

# Compuestos Bioactivos de frutales Nativos Latinoamericanos.

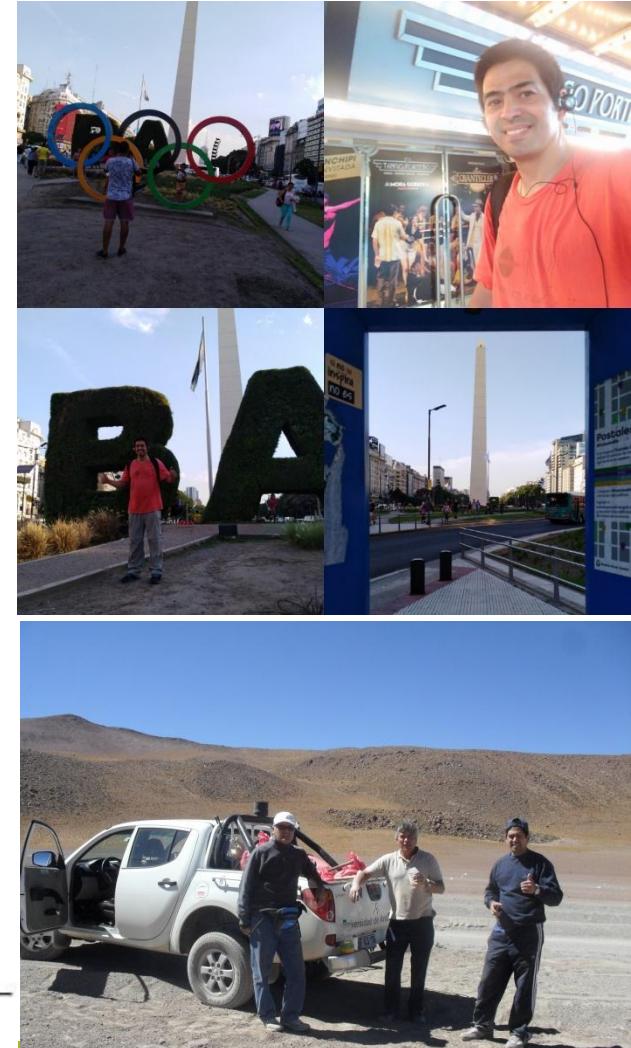
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Conocimiento y Naturaleza



# Introducción:

## Porque estudiar frutas endémicas Americanas? caso de Chile



- Chile: pequeño número de especies pero alto en plantas endémicas casi el 50 % de 5082 especies chilenas, 2561 son endémicas!
- (C. Marticorena, "Composición de la flora vascular de Chile," Flora silvestre de Chile (Jürke Grau & Georg Zizka (eds.), Sonderheft 19, Palmengarten, 1992), p. 74).



Esto es simple:

CHILE ES COMO UNA ISLA, no tiene contacto con el continente por las barreras físicas, además de tener ecosistemas especiales. Podría ser este el caso de la Amazonia?

# Ejemplo ecosistema único: Selva fría Valdiviana



# Como se pueden analizar rápido plantas y frutas? Qué es la técnica de HPLC-PDA-MS ?

Es una combinación de:

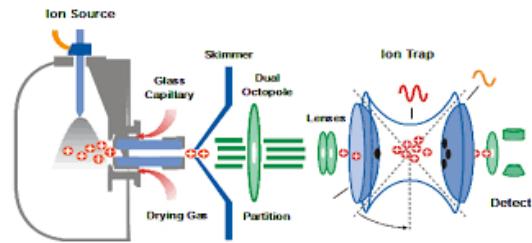
- a) liquid chromatography (HPLC  
o UHPLC) con detectores de  
UV visible (UV o diode array),  
y
- b) un espectrómetro de masas
- c) Conectados por una interfase  
(APCI, ESI)



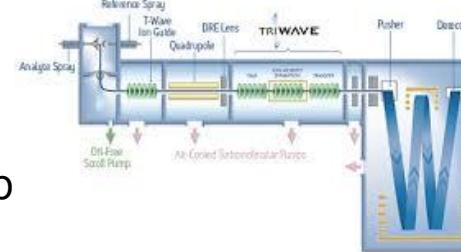
# Espectrómetros usados ahora



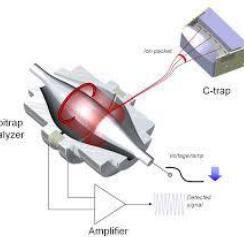
1-ION TRAP  
Muchos hijos MSn  
baja resolución



2-TOF  
alta resolución  
Muy Sensible a la temp



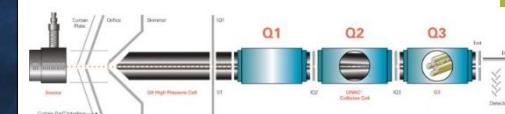
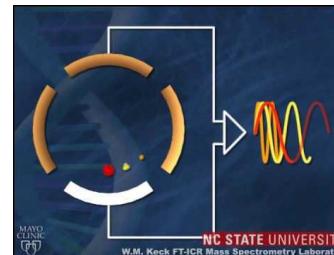
3-ORBITRAP,  
Alta resol,  
Baja velocidad  
Scaneo



5-Triple quad,  
baja resol,  
Alta  
sensibilidad



4-Ion cyclotron,  
muy alta resolución,  
Muy caro!



# Análisis de HPLC-MS

Puede ser quali (saber que hay) o  
quanti (Medir lo que hay)



- Para esto hay que:
- Remover las interferencias y column killers.
- Concentración de analitos
- Desalado
- Cambio de solventes
- Traslado y guardado de muestras



