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# 1    **Functional and sensory properties of phenolic compounds from unripe grapes in vegetable** 2    **food prototypes**

3

4    Ginevra Bucalossi<sup>a</sup>, Giovanna Fia<sup>a</sup>, Caterina Dinnella<sup>a</sup>, Alessandra De Toffoli<sup>a</sup>, Valentina Canuti<sup>a</sup>,  
5    Bruno Zanoni<sup>a</sup>, Maurizio Servili<sup>b</sup>, Ella Pagliarini<sup>c</sup>, Tullia Gallina Toschi<sup>d</sup>, Erminio Monteleone<sup>a\*</sup>

6

7    <sup>a</sup> Department of Agricultural, Food, Environmental and Forestry Sciences and Technologies  
8    (DAGRI) ,University of Florence, Piazzale delle Cascine, 18 - 50144 - Firenze, Italy

9    <sup>b</sup> Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX  
10    Giugno, 74 - 06121 - Perugia, Italy

11    <sup>c</sup> Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Via  
12    Giovanni Celoria, 2, - 20133 – Milano, Italy

13    <sup>d</sup> Department of Agricultural and Food Sciences (DiSTAL), Alma Mater Studiorum - University of  
14    Bologna, Italy

15    [ginevra.bucalossi@unifi.it](mailto:ginevra.bucalossi@unifi.it), [giovanna.fia@unifi.it](mailto:giovanna.fia@unifi.it), [caterina.dinnella@unifi.it](mailto:caterina.dinnella@unifi.it),  
16    [alessandra.detoffoli@unifi.it](mailto:alessandra.detoffoli@unifi.it), [valentina.canuti@unifi.it](mailto:valentina.canuti@unifi.it), [bruno.zanoni@unifi.it](mailto:bruno.zanoni@unifi.it),  
17    [maurizio.servili@unipg.it](mailto:maurizio.servili@unipg.it), [ella.pagliarini@unimi.it](mailto:ella.pagliarini@unimi.it), [tullia.gallinatoschi@unibo.it](mailto:tullia.gallinatoschi@unibo.it)

18

19    \* Corresponding author: [erminio.monteleone@unifi.it](mailto:erminio.monteleone@unifi.it)

20

## 21    **Abstract**

22    Unripe grapes (UGs) from thinning are an unexploited source of phenols useful as functional  
23    ingredient. However, phenols may negative affect sensory quality of food. Chemical and sensory  
24    properties of UG phenols in plant-based foods were not investigated before.

25 With this aim, an extract from UGs, obtained by a green extraction technique, was used to fortify  
26 three plant-based food models: carbohydrates/acidic pH/sweet - beetroot purée, proteins/neutral  
27 pH/sweet - pea purée and starch/neutral pH - potato purée.  
28 Functional and sensory properties of phenol-enriched foods varied as a function of their  
29 composition and original taste. The amount of UG phenols recovered from potato purée was higher  
30 than that recovered from beetroot and pea purée, while the antioxidant activity detected in beetroot  
31 purée was higher than that in potato and pea purée. Significant variations of sourness, saltiness,  
32 bitterness and astringency were induced by UG phenols added to food models. Beetroot purée  
33 resulted more appropriate to counteract the negative sensations induced by UG phenols.

34

35 **Keywords:** functional food; unripe grapes; polyphenols; antioxidant activity; sourness; sweetness.

36

## 37 1. Introduction

38 By-products of the wine industry are rich in phenols and other valuable elements for the human diet  
39 such as mineral salts, fibres and vitamins. There are emerging evidences of the potential preventive  
40 effects of grape polyphenols towards cardiovascular diseases, diabetes, and degenerative diseases  
41 such as cancer (Guilford & Pezzuto, 2011; Mihaylova, Popova, Alexieva, Krastanov & Lante,  
42 2018). The role of phenols from grapes in the prevention of various diseases associated with  
43 oxidative stress is primarily related to their antioxidant properties (Guilford & Pezzuto, 2011;  
44 Villaño, Fernández-Pachón, Moyá, Troncoso, & García-Parrilla, 2007; Rasines-Perea & Teissedre,  
45 2017).

46

47 The sustainability of the winemaking process could be improved by the recovery of high-value  
48 bioactive compounds from by-products. Indeed, extensive studies have been made of the biological  
49 properties, extraction techniques and applications in the food system of phenols from grape pomace,  
50 the main by-product of the wine industry (Beres et al., 2017; Yu & Ahmedna, 2013).

51 Unripe grapes (UGs) discarded during thinning are an undervalued by-product of vineyard  
52 management for the production of high-quality wine (Gatti, Bernizzoni, Civardi, & Poni, 2012;  
53 Keller, Mills, Wample, & Spayd, 2005; Ough Cs, 1984). In unripe berries, the most important  
54 classes of grape antioxidants (phenolic acids, flavan-3-ols, flavonols, anthocyanins, stilbenes and  
55 glutathione) are present to variable extents in function of some factors such as variety, maturity  
56 level and season (Adams, 2006) but their anti  
57  
58  
59 oxidant activity and potential application have received scarce scientific attention (Fia, Gori,  
60 Bucalossi, Borghini, & Zanoni, 2018; Tinello & Lante, 2017). Low-quality unripe grapes are  
61 processed into various traditional juices and sauces with a low pH and variable levels of antioxidant  
62 activity ( (Dupas de Matos, Magli, Marangon, Curioni, Pasini & Vincenzi, 2018; Öncül &  
63 Karabiyikli, 2015). The added value of thinned grapes is higher than the one of other by-products of  
64 wine industry that were largely studied and proposed as source of antioxidants. That is because, the  
65 thinned grapes have not been exploited to make wine and therefore contain an intact complex of  
66 bio-active compounds. Recently, a green extraction technique (i.e. performed without solvents and  
67 preservatives) was patented (Fia & Gori, 2016) and applied at an industrial level with the aid of a  
68 patented oenological machine (Gori, C., Menichetti, S., & Fia, G. 2014) to obtain an extract from  
69 unripe grapes.

