DEGRADATION KINETICS OF ENCAPSULATED GRAPE SKIN PHENOLICS AND MICRONIZED GRAPE SKINS IN VARIOUS WATER ACTIVITY ENVIRONMENTS TO IMPROVE WIDE-RANGING AND TAILOR-MADE FOOD APPLICATIONS

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ABSTRACT

Micronized grape skin powder (GS) and maltodextrin-encapsulated grape skin phenolics (eGSP) were recovered from winemaking byproducts as potential food ingredients. Hygroscopicity was higher in eGSP than in GS. Both eGSP and GS had intense color and less fermented odor than the wet GS. Phenolic content, antioxidant activity and inhibitory effectiveness towards enzymes related to hyperglycemia damage were ~ double in eGSP than in GS. During storage, the rate of phenolic degradation diminished with decreasing a_w from 0.75 to 0.11. Anthocyanins and proanthocyanidins were less stable than monomeric flavanols and flavonols. The rate of decrease in antioxidant activity was lower compared to the extent of phenolic degradation. At a_w 0.11 no degradation was observed in eGSP, while anthocyanin and proanthocyanidin contents slightly decreased in GS ($k*10^3$ in the range 0.69 – 2.94 d^{-1}). Criteria for GS and eGSP storage were defined in relation to their final uses.

Industrial relevance. The conversion of winemaking by products into value added products is considered the unique strategy to overcome the cost of not recycling, including waste disposal and decontamination of affected areas. As winemaking is a seasonal activity, long-term stability of recovered byproducts is needed for their further utilization. GS and eGSP represent potential value-added food ingredients for wide-ranging applications (antioxidant, colorant, phenolic sources) and tailor-made functionalities (inhibitors of enzymes related to hyperglycemia). The results obtained led to the definition of criteria for GS and eGSP storage, which depend on their final use in foods, as illustrated by two discussed scenarios.

KEYWORDS: grape pomace; water activity; phenolic compounds; antioxidant activity; byproduct

ABBREVIATIONS

Abbreviations for chemicals: C, catechin; Cy-glc, cyanidin 3-O-glucoside; Dp-glc, delphinidin 3-O-glucoside; EC, epicatechin; GAE, gallic acid equivalents; K, kaempferol; Mv-glc, malvidin 3-O-glucoside; Mv-*pc*-glc, malvidin *p*-coumaroyl glucoside; Pn-glc, peonidin 3-O-glucoside; Pt-glc, petunidin 3-O-glucoside; Q, quercetin; Q-glc, quercetin 3-O-glucoside; Q-gln, quercetin 3-O-glucuronide. *Other abbreviations*: ADF, antioxidant dietary fiber; a_w, water activity; k, rate constant; eGSP, grape skin phenolics encapsulated into maltodextrins; GS, grape skins; GSP, grape skin phenolics; $t_{½}$, half-time; M_o, monolayer moisture content; T_g, glass transition temperature.

Main chemical compounds studied in this article:

- delphinidin-3-O-glucoside, PubChem CID 443650
- malvidin-3-O-glucoside, PubChem CID 443652
- petunidin-3-O-glucoside, PubChem CID 443651
- cyanidin-3-O-glucoside, PubChem CID 441667
- peonidin 3-O-glucoside, PubChem CID 443654

1. INTRODUCTION

Wine production is one of the most important agricultural activities throughout the world, causing the generation of large amount of byproducts including grape skins, seeds and stems. The management of all of the aforementioned byproducts poses serious environmental concerns because these residues have a low pH, high organic matter content and may exert phytotoxic effects if applied to crops or wetlands. Byproduct recovery and conversion into value added products is considered to be the unique strategy to overcome the cost of not recycling, including waste disposal and decontamination of affected areas (Devesa-Rey, Vecino, Varela-Alende, Barral, Cruz & Moldes, 2011).

Grape skins (GS) are potential sources of dietary fibre and phenolics, particularly, flavonols, flavanols, anthocyanins and proanthocyanidins (Perez-Jimenez et al., 2008; Teixeira et al., 2014). Hence, a great deal of interest has been expressed in the possibility to convert GS into value-added food ingredients due to their ability to provide advanced technological properties and/or health claims, to the final product (Galanakis, 2015). In some applications, GS are dried and micronized to obtain the "antioxidant dietary fiber (ADF)", a product that delivers fiber along with soluble and insoluble antioxidants. Grape ADF was demonstrated to have a positive effect in the prevention of cardiovascular diseases (Perez-Jimenez et al., 2008). Alternatively, GS are extracted using conventional or emerging technologies such as ultrasonics, high hydrostatic pressure, pulsed electric fields (Corrales, Toepfl, Butz, Knorr & Tauscher, 2008) and high voltage electrical discharges (Boussetta et al., 2012) to recover soluble phenolics. Phenolic extracts are then used as such or after encapsulation with various carriers that improve their solubility in alcohol-free water (Souza et al., 2014). Many studies have shown that phenolic can act as antioxidants and inhibitors of enzymes involved in oxidative stress, type-2 diabetes, hypertension and inflammation (Apostolidis, Kwon, Shetty, K., 2007; Teixeira et al., 2014).

Hence, both GS and GSP have been proposed for wide-ranging applications in various food products. In meat- and fish-based products GS and GSP have been applied as antioxidants (Sáyago-Ayerdi, Brenes, & Goñi, 2009; Sanchez-Alonso, Jimenez-Escrig, Saura-Calixto, & Borderias, 2008; García-Lomillo, González-SanJosé, Skibsted, & Jongberg, S., 2016) and antimicrobials (García-Lomillo, González-SanJosé, Del Pino-García, Rivero-Pérez, & Muñiz-Rodríguez, 2014). In beverages and gel products, GS and GSP have been proposed as natural colorants (Maier, Fromm, Schieber, Kammerer, & Carle, 2009; Lavelli, Sri Harsha, & Spigno, 2016a) and texturizing agents (Lavelli, Sri Harsha, Mariotti, Marinoni, & Cabassi, 2015). In dairy products, GS and GSP have been used to increase fibre and/or phenolic contents (Tseng & Zhao, 2013; Marchiani, Bertolino, Ghirardello, McSweeney, & Zeppa, 2015); moreover their effect on curd microstructure has been investigated to modulate cheese rheology (Han et al., 2011). In bakery products, GS and GSP have been used as fortifying

85 agents to improve phenolic and fibre contents (Walker, Tseng, Cavender, Ross, & Zhao, 2014;
86 Sant'Anna, Christiano, Marczak, Tessaro, & Thys, 2014). GS and GSP have also been utilized for
87 specific functionality. In fact, they have been found to decrease the level of N-(carboxymethyl)lysine,
88 an advanced glycated end-product related to health risk (Mildner-Szkudlarz, Siger, Szwengiel, &
89 Bajerska, 2015), to increase the antiglycation activity of foods (Lavelli, Sri Harsha, Torri & Zeppa,
90 2014) and to inhibit starch digestion enzymes, thus leading to food that could address the needs of
91 diabetic people (Lavelli, Sri Harsha, Ferranti, Scarafoni, & Iametti, 2016b.)
92 It is worth considering that winemaking is a seasonal activity and thus byproduct accumulation is
93 typically concentrated to a limited time frame. Hence, long-term stability of GS and GSP is needed
94 for further utilization. Previous studies have pointed out that GSP in the liquid form lack in long-term
95 stability, as the half-life of anthocyanins is about 30 d at 20-25 °C (Cardona, Lee, & Talcott, 2009;
96 Souza et al., 2014). The freeze-dried GSP showed higher anthocyanin stability; moreover,
97 encapsulation of the GSP with maltodextrins further increased anthocyanin stability (Souza et al.,
98 2014). The stability of foods in the dry and intermediate moisture state is critically dependent on the
99 water activity level (a_w). In fact, progressive decrease in a_w slows down the rates of microbial growth,
100 microbial production of toxins, and enzyme activities. Moreover, at low a_w levels, water limits reagent
101 mobility and becomes unavailable as a solvent to support chemical reactions (Lavelli & Vantaggi,
102 2009). With decreasing a_w, the glass transition temperature (T_g) increases. T_g is the temperature below
103 which a soft, rubbery material will transform into hard amorphous solid (glassy state). This leads to
104 a marked increase in viscosity and a decrease in molecular mobility. Foods are often considered very
105 stable below their T_g, as compounds involved in deterioration reactions take many months or even
106 years to diffuse over molecular distances and approach close enough to each other to react (Roos &
107 Karel, 1991; Nurhadi, Roos & Maidannyk, 2016).
108 There is no detailed information on the effect of intermediate and low moisture levels (a_w in the range
109 0.75 – 0.11) on the stability of GS and encapsulated GSP (eGSP). Hence, in the current study
110 micronized GS and eGSP were obtained from winemaking byproducts with the aims to: a) investigate

some properties relevant for wide-ranging applications, namely: hygroscopicity, color, odor, phenolic composition and antioxidant activity, as well as a target functionality, such as inhibition of starch digestion enzymes; b) model the degradation kinetics of individual phenolic compounds as a function of a_w in the range 0.11 – 0.75 at 30 °C; and c) define criteria for GS and eGSP storage in relation to their final uses.

