Optimized methodology for the extraction of free and bound phenolic acids from Chilean Cristalino corn (Zea mays L.) accession

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ABSTRACT

The aim of this study was to optimize the extraction of free and bound phenolic acids from Chilean Cristalino corn by response surface methodology based on the total phenolic contents (TPCs) and the 2,2-diphenyl-1-picrylhydrazyl scavenging-linked antioxidant capacity (AC) as response variables. Central Composite 2° + axial points experimental designs were applied. The best extraction conditions for the free and bound phenolic fraction were acetone 69% in water for 63 min and a hydrolysis with 3 M NaOH for 90 min, respectively. Under these conditions, TPCs in free and bound forms were 59.9 ± 0.7 and 172.9 ± 1.1 mg gallic acid equivalents/100 g, respectively. Further, AC found in free and bound fractions was 186 ± 3 and 694.5 ± 3.3 µmol Trolox equivalents/100 g, respectively. The experimental and predicted values of TPC and AC were similar in case of free phenolic fraction indicating that the model was adequate and reproducible. Major phenolic acids were found in the bound fraction and were ferulic and p-coumaric acids.

Metodología optimizada para la extracción de ácidos fenólicos libres y ligados de la accesión de maíz (Zea mays L.) chileno Cristalino

RESUMEN

El objetivo de este estudio fue optimizar la extracción de los ácidos fenólicos libres y ligados a partir de maíz chileno Cristalino a través de la metodología de superficie respuesta. Para la optimización se utilizó como variables respuesta el contenido de fenólicos totales (TPC) y la capacidad antioxidante (AC) a través de la inhibición del radical libre DPPH. Se aplicó un diseño Compósito Central 2° + puntos axiales. Las mejores condiciones para la extracción de los fenólicos de la fracción libre y ligada fueron el uso de acetona al 69% en agua por 63 min y una hidrólisis con NaOH 3 M por 90 min, respectivamente. Bajo estas condiciones, los TPC en la fracción libre y ligada fueron 59.9±0.7 y 172,9±1,1 mg GAE/100g, respectivamente. Además, las AC observadas para la fracción libre y ligada fueron 186±3 y 694,5±3,3 µmol TE/100g, respectivamente. Los valores experimentales de TPC y AC fueron similares a los predichos para el caso de la fracción libre, indicando que el modelo fue adecuado y reproducible. Los ácidos fenólicos más importantes encontrados en la fracción ligada fueron el ácido ferúlico y p-cumaríaco.

Introduction

Corn (Zea mays L.) belongs to grass family Gramineae (Poaceae) (Canadian Food Inspection Agency, 1994). This grain is the staple food in many countries especially in Central and South America (Poutanen, 2012). Corn has been cultivated for centuries in Mexico, Peru and Bolivia and consumed as traditional staple food products such as tortilla, tortilla chips and typical beverages and desserts (Lopez-Martinez et al., 2009; Montilla, Hillebrand, Antezana, & Winterhalter, 2011). Besides above countries, Chile is also an important center of corn diversity with around 23 local races or landraces encompassing hundreds of native corn accessions, most of them pigmented and cultivated in Andean regions (Salazar, Lobos, Rosas, & Muñoz, 2006). However, limited information exists about their nutritional and potential bioactive health-relevant properties, which have important relevance for potential applications in functional foods development.

Phenolic compounds are widely distributed among plant-derived foods such as grains, including corn, and contribute to crucial functional aspects of plant life such as structural roles, involvement in defense strategies and signaling properties (Sharma, Srivastava, & Prakash, 2011). However, the most important function assigned to phenolic compounds is their antioxidant activity (Nile & Park, 2014). Numerous studies have shown that regular consumption of whole grains rich in phenolic antioxidants and other grain phytochemicals such as carotenoids, phytosterols, lignans, β-glucans and fiber is beneficial to human health with potential for management and reduction of type 2 diabetes, cardiovascular disease and colon cancer (Bernstein, Titgemeier, Kirkpatrick, Golubic, & Roizen, 2013; Hansen et al., 2012). These phytochemicals, especially phenolic compounds, may potentially...
act as agents of oxidation-linked degenerative diseases such as cancer, atherosclerosis, aging and rheumatoid arthritis among others (Liu et al., 2011; Nile & Park, 2014).

