp.

## 2. MATERIALS AND METHODS

### 2.1. Collection and preparation of shrimp

Pacific white shrimp (*Litopenaeus vannamei)* (55-60 shrimp/kg) were purchased from a local market in Hat Yai, Songkhla province, Thailand. Shrimp with storage time less than 6 h after capture were stored in the insulated box containing ice using a shrimp/ice ratio of 1:2 (w/w). The samples were transported to the Department of Food Technology, Prince of Songkla University within 2 h. Upon arrival, shrimp were cleaned using tap water. Shrimp were peeled and deveined manually. Prepared shrimp were placed in polyethylene bag and stored in ice until use.

**2.2 Effects of amino acids at various concentrations in alkaline soaking solution on weight gain, cooking loss and cooking yield of shrimp**

2.2.1 Preparation of treated shrimp

Shrimp (peeled and deveined) samples were mixed with alkaline soaking solution (ASS; 0.75% NaOH containing 2.5% NaCl, pH 11.5) in the presence of glycine, glutamic acid or arginine at levels of 1, 2 and 3% (w/v) using the shrimp/solution ratio of 1:2 (w/v). The mixtures were stirred gently for 30 min at 4 °C and allowed to stand at 4 °C for 30 min. After treatment, the shrimp samples were placed on the plastic screen for 5 min (4 °C) to drain off solution. Sample soaked in 2.5% NaCl containing 3% mixed phosphates (sodium tripolyphosphate+tetrasodium pyrophosphate; 1:2, w/w) (MANHEEM, 2013) and those treated with 2.5% NaCl containing 0.75% NaOH (pH 11.5) were used as the positive controls. Those without soaking were used as the control.
All shrimp samples without and with different treatments were divided into two portions. The first portion was used as raw shrimp and another portion was subjected to cooking. To prepare cooked shrimp, the samples were subjected to steaming until the core temperature of the second segment of shrimp reached 85°C. The samples were cooled rapidly in iced water for 1 min, and drained on a screen for 5 min at 4 °C. Both raw and cooked shrimp samples were weighed and the weight gain, cooking yield and cooking loss were calculated.

2.2.2. Analyses

*2.2.2.1 Determination of weight gain*

Weight gain was determined by weighing the shrimps before and after soaking in the solutions. Weight gain was calculated as follows:

$$\text{Weight gain (\%)} = [(B\text{-}A)/A] \times 100$$

where: A = initial weight (before soaking)
B = weight after soaking, followed by draining

*2.2.2.2 Determination of cooking loss and cooking yield*

Cooking loss and cooking yield were measured by weighing the shrimps before and after heating according to method of MANHEEM *et al*. (2012). Cooking yield and cooking loss were calculated by the following equations:

$$\text{Cooking loss (\%)} = [(B\text{-}C)/B] \times 100$$

$$\text{Cooking yield (\%)} = (C/A) \times 100$$

where: A = initial weight (without soaking and steaming)
B = weight after soaking, followed by draining
C = weight after steaming, followed by cooling in iced water

### 2.3 Effect of glutamic acid at various concentrations in ASS adjusted to different pHs on shrimp treatment

Shrimp (peeled and deveined) samples were mixed with ASS (0.75% NaOH containing 2.5% NaCl) in the presence of glutamic acid at various concentrations (1, 2, and 3%) having pH 7 and 11.5. To adjust the pH of ASS, 6 M and 2 M HCl was used. Shrimp samples were treated with solutions and analysed for weight gain, cooking loss and cooking yield as described previously.

### 2.4 Comparative study of glutamic acid and monosodium glutamate in ASS on shrimp treatment

#### 2.4.1. Preparation of treated shrimp

Shrimp (peeled and deveined) samples were mixed with ASS (pH 11.5) in the presence of glutamic acid or monosodium glutamate (MSG) at various concentrations (1, 2, and 3%) with pH of 11.5. MSG was used at the same mole equivalent to glutamic acid. Samples were treated as mentioned above. Both raw and cooked shrimp were analysed for weight gain, cooking loss and cooking yield. Additional analyses were performed as follows:

#### 2.4.2. Determination of color

Color of raw and cooked shrimp were determined and expressed as $L^*$ (lightness), $a^*$ (greenness/ redness) and $b^*$ (yellowness/ blueness). The second segment of shrimp was subjected for measurement using a Hunterlab colorimeter (Hunter Associates Laboratory, Inc., Reston, Virginia, USA) using a CIE Lab scale (YOUNG and WHITTLE, 1985).

#### 2.4.3. Determination of shear force

Shear force of raw and cooked shrimp was measured using the TA-XT2i texture analyzer (Stable Micro Systems, Surrey, England) equipped with a Warner-Bratzler shear apparatus (BRAUER *et al*., 2003). The operating parameters consisted of a cross head speed of 10 mm/s and a 25 kg load cell. The shear force, perpendicular to the axis of the second segment muscle fibers, was measured.

#### 2.4.4. Determination of protein pattern of soaking solutions

After being soaked with shrimp, the resulting soaking solutions were subjected to SDS-PAGE to determine the patterns of proteins leached out into solutions. SDS-PAGE was performed using 10% running and 4% stacking gels as described by LEAMMLI (1970). Soaking solution (20 ml) was mixed with 10 ml of 10% (w/v) SDS solution. The mixture was then homogenized at 11,000 rpm for 1 min. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The sample was then centrifuged at 7,500 *xg* for 15 min to remove undissolved debris using a microcentrifuge (MIK-RO20), Hettich Zentrifugan, Tuttlingen, Germany). Protein concentration of the supernatant was determined by the Biuret method (ROBINSON and HOGDEN, 1940). Sample (10 $\mu$g protein) was loaded onto the gel consisting of 4% stacking gel and 10% separating gel. Separation was performed by electrophoresis apparatus (Mini-Protein III, Bio-Rad, USA) using 30 mA. Protein was fixed and stained for 3 h in 1.25% Coomassie Brilliant Blue R-250 in 40% methanol and 10% glacial acetic acid. Gels were destained for 15 min with destaining

solution I (50% methanol and 7.5% glacial acetic acid) and with the destaining solution II (5% methanol and 7.5% gracial acetic acid) for 3 h. Wide range molecular weight standards were used and the molecular weight of protein was estimated.

