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1 **Modification of heat-induced whey protein gels by basic amino acids**

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14

15 **ABSTRACT**

16           Various amino acids have been studied as gelation enhancers. This study investigated the  
17 effect of histidine, lysine, and arginine on gelling properties of heat-induced whey protein isolate  
18 (WPI) gels at different pHs. Basic amino acids modified WPI gels in a pH- and amino acid-  
19 dependent manner. Hardness and gumminess of the WPI gel was improved by arginine at pH  
20 7.59 while springiness was enhanced by histidine at pHs 7.59 and 9.74 and by lysine at pH 7.59  
21 ( $P < 0.05$ ). At pH 2.0, WPI formed a weak gel. Lysine and arginine facilitated  $\beta$ -lactoglobulin  
22 cross-linking at pH 2.0 and reduced protein leach out from the gel ( $P < 0.05$ ). At pH 5.2, WPI  
23 formed a particulate gel with poor water holding capacity (WHC). Lysine improved WHC of the  
24 WPI gel at pH 5.2 by changing the structure of the gel network. At pHs away from 5.2, basic  
25 amino acid treatments resulted in a more uniform and porous gel matrix and a greater WHC ( $P <$   
26  $0.05$ ). In conclusion, different basic amino acids may be applied as WPI gel enhancers depending  
27 on the pH and desired attributes of the product.

28 **Keywords:** Whey protein; Basic amino acids; Isoelectric point; Gelling properties

29

## 30 **1. Introduction**

31           Recently, the application of amino acids as gelation enhancers has attracted considerable  
32 attention. Amino acids such as arginine, cysteine, histidine, lysine, proline, and  $\gamma$ -aminobutyric  
33 acid (Cando, Herranz, Borderías, & Moreno, 2016; Liu et al., 2015; Primacella, Fei, Acevedo, &  
34 Wang, 2018; Wang, Liu, Ma, & Zhao, 2019; Wang, Zhao, Liu, & Li, 2019; Zhang, Wu, Jamali,  
35 Guo, & Peng, 2017) have been reported to improve gelling properties of a myriad of food protein  
36 gels. Among these novel additives, basic amino acids, particularly lysine and arginine, have been  
37 studied extensively. Adding basic amino acids resulted in gels with improved water holding  
38 capacity, viscoelasticity and texture profile, and sensory attributes (Cando et al., 2016; Fu,  
39 Zheng, Lei, Xu, & Zhou, 2017; Hayakawa et al., 2012; Lei, Fu, Xu, Zheng, & Zhou, 2016; Lei,  
40 Fu, Zheng, Xu, & Zhou, 2017; Qin, Xu, Zhou, & Wang, 2015; Zhang et al., 2017; Zhou, Li, &  
41 Tan, 2014; Zhou, Li, Tan, & Sun, 2014; Zhu et al., 2018). The underlying mechanisms include  
42 pH modulation, reduced water mobility, increased protein solubility, suppressed protein  
43 aggregation, altered protein thermal stability, facilitated protein unfolding and exposure of buried  
44 hydrophobic groups and sulfhydryls, and formation of a fine gel network (Cando et al., 2016;  
45 Chen et al., 2016; Fu et al., 2017; Gao, Wang, Mu, Shi, & Yuan, 2018; Guo, Peng, Zhang, Liu,  
46 & Cui, 2015; Hayakawa et al., 2012; Lei et al., 2016; Lei et al., 2017; Li et al., 2019; Li, Zheng,  
47 Xu, Zhu, & Zhou, 2018; Qin et al., 2015; Zhang et al., 2017; Zhou, Li, & Tan, 2014; Zhou, Li,  
48 Tan, & Sun, 2014). In addition, basic amino acids can improve emulsion stability (Zhu, Li, Li,  
49 Ning, & Zhou, 2019; Zhu et al., 2018), inhibit lipid and protein oxidation (Xu, Zheng, Zhu, Li, &  
50 Zhou, 2018), and stabilize heme color (Zhou, Li, & Tan, 2014; Zhou, Li, Tan, & Sun, 2014;  
51 Zhou, Ye, Nishiumi, Qin, & Chen, 2014; Zhou, Ye, Wang, Qin, & Li, 2015), and are particularly  
52 useful in emulsified gel systems such as sausages.

53           Although extensive evidence has demonstrated that basic amino acids are effective in  
54 improving quality of muscle protein gels (Fu et al., 2017; Hayakawa et al., 2012; Lei et al., 2016;  
55 Lei et al., 2017; Qin et al., 2015; Zhang et al., 2017), egg yolk gel (Primacella et al., 2018), and  
56 complex gel systems such as sausages (Zhou, Li, & Tan, 2014; Zhou, Li, Tan, & Sun, 2014; Zhu  
57 et al., 2018), surimi (Cando et al., 2016), and cheese (Felicio et al., 2016), to the best of our  
58 knowledge, no study has investigated the effect of basic amino acids on gelling properties of  
59 whey protein isolate (WPI) gels. WPI is a widely used gelling and thickening agent in a variety  
60 of foods such as processed meat, bakery products, and dairy products (Havea, Watkinson, &  
61 Kuhn-Sherlock, 2009). WPI gels can also serve as a carrier of bioactive substances or flavors  
62 (Gunasekaran, 2008; Weel, Boelrijk, Alting, van Mil, Burger, Gruppen, Voragen, & Smit, 2002).  
63 The goal of this study was to investigate how histidine, lysine, and arginine would influence the  
64 gelation of WPI. We hypothesized that addition of basic amino acids would result in changes in  
65 properties of WPI gels such as gel strength and water holding capacity. This could serve as an  
66 alternative method to pH-based manipulation of gel properties, which are particularly  
67 advantageous for foods produced at a given pH. Several studies attributed part of the gelation  
68 promoting effects of the basic amino acids to their ability to increase the pH (Fu et al., 2017; Qin  
69 et al., 2015; Zhou, Li, & Tan, 2014; Zhou, Li, Tan, & Sun, 2014). Since there are more effective  
70 and economic ways to adjust pH, we controlled the pH in the current investigation and examined  
71 the effectiveness of other mechanisms. Moreover, it is evident that the electrostatic interactions  
72 between the basic amino acids and the proteins play an important role in the gelling process  
73 (Cando et al., 2016; Lei et al., 2016; Lei et al., 2017). To uncover how the charge state would  
74 affect the efficacy of the basic amino acids and to test the versatility of the application at

75 different pHs, we performed the experiments at pH 2.0 and at the isoelectric point (pI) of  
76 histidine (pH 7.59), lysine (pH 9.74), arginine (pH 10.76), and  $\beta$ -lactoglobulin (pH 5.2).

77

## 78 **2. Materials and methods**

### 79 *2.1. Materials*

80 Whey protein (WPI-90) was obtained from Hilmar Ingredients (Hilmar, CA, USA).  
81 Histidine, lysine, and arginine were purchased from Sangon Biotech (Shanghai) Co., Ltd.  
82 (Shanghai, China). All other chemicals were purchased from Sigma Aldrich (St. Louis, MO,  
83 USA) or Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were of analytical grade.

84

### 85 *2.2. Preparation of WPI sols*

86 WPI was dissolved in deionized water to a concentration of 12% (w/v). Histidine, lysine,  
87 or arginine was added to the WPI sol to a concentration of 0.5% (w/v). The pH of the WPI sols  
88 in the absence and presence of the basic amino acids was adjusted to 2.0, 5.2, 7.59, 9.74, and  
89 10.76. WPI sols were stored at 4 °C until further use.

90

### 91 *2.3. Particle size and $\zeta$ -potential*

92 Particle size and  $\zeta$ -potential of the basic amino acids-conditioned WPI sols were  
93 determined according to Cheng, Chen, & Xiong (2010) with modifications. The sols were diluted  
94 to 1% WPI (w/v) in deionized water prior to the analyses. The particle size and  $\zeta$ -potential of the  
95 samples were measured using a BT-90 Nano Laser Particle Size Analyzer (Bettersize  
96 Instruments Ltd., Dandong, Liaoning, China) and a NanoPlus-2 Zeta Potential Analyzer  
97 (Particulate Systems, Norcross, GA, USA), respectively.

98

99 *2.4. Preparation of WPI gels*

100 Twenty-five milliliters of WPI sols (12% w/v) in the absence and presence of 0.5% (w/v)  
101 basic amino acids were added to cylindrical containers with an internal diameter of 35 mm and a  
102 height of 30 mm and were heated in a water bath at 90 °C for 30 min. Samples were  
103 subsequently cooled to room temperature (RT, 23 °C) in an ice water bath followed by an  
104 overnight incubation at 4 °C.

