Impact of sodium citrate on structural properties of gluten

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Abstract The effect of sodium citrate on gluten–starch separation and physicochemical properties of gluten was studied. The results showed that the addition of sodium citrate to the dough caused to an improvement in the aggregation of gluten and increased gluten yield in comparison to the control by augmentation pH and approaching to the isoelectric point of glutenin and gliadin. Also, sodium citrate led a shift to larger particle size distribution of glutenin macropolymer (GMP). These observations demonstrated that under the influence of sodium citrate more thiol groups were oxidized and formed disulfide bonds, which increased the storage modulus and resistance to extension. Furthermore, in this sample the GMP gel was more elastic and stiffer.

Keywords Gluten yield · GMP gel · Isoelectric point · Separation · Sodium citrate

Introduction

Wheat flour is a multicomponent system mainly containing carbohydrates and proteins which are responsible for creating the appropriate sensory and physicochemical properties of final product (Kohler 2003). Wheat flour protein includes water insoluble (gluten) and water-soluble substances (Amiri et al. 2016, 2017). Gluten can be defined as

an elastic network that remains after dough washing. The remaining part of starch granules contains about 80% of protein and 10% of lipid. Gluten plays an important role in quality of bakery products and involves in the rheological properties of dough. According to solubility in alcohol, gluten contain two components; the soluble gliadin and the insoluble glutenin. Glutenin proteins were classified into two subunits: high molecular weight (HMW) glutenin subunits and low molecular weight (LMW) glutenin subunits and gliadins can be divided into four main groups (α , β , γ , δ) on the basis of mobility at low pH in gel electrophoresis (Romano et al. 2007; Tseng and Lai 2002). The structural of gluten is formed when mixing through the hydrated wheat flour protein and forming the bonds between gliadin and glutenin (Wieser 2007). Gluten is used in many industries (such as meat and bakery) and separating the wheat into gluten and starch by using the diluted salty solutions is an important process in the industry (Day et al. 2006). Under the influence of sodium chloride and reducing the electrostatic repulsion force, the strong bonds are formed between the protein branches and the process of separation improves, although it causes the wastewater pollution and equipment corrosion (Da-hai et al. 2012; Tuhumury et al. 2014). Our previous studies showed that glucose oxidase, transglutaminase, can increase gluten yield and improve its structural properties. Xylanase also has an appropriate potential to replace the sodium chloride. However, considering the change in the effect of enzyme at different process temperatures and the lack of access to constant enzyme activity in different conditions of the industry of separating gluten from starch, as well as the different effects of various concentrations of glucose oxidase and transglutaminase on the yield and structural properties of gluten, its use in industry is not controlled (Amiri et al. 2016, 2017). Some compounds (potassium

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bromate and azodicarbonamide) could be used to form a strong gluten structure; however, the usage of these compounds is restricted because of their carcinogenic characteristics (Rosell et al. 2002). Other study on the effect of physical factors such as mixing speed and the amount of water used to produce the dough on the gluten separation have been investigated and the desirable amounts have been reported (Broght et al. 2005). The study aims to investigate the effect of sodium citrate on gluten yield and its physical properties.

Experimental section

Commercial wheat flour was obtained at Atlas Company, Isfahan, Iran (Moisture content $12/19 \pm 0/36\%$ and protein $11/1 \pm 0/17\%$). Optimal water adsorption for flour was determined by Farinograph. Sodium citrate was purchased from Merck, Germany and its used doses were 0.5, 1 and 1.5 g/100 g of wheat flour. The pH of dough samples was measured after adding 10 g of each sample to 100 g of distilled water and blending by pH meter probe (Jenoy 3330, USA). At first, we determined the gluten yield and water binding in wet gluten of the samples according to AACC 38-12A method (American Association of Cereal Chemists 2000). The rheological properties of gluten were measured by using a Kieffer extensibility rig fitted to a Texture Analyser as described by Kieffer et al. (1998). Two determined parameters, $R_{\max}$ (maximum resistance to extension) and E at $R_{\max}$ (extensibility) are the indicators for the gluten strength and the deformation of the gluten before its rupture, respectively. Also, the free Sulfhydryl (SHf) content of the gluten samples was measured according to Tuhumury et al. (2014). To get defatted gluten, freeze-dried gluten sample (3 g) was dispersed in 75 ml of petroleum ether, mixed for 20 min and centrifuged by 18,900g for 10 min at 5 °C. All processes were repeated in the same previous conditions and petroleum ether residues were evaporated in a fume hood overnight. Defatted gluten sample (80 mg) was dispersed in 10 ml of

1.5% (w/v) SDS solution and ultracentrifuged with 69,000g for 30 min at 20 °C. The supernatant was discarded and the gel-like layer found on top of the starch pellet was weighed as GMP wet weight. Just then, the protein content of GMP gel was determined by Dumas method after drying at 80 °C for 2 h. Moreover, the size distribution of the dispersed glutenin particles and rheological measurements were conducted according to Amiri et al. (2016).

