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► To cite this version:

Emilie Isidore, Gaëlle Willig, Fanny Brunissen, Christian Magro, Charles Monteux, et al.. Selective recovery of glycosylated phenolic compounds from nectarine tree branches (*Prunus persica* var. *nucipersica*). *Food Chemistry Advances*, 2024, 4, pp.100585. 10.1016/j.focha.2023.100585 . hal-04431326

HAL Id: hal-04431326

<https://hal.science/hal-04431326>

Submitted on 1 Feb 2024

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1 Selective recovery of glycosylated phenolic compounds from nectarine
2 tree branches (*Prunus persica* var. *nucipersica*)

3
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10
11 **Abstract:**

12 Nectarine tree branches, by-products from the arboriculture, contain phenolic compounds. Six
13 main components were revealed by LC-MS and divided into 3 aglycones (A) (catechin,
14 chlorogenic acid, naringenin) and 3 glycosylated (G) (rutin, isoquercetin and prunin) molecules.
15 To maximize their content in nectarine tree branches extract, the extraction process was
16 optimized. The optimum conditions were found at 55% ethanol and 90 °C, leading to $9.05 \pm$
17 0.80 mg/g_{DM} of catechin, 3.17 ± 0.31 mg/g_{DM} of chlorogenic acid, 1.15 ± 0.01 mg/g_{DM} of
18 naringenin, 1.56 ± 0.08 mg/g_{DM} of rutin, 0.57 ± 0.02 mg/g_{DM} of isoquercetin, and 1.73 ± 0.11
19 mg/g_{DM} of prunin. The glycosylated-aglycone ratio is equal to 0.276 ± 0.006 , meaning that
20 aglycones are predominant. Purification of the extract was carried out by adsorption. A
21 screening of several resins led to the choice of the XAD-7 resin that provides a recovery rate
22 of 66-84% of glycosylated compounds and 9-46 % of aglycones. The effect of the adsorption
23 process on the evolution of the G/A ratio was studied. Under the studied conditions, this
24 parameter increased by 123%, mainly due to a good performance of the XAD-7 towards
25 glycosylated components and a low recovery rate of chlorogenic acid.

26
27 **Keywords:** Nectarine tree branches, glycosylated components, extraction, adsorption,
28 phenolic compounds

1. Introduction

Nectarine (*Prunus persica* var. *nucipersica*) is a variety of peach (*Prunus persica*), a species native from China. The culture of peach trees, including nectarine trees, represented 11,700 ha in France in 2021. The nectarine fruit is usually smaller than that of the conventional peach with the particularity of having a smooth skin. They are intended for human nutrition and generate many by-products during cultivation, harvest (leaf, twig) or after processing (kernel, stem) (Agreste, *La Statistique Agricole*, n.d.; Siddiq et al., 2012; Sodeifian & Sajadian, 2021). Although very few studies have been published on the valorization of *Prunus persica* by-products, peach leaves and stems, as well as nectarine kernel oil, represent an interesting source of natural molecules with antioxidant activity and thus a great potential for the food, pharmaceutical or cosmetics industries (Maatallah et al., 2020; Murata et al., 2022; Sodeifian & Sajadian, 2021).

Tree pruning is performed to obtain fruits of better quality and facilitate the harvest. The wood coming from pruning, produced in large volumes, are often sent back on the land or burned (Aliaño-González et al., 2022; Siddiq et al., 2012). For example, the annual pruned biomass of Croatian nectarine trees has been estimated to 2.9 T/ha, which represents a significant amount of underutilized biomass (Bilandzija et al., 2012). Branches of arboriculture, whatever the fruit production, are rich in bioactive secondary metabolites. Phenolic acids, flavonoids have been identified in peach by-products, and several studies have shown the existence of bioactivities from peach branch extracts (Aliaño-González et al., 2022; Maatallah et al., 2020; Nakagawa et al., 2018; Yi et al., 2008). Therefore, *Prunus Persica* var. *nucipersica* branches seems an interesting source of phenolic compounds and it is worth considering studying a potential valorization of this biomass. To that end, a more in-depth knowledge of the phenolic compounds profile is necessary. The extraction of these molecules from their matrix remains a mandatory step for their identification and quantification. Conventional extraction process was mainly used to achieve this goal (Maatallah et al., 2020; Raturi et al., 2012; Yi et al., 2008). The process used in this study is an accelerated solvent extraction based on the use of a pressure fixed at 100 bar. In such working condition, it is possible to exceed the saturation pressure of the solvent and speed up the extraction. The process is fully automated, and the extract is filtered in-line at the end of the extraction process, allowing a quick and efficient screening of the extraction conditions (Mendiola et al., 2007).

However, phenolic compounds are often recovered at low concentrations in a complex mixture and the selectivity of the extraction process with respect to these molecules remains low (Kühn et al., 2014). Among the various purification technologies, our attention has focused on adsorption process. Indeed, synthetic resins have been successfully used to separate and/or concentrate phenolic compounds from crude vegetal extracts. Moreover, adsorption is economic, scalable, simple to operate, and the adsorbent can be regenerated (Conde et al., 2013; Kammerer, Kammerer, Jensen, et al., 2010; Kühn et al., 2014; Soto et al., 2011). This technology offers high yields after optimization which makes it popular (Kammerer, Kammerer, Jensen, et al., 2010; Kinoshita et al., 2015; Soto et al., 2011). Adsorption is impacted by the physical characteristics of the resin (Conde et al., 2013; dos Santos et al., 2022; Kinoshita et al., 2015; Silva et al., 2007) and the operating conditions such as the temperature or the pH of the solution (Conde et al., 2013; Kammerer, Kammerer, Jensen, et al., 2010; Tomás-Barberán et al., 1992). However, the large variety of molecules (phenolic compounds, sugars, proteins) present in the crude extract may affect the affinity of a given compound with the adsorbent, and even compete for adsorption (Conde et al., 2013; Kammerer, Kammerer, & Carle, 2010; Silva et al., 2007). For this reason, a follow-up of each target component is mandatory to assess the process efficiency.

77 Therefore, the objective of this work is to selectively recover phenolic compounds from
78 nectarine tree branches. A first part is dedicated to the identification and quantification of the
79 phenolic compounds present in the raw extract of nectarine tree branches. Then, a second
80 part will be devoted to the study of the impact of the extraction process on the molecules of
81 interest. The last part will be focused on the recovery of phenolic compounds using the
82 adsorption technology, where the behavior of each single component will be studied.

83 **2. Material and Methods**

84 **2.1. Plant material and chemicals**

85 Nectarine tree branches var CARENE® Monecar cov, harvested in March 2020 were kindly
86 provided by the company ChestNut (Valence, France). The plant material was dried outside at
87 ambient temperature before shipment, which is beneficial for the extraction of phenolic
88 compounds (Babiker et al., 2021). Nectarine tree branches' preparation consisted in a grinding
89 step with a SM300 Retsch cutting mill (Retsch, Haan, NRW, Germany). The equipment was
90 set at 1500 rpm and the nectarine tree branches were continuously crushed and sieved at 4
91 mm particle size. The ground sample was stored in the dark at room temperature. The dry
92 matter of the plant material was $90.2 \pm 0.1\%$ w.

93 The standards catechin and isoquercetin were purchased from Sigma Aldrich (Saint-Louis,
94 Missouri, USA) while chlorogenic acid and rutin were supplied from Fischer Scientific (Illkirch,
95 France). Naringenin and prunin are provided by VWR (Radnor, Penn., USA) and Carbosynth
96 (Berkshire, United Kingdom), respectively. For HPLC analysis, acetonitrile was purchased
97 from VWR (Radnor, Penn., USA) and formic acid was from Fischer Scientific (Illkirch, France)
98 as well as the 3 Amberlite resins XAD-4, XAD-7 and XAD-16. Ethanol for the extractions was
99 supplied from VWR (Radnor, Penn., USA). The ultrapure water used in this study was
100 produced with a water filtration equipment (MilliQ Integral 5, Millipore, Burlington,
101 Massachusetts, USA).

