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Recovery and antioxidant activity of phenolic compounds extracted from walnut press-cake using various methods and conditions

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Abstract.

Walnut press-cake is a residue of oil processing from which the phenolic compounds have been extracted using ethanol 60% by batch and semi-continuous operations, or by ultrasound-assisted maceration in the case of a de-oiled cake, with a particular emphasis on the variables time and solid-solvent ratio. For batch extraction, the conditions maximizing total phenolic yield (TPC) and radical scavenging activity (DPPH) were 60°C without the addition of phospholipids, with, however, for TPC, an extraction time of 60 min and a ratio of 25 mL of solvent per g of cake. Values of up to 14.8 mg GAE/g_{cake} and 136.9 µmol Trolox Equiv./g_{cake} were obtained. The semi-continuous variant resulted in a TPC of 19.8 mg GAE/g_{cake} and an overall extract containing 8% of phenolic compounds and 46% of oil. After de-oiling, the cake was subjected to ultrasound-assisted extraction. TPC and DPPH varied slightly with the conditions applied, between 10.4 and 13.3 mg GAE/g_{cake} and 83 to 107 µmol Trolox Equiv./g_{cake} respectively, unless the solid/solvent ratio increased to 1/200 (TPC of 29 mg GAE/g_{cake}). It has been also shown that the oxidative stability of camelina oil can be increased from 24 to 72 h by enrichment with walnut extract.

26

27 **Key-words.** Walnut press-cake; phenolic compounds; oil oxidative stability; batch and semi-
28 continuous extraction; ultrasound-assisted extraction; response surface methodology

29

30 **I. Introduction**

31 In the general context of the United Nations Agenda 2030 for Sustainable Development, the
32 recovery of bioactive compounds from food processing by-products and their reuse in the food chain
33 contribute to the optimal use of the raw materials produced (Galanakis, 2020). The recovery of
34 bioactive compounds such as vitamins, lipids, phenolic compounds furthermore corresponds to the
35 growing consumer interest in nutrients that play a role in improving health, maintaining well-being,
36 improving immunity and thus preventing and treating specific diseases (Kumar et al., 2017,
37 Galanakis, 2020).

38 Walnut (*Juglans regia* L.) is the most widespread tree nut in the world, presently cultivated
39 commercially throughout southern Europe, northern Africa, eastern Asia, United States and western
40 South America (Martinez et al., 2010). The walnut production is increasing by 5% per year and
41 reached 3.6 million tons in 2018, half of which produced in China, 30% in USA, and 11% in France.
42 (Persistence Market Research 2018, FAOstat). The walnut is a kernel categorized as a strategic
43 species for human nutrition since it is a rich source of different valuable chemicals like proteins, oil,
44 fibers, and minerals (Jahanban-Esfahlan et al., 2019, Ozcan, 2009).

45 Walnut oil, rich in ω -3, ω -9, and ω -6 fatty acids, has long been known for its nutritional
46 qualities but is also used in a variety of health and personal care products and in aromatherapy
47 (Persistence Market Research 2018). The oil extraction process consumes twice as many walnut
48 kernels in mass as it produces oil in volume. The production therefore releases huge quantities of
49 partially defatted walnut cake which are often disposed of without any economic benefit or used as
50 animal feed or fertilizer (Li et al., 2017). As with the composition of walnut kernels (Zheng et al.,

2020), the composition of the press-cake will depend on the cultivar, environmental factors, cultivation practices, and on the oil recovery processes as well, but it is still remarkably rich in oil (20-36%), proteins (30-42%), dietary fibre, phenolic substances and minerals (Bakkalbasi et al., 2015, Li et al., 2017, Santos et al., 2018, Martinez et al., 2010). Attempts have been made to increase the valorization level of walnut cake, e.g. by incorporating it into meat, dairy and bakery products (Martinez et al., 2010, Bakkalbasi et al., 2015), by using their proteins as carriers of water-insoluble bioactive compounds (Moghadam et al., 2020) or as precursors of active peptides (Liu et al., 2013; Li et al., 2017; Gu et al., 2015). Due to the polyunsaturated fatty acids of the oil, the press cake is sensitive to oxidation, especially when ground into a fine powder. However, it has been shown that flour could be stored for up to 26 weeks without major changes in peroxides levels (Vanhanen and Savage, 2006). This behavior was attributed to the high vitamin E content of the cake which can reach 174 mg/kg (Bakkalbasi et al., 2015) but other antioxidants such as phenolic compounds may contribute to oxidative stability (Fukuda et al., 2003) since most of the phenolics initially present in the walnut kernels remain in the cake after cold pressing (Bakkalbasi et al., 2015, Arranz et al., 2008, Fukuda et al., 2003, Santos et al., 2018, Fregapane et al., 2020). **Walnut press-cake could therefore be harnessed as a source of phenolic antioxidants.** The concentration of phenolics in press-cakes is generally between 10 and 20 g GAE/kg (Ojeda-Amador et al., 2018, Arranz et al., 2008, Santos et al., 2018) although a value of 58 g GAE/kg has been reported once (Bakkalbasi et al., 2015). The phenolic compounds present in the walnuts are mainly hydrolysable tannins and phenolic acids, the former accounting for 60-80% depending on the nut variety, while the flavonols vary from 26-35% (Ojeda-Amador et al., 2018). The ranking of phenolic families identified in kernels is kept in the corresponding press-cake (Ojeda-Amador et al., 2018, Fregapane et al., 2020). **More information on phenolic compounds in nuts (almonds, walnuts, hazelnuts, peanuts, pistachios, macadamia and Brazil nuts) is available in Bodoira and Maestri, 2020, while the case of the walnut is more specifically described by Jahanban-Esfahlan et al. (2019), including the distribution of phenolic compounds in**

the different parts of the fruit. In addition, broader overviews in extraction and bioactivity of phenolic compounds can be found in Mojzer et al., 2016 and Kumar et al., 2017.

The performance of an extraction depends on several external parameters such as temperature, time, solid-solvent ratio, and on parameters inherent to the matrix and the solutes to be extracted such as solubility, particle size and porosity, or chemical solute-matrix interactions. Methanol, ethanol, acetone, ethyl acetate, water and mixtures thereof are common solvents for the extraction of phenolic compounds from plant materials (Dai and Mumper, 2010), including for the preparation of walnut extracts (Jahanban-Esfahlan et al., 2019, Bodoira and Maestri, 2020). Macerations at room temperature in methanol:water (Bakkalbasi, 2019, Labuckas et al., 2008) or ethanol:water (Labuckas et al., 2008, Fregapane et al., 2020), possibly at higher temperature (Santos et al., 2018) have been used to quantify phenolics in walnut by-products. However, the optimization of extraction conditions has rarely been addressed. Extraction efficiency can be improved by using innovative technologies (Chemat et al., 2020, Martinez et al., 2021) based, for example, on the application of electromagnetic waves (microwave assisted extraction MAE, Rosa et al., 2017), ultrasonic waves (ultrasound assisted extraction UAE; Fadimu et al., 2020) and pulsed electric fields or high-voltage electric discharges (Barba et al., 2015). Ultrasound-assisted extraction consists of the application of ultrasonic waves that cause expansion and compression cycles in which the expansion creates liquid bubbles while the compression leads to bubbles collapse, causing cavitation. Cavitation causes cell rupture near the solid surface, which improves solvent penetration and can also break down cell walls. Ultrasounds assistance has been shown to intensify the recovery of phenolic compounds from walnut shell (Han et al., 2018) or walnut husk (Tabaraki and Rastgoo, 2014) but has not increased the phenolic content of extracts obtained from pistachios (Fregapane et al., 2020). On a defatted walnut flour, Luo et al. (2017) combined ultrasound and microwave-assisted extraction to obtain a maximum yield of 34.91 mg GAE/g of flour. Other intensification techniques such as pressurized solvents or supercritical carbon dioxide have been marginally investigated for nuts, although they are increasingly implemented for the recovery of phenolics from fruits or by-products (Gallego et al., 2019, Garcia-

102 Salas et al., 2010, Espinosa-Pardo et al., 2017, Garcia-Mendoza et al., 2017, Massias et al., 2015,
103 Garcia-Mendoza et al., 2015, Bursać Kovačević et al., 2018, Goncalves Rodrigues et al., 2019).
104 Extractions by supercritical CO₂ (SCE), ultrasound and microwave were optimized for the recovery
105 of phenolics from hazelnut skins (Odabas and Koca, 2015). The extracts obtained by UAE under
106 optimal conditions had the highest total phenolic content and antioxidant activity compared to the
107 extracts obtained by MAE, SCE and reference maceration under maximal conditions. More
108 specifically for walnut by-products, phenolic compounds were extracted from the green husk of the
109 nuts by CO₂ technology (Seabra et al., 2019) and from the wooden layer between the nut seeds by
110 pressurized hot water or methanol (Kamali et al., 2018).

