Box-Behnken design for optimal extraction of phenolics from almond by-products

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Abstract

Response Surface Methodology (RSM) was chosen to optimize the influence of solvent pH and relative proportion, and time of extraction, regarding polyphenols and radical scavenging capacity of almond (*Prunus dulcis* (Mill.) D.A. Webb) by-products (hulls, shells, and skins) from an almond orchard located in the North of Portugal (Lousa, Torre de Moncorvo). The RSM model was developed according to a Box-Behnken design and the optimal conditions were set for pH 6.5, 250.0 min, and 90.0% of food quality ethanol, pH 1.5, 235.0 min, and 63.0% ethanol, and pH 1.5, 250.0 min, and 56.0% ethanol for hulls, shells, and skins, respectively. The optimal conditions were obtained applying spectrophotometric techniques because of their versatility, while the chromatographic profile of extracts obtained when applied the optimal conditions indicated the presence of 3-caffeoylquinic acid, naringenin-7-*O*-glucoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, and isorhamnetin aglycone in hulls and skins. The model designed allowed the optimization of the phenolic extraction from almond by-products, demonstrating the potential of these materials as sources of antioxidant compounds with potential industrial, pharmaceutical and food applications.

Keywords: Almonds; By-products; Phenolics extraction; Optimization process; Antioxidants; RSM

1. Introduction

Among diverse nuts consumed around the world, almonds (*Prunus dulcis* (Mill.) D.A. Webb) constitute a relevant production due to its organoleptic properties and content of healthy nutrients, being nowadays promoted as healthy foods because of their capacity to lower the prevalence of diverse pathophysiological processes; in specific reducing the plasma level of low density lipoproteins (LDL)-cholesterol and risk of colon cancer, and displaying cardioprotective and antidiabetic effects (Davis and Iwahashi 2001; Ros 2010; Vadivel et al. 2012).

Almond orchards are extensively implanted in geographic areas with a Mediterranean climate and the industrial processing of almonds is addressed to the consumption as edible kernel, while producing amounts of by-products that represent up to 80% of the unprocessed production material, with high environmental impact. Such residues include hulls (40–60% of total weight), shells (20–30% of total weight), and skins (4–8% of total weight) (Prgomet et al. 2017). Between 0.8 and 1.7 Mt of shells are annually discarded, while some of them are used as activated carbons and in particleboard production (Pirayesh and Khazaeian 2012) or for energy production. On the other hand, hulls are mainly used for the development of feeds (Takeoka et al. 2000) and skins as biofuel in processing plants (Harrison and Were 2007).

Based on the composition, almond by-products are candidates to be sustainable sources of phytochemicals, such as triterpenes, flavonoids, phenolic acids, and phytoprostanes (Carrasco del Amor et al. 2015; Prgomet et al. 2017; Bottone et al. 2018). The concentration of these bioactive compounds is strongly conditioned by agro-environmental conditions (Bolling et al. 2010; Čolić et al. 2017; Prgomet et al. 2017; Prgomet et al. 2019), especially regarding abiotic stress factors of growing interest under the current climate change (Brito et al. 2019). Based on previous reports characterizing the biological interest of phytochemical compounds, such as prebiotic, anti-inflammatory,

antimicrobial and neuroprotective properties (Mandalari et al., 2010a, 2010b, 2011), these have been suggested as competent to develop interesting potential applications in the development of functional products, for instance, as antimicrobial agents against human pathogens or as phytopharmaceuticals (Takeoka et al. 2000; Wijeratne et al. 2006; Prgomet et al. 2019). Besides, the valorization of plant materials as sources of bioactive phytochemicals would contribute to enhance the waste reduction. Indeed, the descriptions available in the literature on functional compounds present in these materials have focused the attention of pharmaceutical, food, and biomedical industries, which has contributed to boosting further research aimed at providing rational support to new applications. The practical implementation of these advances would reduce the environmental impact of almond production and processing (Smeriglio et al. 2016), and improve the economic returns, with the implementation of green solvents and use of non-thermal technologies in the recovery protocols.

In order to design successful valorization alternatives for almond by-products as sources of bioactive phenolics, optimizing extraction constitutes a crucial stage, while to date, the extraction of phenolic compounds present into these by-products has been reported based on the use of diverse solvents of analytical grade (and therefore no usable by the pharma and food industries), and regarding acidity, and extraction times, upon different extraction technologies (Pinelo et al. 2004; Wijeratne et al. 2006; Rubilar et al. 2007; Garrido et al. 2008; Mandalari et al. 2010c; Valdés et al. 2015). Therefore, further optimization procedures are still required on all three solid almond by-products, given the lack of information existing and diverse extraction technologies applicable to these materials. In this regard, Response Surface Methodology (RSM) integrates a collection of mathematical and statistical algorisms and allows to reduce time and resources needed for the optimization of processes influenced by independent factors (Baş and Boyacı 2007; Domínguez-Perles et al. 2014), providing also valuable information on interactions between

them. From the different models described in the literature, Box and Behnken developed a class of nearly rotatable second-order designs based on the three-level incomplete factorial design, providing a model featured by high efficiency (Box and Behnken 1960).

The aim of this study was to optimize the extraction of total phenolics, *ortho*-diphenols, and flavonoids from solid almond hulls, shells, and skins, concerning solvent (food quality ethanol) percentage, pH, and extraction time by using RSM and to profile the extracts obtained when applying the optimal conditions by HPLC-DAD/UV-*Vis*. Polyphenolic extracts were also assessed on radical scavenging power against 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, in order to define the optimal conditions for obtaining functional extracts through a simple and non-toxic process.

2. Materials and Methods

2.1. Chemicals

The reagents Folin-Ciocalteau, Trolox (6-hydroxy-2,5,7,8-tetremethychroman-2-carboxylic acid), ABTS, DPPH, gallic acid, catechin, sodium carbonate, sodium molybdate, and potassium persulfate were purchased from Sigma-Aldrich (St. Louise, MO, USA). The reagents of aluminum chloride, sodium nitrite, sodium hydroxide, and acetic acid were purchased from Merck (Darmstadt, Germany). Food quality ethanol was from Panreac (Castellar del Vallès, Barcelona, Spain). The phenolic standards (3-caffeoylquinic acid, (+)-catechin, (+)-epicatechin, naringenin-7-*O*-glucoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, and isorhamnetin) were purchased from Extrasynthese company located at Genay, Lion Nord, France. All the chemicals used were of analytical grade. Water was treated with SGS water purification system.

2.2. Orchard location and climatic conditions of the site

Almond fruit and its by-products were obtained from a 6 years old almond orchard located in the North of Portugal (Lousa, Torre de Moncorvo, Portugal (41°11'25" N and 7°10'27" W), in 2014. Climatic data observed in the months when the study was developed were within the reported long-term average (448.9 mm), with the average daily temperatures ranging from 6.0 °C (December) to 23.5 °C (July) (Fig. 1). In the summer months, rainfall was higher than average in July (23 mm), while August was less rainy (2.5 mm) than the average. Data on the average annual rainfall and mean temperatures were obtained from the E-OBS gridded dataset (Haylock et al. 2008).