70  
71 Functional food is essentially a marketing term with different definitions and regulations depending  
72 on the country (Henry, 2010). Recently in Europe, there has been a growing interest in functional  
73 foods. A scientific consensus document was drafted to develop a science-based approach for the  
74 emerging concepts in functional food (Europe, 1999). Foods that have been modified by enrichment  
75 with bioactive substances are included in the functional food categories and the health benefits of

76 phenols, beyond basic nutritional values of plant-based food and beverages containing phenols, are  
77 reported in a recent review (Shahidi & Ambigaipalan, 2015).

78 Phenols from plant by-products have been proposed as ingredients for functional foods and  
79 beverages preparation to improve their nutritional characteristics (De Toffoli et al., 2019; Torri et  
80 al., 2015; Nirmala, Bisht, Bajwa, & Santosh, 2018; Świeca, Gawlik-Dziki, Sęczyk, Dziki, & Sikora,  
81 2018). Some examples of functional food enriched with phenols from tea and Guava are already  
82 included in the “food for specified health uses” (FOSHU) and regulated as functional food in Japan  
83 (Iwatani & Yamamoto, 2019).

84 In developing a phenol-enriched functional food, two main aspects need to be investigated: the first  
85 concerns the phenols’ stability after their addition to the food system, affecting the preservation of  
86 their biological activities; the second concerns oral sensations, such as astringency, bitterness and  
87 sourness, which can arise after the addition of phenols to food and impair the acceptability of the  
88 product to consumers.

89

90 From a sensory point of view, it is well documented that phenolic compounds contribute to the  
91 bitter and astringent oral sensation of food and beverages (Hufnagel & Hofmann, 2008) and this  
92 significantly affects the preference and choice of phenol-rich vegetable foods (Dinnella, Recchia,  
93 Tuorila, & Monteleone, 2011). Monomeric and polymeric phenols have been widely studied  
94 because of their contribution to wine sensory perception. Monomeric flavan-3-ols, procyanidin  
95 dimers and trimers seem to be involved in the perception of astringency and bitterness in red wine  
96 (Peleg, Gacon, Schlich, & Noble, 1999). Several authors have studied the bitterness of polyphenols  
97 in red wine, demonstrating that larger molecules tend to be less bitter and more astringent (Peleg et  
98 al., 1999). More recently, in reconstruction studies it was observed that the puckering astringent  
99 offset was caused by a polymeric fraction exhibiting molecular masses above >5 kDa and it was  
100 found to be amplified by organic acids (Hufnagel & Hofmann, 2008). Some factors such as pH,  
101 acidity, carbohydrate content and saliva characteristics could affect oral sensations (Dinnella,

102 Recchia, Fia, Bertuccioli, & Monteleone, 2009; Fia, Dinnella, Bertuccioli, & Monteleone, 2009; de  
103 Freitas & Mateus, 2012).

104

105 To mitigate functional phenol's bitter and astringent potential, the naturally occurring interactions  
106 phenols/biopolymers in vegetable foods (Zhang et al., 2014) are an effective strategy (De Toffoli et  
107 al., 2019). Plant biopolymers can act as a physical barrier for the phenol stimuli utilized, thus  
108 hindering their interactions with sensory receptors and saliva. Many factors affect  
109 phenol/biopolymer binding, including pH and reagent features such as chemical compositions,  
110 structure, and hydrophobic/hydrophilic characteristics (Kroll, Rawel, & Rohn, 2003). Furthermore,  
111 several studies have investigated the chemical features of phenol/biopolymer interactions and their  
112 consequences on sensory attributes (Jakobek, 2015).

113

114 The health effects of phenols depend on the consumed amount and on their bioavailability. The  
115 bioavailability of phenols may vary depending on their bioaccessibility, referred as the release from  
116 the food matrix, their stability against several biochemical factors, and their later intestinal  
117 absorption (Sengul, Surek & Nilufer-Erdil, 2014). The bioavailability of phenols from many  
118 different vegetable sources, including grapes, was systematically studied by Manach, Scalbert,  
119 Morand, Rémésy, & Jiménez (2004). In humans, among the most well absorbed phenols there are  
120 gallic acid, catechins and quercetin glucosides (Manach et al., 2004). Recently, a phenol extract  
121 from grape pomace was included in the diet of Wistar rats by Olivero-David et al., (2018). The  
122 same authors observed a partial bioavailability of the phenol extract and an improvement in lipid  
123 metabolism of rats.

124 During food processing, bioactive compounds may undergo chemical degradation and lose their  
125 activities. Thermal processing and long-term storage can lead to a decrease in both polyphenol  
126 content and antioxidant activity (Yu & Ahmedna, 2013). Other factors such as pH and interactions  
127 with other macromolecular food constituents can affect the chemical stability and antioxidant

128 activity of phenolic compounds (Jakobek, 2015). It is emerging that the bioaccessibility and  
129 bioavailability of phenolic compounds are affected by interaction with other macromolecules such  
130 as proteins, carbohydrates and lipids. These interactions could give phenolic compounds protection  
131 from oxidation during their passage through the gastrointestinal tract (Saura-Calixto, 2011). On the  
132 other hand, phenol/protein interactions can lead to a loss of nutritional values due to protein  
133 precipitation and enzyme inactivation (Rohn, Petzke, Rawel & Kroll, 2006).

134

135 Variations in chemical composition, antioxidant activity and sensory profiles in food-base  
136 vegetables with added phenols from unripe grapes have never been investigated before.

137

138 This paper explores the chemical and sensory properties of phenols extracted from UGs and the  
139 consequences of phenol/biopolymer interactions on the chemical and sensory properties of plant-  
140 base foods. With this aim, three food models with variable macro-compositions in which different  
141 phenol/biopolymer interactions might occur were functionalised with an extract from unripe grapes  
142 (UGs).

