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Effect of pH on Structural Properties of Heat-Induced Whey Protein Gels

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ABSTRACT: Formation and structure of whey protein heat-induced gels (100 mg mL^{-1}) through heat treatment at 80 °C and pH modifications at three pH values of acidic (2), isoelectric (5.6) and neutral (7) were studied. The obtained results indicated that the nature of the primary gel networks was different at each pH value. The heat-induced gels produced at pH of 2 and 7, had acceptable overall shape and consistency which confirmed the significant effect of pH on the regular gel structures. Conversely, the amorphous gel structure was observed at isoelectric pH. According to Atomic Force Microscopy images, the structural unfolding of the protein during denaturation and formation of the fibrillar structures was observed in gel at pH of 2 and stranded aggregates at pH of 7. In terms of textural analysis, in samples at pH of 2 and 7, the required force to fracture gel structure was approximately equal and less than that of the sample at pH of 5.6 which goes back to the amorphous large protein aggregates in gel network at isoelectric pH. The ordered, regular and stable gel structures and high ionic balance were reflected in color parameters and lower amounts of moisture content in the gel matrix of samples at pH of 2 and 7 ($P<0.05$). The well-organized structure and stable gels network along with their desirable functional characteristics, might reinforce their application as food ingredients in terms of improving the qualitative and textural qualities of food products. This property can facilitate the use of these protein gels in novel food systems.

Keywords: *Atomic Force Microscopy (AFM), Heat-Induced Whey Protein Isolate Gels, pH Modifications, Structural Properties, Textural Analysis.*

Introduction

Whey protein isolate (WPI) is highly unique in terms of nutritional, physicochemical and functional properties in food industry like dairy, meat and bakery products (Jovanović *et al*., 2005; Wijayanti *et al*., 2014). The increase in use of WPI may partially be attributed to their ability to form heat-induced gels capable of immobilizing large quantities of water that govern their utility as food ingredients (Jiménez *et al*., 2012; Ryan *et al*., 2013). The characteristic of gel formation by whey protein is important in creating consistency and increasing the concentration of certain foods, such as some beverages, desserts, soups and sauces (Jiménez *et al*., 2012). Under appropriate thermal conditions, whey

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proteins make irreversible gels by rebuilding proteins in a three-dimensional network. The gelling process involves the accumulation of proteins that results in the formation of gels (Lorenzen & Schrader, 2006).

Heat-induced whey protein gelation occurs during heating above 65 °C in protein concentration above 7% (Clark *et al*., 2001; Jovanović *et al*., 2005). The formation of the gel is not also reversible by temperature and pH. Among whey proteins, β-lactoglobulin (β- lg) plays the utmost importance role in gel formation, but other proteins are also involved in gel formation (Durand *et al*., 2002). In this way, the properties of heat-induced whey protein gels are affected by numbers of interrelated factors: protein concentration, gelation temperature, heating time, pH, ionic strength, divalent ions, mechanical stirring and shear force (Boye *et al*., 2000). The higher the percentage of protein, the better the gel is formed. Low amounts of calcium and sodium chlorides lead to a stronger gel (Havea *et al*., 2009).

The gelation consists of two steps. First, a solution of native globular proteins is heated at proper pH, low ionic strength and appropriate protein concentration. Unfolding of the native proteins is followed by aggregation into disulfide cross-linked aggregates. Therefore, the proteins have a net surface charge and repulsive forces will prevent random aggregation, resulting in the formation of soluble aggregates (Boye *et al*., 2000; Jovanović *et al*., 2005). After cooling, a stable dispersion of aggregates is obtained. Usually, acid-induced gels are stronger than salt-induced gels, for the same protein concentration (Boye *et al*., 2000; Jovanović *et al*., 2005).

Generally globular proteins gel networks can be categorized into transparent gels with fine stranded structure, non-transparent coarse networks and intermediate structures (Durand *et al*., 2002; Loveday *et al*., 2009). It should be noted that the selection of a protein concentrate in food formulation depends on the desired characteristics in the final product. In a food product, a protein concentrate characterized by the formation of strong or weak gel, may be desired to be achieved in final consumer.