2.3. Plant material

Complete production of healthy almonds per tree (*Prunus dulcis* (Mill.) D.A. Webb; late blooming variety Ferraduel) were collected from 10 different trees of comparable age and vigor, located at distinct points in the same growing area. Almond trees were all grafted on GF-677 rootstock and spaced 6 x 4 m. Almond hulls were separated from the rest of the fruit by hand and freeze-dried. Kernels, still within shells, were air dried at room temperature (23 °C) and outer shells were separated from the kernel using a nutcracker and kernels were blanched in deionized boiling water for 3 min, in accordance to the previous descriptions available in the literature (Milbury et al. 2006), based on the processes currently used in the almond processing industry. Skins were removed by hand and oven-dried at 60 °C for 72 hours. All samples were ground to powder and stored protected from humidity and light until phenolic extractions.

2.4. Extraction procedure

Dried powder (50 mg) was extracted in 2 mL of different combinations of solvent percentage under a panel of pH and extracting time conditions. All extraction solvents used contained citric acid (1 g L^{-1}) according to Karvela et al. (2011) and were further adjusted to the desired pH according to the experimental design by adding NaOH/HCl. Extractions were performed using an orbital shaker, at room temperature, during different time periods. Polyphenolic extracts were centrifuged at 5000 rpm, for 10 min at 4 °C (Sigma 2-16K, Germany), and the supernatants collected for analysis.

2.5. Experimental design

The effect of extraction parameters (pH of the extraction solvent (X_1), extraction time (min, X_2), and food quality ethanol concentration (%, X_3)) on the efficiency of the extraction of almond by-products phenolics was assessed by applying a Box-Behnken design for which each variable was coded at the levels, -1, 0, and 1 (Table 1).

For this study, fifteen experiments were developed under specific conditions for each plant material (Tables 2-4). Extracts were assessed on the content of total phenolics, *ortho*-diphenols, and flavonoids, as well as on their radical scavenging power (DPPH and ABTS tests). The model design included three replicates at the central point, randomly spread within the experimental design (experiments 4, 13, and 15; Tables 2-4), in order to maximize the control on unexplained variability due to the inessential factors. All the experiments were performed in triplicate (n=3).

2.6. Total phenolics, flavonoids, and ortho-*diphenols*

The total phenolic content was determined by spectrophotometric analyses using the Folin-Ciocalteau reagent, following the methodology previously described with minor modifications, and adapted at the 96-microplates scale (Domínguez-Perles et al. 2014; Machado et al. 2017). Briefly, after 30 min at 40 °C, samples absorbance was measured at

750 nm using a spectrophotometric microplate reader (Thermo Scientific Multiskan GO Microplate Spectrophotometer) and the total phenolic content was achieved using a gallic acid calibration curve (concentration range of 5-200 mg L^{-1}). Final contents of total phenolics were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} dw).

The *ortho*-diphenol content was determined also by spectrophotometric analyses, following the methodology previously described (Domínguez-Perles et al. 2014), adapted at the 96-microplates scale (Machado et al. 2017). Absorbance was measured at 375 nm using a spectrophotometric microplate reader. Gallic acid (in the concentration range 5-200 mg L^{-1}) was used as the standard compound for the quantification of the *ortho*-diphenols content. Final concentrations were expressed as mg GAE g^{-1} dw.

The flavonoid content of almond residues was determined using the methodology described in the literature and adapted at the 96-microplates scale (Domínguez-Perles et al. 2014; Machado et al. 2017). In detail, to the 24 µL of sample, 28 µL of $NaNO_2$ was added. Five (5) min later 28 µL of $AlCl_3$ was placed and after additional 6 min, 120 µL of NaOH was added to conclude the reaction. Absorbance was measured at 510 nm using a spectrophotometric microplate reader and flavonoids concentration were calculated resorting to freshly prepared catechin standard curves (in the concentration range of 5-200 mg L^{-1}). The results were expressed as mg of catechin equivalents per gram of dry weight (mg CE g^{-1} dw).

2.7. Radical scavenging capacity

The free radical scavenging activity was determined by DPPH and ABTS methods adapted to a microscale, according to the method previously described (Barros et al. 2014). Absorbance was measured at 520 nm after 15 min of reaction for $DPPH^{\bullet}$ and at 734 nm after 30 min for $ABTS^{\bullet+}$, using 96-well microplates and Multiscan FC microplate reader.

211 Results on radical scavenging capacity were expressed as millimoles of Trolox equivalent
212 per gram of dry weight (mmoles TE g^{-1} dw).
213
214 *2.8. HPLC-DAD*-Vis *analysis*
215 The phenolic profile of the separate solid residues of the almond industry was
216 achieved by an HPLC-DAD/UV-*Vis* system, equipped with a C18 column (250 × 4.6 mm,
217 5 µm) (ACE®-HPLC columns, Ltd., Aberdeen, Scotland), by applying a method developed
218 and validated by Aires et al. (2016). Briefly, individual phenolics were eluted using
219 ultrapure water/trifluoroacetic acid (99.9:0.1, v/v) (solvent A) and
220 acetonitrile/trifluoroacetic acid (99.9:0.1, v/v) (solvent B), upon the linear gradient scheme
221 (t in min; %B): (0; 0%B), (5; 0%B), (20; 20%B), (35; 50%B), (40; 100%B), (45; 0%B),
222 and (65, 0%B). The flow rate and the injection volume were 1.0 mL min^{-1} and 10 µL,
223 respectively, and the chromatograms were recorded at 360 nm. The individual phenolic
224 acids were identified resorting to the peak retention time, UV spectra, and UV max
225 absorbance bands, and through comparison with external commercial authentic standards
226 (Extrasynthese, CEDEX, France, and Sigma-Aldrich, Tauferkichen, Germany) that were
227 freshly prepared and run in HPLC-DAD/UV-*Vis* at the same time with samples.
228
229 *2.9. Statistical analysis*
230 Means and standard deviations (n=3) and the coefficients corresponding to the
231 models' equations were calculated resorting to Statgraphics Centurion XVI (StatPoint
232 Tecnhologies, Inc., 2010, USA). This statistical package was also used for the experimental
233 design and to determine the regression coefficients and the statistical significance of each
234 factor within the models, which was set up at $p<0.05$.
235
236 **3. Results and discussion**

