

1 **Sensory and chemical profile of a phenolic extract from olive mill waste waters in plant-base**
2 **food with varied macro-composition**

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15 ***Abstract***

16 *Phenols from olive mill waste water (OMWW) represent valuable functional ingredients. The*
17 *negative impact on sensory quality limits their use in functional food formulations. Chemical*
18 *interactions phenols/biopolymers and their consequences on bioactivity in plant-base foods have*
19 *been widely investigated, but no studies to date have explored the variation of bitterness, astringency*
20 *and pungency induced by OMWW phenols as a function of the food composition.*

21 *The aim of the paper was to profile the sensory and chemical properties of phenols from OMWW in*
22 *plant-base foods varied in their macro-composition.*

23 *Four phenol concentrations were selected (0.44, 1.00, 2.25, 5.06 g/kg) to induce significant*
24 *variations of bitterness, sourness, astringency and pungency in three plant-base food:*
25 *proteins/neutral pH - bean purée (BP), starch/neutral pH - potato purée (PP), fiber/low pH - tomato*
26 *juice (TJ). The macro-composition affected the amount of the phenols recovered from functionalized*
27 *food. The highest recovery was from TJ and the lowest from BP. Two groups of 29 and 27 subjects,*
28 *trained to general Labelled Magnitude Scale and target sensations, participated in the evaluation*
29 *of psychophysical curves of OMWW phenols and of functionalized plant-base foods, respectively.*
30 *Target sensations were affected by the food macro-composition. Bitterness increased with phenol*
31 *concentration in all foods. Astringency and sourness slightly increased with concentration, reaching*
32 *the weak-moderate intensity at the highest phenol concentration in PP and TJ only. Pungency was*
33 *suppressed in BP and perceived at weak-moderate intensity in PP and TJ sample at the highest*
34 *phenol concentration.*

35 *Proteins/neutral pH plant-food (BP) resulted more appropriate to counteract the impact of added*
36 *phenol on negative sensory properties thus allowing to optimize the balance between health and*
37 *sensory properties.*

38

39 **Key-words:** functional foods, by-products, bitterness, pungency, astringency, proteins,
40 carbohydrates

41

42 **Highlights**

- 43 • Food macro-composition affects the amount of recovered phenols
- 44 • The lowest recovery was from proteins/neutral pH plant-food
- 45 • Intensities of sensations depend by phenol concentration and food macro-composition
- 46 • Proteins/neutral pH food counteracted phenol induced “warning” sensations.

47

48 **Introduction**

49 Plant phenolics are powerful antioxidants and free radical scavengers whose protective effects
50 against cardiovascular diseases and oxidative stress related pathologies have been demonstrated
51 (Shahidi & Ambigaipalan, 2015). Plant by-products represent a valuable source of these natural
52 antioxidants and the recovery of such high-value bioactive compounds may have beneficial effects
53 on the economic and environmental sustainability of agro-industry (Kowalska, Czajkowska,
54 Cichowska, & Lenart, 2017).

55

56 Phenolic compounds from olive fruit belong to the class of secoiridoids. Oleuropein, ligstroside,
57 demethylcarboxyoleuropein and nüzhenide are the most abundant glucoside forms of secoiridoids
58 in olive drupe (Servili et al., 2004). Because of the enzymatic and non-enzymatic phenomena along
59 the oil extraction process (Trapani et al., 2017), phenolic compounds in virgin olive oils are mainly
60 represented by the secoiridoid aglycon forms such as 3,4-DHPEA-EDA, *p*-HPEA-EDA, *p*-HPEA-
61 EA and 3,4-DHPEA-EA, and phenolic alcohols (3,4-DHPEA and *p*-HPEA). These phenols are
62 abundant in olive mill waste water (OMWW), the main waste of the virgin olive oil production
63 industry. The phenolic compounds from virgin olive oils and from their by-products are
64 characterized by antioxidant, antimicrobial, anti-inflammatory, chemo-preventive properties
65 (Bendini et al., 2007; Servili et al., 2014). Moreover, OMWW disposal represents a major cost in
66 olive oil production, and the recovery of bioactive phenols may greatly help the sustainability of the
67 olive oil industry.

68

69 Phenols from plant by-products (Torri et al., 2016; Świeca, Gawlik-Dziki, Sęczyk, Dziki, & Sikora,
70 2018; Nirmala, Bisht, Bajwa, & Santosh, 2018), including OMWW (Araújo, Pimentel, Alves, &
71 Oliveira, 2015; Esposito et al., 2015; Servili et al., 2011a; Servili et al., 2011b), have been proposed
72 as functional ingredients that are able to enhance food and beverage antioxidant activity and its
73 potential pro-health effects. Unfortunately, phenol compounds are mainly responsible for the
74 bitterness, astringency and pungency in phenol rich foods (Lesschaeve & Noble, 2005). For
75 instance, secoiridoid aglycons 3,4-DHPEA-EDA and *p*-HPEA-EDA induce intense bitter taste and
76 pungent sensations (Vitaglione et al., 2015). The intensity of these phenol-induced ‘warning’
77 sensations significantly affects preference and choice of phenol rich vegetable foods (Dinnella,
78 Recchia, Tuorila, & Monteleone, 2011).

79

80 Developing a phenol-enriched functional food can be a challenging task since consumers are not
81 willing to compromise on sensory quality when it comes to functional foods (Verbeke, 2006;
82 Krystallis, Maglaras, & Mamalis, 2008; Jaeger, Axten, Wohlers, & Sun-Waterhouse, 2009). Hence,
83 strategies to control for the intensity of warning sensations need to be considered when developing
84 phenol enriched functional foods. Three main strategies can be envisaged to reduce the intensity of
85 the unacceptable sensory properties of phenols (Ares, Barreiro, Deliza, & Gámbaro, 2009; Gaudette
86 & Pickering, 2012; Keast, 2008).

87

88 The first of these is to take advantage of common perceptual interaction in which the suppression of
89 the target sensations occurs through the addition of a counteracting tastant. Sweeteners, fats and salt
90 can lead to perceptual interactions that reduce the impact of phenols on sensory properties of
91 functional food, but these sensory stimuli may also negatively impact on functional food pro-health
92 properties due to the energy and salt intake. Furthermore, the perceived level of healthiness in food
93 is frequently linked to naturalness which may also imply the absence of unnecessary ingredients
94 (Román, Sánchez-Siles, & Siegrist, 2017). Functional foods perceived as natural are more likely to
95 be consumed (Carrillo, Prado-Gascó, Fiszman, & Varela, 2013). Thus, the appropriate strategy to
96 mitigate the impact of phenols on sensory properties of functional food should be to lower the
97 intensity of phenol-induced sensations and limit the use of ingredients that can compromise the pro-
98 health expectations for this food product category.

99

100 Secondly, tasteless ingredients that compete for phenol receptor binding, such as cyclodextrin
101 derivatives, can be employed (Gaudette & Pickering, 2012).

102

103 Finally, the chemical interactions between phenols and biopolymers naturally occurring in vegetable
104 foods (Zhang et al., 2014) can be seen as an appropriate strategy to lower functional phenol bitter
105 and astringent potential. Plant biopolymers can act as a physical barrier for phenol stimuli utilized,
106 thus hindering their interactions with sensory receptors and saliva. Many factors affect
107 phenol/biopolymer binding including pH and reagent features such as chemical compositions,
108 structure, hydrophobic/hydrophilic character (Kroll, Rawel & Rohon, 2003). Several studies have
109 investigated the chemical features of phenol/biopolymer interactions and their consequences on
110 bioactivity (Jakobek, 2015; Ozdal, Capanoglu, & Altay, 2013) but no studies to date have explored
111 the systematic variation of target sensations induced by functional phenols in plant-base food.

