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Use of response surface methodology to describe the effect of time and temperature on the production of decoloured, antioxidant and functional peptides from porcine haemoglobin by sub-critical water hydrolysis

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ABSTRACT

Sub-critical water hydrolysis coupled to oxygen injection was used to hydrolyse porcine haemoglobin. The effect through process time of three different temperatures were tested (120–180 °C), under medium pressures (40 atm.). Peptides obtained at 180 °C showed good antioxidant and functional properties, and the best yield (83%). Moreover, the final product presents almost complete discolouration, thus increasing the range of haemoglobin hydrolysates uses in food industry. Regression modelling was used to investigate the main effects of hydrolysis time and temperature. Both were found to be significant. Predicted models were found to be significant ($p < 0.05$) with high coefficients of determination (R^2). This study found that the average peptide size can be predicted from the temperature and process time; besides, evidence seems to show that preferential targets exist to break down the haemoglobin. This method can transform large amounts of proteins into appreciable low-molecular-weight peptides in a simple and fast way.

1. Introduction

It is widely known that blood represents the most problematic by-product of the meat industries (Moure, Rendueles & Díaz, 2003) due to the high volumes generated and its major polluting power. Besides, proteins constitute one of the main components of blood and given that these molecules have an important economic value, it would seem obvious to consider the feasibility of recovering the proteins contained in slaughterhouse blood, which have been recently classified as a target compound to be recovered from food waste (Mullen, Álvarez, Pojić, Hadnadev, & Papageorgiou, 2015). The most abundant protein in blood is haemoglobin, which represents 12–18% of the total composition of blood. Haemoglobin and other blood proteins can be easily purified and processed to produce several products: heme iron supplement (Vaghefi et al., 2002); peptides with different applications, such as antimicrobial effect (Catiau et al., 2011), antioxidant effect (Chang, Wu, and Chiang, 2007) , iron-biding peptides (In, Chae, & Oh, 2002; Lee & Song, 2009) inhibitors of hypertension or regulators of glucose absorption (Yike et al., 2006; Lafarga & Hayes, 2014); or simply as an ingredient to increase the protein and amino acid content of food proteins. In spite of its potential for use in human nutrition, the dark brownish colour that haemoglobin imparts on food formulations greatly restricts its use as a raw material or ingredient in the food industry (Pereira et al. 2014). In this sense, multiple methods have been developed aiming to reduce the colour of whole blood or haemoglobin: heme sequestration, cold acetone precipitation or hydroxide peroxide (Yang & Lin, 1998). Most of these methods require the use of solvents or chromatographic techniques.

Research into the production of peptides is related to the enhancement of functional and antioxidant properties when they are used as an ingredient in food products. It has been reported (Bautista et al., 2000; Clemente, 2000) that low molecular weight peptides with free amino acids, especially di- and tripeptides, have high nutritional and therapeutic properties (Vijayalakshmi,

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Lemieux & AMoit, 1986; Williams, 1995). Some authors (Moure, Dominguez & Parajo, 2006; Suetsuna, Ukeda, & Ochi, 2000) have reported that peptides minors than 1 kDa present the best antioxidant properties. On the other hand, large molecular weight peptides (more than 20 amino acid residues) are believed to be associated with an improvement in the functional properties of hydrolysates.

Furthermore, interest in peptides has increased in recent years, focussing mainly on peptides that present antioxidant activity. These have drawn attention due to their low molecular weight, which facilitates their easy absorption and high antioxidant activity, thus making low molecular size peptides desirable products (Di Bernardini et al., 2011).

Hydrolysis of proteins to obtain peptides is currently carried out using different enzymatic methods, employing enzymes such as alcalase, trypsin or pepsine, the high cost of which makes the process more expensive. Besides, these methods require stringent control of reaction parameters such as pH, temperature and the enzyme/protein ratio. The use of sub-critical water hydrolysis (SWH) is presented as an alternative for protein hydrolysis (Esteban, García, Ramos, & Márquez, 2010; Marcet, Álvarez, Paredes, & Díaz, 2014; Rogalinski, Herrman & Brunner, 2005; Xian, Chao, Liang, & Cheng, 2008). In the aforementioned studies, the protein was hydrolyzed under very high pressures (15–27 MPa) and very high temperatures (250–300 °C), highly aggressive conditions which completely degrade the protein into amino acids with no peptide production.

