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Physicochemical and functional properties of Chinese soft-shell turtle (*Pelodiscus sinensis*) egg

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The physicochemical properties of Chinese soft-shell turtle egg were characterized for functional use in the food industry. The egg yolk of un-fertilized soft-shell turtle eggs was separated and fractionated into granules and plasma. Then, the egg yolk, albumen, granules, and plasma were freeze-dried for further analysis. Results showed that the Chinese soft-shell turtle egg typically comprised 50% egg yolk, 34% albumen, and 16% shell in average. The egg yolk composed of 61% granules and 39% plasma. The granules contained most of the protein, while the plasma contained most of the lipid in egg yolk. The albumen contained about 26% ash on a dry weight basis. Lysozyme was the major component in turtle egg albumen. The protein solubility of egg yolk, granules, and plasma was affected by the changes in pH, while that of albumen remains constant. The emulsifying and foaming properties increased when the concentration increased for all samples. Both the yolk and the albumen in turtle egg exhibited better functional properties than those in normal hen egg. These physicochemical and functional properties of Chinese soft-shell turtle egg are fundamental and essential for future study and food applications.

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1. Introduction

The Chinese soft-shell turtle, *Pelodiscus sinensis*, is the most common species used in Taiwan and China for food and medicine, or sold as pet (Chen, Chang, & Lue, 2009). In recent years, soft-shell turtle became economically important and is large-scale produced in East Asia. In addition, the turtle egg is also recognized as a healthy food (Shen, Hsu, & Chang, 2008; Yu, Li, Wang, Zheng, & Yan, 2005). Researches show that the turtle eggs had cholesterol-lowering effect and can prevent hypertension (Rawendra et al., 2013; Yu et al., 2005). However, most of the un-fertilized turtle eggs were not consumed as food but sold as animal feed for the price of US$6 per kg.

The high protein turtle egg could be a good quality but less expensive protein source of some food product, either for human or companion animal. The most important functional properties of protein in food applications are solubility, emulsifying properties, foaming properties, water-holding capacity, fat-absorption capacity, and gelation property. These properties significantly affect the physical behaviors and sensory attributes of foods during preparation, processing, and storage. Hen egg yolk and egg white have already been used extensively in food preparation for emulsification, foam formation, and gelation. These functional properties are critical in food products such as desserts, puddings, breads, mayonnaise, reformulated meat products, and tofu. Soft-shell turtle egg's unique properties make it a potential ingredient to fulfill the requirement of specific function in the final food product. The objective of the present study was to characterize the physicochemical and functional properties of Chinese soft-shell turtle egg for assessment in future application.

2. Materials and methods

2.1. Materials

Un-fertilized soft-shell turtle eggs laying by the 3–4 years old turtles were provided by a turtle farm at Pingtung City in Taiwan. A total of 2400 fresh eggs were collected between March and August in 2012. Once arrived, the eggs were stored at 4 °C in the walk-in refrigerator. The cleaning and fractionation of eggs were done within 3 days.

2.2. Egg fractionation

The separation of soft-shell turtle egg yolk and albumen was performed according to the methods of Yan et al. (2010) and Laca, Paredes, and Díaz (2010a) with modification. The eggs were washed with tap water to remove sands first. The clean eggs were then broken to obtain the liquid portion. The dropper was used to suck up the albumen from the liquid portion of eggs. The remaining egg yolk was put on the filter paper to adsorb the traces of albumen on the membrane of egg yolk. The membrane was then ruptured to obtain the liquid egg yolk. All the collected egg yolk and albumen were placed in an ice bath.

The egg yolk was further fractionated into granules and plasma based on the methods of Laca et al. (2010a) and Laca, Sáenz, Paredes, and Díaz (2010b) with modification. Yolk was diluted 1:1.5 with

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distilled water under continuous stirring. After adjusting pH to 7, the diluted solution was stored at 4 °C for one day in the refrigerator. The solution was then centrifuged at 10,000 ×*g* for 40 min to separate plasma (supernatant) from granules (sediment). The egg yolk, albumen, granules, and plasma were freeze-dried and stored at −20 °C in the freezer for further analysis.

2.3. Composition analysis

The composition analysis of soft-shell turtle egg yolk, granules, and albumen was performed according to Chinese National Standards (2013). Moisture content was determined by drying the samples at 100 ± 2 °C in an oven for 6 h. Moisture content was estimated by weighing samples before and after drying. Results were expressed as g moisture/100 g sample. Ash content was determined by ashing the samples at 700 °C in a furnace for 12 h. Results were expressed as g ash/100 g dried matter. Crude lipid was measured by acid hydrolysis and extraction with diethyl ether/petroleum ether (1:1, v/v) mixture. Lipid content was estimated by weighing lipid extract after solvent evaporation. Results were expressed as g lipids/100 g dried matter. Protein content was determined by micro-Kjeldahl method with the conversion factor of 6.25. Results were expressed as g proteins/100 g dried matter.

