Valorization Strategy of Banana Passion Fruit Shell Wastes: An Innovative Source of Phytoprostanes and Phenolic Compounds and Their Potential Use in Pharmaceutical and Cosmetic Industries

Sonia Medina[1,2,#], Jacinta Collado-González[1,#], Federico Ferreres[1], Julián Londoño-Londoño[2,*], Claudio Jiménez-Cartagena[2], Alexandre Guy[3], Thierry Durand[3], Jean-Marie Galano[3], Angel Gil-Izquierdo[1,*]

[1]Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), Espinardo, Murcia, Spain
[2]Faculty of Engineering, Food Engineering Program, Corporación Universitaria Lasallista, Caldas-Antioquia, Colombia
[3]Institut des Biomolécules Max Mousseron, UMR 5247 CNRS, University of Montpellier, ENSCM, Montpellier, France
[#]These two authors have contributed equally to this work.
*Corresponding author: julondono@lasallistadocentes.edu.co, angelgil@cebas.csic.es

Abstract At present, people with today's busy lifestyle have a general trend of consuming fruit like banana passion fruit (*Passiflora tripartita* var. mollissima) as juices or smoothies, being an increasing tendency that can generate a large amount of agro-industrial residues. Whereby, food industries have a significant investment to minimize these wastes generated or devise alternatives of residue use. So, food researches are aimed to exploit these fruit waste. In this sense, this study has allowed the detection for the first time, in banana passion fruit shell, phytoprostanes (PhytoPs), oxylipins with multiple biological activities in humans. Furthermore, this study, with methodology used (LC-MS) allowed us to detect higher amount of these compounds (2318.63 ± 71.51 μg/100 g DW) than other vegetable matrices previously studied (macroalgae, olives or almonds, among others). In addition, we were able to identify 14 phenolic compounds (including cinnamoyl acid derivatives, flavonoid-O-glycoside, flavonoid-C-glycosides), not previously described in this matrix. Hence, this work increased the knowledge about the bioactive compounds profile of banana passion fruit shells and thereby to achieve a product with added value that may be used as natural source of bioactive compounds as alternative of synthetic substances in several industries of pharmaceutic or cosmetic fields.

Keywords: Passiflora tripartita var. mollissima, fruit shells, agro-industries wastes, food analysis, phytoprostanes, polyphenols

Cite This Article: Sonia Medina, Jacinta Collado-González, Federico Ferreres, Julián Londoño-Londoño, Claudio Jiménez-Cartagena, Alexandre Guy, Thierry Durand, Jean-Marie Galano, and Angel Gil-Izquierdo, "Valorization Strategy of Banana Passion Fruit Shell Wastes: An Innovative Source of Phytoprostanes and Phenolic Compounds and Their Potential Use in Pharmaceutical and Cosmetic Industries." *Journal of Food and Nutrition Research*, vol. 5, no. 11 (2017): 801-808. doi: 10.12691/jfnr-5-11-2.

1. Introduction

The banana passion fruit or also known as "curuba" (*Passiflora tripartita* var. mollissima) is a native plant from the Andean region of America and is cultivated in Colombia between 2000 and 3000 m above the sea level, where the fruit is mainly consumed as juices or smoothies, generating a large amount of residues (mostly, fruit shells) [1]. So, nowadays, in the food industries, the processing procedures can lead to one third of the production being discarded. It results in high sums of waste materials or by-products (shells, seeds, leaves, stones, among others), disposal of these materials can be costly for the manufacturer and also may have a negative impact on the environment [2]. The increase of these by-products generated by food companies had led researchers to search for viable alternatives in generating new products (additives or supplements) with high nutritional value [3]. Currently, by-products are used for limited purposes such as agriculture application, animal feed, compost, vermiculture, biofuels or manufacture of chemical compounds [4].

In the same sense, it is well known that by-products represent an important source of sugars, minerals, organic acids, dietary fiber and bioactive compounds such as phenolic compounds. The multiple biological activities of polyphenols and their potential used in the prevention of many humans' disorders (cardiovascular disease, obesity, diabetes, among others) have widely been recognized [5,6]. Consequently, recycling of the by-products has been supported by the fact that these compounds (polyphenols) have been located specifically in the fruit shells [7].

However, recent works have reported the presence of compounds (less well studied) in plant matrices namely phytoprostanes (PhytoPs)-which have not been described previously in banana passion fruit. The PhytoPs (oxylipins) are formed by non-enzymatic peroxidation of α-linolenic acid (C18: 3, n-3, ALA) -the predominant polyunsaturated fatty acids (PUFA) in plants- [8]. In addition, several researchers have proposed that PhytoPs have multiple functions in both plants and humans [8,9,10]. In plants, these bioactive compounds participates in an archaic signaling system that serves to protect plants form oxidative stress (OS), detoxification pathways and they contributes on phytoalexins accumulation in a variety of vegetables species [11]. Likewise, these compounds have a wide range of activities in humans, like immune function regulation, anti-inflammatory activity, neuroprotection and they can reach the gastrointestinal tract and interact with gut microflora [10,12,13,14].

The PhytoPs have been analyzed in a large variety of plant species and foodstuffs. They have been found in *Arabidopsis thaliana* (leaves), *Helianthus annuus* (sunflower, seed), Olea europaea (olive oil), *Vitis vinifera* (grape seed oil, wine and must), *Prunus dulcis* (almond), among others [11,15,16,17,18,19]. However, there is not enough information collected about phytochemical composition (polyphenols and PhytoPs) in a banana passion fruit shells. For those pertinent reasons, the main purpose of this study was the analysis of the phenolic compounds and PhytoPs to valorize the wastes from banana passion fruit shells in order to promote its use in the nutraceutical, pharmaceutical and cosmetic industries as an alternative to synthetic substances.