In previous research, the optimization of the extraction conditions for phenolics of plant food by-products was developed by applying different extraction conditions and technologies (Pinelo et al. 2004; Valdés et al. 2015). However, the separate optimization procedures described have not been developed on the three diverse solid by-products using the same experimental approach, which causes a gap of knowledge that is essential to explore in order to design rational valorization procedures on the solid almond by-products. So the settings of the optimization processes described in the present work were established using ranges of values according to that information available in the literature, for the first time, on the three solid almond by-products. Firstly, when undertaking a screening experiment, to identify the most relevant variables to explain the effectiveness of the phenolics extraction, pH, extraction time, and percentage of ethanol were identified as the most influential factors, being found of minor relevance the liquid-solid ratio and the temperature of extraction. Once selected the variables, to check if the levels currently accepted are consistent with optimum performances, the set of adjustments towards optimal extractions needed to be determined. This situation made mandatory to develop sequential rounds to fine-tune the experimental ranges through the evaluation of experimental responses, so called the method of steepest ascent. Hence, in the first round, the following symmetric ranges of values were considered: X_1 (pH): 1.5–6.5, X_2 (extraction time): 5–90 min, and X_3 (percentage of ethanol): 50–90%. Since the development of the first round provided optimal conditions exceeding the range of values considered for extraction time and ethanol concentration, it was needed to enlarge them, until reaching the optimal limits (Table 1) that fit appropriately the values providing the highest yield of phenolic compounds and radical scavenging activity.

3.1. Yield of the assayed extraction conditions

The comparison of the values obtained on the content of total phenolics, flavonoids, and *ortho*-diphenols upon the panel of extraction conditions tested, as well as their $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging capacities (Tables 2-4), revealed the close agreement between experimental and theoretical data.

When analyzing the results obtained for hulls, the highest level of total phenolics and flavonoids corresponded to extractions developed at pH 6.5, 150.0 min, using 90.0% of ethanol concentration (Table 2). On the other hand, for *ortho*-diphenols, the best result was obtained on extractions developed at pH 4.0, during 50 min, using 90.0% concentration of ethanol. In respect to radical scavenging, the highest efficiency was observed on extracts obtained at pH 4.0, during 250.0 min, and using 90.0% ethanol for ABTS, and on extracts obtained at pH 1.5, during 150.0 min, with 90.0 ethanol percentage for DPPH (Table 2).

The assessment of the influence of the diverse factors on the efficiency of the phenolics extraction in shells showed that the highest values for total phenolics and *ortho*-diphenols were obtained at pH 1.5, 250.0 min, and 60.0% ethanol concentration. These conditions also provided the highest $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging power. For flavonoids, the most efficient extraction was achieved at pH 4.0, 150.0 min, and 60.0% food quality ethanol (Table 3).

Regarding skins, the analysis of the influence of the different factors evaluated on the concentration of total phenolics, flavonoids, and *ortho*-diphenols, as well as on $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging capacity evidenced that the best results on total phenolics, *ortho*-diphenols, and ABTS-based antioxidant activity corresponded to extractions developed at pH 1.5, during 250.0 min, using 60.0% ethanol (Table 4). Moreover, the highest efficiency concerning flavonoids extraction was achieved at pH 6.5, 250.0 min, using 60.0% ethanol (Table 4). Finally, for $DPPH^{\bullet}$ scavenging activity the best value was obtained at pH 1.5, 150.0 min, using 30.0% ethanol (Table 4).

288 *3.2. Model fitting*

289 Data retrieved were subjected to multiple regression analysis to get a detailed
290 description of the relative influence and significance of each factor. Moreover, the
291 significance of the regression coefficients relatively to linear, quadratic, and interception
292 interactions were evaluated by analysis of variance (ANOVA). The evaluation of residues
293 with distinct physical features (hulls, shells, and skins) provided coefficients that noticed
294 well-fitting models, while informing on the factors that need to be considered for each
295 matrix.

296 The coefficients of determination (R^2) of the model developed regarding hulls, shells,
297 and skins for total phenolics were 0.762, 0.955, and 0.976, respectively, regarding
298 flavonoids were 0.980, 0.966, and 0.945, respectively, and finally, for *ortho*-diphenols
299 ranged from 0.837 to 0.976. These results inform on an adequate fitting of the model
300 already indicated by the close relationship between observed and theoretical values (Tables
301 2-5).

302 Almond by-products differ one to another on physical and compositional features
303 and, based on these divergences, the polyphenolic content and the factors influencing the
304 efficiency of the extraction procedure are also expected to differ. Almond hulls extracts had
305 a total phenolic content five and sixteen folds higher compared to almond skins and shells
306 extracts, respectively (Tables 2-4). Furthermore, in all three extracts, the concentration of
307 ethanol was the most important variable affecting the efficiency of the extractions, as well
308 as the antiradical power of the extracts. For almond hulls, the polyphenolic yield increased
309 in parallel to the augment of the ethanol percentage (Table 2). On the other hand, the
310 augment of ethanol promoted a comparable improvement of the extraction efficiency
311 between 30 and 60% in skins and shells, while percentages higher than 60% food quality
312 ethanol caused a decrease of the polyphenolic extraction. These results are in agreement
313 with previous works demonstrating aqueous acetone, methanol, and ethanol as the best

314 solvents to extract phenolic compounds from almond by-products relatively to their pure
315 state (Sarwar et al. 2012; Meshkini 2016).

316 Apart from the optimization of the polyphenols extraction, the success of the
317 procedures was monitored by assessing the extracts obtained on the radical scavenging
318 activity that allowed to identify the most relevant factors for ensuring a high ABTS and
319 DPPH-based antioxidant activity and to set existing correlations with phenolic composition,
320 as previously was reported the existence of a direct relationship between antioxidant tests
321 with radicals and the total phenolic content values (Koch et al. 2015). In this concern,
322 significant differences were observed between the separate almond by-products under
323 evaluation and are shown in the Table 5. The R^2 for ABTS and DPPH antiradical activity
324 ranged from 0.924 and 0.995 for both techniques supporting the consistency of the
325 optimization process.

326 The high F-value obtained for the model (of up to 60.41) and low Mean Absolute
327 Errors (MAE≤0.32), with exception of total phenolics for hulls for which MAE was 3.34,
328 further strengthened the reliability of the models developed.

329 Thus, the highest phenolic contents and antioxidant capacities of polyphenolic
330 extracts of hulls were obtained at the highest food quality ethanol concentrations. However,
331 in shells and skins the most appropriate ethanol concentration ranged between 54.0 and
332 72.0% (v/v). These findings agree with the information available in the literature on the
333 capacity of aqueous ethanol to extract greater amounts of phenolic compounds regarding
334 almond shells (Sarwar et al. 2012), as well as in other nuts by-products (Odabaş and Koca
335 2016), relatively to absolute ethanol. This fact could be due to increased solubility of
336 phenolic compounds because of the occurrence of glycosylated (more polar) derivatives. In
337 addition, different structure and composition of the plant matrices under study and the
338 chemical features of solvents conditioned the distinct behaviors for each plant material-
339 solvent system (Pinelo et al. 2005).

