Extraction and Fractionation of Phenolic Acids and Glycoalkaloids from Potato Peels Using Acidified Water/Ethanol-based Solvents

Alma Fernanda Sanchez Maldonado[1], Elizabeth Mudge[1], Michael G. Gänzle[1], Andreas Schieber[1,2*]

[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, 4-10 Agriculture/Forestry Centre, Edmonton, AB T6G 2P5, Canada

[2]Institute of Nutritional and Food Sciences, Chair of Food Technology and Food Biotechnology, University of Bonn, Römerstrasse 164, D-53117 Bonn, Germany

*Corresponding author:
Prof. Dr. Andreas Schieber
Institute of Nutritional and Food Sciences
Chair of Food Technology and Food Biotechnology
University of Bonn
Römerstrasse 164
D-53117 Bonn, Germany
Phone: +49-228-73-4452
Fax: +49-228-73-4429
E-Mail: Schieber@uni-bonn.de

Abstract

Potato processing generates potato peels as byproducts. Methanolic extracts from the peels result in mixtures of phenolic acids and glycoalkaloids. Phenolic acids have potential for food applications owing to their antioxidant and antibacterial properties. However, when extracted from potatoes, their separation from toxic glycoalkaloids is needed prior to their applications in foods. Moreover, glycoalkaloids may be used as feedstock for synthesis of pharmaceuticals. This study aimed to develop a method for the extraction and fractionation of phenolic acids and glycoalkaloids from potato peels using food grade water/ethanol-based solvents. Samples were analyzed by ultrafast liquid chromatography (UFLC) and/or ultrafast liquid chromatography-mass spectrometry (UFLC-MS). A methanol-based solvent for extraction was used as a control to be compared with two aqueous ethanolic solvents acidified with acetic acid. The recovery of the predominant compounds from potato peels was comparable for all three solvents. Extraction yielded per 100 g of potato peel fresh weight 17.0 mg α-chaconine, 7.1 mg α-solanine, 0.1 mg solanidine, 4.8 mg caffeic acid, 13.3 mg neochlorogenic acid acid, and 77.6 mg chlorogenic acid. More than 90% of these compounds were recovered after two consecutive extractions. The crude extract was fractionated by solid-phase extraction at pH 7 and eluted with aqueous ethanol. Quantitative recovery of the phenolic acids and glycoalkaloids was achieved in their corresponding fractions. Hydrolysis followed by solid-phase fractionation of the crude extract allowed recovery of 139 μmol caffeic acid /100 g potato peel fresh weight. Partial degradation of caffeic acid and glycoalkaloids occurred during the process. Degradation of caffeic acid can be likely mitigated by the addition of antioxidants and metal chelators. The method developed in this study allows the sustainable recovery of secondary plant metabolites from potato peels and their fractionation using food grade water/ethanolic solvents for application of phenolic extracts free of toxic glycoalkaloids for food preservation, and of glycoalkaloid extracts for synthesis of pharmaceuticals.

Keywords

Potato peels, phenolic acids, glycoalkaloids, water/ethanol-based extraction, solid-phase extraction, UFLC-MS

1. Introduction

Potatoes (*Solanum tuberosum* L.) are among the most important staple crops consumed by humans (Mattila & Hellstrom, 2007). The production of value-added potato products has increased to satisfy the demand of consumers for convenience foods, whereas fresh potato consumption is continuously decreasing. Processing leads to the production of significant amounts of waste (FAO, 2008; Schieber & Aranda Saldaña, 2009). Processed potato products account only for 50 to 60% of the raw material. The byproducts include cull potatoes and processing waste (Charmley, Nelson, & Zvomuya, 2006). Peels constitute the main fraction of the processing waste. While considered waste, potato peels also contain valuable components (Mäder et al., 2009). Phenolic compounds and glycoalkaloids are particularly interesting because they are suitable for application in the food and pharmaceutical industries after extraction and purification (Schieber & Aranda Saldaña, 2009; Mäder, Rawel & Kroh, 2009).

Phenolic acids are the main phenolic compounds in potatoes (Schieber & Aranda Saldaña, 2009; Mäder et al., 2009; Singh and Saldana, 2011). They have shown antioxidant and antibacterial activities (Rodriguez de Soltillo, Hadley, & Wolf-Hall, 1998; Sánchez-Maldonado, Schieber, & Gänzle, 2011). Therefore, these compounds hold promise for application as preservatives in foods, feeds, and packing materials. Plant extracts containing phenolic acids were suitable as food preservatives (Corrales, Han, & Tauscher, 2009; Ejechi & Akpomedaye, 2005; Elegir, Kindl, A., Sadocco, & Orlandi, 2008). However, chlorogenic acid constitutes 90% of the phenolic compounds in potato peels (Im et al., 2008; Schieber & Aranda Saldaña, 2009). Chlorogenic acid exists in the form of three main isomers, which include chlorogenic acid (5-*O*-caffeoylquinic acid), neochlorogenic acid (3-*O*-caffeoylquinic acid) and cryptochlorogenic acid (4-*O*-caffeoylquinic acid) (Lee & Finn, 2007; Nandutu, Clifford, & Howell, 2007; Shui, Leong,

& Wong, 2005). Chlorogenic acid isomers do not have strong antibacterial activity but can be hydrolysed to quinic and caffeic acids (**Fig. 1**). Caffeic acid shows antimicrobial activity against gram positive and gram negative bacteria at concentrations ranging from 0.1 – 1 g / L (Rodriguez de Soltillo et al., 1998; Sánchez-Maldonado et al., 2011). Quinic acid, the second product of chlorogenic acid hydrolysis, is a starting material for the synthesis of drugs such as Oseltamivir for influenza treatment (Yeung, Hong, & Corey, 2006).

Glycoalkaloids are plant steroids that contain nitrogen and a sugar moiety attached to the 3-OH position (**Fig. 2**). α-Chaconine and α-solanine are the main glycoalkaloids found in potatoes (Friedman, 2004). They are suitable for utilization in pharmaceutical industry. The aglycone solanidine is an intermediate for the synthesis of hormones such as progesterone and cortisone derivatives (Nikolic & Stankovic, 2003). Additionally, glycoalkaloids and their aglycones have been shown to possess anti-allergic, antipyretic, anti-inflammatory, hyperglycemic, and antibiotic properties (Friedman, 2006). Furthermore, potato glycoalkaloids have antifungal activities (Fewell & Roddick, 1993; Fewell & Roddick, 1997). However, they are toxic for humans and should be absent in potato products or potato extracts used for food applications (Rodriguez-Saona, Wrolstad & Pereira, 1999). For fresh potatoes, a maximum of 200 mg of glycoalkaloids per kilogram is acceptable for human consumption (Fewell & Roddick, 1993; Friedman, 2006).

Conventional methods for the extraction of phenolic compounds from plant material use organic solvents such as methanol, acetone, ethanol, and ethyl acetate (Dai & Mumper, 2010). Glycoalkaloids from potatoes are traditionally extracted with chloroform/methanol mixtures (Bushway & Ponnampalam, 1981; Friedman, Roitman & Kozukue, 2003). These methods are detrimental for the environment. Water and ethanol are alternatives for the recovery of phenolic

compounds from potato peels, facilitating food applications (Kannat, Chander, Radhakrishna, & Sharma, 2005; Onyeneho & Hettiarachchy, 1993; N. Singh & Rajini, 2004). Water/acetic acid mixtures have been used to extract glycoalkaloids (Friedman, Roitman & Kozukue, 2003; Machado, Toledo & Garcia, 2007; Sotelo & Serrano, 2000). However, to our knowledge there is no method for the simultaneous recovery and subsequent separation of phenolic acids and glycoalkaloids to obtain food grade phenolic extracts free of toxic glycoalkaloids and the corresponding glycoalkaloids fraction for pharmaceutical purposes. In addition, recovery of these compounds from potato peels using food grade solvents would be an advantage for the food industry since it reduces the organic waste that causes disposal problems (Kim & Kim, 2010) and minimizes the environmental impact of toxic solvents. Therefore, this study aimed to develop a sustainable method for the simultaneous extraction of these compounds from potato peels using food grade acidified water/ethanol based solvents. Furthermore, experiments aimed to achieve separation of polyphenols and glycoalkaloids from potato peels to allow applications of both fractions in the food and pharmaceutical industries, respectively.