102 **2.2. Identification and quantification of phenolic compounds**

103 **2.2.1. Phenolic compounds identification by LC-MS**

104 The composition in phenolic compounds of nectarine tree branches extract was investigated
105 by LC-MS. The extract sample was diluted in ethanol then filtered with a 0.2 μ m RC filter. The
106 LC-MS equipment was an Agilent 1290 coupled with a 6545 Q-TOF mass spectrometer
107 (Wilmington, DE, USA) and a PDA UV detector. The Dual Agilent JetStream ESI source was
108 run at atmospheric pressure in positive ionization mode. The Zorbax Eclipse plus C18 column
109 (50 mm \times 2.1 mm, 1.8 μ m, Agilent, Santa Clara, CA, USA) was heated at 40 °C and the
110 injection volume was fixed at 1 μ L. The mobile phase was composed of 0.1% (v/v) formic acid
111 in water (solvent A) and 0.1% (v/v) formic acid in acetonitrile (solvent B). The elution gradient,
112 flow rate, acquisition wavelength and MS source were defined as described by (Willig et al.,
113 2022). MassHunter software (version 8.0.0, Agilent, Santa Clara, CA, USA) was used to
114 process the results. The most responding phenolic compounds were identified with an internal
115 database as well as NIST and Metlin commercial databases. The resulting propositions were
116 then validated with commercial standards.

117 **2.2.2. Phenolic compounds quantification by HPLC**

118 The preparation of the extract samples consisted in a suitable dilution in ethanol followed by a
119 filtration with 0.2 μ m RC filters prior HPLC analyses. The phenolic compounds were quantified
120 with a Dionex Ultimate 3000 (Dionex Corporation, Sunnyvale, Californie, USA) system
121 equipped with a diode array detector (DAD). The Halo 90 Å AQ-C18 column (150 mm \times 2.1 mm
122 \times 2.7 μ m, Advanced Material Tech, Wilmington, DE, USA) was heated at 48 °C. A volume of
123 1 μ L was injected, the phenolic compounds were eluted with a mobile phase composed of 1%

124 (v/v) formic acid in water (solvent A) and acetonitrile (solvent B). The elution gradient lasted
125 16 min at a flow rate of 0.6 mL/min as followed: 2 to 20% of solvent B from 0 to 6 min, 20% of
126 (B) from 6 to 8 min, 20 to 50% of (B) from 8 to 11 min, 50% of (B) from 11 to 12 min, 50 to 2%
127 of (B) from 12 to 12.5 min, and 2% of (B) from 12.5 to 16 min. UV detection was set at (i) 285
128 nm to detect catechin, naringenin and prunin, (ii) 254 nm to detect rutin and isoquercetin, and
129 (iii) 325 nm for chlorogenic acid. Chroméléon software (version 7.3) was used to record
130 chromatograms and related data. Stock solutions of chlorogenic acid, catechin, naringenin,
131 rutin, isoquercetin and prunin were prepared in ethanol as well as the corresponding dilutions
132 to draw proper calibration curves. The yield of individual phenolic compounds was determined
133 in mg/g_{DM}.

134 A distinction between glycosylated (G) and aglycone (A) phenolic compounds appeared
135 interesting because these two types of compounds might have different behaviors during the
136 extraction and purification processes. Thus, the G/A ratio parameter, representing the ratio
137 between the glycosylated and aglycone forms was defined in order to monitor the effect of the
138 extraction and purification on the two classes of molecules throughout the study (Equation 1).

139
$$\text{Equation 1: } G/A = \frac{\sum_{i=1}^n C_i}{\sum_{j=1}^n C_j}$$

140 Where G/A is the glycosylated-aglycone ratio (no unit), and C_i and C_j are the respective yields
141 or concentrations of glycosylated and aglycone phenolic compounds, respectively, extracted
142 from nectarine tree branches (mg/g_{DM} or mg/L).

143 **2.3. Study of the operating conditions on the selectivity of the extraction process**
144 **2.3.1. Optimization of the extraction of phenolic compounds**

145 The extraction of phenolic compounds was studied using response surface methodology. A D-
146 optimal design of experiment was set with MODDE software (version 12.0.1, Sartorius,
147 Goettingen, Germany). Accelerated solvent extractor (ASE 350, Dionex Corporation,
148 Sunnyvale, California, USA) is used to extract phenolic compounds from nectarine tree
149 branches. Ten milliliter extraction cells were used. Time (including a 5 min heating period) and
150 sample mass were fixed parameters respectively set at 35 min and 2 g. The pressure is fixed
151 at 100 bar. The extract is filtered online at the end of the extraction. After each extraction, the
152 samples were stored at -20 °C.

153 The first factor studied (X₁) is the ethanol concentration in water (0-30-70-100%, v/v) and the
154 second factor (X₂) is the temperature (40-65-90 °C). Y_i are the responses, expressed in mg/g_{DM}
155 and the glycosylated-aglycone ratio (G/A). The design of experiments, showed in table A.1
156 (supplementary material), consisted in 12 assays including 3 centered points.

157 The experimental results were used to model the responses with a second order polynomial
158 equation as described in Equation 2.

159
$$\text{Equation 2: } Y_i = a_0 + a_1 \cdot X_1 + a_2 \cdot X_2 + a_{11} \cdot X_1^2 + a_{22} \cdot X_2^2 + a_{12} \cdot X_1 \cdot X_2 + e$$

160 Where Y_i are the responses (Y₁ to Y₇), a₀ is the constant, a_i the linear (a₁ and a₂), quadratic
161 (a₁₁ and a₂₂) and interaction (a₁₂) coefficients of the model respectively, X₁ is the variable
162 "ethanol concentration", X₂ is the variable "temperature" and e is the lack of fit.

163 The extraction parameters were optimized using the software optimizer tool and validated
164 through experimental assays carried out in the optimal conditions.

165

166

167 2.3.2. Kinetic study of the extraction in optimal conditions

168 A kinetic study was conducted at 90 °C and 55% ethanol, these conditions are the optimum
169 defined by the aforementioned design of experiments. Different 10 mL extraction cells were
170 filled with 2 g of nectarine tree branches and loaded in the ASE. An extraction was performed
171 on every cell and stopped after the heating time (5 min), and 10, 20, 25 and 35 min including
172 the 5 min heating time. It is not recommended to heat more than 30 minutes with the ASE
173 because of the potential degradation of the phenolic compounds. Y_t were determined by HPLC
174 and plotted according to the extraction time.

175 The extraction kinetics were drawn for each phenolic compound and different mathematical
176 models were applied and compared to find the best fit with the experimental data:

- 177 • 1st order kinetic model (Poojary & Passamonti, 2015)

178
$$\text{Equation 3: } Y_t = Y_{eq} \cdot (1 - e^{-kt})$$

179 Where Y_t and Y_{eq} are the phenolic compound's concentrations at time t and at equilibrium
180 (mg/g_{DM}), k the extraction rate constant (min⁻¹), and t the time of extraction (min).

- 181 • Peleg's model (Peleg, 1988)

182
$$\text{Equation 4: } Y_t = Y_0 + \frac{t}{k_1 + k_2 \cdot t}$$

183 Where Y_0 and Y_t (mg/g_{DM}) are the phenolic compound's concentrations at time $(t) = 0$ and time
184 t (min), k_1 Peleg's rate constant (min g_{DM}/mg), k_2 Peleg's capacity constant (g_{DM}/mg). Y_0 is
185 assumed to be 0 at $t = 0$ min. Equation 4 is thus simplified as follows:

186
$$\text{Equation 5: } Y_t = \frac{t}{k_1 + k_2 \cdot t}$$

187 It should be noted that Peleg's model is very close to a 2nd order kinetic model. For this reason,
188 the latter is not presented in this study.

- 189 • Logarithmic model (Anbalagan et al., 2019)

190
$$\text{Equation 6: } Y_t = b + a \cdot \log(t)$$

191 Where Y_t is the phenolic compound's concentration at time t , a and b are the logarithmic model
192 parameters.

- 193 • Two-sites kinetic model (Karacabey et al., 2013)

194
$$\text{Equation 7: } Y_t = Y_{eq} \cdot [1 - f \cdot e^{-k_1 t} - (1 - f) \cdot e^{-k_2 t}]$$

195 The two-sites kinetic model is a succession of two 1st order terms, that describe the fast release
196 of components in a first step, then the slow diffusion in a second step. In Equation 7, Y_t is the
197 phenolic compound's concentration at time t (min), f is the fraction of the component during
198 the 1st step, k_1 is the corresponding extraction rate constant (min⁻¹); while $(1 - f)$ is the fraction
199 of the component during the 2nd step, and k_2 the corresponding extraction rate constant (min⁻¹).
200

201 Every model was tested thanks to Excel's Solver tool. Model fitting was evaluated by
202 calculation of the coefficient of determination, R^2 and the root mean square error, RMSE.

