Effects of lysine and arginine on the properties of low-salt mince gel from striped catfish (*Pangasianodon hypophthalmus*)

Natchaphol Buamard, Mohammad Akram Javith, Amjad Khansaheb Balange, Gopal Krishna, and Soottawat Benjakul

Abstract: Effects of basic amino acids, lysine (Lys) and arginine (Arg), at different levels (0%, 0.5%, and 1%, based on mince weight) on properties of striped catfish (*Pangasianodon hypophthalmus*) mince gel containing low salt (LS) and high salt (HS) were investigated. Without Lys or Arg addition, HS gel had the higher textural properties including hardness, chewiness, as well as cohesiveness than LS gel ($P < 0.05$) and the highest values were achieved when 1% Arg was incorporated in both LS and HS gels ($P < 0.05$). Arg had no effect on acceptability of mince gel. However, whiteness was decreased in HS gel when Arg was applied. Autolysis of gel was lower in HS gel containing Arg. No differences in protein patterns among all gel samples were found. Addition of Arg could increase the gelling ability of both LS and HS mince during heating as evidenced by higher storage modulus (G') and viscous modulus (G''). Mince gel added with Arg had orderly interconnected structure and their microstructure was finer than that without Arg. Therefore, Arg could be used in LS gel from striped catfish mince, in which quality of gel was equivalent to HS counterpart.

Keywords: arginine, gelation, low salt, lysine, mince, Pangas, striped catfish

Practical Application: Basic amino acid, especially arginine (Arg) with guanidinium group, could increase repulsive force between protein molecules at low-salt concentration (0.5%). This resulted in high solubilization of muscle proteins, whereas gel formation or gel strength was higher than that containing high salt (2.5% to 3.5%). Thus, Arg could be used for production of fish mince gel containing low salt with lowered health risk.

1. INTRODUCTION

Fish has gained increasing interest in the form of flesh for direct consumption or making various products, especially gel-type products. Gelation of muscle proteins includes denaturation and subsequently non-reversible aggregation of myosin heads via disulfide bond and helix-coil transitions in tail portion, leading to three-dimensional interconnections inside the network (Stone & Stanley, 1992; Sun & Holley, 2011). Gelation process is governed by numerous factors involving type and concentration of protein, processing conditions, ionic strength, heating rate, pH, endogenous enzymes, and so forth (Benjakul, Visessanguan, Thongkaew, & Tanaka, 2005).

Salt is an essential ingredient to provoke structural alternations via electrostatic interactions between muscle proteins and the ions of salt, sodium, and chlorides; these bring about dissociation of myofilaments and solubilization of proteins (Totosaus & Perez-Chabela, 2009). Salt at a concentration of 2.5% to 3.5% is commonly used for fish muscle gelation to enhance solubilization of proteins. However, high-sodium content is one of the undesirable concerns in gel-based product associated with the health risk (Kim & Park, 2008; Cando, Herranz, Borderías, & Moreno, 2016). Recently, numerous consumers have paid attention on low-sodium products. Intake of salty diet has the adverse effects on kidneys and urinary systems and is related to high blood pressure as well as cardiovascular diseases (Ha, 2014). Nevertheless, it is a tremendous challenge for producers to reduce the NaCl content in gel-based products, in which NaCl is generally implemented as the main ingredient, particularly for solubilization of myofibrillar proteins (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2014). Under physiological condition, lysine (Lys) and arginine (Arg) are positively charged and mainly exposed to the surface of protein molecule. These two amino acids are considered as Generally Recognized as Safe (GRAS). However, the excessive intake of amino acid consumption could lead to adverse effects (e.g., nausea, vomiting, and diarrhea) (Grimble, 2007). Lys and Arg play significant roles in ionic interactions and hydrogen bonds, and favor water binding of protein chains (Sokalingam, Raghunathan, Soundrarajan, & Lee, 2012). Both Arg and Lys could augment solubility of pork and chicken meat in the presence of low salt (LS) (Zhou, Li, & Tan, 2014). They also effectively improved the water retention ability, textural properties, and color, while minimizing the cooking loss of pork sausage (Zheng et al., 2017; Zhou, Li, Tan, & Sun, 2014). In addition, Arg and Lys were able to change the pH, interact with metallic ions, and prevent fat and protein from oxidation processes (Baker, 2007). In addition, both amino acids have been shown to be nutritious and beneficial for health. Lys is essential in converting fatty acids into energy and lowering the cholesterol in human blood (Douša, Břicháč, Gibala, & Lehnert, 2011). Arg has the ability to dilate blood vessels and inhibit tumor cell proliferation and can also act as a nerve conduction factor related to brain memory (Zhou, Li, Tan, & Sun, 2014).

JFDS-2020-0263 Submitted 2/16/2020, Accepted 6/16/2020. *Authors Buamard and Benjakul are with Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand. Authors Javith, Balange, and Krishna are with Department of Post-Harvest Technology, FRHPHM Division, ICAR-Central Institute of Fisheries Education, Mumbai, Maharashtra 400061, India. Direct inquiries to author Benjakul (E-mail: soottawat.b@psu.ac.th).*

doi: 10.1111/1750-3841.15368

Journal of Food Science A Publication of the Institute of Food Technologists

Striped catfish (Pangas) can be cultured worldwide, especially in the tropical countries (Singh & Lakra, 2012). To increase the value of the flesh of Pangas, making the gel with LS could be a means to increase the utilization of this species as well as to produce the healthy fish products for consumers. Nevertheless, no report concerning the effect of Lys and Arg on LS gel from striped catfish mince exists. The investigation was aimed at studying the influence of Lys and Arg at different levels on properties and acceptability of gels from striped catfish mince with LS content.

2. MATERIALS AND METHODS

2.1 Chemicals

Arg and Lys were obtained from Himedia Laboratories Pvt. Ltd. (Mumbai, India). All the chemicals used for microstructure analysis and electrophoresis were of analytical grade.

2.2 Raw material

Striped catfish (*Pangasianodon hypophthalmus*) (10 to 12 kg/fish) were procured from fresh fish market in Hat Yai, Thailand and delivered on ice to the laboratory within 30 min. Fish were then washed with cold water (approximately 10 °C). After being headed, eviscerated, and washed, fish were subsequently filleted and deskinned manually. The flesh was then minced using a blender until uniformity was obtained (Panasonic, Model MK-5087M, Berkshire, UK).

