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Effects of *in vitro* gastrointestinal digestion on phenolic compounds and antioxidant activity of different white winemaking byproducts extracts

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Abstract

The effect of *in vitro* gastrointestinal digestion on phenolic composition and antioxidant activity of different white winemaking byproducts extracts (grape pomace and its parts: seeds, skins and stems) was evaluated. Fourteen individual phenolic compounds were evaluated by UHPLC. The antioxidant activity was measured by DPPH and ORAC assays. Differences on phenolic profile and antioxidant activity were observed depending on the digestion phase, the type of byproduct, the phenolic group and the antioxidant activity method. In general, digestion had a reducing effect on TPC and antioxidant activity; however, ORAC values of seed and stem extracts increased after digestion and some recovery indexes of the phenolic groups were very high. Results indicate that extracts from white winemaking byproducts are a reliable source of bioaccessible antioxidant compounds, which could be used as functional food ingredients.

Keywords

Winemaking byproducts; phenolic compounds; *in vitro* gastrointestinal digestion; antioxidant activity

Abbreviations

AAPH: 2,20-azobis(2-methylpropionamidine) dihydrochloride; DPPH: 1,1-diphenyl-2-picrylhydrazyl; LOD: limit of detection; LOQ: limit of quantification; ORAC: oxygen radical absorbance capacity; PCA: Principal Component Analysis; RI: Recovery index; SGF: Simulated gastric fluid; SIF: Simulated intestinal fluid; TE: Trolox equivalents; TPC: Total phenolic content.

1. **Introduction**

Nowadays, consumers are very interested in leading a correct and balanced diet that includes healthy and natural foods. Therefore, food industry has become well aware of the importance of producing foods with health beneficial properties. In this sense, the incorporation of bioactive compounds extracted from natural products into foods as functional ingredients is rising (García-Lomillo & González-SanJosé, 2017; Kowalska, Czajkowska, Cichowska, & Lenart, 2017; Martins, Pinho, & Ferreira, 2017; Nunes et al., 2016).

Grape pomace, a byproduct obtained from winemaking in large amounts, is an important source of bioactive compounds. This fact and the potential market interest of this product have led food researchers searching new alternatives for its exploration (García-Lomillo & González-SanJosé, 2017). The extraction of phenolic compounds from this byproduct is an attractive, sustainable and cost-effective source of high-value bioactives. Phenolic compounds are very abundant in grape pomace and they are interesting due to their antioxidant activity and other biological effects, which have been related to the prevention of common disorders (Del Pino-García et al., 2017; Falchi et al., 2016; Fraternale et al., 2016). The phenolic composition and antioxidant activity of grape pomace and its constituents, such as seeds, skins and stems, have been widely studied. Previous studies of our group indicated that phenolic rich extracts from grape pomace attenuate reactive oxygen species (ROS) accumulation and increase the resistance against oxidative stress in *Caenorhabditis elegans* (Jara-Palacios et al., 2013), exert antiproliferative activity in colon cancer cells (Jara-Palacios et al., 2015), and induce apoptosis in leukemia cells (León-González, Jara-Palacios, Abbas, Heredia, & Schini-Kerth, 2017). Moreover, the addition of seeds and skins from grape pomace during the fermentative step of the winemaking process increase the phenolic content

(Gordillo et al., 2014) and antioxidant potential of wines (Jara-Palacios, Hernanz, Escudero-Gilete, & Heredia, 2016). Phenolic extracts from winemaking byproducts have also been added to diverse food products, e.g. cheese (Han et al., 2011), tomate puree (Lavelli, Sri Harsha, Torri, & Zeppa, 2014), yogurt (Marchiani et al., 2016) and milk (Dos Santos et al., 2017), to improve their bioactive properties.

Grape pomace appears to be a promising source of functional ingredients to improve the phenolic profile and the antioxidant properties of food products. However, to evaluate the real bioactive potential of extracts from grape pomace is important to determine the effect of gastrointestinal digestion on phenolic compounds and to establish the bioaccessibility (Garbetta et al., 2018). As reported previously (Saura-Calixto, Serrano, & Goñi, 2007), bioaccessibility is defined as the amount of a food constituent that is released from a complex food matrix in the lumen of the gastrointestinal tract and could potentially be available for absorption into the body and promote biological actions (bioavailability). Therefore, the first step in determining the possible effects of a compound is to evaluate its stability during gastrointestinal digestion. *In vitro* methods simulating digestion process can be used to study the bioaccesibility from food sources. These methods have some advantages compared to *in vivo* methods: are more rapid, economic, safe and do not have ethical restrictions (Hur, Lim, Decker, & McClements, 2011).

Despite the importance of bioaccesibility, studies to determine the effect of digestion on phenolic composition and biological activity of extracts from winemaking byproducts are scarce (Corrêa et al., 2017; Fernández & Labra, 2013; Gil-Sánchez et al., in press; Janisch, Ölschläger, Treutter, & Elstner, 2006). As far as we know no reports on stem grape extracts were found in the literature and no previous studies regarding grape pomace extracts from white grape have been published in this respect. In this sense, the

aim of this study was to evaluate the effect of *in vitro* gastrointestinal digestion on phenolic composition of extracts from different white winemaking byproducts. Phenolic profile of extracts from grape pomace, seeds, skins and stems before and after digestion were analyzed by UHPLC. In addition, changes in the antioxidant activity of the extracts were investigated also during the digestion process.