203

204

205 **2.4. Study of the extract's fractionation by adsorption**
206 **2.4.1. Preparation of an aqueous extract concentrate**

207 ASE is an interesting extraction technique with many advantages such as time reduction or
208 operating at pressure above solvent's boiling point. However, ASE has also drawbacks since
209 it produces small volumes of extract (around 100 mL). For this reason, hot maceration is
210 preferred to scale-up the process. The optimal temperature of phenolic compound's extraction
211 with ASE is 90 °C, above ethanol boiling point. This condition cannot be respected with
212 maceration, thus a temperature of 70 °C and an ethanol concentration of 70% v/v are chosen
213 for this technique.

214 Hot maceration was performed in erlenmeyers. The nectarine tree branches were soaked
215 during 6 h with a solvent composed of 70% ethanol in water (v/v). The solvent to material ratio
216 was 30 mL/g and the temperature was set at 70 °C. A magnetic stirrer was used for the
217 agitation; the power was adjusted to create a vortex and observe the mixture in motion. The
218 final extract is separated from the plant material by filtration through a 20 µm nylon filter and
219 stored at -20 °C.

220 An aqueous extract is preferred to study the fractionation by an adsorption process. The
221 hydroalcoholic solvent of nectarine tree branches extract has been evaporated under vacuum
222 and solubilised in a volume of water in order to concentrate 4 times the final extract. A smaller
223 volume is used to get a higher phenolic compound concentration in the solution and to save
224 solvent with negligible loss of the target molecules. This aqueous extract concentrate (AEC) is
225 used as starting solution for adsorption assays.

226 **2.4.2. Selection of macroporous resin**

227 The three amberlite resins XAD-4, XAD-7 and XAD-16, described in table A.2, are employed
228 to separate phenolic compounds from AEC. Prior their use, resins were soaked in methanol
229 then rinsed with ultrapure water in order to activate the resins and eliminate chemical
230 conservatives. Dry weight of wet resins was measured with moisture analyser (MB23, OHAUS,
231 Parsippany, New Jersey, USA). The adsorption is studied in a static mode. A volume of 50 mL
232 of AEC is introduced in a beaker with an equivalent of 1 g_{DW} resin at room temperature (22 °C).
233 After 120 min, the resin is separated from the extract with a nylon filter and rinsed with ultrapure
234 water. Phenolic compounds are desorbed with 50 mL of 70% ethanol in water (v/v) during 2 h
235 at ambient temperature. The phenolic compounds contained in the extract after adsorption and
236 in the desorption eluent were quantified by HPLC. Adsorption and desorption rates, as well as
237 adsorption capacity and recovery rate, were calculated for each phenolic compound according
238 to the following equations:

$$\text{Equation 8: } A_i = \left(1 - \frac{C_t}{C_0}\right) \times 100$$

$$\text{Equation 9: } D_i = \frac{C_d \times V_d}{(C_0 - C_t) \times V_{AEC}} \times 100$$

$$\text{Equation 10: } Q_{a,i} = \frac{(C_0 - C_t) \times V_{AEC}}{W}$$

$$\text{Equation 11: } R_i = \frac{C_d \times V_d}{C_0 \times V_{AEC}} \times 100$$

239

240 With A_i , D_i , $Q_{a,i}$, and R_i the adsorption rate, desorption rate, adsorption capacity, and recovery
241 rate of individual phenolic compound (%), C_0 the initial phenolic compound's concentration in
242 AEC (mg/L), C_t the phenolic compound concentration in AEC after 120 min adsorption (mg/L),

243 C_d the phenolic compound concentration in eluent after 120 min desorption (mg/L), V_d and
244 V_{AEC} the eluent and AEC volumes (L), and W the mass of resin (g) on a dry matter basis.

245 The best resin was selected based on A, D and R, but also on the evolution of the G/A ratio
246 after the process. The most satisfying resin was used to study the impact of the desorption
247 solution, where different ethanol concentrations (30, 50, 70 and 90%) were tested.

248 **2.4.3. Modelling of adsorption kinetic**

249 The adsorption kinetic corresponds to the adsorption capacity plotted according to the
250 adsorption time. This one is studied using the resin chosen previously (XAD-7 resin), in the
251 same conditions as described above. The samples are collected after 5, 15, 30, 45, 60, 90 and
252 120 min. The adsorption capacity is calculated for each phenolic compound according to
253 Equation 10, and is plotted against time. Pseudo-first order and pseudo-second order kinetic
254 models were applied to determine the best fit to the experimental data:

- 255 • Pseudo 1st order kinetic model (Shen et al., 2022; Yu et al., 2020)

256
$$\text{Equation 12: } Q_t = Q_{eq} \cdot (1 - e^{-k_1 t})$$

- 257 • Pseudo 2nd order kinetic model (Shen et al., 2022; Yu et al., 2020)

258
$$\text{Equation 13: } Q_t = \frac{k_2 \cdot t \cdot Q_{eq}^2}{1 + k_2 \cdot t \cdot Q_{eq}}$$

259 With Q_t and Q_{eq} the adsorption capacities at time t and at equilibrium (mg/g_{DM} resin), k_1 and k_2
260 the adsorption rate constants (min⁻¹) of the pseudo 1st and pseudo 2nd order kinetic models
261 respectively, and t the time of adsorption (min).

262 **2.5. Statistical analysis**

263 Experiments related to the design of experiments were treated with MODDE software (version
264 12.0.1, Sartorius, Goettingen, Germany).

265 All other experiments were conducted in triplicate. Means and standard deviations were
266 calculated with Excel. The analyses of variance (ANOVA) were performed using R software
267 (version 4.0.2). First, the normal distribution of data was validated with a Shapiro test, then a
268 Bartlett test was used to check the variance equality. After validation, the differences in means
269 were tested by a Tukey test. Student tests were also carried out to show the non-significant
270 differences between two means. All the risks used are equal to 5%.

271 **3. Results**

272 First, the phenolic compounds of extracts from nectarine tree branches were identified and
273 quantified. The operating conditions of the extraction process were then optimized and the
274 extraction kinetic was monitored and modelled. In a third part, an adsorption process was
275 applied to separate the phenolic compounds from the extract. Glycosylated-aglycone ratio was
276 monitored along this study to define a potential selectivity of the processes on these classes
277 of phenolic compounds.

278 **3.1. Phenolic compounds identification and quantification**

279 The phenolic compound composition of nectarine tree branches was determined by LC-MS.
280 Thirteen components were identified with databases according to their fragment mass. The
281 corresponding standards allowed to confirm their presence. The results are presented in table
282 A.3.

283 Although the composition in phenolic compounds in nectarine branches had never been
284 studied before, there are a number of studies on fruit trees of the genus *Prunus*. (Willig et al.,
285 2022) have identified from cherry tree branches the same phenolic compounds as found in this
286 study. Other studies dealing with *Prunus persica* branches have identified chlorogenic and
287 neochlorogenic acids, catechin, epicatechin, naringenin, prunin, quercetin and derivatives, and
288 anthocyanins (Aliaño-González et al., 2022; Maatallah et al., 2020; Murata et al., 2022; Raturi
289 et al., 2012). To the best of our knowledge, genistein, apigenin and chrysin were identified for
290 the first time in *Prunus persica* branches.

291 According to a first quantification on LC-MS (table A.4), 6 major compounds were identified.
292 Then, a quantification method has been developed on an UHPLC. The resulting chromatogram
293 is shown in figure A.1 and their respective formula are visible in figure 1.

294 In this study, the most abundant phenolic compounds of nectarine tree branches are divided
295 in 2 categories: the aglycone components (catechin, chlorogenic acid and naringenin) and the
296 glycosylated phenolic compounds (rutin, isoquercetin and prunin). Among the compounds
297 identified, there are two glucosides (prunin and isoquercetin) and one rutoside (rutin), the
298 latter bearing a disaccharide (rhamnose-glucose). It is also interesting to note that prunin is a
299 glycosylated form of naringenin, whereas rutin and isoquercetin are glycosylated forms of
300 quercetin.

301 The interest in glycosylated components lies in their higher solubility in water and higher
302 bioavailability in comparison with their corresponding aglycone, the glycoside moiety inducing
303 a higher polarity and a better hydrophilicity. They are also more stable and are assumed to be
304 the storage form of their corresponding aglycone molecules. However, their bioactivities are
305 usually decreased compared to aglycone forms, and the different sugars and linkages involved
306 can modulate their bioactivities (Johnson et al., 2021; Slámová et al., 2018).