105

106 *2.5. Color measurement*

107 Color of the WPI gels were measured using an SC-10 portable colorimeter (Shenzhen  
108 Threenh Technology Co., Ltd., Shenzhen, Guangdong, China). L\*, a\*, and b\* values of the  
109 samples were determined using 4 mm aperture, 8/d geometry, and D65 illuminant.

110

111 *2.6. Texture profile analysis*

112 WPI gels were subjected to texture profile analysis using a TA.XT plus texture analyzer  
113 (Stable Micro Systems Ltd., Godalming, United Kingdom) with a 5 kg load cell, 3.5-inch  
114 diameter metal compression platen, 1 mm/s pre-test speed, 2 mm/s compression speed, 10 mm  
115 compression distance, and 5 g trigger force (Cheng et al., 2019). Hardness was defined as the  
116 maximum force of the first compression. Resilience was defined as ratio of upstroke-to-  
117 downstroke energy of the first compression. Springiness was defined as the ratio of the second  
118 compression distance to the first compression distance. Cohesiveness was defined as ratio of the  
119 second compression energy to the first compression energy. Gumminess was defined as hardness

120 × cohesiveness. Chewiness was defined as hardness × cohesiveness × springiness (Bourne,  
121 2002).

122

### 123 2.7. *Water holding capacity (WHC)*

124 WHC was measured by centrifuging 2 g of WPI gel samples in centrifuge tubes with a  
125 small piece of filter paper at 3000 × g for 20 min. WHC was calculated according to Equation  
126 (1).

$$\text{WHC (\%)} = \frac{W_2}{W_1} \times 100\% \quad (1)$$

127 Where  $W_1$  is the initial weight of the gel and  $W_2$  is the gel weight after centrifugation  
128 (Wu, Xiong, Chen, Tang, & Zhou, 2009).

129

### 130 2.8. *Swelling ratio*

131 A cylindrical gel (diameter × height = 8 mm × 10 mm) was cored from the center of the  
132 gel samples and heated at 50 °C in deionized water. The gel was blotted dry and weighed, and  
133 the swelling ratio was calculated according to Equation (2).

$$\text{Swelling ratio (\%)} = \frac{W_2 - W_1}{W_1} \times 100\% \quad (2)$$

134 Where  $W_1$  is the initial weight of the gel and  $W_2$  is the weight of the swollen gel (Ozel,  
135 Cikrikci, Aydin, & Oztog, 2017).

136

### 137 2.9. *Protein leachability*

138 Two grams of WPI gel was immersed in 8 mL of 0.05 M sodium phosphate buffer (pH  
139 7.0) at room temperature for 2 h with manual shaking every 30 min. Subsequently, samples were  
140 centrifuged at 3000 × g, room temperature for 10 min. Soluble protein concentration in the



141 supernatant was determined by the Biuret method, and protein leachability was measured as the  
142 percentage of protein that leached out of the gel (Wang, Xiong, Rentfrow, & Newman, 2013).  
143 The leached-out proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel  
144 electrophoresis (SDS-PAGE).

145

#### 146 *2.10. SDS-PAGE*

147 The supernatant from the protein leachability test was mixed with sample buffer (10 mM  
148 Tris-HCl, 10% v/v glycerol, 2% w/v SDS, 0.02% bromophenol blue, pH 8.0) with and without  
149 5% (v/v)  $\beta$ -mercaptoethanol ( $\beta$ ME) at 1:1 ratio and boiled for 3 min. For samples without  $\beta$ ME,  
150 0.5 mM N-ethylmaleimide (NEM) was added to prevent artificial disulfide bond formation.  
151 Samples (20  $\mu$ L) were loaded along with molecular weight standards and electrophoresed on a  
152 5% polyacrylamide stacking gel (20 mA/gel) and a 12.5% polyacrylamide resolving gel (40  
153 mA/gel). The gels were stained using Coomassie Brilliant Blue R250 for 3 h and de-stained with  
154 7.5% (v/v) acetic acid and 10% (v/v) methanol until the background was clear (Laemmli, 1970).

155

#### 156 *2.11. Scanning electron microscopy*

157 A Quanta-200 scanning electron microscopy (FEI Company, Eindhoven, Netherlands)  
158 was used to examine the microstructure of the WPI gels. A sharp razor was used to cut the WPI  
159 gels. Cross-sections of the gels were mounted on a bronze stub and sputter-coated with gold prior  
160 to microscopic observation (Wang et al., 2013).

161

#### 162 *2.12. Statistical analysis*

163 All experiments were replicated at least twice with triplicate measurements in each  
164 replication. One-way ANOVA was used to compare means for difference with Statistix 9.0  
165 (Analytical Software, Tallahassee, FL, USA). Fisher's least significant difference (LSD) test was  
166 used as *post-hoc* test at  $P \leq 0.05$ .

167

### 168 **3. Results and discussion**

#### 169 *3.1. Particle size and $\zeta$ -potential of WPI sols*

170 The aggregation and gelation of the WPI are largely dependent on the pH and surface  
171 charge of the proteins (Brodkorb, Croguennec, Bouhallab, & Kehoe, 2016). To test whether the  
172 addition of basic amino acids would influence these important properties, particle size and  $\zeta$ -  
173 potential of the WPI sols were determined at different pHs in the absence and presence of basic  
174 amino acids. WPI sols registered similar average particle size (397-427 nm) at pH 2.0, 7.59,  
175 9.74, and 10.76 (Fig. 1A). At pH 5.2, WPI formed significantly ( $P < 0.05$ ) larger particles (1728  
176  $\pm 30$  nm). These results were in agreement with previously observations that heat-induced  
177 aggregation of  $\beta$ -lactoglobulin at its pI led to large particle formation whilst smaller particles  
178 were obtained at pHs far from the pI (Guo, Harris, Kaur, Pastrana, & Jauregi, 2017). The lack of  
179 net charge on the protein surface at the pI promoted protein aggregation while strong repulsive  
180 interactions between charged protein molecules at pHs far from pI hindered aggregation. At all  
181 the pH values tested, the addition of basic amino acids did not change the particle size of the  
182 WPI to a great extent. As shown in Fig. 1B, the WPI sol exhibited a  $\zeta$ -potential of  $-4.52 \pm 0.40$   
183 mV at pH 5.2. The addition of basic amino acids changed the  $\zeta$ -potential to slightly positive  
184 (0.31-2.05 mV). At pH 2.0, the WPI sol had a  $\zeta$ -potential of  $8.73 \pm 0.98$  mV due to the  
185 protonation of the carboxyl and amine groups. The  $\zeta$ -potential increased in the presence of lysine

186 (11.55 ± 0.52 mV) and arginine (13.95 ± 0.04 mV), while decreased slightly in the presence of  
187 histidine (5.50 ± 0.11 mV). At pH 7.59, the ζ-potentials of the control (-26.71 ± 0.29 mV) and  
188 histidine added sample (-26.32 ± 0.23 mV) were not significantly different ( $P > 0.05$ ), while the  
189 samples with the addition of lysine and arginine had lower ( $P < 0.05$ ) negative ζ-potentials (-  
190 20.55 mV to -20.96 mV). Since lysine and arginine are strongly cationic at pHs 2.0-7.59, these  
191 results are expected. At pH 9.74 and 10.76, no difference in ζ-potential (-30.09 mV to -31.84  
192 mV) was found between samples possibly due to extensive deprotonation of the WPI and amino  
193 acids (Miyatake, Yoshizawa, Arakawa, & Shiraki, 2016).

194

### 195 *3.2. Appearance and color of WPI gels*

196 Appearance and color are important quality indicators of gels. During gel preparation,  
197 substantial color differences between treatments were noticed (Figure 2). The distinct differences  
198 in the surface charge and particle size of WPI at pH 5.2 in comparison to the other pH values  
199 were reflected on the appearance of the WPI gels. The WPI formed white, opaque gels at pH 5.2  
200 and translucent gels at the other pHs regardless of the absence or presence of the basic amino  
201 acids (Fig. 2). It has been well documented that WPI forms a coarse particulate gel when there is  
202 limited electrostatic repulsion and a fine-stranded gel when the repulsive forces are dominant  
203 (Langton & Hermansson, 1992). The control WPI gel at pH 2.0 was not able to withhold its  
204 shape. Although WPI is capable of forming fine-stranded gels at low pHs, such gels are weak  
205 due to the lack of disulfide bond formation (Shinya Ikeda & Morris, 2002). The addition of the  
206 basic amino acids improved gel rigidity at pH 2.0. The basic amino acids also enhanced the  
207 gelation of the WPI at pH 7.59 and 9.74 based on the appearances of the gels. It has been  
208 reported that the addition of basic amino acids can expose buried hydrophobic groups and

209 reactive sulfhydryl groups and contribute to an enhanced protein gelation (Guo et al., 2015; Lei  
210 et al., 2016; Lei et al., 2017).

