Food Chemistry 357 (2021) 129729

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Multi-response optimal hot pressurized liquid recovery of extractable polyphenols from leaves of maqui (*Aristotelia chilensis* [Mol.] Stuntz)

Pamela Raquel Rivera-Tovar [a], María Dolores Torres [b], Conrado Camilo [c], María Salomé Mariotti-Celis [d], Herminia Domínguez [b], José Ricardo Pérez-Correa [a,*]

[a] Chemical and Bioprocess Engineering Department, School of Engineering, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, P.O. Box 306, Santiago 7820436, Chile
[b] CINBIO, Department of Chemical Engineering, Faculty of Sciences, University of Vigo, Edificio Politécnico, As Lagoas s/n, 32004 Ourense, Spain
[c] Centro de Aromas y Sabores (DICTUC S.A.), Vicuña Mackenna 4860, P.O. Box 306, Santiago 7820436, Chile
[d] Universidad Finis Terrae, Santiago 7501015, Chile

ARTICLE INFO

Keywords:
Accelerated solvent extraction (ASE)
Extract's polyphenol purity
Polyphenol extraction yield
Response surface methodology (RSM)
Desirability function (DF)
Number of cycles

Chemical compounds:
Gallic acid (PubChem CID370)
Chlorogenic acid (PubChem CID1794427)
protocatechuic acid (PubChem CID72)
coumaric acid (PubChem CID637542)
cinnamic acid (PubChem CID444539)
resveratrol (PubChem CID445154)
hydroxytyrosol (PubChem CID82755)
catechin (PubChem CID9064)
quercetin (PubChem CID5280343)
kaempferol (PubChem CID5280863)

ABSTRACT

Multi-response optimization of hot pressurized liquid extraction (HPLE) was applied for the first time to obtain maqui (*Aristotelia chilensis* [Mol.] Stuntz) leaf extracts. The total polyphenol content (TPC), the antioxidant capacity (AC) as well as the total polyphenol purity of the maqui leaf extracts were accurately predicted (RSD < 8%) at the evaluated extraction scales. The optimum HPLE conditions that prioritized TPC and AC equally (OPT1) recovered ~3 times more TPC (205.14 mg GAE/g leaves) than maqui leaf extracts obtained by maceration, while the extract that prioritized purity over TPC and AC presented the highest purity (36.29%) and an EC_{50} ~3 times lower than currently reported values. It was found by multi-response optimization that maqui leaves and HPLE are among the best natural sources and extraction techniques, respectively, to recover protocatechuic acid, quercetin, and catechin.

1. Introduction

Maqui (*Aristotelia chilensis* [Mol.] Stuntz) is a native evergreen shrub that mainly grows in central and southern Chile. Its fruits have been used in food, pharmaceutical, nutraceutical, and cosmeceutical products due to their potent antioxidant capacity demonstrated in several *in vitro* and *in vivo* studies. The total polyphenol content (TPC) values reported from maqui fruit are higher than those of other berries such as blueberry, strawberry, cherry, blackberry, and raspberry; the antioxidant capacity (AC) of maqui, as measured with the oxygen radical absorbance capacity (ORAC) method, was also higher than the berries mentioned above. Moreover, berries present higher TPC and AC levels than other widely studied natural sources such as vegetables, pome fruits, citric fruits, and grapes (Rivera-Tovar, Mariotti-Celis, & Pérez-Correa, 2019). Maqui fruit extracts have shown bioactivities related to: i) prevention of atherosclerosis, ii) promotion of hair growth, iii) anti-photoaging of the skin, iv) inhibition of low-density lipoprotein oxidation (Avello et al., 2009; Rivera-Tovar et al., 2019; Zúñiga, Tapia, Arenas, Contreras, & Zúñiga-Libano, 2017), v) anti-hemolytic protection, vi) inhibition of α-glucosidase and α-amylase, vii) obesity control, viii) diabetes control and ix) cardioprotection. In addition, maqui extracts have shown anti-bacterial, anti-inflammatory, nematocidal, and antiviral activities (Rivera-Tovar et al., 2019; Zúñiga et al., 2017).

Maqui leaves, usually discarded in the agro-industrial production of fruits, could also be used to obtain bioactive ingredients since Rubilar et al. (2011) and Muñoz et al. (2011) showed that they have even higher

* Corresponding author.
E-mail address: perez@ing.puc.cl (J.R. Pérez-Correa).

https://doi.org/10.1016/j.foodchem.2021.129729
Received 7 October 2020; Received in revised form 23 March 2021; Accepted 26 March 2021
Available online 29 March 2021

total polyphenol content and antioxidant capacity than maqui berries (Rivera-Tovar et al., 2019). Aqueous, ethanolic, and methanolic leaf extracts have anti-inflammatory and analgesic properties (Muñoz et al., 2011) and the potential to control diabetes by inhibiting α-glucosidase and α-amylase (Rubilar et al., 2011). Several polyphenols have been identified in maqui leaves: two phenolic acids (gallic acid and coumaric acid), four flavonols (quercetin, isoquercetin, myricetin, and rutin), two anthocyanins (pelargonidin and peonidin), one flavanol (catechin), and one stilbene (resveratrol) (Vidal et al., 2013). Maqui leaves contain indole and quinoline alkaloids (aristoteline, serratoline, aristone, horbatine, horbatinol, protopine, aristoquinoline, 3-fromylindole), as well as minerals, such as calcium, phosphorus, iron, and potassium (Zúñiga et al., 2017). Despite these appealing properties, no previous research has focused on optimizing the polyphenol's extraction process from maqui leaves, and few studies discussed their possible applications.

Rubilar et al. (2011) extracted maqui leaves by maceration with 50% water/ethanol at room temperature and a solvent-to-solid ratio of 5:1. The TPC of the obtained extracts was 69.0 ± 0.9 mg GAE/g dry weight, and the antiradical scavenging capacity against DPPH showed an IC_{50} = 8.0 ± 0.1 mg of extract/L. Hot pressurized liquids extraction (HPLE), also known as accelerated solvent extraction (ASE), can yield extracts with higher polyphenol content in less time and using lower amounts of solvent (Mustafa & Turner, 2011). This green extraction technique is efficient for the extraction of plant bioactives, where extraction temperature, solvent composition, and the number of cycles are the factors that have the most influence on TPC, AC, and the polyphenolic profile of the extracts (Díaz-de-Cerio et al., 2018; Putnik et al., 2017; Tripodo, Ibáñez, Cifuentes, Gilbert-López, & Fanali, 2018).

This work hypothesizes that by applying multi-response optimization to HPLE, it is possible to find operating conditions that yield several polyphenol extracts of maqui leaves with outstanding features. The optimization of the process was focused on maximizing the polyphenols' extraction yield. Three optimization objectives associated with the extracts obtained in an ASE 200 device (5 mL extraction cell) were considered: i) total polyphenol content (TPC) according to the Folin-Ciocalteu method; ii) antioxidant capacity (AC) as measured by ABTS radical scavenging activity assay; and iii) purity (g of gallic acid equivalent/100 g dry extract, %). Additional extractions were carried out under optimal conditions in an ASE 150 device (100 mL extraction cell). These extracts were evaluated in terms of total polyphenol content of maqui leaves, the extract's purity, *in vitro* antioxidant capacity (DPPH and ORAC based on the dry mass of leaves and extracts), and the low molecular weight polyphenol profile.

2. Materials and methods

2.1. Plant materials

Adult maqui (*Aristotelia chilensis*) leaves (four-year-old) were obtained from the Región de La Araucanía, Chile on May 18–20, 2016. They were air-dried at 5–20 °C and relative humidity of 83% for seven days, ground in a meat mincer, and stored in sealed plastic bags in a dry, dark place at −18 °C before use. The main properties of the raw material were obtained using standard analytical procedures, among them: moisture (10.54 ± 0.09%), protein (15.15 ± 0.18 g/100 g leaves), and ash (0.07 ± 0.00 g/100 g leaves). Mineral content was measured after microwave-assisted acid digestion with nitric acid at 1600 W, 15 min, and 200 °C for 10 min (Marsxpress-CEM Corporation, USA). Sodium and potassium were determined by atomic emission spectrophotometry. Calcium, copper, magnesium, cadmium, iron, and zinc were determined by atomic absorption spectrophotometry in a 220 Fast Sequential Spectrophotometer (Varian, USA). The mineral content was potassium (10.50 ± 0.38 mg/g), calcium (21.23 ± 0.30 mg/g), magnesium (1.98 ± 0.01 mg/g), sodium (20.14 ± 0.99 mg/kg), iron (237.93 ± 4.46 mg/kg), zinc (12.33 ± 0.14 mg/kg), copper (<7.00 mg/kg), cadmium (<5.00 mg/kg) and lead (<10.00 mg/kg).

2.2. Solvents and standards

Ethanol 96% (reagent grade Solvents, Scharlau) was used as a co-solvent to prepare the ethanol/water solvent mixtures (5, 15, 20, 25, 50, and 80% v/v). Methanol and acetone (≥99.9%) (HPLC, Sigma Aldrich) were used to prepare aqueous solvents for successive extraction at ambient conditions (20 °C, 1 atm). For the analytical determinations, the following chemical reagents and standards were used: Folin-Ciocalteu reagent (D ≈ 1.24 g/mL); 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) or ABTS (>98%, Aldrich Chemistry); 2,2-diphenyl-1-picrylhydrazyl or DPPH (Sigma Aldrich); 2,2′–azobis(2-methylpropionamidine) dihydrochloride or AAPH (97%, Sigma Aldrich); sodium carbonate; sodium chloride; potassium chloride; potassium dihydrogen phosphate; disodium phosphate; potassium persulfate; dipotassium phosphate; fluorescein sodium salt; (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid or Trolox reagent (97%, Aldrich Chemistry), gallic acid monohydrate (≥99%, Sigma Aldrich), quercetin-3-β-D glucoside (≥90%, Sigma Life Science), hydroxytyrosol (≥98%, Sigma Aldrich), oleuropein (≥98%, Sigma Aldrich). Catechin (≥98%), epigallocatechin (≥98%), epicatechin (≥98%), kaempferol (≥98%), resveratrol (≥98%), quercetin (≥97%), caffeic acid (≥99%), chlorogenic acid (≥98%), vanillic acid (≥99%), protocatechuic acid (≥99%) and ferulic acid (≥98%). These were purchased from Xi'an Haoxuan Bio-Tech Co., Ltd. (Baqiao, China). All solutions were prepared and stored cold and in the dark.

