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Effect of hydrolysis time on the physicochemical and functional properties of corn glutelin by Protamex hydrolysis

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ABSTRACT

The physicochemical and functional properties, such as surface hydrophobicity, disulphide bond content, thermal properties, molecular weight distribution, antioxidant properties, of corn glutelin hydrolysates catalysed by Protamex at different hydrolysis times were evaluated. The hydrolysis influenced the properties of corn glutelin significantly, and not only decreased its molecular weight and disulphide bond content, but also eventually transformed its insoluble native aggregates to soluble aggregates during the hydrolysis process. Corn glutelin hydrolysates were found to have a higher solubility, which was associated with their relatively higher foaming and emulsifying properties compared to the original glutelin. Corn glutelin and its hydrolysates maintained a high thermal stability. In addition, the hydrolysates exhibited excellent antioxidant properties measured through *in vitro* assays, namely DPPH and OH radical scavenging activity, Fe^{2+}-chelating capacity and reducing power; the values were 58.86%, 82.64%, 29.92% and 0.236% at 2.0 mg/mL, respectively.

1. Introduction

Proteins are important nutrients in human and animal diet, whereas the lack of protein rich diet causes malnutrition. There are many people who do not get adequate amounts of protein (Chambal, Bergenståhl, & Dejmek, 2012). Cereals and legumes, which are the cheapest and most abundant protein food, have become a major source of dietary protein in many developing countries (Du et al., 2012). Corn, which is the third most widely cultivated cereal in the world after wheat and rice, supplies approximately 42 million tons of protein per annum (Malumba, Vanderghem, Deroanne, & Béra, 2008). China is the second largest country for production and consumption of corn (http://www.igc.int/en/grainsupdate/sd.aspx?crop=Maize). Corn gluten meal (CGM), a major co-product of corn wet-milling process, contains 62–71% (w/w) protein (Hardwick & Glatz, 1989). Apart from its rich protein content, it has been reported that CGM proteins also have special functionalities, such as antioxidative activity (Zheng, Liu, Wang, Lin, & Li, 2006; Zhou, Sun, & Canning, 2012), angiotensin I converting enzyme-inhibitory activity (Suh, Whang, Kim, Bae, & Noh, 2003) and alcohol metabolism activity (Ma, Zhang, Yu, He, & Zhang, 2012). The major protein fractions of CGM consist of approximately 65% (w/w) of zein and 30% (w/w) of glutelin (Hardwick & Glatz, 1989). Zein contains a high proportion of hydrophobic amino acids, such as glutamine (21.4%), leucine (19.3–21.1%), alanine (8.3–10.5%), proline (9.0–10.5%), and isoleucine (5.7–6.2%) (Pomes, 1971), so it is soluble in alcohol-aqueous solution. The glutelin molecule has excessive intra and intermolecular disulphide bonds and hydrophobic interactions (Paraman, Hettiarachchy, Schaefer, & Beck, 2006), and it is soluble only in dilute acid or alkali solutions. Due to these characteristics, glutelins have limited solubility in aqueous systems under the conditions of pH occurring in most food products, and hence CGM has rarely been applied in food industry.

Studies have been performed for improving CGM protein's solubility and increasing its physiological functional properties. Limited hydrolysis of corn gluten by using commercial proteases not only improved its water solubility, but also the prepared hydrolysates showed powerful angiotensin converting enzyme inhibitory (ACEI) activity (Kim, Whang, Kim, Koh, & Suh, 2004; Suh et al., 2003). Several CGM hydrolysates with improved processing functionality and antioxidative activities also have been reported (Zhang, Luo, & Wang, 2011; Zheng, Liu, & Liu, 2012; Zheng et al., 2006; Zhou et al., 2012). Zheng et al. reported that the aqueous solubility of two kinds of extruded corn gluten meal hydrolysates was increased by using a commercial protease Alcalase and fermentation with *Bacillus natto*, respectively; the hydrolysates also

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exhibited antioxidative activity (Zheng et al., 2006, 2012). The effect of acid and base treatments on the structural, rheological, and antioxidant properties of α-zein was investigated, and the antioxidant activity of treated α-zein was improved (Zhang et al., 2011). Corn protein hydrolysates, prepared by three types of microbial proteases, were separated by sequential ultra-filtration into several fractions, and the fraction of 1–3 kDa showed the highest activity in scavenging peroxyl radicals. Additionally, this fraction inhibited lipid oxidation in ground beef both at 250 and 500 μg/g levels (Zhou et al., 2012).

There are several reports on modifying the functionality of isolated glutelins from barley. Zhao et al. found that the structure and functional properties of a deamidated barley glutelin could be improved (Zhao, Tian, & Chen, 2011). In addition, barley glutelin hydrolysates with antioxidant capacity were obtained by enzymatic hydrolysis (Xia, Bamdad, Gänzle, & Chen, 2012). However, in the available literature, no reports could be found about the use of enzymatic proteolysis for modifying the functional properties of corn glutelin. The aim of the present work was to evaluate the feasibility of improving the functional properties of corn glutelin in an aqueous solution by Protamex hydrolysis, investigate the effects of hydrolysis time on the physicochemical and functional properties (solubility, emulsification, foaming properties and antioxidative activity) of corn glutelin, thus providing useful information regarding its potential commercial applications.