Corn has been shown to contain a wide variety of phenolic phytochemicals and such compounds may exist in free, soluble conjugates and in insoluble bound forms associated to dietary fiber. The predominant group of phenolic antioxidants in cereal grains such as corn is phenolic acids which are mostly hydroxyxyanic acid derivatives found in insoluble bound forms, mainly as ferulic acid and its oxidized forms, esterified to arabinose residues in primary cell wall and to arabinoxylan and arabinoogalactan in the aleurone layer and pericarp (Dewanto, Wu, & Liu, 2002; Guo & Beta, 2013; Mattila, Pihlava, & Helström, 2005; Nazck & Shahidi, 2006). Also, Montilla et al. (2011) and Zilić, Serpen, Akilioğlu, Gökmen and Vančetović (2012) have found that p-coumaric acid and ferulic acid were the main bound phenolic compounds in different corn cultivars whereas the soluble forms were represented by some phenolic acids, flavonoids and quinones (Cabrera-Soto, Salinas-Moreno, Velazquez-Cardelas, & Trujillo, 2009). The growing interest in bioactive food phenolic compounds with the purpose of improving human health has created the need to develop new techniques for their extraction, fractionation, separation and analysis (Pérez-Jiménez & Torres, 2011).

The extraction of phenolic compounds from corn and other cereals is strongly influenced by the solvent type, solvent concentration, time and temperature among other factors. Many studies have focused only on the determination of the soluble-free phenolic fraction of corn grains (Gutierrez et al., 2009; Mohsen & Ammar, 2009; Salinas-Moreno, Lopez-Reynoso, Gonzalez-Flores, & Vazquez-Carrillo, 2007), underestimating the real phenolic content attributed mainly to the bound phenolic forms (Montilla et al., 2011). On the other hand, other reports used variable alkaline hydrolysis conditions for the quantification of the bound phenolic fraction in corn/cereal samples (Dewanto et al., 2002; Lopez-Martinez et al., 2009; Xu et al., 2010; Zilić et al., 2012). However, no studies exist to date on the optimization of extraction conditions for both free and bound phenolic acids in corn and this would be helpful for appropriate analysis and quantification of this group of phenolic compounds from Chilean native corn accessions. Proper statistical and mathematical tools are used for developing, improving and optimizing processes (Turabi, Sumnu, & Sahin, 2008). The most commonly used is the response surface methodology (RSM) that enables the simultaneous evaluation of the effects of several variables and their interactions on response variables (Liyanag-Pathirana & Shahidi, 2005).

Since the overall major phenolic class found in cereals such as corn is the phenolic acid group, the objective of current study was to optimize the extraction conditions of both free and bound phenolic acids from a Chilean native corn accession (Cristalino Chileno INIA CHZM 13005) by using the RSM. The solvent type, solvent concentration in water and extraction time were evaluated as the factors influencing the free phenolic acids extraction whereas the time of alkaline hydrolysis and alkali concentration was studied as the factors influencing the extraction of bound phenolic acids. Selected response variables were the TPC and the 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical scavenging-linked AC. In addition, the phenolic acids composition in the extracts obtained at optimal conditions were analyzed by High Performance Liquid Chromatography (HPLC). Results from current study are the critical first steps for understanding the potential functional bioactive health benefits of several Chilean corn accessions and are focused on the extraction optimization of the phenolic acid contents.

