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HPLC-DAD optimization of quantification of vescalagin, gallic and ellagic acid in chestnut tannins

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ABSTRACT

The quantification of hydrolysable polyphenols such as gallic, ellagic acid and vescalagin by HPLC-DAD is classically run after methanol extraction as a reference solvent. Water extraction is usually discarded because of a lower obtention of total polyphenol content compared to methanol extraction. In our study, methanol was compared to water extraction in both the total polyphenol content method and the HPLC-DAD analysis. Total polyphenol content in water extraction was lower than in methanol extraction, but water extraction gave better results on HPLC-DAD. In conclusion, total polyphenol content cannot be used as reference to choose the solvent of extraction to quantify some polyphenols by HPLC-DAD because of the specific properties of each polyphenol. Indeed, recovery results obtained on hydrolysable polyphenols with water extraction were better and with a lower variability than following methanol extraction.

1. Introduction

Tannins are natural extracts frequently considered as a valuable alternative to antibiotics in intensive animal farming. They are rich in polyphenols, organic compounds naturally secreted by plants for their own protection against UV radiation and aggression by pathogens. Polyphenols regroup 3 categories: flavonoids, stilbenes and phenolic compounds, to which tannins belong to. There are, at least, 2 categories of tannins: 1) the hydrolysable and non-hydrolysable (also called condensed) tannins; 2) the proanthocyanidins and procyanidins. Chestnut tannins, coming from chestnut wood extraction are more and more used in animal feed because of their health properties and more especially of their antimicrobial [1] and anti-oxidant activity [2] *in vitro*. They, indeed, contain high amounts of hydrolysable polyphenols (HP) which include gallotannins (among which gallic acid (Fig. [1(A)])) and ellagitannins (such as vescalagin (Fig. [1(C)]), the major one), or ellagic acid (Fig. [1(B)]). The interest of tannins may come from their ability to scavenge free radicals thus forming stabilized chemical complexes and negating their effects [3]. Several studies have also demonstrated these anti-microbial and antioxidant properties during industrial extraction [4], transformation process [5], or after their inclusion in pig skin gelatin [6].

However, to be used for animal feed, the maximal amount of hydrolysable polyphenols shall be present in the chestnut tannins. It has already been shown that several parameters of the extraction process such as the raw material (peels, wood, bark, etc …) the type of solvent (water, methanol, ethanol) [7], and the time/temperature couple, may affect, not only, the composition of polyphenols

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present in the tannins but also their properties [8].

Most of the time, the quantification of tannins polyphenols starts by an extraction step in which the solvent used to measure hydrolysable polyphenols from the tannin matrix relies on the best result obtained from the analysis of total polyphenols by the method of Folin-Ciocalteu. From the literature, hydrolysable tannins seem to be more efficiently extracted in water thanks to their water solubility [9] while total polyphenols are usually extracted in methanol. Thus, the choice of the extraction solvent is essential to quantify HP since it will condition the results obtained and will change interpretation of the data obtained. To our knowledge, and so far, none of the methods described in the literature based the choice of this extraction method on the one used to extract the tannins from the wood co-product apart from a recent paper from Fulcrand's group [7]. In this work, the authors used for polyphenol extraction the same solvent as the one used to extract the tannins (methanol or water). However, most of the time, the extraction method of tannins is not specified by the supplier. This does not allow to use this approach most of the time.

As thus, it seems to be important to develop a new approach which does not rely on the method of extraction of the tannins from the wood and which is adapted to the type of polyphenol to be measured. This is especially important when studying the evolution of its composition to obtain a correct quantification of this hydrolysable polyphenols present in tannins (obtained from a previous extraction). The aim of this study was to improve the extraction method to quantify 3 main hydrolysable polyphenols of chestnut tannins: gallic acid, ellagic acid and vescalagin.

2. Materials and methods

2.1. Chemicals and samples

Methanol and acetonitrile HPLC Grade, formic acid (assay 98%), sodium carbonate (>99%), sodium hydroxide solution (NaOH) 0.1 M, vescalagin (assay ≥95%) (PubChem CID:5458626), gallic acid (assay ≥98.5%) (PubChem CID:370) and ellagic acid (assay ≥95%) (PubChem CID:5281855), Folin-Ciocalteu reagent were provided by Sigma-Aldrich (Saint-Quentin-Fallavier, France). Vescalagin was prepared in ultra-pure water at a concentration of 500 μg/mL and stored at - 80 °C. Standard working solution were prepared daily at the appropriate dilution to make a five-point regression curve. Vescalagin was prepared and diluted in ultra-pure water, gallic acid was dissolved in methanol and then diluted in ultra-pure water. Finally, ellagic acid was dissolved in ultra-pure water and NaOH solution and then the final dilution was carried out in ultra-pure water.