2. Materials and methods

2.1. External standards

Chlorogenic acid (5-*O*-caffeoylquinic acid) and caffeic acid were purchased from Sigma (St. Louis, MO, USA). α-Chaconine, α-solanine and solanidine were obtained from Extrasynthese (Genay, France).

2.2. Extraction of potato peels

Potatoes from the cultivar ´Russet´ purchased in a local grocery store in Edmonton, Alberta, Canada were used for this study. After manual peeling, 30 g of fresh peels were simultaneously crushed and mixed with 75 mL of extraction solvent in a domestic blender. Peels

and solvent were left in the dark for 30 minutes, stirred for an additional 30 min, sonicated for 20 minutes, and centrifuged at 4696 *g*. The supernatant was recovered and filtered. Extraction was performed three times per batch and samples from each extraction were collected. Three different solvents were used for extraction; acetic acid was used to equal the pH to that of the control solvent (3.2). Solvent A contained 25% water, 70% methanol, and 5% acetic acid; solvent B contained 24% water, 67% ethanol, and 9% acetic acid; solvent C contained 46% water, 51% ethanol and 3% acetic acid. The organic solvent was evaporated under vacuum at 40 °C using a Rotavapor RE21 (Büchi, Flawil, Switzerland). The dry potato peel extract was re-suspended in 15 mL of water. A 40 mg/L standard solution of chlorogenic acid was extracted under the same conditions as the potato peels in order to evaluate stability of chlorogenic acid during the process.

2.3. Fractionation of phenolic acids and glycoalkaloids by solid-phase extraction

Phenolic acids were fractionated from the glycoalkaloids using a Sep Pak Vac 6 cc C18 cartridge. Solvents and water were adjusted to pH 7. Prior to use, the column was conditioned by elution with 5 mL of ethanol followed by 5 mL of water. Two mL of the extract previously re-suspended in water was passed through the column and washed with 5 mL of water (pH 7). Subsequently, 20 mL of the corresponding solvent was added, phenolic acids were eluted with water/ethanol (80:20, v/v) and glycoalkaloids were eluted with water/ethanol (20:80, v/v). To determine the volume of solvent required for complete elution, the fractions were collected successively in 2 mL tubes, and the concentration of phenolic acids and glycoalkaloids was determined subsequently.

2.4. Alkaline hydrolysis of chlorogenic acid

Three mL of the extract obtained from solvent C, previously dissolved in 15 mL of water, was centrifuged and the supernatant was mixed with 750 µL of NaOH solution (10 M) and flushed under nitrogen for 2 min. The vial was hermetically closed and the solution stirred for 4 hours at room temperature. Subsequently, the solution was adjusted to pH 4 with HCl and used for fractionation as described in section 2.3. To evaluate whether alkaline hydrolysis results in the loss of caffeic acid, a 40 mmol/mL standard solution of chlorogenic acid was subjected to alkaline hydrolysis under the same conditions as previously mentioned.

2.5. Quantification of phenolic acids and glycoalkaloids

The separation and quantification of phenolic compounds from potato peels was performed using an ultrafast liquid chromatography (UFLC) system consisting of a LC 20 AD XR pump, SIL-20 AC XR Prominence autosampler, a Prominence column oven and a Prominence SPD-M20 diode array detector (Shimadzu, Kyoto, Japan). Separations were performed on a Kinetex PFP column (100 x 3.0 mm, 2.6 µm). The injection volume was 5 µL and the flow rate was 0.9 mL/min. The temperature of the oven was 25 °C. The mobile phase consisted of (A) 0.1% (v/v) formic acid in water and (B) 0.1% (v/v) formic acid in water/acetonitrile (10:90), according to the method of Cruz, Novak, and Strnad (2008). The gradient program was as follows: 0–20% B (0-1.5 min), 20% B (1.5-4.5 min), 20–90% B (4.5-7.5 min), 90% B (7.5-8 min) and 90–0% B (8-14 min). Phenolic acids were detected at 280 and 320 nm. Quantification of chlorogenic and caffeic acids was performed using external standards dissolved in a mixture of methanol/water/formic acid (80:20:0.1). Chlorogenic acid isomers, neochlorogenic acid and chlorogenic acid, were quantified based on the standard curve of chlorogenic acid. Calibration curves, with a correlation coefficient ≥ 0.99, were established using concentration ranges from 0.005 to 0.15 and from 0.01 to 0.32 g/L for caffeic acid and

chlorogenic acid, respectively. Fresh standard solutions were prepared on the same day of the analysis for each run.

Phenolic compounds in the extracts were characterized by ultrafast liquid chromatography-mass spectrometry (UFLC-MS) under the same LC conditions mentioned above. The UFLC system was coupled to an Applied Biosystems MDS SCIEX 4000 Q TRAP LC/MS/MS System (AB Sciex, Concord, Ontario, Canada) equipped with an ESI Turbo V™ source operating in negative mode with the pneumatically assisted electrospray probe using high-purity nitrogen gas (99.995%) as the nebulizing (GS1) and heating gas (GS2). The values for optimum spray voltage, source temperature, GS1, GS2, and curtain gases were -4 kV, 600 ºC, and 50, 30, and 25 psi, respectively. Identification of phenolic compounds was performed using an information-dependent acquisition (IDA) method, **enhanced mass spectrometry-enhanced** product ion (EMS-4EPI). Q1 and Q3 were operated at low and unit mass resolution. The spectra were obtained over a range from *m/z* 50 to 1300 in 2 s. LIT fill time was 20 ms. The IDA threshold was 100 cps. EPI spectra were collected from the eight most intense peaks above this parameter. The EPI scan rate was 1000 amu/s. Collision-induced dissociation (CID) spectra were acquired using nitrogen as the collision gas under two different collision energies. The collision energy (CE) was -20 eV and collision energy spread (CES) 0 eV. Declustering potential (DP), entrance potential (EP), and collision exit potential (CXP) were -70 V, -10 V and -7 V, respectively.

The analysis of glycoalkaloids was performed using the same UFLC-MS system described above, performed in positive MS mode. Quantification was done by MS using the Multiple Reaction Monitoring mode; **UFLC was only used to achieve separation**. A Kinetex C18 100A (100 x 3.0 mm, 2.6 μm) column was used as the stationary phase. The injection

194 volume was 5 μL and the flow rate 0.6 mL/min. The temperature of the oven was 25 °C. The
195 mobile phase consisted of (A) 0.5% (v/v) formic acid in water/acetonitrile (95:5) and (B) 0.5%
196 (v/v) formic acid in water/acetonitrile (5:95). The gradient was as follows: 20% B (0-12.5 min),
197 20-90% B (12.5-13.5 min), 90% B (13.5-14.5 min), 90-20% B (14.5-16 min) and 20% B (16-20
198 min). An information-dependent acquisition (IDA) method, MRM - EPI, was used to profile and
199 quantify the glycoalkaloids. Q1 and Q3 were operated at low and unit mass resolution. The
200 spectra were obtained over a scan range from *m/z* 50 to 1000 in 2 s. LIT fill time was set at 20
201 ms. The IDA threshold was set at 100 cps, above which enhance product ion spectra were
202 collected from the eight most intense peaks. For the MRM the values for optimum spray voltage,
203 source temperature, GS1, GS2, and curtain gases were +4.5 kV, 600 ºC, 60, 45, and 15 psi,
204 respectively. The MRM scan rate was 1000 amu/s. Optimization of DP, EP, CE and CXP was
205 done specifically for each transition and the values used were in the range of 55-70 V, 8-14 V,
206 60-100 eV and 10-40 V, respectively. The two most abundant transitions for each compound
207 were selected (Q1→Q3), for quantification and confirmation. For α-chaconine, α-solanine and
208 solanidine the transitions for quantification were (Q1 852→Q3 706), (Q1 868→Q3 398) and (Q1
209 398→Q3 98), respectively. For the EPI, the scan rate was 4000 amu/s and the values for
210 optimum spray voltage, source temperature, GS1, GS2, and curtain gases were +5 kV, 600 ºC,
211 50, 30, and 10 psi, respectively. Standard solutions dissolved in methanol/water/formic acid
212 (80:20:0.1) were used for the calibration curves that gave a correlation coefficient ≥ 0.99. The
213 concentration ranges were 100 to 10000 ppb for α-chaconine, and 50 to 5000 ppb for both α-
214 solanine and solanidine.