2. Materials and Methods

2.1. Chemicals and Reagents

Ten PhytoP standards (9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, ent-16-*epi*-16-F_{1t}-PhytoP, 9-D_{1t}-PhytoP, 9-*epi*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, *ent*-16-B_1-PhytoP, 9-L_1-PhytoP, and *ent*-9-L_1-PhytoP) were synthesized according to previously described methods [20,21,22]. Their chemical structures are shown in Figure 1. The PhytoPs are very stable compounds in their frozen form; they were maintained at -80 ºC. Additionally, quercetin-3-O-rutinoside (rutin), 5-O-caffeoylquinic acid, bis-Tris (bis (2-hydroxyethyl)amino-tris(hydroxymethyl)methane), and BHA (tert-butylhydroxyanisole) were acquired from Sigma Aldrich (St. Louis, MO, USA). Hexane, methanol, and acetonitrile were purchased from J.T. Baker (Phillipsburg, New Jersey, USA). The solid-phase extraction (SPE) cartridges used (Strata X-AW; 100 mg, 3 mL) were obtained from Phenomenex (Torrance, CA, USA) and the water used was obtained from a Milli-Q® water purification system (Millipore, Bedford, MA, USA).
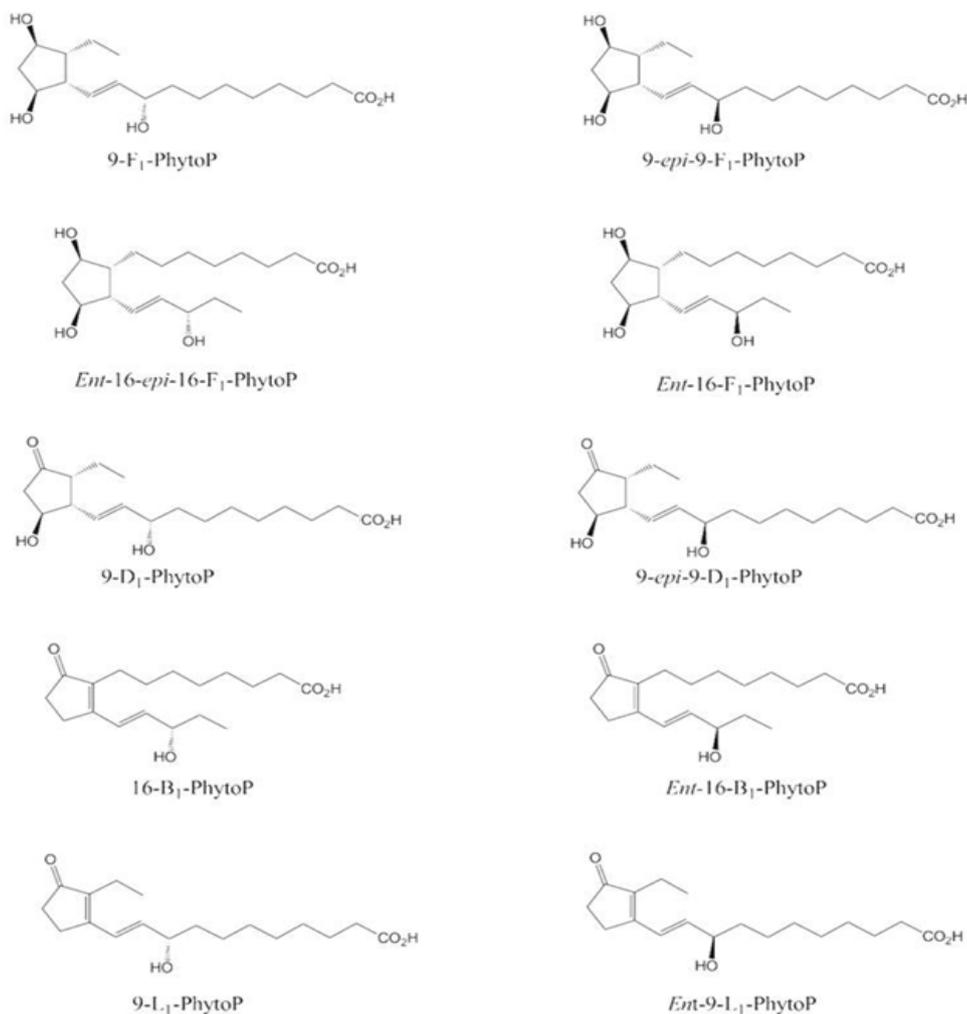

Figure 1. Chemical structures of phytoprostanes

2.2. Banana Passion Fruit Shell Samples

The banana passion fruit (*Passiflora tripartita* var. mollissima) were purchased in a local market in Antioquia, Colombia. 100 g of shell were separated from whole banana passion fruit and subjected to dehydration using a freeze-drying system with manifold (Eyela FDU-1100). The samples were frozen at a rate of 0.05°C min^{-1} at -20°C. Once the sample was frozen, a vacuum pressure of 1.6 mbar for the sublimation process was applied. The shell samples were heated from -20 to 30°C for a period of time of 8 h.

2.3. Extraction and Determination of Phytoprostanes

The analyses of PhytoPs from banana passion fruit shells were performed following the method developed by Collado-Gonzalez and colleagues [16] with slight modifications. Concisely, 1 g of curuba shells were ground with 15 mL MeOH with 0.1% BHA and they were vortexed during 5 minutes at 2000 g. The supernatants were filtered by Sep.pack® cartridges (100 mg, 3 mL) and were employed for the SPE of PhytoPs following the protocols described in several reports [16,19,23]. Each banana passion fruit shell sample was analyzed in triplicate using a UHPLC-QqQ-MS/MS (Agilent Technologies, Waldbronn, Germany) as previously described [16]. For chromatography separation to column BEH C$_{18}$ 2.1 x 50 mm, 1.7 µm (Waters, Milford, MA, USA) was utilized at 6°C. The mobile phases employed were solvent A (H$_2$O/acetic acid; 99.99: 0.01, *v/v*) and B (MeOH/acetic acid; 99.99: 0.01, *v/v*), at a flow rate of 0.2 mL min^{-1} and using the following gradient: (% B; min): (60; 0), (62; 2), (62.5; 4), (65; 8) and (60; 8.01). The MS analyzes were applied in the multiple reaction monitoring (MRM) mode in the negative ionization option of the ESI. Data acquisition and processing were performed using MassHunter software version B.04.00 (Agilent Technologies, Germany).