111 The addition of natural antioxidants to lipid-rich food is a promising strategy to avoid lipid
112 deterioration without using synthetic compounds (Jacobsen et al. 2008, Galanakis, 2018, Kalogianni
113 et al., 2020). The enrichment of oils with antioxidant extracts has attracted attention (Reboredo-
114 Rodríguez et al. 2017, Flori et al. 2020, Romeo et al. 2020). For example, the addition to virgin olive
115 oils of a phenolic extract recovered from olive leaf (Delgado-Adamez et al., 2014) or from olive cake
116 (Suarez et al., 2011) increased oxidative stability by 50% and 65% compared with the control oil. In
117 both cases, oxidative stability was further increased by about 40% and 12%, respectively, by
118 incorporating lecithin into the oils. With regard to the potential of nut extracts, Fregapane et al. (2020)
119 found that olive, pistachio and walnut oils enriched with pistachio or walnut extracts had significantly
120 higher oxidation stability than virgin oils. A 2.4-fold higher oxidation stability was obtained for
121 walnut oil enriched with walnut cake extracts with a concentration of 518 mg phenolics/kg oil. The
122 authors also reported that walnut extracts had a higher protective effect against lipid oxidation than
123 pistachio extracts. Although each polyphenol has its own activity (Ho et al., 2020), it is generally the
124 synergy between compounds, even minority ones, that is responsible for the overall activity of an
125 extract. Each extract can therefore, by its variable composition, induce a variable response. The effect
126 of the enrichment level on the oxidation stability of walnut oil was evaluated by Bakkalbasi (2019).
127 A significant decrease in oxidation markers was observed in oils enriched with 50 to 200 mg phenolic

128 extract/kg oil, and the protective effect was found to be dependent on the walnut extract concentration.
129 Also dealing with walnut oil, Grosso et al. 2018 evaluated the performance of walnut extracts when
130 separated into two fractions, ethyl acetate-soluble polyphenols on the one hand, and water-soluble
131 polyphenols on the other hand. The two extracts added at 1g extract/kg oil helped to preserve the
132 walnut oil against deterioration processes, and in some cases, showed a better performance for quality
133 preservation compared with synthetic BHT. The effectiveness of the ethyl acetate-soluble extract has
134 also been demonstrated when incorporated into an edible coating made from walnut cake protein to
135 protect a solid food, in this case walnut kernels (Grosso et al., 2020).

136 Our team is currently developing biorefinery schemes for oleaginous by-products (Espinosa-
137 Pardo et al., 2020). Walnut press-cake is emerging as a cheap source of phenolic compounds that also
138 contains a significant amount of nutritional grade oil. The press-cake used in this work came directly
139 from the oil mill and was only partially defatted. It was further de-oiled by solvent to recover the
140 residual oil and the residue was subjected to ultrasounds extraction (UAE) in prospects of recovering
141 the phenolic compounds. In a context of circular economy, the more stages of treatment, the higher
142 the costs. De-oiling may not be necessary. The extraction of the phenolic compounds was therefore
143 carried out on the raw press-cake by conventional maceration, and by a semi-continuous process in
144 order to highlight different limitations to mass transfer, if any. An overview of the investigations is
145 provided in Fig.1. The efficiency of extraction depends on many factors. In this work, different
146 conditions were investigated with special attention to time and solid-solvent ratio parameters, which
147 are parameters rarely studied in the literature related to nuts. All extracts were characterized for the
148 total phenolics content while antioxidant activity was evaluated for extracts obtained by maceration
149 or ultrasound assistance. In addition, the ability of extract to prevent oxidation of camelina oil was
150 evaluated.

151 *Fig 1*

152

153 **2. Materials and methods**

154

155 2.1. Walnut cake

156 The pressed cake was kindly donated by the Moulin de la Veyssière, Dordogne, France
157 (www.moulindelaveyssiere.fr). The fruits (Lara variety) were grown locally and the kernels were
158 pressed under mild heating to recover the oil. The partially defatted cake was collected the day after
159 pressing and was stored at -18°C after having spent about 48 h at room temperature. When extraction
160 campaigns were planned, 100 g were grounded with a coffee grinder, sieved below 600 µm (Retsch,
161 Germany) and divided into smaller batches stored at -18°C until use. A sample of the freshly pressed
162 cake was stored at room temperature in a food-grade kraft paper bag after gentle deagglomeration to
163 mimic the storage conditions of flour in mills. The proximate composition of walnut press-cake was
164 analyzed and the results were as follows: moisture (3%), protein (27%), oil (37%), ash (5%),
165 carbohydrates (28%). The oil content was determined by Soxhlet extraction, as described in 2.3.1.,
166 while the moisture content was measured by weight loss during drying at 105°C by halogen heating,
167 the ash content by incineration at 500°C for 24h, protein content by the Kjeldahl method using a
168 conversion factor of 5.3, and the carbohydrate content was estimated by mass difference.

169

170 2.2. Solvents and reagents

171 Hexane used for de-oiling cakes and ethanol used for phenolics extractions were purchased from
172 Atlantic Labo (Bruges, France). Deionized water with a resistivity of 15 mΩ.cm (ELGA, Purelab®
173 Option, UK) was used to prepare ethanol:water mixture. Folin reagent, sulfuric acid, anthrone,
174 glucose, gallic acid, were from Sigma Aldrich (St Quentin Fallavier, France). The phospholipids used
175 in this work (Lipoid P100) was purchased from Lipoid GmbH, France. Lipoid P100 are natural
176 phospholipids derived from non-GMO soybean plants comprising 95 wt% of phosphatidylcholine.

177

178 2.3. Extraction methods

179

180 2.3.1. Complete defatting

181 The walnut pressed cake WAC was further defatted by using boiling hexane in a commercial
182 enhanced design Soxhlet apparatus (Tecator Soxtec™ HT2, FOSS, Denmark). A sample of 14 g of
183 raw material was loaded in a Soxhlet thimble and extracted for 4 hours. The solvent was then
184 evaporated from the aluminium extraction beaker and the recovered oil was weighed. The defatted
185 walnut cake (DEWAC) was left in a fume hood overnight to evaporate the remaining solvent and
186 then stored in sealed containers at -18°C until use. The oil content of the walnut cake (NLC_{WAC}) was
187 $37.25 \pm 0.8\%$.