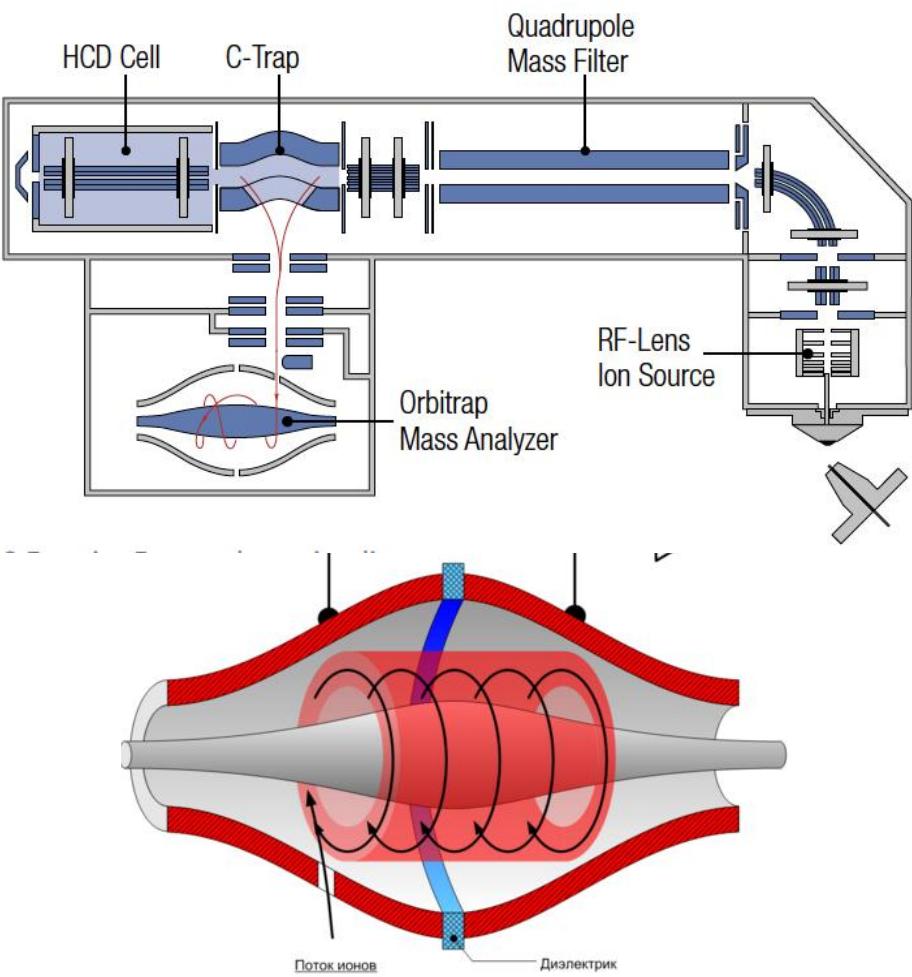
material

extracto

HPLC-DAD-MS analysis  
and quantification

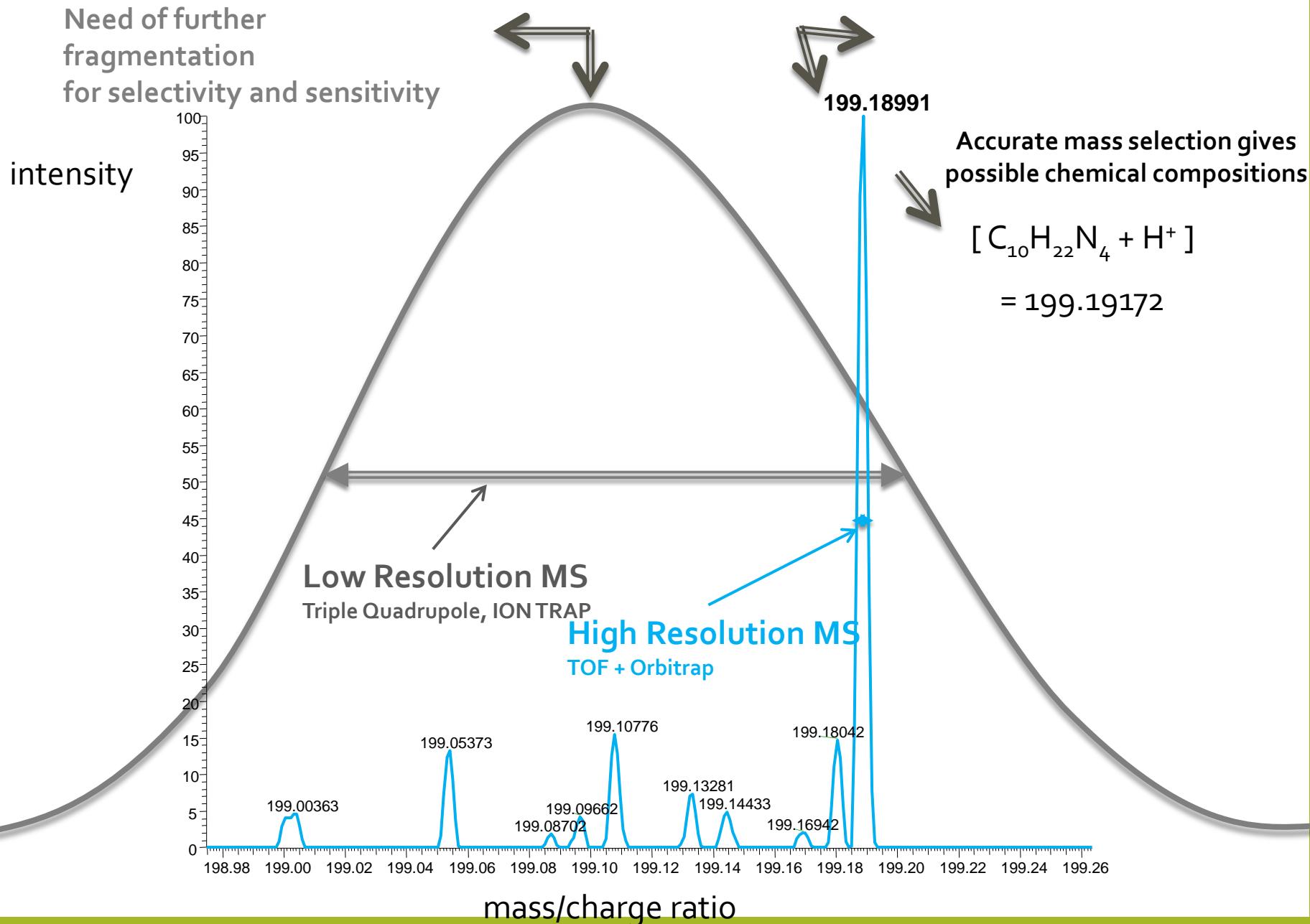


# Thermo orbitrap Q-EXACTIVE FOCUS



<http://planetorbitrap.com/q-exactive#.V-riYvhCMg>

# Baja versus Alta Resolución



# Primer ejemplo: Palo negro (*Leptocarpha rivularis* DC. – Asteraceae)



Agrícola  
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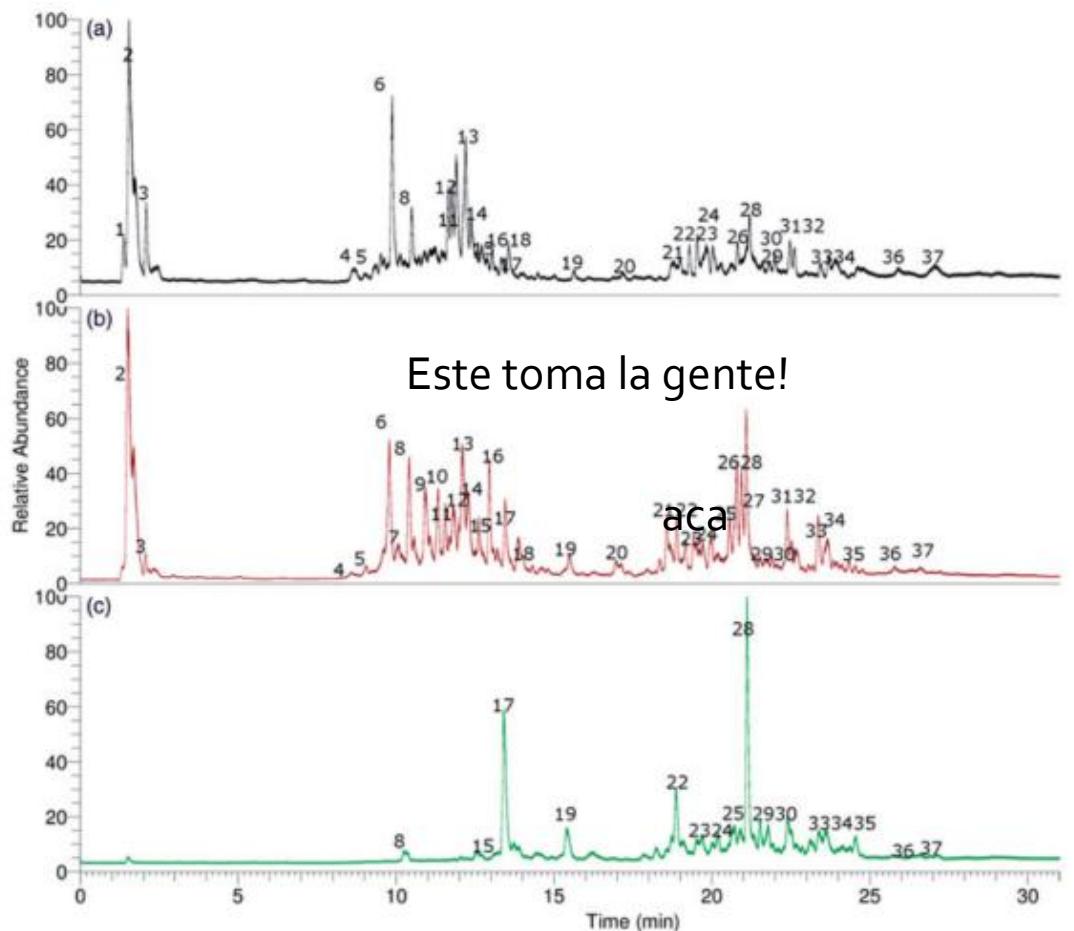
INICIO NOSOTROS TIENDA PUNTOS DE VENTA CONTACTO TESTIMONIOS BLOG VIDEOS 0 PRODUCTOS

Ver más

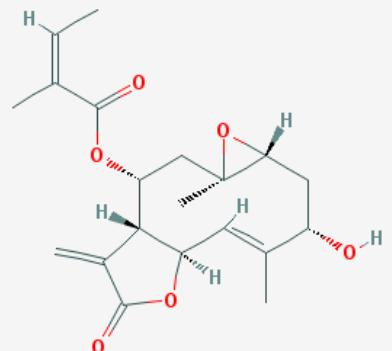
ORIGINAL 100%



# Cromatogramas de *L. rivularis* (a) extracto etanólico, (b) infusión, (c) acetato de etilo



## Leptocarpin



LTC induces an apoptotic process and inhibition of NF-kappa B.

CHEM-BIOL INTERACT., 2015 242: 415-421  
DOI: 10.1016/j.cbi.2015.11.006

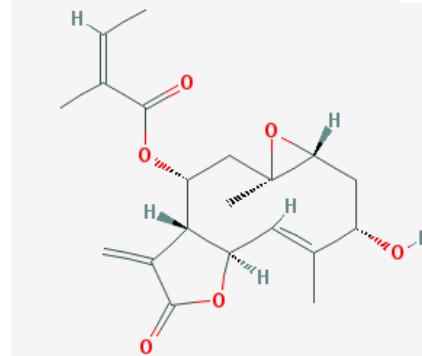
# Identification of compounds in *L. rivularis*.



2	3.87	-	Citric acid*
3	5.21	210–272	Chebulic acid
4	7.55	172	Gallic acid*
5	9.79	207–309	Protocatechuic acid 4-O-glucoside
6	10.35	236–329	1,6-Dicaffeoyl-glucose
7	11.31	236–329	Chlorogenic acid (3-O-caffeyl quinic acid)*
8	11.45	236–329	Cryptochlorogenic acid, (4-O-caffeyl quinic acid)
9	11.82	236–329	Neo-Chlorogenic acid, (5-O-caffeyl quinic acid)
10	12.01	254–361	Kaempferol 3-O-glucose
11	12.28	275–324	Isorhoifolin (apigenin 7-O-rutinoside)
12	12.45	255–355	Quercetin 3-O-glucose
13	12.86	255–355	Lonicerin (luteolin-7-O-neohesperidose)
14	13.28	236–329	3,4-Di-caffeyl-quinic acid
15	13.48	236–329	Isochlorogenic acid A; 3,5-Dicaffeoylquinic acid
16	13.98	254–361	Luteolin 3-O-rhamnose
17	14.34	236–329	Caffeic acid*
18	14.87	255–355	3',5'Di-O-methyl-myricetin
19	15.50	215	9,10,12-Trihydroxy-octadecadienoic acid
20	16.98	255–355	3, 3Di-O-methyl-myricetin
21	18.57	210	9,10,12-Trihydroxyoctadeca-9,15-dienoic acid
22	19.25	235	11-Hydroxy-12-oxooctadeca-9,15-dienoic acid
23	19.43	235	11-Hydroxy-12-oxooctadeca-7, 9,15-trienoic acid
