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

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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.

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.

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

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

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

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

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 ($p \leq 0.001$) 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 ($p \leq 0.001$). The lowest recovery of OMWW phenols was from functionalized bean purée samples irrespective to the amount initially added. The highest recovery was from tomato juice added with 0.44, 2.25 and 5.06 g/kg of phenols. Potato purée showed the highest recovery when 1.00 g/kg of phenols was used.

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

2. Sensory evaluation

2.1 OMWW extract solutions

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

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

2.2 Functionalized foods

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

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

The effect of vegetable matrix composition on the intensity of sensations contributed by OMWW phenols was further explored and the intensities of target sensations in the three matrices at different added phenol concentration were compared (Tab.4). The vegetable matrix significantly affected the intensity of sourness. The concentration of added phenol significantly affected the intensity of target sensations, with the greatest effect on bitterness. The vegetable matrix*concentration interaction was significant only for pungency, due to the suppression of this sensation in bean purée samples. No significant differences were found comparing bitterness from 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 stomach followed by a delayed phenol release has been observed (Ozdal et al., 2013). Furthermore, after *in vitro* digestion of protein/phenol aggregates, the recovery of phenol related antioxidant activity was reported (Drummond e Silva et al., 2017; Kroll et al., 2003). Thus, it is possible to hypothesize that the interactions between food proteins and phenols do not lower the functional potential of the phenols, but rather influence their kinetic of phenol adsorption and bioactivity (Zhang et al., 2014).

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

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

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

Bitterness was the most intense sensation induced by OMWW extracts, astringency and pungency were perceived at lower intensities, while sourness represented a marginal sensation. The observed sensory properties are consistent with the phenol profile of the extract. Secoiridoid derivatives of hydroxytyrosol are considered the main contributors to olive oil bitterness (Bendini et al., 2007). 3,4-DHPEA-EDA represents the main extract component and has been described as mainly bitter and slightly pungent (Taticchi, Esposto, & Servili, 2014). Pungency is instead mainly attributed to *p*-tyrosol derivatives which, when tested at the same concentration 3,4-DHPEA-EDA, primarily produced bitter tastes and low pungency, while *p*-HPEA-EDA mainly induced pungency (Andrewes, Busch, De Joode, Groenewegen, & Alexandre, 2003). Bitterness represents the main contribution of OMWW phenols to sensory profile of functional prototypes. The vegetable matrix macro-composition did not significantly affect the perceived intensity of this sensations. Thus, the strong interactions of OMWW phenols with vegetable biopolymers prevent the chemical extraction of phenols, and in particular of 3,4-DHPEA-EDA, but do not suppress the bitter taste of phenol compounds. In line with the documented *in vivo* release of phenols from biopolymer aggregates (Ozdal et al., 2013) and in vitro action of saliva enzymes on phenol structures (Walle et al., 2005), it might be possible to speculate about their possible release in the oral environment. The relatively high temperature of oral environment, and the presence of salts and hydrolytic enzymes in saliva, may favor phenol release from biopolymer aggregates, their diffusion across bitter taste receptors and a consequent stimulation of these receptors. Moreover, the contribution to bitter taste of 3,4 DHPEA, verbascoside and p-HPEA should be reconsidered. The vegetable matrix composition affected the perceived intensity of pungency and sourness. Pungency perception is suppressed in the protein rich prototype, and this could be tentatively related to 3,4-DHPEA-EDA/protein binding. This could lower the 3,4-DHPEA-EDA concentration so that bitterness is not affected, but the capacity to induce these secondary sensations is instead inhibited.

Conclusions

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

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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 ($p \leq 0.001$).

Table 1: Recovery (mean 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.

							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

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.