Materials and methods

Materials

The Chilean corn accession Cristalino Chileno (INIA CHZM 13005) (yellow/orange-colored kernel) was used for all analyses and was supplied by the Germplasm Bank of the National Institute of Agronomic Research (INIA, acronym in Spanish) located in La Platina, Santiago, Chile. The DPPH radical and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St Louis, MO, USA). All reagents were analytical grade and purchased from Merck (Darmstadt, Germany) whereas for HPLC analyses, all chemicals were HPLC grade (Sigma, St Louis, MO, USA).

Sample preparation

Corn grains (100 g) were milled in an electric grinder under refrigeration (Staufen, Germany) to a size of 0.5 mm, packaged and stored at −20°C until analysis.

General extraction procedure

Free phenolic acids

The extraction was performed according to Dewanto et al. (2002) and Lopez-Martinez et al. (2009) with some modifications as follows: a sample of 2.5 g of powdered sample corn was mixed with 10 mL of solvent/water mixture in a glass flask for different extraction times under agitation at 230 rpm in an orbital shaker. The supernatant was recovered by centrifugation at 5000 g for 15 min. A second extraction was performed on the residue under the same solvent conditions for 30 min. Both supernatants were pooled, then vacuum-evaporated to dryness at 40°C, and the residue was reconstituted in 10 mL with distilled water. The extracts were stored at −20°C until analysis.

Bound phenolic acids

The extraction was performed by applying an alkaline hydrolysis according to De la Parra, Saldivar and Liu (2007) and Lopez-Martinez et al. (2009). A sample of 0.5 g of powdered corn was mixed with 2 mL of acetone 69% in water and the free phenolic fraction was extracted under the optimized conditions following same procedure stated above. Supernatants containing the free phenolic fraction were discarded and the whole residue was used for the next hydrolysis step. The residue was suspended in 20 mL of NaOH at several concentrations and the alkaline hydrolysis was performed under agitation at room temperature for different times. The mixture was then neutralized with concentrated HCl and then extracted seven times with 10 mL of ethyl acetate. The supernatant was recovered by centrifugation.
at 5000 g for 5 min. The ethyl acetate fractions were mixed and vacuum-evaporated at 35°C. The extract from the residue was finally reconstituted in 10 mL of distilled water and stored at −25°C until further analysis.

**Total phenolic contents**

The analyses of total phenolic contents (TPCs) from free and bound fraction extracts were performed according to Shetty, Curtis, Levin, Witkowski, and Ang (1995). Briefly, 0.5 mL of corn extract was transferred into a test tube and mixed with 1 mL of 95% ethanol and 5 mL of distilled water. To each sample, 0.5 mL of Folin–Ciocalteu (1N) was added and mixed. After 5 min at room temperature, 1 mL of sodium carbonate (Na₂CO₃) solution was added to the reaction mixture and allowed to stand for 1 h in a dark place. The absorbance was read at 725 nm. The standard curve was built using various concentrations of gallic acid in 95% ethanol. The analyses were performed in triplicate and results were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample (wet basis).

**Antioxidant capacity by the DPPH radical inhibition assay**

The antioxidant capacity (AC) of free and bound phenolic fractions was determined by the DPPH radical scavenging method according to Pérez-Jiménez and Saura-Calixto (2006). An aliquot of 3.9 mL of 60 µM DPPH in 95% methanol was mixed with 100 µL of extract or standard and the decrease in absorbance was measured at 515 nm after 25 min of reaction. A control of solvent was also included. The calibration curve consisted of a solution of Trolox at different concentrations of gallic acid in 95% ethanol. The analyses were performed in triplicate and results were expressed as µmol Trolox equivalents (TE)/100 g of sample (wet basis).

**Experimental design for the optimization of free and bound phenolic acids extraction**

Evaluated factors and selected levels for each factor are shown in Tables 1 and 2 for the optimization of the extraction of total free and bound phenolic acids, respectively.