2. Materials and Methods

2.1. Reagents and standards

Formic acid, HPLC-grade acetonitrile, methanol, ethanol, sodium carbonate and Folin-Ciocalteau reagent were obtained from Panreac (Barcelona, Spain). Gallic acid, protocatechuic acid, catechin, epicatechin, quercetin, kaempferol and quercetin-3-*O*-rutinoside (rutin) were purchased from Sigma-Aldrich (Madrid, Spain). Quercetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside were obtained from Extrasynthese (Genay, France). 2,2-diphenyl-1-picrylhydrazyl (DPPH), phosphate-buffered saline (PBS), pepsin from porcine gastric mucosa (CAS:9001-75-6) and pancreatin from porcine pancreas (CAS: 8049-47-6) and all other reagents used to prepare simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were purchased from Sigma-Aldrich (Steinheim, Germany). 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and 2,20-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and fluorescein were acquired from Acros Organics (Geel, Germany).

2.2. Samples and extraction procedure

The white winemaking byproducts from Zalema grapes (*Vitis vinifera* sp.) used in this study were grape pomace (including a mixture of seeds, skins, stems and rests of pulp), and skins, seeds, and stems separated manually from the pomace. Grape pomace samples were supplied by a winery located in Condado de Huelva Designation of Origin (southwestern Spain).

Samples were lyophilized and powdered in a blender to achieve a mean particle size less than 2 mm. Extraction of phenolic compounds was performed using a water and ethanol mixture (1:1). The plant material (5 g) was soaked overnight at room temperature in 200 ml of solvent and the resulting extract was filtered through a 5-13 µm membrane. Finally, seed, skin, stem and pomace extracts were concentrated to dryness in a rotary evaporator and stored at -20 ˚C.

2.3. Gastrointestinal *in vitro* digestion

The *in vitro* digestion consisted of an initial gastric phase followed by an intestinal phase using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), respectively, prepared with KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$ and $(NH_4)_2CO_3$, as previously described by Minekus et al. (2014). For gastric phase, 5 mL of each extract (50 mg/mL) were mixed with 3.75 mL of SGF, 0.8 mL porcine pepsin solution (25000 U/mL) and 2.5 µL of 0.3 M $CaCl_2$. The pH of the reaction mixture was adjusted to 3 by 1 M HCl and SGF was added to obtain a final volume of 10 mL. Mixture was incubated in a water bath at 37 °C for 2 h with stirring. Immediately, aliquots (gastric digests) were taken, stored at -20ºC and were analyzed within 2 weeks.

For intestinal phase, 7 mL of gastric digest was mixed with 3.85 mL of SIF, 1.75 mL of a pancreatin solution (800 U/mL), 20 mg of bovine bile extract, 20 mg of porcine bile extract and 14 µL of 0.3 M $CaCl_2$. The pH of the reaction mixture was adjusted to 6 by 1 M NaOH and SIF was added to obtain a final volume of 14 mL. Mixture was also incubated in a water bath at 37 °C for 2 h with stirring. Immediately, aliquots (intestinal digests) were taken, stored at -20ºC and were analyzed within 2 weeks.

Three independent experiments were performed for extracts of each type of byproduct and in each experiment three replicates were evaluated.

2.4. Total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay with some modifications (Gonçalves, Grevenstuk, Martins, Romano et al., 2015). Samples (undigested extracts, gastric and intestinal digests) were appropriately defrosted and stirred; then, the samples were diluted to an adequate concentration in relation to the standard (gallic acid). Briefly, 100 µL of sample, 200 µL of 10% (v/v) Folin-Ciocalteu reagent, and 800 µL of a solution of sodium carbonate (700 nM) were mixed and the solution was left to stand for 120 min for the reaction to take place and stabilize. Absorbance was measured at 765 nm. Gallic acid was employed as a calibration standard and results were expressed as gallic acid equivalents (mg GAE/g extract).

2.5. Individual phenolic compounds

Chromatographic analyses were carried out in an Agilent 1290 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode-array detector, which was set to scan from 200 to 770 nm, and a C18 Eclipse Plus 120 column (1.8 µm, 50 x 2.1 mm). The solvents were 0.01% formic acid in water (solvent A), and acetonitrile (solvent B) at the following gradient: 0-5 min, 5% B linear; 5-20 min 50% B linear; 20-25 min, 100% A linear, washing and re-equilibration of the column. The flow-rate was 0.8 mL/min, and the temperature of the column was set at 25 ºC. For analysis, samples (undigested extracts, gastric and intestinal digests) were defrosted, stirred and filtered through a 0.45 µm pore size membrane filter, and 1 µL of sample was injected in the column.

Gallic acid, protocatechuic acid, catechin, epicatechin, procyanidins B1 and B2, quercetin-3-O-rutinoside, quercetin-3-Oglucoside, kaempferol-3-O-glucoside were identified by their retention time and UV-vis spectra and by comparison with our data library and standards. The identity of these compounds was confirmed by mass spectrometry (Jara-Palacios et al., 2014). B2-O-gallate, trimer, tetramer, kaempferol-3-

O-galactoside and isorhamnetin-3-O-glucoside) were directly identified by mass spectrometry. The compounds were quantified with phenolic standards using peak area data of resolved peaks at 280 nm for flavanols and phenolic acids and 370 nm for flavonols. The corresponding calibration curves were made up for the following phenolic compounds: catechin (r2 = 0.999), gallic acid (r2 = 0.994), protocatechuic acid (r2 = 0.996) and quercetin (r2 = 0.996). The limits of detection (LOD) and quantification (LOQ) were calculated from calibrates curves. The LOD were calculated as three times the relative standard deviation of the analytical blank values calculated from the calibration curve. The LOQ were calculated as ten times the relative standard of the analytical blank values calculated from the calibration curve. The LOD and LOQ were: catechin (LOD: 1.15 ng and LOQ: 3.83 ng), gallic acid (LOD: 3.35 ng and LOQ: 11.18 ng), protocatechuic acid (LOD: 1.31 ng and LOQ: 4.37 ng) and quercetin (LOD: 0.91 ng and LOQ: 3.02 ng).