2.4.5. Sensory evaluation

Cooked samples were subjected to sensory analysis. The samples were evaluated by 30 panelists from the Department of Food Technology with the age of 25-35, using the 9-point hedonic scale, where 9 = like extremely; 7= like moderately; 5 = neither like or not dislike; 3 = dislike moderately; 1 = dislike extremely (MEILGAARD *et al*., 1990). Panelists were acquainted with shrimp consumption and had no allergies to shrimp. All panelists were asked to evaluate for color, appearance, flavor, taste, texture and overall likeness. Samples were presented in the plates coded with three-digit random numbers.

### 2.5. Statistical analyses

A completely randomized design (CRD) was used for the whole experiments. Experiments were run in triplicate using different lots of shrimp samples. Data were subjected to analysis of variance and mean comparison was carried out using Duncan's multiple range test. Statistical analyses were performed using the Statistical Package for Social Science (SPSS 11.0 for window, SPSS Inc., Chicago, IL, USA).

## 3. RESULTS AND DISCUSSION

### 3.1 Weight gain, cooking loss and cooking yield of Pacific white shrimp treated with ASS containing amino acids at various concentrations

Weight gain of Pacific white shrimp treated with ASS (pH 11.5) in the presence of amino acids at different concentrations (1, 2 and 3%) is shown in Fig. 1a. Weight gain of the treated shrimp increased, when the concentrations of amino acids in ASS increased ($P<0.05$), except those treated with ASS containing glutamic acid, in which weight gain decreased with increasing concentration ($P<0.05$). Amongst all amino acids, glutamic acid showed the higher increasing effect on weight gain at low level (1%). Under the alkaline condition, carboxyl groups, both at $\alpha$-carbon and $\gamma$-carbon, were deprotonated and $COO^-$ became dominant. Those negatively charged residues might penetrate into the swollen muscle along with ASS. Some $COO^-$ groups of glutamic acid could interact with positively charged domain of proteins via ionic interaction, while the rest of $COO^-$ groups were able to bind with water. As a result, the water could be retained in the muscle after treatment. Nevertheless, in the presence of an excessive glutamic acid, those $COO^-$ groups in the solution (aqueous phase) more likely competed with muscle proteins in binding with water. As a consequence, the less water was retained in the shrimp muscle as indicated by the lowered weight gain, when the level of glutamic acid in ASS was higher than 1%. Glycine has been known as the smallest amino acid and has H atom as the side chain. Due to its small molecule, it could migrate easily into shrimp muscle and subsequently interact with water by hydrogen bonding via side chains within muscle. Additionally, carboxyl groups at $\alpha$-carbon of glycine, which was deprotonated under the alkaline condition, could interact with water via ionic interaction. Consequently, water could be more imbibed, particularly when the level of glycine increased. This was reflected by the increased weight gain of shrimp after being treated with glycine at higher concentrations.

For shrimp treated with ASS containing arginine, weight gain increased as the levels of arginine increased ($P<0.05$). At pH 11.5, some carboxyl groups of arginine (pI=10.76) became deprotonated. Those groups could interact with muscle proteins and simultaneously bound with water. It was noteworthy that pH of solution (11.5) was close to pI of arginine. Therefore, $COO^-$ group of arginine in ASS was present at low intensity. Owing to the lower abundance of $COO^-$ group in ASS containing arginine, water was less bound with proteins, in comparison with that containing glutamic acid. WOLFENDEN *et al*. (1981) found that hydration potential of arginine was high at pH 7. At neutral pH, $NH_2$ group mostly became protonated, in which $NH_3^+$ was formed. Those positively charged groups effectively bound with water. However, in the present study, the pH of solution was 11.5, which was above pI (10.76). As a result, $NH_3^+$ was not present in ASS, and $NH_2$ group at the side chain became abundant. Arginine is also reported frequently to form hydrogen bonds with other side chains and water molecule (BORDERS *et al*., 1994). Thus, charge of amino acid under the alkaline condition and the way those amino acids interacted with water and proteins in muscle more likely affected weight gain of treated shrimp.

Differences in weight gain among all treatments were plausibly governed by the differences in water binding or water holding capacity of different amino acids under the alkaline conditions. It was found that weight gain of shrimp treated with ASS containing 3% arginine was higher than those treated with mixed phosphates and other samples ($P<0.05$). It is noted that treatment of shrimp with ASS containing 1% glycine, 1% arginine or 3% glutamic acid led to the lower weight gain, compared with ASS without amino acids. Thus, amino acids in alkaline solution had varying impact on weight gain of shrimp after treatments.

Cooking loss and cooking yield of shrimp samples treated with ASS containing different amino acids at various concentrations are shown in Figs. 1b and 1c, respectively. Cooking loss of shrimp decreased when the concentration of glutamic acid in ASS increased ($P<0.05$). However, no differences in cooking yield were noticeable, when glycine at different levels was used ($P\geq0.05$). Overall, when shrimp samples were treated with ASS in the presence of all amino acids, the decrease in cooking loss was obtained, in comparison with those treated with only ASS ($P<0.05$). The weight loss was in the descending order in samples treated with ASS containing glycine, arginine and glutamic acid, respectively. Shrimp treated using ASS comprising 3% glutamic acid had the lower cooking loss than that treated with M-P ($P<0.05$). Generally, the lowest cooking loss was found with the sample treated with ASS containing 3% glutamic acid ($P<0.05$). When heat was applied, denaturation and coagulation of proteins were augmented, which in turn lowered water-holding capacity. Moreover, the increased protein-protein interaction was obtained (NIAMNUY *et al*., 2008), leading to the enhanced release of water from shrimp muscle.

