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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

contained inside, the nondialysable fraction, was separated and stored, representing the material that remained in the gastrointestinal tract, which would reach the colon (potentially colon available fraction). On the other hand, the dialysate (fraction passing through the dialysis membrane), was separated and stored, representing the fraction available for absorption into the circulatory system by passive diffusion (serum available compounds or potentially bioavailable).

2.3.4. Further sample preparation for analysis

Both fractions obtained after intestinal digestion were acidified with formic acid to pH = 2 to neutralise $NaHCO_3$. Aliquots arising from mouth, stomach and intestine (nondialysable fraction) digestions were centrifuged at 13,000*g* for 10 min. All samples were immediately filtered through 0.45 µm pore filters, fractionated in microtubes, and stored at −80 °C until analysis.

2.4. Chemical extraction

Three independent extractions from red grape samples were carried out as described by Lingua et al. (2016a). Briefly, grapes were lyophilised and grounded in presence of liquid nitrogen, obtaining a fine powder. Afterwards, 1 g (DW) of grape powder was extracted with 15 mL of acidified methanol (HCl 0.1% v/v), using an Ultra-Turrax T18 blender (Ika-Labortechnik, Germany). This homogenate was incubated with agitation for 2 h at 4 °C, and centrifuged at 2058*g* for 10 min. The supernatant was separated and the solid pellet re-extracted with 5 mL of acidified methanol as previously described. The combined extracts were filtered, fractionated in microtubes, and stored at −80 °C until analysis.

The phenolic compounds and AC of wine samples were analysed without previous treatment.

2.5. Analysis of phenolic compounds

2.5.1. Determination of total polyphenols (TP)

The TP content of samples was determined by the Folin Ciocalteu method, according to Singleton and Rossi (1965). The absorbance of properly diluted samples was read at 750 nm. TP was calculated by linear regression from a calibration plot constructed with gallic acid. Results for grape samples are expressed as milligrams of polyphenols (equivalent to gallic acid) per kg of fresh grape (mg GAE/kg, FW). Wine results are reported as milligrams of polyphenols (equivalent to gallic acid) per litre of wine (mg GAE/L). All samples were analysed in triplicate.

2.5.2. Determination of phenolic profile

The phenolic profile of samples was determined by HPLC-DAD-MS/MS, using an Agilent Series 1200 LC System (Agilent, Santa Clara, CA, USA), coupled to a DAD detector (Agilent Series 1200) in tandem with an ESI source, connected to a mass spectrometer (Micro-QTOF II; Bruker Daltonics, Billerica, MA, USA), according to Lingua et al. (2016a). Polyphenols present in samples were identified according to their retention times, UV/Vis spectra, high-resolution MS and MS/MS spectra in addition to comparison with authentic standars when available. Alternatively, when authentic standards were not available, a tentative identification was performed considering UV/Vis, high resolution MS and MS/MS spectra reported in the literature (see Supplementary material, Tables S1 and S2). MS was used for quantification of the polyphenols with external calibration plots, constructed by linear regression from available phenolic standards (using the mass peak areas obtained from the extracted ion chromatograms). When reference compounds were not available, a calibration plot from structurally related compound was used. Anthocyanins were quantified as malvidin-3-glucoside; myricetin, laricitrin, syringetin, quercetin and isorhamnetin as quercetin; kaempferol as kaempferol; flavonol glycoside compounds as isoquercetin; flavanol compounds as (+)-catechin; hydroxycinnamic acids compounds as caffeic acid; hydroxybenzoic acid compounds as gallic acid. The calibration plots were prepared by appropriate dilution from stock solutions in methanol (containing 1 g/L of the pure compound). The limits of detection (LOD) and quantification (LOQ) of the method were calculated considering the signal to-noise ratio (LOD: S/N ≥ 3; LOQ: S/N ≥ 10). The precision of the method was evaluated by calculating the coefficients of variation (CV) from at least nine determinations, covering the observed range for the samples. LOQ ranged from 0.023 to 0.066 mg/L. CV were below 13%. All samples and standards solutions were diluted (when required), filtered (0.45 µm) and injected in the HPLC-DAD-MS/MS system. All HPLC runs were performed in triplicate. The results are expressed as mg/kg FW for grape samples, and as mg/L for wine samples.

2.6. Estimation of the in vitro antioxidant capacity (AC)

The estimation of the *in vitro* AC was measured by three methods, and, in all cases, the results were obtained from a linear regression plot constructed using Trolox (linear range between 0 and 0.02 mmol Trolox/L). Results are expressed in mmol Trolox equivalents per kg fresh weight in the case of grapes (mmol TE/kg FW), or mmol TE/L for wine. All samples were analysed in triplicate.

2.6.1. Ferric-reducing antioxidant power assay (FRAP)

The FRAP assay was carried out in according to Benzie & Strain, (1996). Briefly, 100 µL of appropriately diluted sample was added to 3 mL of the FRAP reagent, and the absorbance at 593 nm was measured after 6 min of incubation in the dark at room temperature.

2.6.2. ABTS radical-scavenging capacity assay

The ABTS assay developed by Re et al. (1999) was used in this study. Briefly, 100 µL of appropriately diluted sample was mixed with 3 mL of $ABTS^{\cdot+}$ (dissolved in methanol), measuring the absorbance at 734 nm after 4 min reaction.

2.6.3. DPPH radical-scavenging capacity assay

The DPPH assay was performed in accordance with Brand-Williams, Cuvelier, and Berset (1995). Briefly, 100 µL of appropriately diluted sample were added to 3 mL of 60 µM $DPPH^{\cdot}$ (dissolved in methanol), incubated for 15 min in dark conditions and measured at 515 nm.

