Green solvents for the recovery of phenolic compounds from strawberry (*Fragaria* x *ananassa* Duch) and apple (*Malus domestica*) agro-industrial bio-wastes

Uso de solventes verdes para la extracción de compuestos fenólicos a partir de residuos agroindustriales de frutilla (*Fragaria* x *ananassa* Duch) y manzana (*Malus domestica*)

Esteban Villamil-Galindo [1, 2], Andrea Piagentini [1*]

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Abstract

We aimed to study the obtention of valuable phenolic compounds from tissue by-products of agro-industrial processing of apple (GS) and strawberry (RF) using green solvents and Soxhlet extraction methodology. The effects of solvent type [water (W); 80% ethanol, (EtOH)] and extraction ratio (1:10, 1:20, 1:30, and 1:40 p/v) were determined on total phenolic content (TPC), antioxidant capacity (DPPH), and the profile of phenolic compounds of the GS and RF extracts. The solvent type and the extraction ratio significantly affected TPC and DPPH of GS and RF extracts. Extraction with EtOH and 1:40 ratio produced the highest yields, obtaining an RF extract with 15.8 g GAE/Kg (TPC) and 19 mmol TE/Kg (DPPH). The tetra-galloyl glucose isomer and agrimoniin (0.8-0.4 g/Kg) were the main RF phenolic compounds of the eight identified. GS extract, obtained with EtOH and 1:40 ratio, had 11.9 g GAE/Kg (TPC) and 20.5 mmol TE/Kg (DPPH), having quercetin -3-o-glucuronide (0.43 g/Kg) the highest concentration among the eight phenolic compounds identified. The results highlight the potential of green solvents to obtain valuable compounds from low-cost raw materials, like the high-antioxidant capacity phenolic compound extracts obtained herein.

Keywords
green extraction • bioactive compounds • hydrolysable tannins • flavonols • natural antioxidants

1 Universidad Nacional del Litoral. Facultad de Ingeniería Química. Instituto de Tecnología de Alimentos. Santiago del Estero 2829. 3000. Santa Fe. Argentina.
* ampiagen@fiq.unl.edu.ar
2 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). 3000. Santa Fe. Argentina.

Resumen

El objetivo fue estudiar la extracción de compuestos fenólicos con alto valor bioactivo, a partir de subproductos del procesamiento de manzana (GS) y frutilla (RF), utilizando solventes amigables con el medio ambiente y la extracción Soxhlet. Se determinaron los efectos del tipo de solvente [agua (W); etanol 80% (EtOH)] y la relación de extracción (1:10, 1:20, 1:30 y 1:40 p/v) sobre el contenido de fenoles totales (TPC), capacidad antioxidante (DPPH) y perfil de compuestos fenólicos. El tipo de solvente y la relación de extracción afectaron significativamente el TPC y la DPPH. La extracción con EtOH a 1:40 produjo los rendimientos más altos, obteniendo un extracto de RF con 15.8 g GAE/Kg (TPC) y 19 mmol TE/Kg (DPPH). El isómero de tetra galoil glucosa y agrimoniin (0.8-0.4 g/Kg) fueron los principales compuestos fenólicos de RF, entre los ocho identificados. El extracto de GS, obtenido con EtOH a 1:40, tuvo 11.9 g GAE/Kg (TPC) y 20.5 mmol TE/Kg (DPPH), con quercetin-3-o-glucuronido (0.43 g/Kg) como el principal compuesto fenólico entre los ocho identificados. Los resultados destacan el potencial de los solventes verdes para obtener compuestos fenólicos de alta capacidad antioxidante potencialmente bioactivos a partir de materias primas de bajo costo.

Palabras claves

extracción verde • compuestos bioactivos • taninos hidrolizables • flavonoles • antioxidantes naturales

Introduction

One-third of fruit and vegetable production is wasted or lost in the production chain, producing avoidable and non-avoidable waste (3, 31). The former includes the losses generated by wrong handling during postharvest, processing, transport, storage, and distribution, and the non-avoidable waste is that part of the vegetable or fruit that must be eliminated for processing and sale (peels, seeds, core, and inedible parts). Therefore, the appropriate disposal of this waste is essential to reduce the environmental and economic impact of agro-industrial activity. The circular economy proposes different solutions to prevent this waste plant tissue from ending up in city landfills (35). Although the composition of fruit and vegetable by-products includes vitamins, minerals, and carbohydrates (27), as well as different bioactive compounds like phenolic compounds, these residual plant tissues are mainly used as ingredients in animal feed or as a source of energy for boilers. Waste fruit and vegetable tissues are sources of phenolic compounds with significant health benefits for consumers and antioxidant properties (24, 33). Phenolic compounds are synthesised in the plant´s secondary metabolism during the normal development of plants. Their chemical structure has at least one aromatic ring with one or more hydroxyl groups, showing different biological activities according to their carbon bridges and hydroxyl substitution.

The production, consumption and industrial processing of apples and strawberries continue to increase worldwide, and so do the wasted tissues associated (12). Non-edible or non-processable fruit parts may have better composition and bioactivity than edible tissues (5, 34). The main phenolic compounds of strawberries are ellagitannins, phenolic acids and flavonoids like anthocyanins (32), and the principal apple phenolic compounds are catechins and proanthocyanidins (17), depending on the cultivar, the area, and the type of production of these crops. The extraction method of these compounds significantly impacts the extraction yields and their bioactive potential. Considering the structural diversity of the phenolic compounds, no single extraction system could produce the total recovery of these metabolites of interest from all vegetable tissues.