Heat denaturation, gel formation and resulting gel structures of globular proteins such as whey protein, *α*-Lactalbumin, β-lactoglobulin and soy bean have been studied extensively and reported in the literatures (Clark, *et al*., 2001; Kinekawa & Kitabatake, 1995; Ryan *et al*., 2013; Verheul *et al*., 1998). Yet at pH values at three different ranges which are more common for food products, there are few literature which evaluated formation and structure of whey proteins heat-induced gels through pH modifications at three pH values. The objective of this study was to investigate the whey proteins gelation conditions. The protein network structure formed was also evaluated.

Materials and Methods

- *Materials*

WPI was purchased from Gallo Global Nutrition Inc., USA, and contained 92% protein, 3 % lactose, 2.5 % moisture, 1 % fat and 3.5 % ash. All chemicals used were of analytical grade and obtained from Sigma-Aldrich Co., USA.

- *Methods*

- *Preparation of whey protein gel*

Preparation of whey protein isolate gels was carried out according to Havea *et al*. (2009) with some modifications. Whey protein isolate (WPI) dispersions (100 mg mL^{-1}) were prepared by suspending an appropriate amount of WPI powder in double-distilled water. The pH values of the dispersions were adjusted at three individual pH values of acidic (2), isoelectric (5.6) and neutral (7), separately using HCl (37%, 1N) and NaOH (1N). Each of the dispersions was initially heated at 80 °C using a temperature-controlled heater for 2 hours at the stirring

speed of 2000 rpm, followed by heating at 80 °C for 2 hours without stirring. The termination of gelation was carried out by rapid cooling in an ice bath down to room temperature, followed by refrigerating at 4 °C before the following experiments.

- *Atomic force microscopy (AFM)*

Morphology and microscopic structure of whey protein isolate gel were investigated using atomic force microscopy (AFM) equipped with a platinum coated scanning probe (Dualscope (tm) /Rasterscope (C26)/ DME, Denmark). The cantilever tip had a diameter of less than 10 nm and a spring constant of 42 N/m. The needle at the free end of a cantilever had a length between about 100 µm and 450 µm. The scanning was conducted at the non-contact force constant DC mode. Aliquot (2 µL) of each previously diluted gel dispersions at a concentration of 10 ppm, was spread onto a freshly cleaved mica disk. After 5 min, the mica was rinsed with filtered milli-Q water. Prior to imaging the samples were air-dried for 2 h at ambient temperature. The resulting 1024 pixel images in three scan sizes of 3×3, 5×5 and 10×10 µm were acquired with 0.5 Hz scan rate and were investigated using DME-SPM software, version 2.1.1.2. (Jagtap & Ambre, 2006).

- *Texture analysis*

The evaluation of WPI gel texture was performed according to the previously described method (Lorenzen & Schrader, 2006) with some modifications, using an Instron Universal Testing Machine (Hounsfield, UK) equipped with 50 N load cell. Each of the gel samples prepared in containers with a diameter of 25.24 mm and a height of 50 mm, were initially placed at ambient temperature for 2 hours before the experiment. Gels were penetrated using a 34 mm diameter aluminum cylinder probe at a constant speed of 50.00 mm / min. The maximum force in Newton was obtained from force-deformation curves.

- *Moisture content*

Moisture content of whey protein gels was determined at 105°C according to the moisture measurement assay (AOAC, 2000). Gel samples were first placed at room temperature for 2 hours and then they were individually tested in at least three replicates.

- *Colorimetric parameters*

The reflectance of surface color for all gel samples analyzed using the Minolta Chroma meter (CHROMA METER CR-400, Konica Minolta, SENSING INC., Japan) based on the standard CIELAB color system: L* (the value on the white/black axis), a* (the value on the red/green axis) and b* (the value on the yellow/ blue axis). Colorimeter was calibrated by standard white and black calibration plates, according to the manufacturer's procedure. The opacity or the quality of lacking transparency which specifies the opacity/transparency was determined. Hue and chroma were individually defined according to equations 1 and 2, respectively (Jones *et al*., 2010).

$$\text{Metric Chroma } (C*) = \sqrt{(a*)^2 + (b*)^2} \quad (1)$$

$$\text{Metric Hue} - \text{Angle } (h) = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (2)$$

- *Statistical analysis*

The experiments were performed at least in triplicate via a completely randomized design. Analysis of the results (which were subjected to ANOVA one-way analysis of variance) was carried out using SPSS statistical software version 22 at probability value of 5% ($p<0.05$). The results were expressed as means ± standard error and the mean significant difference was assessed using Duncan's multiple range tests.