143

## 144 **2. Material & Methods**

### 145 *2.1. UG extract and UG-water solutions preparation*

146 The unripe grapes (UGs), cv Merlot, were hand-picked in August 2017 in a commercial vineyard  
147 located in Velletri, Rome, Italy. To obtain the UG extract, maceration was performed as previously  
148 described by Fia et al. (2018), with some modifications (**Fig. S1**). After decantation and filtration of  
149 the liquid extract, sugar was eliminated by ultrafiltration, using a spiral wound configuration  
150 membrane, with a molecular weight cut-off of 2500 Dalton (General Electrix, Boston,  
151 Massachusetts, United States). The liquid extract was dehydrated by lyophilization with the addition  
152 of arabic gum (2% w/v) (Nexira Food, Rouen Cedex, France) as a support and stored in  
153 polyethylene pouches under vacuum, in a desiccator, at room temperature, protected from the light.

154 The UG extract (334 g) was diluted in distilled water to a total volume of 1L. This suspension was  
155 centrifuged at 1646 g, for 10 min, to eliminate the excess arabic gum. The phenol concentration in  
156 the supernatant UG stock solution (SS) was 6.81 g/L. The SS was daily prepared and used to  
157 prepare UG-water solutions at different phenol concentrations to be added to the plant-based food  
158 models (**Fig. S1**).

159 The UG-water solutions were filtered through a membrane ( $\varnothing$  0.45  $\mu$ m) and the phenolic  
160 compounds were purified using a C18 Sep-pak cartridge (1 g) (Waters, Milan, Italy) before the  
161 evaluation of the total polyphenol content.

162

## 163 *2.2 Food models*

164 Three food models were selected on the basis of their composition (**Table S1**) and taste: beetroot  
165 purée (BP) characterized by high carbohydrate content, acidic pH and sweet taste; pea purée (PeP)  
166 characterized by high proteins content, neutral pH and sweet taste; potato purée (PoP) characterized  
167 by high carbohydrates content and neutral pH. Canned or powdered ingredients produced by large  
168 food companies were used to prepare the food models, since they are not subject to seasonal  
169 restriction and their composition is constant. Purées of beetroot, pea and potato were prepared as  
170 following: a) 500 g of peeled and steamed beetroots were blended at maximum speed, for about 1  
171 min, using a Kenwood FDM 780 mixer (Kenwood, Treviso, Italy), until it was obtained a  
172 homogeneous product; b) 310 g of steamed peas were rinsed under cold water for 30 sec and  
173 drained for 30 sec to eliminate the water, then 7 g of water were added and the mix was blended at  
174 maximum speed for 2 min in a mixer Kenwood; c) 75 g of dehydrated potatoes were added to 340 g  
175 of water brought to 80°C and the product was mixed until it became homogeneous, then it was  
176 cooled for 30 min before using. Each food model was prepared at five levels of phenol  
177 concentration (0.00, 0.21, 0.44, 1.11 and 1.93 g/kg) (**Fig. S1**).

178

## 179 *2.3. Chemicals*



180 All solvent and reagents were supplied from Sigma-Aldrich (Milan, Italy), except for methanol and  
181 ethanol which were supplied by Carlo Erba (Milan, Italy). Ultrapure water was obtained using a  
182 Milli-Q Gradient water purification system (Thermo Scientific, Waltham, Massachusetts, USA).

183

## 184 2.4. *Physical-chemical analysis*

### 185 2.4.1 *General analysis*

186 Total acidity and pH were evaluated according to the methods recommended by the International  
187 Organization of Vine and Wine (OIV) (International Organization of Vine and Wine Website,  
188 2014).

### 189 2.4.2. *Moisture content and water activity*

190 The powder moisture content was determined gravimetrically by drying in a vacuum oven, at 70°C,  
191 until a constant weight was reached (A.O.A.C. , 1990 ) . Powder water activity ( $A_w$ ) was measured  
192 using a Rotronic Hygroskop *DT* hygrometer (Michell Italia Srl, Milan, Italy).

### 193 2.4.3. *Solubility*

194 Water solubility was determined according to (Cano-Chauca, Stringheta, Ramos, & Cal-Vidal,  
195 2005). A volume of 100 mL of distilled water was transferred into a blender jar. The sample (1g,  
196 dry basis) was carefully added to the blender while operating at high speed for 5 min. The solution  
197 was centrifuged at 3000 g for 5 min. An aliquot of 25 mL of the supernatant was transferred to pre-  
198 weighed Petri dishes and immediately oven-dried at 105°C for 5 h. The solubility (%) was  
199 calculated by weight difference.

### 200 2.4.4. *Hygroscopicity*

201 Hygroscopicity was evaluated following the method described by Callahan et al. (1982), with some  
202 modifications. The equilibrium moisture content (EMC) of the samples (1 g, dry basis) was  
203 evaluated following storage in desiccators containing saturated salt solutions with a relative  
204 humidity ranging from 8% to 84% at 25°C until a constant weight was reached (approx. 21 days).  
205 The hygroscopicity was expressed as g of adsorbed water per 100 g of dry matter (g/100g dm).

#### 206 2.4.5. Phenol extraction

207 Extracts were obtained from the food models (FMs) following the method described by Turkmen,  
208 Sari, & Velioglu (2005). For each food matrix, 1 g was homogenized and extracted twice with 4.5  
209 mL of 80% aqueous methanol solution in a mechanical shaker, for 2 h. The mixture was centrifuged  
210 at 13440 g, for 15 min, at room temperature, and the supernatant decanted into polypropylene tubes.  
211 The supernatant was filtered through Whatman No.1 filter paper. The extraction procedure was  
212 performed in triplicate.

#### 213 2.4.6. Total polyphenol

214 The total polyphenols (TP) were quantified according to the Folin-Ciocalteu method (Singleton,  
215 Rossi Jr., & Rossi J A Jr., 1965). A Perkin Elmer Lambda 10 spectrophotometer (Waltham, MA,  
216 USA) was used to measure the absorbance of the reaction mixture at 700 nm. A standard curve was  
217 obtained with (+)-catechin solutions at concentrations ranging from 5 to 500 mg/L. The TP was  
218 expressed as mg of (+)-catechin equivalents/L of the UG-water solution or kg of the food model  
219 extracts.

