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Effect of oxidation on the emulsifying properties of soy protein isolate

Nannan Chen [a], Mouming Zhao [a,b], Weizheng Sun [a,*], Jiaoyan Ren [a], Chun Cui [a]

[a] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China
[b] State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China

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ABSTRACT

Soy protein isolate (SPI) was oxidized by peroxyl radicals derived from 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) and the structural and emulsifying properties of oxidized SPI were evaluated. Increasing extent of oxidation resulted in gradual carbonyl group generation, free sulfhydryl group degradation and dityrosine formation. Moderate oxidization could generate soluble protein aggregates with more flexible structure while over-oxidization would induce the formation of insoluble aggregates. Compared with the control, emulsions stabilized by moderately oxidized SPI had smaller droplet size and better thermal stability. Results from creaming index and microstructure measurement after 15 days indicated that emulsions stabilized by SPI of over-oxidation underwent severe droplet aggregation during storage while moderate oxidation had a positive effect on the emulsion stability.

1. Introduction

Soy protein is an important food ingredient used in a lot of protein-based food formulations, since it possesses high nutrition and good ability to improve the food quality (Tang, Wang, Yang, & Li, 2009). Soy protein isolate (SPI) is the most refined commercially available soy protein powder, containing 90% protein on a moisture-free basis, and possesses some desirable functionalities (Morr, 1990). One of the most important applications of SPI is being used as an emulsifier in the manufacture of food, such as meat products (Chen, Chen, Ren, & Zhao, 2011).

As an emulsifier, SPI can form and stabilize small droplets. When proteins adsorb to oil–water interface in emulsion, they reduce the interfacial tension and therefore promote droplet generation and form an interfacial layer which stabilizes droplets against flocculation or coalescence via electrostatic repulsion (Walstra, 1993). The emulsifying capability of proteins depends on their molecular structure and physicochemical characteristics (Keerati-u-rai, Miriani, Iametti, Bonomi, & Corredig, 2012). Under quiescent conditions, the most obvious manifestation of instability is creaming (visible separation of bulk dispersed phase), and the rate of creaming is very sensitive to droplet-size distribution. Stability against creaming is usually correlated with coalescence and flocculation of the droplets (Dickinson, 2001). Environment conditions like pH and temperature also affect the emulsion stability (McClements, 2004).

Soy protein, similar to other components such as lipids and pigments, is vulnerable to oxidative attack during processing and storage (Harel & Kanner, 1985). Protein oxidation is the structural modification induced directly by reactive oxygen species or indirectly by reaction with the by-products of lipid peroxidation (Shacter, 2000). Oxidative modification can trigger a number of changes in amino acid residue side-chains and protein polypeptide backbone, resulting in protein fragmentation, cross-linking, unfolding, and conformational changes (Davies, 2005; Stadtman & Berlett, 1998). Radical oxidant will attack almost all kinds of amino acid side chains while aromatic and sulfur-containing amino acid side chains are particularly vulnerable to oxidation (Davies, 2005). Currently, oxidation that happens in animal meat during storage have been extensively investigated. And it turns out that oxidation has both positive and negative influences on the meat quality (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004; Santé-Lhoutellier, Engel, Aubry, & Gatellier, 2008). Some industry processes will also induce protein oxidation. Researchers have found that heat-induced oxidation would reduce SPI's nutritional qualities (Tang, Wu, Le, & Shi, 2012) and protein oxidation accelerated during the first stage production of Parma Ham (Koutina, Jongberg, & Skibsted, 2012).