Given the particular features of the separate almond residues, in some cases it could be required longer extraction times that lead to a longer contact between the plant material and extracting solvent and thus, increase the diffusion of phenolic compounds. On the other side, excessively prolonged extractions could cause a deleterious impact on the final concentration of phenolics due to a parallel increase of oxidation reactions, which entail a decrease in the final concentration (Naczk and Shahidi 2006). In this regard, Chew et al. (2011) reported that extractions longer than 240.0 min are not appropriate for phenolic compounds from *Orthosiphon stamineus*. In addition, Pompeu et al. (2009) fixed the extraction time for phenolics present in *Euterpe oleracea* fruits around 240.0 min., since longer times degrade polyphenols. Thus, even though some optimal extraction times in the herein presented study was on the limit, i.e. 250.0 min, no longer extractions were considered according to the phenolics degradation occurring when using higher times. Additionally, the extraction time is crucial for reducing energy requirements and costs. So, the use of extraction time longer than 250 min would be no economically advantageous and could constitute a serious drawback for the practical implementation of the optimized conditions.

In addition to the features of the solvent and the length of the extraction, the solvent pH is mostly known to increase phenol stability. In this sense, most of the studies carried out to date have reported pH lower than 5 to be responsible for increasing phenolic yield and preserving antioxidant activity (Ruenroengklin et al. 2008; Amendola et al. 2010). In fact, the results retrieved from the present work are in agreement with such situation, as well as with higher radical activities that were featured by optimal pH at 1.5 for DPPH in all by-products, and at 1.5, 3.3, and 4.8 for ABTS concerning skins, hulls, and shells, respectively. Interestingly, the results revealed that the highest yield of total phenolics and *ortho*-diphenols in shells and skins were obtained under acid pH (pH 1.5), while the most appropriate extraction of flavonoids was retrieved at pH ranging from 4.9 to 6.5. This is in

concordance with Malovaná et al. (2001) that reported a decrease of the content of non-flavonoids at pH between 2.0 and 7.0, while for flavonoids was recorded an opposite behavior (Chethan and Malleshi 2007). On the other hand, in the present study almond hull extracts displaying the highest contents of total phenolics, flavonoids, and *ortho*-diphenols were obtained using pH ranging from 5.7 to 6.5. This a priori controversial results that point out different optimal pH for the same phenolic types could be due to the specific effect of pH depending on the features of the raw material from which phenolic compounds are extracted. In addition, Librán et al. (2013) reported that the influence of the pH of the solvent on the phenolic yield cannot be considered independently, but in combination with ethanol concentration, since concerning extraction of grape marc phenolics, basic pH led to better yields in solvent with lower ethanol percentage, while acidic pH was the best choice when using high percentages of ethanol. Similarly, Ruenroengklin et al. (2008) reported the influence of the combined effects of temperature and pH to the phenolic yield in lichi extraction.

Hence, from the results obtained from the combination of factor levels which maximizes each response over the indicated region, the model has provided predicted values that could be obtained under specific extraction conditions (Table 6).

3.3. Verification of the predictive models developed

The second order polynomial equations provided by the RSM model allowed to obtain theoretical contents of studied parameters. Optimized parameters were obtained by computation for hulls, shells, and skins with the aim of maximizing each factor for the separate variable (Table 6).

Summarizing, the best combinations of parameters regarding each residue were pH 6.5, 250.0 min, and 90.0% ethanol for hulls, pH 1.5, 235.0 min, and 63.0% ethanol for shells, and pH 1.5, 250.0 min, and 56.0% ethanol for skins. The optimal condition for each

residue was obtained according to the optimal settings provided by the model for each variable that were monitored upon a final set of assessments allowing to make decisions based on the limiting variable (those presenting the lower response) and taking into consideration that the single final optimal conditions for each residue was within the 95% upper/lower limits for all of them. The application of these settings to the polynomial equations obtained by the model provided the theoretical results that are shown in the Table 7.

In order to estimate the consistency of the model and thus, the suitability of theoretical values retrieved, it was developed a final panel of extractions applying the optimized settings (Table 7). As expected, the values obtained were within the 95.0% lower and upper limits of the predicted values, except for activity assays and *ortho*-diphenolic content for almond shells. Even though different authors already reported diverse optimal conditions for phenolic extraction from solid almond by-products, the only information available on the relative importance of the parameters influencing the efficiency of phenolic extraction by applying optimization models on almond by-products so far is on almond skin (Valdés et al. 2015), however, with different studied factors compared to the present study. Therefore, results in the present study confirm that the response surface models developed allowed to optimize successfully the most critical parameters involved in the efficiency of phenolic extractions of almond skins and hulls, using food quality ethanol.

The lack of appropriate optimization of *ortho*-diphenols and radical scavenging capacity in shells extracts could be a consequence of the reduced values obtained for such variables that turns the variation of the absolute values retrieved from the experimental determinations in high percentage changes. However, the aim of this work was the optimization of extraction conditions of matrices that are potential source of antioxidants and, in this perspective, almond shells, that exhibited low phenolics concentration, would be a candidate to be addressed to other valorization processes, mainly focused in the energy

418 production, obtaining wood-based composites, production of activated carbons, and in
419 agriculture as soil ameliorants, potential substrate for production of other plant species and
420 mulch (Prgomet et al. 2017).
421 Finally, almond by-products are potential source of bioactive compounds, which
422 extracts can be used in industries, such as cosmetic and pharmaceutical ones.
423
424 *3.4. Phenolic profile of extracts*
425 The HPLC analysis of the almond by-products revealed a limited number of phenolic
426 compounds (Fig. 2, Table 8) that were monitored at 360 nm, at which all phenolic classes
427 in solid almond residues show operative absorbance. This approach leads to obtain
428 chromatograms that represent the overall polyphenolic profile of the extracts, which were
429 identified by comparing their UV-*Vis* spectra with the information available in the literature
430 and the retention time of authentic standards (Fig. 2).
431 Concerning almond hulls, it was identified the presence of the phenolic acid 3-
432 caffeoylquinic acid and the flavanone naringenin-7-*O*-glucoside at the retention times 20.03
433 and 20.26 min, respectively. On the other hand, when assessing almond skins on the profile
434 of phenolic compounds it was observed that this is the solid by-product featured by the
435 widest diversity. Indeed, almond skins exhibited the presence of the flavonol kaempferol-3-
436 *O*-glucoside (23.67 min) and the flavanones isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-
437 *O*-glucoside, and isorhamnetin aglycone (retention times 23.83, 24.69, and 25.24 min,
438 respectively) (Fig. 2, Table 8). Interestingly, although some peaks were observed in the
439 chromatograms corresponding to almond shells, their relative abundance was very low
440 compared to hulls and skins, and no clear identification of the compounds was obtained,
441 which is in agreement with the abundance observed for total phenolics, flavonoids and
442 *ortho*-diphenols. Even though compounds at retention time 18-25 probably correspond to

proanthocyanidins, due to the lack of standard compounds available for their identification, it was not possible to identify nor quantify them properly.

