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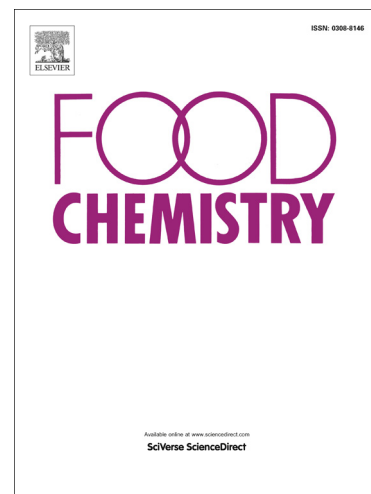
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1 **Functional and sensory properties of phenolic compounds from unripe grapes in vegetable**

2 **food prototypes**

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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

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).

Unripe grapes (UGs) discarded during thinning are an undervalued by-product of vineyard

management for the production of high-quality wine (Gatti, Bernizzoni, Civardi, & Poni, 2012;

Keller, Mills, Wample, & Spayd, 2005; Ough Cs, 1984). In unripe berries, the most important

classes of grape antioxidants (phenolic acids, flavan-3-ols, flavonols, anthocyanins, stilbenes and

glutathione) are present to variable extents in function of some factors such as variety, maturity

level and season (Adams, 2006) but their anti

oxidant activity and potential application have received scarce scientific attention (Fia, Gori,

Bucalossi, Borghini, & Zanoni, 2018; Tinello & Lante, 2017). Low-quality unripe grapes are

processed into various traditional juices and sauces with a low pH and variable levels of antioxidant

activity ((Dupas de Matos, Magli, Marangon, Curioni, Pasini & Vincenzi, 2018; Öncül &

Karabiyikli, 2015). The added value of thinned grapes is higher than the one of other by-products of

wine industry that were largely studied and proposed as source of antioxidants. That is because, the

thinned grapes have not been exploited to make wine and therefore contain an intact complex of

bio-active compounds. Recently, a green extraction technique (i.e. performed without solvents and

preservatives) was patented (Fia & Gori, 2016) and applied at an industrial level with the aid of a

patented oenological machine (Gori, C., Menichetti, S., & Fia, G. 2014) to obtain an extract from

unripe grapes.

Functional food is essentially a marketing term with different definitions and regulations depending

on the country (Henry, 2010). Recently in Europe, there has been a growing interest in functional

foods. A scientific consensus document was drafted to develop a science-based approach for the

emerging concepts in functional food (Europe, 1999). Foods that have been modified by enrichment

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,

4

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

5

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 (Ø 0.45 μm) 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*

7

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).

8

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-

9

232 glucoside which was supplied by Analytik GmbH (Kulzheim, Germany). Coumaric and ferulic

233 acids were used as standards for coutaric and fertaric acids due to the lack of reference materials.

234 Data were expressed as mg of phenols/kg of the UGs or food models.

235

236 *2.5. Sensory evaluations*

237 The present data were collected as part of a larger study aimed at investigating factors affecting the

238 acceptability of health foods (PRIN 2015: Individual differences in the acceptability of health

239 foods: focus on phenol and fat content). This multisession study consisted of a home questionnaire

240 session and one-on-one testing in a sensory laboratory across two days. This paper will only present

241 a selection of these data. The sensory tests are further detailed in De Toffoli et al. (2019). Two

242 respondent groups were recruited to evaluate the UG extract (Group 1: n=29; 59% females; mean

243 age 27.5 ± 7.1) or functionalized food prototypes (Group 2: n=27; 70% females; mean age 31.5 ±

244 9.4). The participants received a gift to compensate for their time. The respondents gave their

245 written informed consent at the beginning of the test according to the principles of the Declaration

246 of Helsinki. In brief, training was performed as described by Monteleone et al., (2017) using the

247 general Labelled Magnitude Scale - gLMS (0: no sensation-100: the strongest imaginable sensation

248 of any kind) (Green et al., 2007). Eight water solutions of UG extract were prepared as sensory

249 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

250 phenol (**Fig. S1**). The data were collected using Fizz software (ver.2.51. A86, Biosystèmes,

251 Couternon, France).

252

253 *2.6. Data analysis*

254 A one-way ANOVA model was used to assess the storage effect on the variation of phenol content

255 and antioxidant activity of the UG extract. Two-way ANOVA models were used to assess the effect

256 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°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°C was determined (**Fig**. S**2**). 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 $$A_w = exp\left(-\frac{B}{n_s^A}\right) \qquad (r^2 = 0.98)$$

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 ± 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 ± 949 TEAC μmol/kg, and the specific

299 activity of the phenols was 1.66 ± 0.04 TEAC μmol/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 ± 0.04 g/L. The stock solution was

305 characterized for total acidity (7.6 ± 0.26 g/L as tartaric acid) and pH (3.21 ± 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

12

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

13

and potato purees. Phenolic acids represented the most abundant class of phenols in the UG extract

(90.3%) and the beetroot purée almost retained this same high percentage (88.9%), while in the pea

and potato purées a slight loss was observed (80.6 and 83.9%, respectively). The proportion of other

phenolic classes (flavonols, flavan-3-ols, procyanidins and stilbenes) was slightly higher in the pea

and potato purées compared to the figure observed in the UG extract and the beetroot purée.

