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Total antioxidant capacity of new natural powdered seasonings after gastrointestinal and colonic digestion

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ABSTRACT

New powdered seasonings, rich in natural antioxidant compounds, have successfully been applied recently in different food matrices. Once ingested, the antioxidants contained in these seasonings may exert protective effects against oxidative stress along the gastrointestinal tract. This fact was evaluated by submitting the different seasonings under study to simulated digestion followed by assessing the reducing and antiradical capacities of the digested fractions. Enzymatic gastrointestinal digestion enhanced 2–3 times both antioxidant activities and colonic fermentation increased more than 10-fold the radical scavenging ability of digested fractions compared with undigested seasonings. Digested fractions derived from the seedless wine pomace seasoning presented generally the highest antioxidant properties. The results were evaluated considering bioaccessibility factors to have a more realistic overview of the potential antioxidant capacities of the seasonings and of the probable beneficial effects of their consumption on the prevention of oxidative damage along the gut.

1. Introduction

Epidemiological studies and associated meta-analyses strongly suggest that long term consumption of fruits and vegetables plays a pivotal role in the prevention against numerous chronic diseases such as cancer (Pandey & Rizvi, 2009; Sun, Chu, Wu, & Liu, 2002). In the gastrointestinal tract, these health-protective effects are partially attributed to their antioxidant properties (Halliwell, Zhao, & Whiteman, 2000), which have been associated with their high phytochemical (mainly phenolic compounds and carotenoids) and antioxidant dietary fibre contents (Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2013; Saura-Calixto et al., 2010).

An adequate bioavailability of bioactive substances is a prerequisite for potential systemic effects *in vivo* (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). However, many antioxidants remain in the intestinal luminal contents and may exert a local beneficial effect within the gut by protecting possible oxidisable molecules and the intestinal epithelium from oxidative damage occurring during digestion (Goñi & Serrano, 2005; Halliwell et al., 2000). In this regard, the chemical alterations and the bioaccessibility of antioxidant compounds in the gastrointestinal tract are key aspects that determine their bioavailability (Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014; Rein et al., 2013), especially for those foods rich in antioxidant dietary fibre due to its low digestibility (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011). Gastrointestinal digestion is able to release, from food matrices, some entrapped antioxidants that might be absorbed in the small intestine, whereas other antioxidants remain enclosed in the indigestible fraction and reach the large intestine (Scalbert & Williamson, 2000). These bioactive substances and the metabolites formed after their fermentation by gut microbiota could exert their antioxidant activity *in situ* or, to some extent, be absorbed in the lower regions of the colon (Delgado-Andrade, Conde-Aguilera, Haro, Pastoriza de la Cueva, & Rufián-Henares, 2010; Saura-Calixto et al., 2010). Similarly, the insoluble

Abbreviations: ABTS, 2,2′-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid; CF, colonic fermented; CFr, colonic fermented residue; CFs, colonic fermented supernatant; FC, Folin-Ciocalteu; GAR, global antioxidant response; GID, gastrointestinal digested; GIDD, gastrointestinal digested+dialysed; Q-, QUENCHER; RWPS, red wine pomace seasoning; Sd-S, seasoning obtained from seeds; Sk-S, seasoning obtained from seedless red wine pomace, in which grape skins are the main component; TAC, total antioxidant capacity; W-S, seasoning obtained from whole red wine pomace.

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matter in ingested food, which remains in the gastrointestinal tract for a long time, may help to counteract the free radicals that are continuously formed in the gut (Pérez-Jiménez et al., 2013; Tabernero, Venema, Maathuis, & Saura-Calixto, 2011).

In view of the above, the effects of the digestive process on the phytochemicals contained in foods, and on their antioxidant activity, have attracted great attention from the scientific community over the last years (Heim, Tagliaferro, & Bobilya, 2002; Rein et al., 2013). Thus, several *in vitro* digestion models to assess bioaccessibility that allow the study of changes in dietary components during the gastric and intestinal stages have been implemented (Carbonell-Capella et al., 2014; Hur, Lim, Decker, & McClements, 2011; McDougall, Fyffe, Dobson, & Stewart, 2005). Despite the limitations of *in vitro* digestion models, especially those comprising only a static simulated digestion, the good correlation of the results obtained with those from several animal and human studies has been established (Alminger et al., 2014; Saura-Calixto et al., 2010). Furthermore, the combination of *in vitro* digestion models with total antioxidant capacity (TAC) assays for the digested fractions obtained has been suggested as a first approach to predict the *in vivo* antioxidant activity of foods (Goñi, Martín, & Saura-Calixto, 2005; Rufián-Henares & Delgado-Andrade, 2009). Consequently, Delgado-Andrade et al. (2010) have proposed a methodology to determine the global antioxidant response (GAR) of food, which is defined as the sum of the antioxidant activities of the soluble and insoluble fractions obtained after a simulated gastrointestinal digestion. According to this method, the TAC of these digested fractions is measured separately, using classical and QUENCHER (Gökmen, Serpen, & Fogliano, 2009) assays, respectively, and then combined to estimate the GAR of foodstuffs. Thus far, several plant-based foods have been assessed following the GAR method, and important variations in the antioxidant activities exhibited by the different food matrices tested have been detected (Papillo, Vitaglione, Graziani, Gokmen, & Fogliano, 2014; Pastoriza, Delgado-Andrade, Haro, & Rufián-Henares, 2011).