188

189 2.3.2. Maceration (MA) on walnut press-cake WAC

190 A 2^{4+1} fractional factorial design was employed to determine the influence of two levels (-1, +1) of
191 extraction time (Z_1), temperature (Z_2), solid-to-solvent ratio (Z_3) and percentage of
192 phosphatidylcholine in the solvent (Z_4) on the extraction of total phenolic compounds (TPC) and free
193 radical scavenging activity (DPPH) of extracts. The addition of phospholipid to the extracting solvent
194 was motivated by the fact that phospholipids could enhance the recovery of hydrophilic antioxidants
195 (Li et al., 2015) or improve their solubility or dispersion in oil (Garcia-Mendoza et al., 2021, Suarez
196 et al., 2011). The Z_4 factor was aliased with the second order interaction Z_{123} giving an experimental
197 design of resolution IV. Additional center points (level 0) were added to assess the reproducibility of
198 experiments, to check the absence of drift and to verify the linearity of the responses. The current
199 design comprised 11 experimental runs including 3 center points (level 0) as shown in Table 1.
200 Globally, WAC (3 g) was extracted in a sealed glass flask with 39, 57 or 75 mL of EtOH60 that
201 provided mass-to-solvent ratios of 1:13, 1:19 or 1:25 (m/v), respectively. The mixture was
202 continuously agitated in a digital magnetic hotplate stirrer with temperature control (Model C-MAG
203 HS 7, Ika®, Germany) for 15, 37.5 or 60 min and the temperature was maintained at 28°C, 44°C or

204 60°C. Prior to extraction, various amounts of soy phosphatidylcholine were dissolved in EtOH60 for
205 providing the concentrations of Lipoid of 0, 0.75% or 1.5% (m/v).

206 At the end of the extraction, the sample was transferred to Falcon graduated tubes and centrifuged
207 (Rotanta 460 RF, Hettich, Germany) at 13 300 g for 6 min. The supernatant was then filtered on a
208 0.45 µm PVDF syringe filter (Rotilabo-syringe filters, Carl Roth, Germany) and finally stored in
209 amber flask at -18°C until analysis of TPC and antioxidant activity.

210

211 2.3.3. Ultrasound assisted extraction (UAE) on defatted walnut press cake (DEWAC)

212 DEWAC (5 g) was placed into a 100 mL plastic beaker containing 64 mL of EtOH60 and the
213 extraction was then conducted in an Ultrasonic processor (Sonifier Analog 450, Branson, USA)
214 provided with a 12.7 mm diameter probe. The probe was immersed to half the height of the sample.
215 After extraction procedure, the sample was centrifuged (Rotanta 460 RF, Hettich, Germany) at 13
216 300 g for 6 min, the supernatant was then filtered on a 0.45 µm PVDF syringe filter (Rotilabo-syringe
217 filters, Carl Roth, Germany) and finally stored in amber flask at -18°C until further analysis.

218 A central composite design was chosen to study the influence of theoretical power (W) (X_1), time
219 (min) (X_2) and duty cycle (%) (X_3) on the extraction of total phenolic compounds (TPC) and the free
220 radical scavenging activity (DPPH) of extracts. The current design comprised 20 experimental runs
221 with 5 levels ($-\alpha$, -1, 0, $+\alpha$, +1) for each factor as shown in Table 2. Star points were done at -1.68
222 level in order to have a rotatable design. The levels of the independent factors were chosen according
223 to the operational limitations of the sonifier and the duty cycle was set as the fraction of a period of
224 one second in which the system is active. All ultrasound assisted extractions were carried out at room
225 temperature ($24 \pm 1^\circ\text{C}$) and at a fix solid-to-solvent ratio (1:13 m/v). Room temperature was selected
226 to limit energy consumption. For avoiding a temperature rise during extraction, the plastic beaker
227 containing the sample was immersed in ice.

228 A second set of experiments was carried out to investigate the solid:solvent ratio, increasing it from
229 1:13 to 1:200 m/v. Other conditions were set at 220 W, 20min and 40% for ultrasounds power,
230 extraction time and duty cycle, respectively.

231

232 2.3.4. Statistical analysis of data from Maceration on WAC and DEWAC walnut cake

233 The evaluation of the predicted model was done adjusting the response variable to a second-order
234 polynomial model equation by response surface methodology using the freeware RStudio (RStudio®,
235 USA).

$$236 \quad Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=1}^3 \beta_{ij} X_i X_j$$

237 where Y represents the response variable (total phenolic extraction or free radical scavenging
238 activity); β_0 , β_i , β_{ii} and β_{ij} are the intercept, linear, quadratic and interaction coefficient, respectively;
239 X_i and X_j are the independent factors affecting the response. In order to identify the significant terms,
240 an analysis of variance (ANOVA) has been performed with a confidence level of 95%.

241

242 2.3.5. Semi-continuous extraction (COL) on walnut press-cake (WAC).

243 *Extraction*

244 The semi-continuous extractions were carried out by flowing the solvent through a bed of walnut
245 cake particles and collecting periodically the effluent in graduated tubes. Conditions of temperature
246 (60°C) and total volume of solvent (25 mL per gram of WAC) were selected from the WAC-MA
247 batch investigations. Five grams of walnut cake were mixed with an inert filler made of glass beads
248 of 2 mm or 0.5-0.75 mm prior being loaded in a column of 19.8 cm height x 0.9 cm internal diameter.
249 The bed was overtopped by glass beads to completely fill the column. The column was placed in an
250 oven (Shimadzu CTO-SA, Japan) to control the temperature. The solvent, heated at 60°C by a
251 magnetic hotplate stirrer with temperature control (model C-MAG HAS, Ika®, Germany), was

252 delivered at 1.0 ± 0.05 mL/min by a dual pump (Waters 515 pump, Saint-Quentin-en-Yvelines,
253 France). At the column exit, a capillary drove the effluent to a graduated tube (10 mL, accuracy 0.2
254 mL or 25 mL, accuracy 0.5 mL) immersed in ice. The graduated tube was changed periodically to
255 collect 6 fractions of 7 mL, 7 mL, 7 mL, 22 mL, 44 mL and 44 mL (V_i mL), respectively, for the
256 entire extraction time of 140 min.

257

258 *Extracts processing and characterization*

259 Once collected, the fractions were transferred to round flasks. An aliquot of 2 mL was sampled for
260 quantification of total phenolic compounds, while the remainder was vacuum desolvated at 95kPa.
261 The amount extracted in each fraction (Q_i , g) was quantified by the weight obtained after rotavapor
262 multiplied by the volume factor $V_i/(V_i-2)$ to account for the 2 mL aliquots. The results are given as
263 cumulative extracted quantities per g of walnut cake.

264 The 2 mL aliquots were analyzed for total phenolic content (TPC) according to the procedure
265 described in 2.4. The aliquots were first vortexed and, depending on the presence of agglomerates or
266 oil, the samples were filtered on PVDF 0.45 micron or/and centrifuged at 10 000 g for 20 min. Each
267 sample was analyzed in duplicate.

268

269 *Modeling extraction kinetics*

270 The extraction kinetics of the phenolic compounds and the total extracted amount were modeled by
271 a two-sites kinetic desorption model (Kubatova et al., 2002). The model considers that a fraction of
272 the extractable compounds is located close to the surface of particles and is therefore easily ‘washed’
273 by the solvent, while the remaining fraction, located inside the matrix particles, is less accessible and
274 is therefore desorbed at a slower rate. The model is derived from the Fick’s law of diffusion like many
275 others (Dagostin et al., 2015, Mosca et al., 2018, Subra et al., 1998) but here an overall rate k is used

276 in the exponential term instead of the diffusion coefficient because the particles are likely not uniform
277 in shape or in size. Extraction kinetics were fitted to the following model:

$$278 \quad y_t = y_{\infty} [1 - fe^{-k_1 t} - (1 - f)e^{-k_2 t}]$$

279 where y_t is the yield (cumulated extracted amount/g_{WAC}) or TPC (mg GAE/g_{WAC}), y_{∞} is the
280 concentration at saturation (g extract/ g_{WAC} or mg GAE/g_{WAC}), f is the fraction of extractable material
281 of easy access, t is the extraction time, k_1 is the first-order rate constant describing the quickly released
282 fraction (min⁻¹), and k_2 is the first-order rate constant describing the slowly released fraction (min⁻¹).
283 The model was fitted to the experimental data using Microsoft Excel Solver, minimizing the mean
284 relative percent deviation.

