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Effect of digestive process on Maillard reaction indexes and antioxidant properties of breakfast cereals

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ABSTRACT

Extrusion, drying and toasting are the most representative manufacturing processes suffered by breakfast cereals, conjugating thermal/moisture conditions that allow the Maillard reaction (MR) and caramelisation development, as well as the destruction of thermally labile antioxidant compounds. However, other compounds – like Amadori products and hydroxymethylfurfural (HMF) – are originated from the MR and caramelisation, showing different biological activities, as the antioxidant activity. But breakfast cereals are ingested and then affected by the digestive process, and so that the aim of this work was to analyse the effects of the digestion on the bioaccessibility of certain MR products (Amadori compound and HMF) and on the antioxidant activity of corn-based breakfast cereals, using a standardized *in vitro* gastrointestinal digestion. After digestion approx. 90% HMF remained soluble, but in some cases HMF distribution between soluble/insoluble fractions was higher than the initial HMF measured in the raw cereal, suggesting a release of initially bound Amadori products and its conversion to HMF during the digestion process. The Amadori compound was uniformly distributed between both fractions. The antioxidant activity of the soluble fraction was always higher than that from the raw cereal in any of the antioxidant method employed; therefore the digestion increased the solubility of the antioxidant compounds.

1. Introduction

Breakfast cereals have currently become an important source of energy in human nutrition. Approximately 50% of the Spanish infant population consumes this food in the breakfast (Montaner, 2004), since its high carbohydrate, bran, mineral and vitamins content, together with a low fat amount, makes it suitable for a healthy breakfast (Bertais et al., 2000).

The main manufacturing process suffered by these foods in the industry is extrusion, which adequately modifies the texture of the raw material. Drying and toasting are also conducted during the final steps of breakfast cereals elaboration, where the application of high temperature (>180 °C) in combination with low moisture (<15%) favour the development of Maillard reactions (MR) (Cheftel, 1986). The MR is a complex chain of chemical reactions that involves reducing sugars and protein or amino acids, and whose products (MRP) are responsible of the brown colour and many of the organoleptic properties of foods (Borrelli et al., 2003; Rufián-Henares, Guerra-Hernández, & García-Villanova, 2004). Besides the MR, during the extrusion and the drying-toasting steps the sugar caramelisation takes also place. Moreover, starch and non-reducing sugars may be hydrolysed to form reducing sugars that can participate in both, Maillard and caramelisation reactions. Therefore, the heating load applied to the raw cereal leads to the formation of some new compounds; among others, the Amadori compounds, formed exclusively via MR, and hydroxymethylfurfural (HMF), coming both from the MR and caramelisation. The first one is analytically determined as furosine, the amino acid formed during acid hydrolysis of the Amadori compounds, originated in the early stage of the Maillard reaction from the interaction of the ε-amino groups of lysine with glucose, lactose and maltose (Nursten, 1980). Furosine has been considered a useful indicator of the degree of damage during the initial steps of the MR in cereal products such as pasta (Resmini, Pagani, & Pellegrino, 1991), commercial baby cereals (Guerra-Hernández, Corzo, & García-Villanova, 1999), bread (Ramírez-Jiménez, Guerra-Hernández, & García-Villanova, 2000) and breakfast cereals (Delgado-Andrade, Rufián-Henares, & Morales, 2007). Although less valuable as index of thermal damage in cereal-derived products but getting important due to the possible mutagenic activity of its *in vivo* metabolites (Surh & Tannenbaum, 1994), HMF has also been detected in these foodstuffs (García-Villanova, Guerra-Hernández, Martínez-Gómez, & Montilla, 1993; Guerra-Hernández, García-Villanova, & Montilla-Gomez, 1992; Resmini, Pellegrino, Pagani, & De Noni, 1993; Ramírez-Jiménez, García-Villanova, & Guerra-Hernández, 2000; Rufián-Henares, Delgado-Andrade, & Morales, 2006a).

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As it is well-established, cereals are an important source of natural antioxidants mainly due to their fibre content (Bertais et al., 2000). Among cereals grains, barley and oats are rich in soluble fibre components, and the maize fibre contains heteroxylans, cellulose and approximately 4% of phenolic acids (essentially ferulic and diferulic acids bound to the cell wall polysaccharides) (Fry, 1986; Carvajal-Millan, Rascón-Chu, Márquez-Escalante, Ponce de León, & Garcea, 2007). But, as in many other foodstuffs thermally treated, a portion of the natural antioxidant compounds present in the raw material can be destroyed during the cereal processing. However, the possible contribution of neo-formed compounds during cereal manufacturing to the final overall antioxidant activity must be valued. In this sense, MRP are key factor since is antioxidant activity is demonstrated both *in vitro* (Morales & Jiménez-Pérez, 2001) and *in vivo* (Goya, Delgado-Andrade, Rufián-Henares, Bravo, & Morales, 2007). Thus, previous assays of our research group working with coffee brews obtained from coffee beans with different roasting degree have shown the maintaining of the antioxidant activity due to the appearance of antioxidant MRP that counteracts the phenolic compounds destruction (Delgado-Andrade & Morales, 2005; Delgado-Andrade, Rufián-Henares, & Morales, 2005a).

