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Analytical Methods

A physiologic approach to test the global antioxidant response of foods. The GAR method

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ABSTRACT

Several methods have been applied to measure antioxidants in foods. Extraction methods have previously relied on chemical methods which are non-physiological or based on enzymatic hydrolysis. Whatever the method used, the insoluble fraction is systematically excluded. The global antioxidant response (GAR) method use an *in vitro* approach with enzymatic digestion, designed to mimic digestion through the gastrointestinal tract, aimed to release antioxidants from foods. A total of 27 samples were analysed using the ABTS and FRAP assays applied to the soluble and insoluble fractions. The GAR method showed a higher antioxidant activity compared with the usual chemical extraction and the Quencher (direct) method. The soluble fraction was more antioxidant than the chemical extracts due to the release of compounds from the hydrolysis of carbohydrates and proteins. In addition, the GAR method allowed the measurement of the antioxidant activity in the insoluble fraction, which was important in fibre-rich samples.

1. Introduction

In the last decades there is a growing interest in the study of the antioxidant activity of foods and diets due to the known implications of oxygen free radicals in the progress and development of cardiovascular and neurodegenerative disease, aging, cancer, etc. (García-Parrilla, 2008). On this purpose, many different procedures have been developed to test the total antioxidant capacity (TAC) of foodstuffs (Pellegrini et al., 2003; Pérez-Jiménez et al., 2008). The different studies show that there are two key issues: (i) the antioxidant method applied and (ii) the extraction procedure of the antioxidant compounds. Regarding the first question, it is well-established that in the TAC determination it is mandatory the use of measurement procedures that include different mechanisms of action of antioxidant compounds, whether radical scavenging or metal-reducing activities. In relation to the second issue, the importance of the extraction procedure of the antioxidants (chemical aqueous–organic solvents or enzymatic hydrolysis) in the final results is also known. Thus, the solubility of the compounds responsible for the antioxidant activity in the reaction media uses to be a limiting step (Pérez-Jiménez et al., 2008), since whatever the extraction procedure, there is always an insoluble fraction of antioxidant material present in the food that is systematically discarded, among which insoluble proteins, tannins and melanoidins (final products of the Maillard reaction) must be considered (Gokmen, Serpen, & Fogliano, 2009). To overcome this problem, Gokmen et al. (2009) have recently 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 in solid stage.

From a physiologic point of view, the biological properties of antioxidants will depend on their release from the food matrix during the digestion process. Moreover, the non-extracted antioxidants after digestion will follow the intestinal transit, being at some extent released and metabolised by the microflora action, and then producing significant biological effects (Delgado-Andrade, Conde-Aguilera, Haro, de la Cueva, & Rufián-Henares, 2010).

Thus, the best approach to the physiologic conditions is the application of a gastrointestinal *in vitro* digestion to the foods before determining the TAC by any antioxidant assay. Previous studies of our research group have shown the increased antioxidant activity detected in the bioaccessible fraction after *in vitro* digestion of breakfast cereals when compared with the solvent extraction procedure (Rufián-Henares & Delgado-Andrade, 2009). The new QUENCHER procedure provides the possibility of evaluating the antioxidant activity corresponding to the inaccessible fraction after digestion, as it has been recently applied in pre-baked bread (Delgado-Andrade et al., 2010). The previous work establishes that the TAC of bread can be estimated by adding the antioxidant

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activity measured in the bioaccesible fraction after digestion, with a traditional antioxidant assay, and the antioxidant activity detected in the insoluble fraction (solid stage) by applying the QUENCHER procedure, in a the method called GAR (global antioxidant response).

The aim of the present work is to demonstrate the validity of the GAR method, a more physiologic approach, to determine the TAC of different fresh and cooked food matrixes. The daily intake of antioxidant compounds, as well as the amount per serving, from different food categories is also established.

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). For the ABTS and FRAP methods, the standard antioxidant used was 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), obtained from Sigma–Aldrich. 2,2′-azobis-(3-ethylbenzothiazoline-6-sulphonic acid) for the ABTS method and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) for the FRAP method were obtained from Fluka Chemicals (Madrid, Spain).

