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Research Article

Extraction Techniques for Bioactive Compounds and Antioxidant Capacity Determination of Chilean Papaya (*Vasconcellea pubescens*) Fruit

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The aim of this work was to assess and compare different extraction methods by using high hydrostatic pressure (HHPE), ultrasound (UE), agitation (AE), and their combinations for the extraction of bioactive compounds of Chilean papaya. Extract antioxidant capacity was evaluated by three methods (i.e., DPPH, FRAP, and Voltammetry) and phenolic compounds and vitamin C were determined by HPLC. Papaya sample extraction was performed by HHPE at 500 MPa for 10 min and UE and AE for 30 min, respectively. The combined-extractions: HHPE-UE and HHPE-AE, were carried out for 5 min and 15 min, respectively. The highest values found were total phenolic 129.1 mg GAE/100 g FW, antioxidant capacity by DPPH 20.6 mM TE/100 g FW, and voltammetry 141.0 mM TE/100 g FW for HHPE-UE method in free compound extraction. Regarding vitamin C content, its highest value was found by HHPE-UE (74 mg/100 g FW) a combined extraction method. The phenolic compounds rutin and *p*-coumaric acid were found in all the extracts, both in free and bound forms, respectively. Besides, the combined techniques improved the extraction of bioactive compounds.

1. Introduction

Fruit and vegetable bioactive compounds interest has greatly increased in the last few years. Fruits being rich in bioactive compounds help to lower the incidence of degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction, and acceleration of the aging process [1, 2]. The protection that fruits and vegetables may provide has been attributed to the presence of several antioxidants, such as ascorbic acid (vitamin C) or vitamin E; nevertheless, recent studies seem to indicate that (poly)phenolic compounds largely contribute to antioxidant properties [3].

Chilean papaya is a native fruit from South America and has been widely distributed throughout the Andean countries; this species was introduced in Chile over 50 years ago and is cultivated in Coquimbo and Valparaiso valleys as well as in Maule Region coast. Chilean papaya belongs to the Caricaceae family and corresponds to *Vasconcellea pubescens*. This fruit is characterized by its intense aroma, yellow color, and oblong shape [4]. Furthermore, ripe papaya is an excellent source of carotenoids, vitamins, proteins, and polysaccharides [5].

The modern chromatographic development and spectrometric techniques have made bioactive compound analysis easier than before but their success is still dependable on the extraction methods, input parameters, and accurate nature of material to be studied [6]. Liquid-solid extraction can be defined as a mass transport phenomenon where solids contained in plant structures migrate into the solvent up to equilibrium [7]. Thus, matrix properties, extraction solvent, temperature, pressure, and time are factors that affect the extraction methods [6]. Owing to these reasons, it is interesting to compare the conventional methods to a new technology that

involves different conditions. It is known that conventional extraction methods have some limitations regarding the high solvent consumption, the long extraction time required, and quality of the extracts [8]. Recently, new extraction methods have been employed, where the most noticeable ones are the ultrasound-assisted extraction and extraction by high hydrostatic pressure. These methods have been reported to be efficient when compared to a conventional extraction, like agitation [9, 10]. These extraction techniques have been used to extract bioactive compounds from various plant materials and thus to evaluate which of the extraction methods contributes more efficiently to improve compound analyses [11].

Different antioxidants can be found in a wide range of concentrations in vegetal tissues, to quantify antioxidant capacity. Several assays are known such as the ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH); these ones are simple, cost effective, and easy to interpret [12]. One method disadvantage is based on its color formation, which can be problematic when being applied to colorful extracts (i.e., carotenoids in fruits). An electrochemical method as cyclic voltammetry is an interesting alternative since it has a lot of advantages; for example, it does not need a laborious preparation and can be performed rapidly with relatively simple equipment. It can be used for both lipophilic and hydrophilic extracts and can be carried out on intensely colored or even turbid extracts [13].

The aim of the present study was to evaluate different extraction methods related to bioactive compounds, antioxidant capacity, and vitamin C content from Chilean papaya and to obtain the best extraction method. The extraction methods being considered were agitation, ultrasound, high hydrostatic pressure, and a combination of them like high hydrostatic pressure-agitation and high hydrostatic pressure-ultrasound extractions.