215 The limits of detection (LOD) and quantitation (LOQ) of phenolic acids and
216 glycoalkaloids were determined according to the International Conference on Harmonization

(ICH) (Chandran & Singh, 2007; Nandutu et al., 2007) as LOD=3σ/S and LOQ=10σ/S, where σ is the standard deviation of response and S is the slope of the calibration curve. The LOD and LOQ of chlorogenic acid were determined as 0.36 and 1.20 ng/L, respectively. The LOD and LOQ for caffeic acid were 0.16 ng/L and 0.55 ng/L, respectively. The LOD for α-chaconine, α-solanine and solanidine were 3.22, 5.42 and 0.01 µg/L, respectively. The respective LOQ were 10.7, 18.07 and 0.033 µg/L, in the same order. Data are reported as means ± standard deviations of triplicate independent experiments.

2.6. Quantification of quinic acid

After alkaline hydrolysis, quinic acid was quantified according to the method for organic acids published by Teixeira, McNeill and Gänzle (2012), using an Agilent 1200 series HPLC unit comprising of a degasser, binary pump, autosampler, thermostated column compartment, and diode array detector (Agilent Technologies, Palo Alto, CA, USA). Separation was performed using an Aminex HPX-87 column (Bio-Rad, Mississauga, ON, Canada) at 70 ºC. Quinic acid was detected at 210 nm. Isocratic elution with a flow rate of 0.4 mL/min during 60 min was used. The solvent consisted of 5 mM H_2SO_4. No peaks were detected, indicating that the amount of quinic acid in the samples was below the detection and quantification limits of the method. For the standards, only concentrations above 1 mmol/L were detected.

2.7. Statistical Analysis

Data are reported as means ± standard deviations of triplicate independent experiments. SigmaPlot software (Systat Software, Inc., San Jose, CA, USA) was used to perform all statistical analyses. To determine statistically significant differences between the three extraction methods, data were subjected to two-way analysis of variance (ANOVA). For the rest of the experiments, the recovery of each compound was statistically analyzed by one-way ANOVA

followed by the Holm–Sidak method for multiple pairwise comparisons when required. For all analyses statistical significance was based on $P < 0.05$.

3. Results

3.1. Extraction of potato peels using three different solvents

Three solvents were tested for the extraction of phenolic acids and glycoalkaloids from fresh potato peels to compare their recovery using acidified aqueous methanol and ethanol-based mixtures. There was no significant difference between the phenolic compounds and glycoalkaloids extracted from potato peels with any of the solvents (**Fig. 3**). To evaluate possible hydrolysis of chlorogenic acid into caffeic acid during the extraction, a standard solution of chlorogenic acid was extracted using the same conditions as for potato peels (**Fig. 3(1)).** No hydrolysis into caffeic acid was observed and the extraction efficiency was higher than 99%.

UFLC-MS analysis of constituents showed four main phenolic compounds and three alkaloids in the potato peel extract **(Table 1).** Chlorogenic and caffeic acids and the three alkaloids were identified using standards. Mass spectra of the three first peaks in the phenolics extract matched those of caffeic acid, chlorogenic acid and neochlorogenic acid. Their maximum absorption wavelength was 324, 326 and 322 nm, respectively, which is typical for hydroxycinnamates (Nandutu et al., 2007). Chlorogenic acid and neochlorogenic acid were distinguished by the order of elution under reversed-phase HPLC conditions and peak intensity as previously reported by (Clifford, Johnson, Knight, & Kuhnert, 2003; Matsui et al., 2007; Nandutu et al., 2007). The fourth compound with a mass spectrum showing *m/z* 529 as parent ion and base peak, and a maximum wavelength of 322 nm, might correspond to a caffeoylferuloylquinic acid (Nandutu et al., 2007). However, no fragmentation was observed to

support the identity of this compound. The glycoalkaloids were identified as α-chaconine, α-solanine and solanidine.

3.3. Recovery of phenolic acids and glycoalkaloids from potato peels

The amount of neochlorogenic, chlorogenic and caffeic acids recovered from potato peels was 13.3, 77.6, and 4.8 mg/100 g of potato peel fresh weight, respectively. The recovery of α-chaconine, α-solanine and solanidine was 17.0, 7.1 and 0.1 mg/100 g of potato peel fresh weight, respectively. The recovery was calculated as average of the yield obtained with three different solvents.

3.4. Consecutive extractions of bioactive metabolites from potato peels

To determine how many extraction steps are needed for the quantitative recovery of secondary metabolites from potato peels, three consecutive extractions were performed with the same batch of fresh potato peels. Samples from each extraction were collected and investigated by UFLC-MS (**Fig. 4**). After the second extraction, 97% of chlorogenic acid, 94% of neochlorogenic acid and 89% of caffeic acid were recovered. The recovery of α-chaconine and α-solanine after the second extraction was higher than 99%. In addition, 95% of solanidine were recovered after the second extraction.

3.5. Fractionation of phenolic acids and glycoalkaloids by solid-phase extraction

To accomplish fractionation of phenolic acids and glycoalkaloids from the potato peels extract, solid-phase extraction with a Sep Pak Vac 6cc C18 cartridge was used. The extract obtained using solvent C was employed for this purpose (**Fig. 5**). Statistical analysis showed that the solid-phase extraction achieved quantitative recovery of chlorogenic acid. However, there was a significant difference between the amounts of neochlorogenic acid and caffeic acid before

and after fractionation. The amount of neochlorogenic acid decreased, while caffeic acid increased.

For complete elution, phenolic compounds and glycoalkaloids required 10 and 8 mL of solvent, respectively (**Fig. 6**). The fractionation allowed complete recovery of glycoalkaloids and no hydrolysis to solanidine was observed. The concentration of glycoalkaloids in the fraction containing phenolic acids was below the detection limit of 3.2, 5.4 and 0.01 µg/L of α-chaconine, solanine and solanidine, respectively. *Vice versa*, the concentrations of phenolic acids in the glycoalkaloids fraction were below their respective detection limits.

3.6. Alkaline hydrolysis of the potato peel extract followed by fractionation of phenolic acids and glycoalkaloids

The extract obtained from solvent C was subsequently subjected to alkaline hydrolysis and fractionated by solid-phase extraction. The crude extract, the hydrolyzed extract and the recovered fractions were analyzed by UFLC-MS (**Fig. 7**). The initial crude extract contained 226, 37 and 29 µmol/100 g of potato peel FW of chlorogenic, neochlorogenic and caffeic acids, respectively. After alkaline hydrolysis, no chlorogenic acid isomers were detected and the extract contained 179 µmol caffeic acid/100 g potato peel FW. After fractionation, 139 µmol caffeic acid/100 g potato peel FW were recovered. To evaluate the efficiency of the hydrolysis and the recovery after fractionation, the amounts in µmol/100 g potato peel FW of chlorogenic acid, neochlorogenic acid and caffeic acid present in the initial extract were summarized and compared to the yield of caffeic acid after hydrolysis and after hydrolysis and fractionation. There was a significant difference between the sum of the three initial compounds and the yield of caffeic acids after hydrolysis. However, no significant difference was observed between caffeic acid after hydrolysis and after hydrolysis and fractionation. To evaluate whether alkaline

hydrolysis results in the loss of caffeic acid, a chlorogenic acid standard was subjected to alkaline hydrolysis (**Fig 7(1)).** After hydrolysis, 44% of the molar concentration of chlorogenic acid was recovered as caffeic acid, indicating high losses of caffeic acid during hydrolysis.

Following alkaline hydrolysis, the amount of α-chaconine and α-solanine significantly decreased to about 50% of their initial quantity (**Fig. 7**). Moreover, no solanidine was detected in the hydrolyzed extract, indicating not only hydrolysis but also degradation of these alkaloids. However, the recovery of glycoalkaloids did not change significantly between after hydrolysis and after hydrolysis and fractionation.

