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Effects of pH changes on functional properties of native and acetylated wheat gluten

*Majzoobi, M. and Abedi, E.

Department of Food Science and Technology. School of Agriculture, Shiraz University, Shiraz, I.R. Iran. Post code: 7144165186

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Abstract

Wheat gluten is an abundant plant protein with many applications in food and non-food products. However, hydrophobicity of the gluten has limited its applications. Acetylation as a common method for protein modification, can improve gluten hydrophilicity. The functional properties of the native and acetylated gluten are affected by the pH of the environment. The main aim of this study was to determine the effects different pH values of 3, 6 and 9 on the functional characteristics of the native and acetylated gluten. Acetylation of the gluten using acetic anhydride under alkaline condition resulted in 44.58% acetylation. The isoelectric point of the native gluten was 6.2 that reduced to 4.4 after acetylation. Upon acetylation water solubility, water absorption, water holding capacity, foaming and emulsifying properties of the gluten improved, significantly ($p < 0.05$). Increasing the pH from 3 to 6 weakened, while further increase of the pH from 6 to 9 enhanced the functional properties of the native gluten. Nevertheless, increasing the pH from 3 to 9 enhanced the functional properties of the acetylated gluten. In total, native gluten can be used for its functional properties in products of acetic or alkaline pH, while acetylated gluten is more suitable in alkaline products.

Introduction

Wheat gluten is an industrial by-product of wheat starch production. Approximately 80% of the gluten consists of wheat storage proteins and the reset is composed of traces of polysaccharides, lipids and minerals (Belton *et al.*, 1995; Shewry and Halford, 2002; Wieser, 2007). The wheat storage proteins are divided into two proteins classes: gliadins and glutenins. Gliadins are alcohol-soluble, while glutenins are dispersible in dilute acid or alkaline solutions. The molecular weight of gliadins ranges from $30\text{-}74\times10^3$, while the estimated molecular weight of glutenin is over 10^7. Gliadins are mainly monomeric with intra-molecular linkages, while glutenins are polymeric with inter- and intra-molecular linkages. Gluten is considered as one of the most important plant protein (Damodaran, 1996; D'Ovidio and Masci, 2004) and has been many applications in bakery, meat, meat mimic products, drugs and chemical products (Hernández-Muñoz *et al*., 2004; Wieser, 2007). The numerous applications of gluten are mainly because of its varying functional properties particularly water absorption and texturizing effects. Nevertheless, low interaction of the gluten with water has limited its applications. Despite other plant proteins, gluten has weak water solubility, emulsifying and foaming properties. The main reasons for low interactions of the gluten with water are the large molecular weight of the gluten and the lack of ionizable groups. Factors which promote the disaggregation of the gluten or ionization of residues can increase water interaction and consequently improve gluten functional properties. Many studies have been performed in order to improve the functional properties of the gluten. Acid, enzyme (Babiker *et al*., 1996; Drago and Gonzalez, 2001; Kong *et al.*, 2007), chemical modifications (Barber and Warthesen, 1982; Żukowska *et al.*, 2008; Saberi *et al*., 2008; Liao et al., 2010) have been used successfully. A common method for chemical modification of the proteins is acetylation. This method involves the reaction of acetic anhydride with the ε-amino group of lysine, other nucleophilic groups such as phenolic (e.g. tyrosine) and aliphatic hydroxyl groups (e.g. serine and threonine). However, ε-amino group of lysine is the most readily reactive group compared to others (Barber and Warthesen, 1982). It can improve water solubility, emulsification, and foaming of the gluten (Barber and Warthesen, 1982; Żukowska *et al.*, 2008; Saberi *et al.*, 2008). Although the methods for gluten acetylation have been studied in some depth, conditions suitable for the modified gluten to show its functional properties have less discussed in the previous literature. The functional properties are affected by other components present in the environment such as acids, salts and polysaccharides. Therefore, for appropriate application of the gluten

*Corresponding author.
Email: *majzoobi@shirazu.ac.ir*
Tel: +987116138356; Fax: +987112286110

and its derivatives, it is important to understand how other food components may affect their characteristics. Accordingly, this study was carried out to determine the effect of varying pH values of 3 (acetic), 6 (near neutral) and 9 (alkaline) on the functional properties of the native (N) and acetylated (AC) gluten.

Materials and Methods

Materials

Commercial wheat gluten powder was obtained from Fars Glucosin Starch Factory, Marvdasht, Iran. This sample is referred to as N gluten. Chemical composition of the gluten including protein, moisture, fat, ash and carbohydrate content was determined according to the Approved Methods of the AACC (2000). Sunflower oil was purchased from local market. Other chemicals used in this study were obtained from Merck, Darmstadt, Germany.