There are only limited data about the effects of the digestive process on the MRP contained in foods and their availability, as well as on the effects of digestion on their antioxidant activity. A recent research on HMF availability from digested breakfast cereals using the Caco-2 cell line has established a low HMF availability from these foods, highly influenced by the composition. It must be underlined that HMF recovery after the *in vitro* digestion of the ready-to-eat cereal employed was incomplete, suggesting some type of transformation of the compound during the digestion process (Delgado-Andrade, Seiquer, Navarro, & Morales, 2008). In the same way as MRP can be transformed in the digestive tract, their MRP-associated antioxidant activity can be also affected, as Rufián-Henares and Morales (2007a) demonstrated working with *in vitro* digested coffee melanoidins (final and high molecular weight MRP). The use of a previous *in vitro* gastrointestinal digestion of the samples is a first approach to predict the *in vivo* antioxidant activity of a food, despite with this procedure only the soluble fraction is considered while the possible action of the insoluble portion is dismissed. However, since it is not very realistic, from a physiological point of view, to employ some of the drastic extraction procedures using solvent mixtures or physical treatments in order to asses the antioxidant activity of insoluble food components (Emmons, Peterson, & Paul, 1999; Zielinski & Kozłowska, 2000; Martínez-Tomé et al., 2004; Serpen, Capuano, Fogliano, & Gökmen, 2007), the *in vitro* gastrointestinal digestion is a staring point to predict the *in vivo* antioxidant power of a food, taking into account that this procedure underestimates it in some extent.

The aim of the present work was to establish the effect of the *in vitro* gastrointestinal digestion on the bioaccessibility of certain MRP as the Amadori compounds (measured as furosine) and HMF, and on the antioxidant activity of corn-based breakfast cereals. The effects of composition, especially regarding to the fibre content, were analysed.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), potassium persulphate, pepsin, pancreatin and bile salts were purchased from Sigma (St. Louis, MO, USA). 2,2′-azobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were from Fluka Chemicals (Madrid, Spain). $FeCl_3 \cdot H_20$, $NaHCO_3$, potassium ferrocyanide, zinc acetate, HCl and methanol were purchased from Panreac (Barcelona, Spain).

2.2. Samples

Six commercial packaged breakfast cereals products were collected from supermarkets. Three samples were corn flaked breakfast cereals (named standard cereals) and another three samples were fibre-enriched corn breakfast cereals (named fibre-enriched). The whole product contained in each package was powdered and homogenised, portions were placed in polyethylene containers, sealed under vacuum and stored at 4 °C prior to analyses. Given the importance of protein content for the furosine analysis, protein determination was performed in our laboratory, although no significant differences were found between our data and the nutritional information obtained from manufacturers.

2.3. In vitro digestion of breakfast cereals

The technique of Miller, Schricker, Rasmussen, and Van Camper (1981), modified to our requirements, was followed. It comprised two stages: gastric digestion and intestinal digestion. Shortly before use, 0.4 g of pepsin was dissolved in 2.5 ml of 0.1 M HCl. For intestinal digestion, 0.1 g of pancreatin and 0.625 g of bile salts were dissolved in 25 ml of 0.1 M $NaHCO_3$. One gram of each breakfast cereal was brought to a final volume of 10 ml of milli-Q water. pH was adjusted to 2 with HCl 6 N, a pepsin solution was added at a proportion of 0.05 g of pepsin/g of sample, and samples were incubated at 37 °C in a shaking water bath at 110 oscillations/min for 2 h for the gastric digestion. For the intestinal digestion, the pH of the digest was raised to pH 6 with 1 M $NaHCO_3$ dropwise, and 2.50 ml of pancreatin + bile salts mixture was added. The pH was then adjusted to pH 7.5 with 1 M $NaHCO_3$, and samples were incubated at 37 °C at 110 oscillations/min for 2 h. After gastrointestinal digestion, the digestive enzymes were inactivated by heat treatment for 4 min at 100 °C in a polyethylenglycol bath. The samples were then cooled by immersion in an ice bath and centrifuged at 3200*g* for 60 min at 4 °C (CS-6R centrifuge, Beckman) to separate soluble and non-soluble fractions. In both of them furosine, HMF as well as the antioxidant activity were measured.

2.4. Furosine assay

Furosine determination was performed following the method described by Resmini and Pellegrino (1991) in order to avoid some interferences with other furoylmethyl-aminoacids, which could interfere in the faster capillary electrophoresis method (Delgado-Andrade, Rufián-Henares, & Morales, 2005b). Samples containing 6.5 mg of protein per ml of 7.95 M HCl were bubbled with high-purity N_2 gas for 2 min and then hydrolyzed at 120 °C for 23 h in a Pyrex screw-cap vial with PTFE-faced septa. The hydrolysate was filtered with a medium-grade paper filter. A 0.5 ml portion of the filtrate was applied to a Sep-pack C_{18} cartridge (Millipore, MA) prewetted with 5 ml of methanol and 10 ml of deionized water and was then eluted with 3 ml of 3 M HCl. Fifty microliters of the solution was analysed by ion-pair RP-HPLC, which consisted on a Waters model 600 quaternary gradient bomb (MA, USA) and a UV/Vis detector model 200 from Konik (Barcelona, Spain) set at 280 nm. The analytical column was a C_8 column (250 mm × 4.6 mm i.d.) from Altech (Furosine dedicated) thermostated at 32 °C. Quantisation was performed by the external standard method within the range 0.01–1000 mg/l, using a commercial standard of pure furosine (Neosystem Laboratoires, Strasbourg, France). The analysis was performed in duplicate and the data are the mean values expressed as milligrams per 100 g of protein.

2.5. HMF assay

The analysis of HMF in undigested breakfast cereals was based on the method of Rufián-Henares, Guerra-Hernández, and García-