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Effect of cooking methods and intestinal conditions on lipolysis, proteolysis and xanthophylls bioaccessibility of eggs

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ABSTRACT

Digestibility of macro and micronutrients depends on the ingested food as well as on gastrointestinal conditions, being those suboptimal in exocrine pancreatic insufficiency (EPI) patients. Under this scenario, oral enzyme supplementation improves enzymatic hydrolysis of nutrients. In this study, a static in vitro model was used to assess lipids and protein digestibility as well as lutein and zeaxanthin bioaccessibility of eggs cooked differently and submitted to different intestinal conditions. Boiled, poached and omelette eggs were digested under different intestinal conditions of pH (6 or 7), bile concentration (1 or 10 mM) and doses of the enzyme supplement (1000–4000 LU/g fat). Results showed that poaching resulted in higher digestibility of lipids and proteins, compared to boiling or omelette preparations, under gastrointestinal conditions of EPI (pH 6, bile 1 mM). Concerning xanthophylls bioaccessibility, boiling and poaching led to higher bioaccessibility of lutein and zeaxanthin than omelette under EPI conditions.

1. Introduction

Egg has lately gained attention as a food to be considered into a healthy diet mainly due to its high protein content together with egg yolks antioxidant composition. Concretely, egg white contains around 10% of high quality protein with a Protein Digestibility Corrected Amino Acid Score (PDCAAS) value of 1. It includes albumins, mucoproteins and globulins, being ovalbumin (OVA) the main protein of egg white which represents 54% of egg white protein (Sponton, Perez, Carrara, & Santiago, 2015; Weijers, Sagis, Veerman, Sperber, & van der Linden, 2002). Egg yolk, on the other hand, is considered among many food types, one of the most important sources of xanthophylls with higher bioavailability than other common sources such as dark-green leafy vegetables (Nimalaratne & Wu, 2015; Nimalaratne, Lopes-Lutz, Schieber, & Wu, 2012; Seuss-baum, 2007; Sunwoo & Gujral, 2015). Xanthophylls, the yellow pigments of egg yolk, are oxygenated carotenoids which all-E-isomeric form predominates in nature. However, processing conditions such as stirring, heating, light, and oxygen exposure may lead to some different changes in protein and lipid digestibility, which may result in changes of the functionality and bioavailability of egg nutrients (Dugave & Demange, 2003; Nimalaratne et al., 2012; Schieber & Carle, 2005). Although the main xanthophylls present in egg yolk are lutein and zeaxanthin, other bioactive compounds such as vitamin E and omega-6/3 polyunsaturated fatty acids are also present (Sunwoo & Gujral, 2015). Due to the lipophilic nature of these compounds, their absorption is closely related to the digestion of lipids. Thus, egg yolk can be an ideal food matrix to deliver highly bioavailable xanthophylls; indeed, bioavailability of lutein from lutein-enriched egg yolk was found to be greater than from lutein supplements or spinach (Chung, Rasmussen, & Johnson, 2004; Handelman, Nightingale, Lichtenstein, Schaefer, & Blumberg, 1999; Nimalaratne, Savard, Gauthier, Schieber, & Wu, 2015).

In order to be bioavailable, lipophilic compounds will have to be released from their food matrix and micellarized, becoming then absorbable (bioaccessible), which means they can be absorbed by intestinal cells and be metabolized (Faulks & Southon, 2005; Nimalaratne et al., 2015). Bioaccessibility of nutrients will depend on different factors related to the food itself such as food matrix, its composition, type of nutrients, processing and cooking methods (Granado-Lorencio et al., 2007; Nimalaratne et al., 2015; Pineda-Vadillo et al., 2017; Ryan, O'Connell, O'Sullivan, Aherne, & O'Brien, 2008). Thus, analyzing the extent to which food matrix and processing can modify the stability, and the bioaccessibility of bioactive compounds is an essential first step for better understanding the actual biological activity of food constituents (Rodríguez-Roque et al., 2015). Furthermore, absorption of this lipophilic bioactive compounds will also depend on individual factors such as gastrointestinal conditions (pH, secretion and composition of the digestive fluids, transit time…) (Ryan et al., 2008; Whitcomb et al., 2010). All this might modify the extent of digestion

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and micellarization in the small intestine, and therefore, to absorb liposoluble compounds (Panozzo et al., 2013; Pineda-Vadillo et al., 2017).

Gastrointestinal environment will vary within different individuals depending on their age, gender, diet, etc. (Shani-Levi et al., 2017); these differences however, can become even more relevant under specific digestive disorders. **It** is the case of Exocrine Pancreatic Insufficiency (EPI), which is a disorder associated to several diseases such as pancreatic cancer, chronic pancreatitis (CP) or cystic fibrosis (CF). The obstruction of the pancreatic duct in EPI, produces an insufficient secretion of sodium bicarbonate and pancreatic juice, containing digestive enzymes. Besides this lack of digestive enzymes, the decrease of pancreatic juice may also decrease the intestinal pH, leading to nutrients mal-digestion and mal-absorption (Layer & Keller, 2003; Naikwade, Meshram, & Bajaj, 2009; Whitcomb et al., 2010). Due to pancreatic lipase is the main responsible of lipolysis (Carrière et al., 2000; Sikkens, Cahen, Kuipers, & Bruno, 2010), this scenario compromises lipids' hydrolysis and absorption, leading therefore to a deficit in fat-soluble vitamins (A, D, E and K) as well as other bioactive compounds, causing malnutrition. The current treatment for EPI involves oral administration of an enzymatic supplement in order to improve nutrients digestion and absorption (Armand, Fieker, & Philpott, 2011). Nowadays, the current guidelines for EPI recommend an enzyme dose of 2000–4000 Lipase Units (LU)/g fat intake, being the only available parameters to guide health professionals on adjusting the prescribed doses, based on the overall fat content of the meals or on patients body weight (Turck et al., 2016). However, the optimal doses are still uncertain since satisfactory levels of fat absorption are not often achieved as they depend on food factors as well as on gastrointestinal (GI) conditions.