307 **3.2. Optimization of the phenolic compounds' extraction**

308 A D-optimal design was performed to find the operating conditions affecting the extraction of
309 phenolic compounds from nectarine tree branches. For this, the extraction yield of the 6 main
310 phenolic compounds and the G/A ratio (Equation 1) were modelled according to the ethanol
311 concentration (X_1) and the extraction temperature (X_2). Values obtained for the 7 responses
312 are shown in table A.5.

313 The extraction yields differ widely between each phenolic compound. Catechin, varying from
314 1.35 to 7.84 mg/g_{DM}, is the most abundant component among the phenolic compounds
315 investigated in nectarine tree branches. This result is supported by other works, where catechin
316 was found to be abundant in *Prunus persica* and *Prunus avium* stems (Maatallah et al., 2020;
317 Willig et al., 2022). On the opposite, naringenin and isoquercetin show the lowest yields, in the
318 range 0-0.84 mg/g_{DM} and 0.14-0.58 mg/g_{DM}, respectively. The aglycone molecules, particularly
319 catechin and chlorogenic acid, are predominant. Glycosylated phenolic compounds are found
320 in a lower quantity, thus leading to a G/A ratio below 1.

321 For all components, the lowest yields are reached at low temperature (40 °C) with a solvent
322 composed of pure water (N1) or pure ethanol (N3). Ethanol mixed with water (70% (v/v)) is the
323 solvent of choice to obtain the highest yields. The minimum values are thus obtained in very
324 close operating conditions, independently of the phenolic compounds. This is also the case for
325 the maximum values. The G/A ratio does not change significantly between each run (0.237 to
326 0.287). The minimum and maximum values are obtained with water, at 65 °C (N4) and 40 °C
327 (N1), respectively.

328

329

3.2.1. Modelling of the extraction yield of phenolic compounds

330 From experimental data, coefficients of the polynomial equations were determined by MODDE
331 software. The analysis of their significance enables to determine the effect of the extraction
332 temperature, the ethanol concentration, their quadratic and interaction terms on the responses.

333 For each response Y_i , the relevant terms are kept in the prediction equations of the results and
334 to shape the response surfaces (figure A.2). Table A.6 gathers the coefficients calculated by
335 MODDE for the reduced models.

336 The adjusted coefficients of determination, p -values of the regressions and p -values for lack
337 of fit of all phenolic compounds indicate a good fit of the models to the experimental data. In
338 the case of G/A ratio, the adjusted R^2 is very low and the p -value exceeds 0.05 suggesting
339 that the model is not satisfactory. This means that in the studied conditions, ethanol
340 concentration and temperature do not allow a selectivity of a category of components over
341 another.

342 Ethanol (X_1) has a significant positive effect on naringenin and prunin yields and a significant
343 negative effect on chlorogenic acid. This term has also non-significant effects on catechin, rutin
344 and isoquercetin yields. As the quadratic term of ethanol (X_1^2) negatively impacts the
345 extraction of every phenolic compounds, the variable ethanol must be kept in the models. The
346 quadratic term of ethanol has the highest effect on the extraction of phenolic compounds from
347 nectarine tree branches. According to (Barwick, 1997), it is difficult to predict the solubility of a
348 given compound in a solvent. Nevertheless, polarity is used to explain this parameter because
349 this is the most influential factor. Rohrschneider polarity scale or Hildebrand solubility
350 parameter are recommended to calculate solvents' polarity (Barwick, 1997). In the work of
351 (Muhamad et al., 2014) and (Rahman et al., 2013), the Rohrschneider polarity scale is used
352 to demonstrate that the addition of water modifies the polarity of a solvent like ethanol and
353 offers a better solubility thus favoring the extraction of phenolic compounds. Indeed, solvent
354 and solute must be in the same polarity range to obtain a good solubility although other
355 interactions influence the solubility of phenolic compounds (Barwick, 1997; Muhamad et al.,
356 2014; Rahman et al., 2013). In the design of experiment, the influence of the water/ethanol
357 mixture varies according to the phenolic compounds, probably due to their own polarity range
358 and interactions with the solvent. These results prove the necessity to use a solvent composed
359 of ethanol and water to increase the extraction yield of phenolic compounds from nectarine
360 tree branches instead of the sole ethanol or water.

361 Temperature (X_2) is the second factor investigated in this study. Temperature has a positive
362 effect on all the phenolic compounds studied indicating that an increase of this parameter
363 benefits the recovery of every phenolic compound. Despite the non-significance for the
364 extraction of chlorogenic acid, rutin and isoquercetin, equations including the temperature's
365 coefficients result in a stronger model. An increase of temperature improves the solubility of
366 phenolic compounds and thus their diffusion from the plant matrix to the solvent (Muhamad et
367 al., 2014). However, a temperature too high may result in compounds degradation and lower
368 recovery. This phenomenon is not observed in the tested conditions. The extraction duration
369 chosen in this work, 35 min, is indeed quite short. Such extraction period is thus not long
370 enough to observe phenolic compound degradation even at the higher temperatures tested.
371 Only prunin is affected by the quadratic term of temperature (X_2^2), indicating a potential
372 degradation of this molecule to a small measure that is not noticeable on the responses' values
373 (table A5).

374 The interaction between the factors ($X_1 * X_2$) has a significant positive impact on naringenin
375 recovery. The best yields are obtained at highest temperatures and ethanol concentrations.
376 The ethanol-temperature interaction does not have such effect on the other responses.

377 The effect of ethanol concentration and temperature described above can be observed on the
378 response surfaces presented in figure A.2.

379 All extraction yields, except that of naringenin, follow the same trend as the ethanol
380 concentration changes in the solvent. As shown in table A.6, this factor has a great impact on
381 the recovery of phenolic compounds, with an optimal concentration around 50%. Naringenin
382 extraction benefits most from higher ethanol concentrations in the solvent, especially at high
383 temperatures. Temperature has a lower impact than the ethanol concentration on the recovery
384 of phenolic compounds. The maximum values of extraction yields were obtained for
385 temperatures above 70-75 °C. As the extraction temperature is limited to 90 °C in this work,
386 studying the effect of temperatures superior to 90 °C could be interesting to possibly reach
387 higher recovery in phenolic compounds.

388 **3.2.2. Validation of the prediction models**

389 The optimal extraction conditions were determined with MODDE software to recover efficiently
390 the phenolic compounds from nectarine tree branches. All the phenolic compounds, except
391 naringenin, have their maximum predicted yield in the same ranges of temperature and ethanol
392 concentration, meaning that they are in close polarity ranges. Furthermore, as mentioned
393 before, the G/A ratio is not affected by the variables. A selection of glycosylated or aglycone
394 phenolic compounds during the extraction is thus not possible. For this reason, the chosen
395 scenario for optimizing the extraction conditions is the maximisation of all the phenolic
396 compounds. A separation step will be performed in the following sections to attempt the
397 fractionation of the glycosylated and aglycone phenolic compounds. The MODDE optimizer
398 tool was used to define the optimum conditions, an ethanol concentration of 55% v/v and an
399 extraction temperature of 90 °C. To validate the design of experiments, these extraction
400 conditions were applied on nectarine tree branches to compare the observed and predicted
401 values (table 1). The observed values are 9.05 ± 0.80 mg/g_{DM} of catechin, 3.17 ± 0.31 mg/g_{DM}
402 of chlorogenic acid, 1.15 ± 0.01 mg/g_{DM} of naringenin, 1.56 ± 0.08 mg/g_{DM} of rutin, 0.57 ± 0.02
403 mg/g_{DM} of isoquercetin, and 1.73 ± 0.11 mg/g_{DM} of prunin. A solvent composed of 55% ethanol
404 gives the best solubility for most phenolic compounds from nectarine tree branches, and a
405 temperature of 90°C is better to enhance their solubility.

406 According to a Student's test, the observed catechin, chlorogenic acid, naringenin, rutin and
407 isoquercetin yields are equivalent to the predicted values (p -value > 0.05). Only the extraction
408 yield of the prunin is not correctly predicted by the model. Acquisition of supplementary data
409 would be useful to increase the prediction precision of this model. After the optimisation of the
410 extraction, G/A ratio is 0.276 ± 0.006 . This value is in the range of results obtained in the design
411 of experiments.