2.7. Statistical analysis

Data are expressed as mean ± SD. Normal distribution of data was tested (Shapiro Wilks test) and satisfied the assumptions of an analysis of variation (ANOVA). For multiple comparisons, analysis of variance (ANOVA) was performed with each variable to evaluate differences among samples and, in the case of significance ($p < 0.05$), a DGC (Di Rienzo, Guzmán & Casanoves) comparison test was performed to reveal paired differences between the means. The statistical analyses were performed using the Infostat software package. In all figures and tables, different letters mean statistically significant differences. For all measurements (phenolic compounds and AC), comparisons following various digestion steps, concentrations corrected for the respective dilutions comprised within the digestion procedure were compared.

3. Results

Previous studies have shown that the higher AC, both *in vitro* and *in vivo*, of different grape and red wine varieties was correlated with their phenolic profile, and was specially influenced by higher anthocyanin content (Lingua et al., 2016a; Lingua et al., 2016b; Jiménez et al., 2010). In this new study, we were interested in evaluating the behaviour of these natural antioxidants at different phases of a simulated digestion, using an extended *in vitro* model.

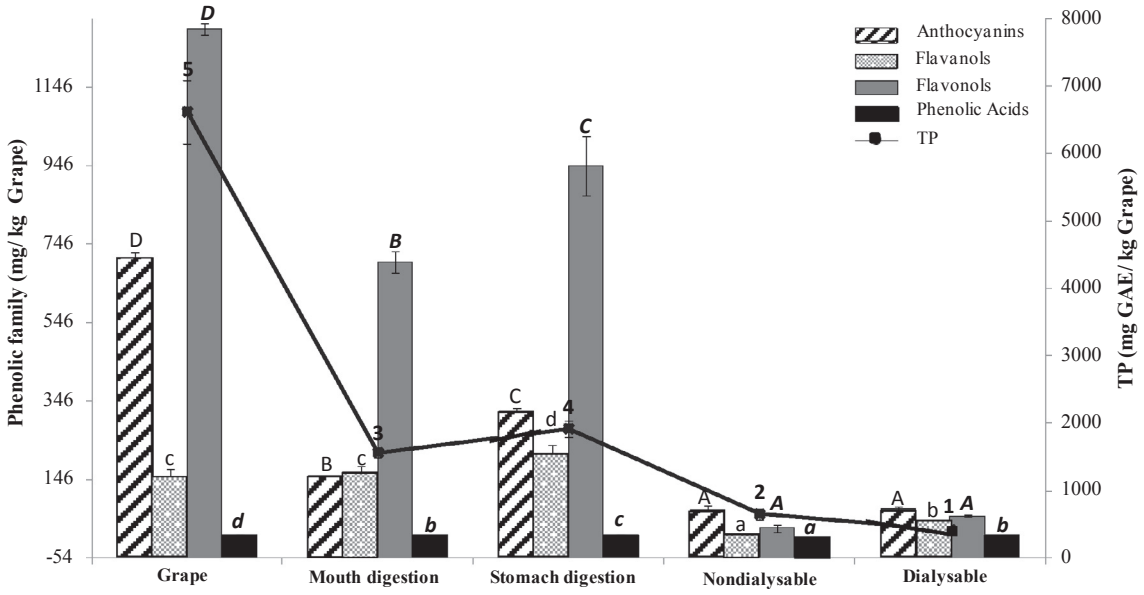

Fig. 1. Changes in total polyphenols content (TP) and polyphenolic compounds, grouped by families, along the simulated GI digestion of red grapes. Results are expressed based on the fresh grape weight. Different letters and numbers indicate statistically significant differences ($p < 0.01$) in each phenolic family among steps of difestive process.

3.1. Bioaccessibility of polyphenols along simulated GI digestion including dialysability

3.1.1. Red grapes

Fig. 1 shows the impact along *in vitro* GI digestion on TP content and polyphenolic compounds, grouped by families. Table 1 shows the amounts of individual polyphenols at each step of this assay.

After simulated mastication, only 24% of TP from grapes (average 6617 mg GAE/kg FW) were bioaccessible, that is to say, the amount of released phenolics from the food matrix after mouth digestion compared to the methanolic extract of grapes (Fig. 1). Conversely, the analysis of polyphenol families showed that 100% of flavanols became bioaccessible after simulated mastication, while 54%, 52% and 22% of flavonols, phenolic acids and anthocyanins were released from grapes at this same stage (Fig. 1).

Following, we observed that the stomach digestion increases ($p < 0.05$) the bioaccessibility of polyphenols compared to mouth digestion up to 29% of TP from grapes. Likewise, all families of compounds showed a significant increase ($p < 0.05$). In this sense, 45%, 73% and 80% of total grape anthocyanins, flavonols and phenolic acids, respectively, became bioaccessible after this step. It was observed that the increase (38%) of flavanols after this stage resulted in a content that exceeded the content initially found in the grapes (Fig. 1).

Finally, the amount of TP decreased significantly ($p < 0.05$) stepwise from stomach to intestinal digestion, with a consequent decrease in all polyphenol families ($p < 0.05$). Only 10% of grape TP was found in the nondialysable fraction, representing those potentially colon available compounds, and 6% in the dialysable fractions, constituted by compounds that passed through the dialysis membrane, and therefore representing those potentially bioavailable compounds. Individual results for each of the polyphenol compounds studied and their recovery percents (R%) along the simulated GI digestion are presented in Table 1. We observed substantial losses in some of the polyphenol compounds after dialysis in relation to their initial content in grape samples. Thus, only 16 out of 33 compounds quantified in the grapes were quantified in dialysed fractions. Among them, the most abundant compounds were monomeric (+)-catechin and (−)-epicatechin flavanols with R% by about 88% and 63%, respectively, and the anthocyanins peonidin-3-glucoside, with 26% of R%; petunidin-3-acetylglucoside, 58% of R%; and peonidin-3-acetylglucoside, 57% of R%. Regarding the nondialysed fractions, only 16 compounds were quantified. Among these, the most abundant ones were the anthocyanins delphinidin-3-glucoside, peonidin-3-glucoside and peonidin-3-acetylglucoside with R% by about 44%, 26% and 53%, respectively.

