Total phenolics content, bioavailability and antioxidant capacity of 10 different genotypes of walnut (*Juglans regia* L.)

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Summary
Walnuts have high nutritional value, owing to their chemical composition. They are an excellent source of phenolic compounds and are known to exert numerous beneficial health effects both in terms of prevention and promotion of health. However, it is necessary to know the bioavailability of phenolic compounds to understand their effects on the organism. Therefore, we analysed 10 different genotypes of walnut, in order to check the availability of total phenolic compounds and antioxidant capacity. In vitro bioavailability and intestinal absorption process were simulated by using digestive enzymes. The total polyphenolics content values of walnut extracts was determined using the Folin–Ciocalteu method and the antioxidant capacity was determined by three different methods. After in vitro digestion, the dialysate of Algaida variety was the one with the highest total phenolics content compared to other varieties (3.733 ± 0.205 g·kg^{-1}). The antioxidant capacity was different depending on the method used to determine it. However, Algaida, Eureka, Sundland and Tehama were the most antioxidant varieties as a whole. In vitro availability of total polyphenolics and antioxidant capacity of walnut decreased after in vitro digestion, showing different trends regarding the genotype.

Keywords
total polyphenolics; antioxidant capacity; walnuts; availability; genotypes; health

Fruits of walnut (*Juglans regia* L.) are essential food in Mediterranean diet, consumed as snacks, desserts or part of a meal. They originate in central Asia and the Mediterranean region. Nowadays, walnuts are cultivated commercially throughout southern Europe, northern Africa, eastern Asia, USA and western South America [1].

Several epidemiological studies suggest that walnuts consumption is inversely associated with the incidence of different diseases as cardiovascular disease, obesity, diabetes, brain illness and some kinds of cancer [2–4]. Walnuts showed numerous beneficial health effects both in terms of prevention and promotion of health. Owing to this evidence, walnuts were incorporated into recommended dietary guidelines in countries as United States, Canada or Spain [5]. Walnuts comprise significant amounts of phytochemicals, particularly phenolic compounds, including hydrolysable and condensed tannins, flavonoids or phenolic acids [6–8]. Recently, different phenolic compounds as ellagitannins, were found to provide potential health benefits of walnut phenolics. Similarly, urolithins (gut microbiota ellagitannin metabolites) were also proposed as determining factors for the improvement of health [9–12].

Knowing the bioavailability of phenolic compounds is essential to know their effect on the organism, in addition to determine the amount needed to establish the range of action. Therefore, establishing bioavailability of phenolic compounds is essential for evaluation of their effect on the organism. A major challenge in the evaluation of the role of health-promoting components in walnuts is the lack of information about their bioavailability regarding the gastrointestinal track. Unlike in vivo bioavailability in humans, there is a lack of previous scientific reports evaluating the in vitro availability of total polyphenolics. The interest in the study of bioactive compounds in foods and their contribution to antioxidant activity [13] has increased the number of investigations in which

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this activity was measured, among the consequent protective effect against various diseases [14, 15]. Numerous studies focused on analysis of antioxidant activity of nuts [16, 17].

The main objective of the present study was to determine the total polyphenolics content, the antioxidant capacity and bioavailability analysing 10 different genotypes of walnut cultured in Murcia, Spain.

MATERIALS AND METHODS

Source of the walnuts and sample preparation

Ten different varieties of walnut samples were obtained from experimental orchard of the Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA; Torre Pacheco, Spain). These commercial walnut varieties were: Algaida, Amigo, Chico, Eureka, Franquette, Payne, Pedro, Serr, Sunland and Tehama. All varieties were grown under the same agricultural conditions. Cultivation references were 6 × 8 m^2. The soil had a clay loam texture according to the classification criteria of US Department of Agriculture. According to the analysis of soil saturation extract, field had the following grading: 31.8 % sand, 32.5 % silt and 35.7 % clay. For each variety, 2 kg of walnuts were harvested. Mesocrap-striped walnuts were obtained at optimum ripeness, discarding those with defects like cracks or microbiological contamination symptoms. From each batch, 50 randomly selected fruits were shelled to remove kernels. Finally, walnut kernels were vacuum-packed in plastic bags and stored at –80 °C until analysis. Prior to analysis, 12 g of walnuts were homogenized with and Ultraturrax IKA T18 Basic (IKA, Staufen, Germany) at 400 Hz with 60 ml of distilled water.