2.4. Extraction and Determination of Phenolic Compounds

The freeze-dried samples of banana passion fruit were powdered and 0.16 g each sample was extracted with 1.5 mL of MeOH/H$_2$O/formic acid (25: 24: 1, *v/v/v*) according to a previous study by Herraiz and colleagues with slight amendments [24]. The extract samples were vortexed and subsequently sonicated in an ultrasonic bath for 60 min and then they were centrifuged at 10.000 g for 10 min. The supernatant was filtered through a 0.22 µm filter (PVDF) for further analysis by HPLC-DAD-ESI/MSn as a previous study by Sanchez-Rodriguez and colleagues [25]. Chromatographic analyses were carried out in an Agilent 1200 Series HPLC equipped with a diode array detector and mass detector in series (Agilent Technologies, Waldbronn, Germany) using a Kinetex column (5 µm, C18, 100 A, 150 x 4.6 mm; Phenomenex, Macclesfield, UK). The mobile phase consisted of two solvents: H$_2$O-formic acid (1%) (A) and acetonitrile (B), starting with 10% B and using a gradient to obtain 25% B at 20 min and 50 % B at 25 min. The flow rate was 800 µL min^{-1},

and the injection volume was 5 µL. Spectral data from all peaks were accumulated in the range of 240-600 nm, and chromatograms were recorded at 330 nm. The mass detector was a Bruker ion trap spectrometer (model HCT Ultra) equipped with an electrospray ionisation interface. The ionisation conditions were adjusted at 350°C and 4.0 kV for capillary temperature and voltage, respectively. The nebulizer pressure and flow rate of nitrogen were 65.0 psi and 11 L/ min, respectively. The full scan mass covered the range from *m/z* 100 up to 1200 *m/z*. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 up to 2 V. Mass spectrometry data were acquired in the negative ionisation mode. MSn was carried out in the automatic mode on the more abundant fragment ion in MS$^{(n-1)}$ and was controlled by LCMSD software (Agilent, v. 6.1).

The different phenolic compounds were identified by comparing their elution order and UV-Vis spectra with those of external standard of quercetin-3-*O*-rutinoside (rutin) and phenolic acids as 5-caffeoyl quinic acid (chlorogenic acid) at 330 nm and with data obtained previously in other matrix [26].

3. Results and Discussion

3.1. Qualitative and Quantitative Analyses of Phytoprostanes

To the best of our knowledge, no information has been published before on the content of PhytoPs in banana passion fruit. In this study, a total of seven PhytoPs were detected in shell samples of this fruit (Table 1), some of these compounds are formed as mixture of regio- and stereoisomers and these epimeric mixture were quantified as the sum of the enantiomers according with the methodology followed [16] using a UHPLC-QqQ-MS/MS. The PhytoPs detection was carried out by their MS and MS/MS fragmentation (quantification and confirmation transitions) and their corresponding retention times. In this work, we detected the 9 series of the F$_1$-, D$_1$- and L$_1$-class and 16 series of B$_1$- and F$_1$-class that oscillated from 0.23 to 1978.60 µg/ 100 g DW, being the 9 series of F$_1$-PhytoP the dominant class of PhytoPs and the 9 series of the D$_1$-PhytoP class the minor amount (Table 1). These results are in accordance with those quantified in other vegetables matrices like macroalgae samples [23] or red wine/must [19] or gulupa shell [27] that were carried out using UHPLC-QqQ-MS/MS. The F$_1$-PhytoP class occurred predominantly esterified in plant lipids where plant membranes are the storage place of this class of PhytoPs representing the first line of the plant defense. In the current study, the total PhytoPs content in banana passion fruit shell samples (2318.63 ± 71.51 µg/ 100 g DW) was in the range higher than dry macroalgae species (from 0.0056 to 1.38 µg/ 100 g DW) [23] or in almonds samples of different cultivars (from 4.0 to 23.8 µg/ 100 g DW) [15] or in gulupa shell (from 0.13 to 6.76 µg/ 100 g DW) [27]. This fact is very important due to these wastes from banana passion fruit represented a valuable source of PhytoPs - with higher content than other matrices studied - and it could have a number of repercussions, especially for

the future applications of these wastes in pharmaceutical and cosmetic industries because their high content in these compounds with multiple biological activities [8,9].

Table 1. Phytoprostanes content (μg/ 100 g DW) in *Passiflora tripartita* var. mollisima shells.

Phytoprostanes	μg/ 100 g DW
9-*epi*-9-F_{1t}-PhytoP	1978.60 ± 70.16
9-F_{1t}-PhytoP	318.88 ± 13.77
16-B_1- PhytoP + *ent*-16-B_1- PhytoP	11.98 ± 1.16
9-L_1- PhytoP + *ent*-9-L_1- PhytoP	8.27 ± 0.77
9- *epi*-9-D_{1t}-PhytoP	0.35 ± 0.04
9-D_{1t}-PhytoP	0.32 ± 0.06
ent-16-*epi*-16-F_{1t}-PhytoP + *ent*-16-F_{1t}- PhytoP	0.23 ± 0.02

Results are expressed as mean ± standard deviation of three determinations

On the other hand, in previous reports, GC-MS has been the most widely used analytical technique to detect PhytoPs. Hence, these compounds have been measured in tissues of several plant species (tomato leaves, peppermint leaves, valerian root, birch pollen and leaves, among others) where they were ranged from 43 to 1380 ng/ g DW [28]. Additionally, PhytoPs have also been detected in edible vegetables oils (soybean, olive, walnut and grape) whose concentrations were from 0.09 up to 99 mg/ L [29].

In the same sense, these bioactive compounds were also quantified in cell culture of four taxonomically distant plant species (*Nicotiana tabacum, Glycine max, Rauvolfia serpentine and Agrostis tenuis*) using GC-MS and found to be in the range of 4.5 to 60.9 ng/ g DW [30]. All these studies had a drawback, they were not able either to quantify individual PhytoPs or detect the series of these compounds. In addition, the methods by GC-MS used in these experiments were slow because of the use of a derivatization process.