412 **3.3. Modelling the extraction kinetic in optimal conditions**

413 The evolution of phenolic compound yields was investigated during the extraction in the optimal
414 conditions (90 °C, 55% EtOH). The kinetics for each phenolic compound are shown in figure 2.

415 Different kinetic models (Peleg's model, first order model, logarithmic model, 2-sites kinetic
416 model) were used to explain the extraction kinetics of every phenolic compounds. The R^2 and
417 RMSE were used to evaluate the model adequacy. Table A.7 gathers these parameters for
418 each model and each phenolic compound.

419 A model is assumed to describe properly the kinetic when the calculated R^2 is close to 1 and
420 the RMSE is low. In table A.7, all the tested models lead to a high R^2 and a low RMSE meaning
421 that they give a good description of the extraction of the phenolic compounds from nectarine
422 tree branches. However, the best kinetic description is achieved with the 1st order and the 2-
423 sites kinetic models for the extraction of catechin, chlorogenic acid, naringenin and rutin. The
424 2-sites kinetic model described better the extraction of isoquercetin and Peleg's model was
425 better for prunin. It should be noted that 1st order kinetic model and 2-sites kinetic model gave
426 very close or equivalent results. As described in Equation 7, the 2-sites kinetic model is
427 composed of two 1st order terms, corresponding to a fast washing of the phenolic compounds
428 from the plant material followed by a slow diffusion. According to (Dias et al., 2017), when the
429 washing does not occur, only the slow diffusion is considered. Equation 7 is simplified and
430 becomes a pseudo 1st order kinetic. An absence of washing step can be thus assumed when
431 1st order kinetic and 2-sites kinetic models give very close evaluation parameters. For this
432 reason, the 2-sites kinetic model is preferred to describe the extraction of phenolic compounds
433 from nectarine tree branches.

434 According to figure 2, the extraction of phenolic compounds from nectarine tree branches is
435 fast, 90% of the theoretical equilibrium yield (C_{eq}) is obtained in less than 10 min for all the
436 phenolic compounds except prunin. The extraction kinetic of the latter is slower and 17 min
437 are needed to achieve 90% of C_{eq} . The 2-sites model predicts an equilibrium reached in 12 to
438 34 min during the ASE extraction for all phenolic compounds except prunin, whose equilibrium
439 yield is expected to be reached in 64 min. The observed values of C_{eq} reach the predicted C_{eq}
440 after 35 min of extraction for each phenolic compound.

441 **3.4. Selective recovery of glycosylated phenolic compounds by adsorption**

442 As we have shown, the phenolic compounds from nectarine tree branches are successfully
443 extracted but the extraction is not selective towards glycosylated or aglycone components. By
444 the implementation of adsorption technology, the following step focuses on increasing the G/A
445 ratio. Prior adsorption, the extract solvent was evaporated, and the extract was dissolved in
446 water to decrease the solubility of the target molecules in the extract and increase their affinity
447 with the resin. There is indeed a competition between the two phenomena (Kammerer,
448 Kammerer, Jensen, et al., 2010; Silva et al., 2007). A selection is performed among 3 resins
449 available in the laboratory. The concentration of ethanol in the desorption solvent was then
450 studied on the most satisfying resin.

451 **3.4.1. Screening of resins**

452 An adsorption process has been used to separate glycosylated and aglycone phenolic
453 compounds from the aqueous extract concentrate (AEC). Macroporous resins XAD-4, XAD-7
454 and XAD-16 have been screened to find the most suitable one. Adsorption, desorption and
455 recovery rate of each phenolic compound were calculated with equations 8, 9, 11, and are
456 given in figure 3 (A, B, C).

457 The material, pore size and surface area of a given resin affect the molecules' affinity and
458 result in different adsorption rates, as it can be observed with phenolic compounds from
459 nectarine tree branches after 120 min in contact with the 3 resins (Conde et al., 2013; dos
460 Santos et al., 2022). Chlorogenic acid is the component with the lowest adsorption rate on all
461 resins ($\leq 33\%$) while naringenin has a good affinity with resins XAD-7 ($96 \pm 2\%$) and 16 ($94 \pm$
462 2%) and is one of the best adsorbed molecules with XAD-4 ($51 \pm 3\%$). In general, XAD-7 offers
463 the best adsorption rates except for chlorogenic acid that is better adsorbed with XAD-16. On
464 the opposite, XAD-4 has a poor performance, with low adsorption of phenolic compounds
465 compared to the other resins. XAD-7, made of acrylic resin, is more polar than XAD-4 and

466 XAD-16, composed of styrene-divinylbenzene, and has higher affinity with glycosylated
467 compounds. However, XAD-16 allows a good adsorption of these molecules as well. A reason
468 for this observation could be the higher pore size of XAD-16 (100 Å) compared to XAD-4 (50
469 Å) as shown in table A.2, inducing a better penetration of the molecules into the resin. The
470 larger molecules, such as glycosylated phenolic compounds, might also have more chance to
471 be adsorbed. A similar observation was made by *Um et al.* (2017), who compared XAD-4 and
472 XAD-16 during the adsorption of phenolic compounds from yellow poplar hydrolysate (*Um et*
473 *al.*, 2017).

474 Furthermore, the average adsorption rate is higher for glycosylated than for aglycone
475 compounds with XAD-7 and 16. However, glycosylated molecules should have a higher
476 solubility in water than the aglycone ones, resulting in a lower affinity with the resins. Indeed,
477 hydrophobicity is important to induce the adsorption, but this parameter does not seem to
478 predominate. This phenomenon has already been described before (*Kammerer, Kammerer, &*
479 *Carle, 2010; Kammerer, Kammerer, Jensen, et al., 2010; Pompeu et al., 2010*). Other
480 interactions, such as hydrogen bonds, might explain the higher binding of glycosylated
481 phenolic compounds. In particular, the low adsorption rate of chlorogenic acid can be explained
482 by the pH of AEC. The pKa of this phenolic acid has been estimated at 3.95 ± 0.50 by
483 *Kammerer et al.* (2010) and is lower than the pH of AEC (4.85 ± 0.14). Indeed, chlorogenic
484 acid was better adsorbed at pH = 1-3, where the neutral form of the molecule predominates
485 (*Kammerer, Kammerer, & Carle, 2010; Rodriguez-Lopez et al., 2021; Soto et al., 2011*). The
486 pH might have a lower effect on other target phenolic compounds that does not display
487 carboxylic acid moiety. The higher pKa of flavonoids makes it possible to operate in a wider
488 pH range (*Kühn et al., 2014*). For example, the pKa of rutin and catechin have been estimated
489 at 6.84 ± 0.60 and 9.54 ± 0.10 , respectively (*Kammerer, Kammerer, & Carle, 2010*). It should
490 be noted that, probably because they are more hydrophilic, rutin (being described as an
491 isoquercetin with a rhamnose) and prunin (as a naringenin with a glucose) exhibit lower
492 adsorption rates than isoquercetin and naringenin, respectively. Other compounds from the
493 AEC might also affect the adsorption, however they are not studied here. For example, the
494 adsorption of phenolic compounds is influenced by the presence of sugars or amino acids
495 (*Kammerer, Kammerer, & Carle, 2010*).

496 The phenolic compounds from the 3 resins have good desorption rates for glycosylated
497 molecules (> 80%) and variable desorption performances for aglycones. Glycosylated
498 compounds have a greater affinity with the eluent than their aglycone or mono-glycosylated
499 homologs, but this affinity is not the only factor affecting the rate of desorption. Indeed, the
500 small molecules could have diffused into the smallest pores of the resin and they would be
501 less accessible during desorption (*Ferri et al., 2011; Kinoshita et al., 2015*). However, prunin
502 shows a better desorption rate than its aglycone (naringenin) with all the resins, while rutin has
503 a better desorption than isoquercetin with XAD-4 and XAD-16 and an equivalent value with
504 XAD-7. Thus, the polarity of phenolic compounds is the predominant factor in explaining the
505 results observed during the desorption.

506 The recovery rate results from the combined efficiency of the adsorption and desorption steps.
507 The XAD-7 and XAD-16 resins exhibit a recovery of aglycone phenolic compounds ranging
508 from 9 to 69% while that of glycosylated compounds can reach 84%. XAD-4 resin offers less
509 than 50% of recovery rate for each phenolic compound. The aglycone phenolic compounds
510 are less recovered under the tested conditions.