3.1.2. Red wines

Fig. 2 shows the impact along *in vitro* GI digestion on TP content and polyphenolic compounds, grouped by families. Table 2 shows the amounts of individual polyphenols in each step of this assay.

The results obtained showed that, compared to the increase observed in the bioaccessibility of grape phenolic compounds after mastication and stomach digestion, the TP content after these two steps did not show statistical differences ($p > 0.05$) with respect to the initial TP content in wine (average 1583 mg GAE/L) (Fig. 2).

After mouth digestion, the content of flavonols and phenolic acids did not show statistical differences ($p > 0.05$) with respect to the initial content in wine. On the other hand, anthocyanins and flavanols were detected in a higher content (an increase of 26% and 39%, respectively) with respect to wine.

After the stomach phase, the flavonols content did not change; while flavanols and phenolic acids were detected in higher concentrations compared to mouth digestion (80% and 43% increase with respect to wine, respectively). In the case of anthocyanins, the detected content in this step was slightly lower ($p < 0.05$) than the concentrations found in the previous step.

Finally, intestinal digestion significantly decreased ($p < 0.05$) the amount of bioaccessible TP, with a consequent decrease in all polyphenol families ($p < 0.05$). Only 31% of wine TP was found in the nondialysable fractions, and 21% in the dialysable fractions. Individual results for each of the polyphenol compounds investigated and their recovery percents (R%) along the simulated GI digestion are presented in Table 2. We observed substantial losses in some of the polyphenol compounds after dialysis in relation to their initial content in wine samples. Thus, only 14 out of 35 compounds quantified in wine were quantified in dialysed samples. Among them, the most abundant compounds were coutaric and fertaric acids with R% by about 37% and 68%, respectively, and the anthocyanins petunidin-3-acetylglucoside and peonidin-3-glucoside, with R% by about 40% and 36%, respectively. Regarding the non-dialysed fractions, only 13 compounds were quantified. Among these, the most abundant were coutaric and fertaric acids with R% by about 45% and 70%, respectively.

Table 1
Individual polyphenolic content (mg/kg fresh weight grape) in grape and in the different stages of GI digestion of said food. Recovery percents (R%) with respect to grape.

Compound	Grape	Mouth	Stomach	Nondialysable (R%)	Dialysable (R%)
Anthocyanins					
Delphinidin-3-glc	4.22 ± 1.14 c	1.66 ± 0.16 b	3.43 ± 0.35 c	1.87 ± 0.41 b (44)	< LOC a
Cyanidin-3-glc	17.17 ± 11.42 b	0.47 ± 0.17 a	< LOD a	1.21 ± 0.09 a (7)	< LOD a
Petunidin-3-glc	13.25 ± 1.92 d	4.39 ± 0.09 b	7.32 ± 0.65 c	0.83 ± 0.30 a (6)	1.29 ± 0.22 a (10)
Peonidin-3-glc	49.81 ± 5.83 b	8.76 ± 1.13 a	15.73 ± 0.23 a	12.75 ± 0.10 a (26)	12.78 ± 6.05 a (26)
Malvidin-3-glc	334.63 ± 22.53 d	105.18 ± 1.28 b	185.69 ± 1.50 c	30.75 ± 6.15 a (9)	31.45 ± 3.81 a (9)
Petunidin-3-acglc	2.51 ± 0.40 d	1.03 ± 0.04 b	1.67 ± 0.19 c	< LOD a	1.46 ± 0.16 c (58)
Malvidin-3-acglc	166.35 ± 15.79 d	29.85 ± 0.61 b	93.76 ± 3.90 c	15.19 ± 1.97 a (9)	14.57 ± 0.90 a (9)
Peonidin-3-acglc	11.89 ± 0.66 b	5.56 ± 1.00 a	11.9 ± 0.72 b	6.34 ± 0.27 a (53)	6.73 ± 3.41 a (57)
Malvidin-3-cafglc	4.43 ± 0.36	< LOD	< LOD	< LOD	< LOD
Delphinidin-3-cmglc	1.87 ± 0.05	< LOD	< LOD	< LOD	< LOD
Petunidin-3-cmglc	5.32 ± 0.46	< LOD	< LOD	< LOD	< LOD
Malvidin-3-cmglc	86.47 ± 5.30 b	< LOD a	< LOD a	0.86 ± 0.07 a (1)	2.53 ± 0.52 a (3)
Peonidin-3-cmglc	10.96 ± 0.71 c	< LOD a	< LOD a	< LOC a	1.5 ± 0.50b (14)
Flavanols					
(+)-Catechin	25.57 ± 4.76 b	79.87 ± 19.41 c	83.24 ± 3.04 c	4.78 ± 0.09 a (19)	22.41 ± 0.24b (88)
(−)-Epicatechin	31.49 ± 7.32 b	76.4 ± 4.63 c	119.64 ± 19.11 d	4.01 ± 0.60 a (13)	19.82 ± 0.72b (63)
Epicatechin gallate	88.35 ± 12.91 b	1.95 ± 0.93 a	< LOD a	< LOD a	< LOD a
Procyanidin dimer	5.62 ± 0.58 b	6.72 ± 0.56 b	7.88 ± 1.19 c	< LOD a	< LOD a
Procyanidin dimer monogallate	1.46 ± 0.37 c	0.70 ± 0.06 b	0.60 ± 0.21 b	< LOD a	< LOD a
Flavonols					
Myricetin	0.86 ± 0.06	< LOD	< LOD	< LOD	< LOD
Quercetin	4.38 ± 0.41	< LOD	< LOD	< LOD	< LOD
Kaempferol	0.84 ± 0.01	< LOD	< LOD	< LOD	< LOD
Syringetin	1.45 ± 0.25	< LOD	< LOD	< LOD	< LOD
Isorhamnetin	3.41 ± 0.26	< LOD	< LOD	< LOD	< LOD
Myricetin-3-glc	46.65 ± 4.24 d	15.96 ± 0.55 b	23.82 ± 1.43 c	< LOD a	< LOD a
Astilbin	5.24 ± 0.49 b	6.51 ± 1.55 b	8.35 ± 0.55 c	< LOD a	< LOD a
Laricitrin-3-glc	36.57 ± 8.40 c	18.17 ± 1.98 b	20.75 ± 3.50 b	< LOC a	< LOC a
Quercetin-3-glcr	37.30 ± 6.46 c	36.78 ± 0.47c	29.65 ± 4.46 b	3.63 ± 1.63 a (10)	6.79 ± 1.05 a (18)
Isoquercetin	494.36 ± 47.34 c	381.93 ± 13.13 b	506.74 ± 51.73 c	7.94 ± 2.96 a (2)	17.19 ± 9.06 a (4)
Kaempferol-3-glc	61.66 ± 6.62 d	19.07 ± 0.32 b	26.11 ± 0.81 c	3.39 ± 1.52 a (6)	12.04 ± 7.06 b (20)
Syringetin-3-glc	183.25 ± 15.25 c	28.78 ± 3.15 b	30.36 ± 3.58 b	2.15 ± 0.58 a (1)	3.66 ± 0.18 a (2)
Isorhamnetin-3-glc	417.07 ± 6.92 d	193.59 ± 16.09 b	299.61 ± 25.95 c	4.79 ± 1.74 a (1)	14.01 ± 3.03 a (3)
Phenolic Acids					
Gallic acid	5.3 ± 0.55 c	0.48 ± 0.06 b	0.59 ± 0.06 b	< LOD a	< LOD a
Fertaric acid	< LOD a	2.29 ± 0.40 c	3.66 ± 0.40 d	1.31 ± 0.37 b	3.19 ± 0.11 d