Total content of three phenolic groups (phenolic acids, flavanols and flavonols) were also estimated by summing the content of each individual phenolic compound quantified by UHPLC. Samples (undigested extracts, gastric and intestinal digests) of each type of byproduct were analyzed in triplicate, and the results were expressed as mg of phenolic compounds per gram of extract (mg/g).

2.6. Assessment of recovery index of phenolic compounds

To evaluate the effect of each digestion phase (gastric and intestinal) on the three phenolic groups (phenolic acids, flavanols and flavonols), the recovery index (RI) was calculated according to the equation below (Martínez-Las Heras, Pinazo, Heredia, & Andrés, 2017):

$$RI\ (\%) = (A/B) \times 100$$

where A is the phenolic content (mg/g extract) quantified in each tested winemaking byproduct extract at each digestion time, and B is the phenolic content in the tested extract before digestion and expressed in the same units.

2.7. DPPH assay

The capacity of samples to scavenge DPPH radicals was evaluated according to the previous protocol (Soler-Rivas, Espín, & Wichers, 2000) with some modifications. Samples (undigested extracts, gastric and intestinal digests) were appropriately defrosted and stirred; then, the samples were diluted to an adequate concentration in relation to the standard (trolox). Firstly, 300 µL of 90 µM DPPH methanolic solution was added to 30 µL of diluted sample , standard or methanol 80% (blank), and the mixtures were diluted with 570 µl of methanol. After 30 min, the reduction of DPPH radicals was measured at an absorbance of 515 nm. The radical scavenger activity was expressed as µmol TE (Trolox equivalents) per gram of extract (µmol TE/g).

2.8. ORAC assay

The oxygen radical absorbance capacity was evaluated as previously described (Gillespie, Chae, & Ainsworth, 2007). Samples (undigested extracts, gastric and intestinal digests) were appropriately defrosted and stirred; then, the samples were diluted to an adequate concentration in relation to the standard (trolox). Fluorescein was used as the fluorescent probe and AAPH as peroxyl radical generator. In each well of a black microplate, 150 µL of FL (80 mM) and 25 µL of diluted sample, blank (PBS), or standard were placed, and the reaction was initiated by adding 25 µL of AAPH (140 mM) to each well after incubating for 10 min at 37 ºC. The reduction in fluorescence was determined by reading fluorescein excitation at 485 nm and emission at 530 nm every minute for 90 min. The ORAC values were calculated using the area under the

curve (AUC) and the results were expressed as µmol TE per gram of extract (µmol TE/g).

2.9. Statistical analysis

Statistical treatment of the data was performed using the Statistica v.8.0 software. Statical analysis were performed to establish if phenolic composition (TPC, individual phenolic compounds and phenolic gropus) and antioxidant activity (DPPH and ORAC values) differed significantly between undigested extracts, gastric digests and intestinal digests for each type of winemaking byproduct. The data were presented as the mean ± standard error and were processed by one-way analysis of variance (ANOVA). Significant differences between means were identified using Tukey's New Multiple Range Test ($p<0.05$). Moreover, correlations between the phenolic composition (TPC, phenolic groups or individual phenolic compounds), and the antioxidant activity during digestion were performed by linear and multiple regressions. In all cases, statistically significant level was considered at $p<0.05$. A Principal Component Analysis (PCA) was also performed (with Table 2 data) in order to attribute which phenolic compounds are more important for each type of white winemaking by-product.

3. Results and Discussion

3.1. Effect of gastrointestinal digestion on phenolic composition

The TPC of seed, skin, stem and pomace extracts before and after *in vitro* digestion are shown in Figure 1. Before digestion, seed extracts showed the highest TPC (593.32 mg GAE/g extract), followed by pomace, stem, and skin extracts (237.15, 116.46 and 91.47 mg GAE/g extract, respectively). Changes in the TPC were found between digested and undigested samples. These changes varied depending on the type of winemaking byproduct and the digestion phase (gastric and intestinal) (Figure 1). The pattern observed for stems is similar to skins, TPC after the gastric phase were lower than in

undigested samples (109.25 and 72.56 mg GAE/g extract, respectively), however after the intestinal phase the values slightly increased to values similar or higher of those of undigested samples (131.52 and 92.31 mg GAE/g extract, respectively). TPC of seed and pomace extracts also decreased significantly ($p<0.05$) after the gastric phase (205.28 and 74.15 mg GAE/g extract, respectively), and although a slight increase was observed after intestinal phase, the values were much lower than in undigested samples (229.81 and 118.04 mg GAE/g extract, respectively).

For all types of byproducts, TPC decreased from undigested samples to intestinal digests. This decrease in TPC after *in vitro* gastrointestinal digestion was also reported by other authors for other fruits extracts (Fawole & Opara, 2016; Martínez-Las Heras et al., 2017; Pavan, Sancho, Pastores 2014; Perez-Vicente, Gil-Izquierdo, & García-Viguera, 2002). Nevertheless, some authors reported the maximum level of polyphenols in grapes after *in vitro* intestinal digestion (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010), which was observed in our study only for stem extract. Phenolics are highly sensitive to pH changes and, thus different changes in TPC after digestion depending on the medium conditions tested could be due to the stability of each type of phenolic compound present in the food matrix (Bouayed, Hoffmann, & Bohn, 2011; Pavan, Sancho, Pastores 2014).

