LWT - Food Science and Technology 73 (2016) 630–639

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Improving albumen thermal stability using succinylation reaction with octenyl succinic anhydride

Charlwit Kulchaiyawat [a], Tong Wang [a, *], Zhaopeng Han [b]

[a] Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, 50011, United States
[b] Chinese National Egg Engineering Research Center, Beijing DQY Agriculture Technology Co., Ltd, Beijing, 100125, China

ARTICLE INFO

Article history:
Received 6 September 2015
Received in revised form
11 June 2016
Accepted 3 July 2016
Available online 4 July 2016

Keywords:
Albumen
Egg white protein
Succinylation
Thermal stability

ABSTRACT

Modification of egg white proteins with octenyl succinic anhydride (OSA) was investigated for improving thermal stability of the albumen. The reaction occurred at pH 8.5 for 3 h at 35 °C, and the degree of reaction was quantified by determining the amine group quantity. The effect of sonication pretreatment and OSA addition level on degree of reaction and product heat stability were evaluated, and they were found to have significant impact on product quality. Three different egg white materials were tested; they were rehydrated commercial egg white protein (EWP), fresh egg white (FEW), and commercial de-glucosed egg white (CFEW). They behaved differently toward this chemical modification. At 5% OSA addition, CFEW did not show significant change in amine group concentration, but at 4% OSA use EWP and FEW had 30–40% reduction of amine group. The use of sonication for the FEW at 4% OSA led to 60% amine reduction. At higher temperature of thermal stability evaluation, 95 °C compared to 75 °C, the sonicated and 10% OSA succinylated FEW showed significant stability improvement with a turbidity 30% of that of the control. For the 4% OSA succinylated EWP, its turbidity was only 20% of the control. Therefore, thermal stability improvement was achieved by OSA modification of egg albumen.

1. Introduction

Chemical modification of proteins is effective in improving the functionality of food proteins. Egg white proteins have been chemically modified through acylation with oleic acid or succinylation with succinic anhydride to improve its functionality (Gandhi, Schultz, Boughey, & Forsythe, 1968; King, Ball, & Garlich, 1981; Ma & Holme, 1982; Montejano, Hamann, & Ball, 1984). Succinylated milk, soy, fish myofibrillar, and egg proteins have shown to have an improvement in solubility, gelation, emulsification, foaming, or heat stability, and these modified proteins are intended for food uses (Franzen & Kinsella, 1976; Ma & Holme, 1982; Miller & Groninger, 1976; Montejano et al., 1984; Shilpashree, Arora, Chawla, & Tomar, 2015; Yang, Yang, Zhang, & Zhang, 2016). Palacian, Gonzalez, Pineiro, and Hernandez (1990) suggested that succinylation produced a more stable protein than acylation. As the demand for consumption of protein increases, there is a need to improve functional properties of egg white proteins, especially its heat stability, to expand its application in the food processing industry (Daniells, 2014).

The functionality improvements from succinylation are attributed to the change in protein's secondary and tertiary structure, because the charges generated from succinic anhydride affect the electrostatic and steric hindrance of the protein (Achouri & Zhang, 2001; Achouri, Zhang, & Shiying, 1998; Shilpashree et al., 2015; Yang et al., 2016). The succinylation of proteins occurs by nucleophilic substitution of the free amine, hydroxyl, or sulfhydryl groups of proteins with the succinylating agent (Zhao, Ma, Yuen, & Phillips, 2004). The reactivity of each functional group varies and is affected by different reaction conditions. A limited degree of sulfhydryl modification occurs, but the hydroxyl containing amino acids are more reactive with serine being more reactive than threonine (Gounaris & Perlmann, 1976). Achouri and Zhang (2001) found that the highest succinylation reactivity occurred on the secondary

Abbreviations used: BCA, bicinchoninic acid; BSA, Bovine serum albumin; CFEW, commercial de-glucosed fresh egg white; EWP, egg white power; FEW, fresh egg white; NS, not significantly different; OSA, octenyl succinic anhydride; OSACF, octenyl succinylated CFEW; OSAE, octenyl succinylated EWP; OSAF, octenyl succinylated FEW; S, significantly different; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Son, sonication or sonicated; TNBS, 2, 4, 6-trinitrobenzene sulfonic acid picrylsulfonic; WPI, whey protein isolate.

* Corresponding author. 2312 Food Sciences Building, Ames, IA, 50011, United States.
E-mail address: tongwang@iastate.edu (T. Wang).

http://dx.doi.org/10.1016/j.lwt.2016.07.003
0023-6438/

amino groups of amino acids such as lysine. Ong, Arumugam, and Tayyab (2009) reported that succinic anhydride can expose the buried lysine residues for the succinylation reaction. Although succinic anhydride has been used successfully to modify proteins, the study of octenyl succinic anhydride (OSA) modified proteins is limited. OSA contains two dicarboxylic acid groups similar to succinic anhydride if hydrolyzed, and it also contains a hydrophobic tail. OSA has been used to dissociate protein aggregates due to such charge-charge repulsion (Polyanovsky, 1965; Rossi, Menezes, & Pudles, 1975), and it was used to modify corn protein zein in two recent studies (Biswas, Sessa, Lawton, Gordon, & Willett, 2005; Biswas, Selling, Woods, & Evans, 2009). We hypothesized that in addition to the charged groups, the presence of the hydrophobic tail may also offer hydrophobic interactions with the unfolded proteins during mild heating, thus the protein-protein hydrophobic interactions could be prevented and protein aggregation and precipitation can be reduced.