2. MATERIALS AND METHODS

2.1. Chemicals

Malvidin-, cyanidin-, delphinidin-, peonidin- and petunidin- 3-O-glucosides were obtained from Polyphenols (Sandes, Norway). Catechin, epicatechin, quercetin 3-O-glucuronide, quercetin 3-O-glucoside, quercetin and kaempferol were purchased from Extrasynthese (Lyon, France). All other standards and chemicals were purchased from Sigma Aldrich (Milan, Italy).

2.2. Grape skins (GS) and grape skin phenolics encapsulated in maltodextrin (eGSP)

Grape pomace of the Barbera variety was kindly provided by a winery located in Northern Italy. At the winery, grapes were processed according to red vinification and the fermented pomace was recovered and sieved (with a 5-mm sieve) to separate the skins from the seeds. The seeds were also removed manually. Like all vegetable materials, the GS rapidly undergo spontaneous fermentation (Lavelli, Pagliarini, Ambrosoli, Minati, & Zanoni, 2006). Hence GS were put in a 1cm-high steel plate and then frozen in a freezer at -20 °C. The frozen GS were transported to the lab and dried at 55 °C for approximately 3 h. After drying, GS were milled and sieved by using the Octagon Digital sieve shaker (Endecotts Ltd., United Kingdom), with a certified sieve (openings: 125 μm) for 10 min at amplitude 8. The sieved GS were stored under vacuum, in the dark, at 4 °C.

The eGSP were obtained as described previously (Lavelli et al., 2016a). Briefly, GS were extracted with 60 % aqueous ethanol with continuous stirring for 2 h at 60 °C. The drying process was performed in a laboratory scale spray dryer (Buchi Mini Spray Dryer B-290, Switzerland), with the following operation conditions: 0.7-mm diameter nozzle, 4 mL/min feeding rate with 6.5 % w/v maltodextrin (dextrose equivalence 12); 700 L/h drying air flow rate; 150 °C inlet air temperature.

2.3. Phenolic Solubility at pH 3.5 and pH 6.5

An amount of 1.25 g of eGSP or GS was added to 15 mL of 0.15 M citrate buffer, pH 3.5 or to 15 mL of 0.15 M citrate buffer, pH 6.5. The mixtures were incubated in a water bath at 30 °C for 30 min and then centrifuged at 10000 x *g* for 20 min. The residues were discarded, while the supernatants were collected, and the total phenolic content was measured as described under Section 2.6. The amount of soluble phenolics extracted from GS and eGSP was evaluated in duplicate and expressed as grams of gallic acid equivalents (GAE) per litre of buffer.

2.4. Storage Study.

The powders were weighed into Petri dishes (6 cm diameter, 5.5 g of product in each dish). The dishes were placed inside airtight plastic chambers on wire-mesh racks situated above saturated salt solutions. The chambers were stored for 6 months at 30 °C in a thermostated cabinet. These time-temperature conditions were chosen as they resemble a practical handling for ingredients derived by winemaking byproduct. In fact, in the Mediterranean countries the winemaking season starts in August and ends in January. Hence, for a convenient management of these ingredients, stability for 6-months at room temperature should be advisable. Cold storage is not appropriate for byproduct since it is too energy-consuming, hence the effect of storage at lower temperatures was not investigated. To create different relative humidity environments, the following saturated salt solutions were used: LiCl (a_w = 0.113 ± 0.002), CH_3COOK (a_w = 0.216 ± 0.005), $MgCl_2$ (a_w = 0.324 ± 0.002), NaBr (a_w = 0.560 ± 0.004), and NaCl (a_w = 0.7509 ± 0.0011). Duplicate chambers were incubated for each a_w level. At five time intervals (0, 28, 60, 112 and 175 days), samples were extracted in duplicate as described in Section 2.6 and analyzed for phenolic content, antioxidant activity and color, as described in Sections 2.7 -2.11. Antioxidant contents, antioxidant activity and color degradation as a function of time (at each a_w level and fixed temperature of 30 °C) was analyzed according to a first-order kinetics, as follows:

$$\ln(A) = \ln(A_0) - \int (dt * k) \qquad (1)$$

where A_o is the initial antioxidant content, antioxidant activity or color, A is the antioxidant content, antioxidant activity or color at time t, and t is the storage time and k is the rate constant.

2.5. Moisture Content and a_w.

Moisture content of GS and eGSP after equilibration at the various relative humidity conditions was determined using a vacuum oven at 70 °C and 50 Torr for 18 h.

The a_w of samples and saturated salt solutions was checked using a dew point hygrometer (Aqualab, Decagon Devices, Pullman, WA, USA). Duplicate determinations were made for each sample. Moisture isotherms were developed for the eGSP and GS samples by plotting the equilibrium moisture content (M) versus the storage a_w. The Guggenheim-Anderson-de Boer (GAB) equation was used to fit the experimental data:

$$M = \frac{M_o C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)} \quad (2)$$

where M is the equilibrium moisture content on a dry basis (g of water/g of dry solids); M_o is the monolayer moisture content on a dry basis; C and K are constants (Nurhadi et al., 2016).

2.6. Phenolic extraction

For phenolic extraction from GS and eGSP, an amount of 100 mg was added with 8-mL methanol:water:HCl (80:20:0.1, v/v/v), for 2 h at room temperature with continuous stirring. The mixture was centrifuged at 10000 x *g* for 10 min, the supernatant was recovered, and the solid residue was re-extracted using 6 mL of the same solvent twice (Sri Harsha, Gardana, Simonetti, Spigno, & Lavelli, 2013). Duplicate determinations were performed for each sample. The extracts were stored in the dark, at -20 °C, until further characterization studies.

2.7. Total phenolics

The Folin–Ciocalteu assay was performed on sample extracts according to Sri Harsha et al. (2013). Briefly, the reaction mixture contained 0.5 mL of the extracts diluted with methanol:water:HCl (80 : 20 : 0.1, v/v/v), 6.0 mL of distilled water, 0.5 mL of Folin–Ciocalteu reagent and 3 mL of 10 % Na_2CO_3. The mixtures were incubated for 90 min at room temperature and the absorbance was

187 recorded at 760 nm against a blank with no extract addition. For each extract, 2 - 4 dilutions were
188 assessed in duplicate. A calibration curve was built using gallic acid as a standard and the total
189 phenolics were expressed as gram GAE per kilogram of dry product.

190 ***2.8. Soluble proanthocyanidins***

191 Proanthocyanidin content was analysed as described previously (Sri Harsha et al., 2013). Briefly, for
192 evaluation of soluble proanthocyanidins 1 mL of the sample extract diluted with methanol:water:HCl
193 (80:20:0.1, v/v/v) was added to 6 mL of *n*-butanol:HCl (95:5, v/v) and 0.2 mL of 2 % $NH_4Fe(SO_4)_2.12$
194 H_2O in 2 M HCl. For evaluation of insoluble proanthocyanidins, 10 mg of the extraction residue was
195 weighted in quadruplicate and added to 20 mL methanol, 120 mL *n*-butanol:HCl (95:5, v/v) and 4 mL
196 of 2 % $NH_4Fe(SO_4)_2.12$ H_2O in 2 M HCl. Hydrolysis was carried out at 95 °C for 40 min. The reaction
197 mixtures were cooled and the absorbance was recorded at 550 nm by a Jasco UVDEC-610
198 spectrophotometer (Jasco Europe, Cremella, Italy) against a blank made as for the sample but
199 incubated at room temperature. For each sample extract, 2 - 4 dilutions were assessed in duplicate.
200 Proanthocyanidin content was determined using 0.1736 (mg/mL) as conversion factor (Sri Harsha,
201 Lavelli & Scarafoni, 2014) and expressed as grams per kilogram of dry product.

202 ***2.9. HPLC analysis of phenolics***

203 The phenolic profile of the extracts was analysed as described previously (Lavelli et a., 2016a), using
204 a model Shimadzu LC-20 AD pump coupled to a model Shimadzu SPD-M20A photodiode array
205 detector and an RF-20 AXS operated by Labsolution Software Shimadzu, Kyoto, Japan). A 2.6 μm
206 Kinetex C18 column (150 x 4.6 mm; Phenomenex, Bologna, Italy) was used for the separation, at a
207 flow-rate of 1.5 mL/min. The column was maintained at 40 °C. The separation was performed by
208 means of a linear gradient elution. Eluents were: (A) 0.1 % H_3PO_4; (B) acetonitrile. The gradient was
209 as follows: from 6% B to 20% B in 18 min; from 20 % B to 60 % B in 7 min; from 60 % B to 90 %
210 B in 19 min; 90 % B for 10 min and then 6 % B for 5 min. DAD analysis was carried out in the range
211 of 200 - 600 nm. Anthocyanins and flavonols were quantified by calibration curves built with external
212 standards, namely, malvidin-3-O-glucoside at 520 nm for anthocyanins and quercetin-3-O-glucoside

213 at 354 nm for flavonols. Flavanols were quantified by catechin and epicatechin standard with the
214 fluorimetric detector set at λ_{ex} 230 and λ_{em} 320. Results were expressed as gram per kilogram of dry
215 product.

216 ***2.10. Color***

217 For color determination, each sample extract was diluted in 0.025 M potassium chloride buffer, pH
218 1.0. Under these conditions, color is due to both monomeric and polymeric anthocyanins (Cheynier
219 et al., 2006). Dilution factor was chosen until absorbance at 520 nm was within the linear range (0.100
220 – 0.800 absorbance units). For each sample extract, 2 dilutions were assessed in duplicate. The
221 variation in color due to total anthocyanins at every sampling time was determined as the difference
222 between the absorbance at 520 nm (A_{520nm}) and that at 700nm (A_{700nm}) to correct for turbidity.