Results and Discussion

- *Overall characterization of whey protein gels*

Apparent comparison of the obtained WPI heat-induced gels in three pH values is shown in Figure 1. The protein gels produced in the acidic range (pH of 2) exhibited a consistent, clear and uniform appearance. The same apparent structure was found for protein gel at pH of 7, except that the gel was more opaque. However, the gel produced at isoelectric range (pH of 5.6) had a different appearance in such a way that there was less coherence and uniformity and more opacity than the other two gel samples.

Protein solutions ability to form gels is related to a suitable balance of repulsive and attractive forces between protein molecules. Both covalent (e.g., disulfide bonds) and non-covalent (e.g., electrostatic interactions, hydrophobic and hydrogen bonding) forces are responsible for protein gelation. Factors including pH, temperature and ionic strength can increase protein-protein interactions and consequently favorable gelation. Appropriate unfolding can improve sufficient protein-protein interactions, including disulfide bond formation to gelation (Monahan *et al*., 1995).

Generally, gel formation in whey protein due to heat treatment applying consists of four stages. The first step involves unfolding the intact structure of the native protein. At this stage, due to heat treatment and denaturation, some changes in protein third structure lead to unfolding of the initial interconnected structure. The second stage involves the placement of unfolded structures behind each other. The third step involves the formation of the initial aggregates and protein strands and the fourth involves connecting the strands together in the form of a three-dimensional matrix (Clark *et al*., 2001; Havea *et al*., 2009; Wijayanti *et al*., 2014).

During heat treatment process, whey proteins experience irreversible denaturation and heat-induced gel formation occur due to the interactions between the disulfide bridges and hydrophobic bonds (Havea *et al*., 2009). In this way, the spherical parts of whey protein, mainly α-lactalbumin and β-lactoglobulin, would be unfolded and by trapping large amounts of water would form three dimensional gel matrix (Havea *et al*., 2009). Three factors including gel critical protein concentration (Cg), gel critical temperature (Tg) and pH are critical for forming the structure and coherence of whey protein gels (Jovanović *et al*., 2005; Tobitani & Ross-Murphy, 1997).

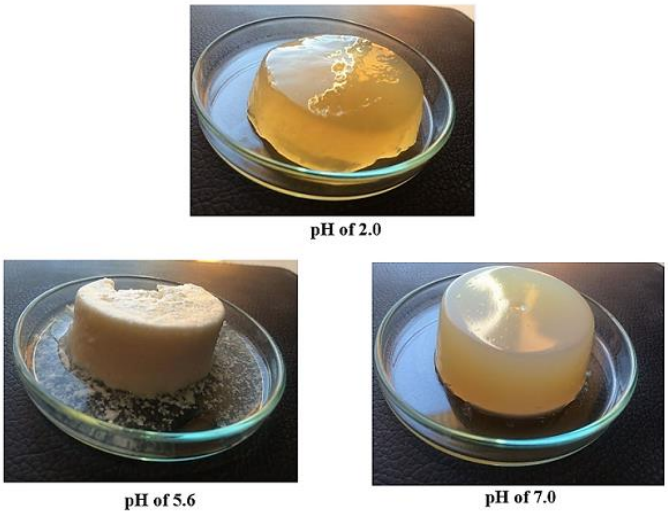

Fig. 1. Apparent comparison of WPI heat-induced gels obtained from the heat treatment process (4 hours at 80 °C) at the concentration of 100 mg mL^{-1}, at three different pH values of acidic (2), Isoelectric (5.6) & Neutral (7).

The concentration of more than 7% protein and the heating above 65 °C lead to form a gel. But in practice, the ideal condition for a perfect gel formation is a concentration of 10 to 15% and the application of a favorable temperature above 75 °C (Boye *et al*., 2000; Jovanović *et al*., 2005; Tobitani & Ross-Murphy, 1997). However, the effect of pH is very important, to the extent that the formation rate of the initial structure of aggregates in gel is independent on the reaction temperature, but depends on the system pH (Boye *et al*., 2000; Jovanović *et al*., 2005; Tobitani & Ross-Murphy, 1997). This indicates that the formation of aggregates at this stage is caused by confrontations and interactions that are influenced by bonding in each region of pH.