3.7. Purity of extracts

To obtain an approximation of the purity of the extracts regarding the amount of the phenolic compounds before and after hydrolysis, the percentages of the compounds were calculated related to the total peak area of the chromatograms at 280 nm and 210 nm. These wavelengths were used because a wide range of compounds show absorbance there. Before alkaline hydrolysis, the summarized amounts of neochlorogenic acid, chlorogenic acid and caffeic acid accounted for 75% and 69% of the material absorbing at 280 nm in the crude extract and phenolic acids fraction, respectively. In the hydrolyzed phenolic acids fraction, caffeic acid accounted for 80% of the UV absorbance at 280. At 210 nm and before alkaline hydrolysis, neochlorogenic acid, chlorogenic acid and caffeic acid together accounted for 74% and 59% in the crude extract and the phenolics fraction. After alkaline hydrolysis, caffeic acid in the phenolic acids fraction was equivalent to 72% of the total material absorbing at 210 nm.

4. Discussion

This study compared the recovery of phenolic acids and glycoalkaloids from fresh potato peels comparing a water/methanol-based solvent and two water/ethanol-based solvents.

Additionally, fractionation of phenolic acids and glycoalkaloids was achieved with solid-phase microextraction and water/ethanol-based solvents. The recovery of phenolic acids without modification or after alkaline hydrolysis of chlorogenic acid isomers into caffeic acid was determined.

The three solvent systems resulted in comparable recoveries of bioactive compounds. Among the solvents that were evaluated in this study, solvent C with the highest proportion of water is the most environmentally benign and least costly alternative. The recovery of chlorogenic, neochlorogenic and caffeic acids reported in this study is two- to threefold higher compared to literature data. (Rodriguez de Soltillo, Hadley, & Holm, 2006) found 24-30 mg/100 g of chlorogenic acid and 1.4-2.7 mg/100 g of caffeic acid from fresh potato peels and also reported the presence of protocatechuic and gallic acids. (Mattila & Hellstrom, 2007) detected mainly chlorogenic acid (15 to 26 mg/100 g) and caffeic acid (4.1 mg/100 g to 4.4 mg/100 g) from fresh potato peels. Because the profile and quantity of phenolic compounds varies with the plant source, variety, season, climate, and several other factors, these differences likely represent different levels of phenolic compounds in the raw material used. The recovery of glycoalkaloids obtained in this study (**Fig. 3**) is well in agreement with previous studies, which achieved between 0.9 to 37 mg/100 g of α-chaconine and from 0.4 to 17 mg/100 g of α-solanine in fresh potato peels (Friedman, Roitman, & Kozukue, 2003).

Consecutive extractions of potato peels revealed that 97%, 94 and 89% of chlorogenic acid, neochlorogenic acid, and caffeic acid, respectively, were recovered after the second extraction, indicating that the extraction is more efficient for chlorogenic acid than for caffeic acid. Higher amounts of caffeic acid in the third extraction are not likely to be resultant of the hydrolysis of bound phenolic components to hydroxycinnamates as reported by Nara, Miyoshi,

354 Honma & Koga (2006), since no hydrolysis was observed when a chlorogenic acid standard was
355 extracted under the same conditions as the samples (**Fig 3(1)**). Therefore, the higher efficiency
356 for extraction of chlorogenic acid is attributable to the higher capability of the solvents to
357 dissolve this compound, which is more polar than caffeic acid. Between 95 and 99% of all
358 glycoalkaloids were extracted after the second extraction. This indicates that in general, two
359 consecutive extractions are sufficient for recovery of more than 90 % of the secondary
360 metabolites from potato peels.

361 Alternative procedures for the extraction of bioactive compounds from plants include
362 subcritical water extraction, which eliminates the need for organic solvents. Subcritical water
363 was used to extract phenolic compounds from bitter melon (Budrat & Shotipruk, 2009),
364 rosemary plants (Ibañez et al., 2003) and oregano (Rodriguez-Meizoso et al. 2006). This process
365 employs high pressure and high temperature and thus accelerates chemical reactions including
366 the release of bound phenolic compounds and the degradation of caffeic and chlorogenic acid.
367 (Singh & Saldaña, 2011) compared the recovery and profile of phenolic acids extracted from
368 potato peels using subcritical water extraction to the recovery achieved with methanol extraction.
369 The total amount of phenolic acids obtained from subcritical water extraction was approximately
370 twofold higher compared to the methanol extracts and ethanol extracts. However, the recovery of
371 chlorogenic and caffeic acids with subcritical water was only 50% and 75%, respectively,
372 compared to methanol extraction; subcritical water extracted hydroxybenzoic acids which were
373 not recovered with methanol. Similarly, catechin was extracted from bitter melons with
374 subcritical water but not with solvent extraction (Budrat & Shotipruk 2009). Our study
375 additionally demonstrates that the use of acidified ethanolic solvents avoids side reactions and
376 the resulting extract is relatively pure and stable. As shown by purity analysis, the methods

377 utilized in this study generate relatively pure mixtures, consisting mainly of chlorogenic and
378 caffeic acids. A major advantage of the method developed in this study compared to subcritical
379 water extraction is the low cost, since sophisticated equipment is not required. The use of
380 acidified ethanolic solvents was also shown to allow high yields of phenolic compounds from
381 onion waste (Khiari, Makris & Kefalas, 2009).
382 Solid-phase extraction of the crude extract containing phenolic compounds and glycoalkaloids
383 allowed separation and complete recovery of all target compounds. Fractionation was carried out
384 at pH 7, which may account for the slight increase in caffeic acid after fractionation; ester
385 hydrolysis occurs faster under alkaline conditions (Kim, Tsao, Yang & Cui, 2006).
386 Glycoalkaloids were stable during fractionation. Previous attempts to separate glycoalkaloids
387 and phenolic acids from potato peel extract by alkaline precipitation resulted in degradation of 30
388 % of phenolic compounds and 90% of glycoalkaloids (Rodriguez-Saona, Wrolstad & Pereira,
389 1999). In addition, the amount of solvent required for elution of both fractions is relatively small
390 and comparable with other protocols carried out with acetonitrile (Machado, Toledo & Garcia,
391 2007; Abreu, Relva, Matthew, Gomes & Morais, 2007). Therefore, solid-phase extraction
392 performed in this study is a significant improvement in the recovery of phenolic compounds and
393 glycoalkaloids as separate fractions.
394 Alkaline treatment of the crude extract achieved virtually quantitative hydrolysis of
395 chlorogenic and neochlorogenic acids. However, the yield of caffeic acid was only 57%.
396 Degradation of caffeic acid was also observed during alkaline hydrolysis of a standard in the
397 same conditions as performed for the extract. Although alkaline hydrolysis is a common method
398 for the determination of bound phenolic acids (Kim, Tsao, Yang & Cui, 2006; Mattila &
399 Kumpulainen, 2002), degradation of more than 50% of caffeic acid during hydrolysis of

chlorogenic acid has been reported (Krygier et al. 1982; Maillard & Berset, 1995; Nardini et al. 2002). Under alkaline conditions, *o*-dihydroxy benzenes are oxidized to their corresponding quinones when oxygen is present. The degradation of caffeic acid during alkaline hydrolysis can be mitigated by the addition of antioxidants such as ascorbic acid, or by chelating metal ions with EDTA (Nardini et al., 2002). Enzymatic hydrolysis with bacterial esterases is also an alternative to increase caffeic acid recovery. Lactobacilli have the strain-specific capacity to hydrolyze chlorogenic acid (Rodriguez de Soltillo et al., 1998; Sánchez-Maldonado et al., 2011) and hydroxycinnamoyl esterases of lactic acid bacteria were recently characterized (EstebanTorres, Reveron, Mancheno, de las Rivas & Munoz, 2013).

Glycoalkaloids and solanidine were also degraded during alkaline hydrolysis. However, all glycoalkaloids present in the hydrolyzed extract were recovered using solid-phase extraction, indicating no degradation at pH 7. Rodriguez-Saona, Wrolstad and Pereira (1999) reported minimum precipitation of glycoalkaloids in a potato peel extract at pH 7 but increased precipitation above pH 8. Quantitative recovery of glycoalkaloids thus requires solid-phase extraction prior to alkaline hydrolysis of chlorogenic acid isomers.