2. Material and Methods

2.1. Reagents and Standards. All reagents were of analytical grade. Methanol and potassium dihydrogen phosphate (Merck, Darmstadt, Germany), 2,4,6-tripyridyl-S-triazine (TPTZ), 6-hydroxy-2,5,7, 8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picryhydrazyl (DPPH) and hexadecytrimethyl-ammonium bromide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Standards of *p*-coumaric, *trans*-ferulic acids and rutin were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and ascorbic acid from Fluka-Guarantee (Buchs, Switzerland). To HPLC analyses only analytical HPLC grade from Merck were used. Ultra-pure water for analyses was prepared by a Water System (Heal Force, Shanghai Canrex Analytic Instrument Co. Ltda.).

2.2. Raw Material and Sample Preparation. Chilean papaya (*Vasconcellea pubescens*) was provided from a local market (La Serena, Chile), in November 2013. Chilean papayas (the starting mass material was approximately 1 kg) were washed and selected to provide a homogeneous group, based on date of harvest, color, size, and freshness according to a visual analysis. Both the upper and lower ends of the fruits were rejected and then cut into longitudinal sections. Both seeds and mucilage were removed, and thereafter the fruit with skin was homogenized in a blender (Philips, HR1720, Amsterdam) for further analyses.

2.3. Physicochemical Analysis. Moisture content was determined by AOAC method number 934.06 [14] employing a vacuum oven (Gallenkamp, OVL570, Leicester, UK) and an analytical balance with an accuracy of ±0.0001 g (CHYO, Jex120, Kyoto, Japan). Crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25 (AOAC number 960.52) using a digestor and distillation unit (DK 20 and UDK 129, VELP Scientifica, Italy). Lipid content was determined gravimetrically following Soxhlet extraction (AOAC number 960.39) using an extraction unit (Gerhard, Königswinter, Germany). Crude fibre was estimated by an acid/alkaline hydrolysis of insoluble residues (AOAC no. 962.09) by using a reflux unit. Crude ash content was estimated by incineration in a muffle furnace (Felisa, FE341, Jalisco, Mexico) at 550°C (AOAC number 923.03). All methodologies followed the recommendations from Association of Official Analytical Chemists (AOAC, 1990) [14]. All measurements were done in triplicate.

2.4. Extraction Methods to Determine Antioxidant Capacity and Phenolic Compound Content

2.4.1. Agitation Extraction (AE). Agitation extraction was carried out weighing 5 g of sample and putting them into an Erlenmeyer flask containing 80% aqueous methanol using a solid/liquid ratio of 1 : 4. An orbital shaker (Boeco, OS 20, Hamburg, Germany) was used to perform the extraction at 200 rpm for 30 min at room temperature (a convenient time to solubilize bioactive compounds [15, 16]). This extraction was fractionated into free and bound phenolic compound forms. After centrifugation at 10000 ×g for 3 min, the supernatant was removed and the extraction was repeated one more time in a similar way. The combined extracts were evaporated in a rotary evaporator (Büchi RE12, Flawil, Switzerland) at 37°C and dissolved in 10 mL MeOH. The phenolic compounds obtained by this procedure were designated as free phenolic compounds "free fraction".

After extraction of free forms, 20 mL of a solution of 3 M NaOH was added directly to the residue and agitated in an orbital shaker for 4 h. The alkaline hydrolysis was acidified to pH 2 with 4 M HCl. The liberated compounds in the clear solution were extracted three times with 10 mL of ethyl acetate. The pooled ethyl acetate extracts were evaporated to dryness under vacuum in a rotary evaporator at 37°C. The dried residue was dissolved in 10 mL MeOH to determine the bound phenolic compounds. All extractions were done in triplicate. The phenolic compounds obtained by this last procedure were designated as bound phenolic compounds "bound fraction". Both free and bound compounds were determined by HPLC and further their antioxidant capacities were determined by spectrophotometry (DPPH and FRAP) and voltammetry, respectively (see Section 2.5).