Gluten acetylation

Acetic anhydride was used to produce acetylated gluten according to the method described by Barber and Warthesen (1982) with slight modification. Slurry of each protein in distilled water (1:4, w/w) was made and the pH was adjusted to 8.5 using 1 N NaOH. Acetic anhydride was gradually added to the slurry (0.5 g increment) and the pH was re-adjusted to 8.5 with 2 N NaOH. Then the solution was left stirring by a magnetic stirrer at room temperature overnight. The mixture was centrifuged at 10000 g for 10 min and the pellet was recovered, washed with 2-propanol until clear supernatant was obtained, then freeze-dried to obtain AC samples. The reaction that occurs during acetylation of proteins (e.g. gluten) is given in Figure 1. Chemical composition of the AC gluten including protein, moisture, fat, ash and carbohydrate content was determined according to the Approved Methods of the AACC (2000).

The effectiveness of the acetylation method used in this study was determined using a Perkin-Elmer FT-IR SPECTRUM 2000 spectrometer. Fourier transform infrared spectroscopy (FTIR) spectra were obtained from KBr pellets (Żukowska *et al.*, 2008). The extent of acetylation was determined using 2, 4, 6-trinitrobenzenesulfonic acid (TNBS) method as described by Fields (1972). Acetylation was expressed as percent reduction in available lysine residue.

Determination of the isoelectric point (IEP)

The pH values in which proteins had the least solubility were selected as the IEP following the method described by Majzoobi *et al.* (2012).

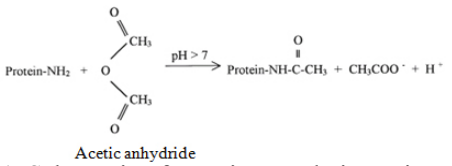

Figure 1. Schematic of protein acetylation using acetic anhydride (Coffman and Garcia, 1977).

Determination of the physicochemical properties of N and AC gluten at different pH values

To determine the effects of pH on the functional properties of the samples, two separate sets of samples were prepared by suspending N and AC samples in sodium acetate buffer (0.1 mol/L), separately to make concentration of 1 mg/mL. These samples were used to obtain pH values of 3 and 6 by addition of 0.5 mol/L HCl. Another set of sample was prepared by suspending N and AC samples in sodium phosphate buffer (0.1 mol/L), separately to make concentration of 1 mg/mL. The pH of these samples was adjusted on 9 by using 0.5 mol/L NaOH. Then following test were performed on the samples.

Water solubility

To determine water solubility of the samples, the protein dispersions were mixed for 1 h using a magnetic stirrer at ambient temperature and then centrifuged at 7000g for 20 min. The protein content (N×5.7) of the supernatant was determined using micro-Kjeldahl method (AACC, 2000). The solubility (%) was determined to using Equation 1 (Beuchat, 1977).

$$\text{Solubility (\%)} = \frac{\text{Protein content in the supernatant}}{\text{Protein content in the suspension}} \times 100 \quad (1)$$

Water absorption

The protein suspensions were stirred using a magnetic stirrer at room temperature for 30 min. Then the samples were left still for 10 min and the weight of separated water was determined. The water absorption was calculated according to the Equation 2 (Beuchat, 1977).

$$\text{Water absorption (g } H_2O\text{/g protein)} = \frac{\text{Weight of the protein suspension} - \text{Weight of the separated water}}{\text{Weight of protein in the suspension}} \quad (2)$$

Emulsifying activity and emulsion stability

Five mL of protein solutions were mixed with 5 mL sunflower oil using a laboratory homogenizer (Digital Ultra-Turrax, IKA, Germany) at 2000 rpm for 5 min and were centrifuged at 1100g for 5 min. The height of the emulsified layer in a glass measuring

cylinder and that of the total contents was measured. The emulsifying activity was calculated according to the Equation 3 (Neto *et al.*, 2001).

$$\text{Emulsifying activity (\%)} = \frac{\text{Height of emulsified layer in the tube}}{\text{Height of total contents in the tube}} \times 100 \quad (3)$$

To determine the emulsion stability the emulsions were heated at 80°C for 30 min. They were then centrifuged at 1100g for 5 min and the emulsion stability was calculated using the Equation 4 (Neto *et al.*, 2001).

$$\text{Emulsion stability (\%)} = \frac{\text{Height of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \times 100 \quad (4)$$

Water holding capacity (WHC)

Suspensions of each sample at varying pH values were centrifuged at 500g for 25 min at room temperature, the supernatant was removed and the weight of the pellet was determined. The WHC was determined using Equation 5 (Childs and Park, 1976).

$$\text{WHC \%} = \frac{\text{Weight of pellet}}{\text{Weight of protein suspension}} \times 100 \quad (5)$$

Foaming volume and foam stability

To produce foam, suspensions of N and AC samples in buffers were placed in glass measuring cylinders and mixed vigorously using a laboratory homogenizer at 20×10^3 rpm for 2 min at ambient temperature. Then the foam volume of each sample was measured in a glass measuring cylinder. The time spent until the foam disappeared completely was determined as foam stability time (Popineau *et al.*, 2001).