2.3 Preparation of frozen mince

To fish mince, 4% sorbitol and 4% sucrose were added as cryoprotectants and mixed thoroughly. Sample (500 g) was placed in a polyethylene bag, sealed, and stored in freezer at −18 °C. Before use, the sample was thawed in running water (26 to 28 °C) until core temperature reached 1 to 2 °C.

2.4 Preparation of mince gels containing salt at different levels in the presence of Arg and Lys at various levels

Salt at two levels (0.5% and 2.5%, based on mince weight) was added into the mince and mixed for 2 min. During blending, the temperature was maintained below 10 °C. Thereafter, Lys or Arg was added to the mince paste at 0.5% or 1.0% (based on mince weight) and then mixed for 2 min. The mixture was adjusted to obtain 80% moisture content using distilled water. The mixed paste was stuffed into a polyvinylidene casing (2.5 cm diameter) and sealed. The gels were subjected to heating at 40 °C for 30 min, followed by at 90 °C for 20 min (Balange & Benjakul, 2009). Thereafter, all the samples were soaked in iced water and the gels were then stored overnight at 4 °C before being analyzed. Gels prepared by addition of only salt, both low and high level, without Arg or Lys were named as "control."

2.5 Analyses

2.5.1 Texture profile. Texture profile analysis (TPA) of the gel sample was run using a texture analyzer. Prepared samples with 2.5 cm in both length and diameter were equilibrated at room temperature (26 to 28 °C) and put on flat plate of the texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) with a cylinder probe P/50 (diameter of 50 mm). Samples were compressed twice to 50% height of sample with a trigger force of 0.1 kg·f and speed of 1 mm/s. TPA parameters including hardness, springiness, cohesiveness, and chewiness were recorded.

2.5.2 Expressible moisture content. Expressible moisture content (EMC) was analyzed as tailored by Benjakul, Visessanguan, and Srivilai (2001). A gel sample (0.5-cm thickness and 2.5-cm diameter) was weighed (approximately 3 ± 0.1 g) and inserted between three pieces of Whatman filter papers No. 93. Weight of samples after being compressed with 5 kg standard weight for 2 min was recorded. EMC was calculated and reported as %.

2.5.3 Color. Color parameters of mince gels after heating and cooling were measured using Hunterlab (ColorFlex, Hunter Associates Laboratory, Reston, VA, USA). Lightness ($L^\star$), redness/greenness ($a^\star$), and yellowness/blueness ($b^\star$) were determined. Whiteness was calculated as follows (Park, 1994):

$$\text{Whiteness} = 100 - \left[\left(100 - L^*\right)^2 + a^{*^2} + b^{*^2}\right]^{1/2}$$

2.5.4 Trichloroacetic acid-soluble peptide content. Gel sample (2 g) was added with 18 ml of 5% trichloroacetic acid (TCA) solution, followed by homogenization for 2 min at 11,000 rpm with the aid of a homogenizer (IKA Labortechnik, Selangor, Malaysia). The homogenate was stood for 1 hr at 4 °C and subsequently centrifuged at 8,000 × *g* for 5 min at room temperature (Balange & Benjakul, 2009). TCA-soluble peptide content (TCA-SPC) was examined according to the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) and was reported as μmol tyrosine equivalent/g sample.

2.5.5 Protein pattern. Protein pattern of the mince gel was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Laemmli, 1970). The sample was prepared by mixing gel (3 g) with 5% sodium dodecyl sulfate solution (27 mL), followed by homogenization, heating at 95 °C for 10 min, and centrifugation as detailed by Balange and Benjakul (2009). The samples (15 μg protein) were loaded into the polyacrylamide gel (10% running gel; 4% stacking gel). After separation at 15 mA per gel, the gels were stained and destained.

2.6 Characterization of selected mince gel

Gels with high-salt (HS) and LS contents without and with 1% Arg were prepared as tailored above and subjected to analysis.

2.6.1 Microstructure of gel. Gel sample was prepared for scanning electron microscope (SEM) analysis as detailed by Petcharat and Benjakul (2018). Samples (2- to 3-mm thickness) were fixed using 2.5% glutaraldehyde solution and washed with distilled water prior to dehydration using serial concentration of ethanol (25%, 50%, 70%, 80%, 90%, and 100%). CO_2 was used as a transition fluid for critical point drying. After gold coating, visualization was made using SEM (Quanta 400, FEI, Eindhoven, the Netherland).

2.6.2 Dynamic rheology. The selected paste samples were used for dynamic rheological measurements (Buamard & Benjakul, 2015). A rheometer (HAAKE RheoStress1, Thermo Fisher Scientific, Karlsruhe, Germany) with 35-mm parallel plate geometry and the gap set at 1.0 mm was used to examine the elastic or storage modulus (G') and viscous modulus (G'') changes. During the analysis, sample was covered with a transparent sample hood to minimize the evaporation of water from mince pastes. For testing, 1% deformation and 1 Hz oscillation were used. Aforementioned condition gave a linear response. During heating from 20 to 90 °C, the temperature sweeps (1 °C/min) were recorded.

2.6.3 Acceptability. The selected gel samples (2.5-cm diameter and 1-cm thickness) were equilibrated for 30 min at room temperature. A random single-digit code was assigned to each

Table 1–Texture profiles of striped catfish mince gel containing low and high salt without and with lysine or arginine at different concentrations.

