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

(Article begins on next page)

28 April 2024

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

Corresponding Author: Professor Erminio Monteleone,

Corresponding Author's Institution: Università degli Studi di Firenze (Florence)

First Author: Alessandra De Toffoli

Order of Authors: Alessandra De Toffoli; Erminio Monteleone; GInevra Bucalossi; Gianluca Veneziani; Giovanna Fia; Maurizio Servili; Bruno Zanoni; Ella Pagliarini; Tullia Gallina Toschi; Caterina Dinnella

Abstract: Phenols from olive mill waste water (OMWW) represent valuable functional ingredients. The negative impact on sensory quality limits their use in functional food formulations. Chemical interactions phenols/biopolymers and their consequences on bioactivity in plant-base foods have been widely investigated, but no studies to date have explored the variation of bitterness, astringency and pungency induced by OMWW phenols as a function of the food composition.
The aim of the paper was to profile the sensory and chemical properties of phenols from OMWW in plant-base foods varied in their macro-composition.
Four phenol concentrations were selected (0.44, 1.00, 2.25, 5.06 g/kg) to induce significant variations of bitterness, sourness, astringency and pungency in three plant-base food: proteins/neutral pH - bean purée (BP), starch/neutral pH - potato purée (PP), fiber/low pH - tomato juice (TJ). The macro-composition affected the amount of the phenols recovered from functionalized food. The highest recovery was from TJ and the lowest from BP. Two groups of 29 and 27 subjects, trained to general Labelled Magnitude Scale and target sensations, participated in the evaluation of psychophysical curves of OMWW phenols and of functionalized plant-base foods, respectively. Target sensations were affected by the food macro-composition. Bitterness increased with phenol concentration in all foods. Astringency and sourness slightly increased with concentration, reaching the weak-moderate intensity at the highest phenol concentration in PP and TJ only. Pungency was suppressed in BP and perceived at weak-moderate intensity in PP and TJ sample at the highest phenol concentration. Proteins/neutral pH plant-food (BP) resulted more appropriate to counteract the impact of added phenol on negative sensory properties thus allowing to optimize the balance between health and sensory properties.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

1 **Sensory and chemical profile of a phenolic extract from olive mill waste waters in plant-base**

2 **food with varied macro-composition**

3

4 [1]De Toffoli A., [1]Monteleone E.*, [1]Bucalossi G., [2]Veneziani G., [1]Fia G., [2]Servili M., [1]Zanoni B.,

5 [3]Pagliarini E., [4]Gallina Toschi T., [1]Dinnella C.

6

7 [1]Dept.GESAAF-University of Florence, Italy

8 [2]Dept. Agricultural, Food and Environmental Sciences -University of Perugia, Italy

9 [3]Dept. DeFENS-University of Milan, Italy

10 [4]Dep. DiSTAL, Alma Mater Studiorum - University of Bologna, Italy

11

12

13 ***Corresponding author:** erminio.monteleone@unifi.it

14

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,*

20 *astringency 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*

22 *in 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 -*

26 *tomato juice (TJ). The macro-composition affected the amount of the phenols recovered from*

27 *functionalized food. The highest recovery was from TJ and the lowest from BP. Two groups of 29*

28 *and 27 subjects, trained to general Labelled Magnitude Scale and target sensations, participated*

29 *in the evaluation of psychophysical curves of OMWW phenols and of functionalized plant-base*

30 *foods, respectively. Target sensations were affected by the food macro-composition. Bitterness*

31 *increased with phenol concentration in all foods. Astringency and sourness slightly increased with*

32 *concentration, reaching the weak-moderate intensity at the highest phenol concentration in PP*

33 *and TJ only. Pungency was suppressed in BP and perceived at weak-moderate intensity in PP and*

34 *TJ sample at the highest phenol concentration.*

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

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

Highlights

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

Introduction

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

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

69 Phenols from plant by-products (Torri et al., 2016; Świeca, Gawlik-Dziki, Sęczyk, Dziki, &
70 Sikora, 2018; Nirmala, Bisht, Bajwa, & Santosh, 2018), including OMWW (Araújo, Pimentel,
71 Alves, & Oliveira, 2015; Esposto et al., 2015; Servili et al., 2011a; Servili et al., 2011b), have
72 been proposed as functional ingredients that are able to enhance food and beverage antioxidant
73 activity and its potential pro-health effects. Unfortunately, phenol compounds are mainly
74 responsible for the bitterness, astringency and pungency in phenol rich foods (Lesschaeve &
75 Noble, 2005). For instance, secoiridoid aglycons 3,4-DHPEA-EDA and p-HPEA-EDA induce
76 intense bitter taste and pungent sensations (Vitaglione et al., 2015). The intensity of these phenol-
77 induced 'warning' sensations significantly affects preference and choice of phenol rich vegetable
78 foods (Dinnella, 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).
83 Hence, strategies to control for the intensity of warning sensations need to be considered when
84 developing phenol enriched functional foods. Three main strategies can be envisaged to reduce the
85 intensity of the unacceptable sensory properties of phenols (Ares, Barreiro, Deliza, & Gámbaro,
86 2009; Gaudette & Pickering, 2012; Keast, 2008).