Statistical analysis

Experiments were carried out in triplicates by factorial experiment in completely randomized design and the data were analyzed for comparison of significant differences between means by using the Multiple Range Duncan's test ($p < 0.05$). Data analysis was performed by means of the statistical software of SPSS 16.

Results and Discussion

Chemical composition

Determination of the chemical composition of the N gluten showed that this sample contained 86.27 ± 0.06% protein, 6.70 ± 0.05% moisture, 1.68 ± 0.03% fat, 1.07 ± 0.01% ash and 4.28 ± 0.53% total carbohydrate content. The AC gluten contained 82.27 ± 0.37% protein, 5.35 ± 0.03% moisture, 1.42 ± 0.03% fat, 1.82 ± 0.03% ash and 9.14 ± 0.81% total carbohydrate content. The protein, carbohydrate and fat content of the AC gluten was significantly ($p < 0.05$) lower than those of the N gluten. These components may be removed during centrifugation and washing steps of the acetylation process. Similarly, Saberi *et al.* (2008) reported lower protein content for the AC gluten compared to the N gluten. The ash content of the AC gluten was significantly ($p < 0.05$) higher than that of N gluten which is in agreement with Majzoobi *et al.* (2012) for AC glutenin and gliadin.

FTIR results and the extent of acetylation

The FTIR spectra for the N and AC gluten are presented in Figure 2. According to Żukowska *et al.* (2008) a new peak at 1742 cm^{-1} and elongation of the band 1235 cm^{-1} in the spectra are evidences of the success of the acetylation process. In the FTIR of the AC gluten of this study, these changes were also observed (arrows on the graph). Under the condition used the extent of gluten acetylation was 44.58%. Barber and Warthesen (1982) reported that the extent of gluten acylation was 40% that depends on the reaction condition. The extent of gluten acetylation is generally low since gluten contains low amount of reactive amino acids (particularly lysine) for acetylation.

The results of determination of IEP

The results (data not given) showed that the least protein solubility of the N gluten was at pH 6.2. Therefore, this pH value was considered as the IEP of the N gluten. The IEP of the N gluten obtained in this study was close to the values reported by El-Hawwary *et al.* (1989) and Mejri *et al*. (2005). After acetylation, however, the IEP of the gluten decreased from 6.2 to 4.4 as a result of the reaction of basic amino acids with acetyl groups.

Water absorption results

As expected, acetylation increased the water absorption (Figure 3) of the samples. The maximum water absorption of the N gluten was 4.2 (g H_2O/g sample) at pH 9, while the minimum water absorption of this sample was 1.5 (g H_2O/g sample) at pH 6. For AC sample, the maximum was absorption of 8.6 (g H_2O/g sample) was obtained at pH 9, while the minimum value of 6.2 (g H_2O/g sample) was obtained at pH 3. It was noted that at pH 3 and 9, acetylation could almost double the water absorption of the gluten, while at pH 6, it could increase the water absorption up to seven times. The interactions of most

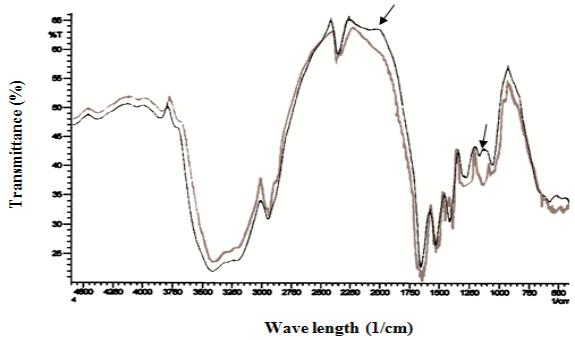

Figure 2. FTIR spectra of control (black line) and acetylated gluten (gray line).