2.2. Samples

A total amount of 27 foods, fresh and cooked, were selected to cover different matrixes in which the GAR method, initially proposed for cereal products, would be tested. They were as follow: bran breakfast cereals, boiled spaghetti and boiled rice as cereal derivatives; fresh tomato, carrot, kiwifruit and apple and steamed spinach as vegetables and fruits group; grilled hake, grilled beef steak, fried sausages, salami-type sausage and boiled jam as meat and fish group; yoghurt and cheese as dairy products; dark chocolate and tiramisu as desserts; boiled lentils, red beans and chickpeas as legumes group; fried peanuts and roasted almonds as nuts group; olives and chips as snacks group; and boiled egg. In addition, wheat bread and wheat-bran bread, analysed in our previous study (Delgado-Andrade et al., 2010) were included again. Samples were freshly crushed, homogenised and frozen until analyses of antioxidant activity were carried out.

2.3. In vitro digestion of samples

The technique of Miller, Schricker, Rasmussen, and Van Camper (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). Once finished the *in vitro* gastrointestinal digestion the bioaccesible (soluble) and the non-accessible (or insoluble) fractions were separated. All these steps are summarised in Fig. 1.

2.4. Chemical extraction

The chemical extraction of antioxidants was performed following the procedure described by Pérez-Jiménez and Saura-Calixto (2005). Briefly, 0.5 g of fresh sample was placed in a tube and 5 ml of acidic methanol/water (50:50 v/v, pH 2) were added. The tube was thoroughly shaken at room temperature for 1 h and centrifuged at 2500*g* for 10 min, and the supernatant was recovered. Five millilitres of acetone/water (70:30, v/v) were added to the residue, and the shaking and centrifugation steps were repeated. The methanolic and aqueous–acetone extracts were then combined and the volume made up to 10 ml. A brief summary of the chemical extraction is shown in Fig. 1.

2.5. Antioxidant activity assays

The antioxidant activity was measured in the fresh soluble and the lyophilised insoluble fractions after the *in vitro* digestion, in the chemical extracts obtained as previously described and directly on the solid samples.

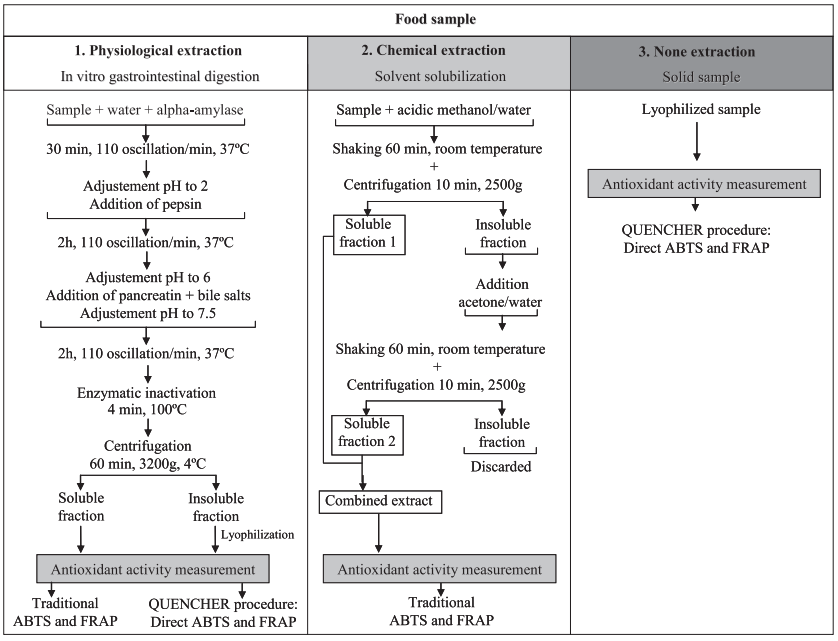

Fig. 1. General procedure of the chemical extraction, QUENCHER and GAR methods.