Walnut (*J. regia*) Agro-Residues as a Rich Source of Phenolic Compounds

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**Simple Summary:** Agro-residues are usually discarded as landfill, or burnt or left to decompose in the orchard. The efficient use of these walnut agro-residues would be a strategy that simultaneously helps to preserve the environment and boosts the economic outcome for farmers and companies. While some studies have reported on the content of bioactive compounds in walnut husks, little or nothing is known to date about the bioactive compounds in the buds and bark. Potentially, if walnut parts are used as a valuable source of bioactive compound, they might still be reused for other purposes. The identification and quantification of new phenolics between the different parts of the plant was carried out. It provided valuable data on their phenolic contents, and demonstrated where the extraction of individual phenolics would be meaningful. These data also show origin-related phenolic contents across the cultivars, and thus these phenolic profiles might serve to define the origins of different walnut cultivars. The study will help to propose new directions for further studies essential for agro-food, cosmetics and pharmacy industries.

**Abstract:** The present study was designed to identify and quantify the major phenolic compounds (phenolics) in the inner and outer husks, buds and bark of the Persian walnut, *Juglans regia* L. A comparison across six different cultivars grown in Slovenia was also carried out: ‘Fernor’, ‘Fernette’, ‘Franquette’, ‘Sava’, ‘Krka’ and ‘Rubina’. A total of 83 compounds were identified, which included 25 naphthoquinones, 15 hydroxycinnamic acids, 8 hydroxybenzoic acids, 13 flavanols, 2 flavones, 1 flavanone and 19 flavonols. For the first time, 38 phenolics in the husks, 57 phenolics in the buds and 29 phenolics in the bark were presented in *J. regia* within this study. Naphthoquinones were the major phenolics determined, approximately 75% of all analysed phenolics in the inner husk, 85% in the outer husk, 50% in buds and 80% in bark. The highest content of phenolics was found in the walnut buds, followed by the bark, the inner husk and the outer husk. On the basis of these high phenolic contents, walnut husks, buds and bark represented valuable by-products of the walnut tree. These data also show origin-related phenolic contents across the cultivars, and thus these phenolic profiles might serve to define the origins of different walnut cultivars.

**Keywords:** hydrojuglone; juglone; naphthoquinones; phenolic compounds; 1,4–naphthoquinone; bark; buds; husk

1. **Introduction**

Persian or English walnut (*Juglans regia* L.) is the second most cultivated tree nut worldwide. Walnuts are native to the region stretching from the Balkans eastward to the Himalayas and southwest China. Nowadays, it is widely cultivated across Europe [1]. Walnuts are the third most consumed nut in the world, after almonds (*Prunus amygdalus* Batsch) and hazelnuts (*Corylus avellana* L.) [2]. The walnut kernel represents 50% of the total fruit weight, and it is the only edible part; therefore, it is clear that a lot of walnut agro-residues are generated every year that might have the potential for further, alternative...
use [3]. Walnut agro-residues include: (i) the hard woody shell that protects the seed; (ii) the green husk that is rich in phenolic compounds (phenolics) and that further protects the woody shell in early stages of development; and (iii) the twigs and branches that are usually mulched after the winter pruning, with the bark and buds potentially also containing high levels of phenolics. All of these walnut agro-residues are usually discarded as landfill, or burnt or left to decompose in the orchard [4]. The efficient use of these walnut agro-residues would be a strategy that simultaneously helps to preserve the environment and boosts the economic outcome for farmers and companies [3].

As many different natural agro-residues are inexpensive and available in large quantities, there have been increasing tendencies over the last few decades towards their reuse as natural ingredients instead of chemical treatments [5]. In more recent years, several new research ideas for using walnut agro-residues have been proposed, with most studies focusing on the woody shells as a sorbent for oil [6], for hazardous material removal [7], as an ingredient in the cosmetics industry and as a blasting medium, among others [3].

The situation is quite different for walnut husks, and in particular the branches that include the buds and bark. While some studies have reported on the content of bioactive compounds in walnut husks, which have demonstrated antiradical and antimicrobial effects [3], little or nothing is known to date about the bioactive compounds in the buds and bark. Potentially, if walnut husks are used as a valuable source of bioactive compounds (i.e., mostly phenolics), they might still be reused for the removal of heavy metals from contaminated wastewaters [3,8], as a biofuel [9], for cosmetics [10] and as a natural dye [10,11]. Similarly, walnut bark might be reused as a natural dye [12], to truly make the most of these agro-residues.

Bioactive compounds are extra nutritional constituents that naturally occur in plant and food products. Most of these are secondary metabolites, such as alkaloids, pigments, mycotoxins, plant growth factors and phenolics. In recent years, numerous studies have been carried out that have promoted the benefits of such bioactive compounds for human health, in terms of potential protection against some degenerative diseases, like cancers and diabetes, and against cardiovascular diseases, and as anti-allergens, anti-microbials, anti-inflammatory medicaments and antioxidants, among others [13]. Phenolics have also been used effectively as functional ingredients in foods, as they can prevent mould and bacterial growth, and lipid oxidation [14].

Due to these countless benefits of such bioactive compounds, and of the phenolics in particular, studies have intensified to identify vegetables, fruit, plants and agricultural and agro-industrial residues as sources of phenolics. For walnuts, naphthoquinones and flavonoids have been reported to be the major phenolics [15].

Naphthoquinones are secondary metabolites that have been identified in about 20 plant families, they are most commonly found in Bignoniaceae, Droseraceae, Plumbaginae, Boraginaceae and Juglandaceae families, and they comprise a wide variety of chemical structures based on the naphthalene skeleton. They participate in multiple oxidative processes, serve as important links in electron transport chains, and might also act as defensive compounds in interspecies chemical warfare (i.e., alelopathy). On the basis of these traits, many studies have been exploring the biological and toxicological activities of naphthoquinones, to potentially discover and develop new drugs (e.g., antibacterial, antifungal, antiviral, antiparasitic and antitumor) [16].

Over time, microorganisms tend to develop resistance to antimicrobial agents that are used as therapeutics, which has prompted the search for new effective antimicrobials. There are numerous studies that have documented activities of a variety of naphthoquinones against an array of microorganisms, including viruses, bacteria, fungi and parasites [16]. The most studied naphthoquinones are vitamin K (e.g., anti-inflammatory, decrease of gap-junctional intercellular communication), juglone (e.g., apoptotic, cell-cycle arrest, anti-inflammatory) and plumbagin (e.g., apoptotic, cell-cycle arrest, inhibition of cell invasion, migration and proliferation, anti-inflammatory and neuroprotection) [17].
Therefore, the recovery of these secondary metabolites from walnut agro-residues might generate functional ingredients, and might also add more value to the walnut industry. To effectively recover and use the phenolics from walnut husks, buds and bark, the chemical profiles of each of these agro-residues need to be defined, especially in terms of their individual phenolics. Due to their non-specific mechanisms of action, naphthoquinones also show significant toxicity [16], which can be seen for juglone and its allelopathic effects [18]. However, adequate modifications to naphthoquinone structures might instead produce new and valuable drugs [16].

Therefore, the objective of this study was to define the phytochemical compositions of walnut husks, buds and bark, and expand the discussion on the use of bioactive molecules in these walnut agro-residues. The identification of new phenolics such as naphthoquinones, in particular, and their quantification between the different parts of the plant will provide valuable data on their phenolic contents, and it will demonstrate where the extraction of individual phenolics would be meaningful. Thus, the identification and quantification of the phenolics in different parts of walnut agro-residues might help to propose new directions for further studies essential for agro-food, cosmetics and pharmacy industries. This study follows and upgrades an earlier study by Medic et al. [19] on walnut (peeled kernel and pellicle) on the phenolic and dicarboxylic acid content of the same six cultivars.

2. Materials and Methods

2.1. Plant Materials

Samples of walnut husks, buds and bark were obtained for six walnut cultivars three French cultivars: ‘Fernor’, ‘Fernette’, ‘Franquette’ and three Slovenian cultivars: ‘Sava’, ‘Krka’ and ‘Rubina’. All of these samples were collected on 23 September 2019, at the Experimental Field for Nut Crops in Maribor (Slovenia; 46°34′01″N; 15°37′51″E; 275 m a.s.l.). They were obtained from 24-year-old trees at a planting density of 10 m × 10 m, with all under the same agronomical management and soil and climate conditions. The samples were collected from four trees for each cultivar, for a total of four repetitions per analysis. The samples were collected from the middle third of the branches on the east side of the trees, put in plastic bags and frozen immediately. The inner and outer husks were separated using a peeler, where ~1 mm of the husk was peeled away as outer husk, with the remaining peeled husk as the inner husk. The terms inner and outer husk are used here because the exocarp and mesocarp of the walnut husk cannot be separated completely. Therefore the inner husk represents only the husk mesocarp, while the outer husk represents the exocarp and part of the mesocarp. The samples were then transported to the laboratory of the Department of Agronomy in the Biotechnical Faculty, of the University of Ljubljana (Slovenia), where they were lyophilised, ground into a powder with liquid nitrogen, and stored at −20 °C prior to further analysis.

2.2. Extraction of the Individual Phenolic Compounds

Briefly, 0.25 g of inner and outer husk and bark, or 0.1 g of buds, were extracted using 100% methanol (Sigma-Aldrich, Steinheim, Germany) at a 1:20 (w/v) tissue:methanol ratio. The protocol followed that described by Medic et al. [19].

2.3. HPLC–Mass Spectrometry Analysis of Individual Phenolic Compounds

The phenolics were analysed on an UHPLC system (Surveyor Dionex UltiMate 3000 series; Thermo Finnigan, San Jose, CA, USA) with a diode array detector at 280 nm for hydroxycinnamic acids, hydroxybenzoic acids, flavanols, flavanones and naphthoquinones, and at 350 nm for flavones and flavonols. The spectra were recorded between 200 nm and 600 nm. A C18 column (Gemini 150 × 4.60 mm; 3 µm; Phenomenex, Torrance, CA, USA) was used to separate the phenolics, at 25 °C, as previously described by Medic et al. [19].

The identification of the phenolics was done by tandem mass spectrometry (LCQ Deca XP Max; Thermo Scientific, Waltham, MA, USA) with heated electrospray ionisation
operating in negative ion mode, using the parameters as described by Medic et al. [19]. The mass spectrometry (MS) scanning for analysis was from m/z 50 to 2000, with data acquisition using the Xcalibur 2.2 software (Thermo Fischer Scientific Institute, Waltham, MA, USA). The phenolics were fragmented, with external standards used for the identification and quantification of known compounds. Literature data and MS fragmentation were used for identification of the unknown compounds, which were quantified using similar standards. The levels of the individual phenolics are expressed as mg/100 g dry weight, with their quantification according to the most relevant standard.

2.4. Analysis of Total Phenolics Content

For the full comparisons across the different walnut cultivars, the total phenolics content is represented first as the sum of all of the individual identified phenolics, each of which is expressed in mg/100 g dry weight according to the most relevant standard. A second determination of the total phenolics content was also carried out for the different walnut samples from the different walnut cultivars, with the extractions according to the same protocol as for the individual phenolics. These values for the total phenolics content of the extracts were determined using the Folin–Ciocalteau phenol reagent, as described by Singleton et al. [20], and then processed as described by Medic et al. [19] and Zamljen et al. [21]. These values are expressed in mg gallic acid equivalents/100 g dry weight.

2.5. Chemicals

The following standards were used to identify and quantify the phenolics: apigenin 7-glucoside, kaempferol-3-glucoside, procyanidin B1, quercetin-3-glucoside, ferulic acid, p-coumaric acid (Fluka Chemie GmbH, Buchs, Switzerland); (+) catechin (Roth, Karlsruhe, Germany); 4-O-caffeoylquinic acid, neochlorogenic acid (3-caffeoylquinic acid), myricetin-3-galactoside, quercetin-3-galactoside, quercetin-3-rhamnoside, juglone (5-hydroxy-1,4-naphthoquinone), 1,4-naphthoquinone, caffeic acid, gallic acid, ellagic acid, naringenin, (−)epicatechin (Sigma–Aldrich Chemie GmbH, Steinheim, Germany); and myricetin-3-rhamnoside, quercetin-3-arabinofuranoside, quercetin-3-arabinopyranoside (Apin Chemicals, Abingdon, UK).

The water used for all sample preparation, solutions and analyses was bi-distilled and purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). The acetonitrile and formic acid for the mobile phases were HPLC-MS grade (Fluka Chemie GmbH, Buchs, Switzerland).

2.6. Statistical Analysis

The data were collated using Microsoft Excel 2016, and analysed using R commander. Samples of the inner and outer husks, buds and bark were assayed as four repetitions. The data are expressed as means ± standard error (SE). For determination of significant differences between the data, one-way analysis of variance (ANOVA) was used, with Tukey’s tests. Statistical means at 95% confidence level were calculated to determine the significance of the differences.