Caffeic acid, a product of chlorogenic acid hydrolysis, has demonstrated substantial antimicrobial activity (Sánchez-Maldonado, Schieber, & Gänzle, 2011), and both chlorogenic and caffeic acids have been highly correlated to the antioxidant activity of potato peel extracts (Nara, Miyoshi, Honma & Koga, 2006). Therefore, due to their purity and stability, the phenolic acid fractions obtained in this study before or after hydrolysis can successfully be applied as food preservatives. In addition, solid phase fractionation provided a high recovery of glycoalkaloids from potato peels, allowing their utilization as raw materials in the pharmaceutical industry.

In conclusion, this study demonstrates that acidified ethanol-based solvents recover phenolic acids and glycoalkaloids from potato peels and are thus suitable alternatives to the use of environmentally harmful solvents. Simultaneous fractionation and hydrolysis of esterified phenolic acids was also achieved. However, use of antioxidants during alkaline hydrolysis or enzymatic hydrolysis should be considered to allow quantitative recovery of caffeic and quinic acids, and hydrolysis of phenolic acids after fractionation may avoid degradation of glycoalkaloids. Solid-phase extraction of phenolic acids and glycoalkaloids is a suitable method that will allow the use of phenolic acids extracts as food preservatives without any toxicological concerns, while recovered glycoalkaloids can be utilized for pharmaceutical purposes. Thus, this study provides a valuable contribution to sustainable production through utilization of by-products as a source of biologically active compounds.

Abreu, P., Relva, A., Matthew, S., Gomes, Z., & Morais, Z. (2007). High-performance liquid chromatographic determination of glycoalkaloids in potatoes from conventional, integrated, and organic crop systems. *Food Control, 18*, 40-44.

Budrat, P., & Shotipruk, A. (2009). Enhanced recovery of phenolic compunds from bitter melon *(Momordica charantia*) by subcritical water extraction. *Separation and Purification Technology, 66*, 125-129.

Bushway, R. J., & Ponnampalam, R. (1981). α-Chaconine and α-solanine content of potato products and their stability during several modes of cooking. *Journal of Agricultural and Food Chemistry, 29*, 814-817.

444 Chandran, S., & Singh, R. S. P. (2007). Comparison of various international guidelines for
445 analytical method validation. *Pharmazie, 62*, 4-14.

446 Charmley, E., Nelson, D., & Zvomuya, F. (2006). Nutrient cycling in the vegetable
447 processing industry utilization of potato by-products. *Canadian Journal of Soil Science, 86*,
448 621-629.

449 Clifford, M. N., Johnson, K. L., Knight, S., & Kuhnert, N. (2003). Hierarchical scheme for
450 LC-MS^n identification of chlorogenic acids. *Journal of Agricultural and Food Chemistry, 51*,
451 2900-2911.

452 Corrales, M., Han, J. H., & Tauscher, B. (2009). Antimicrobial properties of grape seed
453 extracts and their effectiveness after incorporation into pea starch films. *Journal of Food*
454 *Science and Technology, 44*, 425-433.

455 Cruz, J., Novak, O., & Strnad, M. (2008). Rapid analysis of phenolic acids in beverages by
456 UPLC-MS/MS. *Food Chemistry, 111*(3), 789-794.

457 Dai, J., & Mumper, R. S. (2010). Plant phenolics: Extraction, analysis and their antioxidant
458 and anticancer properties. *Molecules, 15*, 7313-7352.

459 Ejechi, B. O., & Akpomedaye, D. E. (2005). Activity of essential oil and phenolic extracts of
460 pepperfruit (*Dennettia tripetala* G. Baker; Annonaceae) against some food-borne
461 microorganisms. *African Journal of Biotechnology, 4*(3), 258-261.

462 Elegir, G., Kindl, A., Sadocco, P., & Orlandi, M. (2008). Development of antimicrobial
463 cellulose packing through laccase-mediated grafting of phenolic compounds. *Enzyme and*
464 *Microbial Technology, 43*, 84-92.

465 Esteban-Torres, M., Reveron, I., Mancheno, J. M., De las Rivas, B., & Munoz, R. (2013).
466 Characterization of a feruloyl esterase from *Lactobacillus plantarum*. *Applied and*
467 *Environmental Microbiology, 79*(17), 5130-5136.

468 FAO (2008). Retrieved November/11th, 2013,
469 from ftp://ftp.fao.org/docrep/fao/011/i0500e/i0500e02.pdf

470 Fewell, A. M., & Roddick, J. M. (1993). Interactive antifungal activity of the glycoalkaloids
471 α-solanine and α-chaconine. *Phytochemistry, 33*(2), 323-328.

472 Fewell, A. M., & Roddick, J. M. (1997). Potato glycoalkaloid impairment of fungal
473 development. *Mycological Research, 101*(5), 597-603.

474 Friedman, M. (2004). Analysis of biologically active compounds in potatoes (*Solanum*
475 *tuberosum*), tomatoes (*Lycopersicon esculentum*), and jimson weed (*Datura stramonium*)
476 seeds. *Journal of Chromatography A, 1054*(1-2), 143-155.

477 Friedman, M. (2006). Potato glycoalkaloids and metabolites: Role in the plant and in the
478 diet. *Journal of Agricultural and Food Chemistry, 54*, 8655-8681.

479 Friedman, M., Roitman, J. N., & Kozukue, N. (2003). Glycoalkaloid and calystegine contents
480 in eight potato cultivars. *Journal of Agricultural and Food Chemistry,51*, 2964-2973.

481 Ibañez, E., Kubátová, A., Señoráns, F. J., Cavero, S., Reglero, G., & Hawthorne, S. B.
482 (2003). Subcritical water extraction of antioxidant compounds from rosemary plants. *Journal*
483 *of Agricultural and Food Chemistry, 51*, 375-382.

484 Im, H. W., Suh, B. S., Lee, S. U., Kozukue, N., Ohnisi-Kameyama, M., Levin, C. E., &
485 Friedman, M. (2008). Analysis of phenolic compounds by high-performance liquid
486 chromatography and liquid chromatography/mass spectrometry in potato plant flowers,
487 leaves, stems, and tubers and in home-processed potatoes. *Journal of Agricultural and Food*
488 *Chemistry, 56*(9), 3341-3349.

489 Kannat, S., Chander, R., Radhakrishna, P., & Sharma, A. (2005). Potato peel extracts, a
490 natural antioxidant for retarding lipid peroxidation in radiation processed lamb meat. *Journal*
491 *of Agricultural and Food Chemistry, 53*, 1499-1504.

492 Khiari, Z., Makris, D. P., & Kefalas, P. (2009). An investigation on the recovery of
493 antioxidant phenolics from onion solid wastes employing water/ethanol-based solvent
494 systems. *Food and Bioprocess Technology, 2*, 337-343.

495 Kim, K. H., Tsao, R., Yang, R., & Cui, S. W. (2006). Phenolic acid profiles and antioxidant
496 activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry, 95*,
497 466-473.

498 Kim, M., & Kim, J. (2010). Comparison through a LCA evaluation analysis of food waste
499 disposal options from the perspective of global warming and resource recovery. *Science of*
500 *the Total Environment, 408*, 3998-4006.

501 Krygier, K., Sosulsky, F., & Hogge, L. (1982). Free, esterified and insoluble-bound phenolic
502 acids. 1. extraction and purification procedure. *Journal of Agricultural and Food*
503 *Chemistry, 30*, 330-334.

504 Lee, J., & Finn, C. E. (2007). Anthocyanins and other polyphenolics in American elderberry
505 (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. *Journal of the Science*
506 *of Food and Agriculture, 87*, 2665-2675.

507 Mäder, J., Rawel, H. & Kroh, L.W. (2009). Composition of phenolic compounds and
508 glycoalkaloids α-solanine and α-chaconine during commercial potato processing. *Journal of*
509 *Agricultural and Food Chemistry*, 57, 6292–6297.

510 Maillard, M. N., & Berset, C. (1995). Evolution of antioxidant activity during kilning: Role
511 of insoluble bound phenolic acids of barley and malt. *Journal of Agricultural and Food*
512 *Chemistry, 43*, 1789-1793.