2.4.2. Ultrasound Extraction (UE). Five grams of papaya sample was put into an Erlenmeyer flask containing 80% aqueous methanol using a solid/liquid ratio of 1 : 4. An ultrasound bath (Branson 2510 E-MT, Danbury, USA) was used to carry out the extraction at 60 Hz for 30 min. This extraction was also fractionated into free and bound fractions as was mentioned above. All extractions were done in triplicate.

2.4.3. High Hydrostatic Pressure Extraction (HHPE). A high hydrostatic pressure system was used for extraction, where water was the pressure-transmitting medium at a ramp rate of 17 MPa s^{-1}; the decompression time was less than 5 s. A 2 L processing unit (Avure Technologies Inc., Kent, WA, USA) was used to pressurize the samples. Five grams of samples was packed individually with extraction solution (80% methanol) and hermetically sealed in high density polyethylene bags. The packaged samples were placed in a cylindrical loading container at room temperature and pressurized at 500 MPa for 10 min (previous work). Afterwards, this extraction was also fractionated into free and bound fractions as was mentioned above. All extractions were done in triplicate.

2.4.4. High Hydrostatic Pressure-Agitation Extraction (HHPE-AE). A combined extraction technique formed by high hydrostatic pressure equipment and an orbital shaker was used to perform the extractions. Five grams of sample was packed individually with an extraction solution (80% methanol) and hermetically sealed in a high density polyethylene bag. The packaged samples were placed in a cylindrical loading container at room temperature and pressurized at 500 MPa 5 min. Then, agitation was performed on an orbital shaker at 200 rpm for 15 min. This extraction was also fractionated into free and bound fractions as it was mentioned above. All extractions were done in triplicate.

2.4.5. High Hydrostatic Pressure-Ultrasound Extraction (HHPE-UE). A combined extraction technique made up by high hydrostatic pressure equipment and an ultrasound bath was used to carry out the extractions. Five grams of samples was packed individually with an extraction solution (80% methanol) and hermetically sealed in high density polyethylene bags. The packaged samples were placed in a cylindrical loading container at room temperature and pressurized at 500 MPa 5 min. Then, a second extraction was made by ultrasound for 15 min at 60 Hz. This extraction also was fractionated into free and bound fractions as was mentioned above. All extractions were done in triplicate.

2.5. Antioxidant Capacity Determination

2.5.1. DPPH Radical Scavenging Assay. The DPPH assay was performed according to the method developed by Brand-Williams et al. [17]. A solution of 50 μM DPPH in methanol was stirred for 40 min. Then, 0.1 mL of standard or sample was mixed with 3.90 mL of DPPH solution and incubated for 30 min in the dark. The concentration of DPPH in the reaction medium is calculated from a calibration curve (Trolox, used as a reference synthetic antioxidant at concentrations ranging from 0.08 to 1.28 mM) obtained by linear regression. Total antioxidant capacity by DPPH assay was expressed as mM TE/100 g FW (millimolar Trolox equivalents per 100 grams of fresh weight). All measurements were done in triplicate and the absorbance was read at 517 nm.

2.5.2. Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP assay procedure described by Benzie and Strain [18] was employed, with some modifications. This method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 2.5 mL of a 10 mM TPTZ (2,4,6-tripyridy-s-triazine, Sigma-Aldrich Company Ltd., St. Louis, MO, USA) solution in 40 mM HCl with 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3 M acetate buffer at pH 3.6; the solution was freshly prepared and warmed to 37°C. Sample aliquots of 30 μL were mixed with 90 μL distilled water and 900 μL FRAP reagent, and the absorbance of the reaction mixture was read spectrophotometrically (Spectrophotometer, Spectronic Instruments, 20 Genesys, USA) at 593 nm after incubation at 37°C for 30 min. The antioxidant capacity was calculated from a calibration curve obtained by linear regression, using Trolox as a synthetic antioxidant at concentrations ranging from 100 to 1500 μM in methanol. The results were expressed as mM TE/100 g FW (millimolar Trolox equivalent per 100 grams of fresh weight).

