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Analytical Methods

In vitro bio-accessibility and antioxidant activity of grape polyphenols

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ABSTRACT

The bio-accessibility (the release of compounds from solid food matrices) of grape polyphenols using an in vitro model simulating gastro-intestinal conditions has been investigated. In vitro studies are needed to unravel factors affecting the release of antioxidants during digestion. The amount of bio-accessible polyphenols, flavonoids and anthocyanins increases during gastric digestion. The transition in the intestinal environment causes a decrease in all the analyzed classes of polyphenols followed by a renewal in the extraction of polyphenols and flavonoids but not of anthocyanins. The stability under gastro-intestinal conditions of pure phenolic acids, flavonoids and resveratrol has been analysed. Gastric digestion had no effect on any phenolic tested. Phenolic acids and resveratrol were degraded under pancreatic conditions whereas catechin and quercetin were not. Changes in antioxidant activity during digestion were correlated to the changes in polyphenols concentration as well as to the pH. Our results suggest that the gastro-intestinal tract may act as an extractor where polyphenols are progressively released from solid matrix and made available for the absorption or to exert their biological effects in the gastro-intestinal tract.

1. Introduction

High intakes of fruit and vegetables have been associated with a lower incidence of coronary heart diseases (Ness & Powles, 1997) and cancer, especially from the gastro-intestinal tract (Johnson, 2004). It is now widely accepted that the protection supplied by fruit and vegetables against diseases is due to the presence of various antioxidants (Gey, 1990) and in particular of polyphenols.

Grape constitutes one of the major source of phenolic compounds among different fruits (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). From a qualitative point of view, grape polyphenols belong to different classes distributed in every part of fruit. The skin contains the highest amount of polyphenols and in particular of condensed tannins (Soquet, Cheynier, Brossaud, & Moutounet, 1996), monomeric flavanols and flavonols, phenolic acids and resveratrol (Mané et al., 2007; Pinelo, Arnous, & Meter, 2006). In red grape varieties the skin contains also anthocyanins, which are responsible for the red colour of the berries. The major constituents of pulp are phenolic acids and monomeric flavonoids, such as flavanols, although at lower concentrations than in the skin (Mané et al., 2007). The seeds contain polymeric condensed tannins with minor quantities of procyanidins and monomeric flavonoids (Pinelo et al., 2006).

One of the principal topics concerning the beneficial effects of polyphenols is their bio-availability and metabolic fate. The bio-availability of a dietary compound is dependent upon its digestive stability, its release from the food matrix (referred as bio-accessibility), and the efficiency of its transepithelial passage. Bio-availability differs greatly from one polyphenol to another, and for some compounds it depends on dietary source (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005). The absorption of phenolic compounds is considered to be low, not exceeding the plasma concentrations of 10 μM. This low absorption may be attributed, at least partially, to the chemical structures of the different polyphenols that determine their gut absorption (Manach et al., 2005). It is believed that the absorption of polyphenols happens through passive diffusion across the membranes of the gut epithelial cells. In this contest, most polyphenols are probably too hydrophilic to penetrate the gut wall by passive diffusion (Manach et al., 2004). In addition, most polyphenols exist in food in the form of esters, glycosides or polymers that cannot be absorbed in their native form. Only aglycones and some glucosides can be absorbed in the small intestine (Manach et al., 2005). Other factors involved in determining the bio-availability of polyphenols are the stability under gastro-intestinal condition and the release from the food matrix, especially from the solid one. For example, the very low bio-availability of anthocyanins can be attributed, at least partially, to the high instability of these molecules in the mild alkaline condition of the small intestine (Pérez-Vicente, Gil-Izquierdo, & García-Viguera, 2002).

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Independently from their bio-availability, food polyphenols can play an important role in protecting the gastro-intestinal tract itself from oxidative damage and cancer (Halliwell, Zhao, & Whiteman, 2000). Compounds that are present in plasma at low concentrations may be present in the gastro-intestinal lumen at much greater concentrations after direct consumption of a meal rich in vegetables, fruit and their derivatives. In this case, the most important factors in determining the potential beneficial effects of polyphenols on the gut epithelial cells are their stability and their bio-accessibility under gastro-intestinal conditions.

While studies regarding the transepithelial passage of polyphenols require in vivo experiments, in vitro methods are useful to study their stability under gastro-intestinal conditions and their release from food matrices. Various studies report the effect of in vitro gastro-intestinal digestion on the stability of pure phenolic compounds (McDougall, Dobson, Smith, Blake, & Stewart, 2005a; Zhu et al., 2002) and on the stability and release of polyphenols from beverages (McDougall, Fyffe, Dobson, & Stewart, 2005b; Pérez-Vicente et al., 2002; Record & Lane, 2001; Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007), but only few studies have been carried out on the solid food matrices (Saura-Calixto, Serrano, & Goñi, 2007; Vallejo, Gil-Izquierdo, Pérez-Vicente, & García-Viguera, 2004). Researches concerning the bio-accessibility of polyphenols from the solid matrices are important since only the compounds released from the food matrix are potentially bioavailable and in condition to exert their beneficial effects on the gastro-intestinal tract.