Three commercial chestnut wood samples (TAN A to TAN C) were obtained from different European suppliers and regions, and analyzed in this study. All samples were stored as powder in the dark at room temperature.

2.2. Total phenolic (TP) content

Total polyphenol content was determined by the Folin-Ciocalteu method with slightly adapted modifications [10]. Briefly, 150 mg of tannin were dissolved in 25 mL of methanol or ultra-pure water and centrifuged at 13 000×*g* for 15 min and then filtered with 0.45 μm PTFE (methanol solvent) or 0.45 μm PET (water solvent) filter. Then, 100 μL of extract of the previous extract were added to 100 μL of methanol: water solution (80:20 - v:v) and 100 μL of Folin-Ciocalteu reagent. The mixture was allowed to stand for 10 min at room temperature before adding 700 μL of sodium carbonate 7.5% (m:v) were added to the mix and placed 20 min at 40 °C in a water bath.

After centrifugation, absorbance has been read at 750 nm with an Evolution 201 UV–visible spectrophotometer (ThermoFisher-Scientific, Les Ulis, France).

The TP content has been expressed in mg of gallic acid equivalent (GAE)/g of tannin extract. Each result of both methods has been expressed as a mean of triplicates.

2.3. Polyphenols extraction from chestnut wood sample

To quantify vescalagin, gallic and ellagic acid, 10 mg of chestnut wood sample were dissolved in 5 mL of methanol or in water following a protocol adapted from Comandini and collaborators [11]. After preparing the mixes, they were vortexed for 1 min, and then homogenized by rotation at 30 rpm for 30 min at room temperature before being centrifuged at 5500×*g* for 10 min. All samples were then filtered with 0.45 μm PTFE filters and then diluted at a ratio of 1:2 and 1:4 in methanol or water.

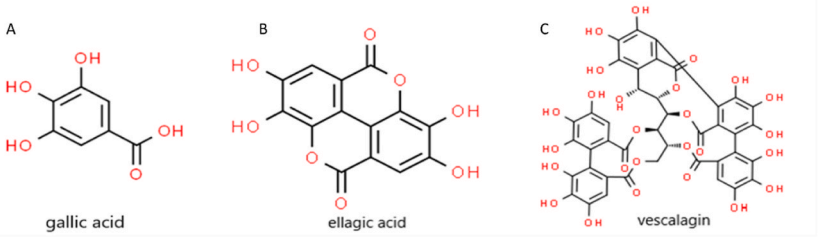

Fig. 1. Chemical structures of gallic acid (A), ellagic acid (B) and vescalagin (C).

2.4. HPLC-DAD analysis

The analysis was performed using a high-performance liquid chromatography (HPLC) system (Ultimate 3000, ThermoFisher-Scientific, Les Ulis, France) coupled with a PDA detector. A C18 Luna column (size 250 × 4.6 mm, 5 μm) from Phenomenex (Le Pecq, France) was used, equipped with precolumn with the same phase and thermostated at 40 °C. The mobile phase consisted of HPLC water with 0.05% formic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.7 mL/min. The gradient was: 0–5 min 0% B; 5–25 min 5% B; 25–40 min 30% B. Ultraviolet chromatograms were recorded at 234 nm, 250 nm and 280 nm to respectively detect vescalagin, ellagic acid and gallic acid. The identification by UV of gallic acid, vescalagin and ellagic acid was performed thanks to commercial standards. A five-point regression curve has been realized for each standard: gallic acid ($r^2 = 0.99$), vescalagin ($r^2 = 0.99$) and ellagic acid ($r^2 = 0.99$).

Limits of detection (LODs) and limits of quantification (LOQs) have been evaluated for each hydrolysable polyphenol. They were obtained by serial dilution of each standard. The LODs and LOQs were determined by the signal to noise (S/N) ratio of respectively 3 and 10.