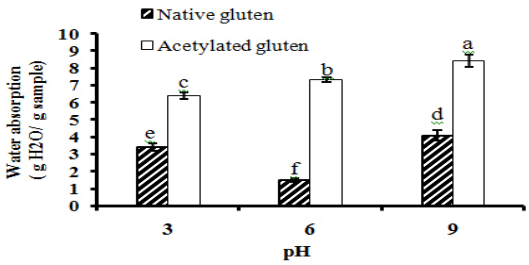

Figure 3. Water absorption of the native and acetylated gluten at varying pH values. Different letters on the bars indicate the statistical difference ($p < 0.05$) between the values.

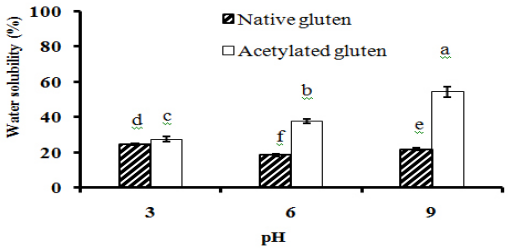

Figure 4. Water solubility of the native and acetylated gluten at varying pH values. Different letters on the bars indicate the statistical difference ($p < 0.05$) between the values.

of the proteins with water are minimized at IEP due to the minimum repulsion among the constituent amino acids. On both sides of the IEP, the water interactions of the proteins increased resulting in the increase of total charge of the ionized protein.

Water solubility results

Addition of hydrophilic acetyl groups on the gluten molecules could improve its water solubility (Figure 4). The water solubility of the gluten is generally low at neutral pH values since the IEP point of the gluten is close to neutral pH. The low water solubility of the gluten is mainly related to the lack of ionizable groups and the increase of molecular weight. Factors which promote the ionization of the gluten or the disaggregation of protein molecules, can enhance the water solubility of the gluten (Bondas and Bicknell, 2003). In this study the lowest water solubility of the N gluten was about 18% at pH 6. Decreasing the pH

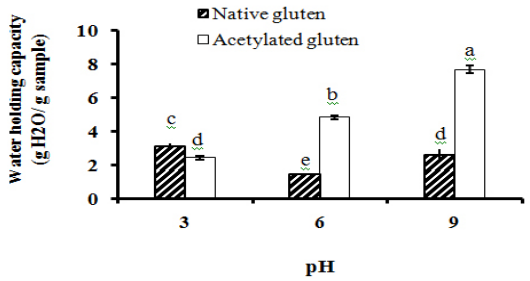

Figure 5. Water holding capacity of the native and acetylated gluten at varying pH values. Different letters on the bars indicate the statistical difference ($p < 0.05$) between the values.

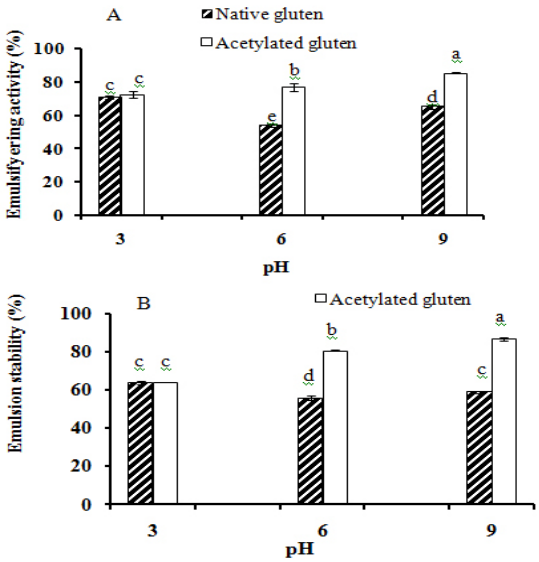

Figure 6. Emulsifying activity (A) and emulsion stability (B) of the native and acetylated gluten at varying pH values. Different letters on the bars indicate the statistical difference ($p < 0.05$) between the values.

from 6 to 3 increased the water solubility to 23.3% that might be related to slight degradation of the gluten in the acetic condition. Furthermore, at above or below the IEP of the gluten, the water solubility increased (e.g. 21.5% at pH 9). The results are in agreement with Mejri *et al.* (2005).

