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The effect of deamidation on the structural, functional, and rheological properties of glutelin prepared from *Akebia trifoliata* var. *australis* seed

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ABSTRACT

The characteristics of glutelin samples from *Akebia trifoliata* var. *australis* seeds (AG) that had been deamidated by malic acid (MDAG) and by citric acid (CDAG) were investigated. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis showed high-molecular-weight subunits that were degraded into smaller fragments, and FTIR indicated a decrease in the number of β-sheet groups and an increase in the amount of β-turns in the deamidated samples. These results could be caused by the cleaving of partial disulfide bonds to form new sulfhydryl groups during deamidation. Citric acid was found to be more effective at deamidation and hydrolysis, resulting in a higher solubility and emulsifying activity for CDAG, and MDAG also exhibited some improvement in terms of surface hydrophobicity and emulsion ability. Rheology showed that the gelation point for deamidated samples was increased, and the gel network was strengthened. The amounts of essential amino acids that were well-preserved and the improved solubility, emulsification, and rheology properties of AG after acid-heating deamidation show that this technique can be useful for treating other plant-based food ingredients in the future.

1. Introduction

Plant proteins have played an increasingly important role as an alternative source of protein to replace the more expensive animal proteins in protein-rich foods in order to meet human dietary needs in developing countries. Thus, the search for new plant protein sources has attracted significant attention (Day, 2013). *Akebia trifoliata* (Thunb.) Koidz. var. *australis* (Diels) Rehd. is a liana commonly known as "Bai Mu Tong" in China (Gao & Wang, 2006). It is a fast-growing plant that bears many seeds (up to 200) and is widely available in Japan, China, and Korea (Liu, Ma, Zheng, Zhang, & Lin, 2007). Many parts of the plant are edible; for example, the fruits and stems are used as folk medicinal herbs for diuretic and antiphlogistic purposes (Kawata, Kameda, & Miyazawa, 2007), the dried young leaves are used as a tea substitute, and the fresh fruits can be directly consumed or processed into juice or fruit vinegar (Jiang et al., 2012). The oil pressed from *A. trifoliata* var. *australis* seeds (AS) is commonly used as edible oil in southern China (Kitaoka et al., 2009). The protein-rich AS meal left after oil extraction is a by-product for livestock, and it could be a potential new source of low-cost plant protein for human consumption.

We previously reported that AS contains 38.83% oil and 17.23% protein, the primary fraction of which is glutelin and accounts for 46.40% of the protein fractions with great nutritional properties (Du et al., 2012). However, the poor solubility of glutelin in aqueous solution because of its high contents of glutamines, asparagine residues, and non-polar amino acid residues restricts its applications in food processing. It is thus necessary to improve the solubility and optimization of its functional properties in aqueous solutions by appropriate modification methods.

Deamidation is a modification method for improving the solubility and other functional properties of proteins by getting rid of amide groups, originally on uncharged amino acids, to form acidic residues (Zhao, Tian, & Chen, 2011). This process can thereby dissociate protein polymers, increasing the electrostatic repulsion among protein molecular chains and the surface hydrophobicity and flexibility of the glutelin molecule (Matsudomi, Kaneko, Kato, & Kobayashi, 1981; Qiu, Sun, Cui, & Zhao, 2013). A number of studies have focused on the deamidation-induced modification of plant proteins, including wheat gluten (Qiu, Sun, Cui, et al., 2013), rice protein (Liu et al., 2011), oat protein (Mirmoghtadaie, Kadivar, & Shahedi, 2009) and barley glutelin (Zhao et al., 2011), to improve their functional properties with different reaction mechanisms by using acids, bases, and enzymes. Among the available acid catalysts for protein deamidation, hydrochloric acid is the most commonly used besides sulfuric acid, formic acid, phosphoric acid, chlorosul-

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fonic acid, and trichloroacetic acid (Liao et al., 2010). However, deamidation by hydrochloric acid also produces potentially carcinogenic substances (such as chloropropanol), and causes the uncontrollable hydrolysis of peptide bonds and the isomerization of some amino acids (Liao et al., 2010).

Several studies have demonstrated that the deamidation of gluten by organic acids could enhance the properties of gluten. Liao et al. (2010) found that wheat gluten that was deamidated by citric acid and succinic acid had a higher molecular flexibility and better nutritional characteristics because of structural changes. Qiu, Sun, Cui, et al. (2013) investigated the effect of deamidating with citric acid on the properties of wheat gliadin and the emulsion characteristics of D-gliadin. Their results showed that citric acid deamidation could effectively deamidate gliadin with little hydrolysis and markedly improved the solubility of wheat gliadin and the emulsifying properties of D-gliadin. Most importantly, organic acids could avoid excessive deamidation because of their mild characteristics (Liao et al., 2010; Qiu, Sun, Cui, et al., 2013). It is therefore of great interest to investigate the influence of acid-heating deamidation with edible organic acids on plant proteins, including glutelin from AS, about which very little information is currently available.