3. Results and Discussion

3.1. Identification of Individual Phenolics in Walnut Inner and Outer Husks, Buds and Bark

Based on the existing literature and the use of standard compounds, a total of 83 phenolics were tentatively identified for the inner and outer husks, buds and bark of these walnuts. Of these 83 phenolics, 14 were identified using standards, with fragmentation of both the standards and the addition of external standards used to confirm their identities. The remaining 69 phenolics were tentatively identified according to their pseudomolecular ions ([M – H]−) and specific fragmentation patterns (i.e., MS2, MS3, MS4, MS5). The selected MS spectra of the compounds can also be found in the Supplementary Material.

Most of the phenolics were identified for the buds, followed by the inner husk, the outer husk and the bark. The majority of naphthoquinones and hydroxycinnamic acids...
were in the inner and outer husks, and the majority of hydroxybenzoic acids and flavanols were in the buds. The only flavanone identified was in the buds.

Seven of the naphthoquinones were identified in all of the plant tissues of all of the cultivars: juglone, hydrojuglone, hydrojuglone β-D-glucopyranoside, hydrojuglone rutinoside, hydrojuglone derivative pentoside 2, hydrojuglone derivative rhamnoside and dihydroxytetralone hexoside. As well as these, the inner and outer husks contained a few hydrojuglone derivatives and mostly other naphthoquinones, while the buds and bark contained mainly hydrojuglone derivatives. To the best of our knowledge, 13 of the phenolics indicated here have not been reported for the walnut *J. regia*, or for any other *Juglans* species, or indeed, for any plant tissues: hydrojuglone, hydrojuglone rutinoside, hydrojuglone dihexoside, hydrojuglone derivative 1, hydrojuglone derivative 2, hydrojuglone derivative 3, hydrojuglone derivative 4, hydrojuglone derivative 5, hydrojuglone derivative pentoside 1, hydrojuglone derivative pentoside 2, hydrojuglone derivative pentoside 3, hydrojuglone derivative rhamnoside, hydrojuglone pentose galloyl derivative and hydrojuglone hexose derivative.

Overall, for the inner and outer husks, 38 phenolics were identified, as 17 naphthoquinones, 11 hydroxycinnamic acids, 3 hydroxybenzoic acids, 3 flavanols, 2 flavones and 2 flavanones. For the buds, 57 phenolics were identified, as 13 naphthoquinones, 6 hydroxycinnamic acids, 6 hydroxybenzoic acids, 12 flavanols, 2 flavones, 1 flavanone and 17 flavonols. For the bark, 29 phenolics were identified, as 11 naphthoquinones, 3 hydroxybenzoic acids, 3 flavanols, 2 flavones and 10 flavanols.

Wherever possible, comparisons with authentic standards were performed. The data for all of these phenolics identified for these walnuts are summarised in Table 1 for the inner and outer husks, in Table 2 for the buds and in Table 3 for the bark.

These include mass spectrometry analysis (*m/z*, MS/MS fragmentation) and the standards according to which they were quantified.

In relation to these walnut naphthoquinones, dihydroxytetralone hexoside was identified by fragmentation ion *m/z* 159 ([M – H]−–H₂O–180), and trihydroxytetralone galloyl hexoside by fragmentation ions *m/z* 331 and 271, as reported previously for walnut leaves [22]. Also, 1,4-Naphthoquinone was identified with the help of the standard at *m/z* 173, which yielded MS² fragments at *m/z* 111, 155, 129 and 145, which were previously mistakenly reported as juglone in *Juglans mandshurica* [23]. Juglone was identified with the help of the standard at *m/z* 189, which yielded an MS² fragment of *m/z* 161 and MS³ fragments of *m/z* 117 and 133. Hydrojuglone β-D-glucopyranoside was identified from its fragmentation that yielded an ion at *m/z* 175, defining the loss of a hexosyl moiety (-162) [24]. The MS³ *m/z* fragment of hydrojuglone β-D-glucopyranoside corresponded to the predicted LC-MS spectrum in a negative scan from the Human Metabolome Database, which yielded fragment ions of *m/z* 131, 157, 103 and 115.

Other phenolics identified through their fragmentation patterns included: hydrojuglone and its derivatives through the distinct fragment ions MS⁶ *m/z* 175 and MS⁹⁺¹ *m/z* 131, 157, 103, 147 and 115, as seen for the fragmentation of hydrojuglone β-D-glucopyranoside; 5-hydroxy-2,3-dihydro-1,4-naphthalenedione through its fragmentation pattern of MS² ions *m/z* 131 [M–H−CO₂]−, 147 [M–H–CO]−, 157 [M–H₂O]− and 129 [M–H–CO–H₂O]−; regiolone through its fragmentation pattern of MS² ions *m/z* 159 [M–H₂O]−, 175 and 131 [M–H₂O–CO]−; 4,5,8-trihydroxynaphthalene-5-D-glucopyranoside through its fragmentation pattern of MS² ions *m/z* 331 [M–H–C₁₀H₁₀O₅]− and 271 [M–H–C₁₂H₁₂O₆]−, and MS⁴ ions *m/z* 211 [M–H–C₁₄H₁₆O₇]− and 169 [M–H–C₁₆H₁₈O₈]−; 1,4,8-trihydroxynaphthalene-1-D-glucopyranoside through its fragmentation pattern of MS² ion *m/z* 327 [M–H–C₁₅H₁₄O₅]− and MS³ ions *m/z* 183 [M–H–C₁₆H₁₅O₇]− and 225 [M–H–C₁₄H₁₄O₆]−; bis-juglone through its fragmentation pattern of MS² ions *m/z* 345 [M–H–H₂O]−, 317 [M–H₂O–CO]− and 301 [M–H₂O]−; and p-hydroxymethoxybenzojuglone through its fragmentation pattern of MS² ions *m/z* 383 [M–H–CH₃O]− and 355 [M–H–CH₂O–CO]−, as reported by Huo et al. [23] in *Juglans mandshurica*. These compounds were previously reported in *J. regia*, but are reported here for the first time in the walnut *J. regia*. 
Table 1. Tentative identification of the 38 phenolics from the walnut inner and outer husks, and the standard equivalents used.

| Phenolic                          | Rt (min) | [M − H]⁻ (m/z) | Fragmentation Pattern (m/z) | Equivalents Expressed                  |
|----------------------------------|----------|----------------|----------------------------|---------------------------------------|
| p-Coumaric acid derivative 2     | 7.06     | 343            | 163, 325, 119              | p-Coumaric acid                       |
| Ferulic acid derivative 1        | 8.20     | 221            | 149, 117                   | Ferulic acid                          |
| Neochlorogenic acid (3-caffeoylquinic acid) | 9.90 | 353            | 191, 179, 135              | Neochlorogenic acid                    |
| Ferulic acid derivative 2        | 11.43    | 489            | 193                        | Ferulic acid                          |
| (+)-Catechin                     | 12.34    | 289            | 245, 205, 179, 125         | (+)-Catechin                          |
| 3-p-Coumaroylquinic acid         | 12.34    | 337            | 163, 191, 173              | 4-O-Caffeoylquinic acid                |
| Dihydroxytetralone hexoside      | 12.69    | 339            | 159, 177                   | Juglone                               |
| Caffeic acid derivative 2        | 13.95    | 251            | 207, 179                   | Caffeic acid                          |
| 5-Hydroxy-2,3-dihydro-1,4-naphthalenedione | 13.97 | 175            | 103, 129                   | Juglone                               |
| p-Coumaric acid derivative 3     | 14.51    | 325            | 163, 191, 119              | p-Coumaric acid                       |
| Regiolone                        | 14.79    | 177            | 159, 175, 131              | Regiolone                             |
| (-)-Epicatechin                  | 14.79    | 289            | 245, 205, 179, 125         | (-)-Epicatechin                       |
| Gallic acid derivative 4         | 15.87    | 263            | 243, 201, 187              | Gallic acid                           |
| Hydrojuglone β-D-glucopyranoside | 16.49 | 337            | 175                        | Hydrojuglone                          |
| Trihydroxyltetralone galloyl hexoside | 17.77 | 507            | 331, 271                   | Trihydroxyltetralone galloyl hexoside |
| Hydrojuglone derivative rhamnoside | 18.02 | 449            | 303, 285                   | Hydrojuglone derivative rhamnoside    |
| Quercetin-3-galactoside          | 18.23    | 463            | 301                        | Quercetin-3-galactoside               |
| p-Coumaric acid derivative 4     | 19.64    | 475            | 265, 163                   | p-Coumaric acid                       |
| Gallic acid derivative 5         | 19.80    | 421            | 313, 169                   | Gallic acid                           |
| (epi)Catechins derivative 5      | 24.04    | 469            | 289                        | (epi)Catechins                        |
| 4,5,8-Trihydroxynaphthalene-5-D-glucopyranoside | 20.55 | 507            | 331, 271                   | 4,5,8-Trihydroxynaphthalene-5-D-glucopyranoside |
| Ferulic acid derivative 3        | 21.03    | 521            | 473, 503, 337              | Ferulic acid                          |
| Hydrojuglone derivative pentoside 2 | 21.42 | 435            | 303, 285                   | Hydrojuglone derivative pentoside 2   |
| Caffeic acid derivative 3        | 21.78    | 519            | 489                        | Caffeic acid                          |
| Gallic acid derivative 3         | 22.10    | 489            | 271, 313                   | Gallic acid                           |
| Quercetin-3-rhamnoside           | 22.60    | 447            | 301                        | Quercetin-3-rhamnoside                |
| 1,4-Naphthoquinone               | 24.11    | 503            | 327                        | 1,4-Naphthoquinone                    |
| Hydrojuglone hexoside derivative | 24.86    | 497            | 335                        | Hydrojuglone hexoside derivative      |
| Hydrojuglone derivative 5        | 26.29    | 517            | 175, 341                   | Hydrojuglone derivative 5            |
| Caffeic acid derivative 4        | 26.62    | 499            | 341, 323, 281, 175         | Caffeic acid                          |
| Hydrojuglone 5                   | 28.57    | 175            | 131, 103, 157, 175         | Hydrojuglone                          |
| 1,4-Naphthoquinone               | 28.57    | 173            | 111, 155, 129, 145         | 1,4-Naphthoquinone                    |
| Hydrojuglone rutinoside          | 29.56    | 483            | 175, 325                   | Hydrojuglone rutinoside               |
| Juglone                          | 30.05    | 189            | 161                        | Juglone                               |
| p-Hydroxymethoxybenzobijuglone   | 31.42    | 363            | 345, 317, 319, 301         | p-Hydroxymethoxybenzobijuglone       |
| 5,7-Dihydroxy-3,4-dimethoxyflavone | 32.37 | 343            | 328                        | 5,7-Dihydroxy-3,4-dimethoxyflavone    |

Rt, retention time; [M − H]⁻, pseudo-molecular ion identified in negative ion mode; bold numbers, fragments further fragmented; first fragment number, fragment that was further fragmented if no bold numbers are given.
Table 2. Tentative identification of the 57 phenolics from the walnut buds, and the standard equivalents used.

| Phenolic                      | Rt (min) | [M – H]− (m/z) | Fragmentation Pattern (m/z) | Equivalents Expressed          |
|-------------------------------|----------|----------------|-----------------------------|------------------------------|
| Gallic acid derivative 1      | 8.01     | 345            | 169, 125, 175               | Gallic acid                  |
| Neochlorogenic acid (3-caffeoylquinic acid) | 9.75     | 353            | 191, 179, 135               | Neochlorogenic acid           |
| (epi)Catechin derivative 1    | 9.90     | 357            | 289, 311                    | (+)-Catechin                 |
| Procyanidin dimer derivative 1| 10.40    | 783            | 301, 275                    | Gallic acid                  |
| Procyanidin dimer 1           | 10.58    | 577            | 425, 407, 451, 289          | Procyanidin B1               |
| Procyanidin dimer 2           | 11.62    | 577            | 425, 407, 451, 289          | Procyanidin B1               |
| Neochlorogenic acid           | 9.75     | 353            | 191, 179, 135               | Neochlorogenic acid           |
| (+)-Catechin derivative       | 12.45    | 289            | 245, 205, 179, 125          | Gallic acid                  |
| 3-p-Coumaroarylquinic acid   | 12.55    | 337            | 163, 191, 173               | 4-O-Caffeoylquinic acid       |
| Dihydroxytetralone hexoside  | 12.76    | 339            | 177, 159                    | Juglone                      |
| (epi)Catechin derivative 2    | 13.33    | 325            | 289, 163, 179               | (+)-Catechin                 |
| Hydrojuglone dixhexoside      | 13.89    | 499            | 175                         | Juglone                      |
| Hydrojuglone derivative 1     | 14.44    | 335            | 175, 169, 265, 193          | Juglone                      |
| Hydrojuglone derivative 2     | 15.33    | 281            | 163, 135, 119               | Juglone                      |
| Hydrojuglone β-D-glucopyranoside | 16.54  | 337            | 175                         | Juglone                      |
| Hydrojuglone derivative 2     | 17.15    | 729            | 289, 163, 179               | Juglone                      |
| Myricetin-3-galactoside       | 18.13    | 479            | 316                         | Myricetin-3-galactoside      |
| Hydrojuglone derivative pentoside 1 | 18.51 | 435            | 285                         | Gallic acid                  |
| Gallic acid derivative 2      | 19.28    | 491            | 271, 331                    | Myricetin-3-galactoside      |
| Quercetin glycolyl hexoside   | 19.71    | 463            | 271, 169, 125               | Juglone                      |
| Myricetin pentoside           | 19.99    | 449            | 317, 316                    | Juglone                      |
| Galloyl-3-(epi)catechin       | 20.36    | 441            | 289                         | (+)-Catechin                 |
| Quercetin-3-galactoside       | 20.63    | 463            | 301                         | Juglone                      |
| Myricetin-3-rhamnoside        | 20.61    | 463            | 316                         | Myricetin-3-rhamnoside       |
| Quercetin-3-glucoside         | 20.80    | 463            | 301                         | Quercetin-3-glucoside        |
| Hydrojuglone derivative rhamnoside | 21.17 | 449            | 303, 285                    | Juglone                      |
| Hydrojuglone derivative pentoside 2 | 21.48 | 435            | 303, 285                    | Gallic acid                  |
| Gallic acid methyl ester      | 21.48    | 465            | 285                         | Quercetin-3-arabinopyranoside |
| Quercetin-3-arabinopyranoside | 21.99    | 433            | 301                         | Gallic acid                  |
| Gallic acid derivative 3      | 22.17    | 489            | 271                         | Quercetin-3-arabinopyranoside |
| Quercetin-3-arabinofuranoside | 22.41    | 433            | 301                         | Quercetin-3-arabinofuranoside |
| Quercetin-3-rhamnoside        | 22.55    | 447            | 301                         | Quercetin-3-rhamnoside       |
| Kaempferol pentoside 1        | 23.22    | 417            | 284                         | Quercetin-3-rhamnoside       |
| Kaempferol pentoside 2        | 23.48    | 417            | 284                         | Quercetin-3-rhamnoside       |
| Caffeic acid hexoside derivative | 23.84   | 501            | 341                         | Caffeic acid                 |
| Kaempferol pentoside 3        | 24.06    | 417            | 285                         | Kaempferol-3-glucoside       |
| Kaempferol-3-rhamnoside       | 24.32    | 431            | 285                         | Kaempferol-3-glucoside       |
| (epi)Catechin derivative 4    | 24.56    | 463            | 289                         | Kaempferol-3-glucoside       |
**Table 2. Cont.**