In order to evaluate the stability of individual phenolic compounds during *in vitro* digestion, a total of fourteen compounds were evaluated by UHPLC: two phenolic acids (gallic and protocatechuic acids), seven flavanols (catechin, epicatechin, procyanidins B1, B2 and B2-*O*-gallate, trimer and tetramer) and five flavonols (quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-galactoside, kaempferol-3-*O*-glucoside and isorhamnetin-3-*O*-glucoside). Table 1 shows the individual phenolic compounds identified and quantified in the byproducts extracts before and after *in vitro*

digestion, and Table 2 shows the total contents of phenolic acids, flavanols and flavonols (as sum of individual compounds) and the RI (%) of each phenolic group. Phenolic acids and flavanols were more abundant in seed extract and flavonols in skin and pomace extracts, which is in accordance with other studies with white winemaking byproducts (Jara-Palacios et al., 2014; Jara-Palacios, Rodríguez-Pulido, Hernánz, Escudero-Gilete, & Heredia, 2016). Contents of phenolic acids decreased at the end of the digestion process for all the extracts (RI of 62, 92 and 75% for seed, stem and pomace extracts, respectively) except for the skin extract that reached a high RI of 355%. Gastrointestinal digestion had no significantly ($p>0.05$) effect on total content of flavanols in seed extract (97-99%). However, digestion increased total contents of flavanols of extracts from stem (from 477 to 961 mg/g, 201%) and pomace (from 443 to 1013 mg/g, 228%). On the other hand, total contents of flavonols decreased significantly ($p<0.05$) after the gastric phase for all byproducts (RI of 0, 47, 27 and 30% for seed, skin, stem and pomace extracts, respectively), followed by an increase after intestinal phase that did not reach initial values (Table 2). Flavanols in skin extract and flavonols in seed extract could not be quantified after digestion because they were degraded or the contents were lower than the limit of quantification.

In a previous study (Corrêa et al., 2017), results indicated that the *in vitro* digestion led to drastic qualitative and quantitative reductions in the phenolic compounds of a red grape pomace extract. However, in a recent work (Garbetta et al., 2018), all the identified compounds showed a good stability to the *in vitro* gastrointestinal conditions.

In this study, the contents of individual phenolic compounds were affected by the digestion process. In relation to phenolic acids, gallic acid values increased during gastric phase but decreased drastically after intestinal digestion, being absent in skin, stem and pomace extracts (Table 1). This fact is in accordance with a previous study

that reported that gallic acid was degraded under pancreatic conditions (Tagliazucchi et al., 2010). Protocatechuic acid had a very different trend to gallic acid in all winemaking byproducts, the content of this compound was higher for intestinal digests than for undigested samples. The gradual increase in its concentration during digestion could indicate a dehydroxylation of gallic acid for generation of protocatechuic acid (Mosele, Macià, Romero, Motilva, & Rubió, 2015) or the degradation of flavonoids (Sánchez-Patán, Monagas, Moreno-Arribas, & Bartolomé, 2011).

In the case of flavanols, the levels were different for monomers, dimers, trimer and tetramer. The monomers catechin and epicatechin were stable after digestion for seed extracts (Table 1), in contrast, these compounds increased significantly ($p<0.05$) and gradually during digestion for stems and pomace. The highest values of dimeric proanthocyanidins (B1, B2 and B2-3-*O*-gallate) were found at the end of digestion whereas trimer and tetramer are absent after digestion. This increase of monomeric and dimeric compounds could be due to the degradation of the trimer and the tetramer, which give rise to compounds with lower molecular weight (Arenas & Trinidad, 2017). Previous studies reported that catechins were increased in the presence of intestinal secretions because of a possible isomerization (Kahle et al., 2011). In a study in which *Vitis vinifera* seeds were subject to simulated gastrointestinal digestion it was observed that more catechin and epicatechin were released at neutral pH in comparison to a digestion in the stomach (Janisch et al., 2006), which is in accordance with our results. These authors also reported a decrease of procyanidins B1 and B2 after intestinal digestion, which was not observed in our work.

In general, the concentrations of all individual flavonols glycosides for all byproducts decreased after digestion in relation to undigested samples (Table 1). Quercetin-3-*O*-glucoside was the most abundant compound in skin, stem and pomace extracts and its

concentration was reduced significantly after intestinal digestion (from 162.60 to 113.26 mg/g, from 142.20 to 67.54 mg/g, and from 174.71 to 80.51 mg/g, respectively). It should be noted that kaempferol-3-*O*-galactoside and isorhamnetin-3-*O*-glucoside were not detected after digestion in stem extracts. This fact is in according with Corrêa et al. (2017), that studied the stability of flavanols from grape pomace and also reported a significant decrease of some flavonols (e.g. quercetin-3-*O*-glucoside and quercetin-O-rhamnoside), and the absence of isorhamnetin-3-*O*-glucoside and other flavonols glycosides after *in vitro* digestion.**3.2. Effect of gastrointestinal digestion on antioxidant activity**

The antioxidant activity of winemaking byproducts extracts before and during the simulated *in vitro* digestion is shown in Figures 2 and 3, for DPPH and ORAC assays, respectively. After digestion, seed extract showed the greater antioxidant activity in both assays followed by stem, pomace and skin extracts. A high and significant correlation (R=0.91, $p<0.05$) was observed between the TPC and radical scavenging activity values obtained with DPPH assay; suggesting that phenolic compounds influence significantly the antioxidant activity of extracts from winemaking byproducts during the whole digestion process. However, correlation between antioxidant activity values measured by ORAC assay and TPC was low and not significant (R=0.43). Also, the correlations between ORAC and TPC were performed for each type of byproduct: R= 0.8, 0.72, 0.60, and 0.43 for pomace, skin, seed and stem, respectively. Correlations regarding pomace and skin were high and significant.

For all byproduct extracts, the radical scavenging activity, measured by DPPH method, decreased from undigested samples to gastric digests (decrease in 48, 32, 26 and 7% for seed, stem, pomace and skin extracts, respectively), and although a slight increase was found from gastric phase to intestinal phase, final values of antioxidant activity were

much lower than those for undigested samples (Figure 2). A previous study reported that the ability of phenolic extracts to scavenge the DPPH· radical increased significantly after intestinal phase (between 5-18%) compared to that observed in the gastric digests, and this activity was also higher in intestinal digests than in undigested samples (Fawole & Opara, 2016), which was not in accordance with our results. Alkaline pH, as in intestinal conditions, has been reported to significantly increase scavenging ability of grape polyphenols (Bouayed et al., 2011). In this study, despite the reduction of antioxidant activity after digestion process, the intestinal digests showed antioxidant activity.