2.5.3. Electrochemical Method. Square wave voltammetry (SWV) experiments were done by using a Princeton Applied Research PG 580 potentiostat and a classical three-electrode setup, consisting of a glassy carbon working electrode (3 mm diameter), a Pt wire auxiliary electrode, and an Ag/AgCl reference electrode. Between the above measurements, the working electrode was polished by using a polishing cloth and diamond paste. Lithium perchlorate (1 gram) was added to each sample as an electrolyte to increase the conductivity of the samples. The conditions for SWV were as follows: frequency, 25 Hz; amplitude, 20 mV step size, 5 mV. The potential was scanned between 0.0 and 1.0 V. To quantify the antioxidant capacity, a calibration curve was performed by using a dilution series of Trolox 0.1–1.0 mM. The AUC (area under the curve) was used as a quantitative parameter of the antioxidant capacity. The results are expressed as mM TE/100 g FW (millimolar Trolox equivalent per 100 grams of fresh weight).

2.6. Total Polyphenolic Content (TPC) Determination. TPC was determined colorimetrically by using the Folin-Ciocalteu (FC) reagent according to previous work with modifications [19]. A 0.5 mL aliquot of the papaya extract solution was transferred to a glass tube; 0.5 mL of reactive FC was added after 5 min, and 2 mL of Na_2CO_3 solution (200 mg/mL) was added and shaken. The sample was then mixed on a vortex mixer and the reaction proceeded for 15 min at ambient temperature. Then, 10 mL of ultrapure water was added and the formed precipitate was removed by centrifugation at 10000 ×g for 5 min. Finally, the absorbance was read in a spectrophotometer (Spectronic 20 Genesys, IL, USA)

at 725 nm and compared to a gallic acid calibration curve. Results were expressed as mg GAE/100 g FW (mg gallic acid equivalents per 100 grams of fresh weight). All reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany). All measurements were done in triplicate.

2.7. Chromatographic Conditions to Identify and Quantify Phenolic Compounds. A HPLC system, Agilent 1200, equipped with a high pressure pump; an automatic injector; an UV-visible-diode array detector (DAD), controlled by ChemStation software, were used for the analysis. The analytical column was a Kromasil 100-5C18 (250 × 4.6 mm; 0.5 μm particle size) (Eka Chemicals, Sweden). The flow rate was 0.7 mL/min, and the eluates were monitored at 280 and 310 nm at 25°C. The mobile phase was composed of solvent A (formic acid 0.1%, pH 3) and solvent B (100% acetonitrile). The elution was as follows. The elution gradient started with 87% solvent A and 13% solvent B; solvent B is to reach 55% at 18 min, 60% at 23 min, and 13% at 25 min and then returned to the initial conditions by 2 min. The phenolic extracts and standard compounds were analyzed under the same analysis conditions. Identification of some of the main phenolic compounds (*p*-coumaric and *trans*-ferulic acids *and* rutin) in MeOH- formic acid (99 : 1) was performed by comparing against the retention times, their spectra, and the peak area of maximum absorption wavelength. The concentration of the main phenolic compounds was expressed as mg/100 g FW.

2.8. Extraction Method for Vitamin C Content. A new extraction was performed to determine vitamin C content (vitamin C extract (VCE)); furthermore this extract will be considered for the determination of antioxidant capacity by cyclic voltammetry. To each extraction technique (HHPE, UE, AE, HHPE-UE, and HHPE-AE), 2.5 g of sample was mixed with 15 mL metaphosphoric acid (MPA) (1%) and their respective extraction time was applied as mentioned above. Then, the sample was centrifuged at 10000 ×g for 15 min at 10°C. The supernatant was collected, filtrated through 0.45 μm membrane filters, and kept at −4°C for further HPLC and voltammetry analyses. All extractions were done in triplicate.

2.9. Vitamin C Determination by HPLC. The chromatographic conditions were performed by using a Zorbax Eclipse XDB-C18, 4.6 × 150 mm, 5 μm reversed-phase column, a mobile phase containing 5 mM cetyltrimethylammonium bromide as the ion-pairing agent, and 50 mM potassium dehydrogenate phosphate as buffer, at pH 3.9, filtered through a 0.22 μm filter. All measurements were done at 20°C with a flow rate of 0.7 mL/min, isocratic elution, and detection at 254 nm. A high-performance liquid chromatography system, Agilent 1200, equipped with a high pressure pump; an automatic injector with a 5 μL loop; a UV-visible-diode array detector (DAD), controlled by a ChemStation software, was used for the analysis. Peak areas were used for quantitative analysis. The calibration curve was prepared using concentrations ranging from 7 to 55 μg of ascorbic acid/mL in 1% MPA. The total vitamin C content was estimated after reduction of dehydroascorbic acid (DHA) with dithiothreitol (DTT), where 1 mg of DTT was added directly into the vial and kept for 2 hours in the dark, before analysis. Results were expressed as mg vitamin C/100 g FW.