The antioxidant activity of the food models with an increasing added amount (0.00, 0.21, 0.44, 1.11

and 1.93 g/kg) of UG phenols was determined after extraction (**Fig. 2A**). The non-functionalized

beetroot and pea purées had similar values of antioxidant activity while it was much lower in the

potato purée. A significant increase in antioxidant activity was observed in the beetroot purée as

function of the UG phenol concentration. No significant difference was observed between the

antioxidant activity of the pea purée functionalized with 0.44 or 1.11 g/kg of UG phenols.

The difference between the antioxidant activity of functionalized food and that of food without

added phenol was calculated to assess the contribution of UG phenols to the food models' final

antioxidant activity. The relationship between the antioxidant activity of UG phenols in the water

solution and in the FM extracts is shown in **Figure 2B**. The antioxidant activity was always

significantly higher in the extracts of beetroot purée compared to that detected in the potato and pea

purée extracts. The mean antioxidant activity was 3794 μmol/kg in the BP, 1722 μmol/kg in the

PoP and 1127 μmol/kg in the PeP extracts.

3.2. Sensory evaluation

3.2.1. UG extract solutions

The phenol concentration of the UG solutions significantly affected the intensity of the target

sensations (**Fig. 3A and Table S2**). According to the F values, the increase in phenol concentration

had the strongest effect on sourness while it influenced the other target sensations much less.

Significant intensity increases were observed in the samples with phenols from the UG extract

14

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.

15

Sourness and sweetness perceived in the food functionalized at different UG concentration were

compared to further explore the effect of food macro-composition on UG phenol sensory properties.

While the vegetable matrix and phenol concentration significantly affected the intensity of sourness

and sweetness, the vegetable matrix*concentration interaction was never significant (**Table S3**).

Significant differences were found upon comparing sourness from the three matrices at phenol

concentrations of 0.41, 1.11 and 1.93 g/L. The highest sourness intensity was rated in the PoP,

whereas no significant differences were found between the BP and PeP (**Fig. 4-A**). Sweetness was

rated as more intense in the BP and PeP than in the PoP across the 0.0 to 0.41 g/kg concentration

range of spiked phenols. At the highest concentration levels, sweetness was perceived at the highest

intensity in the BP (**Fig. 4-B**).

4. Discussion

Physical-chemical characterization was carried out to evaluate the attitude of UG extract towards

rehydration and stability during storage, in terms of phenolic content and antioxidant activity. The

solubility value of the UG extract was similar to those (86% - 88%) obtained by Kuck & Noreña

(2016) on grape skin extracts lyophilized with arabic gum and partially hydrolysed guar gum as

supports.

The moisture content and water activity value of the UG extract were in agreement with the results

obtained on grape skin extracts by Kuck & Noreña (2016). The UG extract showed similar

hygroscopic behaviour to the absorption isotherm of an aqueous solution of salts and simple sugars.

Therefore, the powder has to be protected from humidity during storage to avoid water absorption,

thus preserving the extract's stability.

413   The total phenol content of the UG extract was similar to that obtained by Kuck & Norena (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).

17

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, Kuzmicz, 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 peroxyl and hydroxyl radicals is still far from being fully understood.

474 Valgimigli et al. (2009) described an unexpected dramatic acceleration of phenol-peroxyl 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

5. Conclusions

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

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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≤ 0.038).

692

693 **Table 1.** Phenol profile of the UG extract and phenols detected in the FM extracts. Beetroot puree
694 (BP), pea purée (PeP) and potato purée (PoP) functionalized with 1.93 g/kg of phenols from the UG
695 extract.

696

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

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

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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.

| | F | p | Concentration of phenols from UG (g/kg) | | | | |
			0.00	0.21	0.41	1.11	1.93
Bitterness							
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
Sourness							
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
Saltiness							
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
Sweetness							
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
Astringency							
Beetroot Purée	4.64	0.0017	4.31 bc	4.07 c	3.31 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≤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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715 **Declaration of interests**

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

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

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723
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728 Ginevra Bucalossi: Investigation, Visualization
729 Giovanna Fia: Writing- Original draft preparation
730 Caterina Dinnella: Conceptualization, Methodology, Writing - Review & Editing
731 Erminio Monteleone : Conceptualization, Methodology, Funding acquisition
732 Alessandra De Toffoli: Investigation, Visualization
733 Valentina Canutia: Investigation
734 Bruno Zanoni: Writing - Review & Editing
735 Maurizio Servili: Investigation
736 Ella Pagliarini: Investigation
737 Tullia Gallina Toschi: Investigation

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