285

286 *Determination of oil extracted by COL process*

287 The cake after COL extraction was submitted to hexane Soxhlet extraction to quantify its residual
288 content in oil. The {glass beads + WAC} mix was loaded in the Soxhlet thimble and extracted by
289 hexane as described in section 2.3.1. The oil recovered by Soxhlet (Q'_{oil} , g) was weighted and the
290 amount of oil previously extracted by COL was calculated as the difference between the amount of
291 oil initially present in WAC and the oil recovered by Soxhlet:

292

$$293 \quad Oil_{by\ COL} (g) = NLC_{WAC} M'_{WAC} - Q'_{oil}$$

294

295 in which M'_{WAC} (in g) is the mass of walnut cake submitted to COL extraction, NLC_{WAC} the lipid
296 content of the walnut cake and Q'_{oil} the amount of oil recovered by Soxhlet.

297

298 **2.4 Total phenolic content and antioxidant activity of extracts**

299 2.4.1. Determination of Total Phenolic Content (TPC)

300 TPC of extracts were measured using the Folin–Ciocalteu assay. Firstly, 50 µL of extract or standard
301 solution were added to 800 µL of distilled water and after the addition of 50 µL of Folin–Ciocalteu
302 reagent the samples were vortexed. After 3 min, 100 µL of Na₂CO₃ 1 N solution was added to the
303 mixture. The solutions were incubated for 2 h in absence of light, and the absorbance was then read
304 at 725 nm on a multimode microplate reader (Spark® 10M, Tecan, Switzerland). Gallic acid was
305 used as standard and a calibration curve was plotted in a concentration range of 16–350 mg/L. All
306 analyses were performed in triplicate and the TPCs were expressed as mg of Gallic Acid Equivalents
307 (GAE)/g of raw material.

308

309 2.4.2 Free radical scavenging activity (DPPH)

310 The antioxidant activity of the extracts was evaluated based on the scavenging activity of the 2,2-
311 diphenyl-1-picrylhydrazyl (DPPH) free radical (Espinosa-Pardo et al. ,2017). Solutions consisting of
312 50 µL of extract or standard solution and 150 µL of 0.2 mM DPPH in methanol, were analyzed on a
313 multimode microplate reader (Spark® 10M, Tecan, Switzerland) with absorbance filters for a
314 wavelength of 520 nm. The decoloring process was recorded during 35 min of reaction and the
315 measures were performed in triplicate. The antioxidant activity was calculated using a calibration
316 curve of Trolox® at different concentrations in a range of 7–300 µmol/L and DPPH was expressed as
317 µmol of Trolox Equivalent /g of raw material (Trolox Equiv./g).

318

319 2.4.3 Oil enrichment and oxidative stability assessment

320 The extract n° 10 obtained during WAC maceration was used for enriching camelina oil (Coil) at
321 concentrations of 150 and 1500 mg extract/kg oil, in presence or absence of phospholipids (Lipoid
322 P100). For oil enrichment, 1.4 mL or 14 mL of extract was directly added to 70 g of oil co-enriched
323 or not with phospholipids at 20 mg/g_{oil}, except for the case of 1500 mg extract/kg and 20 mg/g Lipoid

P100 where the extract was first concentrated from 14 to 1.5 mL under nitrogen flux. The samples were gently homogenized and the solvent was then removed in a rotary evaporator (Model RE 300DB, Stuart, UK). The enriched Oil was agitated at room temperature ($24 \pm 1^\circ\text{C}$) for 1 h on a digital magnetic stirrer (Model C-MAG HS 7, Ika®, Germany). After enrichment procedure, the oils were submitted to accelerated oxidation.

The oxidative stability of enriched Oil was studied through the Schaal oven test. For that, 7 g of enriched oil were placed in 40 mL sealed amber flasks and submitted to accelerated oxidation at 60°C in a laboratory oven (DRY-Line, VWR, UK) for several hours. The amber flasks containing the 7 g of oil were periodically collected and stored at -80°C until analysis. Both enriched and non-enriched Oil were oxidized in duplicate and the oxidation was monitored by conjugated dienes (CD) analysis.

3. Results

3.1. Maceration (MA) on walnut cake WAC

The coded values of variables and the responses of the fractional factorial experiments are reported in Table 1. Runs 1, 6 and 11 comprised the three replicates at the central point.

Table 1

For the total phenolic content, values between 2.2 and 14.8 mg GAE/g_{meal} were obtained with a mean value of 8.9 mg GAE/g_{meal}. The highest values are consistent with those reported by Ojeda-Amador et al., 2018 (19 mg/g_{meal}) for a cake from Lara variety kernels, considering that extraction of phenolic compounds was performed at a solid-to-solvent ratio of 1:50 which is a variable influencing the extraction yield. Higher values can be found in literature, as 19-35 mg/g_{meal} for instance (Luo et al., 2017), but the cake was obtained from another variety and extractions were carried out with simultaneous microwave and ultrasonic assistance. The statistical analysis through multiple regression and ANOVA (supplementary data S1) revealed that temperature (Z_2), solid-to-solvent ratio (Z_3), phospholipid concentration in the solvent (Z_4) and binary interactions of temperature with

phospholipid concentration (Z_{24}) and with solid-to-solvent ratio (Z_{23}) were significant at 95% level. The best model fitting the experimental data was determined using a backward-forward AIC criterion (Akaike, 1974). Such model includes time (Z_1) as factor in addition to the significant terms and reads as (expressed in coded values):

$$\text{TPC} = 8.9 + 0.7Z_1 + 2.4Z_2 + 1.4Z_3 - 3Z_4 + 1.4Z_{24} + Z_{23}$$

The model explained 95% of the experimental variance and showed that the effect of phospholipid concentration was negative. Adding PL to the solvent was hence deleterious to the extraction of TPC whereas increasing temperature, ratio and time improved it. The 3D response surface plots (Fig.2) illustrates the trends between TPC response and extraction parameters, as temperature and solid-to-solvent ratio in one hand, temperature and phospholipids concentration in the other hand. The conditions maximizing the TPC were therefore 60 minutes of extraction at 60°C with 1:25 ratio in absence of PL.

For DDPH response, the values ranged from 12 to 137 $\mu\text{mol Trolox Equiv.}/\text{g}_{\text{meal}}$ with a mean of 77 $\mu\text{mol Trolox Equiv.}/\text{g}_{\text{meal}}$. As for total phenolic content, the highest values are consistent with Ojeda-Amador' results that reported a value of 149 $\mu\text{mol}/\text{g}_{\text{meal}}$ for the cake from the Lara variety (Ojeda-Amador et al., 2018). The best model fitting identified phospholipid concentration and temperature as the unique significant factors at 95% level (Supplementary data S2). The relevant model explained 78% of the variability and highlighted the deleterious effect of PL and the positive effect of temperature, as demonstrated by the 3D plots (Fig.2).

$$\text{DPPH} = 77 + 18.6Z_2 - 28.2Z_4$$

The conditions maximizing the DPPH were therefore a temperature of 60°C and no phospholipids added in the extracting solvent leading to a DDPH value of 137 $\mu\text{mol Trolox Equiv.}/\text{g WAC}$.