24	19.58	265–424	7-O-Methyl-8- prenyl-luteolin
25	19.85	225	Leptocarpin
26	19.98	266–419	8-Prenyl-kaempferol      aca
27	21.12	232	9-Hydroxy-octadecatrienoic acid
28	21.24	225	Leptocarpin dehydrated derivative
29	22.39	225	Leptocarpin dehydrated derivative
30	22.31	225	9-Hydroxy-octadecatetraenoic acid
31	22.56	215	9-Hydroxy-octadecadienoic acid
32	22.78	225	8-Methoxy-13-hydroxy-9,11-octadecadienoic acid
33	23.36	246–310	Rosmarinic acid *
34	23.87	-	Ilicic acid
35	24.7	-	Diosmetin (4'-O-methyl-luteolin)*
36	26.32	212	Dihydroxyoctadecadienoic acid
37	27.27	254–354	Isorhamnetin*

\*Identified by spiking experiments with an authentic compound. MS<sup>n</sup>: Daughter ions.

A: Ethanolic extract; B: aqueous extract; C: ethyl acetate extract.



LTC treatment suppressed MG63 cells proliferation, migration, and invasion

MED. SCI. MONIT.

2017, 23: 903427

DOI: 10.12659/MSM.903427

**Table 2.** Content of phenolics and antioxidant capacities of *L. rivularis* extracts evidenced as the bleaching of the DPPH and ABTS radicals, FRAP (ferric reducing antioxidant power), SA (superoxide anion scavenging capacity), Total Phenolic Content (TPC), Total Flavonoids Content (TFC) and inhibitory activity of AChE: acetylcholinesterase and BChe: butyrylcholinesterase enzymes.

Sample	DPPH <sup>a</sup>	ABTS <sup>a</sup>	FRAP <sup>b</sup>	TPC <sup>c</sup>	TFC <sup>d</sup>	SA <sup>e</sup>	AChE <sup>f</sup>	BCHe <sup>f</sup>
Ethanol	168.39 ± 23.87 <sup>a</sup>	186.72 ± 7.09 <sup>b</sup>	201.37 ± 4.91	267.43 ± 8.38	170.78 ± 6.92	62.59 ± 4.42 <sup>c</sup>	1.45 ± 0.06 <sup>c</sup>	1.28 ± 0.08
Aqueous	176.51 ± 28.84 <sup>a</sup>	195.28 ± 4.83 <sup>b</sup>	223.92 ± 2.95	320.49 ± 3.58	230.76 ± 2.5	75.18 ± 9.63 <sup>c</sup>	2.12 ± 0.03 <sup>c</sup>	1.65 ± 0.06
EtOAc	120.86 ± 23.89	132.49 ± 6.12	141.86 ± 6.64	140.98 ± 2.87	150.49 ± 0.46	48.73 ± 7.38	0.89 ± 0.03	0.72 ± 0.04

<sup>a</sup>Antiradical activities are expressed as mmol Trolox/kg dry weight.