Since human studies might give very precise information on the bioaccessibility of nutrients, due to its high cost, technical difficulty and ethical reasons, alternative methods are generally used. *In vitro* digestion methodologies represent therefore, a good approach to mimic *in vivo* luminal digestion and to assess the bioaccessibility of bioactive compounds (Faulks & Southon, 2005; Hur, Lim, Decker, & McClements, 2011; Minekus et al., 2014; Pineda-Vadillo et al., 2017).

To the authors knowledge, there are already some studies focusing on lipids absorption and antioxidants bioaccessibility using egg or egg based food matrices (Chung et al., 2004; Handelman et al., 1999; Nimalaratne et al., 2015; Pineda-Vadillo et al., 2017). However, in all of them, gastrointestinal conditions were simulated according to a standard healthy adult. Therefore, the aim of the present study was to in vitro evaluate the influence of some intestinal factors associated to EPI (intestinal pH, bile concentration and the amount of enzyme supplement), as well as the effect of cooking procedure on lipids digestibility and xanthophylls bioaccessibility in eggs.

2. Materials and methods

2.1. Materials

Pancreatic enzymes supplements (Kreon 10,000 lipase units (LU)) were used to simulate in vitro digestion of an individual with EPI. Each capsule contains 150 mg of gastro-resistant microspheres containing porcine pancreatic enzyme equivalent to 10,000 lipase U., 8000 amylase U., and 600 protease U. The specific lipase activity of the Kreon was usually measured before the experiments (Carrière et al., 2000) and the amount of supplement added to the gastric stage was adjusted always to have the corresponding LU/g fat according to the experimental design.

For the preparation of the simulated digestive fluids (Table 1), the following chemicals were needed: pepsin from porcine gastric mucosa ($\geq$2500 U/g protein), bovine bile extract, KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2$ $(H2O)_6$, $(NH_4)_2CO_3$ and $CaCl_2$ all of them from Sigma-Aldrich Chemical Company (St Louis, MO, USA). NaOH (1N) and HCl (1N), were acquired from AppliChem Panreac. For the analytical determinations, Triton-X 100%, petroleum ether, trichloroacetic acid (TCA), hexane, methanol, acetone, bovine serum albumin (BSA), methyl tert-butyl ether (MTBE), crystalline urea as well as the analytical standards of palmitic acid, lutein and zeaxanthin were all acquired from Sigma-Aldrich.

Table 1
Composition of simulated digestion fluids.

Constituent	SSF mmol/L	SGF mmol/L	SIF mmol/L
KCl	15.1	6.9	6.8
KH_2PO_4	3.7	0.9	0.8
$NaHCO_3$	13.6	25	85
NaCl	–	47.2	38.4
$MgCl_2(H_2O)_6$	0.15	0.1	0.33
$(NH_4)_2CO_3$	0.06	0.5	–
$CaCl_2$	1.5	0.15	0.6

The addition of pepsin, Ca^{2+} solution and water will result in the correct electrolyte concentration in the final digestion mixture.
SSF: Simulated Salival Fluid; SGF: Simulated Gastric Fluid; SIF: Simulated Intestinal Fluid.

2.2. Sample preparation

Eggs were purchased from a local supermarket and divided into four equal sets before their use for the experiments that were performed at least 2 weeks prior to the expiry date. One set was used to characterize the raw product and the other three sets were used to analyse the influence of different cooking ways (boiled, poached and omelette). For the boiling, whole shell eggs were placed in a cooking pan, with boiling water covering the eggs, and they were boiled for 10 min (99 ± 1 °C) (Nimalaratne et al., 2012). After boiling, the whole eggs were placed under running tap water for 5 min, and they were peeled right after. For poaching, eggs were broken into parafilm and then wrapped before boiling them into a pan filled with boiling water for 4 min (99 ± 1 °C). After that, the parafilm wraps were placed under running tap water for 5 min. For omelette, eggs whites and yolks were mixed by stirring for 60 s, placed in a microwavable plate and cooked in a household microwave oven (model GW72N, Samsung) for 80 s at 750 W, 2450 MHz). After cooking, the samples were in vitro digested by using a static system.

2.3. In vitro digestion

Cooked yolks and whites of poached and boiled eggs were separated and sampling was made by weighing both parts in the same proportion as they would appear in a whole cooked egg; in the case of the omelette, raw yolk and white were weighted and added to keep the same proportion of both parts as in the whole egg prior to preparation. The amount of cooked samples to be digested was weighted in order to have 0.35 g fat in each tube (50 ml falcon tubes). Fat content in fresh and cooked eggs was determined previously at the digestion by the official Soxhlet method (AOAC, 2000). The digestion procedure used was based on the standardized static in vitro digestion method for food published by Minekus et al. (2014) with some modifications in order to allow analyzing EPI conditions. Table 1 illustrates the amounts and composition of the fluids required in each of the stages of the digestion process. The digestion fluids were prepared fresh daily from stock solutions, salival (SSS), gastric (SGS) and intestinal (SIS) prepared according to Minekus et al. (2014). The enzymatic activity was tested before each experiment following the protocol proposed by Carrière et al. (2000). Each experimental condition was performed in triplicate. The in vitro digestion process was performed as follows:

Oral stage: Simulated salival fluid (5 ml) (SSF; pH 8) at 37 °C, was added to the egg sample in a ratio 1:1 (v/w) and properly homogenized

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