Abbreviations: glc, glucoside; glcr, glucuronide; ac, acetyl; caf, caffeoyl; cm, coumaroyl; Anthocyanin compounds were quantified as malvidin-3-glc; flavanols as (+)-catechin; myricetin, laricitrin, syringetin, quercetin and isorhamnetin as quercetin; kaempferol as kaempferol; flavonol glycosides as isoquercetin; hydroxycinnamic acids as caffeic acid; hydroxybenzoic acids as gallic acid. < LOD, below limit of detection; < LOQ, below limit of quantification. Different letters indicate significant differences ($p < 0.01$) along the simulated GI digestion.

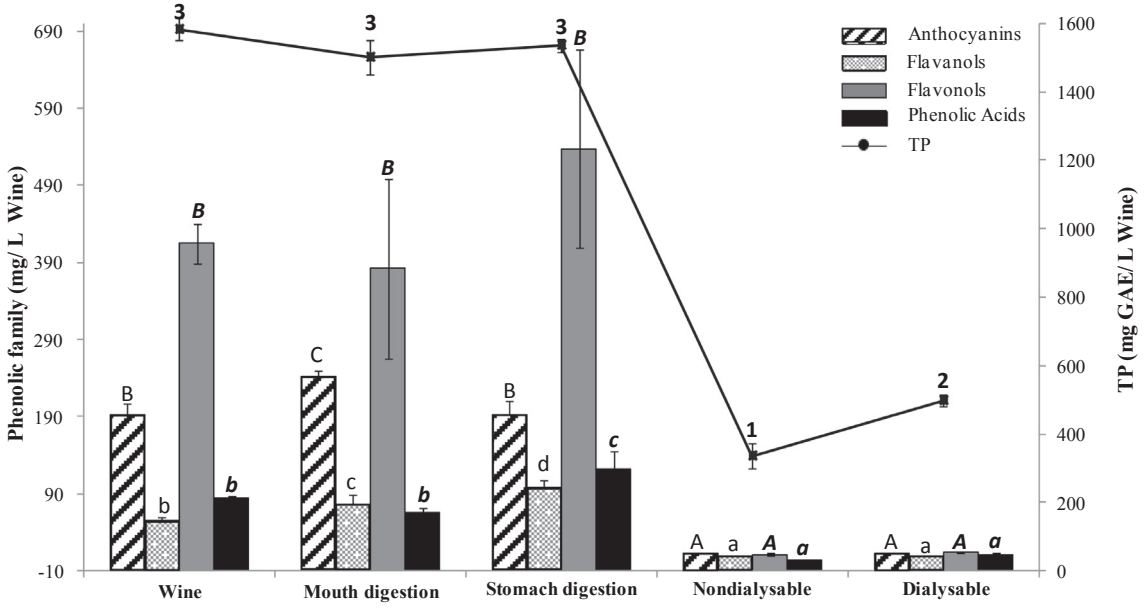

Fig. 2. Changes in total polyphenols content (TP) and polyphenolic compounds, grouped by families, along the simulated GI digestion of red wines. Different letters and numbers indicate statistically significant differences ($p < 0.01$) in each phenolic family among steps of difestive process.

Table 2
Individual polyphenolic content (mg/L wine) in wine and in the different stages of GI digestion of said food. Recovery percents (R%) with respect to wine.

