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ABSTRACT

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

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

1. Introduction

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

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

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

2. Materials and methods

2.1. Materials

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

2.2. Preparation of WPI sols

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

2.3. Particle size and ζ-potential

Particle size and ζ-potential of the basic amino acids-conditioned WPI sols were determined according to Cheng, Chen, & Xiong (2010) with modifications. The sols were diluted to 1% WPI (w/v) in deionized water prior to the analyses. The particle size and ζ-potential of the samples were measured using a BT-90 Nano Laser Particle Size Analyzer (Bettersize Instruments Ltd., Dandong, Liaoning, China) and a NanoPlus-2 Zeta Potential Analyzer (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

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

2.7. Water holding capacity (WHC)

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

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

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

2.8. Swelling ratio

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

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

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

2.9. Protein leachability

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

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

2.10. SDS-PAGE

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

2.11. Scanning electron microscopy

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

2.12. Statistical analysis

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

3. Results and discussion

3.1. Particle size and ζ-potential of WPI sols

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

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

3.2. Appearance and color of WPI gels

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

WPI gel at pH 7.59 in the presence of 0.5% arginine exhibited the highest gumminess. Chewiness is mutually exclusive from gumminess and is not applicable to gels (Bourne, 2002).

Whey proteins agglomerate extensively at pH 5.2 due to weak electrostatic repulsions and form particulate gels that fracture at relatively large stress (Ikeda & Foegeding, 1999; Shinya Ikeda, Foegeding, & Hagiwara, 1999; Stading & Hermansson, 1991). However, such gels are mainly composed of loosely-linked large, spherical particles with fewer junctions as compared to the fine-stranded gels (Ikeda & Morris, 2002; Langton & Hermansson, 1992). During the texture profile analysis, these brittle gels failed to withstand the second compression and resulted in the poor resilience, springiness, and cohesiveness. Although β-lactoglobulins form fine-stranded gels at both low and high pHs, the microstructure and texture of the gels are different. At low pHs, β-lactoglobulin gels are composed of short, stiff strands and are fragile and brittle (Langton & Hermansson, 1992). The low thiolate/thiol ratio and less frequent thiol/disulfide interchange rate at low pHs also contribute to the fragility of the gels (Monahan, German, & Kinsella, 1995; Zhou, Liu, & Labuza, 2008). On the contrary, the high pH gels have extensive disulfide cross-links and curled strands with long junction zones and a rubbery texture (Langton & Hermansson, 1992).

The addition of basic amino acids modified the texture of the WPI gels. Several studies have demonstrated that basic amino acids strongly bind to the charged residues of proteins through electrostatic interactions, which alters the structure and thermal properties of the proteins and in turn affect their gelling properties (Guo et al., 2015; Lei et al., 2016; Lei et al., 2017; Zhou, Li, & Tan, 2014). Gel hardness and gumminess increased significantly ($P < 0.05$) at pH 7.59 when 0.5% arginine was added. Arginine-induced increase in hardness/strength of chicken salt-soluble protein gel (Qin et al., 2015), actomyosin gel (Lei et al., 2016), chicken sausage (Zhu

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

3.4. WHC and swelling ratio

As expected, WPI gels had the lowest WHC at pH 5.2 (Fig. 4A). Particulate gels are known to have poor WHC. As shown in Fig. 2, the WPI gels at pH 5.2 exhibited extensive syneresis. The particulate gels have much larger pore sizes (μm) than the fine-stranded gels (nm) and thus have weaker capillary forces to entrap water (Stading, Langton, & Hermansson, 1993). 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 ($P < 0.0.5$) 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

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

3.5. Protein leachability

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

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

3.6. Gel microstructure

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

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

4. Conclusion

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

Conflict of interest

The authors declare no conflict of interest.