87

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

99

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

102

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

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

Material & Methods

1. OMWW phenol extract preparation

The phenolic fraction was extracted from OMWW of Peranzana, Ogliarola, Coratina and Moraiolo cultivars harvested at ripening in region from Central Italy. The extraction and purification of phenolic fraction from OMWW was carried out as described by Esposto et al., 2015

stages of from OMWW of . Three steps of tangential membrane filtration were applied to obtain a crude phenolic concentrate from OMWW previously treated with an enzymatic solution of pectinase from *Aspergillus niger*, BIODEP (Biotec s.r.l., Roma, Italy) (Servili et al., 2011a).

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

2. Chemical Analysis

2.1 Phenol profile

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

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

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

2.2 Antioxidant activity

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

3. Sensory evaluations

3.1 Subjects

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

3.2 Procedure

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

3.3 Sensory stimuli

3.3.1 OMWW extract

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

3.3.2 Functionalized foods

Three vegetable foods with different macro-composition were selected for the development of phenol functionalized foods: proteins/neutral pH - bean purée (BP), carbohydrates/neutral pH - potato purée (PP), water/low pH - tomato juice (TJ). Canned or powdered ingredients produced by large food companies were used to prepare the functionalized food since their composition is constant, and they are easily available without seasonality restrictions. The three foods had four levels of phenol from OMWW extract added: 0.44, 1.00, 2.25, 5.06 g/kg. A further sample for each series consisting of the vegetable food without OMWW extract added, and indicated as 0.00 g/kg, was considered. In total, five levels of phenol concentration for each vegetable food were considered for evaluation. Samples were evaluated immediately after preparation, within 15 min of extract addition.

3.4 Evaluation conditions

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

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

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

5. Data Analysis

Two-ways ANOVA models were used to assess the effect of phenol concentration and food macro-composition on the amount of phenols extracted from functionalized samples and on their total recovery. Two-way ANOVA mixed models (fixed factor: phenol concentration; random factor: subjects) were used to assess the effect of phenol concentration on the intensity of target sensations in OMWW solutions and food prototype samples. Three-way mixed models (fixed factors: food matrix and phenol concentration; random factor: subjects) with interactions were used to assess the effect of food matrix on the intensity of target sensations. A Fisher LSD post hoc test was applied to test significant differences in multiple comparison test (significant for $P \leq$ 0.05)

The XLSTAT statistical software package version 19.02 (Addinsoft) was used for data analysis.

Results

1. Chemical characterization

1.1 OMWW extract: phenol profile and antioxidant activity

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

1.2 Functionalized foods: OMWW phenol recovery and profile

The amount of OMWW phenols in food samples functionalized with increasing concentrations was determined after extraction and expressed as percentage of recovery (Fig.1). The phenol recovery increased with the added amount (p≤0.001) and ranged from 3.7 to 13. 9 % in bean purée, from 12.6 to 19.9 % in tomato juice and from 5.4 to 17.3 % in potato purée. The recovery was significantly influenced by food macro-composition (p≤0.001). The lowest recovery of OMWW phenols was from functionalized bean purée samples irrespective to the amount initially

270 added. The highest recovery was from tomato juice added with 0.44, 2.25 and 5.06 g/kg of
271 phenols. Potato purée showed the highest recovery when 1.00 g/kg of phenols was used.

272

273 The amount of individual OMWW phenols from functionalized food regularly increased with the
274 total amount initially added ($p \leq 0.0001$) and was affected by food macro-composition ($p \leq 0.001$) in
275 a different extent depending on the specific phenol and the added amount (Tab.1). In general, the
276 lowest amount of each phenol was recovered from bean purée and the largest differences were
277 found among food functionalized with the highest amount of phenols (≥ 2.25 g/kg). Phenol profiles
278 recovered from BP, TJ and PP functionalized with 5.06 g/kg were compared to the profile of
279 OMWW extract (Fig. 2). The relative content of 3,4-DHPEA-EDA, 3,4-DHPEA, *p*-HPEA and
280 verbascoside largely differ between OMWW extract and functionalized food. 3,4-DHPEA-EDA
281 represented the most abundant phenol of OMWW extract (89 %) but its proportion lowered to
282 approx. 27, 35 and 36 % of total OMWW phenols recovered from BP, PP and TJ, respectively.
283 3,4-DHPEA and verbascoside represented 6.4 and 3.5 %, of the total phenol content of OMWW
284 extract respectively, and approximately 40 and 22 %, of the total phenols recovered from
285 functionalized foods. *p*-HPEA was 1 and approximately 4 % of total phenols in OMWW extract
286 and functionalized foods, respectively.

287

2. Sensory evaluation

2.1 OMWW extract solutions

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

301

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

304

2.2 Functionalized foods

305 The impact of OMWW extract on the sensory profile of the three vegetable matrices was
306 independently assessed in each series of prototype as a function of the concentration of added
307 phenols. The intensity of target sensations significantly changed in all the three vegetable
308 prototypes as a function of increasing phenol concentrations, the only exceptions being pungency
309 in bean purée (Tab.3). F values indicated that the increase of phenol concentration induced the
310 strongest effect on bitterness in all the three prototypes. The intensity of sourness, astringency and
311 pungency were influenced by both the increase of phenol concentration and, to a lesser extent, by
312 the matrix macro-composition. All the sensations were barely detectable in bean purée sample
313 without phenol added, while in the rest of samples, bitterness increased from weak to strong/very
314 strong, and sourness and astringency increased slightly from barely detectable to weak/moderate.
315 All sensations were rated as weak in the tomato juice sample without phenol added; in the rest of
316 samples, bitterness increased from weak to strong, and sourness, pungency and astringency
317 increased from weak to weak/moderate as a function of the concentration of added phenols. In the
318 potato purée sample without added phenols, all sensations were rated at barely detectable/weak
319 intensity. Bitterness increased from barely detectable to strong with increasing with phenol
320 concentration, and astringency, pungency and sourness increased slightly, reaching weak/moderate
321 intensity level.