223 ***2.11. Ferric ion reducing antioxidant power (FRAP) assay***

224 The FRAP assay was performed as described previously (Sri Harsha et al., 2013). Briefly, FRAP
225 reagent was prepared by adding 25 mL of 300 mM acetate buffer, pH 3.6; 2.5 mL of 10 mM 2,4,6-
226 tripyridyl-*s*-triazine in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3$. The reaction mixture contained 0.4
227 mL of sample extracts diluted with methanol:water:HCl (80:20:0.1, v/v/v) and 3 mL of FRAP
228 reagent. The absorbance at 593 nm was evaluated on a Jasco UVDEC-610 spectrophotometer (Jasco
229 Europe, Cremella, Italy) after 4 min of incubation at 37 °C against a blank with no extract addition.
230 For each sample extract, 2 - 4 dilutions were assessed in duplicate. A methanolic solution of
231 $FeSO_4 \cdot 7H_2O$ was used for calibration. Results were expressed as millimoles of Fe(II) sulfate
232 equivalents per kilogram of dry product.

233 ***2.12. In vitro α-glucosidase and α-amylase inhibition assay***

234 Inhibition of starch digestion enzymes was carried out as described previously (Lavelli et al., 2016b).
235 A crude α-glucosidase solution was prepared from rat intestinal acetone powder. Briefly, 200 mg of
236 rat intestinal acetone powder was dissolved in 4 mL of 50 mM ice cold phosphate buffer (pH 6.8) and
237 sonicated for 15 min at 4 °C. The suspension was vortexed for 20 min and then centrifuged at 10000
238 x *g* at 4 °C for 30 min. The resulting supernatant was used for the assay. For the α-glucosidase activity

assay, 650 μL of 50 mM phosphate buffer, pH 6.8; 100 μL of the enzyme solution and 50 μL of grape skin extract were added in Eppendorf tubes and pre-incubated for 5 min at 37 °C. Then, 200 μL of 1 mM *p*-nitrophenyl α-D-glucoside was added as substrate and the mixture was further incubated at 37 °C for 25 min. For the pancreatic α-amylase assay, 550 μL of 50 mM phosphate buffer, pH 6.8, 200 μL of the enzyme solution (10 μM in the same buffer) and 50 μL of grape skin extract were added in Eppendorf tubes, pre-incubated for 5 min at 37 °C. Then, 200 μL of 1 mM *p*-nitrophenyl α-D-maltopentaoside was added to the tubes as the substrate and the mixture was further incubated at 37 °C for 55 min. For both enzymatic reactions, the assay mixture was centrifuged at 10,000 *g* for 3 min and the absorbance of the clear supernatant was recorded at 405 nm. The control was run by addition of the extraction solvent replacing the sample. A sample blank and a control blank were run without addition of substrate and without addition of both substrate and sample, respectively. Dose–response curves were built and results are reported as I_{50} ($mg*mL^{-1}$), i.e. amount of samples (mg, d.w.) that inhibited the reaction by 50%.

2.13. Odor

Dried GS (0.06 g and 0.4 g) and eGSP (0.03 g and 0.2 g) were added to 7 mL of 8 mM citric acid buffer, pH 3.0 to obtain final phenolic contents of 0.4 g GAE/L and 2.5 g GAE/L for both samples. For comparison, wet GS and finely homogenized wet GS were prepared at a final phenolic content of 0.4 g GAE/L. Water was used as a control. Samples and control were prepared in 10mL-glass tubes covered with an aluminum foil and marked with 3-digit numbers. In order to evaluate whether these 7 samples differed in terms of fermented odor, the ranking test was performed (ISO 8587, 2006). Twenty-eight selected and trained assessors (43% men, mean age= 22.6 SD =2.4) were involved (ISO 8586). Each assessor was instructed to sniff each sample and to rank the samples from the least intense (1) to the most intense (7) according to fermented odor. A pause of 30 seconds was imposed between samples evaluation. Samples were presented simultaneously to the assessors at room temperature and under red light in order to mask possible difference in appearance. Samples presentation order was

randomized to prevent first-order and carry-over effects. The evaluation was performed in one replicate between 12.30 and 13.30 in sensory booths at the sensory laboratory of the Department of Food, Environmental and Nutritional Sciences (University of Milan). Data were collected using Fizz v2.47b software program (Biosystemes, Couternon, France).

2.13. Statistical analysis of data

Analytical data were analysed using Statgraphics 5.1 (STCC Inc.; Rockville, MD). Simple regression was performed to analyse the relationship between either antioxidant concentration or antioxidant activity or color (in logarithmic scale) as a function of time at fixed temperature for every a_w (equation 1). The output showed the equation of the fitted model giving the value of k, the P-value and R-squared statistic, which was used to estimate the goodness of fit. The model was considered significant when the P-value was less than 0.05 (95% confidence level). Nonlinear regression was performed to analyse the relationship between M and a_w by fitting to the GAB equation (equation 2). The output showed the equation of the fitted model giving the values of Mo, C and K and R-squared statistic, which was used to estimate the goodness of fit. Sensory data were analysed by Friedman test (Analysis of Variance by ranks). In case of significant differences ($P < 0.05$) among the rank orders of the samples, the Least Significant Difference (LSD) was applied to determine which samples were significantly different from others (ISO 8587, 2006).

3. RESULTS AND DISCUSSION

3.1. Phenolic content, FRAP values and water solubility

The total phenolic content of GS (43.9 g GAE/kg d.w.) was in the range of that found previously for grape skins of various varieties recovered from winemaking (Sri Harsha et al., 2013; Sri Harsha et al., 2014). For eGSP the total phenolic content (87.7 g GAE/kg d.w.) was almost two-fold higher than for GS, resulting in higher FRAP values (Table 1). Moreover, in eGSP the total phenolic content encapsulated was almost double than that reported in previous studies of spray drying encapsulation of grape phenolics using maltodextrin (Souza et al., 2014) and that observed upon spray-drying and freeze-drying encapsulation using gum arabic, partially hydrolyzed guar gum, and polydextrose

290 (Kuck & Norena, 2016). This results from the use of a low maltodextrin concentration during spray
291 drying in the current study, i.e. 6.5%, whereas in previous studies carrier concentration was in the
292 range 10 - 30% (Souza et al., 2014) and 10% (Kuck & Norena, 2016).
293 On a dry weight basis, the I_{50} values for α-glucosidase and α-amylase inhibition by eGSP was ~ half
294 with respect to those of GS, i.e., the inhibition effectiveness was double, due to the higher phenolic
295 content in eGSP (Table 1). On a phenolic content basis, I_{50} for α-glucosidase inhibition was ~ 19 μg
296 GAE mL^{-1} for both eGSP and GS. These values were lower with respect to those observed for white
297 grape skins, ranging between 30.9 and 64.8 μg GAE mL^{-1} (Lavelli et al., 2016b) and similar to that
298 of red berries (Boath, Grussu, Stewart, & McDougall, 2012). This result suggested that GS and eGSP
299 had a good phenolic pool, due to their high content in anthocyanins, which have been shown to play
300 a major role in α-glucosidase activity inhibition (Boath et al., 2012). Regarding α-amylase inhibition,
301 GS and eGSP had I_{50} values, on a phenolic content basis, in the range of those observed for white
302 grape skins, which was found to be 12.5 - 27.4 μg GAE mL^{-1} (Lavelli et al., 2016b). Both red grape
303 skin and white grape skins contain proanthocyanidins, which have been considered to play a major
304 role in α-amylase inhibition (Boath et al., 2012). Hence, GS and especially eGSP recovered from
305 winemaking byproducts have the potential to be used in the dietary prevention of hyperglycemia
306 damage, as proposed for other natural sources such as garlic, onion, peppers and cranberry
307 (Apostolidis et al., 2007; Boath et al., 2012).
308 The water solubility of phenolics in eGSP and GS was measured using 0.1 M citrate buffer at pH
309 values 3.5 and 6.5, which represent common pH values of foods in which these ingredients could be
310 incorporated. For GS, phenolic solubility was 458 ± 10 mg GAE/L, while for eGSP it was 8 times
311 higher, equal to 3718 ± 30 mg GAE/L, with no difference related to the pH of the buffer. Accordingly,
312 use of maltodextrins during spray-drying was found to increase solubility of the powder (Goula &
313 Adamopoulos, 2011).
314 ***3.2. Moisture, water sorption and hygroscopicity***