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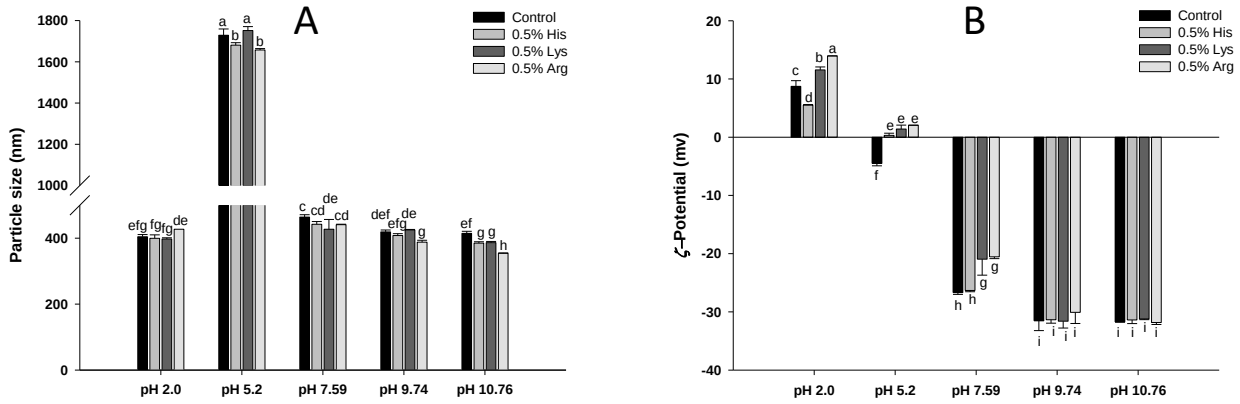
Table 1

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

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

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



525

526 **Fig 1.** Particle size (A) and ζ-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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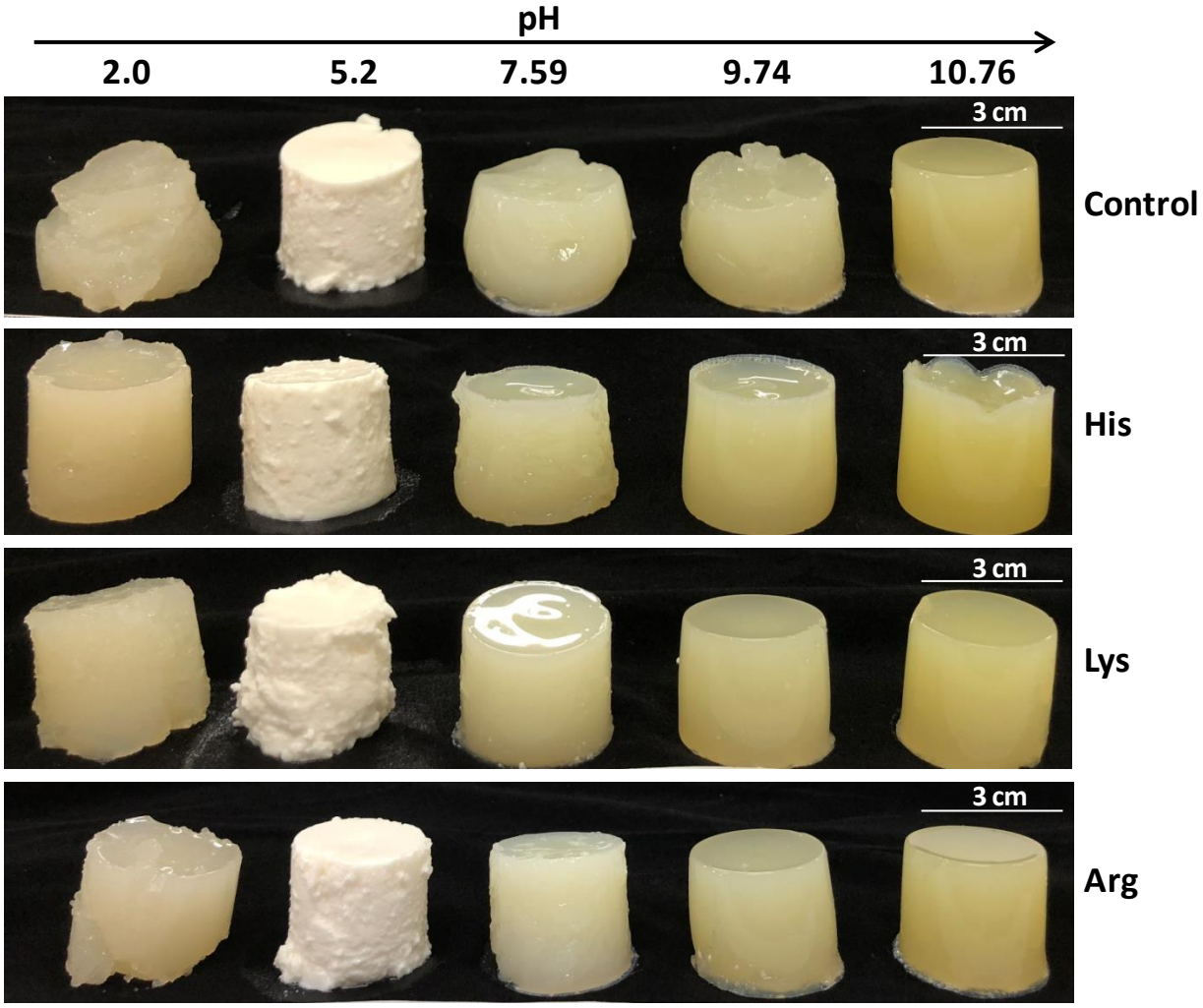