After cooking, the cooking yield of shrimp with different treatments varied (Fig. 1c), in which water molecules might be bound with amino acids or proteins in different fashions. The highest cooking yield was obtained with shrimp treated with ASS containing glutamic acid, compared with other treatments ($P<0.05$). It was suggested that glutamic acid had the potential to bind water in shrimp muscle, when heat was applied. The efficacy in water holding during heating was dependent on concentrations used ($P<0.05$). However, the concentration of glycine had no effect on cooking yield of shrimp ($P>0.05$). Efficiency in increasing cooking yield was in the descending order: glutamic acid, arginine and glycine. The negatively charged residues, especially carboxyl group at $\alpha$-carbon and $\gamma$-carbon of glutamic acid are strongly hydrated (COLLINS *et al*., 2007). The two amino acids that have the highest water-binding ability are aspartic acid and glutamic acid (LOW *et al*., 1978). An

ionic side chain of aspartic acid, glutamic acid and lysine has been claimed to bind 4-7 water molecules (ZAYAS, 1997).

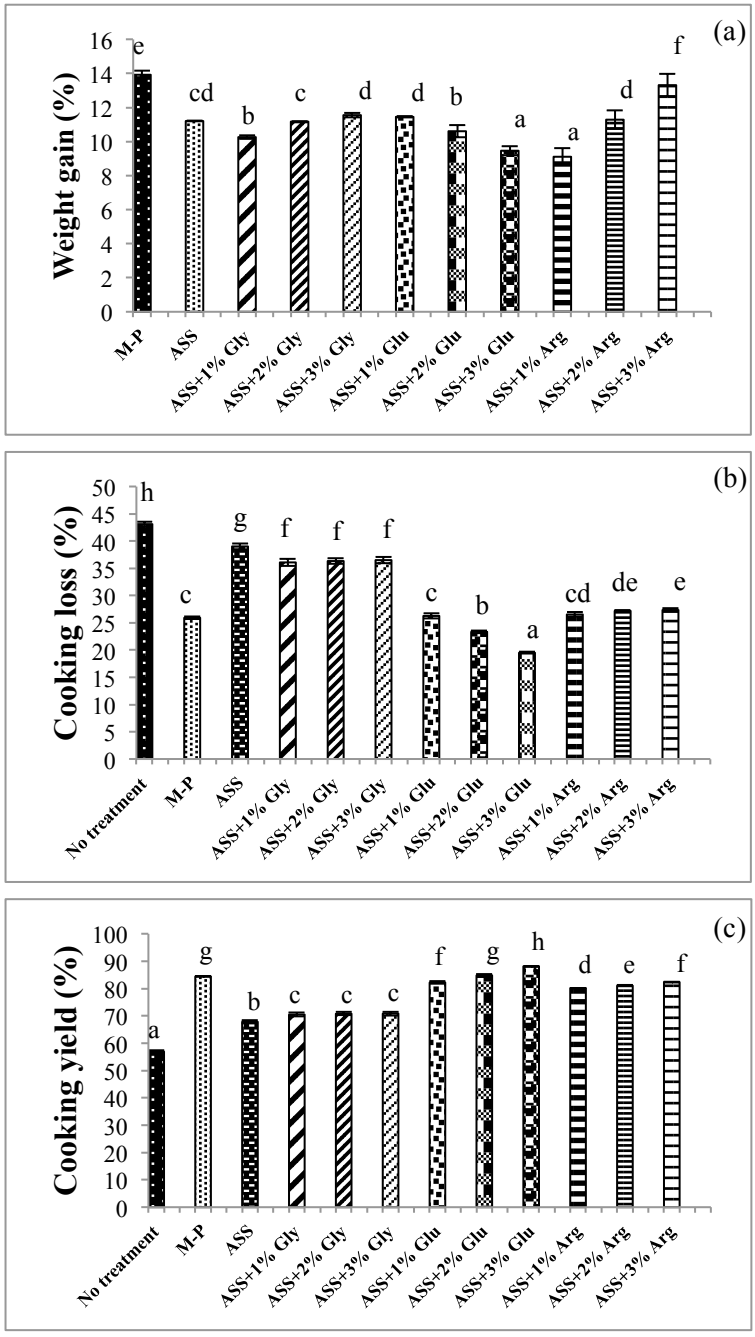


**Figure 1**: Weight gain (a), cooking loss (b) and cooking yield (c) of Pacific white shrimp treated with 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of amino acids at different concentrations. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Gly: glycine Glu: glutamic acid, Arg: arginine. Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent the standard deviation (n=3).

When comparing with the sample treated with ASS alone, all samples treated with amino acids showed the increased cooking yield ($P<0.05$). Only sample treated with ASS containing 3% glutamic acid had the higher cooking yield than that treated with M-P ($P<0.05$). The sample treated with ASS having 2% glutamic acid had similar cooking yield to those treated with mixed phosphate ($P\geq0.05$). Therefore, glutamic acid in ASS was shown to play a vital role in increasing the cooking yield by holding water in muscle during heating.