On the other hand, agronomic factors, agricultural system (conventional versus organic), cultivate, genotype and irrigation conditions or rainfalls, could affect to the PhytoP content [15]. This could be due to abiotic factors, and it could have a significant influence on PUFA levels (including ALA levels) in banana passion fruit and therefore a relevant effect in the PhytoP formation. Similarly, the PhytoP content may be reduced or augmented depending on the storage conditions and processing of the shell samples since inappropriate storage or industrial processing induced autoxidation and modulated PhytoP levels in almonds [31]. In short, good agricultural practices (GAP) and good hygienic practices (GHP) could modulate the PhytoP content of vegetables matrices, including fruit wastes [17].

On the other side, many studies have validated the pharmacological properties of wastes from plants belonging to the family Passifloraceae. So that, it has been reported that flour from passion fruit peel had a positive effect in blood glucose control, antihypertensive effect and an improvement of bowel health [32]. Moreover, in humans, previous works have reported that PhytoPs exhibited an anti-inflammatory and apoptosis-inducing activities, similar to other prostanoids like PGA1 or J_2-IsoP [10] and other recent study has suggested that PhytoP protect

immature neurons from oxidative attack and promote differentiation of oligodendrocytes progenitors through PPAR activation [14]. Accordingly, the large amount of PhytoPs detected in banana passion fruit shells add up to their biological properties could open a new line of business for nutraceutical and pharmaceutical companies that could include these fruit wastes for the preparation of their formulations.

On the other hand, in food applications, pectin is the most substance from passion fruit peels [32]. However, to date, banana passion fruit is the vegetable matrix with the highest content of PhytoPs (especially F_1-class), representing a valuable source of these bioactive compounds that may be used by food companies as a functional ingredient for the nutraceuticals development.

3.2. HPLC-DAD-ESI/MSn Analyses of Phenolic Compounds

Among the Passiflora species extracts analysed, *P. tripartite* var. mollissima displayed the most complex flavonoid profile [33]. In our study, phenolic compounds screening by HPLC-DAD-ESI/MSn of banana passion fruit shells revealed three cinnamoyl acid derivatives (1, 2 and 12) and eleven flavonoid compounds (Figure 2): four *O*-glycosides (8, 10, 11 and 14) and seven *C*-glycosides one of which is a mono-*C*-glycoside (6), three of them are *O*-glycosylated on *C*-glycosylation (7, 9 and 13) and another three present a mixed *O*-glycosylation, on phenolic hydroxyl and on the hexose of *C*-glycosylation (3-5).

3.2.1. Cinnamoyl Acid Derivatives

With respect to the MS analysis of these acid derivatives, the compounds 1 and 2 exhibited a UV spectra of sinapic acid (326 nm), their deprotonated molecular ions were *m/z* 547 and *m/z* 385, with a characteristic fragmentation loss at *m/z* 324 (dihexosyl radical) and 162 amu (hexosyl radical), respectively. Thereby, giving rise to deprotonated ion of sinapic acid ([sinapic acid-H]⁻, *m/z* 223), whereof, these compounds may be labelled as sinapic acid dihexoside (1) and sinapic acid hexoside (2). Moreover, the compound 12 with UV spectrum of *p*-coumaric acid (312 nm) displayed a deprotonated molecular ion at *m/z* 487 amu with a fragmentation loss of 164 amu (*p*-coumaric acid) and an ion at *m/z* 163 ([*p*-coumaric acid-H]⁻) so that it may be considered as *p*-coumaroyl derivative (Figure 2). These compounds are reported herein in dates for the first time in banana passion fruit shells.

3.2.2. Flavonoid-*O*-glycosides

The compounds 8, 10, 11 and 14 exhibited MS fragmentations that are shown in Table 2 where we may observed that these compounds had deprotonated ion of the aglycones as base peak (8: luteolin and 10, 14: quercetin) or aglycone derivatives (11, [pentahydroxy.methoxy.flavone-H-15]⁻). This fragmentation type is representative of flavonoid *O*-glycosides on a unique phenolic hydroxyl [34,35]. In the same sense, the flavonoids 8, 10 and 11 also showed in their MS fragmentations, ions derivatives from the interglycosidic linkage breaking (-162/-180, hexosyl-glycoside, 8) and (-146/-164, rhamnosyl-

glycoside, 10 and 11), with a different linkage of (1→6), it would probably be a linkage (1→2) [34,35]. Consequently, these compounds could tentatively be labelled as luteolin-7-*O*-(2-hexosyl)hexoside (8), quercetin-3-*O*-(2-rhamnosyl)hexoside (10) and pentahydroxy-methoxy-flavone-*O*-(2-rhamnosyl)hexoside and methoxyquercetin-3-*O*-(2-rhamnosyl)hexoside (11). And the compound number 14 was tentative identified as a monohexoside of quercetin, likely quercetin-3-*O*-hexoside (Table 2). In a previous published work, Simirgiotis and colleagues identified four flavonoid-*O*-glycosides in banana passion fruit peels [36], nevertheless, these compounds were different from we detected in our study and the first time reported in banana passion fruit shells.

3.2.3. Flavonoid-*C*-glycosides

These compounds are the most abundant in banana passion fruit, especially, orientin, isoorientin, vitexin and isovitexin and their derivatives [33,36,37].

3.2.3.1. Flavonoid-mono-*C*-glycoside

The compound 6, coeluting with the compound 7, presented a UV spectrum of luteolin (Table 2) and a deprotonated molecular ion of luteolin hexoside (*m/z* 447 amu, 285+162), with a fragmentation losses of 90 and 120 amu (indicating *C*-glycosylation with hexose) corresponded to the ions at *m/z* 357 and 327 amu respectively. In this sense, mono-*C*-flavonoid corresponded to the ions Aglycone+71 and Aglycone+41, the high abundance of

both, indicated a *C*-glycosylation in 6-position [38], therefore these compounds can be considered as luteolin-6-*C*-hexoside (isoorientin). This result was in accordance with other previous study that detected flavonoid-mono-*C*-glycosides including isoorientin in different species of *Passiflora* [33].