Compound	Wine	Mouth	Stomach	Nondialysable (R%)	Dialysable (R%)
Anthocyanins					
Delphinidin-3-glc	2.25 ± 0.28 b	3.84 ± 0.21 d	3.15 ± 0.36 c	< LOC a	< LOD a
Petunidin-3-glc	5.35 ± 0.58 b	8.24 ± 0.44 d	6.49 ± 0.78 c	< LOD a	< LOD a
Peonidin-3-glc	4.44 ± 0.35 b	7.24 ± 0.50 d	5.58 ± 0.74 c	0.67 ± 0.23 a (15)	0.69 ± 0.13 a (16)
Malvidin-3-glc	126.27 ± 14.73 b	161.77 ± 9.34 c	146.88 ± 18.38 c	9.09 ± 0.06 a (7)	6.04 ± 0.26 a (5)
Delphinidin-3-acglc	0.39 ± 0.04 b	0.47 ± 0.04 c	0.39 ± 0.06 b	< LOD a	< LD a
Petunidin-3-acglc	1.17 ± 0.12 d	1.34 ± 0.12 d	0.71 ± 0.06 c	< LOC a	0.47 ± 0.12b (40)
Malvidin-3-acglc	41.1 ± 1.32 c	54.24 ± 1.01 d	25.37 ± 1.58 b	3.46 ± 0.21 a (8)	2.86 ± 0.03 a (7)
Peonidin-3-acglc	4.80 ± 0.39 c	5.18 ± 0.31 c	4.59 ± 0.44 c	< LOC a	1.70 ± 0.15b (36)
Petunidin-3-cmglc	0.28 ± 0.05	< LOD	< LOD	< LOD	< LOD
Malvidin-3-cmglc	2.60 ± 0.43	< LOC	< LOC	< LOC	< LOC
Peonidin-3-cmglc	0.46 ± 0.03	< LOC	< LOC	< LOC	< LOC
Pigment A	2.02 ± 0.25	< LOC	< LOC	< LOC	< LOC
Acetyl Pigment A	0.45 ± 0.06	< LOD	< LOD	< LOD	< LOD
Flavanols					
(+)-Catechin	31.28 ± 3.71 b	47.69 ± 3.94 c	50.18 ± 4.21 c	5.44 ± 0.96 a (17)	6.75 ± 0.45 a (22)
(−)-Epicatechin	15.98 ± 0.82 b	19.64 ± 9.30 b	39.36 ± 3.96 c	3.03 ± 0.57 a (4)	2.25 ± 0.50 a (14)
Procyanidin dimer	7.8 ± 0.42 b	9.05 ± 0.71 c	9.38 ± 0.23 c	< LOD a	< LOD a
Flavonols					
Myricetin	8.69 ± 0.37 c	2.53 ± 1.42 b	< LOD a	< LOD a	< LOD a
Quercetin	45.86 ± 2.37 b	44.23 ± 29.26 b	< LOD a	< LOD a	< LOD a
Laricitrin	0.99 ± 0.05	< LOD	< LOD	< LOD	< LOD
Kaempferol	6.38 ± 0.85	< LOD	< LOD	< LOD	< LOD
Isorhamnetin	16.14 ± 0.60 b	14.33 ± 10.28 b	< LOD a	< LOD a	< LOD a
Myricetin-3-glc	44.93 ± 4.82 c	28.76 ± 9.20 b	98.84 ± 2.93 d	< LOD a	< LOD a
Myricetin-3-glcr	4.33 ± 0.38 b	5.88 ± 1.36 c	7.99 ± 0.81 d	< LOD a	< LOD a
Astilbin	33.30 ± 1.11 c	27.29 ± 1.43 b	26.53 ± 1.54 b	2.48 ± 0.23 a (8)	2.43 ± 0.46 a (7)
Laricitrin-3-glc	31.18 ± 1.30 c	15.93 ± 1.46 b	32.48 ± 3.1 c	< LOC a	1.16 ± 0.03 a (4)
Quercetin-3-glcr	171.54 ± 15.12 b	185.52 ± 77.42 b	219.59 ± 104.93 b	6.01 ± 0.41 a (4)	4.98 ± 0.39 a (3)
Isoquercetin	14.16 ± 0.92 b	16.41 ± 2.74 b	17.32 ± 6.51 b	2.76 ± 1.02 a (20)	< LOC a
Syringetin-3-glc	24.98 ± 4.54 b	29.41 ± 4.77 b	116.48 ± 13.81 c	2.65 ± 0.20 a (11)	2.98 ± 0.35 a (12)
Isorhamnetin-3-glc	12.30 ± 0.90 b	11.59 ± 4.92 b	18.56 ± 0.71 c	< LOC a	< LOC a
Phenolic Acids					
Gallic acid	8.80 ± 0.51 b	7.77 ± 5.52 b	18.8 ± 0.62 c	< LOC a	< LOD a
Ethyl gallate	60.37 ± 1.58 c	36.05 ± 9.66 b	69.44 ± 18.18 c	6.25 ± 1.05 a (10)	< LOC a
Caftaric acid	7.47 ± 0.61 b	10.09 ± 2.73 b	14.02 ± 2.88 c	1.70 ± 0.46 a (23)	0.52 ± 0.10 a (7)
Coutaric acid	3.51 ± 0.23 b	4.22 ± 1.90 b	7.12 ± 0.74 c	1.57 ± 0.43 a (45)	1.29 ± 0.17 a (37)
Fertaric acid	2.81 ± 0.15 a	4.90 ± 0.48 b	8.6 ± 0.84 c	1.96 ± 0.22 a (70)	1.91 ± 0.27 a (68)
Caffeic acid	1.84 ± 0.06 b	2.14 ± 0.75 b	3.38 ± 0.89 c	< LOC a	< LOD a

Abbreviations: glc, glucoside; glcr, glucuronide; ac, acetyl; cm, coumaroyl; Anthocyanin compounds were quantified as malvidin-3-glc; flavanols as (+)-catechin; myricetin, laricitrin, syringetin, quercetin and isorhamnetin as quercetin; kaempferol as kaempferol; flavonol glycosides as isoquercetin; hydroxycinnamic acids as caffeic acid; hydroxybenzoic acids as gallic acid. < LOD, below limit of detection; < LOQ, below limit of quantification. Different letters indicate significant differences ($p < 0.01$) along the simulated GI digestion.