211 The color measurements of the gels corresponded well with the visual appearance (Table  
212 1). The particulate gels at pH 5.2 had considerably higher L\* values than gels at other pHs ( $P <$   
213 0.05). With the exception of the particulate gels at pH 5.2, which reflected most of the colors, the  
214 increase in pH and the addition of lysine and arginine resulted in significantly higher yellowness  
215 values ( $P < 0.05$ ). The yellow color was likely resulted from Maillard browning reaction between  
216 the residual lactose (0.2%) and proteins/amino acids, which was favored at high pHs and with  
217 the addition of free amines.

218

### 219 3.3. *Texture profile analysis*

220 Except for the WPI gel containing 0.5% arginine, the gel strength peaked at pH 5.2,  
221 decreased in the pH range of 5.2 to 9.74, and increased again when the pH reached 10.76 (Fig.  
222 3). In the presence of 0.5% arginine, the highest gel hardness was achieved at pH 7.59. Gel  
223 resilience did not exhibit appreciable changes in acidic pHs, but increased drastically when pH  
224 was raised to 7.59, and then leveled off at higher pHs. The control gel had a higher gel resilience  
225 at pH 2.0, while a lower gel resilience at pHs 7.50-10.76 in comparison to those containing basic  
226 amino acids. The lowest gel springiness was observed at pH 5.2 for all samples. Treatment with  
227 0.5% histidine at pHs 7.59 and 9.74 resulted in the springiest WPI gels followed by the treatment  
228 with 0.5% lysine at pH 7.59. Gel cohesiveness increased in the pH range of 2.0 to 7.59 and  
229 leveled off at higher pHs. The gels were the least cohesive in the presence of 0.5% lysine and  
230 arginine at pH 2.0. The addition of basic amino acids exhibited a trend towards higher gel  
231 cohesiveness at basic pHs. Gumminess of the gel displayed a similar pattern as gel hardness.

232 WPI gel at pH 7.59 in the presence of 0.5% arginine exhibited the highest gumminess.  
233 Chewiness is mutually exclusive from gumminess and is not applicable to gels (Bourne, 2002).  
234       Whey proteins agglomerate extensively at pH 5.2 due to weak electrostatic repulsions  
235 and form particulate gels that fracture at relatively large stress (Ikeda & Foegeding, 1999; Shinya  
236 Ikeda, Foegeding, & Hagiwara, 1999; Stading & Hermansson, 1991). However, such gels are  
237 mainly composed of loosely-linked large, spherical particles with fewer junctions as compared to  
238 the fine-stranded gels (Ikeda & Morris, 2002; Langton & Hermansson, 1992). During the texture  
239 profile analysis, these brittle gels failed to withstand the second compression and resulted in the  
240 poor resilience, springiness, and cohesiveness. Although  $\beta$ -lactoglobulins form fine-stranded gels  
241 at both low and high pHs, the microstructure and texture of the gels are different. At low pHs,  $\beta$ -  
242 lactoglobulin gels are composed of short, stiff strands and are fragile and brittle (Langton &  
243 Hermansson, 1992). The low thiolate/thiol ratio and less frequent thiol/disulfide interchange rate  
244 at low pHs also contribute to the fragility of the gels (Monahan, German, & Kinsella, 1995;  
245 Zhou, Liu, & Labuza, 2008). On the contrary, the high pH gels have extensive disulfide cross-  
246 links and curled strands with long junction zones and a rubbery texture (Langton & Hermansson,  
247 1992).  
248       The addition of basic amino acids modified the texture of the WPI gels. Several studies  
249 have demonstrated that basic amino acids strongly bind to the charged residues of proteins  
250 through electrostatic interactions, which alters the structure and thermal properties of the proteins  
251 and in turn affect their gelling properties (Guo et al., 2015; Lei et al., 2016; Lei et al., 2017;  
252 Zhou, Li, & Tan, 2014). Gel hardness and gumminess increased significantly ( $P < 0.05$ ) at pH  
253 7.59 when 0.5% arginine was added. Arginine-induced increase in hardness/strength of chicken  
254 salt-soluble protein gel (Qin et al., 2015), actomyosin gel (Lei et al., 2016), chicken sausage (Zhu

255 et al., 2018), and pork sausage (Zhou, Li, Tan, & Sun, 2014) have also been reported. Lei et al.  
256 (2016) demonstrated that arginine increased the surface hydrophobicity and reactive sulfhydryl  
257 groups of chicken actomyosin, both of which are critical for the gel network formation. The  
258 addition of histidine (pHs 7.59 and 9.74) and lysine (pH 7.59) significantly increased gel  
259 springiness ( $P < 0.05$ ). Lysine-induced increases in springiness of pork sausage (Zhou, Li, &  
260 Tan, 2014) and chicken sausage (Zhu et al., 2018) have been reported. Lysine enhanced the  
261 thermal stability of the proteins and induced formation of a more compact, uniform, and elastic  
262 gel matrix (Zhou, Li, & Tan, 2014; Zhu et al., 2018). Gao et al. (2018) reported that histidine  
263 suppressed fierce aggregation of carp myosins and induced the proteins to form finer aggregates  
264 and a more ordered network. In addition, charge screening of the WPI by the positively charged  
265 basic amino acids reduced electrostatic repulsion and promoted protein aggregation and gel  
266 formation (Unterhaslberger, Schmitt, Sanchez, Appolonia-Nouzille, & Raemy, 2006). This  
267 explained why lysine and arginine were the most effective at pH 7.59. As shown in Fig. 1, lysine  
268 and arginine significantly ( $P < 0.05$ ) reduced the negative  $\zeta$ -potential of the WPI at pH 7.59. The  
269 charge screening effect diminished at higher pHs due to extensive deprotonation of the WPI and  
270 amino acids (Miyatake, Yoshizawa, Arakawa, & Shiraki, 2016).

271

### 272 *3.4. WHC and swelling ratio*

273 As expected, WPI gels had the lowest WHC at pH 5.2 (Fig. 4A). Particulate gels are  
274 known to have poor WHC. As shown in Fig. 2, the WPI gels at pH 5.2 exhibited extensive  
275 syneresis. The particulate gels have much larger pore sizes ( $\mu\text{m}$ ) than the fine-stranded gels (nm)  
276 and thus have weaker capillary forces to entrap water (Stading, Langton, & Hermansson, 1993).  
277 The addition of basic amino acids increased the WHC at all pHs except for pH 5.2, at which only

278 0.5% lysine resulted in a significantly higher WHC ( $P < 0.05$ ). Histidine, lysine, and arginine  
279 have been reported to improve WHC of chicken salt soluble protein gel (Qin et al., 2015),  
280 chicken myosin gel (Fu et al., 2017), surimi gel (Cando et al., 2016), porcine myosin gel (Zhang  
281 et al., 2017), and pork sausage (Zhou, Li, & Tan, 2014; Zhou, Li, Tan, & Sun, 2014). Some of  
282 the studies attributed the enhanced WHC to basic amino acid-induced pH deviation away from pI  
283 (Fu et al., 2017; Qin et al., 2015; Zhou, Li, & Tan, 2014; Zhou, Li, Tan, & Sun, 2014). However,  
284 since the pH of the samples were controlled in the current investigation, factors other than pH  
285 shifting must have also contributed to the increased WHC. Basic amino acid-induced increase in  
286 protein solubility and hydration capacity (Li et al., 2019; Li et al., 2018), suppression of protein  
287 aggregation (Qin et al., 2015), reduction in water mobility (Fu et al., 2017; Zhang et al., 2017),  
288 and formation of a fine gel structure (Fu et al., 2017; Qin et al., 2015) have been suggested as  
289 possible mechanisms.