During denaturation at pH values above or lower than the isoelectric, the formation of aggregates is in a regular, coherent and, to a large extent, uniform way. But gel formation near the isoelectric point (about 4.6 to 5.6 for whey protein) leads to the formation of irregular, non-transparent gel-like amorphous structures, which are consequents of electrostatic interactions in this range of pH (Ryan *et al*., 2013). As shown in Figure 1, during the formation of whey protein gel under identical conditions (in terms of protein concentration, time, temperature and type of process used), the effect of pH on the apparent structure of the gels is one of the important and determining factors. Although, from a practical point of view, the appearance of the gel does not necessarily reflect the three-dimensional structure and the orientation of the gel and coherence of the aggregates (Schmitt *et al*., 2007), however, the gels produced at acidic (2) and neutral (7) pH values had a better overall shape and consistency. This apparent consistency confirms the significant effect of pH on the appearance of regular structures in the gel.

- *Atomic force microscopy (AFM)*

As shown in Figure 2, the microscopic difference is clearly visible among all three gel samples. In gel sample at pH of 2, protein aggregations are observed in the form of striated and fibrillar structure. These thin fibrillar network structures seemed to be randomly and uniformly distributed throughout the matrix of the gel. This structure of stranded aggregates is not observed in the gel sample at pH of 5.6.

Whey protein heat-induced aggregation at neutral pH includes the formation of granular primary aggregates concurrently followed by the aggregation of these primary aggregates, regardless of the ionic concentration (Ikeda, 2003). Conversely, at acidic pH, heat-induced aggregation leads to the formation of finely stranded aggregates. Secondary structural changes due to heat-induced gelation reflected more clearly transitions in gel network types with an increase in the ionic concentration at neutral pH rather than the shift from two-step aggregation at neutral pH to fine-stranded aggregation at acidic pH (Ikeda, 2003).

Generally, in the pH range of between 2 and 3, fine-stranded structure is formed when the electrostatic repulsion is strong and the protein net charge is positive. This means that gelation occurs mainly through hydrophobic bonds and not through covalent disulfide bridges. High pure net charge leads to the formation of transparent brittle sticky protein gels with regular thin fibrillar structures (Ikeda, 2003; Ikeda & Morris, 2002).

At the isoelectric pH range (4.6 to 5.6), the formation of a regular gel structure is negligible due to low intermolecular bonds and insignificance of pure surface charge and stiff aggregates would be formed (Lorenzen & Schrader, 2006; Tang *et al*., 1994). At pH of 6, there are non-covalent bonds through with disulfide bridges that contribute to the formation of coherent stable elastic gel structure. Non-covalent

bonds lead to larger aggregations, which are the cause of relative non-transparency in the gel. The large aggregates observed at pH of 6 were attributed to secondary, non-covalent interactions of primary, disulfide-linked aggregates (Hoffmann & van Mil, 1999; Lorenzen & Schrader, 2006).