### 3.2 Weight gain, cooking loss, cooking yield and physical properties of Pacific white shrimp treated with ASS containing glutamic acid at various concentrations and pHs

Weight gain, cooking loss and cooking yield of Pacific white shrimp samples after soaking in ASS in the presence of glutamic acid at various concentrations with different pHs (7.0 and 11.5) are shown in Fig. 2. Weight gain and cooking yield of shrimp samples soaked in ASS, pH 11.5 were higher than those treated with ASS at pH 7.0 ($P<0.05$), for all concentrations of glutamic acid used. At pH above pI or very alkaline pH, proteins had more negative charge, in which protein molecules repulsed each other, resulting in the swollen muscle structure (CHANTARASUWAN *et al*., 2011b). As a consequence, water could be more uptaken into shrimp (CHANTARASUWAN *et al*., 2011b). Simultaneously, glutamic acid could be taken into the muscle along with NaCl. Glutamic acid might favor the water binding via its $COO^-$ group. Weight gain was slightly decreased as the concentrations of glutamic acid used increased ($P<0.05$), regardless of pH. Generally, weight gain of all samples was lower than that of samples treated with ASS and with M-P ($P<0.05$), except that treated with ASS containing 1% glutamic acid (pH 11.5) that showed the similar weight gain ($P>0.05$). Among Pacific white shrimp treated with sodium carbonate and sodium bicarbonate at various pHs (5.5-11.5), the highest weight gain and cooking yield were observed in those treated at pH 11.5 (CHANTARASUWAN *et al*., 2011b). Therefore, pH of soaking solution was the prime factor determining weight gain of shrimp.

Cooking loss (Fig. 2b) of shrimp decreased, when the concentration of glutamic acid in ASS increased ($P<0.05$), irrespective of pH used. Lower cooking loss was found as ASS had pH of 11.5, compared with pH 7.0 ($P<0.05$). The decrease in cooking loss was in accordance with the increase in cooking yield. The lowest cooking loss was observed in shrimp soaked with ASS containing 3% glutamic acid (pH 11.5) ($P<0.05$). Glutamic acid could provide negative charge to muscle, thereby enhancing the water binding capacity of muscle proteins. Bound water was held tightly during heating as indicated by the lowered cooking loss and increased cooking yield. AASLYNG *et al*. (2003) suggested that a higher cooking loss was found in the sample with the low pH, whereas high water holding capacity was achieved at medium and high pH. When comparing the cooking loss of shrimp treated with mixed phosphates (M-P), the sample treated with ASS containing glutamic acid at levels of 2 or 3% (pH 11.5) had the lower cooking loss ($P<0.05$). This reflected the high efficiency of ASS containing glutamic acid at a sufficient amount in lowering the cooking loss. The augmented repulsion of muscle compartment also allowed glutamic acid with the high negative charge to penetrate into muscle and bind more water, thus resulting in the lowered cooking loss.

In the presence of glutamic acid at 2 and 3%, no differences in cooking yield were obtained, compared with the sample treated with ASS alone ($P<0.05$), when pH of soaking solution was 7.0 (Fig. 2c). On the other hand, cooking yield of samples treated with ASS containing glutamic acid at all levels were higher than that of sample treated with ASS alone, when pH was 11.5 ($P<0.05$). The result indicated the paramount role of pH in water holding capacity of muscle when heating was implemented. As the shrimp were treated

with ASS (pH 11.5) containing glutamic acid at levels higher than 1%, similar cooking yield was obtained, compared to that found in the sample treated with mixed phosphates ($P<0.05$). Therefore, ASS containing glutamic acid showed the potential in improving the cooking yield, and its efficacy was comparable to mixed phosphates.