For in vitro availability assay, pepsin solution was prepared by adding 4 g of pepsin (P7000; Sigma-Aldrich, St. Louis, Missouri, USA) in 25 ml of distilled water. Pancreatin mixture solution was prepared with 0.42 g of $NaHCO_3$ (Sigma-Aldrich), with 1.25 g of bile salts (Sigma-Aldrich) and 0.2 g of pancreatin (P7545, Sigma-Aldrich), which mixture was dissolved in 50 ml of distilled water.

In vitro availability

In order to represent the in vitro digestion as realistic as possible, extraction from walnuts was carried out in a way simulating gastrointestinal environment. The procedure was adapted from the previous work of Gil-Izquierdo et al. [18]. This method determines the grade of liberation and stability of phenolic compounds. The pepsin solution was prepared as mentioned in the previous work. First, pH was measured and the sample was titrated with 0.6 $mol \cdot l^{-1}$ HCl to pH 2. Then, 6 ml of the solution of pepsin was added and acid digestion was performed for 2 h at 37 °C, in a bath with constant mild agitation, mimicking the peristaltics and human body temperature. During this time, maintenance of pH 2 was checked every half hour. Secondly, an aliquot of 20 ml (Aliquot 1) of the sample was added 5 ml of the solution of bile salts and pancreatin, and titrated with NaOH to pH 7. Another aliquot (Aliquot 2) of approx. 20 ml remained in an ice bath since the acid digestion stopped.

Third, Aliquot 2 was subjected to a second digestion and dialysis, at 37 °C for 2 h in a water bath with constant moderate stirring, simulating human conditions. Membranes were filled with 25 ml of water and a known amount of $NaHCO_3$ was added, equivalent to the acidity value ($NaHCO_3$ equivalents necessary to dialyse the mixture of pepsin and biliary-pancreatic extracts at pH 7.5 of mixture of biliary-pancreatic extracts). The enzyme was allowed to act for 2 h at physiological temperature to obtain a balance between the dialysed fraction (bioavailable) and the non-dialyased fraction (not bioavailable). Finally, the dialysate was collected, filtered through a membrane filter Millex-HV13 (pore size 0.45 μm; Millipore, Billerica, Massachusetts, USA) and stored at –80 °C until analysis. Compounds present in both fractions were then analysed, quantifying the volume of the dissolution. All the analyses were replicated (n = 3) and expressed as mean ± standard deviation.

Sample preparation

A quantity of 5 g of fresh walnuts was homogenized in an Ultraturrax T-18 at 400 Hz for 2 min with 20 ml of formic acid + methanol (3% solution). The extracts were centrifuged at 4000 ×g for 10 min in a centrifuge Heraeus Biofuge stratos (Heraeus, Hanau, Germany). The methanol extract was separated from the precipitate and filtered through a membrane filter Millex-HV13 and collected in opaque bottles. The pellet was subjected to another extraction process under identical conditions, and pooled with the methanol extract obtained after the first extraction.

Determination of total phenolic compounds

The content of total phenolic compounds (TPC) of walnut extracts was determined using the Folin-Ciocalteu method [19]. The reaction mixture contained 1 ml of walnut extracts, 5 ml of the Folin-Ciocalteu reagent and 20 ml of sodium carbon-

ate (200 mg·l^{-1}). The final volume was made up to 50 ml with distilled water. After 30 min of reaction, the absorbance at 765 nm was measured in a spectrophotometer Varian Cary 50 Bio (Varian, Palo Alto, California, USA). A calibration curve was constructed using gallic acid as standard. TPC content was expressed as grams of gallic acid equivalents per kilogram of walnuts.

Antioxidant capacity

When defining antioxidant capacity of foods, it is advisable to use different assays, offering different information due to the diverse chemical reactions employed in different methods [20]. The non-biological radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) are extensively used to test the antioxidant capacity of plant samples.