Table 1: Proximal composition, pH, acidity, soluble solids, and water capacity of fresh Chilean papaya.

Parameters	Content (g/100 g FW)
Moisture	91.6 ± 1.5
Protein	0.9 ± 0.0
Lipid	0.3 ± 0.0
Crude fiber	1.1 ± 0.1
Ash	0.6 ± 0.0
Carbohydrate[a]	4.9 ± 1.4
pH[*]	4.1 ± 0.2
Acidity (%)[b]	0.1 ± 0.0
Soluble solids (°Brix)	5.0 ± 0.0
Water activity[*]	0.997 ± 0.001

[a]Calculated by differences, [*]adimensional, and [b]expressed % citric acid.

2.10. Statistical Analysis. One-way analysis of variance (ANOVA) (Statgraphics software, Statistical Graphics Corp., Herndon, USA) was used to indicate significant differences among samples. A significance testing was performed by using a Fisher's least significant difference (LSD) test; differences were taken as statistically significant when $P \leq 0.05$. The multiple range test (MRT) included in the statistical program was used to test the existence of homogeneous groups within each of the analyzed parameters.

3. Results and Discussion

3.1. Proximal Composition, pH, Acidity, Soluble Solids, and Water Activity of Fresh Chilean Papaya. Table 1 shows physical and chemical properties of fresh Chilean papaya. Such a papaya presents a low lipid and protein content. However, it shows a high moisture content and total carbohydrates were obtained by difference. Similar values were obtained by Nwofia et al. [20] working by other *Carica papaya* (L) morphotypes.

3.2. Total Phenolic Content (TPC) and Antioxidant Capacity. Phenolic compounds are common constituents of the human diet and are found mainly in fruits and vegetables and it has been suggested that the consumption of these compounds renders possible health benefits derived from their antioxidant properties [21].

TPC of Chilean papaya is shown in Table 2. The highest amounts ($P < 0.05$) of total phenolic for free fraction of sample were for HHPE-UE (129.1 mg GAE/100 g FW) followed by HHPE-AE (126.9 mg GAE/100 g FW). HHPE ranked third as to total phenolic content of 28.6 mg GAE/100 g FW. The lowest contents were observed in UE and AE techniques (26.3 and 23.8 mg GAE/100 g FW, resp.). However, the bound fractions were detected only in the combined extractions. Significant differences ($P \leq 0.05$) were observed between

Table 2: Comparison among antioxidant capacity assays and extraction methods from Chilean papaya.

Fraction	Extraction method	Total phenolic content (mg GAE/100 g)	DPPH (mM TE/100 g)	FRAP (mM TE/100 g)	Voltammetry (mM TE/100 g)
Free	AE	23.8 ± 2.0^{b}	$17.6 \pm 0.2^{b.A}$	$100.0 \pm 0.1^{a.B}$	$15.2 \pm 1.3^{a.A}$
	UE	26.3 ± 0.2^{ab}	$15.7 \pm 0.3^{a.A}$	$99.9 \pm 4.8^{ab.B}$	$12.9 \pm 2.2^{a.A}$
	HHPE	28.6 ± 1.1^{a}	$16.2 \pm 0.7^{a.A}$	$101.9 \pm 0.1^{a.B}$	$16.9 \pm 0.3^{a.A}$
	HHPE-AE	126.9 ± 1.9^{c}	$20.5 \pm 0.1^{c.A}$	$101.1 \pm 1.9^{a.B}$	$140.5 \pm 17.9^{b.C}$
	HHPE-UE	129.1 ± 3.8^{c}	$20.6 \pm 0.2^{c.A}$	$97.2 \pm 4.3^{b.B}$	$141.0 \pm 13.8^{b.C}$
Bound	AE	ND*	ND*	$28.1 \pm 2.7^{c.A}$	$2.1 \pm 0.6^{d.B}$
	UE	ND*	ND*	$22.9 \pm 1.4^{d.A}$	$3.3 \pm 1.2^{d.B}$
	HHPE	ND*	ND*	$32.3 \pm 2.7^{c.A}$	$1.6 \pm 0.2^{c.B}$
	HHPE-AE	0.9 ± 0.0^{d}	$2.1 \pm 0.0^{d.A}$	$83.4 \pm 0.2^{e.B}$	$59.4 \pm 4.5^{f.C}$
	HHPE-UE	1.2 ± 0.0^{d}	$1.8 \pm 0.0^{d.A}$	$85.7 \pm 0.9^{e.B}$	$27.7 \pm 8.8^{e.C}$