In the present study we investigated the bio-accessibility of the major classes of polyphenols from Red Globe grapes (a red table grape variety) using an in vitro model that simulated some chemical (pH, temperature and bile salts) and biological (gastric and pancreatic enzymes) gastro-intestinal conditions. In addition, changes in the antioxidant activity during the digestion as well as the digestive stability of some pure phenolic compounds contained in grapes were investigated.

2. Materials and methods

2.1. Materials

Catechin, quercetin, caffeic acid, resveratrol, gallic acid, bile salts (mixture of sodium cholate and sodium deoxycholate), pepsin from porcine gastric mucosa, pancreatin from porcine pancreas, amino-antipyrine (4-AP), horseradish peroxidase (HRP) type II and 2,4,6-tripyridyl-S-triazine (TPTZ) were supplied by Sigma (Milan, Italy). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was supplied by Calbiochem (La Jolla, CA). All the other chemical reagents were from Carlo Erba (Milan, Italy). Sep-Pak cartridges C-18 for solid phase extraction were supplied by Waters (Milan, Italy). The absorbance was read using a Jasco V-550 UV/vis spectrophotometer. Red table grapes (Red Globe variety) were purchased from a local supermarket and stored at −80 °C until used.

2.2. In vitro gastro-intestinal digestion

Red Globe grapes were subjected to successive gastric and pancreatic digestion, following a previously published method (Bermúdez-Soto et al., 2007). Briefly, whole grapes were homogenised in a stomacher laboratory blender for 1 min to simulate mastication. Samples of 20 g were transferred to a volumetric flask and 30 ml of simulated gastric fluid, containing 2 g/l of NaCl and 300 U/ml of pepsin, were added. The pH was adjusted to 2.0 with concentrated HCl and the solution was incubated at 37 °C in a shaking bath for 2 h. At different times aliquots of samples were removed for polyphenols and antioxidant activity analysis, and the reaction was stopped by cooling the test tubes in ice. The test tubes were then centrifuged at 17,500*g* at 5 °C for 10 min and the supernatants were withdrawn for the analysis. At the end of the gastric digestion, the pH was brought to 7.5 with $NaHCO_3$ before adding 0.8 g/l pancreatin and 25 mg/ml bile salts. The solution was then incubated at 37 °C in a shaking bath for further 2 h. At different times aliquots of samples were removed for polyphenols and antioxidant activity analysis, and the reaction was stopped by cooling the test tubes in ice. Samples removed during the pancreatic digestion were immediately acidified to pH 2 to ensure the stability of the phenolic compounds present in the reaction mixture. The test tubes were then centrifuged at 17,500*g* at 5 °C for 10 min and the supernatants were withdrawn for the analysis. Digestions without added enzymes were carried out to differentiate the effects due to the presence of enzymes from those caused by the chemical environment in the assay.

To evaluate the effect of mastication on the extraction of polyphenols from skin, the grapes were homogenised in a stomacher laboratory blender for 1 min to simulate mastication after skin removal.

Selected pure compounds were individually subjected to the same in vitro digestion described above. The polyphenols used were catechin and quercetin as representative of total flavanols and total flavonols, respectively, gallic acid and caffeic acid as representative of total hydroxybenzoic and total hydroxycinnamic acids, respectively, and resveratrol as representative of total stilbenoids. The above indicated phenolic compounds were digested at the typical concentrations found in Red Globe grapes (Cantos, Espín, & Tomás-Barberán, 2002) that was: catechin 4.0 mg/100 g of grapes, quercetin 6.0 mg/100 g of grapes, gallic acid 3.5 mg/100 g of grapes, caffeic acid 0.8 mg/100 g of grapes and resveratrol 0.3 mg/100 g of grapes. All the phenolic compounds were dissolved in water except for quercetin which was dissolved in DMSO so that the final concentration of DMSO in the digestion assay was 5%. At different times samples were removed for antioxidant activity and spectral analysis, and the reaction was stopped by cooling the test tubes in ice. Samples removed during the pancreatic digestion were immediately acidified to pH 2 to ensure the stability of the phenolic compounds.

2.3. Chemical extraction

Whole grapes (20 g) were homogenised in a stomacher laboratory blender for 1 min in 20 ml of water. The mixture was then brought to pH 2 with concentrated HCl and the phenolics were extracted by shaking at room temperature for 30 min. After centrifugation (15 min, 20 °C, 3000*g*) supernatant was recovered (aqueous fraction) and the insoluble fraction was further extracted by using 20 ml of acidified methanol (pH 2 with HCl) and by shaking at room temperature for 30 min. After centrifugation (15 min, 20 °C, 3000*g*) supernatant was recovered (methanolic fraction) and the insoluble fraction was further extracted by using 20 ml of acetone and by shaking at room temperature for 30 min. After centrifugation (15 min, 20 °C, 3000*g*) supernatant was recovered (acetone fraction). The various fractions were used to determine total polyphenolic, flavonoids and anthocyanins content and the antioxidant activity of grapes extracts.

2.4. Determination of total phenolic compounds, flavonoids and anthocyanins

Total polyphenols in the samples at time zero of the gastric digestion, in the digested samples and in the chemically extracted samples were determined using an enzymatic method (Verzelloni, Tagliazucchi, & Conte, 2007). In a 3 ml spectrophotometric cell, 0.1 ml of appropriately diluted sample or standard solutions was