Other widespread method for the evaluation of the antioxidant capacity of plants is oxygen radical absorbance capacity (ORAC) method, based on the ability of peroxyl radical scavenging [21]. The use of various methods can provide a more complete evaluation of the antioxidant capacity of the studied nuts.

DPPH assay

For the analysis of antioxidant capacity by DPPH, the method described by Bondet, Brand-Williams, Berset [21] was used. Briefly, 5 μl of each sample was added to 1 ml of DPPH (Sigma, Steinheim, Germany) solution (0.094 mmol·l^{-1} in methanol). The free radical-scavenging activity using the free radical $^{\bullet}$DPPH reaction was evaluated by measuring the absorbance at 515 nm after 60 min of reaction at 20 °C in a spectrophotometer (Varian Cary 50 Bio). The antioxidant capacity was expressed as millimoles of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalents per kilogram of walnuts. The data are shown on fresh weight (FW) basis.

ABTS assay

The ABTS assay described by Millerand Rice-Evans [22] was used to assess the scavenging capacity of the walnut extracts against the stable ABTS radical (ABTS$^{\bullet+}$). The blue-green ABTS$^{\bullet+}$ was produced through the reaction between ABTS and activated manganese dioxide in water; this solution was stored in the dark for 12–16 h before use. Briefly, the walnut extracts at different concentrations, or the solvent alone, were added to 900 ml ABTS$^{\bullet+}$ solution and the absorbance was measured at 734 nm using spectrophotometer Varian Cary 50 Bio after 6 min incubation in the dark at laboratory temperature. Results were expressed as millimoles of Trolox equivalents per kilogram of walnuts. The data are shown on FW basis.

ORAC assay

The ORAC was determined as described by Dávalos et al. [23]. The ORAC analyses were carried out in a Synergy HT multi-detection microplate reader (Bio-Tek Instruments, Winooski, Vermont, USA), using 96-well polystyrene microplates with black sides and clear bottom. Fluorescence was read through the clear bottom, with an excitation wavelength of 485 nm and an emission wavelength of 528 nm. The reaction was carried out in 75 mmol·l^{-1} sodium phosphate buffer (pH 7.4) and the final volume of the reaction mixture was 200 μl. Fluorescein (FL) (100 μl; 3 mmol·l^{-1}, final concentration) and diluted sample (70 μl) were pipetted in the wells of the microplate. The mixture was pre-incubated for 30 min at 37 °C, before rapidly adding 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) solution (30 μl; 19 mmol·l^{-1}, final concentration) using a multichannel pipette. The microplate was immediately placed in the reader and the fluorescence recorded every 1.14 min for 120 min. The microplate was automatically shaken prior to each reading. A control blank with FL and AAPH (using sodium phosphate buffer instead of the antioxidant solution), besides eight calibration solutions (using Trolox as antioxidant) were also used in each assay. All reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample. In order to avoid a temperature effect, only the inward 60 wells were used for experimental purposes, while the outward wells were filled with 200 μl of distilled water. The antioxidant capacity was expressed as millimoles of Trolox equivalents per kilogram of walnuts. The data are shown on FW basis.

Statistical analysis

For statistical analysis, IBM SPSS Statistics (version 19.0.; IBM, North Castle, New York, USA) statistical package was used. Analysis of variance (ANOVA) was used once the assumption of normality was tested. In variables where significant differences ($p < 0.05$) were obtained, the Tukey's honest significant difference (HSD) test was applied to determine the existence of differences between means, establishing a confidence level of 95%. To study the relationship between quantitative variables, Pearson's correlation was accomplished.

Tab. 1. In vitro availability of total phenolic compounds in walnut.