Regarding the results of ORAC assay, the antioxidant activity trend was different between seed and stem extracts, and skin and pomace extracts. As can be observed in Figure 3, the ORAC values increased significantly ($p<0.05$) during *in vitro* digestion for seed and stems extracts (from 2715.87 to 4048.96, and from 1052.50 to 2311.39 μmol TE/g, respectively). Nevertheless, values for skin and pomace extracts decreased after digestion (from 3384.30 to 1783.64 μmol TE/g, and from 3022.25 to 2279.51 μmol TE/g, respectively). Skin and pomace extracts showed the greatest antioxidant activity before digestion and the lowest one after digestion, which could be related to contents of flavonols in these extracts (higher values than in seed and stem extracts), that also experimented a reduction with the digestion process. In addition, the reduction or increase observed for the antioxidant activity could be linked to the presence of other not analyzed substances in the extracts, such as peptides, that could be involved in this activity (Pavan et al., 2014). Also, the assay employed could also affect the antioxidant activity assessment since pH modifications may alter structure of phenolic compounds affecting the antioxidant activity (Arenas & Trinidad, 2017). According to some

authors, assays carried out with buffer pH 7, such as ORAC and ABTS, are more suitable for the intestinal digests (Bouayed et al., 2011; Guldiken et al., 2016).

A large number of papers have reported the strong correlation between phenolic compounds and antioxidant activity. Thus, different statistical analyses were carried out to study this correlation between our data.

Multiple regressions were performed to check the most influential phenolic compounds (independent variables) total on the antioxidant activity (dependent variable) measured by DPPH and ORAC assay, depending on: undigested samples, gastric and intestinal digests. For these regressions all samples were considered, without differentiating by type of winemaking byproduct.

On the one hand, regarding ORAC assay, catechin, epicatechin, procyanidins B1 and B2, tetramer and kaempferol-3-O-glucoside significantly influenced antioxidant activity for undigested samples; gallic acid for gastric digests; and procyanidins B1 and B2-3-O-gallate, gallic acid and quercetin-3-O-glucoside for intestinal digests. On the other hand, regarding DPPH assay, the most influential phenolic compounds were phenolic acids for undigested samples; gallic acid and flavonols for gastric digests; and gallic acid, catechin and procyanidin B1 for intestinal digests.

Finally, a PCA was also performed in order to attribute which phenolic groups are more important for each type of white winemaking byproduct. After applying PCA to the data, it was seen that the first two principal components (PCs) explained 99.71% of the total variance. It can be seen from Figure 4, the first component (PC1) led to the separation of the seed from skin, stem and pomace samples, mainly due to the flavanol content. The second component (PC2) led to the separation of pomace from the rest of the byproduct samples, being mainly due to flavonol content.

Our results show that the antioxidant activity is strongly influenced by the phenolic profile. Therefore, if phenolic stability is affected by the digestion process, the antioxidant activity could be also affected. In a previous study with white grape skin, authors indicated that the *in vitro* digestion process attenuated the biological effect of skin polyphenols on intestinal cell line (Garbetta et al., 2018). However, other studies indicated an increase of the antioxidant activity of fruit extracts after digestion, which was associated with the release of phenolic compounds after the digestion (Pavan et al., 2014).

The phenolic composition (and therefore the antioxidant activity) of a food source could affect the microbiota and its catabolic activity. In this sense, a previous study suggests that simulated colonic fermentation seems to have a positive effect over the extract's bioactive potential. Therefore, although results from gastric and intestinal digestion provide valuable information on potential bioavailability of polyphenols, it is important to evaluate the effects of colonic fermentation, which would be interesting to look at in future.

4. Conclusions

The present study reported the influence of *in vitro* gastrointestinal digestion on phenolic profile and antioxidant activity of different white winemaking byproducts extracts. The results varied depending on the type of byproduct (seeds, skins, stems and pomace), due to different phenolic profile and different recovery indexes of phenolic groups. Although, gastrointestinal digestion had a reducing effect on TPC and antioxidant activity, digested extracts continued to have high values. In addition, some recovery indexes of the phenolic groups were very high and some individual phenolic compounds showed high stability.In some cases, such as protocatechuic acid, catechin, epicatechin, and dimeric proanthocyanidins, levels of these compounds increased after

the simulated digestion, whichcould indicate high bioaccessibility and potential bioavailability. The main novelty of this work is the study of effects of *in vitro* gastrointestinal digestion using white winemaking by products for the first time. The results obtained showed that white winemaking byproducts extracts studied are a promising source of functional ingredients for food and dietary supplements which can provide potentially bioavailable antioxidants to consumers. In addition, the present work provides information about components of agriculture byproducts with health-promoting, which can be used in the development of new products by the food industry.

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Conflict of interest/Disclosure

All the authors declare no conflict of interest.

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Figure captions

Figure 1. Total phenolic content (TPC) of extracts from the different winemaking byproducts before (undigested samples) and after *in vitro* digestion (gastric and intestinal digests). For the same byproduct different letters indicate significant differences ($p<0.05$) between undigested samples, gastric digests and intestinal digests Tukey's test. .

Figure 2. Antioxidant activity measured by DPPH assay of extracts from the different winemaking byproducts before (undigested samples) and after *in vitro* digestion (gastric and intestinal digests). For the same byproduct different letters indicate significant differences ($p<0.05$) between undigested samples, gastric digests and intestinal digests by Tukey's test. .

Figure 3. Antioxidant activity measured by ORAC assay of extracts from the different winemaking byproducts before (undigested samples) and after *in vitro* digestion (gastric and intestinal digests). For the same byproduct different letters indicate significant differences ($p<0.05$) between undigested samples, gastric digests and intestinal digests by Tukey's test. .