**Free phenolic acids**

The extraction of the free phenolic acids fraction in corn was evaluated using three different solvents: ethanol, methanol and acetone since these are the most common solvents used for extracting bioactive phenolic acids (Adom & Liu, 2002; Dewanto et al., 2002; Lopez-Martinez et al., 2009). For each solvent, a Central Composite Design 2³ + axial points consisting of 12 experimental runs and 4 center points were applied. Two replicates of the model were applied to obtain three blocks; each solvent represented one block. Significant differences were evaluated between the blocks (α = 0.05). The evaluated factors were the solvent concentration in water (X₁ = 67.9–92.1%) and extraction time (X₂ = 15.8–64.2 min) while response variables were the TPC (Y₁) and AC (Y₂).

**Bound phenolic acids**

The residue resulting from the extraction of the free phenolic fraction under the optimized conditions after the statistical analysis was used for this section. A Central Composite Design 2² + axial points consisting of 12 experimental runs with 1 replicate and 4 center points were applied. The evaluated factors were: sodium hydroxide concentration (X₃ = 1.6–4.4 M) and the time of alkaline hydrolysis (X₄ = 47.6–132.4 min). Response variables were the TPC (Y₃) and AC (Y₄).

The analysis of variance (ANOVA) (α = 0.05) and the RSM for the determination of the optimal extraction conditions were done by using Statgraphics Centurion XVI software (StatPoint Inc., Rockvalle, MD, USA).

**Phenolic acid profiles by HPLC analysis**

Corn sample extracts from the free and bound fractions at the optimized or best conditions were filtered (pore size 0.2 µm) and the HPLC analysis was performed according to González-Muñoz, Quesille-Villalobos, Fuentealbalúa, Shetty, and Gálvez-Ranilla (2013). Detected phenolic acids in sample extracts were identified by comparison of their retention times with those of pure standards (vanillin, protocatechuic acid, vanillic acid, p-coumaric acid and ferulic acid), and quantification was performed using the corresponding calibration curves. The results were expressed as mg per 100 g of sample (wet and dry basis considering average moisture of 4.7%).

**Results and discussion**

Several studies have shown a direct correlation between the TPC measured by the Folin–Ciocalteu assay and the total phenolic acid contents measured by HPLC in the free and bound phenolic fractions of corn samples of varying kernel colors (De la Parra et al., 2007; Urias-Peraldi et al., 2013; Žilić et al., 2012). Therefore, the TPC was selected in this study as a response variable for the optimization of the phenolic acids extraction from the free and bound fractions. In addition, the DPPH radical scavenging capacity was selected as a second response variable since it has been shown that this property is generally well correlated to the TPC (Folin–Ciocalteu method) in cereal grains and other plant-derived food samples (Guo & Beta, 2013; Sricharoen, Techaamongpong, & Chanthai, 2015; Yao, Sang, Zhou, & Ren, 2010).

**Effect of evaluated factors on the extraction of the free phenolic acid fraction in corn**

**TPC**

Table 1 shows the TPC found in extracts from the free fraction after the application of a Central Composite Design and using different solvents (ethanol, methanol and acetone). Since the type of solvent is a discrete variable, three blocks were included in the design for evaluating the significance of the type of solvent on the TPC. TPC ranged from 27.5 to 63.7 mg GAE/100 g when aqueous acetone was used. With ethanol and methanol extracts, the total phenolic levels varied from 39.3 to 53.2 mg GAE/100 g, respectively. The type of solvent had no significant (p > 0.05) effect on the TPC among all experimental runs. However, several runs showed higher TPC when acetone was used compared to results obtained with...
methanol and ethanol. In addition, acetone extracts exhibited the highest AC values as it will be further shown.