As SPI is widely used in many lipid-enriched food systems, the effect of lipid on the SPI has aroused scientific attention. Lipid is sensitive to oxidation and will produce lipid peroxidation-derived free radicals as well as lipid hydroperoxides and reactive aldehydes which will interact with protein, leading to protein oxidation (Refsgaard, Tsai, & Stadtman, 2000). Besides, the remaining lipoxygenase in soy protein would catalyze the lipid peroxidation (Wu, Wu, & Hua, 2010). Recently, some researchers had mimicked various kinds of lipid peroxidation-derived by-products and other free radical-generating system such as $FeCl_3/H_2O_2$/ascorbate to trigger protein oxidation and found that oxidation had either positive or negative effects on the gelling properties of soy protein depending on different oxidants (Liu, Xiong, & Butterfield, 2000; Wu, Hua, Lin, & Xiao, 2011). Other investigators have confirmed that lipid peroxidation happened during the storage of emulsion and its by-products may cause

* Corresponding author. Tel./fax: +86 20 22236089.
E-mail address: fewzhsun@scut.edu.cn (W. Sun).

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the protein oxidation (Estévez, Kylli, Puolanne, Kivikari, & Heinonen, 2008; Hu, Mcclements, & Decker, 2003). Despite these prior studies, the relationship between emulsifying properties and the structural characteristics of oxidized SPI is rarely investigated. This relationship is expected to give guidance to better use of SPI in industry process and its storage.

Thermal decomposition of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) can generate peroxyl radicals at a known and constant rate under the stable temperature of 37 °C. The amount of peroxyl radicals from thermal decomposition of AAPH is proportional to its concentration (Gieseg, Duggan, & Gebicki, 2000). Therefore, AAPH-derived peroxyl radicals were selected as a representative by-product of lipid peroxidation in this study to evaluate the effect of oxidation on the emulsifying properties of SPI.

2. Materials and methods

2.1. Samples and materials

Defatted soy flakes were purchased from Yuwang Group (Shangdong, China). 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical reagent grade.

2.2. SPI preparation

Defatted soy flakes powder (200 g) was mixed with 15-fold deionized water, and the mixture (pH 6.7) was adjusted to pH 7.5 with 2.0 M NaOH. After stirring for 2 h, the resulting suspension was centrifuged at 8000 *g* for 20 min at 4 °C to remove the insoluble material. Then the pH of the supernatant was adjusted to pH 4.5 with 2.0 M HCl, and the precipitate was collected by centrifugation at 8000 *g*, for 10 min at 4 °C. The precipitate was then redissolved in 5-fold deionized water and the pH was adjusted to 7.0 with 2.0 M NaOH. The neutral SPI solution was frozen at −18 °C, and then freeze-dried using a freeze drier (Marin Christ, Germany). The freeze-dried samples were then sealed in polyethylene bags and stored at 4 °C and 40% RH until use.

2.3. SPI oxidation

Oxidized SPI was prepared according to the method described by Wu, Zhang, Kong, and Hua (2009). SPI dispersion (40 mg/mL containing 0.5 mg/mL sodium azide, suspended in 10 mM sodium phosphate buffer, pH 7.4) was mixed with a serial concentration of AAPH and then incubated by continuous shaking under air at 37 °C in dark for 24 h. The final concentration of AAPH was 0, 0.05, 0.2, 0.5, 1, 3, and 5 mM. The reaction was stopped by immediately cooling the solution to 4 °C by ice-bathing and then centrifuged at 8000 *g* for 15 min at 4 °C. The supernatant was dialyzed against deionized water at 4 °C for 72 h to remove residual AAPH and salt. The dialysis membrane has a molecular cut off of 14,000 Da (Juyuang Biota Technology Co., Ltd., Shanghai, China). The dialyzed supernatant was frozen at −18 °C, and then freeze-dried using a freeze drier (Marin Christ, Germany). The freeze-dried samples were sealed in polyethylene bags and stored at 4 °C and 40% RH until use.

2.4. Emulsion preparation and heat treatment

The SPI dispersion (20 mg/mL) was prepared in sodium phosphate buffer (10 mM, pH 7.0). Sodium azide was added to the dispersion as an antimicrobial agent with a final concentration of 0.2 mg/mL. Oil-in-water emulsions containing 20% (v/v) corn oil was then prepared at ambient temperature using an APV-1000 homogenizer (APV Gaulin, Abvertslund, Denmark) operating at 30 MPa. Heat treatment involved incubating the emulsion in a water bath set to the desired temperature ranging from 30 °C to 90 °C for 20 min. Heat treatment at 120 °C was placing the emulsion in an autoclave (Zhejiang, China) for 20 min. This approach is widely used when studying the thermal stability of emulsion because it mimics the thermal history that such emulsions undergo during processing.