374 **Fig. 2**

375 Conditions of run 10 provided the highest extraction yield of phenolic compounds and the highest
376 free radical scavenging activity. To evaluate the extract's antioxidant effectiveness at retarding oil
377 oxidation, the run10-extract was added to camelina oil, pure or pre-enriched with
378 phosphatidylcholine. Camelina oil has a high content of polyunsaturated fatty acids (58.7%) in which
379 linolenic acid (C18:3 ω -3) and linoleic acid (C18:2 ω -6), prone to oxidize easily, represent 37.5 and
380 17.3 wt%, respectively. The oil oxidation time-course was monitored by quantifying the conjugated
381 dienes (CD) that are intermediary products of the first stage of lipid oxidation produced by the
382 rearrangement of the double bonds of unsaturated fatty acids during the formation of hydroperoxides.
383 The oxidation curves (Fig.3) showed that the extract did retard the oil oxidation even when added at
384 the lowest level of 150 mg/kg. At that level, the effect was significantly potentiated by the addition
385 of phospholipids whereas at higher concentration of extract (1500 mg/kg), phospholipids exerted only
386 a weak additional benefit. The joint action of extract and phosphatidylcholine could be explained by
387 the ability of phospholipids to stabilize the added phenolic compounds in the oil matrix due to their
388 amphiphile behavior, forming reverse micelles that contain the hydro-alcoholic or the aqueous
389 solution of the extract (Suarez et al., 2011). The oxidation delay can be quantified by considering the
390 conjugated dienes value corresponding to a PV (Peroxide Value) of 15 meqO₂/kg that is the highest
391 PV allowed from the Codex Alimentarius for edible virgin oils, thanks to the linear correlation
392 established between PV and CD for the camelina oil (Garcia Mendoza 2020). The CD value of 0.275
393 was obtained in 40 h to 72 h depending on the formulation, a time which was significantly higher
394 than the 24 h of the non-enriched oil used as control. The positive effect of walnut extract at improving
395 oxidative stability of an enriched oil confirmed the behavior observed for other oils. In Bakkalbasi
396 2019, oils enriched with 50, 100 and 200 mg phenolic extract/kg oil were submitted to accelerated
397 oxidation at 65°C. The total phenol content of the enriched oil varied with the added amount of
398 extract, from 28 to 84 mg GAE/kg oil. A PV value of 12 meqO₂/kg was reached after about 6.5 days
399 of oxidation for the control oil, whereas the enriched oil with 200 mg phenolic extract/kg oil needed

9 days for reaching the same PV, which means 1.4-fold greater oxidative stability of enriched oil. In Grosso et al., 2018, a walnut extract that contains 230 mg phenolic/g extract was added to walnut oil at 0.1% w/w. With an enrichment level of 230 mg GAE/kg oil, a PV of 15 meqO₂/kg was reached in about 6 days which represents a 1.2-fold- increase compared with non-enriched oil. In our camelina oil samples, the enrichment with 150 and 1500 mg extract/kg oil led to a TPC of 42 and 130 mg GAE/kg oil, and to a PV value of 15 meqO₂/kg reached after 41 h and 66 h of oxidation, respectively. It corresponds to an increase in stability of 1.7 and 2.7 times compared with the control sample, values that are therefore of the same order of magnitude as those of literature.

408

409 **Fig. 3**

410 **3.2. Maceration assisted by ultrasounds (UAE) on defatted walnut cake DEWAC**

411 Responses obtained during fractional factorial experiments are reported in Table 2. Experiments were
412 carried out at $T = 24 \pm 1^\circ\text{C}$ with a fixed solid-solvent ratio of 1:13 m/v. Runs 15 to 20 comprised the
413 six replicates at the central point ($X_i=0$). Both TPC and DPPH were found to vary slightly, between
414 10.37 and 13.26 mg GAE/g_{DEWAC} and 83 to 107 $\mu\text{mol Trolox Equiv. /g}_{\text{DEWAC}}$, respectively.

415 The backward-forward method was used for determining the best model fitting the experimental data
416 using AIC criterion and the response surfaces for TPC are shown in Fig. 4. The predicted model for
417 the phenolic extraction is given below (X_1 , ultrasound power; X_2 , time; X_3 , duty cycle, in coded
418 values),

419

$$420 \text{ TPC} = 12.33 + 0.35X_1 + 0.31X_2 + 0.54X_3$$

421

422 and it explained 63% of the variability of phenolic extraction by UAE. It can be stated that the low
423 variation of TPC among the different conditions of UAE influenced significantly the adjusted R^2 of

the fitted model. On the other hand, ANOVA results (Supplementary data as S3) showed that the three factors (power, time and duty cycle) significantly influenced the TPC, nonetheless quadratic coefficients and those related to the interactions among the different factors were not significant. The model also showed that the influence of power, time and duty cycle was positive which means that the higher the power, time and duty cycle the higher the TPC. In this sense, conditions maximizing the TPC extraction corresponded to the maximal value of each factor, e.g. 233.6 W, 23.4 min, 46.8% duty cycle.

Table 2.

Fig. 4

When antioxidant activities of walnut extracts DPPH were attempted to be predicted by a polynomial model, it only explained 36% of the variability of the experimental results significant at $p < 0.05$. Therefore, none of the response surfaces was plotted for the different factors. Although the model had a poor adjusted R^2 , only time and duty cycle appeared as significant factors. Similarly, Odabaş and Koca (2016) also reported a poor adjusted R^2 (0.53) of the model generated for predicting the DPPH radical scavenging activity (1/EC50) of hazelnut skin extracts obtained by UAE.

On the other hand, it is worth mentioning that a strong positive correlation between TPC and DPPH was observed (0.95 Pearson's correlation coefficient, $p < 0.05$). The high and positive correlation between the total phenolic content and the antioxidant activity (DPPH) of several plant extracts has been previously reported by other authors (Piluzza and Bullitta, 2011; Gil et al., 2002).

The second set of experiments, performed at conditions providing the highest TPC yield, aimed to evaluate the influence of the solid-to-solvent ratio (Fig. 5). Other conditions were set at 220 W, 20 min and 40% for ultrasounds power, extraction time and duty cycle, respectively. Results showed that phenolics extraction was significantly influenced by the ratio with a value of 29.0 ± 0.3 mg

449 GAE/g_{cake} obtained at ratio 1:200, which represented a 121.4% increase compared with the TPC
450 obtained at 1:13. Solid-to-solvent ratio influences the concentration gradient between the solid and
451 the solvent phases and this was found to enhance the amount of phenolics recovered at a fix extraction
452 time. Data were satisfactorily fitted with a log_n relationship (R^2 of 0.984, dotted line in Fig.5),
453 indicating that TPC extraction yield will slow down at higher ratio. When estimating the amount of
454 phenolics that would be extracted using the ratio of 1:1000, we get a value of 38 mg/g_{cake}, which
455 would represent a 31% increase in yield but would require a five-fold increase in solvent
456 consumption.

457

458 **Fig. 5**

459

460 **3.4. Semi-continuous extraction (COL) on walnut cake**

461

462 The extraction consisted of flowing the solvent through a bed of walnut cake particles and periodically
463 collecting the extracts to monitor the extraction kinetics. The extraction time was adjusted to obtain
464 the same final volume of solvent per mass of solid as under maceration conditions. The use of a larger
465 quantity of cake (5 g) was motivated by the objective of extracting not only the phenolic compounds,
466 but also the oil, since the cake contains approximately $37.25 \pm 0.8\%$ oil. While hydroalcoholic
467 solvents are suitable for the extraction of phenolic compounds, ethanol is increasingly considered as
468 a potential solvent to replace hexane in the extraction of vegetable oils (Baümeler et al., 2016, Navarro
469 et al., 2016, Citeau et al., 2018) although the presence of water is detrimental to oil solubility (Sampaio
470 Neto et al., 2018, Sawada et al., 2014, Kwiatkowski and Cheryan, 2002).

471 The extraction kinetics, reported as cumulated amounts of total extract or phenolics per g of meal,
472 are given in Fig.6 (WAC load : 5.02 ± 0.02 g, filler of 0.5-0.75 mm or 2 mm, $T = 60^\circ\text{C}$, $F = 1\text{ mL/min}$).