*Not detected. Values are mean ± standard deviation of triplicates; values followed by the same letter in the same column are not significantly different ($P < 0.05$) and the same letter in the same row are not significantly different ($P < 0.05$).

total phenolic levels of Chilean papaya when using different extraction methods.

Antioxidant capacity measured in methanol extracts by using DPPH, FRAP, and SWV assays are shown in Table 2. The free radical scavenging capacity by DPPH assays of Chilean papaya, based on their Trolox equivalent values in free fraction, did not differ widely and ranged from 15.7 to 20.6 mM TE/100 g FW for UE and HHPE-UE, respectively. Likewise, that total phenolic bound fraction was only detected in the combined methods to DPPH assays.

FRAP values of Chilean papaya ranged from 22.9 to 101.9 mM TE/100 g FW in both fractions; the HHPE has the highest FRAP value in the free fraction. The combined methods showed the highest FRAP values in bound fractions (HHPE-UE and HHPE-AE). Gironés-Vilaplana et al. [22] mentioned similar values for FRAP in papaya fruits although with a lesser degree than those in maqui (*Aristotelia chilensis*) fruit. However, our values of DPPH in papaya are higher than those in that study.

Square wave voltammetry (SWV) was expressed in the same terms to represent the antioxidant capacity of sample (mM TE/100 g FW) by comparing it to DPPH and FRAP assays. The square wave voltammetry, and in general all voltammetry techniques, registers a current flow in a working electrode when this is polarized at different potentials. SWV uses a square wave potential scheme; the anodic current versus potential plot gives information on the redox potential of major antioxidants in the sample. Although this technique does not provide information about the individual nature of the antioxidants present in the sample, the area under the curve (AUC) can be used as a measure of antioxidant capacity. Voltammetry values for Chilean papaya were in the range of 12.9–141.0 mM TE/100 g FW in the free fractions and of 1.6–59.4 mM TE/100 g FW in bound fractions. UE and HHPE have the lowest values in both fractions.

In the case of phenolic acids, the antioxidant capacity depends on the numbers and positions of the hydroxyl groups in relation to the carboxyl functional group. That is to say, the higher the hydroxylation degree is, the higher the antioxidant capacity is [3]. Therefore, there is a strong relationship between total phenolic content and antioxidant capacity in different food, such as apricot [23] and pomegranate juice [24].

HHPE increased the extraction yields due to its aptitude to deprotonate charged groups, to disrupt salt bridges and hydrophobic bonds in cell membranes which may lead to a higher permeability that might contribute to the higher levels of total phenolic content and antioxidant capacities [25]. Ultrasound has an effect on acoustic cavitation produced in the solvent by the passage of an ultrasound wave and a mechanical effect allowing a greater penetration of solvent into the sample matrix, thus increasing the contact surface area between the solid and liquid phases as a result, and the solute quickly diffuses from the solid phase [26]. However, the ultrasonic effect on antioxidant capacity was lower than in HHPE and the combined extraction methods. Note that, in the case of combined extractions of both HHPE-UE and HHPE-AE, a better extraction of bioactive compounds can be observed, since total phenolic content and DPPH evaluation allowed bioactive compounds release from their bound fraction that were not detected in the single methods.