<sup>b</sup>Ferric reducing power expressed as mmol Trolox/kg dry weight.

<sup>c</sup>Total phenolic content (TPC) expressed as mmol GAE/kg dry weight.

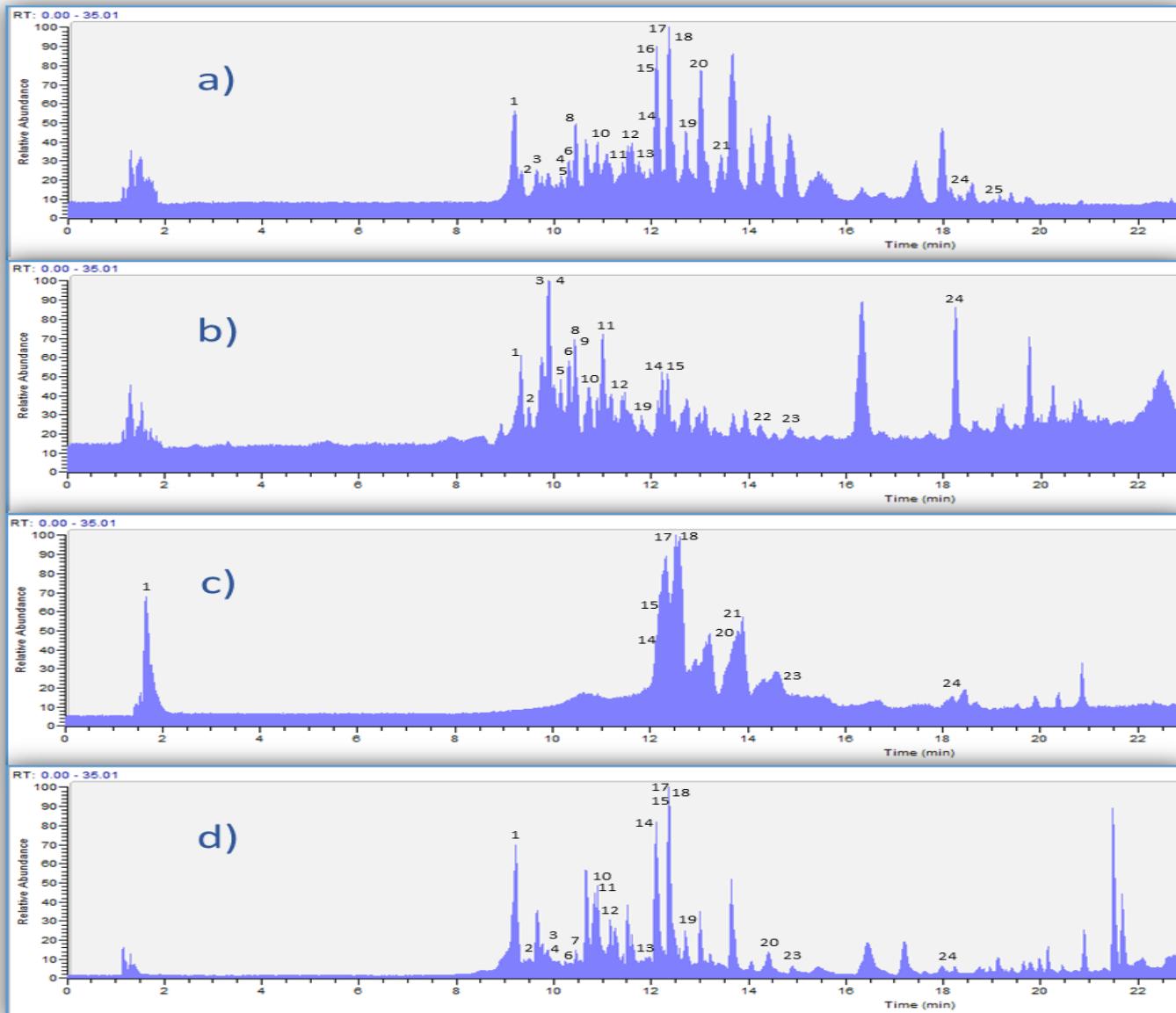
<sup>d</sup>Total flavonoid content (TFC) expressed as mmol QE/kg dry weight.



# Aloe vera del Oasis de Pica



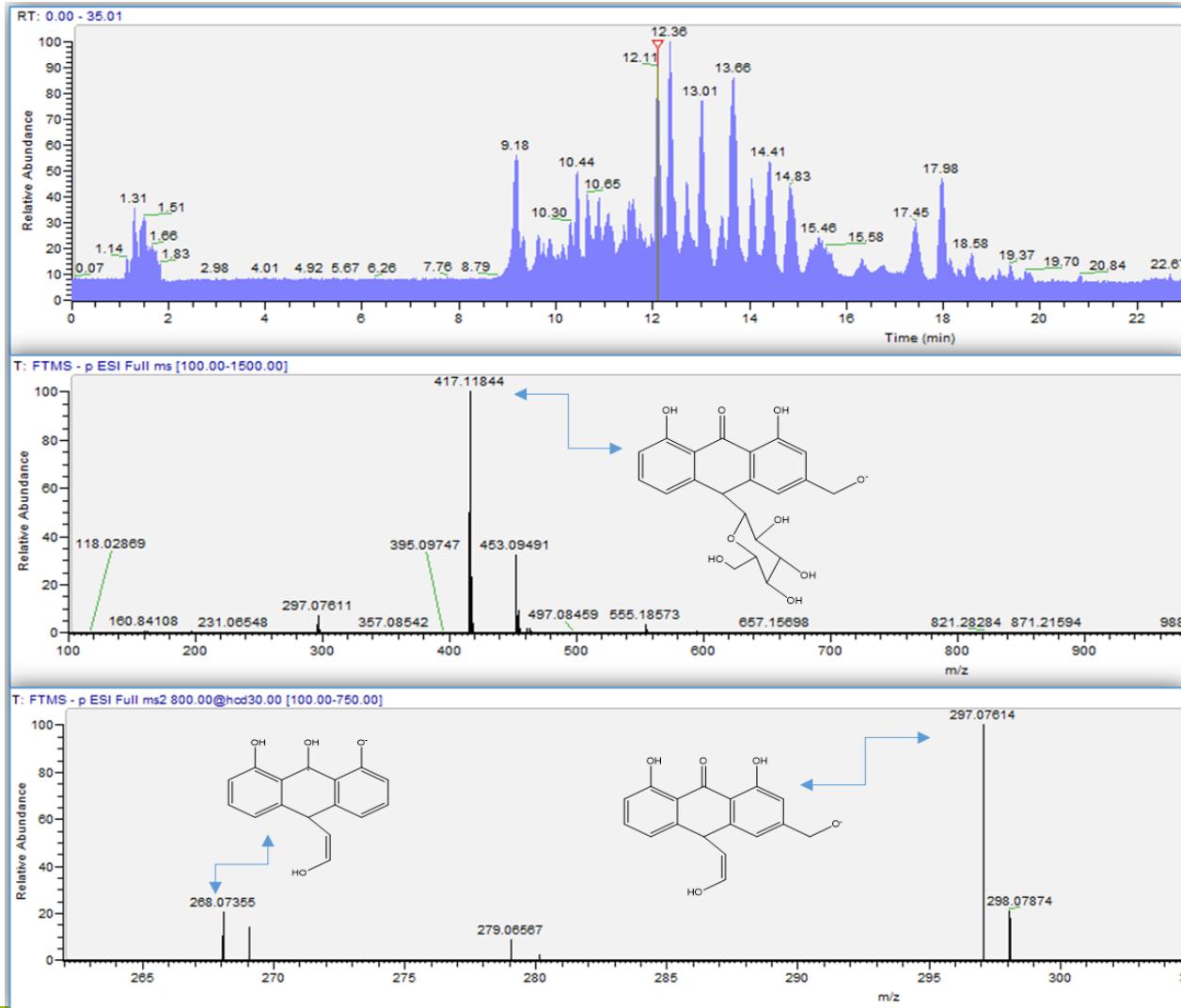
# Cromatogramas de (a) Piel, (b) Flores, (c) Gel y (d) Raíz de *A. vera*.