112

113 The aim of the paper was to profile the sensory and chemical properties of phenols extracted from
114 OMWW in plant-base foods varied in their macro-composition in which different
115 phenol/biopolymer interactions might occur. Selected plant-base foods were proteins/neutral pH -
116 bean purée (BP), starch/neutral pH - potato purée (PP), fibers/low pH - tomato juice (TJ).

117

118 **Material & Methods**

119

120 **1. OMWW phenol extract preparation**

121 The phenolic fraction was extracted from OMWW of Peranzana, Ogliarola, Coratina and Moraiolo
122 cultivars harvested at ripening in region from Central Italy. The extraction and purification of
123 phenolic fraction from OMWW was carried out as described by Esposito et al., 2015
124 stages of from OMWW of . Three steps of tangential membrane filtration were applied to obtain a
125 crude phenolic concentrate from OMWW previously treated with an enzymatic solution of pectinase
126 from *Aspergillus niger*, BIODÉP (Biotec s.r.l., Roma, Italy) (Servili et al., 2011a).

127

128 Phenolic compounds from crude concentrate were recovered by liquid-liquid extraction with ethyl
129 acetate. A rotavapor was used to completely evaporate the ethyl acetate at 35 °C. The phenolic
130 extract obtained was dissolved in ethanol, which was then evaporated using a flow of nitrogen
131 (Servili, et al., 2011b).

132

133 **2. Chemical Analysis**

134 2.1 Phenol profile

135 The analysis of phenolic composition of the extract was performed by HPLC, after sample
136 solubilization with methanol/water (50:50 v/v) and filtration over a 0.2 µm PVDF filter.

137 Extraction of phenols from OMWW from plant-base foods was carried out mixing 2 g of sample
138 and 10 ml of ethanol/acetone (50:50 v/v) with T25 digital Ultra-Turrax (IKA® Works, Wilmington,
139 NC 28405 USA) at 17000 rpm. The sample was centrifuged, made up to volume, filtered over a 0.2
140 µm PVDF filter and directly injected into HPLC system.

141 The HPLC analysis was conducted using an Agilent Technologies Model 1100 following the
142 operating conditions described by Veneziani et al. (2015). DAD with a wavelength of 278 nm was
143 used to detect secoiridoid derivatives and phenolic alcohols. The *p*-HPEA and vanillic acid were
144 purchased from Sigma Aldrich (Milan, Italy), whereas 3,4-DHPEA and verbascoside were provided
145 by Cabru s.a.s. (Arcore, Milan, Italy) and Extrasynthese (Genay, France), respectively. The 3,4-
146 DHPEA-EDA and *p*-HPEA-EDA were extracted from virgin olive oil (VOO) as previously reported
147 by Selvaggini et al. (2014). The data were expressed as mg of phenols kg⁻¹ of extract or foods.

148 2.2 Antioxidant activity

149 Free radical scavenging activity was evaluated by the DPPH assay (Brand-Williams, Cuvelier, &
150 Berset, 1995). A solution of DPPH (6*10⁻⁵ M) was prepared by dissolving 0.236 mg of DPPH in
151 100 mL of methanol. A volume of 0.1 mL of sample was mixed with 3.9 mL of DPPH solution. For
152 the reference sample, 0.1 mL of methanol was added to 3.9 mL of DPPH solution to measure the
153 maximum DPPH absorbance. All samples were left in the dark for 30 min at 30°C then the
154 absorbance decrease was measured at 515 nm with a Perkin Elmer Lambda 10 spectrophotometer
155 (Massachusetts, USA). Free radical scavenging activity was expressed as µmol of Trolox
156 equivalents antioxidant capacity (TEAC). Trolox standard solutions were prepared in ethanol at
157 concentrations ranging from 10 to 600 µmol/L. Each assay was performed in triplicate.

158

159 3. Sensory evaluations

160 3.1 Subjects

161 Participants were recruited on a regional basis by means of announcements published on research
162 unit websites, emails, pamphlet distribution and word of mouth. At the time of recruitment,
163 respondents were asked to complete an online questionnaire on socio-demographic and physical
164 health characteristics. Pregnancy, food allergies and history of perceptual disorders were exclusion
165 criteria. Two respondent groups were recruited to evaluate OMWW extract (Group 1: n=29; 59 %
166 females; mean age 27.5 ± 7.1) or functionalized plant-base foods (Group 2: n=27; 70 % females;
167 mean age 31.5 ± 9.4).

168

169 3.2 Procedure

170 Subjects from group 1 took part in one session for OMWW extract evaluation, group 2 took part in
171 two sessions, held over two days, for the evaluation of three series of functionalized foods. In the
172 first session, participants signed the informed consent according to the principles of the Declaration
173 of Helsinki and were introduced to the general organization of the experiment. Subjects (Ss) were
174 then trained in the use of general Labelled Magnitude Scale (gLMS; 0: *no sensation* - 100: *the*
175 *strongest imaginable sensation of any kind*) (Bartoshuk, 2000; Green et al., 1996; Green, Shaffer,
176 & Gilmore, 1993). Participants were told that the top of the scale - *the strongest imaginable*
177 *sensation of any kind* - represented the most intense sensation that subjects could ever imagine
178 experiencing. Ss were focussed on a variety of remembered sensations from different modalities
179 including loudness, oral pain/irritation and tastes. The Ss were then trained to recognize the
180 following target sensations in water solutions prepared to be at “moderate/strong” intensity on
181 gLMS: bitterness (caffeine 3.00 g/kg), sourness (citric acid - 4.00 g/kg), saltiness (NaCl-15 g/kg),
182 astringency (aluminium potassium sulphate – 0.8 g/kg) and pungency (capsaicin – 1.5
183 mg/kg)(Monteleone et al., 2017). At the end of the training, while all Ss were seated in individual
184 booths, group 1 evaluated OMWW extracts (nine samples), and group 2 evaluated one series of food
185 prototype (five samples). On day two, the gLMS and target sensations were briefly introduced again
186 to group 2, who then they were seated in individual booths to evaluate two series of functionalized
187 foods (five samples each). The two sessions were separated by between 1 and 7 days, according to
188 availability of Ss from group 2. Ss received a gift to compensate them for their time.

189 3.3 Sensory stimuli

190 3.3.1 OMWW extract

191 The OMWW extract was diluted in EtOH 1% to obtain eight solutions at 0.29, 0.44, 0.66, 1.00, 1.50,
192 2.25, 3.37, 5.06 g/L phenol concentrations. These concentrations were chosen based on preliminary
193 informal assessment by expert laboratory personnel to induce bitterness intensity from weak to
194 strong. A further solution consisting of the solvent was considered and indicated as 0.00 g/L phenol.
195 In total, nine OMWW extract solutions were prepared for evaluation. These solutions were stored
196 at room temperature in a tightly closed container protected from light and used within 10 hours.