#### 220 2.4.7. Antioxidant activity

221 Antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-  
222 Williams, Cuvelier, & Berset, 1995). Trolox standard solutions were prepared daily in absolute  
223 ethanol at concentrations ranging from 10 to 600 µmol/L. Antioxidant activity was expressed as  
224 µmol of Trolox equivalent antioxidant capacity (TEAC)/L of the solution or kg of the food model  
225 extract.

#### 226 2.4.8. LC-HRMS analysis

227 Analysis of the phenolic compounds and glutathione was performed via liquid chromatography –  
228 high-resolution mass spectrometry (LC-HRMS), according to Fia et al. (2018) using an Accela  
229 1250 (Thermo Fisher Scientific) coupled with an LTQ OrbitrapExactive mass spectrometer  
230 (Thermo Fisher Scientific) equipped with an electrospray ionization (ESI) source in negative mode.  
231 The standards were purchased from Sigma-Aldrich (Milan, Italy), except for the quercetin 3-O-

glucoside which was supplied by Analytik GmbH (Rülzheim, Germany). Coumaric and ferulic acids were used as standards for coumaric and ferulic acids due to the lack of reference materials. Data were expressed as mg of phenols/kg of the UGs or food models.

## 2.5. Sensory evaluations

The present data were collected as part of a larger study aimed at investigating factors affecting the acceptability of health foods (PRIN 2015: Individual differences in the acceptability of health foods: focus on phenol and fat content). This multisession study consisted of a home questionnaire session and one-on-one testing in a sensory laboratory across two days. This paper will only present a selection of these data. The sensory tests are further detailed in De Toffoli et al. (2019). Two respondent groups were recruited to evaluate the UG extract (Group 1: n=29; 59% females; mean age  $27.5 \pm 7.1$ ) or functionalized food prototypes (Group 2: n=27; 70% females; mean age  $31.5 \pm 9.4$ ). The participants received a gift to compensate for their time. The respondents gave their written informed consent at the beginning of the test according to the principles of the Declaration of Helsinki. In brief, training was performed as described by Monteleone et al., (2017) using the general Labelled Magnitude Scale - gLMS (0: no sensation-100: the strongest imaginable sensation of any kind) (Green et al., 2007). Eight water solutions of UG extract were prepared as sensory stimuli with increasing phenol concentration: 0.14, 0.21, 0.30, 0.41, 0.59, 1.11, 1.27 and 1.93 g/L of phenol (**Fig. S1**). The data were collected using Fizz software (ver.2.51. A86, Biosystèmes, Couternon, France).

## 2.6. Data analysis

A one-way ANOVA model was used to assess the storage effect on the variation of phenol content and antioxidant activity of the UG extract. Two-way ANOVA models were used to assess the effect of both phenol concentration and replicates on the antioxidant activity in the UG solutions and to

257 assess the effect of both the amount of phenol added and replicates on the recovery of UG phenols  
258 from food models.

259 The UG phenols recovered (recovery %) from the functionalized food samples were calculated as  
260 the difference between the total phenol content of the functionalized food and that of the non-  
261 functionalized food, then it was expressed as percentage of the phenols added. Two-way ANOVA  
262 models were used to assess the effect of phenol concentration on the intensity of the target  
263 sensations in UG solutions and food prototype samples (phenol concentration were used as fixed  
264 factor; subjects were considered as random factor). Three-way ANOVA were used to assess the  
265 effect of the food matrix on the perceived intensity of the target sensations models (fixed factors:  
266 food matrix and phenol concentration; random factor: subjects and interactions). A  $p$ -value of 0.05  
267 was considered as the threshold for statistical significance.

268 Data analysis was performed using XLSTAT statistical software package (Addinsoft - version  
269 19.02).

270

## 271 **3. Results**

272

### 273 *3.1. Physical-chemical characterization*

#### 274 *3.1.1. UG extract*

275 The solubility of the UG extract was  $88.1 \pm 1.2\%$ . The moisture content of the UG extract, at  $25^\circ\text{C}$ ,  
276 was  $8.1 \pm 0.3\%$  and the water activity was  $38.7 \pm 0.1\%$ . The adsorption isotherm of the UG extract  
277 at  $25^\circ\text{C}$  was determined (**Fig. S2**). The experimental data for water activity ( $A_w$ ) as a function of the  
278 moisture content fitted well with the Halsey model (Okos et al., 1992), as follows:

279

$$280 \quad A_w = \exp\left(-\frac{B}{n_s^A}\right) \quad (r^2 = 0.98)$$

281

282 where  $n_s$  (g water/g dry matter),  $A = 0.039$  and  $B = 1.461$ .

283 The powder displayed little hygroscopic behaviour up to  $A_w$  values  $< 0.80$ , while for  $A_w$  values  
284 greater than 0.85 the hygroscopicity increased exponentially.

285

286 The total phenol content of the UG extract was  $20403 \pm 943$  mg/kg. The total phenol content of the  
287 UG extract was evaluated monthly until to nine months of storage. After this period, the UG extract  
288 displayed the same phenolic concentration as the outset. No significant differences ( $p = 0.05$ ) were  
289 assessed among phenolic content values during storage.

290

291 The phenolic composition of the UG extract was analysed by LC-HRMS. Nineteen phenolic  
292 compounds were identified in the UG extract (**Table 1**). Phenolic acids were the most abundant  
293 class of phenolic compounds and they accounted for 89% of the amount of phenols identified in the  
294 UG extract. Caftaric acid accounted for 85% of the phenolic acid content. Flavonols, flavan-3-ols,  
295 procyanidins, trans-resveratrol and 2-S-glutathionyl fertaric acid accounted for the remaining 11%  
296 of the amount of phenols detected in the UG extract.