473 The 'zero' time is when the solvent flow was started and it took about 9-10 min for the solvent to exit

the column. The deviation bars are from the duplicate extractions with the exception of the TPC data from the 'storage at room T' essay for which only one of the two extractions was analyzed.

Fig. 6

For phenolic compounds, the highest TPC value of 19.83 ± 0.24 mg/g_{WAC} was higher than the TPC of 14.78 ± 0.37 mg/g_{WAC} obtained by batch maceration. This could be the result of better contact between the solvent and the matrix in COL trials where the solvent flew through the particulate bed, leading to better mass transfer. A slight difference in the composition of the solvent is not excluded since the hydroalcoholic mixture was prepared with EtOH 96% instead of absolute ethanol. The higher extraction rate observed when smaller beads were used as filler could be due to better packing and less wall effect which induced more homogenous velocity distributions (Minhua et al., 2018, Zhang et al., 2018). Storage at room temperature leads to slower extraction, but the particle size may contribute to the behavior since the material has not been sieved below 600 μ m. It should be noted that coarse grinding of the cake and storage of the flour at room temperature, which could mimic the conditions under which flour should be sold, maintained about 77% of the phenolic compound content. Several studies have tested the influence of packaging materials and storage conditions on physicochemical properties and quality of walnut kernels (Adiletta et al., 2020; Ahad et al., 2020). Ahad et al. reported for instance a decrease of TPC from 31.2 mg GAE/g to 18.8 mg GAE/g, which represents a loss of 60% after 270 days of storage at ambient conditions under relative humidity of 80%. In our study, the loss was only 23%. The partially de-oiled flour with a moisture content of 3% therefore seems to better maintain its phenolic potential than fresh kernels.

Data in Fig.6 also show that none of the extraction curves reached a plateau, indicating that extractions were not completed after 140 minutes. Extraction rates nevertheless slowed down after 95 minutes, e.g at a solid/solvent ratio of 1:19. To better address extraction mechanisms, extraction

kinetics were modelled using a two-step approach (Table 3). Although the regression coefficients were greater than 0.98, the model better fit the kinetics of the "room temperature storage" case, probably due to the larger number of experimental data. For the other two conditions, the absence of an experimental plateau in the kinetic curves affected the accuracy of the Y_{∞} estimate. Considering that Y_{∞} corresponds to the maximum extractable quantity (Mosca et al., 2018), the same value of 21.8 mg/g found by optimizing the model variables of the "storage -18°C - filler 0.5 mm" trial was used to model the kinetics of the 2 mm-beads. Differences were observed in the fraction of easily extractable compounds (f) or in the k_I values, but this easy washing step concerned less than 9% of the amount of extractable phenols ($f < 0.09$). Thus, the majority of the phenolic compounds were extracted by a slower diffusion process, which indicated a poor accessibility.

In COL experiments, the overall quantity extracted was also monitored. One of the kinetics reached a plateau at 0.242 ± 0.06 g of extract/g_{WAC}. As for phenolics, the use of 0.5mm beads improved the extraction rate, while the matrix stored at room temperature and not sieved released the extractable compounds more slowly. As shown in Fig.6, the two-sites model using a single Y_{∞} concentration corresponding to the plateau correctly predicted all three kinetics. In contrast to phenolics, the major part of extractable compounds was easily accessed by the solvent at a rate k_I that was significantly higher than the desorption of the less accessible solutes (k_2 values). For the sample stored at room temperature, the f value was lower but the lack of experimental points at short times probably hampered the fit for this step.

518

519 **Table 3**

520

With a fairly polar extracting solvent, a wide variety of molecules such as proteins, carbohydrates, phospholipids can be solubilized. Some triglycerides and free fatty acids can also be extracted, although the high water content of the extraction solvent impairs their solubility. The identification

of the extracted classes was out of scope, however, extraction of lipids can be assessed by analyzing the walnut cakes after COL experiments for their oil content (as described in 2.3.5). The data presented in Fig.7 show that the hydro-alcoholic mixture effectively extracted about 30% of the oil present in the press-cake and that the storage and diameter of the beads had no major effect on the amount of oil extracted. A closer look at the aliquots of the fractions collected during extraction of the test 'storage -18°C; filler 0.5mm' (Fig. 8) showed that the oil was not uniformly extracted during the course of extraction but was especially concentrated in the first fraction. Since the kernels were already crushed to extract the virgin oil, the oil remaining in the press-cake could be easily accessible by the solvent, which explains the high values of the kinetic parameters f and k_l obtained for the overall extracts.

Fig. 7.

Fig. 8

4. Discussion

In this work, the material from which phenols were recovered was a walnut press-cake obtained from an artisanal oil production mill. The cake (WAC sample) was therefore a partially de-oiled material that still contained about 37% oil. It was further de-oiled by hexane extraction to obtain the DEWAC sample.

Phenolic compounds were first extracted from WAC by batch maceration under stirring, changing the temperature, solid-solvent ratio and time from 28°C to 60°C, from 1:13 to 1:25 m/v and from 15 to 60 min, respectively, and considering enrichment of the solvent by phospholipids at 1.5wt% as an additional option. The yield of extracted phenolic compounds ranged from 2.2 to 14.8 mg GAE/g_{cake}, the lowest value being obtained at 28°C in the presence of phospholipids. Otherwise, the yield of TPC was between 6.6 and 14.8 mg GAE/g_{cake}. Statistical analysis showed that extraction was

enhanced by temperature, solid-solvent ratio and time while the presence of phospholipids was deleterious. The effect of the first three parameters is quite common in extraction. A higher temperature increases the solubility and diffusion of the solutes in the solvent while reducing the viscosity of the solvent; a higher solid:solvent ratio gives a higher concentration gradient at the surface of the solid which is the driving force of mass transfer, and reduces the risk of solvent saturation; a longer time would improve recovery when extraction is limited by mass transfer, especially from intra-particle diffusion. The deleterious effect of phospholipids is striking. Either phospholipids act as an antisolvent, i.e. they decrease the solvating capacity of the aqueous ethanolic solvent for the main representative phenols, ellagitannins and flavonols, or they acted as a barrier by trapping the phenols inside the matrix or preventing the solvent from accessing them. On the other hand, phospholipids are amphiphilic molecules commonly used in oil-in-water and water-in-oil emulsions because they are positioned at the water-oil interface. Contrary to the anti-solvent effect, if strong interactions occur between phenolic compounds and the polar fragments of phosphatidylcholine (glyceride skeleton, phosphate and amine groups), the hydrophobic tails of the phospholipids would drag the polar complex close to the oil droplets (part of the oil has indeed been extracted by the hydro-alcoholic solvent, as confirmed by the COL tests), and would form aggregates likely to sediment during centrifugation. The underestimation of TPC was another possible cause as considered by Suarez et al. who hypothesized the formation of small drops of hydro-ethanolic extract solution surrounded by lecithin molecules, a structuring that could prevent the phenols from reacting with the Folin-Ciocalteu reagent (Suarez et al., 2011).

The use of a packed bed of cake particles instead of their suspension allowed a better recovery of phenols (TPC close to 20 mg GAE/g_{cake}) when a filler with a particle size close to that of the cake was used. In a batch extraction process, the concentration gradients between solvent and solid are lower due to the greater homogeneity of the concentration of the extract in the solvent volume and the mass transfer process is completed when the equilibrium concentration is reached (Mosca et al., 2018). In the semi-continuous process, the fresh solvent flow provides higher concentration gradients

575 at the interface and the extraction ends when all the compound is removed (Mosca et al., 2018). The
576 kinetic study highlighted the fact that more phenols could be extracted by increasing the time and,
577 consequently, the solid-solvent ratio. However, from a process point of view, continuing the
578 extraction while the extraction rate slows down is not economically interesting due to the higher
579 solvent consumption and the recovery of increasingly diluted solutions. Quantification of the overall
580 yield and the oil remaining in the residue further confirmed that the hydro-alcoholic solvent was not
581 selective for phenolic compounds alone but extracted up to 32% of the oil present in the press-cake.
582 The phenolics represented about 8% by weight of the total accumulated extract at 140 min, while the
583 oil represented 46 wt%.

