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Pasteurisation of liquid whole egg: Optimal heat treatments in relation to its functional, nutritional and allergenic properties

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ABSTRACT

Pasteurised liquid egg products represent around 70% of total egg products. Pasteurisation, necessary for microbiological safety, is a unit operation undergone by the totality of liquid whole egg commercialized. The present work aims to investigate the impact of a wide range of pasteurisation rates (among those classically used in industry) on the interfacial properties, *in vitro* digestibility and antigenicity of whole egg proteins, in order to optimize the unit operation parameters to ensure all whole egg qualities. Pasteurisation from 4 to 10 min at 60 °C improved protein interfacial properties, *in vitro* digestibility and decreased ovomucoïd antigenicity but did not significantly change ovotransferrin and lysozyme antigenicity. On the opposite, pasteurisation from 4 to 10 min at 66 °C decreased protein *in vitro* digestibility and increased drastically ovotransferrin antigenicity. Pasteurisation from 4 to 10 min at 60 °C enabled to reach all whole egg properties, but for safety reasons, higher pasteurisation rates at 62 or 64 °C are good compromises to ensure microbiological safety without excessively damaging the functional properties.

1. Introduction

Egg is a widely used ingredient in many food products. Especially, whole egg is indeed a major source of high quality proteins and essential nutrients and provides many desirable functional attributes such as foaming, emulsifying, gelling, colouring, flavouring (Yang and Baldwin, 1995). However, hen egg is also one of the leading causes of food allergy in childhood, affecting 1.6–3.2% of young children (Eggesbo et al., 2001).

In the egg product industry, microbiological safety of liquid products is mainly guaranteed by pasteurisation. The USDA requires that liquid whole egg is at least heated at 60 °C for no less than 3.5 min, but in the United Kingdom the recommendations are to pasteurize at least at 64 °C for 2.5 min (Cunningham, 1995). In France, there is no statutory heat treatment; only microbiological results are determined by regulations. To achieve this, the treatments classically used to pasteurize whole egg vary from 65 to 68 °C for 2–5 min in order to ensure 5 to 6 decimal reductions of vegetative micro-organisms and especially *Salmonella* Enteritidis and *Listeria monocytogenes* (Baron et al., 2010). Pasteurisation temperatures used in the egg industry are limited by the sensitivity of egg proteins to heat treatment. Thus, pasteurisation for 2–10 min from 60 to 68 °C modifies whole egg electrophoretic pattern by especially decreasing ovotransferrin, livetin, ovalbumin, apovitellenin, lysozyme and/or ovomucin band intensity (Dixon and Cotterill, 1981; Martinez et al., 1994; Mendes de Souza and Fernandez, 2013; Scholtyssek et al., 1981; Woodward and Cotterill, 1983). Moreover, an increase of whole egg viscosity is observed at temperature as low as 56 °C (Cunningham, 1995), and whereas pasteurisation has little influence on liquid whole egg

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functionality when heated up to 60 °C (Ball et al., 1987; Cunningham, 1995; Herald and Smith, 1988), heating above this temperature decreases foaming and emulsifying properties (Janssen, 1971; Montfort et al., 2012), custard height (Ball et al., 1987; Cunningham, 1995) or pie filling expansion (Herald and Smith, 1988).

The high content of highly digestible proteins in whole egg is of great benefit to human nutrition. However, the effects of industrial processing such as pasteurisation on the nutritional quality of whole egg proteins are poorly documented. Van der Plancken et al. (2002) and Jimenez-Saiz et al. (2011) showed that ovalbumin susceptibility to hydrolysis was increased after heat treatment higher than 10–30 min at 60 °C, whereas the ovomucoid susceptibility was not changed after 30 min at 60 °C. Assuming that pasteurisation results in egg protein denaturation and/or aggregation, whole egg pasteurisation may hide, destroy or unmask protein allergenic epitopes. Moreover, the potential changes in protein digestion can also change the ability to sensitize and elicit the immune response (Jimenez-Saiz et al., 2011). The effect of heat treatment on the allergenic properties of egg proteins has been focused on cooking since some allergic children tolerate cooked eggs (Lemon-Mulé et al., 2008). Moreover, egg white proteins have been mainly studied since they are considered more allergenic than yolk proteins (Kosti et al., 2013), despite apovitellenins I & VI, α-livetine and phosvitin were reported as allergens by Mine and Yang (2008). In most cases, physicochemical changes caused by cooking either enhance a decrease or have no significant effect on allergenicity, depending on the susceptibility of the proteins to unfold and to lose their conformational epitopes. But in some cases, aggregates which are more allergenic and more resistant to digestion can be generated (Thomas et al., 2007). Up to the authors' knowledge, no study has been performed to test the effect of pasteurisation on whole egg antigenicity.