The solid-liquid extraction is commonly used to obtain phenolic compounds, and the success of this process depends on the plant matrix, kind of solvent, temperature, pH, and extraction technology. Polar protic solvents, like ethanol and methanol, can donate hydrogen bonds (33). Otherwise, polar aprotic solvents (acetone and ethyl acetate) have dipoles but do not possess hydrogen atoms bonded with a high electronegativity atom that can be donated in a hydrogen bond. Finally, apolar solvents (hexane and petroleum ether)

have bonds between atoms with similar electronegativities (33). The extraction technique also affects phenolic compound yield. The Soxhlet extraction technique is widely used for obtaining secondary metabolites from different plant tissues, being a reference method for comparing the performance of other extraction techniques. The Soxhlet extraction has good extraction yields without using large quantities of solvent, with a total extraction time of 1-6h, including multiple extraction cycles. This technique commonly employs flammable, hazardous, and toxic organic solvents. The low production cost of ethanol (by fermentation from renewable sources), low energy requirements for final disposal and moderate chronic toxicity make it a sustainable option to replace traditional solvents, along with water, considered the greenest solvent. Water application is generally limited to low-polarity metabolites; nevertheless, it is possible to broaden the solubilisation spectrum of water with suitable parameters (8). Therefore, green extraction with safe and non-toxic solvents, like water, ethanol, and binary ethanol-water mixtures, must be studied. Both solvents are considered safe and acceptable for use in the food industry by regulatory agencies (FDA). The ethanol-aqueous solutions for polyphenol extraction from plant tissues have better yields due to their azeotropic behaviour (1). Additionally, safe solvents in Soxhlet extraction could also yield higher amounts of phenolic compounds from some agro-industrial waste (7, 8, 26).

Complementary treatments with phenolic compounds that strengthen the immune system or their use as antioxidant agents would promote obtaining these phenolic compounds of interest at a low cost, facilitating the accessibility to a larger population and valuing the wasted agro-industrial tissues using green technologies. Therefore, this work aims to study the extraction of the phenolic compounds from strawberry by-products and 'Granny Smith' apple peel, characterise the obtained extracts using the Soxhlet method, evaluate the impact of two green solvents (water and ethanol 80%), and different solid-liquid ratios on the total phenolic content, phenolic profile, and in-vitro antioxidant activity of the extracts.

Materials and methods

Plant material and Experimental design

The by-products of strawberry (RF) (*Fragaria x ananassa Duch*) cv 'Festival', consisting of sepals and stems, with non-processable parts of the fruit (part of the fruit closest to the sepal and peduncle), came from a single field (Coronda, Santa Fe, Argentina) during postharvest preparation for industrial processing. 'Granny Smith' apple peel (GS, 1 mm thickness) was obtained from the minimal processing of apples, according to Rodríguez-Arzuaga and Piagentini (2018). The RF and GS samples (89.2% and 80.4% moisture content, respectively) were weighed, packed in 40 µm polyethylene bags, and stored at -20°C until processing. Before extraction assays, both samples were ground to a particle size <1 mm.

The effect of the extraction solvent (S) and the solid-liquid ratio (R) in the phenolic compound extraction of each vegetable tissue (RF and GS) was studied through a factorial experimental design. The two experimental variables of each factorial design were S and R, with two [S: water (W) and ethanol 80% (EtOH)] and four levels [R: 1:10, 1:20, 1:30 and 1:40 w/v], respectively. The total phenolic content (TPC), phenolic compound profile, and antioxidant capacity (DPPH) were determined on each extract of RF and GS. The extraction times were four hours for EtOH extractions and eight hours for W extractions (extraction times determined in preliminary assays). The extracts were cooled (20°C), filtered, and stored at -20°C for further analysis. Each phenolic compound extraction assay was performed in triplicate.

Total phenolic content (TPC)

Each extract TPC was determined in triplicate by the Folin-Ciocalteu method (34). Gallic acid (Sigma-Aldrich, San Luis, Missouri, USA) was used as the standard reagent to perform the calibration curve, measuring the absorbance of the reaction at 760 nm in a spectrophotometer (Genesys 10s UV-Vis, Thermo Scientific™, Waltham, Massachusetts, USA). The concentration of TPC was reported as g of gallic acid equivalent (GAE) per kilogram of RF or GS (g GAE/Kg).

Phenolic compound profile

The phenolic compound profile of the extracts was performed with an LC-20AT HPLC with a photodiode array detector (PAD), with the software Lab Solutions for data processing and control (Shimadzu Co., Kyoto, Japan). The separation was performed with a hybrid reverse phase column C18 Gemini 5µ 110Å of 250×4.6 mm, attached to a guard column (Phenomenex Inc, CA, USA). The analysis was performed according to Villamil-Galindo *et al.* (2021) for RF and Villamil-Galindo and Piagentini (2022a) for GS. The quantification of the identified compounds was performed using the following external standards (Sigma-Aldrich Inc. St. Louis, Missouri, USA): Ellagic acid (EA), Kaempferol-3-O-glucoside (K3G), Quercetin-3-O-glucoside (Q3G), Chlorogenic acid (ACl), Procyanidin B2 (PACB2), (-) Epicatechin (EPQN), (+) Catechin (CQN), Floretin (FLN), Gallic acid (GA), Coumaric acid (CUA), and Ferulic acid (FRA). The results were expressed in g per Kg of tissue by-product.