3.2.3.2. Flavonoid-*C*-(*O*-glycosyl)glycosides

The compounds 7, 9 and 13 exhibited a characteristic MS fragmentation of *C*-hexosyl derivatives, and as we have already seen, they are characterized by fragment losses of 90 or 120 amu. The high abundance of these ions generated corresponded to base peak in 7 (*m/z* 473, [(M-H)-120]⁻) and less abundant for 9 and 13 (Table 2). This fact may be indicative of *C*-glycosylation position, 6-position in the compound 7 and 8-position in 9 and 13, because the sugar fragmentation in C-6 is easier than in C-8. Moreover, in three compounds, a loss of 164 amu (146+18) –which is characteristic of interglycosidic linkage of rhamnose in 2 position - was observed [39]. The ions *C*-glycosyl derivatives: 357 (aglycone+71) and 327 (aglycone+41), luteolin (7); 341 (aglycone+41), trihydroxy-methoxy-flavone (chrysoeriol/diosmetin) (13) and 293 (aglycone+41-18), apigenin (9) were also detected [39]. On the basis of the above considerations, these compounds can be considered as luteolin-6-*C*-(2-rhamnosyl)hexoside (7), apigenin-8-*C*-(2-rhamnosyl)hexoside (9) and diosmetin/chrysoeriol-8-*C*-(2-rhamnosyl)hexoside (13). This is the first report which identifies these compounds in banana passion fruit shells.

Table 2. Rt, UV and MS: [M-H]-, MS2[M-H]- and MS3 data of flavonoid compounds from Passiflora tripartita var. mollisima shell[a]

| Compounds[b]	Rt (min)	UV (nm)	[M-H]⁻, m/z	MS²[M-H]⁻, m/z (%)			MS³[(M-H)→(M-H-162)]⁻, m/z (%)				
				Flavonoid-*O*-glycosyl-*C*-(*O*-glycosyl)glycosides							
				-162	-162-120	-162-164	-120	-164	Agl+71	Agl+41	Agl+41-18
3 Ap-7-*O*-Hx-8-*C*-(2-*O*-Rh)Hx	8.2	270, 338	739	577(100)		413(5)	457(5)	413(100)	341(2)	311(2)	293(30)
4 Lt-7-*O*-Hx-6-*C*-(2-*O*-Rh)Hx	8.6	---[c]	755	593(100)	473(7)		473(100)	429(40)	357(50)	327(75)	
5 Ch/Dm-7-*O*-Hx-8-*C*-(2-*O*-Rh)Hx	10.3	244, 270, 340	769	607(100)		443(19)	487(4)	443(100)		341(4)	323(15)
				Flavonoid-*C*-(*O*-glycosyl)glycosides							
				MS²[M-H]⁻, m/z (%)							
				-90	-120	-164	Agl+71	Agl+41	Agl+41-18		
7 Lt-6-*C*-(2-*O*-Rh)Hx	11.6	256, 268, 348[d]	593	503(6)	473(100)	429(40)	357(60)	327(60)			
9 Ap-8-*C*-(2-*O*-Rh)Hx	13.2	---[c]	577		457(9)	413(100)			293(25)		
13 Ch/Dm-8-*C*-(2-*O*-Rh)Hx	14.8	252, 270, 348	607		487(10)	443(100)		341(30)			
				Flavonoid-*C*-glycoside							
6 Lt-6-*C*-Hx	11.4	256, 268, 348[d]	447				357(100)	327(90)			
				Flavonoid-*O*-glycosides							
				-146	-162	-164	-180	Agl-H/2H			
8 Lt-7-*O*-(2-*O*-Hx)Hx	12.9	254, 266, 345	609		447(65)		429(5)	285(100)			
10 Qct-3-*O*-(2-*O*-Rh)Hx	13.2	---[c]	609	463(15)		445(20)		300(100)			
11 Pent-OH-MeO-Flv-*O*-(2-*O*-Rh)Hx	13.9	---[c]	639[d,e]	493(3)		475(8)		331(70)			
14 Qct-3-*O*-Hx	15.3	---[c]	463					301(100)			
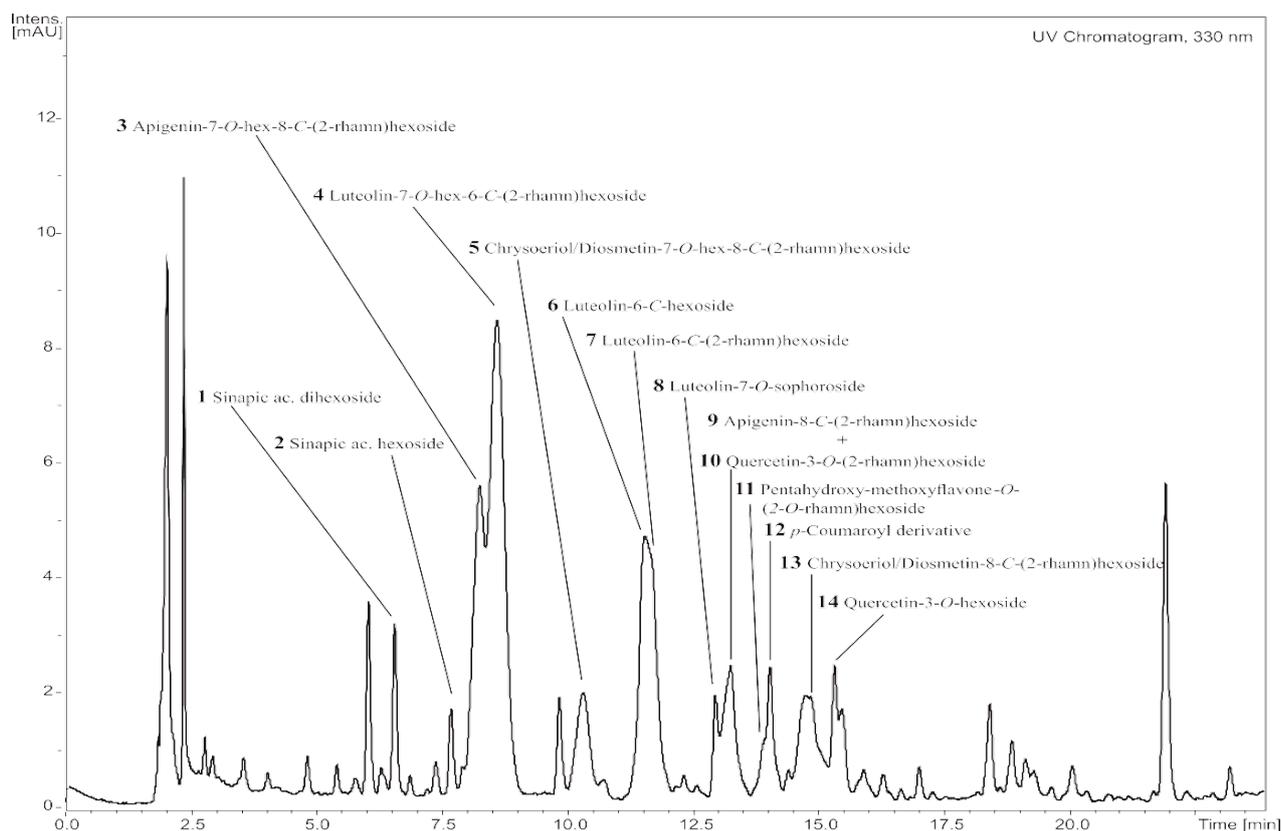
Figure 2. HPLC-UV (330 nm) profile of phenolic compounds from *Passiflora tripartita* var. mollisima shell. The identity of flavonoid compounds was detailed in Table 2
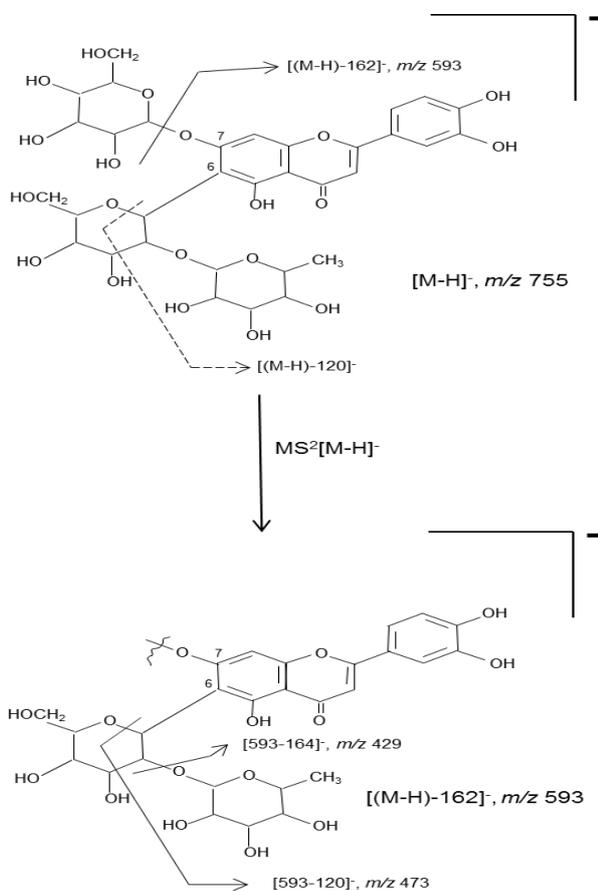