297

298 The antioxidant activity of the UG extract was  $33829 \pm 949$  TEAC  $\mu\text{mol/kg}$ , and the specific  
299 activity of the phenols was  $1.66 \pm 0.04$  TEAC  $\mu\text{mol/mg}$ . The antioxidant activity of the UG extract  
300 was evaluated monthly, up to nine months of storage. After this period, the antioxidant activity of  
301 the UG extract remained at 99.4%. No significant differences ( $p = 0.05$ ) were assessed in the  
302 antioxidant activity values at different times of storage.

### 303 3.1.2. UG water solutions

304 The total phenol content of the stock solution was  $6.81 \pm 0.04$  g/L. The stock solution was  
305 characterized for total acidity ( $7.6 \pm 0.26$  g/L as tartaric acid) and pH ( $3.21 \pm 0.02$ ). The solutions  
306 from the UG extract were tested for antioxidant activity at increasing phenol concentration levels  
307 (0.14, 0.21, 0.30, 0.41, 0.59, 1.11, 1.27 and 1.93 g/L) (**Fig. S3**). The UG phenol concentration  
308 significantly affected the level of antioxidant activity of the water solutions ( $p \leq 0.001$ ) while the

309 replicates were not significant ( $p < 0.05$ ). A significant positive relationship ( $r = 0.978$ ) was found  
310 between the total phenol content and the antioxidant activity of the UG water solutions.

311

### 312 3.1.3. Functionalized food models

313 After the addition of an increasing amount (0.00, 0.21, 0.44, 1.11 and 1.93 g/kg) of UG phenols to  
314 the food models, the phenol concentration in the FM extracts was determined (**Fig. 1A**). The non-  
315 functionalized food models showed different phenolic content, with the highest level detected in the  
316 beetroot purée and the lowest in the potato purée. The amount of phenols added to the food models  
317 significantly affected the concentration of phenols found in the FM extracts ( $p \leq 0.05$ ).

318

319 The phenols recovered from food models significantly varied as a function of both the food model  
320 and the amount of phenols added. The recovered amount ranged from 27.7% to 81.3% in the  
321 beetroot purée, from 34.0% to 53.6% in the pea purée and from 52.7% to 86.4% in the potato purée.  
322 The mean phenol value recovered with the highest added amount of phenols was highest in the  
323 potato purée (68.7%), followed by the beetroot purée (57.8%), and the pea purée (43.3%). (**Fig.**  
324 **1B**).

325

326 The food samples functionalized with the highest amount of phenols (1.93 g/kg) were extracted and  
327 the extracts analysed via LC-HRMS to evaluate their phenol composition. The FM extracts  
328 contained almost all of the phenolic compounds identified in the original UG extract, except for  
329 kaempferol-3-*O*-glucoside, quercetin-3-*O*-hexoside and 2-S-glutathionyl caftaric acid (**Table 1**).  
330 Caftaric acid was the most abundant phenolic compound assayed in the FM extracts of the three  
331 food models. Ferulic acid was not detected in the potato purée. The phenol profiles of the food  
332 model functionalized with 1.93 g/kg of UG phenols were compared to the profile of the UG extract  
333 (**Fig. 1C**). The relative amounts of each phenolic class in functionalized beetroot purée was similar  
334 to that observed in the UG extract, while slight differences were observed in the functionalized pea

335 and potato purées. Phenolic acids represented the most abundant class of phenols in the UG extract  
336 (90.3%) and the beetroot purée almost retained this same high percentage (88.9%), while in the pea  
337 and potato purées a slight loss was observed (80.6 and 83.9%, respectively). The proportion of other  
338 phenolic classes (flavonols, flavan-3-ols, procyanidins and stilbenes) was slightly higher in the pea  
339 and potato purées compared to the figure observed in the UG extract and the beetroot purée.

340

341 The antioxidant activity of the food models with an increasing added amount (0.00, 0.21, 0.44, 1.11  
342 and 1.93 g/kg) of UG phenols was determined after extraction (**Fig. 2A**). The non-functionalized  
343 beetroot and pea purées had similar values of antioxidant activity while it was much lower in the  
344 potato purée. A significant increase in antioxidant activity was observed in the beetroot purée as  
345 function of the UG phenol concentration. No significant difference was observed between the  
346 antioxidant activity of the pea purée functionalized with 0.44 or 1.11 g/kg of UG phenols.

347 The difference between the antioxidant activity of functionalized food and that of food without  
348 added phenol was calculated to assess the contribution of UG phenols to the food models' final  
349 antioxidant activity. The relationship between the antioxidant activity of UG phenols in the water  
350 solution and in the FM extracts is shown in **Figure 2B**. The antioxidant activity was always  
351 significantly higher in the extracts of beetroot purée compared to that detected in the potato and pea  
352 purée extracts. The mean antioxidant activity was 3794  $\mu\text{mol/kg}$  in the BP, 1722  $\mu\text{mol/kg}$  in the  
353 PoP and 1127  $\mu\text{mol/kg}$  in the PeP extracts.

354

### 355 3.2. Sensory evaluation

#### 356 3.2.1. UG extract solutions

357 The phenol concentration of the UG solutions significantly affected the intensity of the target  
358 sensations (**Fig. 3A and Table S2**). According to the F values, the increase in phenol concentration  
359 had the strongest effect on sourness while it influenced the other target sensations much less.  
360 Significant intensity increases were observed in the samples with phenols from the UG extract

361 compared to the sample without added phenol (0.00 g/L). Sourness increased from weak to strong  
362 across the phenol concentration range. Bitterness, astringency and saltiness showed limited intensity  
363 increases, from barely detectable to weak.

364 Four concentration levels, which cover the whole range of significant variations of intensity of  
365 target sensations, were selected to fortify the vegetable matrices: 0.00, 0.21, 0.41, 1.11 and 1.93  
366 g/L.