Citric acid and malic acid are GRAS (Generally Recognized as Safe)-listed compounds and the most common organic acids used as preservatives, acidulants, or flavoring agents. Both acids play an important role in inhibiting the growth of pathogenic microorganisms that cause food spoilage. Citric acid has a mild refreshing acidity and is commonly used in a variety of food products, with almost 70% of the market share of organic acids. Citric acid is a weak organic acid, and malic acid is a better sour stimulus than citric acid. The US FDA (Food and Drug Administration) has limited the application of citric acid as an ingredient in foods for children and the elderly; therefore, L-malic acid has gradually replaced some citric acid applications in the food industry in recent years.

Malic acid and citric acid, which are two types of edible organic acids with different carboxylic groups, were chosen to compare the structure and the functional and rheological properties of glutelin from AS after acid-heating deamidation.

2. Materials and methods

2.1. Materials

Ripe *A. trifoliata* var. *australis* fruits were kindly provided by Jiujiang Regression Biological Technology Development Co., Ltd. (Jiujiang, China). The seeds were pulled out from the fresh fruits to remove the pulp that was adhering to the seeds, and the fruit was dried to a constant weight at 50 °C for 48 h, before storage at −20 °C. The canola oil used for the emulsification study was purchased from a local supermarket. An unstained standard protein molecule marker ranging from 10 to 170 kDa for the SDS–PAGE, an ammonia kit, 1-anilonaphthalene-8-sulfonic-acid (ANS), and 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were of analytical or chromatographic grade.

2.2. Preparing glutelin

Glutelin that was isolated from AS was prepared according to the method in our previous study (Du et al., 2012) with slight modification. In brief, the AS was de-hulled, ground, and defatted to obtain the defatted flours (DAF). The DAF was then passed through a 100-mesh sieve (0.15 mm) before protein extraction. Glutelin isolation from DAF was performed by the sequential extraction of the albumin, globulin, prolamin, and glutelin fractions with deionized water, 0.5 M NaCl, 70% ethanol, and 0.1 M NaOH under magnetic stirring for 2 h with a liquid:solid ratio of 15:1 at room temperature before centrifugation at 4800*g* for 15 min. To remove the other fractions, each extraction step was repeated twice. The glutelin fraction from the combined supernatants of the first and second extractions was isoelectrically precipitated at pH 4.5 with 1 M HCl and kept at 4 °C for 1 h. The precipitate was recovered by centrifugation at 4800*g* for 15 min and washed with deionized water twice. The pH value of the precipitate was adjusted to 7.0 with 1 M HCl and 1 M NaOH before dialysis with deionized water for 24 h at 4 °C. The sample was then freeze-dried for storage. The nitrogen content of glutelin was determined to be 87.9% by the Kjeldahl method at a nitrogen conversion factor of 6.25.

2.3. Glutelin deamidation

The deamidation of the glutelin samples with malic acid (which is abbreviated as MDAG) and by citric acid (CDAG) under hydrothermal treatment was conducted according to the method described by Liao et al. (2010), Qiu, Sun, Cui, et al. (2013) with some modifications. In brief, 8% (w/v) glutelin was mixed with malic acid and citric acid (0.2 M) to form suspensions. The dispersions were hydrated in a water shaker for 12 h at 65 °C. After that, the acid-heating samples were immediately held in an ice water bath for 1 h to stop the reaction. The samples were then centrifuged at 10,000*g* for 10 min at 4 °C, and the soluble fractions were collected using a syringe according to the method described by Du et al. (2012). The collected soluble fractions were neutralized with 0.5 M NaOH and dialyzed in deionized water at 4 °C for 24 h to remove ammonium, and then freeze-dried. Glutelin without acid treatment was used under the same conditions as the control sample (AG).

2.4. Assessing the degree of deamidation and hydrolysis

The degree of deamidation (DD) was determined according to Kato, Tanaka, Lee, Matsudomi, and Kobayashi (1987). DD was referred to as the ratio of ammonia produced from the deamidated sample to the amount of ammonia released from completely deamidated glutelin. Complete deamidation was reached by dissolving 0.8 g of glutelin in 10 ml of 3 M HCl, followed by heating for 3 h at 121 °C. The ammonia content was determined by using an ammonia kit according to the instruction provided.

The degree of hydrolysis (DH) is referred to as the percentage of the free amino groups cleaved from protein, which was the ratio of α-amino nitrogen to total nitrogen by measuring the free amino groups by using TNBS, according to McKellar (1981). A complete hydrolysis was performed by using 3 M HCl at 121 °C for 24 h to determine the total number of amino groups.

2.5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed in a discontinuous buffering system with a 12% separating gel and a 3% stacking gel according to the Laemmli (1970) method by using a Bio-Rad Mini PROTEAN 3 system (Bio-Rad Laboratories, Hercules, CA, USA). AG, MDAG and CDAG were dissolved in deionized water to 5 mg/ml. The protein samples were mixed with loading buffer (0.125 M Tris–HCl (pH 6.8), 4% (w/v) SDS, 20% (v/v) glycerol, 0.5% 2-mercaptoethanol and 1% bromophenol blue (w/v)) and then denatured in boiling water for 10 min, and centrifuged at 10,000*g* for 3 min. After cooling, 10 μl of supernatant from each sample was loaded into a homogeneous PhastGel. Electrophoresis was performed at a constant current of 15 mA for 30 min followed by 25 mA until the tracking dye reached the bottom of the gel. After electrophoresis, the gel was stained for 1 h with the solution (0.25 g of Coomassie