530

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).

533

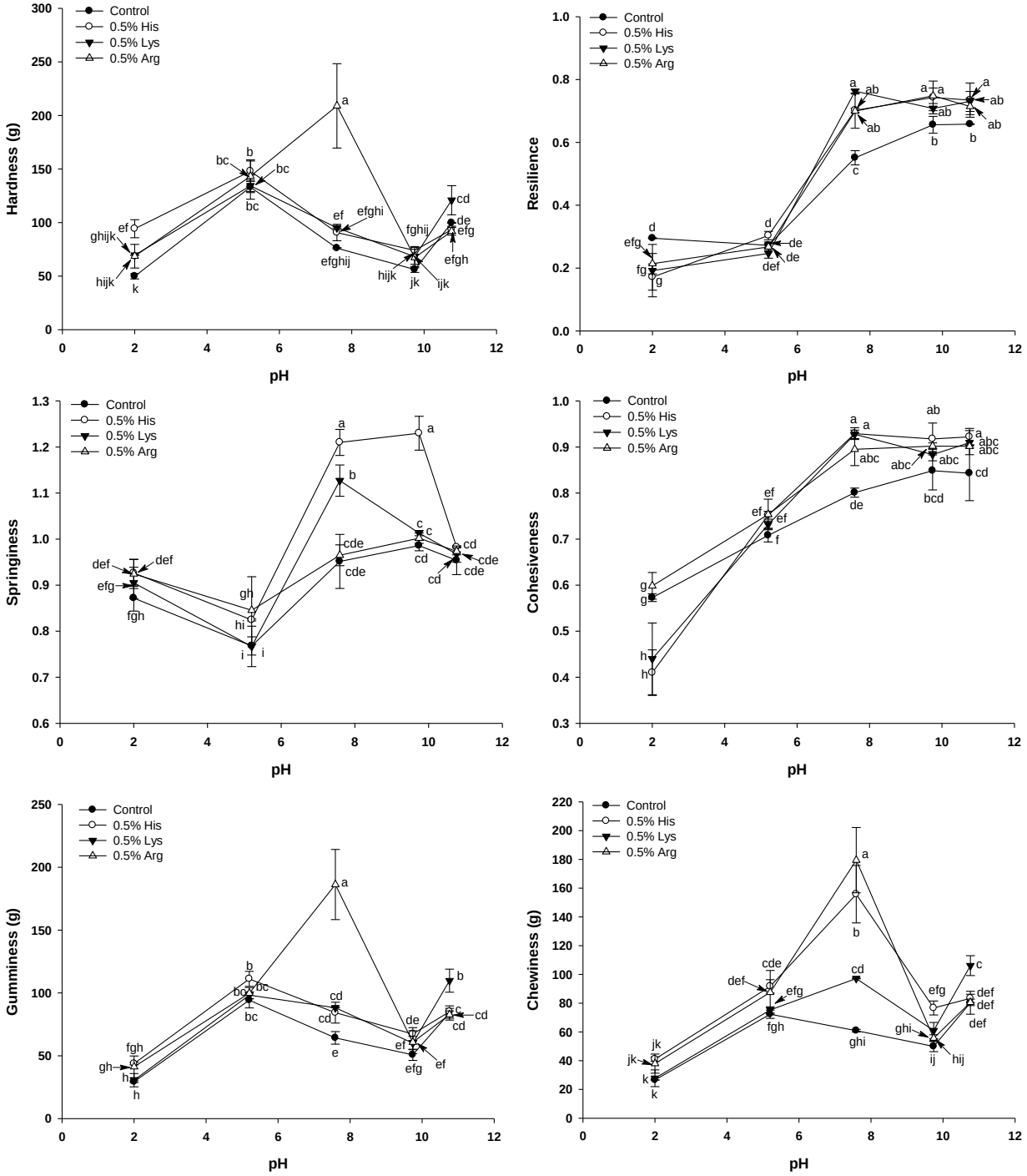

Fig. 3. Texture profile analysis of whey protein isolate gels at different pHs in the absence and presence of 0.5% (w/v) histidine (His), lysine (Lys), or arginine (Arg). Values share no common letters differ significantly ($P < 0.05$).