Figure 3. MS fragmentation scheme of luteolin-7-*O*-hexoside-6-*C*-(2-rhamnosyl)hexoside (compound 4) from banana passion fruit (*Passiflora tripartita* var. mollisima) shell

3.2.3.3. Flavonoid-*O*-glycoside-*C*-(*O*-glycosyl)glycosides

The deprotonated molecular ions of the compounds 3, 4 and 5 were 162 amu higher than those of the compounds 9, 7 and 13, respectively. Additionally, their MS2 fragmentations were based on 162 amu (radical hexosyl) loss, obtaining a base peak which was coincident with those of the compounds 9, 7 and 13. This type of fragmentation is characteristic of *O*-glycosylation on a phenolic hydroxyle. In addition, we can observed, although less frequently, the ions derived from simultaneous losses of 162 and 120 (*m/z* 473(7%), compound 4) (Figure 3) and -162-164 (*m/z* 413(5%), compound 3 and 443(19%), compound 5) (Table 2). The MS3 [(M-H) →(M-H-162)]- of the compounds 3, 4 and 5 was the same that the MS2fragmentation observed in 9, 7 and 13. Hence, they have been assigned to *O*-hexosyl derivatives of 9, 7 and 13 on a phenolic hydroxyle, probably in 7-position [39], consequently, they may be labelled as apigenin-7-*O*-hexoside-8-*C*-(2-rhamnosyl)hexoside (3), luteolin-7-*O*-hexoside-6-*C*-(2-rhamnosyl)hexoside (4), and diosmetin/chrysoeriol-7-*O*-hexoside-8-*C*-(2-rhamnosyl)hexoside (5).

The findings of our study were in agreement with previous reports that have detected *C*-glycosyl flavonoid profile in *P. tripartita* var. mollisima pericarp by HPLC method [37], and in leaves samples [33] or Simirgiotis and colleagues that described in this matrix 31 compounds (flavonoids-*C*-glycosides and orientin derivatives) where the peel presented the highest content of these compounds (56.03 ± 4.34 mg quercetin 100 g-1 DW) than pulp or juice [36]. Nevertheless, the specific compounds detected in our work (exception for compound 6) have not been

reported in previous studies. Consequently, banana passion fruit wastes are a valuable source of phenolic compounds, this fact is very important to justify the investment by companies to exploit these residues. Since, these fruit wastes possess several advantages in contrast to synthetic antioxidant in terms of consumers` acceptance or legal requirements.

4. Conclusion

Residues from fruits usually contain many types of compounds - some of them unknown- with multiple biological activities and with therapeutic effects that most of the time cannot be attributed to an unique compound otherwise many compounds acting in synergy. In this way, this study has allowed to detect and quantify novel compounds in *Passiflora tripartita* var. mollisima shell namely phytoprostanes (seven) and to identify 14 phenolic compounds (including cinnamoyl acid derivatives, flavonoid-*O*-glycoside, flavonoid-*C*-glycosides) in this matrix for the first time.