315 The a_w level of eGSP upon spray-drying with maltodextrins was 0.21. Similarly, by using other
316 carriers it was found that spray-dried and freeze-dried encapsulated GSP had a_w levels in the range
317 from 0.160 to 0.360, with higher values for the freeze-dried samples (Kuck & Norena, 2016).
318 The a_w level of GS decreased during drying at 55 °C from 1 to 0.75 in 100 min, to 0.56 in 120 min
319 and to 0.11 in 180 min (not shown). Microbial growth rate and production of toxins by microrganisms
320 decreased with decreasing a_w. *Aspergillus carbonarius*, which is an ochratoxin A producing fungus,
321 predominantly responsible for the production of this mycotoxin in grapes, cannot grow at a_w levels
322 below 0.85 (Romero et al., 2007), which is higher than the a_w range considered in the current study.
323 Moisture sorption isotherms of eGSP and GS were built at 30 °C and modelled according to the GAB
324 equation (Figure 1). The amount of water absorbed was higher for eGSP than GS, especially at the
325 higher a_w levels. This was probably due to high levels of insoluble fiber and waxy cuticle present in
326 the fruit skins (Bravo & Saura-Calixto, 1998), which limit moisture adsorption.
327 For GS, fitting to the GAB model gave parameters of C = 34 ± 14, k = 0.89 ± 0.02 and M_o = 0.0291
328 ± 0.017 g moisture/g dry solids (R^2 = 0.998). The M_o value of GS corresponded to the a_w level of
329 0.12. For eGSP, the GAB parameters were: C = 17 ± 9, k = 0.94 ± 0.04 and M_o = 0.0629 ± 0.0007 g
330 moisture/g dry solids (R^2 = 0.995), similar to those observed for a mixture of maltodextrin with DE 9
331 -12 (Nurhadi et al., 2016). Due to the presence of numerous hydrophilic sites, M_o was higher for
332 eGSP than for GS and corresponded to the a_w level of 0.18. The M_o value generally relates to the
333 lowest rate of oxidative reactions (Lavelli & Vantaggi, 2009). The visual appearance of eGSP, but
334 not that of GS, changed with increasing the a_w level. In fact, at a_w of 0.56 the powder formed lumps
335 and at a_w of 0.75 the product was a viscous liquid (Figure 1).
336 Besides a_w, the phenomenon of glass transition could be applied as an integrated approach to
337 determine food stability. Maximum food stability occurs when storage temperature is below T_g, i.e.,
338 food is in the glassy state (Roos & Karel, 1991). The reported T_g values for maltodextrins with DE
339 9-12 were 17, 41, 64, 75 and 95 °C at the a_w levels of 0.75, 0.53, 0.33, 0.23 and 0.11, respectively
340 (Nurhadi et al., 2016). Hence, at a_w 0.75 eGSP were in the rubbery state (T_g < storage temperature of

341 30 °C), at a_w 0.56 storage temperature was relatively close to T_g, while eGSP stored at lower a_w levels
342 were in the glassy state (T_g > storage temperature of 30 °C).
343 For GS powder, no physical changes were observed. Based on the literature data, T_g of raisins skins
344 are above 50 °C, even at a_w 0.75 (Georget, Guardo, Ng, Smith, & Waldron, 1997). Hence, it may be
345 assumed that GS skins considered here were in the glassy state. Many studies have related a_w and T_g
346 to the stability of foods. There is often a critical a_w level below which microorganisms do not grow
347 or produce toxins (Romero, Patriarca, Fernández Pinto, Vaamonde, 2007). Regarding chemical
348 degradations, even if in general foods in the glassy state are stable, in some cases degradative
349 reactions have been observed to occur at sub-T_g temperatures (Hung, Horagai, Kimura, & Adachi,
350 2007). Hence, the direct evaluation of the kinetics of food degradation, as shown in Section 3.5, is
351 important since both a_w and T_g could sometimes underestimate the extent of food degradation.
352 ***3.3. Odor***
353 GS and eGSP were obtained from fermented pomace, which is characterized by an intense typical
354 fermented odor. The fermented odor intensity of dried GS and eGSP was then investigated in
355 comparison with wet GS and finely homogenized wet GS (to increase the surface). Considering the
356 specific functionalities of GS and eGSP, their addition level to foods depend on their phenolic content.
357 Accordingly, these samples were analyzed for odor intensity at 0.4 and 2.5 g GAE/L, which represents
358 the range of concentration used in various fortified foods (Maier et al., 2014; Han et al. 2011; Tseng
359 & Zhao 2013; Sant'Anna et al., 2014; Marchiani et al., 2015).
360 According to the Friedman test, the rank sum of each sample was calculated. The higher the sum the
361 more intense the fermented odor. Results are summarized in Table 2. The Friedman test showed
362 significant differences in fermented odor among samples ($F=113.0$, $p<0.0001$). According to LSD
363 test, the water control had the lowest odor intensity, as expected. Interestingly, eGSP at the highest
364 dilution (0.4 g GAE/L) was not significantly different from water, while it was distinguished at the
365 lowest dilution (2.5 g GAE/L), even if it was far less intensively odorous than wet GS. At relatively
366 low maltodextrin concentrations (< 10%) flavor retention upon spray-drying is generally low, while

higher maltodextrin concentrations can entrap flavor compounds (Yoshii et al., 2001). Hence, in eGSP loss of odor probably results from stripping of volatile odorous compounds during spray drying rather than to the odor-masking properties of maltodextrin carriers. GS had a fermented odor intensity significantly higher than water at both dilutions and significantly lower than wet GS, likely due to loss of the volatile compounds during drying. A decrease in odor intensity can be considered as a positive effect, since intense fermented odor could hinder GS utilization for food applications.

3.4. Color

GS and eGSP had high levels of anthocyanins (Table 1), which provide an intense color. However, anthocyanins undergo degradation during processing and storage, thus affecting color characteristic. Degradation of anthocyanins has been studied in model liquid solutions and wine and found to include the formation of both colored and colorless compounds resulting from direct reactions with proanthocyanidins or reactions also involving aldehydes such as acetaldehyde; condensation to form anthocyanin polymers; reaction with yeast metabolites to form pyranoanthocyanins; and addition to caftaric acid through enzymatic oxidation (Revilla, Pérez-Magariño, Gonzalez-SanJose, & Beltrán, 1999; Cheynier et al. 2006). While the patterns of reactions involving anthocyanin are complex, the resulting degradation is generally modelled as a first-order rate reaction (Reyes & Cisneros-Zevallos, 2007; Cardona et al., 2009; Amendola, De Faveri, & Spigno, 2010). Information on anthocyanin degradation at intermediate moisture and dried conditions is lacking. In the current study, during storage at various a_w environments at 30 °C, the decrease in red color of GS and eGSP (measured at pH 1.0 and thus due to total anthocyanins) also followed a first-order kinetics (Figure 2). In GS, the first-order rate constants for red color decrease varied from $3.31*10^{-3}$ to $1.43*10^{-3}$ d^{-1} by diminishing the a_w level from 0.75 to 0.11. In eGSP the first order rate constants for red color degradation decreased from $3.81*10^{-3}$ to $1.71*10^{-3}$ d^{-1} with diminishing the a_w level from 0.75 to 0.32 and the variation was not significant at $a_w \leq 0.22$ (Table 3). Besides drying, extraction of grape pomace (hot or cold pressed) with water containing 3% citric acid (pH 2.1) has also been proposed to recover phenolic-rich colored extracts intended to be used as food ingredients (Cardona et al., 2009).

393 However, color degradation of these water-based extracts is fast, with first-order rate constants of
394 $36.4*10^{-3}$ and $38.0*10^{-3}$ d^{-1} at 30 °C for cold and hot pressed pomace, respectively (Table 3). Removal
395 of sugars, proteins and metal ions from the pomace extracts through Amberlite purification, increases
396 color stability, resulting in first order rate constants at 30 °C of $27.0*10^{-3}$ and $26.1*10^{-3}$ d^{-1} for cold
397 and hot pressed pomace, respectively (Cardona et al., 2009). However, GS and eGSP were more
398 stable than all these water-based pomace extracts (Table 3). Moreover, color of GS and eGSP was
399 more stable than the color of the extract of grape pomace obtained in 60% ethanol and designed to be
400 used as a food colorant, which was found to decrease with a first-order rate constant of $5.8*10^{-3}$ d^{-1} at
401 25 °C (Amendola et al., 2010).

402 ***3.5. Degradation kinetics of single phenolic compounds***

403 The decrease in phenolic compounds during storage in various a_w environments followed a first-order
404 kinetics, as observed previously (Reyes & Cisneros-Zevallos, 2007; Souza et al., 2014; Aizpurua-
405 Olaizola et al., 2016). A representative behavior is shown for malvidin 3-O-glucoside in Figure 3. At
406 the highest a_w, i.e. 0.75, all compounds were degraded (Table 4). Monomeric flavanols, namely
407 catechin and epicatechin were the most unstable with half-time 62 and 76 d for eGSP and 83 and 98
408 d for GS. It is worth noticing that lower half-life for catechin and epicatechin, i.e. 19 and 20 d
409 respectively, at 25 °C, were observed in a grape waste liquid extract (Aizpurua-Olaizola et al., 2016).
410 Similarly, for the most common food antioxidant, i.e., ascorbic acid, half-life is about 23 d at 23°C
411 in liquids (Lavelli, Pompei & Casadei, 2009) and less than few days at a_w 0.75 (Lavelli & Vantaggi,
412 2009).