### 367 3.2.2. *Functionalized foods*

368 The intensity of target sensations significantly changed in all of the three vegetable prototypes as a  
369 function of the increasing phenol concentrations, the only exception being sweetness in the PoP  
370 (**Table 2**). Phenol concentration induced the strongest effect on sourness in all of the three food  
371 models as showed by F-values. The intensity of the other sensations was influenced by both the  
372 increase in phenol concentration and, to a lesser extent, by the macro-composition of the matrix. All  
373 of the sensations were barely detectable in the beetroot purée sample without added phenol, while in  
374 the rest of the samples, sourness increased from weak to strong, sweetness showed a significant  
375 decrease from moderate to weak, while saltiness, astringency and bitterness increased slightly from  
376 barely detectable to weak (**Fig. 3 B-Beetroot purée**). The variation in intensity of the target  
377 sensation in the pea purée as a function of the phenol concentration was similar to that observed in  
378 the beetroot purée (**Fig. 3 C-Pea purée**). The increase in sourness from barely detectable to  
379 moderate was associated with a significant decrease in sweetness, from moderate to weak, while the  
380 rest of the sensations were perceived at a weak intensity or even lower. In the potato purée sample  
381 without added phenols, all the sensations were rated at a barely detectable/weak intensity, while  
382 only sourness showed a remarkable increase from barely detectable to strong as the phenol  
383 concentration increased (**Fig. 3 D-Potato purée**).

384 Bitterness, astringency and saltiness were not further investigated since these sensations were  
385 marginally affected by addition of phenols and perceived at a weak intensity across the whole range  
386 of concentrations.



387

388 Sourness and sweetness perceived in the food functionalized at different UG concentration were  
389 compared to further explore the effect of food macro-composition on UG phenol sensory properties.  
390 While the vegetable matrix and phenol concentration significantly affected the intensity of sourness  
391 and sweetness, the vegetable matrix\*concentration interaction was never significant (**Table S3**).  
392 Significant differences were found upon comparing sourness from the three matrices at phenol  
393 concentrations of 0.41, 1.11 and 1.93 g/L. The highest sourness intensity was rated in the PoP,  
394 whereas no significant differences were found between the BP and PeP (**Fig. 4-A**). Sweetness was  
395 rated as more intense in the BP and PeP than in the PoP across the 0.0 to 0.41 g/kg concentration  
396 range of spiked phenols. At the highest concentration levels, sweetness was perceived at the highest  
397 intensity in the BP (**Fig. 4-B**).

398

#### 399 **4. Discussion**

400

401 Physical-chemical characterization was carried out to evaluate the attitude of UG extract towards  
402 rehydration and stability during storage, in terms of phenolic content and antioxidant activity. The  
403 solubility value of the UG extract was similar to those (86% - 88%) obtained by Kuck & Noreña  
404 (2016) on grape skin extracts lyophilized with arabic gum and partially hydrolysed guar gum as  
405 supports.

406

407 The moisture content and water activity value of the UG extract were in agreement with the results  
408 obtained on grape skin extracts by Kuck & Noreña (2016). The UG extract showed similar  
409 hygroscopic behaviour to the absorption isotherm of an aqueous solution of salts and simple sugars.  
410 Therefore, the powder has to be protected from humidity during storage to avoid water absorption,  
411 thus preserving the extract's stability.

412

413 The total phenol content of the UG extract was similar to that obtained by Kuck & Noreña (2016)  
414 on aqueous extracts of grape skin microencapsulated with different agents while the antioxidant  
415 activity was slightly lower. In general, the phenol content and antioxidant activity of extracts vary  
416 mainly depending on the origin of grape by-products and extraction conditions (Trigo, Alexandre,  
417 Saraiva, & Pintado, 2019). Indeed, when ethanol or methanol were used for the extraction, the  
418 phenolic content and antioxidant activity of the extracts were higher than those detected in aqueous  
419 extracts (Trigo et al., 2019; Tournour, Segundo, Magalhães, Costa & Cunha, 2017). After nine  
420 months, the high percentage of both residual phenols and antioxidant activity in the UG extract  
421 indicated that the adopted storage conditions were suitable to protect the UG phenols from  
422 degradation.

423

424 When a different amount of the UG phenols was used to enrich the food models, the increase of  
425 phenol concentration in the FM extracts was expected. Similar results were obtained by other  
426 authors who studied the addition of phenolic extracts from different by-products to some food and  
427 beverages (Trigo et al., 2019). Chemical-physical characteristics of food models explored in these  
428 study significantly affect phenol recovery thus indicating clear reactivity differences between UG  
429 phenols and food components. The lowest amount of phenols was recovered from the protein-rich  
430 model (pea purée). A similar effect of the interaction phenol/biopolymers on the bioactivity of  
431 phenols from olive mill waste waters in plant-based food has already been observed by other  
432 authors (De Toffoli et al., 2019).

433 The formation of phenol/protein aggregates significantly lowers the phenol bio-activity both in  
434 terms of extractability from raw material and antioxidant activity (Ozdal et al., 2013). Proteins bind  
435 plant polyphenols through hydrophobic and hydrogen interactions; the preferred sites of interaction  
436 plant phenol/food protein in *in vitro* conditions are the proline-rich regions of leguminous proteins  
437 characterized by high basic-residue contents as well as open and flexible structures (Kroll et al.,  
438 2003; Zhang et al., 2014).

439 Phenol chemical structure, size and composition, including number of OH groups, play an  
440 important role in phenol/protein interactions, and phenolic compounds with a low molecular weight  
441 are inefficient to bond proteins (de Freitas & Mateus, 2012). It is known that upon extraction, the  
442 acidic condition of grape juice promotes the depolymerization of proanthocyanidins (Vidal,  
443 Cartalade, Souquet, Fulcrand, & Cheynier, 2002). However, these reactions begin during  
444 maceration and proceed slowly in wine, but they have never been highlighted in grape juice.  
445

446 The quite high percentages of UG phenols recovered, mainly in the carbohydrate-rich potato and  
447 beetroot purée food models, indicated that moderate/weak chemical interactions take place among  
448 UG phenols and food components. These findings, associated with the significant increase in  
449 antioxidant activity detected in the functionalized food models after the addition of UG phenols,  
450 indicate that most of the potential biological activity and the extractability of UG phenols were  
451 maintained after blending.