513 Matsui, Y., Nakamura, S., Kondou, N., Takasu, Y., Ochiai, R., & Masukawa, Y. (2007).
514 Liquid chromatography-electrospray ionization-tandem mass spectrometry for simultaneous
515 analysis of chlorogenic acids and their metabolites in human plasma. *Journal of*
516 *Chromatography B, 858*, 96-105.

517 Mattila, P., & Hellstrom, J. (2007). Phenolic acids in potatoes, vegetables and some of their
518 products. *Journal of Food Composition Analysis, 20*, 152-160.

519 Mattila, P., & Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant
520 derived foods by HPLC with diode-array detection. *Journal of Agricultural and Food*
521 *Chemistry, 50*, 3660-3667.

522 Nandutu, A. M., Clifford, M., & Howell, N. K. (2007). Analysis of phenolic compounds in
523 Ugandan sweet potato varieties (NSP, SPK AND TZ). *African Journal of Biochemistry*
524 *Research, 1*(3), 29-36.

525 Nara, K., Miyoshi, T., Honma, T., & Koga, H. (2006). Antioxidative activity of bound-form
526 phenolics in potato peel. *Bioscience, Biotechnology and Biochemistry,70*(6), 1489-1491.

527 Nardini, M., Cirillo, E., Natella, F., Mencarelli, D., Comisso, A., & Scaccini, C. (2002).
528 Detection of bound phenolic acids: Prevention by ascorbic acid ethylenediaminetetraacetic
529 acid of degradation of phenolic acids during alkaline hydrolysis. *Food Chemistry, 79*, 119-
530 124.

531 Nikolic, N. C., & Stankovic, M. Z. (2003). Kinetics of solanidine hydrolytic extraction from
532 potato (*Solanum tuberosum* L.) haulm in solid-liquid systems. *Journal of the Serbian*
533 *Chemical Society, 68*(1), 9-16.

534 Onyeneho, S. N., & Hettiarachchy, N. S. (1993). Antioxidant activity, fatty acids and
535 phenolic acids compositions of potato peels. *Journal of the Science of Food and*
536 *Agriculture, 62*, 345-350.

Rodriguez de Soltillo, D., Hadley, M., & Holm, E. T. (2006). Phenolics in aqueous potato peel extract: Extraction, identification and degradation. *Journal of Food Science, 59*, 649-651.

Rodriguez de Soltillo, D., Hadley, M., & Wolf-Hall, C. (1998). Potato peel extract a nonmutagenic antioxidant with potential antimicrobial activity. *Journal of Food Science, 63*(5), 1-4.

Rodriguez-Meizoso, I., Marin, F. R., Herrero, M., Señorans, F. J., Reglero, G., Cifuentes, A., & Ibañez, E. (2006). Subcritical water extraction of nutraceuticals with antioxidant activity from oregano. hemical and functional characterization. *Journal of Pharmaceutical and Biomedical Analysis, 41*, 1560-1565.

Rodriguez-Saona, L. E., Wrolstad, R. E., & Pereira, C. (1999). Glycoalkaloid content and anthocyanin stability to alkaline treatment of red fleshed potato extracts. *Journal of Food Science, 64*(3), 445-450.

Sánchez-Maldonado, A. F., Schieber, A., & Gänzle, M. G. (2011). Structure-function relationship of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria. *Journal of Applied Microbiology, 111*(5), 1176-1184.

Satoh, N., Akiba, T., Yokoshima, S., & Fukuyama, T. (2007). A practical synthesis of (-)-oseltamivir. *Angewandte Chemie, 119*, 5836-5838.

Schieber, A., & Aranda Saldaña, M. D. (2009). Potato peels: A source of nutritionally and pharmacologically interesting compounds-A review. *Food, 3*(Special issue), 23-29.

557 Shui, G., Leong, L. P., & Wong, S. P. (2005). Rapid screening and characterisation of
558 antioxidants of *Cosmos caudatus* using liquid chromatography coupled with mass
559 spectrometry. *Journal of Chromatography B, 827*, 127-138.

560 Singh, N., & Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of
561 potato peel. *Food Chemistry, 85*(4), 611-616.

562 Singh, P.P. & Saldaña, M.A. (2011). Subcritical water extraction of phenolic compounds
563 from potato peel. *Food Research International*, 44, 2452–2458

564 Sotelo, A., & Serrano, B. (2000). High-performance liquid chromatography determination of
565 the glycoalkaloids α-solanine and α-chaconine in 12 commercial varieties of Mexican
566 potato. *Journal of Agricultural and Food Chemistry, 48*, 2472-2475.

567 Svensson, L., Sekwati-Monang, B., Lopes Lutz, D., Schieber, A., & Gaenzle, M. G. (2010).
568 Phenolic acids and flavonoids in nonfermented and fermented red sorghum (*Sorghum*
569 *bicolor* (L.) Moench). *Journal of Agricultural and Food Chemistry, 58*, 9214-9220.

570 Teixeira, J., McNeil, V., & Gänzle, M. G. (2012). Levansucrase and sucrose phosphorylase
571 contribute to raffinose, stachyose, and verbascose metabolism by lactobacilli. *Food*
572 *Microbiology, 31*(2), 278-284.

573 Tubtimdee, C., & Shotipruk, A. (2011). Extraction of phenolics from *Terminalia*
574 *chebula* Retz with water-ethanol and water propylene glycol and sugaring-out concentrations
575 of extracts. *Separation and Purification Technology, 77*, 339-346.

Yeung, Y., Hong, S., & Corey, E. J. (2006). A short enantioselective pathway for the synthesis of the anti-influenza neuramidase inhibitor oseltamivir from 1,3-butadiene and acrylic acid. *Journal of the American Chemical Society, 128*, 6310-6311.

FIGURE LEGENDS

Figure 1. Products of alkaline hydrolysis of chlorogenic acid.

Figure 2. Product of hydrolysis of α-chaconine and α-solanine.

Figure 3. Recovery of phenolic acids and glycoalkaloids **(1)** Amount of chlorogenic acid (■) in a standard solution (S) and recovery after extraction from that standard solution (S+E), using solvent C**. (2)** Phenolic acids recovered from potato peels using 3 different solvents (A, B or C): chlorogenic acid (■), neochlorogenic acid (□), caffeic acid (□). **(3)** Glycoalkaloids recovered from potato peels using 3 different solvents (A, B or C): α-chaconine (▨), α-solanine (▨), solanidine (▨). Data are means ± standard deviations (n=3). Significant differences were determined by two-way ANOVA ($P < 0.05$). For **(1)** and **(2)** the yields of the compounds were compared as a function of the solvent used. There were no significant differences.

Figure 4. Recovery of phenolic acids and glycoalkaloids from potato peels in 3 consecutive extractions with different solvents. **(1)** Phenolic acids: chlorogenic acid (■), neochlorogenic acid (□), caffeic acid (□). **(2)** Glycoalkaloids: α-chaconine (▨), α-solanine (▨), solanidine (▨). Data are means ± standard deviations (n=3). Significant differences were determined by two-way ANOVA ($P < 0.05$). The yields of the compounds were compared as a function of the solvent used for each extraction (first, second and third). There were no significant differences.

Figure 5. Recovery of phenolic acids and glycoalkaloids before and after separation by solid phase extraction. **(1)** Phenolic acids; in crude extract: chlorogenic acid (■), neochlorogenic acid (□), caffeic acid (□); recovered in water/ethanol (80:20) fraction: chlorogenic acid (▤), neochlorogenic acid (▤), caffeic acid (▤). **(2)** Glycoalkaloids; in crude extract: α-chaconine (▨), α-solanine (▨),solanidine (▨); recovered in water/ethanol (20:80) fraction: (▩) α-chaconine, α-(▩) solanine, (▩) solanidine. Data are means ± standard deviations (n=3). Significant differences were determined by one-way ANOVA followed by Holm–Sidak method for multiple pairwise comparisons ($P < 0.05$). Comparisons between

extraction and separation were performed for each compound. * Indicates significant difference in the recovery after fractionation compared to the crude extract.

Figure 6. Recovery of phenolic acids in several fractions of the solvents during solid phase extraction. **(1)** Phenolic acids eluted with water/ethanol (80:20); chlorogenic acid (■), neochlorogenic acid (□), caffeic acid (□). **(2)** Glycoalkaloids eluted with water/ethanol (20:80); α-chaconine (▨), α-solanine (▨), solanidine (▨). Data are means ± standard deviations (n=3).