Egg albumen is a heterogeneous mixture that contains over 40 different proteins with ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme (3.4%) representing over 80% of its protein (Mine, 1995). Sonication can thoroughly mix and physically alter the structure of these proteins, thus it may potentially increase the efficiency of a chemical modification reaction of such a heterogeneous system. This physical treatment uses sound waves at low frequency with high power to mix substrates by cavitation (formation and collapse of air bubbles generating pressure and heat indirectly) (Arzeni, Perez, & Pilosof, 2012). The use of ultrasound or sonication could improve the reaction of many chemical modifications, particularly the succinylation of the egg albumen, because it is difficult to obtain a uniform mixture of fresh egg white protein with OSA otherwise. Sonication will not only improve the uniformity of the mixture, but it may also partially denature the proteins and expose reactive sites by unfolding the protein as demonstrated by an increased protein surface hydrophobicity (Arzeni et al., 2012). This partial unfolding of the proteins may assist the succinylation.

Thus, we hypothesized that the thermal stability of egg white proteins can be improved by modification with OSA, creating a more thermally stable protein product. OSA has been safely used to modify food starch in the U.S. and has been widely used in the food industry (Wang, Su, & Wang, 2010). There is currently no published research on succinylating egg white proteins with OSA, especially with the assistance of sonication. Three different types of egg white protein were modified to determine their effectiveness toward such chemical modification, and to simulate actual commercial production. The objectives of this study were to chemically modify egg white proteins with OSA, to study the effects of sonication on reaction efficiency, and to evaluate the changes in thermal stability of the modified protein.

2. Materials and methods

Dried egg white powder (EWP) was purchased from Honeyville Grain Inc. (Brigham City, UT). Large grade A chicken eggs were purchased with similar sell-by dates from a local supermarket (Ames, IA), and the fresh egg white (FEW) was manually separated. Oskaloosa Food Products Corp. (Oskaloosa, IA) provided commercial fresh egg white that was de-glucosed (CFEW) by fermentation. OSA and other chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

2.1. Modification of egg white protein

2.1.1. Succinylation of egg white

Succinylation of commercial dried egg white or fresh egg white was accomplished using a method similar to that of Ball and Winn (1982). The process flow chart is outlined in Fig. 1 and the treatments and acronyms are listed in Table 1. The EWP was mixed as 10% protein dispersion for at least 12 h before centrifugation at 2000*g* for 2 min to remove any insoluble components. The supernatant was then mixed into a large batch and used for OSA modification. The FEW that was manually separated from the egg yolk was mixed gently for at least 12 h. The CFEW was already well-mixed and de-glucosed. For FEW and CFEW, the OSA was added directly to the mixture.

The different egg white dispersions (EWP, FEW, and CFEW) were transferred to a pH-STAT (718 Titrino, Brinkmann, Switzerland) for 1-L batch reactions to control the pH during the reaction. The concentrations of OSA added were 1% (0.0005 M), 2% (0.001 M), 4% (0.002 M), 5% (0.0025 M), and 10% (0.005 M) based on the protein content. Protein content was measured using the bicinchoninic acid assay with BSA as the standard as described below. The pH was maintained at 8.5 with 2 N NaOH for 3 h at 35 °C with mild mixing. To stop the reaction, the pH was adjusted to 6.5 using 2 N HCl and then the mixture was dialyzed (M_w cutoff 6 to 8 KDa) for 3 days in a refrigerated room (4 °C) to remove any salt or unreacted OSA. The water was changed two times a day. The CFEW treatments were not dialyzed due to the large quantity intended for spray drying.

2.1.2. Succinylation of egg white protein with sonication pretreatment

Fresh egg white was prepared as before, but was sonicated using a Sonicator (Fisher Scientific model 500 Ultrasonic Dismembrator, Pittsburgh, PA). Sonication was done before the reaction with OSA in small batches of 150–200 mL for 4 min using a ½ inch horn at 25–35% amplitude (set at 70%) in an ice bath, a condition modified from Gordon and Pilosof (2010). For the CFEW modified final product to be spray dried, the reacted mixture was sonicated again for another 4 min to disperse any precipitated particles.

2.1.3. Drying of succinylated egg white protein

After dialysis, the EWP and FEW products were freeze-dried using a VirTis Genesis 25 EL Pilot Lyophilizer (SP Scientific, Warminster, PA), then finely ground with a mortar and pestle. The CFEW samples were not dialyzed, but underwent filtering using cheesecloth before spray drying. The spray dried products were produced using a conical spin-disk atomizer feed spray dryer (APV Crepaco Inc, Getzville, NY). The inlet and outlet temperatures were between 180–195 °C and 88–110 °C, respectively. The flow rate was adjusted to control the outlet temperature, and it was about 200 mL/min. The powder was collected and stored at 4 °C in plastic bags before analyses.

2.2. Characterization of OSA succinylated protein products

2.2.1. Degree of succinylation determination using a free amine test

The degree of succinylation reaction was determined by using the TNBS (2, 4, 6-trinitrobenzene sulfonic acid picrylsulfonic acid) method that measures the content of free amine according to a method of Habeeb (1966). A TNBS kit was purchased from G-Biosciences (St. Louis, MO). A standard curve was established using glutamic acid and it was used for the free amine quantification. The free amine was calculated as the equivalent of glutamic acid, based on 20 mg of protein.

2.2.2. SDS-PAGE analysis of protein molecular size

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to a method of Laemmli (1970), using a Bio-Rad Mini-PROTEAN® Tetra System to observe changes in molecular size. A 4–20% Mini-Protein® TGX™ precast