2.3. Aqueous-organic successive extraction (SE) at ambient conditions

Successive extractions of maqui leaves with aqueous methanol and aqueous acetone were performed according to Pérez-Jiménez et al. (2008) to obtain reference crude extracts (RCE). Samples of 0.5 g were placed in contact with 20 mL of an acidified (0.8% HCl 2 N) methanol/water solution (50% v/v, pH 2) and vigorously shaken for 1 h; the mixture was then centrifuged at 6000 rpm for 10 min. Subsequently, 20 mL of an acetone/water solution (70% v/v) was added to the remaining solids, and the mixture was then stirred and centrifuged. The methanolic and acetonic extracts were combined to determine the total polyphenol content of the mixture. It can be assumed that the combined extract (RCE1) contains close to 100% of the extractable polyphenols since the natural matrix first comes into contact with a polar and acidified solvent and then with a more non-polar solvent. Additionally, simple individual extractions with both solvents were performed, which were used as reference extracts (RCE2, RCE3) to identify and quantify polyphenols.

2.4. Hot pressurized liquid extraction (HPLE) method

Extraction was performed in an Accelerated Solvent Extraction System ASE 200 (Dionex Corporation, Sunnyvale, CA, USA) using water/ethanol solutions as a solvent. Samples of dried leaves (1 g, dry weight) were mixed with 0.75 g of diatomaceous earth and placed into a 5 mL volume extraction cell. The fixed operating conditions were: 5 min static extraction time, 70% of flush volume, 1 min of purge, and 102.1 atm (1500 psi). The main factors influencing the measured responses (Xynos et al., 2014) were varied within a predefined range: temperature (80 – 200 °C), ethanol concentration (5% – 80% v/v), and the number of cycles (1–5). The obtained extracts were protected from light and stored at −20 °C until analysis. Additionally, extractions at the optimum conditions with the same solid-to-extract ratio (~1:45) were replicated in an ASE 150 (Dionex Corporation, Sunnyvale, CA, USA). AC with two other methods (DPPH and ORAC) and the low molecular weight polyphenol profile were determined to complete the characterization. A sample of 2 g (dry weight) was mixed with 1.8 g of neutral quartz sand (instead of diatomaceous earth) and placed into a 100 mL volume extraction cell previously filled with the sand to reduce the volume of the water/ethanol solution used for the extraction.

2.5. Experimental design and optimization

TPC and AC of a natural extract can be used to assess process yields (expressed in terms of mg per g of dry weight of the natural matrix) or to chemically characterize the extract obtained (expressed in terms of g per g of dry extract). Both global responses are complementary, providing a better description of a given natural matrix's antioxidant properties. Typically, the extraction process optimization goal is to maximize TPC and AC considering the process yield because the extracts are purified in subsequent steps (e.g., microporous resin purification). Consequently, we assessed the impact of the studied factors (temperature (x_1), ethanol concentration (x_2), and the number of cycles (x_3)) on TPC (measured in mg GAE/g maqui leaves) and AC_{ABTS} (measured in mg TE/g maqui leaves). All experiments were carried out in a randomized order to minimize the effect of extraneous factors on measured responses.

Optimization was carried out in three sequential steps, each of them defining a specific experimental region (Fig. 1). In the first two steps, the Box-Behnken experimental design (BBD) was applied to define the experimental points. The third step included a selection of the experimental points of the two previous steps. The response surface methodology (RSM) (Myers, Khuri, & Carter, 1989) was applied in each experimental region defined at each optimization step. The levels of the factors of the first optimization step were defined based on previous research that applied HPLE to different natural matrixes such as olive leaves (Putnik et al., 2017; Xynos et al., 2014), goji berry (Tripodo et al., 2018) and myrtle leaves (Díaz-de-Cerio et al., 2018). The second optimization step's levels resulted from moving the first experimental region towards the steepest ascent direction, although considering ASE 200 device constraints. The final step considered a selection of the experimental points of the two previous regions, discarding outliers. The latter were identified by looking at the residuals vs. order plots and applying the Minitab® Statistical Software v.19 criteria (standardized residuals with absolute values greater than two). Five goodness of fit statistics were calculated for the fitted models, with and without outliers, to determine their deleterious effect on the model performances. The standard error of the regression (S), in response variable units, represents the deviation of the measurements from the modeled response. The lower the value of S, the better the model;

$$S = \sqrt{\frac{\sum \left(y_i - \hat{y}_i\right)^2}{n - p - 1}} \tag{1}$$

y_i is the i^{th} value of the observed response, $\hat{y}_i$ is the i^{th} adjusted response, n is the number of observations, and p is the number of terms in the model.

The determination coefficient (R^2) varies between 0 and 1; the higher the value of R^2, the better the model fit the experimental values (calibration set). R^2 is most useful when comparing models of the same size because its value increases when additional predictors are added to the model, even when there is no real improvement in the model's fit,

$$R^2 = 1 - \frac{\sum \left(y_i - \hat{y}_i\right)^2}{\sum \left(y_i - \bar{y}\right)^2} \tag{2}$$

$\bar{y}$ is the mean response.

The adjusted R-squared (R^2_{adj}) is a modified version of the R^2 that incorporates the number of predictors in the model. The R^2_{adj} increases only if the new term improves the model more than would be expected by chance,

$$R^2_{adj} = 1 - \left[\frac{\sum \left(y_i - \hat{y}_i\right)^2}{\sum \left(y_i - \bar{y}\right)^2}\right]\left(\frac{n - 1}{n - p - 1}\right) \tag{3}$$

The predicted R-squared (R^2_{pred}) is a form of leave-one-out cross-validation that is calculated by systematically removing one observation from the original data set and then estimating the regression equation and determining how well the model predicts the removed observation. Models with an R^2_{pred} value substantially smaller than the corresponding R^2 value may indicate that the model is over-fitted.

$$R^2_{pred} = 1 - \frac{\sum \left(y_i - \hat{y}_{(i)}\right)^2}{\sum \left(y_i - \bar{y}\right)^2} \tag{4}$$

$\hat{y}_{(i)}$ represents the modeled response of the omitted observations.

Akaike's information criterion (AIC) describes the relationship between the accuracy and complexity of the model. If the number of parameters of a model increases, the model gains complexity, but at the same time, the mismatch between model and observations decreases. Therefore, the model with the lowest AIC value is expected to achieve a higher balance between reducing complexity (parsimony principle) and maintaining a minimum mismatch value,

$$AIC = n\ln\sigma^2 + 2(p + 1) \tag{5}$$

σ^2 is the standard error between the model and the experimental values.

The responses of each design were initially adjusted to first-order models (plane) [Eq. 6] and when a curvature was detected, a second-order model [Eq. 7] was fitted, which was expressed as a function of linear, interaction, and second-order terms.

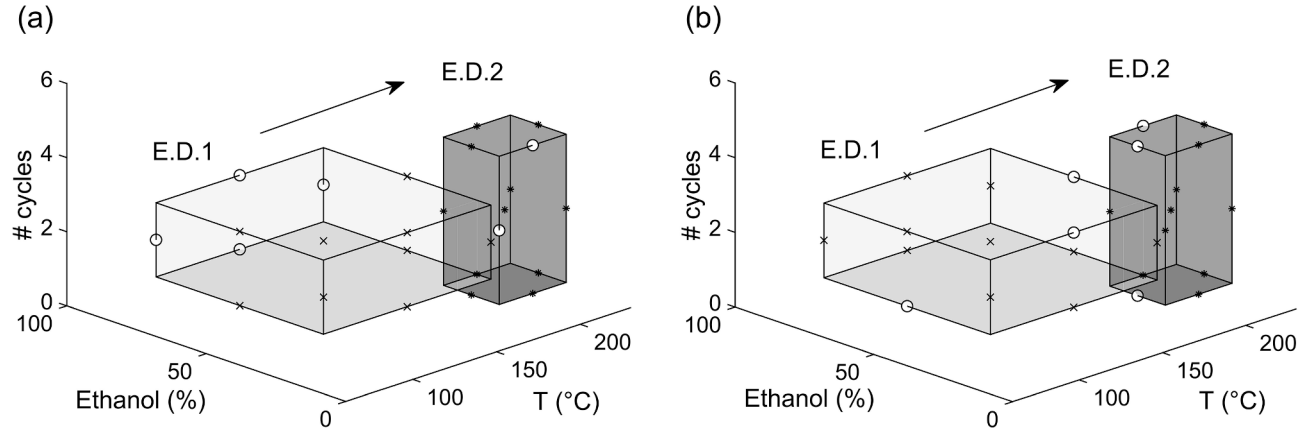

Fig. 1. Graphical representation of the two preliminary experimental designs (BBD) and outliers (round markers in white) not considered on the final experimental region for (a) TPC (left) and (b) AC_{ABTS} (right).

$$y_i = \beta_0 + \beta_1 x_1(^{\circ}C) + \beta_2 x_2(\%) + \beta_3 x_3 \quad (6)$$

$$y_i = \beta_0 + \beta_1 x_1(^{\circ}C) + \beta_2 x_2(\%) + \beta_3 x_3 + \beta_4 x_1^2(^{\circ}C)^2 + \beta_5 x_2^2(\%)^2 + \beta_6 x_3^2 + \beta_7 x_1 x_2(^{\circ}C)(\%) + \beta_8 x_2 x_3(\%) + \beta_9 x_1 x_3(^{\circ}C) \quad (7)$$

y_i are the dependent variables (simple or multi-response), β_i are the regression coefficients (fitted from experimental data), and x_i are the studied factors.

Pareto charts of the effects were used to determine which terms (effect) contribute the most to the response's variability. The p-value effect was compared with the significance level ($\alpha = 0.05$) to determine whether the association between the response and each term in the model was statistically significant. Where $p \leq \alpha$ indicates that the association is statistically significant. These procedures were performed with the Minitab® Statistical Software v.19.