3.2. Effects of simulated GI digestion including dialysability on the AC of both foods

In order to study how the AC of grapes and wine is modified throughout the digestive process, *in vitro* AC was studied by FRAP, ABTS and DPPH assays at each stage of the simulated process. Fig. 3a and b shows the results obtained for grapes and wine, respectively. In general, it was observed that the three different assays (FRAP, ABTS and DPPH) to study the AC gave similar trends for grape and wine along the GI digestion.

In the case of grapes (Fig. 3a), the AC after mastication was significantly lower than the AC of the grape extracts, which is in agreement with the lower TP content extracted after this step (Fig. 1). After stomach digestion, the AC did not change when compared to mouth digestion, although the TP content showed an increase between these stages (Fig. 1). After intestinal digestion, the AC decreased as did the TP content, which was demonstrated in the previously discussed results (Fig. 1).

In the case of wines (Fig. 3b), the AC after mastication was significantly lower than the AC of wines, despite conserving the same TP content in both samples (Fig. 2). From mastication to intestinal digestion, the AC showed the same trend as did the TP content, that is to say, it was maintained from mastication to stomach digestion and then decreased stepwise after intestinal digestion (Fig. 2).

4. Discussion

In the present study, the release and stability of red grape and wine phenolic compounds and AC were monitored stepwise along simulated GI digestion, i.e. mouth, stomach and intestinal phases, including a final stage of dialysis. All results were compared to the polyphenolic profile natively present in grapes and wine. To our knowledge, this is the first study investigating the impact of individual digestion stages (from mouth, a very important step for solid foods such as grape) on the full polyphenolic profile and AC of red grapes, integrating the dialysable and nondialysable fractions and comparing them to those of red wines.

To exert their AC, polyphenols have first to be bioaccessible, i.e., released from the food matrix and solubilised. We observed that red grape bioaccessible TP increased stepwise, from mouth to stomach digestion. Both stages only extracted 29% of the initial grape TP content compared to wine, where in mouth digestion 100% of TP were already accessible. Differences between these results for grapes and wines suggest that amount of bioaccesible polyphenols depend on the food matrix. The processing of food products may have positive or negative effects on bioaccessibility of bioactive compounds (Cilla, Bosch, Barberá, & Alegría, 2016). In this sense, the bioaccesibility of natural antioxidants from fresh and processed products strongly depends on their genuine deposition form on the food matrix (Schweiggert & Carle, 2015). In solid food matrix such as fruit and vegetables, polyphenols are

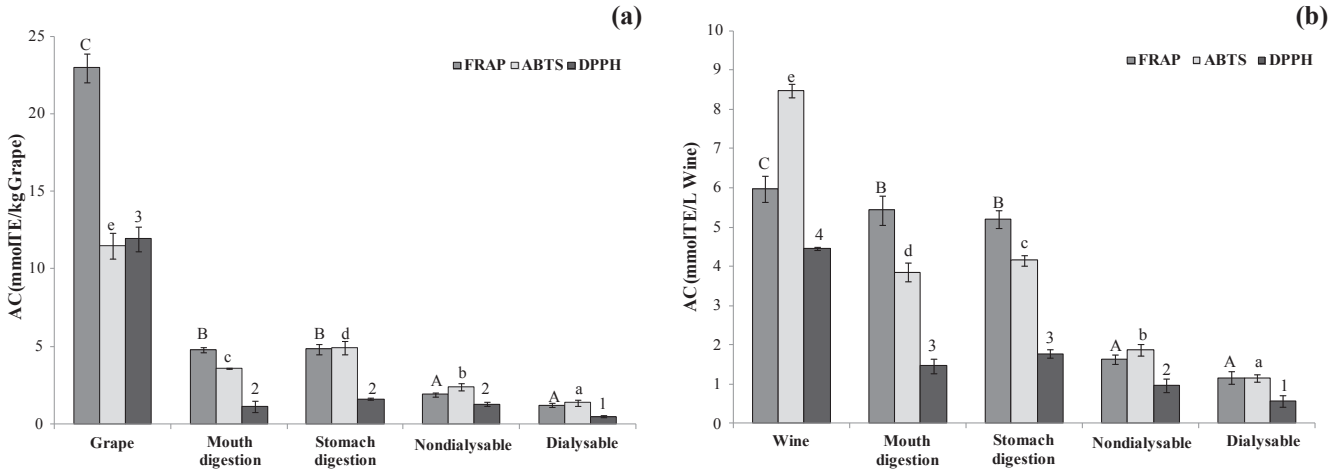

Fig. 3. Changes in *in vitro* antioxidant capacity (AC) along the simulated GI digestion of red grapes (a) and wines (b). CA expressed as: mmol TE/kg grape (fresh weight) or mmol TE/L wine. Different letters and numbers indicate statistically significant differences ($p < 0.01$) in each CA assay among steps of digestive process.

linked to carbohydrates, proteins, and cell walls structural components as well as to other phenolic compounds by covalent bonds, hydrogen bonding, and hydrophobic and hydrophilic interactions. Upon processing (thermal, mechanical, fermentation, etc.) they diffuse out of the vacuolar frontiers to derived product (Dufour et al., 2018; Podsędek et al., 2014). So, while polyphenols contained in the liquid matrix, as in the case of wine, are promptly bioaccessible and ready to exert their beneficial effects on the GI tract, this is not true for those contained in solid matrix such as grape. Therefore, the first steps of digestion may be considered as an extractor where both the mechanical and biochemical (strong acidic environmental and digestive enzymes) action during mastication and stomach digestive phase contribute to disrupt the vegetal tissue and the released of phenolic compounds from solid matrix (Tagliazucchi et al., 2010). Thus, our results showed that mastication and stomach digestion are fundamental steps in the digestion of a solid food matrix, as in the case of grapes.