Antioxidant capacity (DPPH)

The extract DPPH was determined using the 2.2-diphenyl-1-picrylhydrazyl radical (DPPH*) scavenging assay, performed by triplicate (34). A volume (200 µL) of the DPPH* methanolic solution (0.08 mM) reacts with 25 µL of extract or reference reagent 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Sigma-Aldrich), and the absorbance was measured at 515 nm in a microplate reader (Asus UVM 340, Cambridge, England) after 2 h. The results were expressed as mmol Trolox equivalents/Kg of tissue by-product (mmol TE/Kg).

Statistical analysis

All assays were carried out in triplicates, and data were presented as the mean ± standard deviation (SD). The effect of the solid-liquid ratio and the extraction solvent on the total phenolic content, phenolic compound profile and antioxidant capacity of RS and GS extracts were determined by the analysis of variance (ANOVA). Tukey´s test (5% significance level) was used to determine the significant differences among treatment means. Besides, the correlation between the individually identified phenolic compounds and the antioxidant capacity determined in each extract was determined using Pearson's correlation test. The statistical analysis was performed with STATGRAPHICS Centurion XV software (StatPoint Technologies Inc., Warrenton, VA, USA).

Results and discussion

Phenolic compounds recovery from strawberry agro-industrial by-products (RF)

Both Soxhlet extraction parameters [solid-liquid ratio (R) and type of solvent (S)] affected ($p<0.001$) the yields of total phenolic content (TPC), the phenolic compounds profile, and the antioxidant capacity (DPPH) of the strawberry agro-industrial by-products extracts. The interaction term between R and S was also significant ($p<0.001$), meaning that R affected in a different way TPC, DPPH, and the phenolic compounds profile of the extracts depending on the type of solvent used.

The increase of R values improved the TPC yields of RF Soxhlet extraction with water (W), with the highest yield (10.69 g GAE/Kg) obtained at R 1:40, up to 48% higher than those obtained at lower R values (table 1, page XXX). The concentration gradient between the solute and solvent was the driving force for the diffusion process. Therefore, better extraction yields were obtained with the highest R-value (1:40). Therefore, strawberry by-products have great potential as a source of bioactive compounds obtained with water.

The RF Soxhlet extraction with EtOH significantly increased the recovery of TPC as compared with W by 47-170% (table 1, page XXX). Contrary to W extractions, R did not affect the TPC yields of the EtOH extracts (mean value 15.37 g GAE/Kg) (table 1, page XXX). The phenolic compounds of RF could present preferential solvation when using water-ethanol. The compounds would show hydrophobic hydration with the few polar groups they could have in their structure, and a large amount of (-OH) groups would allow more interaction with water (6). Therefore, this phenomenon would produce more effect than the increase in the extraction ratio. However, the use of EtOH on the Soxhlet extraction system significantly

increased the TPC yields compared to RF water extracts, improving extraction yields by up to 170% (table 1). Therefore, the lower the extraction ratio, the smaller the solvent needed, and the lower the production costs of the extracts.

Table 1. Total phenolic content (TPC) and antioxidant capacity (DPPH) of the extracts from strawberry by-products (RF) and 'Granny Smith' apple peel (GS).

Tabla 1. Contenido de fenoles totales (TPC) y capacidad antioxidante (DPPH) de los extractos de sub-productos de frutilla (RF) y cáscara de manzana 'Granny Smith' (GS).

Sample	R	TPC		DPPH	
		W	EtOH	W	EtOH
RF	1:10	5.58 ± 0.08^{cB}	15.10 ± 0.14^{dA}	4.82 ± 0.20^{cB}	9.95 ± 0.08^{aA}
	1:20	6.57 ± 0.23^{bcB}	15.13 ± 0.18^{dA}	10.43 ± 1.16^{bB}	15.44 ± 0.72^{bA}
	1:30	6.27 ± 0.02^{bB}	15.41 ± 0.21^{dA}	10.70 ± 0.21^{bB}	19.01 ± 0.86^{cA}
	1:40	10.69 ± 0.69^{aB}	15.82 ± 1.76^{dA}	15.34 ± 0.12^{aB}	18.99 ± 1.16^{cA}
GS	1:10	4.18 ± 0.02^{aB}	6.33 ± 0.12^{dA}	3.60 ± 0.14^{dB}	7.26 ± 0.20^{dA}
	1:20	3.90 ± 0.31^{abB}	7.27 ± 0.001^{cA}	8.22 ± 0.35^{cB}	12.89 ± 0.38^{cA}
	1:30	3.55 ± 0.13^{bB}	7.92 ± 0.09^{bA}	11.48 ± 0.39^{bB}	13.75 ± 0.15^{bA}
	1:40	4.35 ± 0.24^{aB}	11.90 ± 0.01^{aA}	14.05 ± 1.09^{aB}	20.51 ± 0.79^{aB}

Mean ± standard deviation. R: Solid-liquid ratio. W: water; EtOH: 80% ethanol. Different capital letters and lowercase letters indicate significant differences ($p<0.05$) by Tukey's test, between solvent and among different solid-liquid ratios, respectively.