Samples	Hardness (N)	Springiness (cm)	Cohesiveness	Chewiness (N.cm)
LS	53.37 ± 6.48^{aA}	0.89 ± 0.01^{aAB}	0.44 ± 0.05^{aA}	20.98 ± 5.31^{aA}
LS-0.5L	64.81 ± 3.92^{bB}	0.87 ± 0.02^{aA}	0.62 ± 0.03^{bB}	35.15 ± 4.12^{bB}
LS-1L	63.93 ± 2.28^{bB}	0.88 ± 0.03^{aAB}	0.60 ± 0.02^{bB}	33.63 ± 0.78^{bB}
LS-0.5A	73.90 ± 0.29^{cC}	0.90 ± 0.01^{aAB}	0.74 ± 0.00^{cD}	48.69 ± 0.62^{cCD}
LS-1A	75.20 ± 4.24^{cC}	0.90 ± 0.01^{aAB}	0.77 ± 0.01^{cD}	51.81 ± 3.16^{cD}
HS	65.78 ± 1.74^{aB}	0.88 ± 0.03^{aAB}	0.67 ± 0.01^{aC}	38.82 ± 1.97^{aB}
HS-0.5L	76.45 ± 4.00^{bCD}	0.89 ± 0.01^{aAB}	0.69 ± 0.01^{bC}	46.90 ± 3.19^{bCD}
HS-1L	72.02 ± 2.02^{bC}	0.90 ± 0.02^{aAB}	0.69 ± 0.01^{bC}	44.74 ± 1.70^{bC}
HS-0.5A	72.35 ± 0.83^{bC}	0.90 ± 0.01^{aAB}	0.74 ± 0.00^{cD}	48.21 ± 1.07^{bCD}
HS-1A	82.22 ± 4.81^{cD}	0.91 ± 0.01^{aB}	0.76 ± 0.00^{dD}	56.93 ± 4.03^{cE}

Note. Values are mean ± SD ($n = 3$). Different lowercase superscripts in the same column under the low-salt (LS) level denote the significant differences ($P < 0.05$). Different lowercase superscripts in the same column under the high-salt (HS) level denote the significant differences ($P < 0.05$). Different uppercase superscripts in the same column denote the significant differences ($P < 0.05$).
Abbreviations: LS, low salt (0.5%); HS, high salt (2.5%); 0.5L, 0.5% lysine; 1L, 1% lysine; 0.5A, 0.5% arginine; 1A, 1% arginine.

sample and served under the fluorescent day-light-type illumination on a plastic cup at room temperature. Fifty untrained panelists (aged between 18 and 35) who were regular consumers of the mince gel products were included for the sensory evaluation. The likeness score for sensory attributes was assessed using 9-point hedonic scale as described by Meilgaard, Civille, and Carr (2006).

2.7 Statistical analysis

Completely randomized design (CRD) with triplication was used for entire study. Statistical Package for Social Science (SPSS 16.0 for windows) was used for data analysis. Duncan's multiple range test was applied for comparison of mean at significant level of 0.05 (Steel & Torrie, 1980).

3. RESULTS AND DISCUSSION

3.1 Effect of Arg and Lys at different levels on characteristics and properties of mince gel with LS and HS

3.1.1 Texture profile. Texture is a crucial attribute of gel-based products with respect to consumer acceptability. In the control mince gels containing salt at two different levels, LS and HS contents, namely, LS and HS samples, respectively, the former had the lower score for all TPA attributes than the latter ($P < 0.05$), except for springiness ($P > 0.05$). The addition of Arg and Lys augmented hardness, chewiness, and cohesiveness ($P < 0.05$) of both LS and HS samples (Table 1). At the same level of salt used, no difference in the springiness was noted when Arg or Lys at both levels was added ($P > 0.05$). The hardness ranged from 53 to 75 N and from 65 to 82 N for LS and HS samples, respectively. Hardness is the maximum force used for compression of sample to attain a given deformation. For hardness of LS samples, the addition of Arg increased the value of gel samples more effectively than Lys ($P < 0.05$). Nevertheless, the levels of both amino acids exhibited no difference on the hardness of resulting gels.

HS samples had the increases in hardness when Lys at both levels and Arg (0.5%) was incorporated, in comparison with the HS gel without Lys or Arg ($P < 0.05$). No differences among those samples were detected ($P > 0.05$). Conversely, HS samples added with 1% Arg exhibited the highest hardness ($P < 0.05$). The result was in agreement with the previous findings (Wang et al., 2020; Zhou, Li, & Tan, 2014; Zhou, Li, Tan, & Sun, 2014; Zhu et al., 2018). Gelation of proteins depends mainly on the electrostatic charge of molecules (Sun & Holley, 2011). At alkaline pH, the protein–water interactions increased, whereas protein–protein interactions decreased due to the presence of negative charge on protein surface, thereby enhancing electrostatic repulsion (Nahar, Zakaria, Hashim, & Bari, 2017; Zhu, Li, Li, Ning, & Zhou, 2019). Lys and Arg could augment the pH of mince paste to be higher than pI of myofibrillar proteins. This could favor the solubilization, thus enhancing the entanglement or aggregation of proteins to form the strong gel network. The addition of Arg at both levels in the presence of LS (0.5%) was a potential means to increase hardness, in which the value of gels was higher than control HS gel ($P < 0.05$). The guanidinium group of Arg might allow interactions in three possible directions, which enabled Arg to form a larger number of electrostatic interactions compared to Lys. As a result, gels added with Arg generally showed the better gelling property than those added with Lys (Armstrong, Mason, Anderson, & Dempsey, 2016). No difference in springiness was noted among all the samples, regardless of salt levels used or concentrations of both Arg and Lys. Springiness is recognized as a distance that the food recovered its height during the time that elapsed between the end of the first bite and the start of the second bite. Cohesiveness is the ratio of the positive force area during the second cycle of compression to that of the first cycle. Chewiness is the energy needed for masticating a solid food (Bourne, 2002). Cohesiveness and chewiness of both LS and HS samples showed the same trend with hardness, where Arg at both concentrations was able to augment all aforementioned attributes ($P < 0.05$). Thus, it is possible that Arg helped solubilize the muscle protein in striped catfish meat in the presence of LS. This could contribute to the formation of good-quality gel as shown by the increased hardness, cohesiveness, and chewiness, which were higher than those of the control HS samples (containing 2.5% salt).

3.1.2 Expressible moisture content. Without Arg or Lys addition, gel from HS had lower EMC than that of LS ($P < 0.05$). This might be related with poorer solubilization of myofibrillar proteins when insufficient salt was used in LS sample. EMC was decreased ($P < 0.05$) with addition of Lys and Arg at both concentrations (Table 2) when compared to corresponding control samples (HS and LS). In the absence of Arg and Lys, the highest EMC was found in LS control sample ($P < 0.05$). This result reflected the poor solubilization of proteins, leading to the weak network between the protein chains. Such a gel network was lacking of holding water inside the gel matrix as shown by the augmented EMC. However, EMC between Lys and Arg added samples was different ($P < 0.05$), irrespective of the salt concentration. Samples containing Arg possessed the lower EMC than those containing Lys ($P < 0.05$). These results were in accordance with hardness

Table 2–Color, whiteness, expressible moisture content, and TCA-soluble peptides of low-salt and high-salt minced gel from striped catfish added without and with lysine or arginine at different concentrations.