584 The intensification of the extraction by using ultrasound was studied on a completely defatted nut
585 cake at 24°C using first a mass/solvent ratio of 1:13. The effect of ultrasound is to induce an alteration
586 of the cell structure leading to increased solvent penetration. The yield of phenolic compounds varied
587 by a maximum of 30%, with values ranging from 10.4 to 13.4 mg GAE/g_{cake}. Furthermore, the
588 improvement compared to a maceration carried out without ultrasound for 20 minutes at the same
589 temperature and with the same solid/solvent ratio, which gave a value of 9.7 ± 0.4 mg/cake, was
590 rather small. This could be explained by the relatively narrow range of variables studied here. On the
591 other hand, statistical analysis has shown that increasing the power or duty cycle parameters of the
592 ultrasound should improve extraction efficiency. An additional experiment carried out in another
593 equipment operating at a higher power led to a higher extraction yield, with a TPC value of 19.2 mg
594 GAE/g_{cake} compared to the 14.5 mg GAE/ g_{cake} obtained in the absence of ultrasound (solid-solvent
595 ratio of 1:50). To better appraise the differences between the devices, the ultrasound intensity
596 (W/cm^2) was quantified by calorimetric measurements. For the sonifier used in the UAE experimental
597 design, the intensity ranged from 0.34 (power of 45, duty cycle of 20) to $4 \text{ W}/\text{cm}^2$ (power of 55, duty
598 cycle of 40), while in the additional experiment the intensity was $54.7 \text{ W}/\text{cm}^2$. A higher intensity
599 therefore significantly improved the extraction efficiency. Focusing on UAE of phenolic compounds
600 from defatted hazelnut skins, Odabas and Koca (2016) showed that the most significant variable was

601 the concentration of ethanol in the hydro-alcoholic mixture (range between 50 and 90%, maximum
602 efficiency obtained at 65-70%), followed by the extraction time (ranging between 15 and 45 min),
603 while the extraction temperature, ranging between 20°C and 60°C, was not significant. Compared to
604 a maceration trial, the ultrasound assistance resulted in a much higher efficiency with a TPC of 122.9
605 mg GAE/g under the best conditions instead of 58.8 mg GAE/g, which represents a 2-fold increase.
606 These extractions were carried out at a ratio of 50 mL of solvent/g of solid and at a power of 1500W,
607 which is much higher than the highest power set here. On the other hand, Pingret et al. (2012) obtained
608 a 32% increase over maceration using a power of 0.7 W/cm² (solvent: water, 40°C, 40 min, material:
609 apple pomace, TPC of 5.5 mg catechin equivalent/g versus 4.2 mg/g), an improvement that is within
610 the range of our data in the UAE experimental set. For another by-product (chicory grounds), a
611 smaller increase of 8% and 11% was reported at 20°C and 60°C, respectively, when ultrasounds
612 power was increased from 0 to 100 W (Pradal et al. 2016; solvent : EtOH 60%).

613 Furthermore, the second set of extractions performed under the ultrasound conditions that gave
614 previously the highest TPC yield showed the significant importance of the solid-solvent ratio. By
615 increasing it from 1:13 to 1:200, TPC increased from 13.1 to 29.0 mg GAE/ g_{cake}, an improvement of
616 121%. This behavior was consistent with conclusions drawn from batch and semi-continuous
617 experiments on non-defatted WAC cake that showed the positive effect of either the solid-solvent
618 ratio or time. As previously mentioned, a higher solid-solvent ratio leads to a higher concentration
619 gradient at the surface of the solid, which is the driving force of mass transfer, and decreases the risk
620 of solvent saturation. The fact that both the WAC and DEWAC extractions were influenced by the
621 solid to solvent ratio could suggest that solubility issues are important for the extraction of phenols
622 from walnut and that a ratio of up to 200 mL of solvent per g of solid should be considered as a
623 minimum.

624 Regarding the effect of de-oiling the cake before the extraction of the phenolic compounds, a
625 theoretical TPC was calculated for WAC under the conditions of 24°C, 20 min, 1:13 used for UAE
626 extraction. The theoretical value of 10.0 mg GAE/ g_{cake} was within the range of the value obtained

627 after defatting (9.7 ± 0.4 mg/ g_{cake}) and therefore did not correspond to a concentration of phenolic
628 compounds in the defatted cake proportional to the loss of oil (Ojeda-Amador et al., 2018). On the
629 one hand, some phenols may have been co-extracted with the oil during the Soxhlet treatment, which
630 would result in a decrease in the phenol content of the DEWAC cake. The effect of processing
631 methods (cold pressing and hexane extraction among others in Gao et al., 2021, cold pressing and
632 Soxhlet petroleum ether extraction in Ahmed et al., 2019) on the chemical composition and
633 antioxidant capacity of walnut oil has been studied. In terms of phenolic content, oils extracted by
634 hexane or petroleum ether were the richest, indicating that solvent extraction of the oil leads to co-
635 extraction of phenolic compounds and thus leaves a cake depleted in phenols compared with the
636 untreated cake. On the other hand, a potential degradation of phenolics or a poorer access of the
637 hydro-alcoholic solvent to phenolics after Soxhlet treatment could be also suggested. Meanwhile,
638 ellagic acid was quantified in extracts (data as supplementary S4). It represented about 16% of the
639 total phenolic content, a value consistent with the 15.8% content reported by Bakkalbasi, 2019 and
640 Fukuda et al., 2003.

641

642 5. Conclusions

643 In this study, different modes and specific conditions for the extraction of phenolic compounds from
644 a partially de-oiled walnut cake coming from an oil mill were studied. The results showed that
645 increasing the temperature, time and solid-to-solvent ratio increased the recovery of phenolic
646 compounds, while adding phospholipids to the solvent was not a good option. Defatting of the walnut
647 cake with boiling hexane prior to the extraction did not concentrate the phenolic compounds in the
648 cake and intensified ultrasonic maceration brought only slight improvements in extraction efficiency
649 unless higher power and higher solid/solvent ratio were used. All the methods highlighted the
650 importance of time or solid-to-solvent parameters on extraction efficiency. This work also
651 demonstrated that the extract from walnut cake significantly increased the oxidative stability of an

652 oil sensitive to oxidation, camelina oil. The enrichment of oils with phenolic extracts is therefore a
653 very interesting way of providing nutritional properties while extending the shelf life of the oils.

654

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658

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Table 1.
 Coded and natural values of independent factors (Z) of the fractional factorial design with the results
 of response variables TPC and DPPH for extractions from WAC carried out by maceration.

	Time (min) (Z ₁)	Temperature (°C) (Z ₂)	Solid-solvent ratio (m/v) (Z ₃)	Phospholipid concentration (%) (Z ₄)	TPC mg GAE/g _{cake}	DPPH μmol Trolox/g _{cake}
1	37.5 (0)	44 (0)	1/19 (0)	0.75 (0)	8.23	68.93
2	15 (-1)	28 (-1)	1/13 (-1)	0 (-1)	10.27	90.53
3	60 (+1)	28 (-1)	1/13 (-1)	1.5 (+1)	2.54	42.72
4	15 (-1)	28 (-1)	1/25 (+1)	1.5 (+1)	2.22	12.14
5	60 (+1)	28 (-1)	1/25 (+1)	0 (-1)	12.04	99.72
6	37.5 (0)	44 (0)	1/19 (0)	0.75 (0)	7.85	70.01
7	60 (+1)	60 (+1)	1/13 (-1)	0 (-1)	11.83	104.91
8	60 (+1)	60 (+1)	1/25 (+1)	1.5 (+1)	13.40	91.26
9	15 (-1)	60 (+1)	1/13 (-1)	1.5 (+1)	6.56	60.50
10	15 (-1)	60 (+1)	1/25 (+1)	0 (-1)	14.78	136.92
11	37.5 (0)	44 (0)	1/19 (0)	0.75 (0)	8.27	68.75

899 **Table 2.**

900 Coded and natural values of independent factors (X) of the central composite design with the results
901 of response variables TPC and DPPH for extractions from DEWAC carried out by UAE.