WHC results

The results (Figure 5) showed that the WHC of the gluten was 1.2 (g H_2O/g sample) at pH 6. This parameters increased significantly ($p < 0.05$) to 2.8 and 3.2 (g H_2O/g sample) at pH 9 and 3, respectively. Acetylation of the gluten could improve WHC of the gluten at pH 6 and 9, but at pH 3, WHC of the AC gluten was significantly lower than that of N gluten. The maximum WHC of the AC gluten was 7.8 (g H_2O/g sample) at pH 9 and the lowest value was 2.3 (g H_2O/g sample) at pH 3. pH values of 6 and 3 are close to the IEP of the N and AC gluten, respectively. The neutral global charge of the proteins at the IEP and the increase of association reactions of

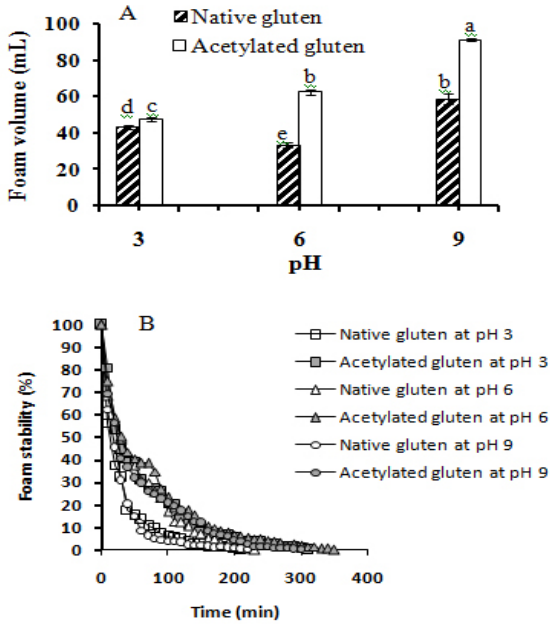

Figure 7. Foam volume (A) and foam stability (B) of the native and acetylated gluten at varying pH values. Different letters on the bars indicate the statistical difference ($p < 0.05$) between the values.

polypeptide chains are the main reason for minimal WHC at this point (Drago and Gonzalez, 2001). As the pH moved from the IEP, the global charge of the proteins increased resulting in higher WHC.

Emulsifying activity and stability results

Figure 6-A showed that the emulsifying activity of the N gluten was 53% at pH 6 and it increased to 65 and 70% at pH values of 3 and 9, respectively. Acetylation of the gluten improved the emulsion activity of the gluten at pH 6 and 9, while at pH 3, there was no significant different between the emulsifying activities of the N and AC samples. Similar trend was observed for the emulsion stability of the samples (Figure 6-B). The least emulsion stability was 68% at pH 6 for the N gluten, while the maximum value of about 63% was obtained at pH 3 and 9. Upon acetylation the emulsion stability increased to about 90% at pH 9. The minimum emulsion stability obtained at pH 3 which was equal to the maximum stability obtained for the N gluten.

Foaming properties and foam stability results

Figure 7-A showed that the AC gluten exhibited improved foaming properties in terms of foam volume and stability compared to the N gluten. Increase in the pH from 3 to 9, enhanced the foam volume of the AC sample from 46 to 92%. For N gluten, minimum value of about 33 mL was obtained at pH 6, which is close to the IEP of N gluten. For this sample maximum foam volume of about 60 mL was determined at pH 9. The Foam stability time of the AC gluten was longer than N gluten at every pH values testes (Figure 7-B).

Table 1. Maximum foam stability time (min) of the native and acetylated gluten at varying pH values*.

Samples	pH		
	3	6	9
Native gluten	210 ± 10^{bB}	230 ± 10^{bA}	220 ± 12^{bB}
Acetylated gluten	350 ± 12^{aA}	310 ± 11^{aB}	300 ± 10^{aB}

*Values are the average of triplicates standard deviation. Values subscripted with different small letters in each column and with capital letters in each row are significantly different ($p < 0.05$).

The results are in agreement with Żukowska *et al.* (2008).

The maximum foam stability of the samples at each pH value was extracted from Figure 7 B and presented in Table 1. For both samples, the maximum foam stability was obtained at the pH value close to their IEP. Maximum foam stability time of 230 min at pH 6 and 310 min at pH 3 were obtained for N and AC gluten, respectively. According to Damodaran (1996) and Yalcin *et al.* (2008), the electrostatic repulsion is minimized at pH values close to the IEP, promoting the cohesive interactions between protein molecules via non-covalent forces leading to more stable foam.

Conclusion

Acetylation can improve some functional properties of the gluten particularly those related to interaction of the gluten with water and hence increase the applications of the gluten. pH changes also affected the functional characteristics of the gluten. On both side of the IEP of the N gluten (i.e. pH 6), its functional properties improved. However, the effect of acetylation on the functional properties of the gluten was more pronounced compared to pH changes. Increasing the pH toward alkaline region significantly increased water solubility, WHC, water absorption, foaming and emulsifying properties of the AC sample. These properties were suppressed at acetic condition. In general, to get advantages from the functional properties, products of high pH values are more appropriate for AC gluten, while acetic or alkaline products are more suitable for N gluten.

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