197

198 3.3.2 Functionalized foods

199 Three vegetable foods with different macro-composition were selected for the development of
200 phenol functionalized foods: proteins/neutral pH - bean purée (BP), carbohydrates/neutral pH -
201 potato purée (PP), water/low pH - tomato juice (TJ). Canned or powdered ingredients produced by
202 large food companies were used to prepare the functionalized food since their composition is
203 constant, and they are easily available without seasonality restrictions. The three foods had four

204 levels of phenol from OMWW extract added: 0.44, 1.00, 2.25, 5.06 g/kg. A further sample for each
205 series consisting of the vegetable food without OMWW extract added, and indicated as 0.00 g/kg,
206 was considered. In total, five levels of phenol concentration for each vegetable food were considered
207 for evaluation. Samples were evaluated immediately after preparation, within 15 min of extract
208 addition.

209

210 3.4 Evaluation conditions

211 The OMWW solutions (7 mL) and functionalized foods (6 g) were presented in 80cc plastic cups
212 identified by a 3-digit random code. Food samples (BP, TJ, PP) were presented with a plastic tea-
213 spoon. Ss from group 1 were presented with a set consisting of the nine OMWW solutions arranged
214 in three subsets of three samples each. Samples were presented in randomized order across Ss. The
215 three series of functionalized foods (BP, PP and TJ) were presented to Ss from group 2 in
216 independent sets, each consisting of five samples of the same food arranged in two subsets of three
217 and two samples each. The presentation order of the three series of foods was balanced across Ss.
218 The presentation order of samples within each series was randomized across subjects. Ss had a 3
219 min break between subsets a 10 min break between the sets.

220

221 During tasting, Ss were instructed to hold the whole OMWW sample in their mouth for 10 s, then
222 expectorate and evaluate the intensity of target sensations (bitterness, sourness, saltiness,
223 astringency and pungency). For the food samples, subjects were instructed to take a spoonful of the
224 sample, wait for 10 s, then swallow and evaluate the intensity of bitterness, sourness, astringency
225 and pungency. The order of sensation evaluation was randomized for the tastes (bitterness, sourness
226 and saltiness), while astringency and pungency were evaluated in penultimate and last position to
227 allow for the full development of their intensity.

228

229 After each sample, Ss rinsed their mouth with water for 30 s, had some plain crackers for 30 s and
230 finally rinsed their mouth with water for a further 30 s. To control for odor cues, Ss were asked to
231 wear nose clips. Evaluations were performed in individual booths under red lights. Data were
232 collected with the software *Fizz* (ver.2.51. A86, Biosystèmes, Couternon, France).

233

234 **5. Data Analysis**

235 Two-ways ANOVA models were used to assess the effect of phenol concentration and food macro-
236 composition on the amount of phenols extracted from functionalized samples and on their total
237 recovery. Two-way ANOVA mixed models (fixed factor: phenol concentration; random factor:

238 subjects) were used to assess the effect of phenol concentration on the intensity of target sensations
239 in OMWW solutions and food prototype samples. Three-way mixed models (fixed factors: food
240 matrix and phenol concentration; random factor: subjects) with interactions were used to assess the
241 effect of food matrix on the intensity of target sensations. A Fisher LSD post hoc test was applied
242 to test significant differences in multiple comparison test (significant for $P \leq 0.05$)
243 The XLSTAT statistical software package version 19.02 (Addinsoft) was used for data analysis.

245 **Results**

247 **1. Chemical characterization**

248 **1.1 OMWW extract: phenol profile and antioxidant activity**

249 Phenols represented approximately 70 % of the OMWW extract. The phenolic composition of the
250 OMWW extract was characterized by the main phenolic compounds of olive fruit and virgin olive
251 oil. The most abundant phenolic compounds were secoiridoid derivatives: 3,4-DHPEA-EDA, the
252 dialdehydic forms of elenolic acid linked to hydroxytyrosol, (605.4 ± 0.5 mg/g of extract),
253 hydroxytyrosol - 3,4-DHPEA, (43.8 ± 0.2 mg/g of extract) and tyrosol - *p*- HPEA (7.6 ± 0.6 mg/g of
254 extract). The OMWW is rich of verbascoside, a phenylethanoid glycoside, which was also present
255 in the purified extract (23.8 ± 1.2 mg/g of extract)(Veneziani, Novelli, Esposito, Taticchi, & Servili,
256 2017). Antioxidant activity of the extract was 3.060 ± 0.071 TEAC eq/mg phenols.

258 **1.2 Functionalized foods: OMWW phenol recovery and profile**

259 The amount of OMWW phenols in food samples functionalized with increasing concentrations was
260 determined after extraction and expressed as percentage of recovery (Fig.1). The phenol recovery
261 increased with the added amount ($p \leq 0.001$) and ranged from 3.7 to 13.9 % in bean purée, from 12.6
262 to 19.9 % in tomato juice and from 5.4 to 17.3 % in potato purée. The recovery was significantly
263 influenced by food macro-composition ($p \leq 0.001$). The lowest recovery of OMWW phenols was
264 from functionalized bean purée samples irrespective to the amount initially added. The highest
265 recovery was from tomato juice added with 0.44, 2.25 and 5.06 g/kg of phenols. Potato purée showed
266 the highest recovery when 1.00 g/kg of phenols was used.

267
268 The amount of individual OMWW phenols from functionalized food regularly increased with the
269 total amount initially added ($p \leq 0.0001$) and was affected by food macro-composition ($p \leq 0.001$) in
270 a different extent depending on the specific phenol and the added amount (Tab.1). In general, the
271 lowest amount of each phenol was recovered from bean purée and the largest differences were found

272 among food functionalized with the highest amount of phenols (≥ 2.25 g/kg). Phenol profiles
273 recovered from BP, TJ and PP functionalized with 5.06 g/kg were compared to the profile of
274 OMWW extract (Fig. 2). The relative content of 3,4-DHPEA-EDA, 3,4-DHPEA, *p*-HPEA and
275 verbascoside largely differ between OMWW extract and functionalized food. 3,4-DHPEA-EDA
276 represented the most abundant phenol of OMWW extract (89 %) but its proportion lowered to
277 approx. 27, 35 and 36 % of total OMWW phenols recovered from BP, PP and TJ, respectively. 3,4-
278 DHPEA and verbascoside represented 6.4 and 3.5 %, of the total phenol content of OMWW extract
279 respectively, and approximately 40 and 22 %, of the total phenols recovered from functionalized
280 foods. *p*-HPEA was 1 and approximately 4 % of total phenols in OMWW extract and functionalized
281 foods, respectively.

282

283 **2. Sensory evaluation**

284 **2.1 OMWW extract solutions**

285 Phenol concentration of OMWW solutions significantly affected the intensity of target sensations
286 (Tab.2). According to F values the increase of phenol concentration had the strongest effect on
287 bitterness and, to a lesser extent, on other target sensations. Significant bitterness and astringency
288 increases were observed in the samples with phenols from OMWW as compared to the sample
289 without phenol added (0.00 g/L). Bitterness increased from weak/moderate to strong/very strong
290 across the phenol concentration range. Sourness showed the same trend of increasing intensity, but
291 only in a narrow range from weak to moderate. Astringency showed a limited intensity increases
292 from moderate to moderate strong on the scale. Pungency did not differ across samples from 0.00
293 and 0.66 g/L of phenols, while higher concentrations induced significant pungency increasing from
294 weak to moderate/strong. Saltiness represents a marginal sensation, its intensity reaching a
295 weak/moderate intensity at the highest phenol concentration, and thus was not considered further.

296

297 Four concentration levels, which cover the whole range of significant variations of intensity of target
298 sensations, were selected to fortify the vegetable matrices: 0.44, 1.00, 2.25 and 5.06 g/L.