Samples	$L^\star$	$a^\star$	$b^\star$	Whiteness	Expressible moisture content (%)	TCA-soluble peptides(μmol/g sample)
LS	62.40 ± 0.67^{aA}	1.82 ± 0.57^{aA}	16.93 ± 0.16^{cE}	58.72 ± 0.68^{bcAB}	43.00 ± 6.08^{aA}	7.09 ± 0.11^{aA}
LS-0.5L	61.88 ± 0.57^{aA}	0.93 ± 0.44^{bB}	15.29 ± 0.35^{bC}	58.92 ± 0.66^{bcAB}	22.00 ± 1.00^{bB}	5.69 ± 0.12^{bC}
LS-1L	62.48 ± 0.31^{aA}	0.91 ± 0.70^{bB}	14.73 ± 0.44^{aB}	59.68 ± 0.46^{cA}	24.00 ± 2.65^{bB}	5.67 ± 0.14^{bC}
LS-0.5A	61.52 ± 1.44^{aA}	0.46 ± 0.14^{bBC}	16.50 ± 0.52^{cD}	58.12 ± 1.12^{bB}	14.00 ± 1.73^{cD}	5.58 ± 0.10^{bC}
LS-1A	58.82 ± 1.03^{bB}	0.33 ± 0.05^{bC}	17.53 ± 0.26^{dF}	55.24 ± 0.87^{aD}	13.00 ± 2.65^{cD}	4.87 ± 0.11^{cD}
HS	59.00 ± 0.38^{aB}	0.43 ± 0.41^{aC}	14.50 ± 0.25^{aAB}	56.51 ± 0.37^{aC}	23.33 ± 2.08^{aB}	6.09 ± 0.15^{aB}
HS-0.5L	59.15 ± 1.22^{aB}	0.21 ± 0.09^{abC}	14.51 ± 0.25^{aAB}	56.65 ± 1.21^{aC}	17.33 ± 0.58^{cCD}	4.95 ± 0.19^{bD}
HS-1L	59.16 ± 0.71^{aB}	0.26 ± 0.06^{abC}	14.27 ± 0.10^{aA}	56.74 ± 0.65^{aC}	20.33 ± 0.58^{bBC}	4.92 ± 0.15^{bD}
HS-0.5A	57.64 ± 0.24^{bC}	0.28 ± 0.06^{abC}	15.34 ± 0.10^{bC}	54.95 ± 0.20^{bD}	16.00 ± 2.00^{cD}	4.68 ± 0.17^{bD}
HS-1A	56.13 ± 0.77^{cD}	0.19 ± 0.06^{bC}	17.02 ± 0.10^{cE}	52.95 ± 0.69^{cE}	13.33 ± 0.58^{dD}	4.34 ± 0.19^{cE}

Note. Values are mean ± SD ($n = 3$). Different lowercase superscripts in the same column under the low-salt (LS) level denote the significant differences ($P < 0.05$). Different lowercase superscripts in the same column under the high salt (HS) level denote the significant differences ($P < 0.05$). Different uppercase superscripts in the same column denote the significant differences ($P < 0.05$).
Abbreviations: LS, low salt (0.5%); HS, high salt (2.5%); 0.5L, 0.5% lysine; 1L, 1% lysine; 0.5A, 0.5% arginine; 1A, 1% arginine.

value of the samples and also suggested that Arg was capable of inducing the development of stronger protein network with high water holding capacity. The cohesiveness, indicating the elasticity of gel, was also correlated with EMC. In the presence of Arg, highly solubilized proteins could align and interact each other to form the strong and ordered network (Wang et al., 2020). The samples containing 1% Arg showed the lowest EMC for both HS and LS samples ($P < 0.05$).

3.1.3 Whiteness. Color is one of the important and highly demanded characteristics by the consumers (Hsu & Chiang, 2002). For control gel (without Arg or Lys addition), the higher lightness and whiteness were noticeable in LS sample than HS samples ($P < 0.05$) (Table 2), plausibly owing to the higher light scattering effect of LS sample. Incomplete solubilization of proteins might be related with non-uniform network, which showed higher light scattering impact of gel, especially at the surface. Decreased lightness ($L^\star$) value was noticeable in the Arg-treated samples (Table 2). Hong, Park, Kim, and Min (2006) reported that the lightness ($L^\star$) values of the meat product was correlated with its moisture content. The lower $L^\star$ values of the meat product were found in meat product having high moisture content. Addition of Arg caused the decrease in EMC, indicating higher water imbibed in network. The yellowness ($b^\star$) value was increased ($P < 0.05$) in both HS and LS samples with addition of Arg or Lys, which in turn decreased the whiteness value of the sample. This might be due to the enhanced browning reaction, especially Maillard reaction between amino groups of Arg and Lys and carbonyl compounds in mince, particularly during thermal-induced gelation process, in which heat could induce the reaction. However, no difference was observed in the Lys-added HS sample at both levels and the control ($P > 0.05$). Zheng et al. (2017) documented that the incorporation of Arg and Lys decreased the lightness ($L^\star$) value of pork sausage. The present results were in line with the findings issued by Zhou, Li, & Tan (2014) and Zhou, Li, Tan, & Sun (2014).

3.1.4 TCA-soluble peptide content. TCA-SPC of both gels from striped catfish mince (HS and LS) with and without addition of Lys and Arg is shown in Table 2. The presence of soluble peptides suggested that proteolytic degradation occurred during setting and gelation (Buamard & Benjakul, 2015). The highest TCA-SPC was found in the gel without Lys or Arg. Greater TCA-SPC was obtained in LS sample than HS sample, irrespective of amino acid addition. Thus, salt at higher level plausibly inhibited indigenous proteases to some degree as shown by the lower TCA-SPC in HS samples. Salt could inhibit autolysis of fish muscle by inactivation of proteases (Taylor, 1921). High TCA-SPC revealed the augmented hydrolysis of protein by endogenous proteases (Rawdkuen, Sai-Ut, Khamsorn, Chaijan, & Benjakul, 2009). This result coincided with the lowest hardness in the control gel sample (Table 1). TCA-SPC of mince gel added with Arg or Lys at both concentrations was lower than that of corresponding control samples (without Arg or Lys). However, there was no difference in TCA-SPC ($P > 0.05$) between the Lys- and Arg-added samples, irrespective of salt concentrations. The addition of Lys and Arg at 0.5% and 1.0% could therefore inhibit the muscle protein degradation. Arg and Lys have been reported to have capability of forming H-bonds with neighbor molecules by the guanidinium and ε-amino groups in their side chains, respectively. Thus, this could in turn hinder the extraction of small peptides as indicated by the lower TCA-soluble peptides (Talavera, Robetson, & Lovell, 2011).

