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Calcium-induced disaggregation of wheat germ globulin under acid and heat conditions

FengRu Liu, Li Wang, Ren Wang, ZhengXing Chen *

State Key Laboratory of Food Science and Technology, Key Laboratory of Carbohydrate Chemistry and Biotechnology Ministry of Education, and School of Food Science and Technology, Jiangnan University, Wuxi 214122, People's Republic of China

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ABSTRACT

This work evaluated the potential of wheat germ globulin (WGG) disaggregation to improve acid and thermal stability in the presence of salts and determine the mechanism of improved solubility. Effects of $CaCl_2$ on disaggregation of wheat germ globulin solution (0.5%, w/w) heat-treated ranging from 55 °C to 95 °C at pH 2.0 were investigated by turbidity, solubility, H_0 (surface hydrophobicity), ζ-potential and microstructure. Maximum turbidity and minimum solubility occurred at the isoelectric point (pI 5.0) and 95 °C. The addition of Ca^{2+} resulted in a steady decrease of the turbidity and increase of the solubility. Both H_0 and ζ-potential of soluble aggregates increased during disaggregation process. The results suggest that the electrostatic repulsion between the particles is sufficiently strong to overcome the attractive forces (hydrophobic interaction), leading to a degradation of large lumps and a homogeneous distribution of WGG particles. Microstructure analysis revealed that the WGG heat-treated contained rough and irregular aggregates at pH 2.0, while the $CaCl_2$-induced disaggregates showed a smooth and loose dispersion, suggesting a dispersive distribution of WGG and a degradation of large lumps. Separating the formation of aggregates may provide a means to manipulate the protein solution properties.

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1. Introduction

Plant proteins are widely used as food ingredients because of their low price, and their nutritional and functional properties. Solubility and aggregability are determinants of functional properties of proteins, and are very sensitive to denaturing treatments (e.g. acid precipitation and high temperature) and ion addition. The denatured proteins would further be associated into aggregates, or even precipitates, thus causing poor solubility. A decrease in solubility greatly limits the practical application of commercial protein in the food industry (Tang, Wang, Yang, & Li, 2009). In some products such as drinks, it is important to keep the proteins in solution. Lowering the pH to around the pI reduces electrostatic repulsion and may also expose hydrophobic groups of the proteins. These changes can lead to protein–protein interactions to form protein aggregates (Ju & Kilara, 1998). Most foods have pH values around or below 6.0. The addition of a protein to such foods may first result in the formation of aggregates. Besides the pH-induced aggregates, heat, enzymes, or salts could also independently induce protein to form aggregates. In the presence of protein aggregates, the protein solution would demonstrate the functionality of the aggregates, and not that of individual protein. Heating destroyed the ordered structure of protein such as the dissociation of proteins into their constituent subunits, unfolding, and exposure of their hydrophobic groups, which caused aggregation (Shah, Shaikh, Peng, & Rajagopalan, 2011). Furthermore, the aggregation of proteins caused by high pressure treatment has also been widely reported (Liu et al., 2011).

Previous observations can provide a potential solution method to improve the solubility of commercial protein. For instance, esterification of milk proteins increases the net negative charge and raises the isoelectric point of proteins, making them more functional at acidic pHs (Sitohy, Chobert, & Haertlé, 2001). Pectin is used to coat casein micelles in acid dairy drinks and thus preventing casein from aggregation by electrostatic and steric stabilization (Parker, Boulenguer, & Kravtchenko, 1993). Partial removal of Ca^{2+} by EDTA and subsequent dialysis resulted in disaggregation of some of the casein micelles (Griffin, Lyster, & Price, 1988). The denatured protein solution containing heat-induced soluble aggregates, could be gelled by the additions of salts (Barbut & Foegeding, 1993). In addition, disulfide bond-reducing agents, thiols (cysteine, mercaptoethanol) and sodium bisulfite, by breaking intermolecular bonds, cause disaggregation of proteins and enhance solubility (Hettiarachchy & Kalapathy, 1998). Therefore, it is very important for appropriate application of protein in a food to manipulate the formation of protein aggregation and the resultant solubility.

Wheat germ as a by-product of the milling industry is a nutrient-dense material. Wheat germ protein (WGP) has been classified with superiorly effective animal protein, and it is rich in 17 amino acids, especially the essential amino acids, lysine, methionine, and threonine, in which many of the cereal grains are deficient (Ge, Sun, & Cai, 1999). Particularly, defatted wheat germ is a kind of natural high-grade protein

* Corresponding author. Tel./fax: +86 510 85197856.
E-mail address: zxchen_2008@126.com (Z. Chen).