299

300 **2.2 Functionalized foods**

301 The impact of OMWW extract on the sensory profile of the three vegetable matrices was
302 independently assessed in each series of prototype as a function of the concentration of added
303 phenols. The intensity of target sensations significantly changed in all the three vegetable prototypes
304 as a function of increasing phenol concentrations, the only exceptions being pungency in bean purée
305 (Tab.3). F values indicated that the increase of phenol concentration induced the strongest effect on

306 bitterness in all the three prototypes. The intensity of sourness, astringency and pungency were
307 influenced by both the increase of phenol concentration and, to a lesser extent, by the matrix macro-
308 composition. All the sensations were barely detectable in bean purée sample without phenol added,
309 while in the rest of samples, bitterness increased from weak to strong/very strong, and sourness and
310 astringency increased slightly from barely detectable to weak/moderate. All sensations were rated
311 as weak in the tomato juice sample without phenol added; in the rest of samples, bitterness increased
312 from weak to strong, and sourness, pungency and astringency increased from weak to
313 weak/moderate as a function of the concentration of added phenols. In the potato purée sample
314 without added phenols, all sensations were rated at barely detectable/weak intensity. Bitterness
315 increased from barely detectable to strong with increasing with phenol concentration, and
316 astringency, pungency and sourness increased slightly, reaching weak/moderate intensity level.

317

318 In general, these intensity data indicate a significant impact of the addition of OMWW extracts on
319 the sensory properties of the three prototypes as a function of the added phenol concentration, and
320 in particular on the perception of bitterness. Sourness, pungency and astringency intensities were
321 significantly modified by OMWW extract, but the extent of these effects appears to be affected by
322 the matrix macro-composition.

323

324 The effect of vegetable matrix composition on the intensity of sensations contributed by OMWW
325 phenols was further explored and the intensities of target sensations in the three matrices at different
326 added phenol concentration were compared (Tab.4). The vegetable matrix significantly affected the
327 intensity of sourness. The concentration of added phenol significantly affected the intensity of
328 target sensations, with the greatest effect on bitterness. The vegetable matrix*concentration
329 interaction was significant only for pungency, due to the suppression of this sensation in bean purée
330 samples. No significant differences were found comparing bitterness from the three matrices at 0.00,
331 0.44, 1.00 and 5.06 g/L phenol concentrations, but at 2.25 g/L, bitterness was significantly higher in
332 tomato juice than in bean purée (Fig.3-A). Sourness was rated as more intense in tomato juice than
333 in either bean purée and potato purée in a concentration range from 0.00 to 2.25 g/L, at 5.06 g/L the
334 lowest intensity was perceived in bean purée and no significant differences were found between
335 tomato juice and potato purée (Fig.3-B). The three vegetable matrices did not differ for the intensity
336 of astringency at 0.44 and 1.00 g/L of added phenol, however in the rest of samples, this sensation
337 was lower in bean purée than in potato purée and no significant differences were found comparing
338 tomato juice and potato purée (Fig.3-C). Pungency was significantly higher in tomato juice (from

339 1.00 to 5.06 g/kg) and in potato puree (5.06 g/kg) than in bean purée, but no significant differences
340 were found between tomato juice and potato purée (Fig.3-D).

341

342 In general, these data indicate that the different composition of vegetable matrices does not affect
343 the contribution to bitterness of phenols from OMWW extract since the same regular trend and the
344 same range of increasing intensity with added phenols was observed in the all three series of
345 prototypes. On the other hand, the increasing intensity range observed for sourness, astringency and
346 pungency differed across the series of prototypes indicating an active role of their macro-component
347 in modulating the sensory impact of phenols from OMWW.

348

349 **Discussion**

350 The amount of OMWW phenols recovered from the functionalized food prototypes was much lower
351 than expected, thus indicating the existence of strong chemical interactions between functional
352 phenols and food components,-the lowest amount was recovered from bean purée, the protein rich
353 food matrix. These findings are in line with the previously documented interactions between phenols
354 and food biopolymers. Proteins strongly interact with plant polyphenols through covalent and non-
355 covalent binding, and high basic-residue content and open and flexible structure are the major
356 features of proteins highly reactive towards phenols (Kroll, et al., 2003; Xiao & Kai, 2012; Zhang
357 et al., 2014). Binding involves hydrophobic and hydrogen interactions, and proline-rich regions of
358 leguminous proteins have been reported as preferred sites of interactions for plant phenol/food
359 protein in *in vitro* conditions (Rawel, Czajka, Rohn, & Kroll, 2002). The formation of aggregates
360 with proteins significantly impacts on the bioactivity of phenols and the reduction of both
361 extractability from raw material and antioxidant activity has been reported (Kroll et al., 2014). The
362 overall bioavailability of phenols from protein aggregates is still a matter of debate, and several
363 sources of evidence indicate a lowering of the blood content of phenols after intake of food protein
364 sources (Ozdal et al., 2013). However, the longer duration of the aggregates in the stomach followed
365 by a delayed phenol release has been observed (Ozdal et al., 2013). Furthermore, after *in vitro*
366 digestion of protein/phenol aggregates, the recovery of phenol related antioxidant activity was
367 reported (Drummond e Silva et al., 2017; Kroll et al., 2003). Thus, it is possible to hypothesize that
368 the interactions between food proteins and phenols do not lower the functional potential of the
369 phenols, but rather influence their kinetic of phenol adsorption and bioactivity (Zhang et al., 2014).

370

371 Phenolic compounds bridge or cross-link with starch and other polysaccharides, and a large fraction
372 of the so called “NEPP” (not extractable polyphenols) consists in phenol associations with

373 polysaccharides (Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2013). The consequences of
374 phenol/carbohydrate interactions on phenol bioactivity depends on phenol and carbohydrate
375 chemical characteristics, and both enhancement or suppression of antioxidant activity and bio-
376 accessibility have been observed (Zhang et al., 2014). The majority of NEPP arrive almost intact to
377 the colon where they are fermented by microflora or depolymerized via enzymes, leading to phenol
378 metabolites being available for adsorption (Pérez-Jiménez et al., 2013).

379

380 Based on these considerations, the low recovery from functionalized prototypes should not be
381 interpreted as the mere loss of the bioactive compounds, and further investigations on phenol
382 bioavailability and bio-accessibility will clarify the potential pro-health effects of experimental food
383 matrices enriched with OMWW phenols.

384

385 The profile of phenol fractions extracted from functionalized foods differed substantially from the
386 profile of the OMWW extract, mainly because of the strong decrease of 3,4-DHPEA-EDA relative
387 to the other phenol compounds. Several phenol features, including their structure, the arrangement
388 of hydroxyl groups, and the planarity of molecules, actively modulate the interactions
389 phenols/environment and might be responsible for the observed differences (Jakobek, 2015; Ozdal
390 et al., 2013). Investigating the associations of the chemical features of OMWW phenols with the
391 strength and the modality of their interaction with biopolymers was behind the aim of the present
392 work but further studies should be encouraged for a deeper understanding of the mechanism
393 underlying phenol/biopolymer interactions in real food systems.