290         During the swelling ratio test, all the pH 2.0 WPI gels collapsed when heated in  
291 deionized water. As shown in Fig. 4B, the swelling ratio of the WPI gels increased significantly  
292 when the pH increased from 5.2 to 7.59 ( $P < 0.05$ ). The swelling property of a gel is largely  
293 dependent on its microstructure (Abaee, Mohammadian, & Jafari, 2017). The particulate gels are  
294 less flexible than the fine-stranded gels and only swell when the interactions within and between  
295 the particulates are disrupted (Li, Chen, & Mercadé-Prieto, 2017; Li, Zhao, Chen, & Mercadé-  
296 Prieto, 2016; Mercadé-Prieto et al., 2016). Basic amino acid treatments either did not result in a  
297 significant change or decreased the swelling ratio. At pH 7.59, the swelling ratio of the control  
298 and histidine-treated WPI gels was considerably higher ( $P < 0.05$ ) than that of the lysine and  
299 arginine-treated WPI gels. The swelling ratio of the control and histidine-treated WPI gels  
300 decreased at higher pHs, while the swelling ratio of the lysine and arginine-treated gels peaked at

301 pH 9.74 and decreased thereafter. Swelling is an equilibrium between water influx-induced gel  
302 stretch and retraction of the cross-linked gel network (Gunasekaran, 2008). Lysine and arginine  
303 have a strong charge screening effect at pH 7.59 and resulted in a lower negative  $\zeta$ -potential of  
304 the WPI as compared to histidine and the control (Fig.1B). Thus, the lysine and arginine-treated  
305 WPI gels had less charged groups and a weaker osmotic pressure to attract water as compared to  
306 the control or histidine-treated gels at pH 7.59 (Wang et al., 2019). At higher pHs, the excessive  
307 electrostatic repulsions resulted in a poorly interconnected gel matrix as evident by the weak gel  
308 strength and springiness (Fig. 3). The declined gel elasticity was likely responsible for the  
309 reduction in swelling ratio at pHs 9.74-10.76.

310

### 311 3.5. Protein leachability

312 Protein leachability of the control WPI gel was the highest at pHs 2.0 and 5.2 and  
313 decreased at higher pHs (Fig. 5). At pH 2.0, the leached-out proteins were predominantly  $\beta$ -  
314 lactoglobulin (Fig. 6). Under acidic condition, the thiolate to thiol reaction and the thiol/disulfide  
315 exchange were inhibited, which hindered the cross-linking of  $\beta$ -lactoglobulins (Monahan et al.,  
316 1995; Zhou et al., 2008). The lysine and arginine treatments significantly reduced protein  
317 leachability at pH 2.0 ( $P < 0.05$ ). Lysine and arginine can alter protein structure and expose  
318 reactive sulfhydryl groups (Guo et al., 2015; Lei et al., 2016; Lei et al., 2017). Therefore, more  
319  $\beta$ -lactoglobulin was retained in the gel network through disulfide cross-linking in the presence of  
320 lysine and arginine. At higher pHs, the leached-out proteins were mostly polymerized  $\alpha$ -  
321 lactalbumins and  $\beta$ -lactoglobulins. These protein polymers were stabilized not only by disulfide  
322 bonds but also by covalent bonds of other kinds (e.g., dityrosine bonds, carbonyl-amine bonds)  
323 as they cannot be completely dissociated by  $\beta$ -mercaptoethanol (Cui, Xiong, Kong, Zhao, & Liu,



324 2012). High molecular weight protein aggregates unable to enter the separating gels were  
325 observed at pHs 7.59 and 9.74 under non-reducing conditions. In the presence of  $\beta$ -  
326 mercaptoethanol, the protein aggregates disappeared with concomitant appearance of  $\beta$ -  
327 lactoglobulin indicating the aggregates were formed through disulfide cross-linking of  $\beta$ -  
328 lactoglobulins. The addition of basic amino acids did not change the protein leachability at pHs  
329 5.2-10.76, except for histidine, which resulted in a higher protein leachability at all pHs. It has  
330 been reported that histidine and more specifically, the imidazole ring, can suppress protein  
331 aggregation by altering the surface charge and structure of the protein (Chen et al., 2016; Gao et  
332 al., 2018; Guo et al., 2015), which explains the elevated protein leachability.

333

### 334 3.6. Gel microstructure

335 The microstructures of the WPI gels are illustrated in Fig. 7. At pH 5.2, WPI formed  
336 particulate gels that are composed of coarsely aggregated spherical particles. The lysine-WPI gel  
337 exhibited a distinct microstructure at pH 5.2, in which the particles were partially fused. Lysine  
338 has been reported to cause unfolding of globular proteins (Cando et al., 2016; Guo et al., 2015),  
339 which may expose more junction zones and promote the formation of stranded structures. The  
340 change in microstructure was likely responsible for the improved WHC at pH 5.2 (Fig. 4A). At  
341 pHs away from the pI, the WPI formed strand-like gels with relatively smooth surface. The  
342 cross-sections of the WPI gels in the presence of basic amino acids displayed a wider distribution  
343 of small cavities and less concave-convex surface in comparison to the control, which were  
344 indicative of a more porous and uniform structure and explained the improved WHC by basic  
345 amino acids (Figure 4A). Similar changes in the gel microstructure as a result of basic amino  
346 acids treatments have been reported (Fu et al., 2017; Lei et al., 2016; Lei et al., 2017; Qin et al.,

347 2015; Zhou, Li, & Tan, 2014; Zhou, Li, Tan, & Sun, 2014). Basic amino acids can act as cationic  
348 surfactants and interact with the oppositely charged protein, provoking the unfolding of the  
349 protein and exposure of hydrophobic regions, which facilitates protein aggregation (Fuda,  
350 Bhatia, Pyle, & Jauregi, 2005).

#### 351 **4. Conclusion**

352 The results from this study suggested that basic amino acids modified WPI gels in a pH-  
353 and amino acid-dependent manner. This was achieved by altering the surface charge and  
354 structure of the whey proteins. At pH 5.2 where proteins carry minimum net charge and form a  
355 particulate gel, basic amino acids had little influence on the gel functional properties except for  
356 lysine, which fused the particulates and resulted in an enhanced water holding capacity. At pHs  
357 away from 5.2, basic amino acid treatments resulted in a more uniform and porous gel matrix  
358 that can better entrap water. Basic amino acids also facilitated  $\beta$ -lactoglobulin cross-linking and  
359 improved texture profile of the gel. In conclusion, basic amino acids can serve as natural,  
360 inexpensive, and non-allergenic additives that can enhance various properties of the WPI gels.  
361 Based on the pH and desired attributes of the product, one can select the appropriate amino acids  
362 as the gel enhancer.

363

#### 364 **Conflict of interest**

365 The authors declare no conflict of interest.

366

#### 367 **Acknowledgment**

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373

#### 374 **References**

375 Abaee, A., Mohammadian, M., & Jafari, S. M. (2017). Whey and soy protein-based hydrogels  
376 and nano-hydrogels as bioactive delivery systems. *Trends in Food Science &*  
377 *Technology, 70*, 69-81.

378 Bourne, M. (2002). *Food texture and viscosity: concept and measurement*: Elsevier.

379 Brodkorb, A., Croguennec, T., Bouhallab, S., & Kehoe, J. J. (2016). Heat-induced denaturation,  
380 aggregation and gelation of whey proteins. In *Advanced Dairy Chemistry* (pp. 155-178):  
381 Springer.

382 Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2016). Different additives to  
383 enhance the gelation of surimi gel with reduced sodium content. *Food Chemistry, 196*,  
384 791-799.

385 Chen, X., Zou, Y., Han, M., Pan, L., Xing, T., Xu, X., & Zhou, G. (2016). Solubilisation of  
386 myosin in a solution of low ionic strength L-histidine: Significance of the imidazole ring.  
387 *Food Chemistry, 196*, 42-49.

388 Cheng, Y., Chen, J., & Xiong, Y. L. (2010). Chromatographic separation and tandem MS  
389 identification of active peptides in potato protein hydrolysate that inhibit autoxidation of  
390 soybean oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 58*(15),  
391 8825-8832.

392 Cheng, Y., Donkor, P. O., Ren, X., Wu, J., Agyemang, K., Ayim, I., & Ma, H. (2019). Effect of  
393 ultrasound pretreatment with mono-frequency and simultaneous dual frequency on the  
394 mechanical properties and microstructure of whey protein emulsion gels. *Food*  
395 *Hydrocolloids*, 89, 434-442.

396 Cui, X., Xiong, Y. L., Kong, B., Zhao, X., & Liu, N. (2012). Hydroxyl radical-stressed whey  
397 protein isolate: Chemical and structural properties. *Food and Bioprocess Technology*,  
398 5(6), 2454-2461.

399 Felicio, T., Esmerino, E., Vidal, V., Cappato, L., Garcia, R., Cavalcanti, R., . . . Silva, M. (2016).  
400 Physico-chemical changes during storage and sensory acceptance of low sodium  
401 probiotic Minas cheese added with arginine. *Food Chemistry*, 196, 628-637.