			Concentration of phenols from OMWW (g/kg)				
			0.00	**0.44**	**1.00**	**2.25**	**5.06**
	F	**p**					
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 ($p \leq 0.001$)

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

Fig.1

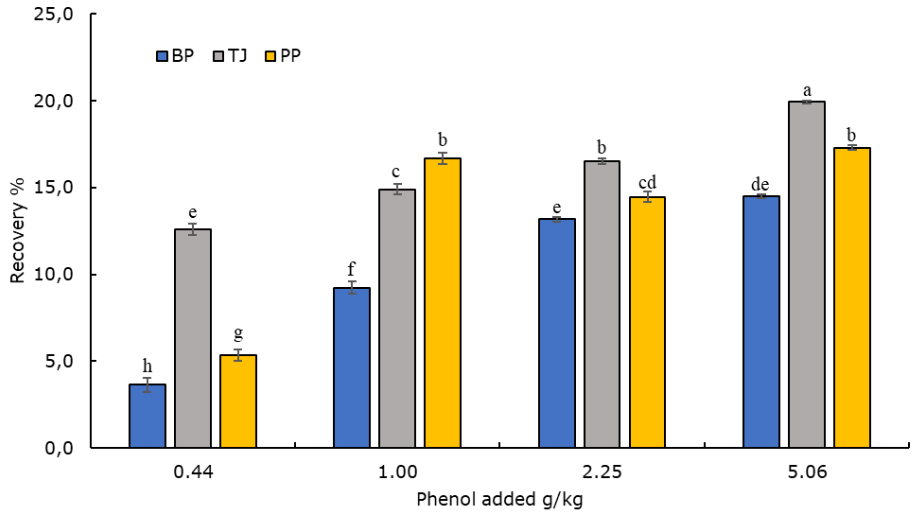

Figure 1: Percentage of OMWW phenols recovered (recovery%) form bean purée (BP), tomato juice (TJ) and potato purée (PP) functionalized with increasing amount of phenols from OMWW extract. Bars represent standard deviation, different letters indicate significantly different values ($p \leq 0.001$)

Fig.2

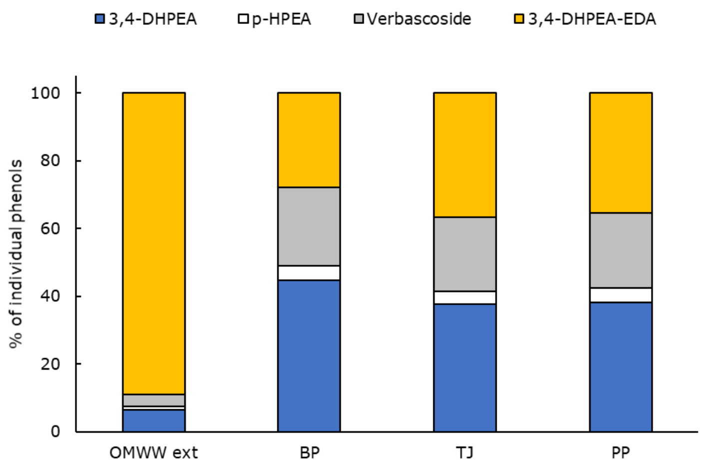

Figure 2: Percentage of individual phenols detected in the OMWW extract (OMWW ext) and in bean purée (BP), tomato juice (TJ) and potato purée (PP) functionalized with 5.06 g/kg phenols from OMWW extract.

Fig.3

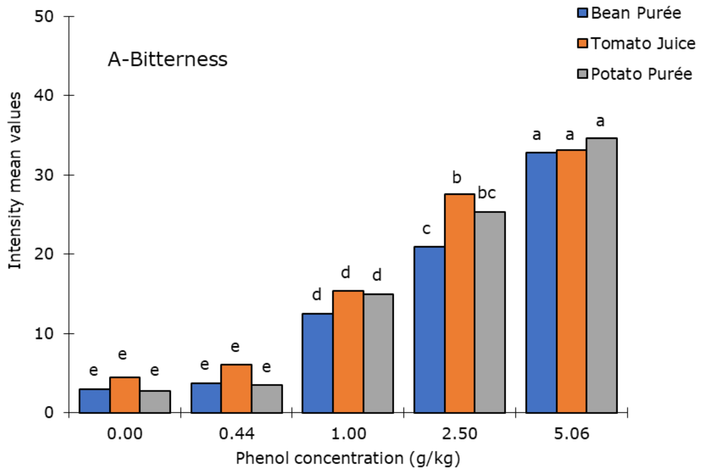

Figure 3A: Effect of the vegetable matrix on the perceived intensity of bitterness in prototypes functionalized with different concentrations of phenols from OMWW extract. Different letters represent significant different values ($p \leq 0.001$).