3.1.5 Protein patterns. Myosin heavy chain (MHC) band intensity was decreased in the samples added with 1% Lys or 1% Arg than that of the corresponding control samples (Figure 1). This might be because of the enhanced polymerization of proteins by endogenous transglutaminase (TGase) in the presence of Lys and Arg, especially at increasing concentration. Myosin plays a major role in the functional properties of meat products (Priyadarshini, Xavier, Nayak, Dhanapal, & Balange, 2017). As discussed above, Arg and Lys were able to increase the solubility of myofibrillar proteins, especially MHCs (Guo, Peng, Zhang, Liu, & Cui, 2015; Qin, Xu, Zhou, & Wang, 2015). As a consequence, indigenous TGase could catalyze acyl group transfer from the donor to the receptor localized on protein chain more potentially. However, similar band intensity between the samples added with 0.5% Lys and Arg was noticeable. This result was concurrent with the increase in hardness (Table 1). Similar actin band intensity was observed for all the samples. Actin could not be polymerized during gelation effectively and it withstood proteolysis (Balange & Benjakul, 2009).

3.2 Characteristics of the selected mince gels

3.2.1 Microstructures. Microstructures of LS and HS striped catfish gel added without and with 1% Arg are illustrated in Figure 2. LS gel possessed a loosen and coarser network with the larger cavities, compared with HS gel. Incomplete solubilization of proteins due to insufficient salt content might be related with non-uniform network. Protein strands of both LS and HS gels added

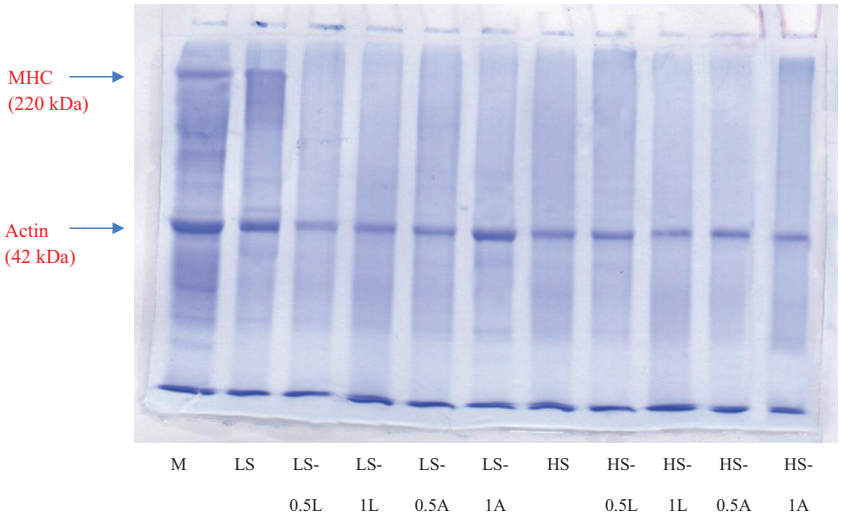

Figure 1–Protein pattern of low-salt and high-salt striped catfish mince gel without and with lysine or arginine at different levels. MHC, myosin heavy chain; M, mince gel; LS, low salt (0.5%); HS, high salt (2.5%); 0.5L, 0.5% lysine; 1L, 1% lysine; 0.5A; 0.5% arginine; 1A, 1% arginine.

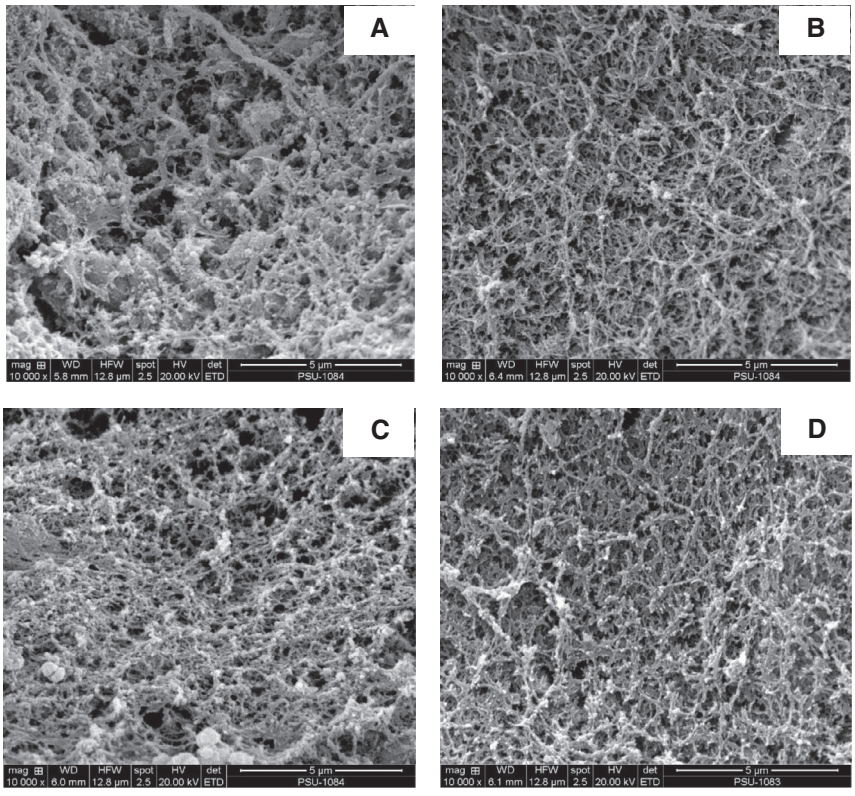

Figure 2–Electron microscopic images of low-salt (LS) and high-salt (HS) striped catfish mince gel without and with 1% arginine (Arg) (magnification: 10,000×): (A) LS gel without Arg, (B) LS gel added with 1% Arg, (C) HS gel without Arg, and (D) HS gel added with 1% Arg.

with 1% Arg had higher density with finer strands, as compared with those found in the LS and HS gel without 1% Arg. Arg was beneficial for the effective solubilization of myofibrillar proteins, in which a compact and uniform gel could be developed during heating (Qin et al., 2015). This contributed to the increased hardness and water holding capacity as evidenced by decreased EMC (Table 2).