402 Fu, Y., Zheng, Y., Lei, Z., Xu, P., & Zhou, C. (2017). Gelling properties of myosin as affected  
403 by L-lysine and L-arginine by changing the main molecular forces and microstructure.  
404 *International Journal of Food Properties*, 20(sup1), S884-S898.

405 Fuda, E., Bhatia, D., Pyle, D. L., & Jauregi, P. (2005). Selective separation of  $\beta$ -lactoglobulin  
406 from sweet whey using CGAs generated from the cationic surfactant CTAB.  
407 *Biotechnology and Bioengineering*, 90(5), 532-542.

408 Gao, R., Wang, Y., Mu, J., Shi, T., & Yuan, L. (2018). Effect of L-histidine on the heat-induced  
409 aggregation of bighead carp (*Aristichthys nobilis*) myosin in low/high ionic strength  
410 solution. *Food Hydrocolloids*, 75, 174-181.

411 Gunasekaran, S. (2008). Whey protein hydrogels and nanoparticles for encapsulation and  
412 controlled delivery of bioactive compounds. In C. I. Onwulata & P. J. Huth (Eds.), *Whey*  
413 *Processing, Functionality and Health Benefits* (pp. 227-284): John Wiley & Sons, Inc.

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

417 Guo, Y., Harris, P., Kaur, A., Pastrana, L., & Jauregi, P. (2017). Characterisation of  $\beta$ -  
418 lactoglobulin nanoparticles and their binding to caffeine. *Food Hydrocolloids*, 71, 85-93.

419 Havea, P., Watkinson, P., & Kuhn-Sherlock, B. (2009). Heat-induced whey protein gels: Protein-  
420 protein interactions and functional properties. *Journal of Agricultural and Food*  
421 *Chemistry*, 57(4), 1506-1512.

422 Hayakawa, T., Yoshida, Y., Yasui, M., Ito, T., Iwasaki, T., Wakamatsu, J., Hattori, A.,  
423 Nishimura, T. (2012). Heat-induced gelation of myosin in a low ionic strength solution  
424 containing L-histidine. *Meat Science*, 90(1), 77-80.

425 Ikeda, S., & Foegeding, E. (1999). Effects of lecithin on thermally induced whey protein isolate  
426 gels. *Food Hydrocolloids*, 13(3), 239-244.

427 Ikeda, S., Foegeding, E. A., & Hagiwara, T. (1999). Rheological study on the fractal nature of  
428 the protein gel structure. *Langmuir*, 15(25), 8584-8589.

429 Ikeda, S., & Morris, V. J. (2002). Fine-stranded and particulate aggregates of heat-denatured  
430 whey proteins visualized by atomic force microscopy. *Biomacromolecules*, 3(2), 382-  
431 389.

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

434 Langton, M., & Hermansson, A.-M. (1992). Fine-stranded and particulate gels of  $\beta$ -lactoglobulin  
435 and whey protein at varying pH. *Food Hydrocolloids*, 5(6), 523-539.

436 Lei, Z., Fu, Y., Xu, P., Zheng, Y., & Zhou, C. (2016). Effects of L-arginine on the  
437 physicochemical and gel properties of chicken actomyosin. *International Journal of*  
438 *Biological Macromolecules*, 92, 1258-1265.

439 Lei, Z., Fu, Y., Zheng, Y., Xu, P., & Zhou, C. (2017). Effects of L-lysine on thermal gelation  
440 properties of chicken breast actomyosin. *Food Science and Biotechnology*, 26(3), 549-  
441 556.

442 Li, H., Chen, X. D., & Mercadé-Prieto, R. (2017). Elastic modulus and equilibrium swelling of  
443 stranded and particulate protein hydrogels at acid pH. *Food Hydrocolloids*, 71, 168-175.

444 Li, H., Zhao, L., Chen, X. D., & Mercadé-Prieto, R. (2016). Swelling of whey and egg white  
445 protein hydrogels with stranded and particulate microstructures. *International Journal of*  
446 *Biological Macromolecules*, 83, 152-159.

447 Li, S., Li, L., Zhu, X., Ning, C., Cai, K., & Zhou, C. (2019). Conformational and charge changes  
448 induced by L-arginine and L-lysine increase the solubility of chicken myosin. *Food*  
449 *Hydrocolloids*, 89, 330-336.

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

453 Liu, M., Wang, Y., Jiang, L., Xia, Q., Qiu, Y., Fan, L., Zhou, J., Zhao, L. (2015). Effect of  $\gamma$ -  
454 aminobutyric acid on the physicochemical, rheological and sensory properties of yoghurt.  
455 *International Journal of Dairy Technology*, 68(4), 503-510.

456 Mercadé-Prieto, R., Zhao, H., Zhang, M., Li, H., Zhao, L., & Chen, X. D. (2016). Dissolution  
457 and swelling of soy protein isolate hydrogels in alkali. *Food Hydrocolloids*, 56, 285-291.

458 Miyatake, T., Yoshizawa, S., Arakawa, T., & Shiraki, K. (2016). Charge state of arginine as an  
459 additive on heat-induced protein aggregation. *International Journal of Biological*  
460 *Macromolecules*, 87, 563-569.

461 Monahan, F. J., German, J. B., & Kinsella, J. E. (1995). Effect of pH and temperature on protein  
462 unfolding and thiol/disulfide interchange reactions during heat-induced gelation of whey  
463 proteins. *Journal of Agricultural and Food Chemistry*, 43(1), 46-52.

464 Ozel, B., Cikrikci, S., Aydin, O., & Oztop, M. H. (2017). Polysaccharide blended whey protein  
465 isolate-(WPI) hydrogels: A physicochemical and controlled release study. *Food*  
466 *Hydrocolloids*, 71, 35-46.

467 Primacella, M., Fei, T., Acevedo, N., & Wang, T. (2018). Effect of food additives on egg yolk  
468 gelation induced by freezing. *Food Chemistry*, 263, 142-150.

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

472 Stading, M., & Hermansson, A.-M. (1991). Large deformation properties of  $\beta$ -lactoglobulin gel  
473 structures. *Food Hydrocolloids*, 5(4), 339-352.

474 Stading, M., Langton, M., & Hermansson, A.-M. (1993). Microstructure and rheological  
475 behaviour of particulate  $\beta$ -lactoglobulin gels. *Food Hydrocolloids*, 7(3), 195-212.

476 Unterhaslberger, G., Schmitt, C., Sanchez, C., Appolonia-Nouzille, C., & Raemy, A. (2006).  
477 Heat denaturation and aggregation of  $\beta$ -lactoglobulin enriched WPI in the presence of  
478 arginine HCl, NaCl and guanidinium HCl at pH 4.0 and 7.0. *Food Hydrocolloids*, 20(7),  
479 1006-1019.

480 Wang, Y., Xiong, Y. L., Rentfrow, G. K., & Newman, M. C. (2013). Oxidation promotes cross-  
481 linking but impairs film-forming properties of whey proteins. *Journal of Food*  
482 *Engineering*, 115(1), 11-19.

483 Wang, Y., Zhao, J., Liu, C., & Li, W. (2019). Influence of  $\gamma$ -aminobutyric acid on gelling  
484 properties of heat-induced whey protein gels. *Food Hydrocolloids*, 94, 287-293.

485 Weel, K. G., Boelrijk, A. E., Alting, A. C., van Mil, P. J., Burger, J. J., Gruppen, H., Voragen, A.  
486 G. J., & Smit, G. (2002). Flavor release and perception of flavored whey protein gels:  
487 Perception is determined by texture rather than by release. *Journal of Agricultural and*  
488 *Food Chemistry*, 50(18), 5149-5155.

489 Wu, M., Xiong, Y. L., Chen, J., Tang, X., & Zhou, G. (2009). Rheological and microstructural  
490 properties of porcine myofibrillar protein–lipid emulsion composite gels. *Journal of Food*  
491 *Science*, 74(4), E207-E217.

492 Xu, P., Zheng, Y., Zhu, X., Li, S., & Zhou, C. (2018). L-lysine and L-arginine inhibit the  
493 oxidation of lipids and proteins of emulsion sausage by chelating iron ion and scavenging  
494 radical. *Asian-Australasian Journal of Animal Sciences*, 31(6), 905-913.

495 Zhang, Y., Wu, J., Jamali, M. A., Guo, X., & Peng, Z. (2017). Heat-induced gel properties of  
496 porcine myosin in a sodium chloride solution containing L-lysine and L-histidine. *LWT-*  
497 *Food Science and Technology*, 85, 16-21.

498 Wang, Y., Liu, C., Ma, T., & Zhao, J. (2019). Physicochemical and functional properties of  $\gamma$ -  
499 aminobutyric acid-treated soy proteins. *Food Chemistry*, 295, 267-273.