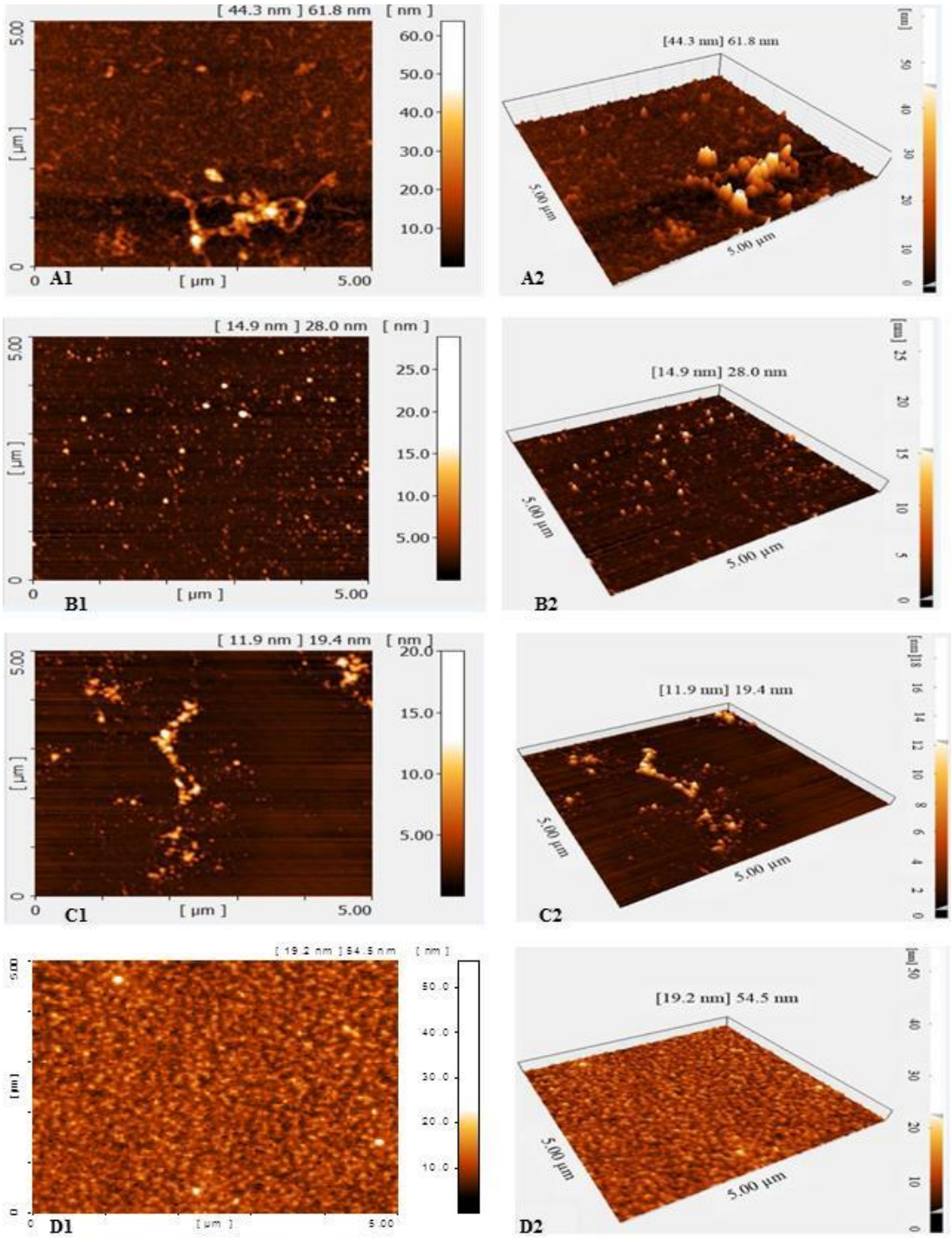

Fig. 2. AFM 2 & 3 Dimensional topographic images of WPI heat-induced gels performed at the protein concentration of 100 mg mL^{-1} (4 hours at 80 °C) related to pH of 2 (A1, A2), pH of 5.6 (B1, B2), pH of 7 (C1, C2) and the native whey protein isolate (D1, D2), respectively. All images prepared at 5µm scale and edited using DME-SPM Version 2.1.1.2.

At all pH values in the range 6 to 8, intermolecular disulphide bonds played an important role in the formation of heat-induced aggregates. At pH range of between 7 and 8, whey protein gel is mainly formed through covalent disulfide bridges. High levels of protein charge prevent large aggregations, therefore, the protein gel has regular thin strings of protein aggregates and a fairly clear appearance (Hoffmann & van Mil, 1999; Lorenzen & Schrader, 2006).