3.3. Phenolic Compounds. The result of the chromatograms of Chilean papaya revealed the presence of 4 different phenolic compounds (caffeic, *trans*-ferulic, and *p*-coumaric acids and rutin) that were identified by their UV spectra. The concentration of the identified free and bound phenolic compounds is shown in Table 3. The main free phenolic compound detected was rutin, obtaining values ranging from 1.9 to 2.8 mg/100 g FW in all the treatments. The free phenolic caffeic and *trans*-ferulic acids were only detected in combined-extraction methods. Regarding bound phenolic compounds, after alkaline hydrolysis two compounds were detected (*p*-coumaric and *trans*-ferulic acids). Thus, the *trans*-ferulic acid was detected in both free and bound fractions of combined extractions, besides the HHPE bound fraction. In samples subjected to hydrolysis, both *p*-coumaric and *trans*-ferulic acids were detected, demonstrating that such compounds are presents as esters. The main phenolic acids being quantified in papaya were also reported by [27]. *trans*-Ferulic acid,

Table 3: Comparison between free phenolic and bound phenolic compounds from Chilean papaya.

Extraction method	Free phenolic (mg/100 g)			Bound phenolic (mg/100 g)	
	Caffeic acid	*trans*-Ferulic acid	Rutin	*p*-Coumaric acid	*trans*-Ferulic acid
AE	ND*	ND*	2.0 ± 0.1^{a}	0.1 ± 0.0^{a}	ND*
UE	ND*	ND*	2.0 ± 0.1^{a}	0.2 ± 0.0^{a}	ND*
HHPE	ND*	ND*	1.9 ± 0.2^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}
HHPE-AE	1.6 ± 0.1^{a}	0.82 ± 0.1^{a}	2.8 ± 0.2^{b}	0.6 ± 0.1^{c}	0.5 ± 0.1^{c}
HHPE-UE	1.5 ± 0.1^{a}	0.86 ± 0.1^{a}	2.8 ± 0.3^{b}	0.4 ± 0.0^{b}	0.3 ± 0.1^{b}

*Not detected. Values are mean ± standard deviation of triplicates; values followed by the same letter in the same column are not significantly different ($P < 0.05$).

caffeic acid, and rutin are the most abundant phenolics in papaya fruit and papaya leaves according to [28]. Gayosso-García Sancho et al. [27] mentioned that caffeic acid and its derivatives exert an anti-inflammatory activity both in vitro and in vivo besides *p*-coumaric acid which was shown to be an intermediate in the synthesis of phenylpropanoids and has also been shown to have antioxidant properties. Rutin is believed to exhibit significant pharmacological activities, including antioxidation, anti-inflammation, antidiabetic, and other activities [29].

The total content sum of phenolic acids in papaya fruits ranged from 0.12 to 3.5 mg/100 g FW. The principal phenolic acid is mainly derived from hydroxycinnamic acid. The most abundant phenol compounds were found in the HHPE-AE treatment followed by HHP-UE. In general, there were significant quantitative differences ($P \leq 0.05$) among HHPE, UE, and AE treatments regarding combined-extraction methods (HHPE-UE and HHPE-AE). Studies carried out in other fruits have determined that hydroxycinnamic acids are generally more abundant than hydroxybenzoic acids [27].

AE and UE obtained lower values of *p*-coumaric acid (0.12 and 0.15 mg/100 g FW, resp.). The rutin showed the lowest values in HHPE, UE, and AE, although it showed no significant differences among them. The positive effect on the phenolic extraction by HHPE can be seen; this modern technology will be regarded as an alternative to reduce solvent consumption and accelerate the extraction process. According to Briones-Labarca et al. [30], HHPE has been shown to be an effective method for extracting bioactive compounds, since HHP has the capability of deprotonating charged groups as it was mentioned above. Ignat et al. [2] described high hydrostatic pressure as a novel method to enhance mass transport phenomena. There are studies that have given importance to the extraction methods to identify phenols such as rutin [29]. The choice of the method is highly dependent on the yield and purity of phenol.

3.4. Vitamin C Content and Antioxidant Capacity Determination Using Voltammetry. The nutritional importance of vitamin C as an essential water-soluble vitamin is well established and is known as a potent antioxidant and has the capacity to eliminate several reactive oxygen species [31]. According to Food and Nutrition Board, Institute of Medicine (2000) suggested Recommended Dietary Allowances (RDA) of 75 mg/day and 90 mg/day for adult women and men, respectively, and 45 mg/day for children (9–12 years old) [32].