Figure 4. Principal Component Analysis (PCA) for phenolic groups and winemaking byproducts

Table 1. Concentrations (mg/g extract) of individual phenolic compounds identified in seed, skin, stem and pomace extracts before (undigested samples) and after *in vitro* digestion (gastric and intestinal digests).

	Seed			Skin			Stem			Pomace		
	Undigested	Gastric digest	Intestinal digest	Undigested	Gastric digest	Intestinal digest	Undigested	Gastric digest	Intestinal digest	Undigested	Gastric digest	Intestinal digest
Phenolic acids												
Gallic acid	100.58 ± 1.49 a	117.44 ± 3.08 b	42.70 ± 0.00 c	13.68 ± 0.75 a	31.64 ± 3.08 b	ND	38.44 ± 3.73 a	53.95 ± 0.66 b	ND	64.59 ± 3.26 a	80.64 ± 3.74 b	ND
Protocatechic acid	22.09 ± 0.37 a	33.14 ± 1.69 b	33.44 ± 0.06 b	8.59 ± 1.04 a	20.37 ± 1.51 b	79.05 ± 0.01 c	16.23 ± 1.70 a	22.85 ± 2.00 b	50.21 ± 3.56 c	20.71 ± 1.23 a	28.17 ± 1.25 b	64.15 ± 3.69 c
Flavanols												
Catechin	196.11 ± 22.79 a	246.66 ± 2.99 b	216.34 ± 7.19 a	22.55 ± 0.58	NQ	NQ	73.10 ± 1.89 a	144.62 ± 10.20 b	241.90 ± 19.80 c	63.08 ± 0.48 a	120.55 ± 0.60 b	213.36 ± 1.20 c
Epicatechin	240.51 ± 7.43 a	247.45 ± 11.99 a	243.38 ± 23.99 a	20.05 ± 1.92	NQ	NQ	53.18 ± 0.24 a	125.98 ± 1.27 b	259.20 ± 21.60 c	60.87 ± 1.68 a	115.90 ± 8.40 b	199.40 ± 0.00 c
Pc B1	173.87 ± 23.03 a	133.11 ± 5.99 b	233.38 ± 3.59 c	31.17 ± 0.12	NQ	NQ	88.87 ± 5.44 a	112.56 ± 2.40 b	223.51 ± 8.16 c	65.54 ± 0.96 a	104.48 ± 1.30 b	196.28 ± 0.24 c
Pc B2	189.04 ± 0.01 a	146.46 ± 10.80 b	205.99 ± 0.12 c	18.09 ± 0.00	NQ	NQ	61.53 ± 2.37 a	145.23 ± 0.00 b	237.07 ± 1.01 c	72.46 ± 0.24 a	109.58 ± 3.60 b	199.44 ± 0.24 c
Pc B2-3-*O*-gall	171.23 ± 8.40 a	175.87 ± 25.79 a	307.58 ± 33.39 b	25.66 ± 0.84	NQ	NQ	62.71 ± 2.84 a	ND	ND	64.06 ± 1.44 a	72.67 ± 0.99 b	204.75 ± 1.62 c
Pc trimer	158.94 ± 4.07 a	146. ± 8.99 b	ND	25.02 ± 2.28	NQ	NQ	76.99 ± 1.89 a	119.94 ± 3.21 b	ND	60.27 ± 0.48 a	ND	ND
Pc tetramer	115.07 ± 2.39 a	132.06 ± 13.19 b	ND	21.25 ± 0.59	NQ	NQ	61.50 ± 0.24 a	99.99 ± 2.05 b	ND	57.46 ± 0.48 a	ND	ND
Flavonols												
Q-3-*O*-rutin	5.73 ± 0.13	NQ	NQ	20.95 ± 2.75 a	12.52 ± 3.64 b	10.17 ± 3.39 b	20.78 ± 1.26 a	9.79 ± 1.88 b	9.63[b] ± 2.75 b	21.21 ± 2.30 a	7.92 ± 0.64 b	9.75 ± 2.05 b
Q-3-*O*-gluc	22.63 ± 0.26	NQ	NQ	161.60 ± 2.91 a	65.56 ± 2.93 b	113.26 ± 5.61 a,b	142.20 ± 14.50 a	31.30 ± 7.83 b	67.54 ± 3.8 b	174.71 ± 11.76 a	47.69 ± 5.11 b	80.51 ± 4.66 b
K-3-*O*-glucu	2.32 ± 0.13	NQ	NQ	5.45 ± 0.19 a	6.28 ± 1.69	7.16 ± 1.79	4.44 ± 0.38 a	1.77 ± 0.00	ND	5.55 ± 0.64 a	3.94 ± 0.77	5.21 ± 1.21

					a	a		b			a	a
K-3-*O*-gluc	2.96 ± 0.05	NQ	NQ	24.15 ± 0.06 a	13.82± 3.84 b	19.65 ± 5.24 a,b	11.71 ± 0.88 a	3.94 ± 0.47 b	11.54 ± 0.56 a	22.52 ± 1.02 a	8.40 ± 0.96 b	11.23 ± 4.54 b
I-3-*O*-gluc	1.25 ± 0.08	NQ	NQ	4.23 ± 0.06 a,b	3.24 ± 1.15 a	5.91 ± 1.41	5.16 ± 0.31 a	2.36 ± 0.00 b	ND	4.84 ± 0.13 a	1.64 ± 0.35 b	1.06 ± 0.00 c

Each value represents mean (n=3) ± SD. For each byproduct, values in the same row followed by different letters indicate significant differences ($p<0.05$) between undigested samples, gastric digests and intestinal digests by Tukey's test ($p<0.05$). NQ: not quantified. ND: not detected. Pc, procyanidin; gall, gallate; Q, quercetin; K: kaempferol; I: isorhamnetin; rutin, rutinoside; gluc, glucoside; glucu, glucuronide

Table 2. Total contents (mg/g extract) of phenolic acids, flavanols and flavonols (as sum of individual compounds), and the Recovery Index (%) of each phenolic group in seed, skin, stem and pomace extracts before (undigested samples) and after *in vitro* digestion (gastric and intestinal digests).