3.2.2 Dynamic rheology. Elastic modulus (G') of LS and HS pastes from striped catfish mince without and with 1% Arg addition was monitored as a function of temperature (Figure 3A). Regardless of Arg addition, HS sample had higher G' than LS counterparts. G', the stored energy of a viscoelastic material, was slightly augmented during heating from 20 to 50 °C, suggesting protein–protein interaction via hydrogen bonds (Lanier et al., 2014). Thereafter, G' was noticeably increased and reached the maximal value at 72 to 80 °C, indicating interaction between protein molecules, in which subsequent formation of a thermo-irreversible gel network took place (Sun & Holley, 2011). Nevertheless, for LS containing Arg, the decrease in G' was observed at 50 to 55 °C, followed by continuous increase up to 75 °C. Indigenous proteases were still active under LS concentration and solubilized proteins were favored for hydrolysis mediated by indigenous

Table 3–Liking score of striped catfish mince gel containing low and high salt without and with 1% arginine.

Samples	Appearance	Color	Texture	Odor	Taste	Overall
LS	6.71 ± 1.05[a]	6.76 ± 1.20[a]	5.41 ± 1.50[a]	6.12 ± 1.50[a]	5.65 ± 1.17[a]	5.41 ± 1.33[a]
HS	7.12 ± 0.86[a]	7.00 ± 0.94[a]	7.12 ± 0.86[b]	6.82 ± 0.95[a]	6.65 ± 1.06[b]	6.76 ± 1.03[b]
LS-1A	7.12 ± 1.11[a]	6.71 ± 0.99[a]	6.94 ± 0.97[b]	6.35 ± 1.50[a]	6.53 ± 1.28[b]	6.53 ± 1.12[b]
HS-1A	6.88 ± 1.11[a]	6.65 ± 1.00[a]	7.24 ± 1.09[b]	6.35 ± 1.32[a]	6.76 ± 0.97[b]	6.94 ± 1.09[b]

Note. Values are mean ± SD ($n = 50$). Different lowercase superscripts in the same column denote the significant differences ($P < 0.05$).
Abbreviations: LS, low-salt (0.5%) mince gel; HS, high-salt (2.5%) mince gel; LS-1A, low-salt mince gel containing 1% arginine; HS-1A, high-salt mince gel containing 1% arginine.

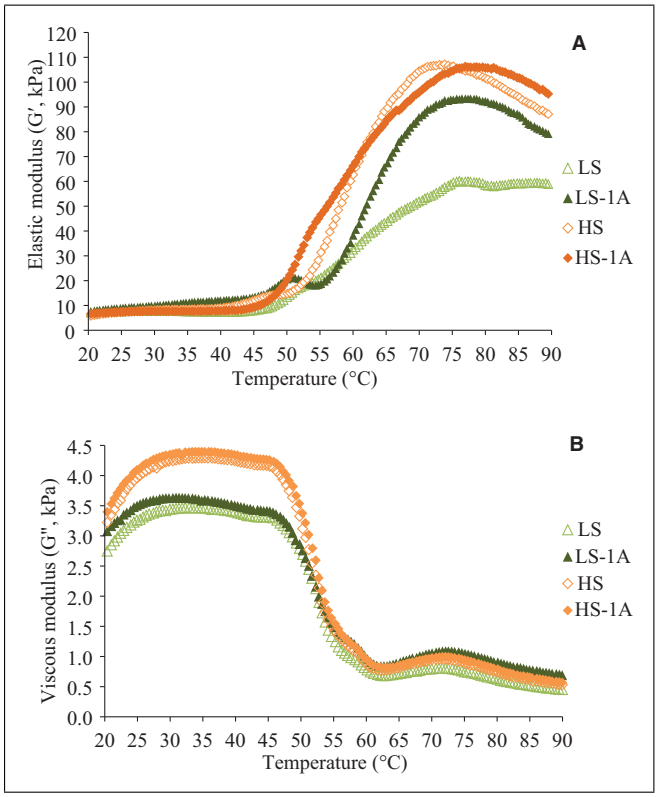

Figure 3–Elastic modulus (G') (A) and viscous modulus (G'') (B) of low-salt (LS) and high-salt (HS) striped catfish mince without and with 1% arginine (Arg) during heating from 20 to 90 °C. LS, low-salt gel without Arg; LS-1A, low-salt gel added with 1% Arg; HS, high-salt gel without Arg; HS-1A, high-salt gel added with 1% Arg.

proteases. However, there was no marked decrease in G' in other samples. This was plausibly due to the formation of lipid oxidation products, mainly aldehydes, which could act as protein cross-linker (Kikugawa & Beppu, 1987). As a result, there was no decrease in G' in the aforementioned samples. Unfolded proteins might facilitate the aggregation of protein chains. Hydrophobic domains preferably underwent aggregation via hydrophobic–hydrophobic interaction (Benjakul et al., 2005), whereas disulfide bond was formed via oxidation of sulfhydryl groups. As a consequence, protein aggregation was promoted. The higher G' represented the higher stiffness or firmness of the gel (Vate & Benjakul, 2017). The lower G' of LS sample was coincidental with the lower hardness due to insufficient salt to solubilize proteins. Thus, ordered and fine networks of surimi proteins were not developed. Additionally, higher G' was observed in both LS and HS pastes added with 1% Arg after heating, compared to corresponding control sample (without Arg). These results were in accordance with a higher hardness of LS and HS samples containing 1% Arg (Table 1). Guanidinium group of Arg, which provides positively charged side chain of amino acid, has been implicated in the effectiveness of Arg in the solubilization of fish myosin and induced rearrangement of myosin network during heating (Takai et al., 2013). Electrostatic repulsion between proteins in surimi paste brought about the higher solubilization of proteins (Li, Zheng, Xu, Zhu, & Zhou, 2018). This indicated that Arg cations preferentially bind myofibrillar protein in surimi and play a role in disruption of electrostatic interactions. As a consequence, the solubility of proteins was increased, and strong gel network could be subsequently attained during heating.