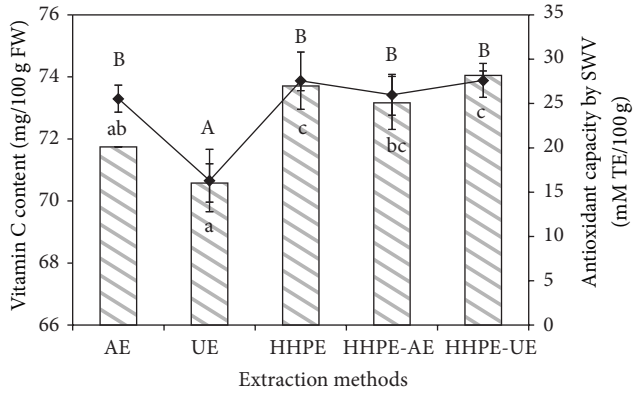


Figure 1: Vitamin C content and capacity antioxidant by SWV of chilean papaya obtained by using different extraction methods. Values are mean ± standard deviation ($n = 3$). Identical letters above the bars indicate no significant difference ($P < 0.05$).

Chilean papaya vitamin C content quantified by HPLC, where the VCE was analyzed, is shown in Figure 1. The content of this vitamin ranged from 70.6 to 74.1 mg/100 g FW whose values are similar to the ones reported by de Souza et al. [33] in three papaya cultivars and slightly lower than those of Othman [34] and Lim et al. [1].

Vitamin C content levels were significantly ($P \leq 0.05$) higher for all high hydrostatic pressure treated samples than for samples using only ultrasound extraction (UE) and agitation extraction (AE). Different extraction methods showed an increase of 1.7%, 3.7%, 4.4%, and 4.9% for AE, HHPE + AE, HHPE, and HHPE + UE, respectively; when being compared against UE, this extraction method showed the lowest vitamin C value (70.6 mg/100 g FW). Vitamin C content of Chilean papaya is higher than that of most fruits, such as orange (67.0 mg/100 g), strawberry (56.4 mg/100 g), and pineapple juice (26.6 mg/100 g) by Lim et al. [1], Nuñez-Mancilla et al. [35], and Hernández et al. [31], respectively. Moreover, antioxidant capacity by the different extraction methods may be related to the amount of vitamin C, owing to the reason that vitamin C is found in high concentration and the papaya fruit is in power full antioxidant, and VCEs were subjected to voltammetry so as to measure its antioxidant capacity as shown in Figure 1.

In the case of SWV, the integrated area under the curve (AUC) coincides with the same tendency as far as vitamin C

content is concerned. The results obtained by voltammetry highlight the combined methods (HHPE-AE; HHPE; HHPE-EU), because these values represent an appraisal of the total antioxidant capacity from papaya fruit extracts ranging from 16.3 to 27.6 mM TE/100 g FW to a potential between 0.0 and 1.0 V.

When comparing the voltammetric results in Table 2 against the ones in Figure 1, it is possible to observe that HHPE methods showed the highest values of antioxidant capacity for both phenolic extractions (free and bound) and VCE extract. The voltammetry has shown to be a convenient methodology for blood plasma, tissue homogenates, and plant extracts antioxidant capacity determination according to Chevion et al. [36] and tropical fruits antioxidant capacity according to Botero et al. [37]. Although it has already been known that vitamin C has antioxidant capacity [31], determined by other methods, the voltammetry can be considered as a new alternative to determine the antioxidant capacity in papaya fruit.

4. Conclusion

A comparison among the five extraction methods in terms of obtaining bioactive compounds was analyzed. Papaya fruit is rich in bioactive compounds such as phenolic compounds and vitamin C contributing to the antioxidant capacity. The HHPE combinations were the extraction techniques that provided the highest amount of bioactive compounds; therefore, this emergent technology can be considered as a useful tool as an extraction method. In addition, it can be inferred that HHPE-UE is the most efficient combined extracting method for bioactive compounds contained in papaya fruits.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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