2.5. Recovery

To calculate the recovery rate, ellagic acid, gallic acid and vescalagin were quantified in each tannin following both the two extraction methods (water or methanol). This quantification has allowed to determine the necessary amount of the targeted polyphenol (adapted to each tannin sample and to the method of extraction) to add to the initial quantity of tannins in order to confirm the validity of the measure. The recovery with water extraction has been measured from samples with either 1/2 or 1/3 of addition depending on the polyphenol of interest, while the recovery measured after methanol extraction followed by methanol or water dilution has only been determined with 1/3 of addition.

2.6. Statistical analysis

All analyses were performed in triplicates. Data were expressed as mean ± standard deviation (SD) and relative standard deviation (RSD in %) established using Microsoft Excel (Microsoft Office 2013).

Data were analyzed using the Kruskal-Wallis non-parametric test. For the comparison of operator incidence on polyphenols measure, a two-way ANOVA was run. Analyses were realized with the GraphPad Prism software (GraphPad Prism V5, GraphPad Software, San Diego, CA, USA). A value of $p < 0.01$ was considered to be significant.

3. Results and discussion

3.1. Total phenolic content

Using methanol extraction, we have observed that TP content were quite similar between samples especially for TAN A and TAN B, where both TP content were around 523 mg GAE/g of dry sample. The TP content of TAN C was slightly lower with 496.0 mg of GAE/g of dry sample but there was no significant difference between the three samples. The TP content obtained with methanol extraction was highly similar to the TP content of chestnut tannins in a previous study [11]. In their work, the results ranged from 23.9 g GAE/100 g sample to 56.1 g GAE/100 g sample. Moreover, compared to other tree species, the chestnut TP content was higher than in Salvia species [12] or Malaysian plant [13].

Using water extraction, the TP content values were basically lower than with the methanol extraction. If we refer to the results from Table 1, methanol extraction should be the one to choose. But this method is not specific enough to identify and quantify the polyphenols present in each tannin sample. Indeed, the intensity of coloration obtained is not specific to HP, since the higher TP content obtained using methanol compared to water solvent is due to the extraction of different types of polyphenols. It does not mean that HP content extracted with methanol will be higher. HPLC-DAD analysis can be more specific and characteristic of the composition of hydrolysable tannins. In previous studies, the use of methanol as an extraction solvent has been recommended based on results obtained with the Folin-Ciocalteu method. Nevertheless, a large part of hydrolysable tannins is hydrosoluble and is degraded when tannins are extracted and conserved in ethanol [14]. It would be interesting to investigate if there is the same impact with methanol. This could explain the interest of using water dilution under methanol extraction in order to reduce any potential degradation [15,16]. In order to quantify HP in chestnut wood samples, we have targeted vescalagin, ellagic and gallic acid, as references. Using this approach, we will confirm if the TP content is correlated to the HP content in each chestnut bark sample, and the impact of extraction solvent.

Table 1
Total phenolic content (mg GAE/g of tannin extract) by the Folin-Ciocalteu method in TAN A, TAN B and TAN C.

	TP content (methanol)	SD	RSD (%)	TP content (water)	SD	RSD (%)
TAN A	523.6[a]	19.2	3.7	243.2[b]	17.3	7.1
TAN B	523.3[a]	14.1	2.7	244.5[b]	8.3	3.4
TAN C	496.0[a]	27.4	5.5	248.5[b]	4.2	1.7

For ellagic acid, the solvent used did not change the regression curve (data not shown) whereas the regression curve of gallic acid and vescalagin were highly influenced by the solvent used (data not shown).

Chestnut tannins have also been extracted in methanol or in water and then analyzed by HPLC-DAD as described previously. The resolution of chromatograms obtained (Figure (2[A,B])) was better under water extraction (Figure (2[A])) than under methanol extraction (Figure (2[B])) for our three samples TAN A, TAN B and TAN C. Furthermore, thanks to water extraction, the separation of peaks was better, and their resolution was enhanced.

To quantify gallic acid, vescalagin and ellagic acid, we have chosen to compare their quantification using the recovery rate after water or methanol extraction.

3.2. Method validation

As water extraction for the quantification of hydrolysable tannin is uncommon, recovery (Tables 2 and 3) and reproducibility (Table 4) have been evaluated to validate the water extraction method.

Recovery percentages after water extraction ranged between 95.6 and 104.6% for gallic acid, between 98.2 and 109.0% for vescalagin and between 88.9 and 109.3% for ellagic acid (Table 2). Means of recovery for each polyphenol were respectively 100.1, 102.5 and 97.6%.