Variety	Total phenolic compounds content	
	Before digestion [g·kg^{-1}]	After in vitro digestion [g·kg^{-1}]
Algaida	10.089 ± 0.555 ^{bcdhj}	3.733 ± 0.205 ^{bcdefghij}
Amigo	7.431 ± 0.238 ^{adfij}	2.826 ± 0.882 ^{acdefg}
Chico	7.624 ± 0.719 ^{adij}	1.448 ± 0.136 ^{abdefghij}
Eureka	13.028 ± 1.395 ^{abcefghi}	2.084 ± 0.223 ^{abcij}
Franquette	8.693 ± 0.238 ^{dj}	1.999 ± 0.054 ^{abcij}
Payne	9.407 ± 1.608 ^{bdj}	1.975 ± 0.337 ^{abcij}
Pedro	8.468 ± 1.204 ^{dj}	2.117 ± 0.301 ^{abcij}
Serr	7.531 ± 0.985 ^{adij}	2.033 ± 0.266 ^{abcij}
Sunland	10.086 ± 1.142 ^{bcdhj}	3.025 ± 0.342 ^{acdefgh}
Tehama	12.969 ± 0.831 ^{abcefghi}	2.982 ± 0.191 ^{acdefgh}

Results are expressed as mean ± standard deviation, as grams of gallic acid equivalents per kilogram of walnuts. The data are shown on fresh weight basis.
Letters in superscript show significant differences ($p \leq 0.05$) compared to: a – Algaida, b – Amigo, c – Chico, d – Eureka, e – Franquette, f – Payne, g – Pedro, h – Serr, i – Sunland, j – Tehama.

Tab. 2. In vitro availability of antioxidant capacity of walnut determined by ORAC method.

Variety	Antioxidant capacity	
	Before digestion [mmol·kg^{-1}]	After in vitro digestion [mmol·kg^{-1}]
Algaida	207.89 ± 17.28 ^{bcdj}	70.68 ± 6.39 ^{bdefgij}
Amigo	165.08 ± 14.76 ^{adij}	61.08 ± 5.46 ^{ad}
Chico	164.76 ± 21.37 ^{adij}	72.49 ± 9.40 ^{degij}
Eureka	270.91 ± 21.08 ^{abcfgh}	116.49 ± 9.06 ^{abcefghij}
Franquette	177.08 ± 21.92 ^{dij}	51.35 ± 6.35 ^{acd}
Payne	190.37 ± 6.57 ^{dj}	62.82 ± 2,16 ^{ad}
Pedro	202.72 ± 16.04 ^{dj}	50.68 ± 4.01 ^{acd}
Serr	187.42 ± 19.29 ^{dj}	63.72 ± 13.35 ^{d}
Sunland	229.17 ± 23.52 ^{bce}	50.41 ± 5.17 ^{acd}
Tehama	265.43 ± 23.15 ^{abcfgh}	58.39 ± 5.09 ^{acd}

Results are expressed as mean ± standard deviation, as millimoles of Trolox equivalents per kilogram of walnuts. The data are shown on fresh weight basis.
Letters in superscript show significant differences ($p \leq 0.05$) compared to: a – Algaida, b – Amigo, c – Chico, d – Eureka, e – Franquette, f – Payne, g – Pedro, h – Serr, i – Sunland, j – Tehama.

RESULTS AND DISCUSSION

TPC of different varieties of walnut is shown in Tab. 1. As a whole, four varieties presented higher TPC than the others. Algaida, Eureka, Sunland and Tehama TPC values were (expressed as grams of gallic acid equivalents per kilogram of walnuts): 10.089 ± 0.555 g·kg^{-1}, 13.028 ± 1.395 g·kg^{-1}, 10.086 ± 1.142 g·kg^{-1} and 1.2969 ± 0.831 g·kg^{-1}, respectively. In turn, Amigo and Chico presented the minor TPC values of all varieties measured (7.437 ± 0.238 g·kg^{-1} and 7.624 ± 0.7198 g·kg^{-1}). The values obtained were similar to other studies. For example the study of SLATNAR et al. [8] on walnut kernels reported approx. 7.7 g·kg^{-1} in fresh kernels.