Results and discussion

The water binding and gluten yield data are tabulated in Table 1. The results indicated that the gluten yield enhanced significantly with augmentation concentration of sodium citrate. The most increase was occurred when the samples were treated by the highest concentration of sodium citrate. However, the moisture content in the separated gluten samples was almost constant. The present observation indicated that under the influence of sodium citrate, more linkages were formed in the gluten network during mixing, resulting in better gluten aggregation. Sodium citrate led to increase the pH and approach to the isoelectric point of gluten components (gliadin: 8.1 and glutenin: 7.1). In this condition, the amount of positive and negative charges in the gluten protein chains were approximately equal and the possibility of formation of covalent and non-covalent interaction increased as a result of reduced repulsive force. In present work, transformation between free sulfhydryl groups was determined to reflect the modification of the gluten structure. From obtained results, sodium citrate changed the protein structure and form more disulfide bond than the control sample. The free sulfhydryl content decreased with increase in sodium citrate concentration which was related to formation of disulfide linkages. It could be assumed that the augmentation of pH and reduction of hydrogen ions cause more oxidation in the thiol groups, which led to the formation of the strong disulfide bonds and changed the three-

Table 1 Effect of sodium citrate (SC) on physicochemical properties of gluten

Sample	Gluten yield	WB in wet gluten (%)	GMP protein (mg/g wet GMP)	$R_{\max}$ (N)	E at $R_{\max}$ (mm)	pH	SHf (mmol/gr)	D[3,2] (μm)
Control	9.51 ± 0.10^c	66.47 ± 0.4^a	10.40 ± 0.01^a	0.36 ± 0.01^c	79.73 ± 0.07^a	5.78 ± 0.03^d	1.86 ± 0.12^a	21.08
SC0.5	9.74 ± 0.21^{bc}	66.43 ± 0.1^a	10.32 ± 0.12^a	0.37 ± 0.005^c	73.67 ± 0.09^b	6.12 ± 0.07^c	1.68 ± 0.2^{ab}	26.11
SC1	10.14 ± 0.2^{ab}	66.5 ± 0.15^a	10.36 ± 0.19^a	0.40 ± 0.01^b	72.34 ± 0.22^c	6.53 ± 0.06^b	1.39 ± 0.18^b	37.67
SC1.5	10.28 ± 0.12^a	66.36 ± 0.2^a	10.49 ± 0.22^a	0.43 ± 0.008^a	72.11 ± 0.18^c	6.82 ± 0.07^a	1.21 ± 0.09^{cb}	77.33

Data are mean $\pm$ S.D. (n = 3)

Letters within a column indicate significantly different values ($p < 0.05$)

Fig. 1 Effect of different concentration of sodium citrate on particle size distribution of GMP dispersion

Fig. 2 Effect of different concentration of sodium citrate on rheological properties of GMP gel

dimensional structure of gluten. As can be seen in Table 1, the different concentration of sodium citrate increased $R_{\max}$ and decreased E at $R_{\max}$, which could explain that sodium citrate led to formation of strong bonds and increased the strength of three-dimensional gluten structure by increasing pH and reducing the repulsion electrostatic force. The impact of sodium citrate on particle size distribution of GMP samples could be seen in Fig. 1. Our observation showed that these treatments had a significant effect on the particle size distribution of the GMP dispersion which led to increase the particle size and the enhancement of particle size distribution was considered as a function of the sodium citrate concentration. The results of previous studies demonstrated that the formation of disulfide bond obtained from ascorbic acid and glucose oxidase treatments, as well as the covalent bond obtained from transglutaminase leads to increase the particle size (Amiri et al. 2016, 2017). Also, Don et al. (2003) had reported that the particle size was decreased by breaking the covalent bond.

The storage module indicates the stiffness of the GMP gel and the delta value represents the ratio of viscous to elastic behavior of the GMP gel (Kaur et al. 2015). It is apparent that the high delta value shows the viscous behavior and the increase in storage module indicates the gel stiffness (Singh et al. 2011). Storage module of GMP gel prepared in presence of sodium citrate was higher than the storage module of control sample and there was a considerable enhancement in the storage module by increasing the sodium citrate concentration (Fig. 2). Also, the reverse trend would be observed in delta pattern and the highest value of delta belonged to the control. The results demonstrated that the GMP gel structure obtained from sodium citrate treatment was more elastic and stiffer than the control. The gel structure was related to the strength and number of protein interaction. It might be due to the formed interaction under influence of sodium citrate in the three-dimensional structure of gluten by changing pH and decreasing the repulsive forces between the protein chains. It was supported by the free sulfhydryl and gluten yield results. In earlier studies, we also demonstrated that more elastic and stiffer structure of GMP gel was obtained in glucose oxidase, transglutaminase and ascorbic acid-treated samples and the formation of covalent bonds was the main reason of this observation.

It seems sodium citrate to be an appropriate substitute for sodium chloride in the industry of separating gluten from wheat flour starch, although more precise studies are needed to discover its exact effect on physicochemical and functional properties of gluten.

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