| Phenolic                      | Rt (min) | [M – H]− (m/z) | Fragmentation Pattern (m/z) | Equivalents Expressed |
|-------------------------------|----------|----------------|-----------------------------|-----------------------|
| Hdrojuglone pentose galloyl derivative | 24.85    | 587            | 455                         | 303, 285, 285, 259, 177, 241, 175 | Juglone               |
| Quercetin hexoside derivative 1 | 25.13    | 669            | 463                         | 301, 179, 151          | Quercetin-3-glucoside |
| Procyanidin dimer derivative 3 | 25.71    | 903            | 729                         | 603, 577               | Procyanidin B1        |
| Quercetin hexoside derivative 2 | 26.15    | 639            | 463                         | 301, 179, 151          | Quercetin-3-glucoside |
| Caffeic acid derivative 1      | 26.62    | 499            | 341, 323, 281, 175          | 251, 221, 179, 179, 135 | Caffeic acid          |
| Diferuoyl hexoside            | 27.73    | 531            | 337                         | 193, 178, 134, 149     | Ferulic acid          |
| Hy drojuglone                 | 28.60    | 175            | 131, 103, 157, 175          |                        | Juglone               |
| Quercetin                     | 29.34    | 301            | 179, 151                    |                        | Quercetin-3-glucoside |
| Hy drojuglone rutinoside      | 29.63    | 483            | 175                         | 131, 157, 103          | Juglone               |
| Hy drojuglone derivative 3    | 29.73    | 513            | 175, 337                    | 131, 157, 103          | Juglone               |
| Juglone                       | 30.14    | 189            | 161                         | 117, 133               | Juglone               |
| Naringenin                    | 30.33    | 271            | 151, 177                    |                        | Naringenin            |
| Kaempferol                    | 30.64    | 285            | 151                         |                        | Kaempferol-3-glucoside|
| Santin                        | 31.97    | 343            | 328                         | 313, 285               | Apigenin-7-glucoside  |
| 5,7-Dihydroxy-3,4-dimethoxylavone | 32.34    | 313            | 298                         | 283, 255               | Apigenin-7-glucoside  |

Rt, retention time; [M – H]−, pseudo-molecular ion identified in negative ion mode; **bold** numbers, fragments further fragmented; first fragment number, fragments that were further fragmented if no **bold** numbers are given.

**Table 3. Tentative identification of the 29 phenolics from the walnut bark, and the standard equivalents used.**

| Phenolic                        | Rt (min) | [M – H]− (m/z) | Fragmentation Pattern (m/z) | Equivalents Expressed |
|---------------------------------|----------|----------------|-----------------------------|-----------------------|
| Procyanidin dimer 2             | 11.67    | 577            | 425, 407, 451, 289          | Procyanidin B1        |
| (+)Catechin                     | 12.43    | 289            | 245, 205, 179, 125          | (+)Catechin           |
| Dihydroxytetralone hexoside     | 12.82    | 339            | 177, 159                    | Juglone               |
| Hydrojuglone β-D-glucopyranoside| 16.58    | 337            | 175                         | Juglone               |
| Procyanidin dimer derivative 2  | 17.16    | 729            | 577                         | Procyanidin B1        |
| Ellagic acid derivative         | 17.47    | 467            | 391, 301                    | Ellagic acid          |
| Hydrojuglone derivative 4       | 17.83    | 451            | 319, 325, 301, 193, 151     | Juglone               |
|                                |          |                | 193, 301, 179, 125          | 165, 175, 121, 131    |
|                                |          |                | 319, 325, 301, 193, 151     | 192, 235              |
|                                |          |                | 319, 325, 301, 193, 151     | 215, 257, 283, 175, 151, 147, 131, 157, 129 |
| Hydrojuglone derivative 2       | 18.02    | 465            | 301                         | 257, 229, 185         | Juglone               |
| Hydrojuglone derivative pentoside 1 | 18.21  | 435            | 285, 301                     | Juglone               |
|                                |          |                | 241, 175, 199, 257, 151     | Juglone               |
|                                |          |                | 285, 301                     | 229, 179, 151, 257, 137 |
| Hydrojuglone derivative pentoside 3 | 18.55  | 435            | 285, 301                     | Juglone               |
| Myricetin pentoside            | 19.20    | 449            | 317                         | Myricetin-3-galactoside|
Table 3. Cont.

| Phenolic                         | Rt  | [M – H]⁻ (m/z) | Fragmentation Pattern (m/z) | Equivalents Expressed |
|----------------------------------|-----|---------------|-----------------------------|-----------------------|
| Gallic acid derivative 2         | 19.32 | 491          | 271 | 211, 169, 125 | 168, 124          | Gallic acid         |
| Quercetin galloyl hexoside       | 19.74 | 615          | 463 | 301           | 179, 151          | Quercetin-3-glucoside |
| Myricetin-3-rhamnoside           | 20.33 | 463          | 316 | 271, 287, 179, 164 | 243, 227, 215, 183 | Myricetin-3-rhamnoside |
| Quercetin-3-galactoside          | 20.64 | 463          | 301 | 179, 151       | Quercetin-3-galactoside |
| Quercetin-3-glucoside            | 20.84 | 463          | 301 | 179, 151       | Quercetin-3-glucoside |
| Hydrojuglone derivative rhamnoside | 21.22 | 449          | 303, 285 | 181, 153, 285 | 503, 285 | 241, 175, 257, 199, 151 | Hydrojuglone |
| Hydrojuglone derivative pentoside 2 | 21.47 | 435          | 285 | 241, 175, 257 | Quercetin-3-arabinopyranoside |
| Quercetin-3-arabinopyranoside    | 22.04 | 433          | 301 | 179, 151       | Quercetin-3-arabinopyranoside |
| Gallic acid derivative 3         | 22.19 | 489          | 271, 313 | 211, 169, 125 | 168, 124          | Gallic acid         |
| Quercetin-3-arabinofuranoside   | 22.39 | 433          | 301 | 179, 151       | Quercetin-3-arabinofuranoside |
| Quercetin-3-rhamnoside           | 22.67 | 447          | 301 | 179, 151, 273, 257, 229 | Quercetin-3-rhamnoside |
| Kaempferol-7-hexoside 1          | 23.06 | 447          | 285 | 165, 119, 93   | Kaempferol-3-glucoside |
| Kaempferol-7-hexoside 2          | 28.34 | 447          | 285 | 165, 119, 93   | Kaempferol-3-glucoside |
| Hydrojuglone                     | 28.56 | 175          | 131, 103, 157, 175 | Juglone |
| Hydrojuglone rutinoside          | 29.64 | 483          | 175 | 131, 103, 157 | Juglone |
| Juglone                          | 30.14 | 189          | 161 | 117, 133       | Juglone |
| Santin                           | 31.98 | 343          | 328 | 313, 285       | Apigenin-7-glucoside |
| 5,7-Dihydroxy-3,4-dimethoxyflavone | 32.34 | 313          | 298 | 283, 255       | Apigenin-7-glucoside |

Rt, retention time; [M – H]⁻, pseudo-molecular ion identified in negative ion mode; **bold** numbers, fragments further fragmented; first fragment number, fragments that were further fragmented if no bold numbers are given.
The 15 hydroxycinnamic acids identified through their fragmentation patterns included: neochlorogenic acid (3-caffeoylquinic acid) through its fragmentation, in addition to an external standard; 3-\(p\)-cumaroylquinic acid through its fragmentation pattern of MS \(m/z\) 337, MS\(^2\) \(m/z\) 163, 191 and 173, as reported by Liu et al. [25] and Vieira et al. [22]; \(p\)-coumaric acid derivatives through the \(p\)-coumaric acid fragmentation pattern after being broken down, through the fragmentation patterns of ions \(m/z\) 163 and 119, as reported by Liu et al. [25] and Vieira et al. [22]; ferulic acid derivatives through their fragmentation patterns of MS\(^n\) ion \(m/z\) 193 and MS\(^{n+1}\) ions \(m/z\) 149 and 117, as reported by Vieira et al. [22] and Šuković et al. [26]; and caffeic acid derivatives through their fragmentation pattern of MS\(^n\) ion \(m/z\) 179 (caffeic acid–H), as reported by Vieira et al. [22].

Seven phenolics were identified for hydroxybenzoic acids: gallic acid derivatives, through the gallic acid fragmentation pattern after being broken down, through the fragmentation pattern of ions \(m/z\) 169 and 125, as reported by Li and Seeram [27] and Šuković et al. [26]; bis-(hexahydroxydiphenoyl)-glucose through its fragmentation pattern of MS\(^2\) ions \(m/z\) 301 and 275, and MS\(^3\) ions \(m/z\) 257, 229 and 185, as reported by Medic et al. [19] and Regueiro et al. [28]; and ellagic acid derivatives through the typical fragmentation ions of ellagic acid at \(m/z\) 257, 229 and 185, as reported by Singh et al. [29].

There were 13 flavanols identified through their fragmentation patterns: (+)catechin and (−)epicatechin through their fragmentation patterns, in addition to an external standard, which produced fragment ions \(m/z\) 245, 205 and 179 for both (+)catechin and (−)epicatechin, thus suggesting that standards are needed when determining either of those compounds; epicatechin and catechin derivatives through the (+)catechin and (−)epicatechin fragmentation patterns after being broken down, through the ions \(m/z\) 245, 205 and 179, as seen in standard fragmentation patterns; and procyanidin dimers and procyanidin dimer derivatives through their characteristic fragmentation of MS\(^n\) \(m/z\) 577 and MS\(^{n+1}\) \(m/z\) 425, 407 and 289 [14,30].

The two flavones identified were santin and 5,7-dihydroxy-3,4-dimetoxyflavone, through their fragmentation patterns according to Yan et al. [30]. Both santin and 5,7-dihydroxy-3,4-dimetoxyflavone have been reported for walnut flowers [30], and now for the first time here for walnut inner and outer husks, buds and bark.

The flavanones included the identification of one compound: naringenin, through its fragmentation in addition to an external standard, through the fragment ions \(m/z\) 151 and 177.

The flavonols included the identification of three groups of compounds: (i) myricetin glycosides through their fragmentation pattern of MS\(^2\) ions \(m/z\) 316, 317 and MS\(^3\) ions \(m/z\) 179, 191; (ii) quercetin and quercetin glycosides through their clear fragmentation pattern of MS\(^2\) \(m/z\) 301 and MS\(^3\) \(m/z\) 179, 151; and (iii) kaempferol and kaempferol glycosides through their fragmentation pattern of MS\(^2\) \(m/z\) 284 and 285 and MS\(^3\) \(m/z\) 255 and 227, as reported by Santos et al. [31] and Vieira et al. [22]. Fragmentation patterns with the loss of hexosyl (-162), pentosyl (-132) and rhamnosyl (-146) residues were seen here, as reported by Vieira et al. [22]. Kaempferol-7-hexosides were identified through their fragmentation pattern of MS\(^2\) ion \(m/z\) 285 and MS\(^3\) ions 165, 119 and 93, as reported by Chen et al. [32]. Kaempferol-7-hexosides have been reported previously for Rhamnus davurica [32], but this is the first time for walnut.