The objective of the first two experimental designs was to maximize TPC and AC_{ABTS} independently. A multi-response optimization problem was formulated for the final design, where TPC, AC_{ABTS}, and extract purity (P) were simultaneously maximized. Purity was defined as the ratio between the total polyphenol content and the total soluble compounds content (SCC) in the extract, expressed as a percentage [Eq. 8]. SCC is affected by temperature, ethanol concentration, and the number of cycles; therefore, including purity in the optimization allowed determining the conditions for the most selective extraction.

$$P(\%) = \frac{\text{grams gallic acid equivalent}}{\text{grams dry extracts(soluble compounds)}} 100 \quad (8)$$

where the grams of dry extracts were obtained by drying the liquid extracts (1 g) in an oven at 105 °C until constant weight (24–72 h).

Multi-response optimization was performed using the desirability function (DF) technique (Derringer & Suich, 1980). The method consists of converting each of the estimated response variables $\hat{y}_i(x)$ into desirable values $d_i(x)$ that can vary between 0 (the response value is "undesirable") and 1 ("completely desirable or ideal" response). The individual desirabilities of each estimated response are then combined using the geometric mean to obtain an overall or composite desirability [Eq. 9].

$$D(x_1, x_2, x_3) = \left(d_{TPC}^{w_{TPC}} \cdot d_{AC_{ABTS}}^{w_{AC_{ABTS}}} \cdot d_P^{w_P}\right)^{1/\left(w_{TPC} + w_{AC_{ABTS}} + w_P\right)} \quad (9)$$

w_i values vary between 0.1 and 10, which are arbitrarily assigned to define the priority of each response variable.

The transformation function for maximization is given by

$$d_i(x_1, x_2, x_3) = \begin{cases} 0 & \hat{y}_i(x_1, x_2, x_3) \leq L_i \\ \left[\dfrac{\hat{y}_i(x_1, x_2, x_3) - L_i}{T_i - L_i}\right]^s & L_i < \hat{y}_i(x_1, x_2, x_3) \langle T_i \\ 1 & \hat{y}_i(x_1, x_2, x_3) \geq T_i \end{cases}$$

L_i, an unacceptable value, is the lower specification bound, and T_i is a target value (a large enough response). Minitab software sets the lower bound and the target value to the minimum value and maximum value of the data, respectively. The exponent s defines how the desirability is distributed on the interval between the lower bound and the target value. The distribution can be convex ($s < 1$) whereby any response that falls within the $L_i - T_i$ interval is highly desirable, concave ($s > 1$) where only responses that fall close to the T_i value take high desirability values, or linear ($s = 1$) where the desirability increases linearly towards the T_i value. This last distribution was used in this study as it represents a neutral configuration that gives equal importance to the T_i and L_i values.

Three composite desirability functions were defined. OPT1 includes the maximization of TPC and AC_{ABTS} (equal priority was assigned to both responses), whereas OPT2 and OPT3 add P as a third response, but with different priorities. OPT2 assigned equal priority to the three objectives, while OPT3 assigned higher priority to P.

2.6. Determination of responses in the extracts

2.6.1. Total polyphenol content (TPC)

The total polyphenol content was spectrophotometrically determined by the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). Aliquots of samples (250 μL) were mixed with Folin–Ciocalteu reagent (125 μL), 10% w/v aqueous sodium carbonate solution Na_2CO_3 (250 μL) and 1875 μL of distilled water, shaken and allowed to react for 1 h at room temperature (20 °C) in darkness; then the absorbance was measured, reading at 765 nm. TPC was calculated from a calibration curve using gallic acid (0.1 g/L maximum concentration), so the results were expressed as mg of gallic acid equivalents (GAE) per g of maqui leaves, dry weight.

2.6.2. Radical scavenging activity: ABTS at a fixed time (AC_{ABTS})

We applied the procedure described in Re et al. (1999). Radical cation $ABTS^{\bullet+}$ was produced by reacting a 7 mM ABTS solution with potassium persulfate (final concentration 2.45 mM). The $ABTS^{\bullet+}$ solution was diluted with phosphate buffer saline (PBS) (pH 7.4) to an absorbance of 0.70 at 734 nm and equilibrated at 30 °C. Then 1 mL of this diluted solution was added to 10 μL of extract or Trolox, and the absorbance was read after 6 min. The percentage inhibition of absorbance was referred to the concentration of extracts and Trolox. AC_{ABTS} was calculated from a calibration curve using Trolox (2.4 mM maximum concentration) and expressed as mg of Trolox equivalents (TE) per g of maqui leaves, dry weight.

2.7. Statistical analysis

All the extractions and chemical analyses were performed in triplicate. The experimental results obtained were expressed as means ± SD. Statistical analysis was carried out using Minitab® Statistical Software v.19. Analysis of variance (ANOVA) at 95% confidence level ($p < 0.05$) was applied to compare all the obtained responses.

2.8. Additional analytical determinations in the optimal extracts (ASE 150 extracts only)

2.8.1. DPPH radical-scavenging activity

The samples' ability to capture free radicals was measured with the DPPH method (Moure, Domínguez, & Parajó, 2005). 50 μL of extract dilutions or absolute methanol (as control) was added to 2 mL of methanolic DPPH (0.06 mM). Soon after vortexing the reaction mixture for 1 min, the tubes were placed in the dark for 16 min, and absorbance was measured at 515 nm. The EC_{50} parameter, which reflects the quantity of antioxidant needed to reduce the initial DPPH concentration by 50%, has been expressed as g maqui leaves in dry weight/g DPPH. This way of expressing DPPH values better reflects the antioxidant capacity because it includes the concentration of the DPPH methanol solution and the sample concentration, which usually vary in each study.

2.8.2. ORAC

The ORAC assay was performed as described in Brescia (2012) with some modifications; 250 mL of phosphate buffer PBS pH 7.4 (75 nM) was prepared with 25 mL K_2HPO_4 solution (581.17 mM) and 25 mL KH_2PO_4 solution (168.72 mM) and distilled water. Briefly, 0.2034 g of AAPH was dissolved in 10 mL of PBS to the final concentration of 75 mM and made fresh daily. A fluorescein stock solution (1 mM) was made in PBS and stored in the dark at 4 °C. The stock solution was diluted sequentially, 1:500 and then 1:250, with PBS. The sample (25 μL), either of Trolox solution or PBS (blank), were added and mixed with 150 μL of sodium fluorescein to a 96-well plate, and they were incubated for 30 min at 37 °C. The reaction was initiated by the addition of 25 μL of AAPH solution. The fluorescence was measured every minute using the

Synergy HTX Operators (BioTek Instruments, Inc.). Excitation was performed at 485/20 nm, and emission was measured at 528/20 nm. The reference calibration curve was performed with Trolox solutions between 4 and 48 μM. The results were expressed as mg of Trolox equivalents (TE) per g of maqui leaves, dry weight.

2.8.3. Identification and quantification of polyphenols

Eighteen polyphenols (8 phenolic acids, 3 flavanols, 3 flavonols, and 4 other polyphenols) were identified and quantified by applying the procedure described in Huaman-Castilla et al. (2019); 5 μL of extract diluted with distilled water (1:10) and filtrated (0.22 mm membrane) was injected (in triplicate) into an ultra-performance liquid chromatography-mass spectrometry (UPLC–MS, Dionex Ultimate 3000 with Detector MS Orbitrap Exactive plus, Thermofisher, Massachusetts, USA) equipped with a reverse-phase Acquity UPLC BEH C18 column (1.7 μm × 2.1 × 100 mm). Gradient elution was conducted at 35 °C with acetonitrile/0.1% formic acid (mobile phase A) and water/0.1% formic acid (mobile phase B) at a constant flow rate of 0.2 mL/min. The gradient elution steps were as follows: the first 6 min, 80% A – 20% B; the next 18 min, 15% A – 85% B; and finally, the last 30 min, 80% A – 20% B. Polyphenol contents were calculated from calibration curves using standards for each compound, and the results were expressed as mg of specific polyphenols per g of maqui leaves (dry weight). Table 1S shows the linearity range, the regression equation, the determination coefficient, the limit of detection (LOD), and the limit of quantification (LOQ) for each standard calibration curve.

3. Results and discussion

3.1. Modelling extraction results of exploratory experimental designs

The TPC (Y_1) and AC_{ABTS} (Y_2) responses of each exploratory experimental design (Table 2S) were fitted to first or second-order models according to the presence or absence of curvature (see Table 3S). The TPC values of both experimental designs (E.D.1 and E.D.2) fit well to linear models, where the three main effects (β_1, β_2, and β_3) were statistically significant (Table 4S). The AC_{ABTS} values of the E.D.1 fit well to a linear model, where the temperature effect (β_1) was the only statistically significant. On the other hand, a second-order model fit well to the AC_{ABTS} values in the E.D.2, where two linear effects (β_2 and β_3), one quadratic effect (β_6) and one interaction effect (β_8) were statistically significant (Table 5S). The determination coefficients, R^2, of these four fitted surfaces ranged between 0.817 and 0.971. The significant effects were identified by analyzing their Pareto charts and their p-values at a significance level of $\alpha = 0.05$.

Fig. 2 shows the relationships between TPC and AC_{ABTS} responses with the three factors studied in E.D.1 and E.D.2. Temperature is the most important factor (steepest slope) and is directly proportional (positive slope) to the two responses for both exploratory regions, except for AC_{ABTS} in E.D.2, where this effect presents a slight curvature. Ethanol concentration is the most important factor affecting AC_{ABTS} in the E.D.2 region. Both responses are inversely proportional to ethanol in E.D.1 and directly proportional in E.D.2. The number of cycles shows a potent effect in TPC and ACABTS in the E.D.2 region. TPC is directly proportional to the number of cycles in both regions, while AC_{ABTS} is directly proportional in E.D.1 and presents a strong curvature in E.D.2.

According to the previous analysis, several changes in the trend of the two original regions' responses were detected. Hence, we decided to define a new experimental region using both exploratory regions. When all 30 observations of the original regions were considered, fitted models with unusual points (outliers) for the TPC and AC_{ABTS} responses were obtained. Therefore, a sequential elimination of outliers was applied until reliable TPC and AC_{ABTS} models were obtained. The goodness of fit statistics calculated with the 30 original points and with the outliers removed are shown in Table 6S. The elimination of 8 outliers for each response yielded second-order models that considerably improved all the statistical indexes considered. These second-order models were used in the optimization. The outliers removed did not present optimal responses, and most of them were in the descent zone of the response surfaces, away from the optimal extraction conditions. In addition, some repetitions of the central points of the experimental designs were identified as outliers (Table 2S).