When measured by every method, the antioxidant capacity of walnut was different depending on the different genotypes (expressed as millimoles of Trolox equivalents per kilogram of walnuts). Briefly, Algaida, Sunland, Eureka and Tehama where the most antioxidant varieties by the three methods (Tab. 2–4). Algaida antioxidant capacity values were 207.89 ± 17.28 mmol·kg^{-1} (ORAC), 155.77 ± 17.18 mmol·kg^{-1} (DPPH) and 166.56 ± 6.76 mmol·kg^{-1} (ABTS). Similar values were found for Sunland variety, showing 229.17 ± 23.52 mmol·kg^{-1} by ORAC assay. In turn, DPPH assay reported 162.93 ± 24.46 mmol·kg^{-1}; meanwhile, 162.87 ± 5.71 mmol·kg^{-1} was found by ABTS method. Eureka and Tehama varieties exerted even higher antioxidant capacity than Algaida and Sundland. According to ORAC assay, antioxidant capacity of Eureka and Tehama was 270.91 ± 21.08mmol·kg^{-1}and265.43 ± 23.15mmol·kg^{-1}, respectively. Similarly, DPPH assay values of Eureka (218.39 ± 53.77 mmol·kg^{-1}) and Tehama (208.54 ± 59.93 mmol·kg^{-1}) were found to be the highest observed for the different varieties. Finally, ABTS assay values were also similar to the values found by ORAC and DPPH methods. In fact, 219.39 ± 41.54 mmol·kg^{-1} and 193.26.7 ± 26.7 mmol·kg^{-1} was found for Eureka and Tehama, respectively.

DPPH assay results from the present study are in agreement with data on walnut (*Juglans regia* L.) kernels [24], Brazil nut (*Bertholletia excels*) kernels and whole nuts [25] and baru nuts (*Dipteryx alata* Vog.) [26]. ORAC assay results were higher than those previously reported for Brazil nut (*Bertholletia excels*) kernel and whole nuts [25], and more than 100 different foods matrices from the United States, including nuts [27]. Finally, ABTS assay values were higher than those found for fresh and dry hazelnuts, walnuts and seed coat pistachios [16]. Moreover, our values were significantly higher than those obtained for walnut, almond, apricot, raisin, fig and hazelnut in another study [28]. Nonetheless, higher antioxidant capacity was reported for many food matrices in scientific lite-

rature. Slightly higher values were reported for tree nut oils found by ABTS method [29], and for roasted peanut, hazelnut and almonds skins determined by ORAC method [30]. Moreover, it was demonstrated that the consumption of walnuts or walnut meal increased the ORAC activity, measured in plasma of 21 healthy men and postmenopausal women [31], and in healthy individuals [32].

In order to confirm the importance of the phenolics content on the antioxidant capacity of walnut, a Pearson's correlation between antioxidant capacity measured by the three methods and the initial TPC was also accomplished. The resulting correlation was strong with DPPH ($R^2 = 0.836$, $p < 0.01$), ABTS ($R^2 = 0.883$, $p < 0.01$) and ORAC ($R^2 = 0.934$, $p < 0.01$) methods. As commented above, phenolic compounds can exert antioxidant activity and be beneficial for the prevention of several diseases [32]. From this point of view, TPC seems to be quite responsible of the antioxidant capacity of the different walnut, independently of the study method.

Importance of availability of phenolic compounds is widely known, as TPC and the subsequent antioxidant capacity of walnut are affected by degradation in the intestinal tract. on the basis of the analysis of TPC of 10 different genotypes of walnut after in vitro digestion, we observed an average decrease of 74.1 % compared to the initial TPC (Fig. 1). Comparing TPC the different varieties before and after in vitro digestion (Tab. 1), it is notable that dialysed Algaida and Amigo varieties showed higher in vitro availability of TPC. In fact, Algaida had an in vitro availability of 37 %, while Amigo's availability was 38 %. In contrast, Eureka variety, exhibiting the highest TPC after in vitro digestion, presented the lowest in vitro availability from all varieties (16 %).

This phenomenon was previously studied with other food matrices. Pérez-Vicente et al. [33] obtained similar results to those obtained in the present study, when determining in vitro bioavailability of TPC in pomegranate juice. After in vitro digestion, pomegranate juice showed only 29 % of TPC in the initial juice fraction. Similarly, in vitro availability of TPC in apple [34], red cabbage [35] and cherry [36] was lower than 30 %, likewise in other fruit matrices [37]. Generally, minor in vitro TPC availability was reported for the majority of food matrices. Some examples are raspberries and red wine, showing 10.3 % and 7.2 % of the initial values of TPC, respectively [38, 39]. Conversely, Tagliazucchi et al. [40] reported a 63 % of TPC availability after subjecting grapes to a simulated digestion. However, these authors used a different enzymatic method for simulation of chewing, besides a different method than Folin-Ciocalteu for measurement of TPC. Similarly, Bouayed et al. [34] reported between 44.6 % and 62.7 % of TPC bioavailability for different varieties of apples. Cilla et al. [41] reported TPC bioavailability

Tab. 3. In vitro availability of antioxidant capacity of walnut determined by DPPH method.