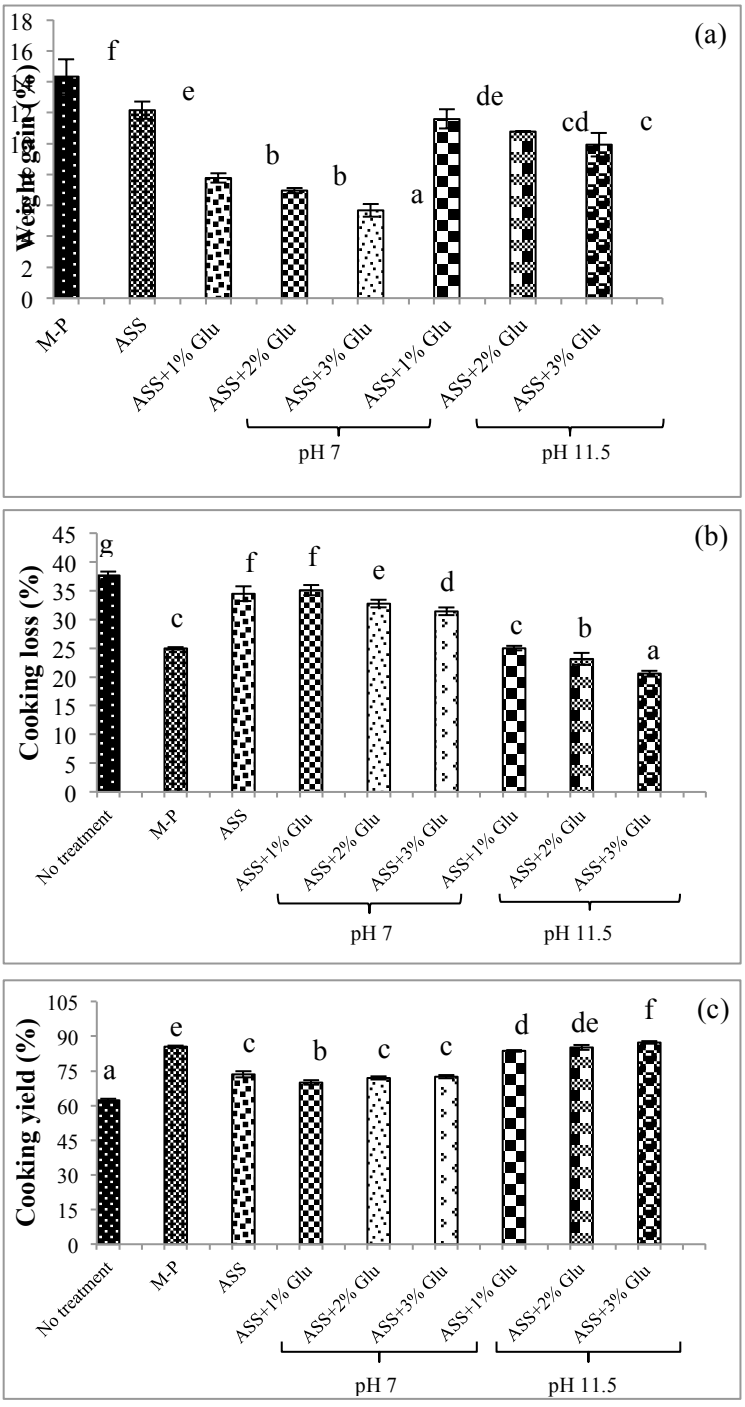


**Figure 2**: Weight gain (a), cooking loss (b) and cooking yield (c) of Pacific white shrimp treated with 0.75% NaOH containing 2.5% NaCl with pHs 7.0 and 11.5 in the presence of glutamic acid at various concentrations. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Glu: glutamic acid. Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent the standard deviation (n=3).

### 3.3 Comparative study of ASS containing glutamic acid and MSG on shrimp treatment

Glutamic acid with high efficacy in increasing cooking yield was selected as the potential additive in ASS. However, glutamic acid had low solubility. Conversely, MSG, a salt form, was cheaper and soluble with ease in water. MSG was used at the same mole equivalent to glutamic acid for shrimp treatment.

#### 3.3.1 Weight gain, cooking loss and cooking yield

Weight gain of shrimp treated with ASS containing glutamic acid at the levels of 1%, 2% and 3% or MSG at the levels of 1%, 2% and 3% mole equivalent of glutamic acid is shown in Fig. 3a. Sample treated with ASS containing 1 or 2% MSG had similar weight gain to that treated with mixed phosphate (positive control) ($P \geq 0.05$). It was postulated that the negatively charged glutamic acids from MSG were able to bind tightly with water molecule via ionic interaction within protein compartment, leading to the increased water holding in shrimp muscle. It was found that MSG showed higher ability in water holding than glutamic acid as indicated by higher weight gain.

For cooking loss (Fig. 3b), shrimp treated with ASS containing MSG had the higher cooking loss than those treated with glutamic acid counterpart, particularly at levels of 2 and 3% ($P<0.05$). The cooking loss was lower in samples treated with ASS comprising both glutamic acid and MSG at higher concentrations ($P<0.05$). For samples treated with mixed phosphate and ASS alone, the cooking losses of 19.19% and 31.07%, respectively were observed. The cooking loss of 13.76-19.62% was obtained for sample treated with ASS having glutamic acid, while cooking loss of 17.51-20.41% was found for the sample treated with ASS containing MSG. During cooking, muscle proteins underwent denaturation to a higher extent, while the amount of water retained in shrimp meat decreased with coincidental increase in fat and protein contents (MANHEEM *et al*., 2012; BENJAKUL *et al*., 2008). The result suggested that shrimp muscle could retain more water when glutamic acid and MSG were incorporated in ASS. However, glutamic acid showed the slightly higher ability in lowering cooking loss of shrimp, compared with MSG, especially at level of 2-3%.

For cooking yield (Fig. 3c), the opposite results were observed, in comparison with cooking loss. The lowest cooking yield was observed in the control (without treatment). Cooking yield of treated shrimp increased, when the concentrations of both glutamic acid and MSG increased ($P<0.05$). Similar cooking yield was found in shrimp treated with ASS containing 2% glutamic acid or 3% MSG ($P>0.05$). It was found that shrimp treated with ASS containing 2 or 3% glutamic acid or 3% MSG had the higher cooking yield than that of sample treated with mixed phosphates ($P<0.05$). Since MSG was more soluble and cheaper than glutamic acid, it was selected for treatment of shrimp. The appropriate concentration of MSG in ASS was 3%.

#### 3.3.2 Shear force

Shear force of raw and cooked Pacific white shrimp treated with ASS (pH 11.5) in the presence of glutamic acid and MSG at different levels (1%, 2% and 3%) is presented in Table 1. For raw shrimp, all treatments had no impact on shear force ($P>0.05$). It was noted that shrimp treated with ASS containing glutamic acid and MSG at all levels (1-3%) had similar shear force ($P \geq 0.05$). All samples treated with ASS containing either glutamic acid or MSG had the similar shear force to those treated with mixed phosphates ($P>0.05$), except those treated with ASS containing 3% glutamic acid, which showed the lower shear force ($P<0.05$). Generally, shrimp tended to have the non-significant decrease in shear

force after treatment, particularly with increasing concentrations of glutamic acid and MSG in ASS used for treatment. When proteins imbibed water within their structure, the muscle had the lower resistance to the force applied.