902

Run	Power (W) (X ₁)	Time (min) (X ₂)	Duty cycle (%) (X ₃)	TPC mg GAE/g _{cake}	DPPH μmol Trolox/g _{cake}
1	180 (-1)	10 (-1)	20 (-1)	10.37	83.0
2	220 (+1)	10 (-1)	20 (-1)	11.55	87.5
3	180 (-1)	20 (+1)	20 (-1)	11.45	91.0
4	220 (+1)	20 (+1)	20 (-1)	12.09	94.8
5	180 (-1)	10 (-1)	40 (+1)	12.35	103.1
6	220 (+1)	10 (-1)	40 (+1)	13.41	104.7
7	180 (-1)	20 (+1)	40 (+1)	12.56	103.3
8	220 (+1)	20 (+1)	40 (+1)	13.12	102.8
9	166.4 (-α)	15 (0)	30 (0)	12.39	102.2
10	233.6 (+α)	15 (0)	30 (0)	13.16	104.9
11	200 (0)	6.6 (-α)	30 (0)	11.66	93.5
12	200 (0)	23.4 (+α)	30 (0)	13.26	106.9
13	200 (0)	15 (0)	13.2 (-α)	12.03	104.2
14	200 (0)	15 (0)	46.8 (+α)	12.86	107.1
15	200 (0)	15 (0)	30 (0)	12.05	97.7
16	200 (0)	15 (0)	30 (0)	12.69	99.3
17	200 (0)	15 (0)	30 (0)	12.16	103.2
18	200 (0)	15 (0)	30 (0)	12.41	103.6
19	200 (0)	15 (0)	30 (0)	12.20	105.5
20	200 (0)	15 (0)	30 (0)	12.84	104.7

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904

905 **Table 3.**
 906 Kinetic parameters of extractions carried out by COL semi-continuous method from walnut press-
 907 cake.
 908

	Storage -18°C Filler 0.5 mm	Storage -18°C Filler 2 mm	Storage room T Filler 0.5 mm
<i>Extraction yield ($g_{extract}/g_{WAC}$)</i>			
Y_{∞}	0.25	0.25	0.25
f	0.947	0.815	0.495
k_1 (min ⁻¹)	0.070	0.071	0.073
k_2 (min ⁻¹)	0.002	0.002	0.004
<i>TPC (mg GAE/g_{WAC})</i>			
Y_{∞}	21.8	21.8	15.5
f	0.087	0.027	0.021
k_1	0.023	0.047	0.030
k_2	0.020	0.015	0.026

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Fig. 1. Overview of extractions methods and variables: batch maceration (MA), maceration assisted by ultrasounds (UAE), semi-continuous extraction (COL).

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919 **Fig. 2.** Response surface plots for the effect of variables of maceration MA from WAC press-cake on
 920 total phenolics recovery TPC (a : slice at $Z_1 = +1$ and $Z_4 = -1$; b : slice at $Z_1 = +1$ and $Z_3 = +1$) and
 921 antioxidant activity DPPH.

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925 **Fig. 3.** Monitoring of CD formation under heating accelerated conditions of (●) pure Coil, (■) Coil
 926 + WAC extract (150 mg/kg), (x) Coil + phosphatidylcholine (20 mg/g) + WAC extract (150 mg/kg),
 927 (◆) Coil + WAC extract (1 500 mg/kg), and (+) Coil + phosphatidylcholine (20 mg/g) + WAC extract
 928 (1 500 mg/kg).

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Fig. 4: Response surface plots for the effect of variables of UAE from DEWAC press-cake on total phenolics recovery TPC (a: slice at $X_1 = 0$; b: slice at $X_3 = 0$; c: slice at $X_2 = 0$).

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943 **Fig.5.** Effect of solid-solvent ratio on TPC recovery in UAE experiments.

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950 **Fig. 6.** Extraction **kinetics** of phenolic compounds and total extract from WAC walnut cake in COL
951 **experiments.**

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956 **Fig. 7.** Amount of oil extracted in COL experiments from 5g of WAC, compared with the extractable
957 amount present in the untreated cake (WAC label).

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962 **Fig. 8.** Aliquots of fractions collected during the semi-continuous COL extraction of WAC (essay
963 ‘storage -18°C, filler 0.5 mm’).

964

	Time (min) (Z ₁)	Temperature (°C) (Z ₂)	Solid-to- solvent ratio (m/v) (Z ₃)	Phospholipid concentration (%) (Z ₄)	TPC mg GAE/g	DPPH μmol Trolox/g
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9	15 (-1)	60 (+1)	1/13 (-1)	1.5 (+1)	6.56	60.50
10	15 (-1)	60 (+1)	1/25 (+1)	0 (-1)	14.78	136.92
11	37.5 (0)	44 (0)	1/19 (0)	0.75 (0)	8.27	68.75

Run	Power (W) (X ₁)	Time (min) (X ₂)	Duty cycle (%) (X ₃)	TPC mg GAE/g	DPPH μmol Trolox/g
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3	180 (-1)	20 (+1)	20 (-1)	11.45	91.0
4	220 (+1)	20 (+1)	20 (-1)	12.09	94.8
5	180 (-1)	10 (-1)	40 (+1)	12.35	103.1
6	220 (+1)	10 (-1)	40 (+1)	13.41	104.7
7	180 (-1)	20 (+1)	40 (+1)	12.56	103.3
8	220 (+1)	20 (+1)	40 (+1)	13.12	102.8
9	166.4 (-α)	15 (0)	30 (0)	12.39	102.2
10	233.6 (+α)	15 (0)	30 (0)	13.16	104.9
11	200 (0)	6.6 (-α)	30 (0)	11.66	93.5
12	200 (0)	23.4 (+α)	30 (0)	13.26	106.9
13	200 (0)	15 (0)	13.2 (-α)	12.03	104.2
14	200 (0)	15 (0)	46.8 (+α)	12.86	107.1
15	200 (0)	15 (0)	30 (0)	12.05	97.7
16	200 (0)	15 (0)	30 (0)	12.69	99.3
17	200 (0)	15 (0)	30 (0)	12.16	103.2
18	200 (0)	15 (0)	30 (0)	12.41	103.6
19	200 (0)	15 (0)	30 (0)	12.20	105.5
20	200 (0)	15 (0)	30 (0)	12.84	104.7

	Storage -18°C	Storage -18°C	Storage room T
	Filler 0.5 mm	Filler 2 mm	Filler 0.5 mm
<i>Extraction yield ($g_{extract}/g_{WAC}$)</i>			
Y_{∞}	0.25	0.25	0.25
f	0.947	0.815	0.495
k_1 (min ⁻¹)	0.070	0.071	0.073
k_2 (min ⁻¹)	0.002	0.002	0.004
<i>TPC (mg GAE/g_{WAC})</i>			
Y_{∞}	21.8	21.8	15.5
f	0.087	0.027	0.021
k_1	0.023	0.047	0.030
k_2	0.020	0.015	0.026

Figure 1

Figure 3

Figure 4

Figure 5

Figure 6a

Figure 6b

Figure 7

Figure 8