# Identificación rápida de fenólicos en piel, flores, gel y raíces de *A. vera* del Oasis de Pica por UHPLC-Q-OT-MS.

Peak #	Tentative identification	Elemental composition [M-H] <sup>-</sup>	Retention time (min.)	Theoretical Mass (m/z)	Measured Mass (m/z)	Error (ppm)	Other Ions (m/z)	Classification of the compound
1	Aloesin or Aloeresin B	C <sub>19</sub> H <sub>21</sub> O <sub>9</sub> <sup>-</sup>	9.23	393.11911	393.11917	-0.15263	203.07106 (C <sub>12</sub> H <sub>11</sub> O <sub>3</sub> <sup>-</sup> ) 245.08168 (C <sub>14</sub> H <sub>13</sub> O <sub>4</sub> <sup>-</sup> )	Chromone (C-glycosylated chromone)
2	Chlorogenic acid	C <sub>16</sub> H <sub>17</sub> O <sub>9</sub> <sup>-</sup>	9.54	353.08781	353.08752	0.82133	191.05534 (C <sub>14</sub> H <sub>11</sub> O <sub>6</sub> <sup>-</sup> )	Cinnamic acid
3	Caffeic acid	C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> <sup>-</sup>	9.92	179.03498	179.03464	1.89907	135.04451 (C <sub>8</sub> H <sub>7</sub> O <sub>2</sub> <sup>-</sup> )	Cinnamic acid
4	Aloe emodin-diglucoside	C <sub>27</sub> H <sub>29</sub> O <sub>15</sub> <sup>-</sup>	9.98	593.15119	593.15063	0.94411	-	Anthracene compound (anthrone)
5	Isoquercitrin	C <sub>21</sub> H <sub>19</sub> O <sub>12</sub> <sup>-</sup>	9.98	463.08820	463.08752	1.46840	301.55185(M-glucosa)	Flavonoid (O-glycosylated flavonoid)
6	Kaempferol-3-O-hexosyl-O-pentoside	C <sub>26</sub> H <sub>27</sub> O <sub>15</sub> <sup>-</sup>	10.29	579.13554	579.13513	0.70795	-	Flavonoid (O-glycosylated flavonoid)
7	6-methyl-1,3,8-trihydroxyanthraquinone (Emodin)	C <sub>15</sub> H <sub>9</sub> O <sub>5</sub> <sup>-</sup>	10.40	269.04555	269.04538	0.63186	225.05438 (C <sub>14</sub> H <sub>9</sub> O <sub>3</sub> <sup>-</sup> ) 241.73468 -----	Anthracene compound (anthrone)
8	Luteolin-8-C-glucoside (Orientin)	C <sub>22</sub> H <sub>19</sub> O <sub>11</sub> <sup>-</sup>	10.41	447.09329	447.09293	0.80520	327.05048 (C <sub>17</sub> H <sub>11</sub> O <sub>7</sub> <sup>-</sup> ) 299.05582 (C <sub>16</sub> H <sub>11</sub> O <sub>6</sub> <sup>-</sup> )	Flavonoid (C-glycosylated flavonoid)
9	Feruloylquinic acid	C <sub>17</sub> H <sub>19</sub> O <sub>9</sub> <sup>-</sup>	10.46	367.10346	367.10309	1.00789	191.05547 (C <sub>9</sub> H <sub>11</sub> O <sub>6</sub> <sup>-</sup> )	Cinnamic acid
10	10-hydroxyaloin A	C <sub>21</sub> H <sub>21</sub> O <sub>10</sub> <sup>-</sup>	10.90	433.11402	433.11392	0.23089	270.05280 (C <sub>15</sub> H <sub>16</sub> O <sub>5</sub> <sup>-</sup> ) 311.05588 (C <sub>17</sub> H <sub>11</sub> O <sub>6</sub> <sup>-</sup> ) 283.06058 (C <sub>16</sub> H <sub>11</sub> O <sub>5</sub> <sup>-</sup> )	Anthracene compound (anthrone) Flavonoid (C-glycosylated flavonoid)
11	Isovitexin	C <sub>21</sub> H <sub>19</sub> O <sub>10</sub> <sup>-</sup>	10.80	431.09837	431.09833	0.09279	-	Flavonoid (O-glycosylated flavonoid)
12	Chrysoeriol-7-O-glucuronide	C <sub>22</sub> H <sub>19</sub> O <sub>12</sub> <sup>-</sup>	11.11	475.08820	475.08856	-0.75775	-	Flavonoid (O-glycosylated flavonoid)
13	Caffeoyl ester of aloesin	C <sub>29</sub> H <sub>29</sub> O <sub>12</sub> <sup>-</sup>	11.74	569.16645	569.16626	0.33382	-	Chromone
14	Aloin A	C <sub>21</sub> H <sub>21</sub> O <sub>9</sub> <sup>-</sup>	12.18	417.11911	417.11908	0.07192	297.07687 (C <sub>17</sub> H <sub>13</sub> O <sub>5</sub> <sup>-</sup> ) 268.07318 (C <sub>16</sub> H <sub>12</sub> O <sub>4</sub> <sup>-</sup> )	Anthracene compound (anthrone)
15	Isoaloeresin D	C <sub>29</sub> H <sub>31</sub> O <sub>11</sub> <sup>-</sup>	11.85	555.18719	555.18677	0.75650	-	Chromone
16	2'-p-methoxycoumaroylaloesin B	C <sub>29</sub> H <sub>29</sub> O <sub>11</sub> <sup>-</sup>	12.29	553.17154	553.17041	2.04277	-	Anthracene compound (anthrone)
17	4,5-dimethyl ether of Aloe emodin	C <sub>17</sub> H <sub>13</sub> O <sub>5</sub> <sup>-</sup>	12.29	297.07685	297.07669	0.53858	-	Anthracene compound (anthrone)
18	Aloin B	C <sub>21</sub> H <sub>21</sub> O <sub>9</sub> <sup>-</sup>	12.36	417.11911	417.11893	0.43153	297.07669 (C <sub>17</sub> H <sub>13</sub> O <sub>5</sub> <sup>-</sup> ) 268.07419 (C <sub>16</sub> H <sub>12</sub> O <sub>4</sub> <sup>-</sup> )	Anthracene compound (anthrone)
19	6'-Malonylnataloin	C <sub>24</sub> H <sub>23</sub> O <sub>12</sub> <sup>-</sup>	12.69	503.11950	503.11911	0.77516	459.12842 (C <sub>23</sub> H <sub>23</sub> O <sub>10</sub> <sup>-</sup> )	Anthracene compound (C-glycosylated anthrone)
20	Naringenin-4'-methoxy-7-O-glucuronide	C <sub>22</sub> H <sub>21</sub> O <sub>10</sub> <sup>-</sup>	14.39	445.11402	445.11401	0.02247	-	Flavonoid
21	7-methylether of 2'-feruloylaloesin	C <sub>30</sub> H <sub>31</sub> O <sub>12</sub> <sup>-</sup>	13.40	583.18210	583.18146	1.09743	-	Chromone
22	Aloe emodin-8-O-glucoside	C <sub>21</sub> H <sub>19</sub> O <sub>10</sub> <sup>-</sup>	14.52	431.09837	431.09793	1.02065	269.04495 (C <sub>15</sub> H <sub>9</sub> O <sub>5</sub> <sup>-</sup> )	Anthracene compound (anthrone)
23	5,3'-dihydroxy-6,7,4'-trimethoxyflavone (Eupatorin)	C <sub>18</sub> H <sub>15</sub> O <sub>7</sub> <sup>-</sup>	14.81	343.08233	343.08231	0.05830	-	Flavonoid
24	Trihydroxy octadecenoic acid	C <sub>18</sub> H <sub>33</sub> O <sub>5</sub> <sup>-</sup>	18.28	329.23335	329.23328	0.21262	-	Oxilipin
25	3,4-di-O-caffeoylequinic acid	C <sub>25</sub> H <sub>23</sub> O <sub>12</sub> <sup>-</sup>	19.04	515.11950	515.11847	1.99954	-	Cinnamic acid