3.2. Modelling extraction results of the final experimental design

The second-order models fit well to TPC and AC_{ABTS} values of the final experimental design (Table 7S), where the three factors (temperature, ethanol concentration, and the number of cycles) showed significant influence on the variation of both responses. Temperature (β_1) was the most statistically significant effect on TPC, followed by the quadratic effect of the ethanol concentration (β_5) and the number of cycles (β_3). AC_{ABTS} was significantly influenced by 7 effects (in decreasing order of statistical significance): temperature (β_1), the quadratic effect of the number of cycles (β_6), the quadratic effect of ethanol concentration (β_5), the interaction effect of temperature-number of cycles (β_9), the number of cycles (β_3), the interaction effect of temperature-ethanol concentration (β_7) and quadratic effect of temperature (β_4) (Table 8S).

Purity, the additional response considered in the final design, was

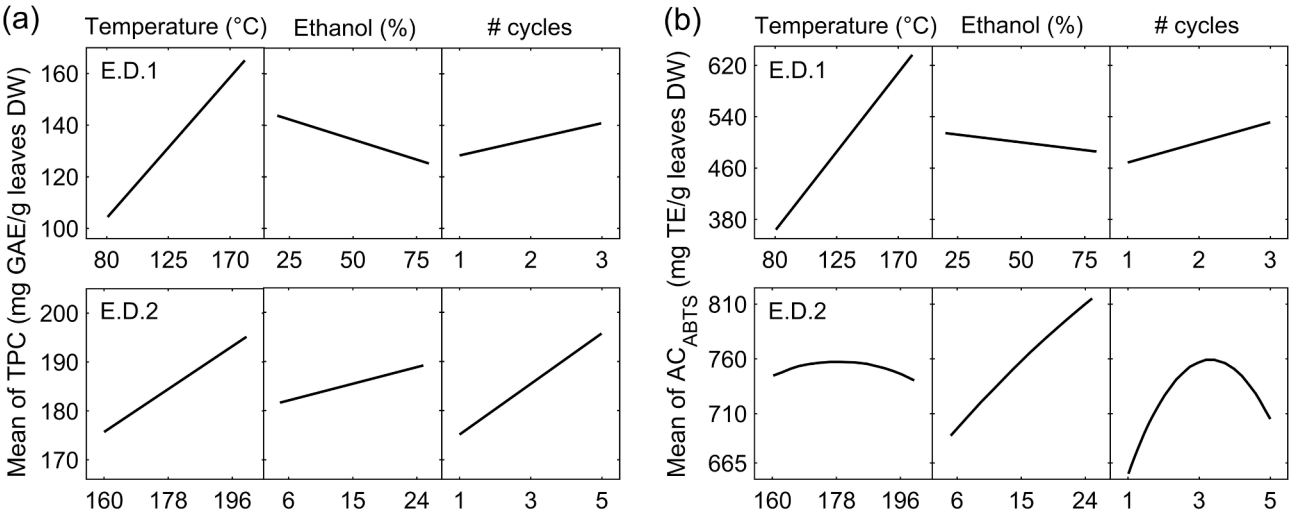

Fig. 2. Main effects plot for (a) TPC and (b) AC_{ABTS} in regions E.D.1 (top row) and E.D.2 (bottom row). DW means dry weight.

only affected by two factors: temperature and ethanol concentration. A second-order model fit well with these data ($S = 1.16$, $R^2 = 0.943$, $R^2_{adj} = 0.904$, $R^2_{pred} = 0.845$), where four effects were relevant (in order of statistical significance): temperature (β_1), the quadratic effect of ethanol concentration (β_5), ethanol concentration (β_2) and the quadratic effect of temperature (β_4) (Table 8S).

Response surface plots for the models are displayed in Fig. 3. Individual maximum values of TPC (208.94 mg GAE/g dry leaves) and AC_{ABTS} (818.02 mg TE/g dry leaves) were achieved at the maximum temperature of the experimental region (200 °C), 20%-26% ethanol and 3–5 cycles. Instead, maximum P (40.07%) was obtained at the lowest temperature (80 °C), 80% ethanol, and the minimum number of cycles (one extraction).

Like in previous HPLE optimization studies, with Croatian olive leaves and Goji berry, we found that temperature is the most significant factor affecting TPC and AC values. High temperatures enhance both mass transfer and polyphenols solubility, as well as reduce solvent viscosity (Putnik et al., 2017; Tripodo et al., 2018). The impact of ethanol concentration is difficult to generalize. Some authors found optimum performance (higher yield of mg GAE/g dry natural matrix) at high ethanol concentrations (71% v/v for myrtle leaves (Díaz-de-Cerio et al., 2018), 86% v/v for Goji berry (Tripodo et al., 2018)), while others at low concentrations (35% v/v for *Moringa oleifera* leaves (Rodríguez-Pérez et al., 2016)). This dissimilar behavior could be attributed to the particular matrix phenolic composition since each polyphenol family responds differently to ethanol as a co-solvent (Huaman-Castilla et al., 2019). It has been reported that the number of cycles tends to increase the extraction efficiency. Despite recovering 87% of the polyphenols in the first cycle of HPLE of black cohosh, the second and third cycles extracted 9% and 4.2%, respectively (Mukhopadhyay, Luthria, & Robbins, 2006). In the optimizations of HPLE of olive leaves, the highest polyphenol content was reached by extracting with 2 and 3 cycles (Putnik et al., 2017; Xynos et al., 2014).

Purity showed a different behavior compared to the other two responses (Fig. 3). The extracts with the highest TPC values reached low P values; this suggests that under these conditions, the soluble compounds content (SCC) increased mainly due to the extraction of non-phenolic compounds (those not reducing the Folin Ciocalteu reagent). Hydroxymethylfurfural (an unwanted compound), generated by the Maillard reaction, was reported as non-interfering in the determination of TPC by the F-C assay (Bastola, Guragain, Bhadriraju, & Vadlani, 2017) and was identified in extracts obtained at temperatures above 130 °C (from *Carménère* grape pomace) (Mariotti-Celis et al., 2018). Similarly, ethanol-soluble compounds such as alkaloids and glucose (identified in maqui leaves (Zúñiga et al., 2017)) were reported as non-interfering in the determination of TPC (Bastola et al., 2017). On the other hand, the extracted non-phenolic compounds (purity determinants) have a particular behavior for the studied factors, which generates differences

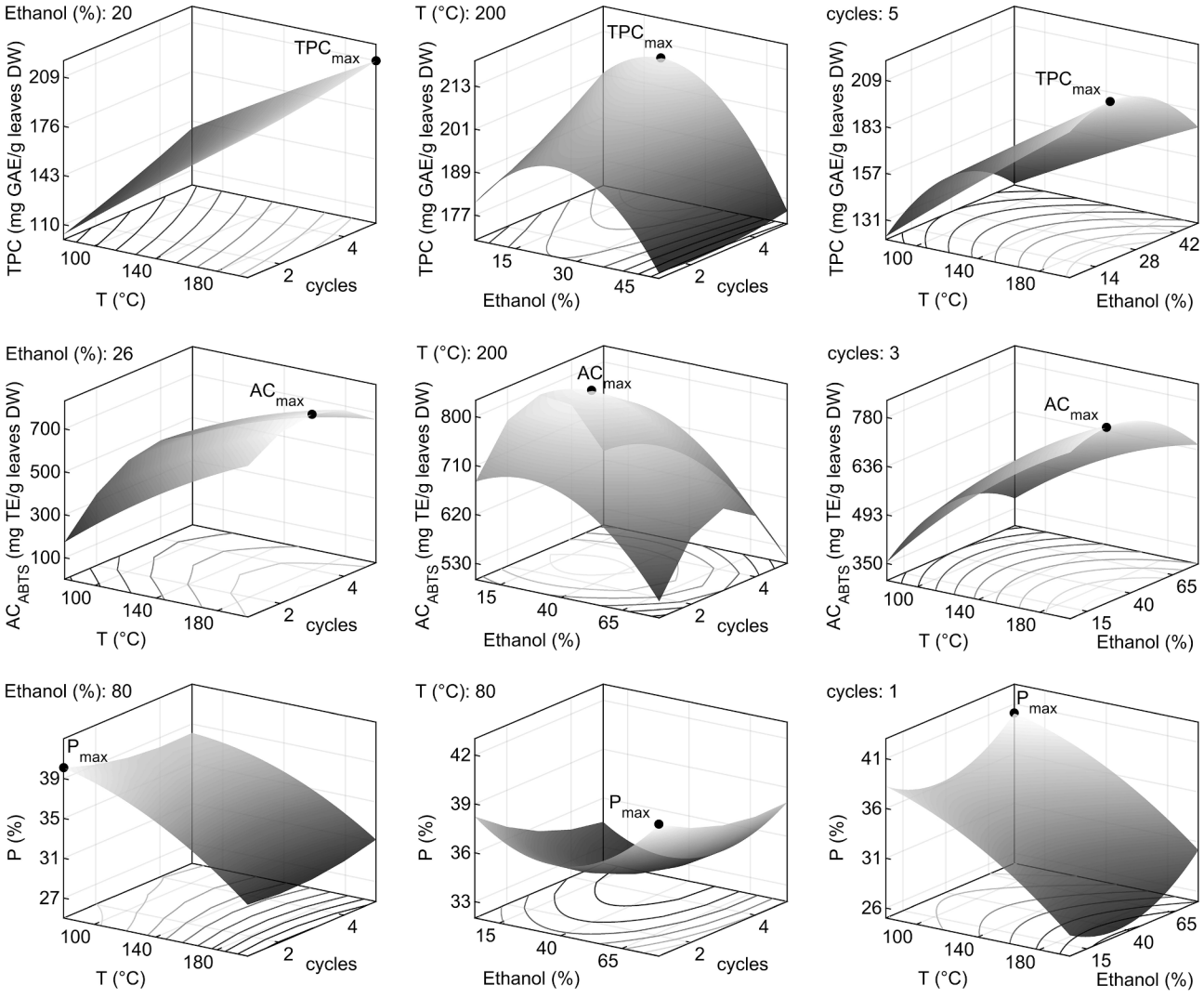

Fig. 3. Response surface plots of the total polyphenol content (first row), antioxidant capacity measured by ABTS method (second row) and extract purity (third row) as a function of the studied factors. Where DW is dry weight.

in their respective TPC and P ratios.