The transition of samples from stomach digestion to the small intestine digestion environment caused a considerable decrease in polyphenolic constituents. The TP content after the small intestine digestion was reduced in 44% and 46% for grapes and wine, respectively, with respect to stomach digestion. According to other authors, this dramatic decrease in TP content may be correlated with pH changes along the GI digestion. The authors consider that phenolics are unstable in a neutral or alkaline medium, as is the case of the small intestine (pH 7.5), displaying better resistance to stomach conditions (pH 2) during the digestion process (Arenas & Trinidad, 2017; Burgos-Edwards, Jiménez-Aspee, Thomas-Valdés, Schmeda-Hirschmann, & Theoduloz, 2017; Celep et al., 2015; Sanz-Buenhombre et al., 2016). Another factor contributing to this drastic decrease is the effect of GI digestive enzymes, since they stimulate the release of phenolics from the sample matrix, then phenolic structure seemed not to be stable, and in consequence, subject to hydrolysis (Bouayed, Hoffmann, & Bohn, 2011; McDougall et al., 2005).

The native phenolic profile of grape and wine was qualitatively and quantitatively modified along the GI digestion assay. For example, (+)-catechin and (−)-epicatechin increased their concentration after mouth digestion of grapes probably because of the vigorous mechanical action, in addition to the chemical action, during mastication determine the breaking of grape seeds with the release of this kind of phenolics which are abundant in the solid parts of the berry, with seeds having the highest concentration (Lingua et al., 2016a).

On the other hand, the higher content (an increase of 26%) observed for anthocyanins could be the result of hydrolysis of their polymeric precursors during the mastication stage. Evidence of this could be, for example, pigment A and acetyl pigment A, compounds quantified in wine, which could not be quantified after mastication, while an increase in their precursors (malvidin-3-glucoside and malvidin-3-acetylglucoside, respectively) was observed after this step of digestion (Table 2). Similarly, the higher content of flavanols observed after gastric digestion for grapes and wine, resulting in contents that exceeded those initially found in both foods, could be explained as a consequence of hydrolysis of proanthocyanidins, which are hydrolysed to their monomeric units after the simulated stage of stomach digestion due to the strong acidic conditions to which they are subjected in said stage according to Fernández & Labra (2013). In our study, an evidence of this instability of proanthocyanidins could be the increase observed, for example, in the (–)-epicatechin monomer in both foods (Table 1 and Table 2). On the other hand, among polyphenols, a fraction of flavanols are known to stron gly bind to grape cell walls structural components (such as cellulose, hemicellulose, lignin, pectin) by covalent bonds (insoluble, bound or nonextractable phenolics) (de Camargo, Regitano-d'Arce, Biasoto, & Shahidi, 2014). So, the drastic conditions of stomach digestion may be enhance the liberation of this flavanols fraction of grapes, being another explanation why we observed an increase in its content after this stage even higher than the initial content of the samples. As regards to the amount of bioaccessible anthocyanins, flavonols and phenolic acids after 2 h of gastric digestion, we observed that was increased for grapes while in wines this amount was reduced for anthocyanins, conserved for flavonols and increased in case of phenolic acids. Differences between these results for grapes and wines suggest again that polyphenols bioaccesibility depends on the food matrix and, on the other hand, also suggest that the phenolic stability strongly depends on the food matrix. In case of grapes, as we mentioned earlier, compounds bound to solid food matrix are released by the action of strong acidic environmental (pH 2) and digestive enzymes which disrupt macromolecules. The low pH in gastric is also a factor increasing high stability of anthocyanins, which occur as stable flavylium cations, stimulating their released from the food matrix (Podsędek et al., 2014). As regards to wine, phenolic compounds showed different stabilities under gastric conditions which are in agreement with those reported for juices by Rodríguez-Roque, Rojas-Graü, Elez-Martínez, and Martín-Belloso (2013). These results suggest that phenolic stability in liquid matrix might depend on some factors such as their physicochemical properties and the interaction with dietary and/or gastric constituents. Additionally, the low pH and enzyme action of gastric digestion could hydrolyse some phenolic substances bound to proteins and carbohydrates from the food matrix, modifying the content of these bioactive compounds (Saura-Calixto, Serrano, & Goñi, 2007). In our study, probably other components of grapes, solid food matrix, at the same time they are disrupt to release the polyphenols from the food

matrix are also those that protect from their degradation under GI digestión, while in wines, liquid food matrix, phenolics already all bioaccesible and, in consequence, are more exposed and more labile to degradation (Podsędek et al., 2014).

Phenolic compounds showed different stabilities along this assay and similar results have been reported in several fruits and foods subjected to simulated digestion (Arenas & Trinidad, 2017; Bouayed, Deuβer, Hoffmann, & Bohn, 2012; Rodríguez-Roque et al., 2013). Food is physically and chemically broken down as it travels along the GI tract as a result of churning motions and hydrolytic enzymatic or chemical reactions. The GI physicochemical environment, with special influence of pH changes and digestive enzymes, is responsible for affect the phenolic stability and improve the bioaccesibility, from liquid and solid food matrix respectively, and in consequence produce changes their phenolic profiles (Bergmann, Rogoll, Scheppach, Melcher, & Richling, 2009).

An *in vitro* digestion model gives an indication as to the availability of foods antioxidants in a biological system, because this model simulates *in vivo* digestion (Podio et al., 2015). Several studies have shown the amount of dialysable polyphenol compounds, which it could be potentially bioavailable in the small intestine (Podio et al., 2015). In this sense, the dialyzable fraction of polyphenolic compounds from different foods has been studied and the R% obtained was in agreement with our observations in this study. Rodríguez-Roque et al. (2013) observed R% between 11% and 36% for different hydroxycinnamic acids, and between 19% and 29% for specific flavonoids compounds from a blended fruit juice. Celep et al. (2015) obtained amounts ranging between 17% and 96% for compounds belonging to the families of hydroxycinnamic acids, flavanols and flavonols in blueberry and blackberry wines. Podio et al. (2015) reported that the most abundant dialysable compounds in instant coffees were quinic acid and coumaroyl tryptophan conjugated with R% between 47–75% and 19–49%, respectively. However, to our knowledge, there are no reports on the dialysable compounds of the full polyphenolic profile in red grapes and wines, using an *in vitro* digestion model. Therefore, assuming that the dialysis during trial allowed simulating the passive absorption of the polyphenolic compounds in the intestine, it could be affirmed that the compounds observed in the dialysed fraction of those foods would be available for their absorption *in vivo*, influencing the cell activity to finally exercise its potential beneficial effect on human health (Podio et al., 2015).