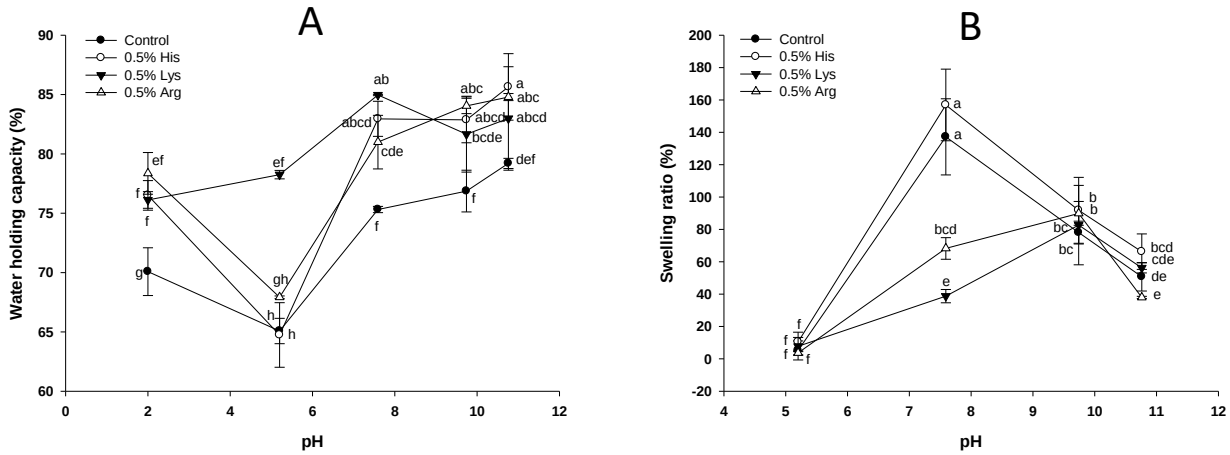

Fig. 4. Water holding capacity (A) and swelling ratio (B) of whey protein isolate gels at different pHs in the absence and presence of 0.5% (w/v) histidine (His), lysine (Lys), or arginine (Arg). Values share no common letters differ significantly ($P < 0.05$).

