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Effect of ultrasound on structure and functional properties of laccase-catalyzed α-lactalbumin

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ABSTRACT

The effects of ultrasound (400 W, for 0, 20, 40, 60, 80, or 100 min) pre-treatment on the physicochemical properties, functionality and structure of laccase-catalyzed α-LA in the presence of ferulic acid (FA), were investigated in this study. Size exclusion chromatography showed that α-LA monomers decreased significantly in the presence of laccase and FA, and its high molecule polymers were formed with increasing ultrasonic time. SDS-PAGE analysis also showed that ultrasound treatment contributed to the formation of oligomers and polymers of α-LA treated with laccase and FA. Surface hydrophobility and gel strength of laccase-catalyzed α-LA-FA conjugates were higher than that of three control systems, and gradually enhanced with the ultrasonic time increased. Moreover, ultrasound-treated α-LA with laccase and FA had greater G′ values than other three control systems according to the rheological measurements. Ultrasound treatment did not significantly changed conformational structure of laccase-catalyzed α-LA-FA conjugates, as demonstrated by CD spectra. Therefore, ultrasound contributed to form greater non-covalent and covalent interactions between α-LA molecules and aggregates, and could remarkably change physicochemical and functional properties of laccase-catalyzed α-LA-FA conjugates.

1. Introduction

Lactalbumin (α-LA) is one of major constituents of whey proteins and widely utilized in the food industry (Outinen and Rantamäki, 2008). It plays crucial effects on the physiological and functional properties of whey proteins (Jakopović et al., 2016; Thalmann and Lötzbeyer, 2002). Functional properties of whey protein can be altered by various treatments, such as chemical modifications that include the succinylation, acetylation, phosphorylation and thiolation (Gianfreda et al., 1999; Lawal and Adebowale, 2004), physical modifications that included the heat treatment (Fitzsimons et al., 2007; Lam and Nickerson, 2015; Wolz and Kulozik, 2015) and enzymatic modifications.

An enzymatic modification has higher specificity of reactions with typical mild reaction conditions, and can induce modest changes in protein molecule conformation. Enzymatic cross-linking potentially belongs to the innovative ways during the processing of food proteins to optimize functional properties in food products. A lot of works have focused on various improvements of food protein functionality induced by transglutaminase (TG), like enhancements of thermal stability and solubility, and improvements in gel properties (Chanarat and Benjakul, 2013; Hu et al., 2015). For milk proteins, casein is a good substrate of TG. However, TG is less efficient to native α-LA and β-lactoglobulin (β-LG), due to their rigid globular structure. Thus, reducing reagents such as DTT are needed during the cross-linking of TG (Bönisch et al., 2007). Therefore, it is of great interest to investigate other possibilities for cross-linking of native whey proteins.

Laccase (E.C.1.10.3.2) can catalyze the oxidation cross-linking of various phenolic compounds with broad specificity. Laccase is an another group of oxidoreductase with potential for α-LA cross-linking. Laccase can utilize atmospheric oxygen as the electron acceptor during its oxidation reaction (Ma et al., 2011). In contrast to tyrosinase, steric effects play a less important role in biotransformation catalyzed by laccase than redox potential differences

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(Thalmann and Lötzbeyer, 2002). It was investigated that laccase enhanced the mixing properties of wheat flour dough (Labat et al., 2000). It was shown that β-LG-sugar beet pectin conjugates catalyzed by laccase had higher solubility over a wide pH range than β-LG (Jung and Wicker, 2012). However, in laccase-catalyzed reactions, small phenolic compounds facilitate laccase catalysis of proteins as electron transfer mediator, acting as bridging agents in heteropolymer structures (Selinheimo et al., 2008). Phenolic substances with low molecular weights such as ferulic acid (FA), caffeic acid and chlorogenic acid, could enhance laccase to mediate the polymerization of casein and WPI (Gauche et al., 2008; Mattinen et al., 2006). For instance, vanillic acid enhances cross-linking of WPI catalyzed by laccase and its conjugates show improved emulsion stability (Chen et al., 2012).

Ultrasound has extensively been used in food industry (Færgemand et al., 1998; Selinheimo et al., 2008). The effect of ultrasound is closely related with the cavitation, shear stress, turbulence and dynamic agitation. Recent studies showed that high intensity ultrasound could be applied to change physicochemical properties of soy protein (Lantto et al., 2005; Yen and Hsieh, 1995). High intensity ultrasound (HIU) can also lead to the improvement in the solubility of soy protein isolate dispersions (Figueroa-Espinoza et al., 1998), and enhancement in the free sulfhydryl group and surface hydrophobicity (Gauche et al., 2008). Moreover, ultrasound has increased the gelation property of the heat-treated SPI gels induced by calcium sulphate (Gauche et al., 2008). Similarly, ultrasonic treatments also had a profound impact on gelation characteristics of acid-induced gels derived from casein solutions (Jakopović et al., 2016) and skim milk (Nguyen and Anema, 2010).