Variety	Antioxidant capacity	
	Before digestion [mmol·kg^{-1}]	After in vitro digestion [mmol·kg^{-1}]
Algaida	155.77 ± 17.18	35.82 ± 3.60^{dj}
Amigo	130.58 ± 15.45^{dj}	27.42 ± 3.24^{cdhj}
Chico	160.28 ± 38.02	51.28 ± 12.29^{b}
Eureka	218.39 ± 53.77^{begh}	63.33 ± 15.59^{ab}
Franquette	124.87 ± 27.23^{dj}	42.45 ± 9.51
Payne	160.42 ± 28.08	49.73 ± 8.70
Pedro	134.21 ± 32.05^{dj}	49.65 ± 11.85
Serr	137.05 ± 35.96^{dj}	54.82 ± 14.38^{b}
Sunland	162.93 ± 24.46	43.99 ± 6.60
Tehama	208.54 ± 59.93^{begh}	70.90 ± 20.37^{abei}

Results are expressed as mean ± standard deviation, as millimoles of Trolox equivalents per kilogram of walnuts. The data are shown on fresh weight basis.
Letters in superscript show significant differences ($p \leq 0.05$) compared to: a – Algaida, b – Amigo, c – Chico, d – Eureka, e – Franquette, f – Payne, g – Pedro, h – Serr, i – Sunland, j – Tehama.

Tab. 4. In vitro availability of antioxidant capacity of walnut determined by ABTS method.

Variety	Antioxidant capacity	
	Before digestion [mmol·kg^{-1}]	After in vitro digestion [mmol·kg^{-1}]
Algaida	166.56 ± 6.76^{dh}	43.30 ± 1.96^{cdefghij}
Amigo	137.41 ± 22.09^{dj}	39.84 ± 6.41^{cdeghij}
Chico	147.95 ± 16.27^{dhj}	60.66 ± 6.67^{abdefghij}
Eureka	219.39 ± 41.54^{abcefgh}	74.59 ± 14.12^{abcefghij}
Franquette	132.94 ± 22.23^{dj}	22.60 ± 3.78^{abcd}
Payne	158.10 ± 12.25^{dh}	30.04 ± 2.32^{acd}
Pedro	139.29 ± 33.96^{dj}	20.89 ± 5.09^{abcd}
Serr	103.30 ± 3.87^{acdfij}	20.66 ± 0.77^{abcd}
Sunland	162.87 ± 5.71^{dh}	21.17 ± 0.74^{abcd}
Tehama	193.78 ± 26.70^{bcegh}	25.19 ± 3.48^{abcd}

Results are expressed as mean ± standard deviation, as millimoles of Trolox equivalents per kilogram of walnuts. The data are shown on fresh weight basis.
Letters in superscript show significant differences ($p \leq 0.05$) compared to: a – Algaida, b – Amigo, c – Chico, d – Eureka, e – Franquette, f – Payne, g – Pedro, h – Serr, i – Sunland, j – Tehama.

