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Double enzymatic hydrolysis preparation of heme from goose blood and microencapsulation to promote its stability and absorption

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ABSTRACT

Iron deficiency anemia (IDA) is the most common nutritional deficiency worldwide. This deficiency could be solved by preparing stable, edible, and absorbable iron food ingredients using environmentally friendly methods. This study investigated enzymatic hydrolysis and microencapsulation process of goose blood. The physicochemical properties, stabilities of the microencapsulated goose blood hydrolysate (MGBH) and a supplement for rats with IDA were also evaluated. The results showed that the synergetic hydrolytic action of neutrase and alkaline protease significantly increased the heme-releasing efficiency. The heme was then microencapsulated using sodium caseinate, maltodextrin and carboxymethyl cellulose (CMC) as the edible wall material, and the encapsulation efficiency of the product reached 98.64%. Meanwhile, favorable thermal, storage and light stabilities were observed for the microencapsulation. It was found that MGBH can significantly improve the body weight and hematological parameters of IDA Wistar rat.

1. Introduction

Iron deficiency is the most common nutritional deficiency in the word. This symptom occurs in 20% of the world's population, particularly in women and children (Kadivar, Yarmohammadi, Mirahmadizadeh, Vakili, & Karimi, 2003). Iron deficiency anemia (IDA) can delay the development of physical and mental functions in infants as well as cause severe fatigue and various health problems in adults (Goddard, James, McIntyre, & Scott, 2011). This phenomenon may be partially induced by plant-based diets that typically contain non-heme iron, which is poorly absorbed. As heme-iron is better absorbed than non-heme iron, fortifying food with heme-iron has been suggested as the most sustainable approach to overcome iron deficiency problems in developing countries (Miret, Tascioglu, van der Burg, Frenken, & Klaffke, 2010). Normally, dietary sources of heme iron are derived from animal origins, such as meat and liver, which are often difficult to obtain in developing areas, as animal products are expensive and not readily available (Martínez-Navarrete, Camacho, Martínez-Lahuerta, Martínez-Monzó, & Fito, 2002). Slaughterhouse blood is an affordable alternative for the fortification of a daily diet. It is a rich source of high-quality heme iron, as well as of nutritional and functional proteins. For example, one adult cattle will yield approximately 10–12 L blood that contains 400–500 mg of iron and 0.1–0.18 g of protein per milliliter (Ockerman & Hansen, 1999). However, animal blood is used as a source of human food, typically in the form of sausages or congealed blood curd, in only a few countries. Generally, the slaughter blood, is discarded or not fully utilized. Coupled with recent advances in blood collection and processing techniques, a myriad of blood ingredients are now available for food and dietary supplements (Ofori & Hsieh, 2012).

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Hemoglobin obtained from slaughtered animal blood can be used as a high quality and quantity source of natural red colorant as well as iron supplements (Hsieh & Ofori, 2011).

However, the occurrence of the hemoglobin auto-oxidation is frequently inevitable during food processing and preservation (Ofori & Hsieh, 2012). The red color that results depends on the oxidation state of the heme iron. Oxyhemoglobin, the dioxygen ferrous form is bright red in color but the heme iron is easily oxidized into ferric form, and the resulting methemoglobin exhibits an undesirable dark-brown color, along with an undesirable odor and a metallic taste in the final food product (Fontes, Gomide, Fontes, Ramos, & Ramos, 2010). Due to the unstable nature of hemoglobin, its usage as a food ingredient is only possible when it is extracted and stabilized to form a complex structure that protects it from oxygen accessibility (Saguer et al., 2003).

Previously, the acidic acetone extraction method was widely used for heme extraction from blood products. Although this method has demonstrated high efficiency, organic solvent pollution is introduced during the extraction and poses a risk as a food ingredient (Lu & Tian, 2007). Proteases, such as trypsin, papain and neutrase have been used on blood heme extraction, which seems the best choice as an environmentally friendly processing technology (Xia & Yang, 2005). Also, microencapsulation has shown positive results as a good envelopment technique in food industry applications (Nazzaro, Orlando, Fratianni, & Coppola, 2012). In addition to providing easy handling, microencapsulation gives the oxidizable substances, volatile additives, enzymes or microbes the chance to become stable and be protected from nutritional loss and oxidation deterioration (Freitas, Merkle, & Gander, 2005).