Quantification of hydrolysable tannins has been also made after methanol extraction coupled with methanol or water dilution (Table 3). Indeed, in the literature, the methanol extraction method is often chosen based on total phenolic content [11,17,18]. In our study, the recovery after methanol extraction has been performed using 1/3 of supplementation from the initial quantification of each tannin. Methanol extraction followed by dilution with the same solvent dilution was adapted to quantify ellagic acid. However, it seemed to be more fluctuant with gallic acid as the mean of recovery ranged from 88.7 to 96.8% and with a high variability (RSD around 24.9%). At last, the recovery for vescalagin was highly overestimated with this method, with recovery percentages ranging from 314.3 to 449.0%.

3.3. HP quantification after water extraction

Quantification of ellagic acid, gallic acid and vescalagin has been evaluated after the validation of the water extraction method (Table 4). TAN B had the highest ellagic acid content with 10.0 mg/g dry matter whereas TAN A and C had respectively 4.3 and 4.8 mg of this polyphenol/g of dry matter. Gallic acid content ranged between 19.2 mg/g and 37.9 mg/g. At last, vescalagin was highly present

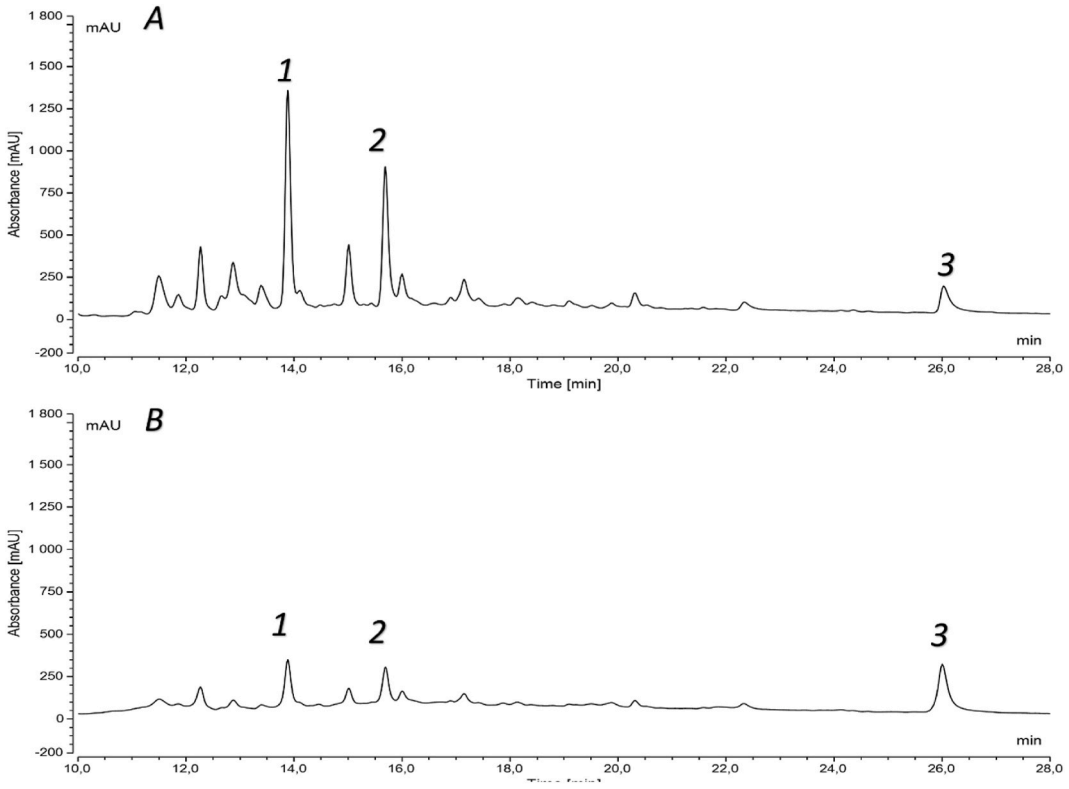

Fig. 2. Chromatograms of TAN A after water (A) or methanol (B) extraction obtained by HPLC-DAD at 250 nm. Peak identification 1: gallic acid, 2: vescalagin, 3: ellagic acid.

Table 2
Recovery rate of gallic acid (1), vescalagin (2) and ellagic acid (3) extracted from chestnut tannins with water (H_2O) as solvent obtained after HPLC-DAD.