511 The evolution of the G/A ratio at the different steps of the adsorption process is presented
512 figure 3 (D).

513 The G/A ratio of AEC before the adsorption is 0.304 ± 0.022 . After the adsorption step, the G/A
514 ratio on XAD-7 and XAD-16 resins increased significantly to 0.483 ± 0.077 and 0.522 ± 0.016 ,
515 respectively. Since both values are significantly different from G/A ratio in AEC, the adsorption
516 is considered to be selective for glycosylated phenolic compounds. This result can be
517 explained by the good binding of these molecules due to hydrogen bounds, and on the lower
518 adsorption of aglycones, especially chlorogenic acid. On the contrary, XAD-4 resin has a G/A
519 ratio similar to AEC (0.288 ± 0.030) because the phenolic compounds have similar adsorption
520 rates. The desorption rates with this resin are also quite close, resulting in a slight but not
521 significant increase in the G/A ratio after elution. Concerning the XAD-7 and XAD-16 resins,
522 the G/A ratios increase after the desorption going from 0.483 ± 0.077 and 0.522 ± 0.016 after
523 the adsorption step to 0.678 ± 0.028 and 0.544 ± 0.020 after desorption, respectively. In
524 addition, XAD-7 has very good recovery rates of phenolic compounds and the best increase
525 in G/A ratio. This increase is significant, showing a selective desorption of glycosylated
526 phenolic compounds. The use of XAD-7 resins allows to increase the selectivity of the
527 purification process with respect to glycosylated phenolic compounds. This resin was thus
528 selected to study the adsorption kinetics and the desorption solution in the following chapters.

529 **3.4.2. Modelling of adsorption kinetics**

530 Pseudo 1st and pseudo 2nd order models are empirical models that consider adsorption as a
531 pseudo chemical reaction. They are frequently used to describe the adsorption kinetics and
532 are employed to fit the adsorption kinetic of each phenolic compound obtained from nectarine
533 tree branches with XAD-7 resin (dos Santos et al., 2022; Soto et al., 2011). The R² and RSME
534 parameters were used to assess the model adequacy; they are presented in table A.8 as well
535 as the equilibrium adsorption capacities and the adsorption rate constants.

536 The pseudo 2nd order model provides the best fit to the adsorption kinetics of all phenolic
537 compounds. The R² and RMSE parameters are indeed more satisfactory with this model. For
538 this reason, the adsorption kinetics of phenolic compounds from nectarine tree branches were
539 modelled with the pseudo 2nd order equation (figure 4).

540 The adsorption of aglycone phenolic compounds is very fast (figure 4 A, B, C). Indeed, the
541 XAD-7 resin reaches 90% of its equilibrium adsorption capacity in less than 20 min. Concerning
542 glycosylated phenolic compounds, 90% are reached in 27 to 107 min. The adsorption kinetics
543 of hydrophobic compounds has already been described and seems to indicate a weaker
544 competition between the aqueous extract and the resin, which would explain why the
545 adsorption of aglycone compounds is fast (Campone et al., 2020). It should be noted that the
546 adsorption of chlorogenic acid is instantaneous with a low rate (table A.8). As explained above,
547 the ionic form of this component is predominant under the working pH, which induces a high
548 solubility in water, and thus a lower affinity with the resin. The predicted equilibrium adsorption
549 capacities are reached at very long times (more than 100 h), however, 120 min is a satisfactory
550 duration for obtaining an efficient adsorption of the phenolic compounds.

551 **3.4.3. Effect of the elution solvent**

552 Hydroalcoholic solutions have been widely used to elute phenolic compounds from resins (dos
553 Santos et al., 2022; Soto et al., 2011). Different concentrations of ethanol in water (30 to 90%,
554 v/v) were tested during the desorption step with XAD-7 resin. Indeed, the 6 phenolic
555 compounds could have different affinities according to the ethanol concentration and induce a
556 change of the G/A ratio. The desorption rates of each phenolic compound and the G/A ratio
557 are given, in figure 5, for all the eluent solutions.

558 The desorption rate increases with higher ethanol concentrations up to 70% and then
559 decreases for catechin, chlorogenic acid, isoquercetin and prunin. A higher ethanol

560 concentration (90%) is needed to solubilize efficiently naringenin during the elution. In the case
561 of rutin, 50, 70 and 90% ethanol give the same desorption performance. All phenolic
562 compounds are desorbed with the lowest ethanol concentration, a partition of the molecules
563 with different eluents is thus not possible. It has been demonstrated that phenolic compounds
564 can be desorbed from acrylic resins with high quantity of water which is in accordance with the
565 results of this study (Tomás-Barberán et al., 1992).

566 The G/A ratio after an elution with 30% ethanol is 0.361 ± 0.017 , which represents a significant
567 decrease compared to the value obtained after the adsorption step. Eluents composed of 50
568 to 90% ethanol desorb glycosylated compounds at a higher rate compared to the aglycone
569 ones which results in higher G/A ratios. The elution with 70% ethanol offers the highest G/A
570 ratio, meaning that the glycosylated phenolic compounds are well recovered while some of the
571 aglycone compounds component remains on the resin after desorption. Some authors have
572 already showed the best elution of phenolic compounds with 70% ethanol among mixtures of
573 ethanol in water ranging from 0% to 95% ethanol (Campone et al., 2020; Jiao et al., 2017).
574 This eluent composition is therefore the best solvent for an efficient recovery and separation
575 of phenolic compounds from AEC.

576 **4. Conclusion**

577 The selective recovery of phenolic compounds from nectarine tree branches has been studied.
578 A preliminary extract allowed the identification of 13 phenolic compounds including 6 major
579 ones. Some components were identified for the first time in this work. The 6 main phenolic
580 compounds were tracked during the extraction and purification. The extraction process is
581 efficient, however there is no selectivity towards glycosylated or aglycone components. An
582 adsorption process was thus applied to the extract and XAD-7 was found to be the best resin
583 to separate them with an elution with 70% ethanol. After the purification process, the G/A ratio
584 increased significantly from 0.304 ± 0.022 in AEC to 0.678 ± 0.028 in the eluent. The
585 glycosylated phenolic compounds are well recovered while a part of the aglycone component
586 (mainly chlorogenic acid) was lost during the adsorption step. The extraction and adsorption
587 kinetics were successfully modelled by the 2-sites and pseudo 2nd order models respectively.
588 An extraction step followed by adsorption is thus an efficient and scalable process to selectively
589 recover a fraction of glycosylated phenolic compounds from nectarine tree branches. A deeper
590 study on purification by adsorption would be necessary to observe the results in dynamic mode
591 and determine the isotherms of adsorption, thus allowing the determination of the adsorption
592 mechanism of each phenolic compound.

593 **Authors contributions**

594 Emilie ISIDORE: investigation, statistical and HPLC analyses, writing-original draft. Gaëlle
595 WILLIG: investigation. Fanny BRUNISSEN: LC-MS analyses, development of HPLC analyses.
596 Christian MAGRO: resources, writing-review and editing. Charles MONTEUX: resources,
597 writing-review and editing. Irina IOANNOU: conceptualization, supervision, writing-review and
598 editing.

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728

729 *Figure 1: Chemical structure of the 6 main phenolic compounds found in nectarine tree*
730 *branches.*

731

732 *Figure 2: Extraction kinetics of the different phenolic compounds (55% ethanol – 90 °C – 35*
733 *min). A: catechin. B: chlorogenic acid. C: naringenin. D: rutin. E: isoquercetin. F: prunin.*
734 *Dashed lines correspond to the models while dots are the observed values.*

735

736 *Figure 3: Comparison of macroporous resins performances. A: Adsorption rates. B: Desorption*
737 *rates. C: Recovery rates. For a given phenolic compound, values with different letters mean a*
738 *significant difference ($p < 0.05$). D: G/A ratio at different adsorption steps. For each step,*
739 *different letters mean a significant difference in the values ($p < 0.05$).*

740

741 *Figure 4: Adsorption kinetic of phenolic compounds from nectarine tree branches with XAD-7*
742 *resin (2h – room temperature). A: catechin. B: chlorogenic acid. C: naringenin. D: rutin. E:*
743 *isoquercetin. F: prunin. Dashed lines correspond to the models while dots are the observed*
744 *values.*