Goose is one of the most important waterfowls in China. Goose consumption, along with the feed industry are booming in recent years (Huang et al., 2012). However, very little research on goose blood processing and comprehensive utilization has been conducted. This study examined the enzymatic extraction and microencapsulation of heme from goose blood, and also evaluated the effects of microencapsulated goose blood hydrolysate (MGBH) supplements on iron deficiency in rats.

2. Materials and methods

2.1. Goose blood preparation, chemicals, and reagents

Fresh goose blood that had passed quarantine inspection was centrifuged at 3000*g* and 4 °C. The sedimentary red blood cells were collected and washed twice with sterile saline. After frozen under −20 °C overnight in refrigerator, a vacuum freeze drier was used to prepare red blood cell powder. The samples were sealed under vacuum conditions and stored at a temperature of −20 °C for further treatment.

Enzymes, including alkaline protease, neutrase, flavourzyme, trypsin and papain were obtained from Sigma-Aldrich. Standard heme was purchased from Sangon Biotech Co., Ltd, Shanghai. The sodium caseinate, gelatin, arabic gum, xanthan gum and soybean protein isolate were of food-grade. Chitosan, sodium alginate, methyl isobutyl ketone (MIBK), carboxymethyl cellulose (CMC), maltodextrin, β-cyclodextrin, sucrose, NaOH, HCl and other chemicals were of analytical grade.

2.2. Enzymatic hydrolysis of goose blood cells

The red blood cells were blended with water at a ratio to of 3:20 (w/w). The mixture was submerged in ice. It was then disrupted with ultrasound for 1 min followed by a 30 s interval for 30 times. Enzymatic hydrolysis was conducted using different proteases with a concentration of 1.5 g enzyme per 100 mL red blood cell mixture. The effect of pH on heme releasing activity was observed at 45 °C with different buffers (Cheng et al., 2012), and the effect of temperature on the heme releasing activity was determined under different temperatures at pH 7.0 (In, Jeong Chae, & Oh, 2002).

2.3. Microencapsulation of goose blood heme

Sodium caseinate (3.76 g), maltodextrin (1.82 g) and CMC (1.86 g) as the wall materials were dissolved in 40.86 mL boiling distilled water to obtain an aqueous solution. The goose blood heme solution was then added dropwise into the aqueous solution to form an emulsion using magnetic stirring. After stirring and high-speed dispersion, the emulsion was frozen overnight and placed into a vacuum freeze dryer to obtain microencapsulation. The resulting product was stored at 4 °C for further experiments. The encapsulation efficiency and heme content were tested as parameters by which to evaluate the microencapsulation capability.

2.4. Surface heme and total heme content assay

The acidic MIBK was used as the dissolvent to extract the heme from the microcapsule surface. A 0.02 g sample was gently and thoroughly mixed with 10 mL MIBK and extracted for 5 min. As for the total heme content, the samples were irreversibly dissolved with 4% (w/v) NaOH solution. The obtained solution was subsequently mixed with 2 mL of acidic MIBK and 1 mL of HCl to extract the total hemoglobin under vigorous vortex shaking conditions for 3 min. Then, 2 mL of water was added into the mixture for the second extraction for 5 min. The surface and total heme extraction solution were then centrifuged at 3000*g* for 5 min to obtain the respective supernatant. The final supernatant volume was fixed to 10 mL by adding MIBK solvent and mixing thoroughly. Heme iron was determined by direct spectrophotometric measurement at 640 nm against MIBK as a blank using a UV spectrophotometer (Thiansilakul, Benjakul, & Richards, 2010) .

2.5. Encapsulation efficiency and stability assay

Encapsulation efficiency (E) was calculated according to the following formula:

$$E(\%) = \left(1 - \frac{S}{T}\right) \times 100$$

where E, S and T stand for the encapsulation efficiency, heme content on microcapsule surface and total heme content, respectively.

The thermostability, light stability, and storage stability of microcapsule were evaluated using preservation rate (P), which was calculated according to the following formula:

$$\mathrm{P}(\%) = \frac{A}{I} \times 100$$

where P, A and I are the preservation rate, heme content after process, and heme content at the initial state, respectively.

To evaluate the thermal stability, 1.0 g of microcapsule powder was placed in an oven at 105 °C. The respective preservation rates were determined every hour. The light stability assay for microcapsule powder was performed in a closed container with a thickness of 0.2 cm under the direct sunlight. The content of goose heme was determined weekly through preservation rate. The storage stability was evaluated in a 14-day storage trial in an open container to maintain the thickness of 0.2 cm at 25 °C under forced ventilation atmosphere. The content of goose heme was evaluated every 2 days through preservation rate.