413 The half-life for anthocyanins at a_w 0.75was in the range 58 – 100 d for eGSP and 107 – 272 d for
414 GS. From the kinetic data obtained by Reyes & Cisneros-Zevallos (2007) it can be calculated that the
415 half-life of monomeric anthocyanins in a grape skin water extract at pH 3.0 is 32 d at 30 °C. Hence,
416 in the current study, higher stability for all individual anthocyanin compounds was observed for GS
417 and eGSP stored at a_w 0.75, consistent with higher color stability discussed in the previous paragraph.
418 The half-life varied among individual compounds. In fact, delphinidin-3-O-glucoside displayed the

419 lowest stability, while malvidin-*p*-coumaroyl-glucoside the highest. Proanthocyanidin oligomers also
420 showed low stability in GS at the a_w level of 0.75 with the half-life 179 d, whereas in eGSP the half-
421 life was 580 d. Proanthocyanidins were found to be very unstable in grape juice, with complete loss
422 after 9 months at 25 °C (Spanos & Wrolstad, 1992). Flavonols were relatively stable in both GS and
423 eGSP at the a_w level of 0.75, with half-life in the range 322 – 492 d for eGSP and 550 – 800 d in GS.
424 The rate of decrease in antioxidant activity (FRAP values) was lower compared to the extent of
425 phenolic degradation.
426 With decreasing the a_w level to 0.54, the rate constant for antioxidant degradation decreased
427 markedly, leading to an increase of half-life for every compounds by approximately two-times. From
428 the observed rate constants of FRAP values decrease, it can be calculated that the antioxidant activity
429 would be retained by 60 % and 70 % upon one year of storage at a_w levels 0.75 and 0.56, respectively.
430 As a general rule, for both GS and eGSP, the stability of all antioxidant classes at intermediate
431 moisture levels (a_w 0.75 and 0.56) was higher than that observed in previous studies in the liquid
432 state. This result could depend on the increased viscosity of the matrix at intermediate moistures with
433 respect to the liquid state, which limits molecular diffusion and hence the reaction rates. Degradation
434 rates for both anthocyanins, flavanols and flavonols were also slower with respect to those observed
435 in apple pulp under the same conditions. This effect could be due to the occurrence of Maillard
436 reaction in apple, as demonstrated by the formation of hydroxymethylfurfural, which can lead to
437 antioxidant degradation (Lavelli & Vantaggi, 2009). In contrast, hydroxymethylfurfural was not
438 detected in both GS and eGSP. Decreasing a_w at intermediate moisture levels could therefore be a
439 mean to limit the energy consumption of the process by decreasing the amount of water to be
440 evaporated and shortening the drying process, while achieving good antioxidant stability. In fact,
441 drying is one of the most energy consuming operations in food technology, with 3200 – 11500 kJ
442 consumed per kilogram of water evaporated, depending on the dryer and conditions used (Sanjua,
443 Stoessel, & Hellweg, 2014). Hence a new scenario of food applications can be proposed, by replacing
444 the use of totally dried GS with partially dehydrated GS ($a_w \approx 0.55$). In fact, partially dehydrated GS

445 could be used for wide-ranging food applications as proposed for dried GS, like as a fortifying
446 ingredient in bakery products (Walker, Tseng, Cavender, Ross, & Zhao, 2014; Sant'Anna, Christiano,
447 Marczak, Tessaro, & Thys, 2014) a natural antioxidant in fish and meat products (Sáyago-Ayerdi,
448 Brenes, & Goñi, 2009; Sanchez-Alonso, Jimenez-Escrig, Saura-Calixto, & Borderias, 2008; García-
449 Lomillo, González-SanJosé, Skibsted, & Jongberg, S., 2016), a texturizing agent in purees (Lavelli,
450 Sri Harsha, Mariotti, Marinoni, & Cabassi, 2015) and natural seasoning in meat (García-Lomillo,
451 González-SanJosé, Del Pino-García, Ortega-Heras, & Muñiz-Rodríguez, 2016). Interestingly, a
452 partially dried grape pomace ($a_w \approx 0.55$) was also found to be an optimal matrix for phenolic
453 extraction by pulse electric fields (Brianceau, Turk, Vitrac & Vorobiev, 2015).
454 Storage at a_w of 0.32 resulted in further increase in antioxidant stability (Table 5). Anthocyanins half-
455 life was higher than one year, except for delphinidin 3-O-glucoside and cyanidin 3-O-glucoside,
456 which were less stable than the other anthocyanins in both GS and eGSP. Similar values were
457 observed by Souza et al. (2014) for total anthocyanins encapsulated in maltodextrins.
458 Proanthocyanidin contents decreased only in GS. The same trend was observed at the a_w level of 0.22,
459 with slower rates. At a_w 0.11, no degradation occurred in eGSP, while in GS the degradation of
460 anthocyanins and proanthocyanidins was still prominent, at slower rate with respect to those observed
461 at higher a_w levels. As observed at higher a_w levels, the decrease of FRAP values was slower than the
462 decrease in anthocyanins and proanthocyanidins.
463 Hence, GS degradation phenomena proceeded in GS at the lowest a_w level, even if the storage
464 temperature was below 50 °C, i.e., the T_g previously reported (Georget, Guardo, Ng, Smith, &
465 Waldron, 1997). This finding confirms results observed in previous studies for ascorbic acid (Hung
466 et al., 2007) and could be related to heterogeneous water distribution within the food matrix. In
467 contrast for eGSP, very slow degradation was observed at a_w 0.22 and no degradation was observed
468 at a_w 0.11. As discussed in 3.2, at these latter a_w values, the storage temperature was below the
469 reported T_g values for maltodextrins (95 °C at a_w 0.11 and 75 °C at a_w 0.23 as reported by Nurhadi et
470 al., 2016). Additionally, as found in the current study, M_o for eGSP corresponded to the a_w level of

0.18. Hence, the high stability found in this a_w range is probably attributable to the limited water mobility and diffusion rate of reagents. Encapsulation of GSP with maltodextrins through spray-drying, followed by storage at $a_w \leq 0.22$, could therefore be a strategy to increase phenolic solubility, and stability, especially for anthocyanins and proanthocyanidins. Hence, use of eGSP stored at $a_w \leq 0.22$ could open up another scenario of food applications with targeted functionalities, related to high anthocyanin and proanthocyanidin contents. For instance, eGSP could be used in foods as a dietary aid to inhibit starch digestion enzymes, in which both anthocyanin and proanthocyanidins display particularly high effectiveness (Apostolidis et al., 2007; Boath et al., 2012) leading to food that could address the needs of diabetic people.

4. CONCLUSIONS

The current study led to a description of relevant technological properties of GS and eGSP, such as hygroscopicity, color, odor, antioxidant profile, antioxidant activity and efficacy towards starch digestion inhibition *in vitro*. Antioxidant changes in GS and eGSP during storage at various a_w levels were investigated. Based on the findings obtained, the criteria for processing and storage conditions for GS and eGSP can be defined, which depend on their final use in foods, as illustrated by two possible scenarios. For tailor-made applications involving anthocyanin and proanthocyanidin functionality, i.e., inhibition of starch digestion enzymes, encapsulation with maltodextrins followed by storage at a_w levels below 0.22 is suggested to provide increased levels of these compounds and maximum stability. In contrast, for uses addressing an antioxidant effect, drying of GS could be ended at intermediate moisture levels (a_w 0.75), followed by storage at room temperature, thus accomplishing a shorter heating process and good retention of the antioxidant activity.

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LITERATURE CITED

497 Aizpurua-Olaizola, O., Navarro, P., Vallejo, A., Olivares, M., Etxebarria, N., & Usobiaga, A. (2016).
498 Microencapsulation and storage stability of polyphenols from *Vitis vinifera* grape wastes. *Food*
499 *Chemistry*, 190, 614–621.
500 Amendola, D., De Faveri, D. M., Spigno, G. (2010). Grape marc phenolics: Extraction kinetics,
501 quality and stability of extracts. *Journal of Food Engineering*, 97, 384–392.
502 Apostolidis, E., Kwon, Y.-I., Shetty, K. (2007). Inhibitory potential of herb, fruit, and fungal-enriched
503 cheese against key enzymes linked to type-2 diabetes and hypertension. *Innovative Food Science*
504 *and Emerging Technologies*, 8, 46–54.
505 Boussetta, N., Vorobiev, E., Reess, T., De Ferron, A., Pecastaing, L., Ruscassié, R., & Lanoisellé, J.
506 L. (2012). Scale-up of high voltage electrical discharges for polyphenols extraction from grape
507 pomace: Effect of the dynamic shock waves. *Innovative Food Science and Emerging*
508 *Technologies*, 16, 129-136.
509 Boath, A. S., Grussu, D., Stewart, D., & McDougall, G. J. (2012). Berry polyphenols inhibit digestive
510 enzymes: a source of potential health benefits? *Food Digestion*, 3, 1–7.
511 Bravo, L., & Saura-Calixto, F. (1998). Characterization of dietary fiber and *in vitro* indigestible
512 fraction of grape pomace. *American Journal of Enology and Viticulture*, *49*, 135-141.
513 Brianceau, S., Turk, M., Vitrac, X. & Vorobiev, E. (2015). Combined densification and pulsed
514 electric field treatment for selective polyphenols recovery from fermented grape pomace
515 *Innovative Food Science and Emerging Technologies*, 29, 2–8.
516 Cardona, J. A., Lee, J.-H. & T. Talcott, S. T. (2009). Color and polyphenolic stability in extracts
517 produced from muscadine grape (Vitis rotundifolia) pomace. *Journal of Agricultural and Food*
518 *Chemistry*, 57, 8421–8425.
519 Cheynier, V., Dueñas-Paton, M., Salas, E., Maury, C., Souquet, J. M., Sarni-Manchado, P. &
520 Fulcrand, H. (2006). Structure and Properties of Wine Pigments and Tannins. *American Journal*
521 *of Enology and Viticulture*, 57, 3, 298-305.