	Phenolic acids	RI	Flavanols	RI	Flavonols	RI
Seed						
Undigested	122.67 ± 1.13 a		1244.77 ± 68.14 a		34.90 ± 0.55	
Gastric	150.58 ± 3.08 b	123	1227.63 ± 79.78 a	99	NQ	0
Intestinal	76.15 ± 0.06 c	62	1206.69 ± 55.67 a	97	NQ	0
Skin						
Undigested	22.29 ± 1.79 a		163.79 ± 1.31		216.38 ± 13.84 a	
Gastric	52.01 ± 4.40 b	233	NQ	0	101.43 ± 3.53 b	47
Intestinal	79.05 ± 0.01 c	355	NQ	0	156.15 ± 5.81 a,b	72
Stem						
Undigested	54.67 ± 5.43 a		477.88 ± 14.91 a		184.28 ± 15.40 a	
Gastric	76.80 ± 2.57 b	140	747.47 ± 8.14 b	156	49.15 ± 9.69 b	27
Intestinal	50.21 ± 3.56 c	92	961.67 ± 14.95 c	201	88.71 ± 3.64 b	48
Pomace						
Undigested	85.30 ± 4.48 a		443.73 ± 0.04 a		228.83 ± 13.51 a	
Gastric	108.81 ± 2.81 b	128	522.98 ± 9.16 a	118	69.60 ± 6.78 b	30
Intestinal	64.15 ± 3.69 c	75	1013.24 ± 2.71 b	228	107.76 ± 7.17 b	47

Each value represents mean (n=3) ± SD. For each byproduct, values in the same row followed by different letters indicate significant differences ($p<0.05$) between undigested samples, gastric digests and intestinal digests by ANOVA (Tukey's test).

Figure 1.

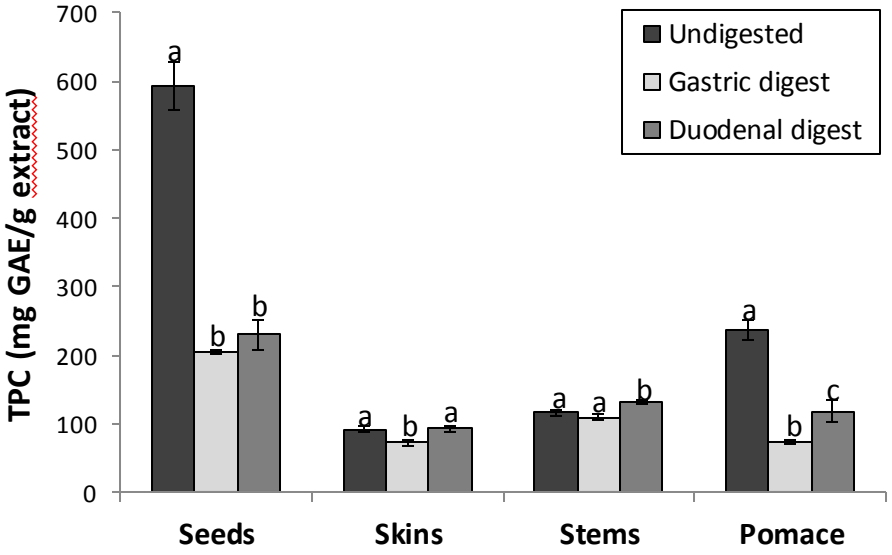


Figure 2.

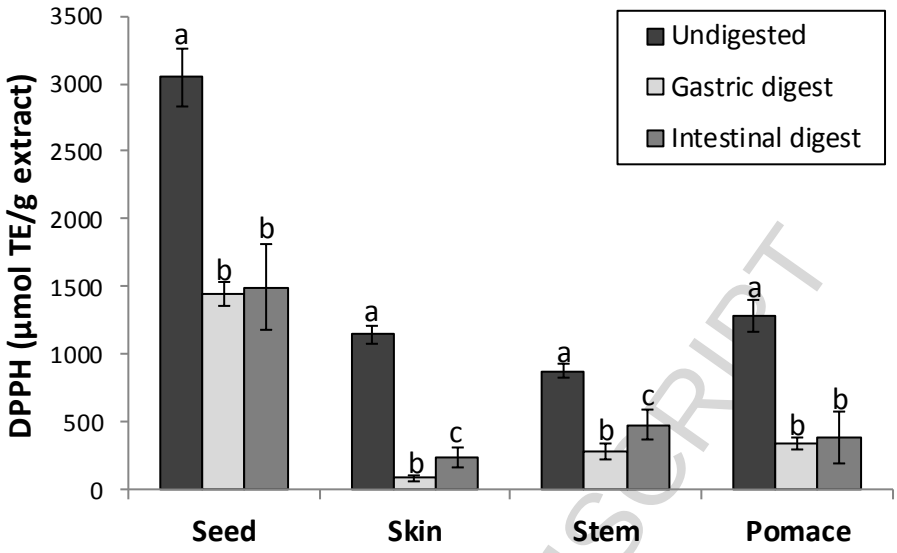


Figure 3.

