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Effect of simulated digestion on the phenolic components of red grapes and their corresponding wines

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ABSTRACT

The aim of this study was to evaluate the effect of the simulated gastro-intestinal (GI) digestion on the phenolic profile and the antioxidant capacity (AC) of grapes and red wines. Mouth and stomach digestion increased the bioaccesibility of TP (total polyphenols) in grapes, while in wine these compounds were already bioaccessible. Intestinal digestion reduced the bioaccessible polyphenols of grapes and wines, mainly due to the alkaline pH of the digestive fluid. Only 16% and 52% of the initial TP in grapes and wine, respectively, were found after assay (dialysed plus nondialysed fractions). Moreover, 21% and 39% of grape and wine AC, respectively, was conserved. In spite of the significant loss of polyphenols during digestion, both grapes and red wine still retain AC. Anthocyanins were less affected by human GI tract. Therefore, they could be the most relevant compounds to explain the AC of both grapes and red wine after GI.

1. Introduction

Vitis vinifera L. grape is one of the most cultivated fruits in the world, and its vinification product, red wine, is widely consumed around the world. In recent years, the possible positive implications for the consumption of red grapes and wines on human health have been of increasing interest (Irita & Varoni, 2014). Epidemiological studies and clinical trials have shown that the consumption of red grapes and wines reduces the risk of chronic diseases such as different types of cancer, cardiovascular and neurodegenerative diseases (Covas, Gambert, Fitó, & de la Torre, 2010; Irita & Varoni, 2014; Martin, Goya, & Ramos, 2017). This beneficial effect has been attributed, at least in the most part, to the high antioxidant capacity (AC) demonstrated by their phenolic compounds (Costa et al., 2017).

Several studies showed that red grapes and wines of different *Vitis vinifera* L. varieties presented a high content and a great variety of polyphenols (Figueiredo-González, Martínez-Carballo, Cancho-Grande, Santiago, & Martínez, 2012; Ivanova et al., 2011), being the anthocyanins the major contributors to both *in vitro* and *in vivo* AC (Lingua, Fabani, Wunderlin, & Baroni, 2016a; Lingua, Fabani, Wunderlin, & Baroni, 2016b; Jiménez et al., 2010). However polyphenols must be bioavailable to exert its bioactivity (AC in this study).

The term bioavailability is used to describe the proportion of the ingested compound that reaches the systemic circulation (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). The bioavailability of polyphenols will depend on their bioaccessibility (referred as the relative amount of compounds released from the food matrix along the digestive system), their digestive stability, and the efficiency of their transepithelial passage (intestinal absorption). Thus, only those compounds that are released from the food matrix, that are able to tolerate the conditions found throughout the gastro-intestinal (GI) tract, and that pass through the intestinal membrane, will be potentially bioavailable to exert their beneficial effects on the human body (Manach et al., 2004; Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). Nowadays, mechanistic studies also suggest that the health-promoting properties of phenolic compounds on the human body may be mediated, in part, by their interaction with the gut microbiota (Marchesi et al., 2016).

Different models of *in vitro* GI digestion have been developed, and were widely used in recent years to mimic human digestion, since they allow studying the bioaccessibility, stability and potential bioavailability of the polyphenolic compounds present in foods (Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014; Minekus et al., 2014). These models mimic the physiochemical and biochemical factors to which foods are exposed in the upper GI tract (addition of digestive enzymes such as pepsin and pancreatin, bile salts, and adjust of pH and temperatures similar to the conditions found *in vivo*). Then dialysis may be performed to simulate the passive intestinal absorption. Despite their

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limitations, such as typically constituting only a static model of digestion, does not include microbiota intestinal and does not include the complex interaction between food and body, this methodology has been proposed as an estimation of bioaccesibility of food components in different food matrices (Carbonell-Capella et al., 2014; Minekus et al., 2014).

Gumienna, Lasik, and Czarnecki (2011) observed that the red wine digestion decreases the content of total polyphenols (TP), with significant quantitative changes in the phenolic profile. McDougall, Fyffe, Dobson, and Stewart (2005) observed that wine anthocyanins are unstable to alkaline conditions found in the gut. Fernández & Labra, (2013) demonstrated that the proanthocyanidins from red grape extracts were degraded throughout the whole digestive process. On the other hand, Podsędek et al. (2014) showed that the recovery of anthocyanins during *in vitro* digestion of cabbage was strongly influenced by the food matrix, and that other constituents present in this food enhanced the stability of anthocyanins during its digestion.

Most studies evaluate the effect of *in vitro* GI digestion on polyphenols from beverages, food extracts, and some of them even use pure phenolic compounds (Corrêa et al., 2017; Gil-Sánchez et al., 2017; Sanz-Buenhombre et al., 2016). However few of them take into account the food as it is ingested, without considering solid foods, as it is the case with grapes (Dufour et al., 2018; Podsędek et al., 2014; Tagliazzuchi et al., 2010). In this sense, to our knowledge, there are no reports on effects of processing of grapes as wine on the bioaccessibility of its polyphenols and AC.

The main goal of this research work was to evaluate the effects of processing of grapes as wine on the bioaccessibility of its polyphenols and AC. For this purpose, bioaccessibility, stability and AC of phenolic compounds from red grapes were studied by *in vitro* GI digestion, including a final stage of dialysis to identify those compounds potentially bioavailable and those potentially colon available, and results were compared to those of their vinification product, red wine.