522 Corrales, M., Toepfl, S., Butz, P., Knorr, D., & Tauscher, B. (2008). Extraction of anthocyanins from
523 grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A
524 comparison. *Innovative Food Science and Emerging Technologies,* 9, 85-91.
525 Devesa-Rey, R., Vecino, X., Varela-Alende, J. L., Barral, M.T., Cruz, J.M., & Moldes, A.B. (2011).
526 Valorization of winery waste vs. the costs of not recycling. *Waste Management*, 31, 2327–2335.
527 Galanakis, C. M. (2015). Separation of functional macromolecules and micromolecules: From
528 ultrafiltration to the border of nanofiltration. *Trends in Food Science & Technology*, *42,* 44-
529 63.
530 García-Lomillo, J., González-SanJosé, M. L., Del Pino-García, R., Rivero-Pérez, M.D. & Muñiz-
531 Rodríguez, P. (2014). Antioxidant and antimicrobial properties of wine byproducts and their
532 potential uses in the food industry. *Journal of Agricultural and Food Chemistry*, 52, 12595-
533 12602.
534 García-Lomillo, J., González-SanJosé, M. L., Del Pino-García, R., Ortega-Heras, M. & and Muñiz-
535 Rodríguez, P. (2016) Effect of a new natural seasoning on the formation of pyrazines in
536 barbecued beef patties. *Journal of Chemistry*, 2016, 1-7.
537 García-Lomillo, J., González-SanJosé, M.L., Skibsted, L.H. & Jongbergb, S. (2016). Effect of skin
538 wine pomace and sulfite on protein oxidation in beef patties during high-oxygen atmosphere
539 storage. *Food and Bioprocess Technology,* 9, 532-542.
540 Georget, D. M. R., Guardo, M., Ng, A., Smith, A. C. & Waldron K. W. (1997). Mechanical and
541 thermal analysis of raisin components. *Thermochimica Acta*, 294, 71-79. (1997) 71-7
542 Goula, A. M., & Adamopoulos, K. G. (2011). A new technique for spray drying orange juice
543 concentrate. *Innovative Food Science and Emerging Technologies*, 11, 342–351.
544 Han, J., Britten, M., St-Gelais, D., Champagne, C.P., Fustier, P., Salmieri, S. & Lacroix, M. (2011).
545 Effect of polyphenolic ingredients on physical characteristics of cheese. *Food Research*
546 *International*, 44, 494-497.

547 Hung., L., Horagai, Y., Kimura, Y., & Adachi, S. (2007). Decomposition and discoloration of L-
548 ascorbic acid freeze-dried with saccharides. *Innovative Food Science and Emerging*
549 *Technologies*, 8, 500-506.
550 Kuck, L. S., & Norena, C. P. Z. (2016). Microencapsulation of grape (*Vitis labrusca* var. Bordo) skin
551 phenolic extract using gum arabic, polydextrose, and partially hydrolyzed gum as
552 encapsulating agents. *Food Chemistry*, 194, 569–576.
553 Lavelli, V., Pagliarini, E., Ambrosoli, R., Minati, J. L., & Zanoni, B. (2006). Physicochemical,
554 microbial, and sensory parameters as indices to evaluate the quality of minimally-processed
555 carrots. *Postharvest Biology and Technology*, 40, 34‑40.
556 Lavelli, V., & Vantaggi, C. (2009). Rate of antioxidant degradation and color variations in dehydrated
557 apples as related to water activity. *Journal of Agricultural Food Chemistry, 57,* 4733−4738.
558 Lavelli, V., Sri Harsha, P.S.C., Torri, L. & Zeppa, G. (2014). Use of winemaking by-products as an
559 ingredient for tomato puree: The effect of particle size on product quality. *Food Chemistry,*
560 162-168.
561 Lavelli, V., Sri Harsha, P.S.C., Mariotti, M., Marinoni, L. & Cabassi, G. (2015). Tuning physical
562 properties of tomato puree by fortification with grape skin antioxidant dietary fibre. *Food and*
563 *Bioprocess Technology*, 8, 1668-1679.
564 Lavelli, V., Sri Harsha, P.S.C. & Spigno, G. (2016a). Modelling the stability of maltodextrin-
565 encapsulated grape skin phenolics used as a new ingredient in apple puree. *Food Chemistry*,
566 209, 323-331.
567 Lavelli, V., Sri Harsha P.S.C., Ferranti, P., Scarafoni, A. & Iametti, S. (2016b). Grape skin phenolics
568 as inhibitors of mammalian α-glucosidase and α-amylase – Effect of food matrix and processing
569 on efficacy. *Food & Function*, 7, 1655-1663.
570 Maier, T., Fromm, M., Schieber, A., Kammerer, D.R. & Carle, R. (2009). Process and storage
571 stability of anthocyanins and non-anthocyanin phenolics in pectin and gelatin gels enriched with
572 grape pomace extracts. *European Food Research and Technology*, 229, 949-960.

573 Marchiani, R., Bertolino, M., Ghirardello, D., McSweeney, P. L. H. & Zeppa, G. (2015).
574 Physicochemical and nutritional qualities of grape pomace powder-fortified semi-hard cheese.
575 *Journal of Food Science and Technology*, 53, 1585-1596.
576 ISO 8586 (2012) Sensory analysis - General guidelines for the selection, training and monitoring of
577 selected assessors and expert sensory assessors. International Standard Organization, Geneva,
578 Switzerland.
579 ISO 8587 (2006) Sensory analysis – Methodology – Ranking. International Standard Organization,
580 Geneva, Switzerland.
581 Mildner-Szkudlarz, S., Siger, A., Szwengiel, A. & Bajerska, J. (2015). Natural compounds from grape
582 by-products enhance nutritive value and reduce formation of CML in model muffins. *Food*
583 *Chemistry*, 172, 78–85.
584 Nurhadi, B., Roos, Y. H., & Maidannyk, V. (2016). Physical properties of maltodextrin DE 10: Water
585 sorption, water plasticization and enthalpy relaxation. *Journal of Food Engineering*, 174, 68-74.
586 Perez-Jimenez, J., Serrano, J., Tabernero, M., Diaz-Rubio, M. E., Arranz, S., Garcia-Diz, L., et al.
587 (2008). Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. *Nutrition*,
588 24, 646–653.
589 Revilla, I., Pérez-Magariño, S., Gonzalez-SanJose, M. L. & Beltrán, C. (1999). Identification of
590 anthocyanin derivatives in grape skin extracts and red wines by liquid chromatography with
591 diode array and mass spectrometric detection. *Journal of Chromatography A*, 847, 83–90.
592 Reyes, L. F., & Cisneros-Zevallos, L. (2007). Degradation kinetics and colour of anthocyanins in
593 aqueous extracts of purple- and red-flesh potatoes (*Solanum tuberosum* L.). *Food Chemistry*,
594 *100*, 885–894.
595 Roos, Y. H., & Karel, M. (1991). Phase transition of mixture of amorphous polysaccharides and
596 sugars. *Biotechnology Progress*, 7, 49–53.

Romero, S. M., A. Patriarca, A., Fernández Pinto, V., Vaamonde, G. (2007). Effect of water activity and temperature on growth of ochratoxigenic strains of Aspergillus carbonarius isolated from Argentinean dried vine fruits. *International Journal of Food Microbiology*, 115, 140–143.

Sanchez-Alonso, I., Jimenez-Escrig, A., Saura-Calixto, F. & Borderias A. J. (2008). Antioxidant protection of white grape pomace on restructured fish products during frozen storage. *LWT - Food Science and Technology*, 41, 42-50.

Sanjua, N., Stoessel, F. & Hellweg, S. (2014). Closing data gaps for LCA of food products: estimating the energy demand of food processing. *Environmental Science & Technology*, 48, 1132−1140.

Sant'Anna, V., Christiano, F.D.P., Marczak, L.D.F., Tessaro, I.C. & Thys, R.C.S. (2014). The effect of the incorporation of grape marc powder in fettuccini pasta properties. *LWT - Food Science and Technology*, 58, 497-501.

Sáyago-Ayerdi, S.G., Brenes, A. & Goñi, I. (2009). Effect of grape antioxidant dietary fiber on the lipid oxidation of raw and cooked chicken hamburgers. *LWT - Food Science and Technology*, 42, 971-976.

Souza, V. B., Fujita, A., Thomazini, M., da Silva, E. R., Lucon, J. F., Genovese, M. I., & Favaro-Trindade, C. S. (2014). Functional properties and stability of spray-dried pigments from Bordo grape (Vitis labrusca) winemaking pomace. *Food Chemistry, 164,* 380–386.

Spanos, G. A.; Wrolstad, R. E. (1992). Phenolics of apple, pear and white grape juices and their changes with processing and storage; A review. Journal of Agricultural and Food Chemistry, 40, 1478–1487.

Sri Harsha, P. S. C., Gardana, C., Simonetti, P., Spigno, G. & Lavelli, V. (2013). Characterization of phenolics, *in vitro* reducing capacity and anti-glycation activity of red grape skins recovered from winemaking by-products. *Bioresource Technology*, 140, 263-268.

Sri Harsha, P. S. C., Lavelli V. & Scarafoni A. (2014). Protective ability of phenolics from white grape vinification by-products against structural damage of bovine serum albumin induced by glycation. *Food Chemistry*, 156, 220–226.