The presence of phenolic acids, flavonols and flavanones, and the phenolic extracts of almond by-products obtained by using solvents compatible with the food and pharma industries, is in agreement to that recently published by Valdés et al. (2015) applying microwave assisted extraction. This coincidence evidences a similar efficiency of both methods, and thus the interest of the optimization reported in the present work to be applied by the industry.

Among the information available in the literature in the respect of polyphenolic profile of almond by-products, it should be stressed that solid almond by-products contain cinnamic acid derivatives, such as caftaric and chlorogenic acids, flavonols, namely kaempferol and quercetin glycosides and aglycones, flavan-3-ols represented by catechin and epicatechin, and flavanone derivatives including naringenin and isorhamnetin derivatives (Valdés et al. 2015; Pasqualone et al. 2018; Prgomet et al. 2019).

Compounds observed in the present study in almond skins, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, and isorhamnetin aglycone, were also identified in a study of influence of a season and irrigation treatment on almond by-products polyphenols (Prgomet et al. 2019). Quercetin, an ubiquitous compound found in almond skins extracts (Smeriglio et al. 2016), was observed in the present study, however, just in traces. On the other side, although flavan-3-ols (catechin and epicatechin) were found in recent studies on polyphenolic composition of almond skins (Pasqualone et al. 2018; Prgomet et al. 2019), the characterization of the extracts obtained using conditions compatible with the food and pharma industry did not allow to found these compounds. However, use of different solvents might be a reason of this diversity in the yield of phenolic extractions.

Despite the limited identification of peaks relative to almond hulls, herein result is in agreement with previous reports available in the literature, which noticed this solid residue of the almond production (hulls) as a source of mainly phenolic acids, and in a lesser extent flavonoids (Rubilar et al. 2007), being chlorogenic acid the most relevant phenolic acid in this plant material (Takeoka and Dao 2003; Prgomet et al. 2019). Furthermore, in a recent study, naringenin-7-*O*-glucoside, identified as well herein, was observed as the predominant flavonoid in almond hulls (Prgomet et al. 2019).

4. Conclusions

In the present study, a RSM dedicated design was set up to optimize the extraction process of phenolic compounds of almond by-products (hulls, shells, and skins), investigating solvent pH, concentration and extraction time. This methodology was successfully employed for the optimization of total phenolics, flavonoids, and *ortho*-diphenols, as well as for achieving the highest antioxidant activities. Factor settled at optimum for the analyzed responses were at pH 1.5 for skins and shells, and 6.5 for hulls; time of 250.0 min for hulls and skins, and 235.0 for shells, and 90.0%, 63.0% and 56.0% of ethanol for hulls, shells and skins, respectively. The relevance of the optimized extraction conditions stated upon the present work is the feasibility of using non-toxic, food grade ethanol to extract phenolic compounds from these underexplored and underexploited plant materials of interest as a source of bioactive phytochemicals with diverse the purpose of developing new functional foods and cosmetics. In this regard, actually, the application of the reported conditions by the agro-food companies would allow an improvement of the valorization alternatives for these residues and their extracts and thus, to take advantage from the biological and biochemical attribution of such compounds; for instance, as natural food preservation additives, dietary and nutraceutical supplements, and active ingredients for skin care products. In this sense, in the present work, extraction conditions susceptible

to be practically implemented by the industry by using green solvents solvents and use of non-thermal technologies upon the recovery procedures were reported, all of the above being of great interest to reduce the environmental impact of the agri-food sector, while enhance its competitiveness and sustainability, allowing to advance decisively forward in the strategy of a zero-waste circular economy. Obviously, since the characterization was done at laboratory scale, further research is needed to scale up the settings reported in the present work to an industrial dimension to finally establish valorization procedures for these materials.

Compliance with Ethical Standards

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Disclosure of potential conflicts of Interest

Author I. P., Author B. G., Author R. D.-P., Author N. P.-S. and Author A. I.R.N.A. B. declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Not applicable

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Table 1. Symbols and coded factor levels for the considered independent variables.

Independent variables	Code	Levels		
		-1	0	1
pH	X_1	1.5	4.0	6.5
Time (min)	X_2	50	150	250
Ethanol concentration (%)	X_3	30	60	90

Table 2. Effect of processing variables on the phytochemical composition and radical scavenging capacity of hydro-ethanolic extracts of almonds hulls by RSM.

Assay	Coded level			Total phenolics (mg GAE g^{-1} dw)		*Ortho*-diphenols (mg GAE g^{-1} dw)		Flavonoids (mg CE g^{-1} dw)		$ABTS^{\bullet+}$ scavenging capacity (mmol TE g^{-1} dw)		$DPPH^{\bullet}$ scavenging capacity (mmol TE g^{-1} dw)	
	pH	Time (min)	Ethanol conc. (%)	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.
1	**0 (4)**	**-1 (50)**	**1 (90)**	91.76	100.62	**131.34**	**131.03**	120.11	116.46	1.43	1.40	1.11	1.13
2	-1 (1.5)	0 (150)	-1 (30)	104.97	112.79	108.08	109.83	36.99	39.64	1.17	1.15	1.32	1.33
3	1 (6.5)	0 (150)	-1 (30)	107.32	113.22	111.06	108.57	88.58	83.72	1.00	0.97	1.00	1.00
4[Z]	0 (4)	0 (150)	0 (60)	118.62	116.51	112.44	113.10	101.78	100.64	1.33	1.33	0.85	0.87
5	1 (6.5)	1 (250)	0 (60)	133.28	136.25	115.30	117.48	109.52	110.73	1.22	1.21	1.08	1.09
6	-1 (1.5)	-1 (50)	0 (60)	123.01	120.04	123.96	121.78	58.87	57.66	1.26	1.27	1.42	1.41
7	-1 (1.5)	1 (250)	0 (60)	125.45	126.50	123.10	121.04	57.86	51.57	1.42	1.41	1.32	1.32
8	**0 (4)**	**1 (250)**	**1 (90)**	137.47	142.32	123.75	123.32	112.85	114.29	**1.54**	**1.52**	0.99	1.00
9	**-1 (1.5)**	**0 (150)**	**1 (90)**	134.50	128.61	120.37	122.86	52.69	57.55	1.28	1.30	**1.46**	**1.45**
10	1 (6.5)	-1 (50)	0 (60)	114.22	113.18	122.12	124.18	101.45	107.74	1.38	1.39	1.12	1.11
11	**1 (6.5)**	**0 (150)**	**1 (90)**	**138.90**	**131.08**	124.70	122.95	**125.35**	**122.71**	1.38	1.40	1.25	1.24
12	0 (4)	-1 (50)	-1 (30)	115.55	110.71	112.90	113.33	88.82	87.39	1.24	1.25	0.88	0.88
13[Z]	0 (4)	0 (150)	0 (60)	104.75	116.51	107.34	113.10	96.67	100.64	1.24	1.33	0.85	0.87
14	0 (4)	1 (250)	-1 (30)	107.42	98.55	113.30	113.61	82.81	86.46	1.07	1.10	0.92	0.90
15[Z]	0 (4)	0 (150)	0 (60)	126.15	116.51	119.53	113.10	103.46	100.64	1.42	1.33	0.91	0.87

[Z] Central point. It was highlighted in bold the best condition for each of the variables monitored.