Promedio ± desviación estándard. R: relación sólido-líquido. W: agua; EtOH: 80% etanol. Letras mayúscula y minúsculas indican diferencias significativas ($p<0,05$) por el test de Tukey, entre solvente y entre diferentes relaciones sólido-líquido, respectivamente.

Furthermore, these results confirm that binary ethanol-water mixtures are suitable bio-solvents for obtaining phenolic compounds due to their polar protic properties. The yields obtained for RF with EtOH using Soxhlet extraction were higher than those reported for pandan leaves (6.6 g GAE/Kg), mango by-products (4.5-6.6 g GAE/Kg), asparagus (2.8-3.7 g GAE/Kg), cauliflower (1.1-1.8 g GAE/Kg), and bergamot lemon (4-10 g GAE/Kg) (4, 14, 15, 27).

Regarding the antioxidant capacity of RF water extracts, they were significantly affected by R-value, comparable to TPC. The highest DPPH value was obtained for the 1:40 ratio (15.3 mmol TE/Kg), being up to 69% higher than that obtained at R 1:10. Otherwise, like with the content of phenolic compounds, EtOH improved the antioxidant capacity of the RF extracts obtained compared with W extracts. The EtOH RF extracts with the highest DPPH values (18.9-19.0 mmol TE/Kg) were those obtained with R 1:40 and 1:30 ($p>0.05$), respectively. The phenolic compounds are excellent antioxidants of natural origin due to their reducing- capacity, shown by the highly significant correlation between the TPC and the antioxidant capacity of the RF extracts.

Eight major phenolic compounds were identified for the strawberry agro-industrial waste tissue (RF), belonging to three main phenolic compound classes: hydrolysable tannins, ellagic acid derivatives, and flavonols (figure 1a and figure 2, page XXX). Tetragalloyl-glucose isomer (TGI) and galloyl-bis-HHDP-glucose dimer (agrimoniin) (AGN) were identified among the hydrolysable tannins; ellagic acid pentoxide (EAP) and free ellagic acid (EA) among the ellagic acid derivatives; and finally, the flavonols were represented by quercetin-3-O-glucuronide (Q3G), quercetin hexoxide (QHS), kaempferol-3-O-glucuronide (K3G), and kaempferol hexoxide (KHS).

TGI: Tetragalloyglucose isomer, **EAP**: Ellagic acid pentoxide, **AGN**: Dimer of galloyl-bis-HHDP-glucose (agrimoniin isomer), **EA**: Ellagic acid, **Q3G**: Quercetin-3-*O*-glucuronide, **QHS:** Quercetin Hexoxide, **K3G**: Kaempferol-3-*O*-glucuronide. **(+) CTQN**: Catechin, **PACB2**: Procyanidin B2, **(-) EPQN**: Epicatechin, **PACT**: Procyanidin tetramer, **QPN:** Quercetin pentoxide.

TGI: Tetragalloyglucosa isomero, **EAP**: pentoxidode ácido Ellagico, **AGN**: Dimero de galloyl-bis-HHDP-glucosa (agrimoniin isomero), **EA**: ácido Ellagico, **Q3G**: Quercetin-3-*O*-glucuronido, **QHS:** Quercetin Hexoxido, **K3G**: Kaempferol-3-*O*-glucuronido. **(+) CTQN**: Catequina, **PACB2**: Procianidina B2, **(-) EPQN**: Epicatequina, **PACT**: Procianidin tetramero, **QPN:** Quercetina pentoxido.

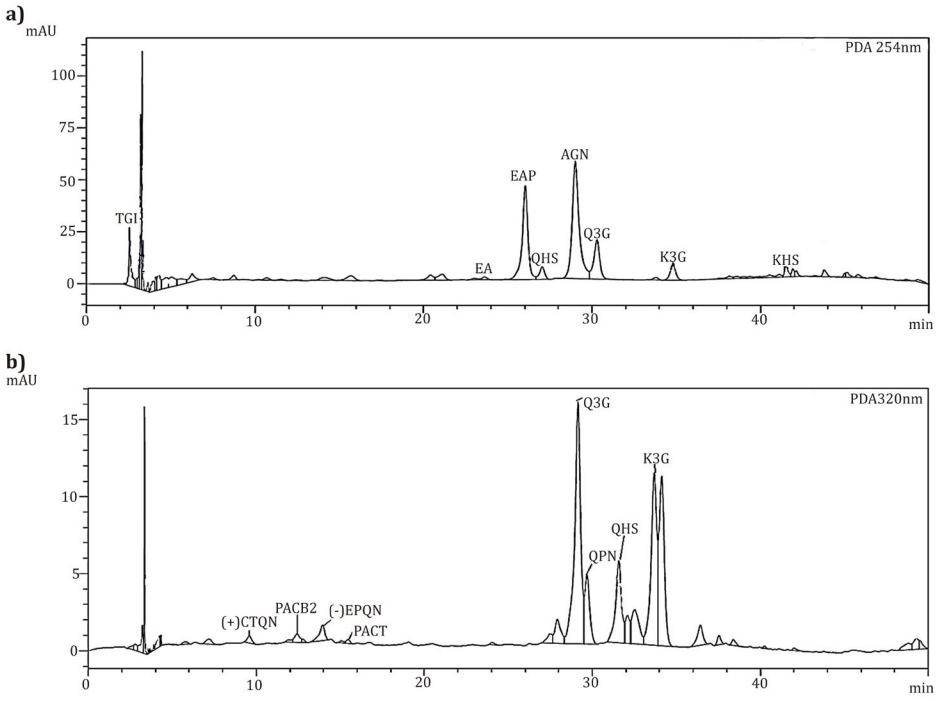

Figure 1. Typical HPLC-UV chromatogram obtained of (a) strawberry by-products at 254 nm and (b) 'Granny Smith' apple peel at 320 nm.

