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A combined procedure to evaluate the global antioxidant response of bread

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ABSTRACT

Baking is the most representative manufacturing process applied to bread, involving thermal and moisture conditions that facilitate the Maillard reaction (MR) and, at the same time, the destruction-formation of natural-labile and thermally-induced antioxidant compounds respectively. In the present paper, the use of a new approach to measure the Global Antioxidant Response (GAR) of cereal derivatives is proposed: a combination of *in vitro* digestion – which enables measurement of the bioaccessible fraction – and the QUENCHER, which makes it possible to determine the antioxidant activity of the insoluble fraction, since it is a simple and direct procedure for determining the total antioxidant capacity of solid products. After digestion, the results obtained by the antioxidant assays are up to 20-fold higher than those reported using the standard extraction methods. The non-extractable residue displayed significant antioxidant activity that accounted for up to 17% of the total antioxidant activity. Moreover, the GAR obtained in some of the assays developed was 10–40% higher than the antioxidant activity registered by the QUENCHER procedure in wheat bread, and the difference was even higher in wheat bran bread. Therefore, the use of the GAR approach could avoid underestimation of the antioxidant activity of cereal derivatives.

1. Introduction

During bread baking, various modifications in the chemical composition and properties of the food matrix take place (Kroh, 1994; Wagner et al., 2002), leading to changes in the nutritional value of the final product. Several authors have shown that caramelisation and the Maillard reaction (MR) are directly involved in these changes, producing newly formed compounds responsible, on the one hand, for different biological activities (Rufián-Henares et al., 2007) and, on the other, for improving organoleptic characteristics of bread, such as its appearance and taste (Michalska et al., 2008; Zielínski et al., 2008).

Among the most interesting biological activities associated with such newly formed compounds are their antioxidant properties (Delgado-Andrade and Morales, 2005; Michalska et al., 2008; Rufián-Henares and Delgado-Andrade, 2009). Of course, other bread components can also contribute to its total antioxidant capacity (TAC), for example, the phenolic compounds (Andreasen et al., 2000), fibre (Bertais et al., 2000) or tocols (including vitamin E) (Shewry, 2009) present in the bread flour. But the outstanding feature of Maillard reaction products (MRP) is their generation during heat treatment, while, on the contrary, many of the former are degraded by heating (Del Castillo et al., 2002).

Many simple procedures have been applied to assess the TAC of bread (Martinez-Villaluenga et al., 2009; Michalska et al., 2008), but the limiting step in all of them is the solubility of the compounds responsible for the antioxidant activity in the reaction media. For this reason, alcoholic and aqueous reaction media, as well as enzymatic hydrolysis, have been applied to extract the active moieties from the food matrix (Pérez-Jiménez and Saura-Calixto, 2005). But whatever the extraction procedure, there is always an insoluble fraction of antioxidant material present in the food, among which insoluble proteins, tannins and melanoidins (final products of the MR) must be considered (Gokmen et al., 2009). Therefore, the TAC of foods is systematically underestimated. To overcome this problem, Gokmen et al. (2009) developed a direct procedure called QUENCHER to evaluate the TAC of foods without the extraction step, and thus, work with the whole antioxidant material present.

However, *in vivo*, the food consumed is conducted toward gastrointestinal digestion, which releases entrapped antioxidant structures of the food matrix. Moreover, other antioxidant molecules may remain enclosed in the non-digestible fraction and they, or their metabolites after the microflora action, could act *in situ* or to some extent be absorbed in the final portions of the intestine. Under laboratory conditions, when food is subjected to *in vitro*

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gastrointestinal digestion, it is usual to analyse only the activity of the bioaccessible fraction obtained and to discard the non-digested fraction (Rufián-Henares and Delgado-Andrade, 2009). The QUENCHER procedure provides the possibility of evaluating the antioxidant activity corresponding to the latter portion.

The goal of the present work is to combine two different procedures to evaluate the Global Antioxidant Response (GAR) of food in general, and bread in particular, under physiological conditions. On the one hand, gastrointestinal digestion is conducted in order to release the *in vivo* available antioxidant compounds; the antioxidant capacity of the bioaccessible fraction is then measured using a conventional procedure. On the other, the non-digestible portion is also considered and its antioxidant activity evaluated by the QUENCHER procedure. The joint use of these procedures ensures the measurement of the GAR of a food as it could occur *in vivo*. Simultaneously, a study of the protein profile during different steps of the *in vitro* gastrointestinal digestion is addressed to evaluate the relationship between the release of proteins during the digestive process and the progress of the GAR.

2. Experimental

2.1. Chemicals

Alpha-amylase (A1031-5KU) was from Sigma–Aldrich (St. Louis, MO, USA), pepsin, pancreatin and bile salts were purchased from Sigma (St. Louis, MO, USA).