Studies related to the quantification of total phenolic compounds in corn generally use methanol or ethanol as solvents for the extraction of phenolic content. In this sense, Cabrera-Soto et al. (2009) reported that phenolic contents (extracted with 80% methanol) of seven types of corn ranged from 86.9 to 118 mg GAE/100 g, whereas Montilla et al. (2011) found levels of 72.6 and 102 mg GAE/100 g (dried weight) in samples of partially pigmented and black corns, respectively, when using 80% ethanol. Such differences may be related to the degree of corn pigmentation, sample origin, agronomic and climatic conditions among other variables. Omwamba and Hu (2010) applied a RSM for optimizing the extraction conditions of phenolic compounds from barley and the highest values of phenolic contents were obtained when acetone was used instead of water. Current study revealed that acetone showed better ability in extracting phenolic compounds from corn instead of water. Current study revealed that acetone showed better ability in extracting phenolic compounds from corn instead of water. Current study revealed that acetone showed better ability in extracting phenolic compounds from corn instead of water.

In case of the effect of the extraction time and solvent concentration in water, higher TPCs were observed when the extractions were performed at lower solvent concentrations and shorter time of extraction (Table 1). According to the ANOVA (Table 3), the model for the TPC using acetone had a high value of coefficient of determination for the fit ($R^2 = 0.9952$) with a standard error of estimation of 0.9999. Among the evaluated independent variables, only the solvent concentration ($X_1$) showed a significant effect ($p < 0.05$) on the total phenolic results while the extraction time ($X_2$) had a significant quadratic effect ($p < 0.05$). Since the $p$ value of the Durbin–Watson test was not significant ($p > 0.05$), there is no indication of autocorrelation in the residuals at the 5% significant level. The nonsignificant lack of fit indicates that the model is adequate for the observed data at 95% confidence level. The predicted model for $Y_1$ (TPC) was

$$Y_1 = -14.2039 + 3.6745X_1 - 2.0830X_2 - 0.0310X_1^2 + 0.0045X_1X_2 + 0.0218X_2^2$$

Table 1. Central composite design for the extraction of free phenolic acids from Chilean native corn using different solvents (ethanol, methanol and acetone) and experimental results of TPCs and AC from the free phenolic fraction extracts.

Table 2. Central composite design for the extraction of bound phenolic acids from Chilean native corn and experimental results of TPC and AC from the bound phenolic fraction extracts.
Table 3. Análisis de varianza (ANOVA) para los efectos de concentración de NaOH (X₁) y tiempo de hidrólisis (X₂) en el contenido de fenoles totales (TPC, mg GAE/100 g base húmeda) y la capacidad antioxidante (AC, µmol TE/100 g base húmeda) para la extracción de ácidos fenólicos libres.

| Source | Sum of squares | Mean squares | F ratio | p Value | Sum of squares | Mean squares | F ratio | p Value |
|--------|----------------|--------------|---------|---------|----------------|--------------|---------|---------|
| X₁     | 860.12         | 860.12       | 860.22  | 0.0000  | 6984.65        | 6984.65      | 169.66  | 0.0000  |
| X₂     | 0.9213         | 0.9213       | 0.92    | 0.3742  | 10.2712        | 10.2712      | 0.25    | 0.6352  |
| X₁²    | 41.3177        | 41.3177      | 41.32   | 0.0007  | 1683.0         | 1683.0       | 40.88   | 0.0007  |
| X₂²    | 326.154        | 326.154      | 326.19  | 0.0000  | 0.0842         | 0.0842       | 0.00    | 0.9654  |
| X₁X₂   | 3.1819         | 3.1819       | 3.18    | 0.1247  | 71.4012        | 44.0294      | 1.73    | 0.2359  |
| Lack of fit | 5.3929 | 1.7396 | 8.89 | 0.0529 | 132.088        | 40.0294      | 1.15    | 0.4558  |
| Pure error | 0.6049 | 0.2021 | 0.6064 | 0.9911 |
| Total (corrected) | 1237.69 | 8996.42 | 8.65 | 0.0000 |

TPC: Total phenolic content; AC: antioxidant capacity; GAE: gallic acid equivalents; TE: Trolox equivalents.