			TAN A			TAN B			TAN C		
	Extraction solvent	HP	Mean (%)	SD	RSD (%)	Mean (%)	SD	RSD (%)	Mean (%)	SD	RSD (%)
Add $\frac{1}{2}$ of [Cmg/g] in sample	H_2O	1	96.5	0.4	0.5	97.3	1.7	1.7	104.6	2.1	2.0
		2	101.2	0.7	0.6	104.1	0.7	0.7	103.4	6.9	6.6
		3	109.3	3.9	3.6	97.2	1.8	1.9	108.0	4.1	3.8
Add $\frac{1}{3}$ of [Cmg/g] in sample	H_2O	1	103.9	1.4	1.3	102.6	2.0	1.9	95.6	2.9	3.0
		2	98.2	1.7	1.7	99.1	2.1	2.1	109.0	6.0	5.5
		3	88.9	2.3	2.6	86.2	3.3	3.8	96.0	10.3	10.8

Table 3
Recovery rate of gallic acid (1), vescalagin (2) and ellagic acid (3) extracted from chestnut tannins with methanol (MeOH) and diluted ½ with methanol or water as solvent obtained after HPLC-UV.

				TAN A			TAN B			TAN C		
	Extraction solvent	Dilution solvent	HP	Mean (%)	SD	RSD (%)	Mean (%)	SD	RSD (%)	Mean (%)	SD	RSD (%)
Add $\frac{1}{3}$ of [Cmg/g] in sample	MeOH	MeOH	1	88.7	7.0	7.9	96.1	3.0	3.1	96.8	24.1	24.9
			2	314.3	5.2	1.7	346.9	14.5	4.2	449.0	21.1	4.7
			3	109.6	4.7	4.3	101.5	3.7	3.7	104.5	4.0	3.8
	MeOH	H_2O	1	130.2	7.7	5.9	203.8	32.0	15.7	130.1	45.2	28.3
			2	69.6	3.3	4.7	158.7	22.6	14.2	84.5	16.3	19.4
			3	114.3	4.9	4.3	140.8	20.5	14.6	131.0	11.5	8.8

Methanol extraction with water as a dilution agent had the highest rate of variation, the recovery ranged from 69.6 to 203.8% for every polyphenol analyzed whatever tannin sample analyzed.

Table 4
Quantification of ellagic acid, gallic acid and vescalagin from the three tannins by two different operators. Data are expressed in mg/g of dry matter obtained by HPLC-DAD.

	ellagic acid			gallic acid			vescalagin		
	Mean (mg/g dry matter)	RD	RSD (%)	Mean (mg/g dry matter)	RD	RSD (%)	Mean (mg/g dry matter)	RD	RSD (%)
TAN A operator 1	3.8^{b}	0.3	6.6	19.3^{c}	0.4	2.3	25.4^{b}	0.4	1.7
TAN A operator 2	4.3^{b}	0.1	1.3	19.2^{c}	0.6	3.1	26.7^{b}	1.0	3.8
TAN B operator 1	9.9^{a}	0.8	7.8	36.2^{a}	0.9	2.5	9.3^{c}	0.4	4.2
TAN B operator 2	10.0^{a}	0.2	1.8	37.9^{a}	1.6	4.3	8.6^{c}	0.3	3.6
TAN C operator 1	4.7^{b}	0.3	6.0	28.9^{b}	0.5	1.8	61.9^{a}	0.4	0.7
TAN C operator 2	4.8^{b}	0.1	2.8	27.8^{b}	0.1	0.3	62.5^{a}	0.2	0.4

Significant differences are represented by different letters in the same means column.

in TAN C with a mean content of 62.2 mg/g of dry matter. No significant differences in polyphenols quantification had been found between the 2 operators.

LOQ and LOD were the lowest concentrations allowed to quantify or detect molecules of interest. LOQs and LODs respectively were 0.05 μg/g and 0.1 μg/g of tannin for gallic acid, 0.04 μg/g and 1.04 μg/g of tannin for vescalagin and 0.07 μg/g and 0.14 μg/g of tannin for ellagic acid.

The determination of TP content by Folin-Ciocalteu method is not sufficiently specific to choose the extraction solvent to use for an HPLC analysis. Indeed, some molecules of interest will have better extraction in a different solvent than the one showing the highest TP content. In this study, the determination of TP content extracted by water was lower than in an assay using methanol. Even if water extraction does not seem to result in a better rate than following methanol extraction when quantified by Folin-Ciocalteu, this solvent can be the best adapted one depending on the polyphenol studied.