2.2. Samples

Wheat and wheat bran breads (W and WB, respectively) commercialised as pre-baked breads to be finally baked at home, were kept in an air oven (Selecta, Spain) for 0, 12, 20 and 30 min at 200 °C, and, after aeration, they were lyophilised. The breads heated for 20 min were selected for the study of *in vitro* digestion and protein profile since the usual aspect of bread was obtained after that time.

2.3. In vitro digestion of bread

The technique of Miller et al. (1981), modified to include a previous oral step, was followed. Besides the usual gastric and intestinal digestion, oral digestion was also performed, using 250 μl of an alpha-amylase solution (32.5 mg of alpha-amylase dissolved in 25 ml 1 mM $CaCl_2$ pH 7.0) per gram of lyophilised sample. The mixture was then incubated at 37 °C for 30 min. After this step, the usual gastric digestion followed by the intestinal one was performed as described in Rufián-Henares and Delgado-Andrade (2009). The soluble fraction of this completely digested sample is termed the total soluble fraction (TSF) and the insoluble fraction, the total insoluble fraction (TIF).

For the study of the protein profile during the different steps of the *in vitro* gastrointestinal digestion and its relationship with the progress of the TAC, the same enzymatic digestion was conducted in the samples but stopping the protocol after the oral digestion or after the oral + gastric digestion. The soluble fraction obtained after the alpha-amylase digestion step is termed the amylase soluble fraction (ASF) and the soluble fraction obtained after the alpha-amylase + pepsin digestion steps, the pepsin soluble fraction (PSF). In the same way, the insoluble fractions are termed AIF and PIF (see Fig. 1).

2.4. Chemical extraction

The chemical extraction of antioxidants was performed following the procedure described by Pérez-Jiménez and Saura-

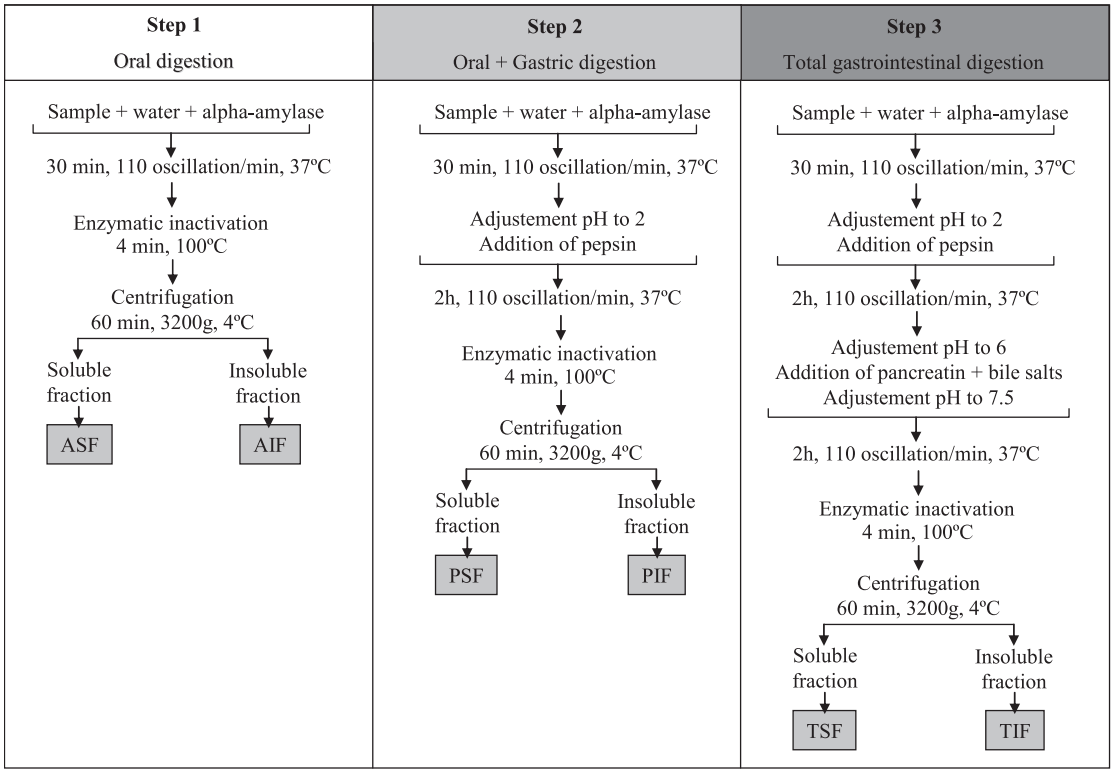

Fig. 1. Fractions obtained from both breads (W and WB) after different steps of the interruption of the *in vitro* gastrointestinal digestion for the study of the progress of the antioxidant activity. (ASF, soluble fraction after alpha-amylase action; AIF, insoluble fraction after alpha-amylase action; PSF, soluble fraction after alpha-amylase and pepsin actions; PIF, insoluble fraction after alpha-amylase and pepsin actions; TSF, total soluble fraction after complete *in vitro* gastrointestinal digestion; TIF, total insoluble fraction after complete *in vitro* gastrointestinal digestion).