Table 4. Análisis de varianza (ANOVA) para los efectos de concentración de NaOH (X₁) y tiempo de hidrólisis (X₂) en el contenido de fenoles totales (TPC, mg GAE/100 g base húmeda) y la capacidad antioxidante (AC, µmol TE/100 g base húmeda) para la extracción de ácidos fenólicos ligados.

| Source | Sum of squares | Mean squares | F ratio | p Value | Sum of squares | Mean squares | F ratio | p Value |
|--------|----------------|--------------|---------|---------|----------------|--------------|---------|---------|
| X₁     | 50.9328        | 50.9328      | 1.24    | 0.3088  | 0.0748         | 0.0748       | 0.00    | 0.9899  |
| X₂     | 5.4425         | 5.4425       | 0.13    | 0.7288  | 1502.98        | 1502.98      | 3.48    | 0.1112  |
| X₁²    | 616.615        | 616.615      | 14.96   | 0.0083  | 6562.3         | 6562.3       | 15.21   | 0.0080  |
| X₂²    | 359.34         | 359.34       | 8.72    | 0.2550  | 3164.52        | 3164.52      | 7.34    | 0.0352  |
| X₁X₂   | 6.5025         | 6.5025       | 0.16    | 0.0704  | 22.7529        | 22.7529      | 0.05    | 0.8260  |
| Lack of fit | 243.576 | 81.192 | 66.31 | 0.0031 | 10.541         | 2.594        | 1.64    | 0.2021  |
| Pure error | 3.1819 | 1.2245 | 3.18 | 0.1247 | 3.1819         | 1.2245       | 0.1247  | 0.2021  |
| Total (corrected) | 1130.62 | 860.22 | 8.65 | 0.0000 |

TPC: Total phenolic content; AC: antioxidant capacity; GAE: gallic acid equivalents; TE: Trolox equivalents.

Effect of evaluated factors on the extraction of the bound phenolic acid fraction in corn

TPC

Results of the TPC in the bound fraction are shown in Table 2. According to the applied experimental design, conditions at the midpoint (3 M NaOH for 90 min) allowed the extraction of higher TPC compared to the other experimental runs. The total phenolic levels varied from 144 to 174 mg GAE/100 g and these values were significantly higher than those found in the free fraction. Lopez-Martinez et al. (2009) also applied an alkaline hydrolysis using 2 M NaOH for the quantification of TPC in orange corn and found comparable results (175 mg GAE/100 g) to those obtained in current study.

Several studies indicate that bound phenolic in cereals such as corn are in greater proportion than phenolic found in the free fraction. This bound phenolic fraction represents over 70% of the TPC and consists of phenolic acids, mainly cinnamic acid derivatives which are covalently bound to cell wall polysaccharides by UV-catalyzed cycloaddition or by coupling reactions by cell wall or intracellular oxidases (Cabrera-Soto et al., 2009). Montilla et al. (2011) pointed out that alkaline conditions favor the extraction of phenolic compounds linked to cell wall. Results from this study indicate that bound phenolic extracted after an alkaline hydrolysis represents the major phenolic fraction in the Cristalino Chileno corn accession; therefore, a proper quantification of phenolic compounds from this fraction is essential for further studies to understand the potential health-relevant properties associated to corn consumption. Bound phenolic compounds, specifically phenolic acids, may be released after colonic microbial fermentation thus potentially exerting important site-specific health benefits at colon level (Chandrasekara & Shahidi, 2011).

The analysis of variance (ANOVA) (Table 4) indicated that both evaluated factors (alkali concentration and time of extraction) had a significant quadratic effect (p < 0.05) on the TPCs. The model had a lower coefficient of determination for the fit (R² = 0.7813) than the obtained for the free phenolic extraction and showed a standard error of estimation of 6.4194. No autocorrelation in the residuals at 5% significant level was observed (Durbin–Watson test with p > 0.05). However, the lack of fit was significant (p < 0.05). Therefore, based on overall statistical parameters, it is not possible to obtain a proper prediction model for the extraction of TPC from the bound fraction. Nevertheless, the model shows a clear tendency about the area where the estimated values of TPC would be.