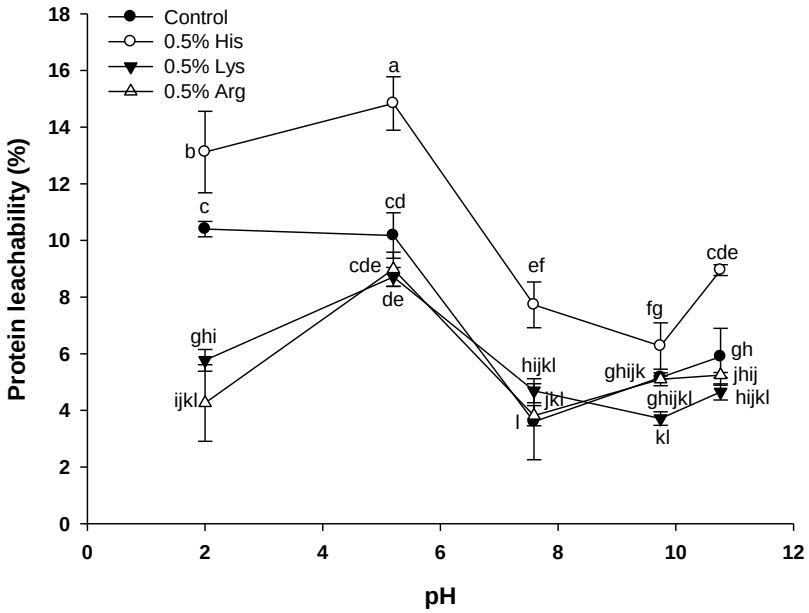

Fig. 5. Protein leachability of whey protein isolate gels at different pHs in the absence and presence of 0.5% (w/v) histidine (His), lysine (Lys), or arginine (Arg). Values share no common letters differ significantly ($P < 0.05$).

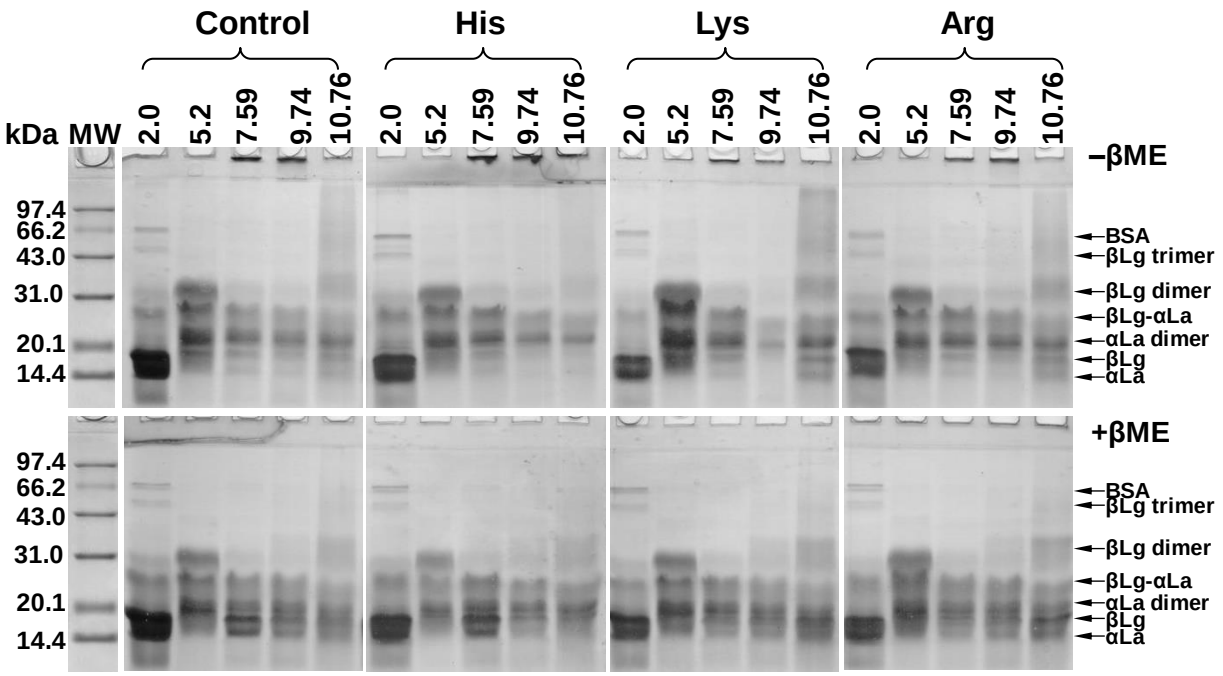

Fig. 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins leached out of whey protein isolate gels at different pHs in the absence and presence of 0.5% (w/v) histidine (His), lysine (Lys), or arginine (Arg). The gels were run under reducing (+βME) and non-reducing (-βME)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