2.4. Gel electrophoresis

The 30–40 mg of sample was dissolved with 1 mL 50 mM Tris–HCl buffer containing 8 mM urea, 140 mM β-Mercaptoethanol and 10% SDS (pH 8). The sample solution was then heated at 95 °C for 30 min and centrifuged at 16,000 ×*g* for 10 min at 20 °C. Protein content of supernatant was analyzed by using Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, USA) and bovine serum albumin (BSA) was used as standard. SDS-PAGE was carried out in a Bio-Rad mini-protein electrophoresis system with a 12% Bis–Tris SDS-PAGE gel. Sample was mixed with SDS-PAGE loading dye, β-Mercaptoethanol, and Tis-HCl buffer to the final concentration of 1 μg/μL. The 10 μL of sample and protein ladder were then loaded into the separated wells in the electrophoresis system. After electrophoresis with MES SDS-running buffer for 2 h, gels were immediately stained with the InstantBlue for one day. Stained gels were then scanned by using a photo scanner (Epson Perfection V200 Photo, Seiko Epson Co., Nagano, Japan).

2.5. Protein solubility analysis

The pH of sample solution (1%) was adjusted to 2, 3, 4, 5, 6, 7, 8 and 9 using 1 N HCl and 1 N NaOH. The sample solution was then agitated for 1 h at room temperature and centrifuged at 10,000 ×*g* for 10 min. Protein content of supernatant was measured by the Biuret reagent. It was calculated based on a standard curve derived from BSA. Protein solubility was calculated as the protein content of supernatant divided by the total protein content of sample in percentage.

2.6. Emulsifying properties analysis

The emulsifying capacity (EC) and emulsifying stability index (ESI) of sample were determined using the turbidimetric methods of Anton and Gandemer (1997) and Yan et al. (2010) with modification. The sample solution was prepared at the concentration of 0.5, 1, 2.5, and 5% and the pH of these solutions was adjusted to 3. Soybean oil (12 mL) was added (oil volume fraction = 0.375) and homogenized at 11,000 rpm for 30 s (Polytron PT-MR-3000, Switzerland). The emulsion was then diluted 1:400 with 0.1% SDS. The absorbance of diluted emulsion was measured at 500 nm using a UV–visible spectrophotometer (SP8001, Metertech Inc., Taiwan) against a blank. The higher the turbidity (absorbance at 500 nm), the higher the EC of sample. The ESI was reported as ESI (min) = $A_0/(A_0 - A_5) \times 5$ min, where A_0 and A_5 were the absorbance of diluted emulsion measured at 0 min and 5 min, respectively.

2.7. Microscopy emulsion

The emulsion was prepared according to the method described in Section 2.6. The emulsion was further diluted 1:400 with 0.1% SDS. A drop of diluted emulsion was placed on the slide and covered with a micro cover glass. The size and distribution of emulsion were observed using a light microscope (G380-LED, Uniso, USA) under 400× magnification.

2.8. Foaming properties analysis

The foaming capacity (FC) and foaming stability (FS) of sample were determined by the method of Wong and Kitts (2003). The sample solution was prepared at the concentration of 0.5, 1, 2.5, and 5% and the pH of these solutions was adjusted to 3. The solution was then blended at 11,000 rpm for 30 s. The volume of foam and liquid were measured in a glass graduated cylinder. FC (mL of foam/mL of initial liquid) and FS (mL of liquid remaining in foam/mL of initial liquid) were calculated as FC = V_f/V_i and FS = $(V_i - V_o)/V_i$, where V_f is the volume of foam after blending, V_i is the initial volume of solution, and V_o is the volume of liquid after 1 h of standing.

2.9. Water-holding capacity and fat-absorption capacity analysis

Water-holding capacity (WHC) and fat-absorption capacity (FAC) were determined according to the centrifugation method of Wong and Kitts (2003) with modification. The 1 g of sample was hydrated with 10 mL distilled water (WHC) or mixed with 10 mL of soybean oil (FAC) for 30 min and then centrifuged at 2200 ×*g* for 30 min. WHC and FAC were calculated as WHC (g H_2O/g sample)/FAC (g fat/g sample) = $(W_b - W_a)/W_a$, where W_a is the weight of sample and W_b is the weight of sediment.

2.10. Gelation behavior analysis

The sample solution was prepared at the concentration of 5, 10, 20, 30, and 44%. Then, 1 mL of the sample solution was added in an Eppendorf tube and heated at 80 °C for 10 min in a waterbath. After cooling, the tubes were flipped over in order to observe the gel formation of samples. A firm gel would stay on the top, whereas a weak gel would break down to the bottom. The appearance of egg gels was observed using a digital camera (EOS 1000D, Canon Inc., Tokyo, Japan) equipped with a Canon EF-S 18–55 mm f/3.5–5.6 lens.

The gelation behavior and the rheological property of egg samples were measured by using a controlled stress dynamic rheometer (AR2000 ex, TA Instruments, Inc., New Castle, USA) equipped with a parallel plate geometry (40-mm diameter, 1-mm gap). The sample solutions at the concentrations of 5, 10, 20, 30, and 44% were placed on the plate of rheometer. The edge of the geometry was sealed with Vaseline oil, and the geometry itself was coved with solvent trap to minimize the moisture loss during the test. The oscillation stress of 10 Pa was applied at the frequency of 1 Hz. The temperature sweep of sample was performed from 25 °C to 80 °C at the heating rate of 5 °C/min. The time sweep of sample was then carried out at 80 °C for 10 min. Finally, the temperature sweep of sample was performed again from 80 °C to 25 °C at the cooling rate of 5 °C/min. Samples were subjected to a small amplitude oscillatory measurement. The storage modulus (G′) and loss modulus (G″) of sample were recorded as a function of time within the linear viscoelastic region which was previously determined by the stress sweep (0.01–1000 Pa) on gels at 25 °C.

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