Figura 1. Cromatograma típico HPLC-UV obtenido para (a) sub-productos de frutillas a 254 nm y (b) cáscara de manzanas 'Granny Smith' a 320 nm.

TGI: Tetragalloyglucose isomer, **EAP**: Ellagic acid pentoxide, **AGN**: Dimer of galloyl-bis-HHDP-glucose (agrimoniin isomer), **EA**: Ellagic acid, **Q3G**: Quercetin-3-*O*-glucuronide, **QHS:** Quercetin Hexoxide, **K3G**: Kaempferol-3-*O*-glucuronide, **TPC_{HPLC}**: Total phenolic compounds analyzed by HPLC. Different lowercase letters indicate significant differences ($p<0.05$) by Tukey´s test, between different solid-liquid ratios.

TGI: Tetragalloyglucosa isomero, **EAP**: pentoxido de ácido Ellagico, **AGN**: Dimero de galloyl-bis-HHDP-glucosa (agrimoniin isomero), **EA**: ácido Ellagico, **Q3G**: Quercetin-3-*O*-glucuronido, **QHS:** Quercetin Hexoxido, **K3G**: Kaempferol-3-*O*-glucuronido, **TPC_{HPLC}**: Compuestos fenólicos totales analizados por HPLC. Diferentes letras minúsculas indican diferencias significativas ($p<0,05$) por el test de Tukey, entre diferentes relaciones sólido-líquido.

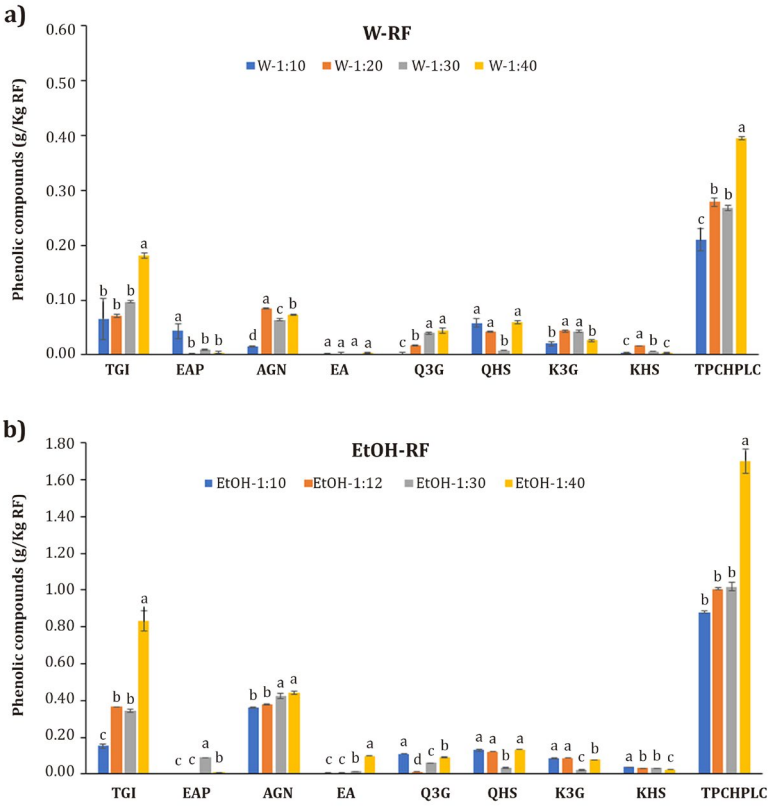

Figure 2. Phenolic compounds from strawberry by-products (RF) extracted with a) water (W) and b) 80% ethanol (EtOH).

Figura 2. Compuestos fenólicos de los sub-productos de frutilla extraídos con a) agua (W) and b) 80% etanol (EtOH).

For the water extracts at R 1:10, hydrolysable tannins represented 38.5% of the total phenolic compounds, similar to flavonols with 39.9%, followed by ellagic acid derivatives with 21.6% (figure 2a, page XXX). However, as the extraction ratio increased, hydrolysable tannins represented 64% of the total phenolic compounds in the extracts obtained with R 1:40, showing that the increase in the concentration gradient in the extraction solvent (W) allowed greater recovery of hydrolysable tannins (33).