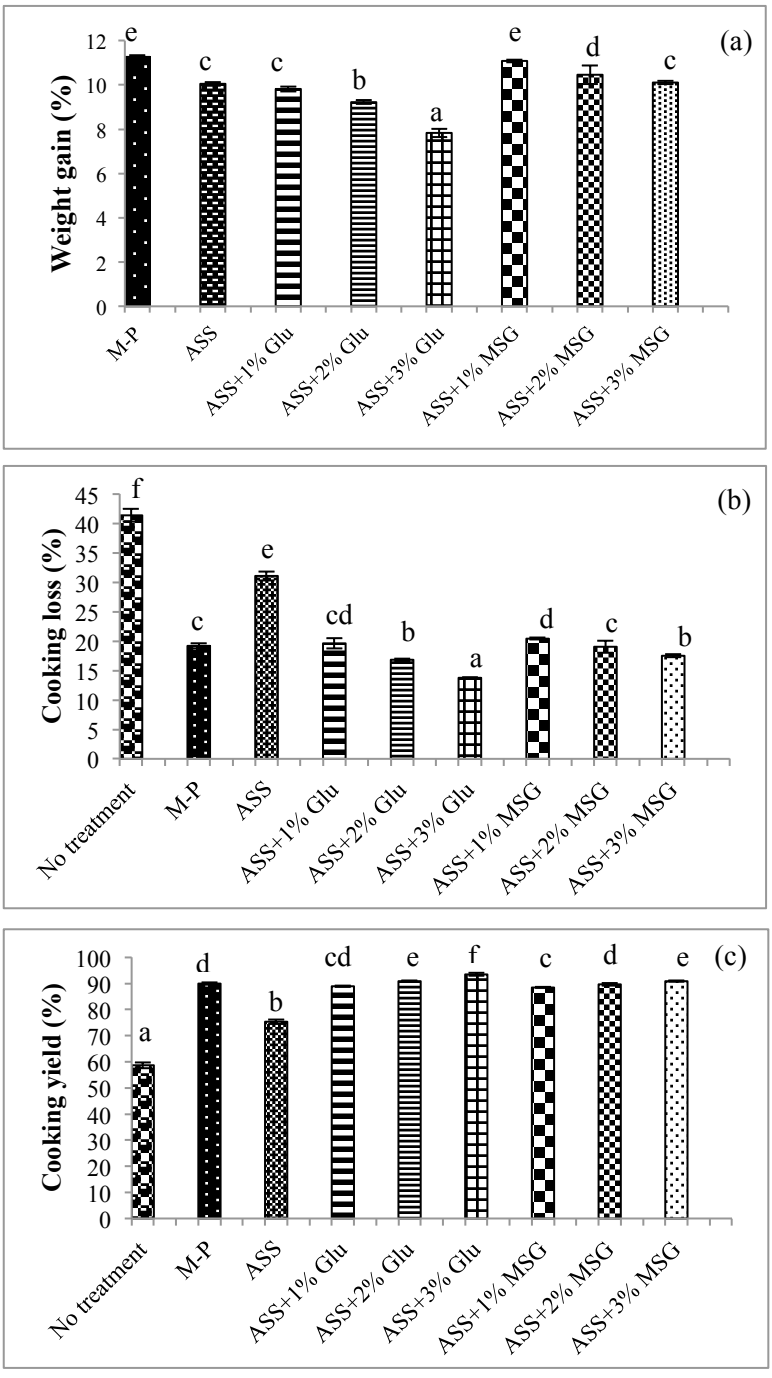


**Figure 3**: Weight gain (a), cooking loss (b) and cooking yield (c) of Pacific white shrimp treated with 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of glutamic acid and MSG at various concentrations. Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Glu: glutamic acid. MSG: monosodium glutamate. Different lowercase letters on the bars indicate significant differences ($P<0.05$). Bars represent the standard deviation (n=3).

**Table 1**: Shear force of raw and cooked of Pacific white shrimp treated with 0.75% NaOH (pH 11.5) containing 2.5% NaCl in the presence of glutamic acid and MSG at various concentrations.

| Treatments | Shear force (g) | |
|---|---|---|
| | Raw | Cooked |
| No treatment | 1970±177 †,abc | 2194±326 b |
| M-P | 2057±212 bc | 1578±341 a |
| ASS | 1890±416 abc | 2031±345 b |
| ASS+1% MSG | 2127±219 c | 1549±130 a |
| ASS+2% MSG | 1995±284 abc | 1525±334 a |
| ASS+3% MSG | 1794±275 abc | 1472±83 a |
| ASS+1% glu | 1972±329 abc | 1555±205 a |
| ASS+2% glu | 1665±253 ab | 1515±126 a |
| ASS+3% glu | 1618±243 a | 1449±118 a |

†Mean±SD (n=3).
Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Glu: glutamic acid. MSG: monosodium glutamate. Different lowercase superscripts in the same column indicate significant differences ($P<0.05$).

For cooked shrimp, lower shear force was observed in all treated samples, in comparison with those without treatment and those treated with ASS alone ($P<0.05$). Nevertheless, all samples had the similar shear force, compared to those treated with mixed phosphates ($P>0.05$). When heat was applied, protein denaturation and aggregation took place. Those phenomena resulted in the toughness as well as high resistance to force. No differences in shear force were found among shrimp treated with ASS containing glutamic acid and MSG at different concentrations used ($P>0.05$). Myofibrillar proteins with increased negative charge favored the repulsion of polypeptide chains, which resulted in the swelling of muscle and became less resistant to shear force applied. When the concentration of glutamic acid or MSG in ASS increased, non-significant decrease in shear force was obtained ($P\geq0.05$). Therefore, treatment of shrimp using ASS containing glutamic acid or MSG did not had the negative impact on textural property and their shear force was comparable to that of shrimp treated with mixed phosphates.

3.3.3 Color

Color parameters ($L^*$, $a^*$ and $b^*$) of raw and cooked shrimp treated with ASS (pH 11.5) in the presence of glutamic acid or MSG at different concentrations are shown in Table 2. For raw shrimp, $L^*$ value increased when concentrations of MSG in ASS increased (3%) ($P<0.05$). The $a^*$ value generally increased as the concentrations of both glutamic acid and MSG in ASS increased ($P<0.05$). Basically, $a^*$ value indicates the reddish color. Conversely, no changes in $b^*$ value were observed as the level of glutamic acid or MSG in ASS increased ($P<0.05$). Thus, raw shrimp turned to be slightly reddish as the levels of glutamic acid or MSG in ASS increased. Appearance of the product plays a significant role in maintaining high consumer acceptance (BONO *et al*., 2012). Glutamic acid or MSG in ASS might induce the change of proteins associated with carotenoid, named carotenoprotein. This led to the enhanced exposure of free carotenoids, especially astaxanthin, as evidenced by more reddish color. Astaxanthin was reported as the major

pigment in shrimp meat (NIAMNUY *et al*. 2008). After cooking, no difference in color was observed among shrimp treated with ASS containing glutamic acid or MSG at all levels (P>0.05). It was obvious that *L**, *a** and *b**-values of cooked shrimp increased, in comparison with raw counterparts. The increase in intensity of red color alter cooking is caused by muscle protein denaturation and the release of carotenoid pigment bound to the protein (carotenoproteins) (LATSCHA 1989; NIAMNUY *et al*. 2008). The *a**- and *b**- values of samples treated with ASS containing 2 or 3% glutamic acid were lower, compared with the control (no treatment). During soaking, some proteins including carotenoproteins were partially solubilized or leached out. As the results, less pigments were retained in the meat. Coincidentally, the soaking solution was more reddish in color as the glutamic acid or MSG concentrations in ASS increased (data not shown). There was no difference in all color parameters between cooked shrimp treated with ASS containing glutamic acid or MSG at all levels and those treated with mixed phosphates.