322

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

328

329 The effect of vegetable matrix composition on the intensity of sensations contributed by OMWW
330 phenols was further explored and the intensities of target sensations in the three matrices at
331 different added phenol concentration were compared (Tab.4). The vegetable matrix significantly
332 affected the intensity of sourness.   The concentration of added phenol significantly affected the
333 intensity of target sensations, with the greatest effect on bitterness. The vegetable
334 matrix*concentration interaction was significant only for pungency, due to the suppression of this
335 sensation in bean purée samples. No significant differences were found comparing bitterness from
336 the three matrices at 0.00, 0.44, 1.00 and 5.06 g/L phenol concentrations, but at 2.25 g/L,

bitterness was significantly higher in tomato juice than in bean purée (Fig.3-A). Sourness was rated as more intense in tomato juice than 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 lowest intensity was perceived in bean purée and no significant differences were found between tomato juice and potato purée (Fig.3-B). The three vegetable matrices did not differ for the intensity of astringency at 0.44 and 1.00 g/L of added phenol, however in the rest of samples, this sensation was lower in bean purée than in potato purée and no significant differences were found comparing tomato juice and potato purée (Fig.3-C). Pungency was significantly higher in tomato juice (from 1.00 to 5.06 g/kg) and in potato puree (5.06 g/kg) than in bean purée, but no significant differences were found between tomato juice and potato purée (Fig.3-D).

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

Discussion

The amount of OMWW phenols recovered from the functionalized food prototypes was much lower than expected, thus indicating the existence of strong chemical interactions between functional phenols and food components,-the lowest amount was recovered from bean purée, the protein rich food matrix. These findings are in line with the previously documented interactions between phenols and food biopolymers. Proteins strongly interact with plant polyphenols through covalent and non-covalent binding, and high basic-residue content and open and flexible structure are the major features of proteins highly reactive towards phenols (Kroll, et al., 2003; Xiao & Kai, 2012; Zhang et al., 2014). Binding involves hydrophobic and hydrogen interactions, and proline-rich regions of leguminous proteins have been reported as preferred sites of interactions for plant phenol/food protein in *in vitro* conditions (Rawel, Czajka, Rohn, & Kroll, 2002). The formation of aggregates with proteins significantly impacts on the bioactivity of phenols and the reduction of both extractability from raw material and antioxidant activity has been reported (Kroll et al., 2014). The overall bioavailability of phenols from protein aggregates is still a matter of debate, and several sources of evidence indicate a lowering of the blood content of phenols after intake of food protein sources (Ozdal et al., 2013). However, the longer duration of the aggregates in the

372 stomach followed by a delayed phenol release has been observed (Ozdal et al., 2013). 373 Furthermore, after *in vitro* digestion of protein/phenol aggregates, the recovery of phenol related 374 antioxidant activity was reported (Drummond e Silva et al., 2017; Kroll et al., 2003). Thus, it is 375 possible to hypothesize that the interactions between food proteins and phenols do not lower the 376 functional potential of the phenols, but rather influence their kinetic of phenol adsorption and 377 bioactivity (Zhang et al., 2014).

379 Phenolic compounds bridge or cross-link with starch and other polysaccharides, and a large 380 fraction of the so called "NEPP" (not extractable polyphenols) consists in phenol associations with 381 polysaccharides (Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2013). The consequences of 382 phenol/carbohydrate interactions on phenol bioactivity depends on phenol and carbohydrate 383 chemical characteristics, and both enhancement or suppression of antioxidant activity and bio-384 accessibility have been observed (Zhang et al., 2014). The majority of NEPP arrive almost intact 385 to the colon where they are fermented by microflora or depolymerized via enzymes, leading to 386 phenol metabolites being available for adsorption (Pérez-Jiménez et al., 2013).

388 Based on these considerations, the low recovery from functionalized prototypes should not be 389 interpreted as the mere loss of the bioactive compounds, and further investigations on phenol 390 bioavailability and bio-accessibility will clarify the potential pro-health effects of experimental 391 food matrices enriched with OMWW phenols.

393 The profile of phenol fractions extracted from functionalized foods differed substantially from the 394 profile of the OMWW extract, mainly because of the strong decrease of 3,4-DHPEA-EDA relative 395 to the other phenol compounds. Several phenol features, including their structure, the arrangement 396 of hydroxyl groups, and the planarity of molecules, actively modulate the interactions 397 phenols/environment and might be responsible for the observed differences (Jakobek, 2015; Ozdal 398 et al., 2013). Investigating the associations of the chemical features of OMWW phenols with the 399 strength and the modality of their interaction with biopolymers was behind the aim of the present 400 work but further studies should be encouraged for a deeper understanding of the mechanism 401 underlying phenol/biopolymer interactions in real food systems.

403 Bitterness was the most intense sensation induced by OMWW extracts, astringency and pungency 404 were perceived at lower intensities, while sourness represented a marginal sensation. The observed 405 sensory properties are consistent with the phenol profile of the extract. Secoiridoid derivatives of

406 hydroxytyrosol are considered the main contributors to olive oil bitterness (Bendini et al., 2007).