2. Materials and methods

2.1. Materials and chemicals

Corn gluten meal (CGM) was obtained from Longfeng Corn Co. Ltd. (Heilongjiang, China), with a total protein content of 56.42% (w/w). Protamex from *Bacillus subtilis* was purchased from Novo Nordisk (Bagsvaerd, Denmark), and α-amylase was purchased from Aoboxing Biotechnology Co. Ltd. (Beijing, China). 1-Anilinonaphthalene-8-sulphonic acid (ANS) and 5,5′-dithiobis-2-nitrobenzoic acid (DNTB) were purchased from Sigma Chemical Co. Ltd. (California, America). Superdex peptide (10/300) column and gel filtration calibration kit were purchased from GE Life sciences (USA). All other chemicals were of reagent grade unless otherwise stated.

2.2. Extrusion of CGM

The CGM was subjected to extrusion in a double-screw-rod extruder (DS32-α, China), which was described in our previous report (Zheng et al., 2006). The moisture content of CGM was adjusted to 16-18% by adding a certain amount of de-ionised water to CGM, mixed and left to sit for 16 h. The moisturised raw material was introduced into the extruder and extruded at 160–180 °C and 1–1.5 MPa. The obtained extrudate was dried at room temperature and then was ground and sieved to collect the fraction of <280 μm.

2.3. Starch removal of extruded CGM

Starch was removed from the extruded CGM with the method described in our previous report (Zheng et al., 2006). Suspensions of extruded CGM of 10% (w/v) were adjusted to pH 6.5 with 1 M NaOH and then incubated with the α-amylase (30 U/g) at 70 °C for 120 min in a shaking apparatus. The reaction mixtures were filtered to remove hydrolysis products of starch and then washed three times with the same amount of water, and dried to obtain extruded and starch-removed CGM.

2.4. Pigment and zein removal of pretreated CGM

CGM pretreated by extrusion and starch removal was extracted with acetone for 30 min (acetone:CGM = 10:1, w/w) to remove pigment, and the extract was centrifuged at 4000×*g* for 15 min at room temperature. The residues were collected and dispersed with 70% ethanol water solution (ethanol:CGM = 10:1, w/w) at 60 °C for 2 h, followed by centrifugation at 4000×*g* for 15 min. The precipitate was collected.

2.5. Extraction of glutelin

The residues were dispersed with 0.1 M NaOH (residues:NaOH = 1:10, w/v) at 60 °C for 2 h, then centrifuged at 4000×*g* for 15 min at room temperature. The process was repeated twice. The supernatants were adjusted to pH 4.8 using 4 M HCl and centrifuged at 4000×*g* for 15 min. The residues were washed three times with 70% ethanol–water solution and distilled water, respectively. Finally, the residues were freeze-dried and then ground and sieved to collect the fraction of <280 μm.

2.6. Preparation of corn glutelin hydrolysates with Protamex

Corn glutelin extracted from pretreated CGM was mixed with 20 mM phosphate buffer (pH 7.0); then the enzyme was added and the mixture was hydrolysed at a specific temperature and pH in a water bath with constant agitation. The degree of hydrolysis (DH) was determined at a hydrolysis time of 15, 30, 60, 90, 120, and 150 min, respectively, and measured by pH-stat method (Adler-Nissen, 1986). The following parameters were varied in the hydrolysis process: pH at 0.5 intervals from 6.5 to 7.5, temperature at 5 °C intervals from 45 to 55 °C, concentration of the raw material at 1% intervals, from 4% to 7%, and enzyme-substrate (E:S) ratio of 0.54, 0.81 and 1.08 g/100 g protein. After hydrolysis, the reaction was terminated by boiling for 15 min. The glutelin hydrolysates were centrifuged at 10,000×*g* for 15 min, and then the supernatants were freeze-dried.

2.7. Determination of protein concentration and recovery

The soluble protein concentration was determined according to the method of Lowry, Rosebrough, Farr, and Randall (1951) using bovine serum albumin as the standard.

The protein recovery is calculated as follows:

$$\text{Protien recovery } (\%) = (A/B) \times 100\% \quad (1)$$

where *A* is the protein content of supernatant and *B* is the protein content of the raw material.

2.8. Characterisations of corn glutelin and the glutelin hydrolysates

2.8.1. Determination of disulphide bond contents

The disulphide bond contents of corn glutelin and its hydrolysates were determined by using Ellman's reagent (DTNB) according to the method of Beveridge, Toma, and Nakai (1974) with some modifications. The Ellman's reagent was prepared by dissolving 4 mg of DTNB reagent in 1 mL of Tris–glycine buffer (0.086 M Tris, 0.09 M glycine, 4 mM EDTA, and pH 8.0).

Samples (30 mg) were suspended in 10 mL of Tris–glycine buffer that contained 10 M urea. 0.03 mL of 2-mercaptoethanol was mixed with 0.5 mL of the suspension and incubated at room temperature for 1 h. Then 5 mL of 12% (w/v) TCA were added and incubated for 1 h, and the mixture was centrifuged at 5000×*g* for 10 min. The residues were washed twice with 12% TCA, and then they were dissolved in 3 mL of Tris–glycine buffer containing 8 M urea and 0.03 mL of DNTB. The resultant suspension was incubated