3.3. Obtaining and evaluating optimal extracts

3.3.1. Multi-response optimization and model validation

Three composite desirability functions were applied to identify the combination of factor values that maximizes the set of responses, assigning different priorities to each response (w in Eq. 9) according to three scenarios (Table 1). Scenario 1 (OPT1) maximized TPC and AC_{ABTS} with equal priority, but neglected purity P. In this case, the composite desirability (0.952) was close to 1, the ideal case, indicating an outstanding overall performance; since purity was not considered in the optimization, it presented a low value (24.91%). The second (OPT2) and third (OPT3) scenarios included the maximization of P but with different priorities. According to Harrington's rating system, these two scenarios showed low composite desirabilities, indicating that they achieved an acceptable but poor overall optimization. This result was expected as TPC and AC_{ABTS} objectives conflict with the purity objective. OPT3 (higher priority to P) seems to be a better option than OPT2 (all responses with the same priority) since it showed higher composite desirability than OPT2.

Extractions at the 3 optimum conditions were performed (in triplicate) on an ASE 200 device to verify them experimentally and assess the predictive ability of the fitting models. The optimal conditions of the first and second scenarios were slightly changed, as shown in Table 2, since the ASE 200 device operates in conjunction with a Solvent Controller product that mixes solvents in a range of 5 to 100%, in 5% increments for the ethanol concentration. The relative standard deviations (RSD) between the estimated and experimental responses were low (<8%), which means that the experimental values were in good agreement with those estimated theoretically. Additionally, extractions were performed under the original optimal conditions (Table 1) in the ASE 150 equipment, which has no constraints on ethanol concentration. The second-order models obtained with ASE 200 were accurate to predict the experimental responses obtained in ASE 150 extractions since the experimental values of TPC and P were in good agreement with those estimated (RSD < 6%) (Table 2).

3.3.2. Characterization of optimal extracts in terms of TPC and AC

As expected, successive extraction (SE) with acidified methanol/water (50% v/v, pH 2) and acetone/water (70% v/v) yielded an extract (RCE1) with the highest TPC values (264.53 ± 8.21 mg GAE/g maqui leaves). Therefore, SE can be considered a reference extraction method that achieved complete recovery of extractable polyphenols (Pérez-Jiménez et al., 2008).

OPT1 conditions achieved the highest TPC, whereas OPT3 conditions reached the highest purity; OPT2 conditions were in a middle ground (ASE 150, Table 2). OPT1 achieved a high recovery of TPC compared to RCE1 (78%). OPT3 extract presented a lower polyphenol extraction yield than RCE1 (44%) but a significantly higher purity (35%) than the OPT1 extract. The intermediate extract (OPT2) achieved 65% of the TPC of RCE1 and 22% more purity than the OPT1 extract. The three optimal extracts yielded higher TPC values (ASE 150, Table 2) than the hydroalcoholic extracts (50% of ethanol) of maqui leaves obtained by maceration in a previous study (Rubilar et al., 2011), which reached only 26% of the TPC of RCE1. At the same time, the best extract (OPT1 extract) presented a TPC ~6 times higher than the average TPC of those reported for maqui fruit (35.14 ± 1.30 mg GAE/g maqui fruit) (Rivera-Tovar et al., 2019).

The three optimum HPLE extractions reached yield values (g dry extract/100 g dry leaves, %) of 72%, 52%, and 34%, respectively. Similar yields were reported in previous work with HPLE of other natural matrices: 75% (Goji berry, 50% ethanol – 180 °C), 56% (*Moringa leaves*, 35% ethanol – 128 °C) and 54% (olive leaves, 50% ethanol – 190 °C) (Tripodo et al., 2018; Rodríguez-Pérez et al., 2016; Xynos et al., 2014, respectively).

The AC of maqui leaves in the optimum extracts were determined by three methods that measure a sample's free radical scavenging capacity (ABTS, DPPH, and ORAC) to provide comprehensive information that considers the different mechanisms of actions of the different antioxidants contained in the natural matrix (Table 9S). The one-way analysis of variance (ANOVA) with Tukey's multiple comparison method showed that the three optimum conditions (OPT1, OPT2, and OPT3) yielded significantly different AC values (ABTS and ORAC) of the MLEs. Whereas using the DPPH method, only OPT2 extracts showed significantly different AC values, lower than OPT1 and OPT3 extracts.

OPT1 extracts presented the highest AC (ABTS, ORAC), between 14% and 39% more than OPT2 and OPT3 (Table 9S). AC_{DPPH} behavior differed from that of AC_{ABTS} and AC_{ORAC}; this could be due to the characteristics of the compounds extracted under optimum conditions. It was previously reported that compounds such as carotenoids and anthocyanins, present in maqui leaves (Vidal et al., 2013; Zúñiga et al., 2017), absorbs at 515 nm (λ_{max} to DPPH radical absorption), which generates an overestimated measurement (Boligon, Machado, & Athayde, 2014). In contrast, the ABTS method is more effective for analyzing plant extracts because the measurement at 734 nm eliminates possible interferers, and also, the radical can interact with a broader range of antioxidants (Mareček et al., 2017).

The antioxidant capacities of the optimum extracts (based on the grams of dry extract) reached values in the ranges of 1225.92 – 1668.20 mg TE/g dry extract (ABTS method), 1204.13 – 2142.50 mg TE/g dry extract (ORAC method), and 0.17 – 0.37 g dry extract/g DPPH (EC_{50}, DPPH method); the MLE with the highest AC was OPT3 followed by OPT2 (Table 10S). Two previous studies on the recovery of polyphenols from maqui leaves determined the antioxidant capacity with the DPPH method using different concentrations of the DPPH methanol solution (400 and 200 μM) (Muñoz et al., 2011; Rubilar et al., 2011). Therefore, to compare adequately our AC results with the literature values, it was necessary to express all DPPH values in the same units (g dry extract/g DPPH). Our best extract in terms of antioxidant capacity and P (OPT3 extract, ASE 150) needed only 0.17 g dry extract/g DPPH for 50% depletion of the free radical, ~3 times lower than the results of previous studies, which showed AC_{DPPH} values of 0.55 g dry extract/g DPPH (Rubilar et al., 2011) and 0.46 g dry extract/g DPPH (Muñoz et al., 2011). This comparison should be made with caution, AC results depend strongly on extraction methods and conditions, solvent, particle size, and pre-treatment (Pérez-Jiménez et al., 2008), as well as on factors related to the plant (genotype, environment, stage of harvesting and storage) (Rivera-Tovar et al., 2019). Nevertheless, our extracts' high AC_{DPPH} values confirm the extraction method's efficiency and the optimization procedure's adequacy.

A correlation between TPC and AC_{ABTS} (R^2 = 0.996) as well as

Table 1
Optimization of the 3 established objective functions.

Extract	Optimization function			Adjusted factors			Estimated responses		
	$w_{TPC=AC}$	w_P	D	T (°C)	Ethanol (%)	# cycles	TPC[a]	AC_{ABTS}[a]	P[a]
OPT1	1	0	0.952	200	23	3	200.71	815.35	24.91
OPT2	1	1	0.524	143	22	3	167.68	664.24	30.83
OPT3	1	10	0.550	122	5	3	126.08	541.52	35.94

[a] TPC: mg GAE/g maqui leaves (dry weight), AC_{ABTS}: mg TE/g maqui leaves (dry weight), and P: %.

Table 2
Predicted and experimental values of the responses measured in the optimal extracts of maqui leaves, processed by ASE 200 and ASE 150 equipment.

	Extract	Optimal conditions	Response	Predicted	Experimental	SD	RSD
ASE 200	OPT1	200 °C	TPC (mg GAE/g[b])	199.87	188.73 ± 3.02	7.90	4.07
		25% ethanol[a]	AC_{ABTS} (mg TE/g[b])	817.03	825.43 ± 51.19	5.64	0.69
		3 cycles	P (%)	24.94	28.03 ± 0.85	2.18	8.04
	OPT2	145 °C[a]	TPC (mg GAE/g[b])	164.03	162.86 ± 1.50	0.80	0.49
		20% ethanol[a]	AC_{ABTS} (mg TE/g[b])	668.21	710.65 ± 21.94	30.06	4.39
		3 cycles	P (%)	30.69	31.41 ± 0.63	0.51	1.64
	OPT3	122 °C	TPC (mg GAE/g[b])	126.08	129.57 ± 0.49	2.49	1.95
		5% ethanol	AC_{ABTS} (mg TE/g[b])	541.52	593.37 ± 8.53	36.40	6.42
		3 cycles	P (%)	35.87	36.34 ± 0.47	0.33	0.92
ASE 150	OPT1	200 °C	TPC (mg GAE/g[b])	200.71	205.14 ± 1.64	3.10	1.53
		23% ethanol	P (%)	24.51	26.92 ± 0.21	1.42	5.48
		3 cycles					
	OPT2	143 °C	TPC (mg GAE/g[b])	167.68	170.74 ± 1.33	2.14	1.26
		22% ethanol	P (%)	30.83	33.06 ± 0.27	1.58	4.94
		3 cycles					
	OPT3	122 °C	TPC (mg GAE/g[b])	126.08	115.79 ± 1.01	7.27	6.01
		5% ethanol	P (%)	35.94	36.29 ± 0.62	0.25	0.79
		3 cycles					

[a] Extraction conditions adjusted to the operating range of the ASE 200 Solvent Controller. [b] Grams of maqui leaves in dry weight.

between TPC and AC_{ORAC} (R^2 = 0.991) was observed, although the correlation was only statistically significant for the first case (p-value = 0.04 and 0.062, respectively).