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



502 Zhou, C., Li, J., Tan, S., & Sun, G. (2014). Effects of L-arginine on physicochemical and sensory  
503 characteristics of pork sausage. *Advance Journal of Food Science and Technology*, 6(5),  
504 660-667.

505 Zhou, C., Ye, H., Nishiumi, T., Qin, H., & Chen, C. (2014). L-histidine enhances stability of  
506 hemoglobin concentrates by coordinating with free iron. *Food Research International*,  
507 62, 637-643.

508 Zhou, C., Ye, H., Wang, H., Qin, H., & Li, J. (2015). Coordination of L-arginine and iron cation  
509 improves stability of hemoglobin concentrates. *European Food Research and*  
510 *Technology*, 240(4), 743-751.

511 Zhou, P., Liu, X., & Labuza, T. P. (2008). Moisture-induced aggregation of whey proteins in a  
512 protein/buffer model system. *Journal of Agricultural and Food Chemistry*, 56(6), 2048-  
513 2054.

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

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

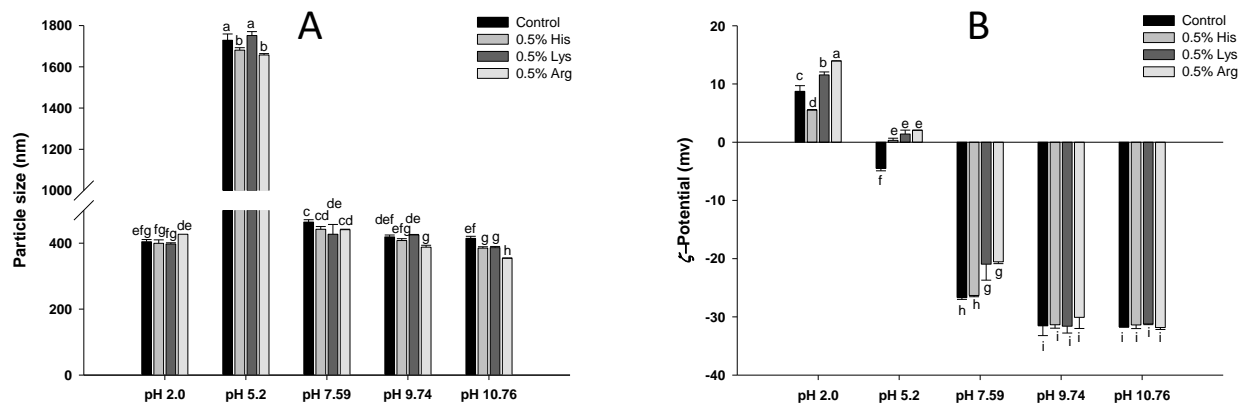
520 **Table 1**

521 Color of whey protein isolate gels at different pHs with and without 0.5% (w/v) histidine (His),  
 522 lysine (Lys), and arginine (Arg).

Gel sample	pH	Color		
		L*	a*	b*
Control	2.0	38.43±0.51 <sup>ghi</sup>	-1.87±0.06 <sup>c</sup>	-2.04±0.05 <sup>gh</sup>
	5.2	90.61±0.57 <sup>b</sup>	-2.07±0.07 <sup>cde</sup>	5.17±0.38 <sup>c</sup>
	7.59	41.47±1.15 <sup>def</sup>	-2.53±0.08 <sup>ghi</sup>	-3.01±0.05 <sup>jk</sup>
	9.74	33.88±0.78 <sup>k</sup>	-2.11±0.09 <sup>cde</sup>	-2.28±0.11 <sup>hi</sup>
	10.76	37.39±1.62 <sup>ij</sup>	-3.64±0.28 <sup>lm</sup>	3.71±0.41 <sup>d</sup>
0.5% His	2.0	43.35±0.74 <sup>cde</sup>	-2.30±0.10 <sup>efg</sup>	-2.91±0.14 <sup>jk</sup>
	5.2	92.40±0.20 <sup>ab</sup>	-1.50±0.02 <sup>b</sup>	6.62±0.04 <sup>a</sup>
	7.59	43.64±1.70 <sup>cd</sup>	-2.86±0.19 <sup>jk</sup>	-4.14±0.19 <sup>l</sup>
	9.74	36.53±0.58 <sup>ij</sup>	-2.66±0.07 <sup>hij</sup>	-1.61 ±0.06 <sup>g</sup>
	10.76	41.18±1.63 <sup>def</sup>	-3.69±0.22 <sup>lm</sup>	3.01±0.80 <sup>e</sup>
0.5% Lys	2.0	42.14±0.75 <sup>def</sup>	-2.18±0.05 <sup>def</sup>	-1.81±0.02 <sup>gh</sup>
	5.2	90.86±0.11 <sup>b</sup>	-2.05±0.01 <sup>cde</sup>	6.00 ±0.11 <sup>b</sup>
	7.59	40.87±1.39 <sup>efg</sup>	-2.75±0.10 <sup>ijk</sup>	-4.47±0.05 <sup>l</sup>
	9.74	44.73±1.22 <sup>c</sup>	-3.48±0.10 <sup>l</sup>	-0.62±0.26 <sup>f</sup>
	10.76	40.03±2.00 <sup>fgh</sup>	-4.16±0.33 <sup>n</sup>	6.15±0.70 <sup>ab</sup>
0.5% Arg	2.0	42.27±2.46 <sup>cdef</sup>	-2.00±0.09 <sup>cd</sup>	-2.61±0.15 <sup>ij</sup>
	5.2	94.50±0.56 <sup>a</sup>	-0.99±0.01 <sup>a</sup>	6.41±0.10 <sup>ab</sup>
	7.59	40.99±0.55 <sup>ef</sup>	-2.42±0.04 <sup>fgh</sup>	-3.39±0.05 <sup>k</sup>
	9.74	35.34±3.98 <sup>jk</sup>	-2.99±0.23 <sup>k</sup>	-1.56±0.41 <sup>g</sup>
	10.76	37.80±1.99 <sup>hij</sup>	-3.88±0.41 <sup>m</sup>	5.94±0.52 <sup>b</sup>

523 Values share no common letters differ significantly ( $P < 0.05$ ).

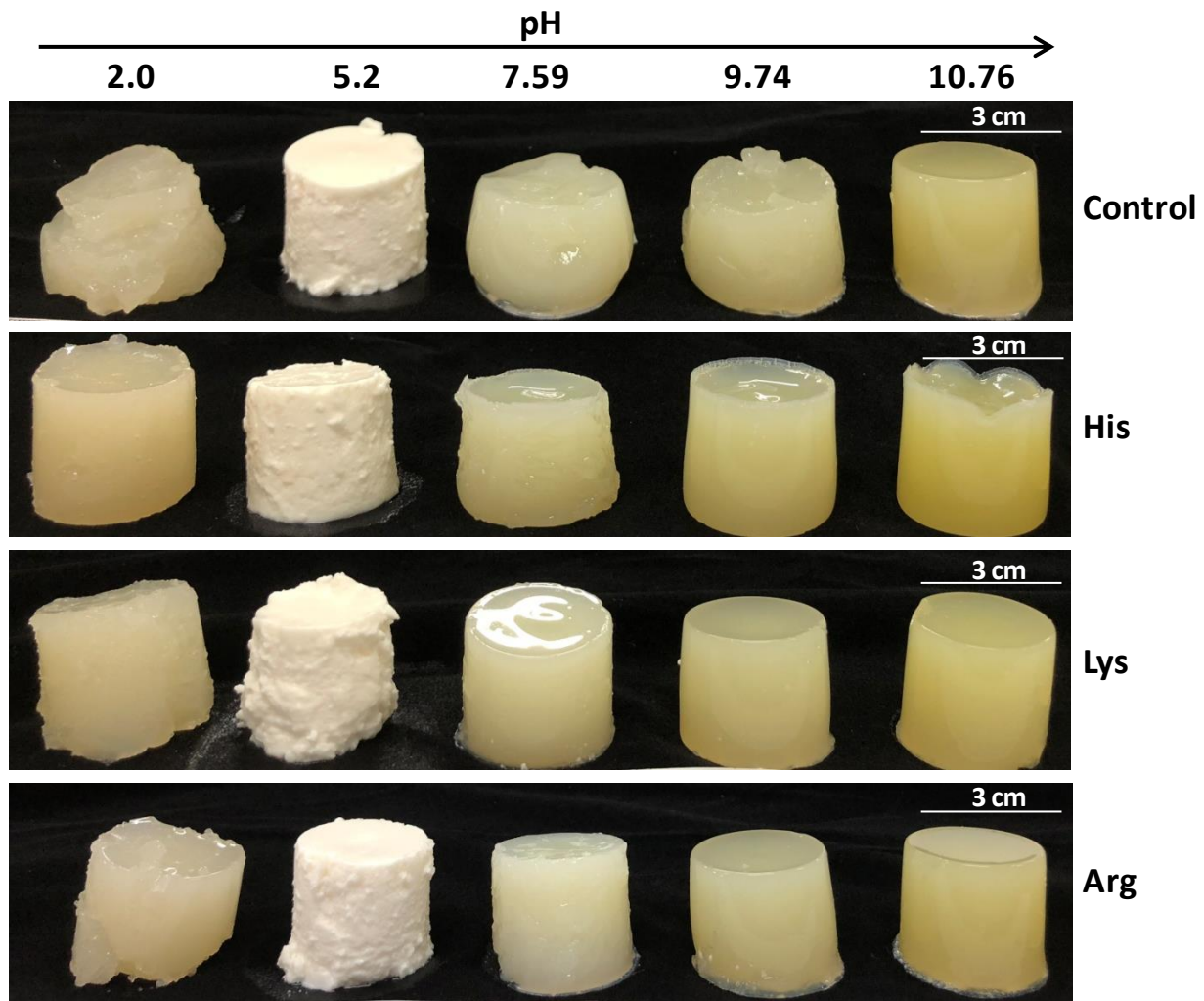
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525