3.2. Quantification of Total and Individual Phenolic Compounds for Walnut Inner and Outer Husks, Buds and Bark

The highest contents of phenolics were in the walnut buds, followed by the bark, the inner husk and the outer husk, as shown in Figure 1B.

The highest relative contents of hydroxycinnamic acids and flavones were seen for the inner husk, with the highest relative contents of hydroxybenzoic acids, flavanoles, flavanones and flavonols for the walnut buds, as shown in Figure 1A. The higher absolute contents of phenolics in the walnut buds compared to the bark was mostly because of the higher content of flavanoles, flavonols, hydroxycinnamic and hydroxybenzoic acids in the buds. The content of naphthoquinones was around 11 to 12 g/100 g plant material in both plant tissues. Therefore, walnut buds and bark represent an excellent source of naphthoquinones.
The highest contents of phenolics were in the walnut buds, followed by the bark, the inner husk and the outer husk, as shown in Figure 1B.

**Figure 1.** Relative contents of the phenolics groups for the different walnut tissues, as proportions of total phenolic compounds identified (A) and as g/100 g walnut tissue defined by the most relevant standards (B).

The highest relative contents of hydroxycinnamic acids and flavones were seen for the inner husk, with the highest relative contents of hydroxybenzoic acids, flavanols, flavanones and flavonols for the walnut buds, as shown in Figure 1A. The higher absolute contents of phenolics in the walnut buds compared to the bark was mostly because of the higher content of flavanols, flavonols, hydroxycinnamic and hydroxybenzoic acids in the buds. The content of naphthoquinones was around 11 to 12 g/100 g plant material in both plant tissues. Therefore, walnut buds and bark represent an excellent source of naphthoquinones.

The total naphthoquinones were the major phenolic group determined for the inner and outer husks, and for the buds and bark as well. These represented approximately just over 50% of all of the identified phenolics in the buds, 75% in the inner husk, 80% in the bark and 85% in the outer husk, as shown in Figure 1A. As mentioned above, various naphthoquinones have shown activities against an array of microorganisms, including viruses, bacteria, fungi and parasites [16]. While the walnut buds were a better source of flavanols, hydroxybenzoic acids and flavonols, the inner and outer husks can also be considered as a source of naphthoquinones, with different naphthoquinones in the walnut buds and bark compared to the inner and outer husks, as seen in Table 1.

While the content of phenolics is usually higher in the peel of fruit compared to the flesh [19], here, interestingly, the content of phenolics for the outer husk was much lower than for the inner husk. When considering further the different tissues of walnut plants, the total phenolic contents (both as the summation and the total extracts; Tables 4–7) were higher than any previously reported for walnut shoots [15], leaves [18] or kernels [19], which further justifies the use of the husk, buds and bark as sources of the phenolics.
| Phenolic                        | 'Fernor' | 'Fernette' | 'Franquette' | 'Sava' | 'Krka' | 'Rubina' |
|--------------------------------|---------|-----------|--------------|--------|--------|---------|
| **Naphthoquinones**            |         |           |              |        |        |         |
| 1,4-Naphthoquinone             | 1148.5 ± 109.0 b | 1067.5 ± 95.2 b | 435.3 ± 73.6 a | 1521.8 ± 47.3 b | 2231.8 ± 233.2 c | 1547.1 ± 105.5 b |
| Juglone                        | 593.2 ± 45.9 a | 776.3 ± 39.8 ab | 533.6 ± 34.3 a | 988.9 ± 61.8 a | 852.1 ± 93.4 b | 852.0 ± 38.8 b |
| Hydrojuglone                   | 143.2 ± 13.6 b | 133.1 ± 11.9 b | 54.3 ± 9.2 a | 189.8 ± 5.9 b | 278.3 ± 29.1 c | 192.9 ± 13.2 b |
| Hydrojuglone β-D-glucopyranoside| 502.9 ± 9.9 b | 250.9 ± 17.4 a | 266.3 ± 21.4 a | 761.9 ± 50.1 c | 624.9 ± 31.8 bc | 734.7 ± 69.4 c |
| Hydrojuglone rutinoside        | 125.2 ± 2.3 ab | 93.4 ± 10.6 a | 90.7 ± 14.7 a | 179.2 ± 14.7 bc | 234.1 ± 17.3 c | 200.8 ± 15.5 c |
| Hydrojuglone derivative 5      | 153.9 ± 7.0 b | 75.3 ± 5.1 a | 58.3 ± 10.3 a | 294.1 ± 31.3 c | 286.2 ± 12.4 c | 187.2 ± 22.2 b |
| Hydrojuglone derivative pentoside 2 | 154.8 ± 16.6 bc | 95.9 ± 9.6 ab | 65.4 ± 11.8 a | 246.7 ± 23.2 d | 236.0 ± 21.7 cd | 274.2 ± 22.2 d |
| Hydrojuglone derivative rhamnoside | 324.2 ± 8.4 bc | 155.7 ± 11.6 a | 208.8 ± 25.2 ab | 572.1 ± 45.0 e | 486.3 ± 45.0 de | 403.8 ± 45.1 cd |
| Hydrojuglone hexoside derivative | 137.9 ± 13.6 ab | 97.6 ± 11.8 a | 85.2 ± 20.2 a | 239.9 ± 16.4 c | 194.5 ± 26.3 bc | 153.6 ± 19.7 ab |
| bis-Juglone                    | 110.7 ± 7.0 a | 125.8 ± 11.0 a | 133.8 ± 17.8 ab | 247.9 ± 16.0 c | 194.4 ± 19.1 bc | 175.6 ± 13.2 ab |
| p-Hydroxymetoxybenzobijuglone  | 680.4 ± 92.1 a | 494.3 ± 85.9 a | 440.1 ± 46.6 a | 490.5 ± 60.1 a | 641.6 ± 8.8 a | 528.1 ± 46.9 a |
| Regiolone                      | 672.3 ± 18.6 ab | 511.3 ± 39.8 ab | 775.1 ± 73.8 bc | 808.6 ± 44.6 bc | 936.3 ± 42.8 c | 920.9 ± 38.8 c |
| 4,5,8-Trihydroxy-3,5-dihydroxy-2-naphthoquinone | 709.0 ± 44.7 bc | 479.5 ± 32.6 ab | 412.6 ± 15.3 a | 1276.7 ± 45.1 d | 1241.5 ± 58.5 d | 913.6 ± 88.1 c |
| 1,4,8-Trihydroxy-4,5-dihydroxy-2-naphthoquinone | 335.7 ± 42.6 bc | 151.1 ± 13.6 a | 156.3 ± 21.3 a | 478.0 ± 50.7 c | 339.1 ± 23.2 bc | 262.1 ± 23.6 ab |
| Dihydroxytetralone hexoside    | 88.2 ± 3.6 b | 21.4 ± 9.3 a | 68.9 ± 14.7 a | 106.8 ± 16.7 b | 113.4 ± 11.9 b | 104.2 ± 12.2 b |
| Trihydroxytetralone galloyl hexoside | 169.6 ± 10.2 a | 61.2 ± 10.4 a | 78.4 ± 20.7 a | 240.8 ± 27.2 b | 182.7 ± 12.7 c | 174.0 ± 23.1 b |
| **Hydroxycinnamic acids**      |         |           |              |        |        |         |
| Neochlorogenic acid (3-cafeoylquinic acid) | 28.5 ± 0.8 ab | 17.0 ± 1.2 a | 21.6 ± 1.0 ab | 47.6 ± 5.3 d | 43.0 ± 2.6 cd | 31.9 ± 3.5 bc |
| 3-p-Coumaroylquinic acid       | 494.7 ± 4.3 a | 496.9 ± 26.7 a | 443.4 ± 18.4 a | 712.2 ± 26.1 b | 628.7 ± 32.1 b | 474.2 ± 17.8 a |
| p-Coumaric acid derivative 2   | 11.6 ± 0.5 ab | 8.8 ± 0.8 a | 10.8 ± 0.8 a | 21.6 ± 1.5 d | 17.7 ± 0.6 cd | 14.4 ± 1.4 bc |
| p-Coumaric acid derivative 3   | 16.7 ± 0.6 ab | 12.5 ± 1.1 a | 17.8 ± 1.3 ac | 20.3 ± 2.5 bc | 22.0 ± 1.0 bc | 23.8 ± 1.5 c |
| p-Coumaric acid derivative 4   | 11.1 ± 0.3 b | 6.5 ± 0.5 a | 4.8 ± 0.6 a | 13.3 ± 0.9 b | 11.8 ± 0.6 b | 12.2 ± 1.2 b |
| Caffeic acid derivative 2      | 9.5 ± 0.4 ab | 5.0 ± 1.4 a | 9.3 ± 1.6 ab | 14.1 ± 1.6 b | 14.9 ± 0.9 b | 13.1 ± 1.3 b |
| Caffeic acid derivative 3      | 52.9 ± 3.0 b | 29.7 ± 2.3 a | 25.1 ± 2.9 a | 85.2 ± 4.2 c | 61.4 ± 4.8 b | 64.3 ± 5.6 b |
| Caffeic acid derivative 4      | 4.8 ± 0.5 ab | 3.2 ± 0.8 a | 2.8 ± 0.9 a | 5.4 ± 0.9 ab | 7.2 ± 1.3 b | 4.9 ± 0.8 ab |
| Ferulic acid derivative 1      | 29.8 ± 3.1 bc | 10.5 ± 1.1 a | 18.3 ± 2.0 ab | 34.8 ± 2.7 cd | 40.3 ± 2.2 cd | 43.7 ± 4.3 d |
| Ferulic acid derivative 2      | 19.8 ± 1.3 ab | 12.4 ± 0.6 a | 18.2 ± 1.3 a | 34.5 ± 3.0 c | 27.0 ± 1.2 b | 17.8 ± 2.0 a |
| Ferulic acid derivative 3      | 15.2 ± 1.5 a | 11.2 ± 0.4 a | 10.5 ± 1.5 a | 28.9 ± 1.8 b | 26.9 ± 1.1 b | 28.6 ± 2.2 b |
| **Hydroxybenzoic acids**       |         |           |              |        |        |         |
| Gallic acid derivative 3       | 335.7 ± 9.9 b | 148.7 ± 10.4 a | 240.6 ± 14.6 ab | 897.8 ± 41.4 d | 766.9 ± 27.5 c | 817.6 ± 31.2 cd |
| Gallic acid derivative 4       | 53.8 ± 3.1 b | 21.3 ± 2.2 a | 30.7 ± 2.5 a | 41.4 ± 7.4 ab | 42.9 ± 5.8 ab | 42.6 ± 6.4 ab |
| Gallic acid derivative 5       | 26.4 ± 1.9 bc | 21.0 ± 2.0 ab | 12.7 ± 2.4 a | 38.8 ± 5.0 cd | 44.8 ± 2.9 d | 31.9 ± 1.6 bc |
| Phenolic                              | Inner Husk Phenolic Content per Cultivar (mg/100 g Dry Weight) |
|--------------------------------------|---------------------------------------------------------------|
|                                      | 'Fernor'           | 'Fernette'         | 'Tranquette'       | 'Sava'            | 'Krka'            | 'Rubina'          |
| (++)Catechin                         | 151.7 ± 1.0 a      | 151.5 ± 8.1 a      | 136.1 ± 5.7 a      | 218.1 ± 8.1 b     | 192.1 ± 10.0 b    | 145.4 ± 5.4 a     |
| (−)Epicatechin                       | 398.9 ± 9.9 ab     | 305.1 ± 23.8 a     | 459.9 ± 44.9 bc    | 480.6 ± 27.0 bc   | 557.7 ± 25.7 c    | 546.6 ± 23.7 c    |
| Flavones                             |                    |                    |                    |                   |                   |                   |
| Santin                               | 61.3 ± 6.1 ab      | 51.8 ± 1.3 a       | 56.1 ± 4.7 a       | 92.5 ± 11.4 c     | 89.5 ± 3.5 bc     | 51.2 ± 6.7 a      |
| 5,7-Dihydroxy-3,4-dimetoxyflavone    | 43.1 ± 1.7 a       | 30.4 ± 5.2 a       | 23.1 ± 5.4 a       | 166.8 ± 5.7 d     | 124.4 ± 11.7 c    | 74.3 ± 6.0 b      |
| Flavonols                            |                    |                    |                    |                   |                   |                   |
| Quercetin-3-galactoside              | 72.3 ± 3.1 abc     | 49.2 ± 6.5 ab      | 40.6 ± 3.5 a       | 115.0 ± 15.3 d    | 91.0 ± 10.1 cd    | 85.1 ± 7.6 bd     |
| Quercetin-3-rhamnoside               | 18.3 ± 0.7 bc      | 7.7 ± 2.1 ab       | 6.3 ± 3.0 a        | 22.1 ± 3.0 c      | 24.5 ± 1.5 c      | 24.4 ± 4.2 c      |
| Total naphthoquinones                | 6049.6 ± 224.4 b   | 4590.5 ± 193.6 a   | 3863.0 ± 294.6 a   | 8253.6 ± 192.3 cd | 9073.1 ± 301.2 d | 7624.8 ± 361.0 c |
| Total hydroxycinnamic acids          | 694.6 ± 5.0 ab     | 613.6 ± 27.4 ab    | 582.7 ± 22.6 a     | 1017.7 ± 35.0 c   | 900.2 ± 40.6 c    | 728.9 ± 39.4 b    |
| Total hydroxybenzoic acids           | 415.8 ± 12.1 b     | 190.9 ± 9.7 a      | 284.0 ± 17.2 a     | 977.9 ± 42.3 c    | 854.6 ± 32.5 c    | 892.1 ± 37.8 c    |
| Total flavanols                      | 550.6 ± 9.4 ab     | 456.6 ± 22.7 a     | 596.1 ± 45.9 bc    | 698.7 ± 31.3 cd   | 749.8 ± 18.6 d    | 692.0 ± 19.9 cd   |
| Total flavones                        | 104.4 ± 7.7 a      | 82.2 ± 5.7 a       | 79.2 ± 9.6 a       | 259.3 ± 16.4 b    | 213.9 ± 13.8 b    | 125.5 ± 2.0 a     |
| Total flavanones                     | nd                 | nd                 | nd                 | nd                 | Nd                 | nd                |
| Total flavonols                      | 90.6 ± 3.8 bc      | 56.9 ± 6.5 ab      | 46.9 ± 6.4 a       | 137.1 ± 15.2 d    | 115.5 ± 9.3 cd    | 109.5 ± 11.5 cd   |
| Total phenolics content (summation; relevant standards) x | 7905.5 ± 236.8 b  | 5990.8 ± 204.2 a   | 5451.8 ± 369.6 a   | 11344.4 ± 298.0 cd | 11907.1 ± 228.9 d | 10172.8 ± 426.0 c |
| Total phenolics content (total extracts; mg gallic acid equivalents/100 g dry weight) y | 1447.2 ± 73.7 ab | 1327.2 ± 92.9 a | 1575.1 ± 92.0 ab | 1842.6 ± 175.9 b | 1804.5 ± 26.5 b | 1788.1 ± 85.5 b |