Regarding the mentioned reasons, altering pH values may lead to different gel structures. The gel structure of globular protein is generally consisted of fibrillar, linear and stranded aggregates, as well as larger spherical aggregates. Reducing the pH leads to reduced ionic strength and the formation of different spaces in the protein network (Bolder *et al*., 2006). Clark *et al*. (2001); Durand *et al*. (2002); Kavanagh *et al*. (2000); Langton & Hermansson (1992) and Stading & Hermansson (1991), described particulate gel networks at different pH values which attributed irregular coarse uneven aggregates to isoelectric and regular uniformly distributed gel structures to pH values far from isoelectric. Below pH of 4 and above pH of 6, fine-stranded gel networks are formed with shorter and apparently stiffer linear strands occurring at the lower pH (Langton & Hermansson, 1992; Stading & Hermansson, 1991). At pH of 2, a combination of short and long linear aggregates can be observed in heat-induced β-lg gels (Kavanagh *et al*., 2000). Also in β-lg gels at pH of 2, rigid linear aggregates and flexible linear aggregates are formed at low and high ionic strength, respectively and generally finely stranded gels would be formed (Durand *et al*., 2002). While the structure at pH of 7 is small elongated primary aggregates which are formed at low ionic strength. Due to electrostatic repulsion, the primary aggregates are inhibited from further association at low ionic strength. Accordingly, at pH of 7, the first step of the aggregation leads to small and rather well-defined size particles, but at pH of 2 it leads to large linear aggregates.

In accordance with Figure 2, at pH of 2 and pH of 7, the lower ionic strength, the greater surface charge and the different types of bonding, formed a gel structure consisting of relatively subtle compounds that are joined together. But at pH of 5.6, the insignificance charge and higher ionic strength led to a network without thin fibrillar structure.

Other researchers' findings about the formation of gel-like network with fibrillar structures in WPI and β-lactoglobulin indicate that in a gel network formed at pH of 2, thin fibrillar stranded structures would be formed, but at pH values close to isoelectric and high ionic strength, spherical aggregates are formed in the gel network which do not have the same structure (Durand *et al*., 2002; Ohgren *et al*., 2004; Verheul *et al*., 1998).

- *Texture properties*

The results of texture analysis of each three gel samples are presented in Figure 3. The maximum force required to penetrate the probe into the gel is generalized to the rigidity and stiffness of the gel texture (Comfort & Howell, 2002). The maximum force required to compress and change the gel texture was significantly different for all gel samples, and the gel at pH of 7 and 5.6 had the lowest and highest required force, respectively ($P<0.05$).

The difference in the required force is related to the gel structure and the conditions of gel formation. The gel networks formed far from isoelectric point typically have regular and coherent structures. This causes that the amount of required force to breakdown and penetrate the gel structure would not be too high (Durand *et al*., 2002). Therefore, in the gel samples at pH of 2 and 7, the required force range was

approximately equal and less than that of the sample at pH of 5.6. This high required force at isoelectric range goes back to the amorphous and coagulated structure and also the presence of large protein aggregates in the gel network (Durand *et al*., 2002).

At pH range close to the isoelectric point, due to the lack of surface charge and a significant reduction in electrostatic intra-molecular repulsive force, the previously formed protein structure would create large, stable and dense aggregates together which are not well-organized and coherent (Cavallieri *et al*., 2007). At pH value above or lower than the isoelectric point, the intra-molecular repulsion resulting from maximum surface charge creates stranded fibrillar structures. This causes the gel structure to be elastic and exhibit less resistance to the applied force and the gel texture stiffness would also be reduced to some extent (Cavallieri *et al*., 2007).

The pH of β-lactoglobulin gels has a significant effect on the formation of aggregates and the gel stability (Ryan *et al*., 2013). At pH values above the isoelectric, electrostatic interactions caused by surface charges lead to a regular and elastic gel structure. Nevertheless, since these interactions are reduced within the isoelectric range, higher energy is necessary for protein unfolding, formation of thiol structures in order to create intra-molecular covalent bonds and also breakdown the gel network (Ryan *et al*., 2013). The higher texture stiffness of sample at pH of 5.6 compared to the other two gels, is greatly related to the presence of amorphous and non-fibrillar protein aggregates. In addition, AFM results also confirmed the fibrillar or non-fibrillar structures present in gel samples, which is somehow related to the gels texture. The results of the gel texture analysis were matched with some investigating on β-lg gel samples (Barbut & Drake, 1997; Boye *et al*., 2000; Durand *et al*., 2002).