DPPH radical scavenging capacity

The AC of the bound fraction is shown in Table 2 and ranged from 600 ± 15 to 699 ± 9 µmol TE/100 g. These levels were significantly higher than those determined in the free fraction and this may be related to its high TPC as shown above. Best results were also observed at the midpoints of the experimental design with an average of 695 ± 3 µmol TE/
100 g. Xu et al. (2010) indicated that in mature corn grains, AC based on the DPPH-free radical inhibition is higher in the bound phenolic fractions than in the other fractions (20.7%, 31%, 82% and 90.2% for soluble ester-bound phenolic, free phenolic, insoluble cell-wall-bound phenolic and bound phenolic, respectively). This difference may be related to the high contents of specific phenolic acids such as ferulic and p-coumaric acids linked to the bound phase (Lopez-Martinez et al., 2009; Zilić et al., 2012).

The model for the AC showed a low value of coefficient of determination for the fit with $R^2 = 0.7904$ and a standard error of estimation of 20.7684 (Table 4). The analysis of variance revealed that the alkali concentration and time of extraction had a significant quadratic effect ($p < 0.05$) on the AC results. Since the $p$ value of the Durbin–Watson test was not significant ($p > 0.05$), there is no indication of autocorrelation in the residuals at the 5% significant level. However, the lack of fit of the model was significant ($p < 0.05$). Similar to the case of the TPC results, the overall statistical parameters indicate that no prediction model is possible to obtain for the AC from the bound fraction.

### Multiple optimizations of the extraction process and comparison of predicted values with experimental data

Experimental data from the free phenolic corn fraction were evaluated through a RSM to find the independent variable combinations that allow conditions for optimum-free phenolic acids extraction and highest AC. Therefore, a multiple optimization of response variables through the maximization of a desirability function (D) was used.

The estimated response surface showing the effects of solvent (acetone) concentration and time of extraction on the desirability function for optimizing extraction conditions from the free phenolic acid fraction in corn is shown in Figure 1. Factor combinations that maximized the desirability function were acetone 69% (solvent concentration) and 63 min (time of extraction). At this optimal point, the predicted TPC and AC values were 63.1 mg GAE/100 g and 193 µmol TE/100 g, respectively.

In case of the bound phenolic fraction, the selection of parameters for the extraction of bound phenolic acids was based on conditions that allowed the highest TPC and AC since no prediction models were possible to obtain based on TPC and AC results. According to the applied experimental design, an alkaline hydrolysis with 3 M NaOH for 90 min (conditions at midpoint) was selected for the extraction of phenolic acids from the bound fraction. At this point, the TPC and AC were 172.9 ± 1.1 mg GAE/100 g and 694.5 ± 3.3 µmol TE/100 g, respectively.

For both extractions, the temperature was not considered as a factor for optimization purpose since other authors found no significant effect on temperature extraction (Lee et al., 2013; Sarkis, Michel, Tessaro, & Ferreira Marczak, 2014).

### Validation of the optimum or best point

To validate the adequacy of the model equation for the free phenolic fraction, experiments in triplicate were performed at the optimal conditions. The TPC and AC were 59.9 ± 0.7 mg GAE/100 g and 186 ± 3 µmol TE/100 g, respectively. The difference between the values obtained by the model and experimentally was lower than 5.3%. These results were acceptable for validation purposes (Sarkis et al., 2014).