407 3,4-DHPEA-EDA represents the main extract component and has been described as mainly bitter

408 and slightly pungent (Taticchi, Esposto, & Servili, 2014). Pungency is instead mainly attributed to

409 *p*-tyrosol derivatives which, when tested at the same concentration 3,4-DHPEA-EDA, primarily

410 produced bitter tastes and low pungency, while *p*-HPEA-EDA mainly induced pungency

411 (Andrewes, Busch, De Joode, Groenewegen, & Alexandre, 2003). Bitterness represents the main

412 contribution of OMWW phenols to sensory profile of functional prototypes. The vegetable matrix

413 macro-composition did not significantly affect the perceived intensity of this sensations. Thus, the

414 strong interactions of OMWW phenols with vegetable biopolymers prevent the chemical

415 extraction of phenols, and in particular of 3,4-DHPEA-EDA, but do not suppress the bitter taste of

416 phenol compounds. In line with the documented *in vivo* release of phenols from biopolymer

417 aggregates (Ozdal et al., 2013) and in vitro action of saliva enzymes on phenol structures (Walle et

418 al., 2005), it might be possible to speculate about their possible release in the oral environment.

419 The relatively high temperature of oral environment, and the presence of salts and hydrolytic

420 enzymes in saliva, may favor phenol release from biopolymer aggregates, their diffusion across

421 bitter taste receptors and a consequent stimulation of these receptors. Moreover, the contribution to

422 bitter taste of 3,4 DHPEA, verbascoside and p-HPEA should be reconsidered. The vegetable

423 matrix composition affected the perceived intensity of pungency and sourness. Pungency

424 perception is suppressed in the protein rich prototype, and this could be tentatively related to 3,4-

425 DHPEA-EDA/protein binding. This could lower the 3,4-DHPEA-EDA concentration so that

426 bitterness is not affected, but the capacity to induce these secondary sensations is instead inhibited.

427

Conclusions

429 Food macro-composition actively impacts on the chemical and sensory properties of phenols from

430 an OMWW extract with the strongest effects observed in protein-based foods. Interactions

431 between food proteins and phenols appear a possible strategy to produce a compromise between

432 the health potential of phenols and sensory acceptability of phenol-enriched foods since lower the

433 intensity of warning sensations, while at the same time avoiding extraneous ingredients in their

434 formulations. Specificities were found between phenol chemical structure and strength of their

435 interactions with food components. Systematic investigations in real food systems would help in

436 clarifying the mechanisms underlying the phenol-biopolymer aggregate formation, thus helping in

437 optimizing functional food formulations.

438

References

440 Andrewes, P., Busch, J. L. H. C., De Joode, T., Groenewegen, A., & Alexandre, H. (2003).

441 Sensory properties of virgin olive oil polyphenols: Identification of deacetoxy-ligstroside

442 aglycon as a key contributor to pungency. *Journal of Agricultural and Food Chemistry*,

443 *51*(5), 1415–1420. https://doi.org/10.1021/jf026042j

444 Araújo, M., Pimentel, F. B., Alves, R. C., & Oliveira, M. B. P. P. (2015). Phenolic compounds

445 from olive mill wastes: Health effects, analytical approach and application as food

446 antioxidants. *Trends in Food Science and Technology*.

447 https://doi.org/10.1016/j.tifs.2015.06.010

448 Ares, G., Barreiro, C., Deliza, R., & Gámbaro, A. (2009). Alternatives to reduce the bitterness,

449 astringency and characteristic flavour of antioxidant extracts. *Food Research International*,

450 *42*(7), 871–878. https://doi.org/10.1016/j.foodres.2009.03.006

451 Bartoshuk, L. M. (2000). Comparing Sensory Experiences Across Individuals: Recent

452 Psychophysical Advances Illuminate Genetic Variation in Taste Perception. *Chemical Senses*,

453 *25*(4), 447–460. https://doi.org/10.1093/chemse/25.4.447

454 Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Segura-Carretero, A.,

455 Fernández-Gutiérrez, A., & Lercker, G. (2007). Phenolic molecules in virgin olive oils: A

456 survey of their sensory properties, health effects, antioxidant activity and analytical methods.

457 An overview of the last decade. *Molecules*. https://doi.org/10.3390/12081679

458 Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to

459 evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30.

460 https://doi.org/10.1016/S0023-6438(95)80008-5

461 Carrillo, E., Prado-Gascó, V., Fiszman, S., & Varela, P. (2013). Why buying functional foods?

462 Understanding spending behaviour through structural equation modelling. *Food Research*

463 *International*, *50*(1), 361–368. https://doi.org/10.1016/j.foodres.2012.10.045

464 Dinnella, C., Recchia, A., Tuorila, H., & Monteleone, E. (2011). Individual astringency

465 responsiveness affects the acceptance of phenol-rich foods. *Appetite*, *56*(3), 633–642.