Hydroxymethylfurfural (HMF) could be present in OPT1 and OPT2 extracts because they were generated at temperatures above 130 °C (Huaman-Castilla et al., 2019; Mariotti-Celis et al., 2018). However, these two extracts were focused on optimizing the polyphenol's yield; in a subsequent process, these extracts can be purified to eliminate or reduce HMF concentration (an undesirable extractable compound) to levels below those that generate carcinogenic effects. Applying solid-phase extraction with HP-20 macroporous resin (Huaman-Castilla et al., 2019), the HMF concentration in grape pomace crude extracts (23.61 mg HMF/g dry pomace) was reduced almost entirely (~95%). In our case, maqui leaves extracts probably contain HMF concentrations similar or lower than those of grape pomace and notably lower than those of natural matrices such as ground coffee or bakery products, which already formed HMF during high-temperature processes before extraction.

3.4. Low molecular weight phenolic compounds

The three optimum ASE 150 (OPT1, OPT2, OPT3) MLEs and two reference extracts (RCE2 = 70% acetone and RCE3 = 50% methanol) were characterized in terms of their low molecular weight polyphenols; 11 phenolics of the 18 analyzed were quantified (Table 3). OPT1, OPT2, and OPT3 extracts contained 54%, 44%, and 58% of the total quantified polyphenols in RCE2, as well as 57%, 46%, and 61% of the total of quantified polyphenols in RCE3, respectively (Fig. 1S **a**). All extracts, except OPT1, contained more flavonoids than non-flavonoids (Fig. 1S **a**). RCE2 contained almost two times more flavonoids than non-flavonoids, while OPT1 contained equal amounts of flavonoid and non-flavonoids. OPT3 showed the highest recovery of quantified flavonoids in HPLE, probably due to the low ethanol content (5% v/v) in the extraction solvent, which has been shown to favor their recovery (Rodríguez-Pérez et al., 2016).

All extracts presented a similar distribution of flavonols (41%–48%), phenolic acid (35%–45%), flavanols (4%–22%), and others (1%–7%) (Fig. 1S **b**). However, only for phenolic acids, the effect of extraction temperature showed a clear trend. OPT3 (122 °C, 5% ethanol) was particularly efficient to recover gallic and cinnamic acids (Table 3). High temperatures (200 °C) have been shown to accelerate these phenolic acids' degradation (Khuwijitjaru et al., 2014). The recovery of gallic acid is significantly enhanced at moderately high temperatures (≤150 °C) compared with extractions at lower temperatures (90 °C) (Huaman-Castilla et al., 2019). In our case, its recovery at 200 °C (OPT1) was

Table 3
Low molecular weight phenolic compounds are quantified in optimum HPLE and reference maqui leaves' extracts (ASE 150).

Compound	mg/g leaves (dry weight)				
	OPT1 (200 °C – 23% – 3 cycles)	OPT2 (143 °C – 22% – 3 cycles)	OPT3 (122 °C – 5% – 3 cycles)	RCE3 (20 °C – 50%)	RCE2 (20 °C – 70%)
Phenolic acids					
Gallic acid[a]	0.07 ± 0.00^{D}	0.22 ± 0.03^{C}	0.36 ± 0.01^{B}	0.23 ± 0.01^{C}	0.64 ± 0.02^{A}
Chlorogenic acid	1.10 ± 0.00^{B}	0.76 ± 0.01^{C}	0.65 ± 0.02^{C}	1.47 ± 0.01^{A}	1.44 ± 0.02^{A}
Vanillic acid	n.d.	n.d.	n.d.	n.d.	n.d.
Caffeic acid	n.d.	n.d.	n.d.	n.d.	n.d.
Ferulic acid	n.d.	n.d.	n.d.	n.d.	n.d.
Protocatechuic acid	1.06 ± 0.03^{A}	0.93 ± 0.05^{A}	1.13 ± 0.07^{A}	1.57 ± 0.02^{A}	0.93 ± 0.01^{A}
Coumaric acid[a]	0.10 ± 0.03^{A}	0.03 ± 0.00^{B}	0.03 ± 0.00^{B}	0.03 ± 0.00^{B}	0.03 ± 0.00^{B}
Cinnamic acid[a]	0.06 ± 0.00^{C}	0.08 ± 0.01^{C}	0.31 ± 0.02^{B}	0.54 ± 0.02^{A}	0.53 ± 0.01^{A}
Other polyphenols (Stilbenes, tyrosols, dihydrochalcones)					
Resveratrol[b]	n.d.	0.08 ± 0.01^{B}	0.13 ± 0.02^{A}	n.d.	n.d.
Hydroxytyrosol	0.38 ± 0.00^{A}	0.12 ± 0.01^{B}	0.17 ± 0.02^{B}	0.18 ± 0.01^{B}	0.07 ± 0.01^{B}
Oleuropein	n.d.	n.d.	n.d.	n.d.	n.d.
Phloridzin	n.d.	n.d.	n.d.	n.d.	n.d.
Flavanols					
Catechin	0.21 ± 0.01^{D}	0.51 ± 0.01^{C}	0.43 ± 0.00^{C}	1.67 ± 0.02^{B}	2.25 ± 0.03^{A}
Epicatechin	n.d.	n.d.	n.d.	n.d.	n.d.
Epigallocatechin	n.d.	n.d.	n.d.	n.d.	n.d.
Flavonols					
Quercetin – 3 glucoside	0.56 ± 0.01^{C}	1.08 ± 0.02^{B}	1.04 ± 0.02^{B}	2.01 ± 0.03^{A}	2.09 ± 0.03^{A}
Quercetin	1.53 ± 0.03^{A}	0.67 ± 0.02^{B}	1.52 ± 0.05^{A}	1.31 ± 0.02^{A}	1.35 ± 0.03^{A}
Kaempferol	0.44 ± 0.01^{C}	0.10 ± 0.01^{E}	0.29 ± 0.01^{D}	0.67 ± 0.02B	0.90 ± 0.01^{A}
∑ Polyphenols identified	5.50 ± 0.13	4.49 ± 0.16	5.93 ± 0.22	9.68 ± 0.15	10.23 ± 0.15

[a] Expressed as ferulic acid.
[b] mg/100 g maqui leaves, and n.d. means not detected.
$^{(A\text{ - }E)}$ Values that do not share a letter are significantly different.

severely affected; hence, we estimated that maximum recovery of gallic acid could be achieved in the range of 120 – 150 °C. Previous studies with other natural matrices have shown that gallic acid can be extracted better with higher ethanol concentrations (15%–50%) (Dhanani, Singh, & Kumar, 2017; Huaman-Castilla et al., 2019). The optimum ethanol concentration to extract gallic acid may depend on interactions between this acid and specific polyphenols of the given matrix.

OPT1 contained higher amounts of chlorogenic acid (~2 times) and coumaric acid (~4 times) than OPT3. These phenolic acids are resistant to thermal degradation, and probably high temperatures are required to weaken their bonds with the matrix (Khuwijitjaru et al., 2014). Also, moderate ethanol concentration (15%) in the extraction solvent favored chlorogenic acid recovery (Huaman-Castilla et al., 2019).

Both temperature and ethanol content did not clearly show an effect on other polyphenols' recovery (Table 3). The catechin and quercetin glucoside recovery showed a slight improvement with OPT2 conditions (143 °C – 22% ethanol). At 200 °C, quercetin 3-glucoside and catechin may have suffered significant degradation, as observed in microwave-assisted extraction at temperatures higher than 125 °C for compounds with many hydroxyl groups (Liazid, Palma, Brigui, & Barroso, 2007). It was previously reported that maximum recoveries of catechin were achieved at 150 °C (evaluated in the range 90–150 °C) from *Carménère* wine pomace (Huaman-Castilla et al., 2019), and at 130 °C (evaluated in the range 100–200 °C) from tea leaves and grape seeds (Piñeiro, Palma, & Barroso, 2004); explaining the good performance of OPT2 conditions.

Resveratrol recovery was favored at low extraction temperatures and ethanol contents; hence, OPT3 conditions yielded the highest recoveries. Hydroxytyrosol and kaempferol contents were higher in the OPT1 extracts. These polyphenols require high temperatures (180 °C and 150 °C, respectively) and low concentrations of ethanol (<50% and 15%,