The promising use as food ingredients of new seasonings obtained from red wine pomace (RWPSs) has recently been demonstrated (García-Lomillo, González-Sanjosé, Del Pino-García, Rivero-Pérez, & Muñiz, 2014). The new powdered vegetal seasonings are antioxidant-rich products, containing mainly phenolic compounds, which may contribute to the intake of exogenous natural antioxidants and reinforce the endogenous redox environment once ingested. In this regard, it has been suggested that consumption of wine pomace may help prevent colon cancer (López-Oliva, Agis-Torres, García-Palencia, Goñi, & Munoz-Martínez, 2006), and its high antioxidant content certainly plays an important role in this protective effect.

On the basis of the previous considerations, the present study was conducted to evaluate the effects of the digestive process on the antioxidant activity of three of these new seasonings, targeting the antioxidant capacities of digested fractions which can mimic those produced in the small and the large intestine after intake of each studied seasoning. For this purpose, the TAC of *in vitro* digested fractions (including both gastrointestinal and colonic phases) was measured using QUENCHER methodologies.

2. Materials and methods

2.1. Chemicals and materials

Ammonium bicarbonate (NH_4HCO_3), 2,2′-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), porcine bile extract, calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), cobalt(II) chloride hexahydrate ($CoCl_2{\cdot}6H_2O$), L-cysteine hydrochloride, gallic acid, hydrochloric acid (HCl), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), magnesium sulphate heptahydrate ($MgSO_4{\cdot}7H_2O$), manganese(II) chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$), maleic acid, porcine pancreas pancreatin, potassium chloride (KCl), potassium phosphate monobasic (KH_2PO_4), resazurin sodium salt, sodium bicarbonate ($NaHCO_3$), sodium hydroxide (NaOH), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4), sodium sulphide nonahydrate ($Na_2S{\cdot}9H_2O$), Tris hydrochloride (Tris), tryptone, enzymes used in enzymatic digestion α-amylase (EC 3.2.1.1), amyloglucosidase (EC 3.2.1.3), lipase (EC 3.1.1.3), and pepsin (E.C 3.4.23.1), and cellulose membrane dialysis tubing (12,000 Da molecular weight cut-off) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Folin-Ciocalteu (FC) reagent and sodium carbonate (Na_2CO_3) were purchased from Panreac Química S.L.U. (Barcelona, Spain).

The seasonings used in this study were made in the pilot plant of the Food Technology Department of University of Burgos (Spain) as previously described (García-Lomillo et al., 2014), applying the process patented by González-Sanjosé, García-Lomillo, Del Pino-García, Muñiz-Rodríguez, and Rivero-Pérez (2013). Three different types of powdered seasonings were used, which were labelled as W-S, corresponding to the seasonings obtained from whole red wine pomace; Sk-S, representing the seasonings obtained from seedless red wine pomace, in which grape skins are the main component; Sd-S, referring to the seasonings obtained from the seeds separated from red wine pomace. The humidity of these seasonings was lower than 8%. Three different batches of each type of RWPS were used in this study.

The inoculums used for colonic fermentation were obtained, following the method described by Saura-Calixto, Serrano, and Goñi, (2007), at the animal-housing unit of the University Hospital of Burgos (Spain) by mixing the caecal content from 5 male Wistar rats (body weight of 250 ± 5 g) fed with standard maintenance diet. All aspects of this procedure were conducted in accordance with the guidelines established by the Ethics Committee at both the University Hospital of Burgos and the University of Burgos.

2.2. In vitro gastrointestinal digestion and colonic fermentation of the seasonings

Simulated complete digestion of the three RWPSs (Sk-S, W-S, and Sd-S) was performed according to the method describe by Saura-Calixto et al. (2007), with only slight modifications in the dialysis step. This *in vitro* static digestion model allows the estimation of the bioaccessibility of dietary antioxidants, and mainly comprises two consecutive stages, an enzymatic gastrointestinal digestion first phase, followed by a colonic microbial fermentation phase. A schematic representation of the main steps performed and the fractions obtained along simulated digestion can be seen in Fig. 1. Briefly, each powdered seasoning, labelled as 'undigested' (UD), was successively incubated with digestive enzymes, as described in detail by Saura-Calixto et al. (2007), yielding the so-called 'gastrointestinal digested' (GID) fraction. This fraction was centrifuged (3000*g*, 15 min, 25 °C) to separate the supernatant and the solid residue. The centrifugation step was repeated twice, washing the residue with 5 mL of Milli-Q water. All the supernatants obtained were then combined, transferred into cellulose membrane dialysis tubing, and dialysed against a total of 2 L of Milli-Q water (changing the water twice). The dialysis retentate was mixed with the GID solid residue to obtain the so-called 'gastrointestinal digested + dialysed' (GIDD) fraction, which contained the compounds hypothetically non-absorbed in the small intestine that may reach the large intestine. The GIDD fraction was the substrate for the action of colonic microbiota, obtaining the 'colonic fermented' (CF) fraction. Finally, the CF fraction was centrifuged (2500*g*, 10 min, 25 °C) to collect the supernatant (CFs) and the residual solid (CFr) respective fractions. All digested fractions were