Furthermore, the viscous modulus (G'') curves had the same trend with those of G'. However, G'' had the lower value than G' over the entire temperature range tested, indicating the formation of viscoelastic network (Figure 3B). All paste samples had noticeable decrease in G'' at 45 to 55 °C, which was plausibly due to phase transition of the paste from solid to liquid phase. At above 75 °C, G'' remained almost constant, indicating the formation of a highly elastic protein gel (Campo-Deaño, Tovar, & Borderías, 2010).

3.2.3 Likeness score. Likeness score of gel prepared from striped catfish mince with LS and HS without and with 1% Arg is presented in Table 3. Similar likeness scores for appearance, color, and odor between both LS and HS samples were found. However, texture, taste, and overall likeness scores were higher in HS sample ($P < 0.05$). This was in line with the higher hardness, cohesiveness, and chewiness of the latter. Addition of 1% Arg enhanced ($P < 0.05$) the texture and taste likeness scores of gel, more likely related with the increased hardness value of the Arg-added sample (Table 1). Also, gel added with 1% Arg had higher taste and overall likeness score than the control ($P < 0.05$). Arg has been reported to possess bitterness-suppressing effect in the presence of sodium chloride (Ogawa et al., 2004). Moreover, when solubilization and unfolding of fish protein were more pronounced by addition of Arg, umami taste substances such as glutamic acid as well as inosine 5′-monophosphate might be easily released during chewing of gel samples and likeness score was then increased (Maruji et al., 2010). Similarly, the increased likeness score of sodium-reduced pork sausage with addition of Arg was found by Zheng et al. (2017). It could be inferred that the addition of 1% Arg was capable of improving the sensory property of LS (0.5%) striped catfish mince gel. The mince could serve as a promising proteinaceous material for production of low-sodium gel along with Arg addition, in which gel with acceptability could be produced.

4. CONCLUSIONS

Salt content of minced gel from striped catfish could be reduced by the addition of basic amino acids, especially Arg. Incorporation of Arg yielded the mince gel with the improved textural and sensory properties. Nonetheless, Arg caused a slight decrease in whiteness in the HS gel. Therefore, Arg could be hence used as a natural salt substitute in mince gel or related products with LS content.

AUTHOR CONTRIBUTIONS

N. Buamard collected experiment data and did draft preparation. M. A. Javith collected experiment data and did draft preparation. A. K. Balange did draft preparation. G. Krishna supervised the project. S. Benjakul provided analytical instruments and chemicals, designed the study, and revised draft.

ACKNOWLEDGEMENTS

The authors would like to thank Prince of Songkla University (grant no. AGR6302013N). The ICAR and World Bank funded National Agriculture Higher Education Project (NAHEP) were also acknowledged. This work was carried out under the components of NAHEP.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Armstrong, C. T., Mason, P. E., Anderson, R., & Dempsey, C. E. (2016). Arginine side chain interactions and the role of arginine as a gating charge carrier in voltage sensitive ion channels. *Scientific Reports*, *6*, 21759.

Baker, D. H. (2007). Lysine, arginine, and related amino acids: An introduction to the 6th amino acid assessment workshop. *The Journal of Nutrition*, *137*(6), 1599S–1601S.

Balange, A., & Benjakul, S. (2009). Enhancement of gel strength of bigeye snapper (*Priacanthus tayenus*) surimi using oxidised phenolic compounds. *Food Chemistry*, *113*(1), 61–70.

Benjakul, S., Visessanguan, W., Thongkaew, C., & Tanaka, M. (2005). Effect of frozen storage on chemical and gel-forming properties of fish commonly used for surimi production in Thailand. *Food Hydrocolloids*, *19*(2), 197–207.

Benjakul, S., Visessanguan, W., & Srivilai, C. (2001). Porcine plasma proteins as gel enhancer in bigeye snapper (*Priacanthus tayenus*) surimi. *Journal of Food Biochemistry*, *25*(4), 285–305.

Bourne, M. (2002). *Food texture and viscosity: Concept and measurement* (2nd ed.). San Diego, CA: Academic Press.

Buamard, N., & Benjakul, S. (2015). Improvement of gel properties of sardine (*Sardinella albella*) surimi using coconut husk extracts. *Food Hydrocolloids*, *51*, 146–155.

Campo-Deaño, L., Tovar, C. A., & Borderías, J. (2010). Effect of several cryoprotectants on the physicochemical and rheological properties of suwari gels from frozen squid surimi made by two methods. *Journal of Food Engineering*, *97*, 457–464.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2016). Different additives to enhance the gelation of surimi gel with reduced sodium content. *Food Chemistry*, *196*, 791–799.

Douša, M., Břichář, J., Gibala, P., & Lehnert, P. (2011). Rapid hydrophilic interaction chromatography determination of lysine in pharmaceutical preparations with fluorescence detection after postcolumn derivatization with o-phtaldialdehyde. *Journal of Pharmaceutical and Biomedical Analysis*, *54*(5), 972–978.

Grimble, G. K. (2007). Adverse gastrointestinal effects of arginine and related amino acids. *The Journal of Nutrition*, *137*(6), 1693S–1701S.

Guo, X. Y., Peng, Z. Q., Zhang, Y. W., Liu, B., & Cui, Y. Q. (2015). The solubility and conformational characteristics of porcine myosin as affected by the presence of L-lysine and L-histidine. *Food Chemistry*, *170*, 212–217.

Ha, S. K. (2014). Dietary salt intake and hypertension. *Electrolyte and Blood Pressure*, *12*(1), 7–18.

Hong, G. P., Park, S. H., Kim, J. Y., & Min, S. G. (2006). The effects of high pressure and various binders on the physico-chemical properties of restructured pork meat. *Asian-Australasian Journal of Animal Sciences*, *19*(10), 1484–1489.

Hsu, C. K., & Chiang, B. H. (2002). Effects of water, oil, starch, calcium carbonate and titanium dioxide on the colour and texture of threadfin and hairtail surimi gels. *International Journal of Food science and Technology*, *37*(4), 387–393.

Kikugawa, K., & Beppu, M. (1987). Involvement of lipid oxidation products in the formation of fluorescent and cross-linked proteins. *Chemistry and Physics of Lipids*, *44*(2-4), 277–296.