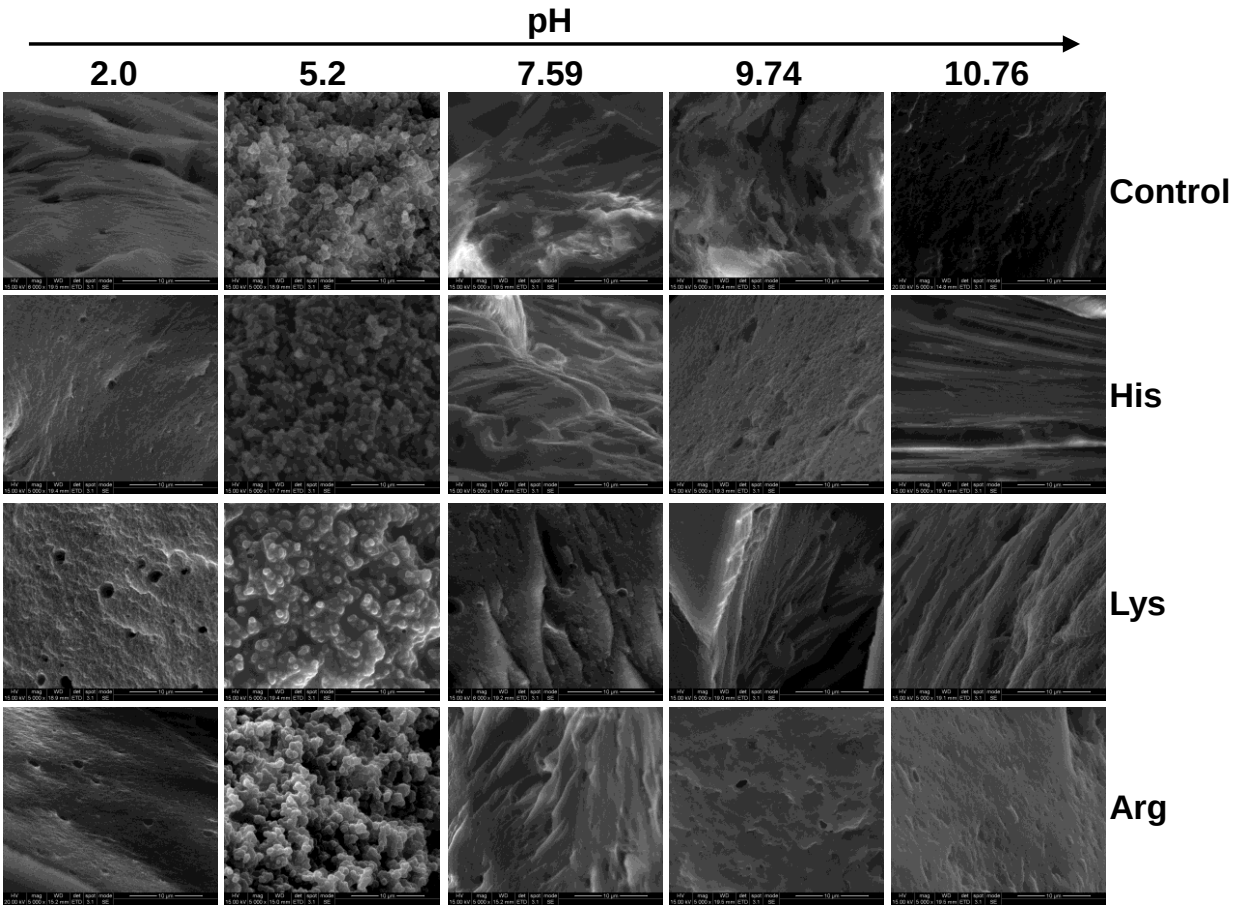

Fig. 7. Scanning electron microscopy image of the cross-section of whey protein isolate gels at different pHs in the absence and presence of 0.5% (w/v) histidine (His), lysine (Lys), or arginine (Arg).