466 https://doi.org/10.1016/j.appet.2011.02.017

467 Drummond e Silva, F. G., Miralles, B., Hernandez-Ledesma, B., Amigo, L., Iglesias, A. H.,

468 Reyes, F. G. R., & Netto, F. M. (2017). Influence of protein-phenolic complex on the

469 antioxidant capacity of flaxseed (Linum usitatissimum L.) products. *Journal of Agricultural*

470 *and Food Chemistry*, *65*(4), 800–809. https://doi.org/10.1021/acs.jafc.6b04639

471 Esposto, S., Taticchi, A., Di Maio, I., Urbani, S., Veneziani, G., Selvaggini, R., … Servili, M.

472 (2015). Effect of an olive phenolic extract on the quality of vegetable oils during frying. *Food*

473 *Chemistry*, *176*, 184–192. https://doi.org/10.1016/j.foodchem.2014.12.036

474   Gaudette, N. J., & Pickering, G. J. (2013). Modifying Bitterness in Functional Food Systems.

475   *Critical Reviews in Food Science and Nutrition*, 53(5), 464–481.

476   https://doi.org/10.1080/10408398.2010.542511

477   Green, B. G., Dalton, P., Cowart, B., Shaffer, G., Rankin, K., & Higgins, J. (1996). Evaluating the

478   "labeled magnitude scale" for measuring sensations of taste and smell. *Chemical Senses*,

479   21(3), 323–334. https://doi.org/10.1093/chemse/21.3.323

480   Green, B. G., Shaffer, G. S., & Gilmore, M. M. (1993). Derivation and evaluation of a semantic

481   scale of oral sensation magnitude with apparent ratio properties. *Chemical Senses*, 18(6),

482   683–702. https://doi.org/10.1093/chemse/18.6.683

483   Jaeger, S. R., Axten, L. G., Wohlers, M. W., & Sun-Waterhouse, D. (2009). Polyphenol-rich

484   beverages: Insights from sensory and consumer science. *Journal of the Science of Food and*

485   *Agriculture*, 89(14), 2356–2363. https://doi.org/10.1002/jsfa.3721

486   Jakobek, L. (2015). Interactions of polyphenols with carbohydrates , lipids and proteins. *Food*

487   *Chemistry*, 175, 556–567. https://doi.org/10.1016/j.foodchem.2014.12.013

488   Keast, R. S. J. (2008). Modification of the bitterness of caffeine. *Food Quality and Preference*,

489   19(5), 465–472. https://doi.org/10.1016/j.foodqual.2008.02.002

490   Kowalska, H., Czajkowska, K., Cichowska, J., & Lenart, A. (2017). What's new in biopotential of

491   fruit and vegetable by-products applied in the food processing industry. *Trends in Food*

492   *Science and Technology*. https://doi.org/10.1016/j.tifs.2017.06.016

493   Kroll, J., Rawel, H. M., & Rohn, S. (2003). Reactions of Plant Phenolics with Food Proteins and

494   Enzymes under Special Consideration of Covalent Bonds. *Food Science and Technology*

495   *Research*, 9(3), 205–218. https://doi.org/10.3136/fstr.9.205

496   Krystallis, A., Maglaras, G., & Mamalis, S. (2008). Motivations and cognitive structures of

497   consumers in their purchasing of functional foods. *Food Quality and Preference*, 19(6), 525–

498   538. https://doi.org/10.1016/j.foodqual.2007.12.005

499   Lesschaeve, I., & Noble, A. C. (2005). Polyphenols : factors influencing their sensory properties

500   and their effects on food and beverage preferences, 81, 330–335.

501   Monteleone, E., Spinelli, S., Dinnella, C., Endrizzi, I., Laureati, M., Pagliarini, E., … Tesini, F.

502   (2017). Exploring influences on food choice in a large population sample: The Italian Taste

503   project. *Food Quality and Preference*, 59, 123–140.

504   https://doi.org/10.1016/j.foodqual.2017.02.013

505   Nirmala, C., Bisht, M. S., Bajwa, H. K., & Santosh, O. (2018). Bamboo: A rich source of natural

506   antioxidants and its applications in the food and pharmaceutical industry. *Trends in Food*

507   *Science and Technology*. https://doi.org/10.1016/j.tifs.2018.05.003

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

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

514 Rawel, H. M., Czajka, D., Rohn, S., & Kroll, J. (2002). Interactions of different phenolic acids and
515 flavonoids with soy proteins. *International Journal of Biological Macromolecules*, *30*(3–4),
516 137–150. https://doi.org/10.1016/S0141-8130(02)00016-8

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

520 Selvaggini, R., Esposto, S., Taticchi, A., Urbani, S., Veneziani, G., Di Maio, I., … Servili, M.
521 (2014). Optimization of the temperature and oxygen concentration conditions in the
522 malaxation during the oil mechanical extraction process of four italian olive cultivars.
523 *Journal of Agricultural and Food Chemistry*, *62*(17), 3813–3822.
524 https://doi.org/10.1021/jf405753c

525 Servili, M., Esposto, S., Veneziani, G., Urbani, S., Taticchi, A., Di Maio, I., … Montedoro, G.
526 (2011)a. Improvement of bioactive phenol content in virgin olive oil with an olive-vegetation
527 water concentrate produced by membrane treatment. *Food Chemistry*, *124*(4), 1308–1315.
528 https://doi.org/10.1016/j.foodchem.2010.07.042

529 Servili, M., Rizzello, C. G., Taticchi, A., Esposto, S., Urbani, S., Mazzacane, F., … Di Cagno, R.
530 (2011)b. Functional milk beverage fortified with phenolic compounds extracted from olive
531 vegetation water, and fermented with functional lactic acid bacteria. *International Journal of*
532 *Food Microbiology*, *147*(1), 45–52. https://doi.org/10.1016/j.ijfoodmicro.2011.03.006

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

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

540 Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and
541 spices: Antioxidant activity and health effects - A review. *Journal of Functional Foods*.

