LWT - Food Science and Technology 72 (2016) 267–276

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Combination of treatments to improve thermal stability of egg albumen

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ARTICLE INFO

Article history:
Received 20 October 2015
Received in revised form
11 April 2016
Accepted 25 April 2016
Available online 26 April 2016

Keywords:
Egg white protein
Octenyl succinic anhydride
Thermal stability
Raman spectroscopy

Chemical compounds studied in this article:
octenyl succinic anhydride (OSA)
albumen
bicinchoninic acid (BCA)
bovine serum albumin (BSA)

ABSTRACT

Protein modification with combined pre-heating, sonication, and the addition of octenyl succinic anhydride (OSA) was performed on egg albumen to improve its thermal stability. The treatments applied to fresh egg white (FEW, or albumen) led to an increased stability. The pre-heating step and high level of OSA addition, such as 20% relative to protein, were beneficial when the stability of protein was evaluated at a higher temperature of 95 °C or 121 °C compared to that of 75 °C. The low level of OSA treatment, such as 5%, showed improved thermal stability evaluated by turbidity and protein solubility at 75 °C. Thus, the optimal level of OSA addition would depend on the degree of thermal stability improvement needed. The use of Raman spectroscopy method revealed that there were strong interactions between OSA and albumen protein and that this complex was more resistant to structural changes by heating. A mechanistic explanation of thermal stabilization based on experimental observations was illustrated. This is the first study that utilizes OSA along with other physical means to complex and stabilize egg proteins.

1. Introduction

The main limitation of a wide utilization of egg white protein in the food industry is its sensitivity to heat. By improving the thermal stability of egg protein, its use in the food processing industry can be expanded as demand for high protein food products continues to increase (Daniells, 2014; Gerrard, 2014; Scott-Thomas, 2013). Past research has focused on modifying the major egg albumen proteins through enzymatic, chemical, and physical methods to improve functionality, however, there has been no significant advance in improving the thermal stability of the protein unless it is significantly hydrolyzed (Arzeni, Martenez et al., 2012; Campbell, Raikos, & Euston, 2003; Mine, 1995).

Ultrasound or sonication is a physical processing method that could be used in egg processing to improve its thermal stability if combined with other treatments. The use of ultrasound generates heat and cavitation through the formation and collapse of air bubbles in a solution with minimal incorporation of air (Ashokkumar et al., 2008, 2009; Gordon & Pilosof, 2010; Mason, Paniwnyk, & Lorimer, 1996). This physical process has been shown to enhance protein's emulsion stabilization effect and increase protein gelation properties (Arzeni, Martenez et al., 2012, Arzeni, Perez, & Pilosof, 2012; Martini & Walsh, 2012). Sonication (Son) is ideal for mixing egg white proteins since it can minimize foam formation during mixing with other food ingredients. In chemical reactions involving succinic anhydride, the solubility or dispersibility of the anhydride can be increased by using ultrasound (Zhao, Ma, Yuen, & Phillips, 2004). Sonication can also partially expose hydrophobic moieties of proteins. In this way, the protein is partially denatured, thus improved interactions with other food additives are expected.

Ashokkumar et al. (2009) improved the heat stability of whey protein through a two-step heat denaturation and sonication

ABBREVIATIONS: BCA, bicinchoninic acid; BSA, bovine serum albumin; FEW, fresh hen egg white or fresh egg white; H, with pre-heating; N, no pre-heating; OSA, octenyl succinic anhydride; PCA, principle component analysis; SDS, sodium dodecyl sulfate; Son, sonication or sonicated; WPI, whey protein isolate.

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http://dx.doi.org/10.1016/j.lwt.2016.04.047
0023-6438/

process. However, Arzeni, Perez et al. (2012) found no improvement in heat stability of rehydrated egg white powder with sonication alone. The use of food ingredients or additives in combination with sonication for the protein has shown some promise. The addition of anionic additives such as 2-decylcitric acid, sodium dodecyl sulfate (SDS), and lauric acid has been shown to improve the thermal stability of ovalbumin in weak alkaline pH regions (Hegg & Löfqvist, 1974). The authors speculated that the hydrophobic core of the protein may be stabilized by the interaction of the hydrocarbon chain of the additive. It has also been shown that adding an amphiphilic phospholipid along with a pre-heating step prior to sonication treatment improved the thermal stability of ovalbumin, and the phospholipids can interact with proteins as shown using sodium caseinate (Istarova et al., 2005; Oshima & Nagasawa, 1973).

Our hypothesis is that the combination of pre-heating, sonication, and with the addition of octenyl succinic anhydride (OSA) to fresh egg white (FEW) can improve the heat stability of the protein dispersion by a stable complex formation. The objectives of this study were to examine the effects of pre-heating treatment with sonication and various levels of OSA addition on heat stability of the albumen dispersion.