394

395 Bitterness was the most intense sensation induced by OMWW extracts, astringency and pungency
396 were perceived at lower intensities, while sourness represented a marginal sensation. The observed
397 sensory properties are consistent with the phenol profile of the extract. Secoiridoid derivatives of
398 hydroxytyrosol are considered the main contributors to olive oil bitterness (Bendini et al., 2007).
399 3,4-DHPEA-EDA represents the main extract component and has been described as mainly bitter
400 and slightly pungent (Taticchi, Esposto, & Servili, 2014). Pungency is instead mainly attributed to
401 *p*-tyrosol derivatives which, when tested at the same concentration 3,4-DHPEA-EDA, primarily
402 produced bitter tastes and low pungency, while *p*-HPEA-EDA mainly induced pungency
403 (Andrewes, Busch, De Joode, Groenewegen, & Alexandre, 2003). Bitterness represents the main
404 contribution of OMWW phenols to sensory profile of functional prototypes. The vegetable matrix
405 macro-composition did not significantly affect the perceived intensity of this sensations. Thus, the
406 strong interactions of OMWW phenols with vegetable biopolymers prevent the chemical extraction

407 of phenols, and in particular of 3,4-DHPEA-EDA, but do not suppress the bitter taste of phenol
408 compounds. In line with the documented *in vivo* release of phenols from biopolymer aggregates
409 (Ozidal et al., 2013) and *in vitro* action of saliva enzymes on phenol structures (Walle et al., 2005),
410 it might be possible to speculate about their possible release in the oral environment. The relatively
411 high temperature of oral environment, and the presence of salts and hydrolytic enzymes in saliva,
412 may favor phenol release from biopolymer aggregates, their diffusion across bitter taste receptors
413 and a consequent stimulation of these receptors. Moreover, the contribution to bitter taste of 3,4
414 DHPEA, verbascoside and p-HPEA should be reconsidered. The vegetable matrix composition
415 affected the perceived intensity of pungency and sourness. Pungency perception is suppressed in the
416 protein rich prototype, and this could be tentatively related to 3,4-DHPEA-EDA/protein binding.
417 This could lower the 3,4-DHPEA-EDA concentration so that bitterness is not affected, but the
418 capacity to induce these secondary sensations is instead inhibited.

419

420 **Conclusions**

421 Food macro-composition actively impacts on the chemical and sensory properties of phenols from
422 an OMWW extract with the strongest effects observed in protein-based foods. Interactions between
423 food proteins and phenols appear a possible strategy to produce a compromise between the health
424 potential of phenols and sensory acceptability of phenol-enriched foods since lower the intensity of
425 warning sensations, while at the same time avoiding extraneous ingredients in their formulations.
426 Specificities were found between phenol chemical structure and strength of their interactions with
427 food components. Systematic investigations in real food systems would help in clarifying the
428 mechanisms underlying the phenol-biopolymer aggregate formation, thus helping in optimizing
429 functional food formulations.

430

431 **References**

- 432 Andrewes, P., Busch, J. L. H. C., De Joode, T., Groenewegen, A., & Alexandre, H. (2003).
433 Sensory properties of virgin olive oil polyphenols: Identification of deacetoxy-ligstroside
434 aglycon as a key contributor to pungency. *Journal of Agricultural and Food Chemistry*,
435 *51*(5), 1415–1420. <https://doi.org/10.1021/jf026042j>
- 436 Araújo, M., Pimentel, F. B., Alves, R. C., & Oliveira, M. B. P. P. (2015). Phenolic compounds
437 from olive mill wastes: Health effects, analytical approach and application as food
438 antioxidants. *Trends in Food Science and Technology*.
439 <https://doi.org/10.1016/j.tifs.2015.06.010>
- 440 Ares, G., Barreiro, C., Deliza, R., & Gámbaro, A. (2009). Alternatives to reduce the bitterness,

441 astringency and characteristic flavour of antioxidant extracts. *Food Research International*,
442 42(7), 871–878. <https://doi.org/10.1016/j.foodres.2009.03.006>

443 Bartoshuk, L. M. (2000). Comparing Sensory Experiences Across Individuals: Recent
444 Psychophysical Advances Illuminate Genetic Variation in Taste Perception. *Chemical Senses*,
445 25(4), 447–460. <https://doi.org/10.1093/chemse/25.4.447>

446 Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Segura-Carretero, A.,
447 Fernández-Gutiérrez, A., & Lercker, G. (2007). Phenolic molecules in virgin olive oils: A
448 survey of their sensory properties, health effects, antioxidant activity and analytical methods.
449 An overview of the last decade. *Molecules*. <https://doi.org/10.3390/12081679>

450 Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to
451 evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30.
452 [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)

453 Carrillo, E., Prado-Gascó, V., Fiszman, S., & Varela, P. (2013). Why buying functional foods?
454 Understanding spending behaviour through structural equation modelling. *Food Research*
455 *International*, 50(1), 361–368. <https://doi.org/10.1016/j.foodres.2012.10.045>

456 Dinnella, C., Recchia, A., Tuorila, H., & Monteleone, E. (2011). Individual astringency
457 responsiveness affects the acceptance of phenol-rich foods. *Appetite*, 56(3), 633–642.
458 <https://doi.org/10.1016/j.appet.2011.02.017>

459 Drummond e Silva, F. G., Miralles, B., Hernandez-Ledesma, B., Amigo, L., Iglesias, A. H.,
460 Reyes, F. G. R., & Netto, F. M. (2017). Influence of protein-phenolic complex on the
461 antioxidant capacity of flaxseed (*Linum usitatissimum* L.) products. *Journal of Agricultural*
462 *and Food Chemistry*, 65(4), 800–809. <https://doi.org/10.1021/acs.jafc.6b04639>

463 Esposto, S., Taticchi, A., Di Maio, I., Urbani, S., Veneziani, G., Selvaggini, R., ... Servili, M.
464 (2015). Effect of an olive phenolic extract on the quality of vegetable oils during frying. *Food*
465 *Chemistry*, 176, 184–192. <https://doi.org/10.1016/j.foodchem.2014.12.036>

466 Gaudette, N. J., & Pickering, G. J. (2013). Modifying Bitterness in Functional Food Systems.
467 *Critical Reviews in Food Science and Nutrition*, 53(5), 464–481.
468 <https://doi.org/10.1080/10408398.2010.542511>

469 Green, B. G., Dalton, P., Cowart, B., Shaffer, G., Rankin, K., & Higgins, J. (1996). Evaluating the
470 “labeled magnitude scale” for measuring sensations of taste and smell. *Chemical Senses*,
471 21(3), 323–334. <https://doi.org/10.1093/chemse/21.3.323>

472 Green, B. G., Shaffer, G. S., & Gilmore, M. M. (1993). Derivation and evaluation of a semantic
473 scale of oral sensation magnitude with apparent ratio properties. *Chemical Senses*, 18(6),
474 683–702. <https://doi.org/10.1093/chemse/18.6.683>

475 Jaeger, S. R., Axten, L. G., Wohlers, M. W., & Sun-Waterhouse, D. (2009). Polyphenol-rich
476 beverages: Insights from sensory and consumer science. *Journal of the Science of Food and*
477 *Agriculture*, 89(14), 2356–2363. <https://doi.org/10.1002/jsfa.3721>

478 Jakobek, L. (2015). Interactions of polyphenols with carbohydrates , lipids and proteins. *Food*
479 *Chemistry*, 175, 556–567. <https://doi.org/10.1016/j.foodchem.2014.12.013>

480 Keast, R. S. J. (2008). Modification of the bitterness of caffeine. *Food Quality and Preference*,
481 19(5), 465–472. <https://doi.org/10.1016/j.foodqual.2008.02.002>

482 Kowalska, H., Czajkowska, K., Cichowska, J., & Lenart, A. (2017). What’s new in biopotential of
483 fruit and vegetable by-products applied in the food processing industry. *Trends in Food*
484 *Science and Technology*. <https://doi.org/10.1016/j.tifs.2017.06.016>

485 Kroll, J., Rawel, H. M., & Rohn, S. (2003). Reactions of Plant Phenolics with Food Proteins and
486 Enzymes under Special Consideration of Covalent Bonds. *Food Science and Technology*
487 *Research*, 9(3), 205–218. <https://doi.org/10.3136/fstr.9.205>

488 Krystallis, A., Maglaras, G., & Mamalis, S. (2008). Motivations and cognitive structures of
489 consumers in their purchasing of functional foods. *Food Quality and Preference*, 19(6), 525–
490 538. <https://doi.org/10.1016/j.foodqual.2007.12.005>

491 Lesschaeve, I., & Noble, A. C. (2005). Polyphenols : factors influencing their sensory properties
492 and their effects on food and beverage preferences, 81, 330–335.