**Table 2**: Color of raw and cooked of Pacific white shrimp treated with 0.75% NaOH (pH 11.5) containing 2.5% NaCl in the presence of glutamic acid and MSG at various concentrations.

| Samples | Treatments | *L** | *a** | *b** |
|---|---|---|---|---|
| Raw | No treatment | 46.46±1.58 $^{ab}$ | -1.64±1.05 $^{a}$ | 0.12±1.91 $^{cd}$ |
| | M-P | 46.33±3.43 $^{ab}$ | -1.69±0.36 $^{a}$ | -3.23±1.79 $^{a}$ |
| | ASS | 44.96±2.10 $^{a}$ | -1.66±0.34 $^{a}$ | -2.01±1.61 $^{ab}$ |
| | ASS+1% MSG | 45.42±2.58 $^{a}$ | 0.27±0.96 $^{b}$ | -1.13±0.48 $^{bc}$ |
| | ASS+2% MSG | 46.81±2.10 $^{ab}$ | 1.28±1.13 $^{bc}$ | -0.88±1.28 $^{bc}$ |
| | ASS+3% MSG | 48.87±2.51 $^{b}$ | 2.30±1.15 $^{d}$ | 0.16±1.96 $^{cd}$ |
| | ASS+1% glu | 45.22±2.39 $^{a}$ | 0.89±1.51 $^{b}$ | -0.02±1.63 $^{cd}$ |
| | ASS+2% glu | 46.09±0.88 $^{a}$ | 2.01±0.74 $^{cd}$ | 0.08±1.95 $^{cd}$ |
| | ASS+3% glu | 46.35±1.22 $^{ab}$ | 2.77±0.87 $^{d}$ | 1.19±2.71 $^{d}$ |
| Cooked | No treatment | 70.02±4.04 $^{c}$ | 13.18±4.40 $^{d}$ | 16.34±3.98 $^{b}$ |
| | M-P | 65.50±4.30 $^{ab}$ | 8.55±2.45 $^{ab}$ | 12.37±4.63 $^{a}$ |
| | ASS | 69.35±5.37 $^{bc}$ | 11.85±4.02 $^{cd}$ | 13.39±3.20 $^{ab}$ |
| | ASS+1% MSG | 65.09±2.40 $^{a}$ | 11.17±2.77 $^{abcd}$ | 14.34±3.86 $^{ab}$ |
| | ASS+2% MSG | 64.77±3.95 $^{a}$ | 8.86±1.31 $^{ab}$ | 11.28±3.63 $^{a}$ |
| | ASS+3% MSG | 63.17±4.55 $^{a}$ | 8.27±1.94 $^{a}$ | 10.92±3.18 $^{a}$ |
| | ASS+1% glu | 65.50±3.75 $^{ab}$ | 11.32±1.91 $^{bcd}$ | 15.64±3.06 $^{ab}$ |
| | ASS+2% glu | 64.94±2.38 $^{a}$ | 9.69±1.60 $^{abc}$ | 14.40±3.13 $^{ab}$ |
| | ASS+3% glu | 64.44±2.05 $^{a}$ | 9.04±1.30 $^{abc}$ | 11.78±2.18 $^{a}$ |

†Mean±SD (n=3).

Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1, (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), Glu: glutamic acid. MSG: monosodium glutamate. Different lowercase superscripts in the same column under the same state of sample indicate significant differences (P<0.05).

### 3.3.4 Protein patterns of soaking solutions

Protein patterns of soaking solutions after shrimp treatments are shown in Fig. 4. Band intensity of myosin heavy chain (MHC) and actin slightly increased as concentration of both glutamic acid and MSG in ASS increased. The result suggested that more MHC and actin were solubilized and leached out to solution to a higher extent when glutamic acid and MSG levels in ASS increased. The increase in MHC band intensity in soaking

solutions was in agreement with the higher cooking yields (Fig. 3c). Protein extraction and dissociation of myofibrillar proteins were mainly due to the ionic effect and pH alteration (BENDALL, 1954). Apart from MHC and actin, protein with MW of 25.6 and 18.2 kDa also increased with increasing levels of glutamic acid and MSG in ASS. CHANTARASUWAN *et al*. (2011) reported that Pacific white shrimp soaked in sodium bicarbonate and sodium carbonate solution had the increase in band intensity of MHC as pH of soaking solution increased. Protein patterns of ASS and ASS with mixed phosphates after shrimp soaking were similar, in which actin and protein with MW of 18.2 kDa were dominant. After those proteins were leached out from shrimp muscle, the soaking solution could be more penetrated to looser muscle compartment and retained inside the muscle as indicated by higher weight gain and cooking yield.