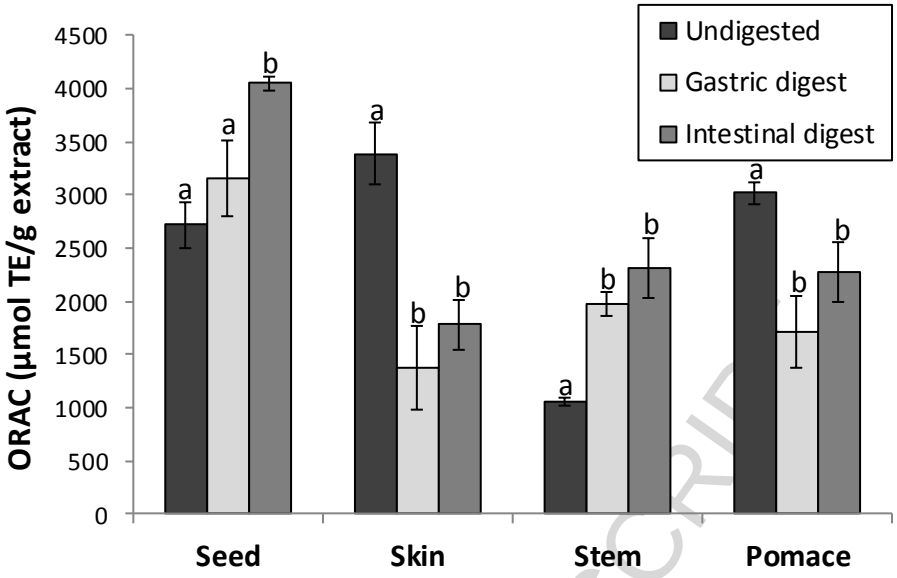
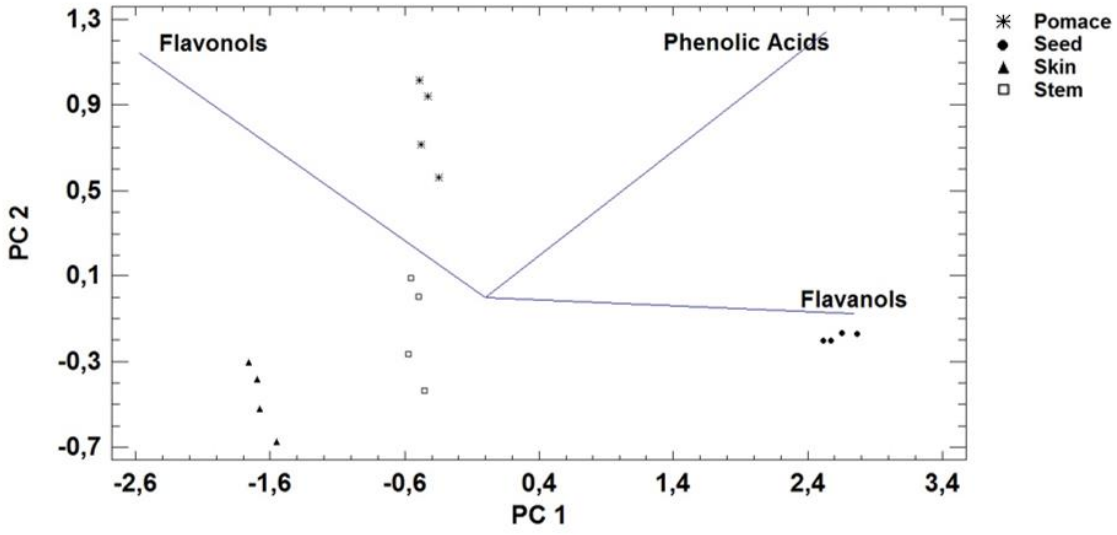
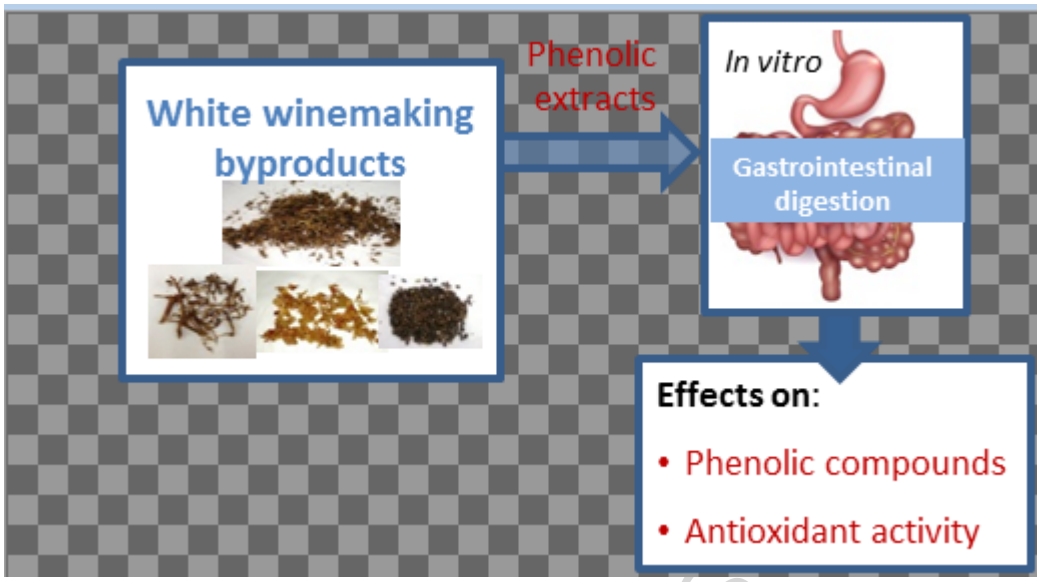


Figure 4.



Graphical abstract



Highlights

- White winemaking byproducts (seed, skin, stem and pomace) were subjected to *in vitro* digestion
- Digestion greatly affected the phenolic composition and antioxidant activity
- Effects of digestion were different according to the type of byproduct
- Total phenolics decreased although some individual phenolics showed high stability
- These byproducts are a reliable source of bioaccessible antioxidant compounds