493 Monteleone, E., Spinelli, S., Dinnella, C., Endrizzi, I., Laureati, M., Pagliarini, E., ... Tesini, F.
494 (2017). Exploring influences on food choice in a large population sample: The Italian Taste
495 project. *Food Quality and Preference*, 59, 123–140.
496 <https://doi.org/10.1016/j.foodqual.2017.02.013>

497 Nirmala, C., Bisht, M. S., Bajwa, H. K., & Santosh, O. (2018). Bamboo: A rich source of natural
498 antioxidants and its applications in the food and pharmaceutical industry. *Trends in Food*
499 *Science and Technology*. <https://doi.org/10.1016/j.tifs.2018.05.003>

500 Ozdal, T., Capanoglu, E., & Altay, F. (2013). A review on protein – phenolic interactions and
501 associated changes Food Research International A review on protein – phenolic interactions
502 and associated changes. *FRIN*, 51(2). <https://doi.org/10.1016/j.foodres.2013.02.009>

503 Pérez-Jiménez, J., Díaz-Rubio, M. E., & Saura-Calixto, F. (2013). Non-extractable polyphenols, a
504 major dietary antioxidant: Occurrence, metabolic fate and health effects. *Nutrition Research*
505 *Reviews*, 26(2), 118–129. <https://doi.org/10.1017/S0954422413000097>

506 Rawel, H. M., Czajka, D., Rohn, S., & Kroll, J. (2002). Interactions of different phenolic acids and
507 flavonoids with soy proteins. *International Journal of Biological Macromolecules*, 30(3–4),
508 137–150. [https://doi.org/10.1016/S0141-8130\(02\)00016-8](https://doi.org/10.1016/S0141-8130(02)00016-8)

509 Román, S., Sánchez-Siles, L. M., & Siegrist, M. (2017). The importance of food naturalness for
510 consumers: Results of a systematic review. *Trends in Food Science and Technology*.
511 <https://doi.org/10.1016/j.tifs.2017.06.010>

512 Selvaggini, R., Esposto, S., Taticchi, A., Urbani, S., Veneziani, G., Di Maio, I., ... Servili, M.
513 (2014). Optimization of the temperature and oxygen concentration conditions in the
514 malaxation during the oil mechanical extraction process of four italian olive cultivars.
515 *Journal of Agricultural and Food Chemistry*, 62(17), 3813–3822.
516 <https://doi.org/10.1021/jf405753c>

517 Servili, M., Esposto, S., Veneziani, G., Urbani, S., Taticchi, A., Di Maio, I., ... Montedoro, G.
518 (2011)a. Improvement of bioactive phenol content in virgin olive oil with an olive-vegetation
519 water concentrate produced by membrane treatment. *Food Chemistry*, 124(4), 1308–1315.
520 <https://doi.org/10.1016/j.foodchem.2010.07.042>

521 Servili, M., Rizzello, C. G., Taticchi, A., Esposto, S., Urbani, S., Mazzacane, F., ... Di Cagno, R.
522 (2011)b. Functional milk beverage fortified with phenolic compounds extracted from olive
523 vegetation water, and fermented with functional lactic acid bacteria. *International Journal of*
524 *Food Microbiology*, 147(1), 45–52. <https://doi.org/10.1016/j.ijfoodmicro.2011.03.006>

525 Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G. F., & Morozzi, G. (2004).
526 Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and
527 technological aspects of production that affect their occurrence in the oil. *Journal of*
528 *Chromatography A*. <https://doi.org/10.1016/j.chroma.2004.08.070>

529 Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Maio, I. Di, ... Economico-
530 estimative, S. (2014). Biological Activities of Phenolic Compounds of Extra Virgin Olive
531 Oil, 1–23. <https://doi.org/10.3390/antiox3010001>

532 Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and
533 spices: Antioxidant activity and health effects - A review. *Journal of Functional Foods*.
534 <https://doi.org/10.1016/j.jff.2015.06.018>

535 Świeca, M., Gawlik-Dziki, U., Sęczyk, Ł., Dziki, D., & Sikora, M. (2018). Interactions of green
536 coffee bean phenolics with wheat bread matrix in a model of simulated in vitro digestion.
537 *Food Chemistry*, 258. <https://doi.org/10.1016/j.foodchem.2018.03.081>

538 Taticchi, A., Esposto, S., & Servili, M. (2014). *The Basis of the Sensory Properties of Virgin Olive*
539 *Oil. Olive Oil Sensory Science*. <https://doi.org/10.1002/9781118332511.ch2>

540 Torri, L., Piochi, M., Marchiani, R., Zeppa, G., Dinnella, C., & Monteleone, E. (2016). A sensory-
541 and consumer-based approach to optimize cheese enrichment with grape skin powders.
542 *Journal of Dairy Science*, 99(1), 194–204. <https://doi.org/10.3168/jds.2015-9922>

- 543 Trapani, S., Migliorini, M., Cecchi, L., Giovenzana, V., Beghi, R., Canuti, V., ... Zanoni, B.
544 (2017). Feasibility of filter-based NIR spectroscopy for the routine measurement of olive oil
545 fruit ripening indices, *I600239*, 1–4. <https://doi.org/10.1002/ejlt.201600239>
- 546 Veneziani, G., Esposito, S., Taticchi, A., Selvaggini, R., Urbani, S., Di Maio, I., ... Servili, M.
547 (2015). Flash Thermal Conditioning of Olive Pastes during the Oil Mechanical Extraction
548 Process: Cultivar Impact on the Phenolic and Volatile Composition of Virgin Olive Oil.
549 *Journal of Agricultural and Food Chemistry*, *63*(26), 6066–6074.
550 <https://doi.org/10.1021/acs.jafc.5b01666>
- 551 Veneziani, G., Novelli, E., Esposito, S., Taticchi, A., & Servili, M. (2017). *Applications of*
552 *recovered bioactive compounds in food products. Olive Mill Waste: Recent Advances for*
553 *Sustainable Management*. <https://doi.org/10.1016/B978-0-12-805314-0.00011-X>
- 554 Verbeke, W. (2006). Functional foods: Consumer willingness to compromise on taste for health?
555 *Food Quality and Preference*, *17*(1–2), 126–131.
556 <https://doi.org/10.1016/j.foodqual.2005.03.003>
- 557 Vitaglione, P., Savarese, M., Paduano, A., Scalfi, L., Fogliano, V., & Sacchi, R. (2015). Healthy
558 Virgin Olive Oil: A Matter of Bitterness. *Critical Reviews in Food Science and Nutrition*,
559 *55*(13), 1808–1818. <https://doi.org/10.1080/10408398.2012.708685>
- 560 Walle, T., Browning, A. M., Steed, L. L., Reed, S. G., & Walle, U. K. (2005). Flavonoid
561 glucosides are hydrolyzed and thus activated in the oral cavity in humans. *The Journal of*
562 *nutrition*, *135*(1), 48-52.
- 563 Xiao, J., & Kai, G. (2012). A review of dietary polyphenol-plasma protein interactions:
564 Characterization, influence on the bioactivity, and structure-affinity relationship. *Critical*
565 *Reviews in Food Science and Nutrition*. <https://doi.org/10.1080/10408398.2010.499017>
- 566 Zhang, H., Yu, D., Sun, J., Liu, X., Jiang, L., Guo, H., & Ren, F. (2014). Interaction of plant
567 phenols with food macronutrients: Characterisation and nutritional-physiological
568 consequences. *Nutrition Research Reviews*. <https://doi.org/10.1017/S095442241300019X>

569

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573 Nazionale – PRIN 2015: “ Individual differences in the acceptability of healthy foods: focus on
574 phenol and fat content”.