Fruit wastes are a part of plant that cannot be consumed directly, consequently, the valorization of fruit waste -rich source of PhytoPs and phenolic compounds- may be a meaningful alternative for many companies that may exploit their vegetable wastes from industry processing to obtain a product with added value. Specifically, wastes from banana passion fruit represent rich natural source of bioactive compounds with multiple biological activities, like anti-inflammatory and antioxidant properties and could act as a potential alternative to the synthetic antioxidant compounds commonly used for food, pharmaceutical and cosmetic industries.

Abbreviations

PhytoPs: phytoprostanes;
DW: dry weight;
ALA: α-linolenic acid;
PUFA: polyunsaturated fatty acids;
OS: oxidative stress;
Bis-Tris: Bis-(2-hydroxyethyl)-amino-tris(hydroxymethyl)-methane;
BHA: tert-butylhydroxyanisole;
SPE: solid phase extraction;
UHPLC-QqQ-MS/MS: ultra-high performance liquid chromatography-triple quadrupole-tandem mass spectrometry;
MRM: multiple reaction monitoring;
PVDF: polyvinylidene difluoride;
HPLC-DAD-ESI-MSn: high-performance liquid chromatography-diode array detection-electrospray ionization-tandem mass spectrometry;
MS/MS: mass spectrometry tandem;
GAP: good agricultural practices;
GHP: good hygienic practices.

Acknowledgement

References

[1]   Chaparro R DC, Maldonado C ME, Franco L MC, Urango M LA (2015). Características nutricionales y antioxidantes de la fruta curuba larga (Passiflora mollisima Bailey). Biotecnología en el Sector Agropecuario y Agroindustrial 13: 120-128.

[2]   O'Shea N, Arendt EK, Gallagher E (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. Innovative. Food Science & Emerging Technologies 16: 1-10.

[3]   Lima H, Corrêa NCF, Santos O, de Fátima Henriques Lourenço L (2015). Use of agroindustrial wastes (açai fiber and glycerol) in the preparation of cookies. Journal of Food Science and Technology 52: 4593-4599.

[4]   Yepes SM, Montoya Naranjo LJ, Orozco Sánchez F (2008). Valorización de residuos agroindustriales - frutas - en Medellín y el Sur del valle del Aburrá, Colombia. Revista Facultad Nacional de Agronomía, Medellín. 61: 4422-4431.

[5]   Działo M, Mierziak J, Korzun U, Preisner M, Szopa J, Kulma A (2016). The Potential of Plant Phenolics in Prevention and Therapy of Skin Disorders. International Journal of Molecular Sciences 17: 160.

[6]   Tomás-Barberán FA, Gil MI (2008). Improving the Health-Promoting Properties of Fruit and Vegetable Products. Elsevier Science.

[7]   Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., Lamuela-Raventós, R., Buxaderas, S., Codina, C, "An industrial approach in the search of natural antioxidants from vegetable and fruit wastes", Food Chemistry, 97 (1). 137-150. July 2006.

[8]   Collado-González J, Durand T, Ferreres F, Medina S, Torrecillas A, Gil-Izquierdo Á (2015). Phytoprostanes. Lipid Technology 27: 127-130.

[9]   Durand, Bultel-Ponce V, Guy A, Berger S, Mueller MJ, Galano JM (2009). New bioactive oxylipins formed by non-enzymatic free-radical-catalyzed pathways: the phytoprostanes. Lipids 44: 875-888.

[10] Durand, Bultel-Poncé V, Guy A, El Fangour S, Rossi JC, Galano JM (2011). Isoprostanes and phytoprostanes: Bioactive lipids. Biochimie 93: 52-60.

[11] Thoma I, Krischke M, Loeffler C, Mueller MJ (2004). The isoprostanoid pathway in plants. Chemistry and Physics of Lipids 128: 135-148.

[12] Barden AE, Croft KD, Durand T, Guy A, Mueller MJ, Mori TA (2009). Flaxseed oil supplementation increases plasma F1-phytoprostanes in healthy men. Journal of Nutrition 139: 1890-1895.

[13] Gilles S, Mariani V, Bryce M, Mueller MJ, Ring J, Jakob T, Pastore S, Behrendt H, Traidl-Hoffmann C (2009). Pollen-derived E1-phytoprostanes signal via PPAR-γ and NF-κB-dependent mechanisms. The Journal of Immunology 182: 6653-6658.

[14] Minghetti L, Salvi R, Lavinia Salvatori M, Antonietta Ajmone-Cat M, De Nuccio C, Visentin S, Bultel-Poncé V, Oger C, Guy A, Galano J-M, Greco A, Bernardo A, Durand T (2014). Nonenzymatic oxygenated metabolites of α-linolenic acid B1- and L1-phytoprostanes protect immature neurons from oxidant injury and promote differentiation of oligodendrocyte progenitors through PPAR-γ activation. Free Radical Biology and Medicine 73: 41-50.

[15] Carrasco del Amor, Collado-Gonzalez J, Aguayo E, Guy A, Galano JM, Durand T, Gil-Izquierdo A (2015). Phytoprostanes in almonds: identification, quantification, and impact of cultivar and type of cultivation. RSC Advances 5: 51233-51241.

[16] Collado-González J, Medina S, Durand T, Guy A, Galano J-M, Torrecillas A, Ferreres F, Gil-Izquierdo A (2015). New UHPLC–QqQ-MS/MS method for quantitative and qualitative determination of free phytoprostanes in foodstuffs of commercial olive and sunflower oils. Food Chemistry 178: 212-220.

[17] Collado-González J, Moriana A, Girón IF, Corell M, Medina S, Durand T, Guy A, Galano J-M, Valero E, Garrigues T, Ferreres, F, Moreno F, Torrecillas A, Gil-Izquiero, A (2015). The phytoprostane content in green table olives is influenced by Spanish-style processing and regulated deficit irrigation. LWT - Food Science and Technology 2015, 64: 997-1003.

[18] Collado-Gonzalez J, Perez-Lopez D, Memmi H, Gijon MC, Medina S, Durand T, Guy A, Galano JM, Ferreres F, Torrecillas A, Gil-Izquierdo A (2015). Water deficit during pit hardening enhances phytoprostanes content, a plant biomarker of oxidative stress, in extra virgin olive oil. Journal of Agricultural and Food Chemistry 63: 3784-3792.