Figure 7. Recovery of phenolic acids and glycoalkaloids after hydrolysis and fractionation. **(1)** Chorogenic acid acid (■) standard solution before (S) and after hydrolysis (S+H). Panels **(1)** and **(2)** show compounds recovered in crude extract (E), hydrolysed crude extract (E+H) and hydrolysed crude extract after fractionation (E+H+F). **(1)** Phenolic acids: chlorogenic acid (■), neochlorogenic acid (□), caffeic acid (□). **(2)** Glycoalkaloids: α-chaconine (▨), α-solanine (▨). Data are means ± standard deviations (n=3).Significant differences were determined by one-way ANOVA followed by Holm–Sidak method for multiple pairwise comparisons ($P < 0.05$). For panel **(2)** since hydrolysis of chlorogenic and neochlorogenic acids releases caffeic acid, the summarized amounts of chlorogenic, neochlorogenic and caffeic acids were compared to the amount of caffeic acid recovered after extraction and alkaline hydrolysis, and after extraction, alkaline hydrolysis and fractionation. For panel **(3)** the yield of each compound was compared between extraction, extraction and alkaline hydrolysis and extraction, alkaline hydrolysis and fractionation (capital letters were used to compare amounts of α-chaconine and non-capital letters to compare α-solanine). Different superscripts in the same panel indicate significant difference. Solanidine was not quantified after hydrolysis, since its concentration was below the lowest concentration of the calibration curve.

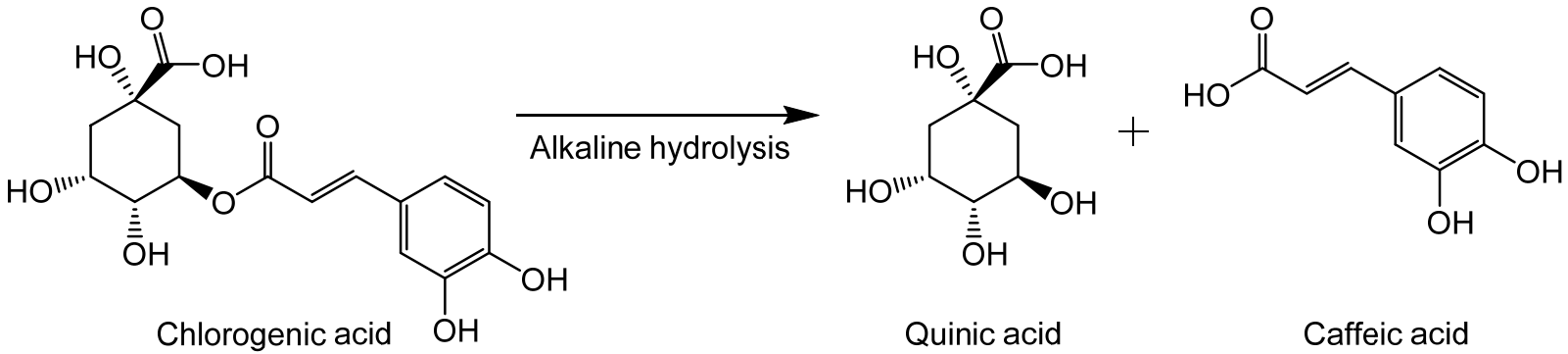

Figure 1 Sánchez-Maldonado et al.

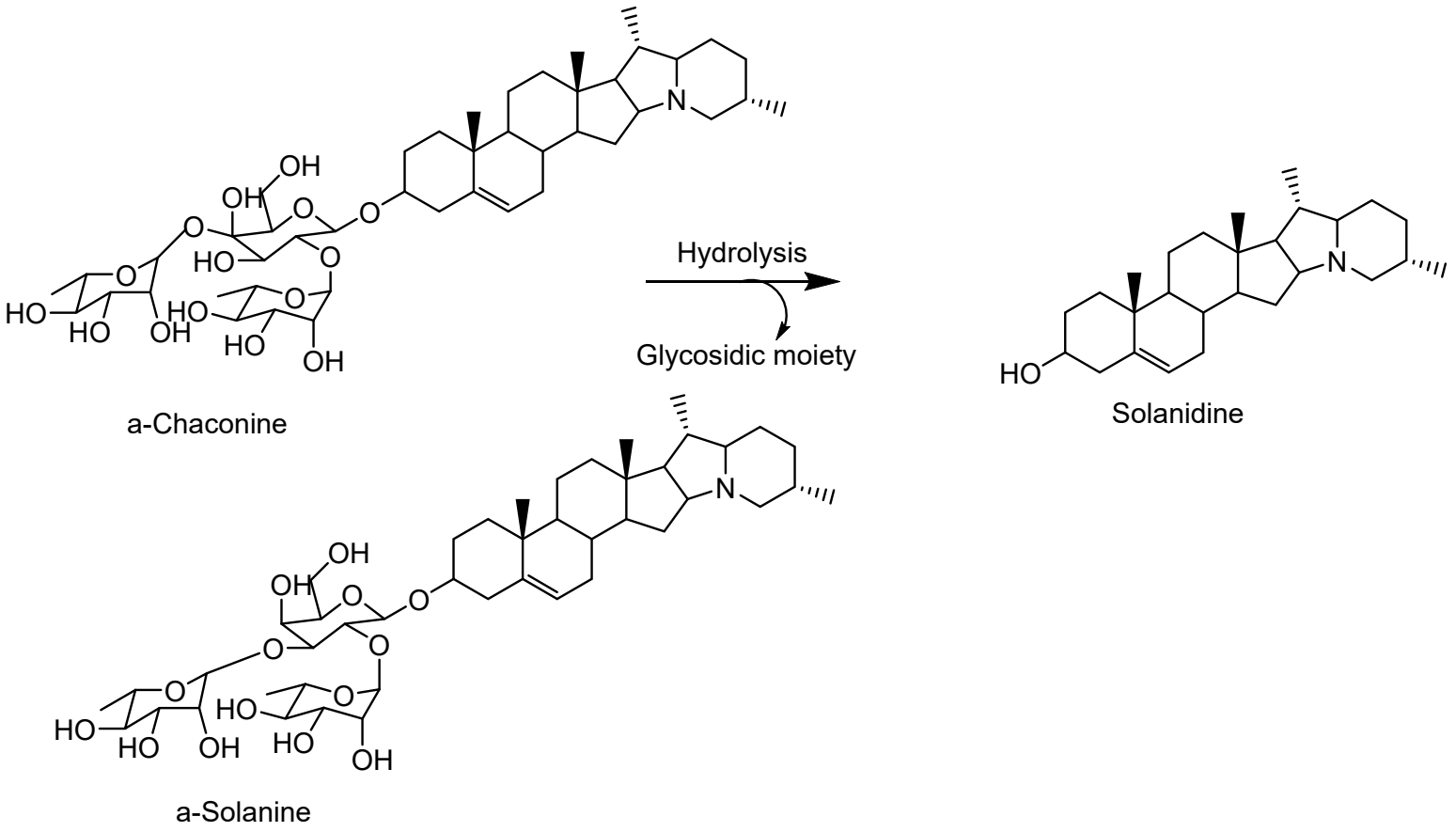

Figure 2 Sánchez-Maldonado et al.