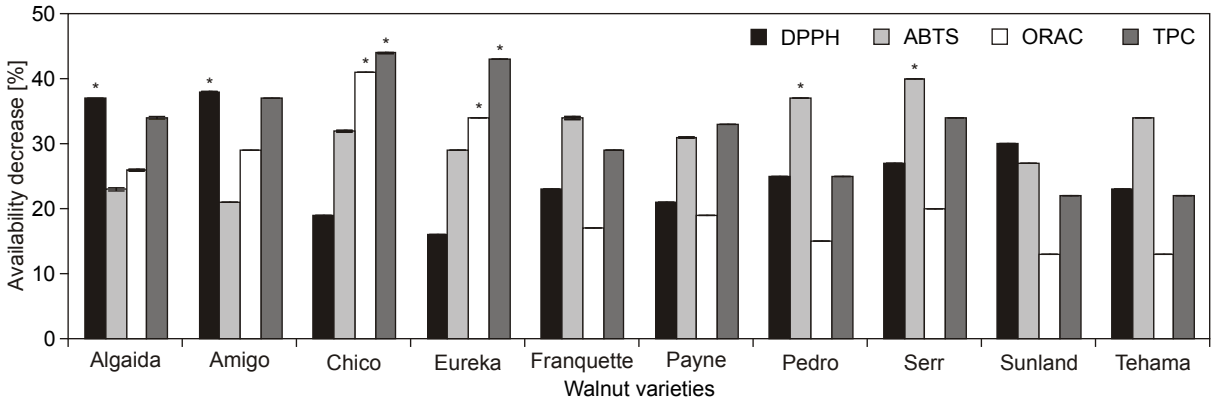

Fig. 1. Total phenolic compounds content and antioxidant capacity availability of the 10 walnut genotypes after in vitro digestion.

The statistic differences were determined by ANOVA and Tukey's Honest Significant Difference. * indicates significant differences. DPPH, ABTS, ORAC – values determined by DPPH, ABTS and ORAC assays, respectively. TPC – total phenolic compounds.

values between 74 % and 96 % for different fruit juices.

The antioxidant capacity after the simulated in vitro digestion was also determined (Fig. 1). ABTS assay was the method with minor availability. On average, antioxidant capacity determined by ABTS method after in vitro digestion was only the 23 % of the that in the initial fraction. Chico and Eureka were the varieties with the highest values, showing availability on the basis of antioxidant capacity found by ABTS method of 41 % and 34 %, respectively. Meanwhile, Franquette, Payne, Pedro, Serr, Sunland and Tehama showed the minor available antioxidant capacity measured by ABTS method, not reaching up to 20 %. Simultaneously, the mean availability of antioxidant capacity after in vitro digestion determined by DPPH method reached up to 31 %. The highest values were with Serr and Pedro, 40 % and 37 %, respectively. Meanwhile, Algaida and Amigo only reached antioxidant availabilities of 23 % measured by DPPH method.

Finally, in vitro availability of antioxidant capacity measured by ORAC method was 32 %. Clearly, the highest antioxidant capacity after simulated digestion was determined for Eureka and Chico varieties, showing a rate of 42 % and 43 %, respectively ($p < 0.05$). In turn, values for Sunland and Tehama were 22 % in both cases, showing the lowest antioxidant capacity availability measured by ORAC method.

Our results are in line with several literature data. PERALES et al. [42] obtained significantly ($p \leq 0.05$) lower values of ABTS and ORAC methods after in vitro gastrointestinal digestion of different fruit juices for children (approx. 19 % of the initial antioxidant activity). In a study conducted for blueberries, TAVARES et al. [43] reported availability of 16 % by ORAC method. Reasonable loss (approx. 25 %) of the antioxidant capacity upon in vitro digestion was reported for green and black tea extracts [44]. However, an increase in the antioxidant activity was reported for different foods like juices, bread and millet, after subjecting them to simulated digestion [41].

CONCLUSION

On the basis of our results, the antioxidant capacity of walnut is dependent on their total phenolics content. This fact was relevant comparing the three different methods for the analysis of antioxidant capacity. Therefore, the dietary intake of walnuts for human health benefits needs to take into account the source, mainly regarding total phenolics content and the high values of antioxidant capacity as determined by three different methods. However, the availability of total phenolics and antioxidant capacity is limited, because of the wide inherent variation in total polyphenol content in different walnut genotypes. After in vitro digestion, the dialysate of Algaida variety was the one with the highest total phenolics content compared to other varieties. The antioxidant capacity was different depending on the method used. However, Algaida, Eureka, Sundland and Tehama were overall the most antioxidant varieties. Despite the high total phenolics content and antioxidant capacity of walnut, more bioavailability studies are required, with the aim to attribute the beneficial effects of walnuts to their phenolics content.

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