[19] Marhuenda J, Medina S, Diaz-Castro A, Martinez-Hernandez P, Arina S, Zafrilla P, Mulero J, Oger C, Galano JM, Durand T, Mulero J, Oger C, Galano JM, Durand T, Ferreres F, Gil-Izquierdo A (2015). Dependency of Phytoprostane Fingerprints of Must and Wine on Viticulture and Enological Processes. Journal of Agricultural and Food Chemistry 63: 9022-9028.

[20] Durand, Guy A, Henry O, Roland A, Bernad S, Fangour S, Vidal JP, Rossi JC (2004). Total syntheses of iso-, neuro- and phytoprostanes: new insight in lipid chemistry. Chemistry and Physics of Lipids 128: 15-33.

[21] El Fangour S, Guy A, Despres V, Vidal JP, Rossi JC, Durand T(2004). Total synthesis of the eight diastereomers of the syn-anti-syn phytoprostanes F1 types I and II. The Journal of Organic Chemistry 69: 2498-2503.

[22] El Fangour S, Guy A, Vidal JP, Rossi JC, Durand T (2005). A flexible synthesis of the phytoprostanes B1 type I and II. The Journal of Organic Chemistry 70: 989-997.

[23] Barbosa M, Collado-Gonzalez J, Andrade PB, Ferreres F, Valentao P, Galano JM, Durand T, Gil-Izquierdo A (2015). Nonenzymatic alpha-linolenic acid derivatives from the sea: macroalgae as novel sources of phytoprostanes. Journal of Agricultural and Food Chemistry 63: 6466-6474.

[24] Herraiz FJ, Villano D, Plazas M, Vilanova S, Ferreres F, Prohens J, Moreno DA (2016). Phenolic profile and biological activities of the pepino (*Solanum muricatum*) fruit and its wild relative S. caripense. International Journal of Molecular Science 17: 394.

[25] Sanchez-Rodriguez E, Ruiz JM, Ferreres F, Moreno DA (2012). Phenolic profiles of cherry tomatoes as influenced by hydric stress and rootstock technique. Food Chemistry 134: 775-782.

[26] Ferreres, Grosso C, Gil-Izquierdo A, Valentão P, Azevedo C, Andrade PB (2014). HPLC-DAD-ESI/MSn analysis of phenolic compounds for quality control of *Grindelia robusta* Nutt. and bioactivities. Journal of Pharmaceutical and Biomedical Analysis 94: 163-172.

[27] Medina S, Collado-González J, Ferreres F, Londoño-Londoño J, Jiménez-Cartagena C, Guy A, Durand T, Galano J-M, Gil-Izquierdo A (2017). Quantification of phytoprostanes–bioactive oxylipins–and phenolic compounds of Passiflora edulis Sims shell using UHPLC-QqQ-MS/MS and LC-IT-DAD-MS/MS. Food Chemistry 229: 1-8.

[28] Imbusch R, Mueller MJ (2000). Formation of isoprostane F2-like compounds (phytoprostanes F1) from α-linolenic acid in plants. Free Radical Biology and Medicine 28: 720-726.

[29] Karg K, Dirsch VM, Vollmar AM, Cracowski JL, Laporte F, Mueller MJ (2007). Biologically active oxidized lipids (phytoprostanes) in the plant diet and parenteral lipid nutrition. Free Radical Research 41: 25-37.

[30] Parchmann S, Mueller MJ (1998). Evidence for the formation of dinor isoprostanes E1 from alpha-linolenic acid in plants. The Journal of Biological Chemistry 273: 32650-32655.

[31] Carrasco-Del Amor AM, Aguayo E, Collado-González J, Guy A, Galano JM, Durand T, Gil-Izquierdo A (2016). Impact of packaging atmosphere, storage and processing conditions on the generation of phytoprostanes as quality processing compounds in almond kernels. Food Chemistry 211: 869-875.

[32] Cheok CY, Adzahan NM, Rahman RA, Abedin NH, Hussain N, Sulaiman R, Chong GH (2017). Current trends of tropical fruit waste utilization. Critical Reviews in Food Science and Nutrition 1-27.

[33] Costa GM, Gazola AC, Zucolotto SM, Castellanos L, Ramos FA, Reginatto FH, Schenkel EP (2016). Chemical profiles of traditional preparations of four South American Passiflora species by chromatographic and capillary electrophoretic techniques. Revista Brasileira de Farmacognosia 26: 451-458.

[34] Cuyckens F, Ma Y, L., Pocsfalvi G, Claeysi M (2000). Tandem mass spectral strategies for the structural characterization of flavonoid glycosides. Analusis 28: 888-895.

[35] Ferreres, Llorach R, Gil-Izquierdo A (2004). Characterization of the interglycosidic linkage in di-, tri-, tetra- and pentaglycosylated flavonoids and differentiation of positional isomers by liquid chromatography/electrospray ionization tandem mass spectrometry. Journal of Mass Spectrometry 39: 312-321.

[36] Simirgiotis MJ, Schmeda-Hirschmann G, Bórquez J, Kennelly EJ (2013). The *Passiflora tripartita* (banana passion) fruit: a source of bioactive flavonoid *C*-glycosides isolated by HSCCC and characterized by HPLC–DAD–ESI/MS/MS. Molecules 18: 1672-1692.

[37] Zucolotto SM, Fagundes C, Reginatto FH, Ramos FA, Castellanos L, Duque C, Schenkel EP (2012). Analysis of *C*-glycosyl flavonoids from South American Passiflora species by HPLC-DAD and HPLC-MS. Phytochemical Analysis 23: 232-239.

[38] Ferreres, Silva BM, Andrade PB, Seabra RM, Ferreira MA (2003). Approach to the study of *C*-glycosyl flavones by ion trap HPLC-PAD-ESI/MS/MS: application to seeds of quince (*Cydonia oblonga*). Phytochemical Analysis 14: 352-359.

[39] Ferreres, Gil-Izquierdo A, Andrade PB, Valentao P, Tomas-Barberan FA (2007). Characterization of *C*-glycosyl flavones *O*-glycosylated by liquid chromatography-tandem mass spectrometry. Journal of Chromatography A 1161: 214-223.