526 **Fig 1.** Particle size (A) and  $\zeta$ -potential (B) of whey protein isolate sols at different pHs in the  
 527 absence and presence of 0.5% (w/v) histidine (His), lysine (Lys), or arginine (Arg). Values share  
 528 no common letters differ significantly ( $P < 0.05$ ).

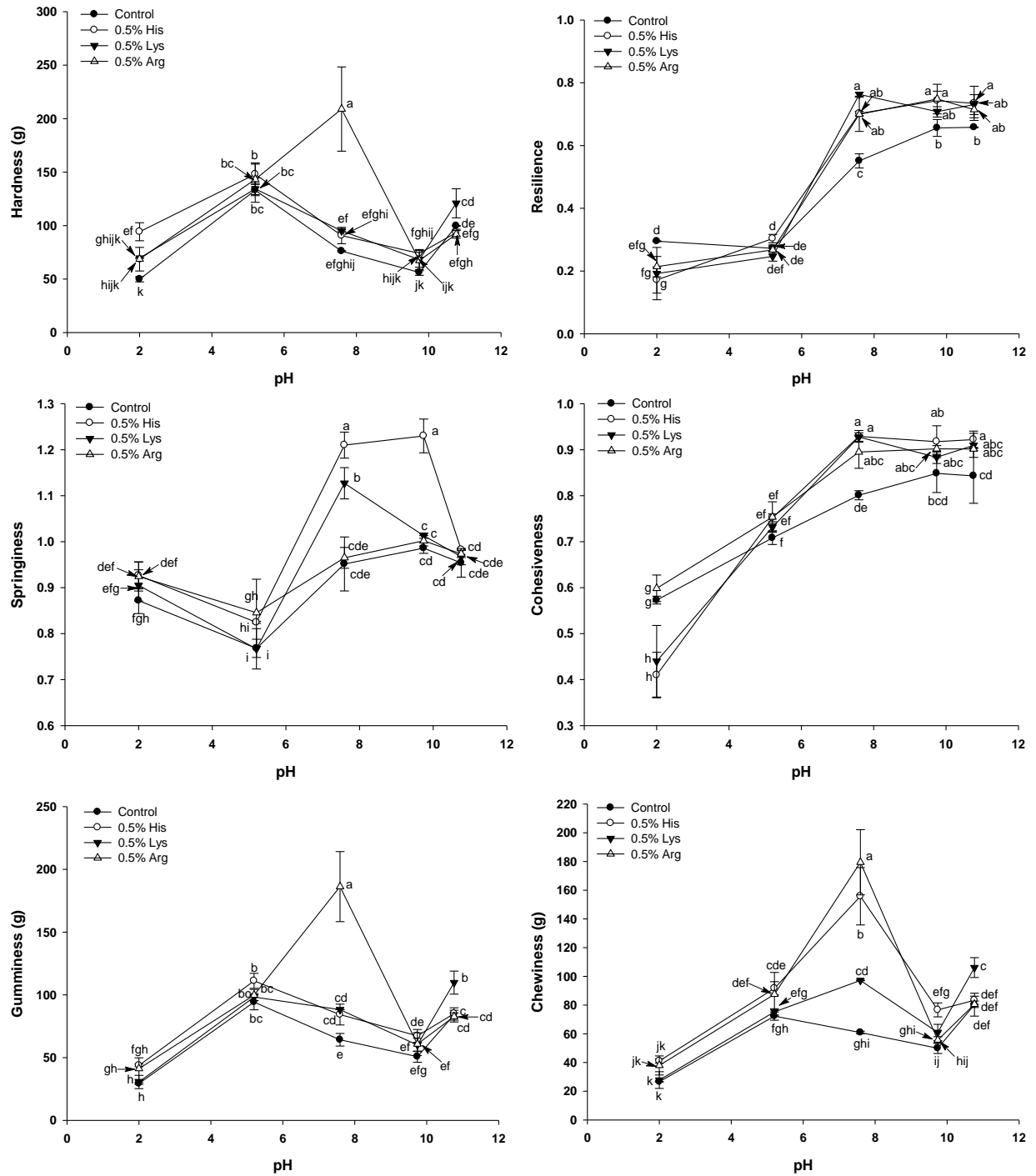
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531 **Fig. 2.** Appearance of whey protein isolate gels at different pHs in the absence and presence of  
 532 0.5% (w/v) histidine (His), lysine (Lys), or arginine (Arg).

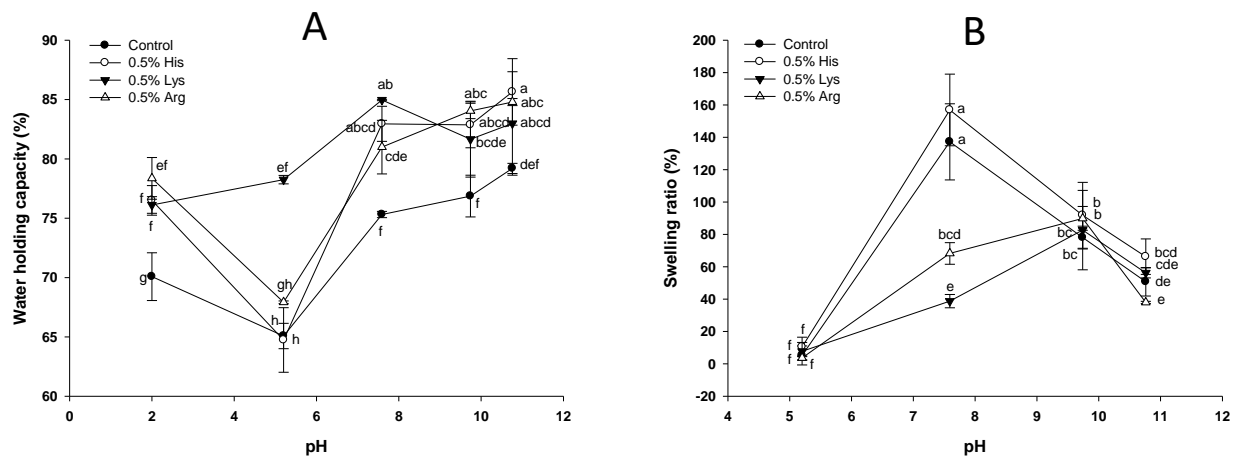
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534

535 **Fig. 3.** Texture profile analysis of whey protein isolate gels at different pHs in the absence and  
 536 presence of 0.5% (w/v) histidine (His), lysine (Lys), or arginine (Arg). Values share no common  
 537 letters differ significantly ( $P < 0.05$ ).