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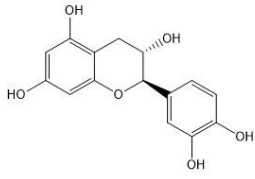
746 *Figure 5: Desorption rate of phenolic compounds from nectarine tree branches using different*
747 *eluent solutions (XAD-7 – 2h adsorption – room temperature – 2h desorption). For a given*
748 *phenolic compound, different letters mean a significant difference in the values ($p < 0.05$).*

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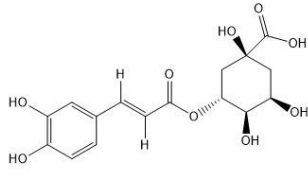
750 *Table 1: Experimental validation of the prediction models of the phenolic compounds'*
751 *extraction yield.*

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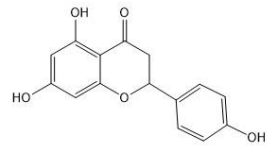
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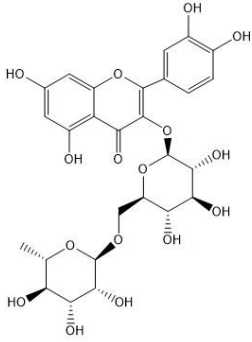
Catechin



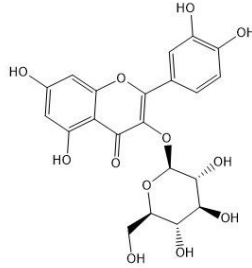
Chlorogenic acid



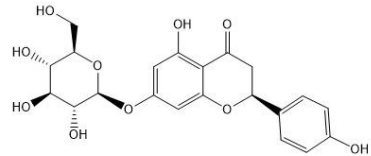
Naringenin



Rutin



Isoquercetin

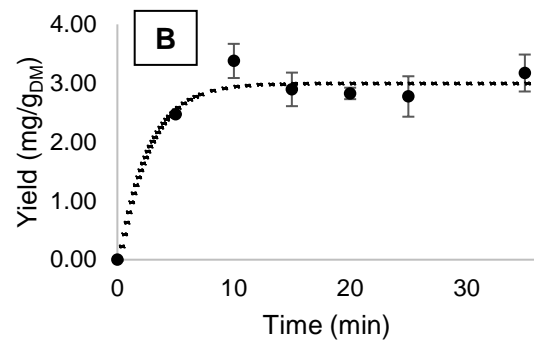
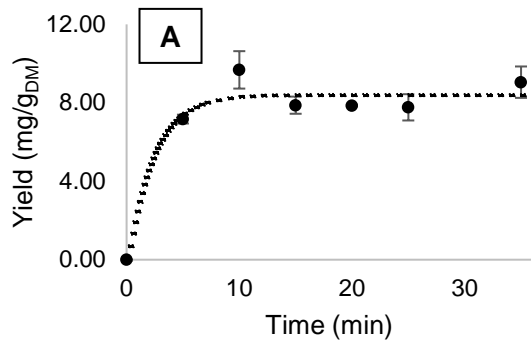


Prunin

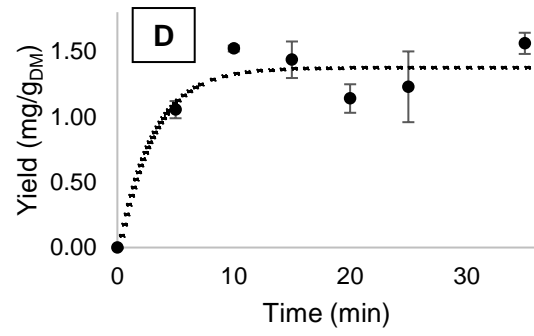
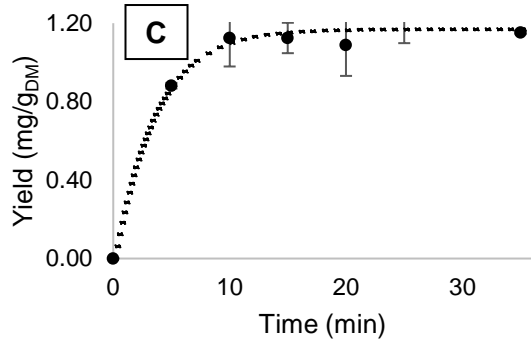
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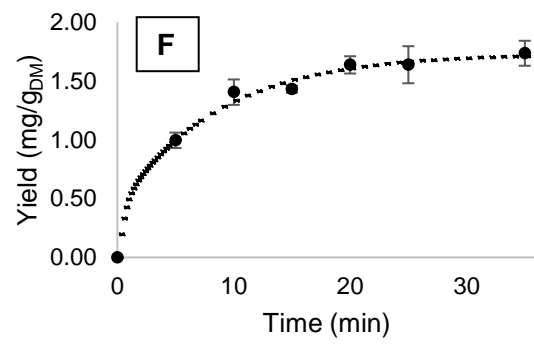
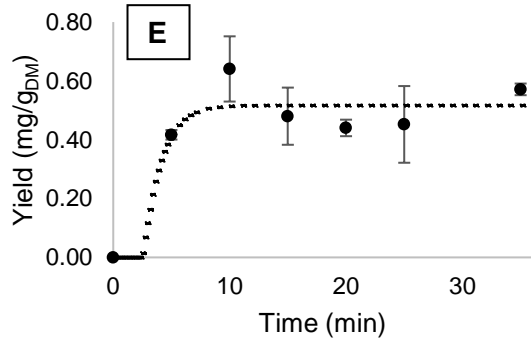
Figure 1



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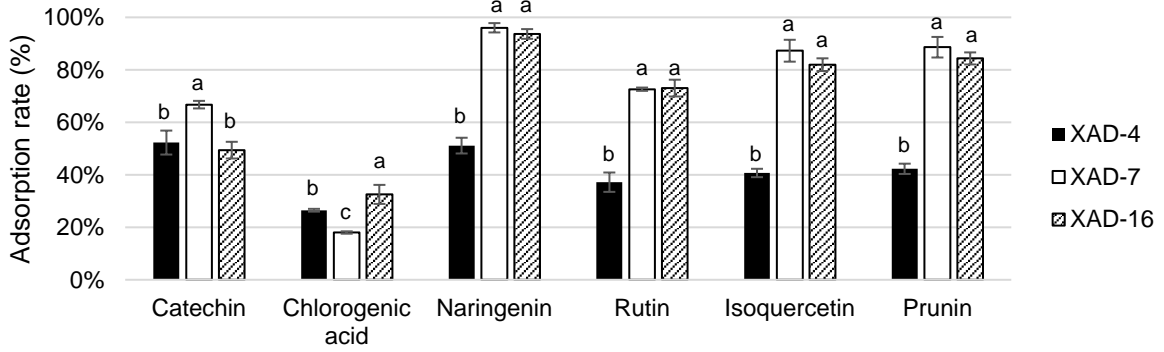
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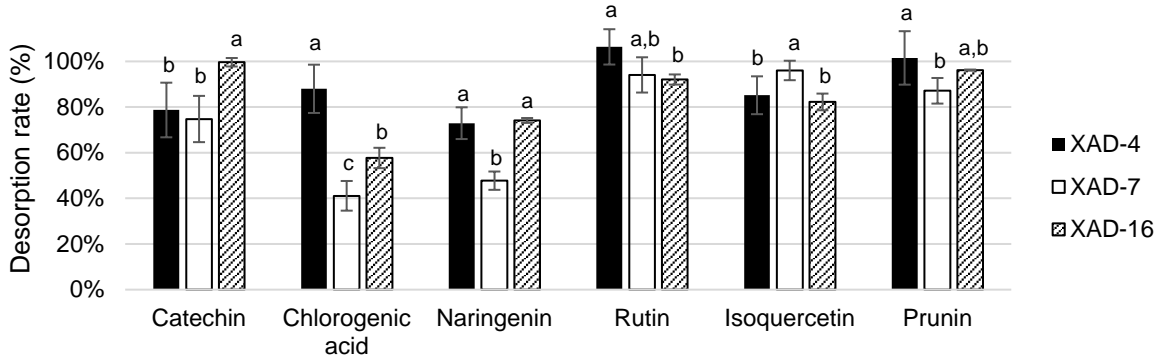
Figure 2