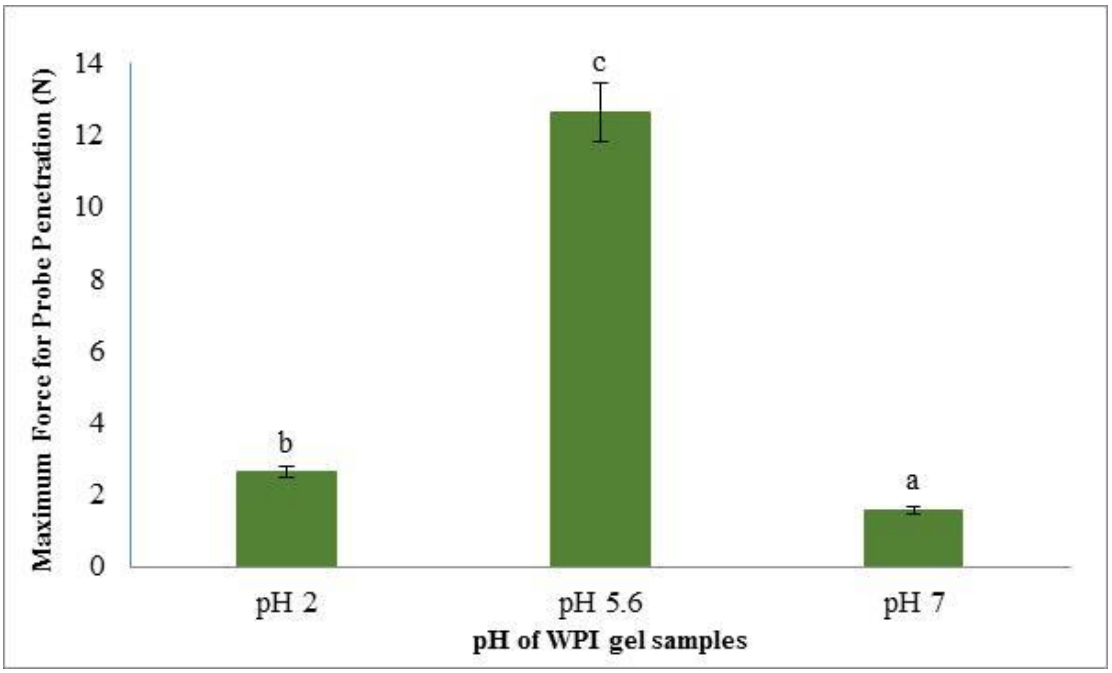

Fig. 3. Texture analysis of WPI heat-induced gels acquired by Instron texture Analyzer. Reported values are the means ± standard deviation of three replicates. Different letters above each column represent significant differences in mean ($P<0.05$).

- *Moisture content*

According to the results in Table 1, the moisture content in the gel at pH of 5.6 and 7, was significantly the highest and lowest, respectively (P<0.05). In gel samples at pH of 2 and 7, the presence of stranded structures in the gel network, as well as forming hydrogen bonds and increased hydrophobicity, respectively lead to more strongly water binding and trap water inside the gel network. Therefore, the gel structure will be soft, flexible and elastic (Morr & Ha 1993).

The higher moisture in the gel sample at pH of 5.6 results from free water in its protein network (Schmitt *et al*., 2007). At the isoelectric pH range, due to the lack of surface charge, the bonding with water molecules is very temporary and superficial, which causes the water to be free-flowing in the gel network and can easily be removed from the gel system (Urbonait *et al*., 2016). In addition, although free water released from the sample at pH of 5.6 would apparently increase its moisture content, yet would not have binding within the gel network matrix. This causes a false increase in moisture content in this sample.

- *Color parameters*

According to Table 1, pH had a significant effect on the color indexes of the whey protein gels (P<0.05). The gel at pH of 5.6 showed the highest L* value and the lowest a* and b* values (P<0.05). Overall, the gel sample at pH of 2 was less white with red and yellowish color, while the gel sample at pH of 7 was slightly whiter with red and yellow color and the gel sample at pH of 5.6 was presented in white with a tendency to green and blue color.

In terms of hue factor, the sample at pH of 2 had the lowest amount (P<0.05). This factor showed that there is significant distinction among all samples color (P<0.05). Chroma measurement showed that the purity and color saturation was the highest in the sample at pH of 5.6 (P<0.05). The opacity also showed that the gel sample at pH of 5.6 had the highest non-transparent appearance compared to the other gels (P<0.05).