The highest concentration of tetragalloylglucose isomer (TGI) in W extracts was obtained at R 1:40 (0.18 g/Kg); for the other R-values, the TGI concentration was similar (0.07-0.1 g/Kg) (figure 2a, page XXX). EtOH extraction significantly improved the TGI yields for all extraction ratios, with the maximum concentration at R 1:40 (0.83 g/Kg). The Ellagic acid pentoxide (EAP) had the highest concentration, 0.088 g/Kg ($p<0.05$), in the EtOH extracts (figure 2, page XXX). EAP concentrations were higher than those reported for the stem of *Sanguisorba Officinalis L* (Rosaceae family) (0.038 g/Kg) (20). Agrimoniin (AGN) is a compound derived from hexahydroxydiphenic acid (HHDP), considered a taxonomic marker of the Rosaceae, with great importance in the nutraceutical industry due to its bioactive properties (30). The AGN yield obtained with water was about 0.064-0.084 g/kg for the higher R values. The AGN yields were significantly improved for all R-values ($p<0.05$) when EtOH was used, with a maximum concentration of 0.44 g/Kg at R 1:40 (figure 2, page XXX). This AGN concentration was higher than the reported for whole strawberry fruit extracts (0.12 g/Kg) obtained with 70% methanol (25), showing this residual tissue as a valuable source of AGN. The antioxidant properties of hydrolysable tannins have been reported *in-vitro* and *in-vivo* (16). Simirgiotis *et al.* (2010) reported that cyanidin glucosides and ellagic acid were the compounds with the highest participation in the antioxidant activity in the edible part of strawberries. For RF, the hydrolysable tannins TGI and AGN had a significant correlation ($p<0.01$) with the DPPH* antioxidant capacity. These compounds have polyhydric alcohol in the centre, and their hydroxyl groups could be partially esterified with ellagic acid or HHDP, having the capacity to yield electrons and thus neutralise the free radicals present (14). The ellagic acid (EA) concentrations were lower in W extracts for any extraction ratio ($p>0.05$). Like AGN, EtOH improved the extraction of EA, with a maximum concentration of 0.10 g/Kg R 1:40 (figure 2, page XXX). The consumption of ellagic acid derivatives was associated with numerous health benefits since, in-vivo conditions, different types of urolithins were metabolised by the microbiota, which had powerful antiproliferative and cancer cell apoptosis-inducing activities (21).

The flavonol Quercetin-3-o-glucuronide (Q3G) concentrations in RF water extracts were 0.002-0.04 g/Kg, obtaining the higher at R 1:40 and 1:30 ($p>0.05$). Nevertheless, using 80% ethanol significantly increased Q3G yields up to 0.11 g/Kg (figure 2, page XXX), similar to chokeberry extracts (11). QHS concentrations obtained with EtOH (0.12-0.13 g/Kg) were higher than those obtained with W (0.04-0.05 g/Kg). Kaempferol is one of the most common flavonols in different botanical species. In the cell vacuoles, Kaempferol tends to glycosidate with some carbohydrates to have more stability in the pH of the medium (28). The highest kaempferol-3-o-glucuronide (K3G) concentration obtained with water was 0.043 g/Kg. EtOH improved the K3G extraction yields by about 50% (figure 2, page XXX). K3G values in RF were close to those reported for green tea (60% methanol), a popular antioxidant infusion (19). Finally, the Kamepferol Hexoxide (KHS) yield increased by up to 91% in EtOH extractions compared to water extracts (figure 2, page XXX).

Therefore, the agro-industrial strawberry by-product showed a large variability of phenolic compounds of interest. The highest recovery of total phenolic compounds (TPC_{HPLC}) with W was achieved with R 1:40 (0.40 g/Kg). As expected, the EtOH increased TPC_{HPLC} up to 425%, obtaining the highest concentration also at R 1:40 (figure 2, page XXX). Similar to the results of TPC and DPPH, the extractions with the ethanol-water binary mixture (80:20) had the highest recovery of phenolic compounds with high antioxidant capacity. The maximum TPC_{HPLC} content (1.70 g/Kg) obtained for RF EtOH extracts was comparable to that reported for strawberry plant leaves (1.95-2.07 g/Kg) obtained with methanol-formic acid (99:1) (30). The phenolic compounds of RF have an excellent antioxidant, anti-inflammatory and anticarcinogenic potential for colorectal cancer (34, 36). Therefore, the high concentration of hydrolysable tannins and ellagic acid derivatives in RF enables the promotion of this kind of agro-industrial by-products as a low-cost source of healthy compounds.

Phenolic compounds recovery from 'Granny Smith' apple peel (GS).

The solid-liquid ratio (R) and the type of solvent (S) affected ($p<0.001$) the content of phenolic compounds and the antioxidant capacity of GS extracts. The interaction term between R and S was also significant ($p<0.001$) for TPC, DPPH, and phenolic compounds profile.

The use of EtOH improved the TPC recovery from GS, like RF (table 1, page XXX). The TPC of GS EtOH extracts increased as R increased, obtaining the highest yield (11.9 g GAE/Kg) for R 1:40. Castro-López *et al.* (2017) reported that R-values higher than 1:20 increased the recovery of phenolic compounds. Binary alcohol-water mixtures offer an eco-friendly solvent system for obtaining phenolic compounds from different wasted plant matrices than those using pure ethanol or other organic solvents. The water and ethanol mixture act synergistically and could provide a suitable polarity range for extracting phenolic compounds (medium-high polarity). The former is fundamental as a swelling agent of the plant matrix, allowing the lower viscosity ethanol to diffuse through the material and break the non-covalent interactions between the solute and the matrix, facilitating the preferential solvation sphere transferring the analyte to the dissolution medium (33).