# Piel de *A. vera* (a) TIC (b) espectro de Full high-resolution mostrando el Aloin A y (c) los iones hijos.





# Scavenging of the DPPH, ABTS, FRAP and extraction yields of parts from *A. vera*.

Parts from <i>A. vera</i>	PEE (%) <sup>a</sup>	DPPH <sup>•+ b</sup>	ABTS <sup>•+ c</sup>	FRAP <sup>d</sup>
Peel	16.2	2.43 ± 0.14 X	34.32 ± 2.60	3.82 ± 0.23
Flower	12.6	1.25 ± 0.03 <sup>(d)</sup>	16.55 ± 2.30 <sup>(e)</sup>	2.01 ± 0.10
Gel	12.3	0.34 ± 0.01	2.06 ± 0.06	0.38 ± 0.01
Root	8.5	1.43 ± 0.08 <sup>(d)</sup>	17.54 ± 0.77 <sup>(e)</sup>	2.67 ± 0.16

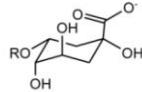
<sup>a</sup> Phenolic-enriched extracted expressed in percent; <sup>b</sup> antiradical DPPH activities are expressed as mM equivalent to Trolox/g fresh weight, for c and d is same Values are not significantly different (at  $p < 0.05$ ). All measurements were repeated three times (n=3).

La cascara es mas antioxidante! Y es un desecho!

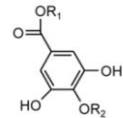
## Mango del desierto (del oasis de Pica)



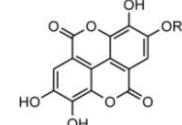
# Ácidos, xantonas y flavonoides....



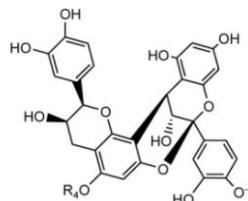
- 1 R = H  
5 R = Caffeic acid  
15 R = Gallic acid



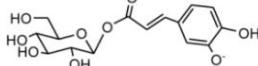
- 2 R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = Gallic acid  
4 R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = H  
8 R<sub>1</sub> = Glucose, R<sub>2</sub> = H



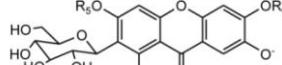
- 3 R<sub>3</sub> = Gallic acid  
20 R<sub>3</sub> = CH<sub>3</sub>  
21 R<sub>3</sub> = H



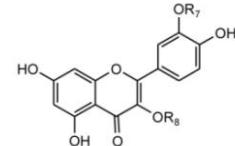
- 6 R<sub>4</sub> = Gallic acid  
7 R<sub>4</sub> = H



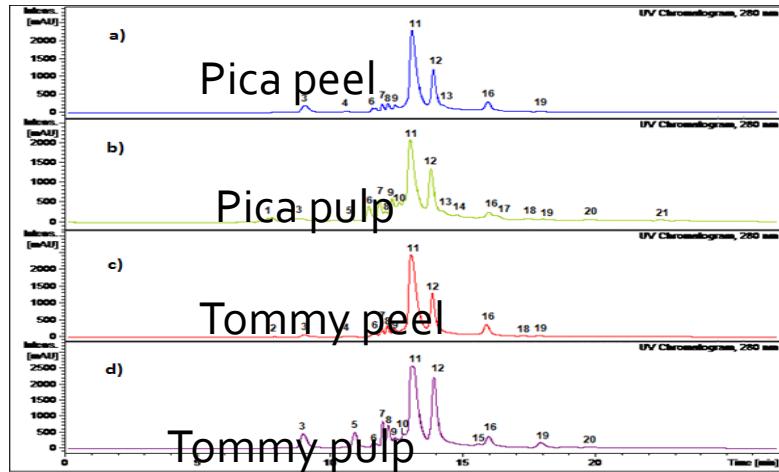
11



- 10 R<sub>5</sub> = H, R<sub>6</sub> = Gallic acid  
12 R<sub>5</sub> = H, R<sub>6</sub> = H  
13 R<sub>5</sub> = CH<sub>3</sub>, R<sub>6</sub> = H  
14 R<sub>5</sub> = CH<sub>3</sub>, R<sub>6</sub> = CH<sub>3</sub>