Employing high hydrostatic pressures at room temperature is known to produce the denaturation of proteins without any change in molecular size (Rodiles-López et al., 2010). If the temperature is increased, the pressure can be decreased to moderate values and the proteins still remain unfolded (Smeller, 2002) and no hydrolysis process is taking place. However, a combination of high pressure and high temperature leads to protein unfolding and further hydrolysis (Quirós, Chichón, Recio, & López-Fandiño, 2007; Toldrá, Parés, Saguer, & Carretero, 2011). The first step in denaturation is the loss of the quaternary structure (if it exists, as in the case of haemoglobin), which is the most pressure-sensitive interaction (Lullien-Pellerin & Balny, 2002). Subsequently, the tertiary structure is unfolded in a direct relation with pressure and temperature. Finally, the completely denatured protein presents more bonds susceptible to be hydrolysed than when in the native state.

In a previous work (Álvarez, Rendueles & Díaz, 2012) it was observed that the use of SWH at mild conditions (40 atm and 130–180 °C) under inert atmosphere was able to produce peptides instead of free amino acids. Besides, the peptides obtained showed certain degree of decolouration. In present work the effect of SWH under an oxidizing atmosphere on the production of peptides from porcine haemoglobin was investigated. As result of the process, an 83% of the haemoglobin was transformed into small decoloured peptides, which shown excellent antioxidant properties and good functional properties when compared to native haemoglobin or enzymatic hydrolysates. The effect of temperature and time on peptide size and peptide production was evaluated by means of response surface methodology, which allows predicting the final molecular weight distribution of the hydrolysate obtained.

2. Materials and methods

All reagents (chloroform, sodium citrate, leucine, Nessler's reagent, sodium hydroxide, hydrochloric acid, sodium chloride, cottonseed oil, potassium ferrocyanide, trichloroacetic acid, ferric chloride, EDTA-Fe^{2+}, safranin O, H_2O_2 and ferrozine) were supplied by Sigma-Aldrich® and were analytical or HPCL reagent grade. Haemoglobin was purified following a method previously reported (Álvarez et al., 2012) using blood from a local slaughterhouse (Junquera-Bobes, Noreña, Spain).

2.1. Sub-critical water hydrolysis coupled to oxygen injection

The hydrolysis was carried out as outlined in Álvarez et al., (2012) involving the use of high purity oxygen (99.8%) instead of nitrogen as gas injected in the reactor (Fig. 1). Samples were withdrawn at regular intervals during the hydrolysis process over a

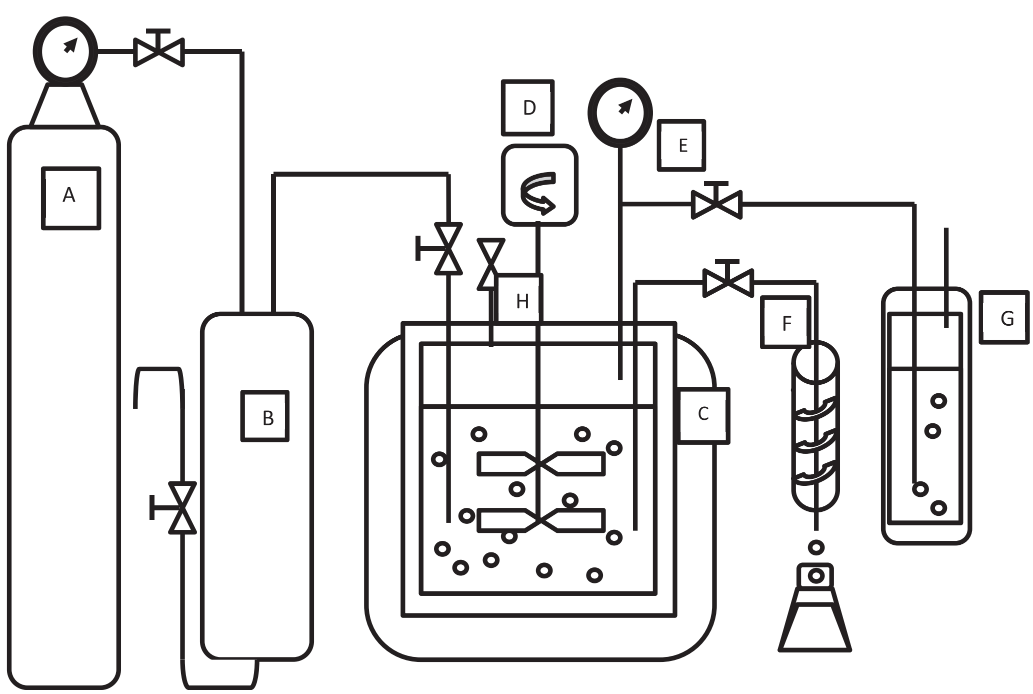

Fig. 1. Scheme of the experimental setup. A: gas tank; B: humidifier; C: jacketed reactor; D: agitation; E: back-pressure valve; F: sample cooler; G: bubbler; H: rupture disk.