Phenolic compounds are the plant-secondary metabolites with the highest reported antioxidant activity. Each compound antioxidant capacity differs due to its oxidation-reduction reactions, phenyl ring structure resonance, and hydroxyl group substitution pattern (32). The antioxidant capacity of GS extracts at R 1:40 is 47% higher in EtOH extract than in W extract. The DPPH values obtained were comparable to those reported for other plant materials by Soxhlet extraction (sugar beet molasses, rapeseed, and flowers of *Jatropha integerrima*) (10, 13). The use of EtOH enhanced the antioxidant capacity of GS extracts as R increased (table 1, page XXX), comparable to TPC, and therefore, showing a strong correlation between TPC and DPPH ($p<0.01$).

The two main classes of phenolic compounds identified and quantified in 'Granny Smith' apple peel (GS) were the flavan-3-ols with (+) catechin [(+) CTQN], Procyanidin B2 (PACB2), (-) epicatechin [(-) EPQN], and Procyanidin tetramer (PACT); and the flavonols with the Quercetin-3-o-glucuronide (Q3G), Quercetin pentoxide (QPN), Quercetin Hexoxide (QHS), and Kaempferol-3-o-glucuronide (K3G) (figure 1b, page XXX, and figure 3, page XXX).

For the GS W extracts, the flavan-3-ols and flavanols represented each 50 % of the total phenolic compounds (R 1:10, 1:20 and 1:30), increasing the flavan-3-ols proportion with R 1:40 up to 76 % of the total of the quantified compounds, showing the affinity of this class of phenolic compounds for a polar solvent like water (figure 3a, page XXX). Nevertheless, flavonols accounted for more than 50% of the total phenolic compounds in all the EtOH extractions, with a maximum of 84% in the extracts with R 1:40 (figure 3b, page XXX).

R did not affect ($p>0.05$) the (+)CTQN extraction yield with water. EtOH extractions increased (+)CTQN yields up to 0.066 g/kg, 77% higher than W extracts (figure 3, page XXX). The (+)CTQN yields obtained were lower than those reported by Almeida *et al.* (2017) for 'Granny Smith' apple peel extracted with 100% acetone (0.17 g/Kg). PACB2 (epicatechin-epicatechin dimer) is the most common proanthocyanidin determined in high concentrations in fruits like peaches, apples, and plums. The PACB2 content in W extracts increased with R; it was at least 71% higher for R 1:40 than for the other extraction ratios. However, ethanol did not improve the PACB2 extraction yields. Procyanidin B2 in a liquid medium from 90°C onwards starts a degradation process by oxidation and epimerisation, lowering the procyanidin B2 recovery. The highest concentration of EPQN, reported as the main phenolic compound in apples (23), was achieved with W and R 1:40 (0.1 g/Kg), being higher than that reported for apple pomace extracts (0.02 g/Kg) (20). The highest PACT concentration in W extracts was 0.04 g/Kg, obtained at R 1:40. The EtOH improved PACT yields ($p<0.05$) for all R values (figure 3, page XXX). Procyanidins are oligomers composed of catechin and epicatechin; their structure and high molecular weight give them different bioactive and functional properties for the food industry.

(+)CTQN: Catechin, **PACB2**: Procyanidin B2, **(-)EPQN**: Epicatechin, **PACT**: Procyanidin tetramer, **Q3G**: Quercetin-3-*O*-glucuronide, **QPN:** Quercetin pentoxide, **QHS:** Quercetin Hexoxide, **K3G**: Kaempferol-3-*O*-glucuronide, **TPC_{HPLC}**: Total phenolic compounds analyzed by HPLC. Different lowercase letters indicate significant differences ($p<0.05$) by Tukey´s test, between different solid-liquid ratios.

(+)CTQN: Catequina, **PACB2**: Procianidina B2, **(-)EPQN**: Epicatequina, **PACT**: Procianidina tetramero, **Q3G**: Quercetina-3-*O*-glucuronido, **QPN:** Quercetina pentoxido, **QHS:** Quercetina Hexoxido, **K3G**: Kaempferol-3-*O*-glucuronido, **TPC_{HPLC}**: Compuestos fenólicos totales analizados por HPLC. Diferentes letras minúsculas indican diferencias significativas ($p<0,05$) por el test de Tukey, entre diferentes relaciones sólido-líquido.

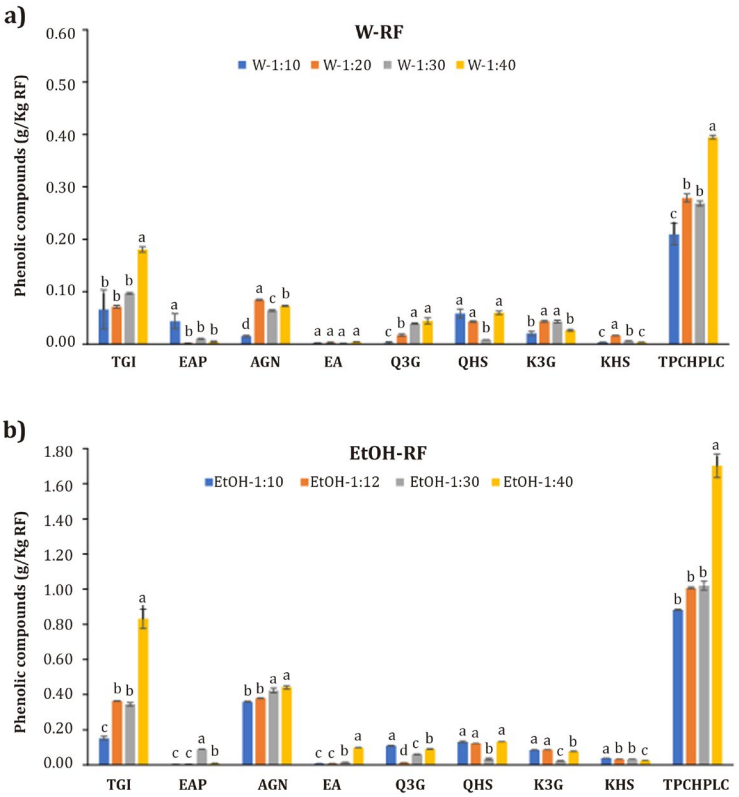

Figure 3. Phenolic compounds from 'Granny Smith' apple peel (GS) extracted with a) water (W) and b) 80% ethanol (EtOH).