Table 3. Effect of processing variables on the phytochemical composition and radical scavenging capacity of hydro-ethanolic extracts of almonds shells by RSM.

Assay	Coded level			Total phenolics (mg GAE g^{-1} dw)		*Ortho*-diphenols (mg GAE g^{-1} dw)		Flavonoids (mg CE g^{-1} dw)		$ABTS^{\bullet+}$ scavenging capacity (mmol TE g^{-1} dw)		$DPPH^{\bullet}$ scavenging capacity (mmol TE g^{-1} dw)	
	pH	Time (min)	Ethanol conc. (%)	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.
1	0 (4)	-1 (50)	1 (90)	3.55	3.56	3.67	3.59	2.77	2.82	0.04	0.04	0.03	0.03
2	-1 (1.5)	0 (150)	-1 (30)	5.76	5.90	5.30	5.43	1.74	1.89	0.05	0.06	0.07	0.06
3	1 (6.5)	0 (150)	-1 (30)	6.53	6.90	6,75	7.44	3.77	4.06	0.06	0.07	0.08	0.08
4[z]	0 (4)	0 (150)	0 (60)	8.23	8.27	7.49	7.51	5.72	5.80	0.08	0.08	0.08	0.08
5	1 (6.5)	1 (250)	0 (60)	7.91	7.55	7,82	7.05	5.55	5.30	0.09	0.08	0.10	0.09
6	-1 (1.5)	-1 (50)	0 (60)	6.48	6.84	5.98	6.75	2.93	3.19	0.07	0.07	0.07	0.08
7	**-1 (1.5)**	**1 (250)**	**0 (60)**	**8.62**	**8.79**	**9.95**	**9.72**	4.59	4.48	**0.09**	**0.08**	**0.10**	**0.10**
8	0 (4)	1 (250)	1 (90)	4.64	5.14	4,81	5.72	3.30	3.70	0.05	0.05	0.05	0.05
9	-1 (1.5)	0 (150)	1 (90)	6.51	6.14	8.92	8.22	3.20	2.91	0.06	0.06	0.07	0.07
10	1 (6.5)	-1 (50)	0 (60)	6.61	6.74	6.28	6.50	4.77	4.87	0.06	0.06	0.09	0.09
11	1 (6.5)	0 (150)	1 (90)	3.95	3.81	3.43	3.29	3.39	3.24	0.05	0.04	0.04	0.04
12	0 (4)	-1 (50)	-1 (30)	5.69	5.19	5.55	4.64	3.14	2.74	0.06	0.05	0.06	0.05
13[z]	**0 (4)**	**0 (150)**	**0 (60)**	8.23	8.27	7.61	7.51	**6.05**	**5.80**	0.09	0.08	0.08	0.08
14	0 (4)	1 (250)	-1 (30)	6.39	6.37	5.96	6.04	3.62	3.58	0.07	0.07	0.06	0.06
15[z]	0 (4)	0 (150)	0 (60)	7.75	8.27	7.45	7.51	5.64	5.80	0.08	0.08	0.08	0.08

[z] Central point. It was highlighted in bold the best condition for each of the variables monitored.

Table 4. Effect of processing variables on the phytochemical composition and radical scavenging capacity of hydro-ethanolic extracts of almonds skins by RSM.

Assay	Coded level			Total phenolics (mg GAE g^{-1} dw)		*Ortho*-diphenols (mg GAE g^{-1} dw)		Flavonoids (mg CE g^{-1} dw)		$ABTS^{\bullet+}$ scavenging capacity (mmol TE g^{-1} dw)		$DPPH^{\bullet}$ scavenging capacity (mmol TE g^{-1} dw)	
	pH	Time (min)	Ethanol conc. (%)	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.
1	0 (4)	-1 (50)	1 (90)	8.91	8.67	8.52	8.82	4.45	5.33	0.09	0.08	0.04	0.02
2	**-1 (1.5)**	**0 (150)**	**-1 (30)**	16.11	16.74	13.28	14.30	5.09	5.98	0.13	0.14	**0.30**	**0.27**
3	1 (6.5)	0 (150)	-1 (30)	15.58	14.65	12.65	11.90	10.96	10.85	0.15	0.14	0.12	0.10
4[Z]	0 (4)	0 (150)	0 (60)	17.38	18.91	14.97	15.56	11.12	12.75	0.17	0.18	0.10	0.10
5	**1 (6.5)**	**1 (250)**	**0 (60)**	21.08	21.77	16.58	17.62	**13.97**	**14.96**	0.21	0.21	0.17	0.17
6	-1 (1.5)	-1 (50)	0 (60)	24.35	23.66	22.87	21.82	12.39	11.40	0.20	0.20	0.24	0.25
7	**-1 (1.5)**	**1 (250)**	**0 (60)**	**25.17**	**24.31**	**23.32**	**22.60**	11.76	11.75	**0.23**	**0.22**	0.26	0.26
8	0 (4)	1 (250)	1 (90)	10.56	10.49	9.94	9.91	7.44	7.35	0.10	0.11	0.06	0.03
9	-1 (1.5)	0 (150)	1 (90)	12.29	13.22	14.62	15.37	5.32	5.42	0.10	0.11	0.08	0.10
10	1 (6.5)	-1 (50)	0 (60)	17.04	17.90	14.23	14.95	11.97	11.98	0.17	0.18	0.15	0.15
11	1 (6.5)	0 (150)	1 (90)	7.62	7.00	6.95	5.93	5.22	4.33	0.09	0.08	0.05	0.08
12	0 (4)	-1 (50)	-1 (30)	13.76	13.83	10.59	10.63	9.13	9.23	0.14	0.14	0.09	0.11
13[Z]	0 (4)	0 (150)	0 (60)	19.14	18.91	15.01	15.26	13.48	12.75	0.17	0.18	0.10	0.10
14	0 (4)	1 (250)	-1 (30)	16.27	16.51	13.29	12.99	11.42	10.54	0.15	0.15	0.10	0.13
15[Z]	0 (4)	0 (150)	0 (60)	20.22	18.91	15.80	15.56	13.66	12.75	0.19	0.18	0.11	0.10

[Z] Central point. It was highlighted in bold the best condition for each of the variables monitored.