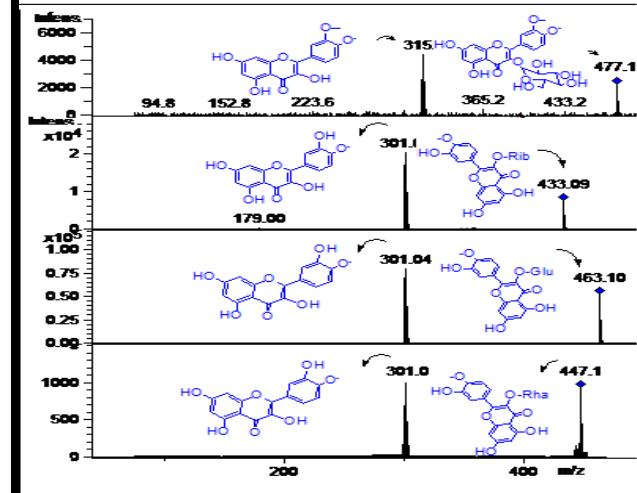
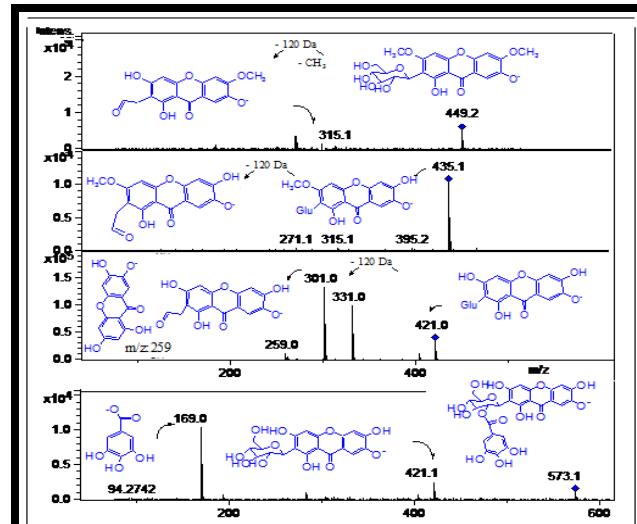


- 16 R<sub>7</sub> = H, R<sub>8</sub> = Glucose  
17 R<sub>7</sub> = H, R<sub>8</sub> = Rhamnose  
18 R<sub>7</sub> = H, R<sub>8</sub> = Arabinose  
19 R<sub>7</sub> = CH<sub>3</sub>, R<sub>8</sub> = Glucose



Los perfiles son similares, pero la actividad antioxidante fue el doble en la cascara de mango de pica!

Por lo tanto se podría hacer un suplemento dietario de la cascara



# dos Peumos...

## German Peumo

(*Crataegus monogyna*) espino albar, usado para ansiedad por los flavonoides, y un alcaloide esparteina uso tonico cardiaco



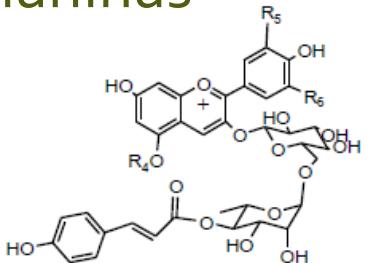
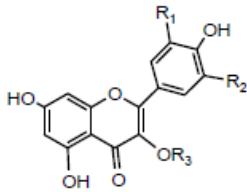
## Chilean Peumo (*Cryptocarya alba*)



Son parecidos los frutos, pero son dos especies diferentes!



# Flavonoides y antocianinas



11      R<sub>1</sub> OH    R<sub>2</sub> OH    R<sub>3</sub> (6'' galloyl)

Galactose

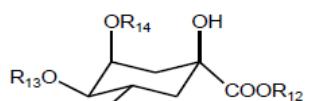
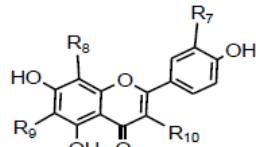
13\*     H       OH     Galactose

17       OH       H     (6'' galloyl)  
Galactose

21       OH       OH    Pentose

22\*     OH       H     Galactose

23\*     OH       H     Glucose



15       R<sub>7</sub> H     Rhamnose    R<sub>9</sub> (2'' glucosyl)  
Glucose

27\*     OH       Glucosyl  
moiety    H       H

34       H       Glucosyl  
moiety    H       H

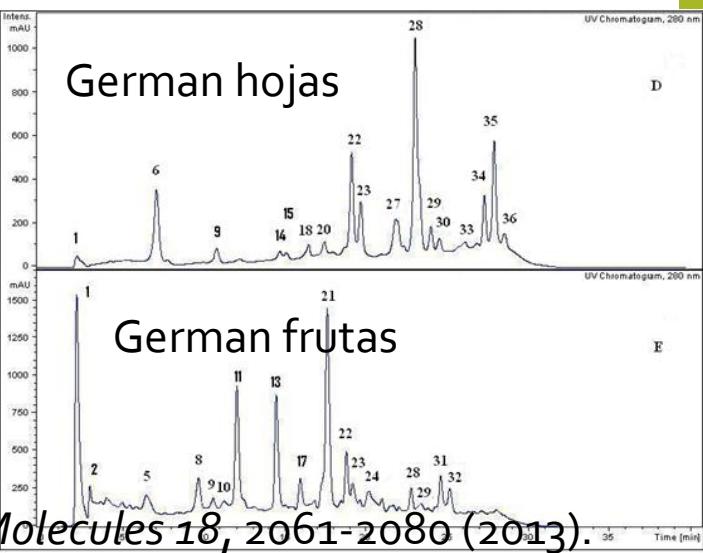
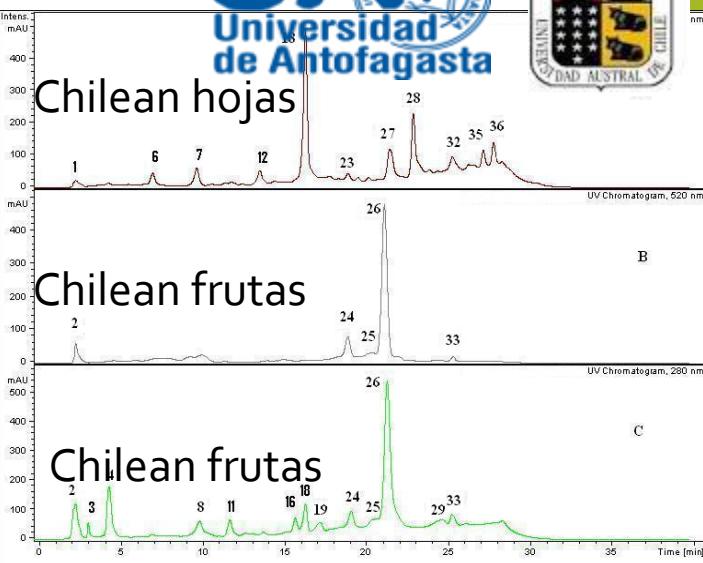
29       H       CH<sub>3</sub>    H       Glucose

30       H       H       H       Pentose

35       H       H       H       Glucose

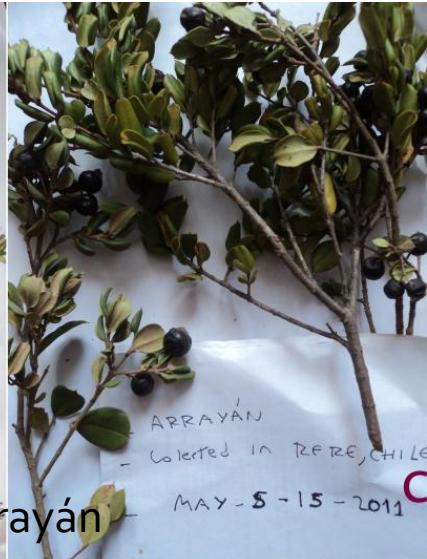
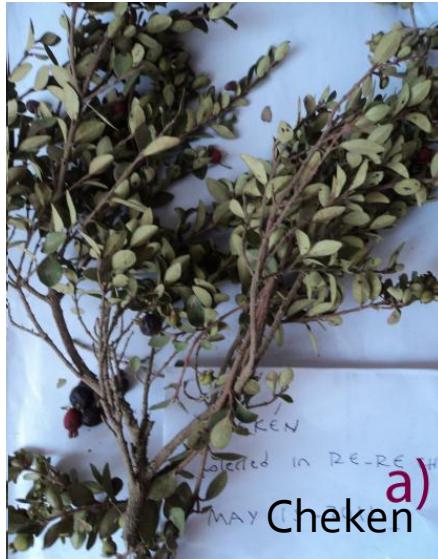
36       H       CH<sub>3</sub>    H       OH

\*Identified by co-elution with standard compound.