Moreover, it seems that some ellagitannins such as vescalagin are not well conserved when extraction is realized with ethanol. It may explain the 1:2 dilution with water after extraction with methanol described in some publications [11,15,16]. Furthermore, the results obtained by recovery using methanol as a solvent are not conclusive except for ellagic acid. For vescalagin, this solvent seems to be not appropriate according to the results obtained by recovery. At last, for gallic acid, methanol may be used, but variability will be higher compared to the extraction with water. On the other hand, results obtained after methanol extraction followed by water dilution have induced a too high recovery and variability for all polyphenols.

After testing the extraction method with water, recovery obtained for vescalagin, gallic acid and ellagic acid were better and the variability lower than following methanol extraction and even better compared to the methanol extraction coupled with water dilution. Reproducibility and repeatability obtained in Table 4 validate this method, and no difference between the operators has been

found.

Here we compared the method of extraction to identify and quantify ellagitannins and more especially vescalagin using either methanol or water. Methanol extraction of tannins is currently the reference method as confirmed by the literature, not only with chestnut tannin [11] but also with several matrices such as wine [16], grape [15], fruit [19] or medicinal plants [20]. To confirm the interest to revisit the method of extraction of the hydrolysable polyphenols, it would be necessary to investigate if the improvement of the quantification obtained with chestnut tannin will be the same with other matrices analyzed. If confirmed, this would improve the characterization of food matrices rich in polyphenols and help to better evaluate the antibacterial and antioxidants properties of its hydrolysable compounds. Furthermore, it would also help to understand how the process could modify the composition of hydrolysable polyphenols but also how this composition may affect the antibacterial and antioxidant properties.

4. Conclusion

From this work, we can conclude that the TP content obtained by the Folin-Ciocalteu method is not representative of the HP content, which explains why it cannot be used to determine the solvent of extraction for quantification of hydrolysable polyphenols by HPLC. Moreover, we have shown that water can be used as an extraction solvent to quantify gallic acid, ellagic acid and vescalagin by HPLC-DAD. This is particularly interesting because this solvent is more accurate and safer than methanol.

To broaden the insights obtained in this study, it would be interesting to run the same protocol with other hydrolysable polyphenols present in other food matrices to see if water extraction fits to most of them.

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Author contribution statement

Charline RICHARD-DAZEUR: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Philippe JACOLOT, Céline NIQUET-LERIDON: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Luc GOETHALS: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nicolas BARBEZIER, Pauline M. ANTON: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

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

References

[1] L. Marín, E.M. Miguélez, C.J. Villar, F. Lombó, Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties, BioMed Res. Int. (2015), https://doi.org/10.1155/2015/905215.

[2] M.K. Roy, M. Koide, T.P. Rao, T. Okubo, Y. Ogasawara, L.R. Juneja, ORAC and DPPH assay comparison to assess antioxidant capacity of tea infusions: relationship between total polyphenol and individual catechin content, Int. J. Food Sci. Nutr. 61 (2010) 109–124, https://doi.org/10.3109/09637480903292601.

[3] H. Cory, S. Passarelli, J. Szeto, M. Tamez, J. Mattei, The role of polyphenols in human health and food systems: a mini-review, Front. Nutr. 5 (2018) 87, https://doi.org/10.3389/fnut.2018.00087.

[4] C. Cravotto, G. Grillo, A. Binello, L. Gallina, M. Olivares-Vicente, M. Herranz-López, V. Micol, E. Barrajón-Catalán, G. Cravotto, Bioactive antioxidant compounds from chestnut peels through semi-industrial subcritical water extraction, Antioxidants 11 (2022) 988, https://doi.org/10.3390/antiox11050988.

[5] B.W. Obiang-Obounou, G.H. Ryu, The effect of feed moisture and temperature on tannin content, antioxidant and antimicrobial activities of extruded chestnuts, Food Chem. 141 (2013) 4166–4170, https://doi.org/10.1016/j.foodchem.2013.06.129.

[6] C. Peña-Rodriguez, J.F. Martucci, L.M. Neira, A. Arbelaiz, A. Eceiza, R.A. Ruseckaite, Functional properties and in vitro antioxidant and antibacterial effectiveness of pigskin gelatin films incorporated with hydrolysable chestnut tannin, Food Sci. Technol. Int. Cienc. Tecnol. Los Aliment. Int. 21 (2015) 221–231, https://doi.org/10.1177/1082013214525429.