Figura 3. Compuestos fenólicos de cáscara de manzana compounds from 'Granny Smith' (GS) extraidos con a) agua (W) and b) 80% etanol (EtOH).

Q3G extraction yields with W were affected by R, obtaining the highest one at R 1:40, 82% higher than the yields obtained at lower R values. Nevertheless, the EtOH improved Q3G extraction yields, as expected for a medium polarity compound. The highest Q3G yield with EtOH (0.43 g/Kg) was obtained at R 1:40, 50% higher than those obtained at lower R values (figure 3). Moreover, Q3G was the GS individual compound with the highest correlation with the antioxidant capacity, mainly for its structure and hydroxyl groups that allowed the donation of electrons, neutralising the free radicals present in the medium (32). Contrarily, RF flavonols did not show a highly significant correlation with the antioxidant capacity determined by DPPH. Consequently, the bioactive potential did not depend only on the bioactive compound concentration but also on the interaction with the food matrix. The values obtained with EtOH were similar and even higher than those reported for 'Granny Smith' apple peel acetone extracts (0.18-0.4 g/Kg) (2).

The highest concentration of QPN, the other quercetin glucoside derivative, was obtained with EtOH at R 1:40 (0.08 g/Kg). The yields of QHS in EtOH extracts were higher than in W, obtaining the highest concentration at R 1:40 (0.14 g/Kg). According to previous reports, quercetin and its glycosides have a low bioavailability (16-25%) due to their low water solubility and crystalline structure at body temperatures (16). Like the other flavonols, EtOH enhanced the recovery of K3G, with the highest concentration (0.25 g/Kg) at R 1:40 (figure 3).

Considering the sum of the compounds identified and quantified, the TPC_{HPLC} obtained for W extracts increased with R, determining the highest concentration in W at R 1:40 (0.55 g/Kg) (figure 3a). Higher TPC_{HPLC} values were obtained with EtOH and higher R values. TPC_{HPLC} extracted with EtOH 1:40 (1.07 g/Kg) was higher than that reported for

the apple pulp (17), showing the bioactive potential of apple peel. The GS TPC_{HPLC} highly correlates with antioxidant activity (R^2 0.87), mainly due to flavonols. These results encourage the integral use of the apple peel as a source of valuable compounds, focusing on green solvents use with low environmental impact and cost (35).

Conclusions

There is a growing demand for nutraceutical products of vegetable origin, as their frequent consumption has been associated with a decreasing risk of having chronic non-transmissible diseases. The market for nutraceutical compounds is booming, and the extraction of bioactive compounds using clean solvents from agro-industrial waste tissues, like the strawberry by-products and apple peel, presents an opportunity to reduce costs and the environmental impact. The conventional Soxhlet extraction technique has good yields, low complexity, and high efficiency, allowing optimal use of natural resources, especially those that are rejected for industrial processing, like the waste tissue produced during the postharvest trimming of the strawberry (about 7-20% of the fruit intended for industrial processing) and Granny Smith apple peel (about 12% of the fruit intended for minimal processing).

This study demonstrates that waste vegetable tissues can be transformed into valuable phenolic compounds with antioxidant properties using eco-friendly solvents such as water and ethanol. The extracts with the highest content of phenolic compounds and antioxidant capacity were obtained for Soxhlet extraction with 80% ethanol and 1:40 extraction ratio for both the strawberry by-products (15.8 g GAE/Kg and 19 mmol TE/Kg) and the 'Granny Smith' apple peel (11.9 g GAE/Kg and 20.5 mmol TE/ Kg). Additionally, eight main phenolic compounds were identified and quantified in both waste tissues. The hydrolysable tannins, like Tetragalloyglucose isomer (TGI: 0.83 g/Kg) and Dimer of galloyl-bis-HHDP-glucose (agrimoniin isomer, AGN: 0.44 g/Kg), were the main phenolic compounds extracted from RF, while flavonols accounted for 83.7% of the total extracted phenolic compounds from GS, obtaining for Quercetin-3-O-glucuronide the highest yield (Q3G: 0.43 g/Kg).

These results demonstrated the importance of by-products as low-cost sources of bioactive compounds with high nutraceutical potential through a circular process approach in the fruit and vegetable industry. Currently, these bio-wastes are disposed of in landfills without any use. The information obtained in this study provides a pathway towards the integral use of strawberry and apple by-products. The challenge is to continue studying the development of a procedure for obtaining bioactive compounds from strawberry by-products and 'Granny Smith' apple peel with higher yields, shorter extraction times and lower energy consumption, using more sustainable and efficient technologies stimulating an integral use of these by-products.

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