Data are means ± standard error. x expressed as the sum of all of the individual identified phenolics (summation), in mg/100 g dry weight of the most relevant standard. y expressed as the separate analysis of the total phenolics for each extract (total extracts), in mg gallic acid equivalents/100 g dry weight. Means followed by different letters within a cultivar are significantly different (p ≤ 0.05; Tukey’s tests); nd, not detected.
Table 5. Individual phenolics for the walnut outer husks across the six selected cultivars.

| Phenolic                  | 'Fenor'   | 'Fernette'  | 'Tranquette' | 'Sava'   | 'Krka'   | 'Rubina'   |
|---------------------------|-----------|-------------|--------------|----------|----------|------------|
| Naphthoquinones           |           |             |              |          |          |            |
| 1,4-Naphthoquinone        | 128.8 ± 20.4 a | 260.6 ± 47.9 a | 296.5 ± 34.3 a | 800.8 ± 55.3 b | 1079.9 ± 44.7 c | 1026.5 ± 117.7 bc |
| Juglone                   | 431.7 ± 25.1 a | 528.5 ± 37.1 ab | 519.8 ± 8.7 ab | 609.2 ± 18.7 b | 608.3 ± 9.6 b | 838.8 ± 19.5 c |
| Hydrojuglone              | 25.7 ± 4.1 a | 52.0 ± 9.6 a | 59.2 ± 6.8 a | 159.8 ± 11.0 b | 215.5 ± 8.9 c | 204.8 ± 23.5 bc |
| Hydrojuglone β-D-glucopyranoside | 234.1 ± 9.8 a | 218.5 ± 21.7 a | 291.3 ± 24.6 ab | 361.7 ± 35.2 b | 278.7 ± 15.9 ab | 370.0 ± 19.5 b |
| Hydrojuglone rutinoside   | 82.8 ± 7.3 ab | 80.3 ± 8.9 a | 70.2 ± 2.9 a | 97.6 ± 11.0 ab | 116.7 ± 4.3 bc | 132.2 ± 8.5 c |
| Hydrojuglone derivative 5 | 70.5 ± 14.6 a | 49.0 ± 3.3 a | 54.8 ± 4.7 a | 165.2 ± 18.8 b | 178.1 ± 16.6 b | 173.2 ± 6.2 b |
| Hydrojuglone derivative pentoside 2 | 545.7 ± 40.8 c | 493.5 ± 54.4 bc | 327.9 ± 27.3 a | 480.2 ± 20.1 bc | 371.9 ± 11.6 ab | 328.8 ± 12.4 a |
| Hydrojuglone derivative rhamnoside | 172.5 ± 17.8 bc | 109.3 ± 11.5 a | 164.4 ± 10.3 a | 202.2 ± 16.7 bd | 224.5 ± 13.1 cd | 244.7 ± 3.8 d |
| Hydrojuglone hexoside derivative | 107.8 ± 6.3 a | 101.8 ± 13.6 a | 134.1 ± 10.5 a | 79.7 ± 15.1 a | 75.9 ± 16.3 a | 124.2 ± 16.7 a |
| bis-Juglone               | 102.1 ± 9.8 a | 150.6 ± 18.4 ab | 132.6 ± 9.4 a | 177.5 ± 14.5 bc | 150.4 ± 9.1 ab | 215.3 ± 19.6 c |
| Regiolone                 | 162.3 ± 11.8 a | 131.7 ± 11.7 a | 153.8 ± 9.1 a | 128.1 ± 9.2 a | 245.8 ± 12.3 b | 326.0 ± 22.0 c |
| 5-Hydroxy-2,3-dihydro-1,4-naphthalenedione | 79.1 ± 11.6 ab | 120.1 ± 6.5 ab | 126.0 ± 19.6 ab | 70.5 ± 9.5 a | 91.5 ± 9.8 ab | 103.9 ± 4.2 ab |
| 4,5,8-Trihydroxynaphthalene-5-D-glucopyranoside | 459.8 ± 15.0 ab | 337.8 ± 30.3 a | 418.8 ± 28.0 ab | 672.3 ± 25.4 c | 591.2 ± 26.3 bc | 392.3 ± 35.4 a |
| 1,4,8-Trihydroxynaphthalene-1-D-glucopyranoside | 87.4 ± 8.4 a | 100.6 ± 14.3 ab | 119.0 ± 15.2 ab | 104.3 ± 19.1 ab | 161.6 ± 14.7 b | 148.5 ± 8.3 bc |
| Dihydroxytetralone hexoside | 58.9 ± 9.0 a | 63.7 ± 7.4 a | 79.5 ± 10.2 a | 70.5 ± 4.4 a | 57.9 ± 3.6 a | 115.3 ± 7.2 b |
| Trihydroxytetralone galloyl hexoside | 68.6 ± 8.7 a | 95.3 ± 15.5 a | 86.5 ± 6.0 a | 73.4 ± 8.6 a | 90.2 ± 9.7 a | 106.7 ± 12.7 a |
| Hydroxycinnamic acids     |           |             |              |          |          |            |
| Neochlorogenic acid (3-cafeoylquinic acid) | 12.2 ± 0.7 a | 17.5 ± 1.7 a | 16.4 ± 2.1 a | 14.3 ± 1.1 a | 16.1 ± 1.4 a | 18.0 ± 1.3 a |
| 3-p-Coumaroylquinic acid  | 66.5 ± 3.6 ab | 101.7 ± 5.5 c | 104.9 ± 4.0 c | 70.1 ± 3.0 ab | 60.4 ± 2.3 a | 82.1 ± 2.9 b |
| p-Coumaric acid derivative 2 | 4.5 ± 0.5 ab | 4.0 ± 0.3 ab | 3.8 ± 0.4 a | 6.7 ± 1.0 ac | 7.8 ± 0.8 c | 6.9 ± 0.6 bc |
| p-Coumaric acid derivative 3 | 9.1 ± 0.6 b | 6.2 ± 0.3 a | 7.2 ± 0.7 ab | 6.0 ± 0.2 a | 14.2 ± 1.0 c | 15.3 ± 0.8 c |
| p-Coumaric acid derivative 4 | 4.9 ± 0.6 a | 5.0 ± 0.3 a | 5.3 ± 0.5 a | 5.4 ± 0.4 a | 6.1 ± 0.3 a | 6.5 ± 0.4 a |
| Caffeic acid derivative 2 | 2.5 ± 0.9 a | 3.2 ± 0.2 ab | 3.1 ± 0.4 ab | 7.7 ± 0.8 c | 6.9 ± 1.4 bc | 9.7 ± 1.0 c |
| Ferulic acid derivative 2 | 15.3 ± 1.0 b | 20.2 ± 1.3 c | 21.8 ± 1.6 c | 15.1 ± 0.5 b | 9.9 ± 0.7 a | 15.1 ± 0.6 b |
| Ferulic acid derivative 3 | 6.3 ± 0.2 a | 8.1 ± 0.8 ab | 11.8 ± 1.0 bc | 8.7 ± 1.1 ab | 13.6 ± 1.0 c | 14.8 ± 1.2 c |
| Hydroxybenzoic acids      |           |             |              |          |          |            |
| Gallic acid derivative 3   | 142.2 ± 14.1 a | 85.9 ± 10.6 a | 132.2 ± 3.5 a | 244.6 ± 18.6 b | 331.3 ± 16.1 c | 366.9 ± 11.8 c |
| Gallic acid derivative 4   | 167.6 ± 2.7 a | 17.6 ± 2.7 ab | 23.6 ± 4.2 ab | 13.0 ± 1.7 a | 16.5 ± 2.6 ab | 25.4 ± 1.7 b |
| Gallic acid derivative 5   | 12.9 ± 2.4 a | 13.6 ± 2.2 a | 14.1 ± 2.8 a | 15.6 ± 1.6 a | 25.7 ± 2.0 b | 17.5 ± 1.3 ab |
Table 5. Cont.

| Phenolic                  | 'Fernor' (mg/100 g) | 'Fernette' (mg/100 g) | 'Tranquette' (mg/100 g) | 'Sava' (mg/100 g) | 'Krka' (mg/100 g) | 'Rubina' (mg/100 g) |
|---------------------------|---------------------|-----------------------|-------------------------|-------------------|-------------------|---------------------|
| Flavanols                |                     |                       |                         |                   |                   |                     |
| (+)-Catechin             | 53.7 ± 2.9 ab       | 82.2 ± 4.4 c          | 84.8 ± 3.2 c            | 56.7 ± 2.4 ab     | 48.9 ± 1.8 a      | 66.4 ± 2.4 b        |
| (-)-Epicatechin          | 69.4 ± 5.0 a        | 56.3 ± 5.0 a          | 65.7 ± 3.9 a            | 54.8 ± 4.0 a      | 105.0 ± 5.2 b     | 139.3 ± 9.4 c       |
| (epi)Catechin derivative 5 | 50.6 ± 8.9 a       | 56.9 ± 8.2 ab         | 68.8 ± 3.3 ac           | 79.4 ± 4.7 bc     | 88.0 ± 0.8 c      | 80.9 ± 1.6 bc       |
| Flavones                 |                     |                       |                         |                   |                   |                     |
| Santin                   | 14.1 ± 1.6 a        | 13.3 ± 1.9 a          | 15.0 ± 1.9 a            | 35.6 ± 4.8 b      | 49.4 ± 1.1 c      | 46.2 ± 3.7 bc       |
| 5,7-Dihydroxy-3,4-dimetoxyflavone | 27.1 ± 0.4 bc | 12.5 ± 1.8 a          | 16.2 ± 2.8 ab           | 20.3 ± 2.0 ab     | 40.5 ± 4.4 c      | 39.8 ± 4.4 c        |
| Flavonols                |                     |                       |                         |                   |                   |                     |
| Quercetin-3-galactoside  | 33.6 ± 1.8 ab       | 39.8 ± 2.5 ab         | 34.7 ± 2.1 ab           | 42.3 ± 3.1 b      | 35.8 ± 1.8 ab     | 31.4 ± 0.6 a        |
| Quercetin-3-rhamnoside   | 12.7 ± 3.1 a        | 22.7 ± 3.5 a          | 20.6 ± 1.5 a            | 16.8 ± 2.4 a      | 17.4 ± 0.4 a      | 16.3 ± 1.2 a        |
| Total naphthoquinones    | 2946.7 ± 116.4 a    | 3026.2 ± 133.4 a      | 3202.6 ± 142.9 a        | 4454.6 ± 235.0 b  | 4670.0 ± 74.7 b   | 5088.8 ± 156.9 b    |
| Total hydroxycinnamic acids | 121.2 ± 4.3 a | 166.0 ± 7.7 b         | 174.3 ± 8.8 b           | 134.0 ± 4.4 a     | 135.0 ± 6.3 a     | 168.4 ± 5.0 b       |
| Total hydroxybenzoic acids | 171.8 ± 17.9 a | 117.1 ± 14.0 a        | 169.9 ± 9.4 a           | 273.2 ± 20.0 b    | 373.5 ± 17.3 c    | 409.8 ± 13.4 c      |
| Total flavanols          | 173.7 ± 11.3 a      | 195.5 ± 15.3 a        | 219.4 ± 8.7 ab          | 190.9 ± 8.3 a     | 241.9 ± 7.2 bc    | 286.6 ± 8.9 c       |
| Total flavones           | 41.1 ± 1.8 ab       | 25.7 ± 3.2 a          | 31.2 ± 4.0 a            | 55.9 ± 6.7 b      | 89.9 ± 5.0 c      | 86.0 ± 7.3 c        |
| Total flavanones         | nd                  | nd                    | nd                       | nd                | nd                | nd                  |
| Total flavonones         | 46.3 ± 4.1 a        | 62.4 ± 5.2 a          | 55.2 ± 3.4 a            | 59.1 ± 4.8 a      | 53.2 ± 2.0 a      | 47.8 ± 1.7 a        |
| Total phenolics content (summation; relevant standards) \(^x\) | 3500.8 ± 137.3 a | 3592.8 ± 155.3 a | 3852.7 ± 173.1 a | 5167.7 ± 275.4 b | 5563.5 ± 81.0 bc | 6087.3 ± 179.6 c |
| Total phenolics content (total extracts; mg gallic acid equivalents/100 g dry weight) \(^y\) | 1156.6 ± 51.4 a | 1532.7 ± 105.6 b | 1398.9 ± 80.2 a | 1241.2 ± 56.6 ab | 1155.2 ± 22.9 a | 1398.5 ± 102.8 ab |