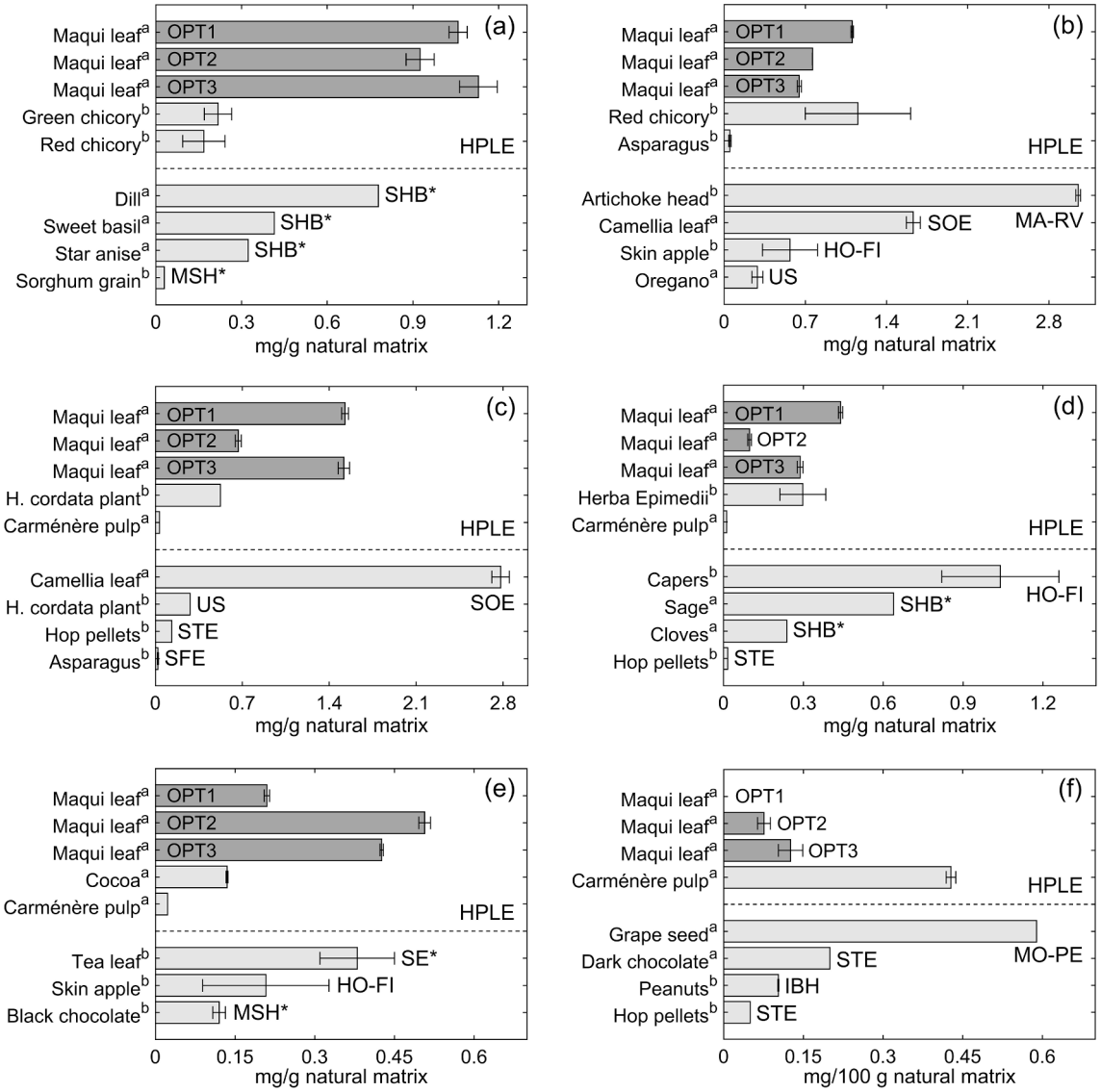

Fig. 4. Comparison of the content of target polyphenols in maqui leaves with those of different natural matrices that were obtained by HPLE – ethanol (above) and by other extraction technologies - ethanol (down). (a) protocatechuic acid (Neveu et al., 2010), (b) chlorogenic acid (Haghi & Hatami, 2010; Neveu et al., 2010; Solana, Boschiero, Dall'Acqua & Bertucco, 2015), (c) quercetin (Callemien, Jerkovic, Rozenberg, & Collin, 2005; Haghi & Hatami, 2010; Huaman-Castilla et al., 2019; Neveu et al., 2010; Solana, Boschiero, & Dall'Acqua, A., & Bertucco, A., 2015; Zhang, Li, & Wu, 2008), (d) kaempferol (Callemien et al., 2005; Chen et al., 2008; Huaman-Castilla et al., 2019; Neveu et al., 2010), (e) catechin (Huaman-Castilla et al., 2019; Neveu et al., 2010; Okiyama et al., 2018; Piñeiro et al., 2004), and (f) resveratrol (Callemien et al., 2005; Huaman-Castilla et al., 2019; Neveu et al., 2010; Shrikanta, Kumar, & Govindaswamy, 2015). [a]: dry weight, [b]: fresh weight, *: methanol instead of ethanol, SHB: shaking bath, STE: Stirring extraction, MSH: mechanical shaking, US: ultrasound-assisted, HO-FI: homogenization and vacuum filtration, SOE: extraction with sonication, MA-RV: maceration and rotary evaporator, SFE: supercritical fluid extraction, SE: static extraction, IBH: ice bath homogenizer and MO-PE: mortar and pestle.

respectively) to optimize their extraction from natural matrices (Cea Pavez et al., 2019; Huaman-Castilla et al., 2019). Resveratrol, hydroxytyrosol, and coumaric acid were extracted better in our optimal HPLE conditions than in the reference extracts, probably because room temperature (20 °C) was not effective.

Quercetin and protocatechuic acid contents did not present significant differences (according to one-way ANOVA, Tukey Pairwise comparisons) between optimum extracts (OPT1, OPT3) and reference extracts (RCE2, RCE3). Only OPT2 extract showed a significantly lower quercetin content, which suggests that these polyphenols were stable in the entire operating range explored in this research.

The observed variability in the recovery of some polyphenols with temperature and ethanol could be caused by some specific interactions between polyphenols and other compounds present in the natural matrix as well as between polyphenols and the extraction solvent. A deep chemical characterization that considers a computational calculation would improve the understanding of these interactions (Huaman-Castilla et al., 2019).

Some of the identified polyphenols present in our MLEs are potentially beneficial for human health, according to *in vitro* and *in vivo* assays and clinical trials (Heleno, Martins, Queiroz, & Ferreira, 2015; Pohl & Kong Thoo Lin, 2018).

The HPLE of maqui leaves has become a new promising alternative for the sustainable extraction of polyphenols (Fig. 4). Compared with other natural matrices and extraction processes (mechanical shaking, sonication, ultrasound-assisted, stirring, and supercritical fluid), our optimal extracts place HPLE of maqui leaves as among the best natural source and extraction technique to obtain protocatechuic acid (1.13 mg protocatechuic acid/g maqui leaves), quercetin (1.53 mg quercetin/g maqui leaves) and catechin (0.51 mg catechin/g maqui leaves) (Fig. 4**a, c, and e**). Chlorogenic acid and kaempferol contents of our MLEs (1.10 and 0.44 mg/g maqui leaves, respectively) were equivalent to those obtained from other natural matrices using non-eco-friendly techniques and were the best among the natural matrices processed by HPLE (Fig. 4**b and d**). Resveratrol, kaempferol, cinnamic acid, and hydroxytyrosol have been identified only in ~14, ~12, ~8, and ~5 solid natural matrices, respectively (Neveu et al., 2010). Therefore, maqui leaves that have been discarded in the maqui fruit industry become an attractive new potential source of these polyphenols.

4. Conclusions

HPLE, an eco-friendly technique, was used to recover polyphenols from maqui leaves currently discarded in the maqui berry industry. RSM and DF were applied for HPLE multi-response optimization. For the first time, HPLE optimal extracts were obtained considering the simultaneous maximization of TPC, AC, and polyphenol purity. Optimal HPLE conditions (OPT3: 122 °C, 5% EtOH and 3 cycles) that prioritized polyphenol purity (P) more than TPC and AC achieved extracts 35% more pure than the other optimum MLEs. In turn, optimal extraction conditions (OPT1: 200 °C, 23% EtOH and 3 cycles) that prioritized TPC and AC equally achieved extracts with the highest AC and a TPC that was 78% of the TPC of the reference extract (RCE1), that supposedly recovered 100% of the polyphenols contained in the original matrix. Eleven polyphenols were identified and quantified by UPLC–MS in the optimum extracts of maqui leaves. Chlorogenic and coumaric acids, hydroxytyrosol, and kaempferol were better recovered at OPT1 conditions. Gallic acid, cinnamic acid, and resveratrol were better recovered at OPT3 conditions since they experience thermal degradation. Many of the polyphenols quantified in this study showed higher contents in our MLE than in other natural sources processed with HPLE or with other extraction technology.

Declaration of Competing Interest

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

Acknowledgments

Financial support from the Xunta de Galicia (Centro singular de investigación de Galicia, ED431G/02 and Grupos de referencia competitiva EDC431C 2018/47); the European Union (European Regional Development Fund - ERDF); FONDECYT Regular project number 1180571; and Programa de Ciencia y Tecnología para el Desarrollo (CYTED), Red Iberoamericana de Alimentos Autóctonos Subutilizados (ALSUB-CYTED, 118RT0543) is gratefully acknowledged. M. Torres thanks the Spanish Ministry of Science, Innovation, and Universities for her postdoctoral grant (RYC2018-024454-I). P. Rivera-Tovar thanks scholarship "Estadía en el Extranjero para Tesistas de Doctorado" awarded by Vicerrectoría de Investigación of the Pontificia Universidad Católica de Chile. We appreciate the text edition by Lisa Gingles.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.129729.

References

Avello, M., Valdivia, R., Sanzana, R., Mondaca, M. A., Mennickent, S., Aeschlimann, V., … Becerra, J. (2009). Antioxidants and antimicrobial extracts of *Aristotelia chilensis* and *Ugni molinae* and its applications as preservatives in cosmetic products. *Bol. Latinoamericano y Del Caribe de Plantas Medicinales y Aromaticas, 8*(6), 479–486.

Bastola, K. P., Guragain, Y. N., Bhadriraju, V., & Vadlani, P. V. (2017). Evaluation of standards and interfering compounds in the determination of phenolics by Folin-Ciocalteu assay method for effective bioprocessing of biomass. *American Journal of Analytical Chemistry, 08*(06), 416–431. https://doi.org/10.4236/ajac.2017.86032.

Boligon, A. A., Machado, M. M., & Athayde, M. L. (2014). Technical evaluation of antioxidant activity. *Medicinal Chemistry, 4*(7), 517–522. https://doi.org/10.4172/2161-et.

Brescia, P. J. (2012). Determination of Antioxidant potential using an Oxygen Radical Absorbance Capacity (ORAC) Assay with Synergy TM H4.

Callemien, D., Jerkovic, V., Rozenberg, R., & Collin, S. (2005). Hop as an interesting source of resveratrol for brewers: Optimization of the extraction and quantitative study by liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 53*(2), 424–429. https://doi.org/10.1021/jf040179n.

Cea Pavez, I., Lozano-Sánchez, J., Borrás-Linares, I., Nuñez, H., Robert, P., & Segura-Carretero, A. (2019). Obtaining an extract rich in phenolic compounds from olive pomace by pressurized liquid extraction. *Molecules, 24*(17), 3108. https://doi.org/10.3390/molecules24173108.

Chen, X. J., Ji, H., Zhang, Q. W., Tu, P. F., Wang, Y. T., Guo, B. L., & Li, S. P. (2008). A rapid method for simultaneous determination of 15 flavonoids in Epimedium using pressurized liquid extraction and ultra-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis, 46*(2), 226–235. https://doi.org/10.1016/j.jpba.2007.09.016.

Derringer, G., & Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology, 12*(4), 214–219. https://doi.org/10.1080/00224065.1980.11980968.