623 Teixeira, A., Baenas, N., Dominguez-Perles, R., Barros A., Rosa E., Moreno D. A., & Garcia-
624 Viguera, C. (2014). Natural bioactive compounds from winery by-products as health
625 promoters: A review. *International Journal of Molecular Sciences, 15,* 15638-15678.
626 Tseng, A. & Zhao, Y. (2013). Wine grape pomace as antioxidant dietary fibre for enhancing
627 nutritional value and improving storability of yogurt and salad dressing. *Food Chemistry*, 138,
628 1, 356-365.
629 Walker, R., Tseng, A., Cavender, G., Ross, A., & Zhao, Y. (2014). Physicochemical, nutritional, and
630 sensory qualities of wine grape pomace fortified baked goods. *Journal of Food Science*, 79,
631 S1811-S1822.
632 Yoshii, H., Soottitantawat, A., Liu, X-D, Atarashi, T., Furuta T., Aishima, S., Ohgawara, M., Linko,
633 P. (2001). Flavor release from spray-dried maltodextrin_gum arabic or soy matrices as a function
634 of storage relative humidity. *Innovative Food Science & Emerging Technologies*, 2, 55-61.
635

Figure captions

Figure 1. Water sorption properties (left) and images of eGSP and GS equilibrated at various a_w levels at 30 °C (right). In the left-side graph, symbols (●) and (▲) indicate the experimental data for GSP and GS, respectively; error bars indicate SD; continuous lines indicate the absorption isotherms obtained by fitting experimental data with the GAB model.

Figure 2. Time course for the decrease in color (A/Ao) in eGSP (left) and GS (right) during storage at the specified a_w levels, at 30 °C. A and Ao represent the difference between the absorbance at 520 nm and that at 700 nm of the samples diluted in 0.025 M potassium chloride buffer, pH 1.0. at time t and 0, respectively. Error bars represent SD. Dotted lines represent fitting of data with a first-order kinetics. For eGSP stored at the a_w levels 0.22 and 0.11 the decrease was not significant. Rate constants are reported in Table 3.

Figure 3. Time course for the decrease in malvidin 3-O-glucoside concentration (A) in eGSP (left) and GS (right) during storage at the specified a_w levels, at 30 °C. Error bars represent SD. Dotted lines represent fitting of data with a first-order kinetics. For eGSP stored at a_w 0.11 the decrease was not significant. Rate constants are reported in Tables 4 and 5.

653 **Table 1**. Total phenolics (g GAE/kg d.w.), proanthocyanidins (g/kg d.w.), monomeric flavanols (g C
654 eq./kg d.w.), monomeric anthocyanins (g Mv-glc eq./kg d.w.) flavonols (g Q-glc eq./kg d.w.), FRAP
655 values (mmol Fe(II) eq./kg d.w.) and α-glucosidase/α-amylase inhibition effectiveness ($I_{50,}$ mg/mL
656 d.w.) of GS and eGSP.
657

Quality index	**GS**	**eGSP**
Total phenolics	43.9 ± 4.2	87.7 ± 3.5
Proanthocyanidins	25.4 ± 4.3	37.7 ± 4.1
Monomeric flavanols	0.26 ± 0.01	1.32 ± 0.01
Monomeric anthocyanins	3.6 ± 0.1	6.9 ± 0.1
Flavonols	1.1 ± 0.1	2.2 ± 0.2
FRAP values	332 ± 40	706 ± 28
α-glucosidase inhibition	0.44 ± 0.02	0.20 ± 0.01
α-amylase inhibition	0.28 ± 0.02	0.15 ± 0.02

658 The results are expressed as the average ± SD.

659 **Table 2**. Fermented odor rank sum for eGSP, dried GS and wet GS.

Sample	Phenolic content	Rank sum
Water	-	46 [e]
Dissolved eGSP	0.4	63 [de]
Dissolved eGSP	2.5	86 [cd]
Dispersed dried GS	0.4	108 [bc]
Dispersed dried GS	2.5	128 [b]
Dispersed wet GS	0.4	166 [a]
Dispersed homogenized wet GS	0.4	183 [a]

660 To achieve the indicated phenolic content, eGSP and GS were added with 8 mM citric acid, pH 3.0.
661 Phenolic content is expressed as g GAE/L. Superscripts indicate significant differences ($P < 0.0001$)
662 according to LSD test.

663 **Table 3.** First-order rate constants ($k*10^3$, d^{-1}) for the decrease in color of GS and eGSP during
664 storage in the a_w range 0.11 – 0.75 at 30 °C, in comparison with literature data on other potential food
665 ingredients recovered from grape pomace.

Recovered ingredient	Storage conditions	$k*10^3$	Reference
Grape pomace extract in 3% citric acid pH 2.1			Cardona et al., 2009
- cold pressed	55 d, 30 °C	36.4	
- hot pressed	55 d, 30 °C	38.0	
Purified grape pomace extract in 3% citric acid pH 2.1			
- cold pressed	55 d, 30 °C	27.0	
- hot pressed	55 d, 30 °C	26.1	
Grape pomace extract in 60% ethanol	47 d, 25 °C	5.8	Amendola et al., 2010
Micronized grape skins			
GS at a_w 0.75	180 d, 30°C	3.31 ± 0.40	This study
GS at a_w 0.56	180 d, 30°C	2.40 ± 0.47	
GS at a_w 0.32	180 d, 30°C	1.47 ± 0.04	
GS at a_w 0.22	180 d, 30°C	1.51 ± 0.28	
GS at a_w 0.11	180 d, 30°C	1.43 ± 0.02	
Encapsulated grape skin phenolics			
eGSP at a_w 0.75	180 d, 30°C	3.81 ± 0.09	This study
eGSP at a_w 0.56	180 d, 30°C	2.54 ± 0.17	
eGSP at a_w 0.32	180 d, 30°C	1.71 ± 0.30	
eGSP at a_w 0.22	180 d, 30°C	n.s.	
eGSP at a_w 0.11	180 d, 30°C	n.s.	

666 Color was measured as the difference between absorbance at 520 nm and absorbance at 700 nm of
667 the samples diluted in 0.025 M potassium chloride buffer, pH 1.0. The rate constants are expressed
668 as the average ± SD. For all the rate constants reported, R^2 values were > 0.92 and P values were <
669 0.05. When rate constants are not reported, changes in color were not significant (n.s., $P \geq 0.05$).

Table 4. First-order rate constants (k *10^3, d^{-1}) and predicted half-life ($t_{½}$, d) for antioxidant degradation and decrease in FRAP values in GS and eGSP during storage at intermediate moisture levels (a_w 0.75 and 0.56).

	a_w 0.75				**a_w 0.56**			
	GS		**eGSP**		**GS**		**eGSP**	
	k *10^3	**$t_{1/2}$**	**k *10^3**	**$t_{1/2}$**	**k *10^3**	**$t_{1/2}$**	**k *10^3**	**$t_{1/2}$**
Anthocyanins								
Dp-glc	6.47 ± 0.09	107	11.98 ± 0.08	58	2.26 ± 0.32	307	4.35 ± 0.08	159
Cy-glc	6.41 ± 0.26	108	8.41 ± 0.10	82	2.48 ± 0.37	280	4.30 ± 0.06	161
Pt-glc	4.41 ± 0.01	157	8.31 ± 0.12	83	2.27 ± 0.43	306	3.93 ± 0.03	176
Pn-glc	4.79 ± 0.26	145	7.99 ± 0.11	87	2.47 ± 0.38	281	3.95 ± 0.16	175
Mv-glc	4.30 ± 0.02	161	7.56 ± 0.10	92	2.27 ± 0.42	305	3.65 ± 0.00	190
Mv-pc-glc	2.55 ± 0.09	272	6.90 ± 0.10	100	1.53 ± 0.40	452	2.71 ± 0.12	255
Flavanols								
C	7.10 ± 0.47	98	9.12 ± 0.01	76	3.38 ± 0.19	205	4.50 ± 0.11	154
EC	8.33 ± 0.07	83	11.18 ± 0.13	62	4.47 ± 0.55	155	5.51 ± 0.16	126
Proanthocyanidins	3.88 ± 0.16	179	1.20 ± 0.56	580	2.32 ± 1.40	299	0.61 ± 0.36	1127
Flavonols								
Q-glc + Q-gln	1.26 ± 0.15	550	2.16 ± 0.36	322	0.79 ± 0.29	882	1.23 ± 0.12	562
Q	0.86 ± 0.05	807	n.s.		0.78 ± 0.28	894	n.s.	
K	1.04 ± 0.01	665	2.24 ± 0.49	309	0.77 ± 0.26	906	n.s.	
Antioxidant activity								
FRAP values	1.43 ± 0.10	486	1.08 ± 0.56	644	1.06 ± 0.30	657	0.38 ± 0.06	1832

The rate constants are expressed as the average ± SD. For all the rate constants reported, R^2 values were > 0.64 and P values were < 0.05.When rate constants are not reported, changes in antioxidant contents were not significant (n.s., $P \geq 0.05$).