Table 5. Corresponding *F*-values and *P*-values for each obtained coefficient and second order polynomial models used to express the content in total phenolics, flavonoids and *ortho*-diphenols, and the ABTS and DPPH-based antioxidant activities as a function of independent variables in almond hulls, shells and skins.

Hulls

Variable	Statistics	X_1	X_2	X_3	$X_{1,2}$	$X_{1,3}$	$X_{2,3}$	X_1^2	X_2^2	X_3^2	Model *F*-value
Total phenolics	*P*-value	N.s.[Z]	N.s.	N.s.	N.s.	N.s.	N.s.	N.s.	N.s.	N.s.	17.68
	F-value	0.03	3.35	4.36	0.53	0.01	5.57	1.78	0.01	0.26	
Flavonoids	*P*-value	***	N.s.	**	N.s.	N.s.	N.s.	**	N.s.	N.s.	27.63
	F-value	149.87	0.12	40.67	0.52	2.79	0.01	44.80	0.99	0.70	
Ortho-diphenols	*P*-value	N.s.	N.s.	**	N.s.	N.s.	N.s.	N.s.	N.s.	N.s.	41.43
	F-value	0.03	1.23	16.77	0.40	0.02	0.71	0.58	6.22	0.19	
ABTS	*P*-value	N.s.	N.s.	**	N.s.	N.s.	N.s.	N.s.	N.s.	N.s.	35.93
	F-value	0.84	0.14	40.25	6.23	4.37	4.77	3.36	2.37	3.53	
DPPH	*P*-value	***	*	***	N.s.	N.s.	*	***	*	**	60.41
	F-value	228.94	9.45	94.18	1.54	5.40	9.64	609.79	10.69	24.25	

Polynomial models[Y]	R^2	MAE[X]
$Total\ phenolics = 146.672 - 12.7611X_1 - 0.248532X_2 - 0.0180583X_3 + 1.26874X_1^2 + 0.016615X_1X_2 + 0.0068X_1X_3 - 4.43875x10^{-5}X_2^2 + 0.00448733X_2X_3 - 0.00334625X_3^2$	0.762	3.338
$Flavonoids = 33.4807 - 8.90213X_1 - 0.135908X_2 + 0.575969X_3 - 3.51668X_1^2 + 0.009088X_1X_2 + 0.0702233X_1X_3 + 0.000326675X_2^2 - 0.000103583X_2X_3 - 0.00305944X_3^2$	0.980	0.320
$Ortho\text{-}diphenols = 117.103 - 1.89279X_1 - 0.139145X_2 + 0.166458X_3 + 0.300087X_1^2 - 0.005958X_1X_2 + 0.00448X_1X_3 + 0.000614479X_2^2 - 0.000665917X_2X_3 + 0.00119921X_3^2$	0.837	0.002
$ABTS = 1.02843 + 0.0643933X_1 - 0.001745X_2 + 0.00607389X_3 - 0.00978667X_1^2 - 0.00032X_1X_2 + 0.000893333X_1X_3 + 5.13333x10^{-6}X_2^2 + 2.33333x10^{-5}X_2X_3 - 6.96296x10^{-5}X_3^2$	0.930	0.002
$DPPH = 2.1395 - 0.496407X_1 - 0.001018X_2 - 0.00528278X_3 + 0.0513133X_1^2 + 6.2x10\text{-}5X_1X_2 + 0.000386667X_1X_3 + 4.24583x10^{-6}X_2^2 - 1.29167x10^{-5}X_2X_3 + 7.10648x10^{-5}X_3^2$	0.995	0.011

Shells

Variable	Statistics	X_1	X_2	X_3	$X_{1,2}$	$X_{1,3}$	$X_{2,3}$	X_1^2	X_2^2	X_3^2	Model *F*-value
Total phenolics	*P*-value	N.s.	*	*	N.s.	*	N.s.	N.s.	N.s.	***	24.54
	F-value	2.60	11.22	11.98	0.95	8.14	0.11	0.08	5.37	67.61	
Flavonoids	*P*-value	**	*	N.s.	N.s.	N.s.	N.s.	*	*	***	25.26
	F-value	19.75	9.38	0.12	1.20	5.32	0.00	13.56	7.88	94.69	
Ortho-diphenols	*P*-value	N.s.	*	N.s.	N.s.	*	N.s.	N.s.	N.s.	**	14.62
	F-value	5.40	7.86	1.18	1.86	15.21	0.17	1.39	1.43	17.92	
ABTS	*P*-value	N.s.	*	N.s.	N.s.	N.s.	N.s.	N.s.	N.s.	**	20.29
	F-value	0.21	11.36	5.83	0.13	2.92	0.08	0.18	2.73	38.84	
DPPH	*P*-value	N.s.	N.s.	*	N.s.	*	N.s.	*	N.s.	**	15.87
	F-value	0.03	3.44	8.08	0.76	7.49	0.67	8.04	0.13	42.39	

Polynomial models	R^2	MAE
$Total\ phenolics = -5.65489 + 0.813067X_1 + 0.0306275X_2 + 0.348711X_3 - 0.0137333X_1^2 - 0.001135X_1X_2 - 0.0111X_1X_3 - 7.04083x10^{-5}X_2^2 + 3.24167x10^{-5}X_2X_3 - 0.00277481X_3^2$	0.955	0.028
$Flavonoids = -9.22957 + 1.72514X_1 + 0.0250407X_2 + 0.294425X_3 - 0.122073X_1^2 - 0.000873X_1X_2 - 0.00612333X_1X_3 - 5.81458x10^{-5}X_2^2 + 3.41667x10^{-6}X_2X_3 - 0.00223995X_3^2$	0.966	0.020
$Ortho\text{-}diphenols = -6.11287 + 0.761553X_1 + 0.0314875X_2 + 0.333559X_3 + 0.0873933X_1^2 - 0.00243X_1X_2 - 0.0231467X_1X_3 - 5.53042x10^{-5}X_2^2 + 6.075x10^{-5}X_2X_3 - 0.00217894X_3^2$	0.914	0.004
$ABTS = -0.0443363 + 0.00557333X_1 + 0.000265X_2 + 0.00320889X_3 - 0.000246667X_1^2 + 5x10^{-6}X_1X_2 - 8.0x10^{-5}X_1X_3 - 6.042167x10^{-7}X_2^2 - 3.33333x10^{-7}X_2X_3 - 2.53241x10^{-5}X_3^2$	0.924	< 0.001
$DPPH = -0.03529 - 0.00476333X_1 + 9.375x10^{-5}X_2 + 0.00404194X_3 + 0.00202667X_1^2 - 1.5x10^{-5}X_1X_2 - 0.000156667X_1X_3 - 1.58333x10^{-7}X_2^2 + 1.16667x10^{-6}X_2X_3 - 3.23148x10^{-5}X_3^2$	0.937	< 0.001