We proposed that pre-heating with sonication may be used in combination with the unique ingredient, OSA, to improve egg white protein's thermal stability through two simultaneous mechanisms. First, the OSA's eight carbon tail or hydrophobic group may interact with egg protein's hydrophobic moieties, particularly if the hydrophobic groups are partially loosened or exposed during pre-heating or sonication. Such complexing may prevent further unfolding of the protein if heated, thus minimizing protein–protein hydrophobic association and large aggregate formation. Secondly, OSA's partially reacted or hydrolyzed dicarboxylic acid polar moiety may provide electrostatic repulsions among the proteins because it has been previously shown (Ma & Holme, 1982) that chemically modifying proteins with a dicarboxylic acid would improve heat stability. Therefore, the system created by OSA-albumen interaction may prevent protein aggregation after heating the protein at high temperatures.

2. Materials and methods

Grade A large fresh eggs were purchased from a local supermarket (Ames, IA) with similar sell-by dates for each replicate. OSA was purchased from Sigma Aldrich (St. Louis, MO). All other materials and chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

2.1. Treatments applied to fresh egg white

This experiment was designed to evaluate the effect of pre-heating and dosage level of OSA on the improvement of protein thermal stability. Therefore, two sets of samples were created, one with different levels of OSA (5, 10, and 20% based on protein (dwb)) without pre-heating and the other with OSA at 2, 5, 10, and 20% with pre-heating treatment. Three replicates of these treatments were conducted. The modification procedure was similar to the method of Ashokkumar et al. (2009) with a different heating temperature and the amount of OSA.

The eggs were broken and the white was separated from the yolk. The fresh egg white was then mixed with 0.02% sodium azide (w/w) and diluted with one part of Milli-Q water to prevent protein gelation during the pre-heating step. If the treatment was pre-heated, the mixed fresh egg white (FEW) was heated in a water bath set at 63 °C for 5 min (after samples reached this temperature) before sonication. OSA was added at 4 min during heating to allow partial structure changes while the system was manually mixed. The sonication treatments were done in 150 mL-volume batches, using an ultrasound or sonicator (20 KHz and 400 W, Fisher Scientific model 500 Ultrasonic Dismembrator, Pittsburgh, PA). A ½ inch horn with flat tip was used and the amplitude was set at 70% with 30 s on and 10 s off for 2 min. No temperature control was used since the sample was sonicated immediately after pre-heating. The different treatment combinations are outlined in Table 1.

2.2. Thermal stability evaluation and effect of pH on complex stability

In addition to the treatment comparisons for the effect of pre-heating and OSA dosage, another pair of comparison was made to evaluate the effect of pH on thermal stability evaluation. One set of samples was evaluated as-is. The other set was adjusted to pH 7 by using pH 7 buffer (0.1M sodium phosphate) so the factor of pH change due to OSA addition can be removed. The treatments were repeated 3 times.

2.2.1. Thermal stability evaluation by measuring turbidity of protein after 75 °C heating

A thermal stability evaluation based on turbidity after heating at 75 °C for 30 min was conducted on the treated FEW samples. The sample was diluted to approximately 1% protein concentration with Milli-Q water or pH 7 buffer and 5 mL of the dispersion was transferred to 15 mL Corning centrifuge tube. The centrifuge tube was placed in the shaking water bath set at 75 ± 2 °C for 30 min. The transmittance of the FEW-OSA was measured using an UV DU720 spectrophotometer (Beckman Coulter Inc., Brea, CA) at a wavelength of 600 nm, according to a modified method of Zhang et al. (2004) and Shimada and Matsushita (1980a). Samples were cooled to ambient temperature and mixed before turbidity measurement. All samples were evaluated twice for each treatment replicate. The turbidity value was obtained by subtracting the % transmittance value from 100.

2.2.2. Thermal stability evaluation by measuring turbidity and protein solubility at 95 °C

Another turbidity method was used to confirm and further test the heat stability of the protein-OSA complex at a higher temperature. The treated FEW was diluted to approximately 2.5% protein concentration (w/w) or 25 mg/mL with pH 7 buffer. The diluted solution, 1 mL, was then transferred to a disposable glass tube containing 5 mL of 95 °C pH 7 buffer (a final 0.4% protein concentration). The test tube was then heated at 95 °C for 1 h, cooled to ambient temperature and mixed before turbidity measurement. The % transmittance of the samples was measured at 600 nm with the turbidity calculated as before. The concentration of dispersible protein was measured using the Biuret protein assay (Torten & Whitaker, 1964) to ensure that a decrease in turbidity was not due to a reduction in soluble protein. Bovine serum albumin (BSA) was used to establish a standard curve and the percent protein retained in supernatant was calculated based on the measured absorbance at 540 nm for the samples before and after heating. A protein dispersion not heated to 95 °C was also determined as the beginning turbidity of the sample.

2.2.3. Thermal stability evaluation by measuring protein solubility at 75 °C over time

A thermal stability evaluation modified from Ball and Winn (1982) was also performed. It is based on protein solubility of a sample heated at 75 °C and measured over time. The treated proteins were diluted to ~1% protein concentration with pH 7 buffer.