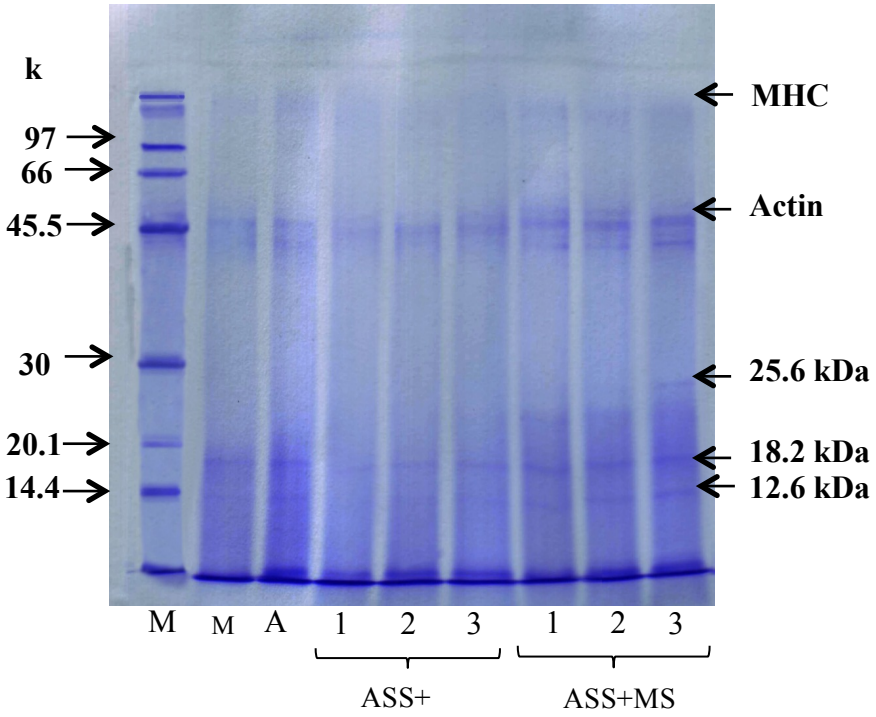


**Figure 4**: Protein patterns of soaking solution (0.75% NaOH and 2.5% NaCl, pH 11.5) in the presence of glutamic acid and MSG solutions at different concentrations after soaking with shrimp. M, Standard marker; M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), MSG: monosodium glutamate, MHC: myosin heavy chain. Numbers indicate the concentration of Glu or MSG (%).

### 3.4 Sensory properties of cooked shrimp treated with alkaline soaking solution containing MSG

Sensory properties of cooked shrimp without and with different treatments are shown in Table 3. The lowest likeness score for all attributes tested except flavor was found in the control sample (without treatment) ($P<0.05$). During heating, the shrinkage of muscle of shrimp occurred, resulting in more compact in microstructure of shrimp with less juiciness. Among all samples, ASS +3%MSG sample showed the highest score for all attributes except for flavor that showed the lower score than the control and M-P sample

(P<0.05). This might be associated with fishy odor/flavor developed in of ASS +3%MSG sample. MSG is sodium salt of glutamic acid and provides a flavoring function similar to naturally occurring free glutamate in foods (YAMAGUCHI and NINOMIYA, 2000). Monosodium L-glutamate (MSG) has been used as a flavor enhancer since 1908, when it was identified as the source of umami taste (pleasant savory taste) (IMADA *et al*., 2014). Since ASS +3%MSG samples had the high water holding capacity, more water was retained in shrimp meat as shown by a high cooking yield (Fig. 3). Decreased toughness with high juiciness contributed to the higher likeness score for texture and appearance. For color likeness, the highest score was observed for ASS +3%MSG sample (P<0.05). Treatment of shrimp using 0.75% NaOH containing 2.5% NaCl and 3% MSG (ASS+3%MSG) rendered the resulting cooked shrimp with the highest overall likeness score (P<0.05). However, the score was similar to that of shrimp treated with mixed phosphates (P>0.05).

**Table 3**: Likeness score of cooked Pacific white shrimp with different treatments.

| Attributes | No treatment | M-P | ASS | ASS+3% MSG |
|---|---|---|---|---|
| Appearance | 5.50±1.25 †,a | 7.76±0.66 c | 7.00±0.98 b | 8.00±0.74 c |
| Color | 6.03±1.56 a | 7.76±0.66 c | 7.07±1.01 b | 7.87±0.82 c |
| Flavor | 7.10±1.03 b | 7.31±1.13 b | 6.80±1.19 ab | 6.40±1.57 a |
| Texture | 5.57±2.10 a | 7.55±0.81 bc | 7.00±1.74 b | 7.80±0.85 c |
| Taste | 5.80±1.85 a | 7.83±0.73 b | 7.73±0.78 b | 8.13±0.73 b |
| Overall | 5.77±1.45 a | 7.76±0.85 bc | 7.40±0.81 b | 8.07±0.69 c |

†Mean±SD (n=3).
Note: M-P: solution containing 2.5% NaCl and 3% mixed phosphates (tetrasodium pyrophosphate and sodium tripolyphosphate, 2:1 (w/w)), ASS: 0.75% NaOH containing 2.5% NaCl (pH 11.5), ASS+3% MSG: 0.75% NaOH containing 2.5% NaCl (pH 11.5) in the presence of 3% monosodium glutamate. Different lowercase superscripts in the same row indicate significant differences (P<0.05).

## 4. CONCLUSION

Amino acids in ASS had the pronounced impact on quality improvement of Pacific white shrimp. Glutamic acid showed the marked effect on increasing cooking yield, while arginine could increase weight gain effectively. pH of ASS played a paramount role in water uptake and lowering the cooking loss of treated shrimp. MSG, the water soluble salt, showed the comparable impact on quality improvement to glutamic acid. Glutamic acid or MSG at higher level in ASS enhanced the solubility of MHC and actin, facilitating the migration of soaking solution and water holding capacity of shrimp muscle. Cooked shrimp treated with ASS containing MSG had the increased overall likeness score, but showed less score in flavor associated with the slightly fishy odor. The use of ASS containing 3% MSG was recommended as the promising soaking solution for shrimp treatment.

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