### HPLC analysis of extracts obtained at optimal or best conditions

The phenolic profile of the free phenolic fraction obtained at the optimized extraction conditions and phenolic profiles of the bound fraction obtained at the midpoint of the experimental design was determined by HPLC. Both free and bound fractions had different phenolic acid profiles and the most abundant phenolic acids were found in the bound form (Figure 2). Phenolic acids such as vanillin (0.35 ± 0.01 mg/100 g wet basis and 0.37 ± 0.01 mg/100 g dry basis),vanillic acid (0.73 ± 0.01mg/100 g wet basis and 0.77 ± 0.01 mg/100 g dry basis) and protocatechuic acid (0.42 ± 0.03 mg/100 g wet basis and 0.44 ± 0.03 mg/100 g dry basis) were detected. In addition, p-coumaric acid (0.66 ± 0.01 mg/100 g wet basis and 0.70 ± 0.01 mg/100 g dry basis) and ferulic acid (0.48 ± 0.01 mg/100 g wet basis and 0.50 ± 0.01 mg/100 g dry basis) were also found in the free fraction. These results are comparable to those found by Hu and Xu (2011) where ferulic acid (0.41–0.89 mg/100 g dry basis), vanillic acid (0.24–0.32 mg/100 g dry basis) and pro-

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**Figure 1.** Estimated response surface showing the effects of solvent (acetone) concentration and time of extraction on the desirability function for optimizing phenolic acids extraction from the free fraction in corn.

**Figura 1.** Superficie respuesta estimada mostrando los efectos de la concentración de solvente (acetona) y tiempo de extracción en la función de deseabilidad para optimizar la extracción de ácidos fenólicos libres en maíz.

**Figure 2.** HPLC phenolic acids profile (Cristalino Chileno, CHZM 13005): (a) free phenolic fraction and (b) bound phenolic fraction. 1: Protocatechuic acid; 2: vanillic acid; 3: vanillin; 4: p-coumaric acid and 5: ferulic acid.

**Figura 2.** Perfil HPLC de ácidos fenólicos (Cristalino Chileno, CHZM 13005): (a) fracción de ácidos fenólicos libres y (b) fracción de ácidos fenólicos ligados. 1: ácido protocatecuico; 2: ácido vanílico; 3: vanilina; 4: p-coumarico; y 5: ácido ferúlico.
tocatechuic acid (0.13–0.60 mg/100 g dry basis) were detected in several colored corn samples. However, they reported lower contents of p-coumaric acid (0.03 mg/100 g dry basis) than those found in this study.

Major phenolic acids in bound fractions were hydroxycinnamic acids such as p-coumaric acid and ferulic acid. Bound ferulic acid content was 191 ± 6 mg/100 g wet basis and 200 ± 6 mg/100 g dry basis meanwhile p-coumaric acid level was 19.6 ± 0.4 mg/100 g wet basis and 20.5 ± 0.4 mg/100 g dry basis. Guo and Beta (2013) reported lower contents of ferulic acid (138 mg/100 g dry basis) in the bound fraction and comparable results (133–298 mg/100 g dry basis) were obtained by Montilla et al. (2011).

Total bound phenolic acid contents obtained by calculating the sum of all detected phenolic acids were 211 ± 6 mg/100 g. This value is slightly higher than the bound TPC analyzed by the Folin–Ciocalteu method (176 ± 3 mg GAE/100 g) confirming that corn phenolic compounds are constituted by bound phenolic acids in evaluated corn accession.

Conclusions

Extraction conditions of free phenolic acids in Chilean Cristalino corn (Zea mays L.) accession were optimized to obtain the highest values of health-relevant bioactive phe- nolic compounds and related AC by using a RSM. According to the statistical analysis of all data, the optimum extraction conditions that maximized the TPC and AC in the free fraction were the use of acetone at 69% (v/v, in distilled water) together with a 63 min of extraction time. No RSM was possible to apply to the bound phenolic fraction data under conducted experimental design; however, the highest values of TPC and AC were observed with 3 M NaOH for 90 min of hydrolysis.

Current study revealed that higher values of TPC and DPPH radical-linked AC were found in the bound fraction and that the Cristalino Chileno INIA CHZM 13005 corn accession may be a potential source of important functional phenolic compounds. Finally, results from current study represent the first stages for further in-depth investigations of several Chilean corn accessions focused on the characterization of bioactive composition and further analysis of their health-relevant functional properties.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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