575 **Figure Legend**

576 **Figure 1:** Percentage of OMWW phenols recovered (Recovery %) form bean purée (BP), tomato
577 juice (TJ) and potato purée (PP) functionalized with increasing amount of phenols from OMWW
578 extract.

579 Bars represent standard deviation, different letters indicate significantly different values ($p \leq 0.001$)

580

581 **Figure 2:** Percentage of individual phenols detected in the OMWW extract (OMWW ext) and in
582 bean purée (BP), tomato juice (TJ) and potato purée (PP) functionalized with 5.06 g/kg phenols
583 from OMWW extract.

584

585 **Figure 3:** Effect of the vegetable matrix on the perceived intensity of target sensations (A-bitterness;
586 B-sourness; C-astringency; D-pungency) in foods functionalized with different concentrations of
587 phenols from OMWW extract. Different letters represent significant different values ($p \leq 0.001$).

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610 **Table 1:** Recovery (mean values g/kg) of individual phenols from foods (BP-bean purée, TJ-tomato
 611 juice, PP-potato purée) functionalized with increasing amount of phenols from OMWW extract.
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		Concentration of phenols from OMWW				
		0	0.44	1.00	2.25	5.06
3.4- DHPEA						
BP	0 h	5.34 gh	45.24 f	112.36 e	283.09 c	
TJ	0 h	7.89 g	48.74 f	127.78 d	378.86 b	
PP	0 h	6.57 gh	51.29 f	122.96 d	333.80 a	
p-HPEA						
BP	0 f	0 f	10.85 e	15.52 d	31.07 b	
TJ	0 f	0 f	15.11 d	23.42 c	38.44 a	
PP	0 f	9.02 e	17.59 d	27.77 b	37.04 a	
Verbascoside						
BP	0 i	10.75 gh	36.15 f	74.62 de	171.09 c	
TJ	0 i	13.75 gh	18.07 g	80.43 d	222.28 a	
PP	0 i	7.96 h	31.35 f	68.58 e	194.24 ab	
3.4-DHPEA-EDA						
BP	0 i	0 i	0 i	93.73 f	203.63 c	
TJ	0 i	34.03 h	67.09 g	140.21 d	368.72 a	
PP	0 i	0 i	66.53 g	106.18 e	310.05 b	

613 Different letters indicate significantly different values ($p \leq 0.0001$)

614 **Table 2:** 2-Way ANOVA mixed model (random effect assessors): Phenol concentration effect on
 615 intensity of target sensations in OMWW extract solutions. Mean, F and p values.

	Concentration (g/L)										
	F	p	0.00	0.29	0.44	0.66	1.00	1.50	2.25	3.37	5.06
Bitterness	106.62	p<0.0001	1.69 f	9.95 e	13.23 de	17.18 d	23.18 c	26.91 c	34.28 b	38.28 ab	40.75 a
Sourness	17.30	p<0.0001	1.65 e	4.47 de	5.37 de	7.17 cd	8.13 bcd	8.75 bcd	10.10 bc	11.98 ab	16.21 a
Saltiness	13.83	p<0.0001	1.83 d	2.56 cd	2.72 cd	4.35 bcd	5.55 bc	5.59 bc	5.78 bc	7.17 b	11.07 a
Astringency	17.69	p<0.0001	1.65 c	14.53 b	14.44 b	17.12 ab	18.26 ab	21.62 a	22.31 a	22.78 a	21.75 a
Pungency	47.79	p<0.0001	1.62 e	1.88 e	2.83 e	4.17 de	8.52 cd	9.34 bc	14.21 b	19.51 a	23.73 a

616 Different letters indicate significantly different values ($p \leq 0.0001$)

617 **Table.3** 2-Way ANOVAs mixed model (random effect: assessors): Phenol concentration effect on
 618 intensity of target sensations in food models. Mean, F and p values.
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	F	p	Concentration of phenols from OMWW (g/kg)				
			0.00	0.44	1.00	2.25	5.06
Bitterness							
Bean Purée	68.09	< 0.0001	2.89 d	3.81 d	12.19 c	21.23 b	33.27 a
Tomato Juice	45.39	< 0.0001	4.22 d	6.00 d	15.15 c	27.00 b	32.67 a
Potato Purée	57.68	< 0.0001	3.15 d	4.08 d	14.92 c	25.69 b	35.15 a
Sourness							
Bean Purée	7.63	< 0.0001	2.70 b	2.50 b	3.35 b	5.08 b	10.00 a
Tomato Juice	4.72	0.002	8.41 c	11.41 bc	10.89 bc	16.70 a	14.74 ab
Potato Purée	12.75	< 0.0001	2.73 c	2.85 c	5.04 bc	8.46 b	14.96 a
Astringency							
Bean Purée	5.14	0.001	2.85 c	5.73 bc	5.42 bc	7.73 ab	9.92 a
Tomato Juice	5.04	0.001	4.89 c	5.11 c	7.07 bc	8.96 ab	11.04 a
Potato Purée	4.62	0.002	6.81 c	8.11 bc	8.35 bc	11.11 ab	14.81 a
Pungency							
Bean Purée	0.26	0.905	1.15 a	1.50 a	1.11 a	1.50 a	1.50 a
Tomato Juice	9.98	< 0.0001	2.41 c	3.11 c	4.89 bc	6.78 b	12.67 a
Potato Purée	12.53	< 0.0001	1.08 b	0.96 b	2.19 b	4.31 b	11.54 a

620 Different letters indicate significantly different values ($p \leq 0.001$)

621 **Table 4:** 3-Way ANOVA mixed model (random effect assessors): Vegetable matrix. phenol
 622 concentration and their interactions effects on intensity of target sensations in food models. F and
 623 p values.
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	Bitterness	Sourness	Astringency	Pungency
Vegetable matrix				
F	2.81	36.02	6.64	23.33
P	0.06	< 0.0001	0.001	< 0.0001
Concentration				
F	147.52	17.61	10.79	20.30
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Vegetable matrix*Concentration				
F	0.56	1.83	0.22	4.85
p	0.81	0.07	0.99	< 0.0001