452 Phenolic compounds can bridge or cross-link with polysaccharides, and a large fraction of the not  
453 extractable polyphenols consist phenol associated with polysaccharides (Pérez-Jiménez, Díaz-  
454 Rubio, & Saura-Calixto, 2013). The consequences of phenol/carbohydrate interactions on phenol  
455 biological activity depends on the chemical characteristics of both phenols and carbohydrates  
456 (Zhang et al., 2014).

457 Other authors have described a competition between the arabic gum and other carbohydrates and  
458 the proteins to bind to the tannin (Gonçalves, Mateus, & de Freitas, 2011). The mechanism was  
459 previously investigated by tasting the influence of several carbohydrates on the formation of  
460 polyphenols/protein complexes. Polygalacturonic acid, arabic gum and pectin prevented the  
461 association of procyanidin B3 with trypsin, and that of salivary proteins with grape seed  
462 procyanidins. The interruption of polyphenol-protein association by carbohydrates can prevent  
463 some of the negative effects of these complexes, such as enzyme activity inhibition, and it can  
464 influence the perceived astringency of some food products.

465

466 The antioxidant activity of UG phenols was influenced by the food composition. The highest level  
467 of antioxidant activity was found in the carbohydrate-rich/acidic pH beetroot purée. The antiradical  
468 capacity of phenols depends on several factors such as their concentration and structures, and the  
469 physical-chemical characteristics of the solvent. The role of acidity in the kinetics of phenol/radical  
470 reactions was previously investigated by (Musialik, Kuzmich, Pawcowski, & Litwinienko, 2009). In  
471 general, it is known that deprotonated flavonoids are more potent electron donors and are better  
472 radical scavengers than neutral molecules. However, the ability of phenols to scavenge reactive  
473 oxygen species such as peroxy and hydroxyl radicals is still far from being fully understood.  
474 Valgimigli et al. (2009) described an unexpected dramatic acceleration of phenol-peroxy radical  
475 reaction with the addition of acid. The best performance, in terms of antioxidant activity, of UG  
476 phenols when added to beetroot purée could be due to the acidic pH of the beetroot food model.

477

478 Sensory profiles of the three matrices were significantly affected by the addition of UG extracts.  
479 Sourness intensity increased as a function of the UG phenol concentration. The natural sweetness  
480 of the beetroot and pea purées was reduced by the spiked phenols due to the intermodal interaction  
481 between sour and bitter tastes, which induced the suppression of perceived sweetness as the  
482 sourness intensity increased (Keast & Breslin, 2002). The bitterness, saltiness and astringency  
483 intensities were significantly modified by the UG extract, but the extent of these effects appears  
484 marginal since these sensations are perceived at a weak intensity across the whole range of  
485 concentrations.

486 The different compositions of the vegetable matrices affect the UG phenols' contribution to  
487 sourness. Furthermore, the observed increasing intensity range differed across the series of samples  
488 indicating that their macro-component plays an active role in modulating the sensory impact of UG  
489 phenols.

490

## 491 5. Conclusions

492

493 An extract from unripe grapes showed suitable physical-chemical characteristics for its inclusion in  
494 plant-based foods. Food composition influenced the functional and sensory properties of phenols  
495 from unripe grapes. The strongest effect in terms of recovered phenol and antioxidant activity was  
496 observed in protein-based food. The use of matrices high in carbohydrates, with acidic pH and  
497 characterized by sweet taste appears a suitable strategy to counteract the impact of the negative  
498 sensory properties of added phenol on plant-based food. The use of phenolic extracts from unripe  
499 grapes can be useful to improve potential health benefits when formulating plant-based functional  
500 food.

501

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## Figure legend

**Figure 1.** Total phenols (A) of food models, mean values of UG phenols recovered (B) from beetroot purée (BP), pea purée (PeP) and potato purée (PoP) functionalized with increasing amounts (0.00, 0.21, 0.44, 1.11 and 1.93 g/kg of food) of phenols and percentage of each phenolic class (C) detected in the UG extract (UG ext) and food models functionalized with 1.93 g/kg phenols from UG extract. The bars represent standard deviation. Different letters represent significant different values ( $p \leq 0.001$ ).

**Figure 2.** Antioxidant activity (A) of beetroot purée (BP), pea purée (PeP) and potato purée (PoP) functionalized with increasing amounts of phenols (0, 0.21, 0.44, 1.11 and 1.93 g/kg of food) from UG extract and antioxidant activity (B) of UG phenols in water solution vs antioxidant activity in the FM extracts. The bars represent standard deviation. Different letters represent significant different values ( $p \leq 0.001$ ).

**Figure 3.** Mean intensity of target sensations (A) in the UG solutions with increasing phenol concentration and food models (B, C and D) functionalized with increasing concentrations of phenols from UG extract. The bars represent standard error.

689 **Figure 4.** Effect of the vegetable matrix on the perceived intensity of sourness (A) and sweetness  
690 (B) in foods spiked with different concentrations of phenols from UG extract. Different letters  
691 represent significant different values ( $p \leq 0.038$ ).  
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**Table 1.** Phenol profile of the UG extract and phenols detected in the FM extracts. Beetroot purée (BP), pea purée (PeP) and potato purée (PoP) functionalized with 1.93 g/kg of phenols from the UG extract.