Data are means ± standard error. \(^x\) expressed as the sum of all of the individual identified phenolics (summation), in mg/100 g dry weight of the most relevant standard. \(^y\) expressed as the separate analysis of the total phenolics for each extract (total extracts), in mg gallic acid equivalents/100 g dry weight. Means followed by different letters within a cultivar are significantly different \((p \leq 0.05; Tukey’s tests); nd, not detected.
Table 6. Individual phenolics for the walnut buds across the six selected cultivars.

| Compound                        | Bud Phenolic Content per Cultivar (mg/100 g Dry Weight) |
|---------------------------------|--------------------------------------------------------|
|                                 | 'Fernor' | 'Fernette' | 'Franquette' | 'Sava' | 'Krka' | 'Rubina' |
| Naphthoquinones                 |          |            |              |        |        |          |
| Juglone                         | 573.8 ± 30.3 c | 407.5 ± 24.1 ab | 373.6 ± 9.9 a | 392.3 ± 5.9 a | 466.0 ± 18.2 ab | 508.8 ± 29.1 bc |
| Hydrojuglone                    | 149.4 ± 6.7 c | 100.4 ± 2.9 ab | 113.4 ± 10.7 bc | 70.7 ± 11.8 a | 88.0 ± 7.0 ab | 111.6 ± 9.0 bc |
| Hydrojuglone β-D-glucopyranoside| 2744.4 ± 58.1 c | 1636.6 ± 11.4 a | 3619.9 ± 100.3 d | 2326.5 ± 25.5 b | 2148.8 ± 37.8 b | 1688.2 ± 6.4 a |
| Hydrojuglone rutinoside         | 314.7 ± 14.4 c | 191.1 ± 4.0 a | 283.0 ± 13.9 c | 189.4 ± 11.9 a | 261.1 ± 13.1 bc | 222.5 ± 6.7 ab |
| Hydrojuglone dihexoside         | 509.0 ± 17.0 b | 404.3 ± 14.8 a | 683.1 ± 20.3 d | 606.5 ± 12.0 c | 454.7 ± 13.7 ab | 444.2 ± 14.0 ab |
| Hydrojuglone derivative 1       | 642.3 ± 27.3 b | 348.1 ± 18.7 a | 951.0 ± 27.1 c | 432.1 ± 15.4 a | 440.7 ± 8.5 a | 432.5 ± 16.1 a |
| Hydrojuglone derivative 2       | 109.1 ± 6.2 a | 57.1 ± 3.6 a | 868.7 ± 14.7 e | 270.9 ± 7.2 b | 526.8 ± 17.8 c | 594.1 ± 12.6 d |
| Hydrojuglone derivative 3       | 301.2 ± 11.5 c | 165.3 ± 8.6 a | 260.3 ± 17.5 bc | 161.0 ± 8.4 a | 180.7 ± 11.3 a | 216.1 ± 10.8 ab |
| Hydrojuglone derivative pentoside 1 | 1038.7 ± 18.2 d | 594.0 ± 18.9 a | 1995.1 ± 30.9 e | 973.0 ± 11.6 cd | 824.0 ± 17.6 b | 916.5 ± 27.1 bc |
| Hydrojuglone derivative pentoside 2 | 3855.8 ± 86.1 d | 2138.6 ± 29.4 ab | 3021.7 ± 34.9 c | 3166.0 ± 50.0 c | 2370.3 ± 46.4 b | 1940.6 ± 34.8 a |
| Hydrojuglone derivative rhamnoside | 1525.3 ± 31.7 c | 861.5 ± 18.3 a | 2429.3 ± 36.9 e | 1297.8 ± 33.8 b | 1933.2 ± 29.1 d | 1646.5 ± 14.1 c |
| Hydrojuglone pentose galloyl derivative | 433.9 ± 25.8 c | 234.5 ± 5.6 a | 525.5 ± 14.7 d | 268.0 ± 11.9 ab | 288.8 ± 24.0 a | 341.7 ± 12.8 b |
| Dihydroxytetralone hexoside     | 285.0 ± 7.8 c | 194.1 ± 13.7 a | 382.4 ± 13.1 d | 281.8 ± 9.0 bc | 219.5 ± 14.6 b | 257.2 ± 20.0 ac |
| Hydroxycinnamic acids           |          |            |              |        |        |          |
| Neochlorogenic acid (3-cafeoylquinic acid) | 74.2 ± 0.9 c | 29.2 ± 2.6 a | 99.4 ± 6.8 d | 58.3 ± 3.1 bc | 62.0 ± 3.9 bc | 51.2 ± 4.3 b |
| 3-p-Coumaroylquinic acid        | 323.1 ± 5.2 c | 165.9 ± 4.7 a | 395.3 ± 10.4 d | 273.4 ± 4.6 b | 257.5 ± 2.2 b | 283.3 ± 4.3 b |
| p-Coumaric acid derivative 1    | 70.8 ± 0.9 c | 45.7 ± 0.8 a | 100.3 ± 1.5 d | 62.1 ± 0.9 b | 65.8 ± 0.6 b | 42.1 ± 0.4 a |
| Caffeic acid hexoside derivative | 20.5 ± 0.7 a | 18.5 ± 0.6 a | 48.5 ± 2.2 b | 22.2 ± 1.8 a | 18.9 ± 0.7 a | 23.0 ± 0.6 a |
| Caffeic acid derivative 1        | 9.0 ± 0.7 b | 6.5 ± 0.3 a | 12.0 ± 0.4 c | 4.9 ± 0.6 a | 6.8 ± 0.5 ab | 6.8 ± 0.4 ab |
| Diferuoyl hexoside              | 8.4 ± 0.5 c | 5.6 ± 0.3 b | 8.0 ± 0.5 c | 2.8 ± 0.1 a | 7.1 ± 0.4 bc | 7.3 ± 0.3 bc |
| Hydroxybenzoic acids            |          |            |              |        |        |          |
| Gallic acid derivative 1         | 55.2 ± 1.5 b | 37.4 ± 1.5 a | 60.7 ± 1.0 b | 41.9 ± 1.5 a | 42.3 ± 1.2 a | 42.2 ± 1.1 a |
| Gallic acid derivative 2         | 705.9 ± 4.3 b | 369.8 ± 157.6 a | 1209.4 ± 263.3 c | 811.7 ± 22.3 b | 655.2 ± 6.3 ab | 685.1 ± 7.2 b |
| Gallic acid derivative 3         | 541.6 ± 6.7 d | 305.1 ± 6.7 a | 424.2 ± 9.9 b | 485.5 ± 6.1 c | 520.6 ± 7.1 cd | 416.0 ± 9.5 b |
| Gallic acid methyl ester        | 90.9 ± 1.6 b | 62.1 ± 5.7 a | 188.9 ± 9.5 c | 92.1 ± 4.5 b | 92.1 ± 4.2 b | 95.7 ± 2.3 b |
| l-H-HDHP-glucose               | 70.7 ± 3.7 bc | 46.1 ± 2.0 a | 80.8 ± 4.9 c | 72.9 ± 2.3 bc | 41.8 ± 1.3 a | 63.6 ± 0.7 b |
| Ellagic acid derivative         | 451.4 ± 3.9 d | 226.0 ± 13.5 a | 942.8 ± 17.0 e | 366.7 ± 7.1 c | 276.5 ± 3.6 b | 333.1 ± 10.4 c |
| Flavanols                       |          |            |              |        |        |          |
| Procyanidin dimer 1             | 303.8 ± 18.4 b | 195.4 ± 21.8 a | 256.1 ± 11.5 ab | 289.7 ± 3.5 b | 275.9 ± 5.8 b | 267.1 ± 10.6 b |
| Procyanidin dimer 2             | 494.3 ± 9.8 cd | 350.9 ± 25.4 a | 527.6 ± 16.2 d | 507.1 ± 17.2 cd | 378.9 ± 19.6 ab | 433.9 ± 7.9 bc |
### Table 6. Cont.

| Compound                  | 'Fenor' | 'Fernette' | 'Tranquette' | 'Sava' | 'Krkα' | 'Rubina' |
|---------------------------|---------|------------|--------------|--------|--------|----------|
| Procyanidin dimer derivative 1 | 655.6 ± 24.0 c | 293.9 ± 10.1 a | 859.0 ± 40.5 d | 604.1 ± 11.7 c | 589.6 ± 2.7 c | 452.4 ± 10.8 b |
| Procyanidin dimer derivative 2 | 431.7 ± 13.9 b | 261.6 ± 3.4 a | 823.9 ± 18.2 d | 445.2 ± 15.1 b | 413.3 ± 7.6 b | 630.5 ± 22.8 c |
| Procyanidin dimer derivative 3 | 122.4 ± 7.3 c | 88.0 ± 5.6 ab | 193.8 ± 8.3 d | 72.4 ± 3.7 a | 116.1 ± 3.6 bc | 116.6 ± 7.9 bc |
| (+)Catechin              | 838.6 ± 34.4 c | 469.0 ± 6.6 a | 1218.0 ± 25.9 d | 666.2 ± 22.9 b | 664.8 ± 16.2 b | 744.9 ± 26.5 bc |
| (−)Epicatechin           | 266.6 ± 6.0 c | 178.3 ± 4.2 a | 347.4 ± 7.0 d | 198.5 ± 5.2 a | 238.5 ± 8.1 b | 192.8 ± 1.2 a |
| (epi)Catechin derivative 1 | 210.9 ± 2.7 b | 160.5 ± 5.0 a | 273.1 ± 4.8 c | 208.8 ± 1.9 b | 205.5 ± 5.3 b | 208.8 ± 2.5 b |
| (epi)Catechin derivative 2 | 267.3 ± 7.1 c | 185.1 ± 15.7 a | 384.7 ± 10.0 d | 269.3 ± 13.2 c | 256.4 ± 13.4 bc | 206.6 ± 19.6 ab |
| (epi)Catechin derivative 3 | 435.9 ± 9.9 c | 291.5 ± 6.8 a | 568.0 ± 11.4 d | 324.5 ± 8.5 a | 390.0 ± 13.3 b | 315.3 ± 2.0 a |
| (epi)Catechin derivative 4 | 94.7 ± 4.1 a | 80.4 ± 4.5 a | 155.5 ± 4.4 b | 91.3 ± 3.2 a | 82.3 ± 3.9 a | 92.8 ± 2.9 a |
| Galloyl-3-(epi)catechin   | 850.0 ± 9.3 d | 461.8 ± 15.3 a | 1396.0 ± 23.6 e | 644.6 ± 20.7 b | 724.0 ± 20.0 bc | 797.5 ± 6.3 cd |

#### Flavones

| Compound                  | 'Fenor' | 'Fernette' | 'Tranquette' | 'Sava' | 'Krkα' | 'Rubina' |
|---------------------------|---------|------------|--------------|--------|--------|----------|
| 5,7-Dihydroxy-3,4-dimetoxy flavone | 45.3 ± 2.3 c | 38.6 ± 2.6 bc | 28.5 ± 1.8 a | 29.2 ± 2.5 c | 35.9 ± 1.7 ac | 58.4 ± 1.0 d |

#### Flavanones

| Compound                  | 'Fenor' | 'Fernette' | 'Tranquette' | 'Sava' | 'Krkα' | 'Rubina' |
|---------------------------|---------|------------|--------------|--------|--------|----------|
| Naringenin                | 83.5 ± 3.5 ab | 65.7 ± 4.7 a | 83.9 ± 4.7 ab | 73.9 ± 2.7 a | 70.3 ± 4.8 a | 98.4 ± 3.3 b |