Our previous report proved that laccase improved gel property and antioxidant capacity of α-LA (Jiang et al., 2017). However, it has not been verified whether combination of ultrasound and laccase inevitably can lead to significant enhancement of functional properties of α-LA. Therefore, in this study, it was investigated that the impact of ultrasound treatment (400 W, from 0 to 100 min) on the functional properties and structure of laccase-catalyzed α-LA, extending the application fields of α-LA and providing excellent ingredients for food industry.

2. Materials and methods

2.1. Chemicals

A commercial α-LA powder (91.6% protein content) was gifted from Davisco Foods International, Eden Prairie, MN, USA. Laccase (from Aspergillus, activity 2000 U/g) and ferulic acid (FA) were purchased from Shanghai Gold Wheat Biological Technology Company (Shanghai, China). 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) and 8-Anilino-1-naphthalenesulfonic acid (ANS) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Molecular weight markers (14–120 kDa) were donated by Novoprotein Company (Shanghai, China). All other chemicals were of analytical grade.

2.2. Ultrasound treatment of α-LA

α-LA suspension was sonicated according to the modified method of Nguyen and Anema (2010). α-LA was dissolved in deionized water at a concentration of 100 mg/mL and stirred at 25 °C for 2 h. Subsequently, this solution was adjusted to pH 6.0 by using 1 mol/L HCl. An ultrasound processor model DY-1200Y (Deyangyibang Instruments Co. Ltd, Shanghai, China) with a 2 cm diameter titanium probe, was used to sonicate α-LA suspensions. The sonication probe was immersed in α-LA suspensions. During the process of ultrasonic treatment, the pulse duration of on-time 2 s and off-time 5 s was used to avoid the probe damage. And the flat bottom beaker containing α-LA samples was put in an ice water bath to keep between 14 and 20 °C. α-LA suspensions were treated at 20 kHz with ultrasound power of 400 W and the corresponding ultrasound intensity was 12.74 W/cm^2 for 0, 20, 40, 60, 80 or 100 min, respectively.

2.3. Laccase-catalyzed cross-linking of ultrasound-treated α-LA

Laccase-catalyzed cross-linking of ultrasound-treated α-LA was carried out according to the method of Jiang et al. (2017). After the α-LA suspensions (100 mg/mL) were treated by the ultrasound, the cross-linking reaction of α-LA was initiated by the addition of laccase at 40 °C in the presence of 15 mmol/L FA and the enzyme concentration was fixed to 80 U/g (U/w protein). Samples were taken after being incubated for 8 h and immediately heated at 70 °C for 15 min to inactivate this enzyme. Subsequently, samples were cooled in the ice water, and lyophilized by a freeze dryer (LGJ-1, Shanghai medical analytical instrument Co. Ltd, China) for further experiments. Three control experiments with only ultrasound-treated α-LA, α-LA incubated with laccase or α-LA incubated with FA were also carried out.

2.4. Molecular size distribution

The Molecular size distribution profiles of ultrasound-treated α-LA incubated with FA and laccase and three control samples, were estimated by size exclusion chromatography (SEC). And a series connection column with TSK G 3000 SW XL (7.5 mm ID × 30 cm L, 5 μm, Japan) and TSK G 2000 SW XL (7.5 mm ID × 7.5 cm L, 5 μm, Japan) were used during the analysis of SEC. Diluted protein samples (15 μL) were injected to the column at a concentration of 2.5 mg/mL. Prior to injection, samples must be filtered through a 0.45 μm membrane. The column was equilibrated and eluted with 50 mmol/L Tris-HCl buffer (pH 7.0) at a flow rate of 0.6 mL/min. The eluate was monitored at 280 nm with a UV detector. The method was calibrated using native α-LA and β-LG standards.

2.5. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Molecular mass distributions of ultrasound-treated α-LA incubated with FA and laccase, and three control samples, were analyzed by SDS-PAGE according to the modified method of Ercili Cura et al. (2009). In this experiment, a stacking gel of 4% acrylamide and a running gel of 12.6% acrylamide were used. Sample solutions were diluted to 2.5 mg/mL. The samples were then mixed with SDS reducing buffer containing 62.5 mM Tris-HCl (pH 6.8), 20% glycerol, 2% SDS, 0.5% bromphenol blue and 5% β-mercaptoethanol, according to the volume ratio of one to four. The mixture was heated in boiling water for 5 min and then stored at 4 °C. Protein bands were stained with Coomassie Brilliant Blue R250 and visualized. Molecular mass standards of 14–120 kDa (Bio-Rad, Hercules, CA) were used for molecular mass estimations. Electrophoresis was carried out at ambient temperature using a constant voltage of 60 V until the sample entered into the stacking gel. The voltage was then adjusted to 120 V until the blue tracking dye reached 1 cm from the bottom of gel.

2.6. Surface hydrophobicity measurement

Surface hydrophobicity (H_0) values of ultrasound-treated α-LA incubated with FA and laccase, and three control samples were determined according to the modified method of Chandrapala et al. (2011). ANS was used as the fluorescence probe. Sample solutions