Kim, Y. S., & Park, J. W. (2008). Negative roles of salt gelation properties of fish protein isolate. *Journal of Food Science*, *73*(8), C585–C588.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*(5259), 680–685.

Lanier, T. C., Yongsawatdigul, J., & Carvajal-Rondanelli, P. (2014). Surimi gelation chemistry. In J. W. Park (Ed.), *Surimi and surimi seafood* (pp. 101–140). Boca Raton, FL: CRC Press.

Li, S., Zheng, Y., Xu, P., Zhu, X., & Zhou, C. (2018). L-Lysine and L-arginine inhibit myosin aggregation and interact with acidic amino acid residues of myosin: The role in increasing myosin solubility. *Food Chemistry*, *242*, 22–28.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Maruji, Y., Shimizu, M., Murata, M., Ando, M., Sakaguchi, M., & Hirata, T. (2010). Multiple taste functions of the umami substances in muscle extracts of yellowtail and bastard halibut. *Fisheries Science*, *76*, 521–528.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2006). *Sensory evaluation techniques* (4th ed.). Boca Raton, FL: CRC press.

Nahar, M. K., Zakaria, Z., Hashim, U., & Bari, M. F. (2017). Effect of pH and salt concentration on protein solubility of slaughtered and non-slaughtered broiler chicken meat. *Sains Malaysiana*, *46*(5), 719–724.

Ogawa, T., Nakamura, T., Tsuji, E., Miyanaga, Y., Nakagawa, H., Hirabayashi, H., & Uchida, T. (2004). The combination effect of L-arginine and NaCl on bitterness suppression of amino acid solutions. *Chemical and Pharmaceutical Bulletin*, *52*(2), 172–177.

Park, J. W. (1994). Functional protein additives in surimi gels. *Journal of Food Science*, *59*(3), 525–527.

Petcharat, T., & Benjakul, S. (2018). Effect of gellan incorporation on gel properties of bigeye snapper surimi. *Food Hydrocolloids*, *77*, 746–753.

Priyadarshini, B., Xavier, K. M., Nayak, B. B., Dhanapal, K., & Balange, A. K. (2017). Instrumental quality attributes of single washed surimi gels of tilapia: Effect of different washing media. *LWT-Food Science and Technology*, *86*, 385–392.

Qin, H., Xu, P., Zhou, C., & Wang, Y. (2015). Effects of L-Arginine on water holding capacity and texture of heat-induced gel of salt-soluble proteins from breast muscle. *LWT-Food Science and Technology*, *63*(2), 912–918.

Rawdkuen, S., Sai-Ut, S., Khamsorn, S., Chaijan, M., & Benjakul, S. (2009). Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process. *Food Chemistry*, *112*(1), 112–119.

Sokalingam, S., Raghunathan, G., Soundrarajan, N., & Lee, S. G. (2012). A study on the effect of surface lysine to arginine mutagenesis on protein stability and structure using green fluorescent protein. *PLoS ONE*, *7*(7), e40410.

Singh, A. K., & Lakra, W. S. (2012). Culture of *Pangasianodon hypophthalmus* into India: Impacts and present scenario. *Pakistan Journal of Biological Sciences*, *15*(1), 19–26.

Steel, R. D. G., & Torrie, J. H. (1980). *Principle and procedures of statistics: A biometrical approach* (2nd ed.). New York, NY: McGraw Hill Co.

Stone, A. P., & Stanley, D. W. (1992). Mechanisms of fish muscle gelation. *Food Research International*, *25*(5), 381–388.

Sun, X. D., & Holley, R. A. (2011). Factors influencing gel formation by myofibrillar proteins in muscle foods. *Comprehensive Reviews in Food Science and Food Safety*, *10*(1), 33–51.

Takai, E., Yoshizawa, S., Ejima, D., Arakawa, T., & Shiraki, K. (2013). Synergistic solubilization of porcine myosin in physiological salt solution by arginine. *International Journal of Biological Macromolecules*, *62*, 647–651.

Talavera, D., Robetson, D. L., & Lovell, S. C. (2011). Characterization of protein-protein interaction interfaces from a single species. *PLoS ONE*, *6*(6), e21053.

Taylor, H. F. (1921). Principles involved in the preservation of fish by salt. *Transactions of the American Fisheries Society*, *50*(1), 120-152.

Totosaus, A., & Perez-Chabela, M. L. (2009). Textural properties and microstructure of low-fat and sodium-reduced meat batters formulated with gellan gum and dicationic salts. *LWT-Food Science and Technology*, *42*(2), 563–569.

Vate, N. K., & Benjakul, S. (2017). Enhancement of gel properties of sardine surimi using squid ink tyrosinase in combination with coconut husk extract. *International Journal of Food Engineering*, *13*(3), 20160163.

Wang, W., Zhao, J., Zhang, J., Liu, C., Jauregi, P., & Huang, M. (2020). Modification of heat-induced whey protein gels by basic amino acids. *Food Hydrocolloids*, *100*, 105397.

Zheng, Y., Xu, P., Li, S., Zhu, X. X., Chen, C. G., & Zhou, C. L. (2017). Effects of L-lysine/L-arginine on the physicochemical properties and quality of sodium-reduced and phosphate-free pork sausage. *International Journal of Nutrition and Food Sciences*, *6*(1), 12–18.

Zhou, C., Li, J., & Tan, S. (2014). Effect of L-lysine on the physicochemical properties of pork sausage. *Food Science and Biotechnology*, *23*(3), 775–780.

Zhou, C., Li, J., Tan, S., & Sun, G. (2014). Research article effects of L-arginine on physicochemical and sensory characteristics of pork sausage. *Advance Journal of Food Science and Technology*, *6*(5), 660–667.

Zhu, X., Li, L., Li, S., Ning, C., & Zhou, C. (2019). L-Arginine/L-lysine improves emulsion stability of chicken sausage by increasing electrostatic repulsion of emulsion droplet and decreasing the interfacial tension of soybean oil-water. *Food Hydrocolloids*, *89*, 492–502.

Zhu, X., Ning, C., Li, S., Xu, P., Zheng, Y., & Zhou, C. (2018). Effects of L-lysine/L-arginine on the emulsion stability, textural, rheological and microstructural characteristics of chicken sausages. *International Journal of Food Science and Technology*, *53*(1), 88–96.