[7] V. Karaseva, A. Bergeret, C. Lacoste, L. Ferry, H. Fulcrand, Influence of extraction conditions on chemical composition and thermal properties of chestnut wood extracts as tannin feedstock, ACS Sustain. Chem. Eng. 7 (2019) 17047–17054, https://doi.org/10.1021/acssuschemeng.9b03000.

[8] T. Gagić, Ž. Knez, M. Škerget, Subcritical water extraction of chestnut bark and optimization of process parameters, Molecules 25 (2020) 2774, https://doi.org/10.3390/molecules25122774.

[9] A. Smeriglio, D. Barreca, E. Bellocco, D. Trombetta, Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects, Br. J. Pharmacol. 174 (2017) 1244–1262, https://doi.org/10.1111/bph.13630.

[10] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158. https://www.ajevonline.org/content/16/3/144. (Accessed 15 December 2022).
[11] P. Comandini, M.J. Lerma-García, E.F. Simó-Alfonso, T.G. Toschi, Tannin analysis of chestnut bark samples (Castanea sativa Mill.) by HPLC-DAD-MS, Food Chem. 157 (2014) 290–295, https://doi.org/10.1016/j.foodchem.2014.02.003.
[12] M. Tosun, S. Ercisli, M. Sengul, H. Ozer, T. Polat, E. Ozturk, Antioxidant properties and total phenolic content of eight Salvia species from Turkey, Biol. Res. 42 (2009) 175–181. S0716-97602009000200005.
[13] S.W. Qader, M.A. Abdulla, L.S. Chua, N. Najim, M.M. Zain, S. Hamdan, Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants, Molecules 16 (2011) 3433–3443, https://doi.org/10.3390/molecules16043433.
[14] J.-L. Puech, C. Mertz, V. Michon, C. Le Guernevé, T. Doco, C. Hervé du Penhoat, Evolution of castalagin and vescalagin in ethanol solutions. Identification of new derivatives, J. Agric. Food Chem. 47 (1999) 2060–2066, https://doi.org/10.1021/jf9813586.
[15] N. de Andrade Neves, P. César Stringheta, I. Ferreira da Silva, E. García-Romero, S. Gómez-Alonso, I. Hermosín-Gutiérrez, Identification and quantification of phenolic composition from different species of Jabuticaba (Plinia spp.) by HPLC-DAD-ESI/MSn, Food Chem. 355 (2021), 129605, https://doi.org/10.1016/j.foodchem.2021.129605.
[16] I. García-Estévez, M.T. Escribano-Bailón, J.C. Rivas-Gonzalo, C. Alcalde-Eon, Development of a fractionation method for the detection and identification of oak ellagitannins in red wines, Anal. Chim. Acta 660 (2010) 171–176, https://doi.org/10.1016/j.aca.2009.10.020.
[17] M. Navarro, N. Kontoudakis, J.M. Canals, E. García-Romero, S. Gómez-Alonso, F. Zamora, I. Hermosín-Gutiérrez, Improved method for the extraction and chromatographic analysis on a fused-core column of ellagitannins found in oak-aged wine, Food Chem. 226 (2017) 23–31, https://doi.org/10.1016/j.foodchem.2017.01.043.
[18] S.A. Vekiari, M.H. Gordon, P. García-Macías, H. Labrinea, Extraction and determination of ellagic acid contentin chestnut bark and fruit, Food Chem. 110 (2008) 1007–1011, https://doi.org/10.1016/j.foodchem.2008.02.005.
[19] D. Fracassetti, C. Costa, L. Moulay, F.A. Tomás-Barberán, Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (Myrciaria dubia), Food Chem. 139 (2013) 578–588, https://doi.org/10.1016/j.foodchem.2013.01.121.
[20] E.Y.A. Salih, R. Julkunen-Tiitto, O. Luukkanen, M.K.M. Fahmi, P. Fyhrquist, Hydrolyzable tannins (ellagitannins), flavonoids, pentacyclic triterpenes and their glycosides in antimycobacterial extracts of the ethnopharmacologically selected Sudanese medicinal plant Combretum hartmannianum Schweinf, Biomed. Pharmacother. 144 (2021), 112264. https://10.1016/j.biopha.2021.112264.