Table 5. First-order rate constants (k *10^3, d^{-1}) and predicted half-life ($t_{½}$, d) for antioxidant degradation and decrease in FRAP values in GS and eGSP during storage at low moisture levels (a_w 0.32, 0.22 and 0.11).

	a_w 0.32				**a_w 0.22**				**a_w 0.11**	
	GS		**eGSP**		**GS**		**eGSP**		**GS**	
	k *10^3	**$t_{1/2}$**	**k *10^3**	**$t_{1/2}$**	**k *10^3**	**$t_{1/2}$**	**k *10^3**	**$t_{½}$**	**k *10^3**	**$t_{1/2}$**
Anthocyanins										
Dp-glc	3.22 ± 0.08	215	3.49 ± 0.34	199	2.73 ± 0.05	254	0.72 ± 0.66	964	2.94 ± 0.14	235
Cy-glc	3.33 ± 0.02	208	3.21 ± 0.10	216	1.32 ± 0.06	523	0.58 ± 0.49	1195	0.79 ± 0.15	880
Pt-glc	1.18 ± 0.45	587	1.12 ± 0.07	617	0.96 ± 0.07	722	0.47 ± 0.48	1487	0.90 ± 0.06	767
Pn-glc	1.31 ± 0.56	530	1.29 ± 0.14	538	0.85 ± 0.08	820	0.50 ± 0.19	1393	0.84 ± 0.03	827
Mv-glc	1.11 ± 0.44	627	1.02 ± 0.03	676	0.83 ± 0.10	837	0.68 ± 0.31	1026	0.77 ± 0.07	896
Mv-pc-glc	0.89 ± 0.45	789	1.93 ± 0.15	359	0.75 ± 0.04	929			0.69 ± 0.05	998
Flavanols										
Proanthocyanidins	2.70 ± 1.8	253	n.s.		2.64 ± 0.92	263	n.s.		1.30 ± 0.7	546
Antioxidant activity										
FRAP values	0.65 ± 0.33	1065	n.s.		0.34 ± 0.09	2054	n.s.		n.s.	

The rate constants are expressed as the average ± SD. For all the rate constants reported, R^2 values were > 0.64 and P values were < 0.05. When rate constants are not reported, changes in antioxidant contents or FRAP values were not significant (n.s., $P \geq 0.05$).

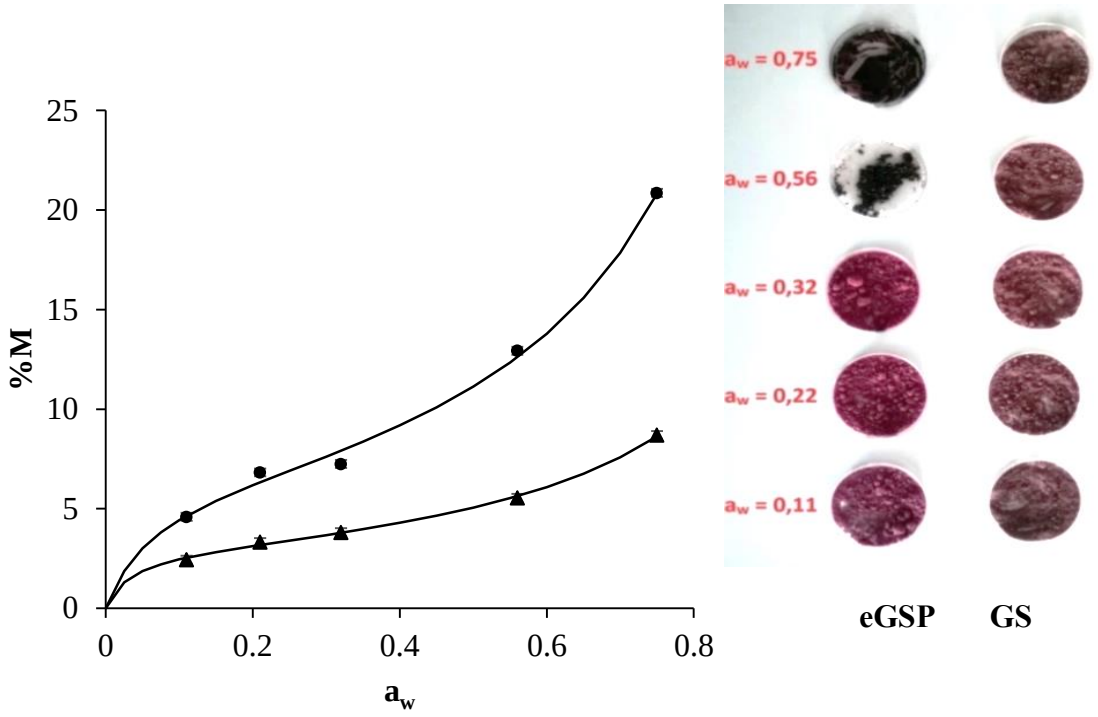

Figure 1. Water sorption properties (left) and images of eGSP and GS equilibrated at various a_w levels at 30 °C (right). In the left-side graph, symbols (●) and (▲) indicate the experimental data for GSP and GS, respectively; error bars indicate SD; continuous lines indicate the absorption isotherms obtained by fitting experimental data with the GAB model.

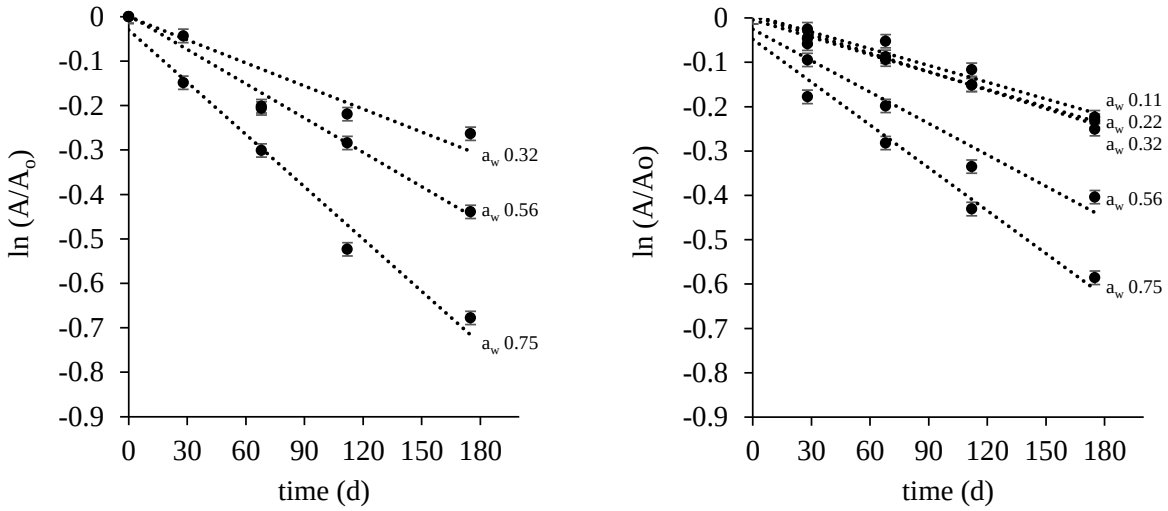

Figure 2. Time course for the decrease in color (A/Ao) in eGSP (left) and GS (right) during storage at the specified a_w levels, at 30 °C. A and Ao represent the difference between the absorbance at 520 nm and that at 700 nm of the samples diluted in 0.025 M potassium chloride buffer, pH 1.0 at time t and 0, respectively. Error bars represent SD. Dotted lines represent fitting of data with a first-order kinetics. For eGSP stored at the a_w levels 0.22 and 0.11 the decrease was not significant. Rate constants are reported in Table 3.

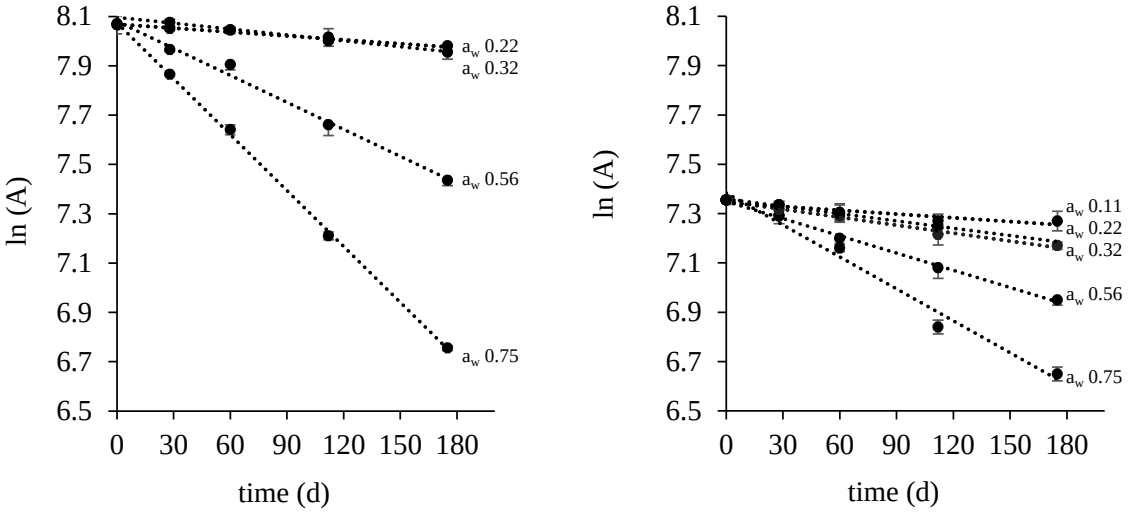

Figure 3. Time course for the decrease in malvidin 3-O-glucoside concentration (A) in eGSP (left) and GS (right) during storage at the specified a_w levels, at 30 °C. Error bars represent SD. Dotted lines represent fitting of data with a first-order kinetics. For eGSP stored at a_w 0.11 the decrease was not significant. Rate constants are reported in Tables 4 and 5.