Dhanani, T., Singh, R., & Kumar, S. (2017). Extraction optimization of gallic acid, (+)-catechin, procyanidin-B2, (–)-epicatechin, (–)-epigallocatechin gallate, and (–)-epicatechin gallate: Their simultaneous identification and quantification in *Saraca asoca*. *Journal of Food and Drug Analysis, 25*(3), 691–698. https://doi.org/10.1016/j.jfda.2016.08.004.

Díaz-de-Cerio, E., Arráez-Román, D., Segura-Carretero, A., Ferranti, P., Nicoletti, R., Perrotta, G. M., & Gómez-Caravaca, A. M. (2018). Establishment of pressurized-liquid extraction by response surface methodology approach coupled to HPLC-DAD-TOF-MS for the determination of phenolic compounds of myrtle leaves. *Analytical and Bioanalytical Chemistry, 410*(15), 3547–3557. https://doi.org/10.1007/s00216-018-0914-0.

Haghi, G., & Hatami, A. (2010). Simultaneous quantification of flavonoids and phenolic acids in plant materials by a newly developed isocratic high-performance liquid chromatography approach. *Journal of Agricultural and Food Chemistry, 58*(20), 10812–10816. https://doi.org/10.1021/jf102175x.

Heleno, S. A., Martins, A., Queiroz, M. J. R. P., & Ferreira, I. C. F. R. (2015). Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. *Food Chemistry, 173*, 501–513. https://doi.org/10.1016/j.foodchem.2014.10.057.

Huaman-Castilla, N. L., Martínez-Cifuentes, M., Camilo, C., Pedreschi, F., Mariotti-Celis, M., & Pérez-Correa, J. R. (2019). The impact of temperature and ethanol concentration on the global recovery of specific polyphenols in an integrated HPLE/RP process on Carménère pomace extracts. *Molecules, 24*(17), 1–16. https://doi.org/10.3390/molecules24173145.

Khuwijitjaru, P., Plernjit, J., Suaylam, B., Samuhaseneetoo, S., Pongsawatmanit, R., & Adachi, S. (2014). Degradation kinetics of some phenolic compounds in subcritical water and radical scavenging activity of their degradation products. *Canadian Journal of Chemical Engineering, 92*(5), 810–815. https://doi.org/10.1002/cjce:v92.510.1002/cjce:21898.

Liazid, A., Palma, M., Brigui, J., & Barroso, C. G. (2007). Investigation on phenolic compounds stability during microwave-assisted extraction. *Journal of Chromatography A, 1140*(1-2), 29–34. https://doi.org/10.1016/j.chroma.2006.11.040.

Mareček, V., Mikyška, A., Hampel, D., Čejka, P., Neuwirthová, J., Malachová, A., & Cerkal, R. (2017). ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. *Journal of Cereal Science, 73*, 40–45. https://doi.org/10.1016/j.jcs.2016.11.004.

Mariotti-Celis, M. S., Martínez-Cifuentes, M., Huamán-Castilla, N., Pedreschi, F., Iglesias-Rebolledo, N., & Pérez-Correa, J. R. (2018). Impact of an integrated process of hot pressurised liquid extraction–macroporous resin purification over the polyphenols, hydroxymethylfurfural and reducing sugars content of *Vitis vinifera* 'Carménère' pomace extracts. *International Journal of Food Science and Technology, 53*(4), 1072–1078. https://doi.org/10.1111/ijfs.2018.53.issue-410.1111/ijfs.13684.

Moure, A., Domínguez, H., & Parajó, J. C. (2005). Antioxidant activity of liquors from aqueous treatments of *Pinus radiata* wood. *Wood Science and Technology, 39*(2), 129–139. https://doi.org/10.1007/s00226-004-0279-8.

Mukhopadhyay, S., Luthria, D. L., & Robbins, R. J. (2006). Optimization of extraction process for phenolic acids from black cohosh (*Cimicifuga racemosa*) by pressurized liquid extraction. *Journal of the Science of Food and Agriculture, 86*(1), 156–162. https://doi.org/10.1002/jsfa.2326.

Muñoz, O., Christen, P., Cretton, S., Backhouse, N., Torres, V., Correa, O., … Delporte, C. (2011). Chemical study and anti-inflammatory, analgesic and antioxidant activities of the leaves of *Aristotelia chilensis* (Mol.) Stuntz, Elaeocarpaceae. *Journal of Pharmacy and Pharmacology, 63*(6), 849–859. https://doi.org/10.1111/j.2042-7158.2011.01280.x.

Mustafa, A., & Turner, C. (2011). Analytica Chimica Acta Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. *Analytica Chimica Acta, 703*(1), 8–18. https://doi.org/10.1016/j.aca.2011.07.018.

Myers, R. H., Khuri, A. I., & Carter, W. H. (1989). Response surface methodology: 1966–1988. *Technometrics, 31*(2), 137–157. https://doi.org/10.1080/00401706.1989.10488509.

Neveu, V., Perez-Jiménez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., Knox, C., … Scalbert, A. (2010). Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. DOI:10.1093/database/bap024.

Okiyama, D. C. G., Soares, I. D., Cuevas, M. S., Crevelin, E. J., Moraes, L. A. B., Melo, M. P., … Rodrigues, C. E. C. (2018). Pressurized liquid extraction of flavanols and alkaloids from cocoa bean shell using ethanol as solvent. *Food Research International, 114*, 20–29. https://doi.org/10.1016/j.foodres.2018.07.055.

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz- Rubio, M. E., Serrano, J., Goñi, I., & Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International, 41*(3), 274–285. https://doi.org/10.1016/j.foodres.2007.12.004.

Piñeiro, Z., Palma, M., & Barroso, C. G. (2004). Determination of catechins by means of extraction with pressurized liquids. *Journal of Chromatography A, 1026*(1–2), 19–23. https://doi.org/10.1016/j.chroma.2003.10.096.

Pohl, F., & Kong Thoo Lin, P. K. T. (2018). The potential use of plant natural products and plant extracts with antioxidant properties for the prevention/treatment of neurodegenerative diseases: In vitro, in vivo and clinical trials. *Molecules, 23*(12), 3283. https://doi.org/10.3390/molecules23123283.

Putnik, P., Barba, F. J., Španić, I., Zorić, Z., Dragović-Uzelac, V., & Kovačević, D. B. (2017). Green extraction approach for the recovery of polyphenols from Croatian olive leaves (*Olea europea*). *Food and Bioproducts Processing, 106*, 19–28. https://doi.org/10.1016/j.fbp.2017.08.004.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying and improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 26*(9–10), 121–137. https://doi.org/10.1016/S0891-5849(98)00315-3.

Rivera-Tovar, P. R., Mariotti-Celis, M. S., & Pérez-Correa, J. R. (2019). Maqui *(Aristotelia chilensis* (Mol.) Stuntz) and Murta *(Ugni molin*ae Turcz): Native Chilean Sources of Polyphenol Compounds. *Mini-Reviews in Organic Chemistry., 16*(3), 261–276. https://doi.org/10.2174/1570193X15666180627120609.

Rodríguez-Pérez, C., Gilbert-López, B., Mendiola, J. A., Quirantes-Piné, R., Segura-Carretero, A., & Ibáñez, E. (2016). Optimization of microwave-assisted extraction and pressurized liquid extraction of phenolic compounds from *Moringa oleifera* leaves by multi-response surface methodology. *Electrophoresis, 37*(13), 1938–1946. https://doi.org/10.1002/elps.201600071.

Rubilar, M., Jara, C., Poo, Y., Acevedo, F., Gutierrez, C., Sineiro, J., & Shene, C. (2011). Extracts of maqui *(Aristotelia chilensis*) and murta *(Ugni molin*ae Turcz.): Sources of antioxidant compounds and α-glucosidase/α-amylase inhibitors. *Journal of Agricultural and Food Chemistry, 59*(5), 1630–1637. https://doi.org/10.1021/jf103461k.

Shrikanta, A., Kumar, A., & Govindaswamy, V. (2015). Resveratrol content and antioxidant properties of underutilized fruits. *Journal of Food Science and Technology, 52*(1), 383–390. https://doi.org/10.1007/s13197-013-0993-z.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods of Enzymology, 299*, 152–178. https://doi.org/10.1007/BF02530903.

Solana, M., Boschiero, I., & Dall'Acqua, A., & Bertucco, A.. (2015). A comparison between supercritical fluid and pressurized liquid extraction methods for obtaining phenolic compounds from *Asparagus officinalis* L. *The Journal of Supercritical Fluids, 100*, 201–208. https://doi.org/10.1016/j.jinf.2020.04.010.

Tripodo, G., Ibáñez, E., Cifuentes, A., Gilbert-López, B., & Fanali, C. (2018). Optimization of pressurized liquid extraction by response surface methodology of Goji berry (*Lycium barbarum* L.) phenolic bioactive compounds. *Electrophoresis, 39*(13), 1673–1682. https://doi.org/10.1002/elps.201700448.

Vidal, J. L., Avello, L. M., Loyola, C. C., Campos, P. J., Aqueveque, M. P., Dungan, R. S., … Guarda, M. A. (2013). Microencapsulation of maqui *(Aristotelia chilensis* [Molina] Stuntz) leaf extracts to preserve and control antioxidant properties. *Chilean Journal of Agricultural Research, 73*(1), 17–23. https://doi.org/10.4067/S0718-58392013000100003.

Xynos, N., Papaefstathiou, G., Gikas, E., Argyropoulou, A., Aligiannis, N., & Skaltsounis, A. L. (2014). Design optimization study of the extraction of olive leaves performed with pressurized liquid extraction using response surface methodology. *Separation and Purification Technology, 122*, 323–330. https://doi.org/10.1016/j.seppur.2013.10.040.

Zhang, Y., Li, S. Fen, & Wu, X. Wen (2008). Pressurized liquid extraction of flavonoids from *Houttuynia cordata* Thunb. *Separation and Purification Technology, 58*(3), 305–310. https://doi.org/10.1016/j.seppur.2007.04.010.

Zúñiga, G. E., Tapia, A., Arenas, A., Contreras, R. A., & Zúñiga-Libano, G. (2017). Phytochemistry and biological properties of *Aristotelia chilensis* a Chilean blackberry: A review. *Phytochemistry Reviews, 16*(5), 1081–1094. https://doi.org/10.1007/s11101-017-9533-1.