542 https://doi.org/10.1016/j.jff.2015.06.018

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

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

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

Trapani, S., Migliorini, M., Cecchi, L., Giovenzana, V., Beghi, R., Canuti, V., … Zanoni, B. (2017). Feasibility of filter-based NIR spectroscopy for the routine measurement of olive oil fruit ripening indices, *1600239,* 1–4. https://doi.org/10.1002/ejlt.201600239

Veneziani, G., Esposto, S., Taticchi, A., Selvaggini, R., Urbani, S., Di Maio, I., … Servili, M. (2015). Flash Thermal Conditioning of Olive Pastes during the Oil Mechanical Extraction Process: Cultivar Impact on the Phenolic and Volatile Composition of Virgin Olive Oil. *Journal of Agricultural and Food Chemistry, 63*(26), 6066–6074. https://doi.org/10.1021/acs.jafc.5b01666

Veneziani, G., Novelli, E., Esposto, S., Taticchi, A., & Servili, M. (2017). *Applications of recovered bioactive compounds in food products. Olive Mill Waste: Recent Advances for Sustainable Management.* https://doi.org/10.1016/B978-0-12-805314-0.00011-X

Verbeke, W. (2006). Functional foods: Consumer willingness to compromise on taste for health? *Food Quality and Preference, 17*(1–2), 126–131. https://doi.org/10.1016/j.foodqual.2005.03.003

Vitaglione, P., Savarese, M., Paduano, A., Scalfi, L., Fogliano, V., & Sacchi, R. (2015). Healthy Virgin Olive Oil: A Matter of Bitterness. *Critical Reviews in Food Science and Nutrition, 55*(13), 1808–1818. https://doi.org/10.1080/10408398.2012.708685

Walle, T., Browning, A. M., Steed, L. L., Reed, S. G., & Walle, U. K. (2005). Flavonoid glucosides are hydrolyzed and thus activated in the oral cavity in humans. *The Journal of nutrition, 135*(1), 48-52.

Xiao, J., & Kai, G. (2012). A review of dietary polyphenol-plasma protein interactions: Characterization, influence on the bioactivity, and structure-affinity relationship. *Critical Reviews in Food Science and Nutrition.* https://doi.org/10.1080/10408398.2010.499017

Zhang, H., Yu, D., Sun, J., Liu, X., Jiang, L., Guo, H., & Ren, F. (2014). Interaction of plant phenols with food macronutrients: Characterisation and nutritional-physiological

576 consequences. *Nutrition Research Reviews*. https://doi.org/10.1017/S095442241300019X

577

Figure Legend

Figure 1: Percentage of OMWW phenols recovered (Recovery %) form 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 ($p \leq 0.001$)

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.

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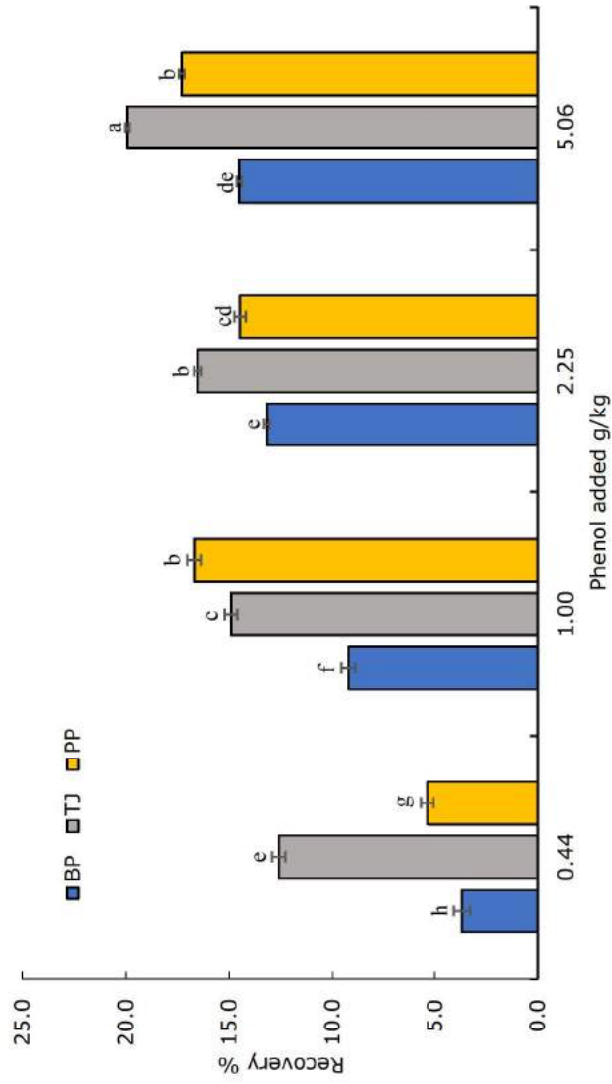
Figure

Fig.1

Figure 1: Percentage of OMWW phenols recovered (recovery%) form 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)