#### Flavanols

| Compound                  | 'Fenor' | 'Fernette' | 'Tranquette' | 'Sava' | 'Krkα' | 'Rubina' |
|---------------------------|---------|------------|--------------|--------|--------|----------|
| Myricetin galactoside     | 259.0 ± 14.1 d | 140.2 ± 3.9 b | 214.0 ± 8.0 c | 141.4 ± 3.2 b | 131.5 ± 5.1 b | 107.7 ± 3.0 a |
| Myricetin pentoside       | 105.4 ± 4.8 b | 54.4 ± 2.1 a | 242.3 ± 8.5 c | 92.4 ± 4.7 b | 95.6 ± 4.1 b | 104.8 ± 6.0 b |
| Myricetin-3-rhamnoside    | 533.3 ± 12.2 c | 356.6 ± 2.1 a | 770.6 ± 14.9 d | 553.9 ± 9.9 c | 452.2 ± 12.6 b | 365.0 ± 7.7 a |
| Quercetin-3-galactoside   | 227.5 ± 2.5 d | 123.6 ± 4.1 a | 373.6 ± 6.3 e | 172.5 ± 5.5 b | 193.8 ± 5.4 bc | 213.4 ± 1.7 cd |
| Quercetin-3-glucoside     | 144.0 ± 4.3 bc | 113.9 ± 4.6 a | 262.0 ± 6.7 d | 147.9 ± 6.5 bc | 158.4 ± 4.1 c | 133.6 ± 3.4 ab |
| Quercetin-3-arabinopyranoside | 293.4 ± 3.6 b | 206.9 ± 3.1 a | 495.9 ± 9.8 c | 287.9 ± 6.5 b | 308.9 ± 2.5 b | 282.5 ± 5.3 b |
| Quercetin-3-arabinofuranoside | 250.8 ± 12.6 a | 207.2 ± 2.8 a | 613.0 ± 19.3 c | 244.3 ± 8.9 a | 335.3 ± 6.0 b | 320.2 ± 5.8 b |
| Quercetin-3-rhamnoside    | 399.1 ± 7.6 bc | 352.5 ± 4.9 a | 745.4 ± 25.7 d | 371.0 ± 7.2 ab | 440.7 ± 8.5 c | 424.7 ± 7.6 bc |
| Quercetin galloyl hexoside | 130.8 ± 4.4 c | 90.5 ± 2.3 a | 177.5 ± 4.6 d | 143.7 ± 1.6 c | 98.4 ± 2.9 ab | 107.2 ± 2.5 b |
| Quercetin hexoside derivative 1 | 54.5 ± 2.8 c | 34.4 ± 1.6 ab | 68.6 ± 4.3 d | 27.2 ± 1.4 a | 50.6 ± 1.2 c | 41.8 ± 3.4 bc |
| Quercetin hexoside derivative 2 | 36.3 ± 1.0 bc | 30.4 ± 1.6 b | 40.2 ± 0.9 c | 19.6 ± 1.3 a | 30.7 ± 1.5 b | 31.5 ± 1.5 b |
| Quercetin                 | 29.0 ± 1.1 c | 21.4 ± 0.9 b | 29.6 ± 1.4 c | 15.6 ± 1.3 a | 21.3 ± 0.8 b | 26.8 ± 1.3 bc |
| Kaempferol pentoside 1    | 39.1 ± 1.0 b | 22.9 ± 1.9 a | 76.6 ± 4.9 c | 26.7 ± 0.9 a | 29.5 ± 2.1 ab | 40.2 ± 1.4 b |
| Kaempferol pentoside 2    | 68.8 ± 2.9 b | 43.1 ± 0.9 a | 114.6 ± 4.7 c | 61.0 ± 3.0 b | 56.5 ± 2.3 ab | 53.4 ± 4.4 ab |
| Kaempferol pentoside 3    | 36.2 ± 1.6 c | 17.9 ± 0.4 a | 66.5 ± 2.2 d | 28.4 ± 1.7 bc | 29.1 ± 2.0 bc | 27.8 ± 1.9 b |
| Kaempferol rhamnoside     | 53.0 ± 1.4 bc | 37.5 ± 3.9 a | 83.4 ± 3.6 d | 45.0 ± 4.2 ab | 46.1 ± 2.5 ab | 60.9 ± 2.0 c |
| Kaempferol                | 21.3 ± 1.2 a | 20.8 ± 1.6 a | 18.5 ± 1.7 a | 20.6 ± 1.7 a | 20.9 ± 1.5 a | 25.1 ± 1.4 a |
Table 6. Cont.

| Compound                                            | 'Fernor'       | 'Fernette'     | 'Tranquette'    | 'Sava'         | 'Krka'         | 'Rubina'       |
|-----------------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Total naphthoquinones                               | 12482.7 ± 126.9 d | 7333.0 ± 102.7 a | 15507.1 ± 122.8 e | 10435.9 ± 42.1 c | 10202.6 ± 31.8 c | 9320.5 ± 91.8 b |
| Total hydroxycinnamic acids                         | 506.1 ± 4.9 c   | 271.2 ± 7.9 a   | 663.4 ± 17.7 d  | 423.7 ± 10.1 b  | 418.1 ± 2.0 b   | 413.7 ± 3.2 b  |
| Total hydroxybenzoic acids                          | 1915.8 ± 2.1 b  | 1046.6 ± 156.6 a | 2906.8 ± 46.2 c | 1870.8 ± 21.0 b | 1628.5 ± 20.1 b | 1635.7 ± 10.9 b |
| Total flavonols                                      | 4971.9 ± 61.0 c | 3016.5 ± 43.1 a | 7003.2 ± 129.6 d | 4318.6 ± 9.9 b  | 4335.3 ± 59.0 b | 4454.1 ± 32.6 b |
| Total flavones                                       | 91.3 ± 4.5 c    | 73.9 ± 4.0 b    | 55.1 ± 4.0 a    | 61.4 ± 0.6 a    | 64.2 ± 1.2 a    | 95.3 ± 0.7 c   |
| Total flavanones                                     | 82.5 ± 3.5 ab   | 65.7 ± 4.7 a    | 83.9 ± 4.7 ab   | 73.9 ± 2.7 a    | 70.3 ± 4.8 a    | 98.4 ± 3.3 b   |
| Total phenolics content (summation; relevant standards) x | 22732.6 ± 189.8 d | 13661.1 ± 283.3 a | 30611.8 ± 130.3 e | 19583.4 ± 74.3 c | 19218.3 ± 21.1 c | 18384.4 ± 13.8 b |

Total phenolics content (total extracts; mg gallic acid equivalents/100 g dry weight) y

7236.4 ± 188.1 c  4270.2 ± 144.4 a  8232.8 ± 57.7 d  5199.6 ± 166.3 b  5017.7 ± 220.7 ab  4853.8 ± 259.2 ab

Data are means ± standard error. x expressed as the sum of all of the individual identified phenolics (summation), in mg/100 g dry weight of the most relevant standard. y expressed as the separate analysis of the total phenolics for each extract (total extracts), in mg gallic acid equivalents/100 g dry weight. Means followed by different letters within a cultivar are significantly different (p ≤ 0.05; Tukey’s tests); nd, not detected.

Table 7. Individual phenolics for the walnut bark across the six selected cultivars.

| Compound                              | 'Fernor'       | 'Fernette'     | 'Tranquette'    | 'Sava'         | 'Krka'         | 'Rubina'       |
|---------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Naphthoquinones                       |                |                |                |                |                |                |
| Juglone                               | 251.0 ± 31.2 ac | 185.8 ± 8.6 ab | 281.2 ± 16.6 bc | 169.4 ± 10.2 a | 219.4 ± 24.1 ac | 299.8 ± 35.6 c |
| Hydrojuglone                          | 72.8 ± 2.2 bc  | 89.9 ± 9.6 c   | 50.7 ± 7.2 ab  | 65.9 ± 5.5 bc  | 21.3 ± 2.4 a   | 66.9 ± 9.8 bc  |
| Hydrojuglone β-D-glucopyranoside      | 239.8 ± 13.0 a | 366.1 ± 18.7 b | 342.3 ± 17.8 ab | 285.5 ± 26.2 a | 255.1 ± 11.9 b | 523.4 ± 52.7 c |
| Hydrojuglone rutinoside               | 47.6 ± 4.8 ab  | 30.9 ± 3.1 a   | 40.6 ± 5.5 a   | 34.9 ± 2.8 a   | 45.1 ± 3.8 ab  | 60.9 ± 2.1 b   |
| Hydrojuglone derivative 2             | 16.6 ± 5.1 a   | 10.5 ± 4.7 a   | 156.3 ± 21.1 b | 32.0 ± 9.2 a   | 143.9 ± 9.4 b  | 143.3 ± 22.0 b |
| Hydrojuglone derivative 4             | 1258.0 ± 73.5 c| 877.8 ± 53.9 b | 639.1 ± 83.0 a | 471.6 ± 35.5 a | 417.4 ± 17.0 a | 535.2 ± 78.9 a |
| Hydrojuglone derivative pentoside 1   | 566.3 ± 25.2 b | 757.7 ± 34.4 c | 401.4 ± 14.1 a | 699.1 ± 35.5 bc| 363.2 ± 21.9 a | 394.7 ± 42.7 a |
| Hydrojuglone derivative pentoside 2   | 6579.7 ± 402.1 b| 7608.0 ± 301.1 b| 4768.3 ± 337.3 a| 7189.0 ± 266.6 b| 4672.8 ± 174.5 a| 4954.1 ± 494.4 a|
| Hydrojuglone derivative pentoside 3   | 605.9 ± 24.3 bc| 743.9 ± 32.8 c | 487.3 ± 24.5 ab| 681.9 ± 36.8 c | 427.7 ± 16.0 a | 460.3 ± 49.8 ab |
| Hydrojuglone derivative rhamnoside    | 2015.2 ± 110.6 a| 2332.2 ± 103.3 a| 2172.8 ± 157.9 a| 2039.4 ± 75.6 b| 2222.2 ± 123.3 a| 2230.2 ± 193.9 a|
| Dihydroxytetralone hexoside           | 43.4 ± 3.0 a   | 54.0 ± 4.7 a   | 48.8 ± 2.8 a   | 47.2 ± 6.8 a   | 37.7 ± 5.7 a   | 49.6 ± 3.5 a   |
| Hydroxybenzoic acids                  |                |                |                |                |                |                |
| Gallic acid derivative 2              | 80.0 ± 5.3 ab  | 89.9 ± 5.3 ab  | 106.7 ± 4.9 b  | 92.5 ± 7.0 ab  | 71.8 ± 3.5 a   | 93.0 ± 8.9 ab  |
| Gallic acid derivative 3              | 29.7 ± 3.1 bc  | 31.1 ± 1.5 c   | 15.6 ± 0.8 a   | 35.2 ± 3.6 cd  | 16.8 ± 2.3 ab  | 44.1 ± 4.3 d   |
| Ellagic acid derivative               | 145.6 ± 12.6 b | 153.9 ± 8.9 b  | 155.8 ± 9.7 b  | 93.2 ± 9.4 a   | 111.0 ± 7.1 ab | 123.0 ± 13.6 ab|

Total phenolics content (total extracts; mg gallic acid equivalents/100 g dry weight) y

7236.4 ± 188.1 c  4270.2 ± 144.4 a  8232.8 ± 57.7 d  5199.6 ± 166.3 b  5017.7 ± 220.7 ab  4853.8 ± 259.2 ab
Total phenolics content (summation; relevant standard) expressed as the sum of all of the individual identified phenolics (summation), in mg/100 g dry weight of the most relevant standard.

| Compound | 'Fernor' | 'Fernette' | 'Franquette' | 'Sava' | 'Krka' | 'Rubina' |
|----------|----------|------------|--------------|--------|--------|----------|
| Flavonols | Procyanidin dimer 2 | 219.8 ± 18.8 a | 277.1 ± 20.2 a | 301.3 ± 25.6 a | 335.3 ± 24.5 a | 288.7 ± 14.6 a | 333.2 ± 45.3 a |
| Flavonols | Procyanidin dimer derivative 2 | 200.9 ± 10.5 ab | 248.2 ± 21.8 b | 213.0 ± 17.1 ab | 245.4 ± 17.8 b | 149.8 ± 7.8 a | 178.4 ± 25.6 ab |
| Flavonols | (+)Catechin | 528.2 ± 56.6 a | 537.9 ± 40.9 a | 675.5 ± 42.0 a | 708.7 ± 49.5 a | 748.8 ± 53.0 a | 797.2 ± 103.9 a |
| Flavones | 5,7-Dihydroxy-3,4-dimetoxyflavone | 2.2 ± 0.6 ab | 0.9 ± 0.1 a | 4.3 ± 0.3 b | 4.4 ± 0.9 b | 2.4 ± 0.2 ab | 3.6 ± 0.5 b |
| Total flavonoids | Santin | 14.4 ± 2.0 b | 6.3 ± 0.4 a | 7.7 ± 0.6 a | 10.6 ± 2.2 ab | 6.6 ± 0.3 a | 7.8 ± 0.1 a |
| Total flavonoids | Kaempferol-7-glucoside | 82.7 ± 5.8 ab | 97.3 ± 4.9 ab | 82.6 ± 7.0 ab | 101.6 ± 6.5 b | 69.5 ± 3.0 a | 76.9 ± 10.0 ab |
| Total flavonoids | Kaempferol-7-hexoside 1 | 150.5 ± 9.7 bc | 237.1 ± 8.6 d | 113.0 ± 18.3 ab | 195.3 ± 11.7 cd | 63.5 ± 6.3 a | 107.7 ± 23.5 ab |
| Total flavonoids | Kaempferol-7-hexoside 2 | 30.2 ± 1.3 b | 42.2 ± 1.5 c | 32.2 ± 3.6 bc | 26.6 ± 2.4 b | 14.8 ± 2.3 a | 34.1 ± 2.7 bc |