On the other hand, our work also demonstrates that a considerable amount of phenolic compounds remain in nondialysable fraction and maybe simulating those potentially colon available. It is a well-known fact that the colon contains a diverse ecosystem of microorganisms that degrade and transform the non-digested components to microbial metabolites. Therefore, phenolic compounds found in the nondialysed fraction may appear as substrate for the community of microorganisms in the colon (Possemiers, Bolca, Verstraete, & Heyerick, 2011). Microbial metabolism of polyphenols includes numerous reactions or cleavages resulting in low molecular weight phenolics, phenolic acids such as caffeic acid, which may themselves have beneficial bioactive effects (Alqurashi et al., 2017; Gumienna et al., 2011). These acids, which are more stable and with comparable AC to their precursor polyphenol, are the phenolic form through which they enter the blood stream to exert their effects. Furthermore, the yielding products of microbial biotransformation can influence the composition and ecosystem activity itself. Several studies have linked the microbial metabolism of phenolic compounds to colorectal cancer prevention because of their influence on the bacterial metabolising enzymes (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013).

Considerable degradation and additional formation of polyphenolic constituents were observed in this work, highlighting that the manifold changes occurring during GI digestion then have an impact on availability and, in consequence, on the bioactivity of polyphenols. The AC is tightly associated with polyphenol content and composition (Lingua et al., 2016a). In the present study, AC in mouth digestion was lower than that observed for both foods, presumably due to the lower concentrations of polyphenols present compared to the chemical extraction in the case of grapes, in addition to the polyphenolic composition derived from mastication in the case of wines. Although the AC from mouth to stomach digestion was not modified, it was reduced after intestinal digestion due to the lower concentrations of polyphenols, in accordance with other studies (Bouayed et al., 2011; Rodríguez-Roque et al., 2013). Only 21% and 39% (average among FRAP, ABTS and DPPH results) of grape and wine AC, respectively, were observed after intestinal digestion (dialysed plus nondialysed fractions). Is important highlight that, in addition to the AC exerted by those dialysed phenolics, we report that the AC was retained by those nondialysed phenolics. Therefore, not only those potentially bioavailable compounds but also those potentially colon available ones would be able to exert their beneficial effects on human health. Further investigations with these fractions employing batch culture fermentation system, used to simulate colonic fermentation (to study the biotransformation of polyphenolic compounds by human microflora), and Caco-2 cells monolayer, used as model system to simulates the small intestinal barrier and study the transepithelial absorption, metabolism and molecular mechanism of intestinal transport, are needed to improve the understanding of bioaccesibility of phenolic compounds in the body (Alqurashi et al., 2017; Carbonell-Capella et al., 2014).

Data from chemical extractions have often been used to estimate the amount of polyphenols intake from human daily diets or portions (Zamora-Ros et al., 2016). Although these studies give valuable information about recommendations on nutritional intake, they are based on mere content data, not taking into account changes happening during GI digestion. Our results emphasize that chemical extraction, or the mere content data in case of wine, could overestimate the availability of polyphenols, since only 16% and 52% of the native polyphenols in grapes and wines, respectively, were found following GI digestion (dialysed plus nondialysed fractions) (Fig. 1 and Fig. 2). When incorporating the limited availability of polyphenols based on the present results, it may be estimated, for example, that the consumption of 100 g (a portion) of red grapes a day could contribute to comparable AC with respect to 125 mL (a cup) of red wine a day, in spite of the lower proportion of red grape TP found after GI digestion in comparison with red wine TP.

5. Conclusions

The results highlight that the bioaccessibility of polyphenols depends on the food matrix. In this sense, the processing of grapes as wine, liquid matrix, allow that all polyphenols are already bioaccessible, while in the grapes, these compounds should be release during mouth and stomach digestion. The GI physicochemical environment, with special influence of pH changes and digestive enzymes, affected the phenolic stability and improve the bioaccesibility, from liquid and solid food matrix respectively, producing changes in the content and composition of their profiles. From the original phenolic content of grapes and wines, only 16% and 52% respectively, were found after GI digestion (dialysed plus nondialysed fractions). Results showed that despite the significant loss of phenolic compounds during digestion, grapes and red wines still have AC. The AC after intestinal digestion showed the same trend as did the TP, and therefore, only 21% and 39% of grape and wine AC, respectively, was conserved. After dialysis, three anthocyanins (peonidin-3-glucoside, petunidin-3-acetylglucoside, and peonidin-3-acetylglucoside,) together with two flavanols ((+)-catechin and (−)-epicatechin) were those compounds found in greater proportion in dialysed fraction for grapes, while in wines there were two anthocyanins (petunidin-3-acetylglucoside and peonidin-3-glucoside) and two hydroxycinnamic acids (coutaric and fertaric acids). With regard to the non-dialysable fraction, the compounds found in higher proportion for grapes were three anthocyanins (delphinidin-3-glucoside, peonidin-

3-glucoside and peonidin-3-acetylglucoside), while in wines there were two hydroxycinnamic acids (coutaric and fertaric acids). These compounds, with special influence of anthocyanins, would be the most resistant to the human GI tract, and therefore the most relevant ones to explain the AC observed in grapes and red wines independently of the food matrix.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2018.02.034.

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