538



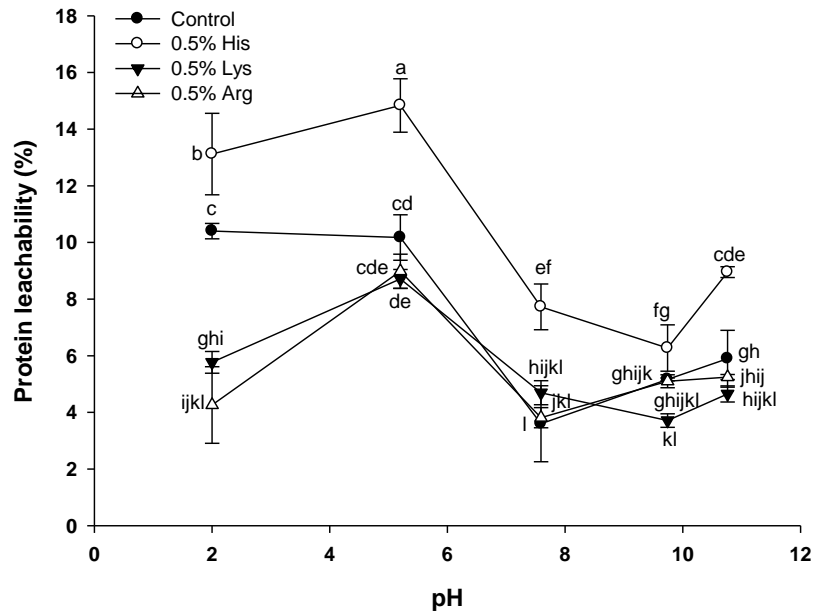
539

540 **Fig. 4.** Water holding capacity (A) and swelling ratio (B) of whey protein isolate gels at different

541 pHs in the absence and presence of 0.5% (w/v) histidine (His), lysine (Lys), or arginine (Arg).

542 Values share no common letters differ significantly ( $P < 0.05$ ).

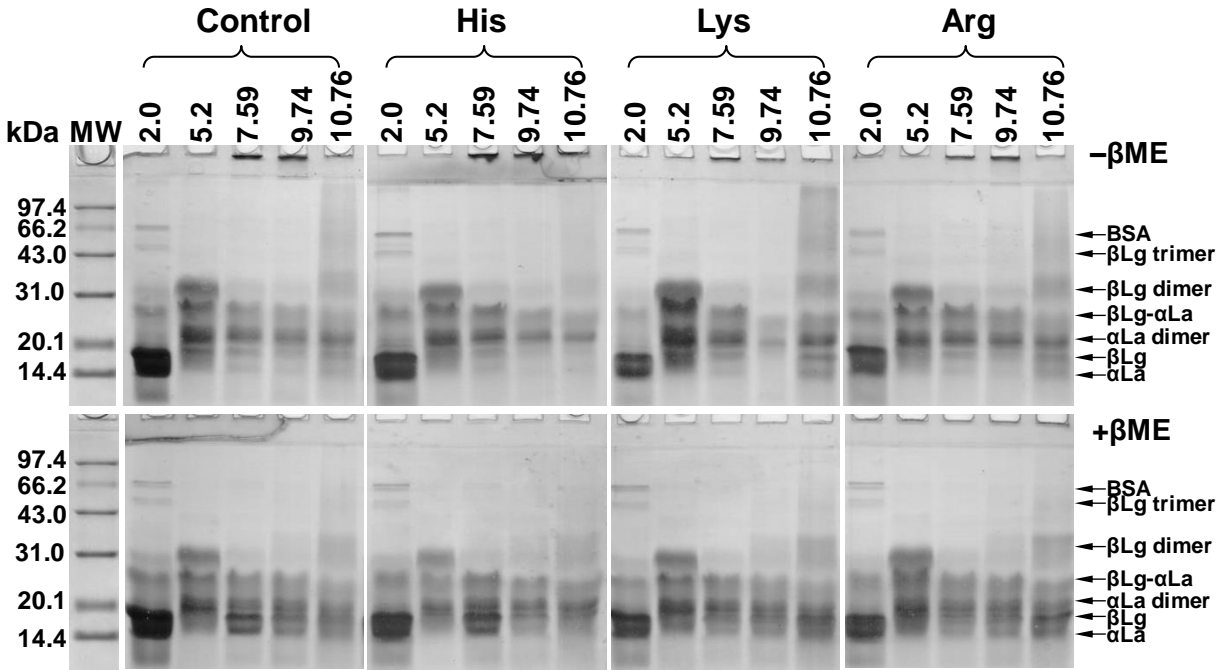
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544

545 **Fig. 5.** Protein leachability of whey protein isolate gels at different pHs in the absence and  
 546 presence of 0.5% (w/v) histidine (His), lysine (Lys), or arginine (Arg). Values share no common  
 547 letters differ significantly ( $P < 0.05$ ).

548



549

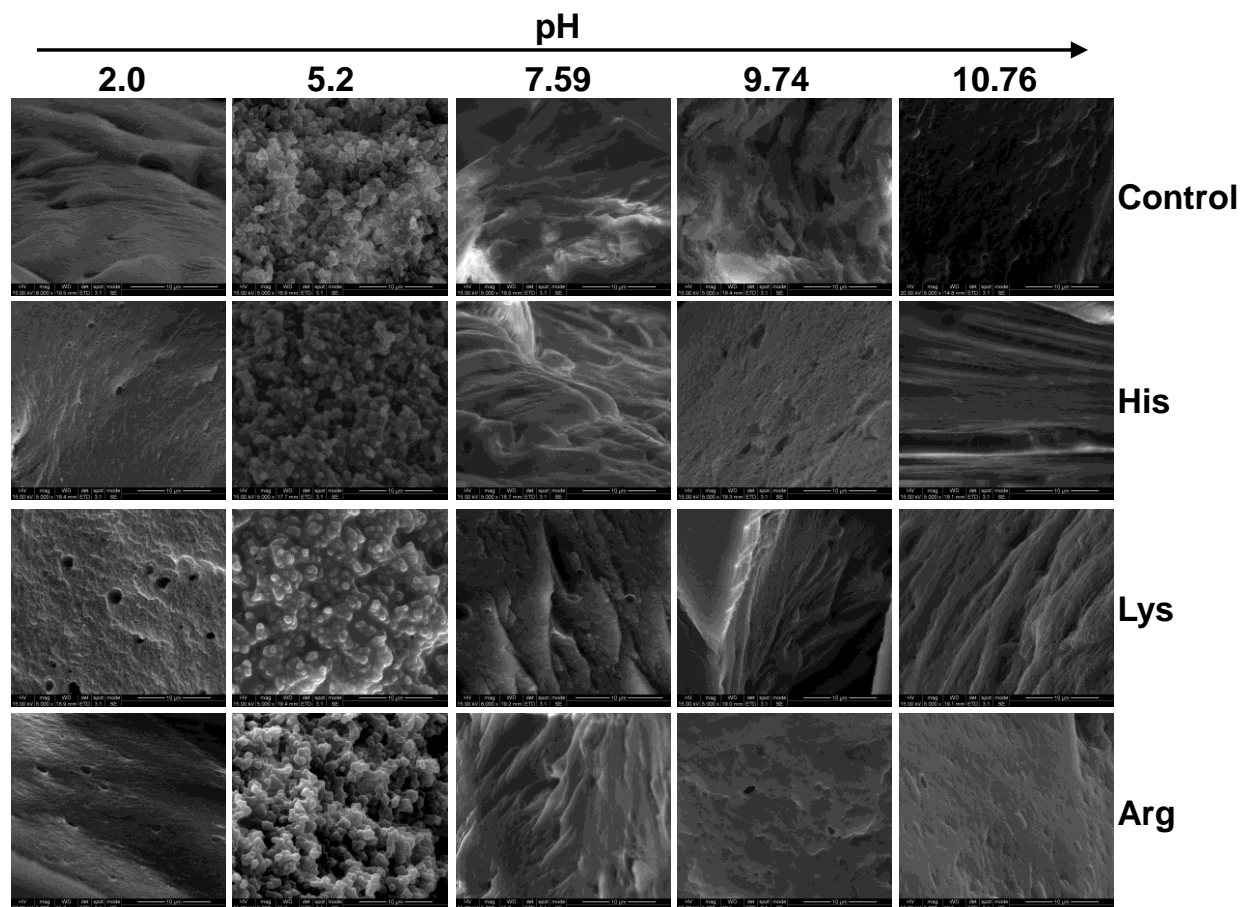
550 **Fig. 6.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins  
 551 leached out of whey protein isolate gels at different pHs in the absence and presence of 0.5%  
 552 (w/v) histidine (His), lysine (Lys), or arginine (Arg). The gels were run under reducing (+βME)  
 553 and non-reducing (-βME) conditions. MW: molecular weight; BSA: bovine serum albumin; βLg:  
 554 β-lactoglobulin; αLa: α-lactalbumin; βME: β-mercaptoethanol.

555

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557





558

559 **Fig. 7.** Scanning electron microscopy image of the cross-section of whey protein isolate gels at  
 560 different pHs in the absence and presence of 0.5% (w/v) histidine (His), lysine (Lys), or arginine  
 561 (Arg).