Generally, there is no explicit relationship between a* and b* values within physical properties and microscopic structure of whey protein gels (Ikeda & Morris, 2002). However, an increase in the L* value indicates an increase in the amount of large and spherical aggregates in protein (Kinekawa & Kitabatake, 1995). Close to the isoelectric point, due to the absence of significant surface charges and the absence

Table 1. Moisture content (%) and color parameters of WPI heat-induced gel samples

Gel Treatment		pH of 2	pH of 5.6	pH of 7
Moisture Content (%)		91.46 ± 0.26^{b}	93.81 ± 0.29^{c}	89.61 ± 0.17^{a}
Color Parameters	L*	25.98 ± 1.18^{a}	68.61 ± 1.25^{c}	57.95 ± 2.87^{b}
	a*	3.87 ± 0.16^{c}	0.73 ± 0.21^{a}	1.11 ± 0.15^{b}
	b*	6.52 ± 0.42^{c}	2.28 ± 0.25^{a}	5.24 ± 0.34^{b}
	Hue	35.86 ± 1.14^{a}	83.67 ± 1.46^{c}	78.06 ± 1.01^{b}
	Chroma	5.36 ± 0.36^{b}	6.56 ± 0.45^{c}	4.85 ± 0.34^{a}
	Opacity	12.40 ± 0.06^{a}	69.51 ± 0.81^{c}	26.27 0.54^{b}

* Reported values are the means ± standard deviation of three replicates.
* Different letters above each row represent significant differences in mean (P<0.05).

of inter and intra-molecular repulsion forces among proteins, the density of large spherical aggregations increases alongside each other. Larger aggregates reflect more light and thus the gels produced at this pH range have more white appearance (Barbut & Drake, 1997; Cavallieri *et al*., 2007). On the other hand, the steady increase in large massive non-stranded aggregates in the protein causes the color of the gel to be highly saturated and the opacity of the gel also increases (Cavallieri *et al*., 2007; Kinekawa & Kitabatake, 1995). These confirm the color appearance of the gel sample at pH of 5.6. Meanwhile, comparing the results of two gel samples at pH of 2 and 7 indicates that both gels are clear and transparent and light can pass through them to some extent. In explaining the cause of the color appearance and transparency in these samples, in addition to the above mentioned reasons, mainly the presence of hydrogen and hydrophobic bonding and the existence of stranded fibril-like structures are also involved (Ikeda & Morris, 2002).

Some scholars regard the difference in color and transparency among β-lg and whey protein heat-induced gels from differences in pH range and the type of dominant bonds. β-lg dispersions often form transparent gels at neutral pH and increasing pH improves gel transparency (Cavallieri *et al*., 2007). The apparent structure and color of the WPI gels depend on the ionic strength and pH of the initial dispersion and consequently different transparent to opaque gels would be formed (Lakemond *et al*., 2003; Kinekawa & Kitabatake, 1995). This occurs in such a way that the granular state and the lack of texture uniformity, as well as the turbidity and non-transparency of the gel, which are associated with coagulated large protein aggregates and particle size, would be reduce by decreasing pH lower than isoelectric range and also reducing ionic strength (Lakemond *et al*., 2003; Kinekawa & Kitabatake, 1995). The turbidity of heated gels is also related to heat-denatured protein aggregation. At low ionic strength, distant pH from isoelectric and controlled heating conditions, cross-linked soluble linear aggregates of denatured molecules can be formed which leads to a transparent gel (Kinekawa & Kitabatake, 1995).

During gelation at pH lower than the isoelectric point, the equilibrium between the hydrophobic and repulsive electrostatic forces due to heat denaturation, leads to stranded structures and relative transparency in the resulting gels (Ikeda & Morris, 2002). On the other hand, the solubility of whey protein is significantly reduced at isoelectric region. This can be attributed to produce relatively clear or non-turbid solutions or gels at pH values lower or higher than the isoelectric (Ryan *et al*., 2013).

Conclusion

Based on the obtained results, the WPI heat-induced gels produced at neutral and acidic pH range had relative acceptable structural properties. The well-organized structure and stable gels network along with their desirable functional characteristics, might reinforce their application as food ingredients in terms of improving the qualitative and textural qualities of food products. This property can facilitate the use of these protein gels in novel and diverse food systems.

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