Fig.3

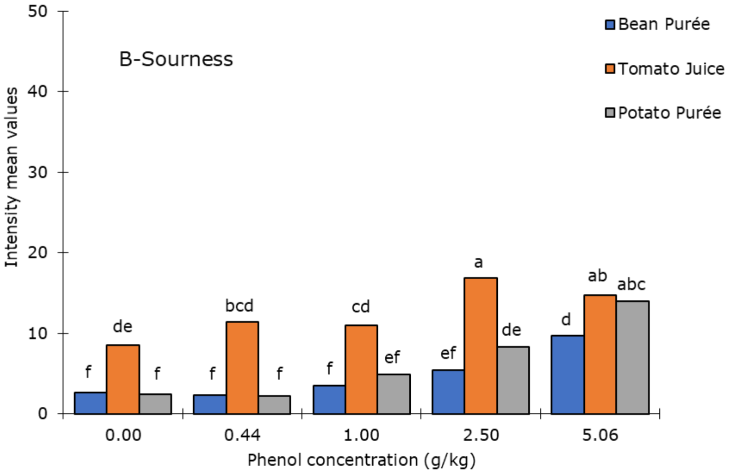

Figure 3B: Effect of the vegetable matrix on the perceived intensity of sourness in prototypes functionalized with different concentrations of phenols from OMWW extract. Different letters represent significant different values ($p \leq 0.001$).

Fig.3

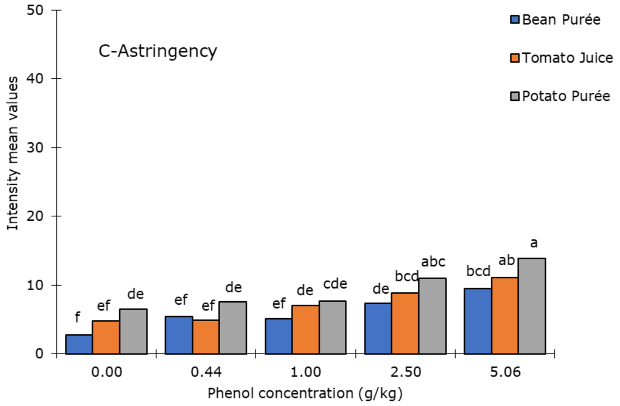

Figure 3C: Effect of the vegetable matrix on the perceived intensity of astringency in prototypes functionalized with different concentrations of phenols from OMWW extract. Different letters represent significant different values ($p \leq 0.001$).

Fig.3

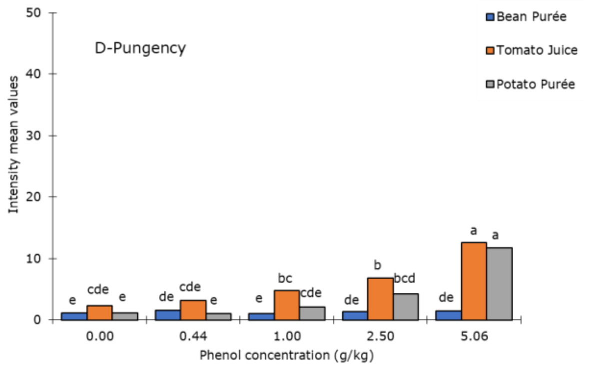

Figure 3D: Effect of the vegetable matrix on the perceived intensity of pungency in prototypes functionalized with different concentrations of phenols from OMWW extract. Different letters represent significant different values ($p \leq 0.001$).