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Fig.1

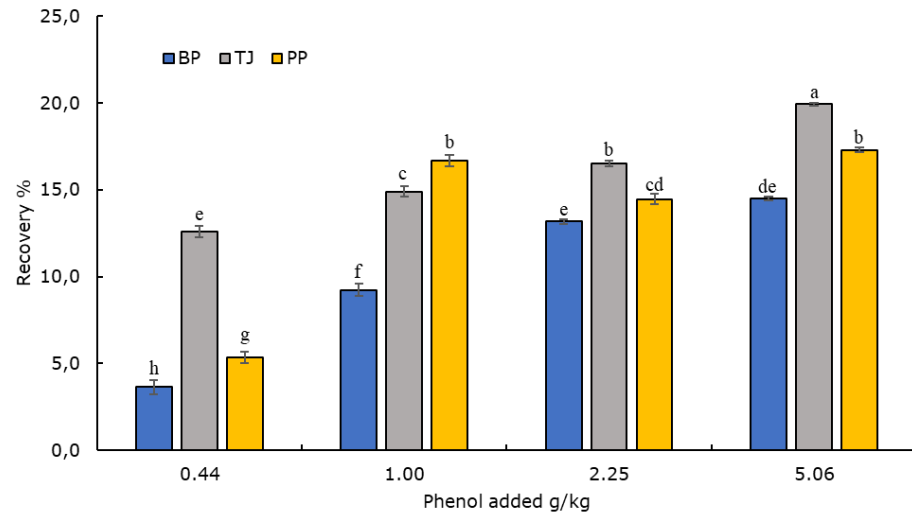


Figure 1: Percentage of OMWW phenols recovered (recovery%) from bean purée (BP), tomato juice (TJ) and potato purée (PP) functionalized with increasing amount of phenols from OMWW extract. Bars represent standard deviation, different letters indicate significantly different values ($p < 0.001$)

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Fig.2

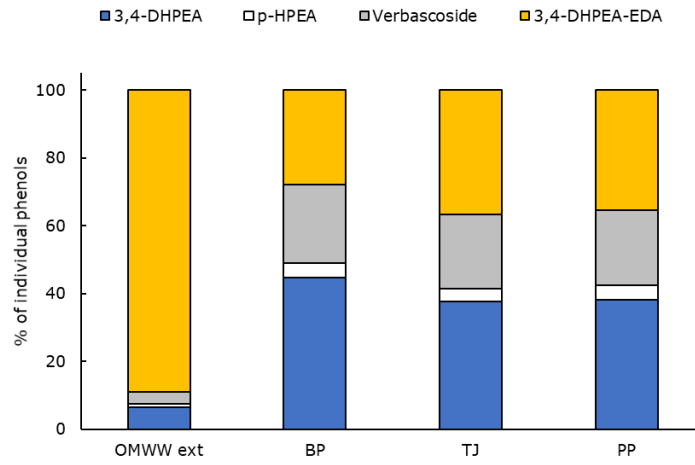


Figure 2: Percentage of individual phenols detected in the OMWW extract (OMWW ext) and in bean purée (BP), tomato juice (TJ) and potato purée (PP) functionalized with 5.06 g/kg phenols from OMWW extract.

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Fig.3

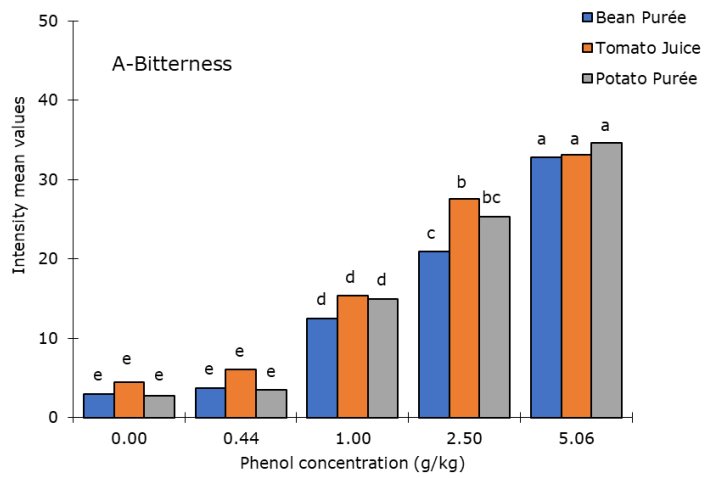


Figure 3A: Effect of the vegetable matrix on the perceived intensity of bitterness in prototypes functionalized with different concentrations of phenols from OMWW extract. Different letters represent significant different values ($p \leq 0.001$).

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Fig.3

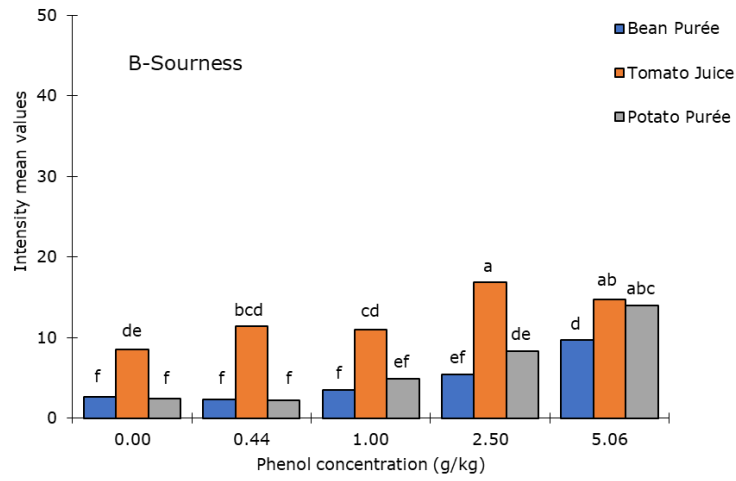


Figure 3B: Effect of the vegetable matrix on the perceived intensity of sourness in prototypes functionalized with different concentrations of phenols from OMWW extract. Different letters represent significant different values ($p \leq 0.001$).

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Fig.3

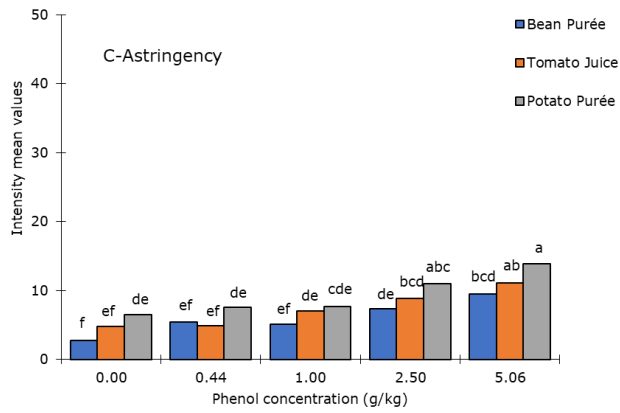


Figure 3C: Effect of the vegetable matrix on the perceived intensity of astringency in prototypes functionalized with different concentrations of phenols from OMWW extract. Different letters represent significant different values ($p \leq 0.001$).

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Fig.3

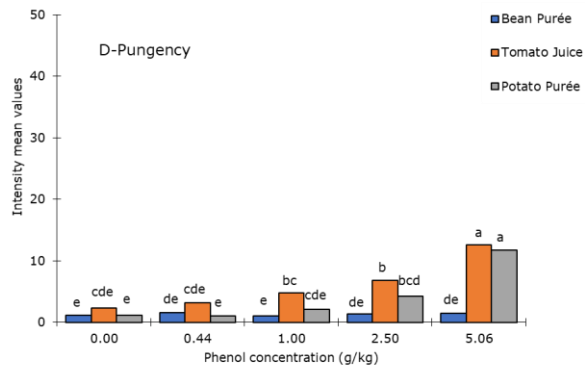


Figure 3D: Effect of the vegetable matrix on the perceived intensity of pungency in prototypes functionalized with different concentrations of phenols from OMWW extract. Different letters represent significant different values ($p \leq 0.001$).