2. Materials and methods

2.1. Chemicals and reagents

Methanol (HPLC grade) and formic acid (puriss. p.a. for mass spectroscopy) were obtained from J. T. Baker (Edo. de México, México) and Fluka (Steinheim, Germany), respectively. Commercial standards of (+)-catechin, malvidin-3-glucoside and caffeic acid were obtained from Extrasynthese (Genay, France). Kaempferol and quercetin were purchased from Fluka (Dorset, U.K.). Isoquercetin was obtained from Sigma-Aldrich (Buenos Aires, Argentina), and gallic acid was purchased from Riedel-de-Hagën (Seelze, Germany). Filters (0.45 µm, HVLP04700) were obtained from Millipore (São Paulo, Brazil). ABTS (2,2′-azino-bis-(3-thylbenzothiazolne-6-sulfonic acid) diammonium salt), DPPH (1,1,-diphenyl-2-picrylhydrazyl radical), TPTZ (2,4,6-tripyridyl-S-triazine), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), Folin-Ciocalteu Reagent, pepsin (P-7000, from porcine stomach mucosa), pancreatin (P-1750, from porcine pancreas) and bile extract (B-8631, from porcine) were purchased from Sigma-Aldrich (Buenos Aires, Argentina). SnakeSkin dialysis bags with a molecular weight cut-off of 10 kDa and a width of 22 mm were obtained from ThermoFisher SCIENTIFIC. All other reagents were of analytical grade.

2.2. Red grape and wine samples

Red grape samples from *Vitis vinifera* L. cv Syrah, and the respective wines obtained from their vinification were studied. The samples were obtained from the "Antonio de la Torre" winery located in the Province of San Juan, Argentina. Grapes were collected in their optimal ripening stage (23–25 g sucrose/100 mL) from vineyard plots located in Valle de Tulum, between 31°39′ south latitude and 68°33′ west longitude. The geological setting of production areas are represented by a clastic sedimentary Tertiary sequence, overlaid by Quaternary alluvial and eolian units. This region is located near the outcrops of the Cambrian-Ordovician thick carbonatic succession of Pre-Andes range area. The weather is dry, the average annual rainfall is 70 mm/year, with average temperatures ranged between 21 °C and 34 °C in summer and average temperatures ranged between 3 °C and 16 °C in winter. All samples, grapes and wines, were obtained directly from producer having both GMP (good manufacturing practices) and traceability systems. Thus, wine samples were obtained from 2014 vintage after stabilisation (4–5 months after primary fermentation) and bottling in 750 mL dark glass bottles with cork plugs. All samples (grapes and wines) were transported to the laboratory at 4–8 °C and protected from the light. In the laboratory, samples were stored at −80 °C until analysis within 6 months.

The Syrah variety was selected because, in previous studies, it showed a greater antioxidant capacity (AC) among the different red varieties, probably due to its phenolic profile, characterised by the highest anthocyanin content among studied varieties (Lingua et al., 2016a; 2016b).

2.3. Simulated in vitro gastro-intestinal (GI) digestion

The assay was performed according to the procedure described by Celep, Charehsaz, Akyüz, Türköz Acar, and Yesilada (2015) and Tagliazucchi et al. (2010) with slight modifications. To mimic the *in vivo* GI digestion, the model consisted of three sequential steps: the digestive process in the mouth, stomach (gastric) and small intestine (duodenal) digestion (Carbonell-Capella et al., 2014; Minekus et al., 2014). Three independent experiments were conducted for each sample type under study. Each experiment involved sampling at the end of each digestive step, enabling the evaluation of both phenolic compounds and AC at each digestive step (as defined previously). Simultaneously, two blank samples (without grape /wine) were processed and analyzed to discard the influence of the digestion reagents on both phenolic compounds and AC.

2.3.1. Mouth digestion

This stage was performed using human saliva collected according to Hu, Nie, Min, and Xie (2013). Red grapes (1 g fresh weight: FW), or red wine samples (2 mL), were homogenised in presence of freshly collected human saliva (2 mL) for 30 s at 24,000 rpm in an Ultra-Turrax T18 blender (Ika-Labortechnik, Germany) to simulate mastication. The pH was immediately adjusted to 2 with 6 M HCl, to stop the action of amylase, and conditioning the medium to further continue with the gastric digestion.

2.3.2. Stomach digestion

The mixture obtained from the mouth digestion was subsequently incubated in the dark, shake for 2 h at 37 °C in the presence of 450 units of pepsin per gram or mL of initial grape or wine, respectively (pepsin solution: 40 mg/mL in 0.1 M HCl).

2.3.3. Small intestine digestion including dialysability

Pancreatin (1.2 mg per g/mL of initial grape/wine) and bile salts (5.6 mg per g/mL of initial grape/wine) (pancreatin-bile salt solution: 5 mg of pancreatin plus 25 mg of bile salts in 1 mL of 0.1 M $NaHCO_3$, pH = 7.5) were added to the homogenate from the stomach digestion to simulate intestinal digestion. This mixture was placed inside a dialysis bag, which allowed simulating the passive absorption of the polyphenolic compounds through the membrane of the small intestine. The full filled, bubble-free and closed dialysis bag was completely immersed in 0.1 M $NaHCO_3$, pH = 7.5 (55 mL per gram or 15 mL per mL of initial grape or wine, respectively; these amounts of 0.1 M $NaHCO_3$ used here correspond to the quantity required to neutralise the titratable acidity in gastric samples). The submerged dialysis bag was incubated in the dark with agitation for 2 h at 37 °C. After this time, the solution