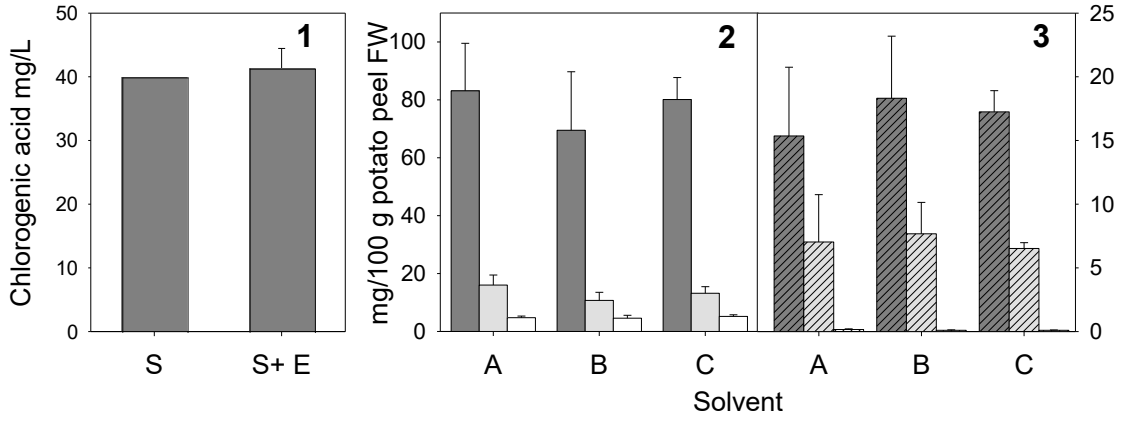

Figure 3 Sánchez-Maldonado et al

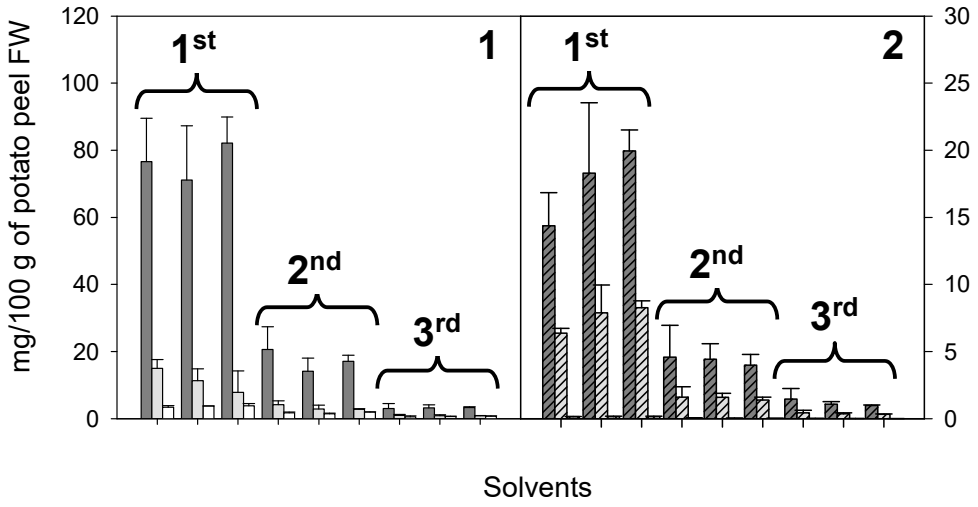

Figure 4 Sánchez-Maldonado et al.

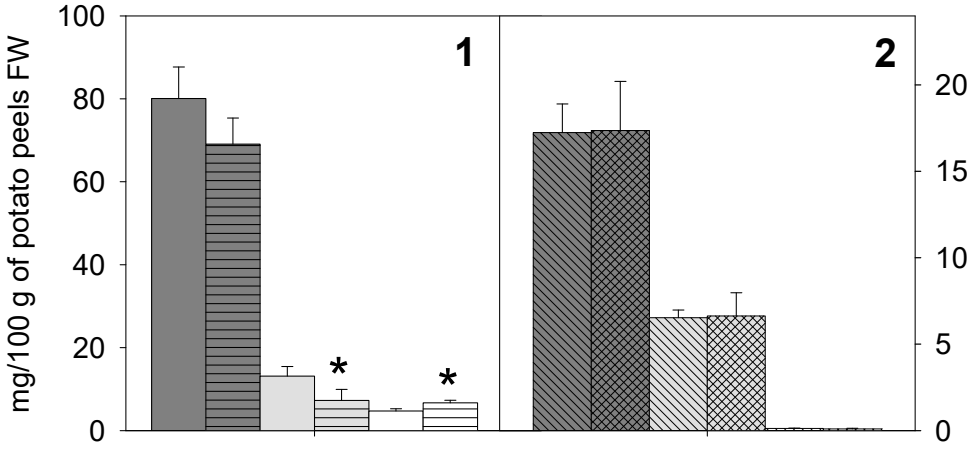

Figure 5 Sánchez-Maldonado et al.

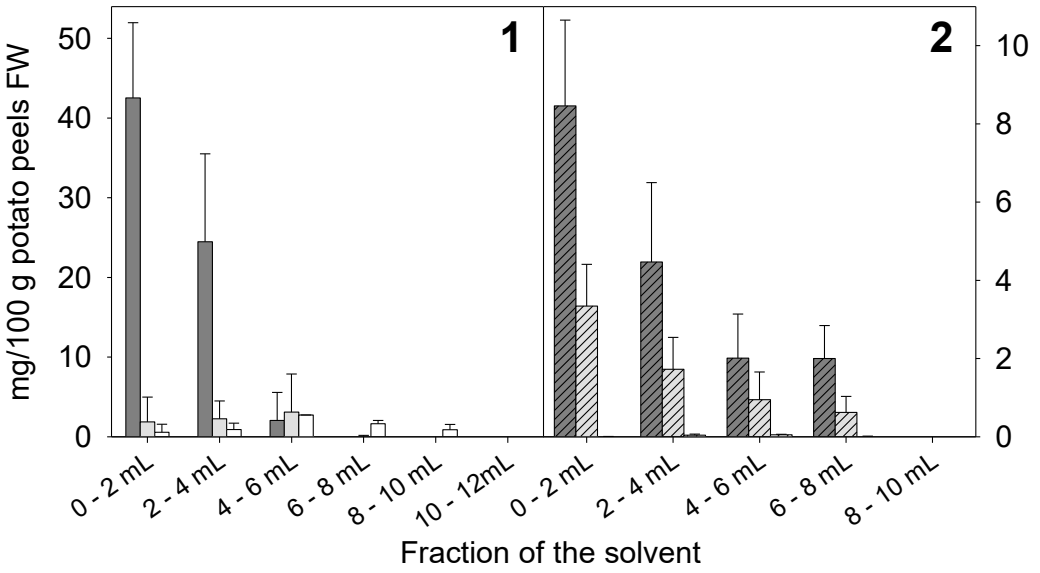

Figure 6 Sánchez-Maldonado et al.

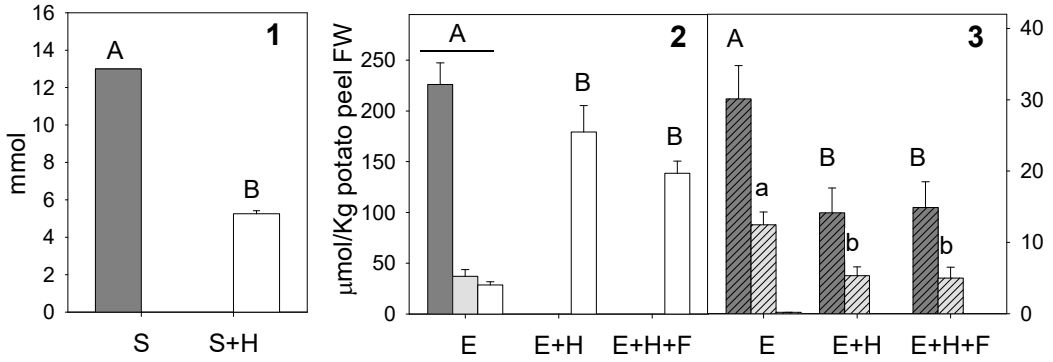

Figure 7 Sánchez-Maldonado et al.

Table 1. Main compounds recovered from potato peels.

Compound	Retention time	*m/z* (% intensity)
Phenolic compounds		
Neochlorogenic acid (3-*O*-caffeoylquinic acid) (Clifford et al. 2003; Nandutu et al. 2007)	2.3	353(59), 191(100), 179(53), 173(4), 135(55)
Chlorogenic acid (5-*O*-caffeoylquinic acid)	2.6	353(41), 191(100), 179(10), 173(18), 135(9)
Caffeic acid	2.8	179(2), 135(100)
Unknown compound	3.2	529 (100)
Glycoalkaloids		
α-Solanine	12.4	868(100), 722(50), 398(37)
α-Chaconine	12.8	852(100), 706(32), 398(28)
Solanidine	15.6	398(100), 382.6(23), 98(8)

Standards of all compounds, except neochlorogenic acid, were analysed under the same conditions and their MS spectrum matched that of the samples.