Consequently, if the effects of pasteurisation on whole egg functional properties have been well documented, there is a lack of knowledge on the effect of this unit operation on whole egg protein digestibility and antigenicity. The present study aimed at investigating the effect of a wide range of pasteurisation rates on the interfacial properties, *in vitro* digestibility and antigenicity of whole egg proteins, and to determine the best conditions to optimize these different functionalities.

2. Material and methods

2.1. Experimental design

Raw liquid whole egg was supplied by Ovoteam (Plaintel, France). Dry matter was adjusted to 22% by adding raw egg white (Ovoteam, Plaintel, France). Homogenised whole egg was obtained from raw liquid whole egg using a two-stage high-pressure homogeniser (Rannie LAB 16/15, APV France, Evreux, France) at 5 MPa. Heat treatments of homogenised whole egg were performed with an indirect tubular processing system (Microthermics UHT/HTST Lab 25 EDH, Microthermics, Inc., Raleigh, NC 27615, USA) according to the following complete factorial design 4^3: whole egg was heated to 60, 62, 64 or 66 °C and held at temperature for 4, 7 or 10 min. The pasteurisation temperature curves are given in Supplementary Data 1. After cooling to 25 °C, glass bottles (volume 250 mL) were filled and then frozen. Homogenised raw whole egg was also frozen as a control. The time-temperature rates applied enables to reach from 23 to 1243 decimal reductions *of Salmonella enteritidis* according to D and z values of these bacteria in whole egg (Jin et al., 2008).

2.2. Interfacial properties

2.2.1. Langmuir isotherms at air-water interface

Measurements of the surface pressure (Π) - surface area (*A*) isotherms have been performed by compression – expansion cycles using the Wilhelmy plate method as described by Lechevalier et al. (2017) except that egg white solutions was replaced by liquid whole egg diluted in 50 mM phosphate buffer pH 7, NaCl 300 mM to a final concentration of 1 mg ml^{-1} after measurement of protein content according to Markwell et al. (1978).

The surface corresponding to an interfacial pressure of 10 mN m^{-1} (S_{10}) was selected as the indicator of the sample behaviour at the air-water interface.

2.2.2. Emulsifying properties

2.2.2.1. Preparation of oil-water emulsions. Emulsions were prepared as described by Rannou et al. (2015) with the aqueous phase being 1.5% (w/v) whole egg protein solutions in phosphate buffer pH7, NaCl 300 mM.

2.2.2.2. Droplet size distribution. The droplet size distribution (DSD) was determined as described by Lechevalier et al. (2017). The volumetric mean diameter ($d_{4,3}$) was selected to measure droplet size distribution.

2.2.2.3. Emulsion stability. Emulsion stability towards creaming was determined as described by Lechevalier et al. (2017). The creaming index (Cr_i) was calculated as follows:

$$Cr_i = \frac{Va}{Vt} * \frac{100}{\phi} \quad (1)$$

where V_a is the volume of separated aqueous phase, V_t is the total volume of sample in the tube after centrifugation, and Φ is the volumic fraction of the aqueous phase used to prepare the emulsion (i.e. 0.7). The higher the creaming index, the lower the emulsion stability, since it assumes a high release of aqueous phase due to a high compaction of droplets with centrifugation.

2.3. Protein in vitro digestion and characterization of digested samples

2.3.1. In vitro digestion

Each pasteurised whole egg sample and the control sample were subjected to an *in vitro* digestion model which simulates successive gastric and intestinal stages of digestion in the adult human as proposed by Dupont et al. (2010) and detailed by Lechevalier et al. (2015). One ml of protein solution was aliquoted before digestion (gast ti), at the end of the gastric stage (gast tf) and at the end of the intestinal stage (int tf).

2.3.2. Degree of hydrolysis (DH)

The DH was calculated from the measurement of amino groups released during *in vitro* digestion using OPA (orthophtaldialdéhyde) according to Church et al. (1983), modified as described by Lechevalier et al. (2017). The hydrolysis degree (DH) was calculated using the following formula:

$$\%\,DH = (L_t - L_0)/L_{tot} \times 100 \quad (2)$$

where L_t is the amount of free NH_2 after t min hydrolysis, L_0 is the amount of initial free NH_2 (before digestion), and L_{tot} is the maximum amount of free NH_2, i.e. after total acid hydrolysis. Free NH_2 was expressed in mg per g of protein. The DH at the end of the gastric and the intestinal stages were kept as results.