Skins

Variable	Statistics	X_1	X_2	X_3	$X_{1,2}$	$X_{1,3}$	$X_{2,3}$	X_1^2	X_2^2	X_3^2	Model *F*-value
Total phenolics	*P*-value	**	N.s.	**	N.s.	N.s.	N.s.	N.s.	N.s.	***	24.14
	F-value	18.72	5.53	33.90	1.41	2.32	0.10	6.21	3.07	121.16	
Flavonoids	*P*-value	N.s.	N.s.	*	N.s.	N.s.	N.s.	N.s.	N.s.	***	16.35
	F-value	3.91	3.04	13.75	0.95	4.88	0.07	1.46	0.77	56.00	
Ortho-diphenols	*P*-value	***	N.s.	*	N.s.	*	N.s.	**	N.s.	***	22.26
	F-value	49.87	4.23	8.48	0.64	8.81	0.29	18.30	4.79	95.25	
ABTS	*P*-value	N.s.	N.s.	**	N.s.	N.s.	N.s.	N.s.	N.s.	***	22.07
	F-value	1.77	4.97	22.80	0.05	0.72	0.10	2.42	3.56	93.12	
DPPH	*P*-value	**	N.s.	**	N.s.	N.s.	N.s.	**	N.s.	*	5.41
	F-value	18.38	0.50	17.26	0.00	4.81	0.00	25.78	1.50	8.56	

Polynomial models	R^2	MAE
$Total\ phenolics = 0.303012 - 2.73963X_1 - 0.034393X_2 + 1.00922X_3 + 0.281667X_1^2 + 0.003222X_1X_2 - 0.0137867X_1X_3 + 0.000123642X_2^2 - 7.18333x10^{-5}X_2X_3 - 0.00863676X_3^2$	0.976	0.066
$Flavonoids = -10.9509 + 2.26159X_1 - 0.024206X_2 + 0.712967X_3 - 0.135787X_1^2 + 0.002634X_1X_2 - 0.01988X_1X_3 + 6.16333x10^{-5}X_2^2 + 5.83333x10^{-5}X_2X_3 - 0.00584352X_3^2$	0.945	0.061
$Ortho\text{-}diphenols = 1.39726 - 3.43979X_1 - 0.033104X_2 + 0.872186X_3 + 0.422387X_1^2 + 0.001896X_1X_2 - 0.0234667X_1X_3 + 0.000135092X_2^2 - 0.000106333X_2X_3 - 0.00669231X_3^2$	0.976	0.006
$ABTS = -0.0103 - 0.0132683X_1 - 0.000369175X_2 + 0.00875419X_3 + 0.00180667X_1^2 + 6.2x10^{-6}X_1X_2 - 7.9x10^{-5}X_1X_3 + 1.36917x10^{-6}X_2^2 + 7.25x10^{-7}X_2X_3 - 7.77593x10^{-5}X_3^2$	0.964	< 0.001
$DPPH = 0.445207 - 0.156996X_1 - 0.000555275X_2 + 0.00304669X_3 + 0.0136127X_1^2 + 2.1x10^{-6}X_1X_2 + 0.000471\ X_1X_3 + 2.05292x10^{-6}X_2^2 + 1.91667x10^{-7}X_2X_3 - 5.44676x10^{-5}X_3^2$	0.940	< 0.001

[Z] N.s.: Not significant. Significant at $p<0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***). [Y] X_1: pH, X_2: Time (min), and X_3: Ethanol concentration (%). [X] MAE: Mean absolute error.

Table 6. Predicted values under optimum conditions based on individual response (total phenolics, flavonoids, *ortho*-diphenols, ABTS, and DPPH).

Matrix	Responses	Process variables			Predicted value
		X_1 pH	X_2 Time (min)	X_3 Ethanol concentration (%)	
Hulls	Total phenolics[Z]	6.5	249.0	90.0	155.33
	Flavonoids[Y]	5.7	50.0	89.9	126.87
	Ortho-diphenols[Z]	6.5	50.0	90.0	134.42
	ABTS[X]	3.3	250.0	90.0	1.53
	DPPH[X]	1.5	50.0	89.2	1.56
Shells	Total phenolics	1.5	219.0	61.1	8.86
	Flavonoids	4.9	180.0	59.1	5.99
	Ortho-diphenols	1.5	250.0	72.1	10.04
	ABTS	4.8	224.0	54.4	0.09
	DPPH	1.5	250.0	63.4	0.10
Skins	Total phenolics	1.5	250.0	56.2	24.43
	Flavonoids	6.5	250.0	51.2	15.41
	Ortho-diphenols	1.5	250.0	60.6	22.60
	ABTS	1.5	250.0	56.7	0.22
	DPPH	1.5	250.0	35.0	0.30

[Z] mg GAE/g dw. [Y] mg CE/g dw. [X] mmol TE/g dw.

Table 7. Predicted and obtained values under overall optimum conditions (total phenolics, flavonoids, *ortho*-diphenols, ABTS, and DPPH).

Matrix	Responses	Process variables			Predicted value	Observed value
		X_1 pH	X_2 Time (min)	X_3 Ethanol concentration (%)		
Hulls	Total phenolics[Z]	6.5	250.0	90.0	155.63	130.03
	Flavonoids[Y]				127.16	129.60
	Ortho-diphenols[Z]				123.16	111.96
	ABTS[X]				1.43	1.67
	DPPH[X]				1.23	1.28
Shells	Total phenolics	1.5	235.0	63.0	8.83	6.30
	Flavonoids				4.58	3.87
	Ortho-diphenols				9.80	5.87
	ABTS				0.08	0.04
	DPPH				0.10	0.05
Skins	Total phenolics	1.5	250.0	56.0	24.43	20.93
	Flavonoids				11.68	13.98
	Ortho-diphenols				22.47	20.49
	ABTS				0.22	0.24
	DPPH				0.27	0.33

[Z] mg GAE/g dw. [Y] mg CE/g dw. [X] mmol TE/g dw.

Table 8. UV-*Vis* features of the main polyphenolic phytochemicals detected in the optimally obtained almond by-products extracts.

Peak	Rt (min)	λ_{max}(nm)	Compound	Almond by-product		
				Hulls	Shells	Skins
1	20.03	326	3-caffeoylquinic acid	+	-	-
2	20.26	283	Naringenin-7-*O*-glucoside	+	-	-
3	23.67	345	Kaempferol-3-*O*-glucoside	-	-	+
4	23.83	358	Isorhamnetin-3-*O*-rutinoside	-	-	+
5	24.69	354	Isorhamnetin-3-*O*-glucoside	-	-	+
6	25.24	358	Isorhamnetin	-	-	+

Peak number and retention time according to Fig. 2

Figures caption:

Fig. 1 Average precipitation (mm) and temperature (°C) in the study year

Fig. 2 Chromatograms of almond by-products recorded at 360 nm. The identity of the compounds associated with the peaks shown here is given in Table 8