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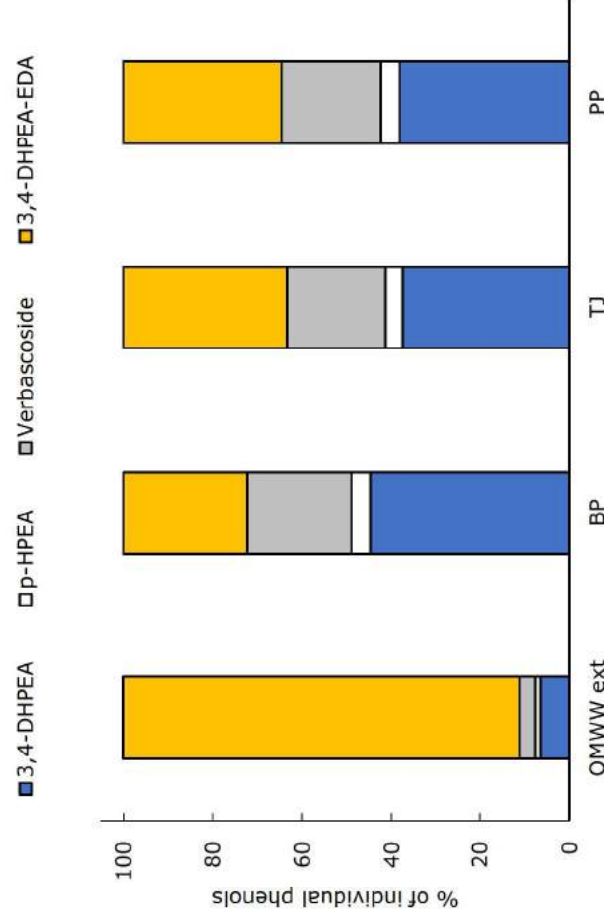
Figure 2: Percentage of individual phenols detected in the OMWW extract (OMWW ext) and in bean purée (BP), tomato juice (TJ) and potato purée (PP) functionalized with 5.06 g/kg phenols from OMWW extract.

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≤ 0.001).

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≤ 0.001).

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≤ 0.001).
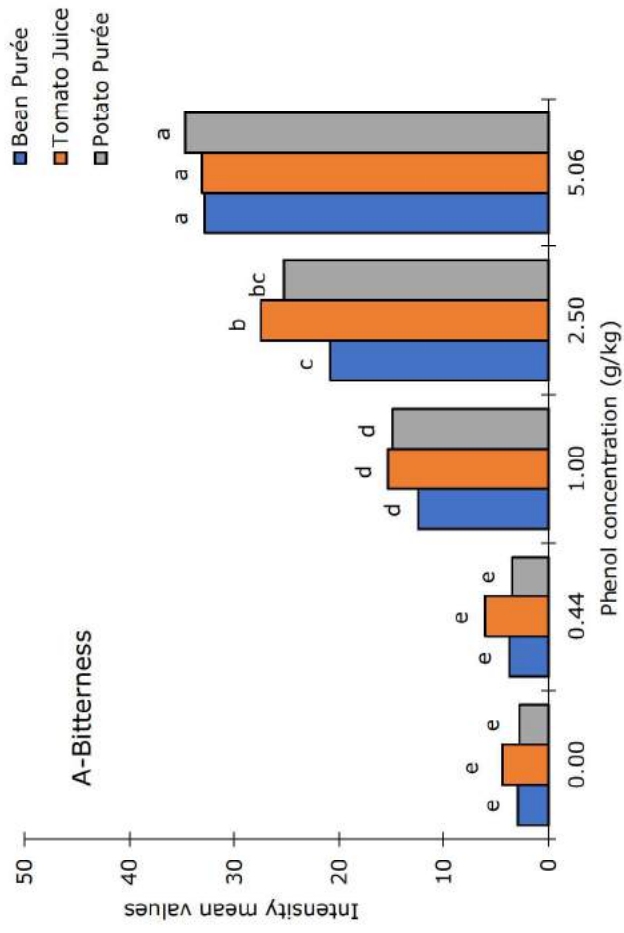
Fig.3