Compound	mg/kg			
	UG extract	BP*	PeP*	PoP*
<i>Phenolic acid</i>				
Caffeic acid	11.0 ± 0.4	1.04 ± 0.07 <sup>c</sup>	1.55 ± 0.14 <sup>a</sup>	1.28 ± 0.14 <sup>b</sup>
Caftaric acid	704 ± 33	48.7 ± 1.2 <sup>a</sup>	35.7 ± 6.5 <sup>b</sup>	36.5 ± 4.0 <sup>b</sup>
Coumaric acid	19.6 ± 0.6	1.80 ± 0.13 <sup>b</sup>	2.30 ± 0.12 <sup>a</sup>	1.79 ± 0.14 <sup>b</sup>
Coutaric acid	34.3 ± 1.1	2.31 ± 0.17 <sup>a</sup>	2.03 ± 0.18 <sup>ab</sup>	1.81 ± 0.15 <sup>b</sup>
Ferulic acid	4.63 ± 0.59	2.51 ± 0.04 <sup>a</sup>	0.44 ± 0.03 <sup>b</sup>	nd
Fertaric acid	52.0 ± 2.0	3.44 ± 0.10 <sup>a</sup>	3.54 ± 0.28 <sup>a</sup>	3.71 ± 0.19 <sup>a</sup>
Gallic acid	1.63 ± 0.03	0.03 ± 0.01 <sup>b</sup>	0.24 ± 0.02 <sup>a</sup>	0.05 ± 0.01 <sup>b</sup>
<i>Flavonols</i>				
Isorhamnetin	1.41 ± 0.03	0.05 ± 0.01 <sup>b</sup>	0.09 ± 0.01 <sup>a</sup>	0.06 ± 0.02 <sup>b</sup>
Kaempferol	0.78 ± 0.04	0.06 ± 0.01 <sup>a</sup>	0.06 ± 0.01 <sup>a</sup>	0.0 ± 0.01 <sup>a</sup>
Kaempferol-3- <i>O</i> -glucoside	0.54 ± 0.03	nd	nd	nd
Myricetin	3.79 ± 0.11	0.39 ± 0.03 <sup>b</sup>	0.47 ± 0.04 <sup>a</sup>	0.45 ± 0.03 <sup>ab</sup>
Quercetin	14.0 ± 0.4	1.26 ± 0.11 <sup>b</sup>	1.48 ± 0.13 <sup>ab</sup>	1.57 ± 0.14 <sup>a</sup>
Quercetin-3- <i>O</i> -hexoside	1.32 ± 0.08	nd	nd	nd
<i>Flavan-3-ols</i>				
(+)-Catechin	13.6 ± 0.8	1.23 ± 0.07 <sup>c</sup>	2.28 ± 0.12 <sup>a</sup>	1.51 ± 0.11 <sup>b</sup>
(-)-Epicatechin	8.23 ± 0.29	0.70 ± 0.03 <sup>c</sup>	1.09 ± 0.08 <sup>a</sup>	0.83 ± 0.05 <sup>b</sup>
<i>Procyanidins</i>				
Procyanidin B1	4.55 ± 0.19	0.44 ± 0.04 <sup>b</sup>	0.56 ± 0.04 <sup>a</sup>	0.47 ± 0.06 <sup>ab</sup>
Procyanidin B2	9.74 ± 0.37	1.13 ± 0.05 <sup>c</sup>	1.66 ± 0.05 <sup>a</sup>	1.33 ± 0.07 <sup>b</sup>
<i>Stilbenes</i>				
Trans-resveratrol	31.3 ± 1.6	2.18 ± 0.13 <sup>b</sup>	3.33 ± 0.48 <sup>a</sup>	2.36 ± 0.36 <sup>b</sup>
2- <i>S</i> -Glutathionyl caftaric acid	16.8 ± 0.6	nd	nd	nd

Data are expressed as mean ± standard deviation (n=3); nd, not detected. Different letters represent significant different values ( $p \leq 0.001$ ) among the columns.

**Table 2.** Two-way ANOVA mixed model (random effect: assessors): phenol concentration effect on intensity of target sensations in food models. Mean, F and p values.

			Concentration of phenols from UG (g/kg)				
			0.00	0.21	0.41	1.11	1.93
	F	p					
<b>Bitterness</b>							
Beetroot Purée	4.92	0.0011	0.97 b	1.34 b	0.62 b	1.34 b	3.31 a
Pea Purée	6.78	< 0.0001	1.28 b	1.31 b	1.41 b	3.72 a	5.28 a
Potato Purée	2.53	0.0445	2.61 b	3.00 b	3.25 b	4.11 ab	5.46 a
<b>Sourness</b>							
Beetroot Purée	26.22	< 0.0001	2.38 c	3.07 c	4.41 c	13.86 b	21.86 a
Pea Purée	39.02	< 0.0001	3.48 b	3.34 b	5.62 b	16.31 a	19.72 a
Potato Purée	48.39	< 0.0001	3.07 e	8.54 d	13.46 c	20.43 b	27.68 a
<b>Saltiness</b>							
Beetroot Purée	4.85	0.0012	1.17 b	1.38 b	2.38 b	2.86 ab	4.55 a
Pea Purée	3.63	0.0081	4.52 c	4.31 c	5.79 bc	7.24 ab	8.55 a
Potato Purée	5.78	0.0003	2.29 bc	1.96 c	3.89 bc	4.00 b	6.14 a
<b>Sweetness</b>							
Beetroot Purée	3.07	0.0194	16.31 a	17.79 a	15.21 ab	13.83 ab	11.28 b
Pea Purée	10.01	< 0.0001	12.72 a	13.69 a	11.41 a	7.31 b	5.52 b
Potato Purée	1.56	0.1865	4.18	3.21	3.43	2.36	2.54
<b>Astringency</b>							
Beetroot Purée	4.64	0.0017	4.31 bc	4.07 c	31 c	7.38 a	6.34 ab
Pea Purée	4.16	0.0035	5.48 bc	3.72 c	3.97 bc	6.76 ab	8.72 a
Potato Purée	6.01	0.0001	2.86 c	4.93 bc	6.86 ab	7.64 a	8.43 a

Different letters indicate significantly different values ( $p \leq 0.05$ ).

## Highlights

- A strategy was outlined for the exploitation of high-quality unripe grapes
- The food composition affected both the phenol recovered and antioxidant activity
- The highest recovery of phenols was from the starch/neutral pH food model
- The highest antioxidant activity was from the carbohydrates/acidic pH food model
- The models' sensory properties are modulated by phenol content and food composition

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**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Ginevra Bucalossi: Investigation, Visualization  
Giovanna Fia: Writing- Original draft preparation  
Caterina Dinnella: Conceptualization, Methodology, Writing - Review & Editing  
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