2.5. Protein carbonyl group measurement

Protein carbonyl groups in native and oxidized SPI were quantified according to the method described by Huang, Hua, and Qiu (2006) using SP-721 UV spectrophotometer (Shanghai, China). SPI samples were suspended in deionized water with a concentration of 5 mg/mL, stirring for 30 min at room temperature (25 ± 2 °C). In 15-ml capped polyethylene centrifuge tubes, 1 mL SPI dispersion was mixed with 3 mL 10 mM 2,4-dinitrophenylhydrazine(DNPH) dissolved in 2 M HCl and incubated at room temperature for 2 h. A matching aliquot was mixed in 3 mL 2 M HCl as an absorbance blank. Then 4 mL 20% trichloroacetic acid was added to each tube and blended. The mixture was centrifuged at 10,000 *g* for 10 min at 4 °C, after standing for 20 min. The supernatant was discarded, and the pellet was washed three times with 5 mL ethanol/ethyl acetate solution (1:1, v/v). The protein, free of DNPH, was then dissolved in 3 mL 6 M guanidine hydrochloride in 0.1 M sodium phosphate buffer (pH 7.0). The absorbance at 367 nm was corrected by the absorbance in the HCl blank. Soluble protein concentration in guanidine hydrochloride solution was evaluated by the Biuret method (Chang, 2010) with bovine serum albumin (BSA) (Dingguo Biota Technology Co., Ltd., Beijing, China) as the standard. The results were expressed as nmole of carbonyl groups per milligram of soluble protein with molar extinction coefficient of 22,000 M^{-1} cm^{-1}.

2.6. Free sulfhydryl group measurement

Contents of free sulfhydryl (free SH) groups in native and oxidized SPI were determined using Beveridge's procedure (Beveridge, Toma, & Nakai, 1974). SPI samples were dissolved with 8 M urea in Trs-Gly buffer (pH 8.0) with a concentration of 5 mg/mL and the mixture was stirred at room temperature for 30 min. The suspension was then centrifuged under 10,000 *g* for 15 min at 4 °C and soluble protein concentration in the supernatant was evaluated by the Biuret method (Chang, 2010) with bovine serum albumin(BSA) (Dingguo Biota Technology Co., Ltd., Beijing, China) as the standard. Then supernatant (3 mL) reacted with 0.02 ml DTNB reagent (4 mg/mL) dissolved in Tris-Gly buffer. After standing at room temperature for 1 h, the absorbance was measured at 412 nm using SP-721 UV spectrophotometer (Shanghai, China). The results were expressed as nmole of SH per milligram of soluble protein with molar extinction coefficient of 13,600 M^{-1} cm^{-1}.

2.7. Dityrosine measurement

Dityrosine formation of native and oxidize SPI was estimated using the method of Morzel, Gatellier, Sayd, Renerre, and Laville (2006) with slight modifications. SPI dispersion (2 mg/mL) was prepared in 10 mM sodium phosphate buffer (pH 7.0). The mixture was then centrifuged at 10,000 *g* for 10 min at 4 °C. Fluorescent intensity of the supernatant was estimated at 420 nm using F7000 fluorescence spectrophotometer (Hitachi Co., Japan), with an excitation wavelength of 325 nm and constant slit of 5 nm for both excitation and emission. Soluble protein concentration in the supernatant was evaluated by the Biuret method (Chang, 2010) with bovine serum albumin (BSA) (Dingguo Biota Technology Co., Ltd., Beijing, China) as the standard. Corrected fluorescence was obtained by dividing measured fluorescence by protein concentration (mg/mL). The results were expressed in arbitrary units (A.U.).