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≤ 0.001).
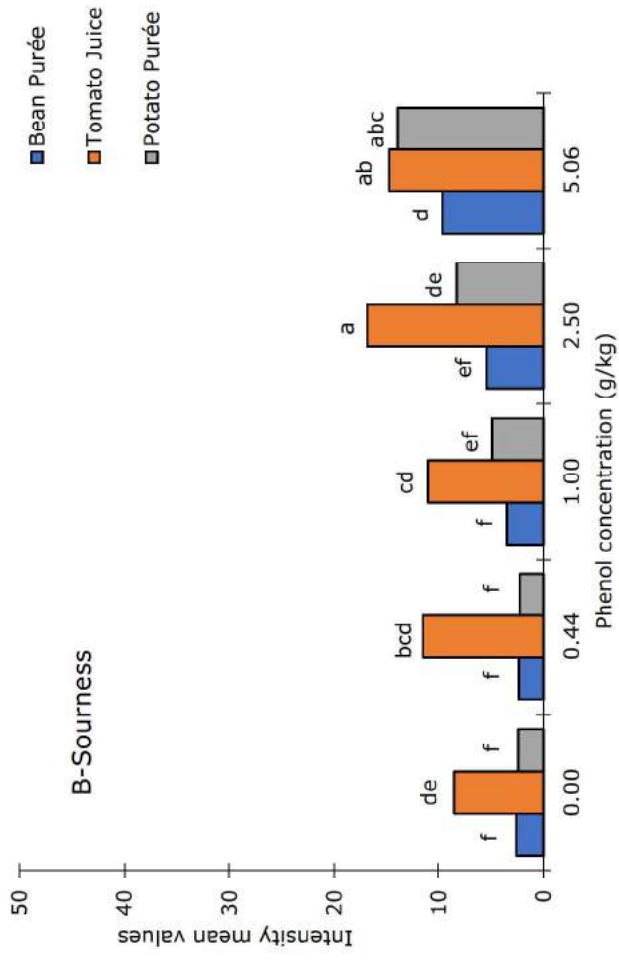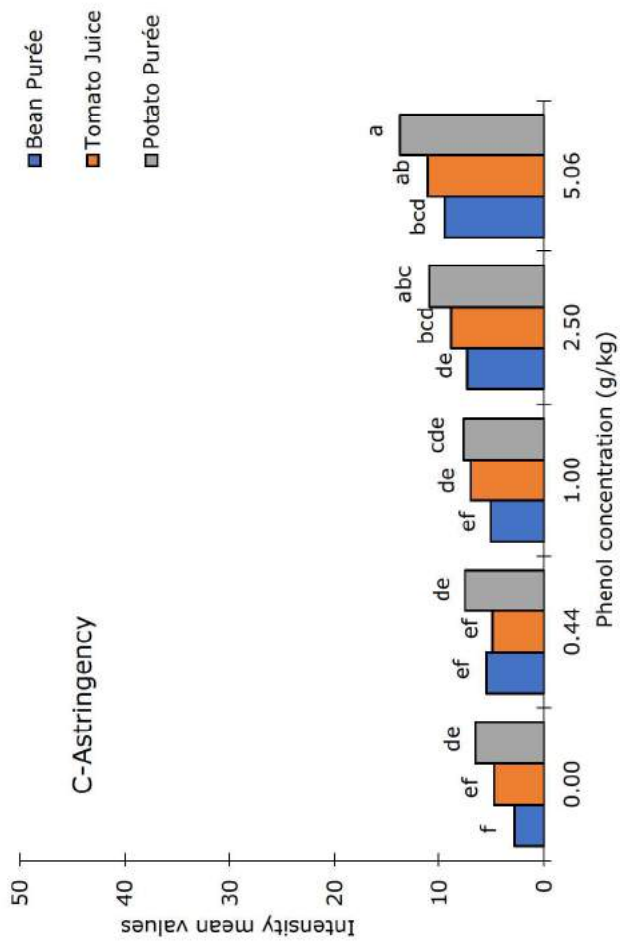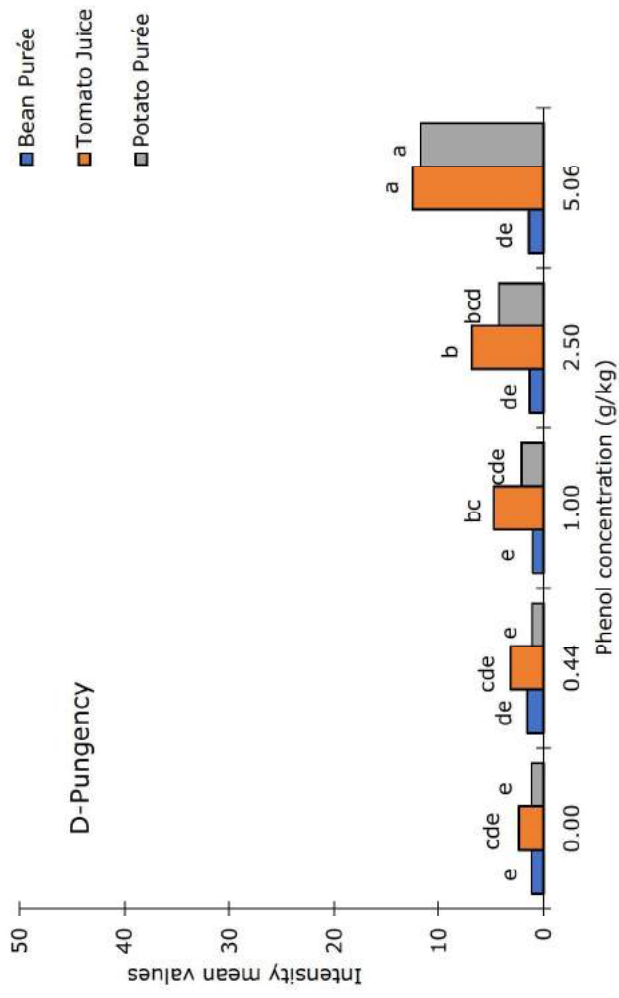
Table

Table 1: Recovery (mMean values

_g/kg) of individual phenols from foods (BP-bean purée, TJ-tomato juice, PP-potato purée) functionalized
with increasing amount of phenols from OMWW extract.

	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

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

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

	F	p	Concentration (g/L)								
			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

Different letters indicate significantly different values (p≤0.0001)

Table.3 2-Way ANOVAs 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 OMWW (g/kKg)				
			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

Different letters indicate significantly different values (p≤0.001)

Table 4: 3-Way ANOVA mixed model (random effect assessors): Vegetable matrix. phenol concentration and their interactions effects on intensity of target sensations in food models. F and p values.

	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

*Graphical Abstract

Olive Mill Waste Water

HPLC and DPPH assay

Set up of functionalized vegetable prototypes added with OMWW

Chemical and Sensory evaluations