A



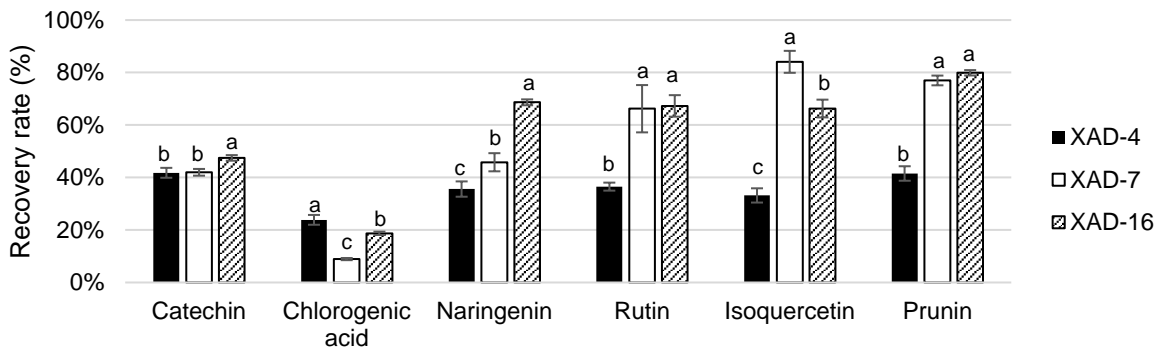
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B



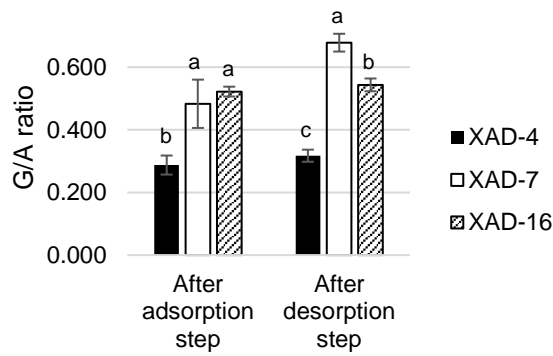
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C



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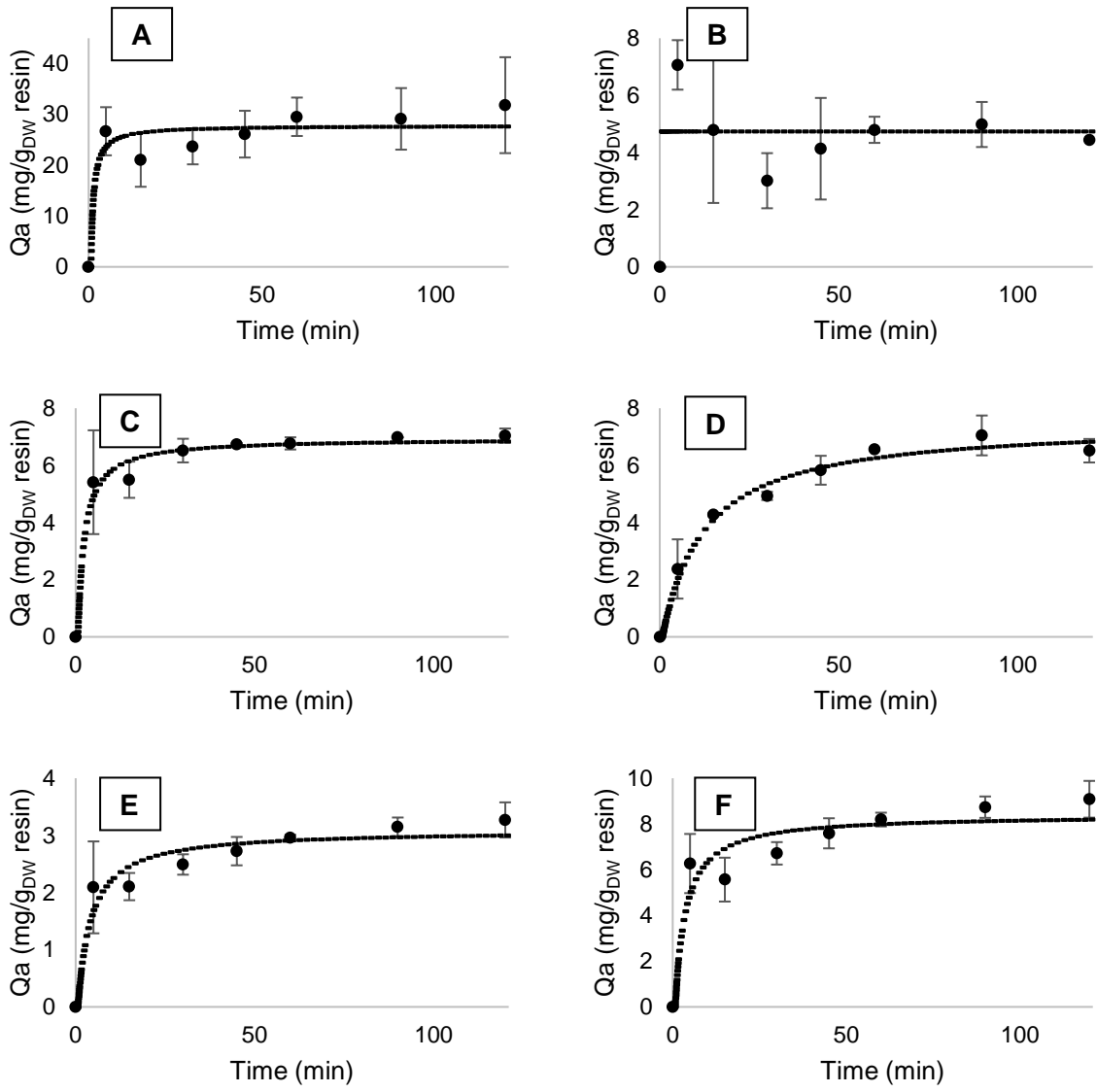
D



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Figure 3



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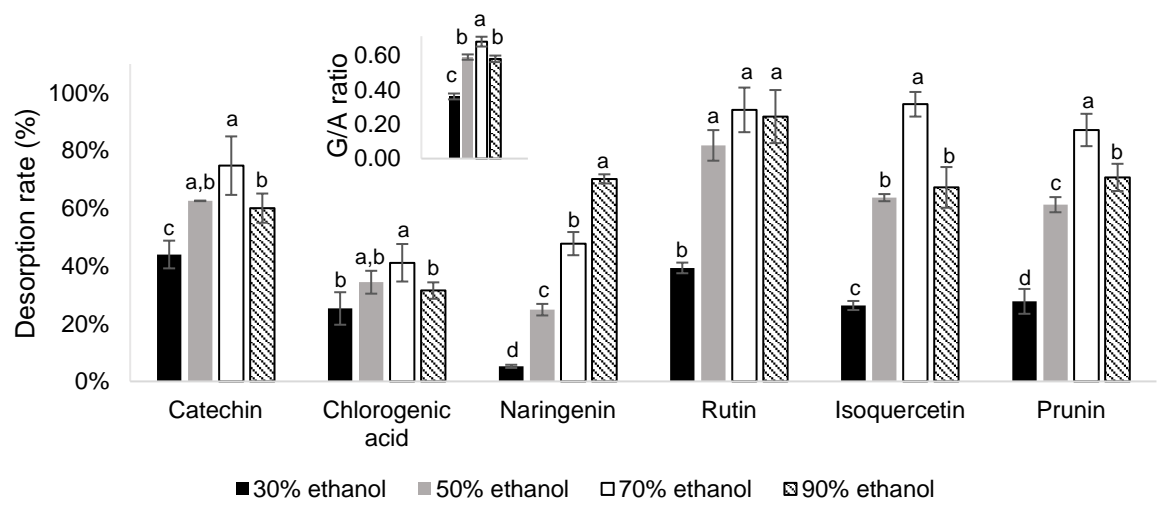
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Figure 4



772

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Figure 5

774

Table 1

	Catechin (mg/g _{DM})	Chlorogenic acid (mg/g _{DM})	Naringenin (mg/g _{DM})	Rutin (mg/g _{DM})	Isoquercetin (mg/g _{DM})	Prunin (mg/g _{DM})
Predicted values	8.01 ± 0.61	3.15 ± 0.38	1.02 ± 0.06	1.35 ± 0.20	0.58 ± 0.06	1.13 ± 0.02
Observed values	9.05 ± 0.80	3.17 ± 0.31	1.15 ± 0.01	1.56 ± 0.08	0.57 ± 0.02	1.73 ± 0.11
p-value	0.15	0.93	0.14	0.30	0.83	0.01

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