Table 7. Cont.

| Compound | 'Fernor' | 'Fernette' | 'Franquette' | 'Sava' | 'Krka' | 'Rubina' |
|----------|----------|------------|--------------|--------|--------|----------|
| Total naphthoquinones | 11696.3 ± 654.3 bc | 13056.9 ± 527.9 c | 9388.8 ± 596.2 ab | 11716.0 ± 488.3 bc | 9825.9 ± 399.7 a | 9718.4 ± 919.8 ab |
| Total hydroxycinnamic acids | nd | nd | nd | nd | nd | nd |
| Total hydroxybenzoic acids | 255.3 ± 15.9 a | 274.9 ± 15.4 a | 272.8 ± 14.1 a | 220.8 ± 19.8 a | 199.6 ± 11.4 a | 260.2 ± 25.9 a |
| Total flavonols | 948.9 ± 84.2 a | 1083.3 ± 78.6 a | 1189.8 ± 81.3 a | 1289.3 ± 88.6 a | 1187.3 ± 72.3 a | 1308.7 ± 174.5 a |
| Total flavones | 16.5 ± 2.0 c | 7.2 ± 0.4 a | 12.0 ± 0.8 ac | 15.0 ± 2.9 bc | 9.0 ± 0.5 ab | 11.4 ± 0.6 ac |
| Total gallic acid equivalents/100 g dry weight | 1956.2 ± 77.6 a | 2236.4 ± 251.1 a | 2544.4 ± 251.3 a | 1898.2 ± 121.7 a | 1979.3 ± 38.7 a | 2740.0 ± 440.6 a |

Data are means ± standard error. * expressed as the sum of all of the individual identified phenolics (summation), in mg/100 g dry weight of the most relevant standard. † expressed as the separate analysis of the total phenolics for each extract (total extracts), in mg gallic acid equivalents/100 g dry weight. Means followed by different letters within a cultivar are significantly different (p ≤ 0.05; Tukey’s tests); nd, not detected.
Interestingly, the three Slovenian cultivars of ‘Sava’, ‘Krka’ and ‘Rubina’ had similar naphthoquinone contents in the walnut outer husk that were also higher than for the French cultivars ‘Fernor’, ‘Fernette’ and ‘Franquette’, which were also similar for their total naphthoquinone contents. The same was seen for the walnut inner husk, where the Slovenian varieties showed higher total naphthoquinone content than the French cultivars. This information that the Slovenian cultivars had higher total naphthoquinone contents than the French cultivars might also be useful in the future determination of the genetic origins of a cultivar, as cultivars that are bred in different climates might have specific naphthoquinone contents, as previously reported by Medic et al. [19]. The summarised total phenolic content of the buds identified in this study was compared with the total phenolic content of pellicle identified by Medic et al. [19], which was similar in terms of the order of phenolic compound content of the selected cultivars, with ‘Franquette’ containing the most phenols, followed by ‘Fernor’, ‘Krka’ and ‘Sava’ and ‘Rubina’ containing the second least phenols and the least ‘Fernette’. Otherwise, no clear picture was seen linking phenolic content to different plant organs, suggesting that the total phenolics analysed may not be related between different parts of walnut, but rather a characteristic of the cultivar that dictates where the majority of phenolics are concentrated. Of note, this was observed only for the inner and outer husks, and not for the buds or bark, where the total naphthoquinone contents were not influenced by the origins of the cultivars.

Looking at individual naphthoquinones, in all these plant tissues, juglone was most abundant in the walnut inner husk, as can be seen in Tables 4–7. Among the walnut cultivars, ‘Rubina’ had the highest juglone content for the inner and outer husks and the bark, and the second highest juglone content for the buds, following ‘Fernor’. This makes ‘Rubina’ an excellent choice for the purpose of juglone extraction. As juglone is used as a natural dye [12] and has anti-inflammatory effects [17], the efficient use of these agro-residues would represent a strategy that simultaneously helps to preserve the environment and potentially to boost the economic outcome for farmers and companies. This calls for further studies on the extraction of juglone from these, and other, plant tissues. The 83 phenolics identified across the different parts of the walnut tissues are shown in Table 8.

Table 8. Overview of the 83 phenolics identified for the walnut inner and outer husks, buds and bark.

| Phenolic Compound                                      | Husk    | Buds   | Bark   |
|--------------------------------------------------------|---------|--------|--------|
| **Naphthoquinones**                                    |         |        |        |
| 1,4-Naphthoquinone                                     | +       | +      |        |
| Juglone                                                | +       | +      | +      |
| Hydrojuglone                                           | +       | +      | +      |
| Hydrojuglone β-D-glucopyranoside                       | +       | +      | +      |
| Hydrojuglone rutinoside                                | +       | +      | +      |
| Hydrojuglone dihexoside                                | +       |        |        |
| Hydrojuglone derivative 1                             | +       |        |        |
| Hydrojuglone derivative 2                              | +       |        |        |
| Hydrojuglone derivative 3                              | +       |        |        |
| Hydrojuglone derivative 4                              | +       |        |        |
| Hydrojuglone derivative 5                              | +       |        |        |
| Hydrojuglone derivative pentoside 1                   | +       |        | +      |
| Hydrojuglone derivative pentoside 2                   | +       | +      | +      |
| Hydrojuglone derivative pentoside 3                   | +       | +      | +      |
| Hydrojuglone derivative rhamnoside                     | +       | +      | +      |
| Hydrojuglone pentose galloyl derivative                | +       |        |        |
| Hydrojuglone hexoside derivative                       | +       | +      |        |
| βis-Juglone                                           | +       | +      |        |
| p-Hydroxymetoxybenzobijuglone                          | +       |        |        |
| Regiolone                                              | +       |        |        |
| 5-Hydroxy-2,3-dihydro-1,4-naphthalenedione             | +       |        |        |
Table 8. Cont.

| Phenolic                  | Inner | Outer | Bark |
|---------------------------|-------|-------|------|
| **Hydroxycinnamic acids** |       |       |      |
| Neochlorogenic acid (3-caffeoylquinic acid) | +     | +     | +    |
| 3-p-Coumaroylquinic acid  | +     | +     | +    |
| p-Coumaric acid derivative 1 | +     |       |      |
| p-Coumaric acid derivative 2 | +     |       |      |
| p-Coumaric acid derivative 3 | +     |       |      |
| p-Coumaric acid derivative 4 | +     |       |      |
| Caffeic acid derivative 1  | +     |       |      |
| Caffeic acid derivative 2  |       |       |      |
| Caffeic acid derivative 3  | +     |       |      |
| Caffeic acid derivative 4  | +     |       |      |
| Diferuoyl hexoside         |       |       |      |
| Ferulic acid derivative 1  | +     |       |      |
| Ferulic acid derivative 2  | +     |       |      |
| Ferulic acid derivative 3  | +     |       |      |

| **Hydroxybenzoic acids**  |       |       |      |
| Gallic acid derivative 1   |       |       |      |
| Gallic acid derivative 2   |       | +     |      |
| Gallic acid derivative 3   | +     | +     | +    |
| Gallic acid derivative 4   | +     |       |      |
| Gallic acid derivative 5   | +     |       |      |
| Gallic acid methyl ester   | +     |       |      |
| *bis*-HHDP-glucose        | +     |       |      |
| Ellagic acid derivative    | +     |       |      |

| **Flavanols**              |       |       |      |
| Procyanidin dimer 1        | +     |       |      |
| Procyanidin dimer 2        | +     | +     |      |
| Procyanidin dimer derivative 1 | +     |       |      |
| Procyanidin dimer derivative 2 | +     |       |      |
| Procyanidin dimer derivative 3 | +     |       |      |
| (+)-Catechin               | +     | +     | +    |
| (−)-Epicatechin            | +     | +     | +    |
| (epi)Catechin derivative 1 | +     |       |      |
| (epi)Catechin derivative 2 | +     |       |      |
| (epi)Catechin derivative 3 | +     |       |      |
| (epi)Catechin derivative 4 | +     |       |      |
| (epi)Catechin derivative 5 |       |       |      |
| Galloyl-3-(epi)catechin    | +     |       |      |

| **Flavones**               |       |       |      |
| Santin                     | +     | +     |      |
| 5,7-Dihydroxy-3,4-dimetoxyflavone | +     | +     |      |

| **Flavanones**             |       |       |      |
| Naringenin                 | +     |       |      |

| **Flavonols**              |       |       |      |
| Myricetin galactoside      | +     |       |      |
| Myricetin pentoside        | +     | +     |      |
| Myricetin-3-rhamnoside     | +     |       |      |
| Quercetin-3-galactoside    | +     | +     | +    |
| Quercetin-3-glucoside      |       |       | +    |
Table 8. Cont.

| Phenolic                        | Husk     | Buds     | Bark     |
|--------------------------------|----------|----------|----------|
|内外                           |          |          |          |
| Quercetin-3-arabinopyranoside  | +        | +        | +        |
| Quercetin-3-arabinofuranoside  | +        | +        |          |
| Quercetin-3-rhamnoside         | +        | +        | +        |
| Quercetin galloyl hexoside     |          |          | +        |
| Quercetin hexoside derivative 1|          |          |          |
| Quercetin hexoside derivative 2|          |          |          |
| Quercetin                      |          |          |          |
| Kaempferol pentoside 1        |          |          | +        |
| Kaempferol pentoside 2        |          |          | +        |
| Kaempferol pentoside 3        |          |          |          |
| Kaempferol rhamnoside         |          |          |          |
| Kaempferol-7-hexoside 1       |          |          | +        |
| Kaempferol-7-hexoside 2       |          |          |          |
| Kaempferol                    |          |          | +        |

+, phenolic identified.

4. Conclusions

A total of 83 individual phenolics and the total phenolics content were identified and quantified for the inner and outer husks, buds and bark of six walnut cultivars. These 83 phenolics comprised 25 naphthoquinones, 15 hydroxycinnamic acids, 8 hydroxybenzoic acids, 13 flavanols, 2 flavones, 1 flavanone and 19 flavonols. Thirteen naphthoquinones have been reported for the walnut *J. regia*, or any other species for the first time, that may be unique to Juglandaceae family. To the best of our knowledge, this is the most complete study to describe the levels of the various phenolics for walnut husk, buds and bark. Furthermore, this is the first report to provide not only characterisation and quantification of the phenolics for walnut buds, but also a detailed characterisation and quantification of the separate husk layers (i.e., inner, outer). These data demonstrate the levels of the phenolics in these different walnut tissues, which are classified as agro-residuals. When considering the different walnut tissues, the total phenolic contents (both for the sum, and for the total extracts) were higher than previously reported for walnut shoots, leaves and kernels. This justifies the use of the husk, buds and bark as sources of phenolics. Furthermore, the Slovenian cultivars showed higher total naphthoquinone contents in the outer and inner walnut husks compared to the French cultivars. This information might be useful for the future determination of the genetic origin of a cultivar and also for the authentication of the walnuts belonging to each region, country, etc., as cultivars bred in different climates appear to show some specific variations in their naphthoquinone contents. Consequently, the present study provides useful information not only for agro-food industry (additives, pesticides) but also for the cosmetic and pharmaceutical industries.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/biology10060535/s1. Figure S1: MS1 spectra of hydrojuglone β-D-glucopyranoside (naphthoquinone) in a negative ion mode., Figure S2: MS2 spectra of hydrojuglone β-D-glucopyranoside (naphthoquinone) in a negative ion mode., Figure S3: MS1 spectra of gallic acid derivative 5 (hydroxybenzoic acid) in a negative ion mode., Figure S4: MS1 spectra of (epi)catechin derivative 5 (flavanol) in a negative ion mode., Figure S5: MS2 spectra of (epi)catechin derivative 5 (flavanol) in a negative ion mode., Figure S6: MS1 spectra of santin (flavone) in a negative ion mode., Figure S7: MS2 spectra of santin (flavone) in a negative ion mode., Figure S8: MS1 spectra of quercetin-rhamnoside (flavonol) in a negative ion mode., Figure S9: MS2 spectra of quercetin-rhamnoside (flavonol) in a negative ion mode., Figure S10: MS1 spectra of p-coumaric acid derivative 4 (hydroxycinnamic acid) in a negative ion mode., Figure S11: MS2 spectra of p-coumaric acid derivative 4 (hydroxycinnamic acid) in a negative ion mode.
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Abbreviations

| Abbreviation | Description                        |
|--------------|------------------------------------|
| MS           | mass spectrometer                  |
| UHPLC        | ultra-high performance liquid chromatography |
| HPLC         | high performance liquid chromatography |

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