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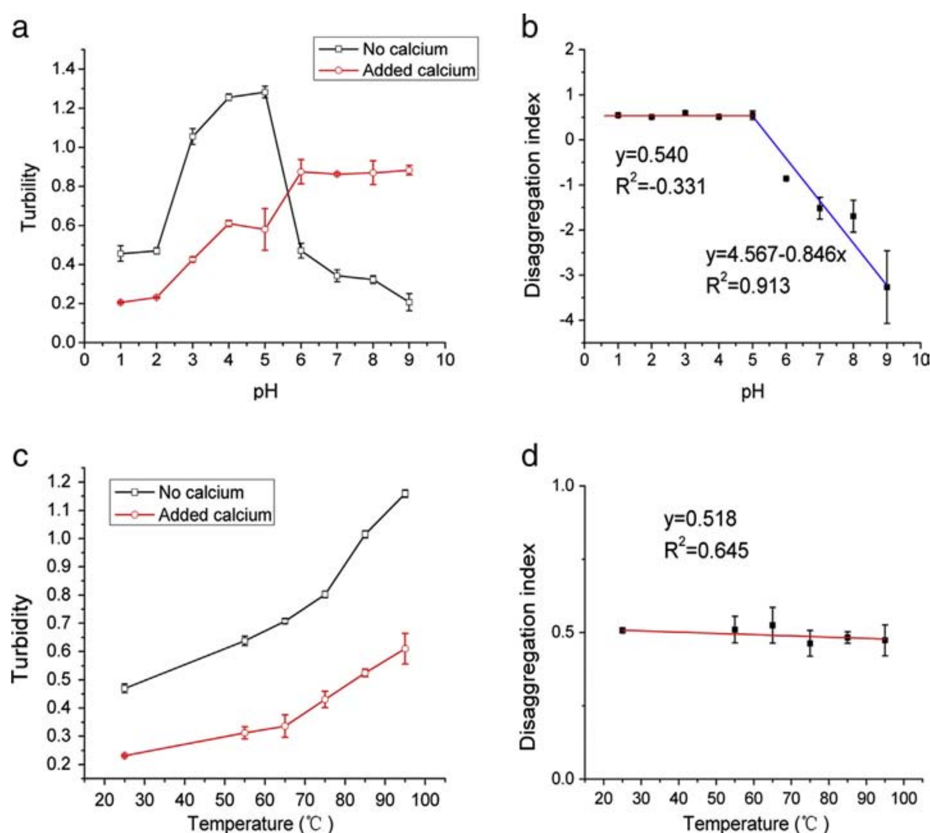

Fig. 1. Turbidity at 400 nm and disaggregation index of 0.5% (w/w) wheat germ globulin dispersions at varied pH values and under heat treatment in the presence of 0 and 50 mM of $CaCl_2$: (a) Turbidity (400 nm) of wheat germ globulin dispersions at pH ranging from 1.0 to 9.0 without (blank) and with $CaCl_2$ (50 mM); (b) the fitting curve of disaggregation index at varied pH values; (c) turbidity (400 nm) of samples heated at 55, 65, 75, 85 and 95 °C for 5 min in the presence of 0 and 50 mM $CaCl_2$ at pH 2.0; and (d) the fitting curve of disaggregation index at varied temperatures. The disaggregation indexes were determined as the reducing rate of turbidity after adding calcium ions. Results are the mean of three determinations. Vertical bars represent standard deviation.

and amino acid fortification substance, which has high protein content (30–32%), is rich in albumin (34.5% of total protein) and globulin (15.6%), and thus presents a well-balanced amino acid profile (Brandolini & Hidalgo, 2012). The use of wheat germ protein in breads (Sidhu, Al-Hooti, & Al-Saqer, 1999), cookies (Arshad, Anjum, & Zahoor, 2007), yogurt (Ayar, Elgün, & Yazici, 2005) and in comminuted meat products (Gnanasambandam & Zayas, 1992) as an extender has been reported. In various food products, different acid and heat treatments were used to treat and to influence the protein subunit composition. In addition, divalent calcium ions have been suggested as a trigger for protein aggregation at elevated temperatures, being involved in intermolecular protein–Ca^{2+}–protein cross-linking, intramolecular electrostatic shielding, or ion-induced protein conformational changes (Simons, Kosters, Visschers, & de Jongh, 2002). The denatured solution of beta-lactoglobulin, containing heat-induced soluble aggregates, could form a microgel by the addition of calcium (Phan-Xuan et al., 2012). However, it is not known whether all these factors including heat, pH, and $CaCl_2$, could independently induce wheat germ protein aggregation and disaggregation. The objective of this research was to investigate the effects of $CaCl_2$ change on the disaggregation of WGP, and on the solubility, surface properties and microstructure properties of protein solution.

2. Materials and methods

2.1. Materials

The wheat germ used in this study was purchased from the Anyang Mantianxue Food Co., Ltd. (Henan, China). The powder contained about 30.6 wt.% protein (Kjeldahl, N × 5.70). 1-Anilino-8-naphthalene-sulfonate (ANS) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All reagents were analytical grade.

2.2. Preparation of wheat germ globulin

The fraction of globulin (salt-soluble) was prepared from wheat germ by following the classical method of Osborne using solubility criteria (Osborne, 1924). The solution used for extraction of WGG was 1% NaCl solution, respectively. Raw wheat germ was defatted with n-hexane for 8 h and air-dried at room temperature. The defatted wheat germ meal was dispersed in deionized water (1:10 w/v) and stirred for 1 h at ambient temperature. The suspension was centrifuged at 9680 *g* for 20 min at 4 °C to remove the albumin-rich suspension. The precipitate was then dispersed in 1% (w/v) NaCl solution and stirred for 2 h at ambient temperature. The globulin-rich suspension was centrifuged at 9680 *g* for 20 min at 4 °C to remove the fiber and other suspended solids and was then adjusted pH to 4.0 with 1.0 mol/L HCl to precipitate the proteins and centrifuged again at 9680 *g* for 20 min at 4 °C. The precipitates were washed several times with distilled water, dispersed in a small amount of distilled water, and adjusted to pH 7.0 with 0.1 mol/L NaOH. The globulin was dialyzed against water using a 100 Da MWCO membrane (Spectra/Por, Spectrum Laboratories, Houston) for 24 h, lyophilized, and analyzed for protein aggregation.

2.3. Turbidity measurements

Aggregation was obtained by adding divalent cations Ca^{2+} to WGG solutions at room temperature (approximately 23 °C). Solution of 1 M $CaCl_2$ was used. Turbidity was measured without Ca^{2+} and after

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