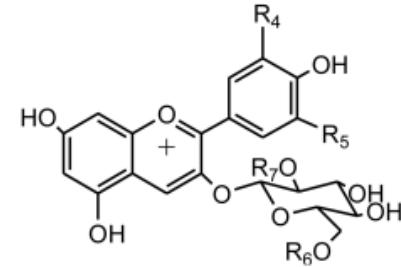
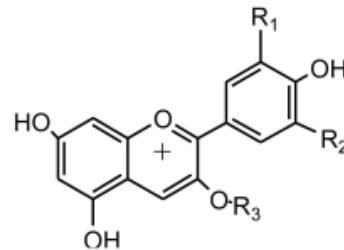


Por los perfiles químicos se pueden diferenciar los frutos similares

# Berries nativas patagónicas...



# Antocianos en berries

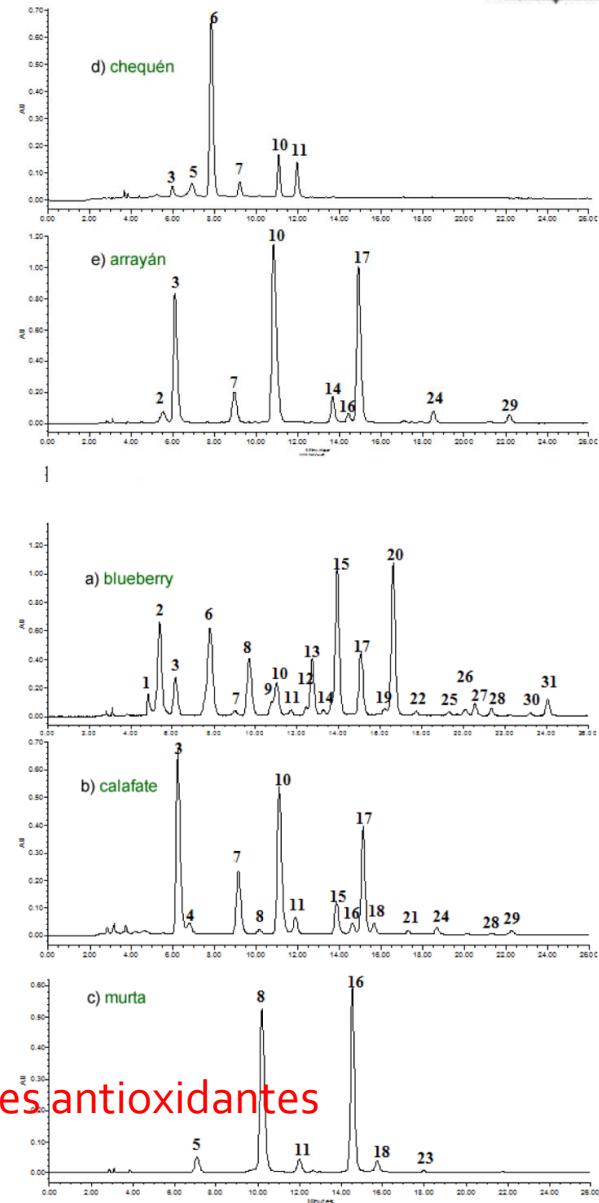


Peak	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Peak	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>
3*	OH	OH	Gal	1	OH	OCH <sub>3</sub>	Glu	H
4	OH	OH	Glu	2	OH	OCH <sub>3</sub>	Glu	H
6*	OH	H	Gal	5	OH	H	Rha	H
7*	OH	H	Glu	8	OH	OCH <sub>3</sub>	Rha	H
9	OCH <sub>3</sub>	OCH <sub>3</sub>	Rha	9	OCH <sub>3</sub>	OCH <sub>3</sub>	Rha	H
10*	OH	OCH <sub>3</sub>	Glu	12	H	OCH <sub>3</sub>	Rha	H
11*	OH	OCH <sub>3</sub>	Gal	21	OCH <sub>3</sub>	OCH <sub>3</sub>	Cou	H
13*	H	OCH <sub>3</sub>	Gal	23	OH	H	Succ	H
14	OCH <sub>3</sub>	OCH <sub>3</sub>	Gal	24	H	OCH <sub>3</sub>	Glu	H
15	OH	OH	Ara	25	OH	OH	Cou-rha	Glu
16*	H	OCH <sub>3</sub>	Glu	26	OH	OH	Ac	H
17*	OCH <sub>3</sub>	OCH <sub>3</sub>	Glu	27	OH	H	Ac	H
18	H	OCH <sub>3</sub>	Ara	28	OH	OCH <sub>3</sub>	Ac	H
				29	OCH <sub>3</sub>	OCH <sub>3</sub>	Ac	H
				30	OH	OH	Caff	H
				31	OCH <sub>3</sub>	OCH <sub>3</sub>	Ac	H

\*Identified using standard compounds. Gal: galactose; Glu: Glucose; Ara: Arabinose; Rha: Rhamnose; Cou: Coumaric acid; Acetyl group; Caff:Caffeic acid.

Molecules 2014 Jul 28;19(8): 10936-55.

Se diferencian por los perfiles y se atribuyen las propiedades antioxidantes de acuerdo a la cantidad de los antocianos detectados





- Estos berries son muy poco conocidos,
- Hay productos que comercializan en China y Europa del Maqui que es el mas conocido. Se puede replicar con los otros lo mismo!



Get the Power of Purple

THE NATURAL SECRET TO WEIGHT LOSS POWERFUL ANTIOXIDANT CLICK HERE TO ORDER NOW

MAQUI BERRY SELECT

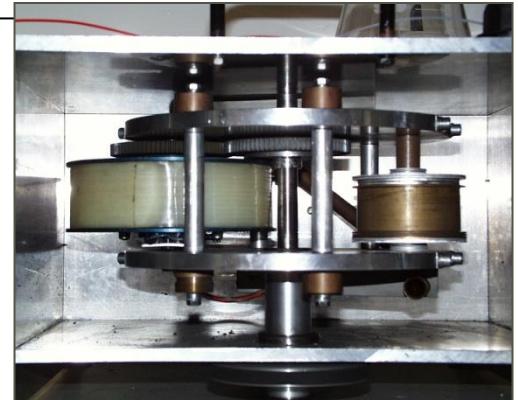
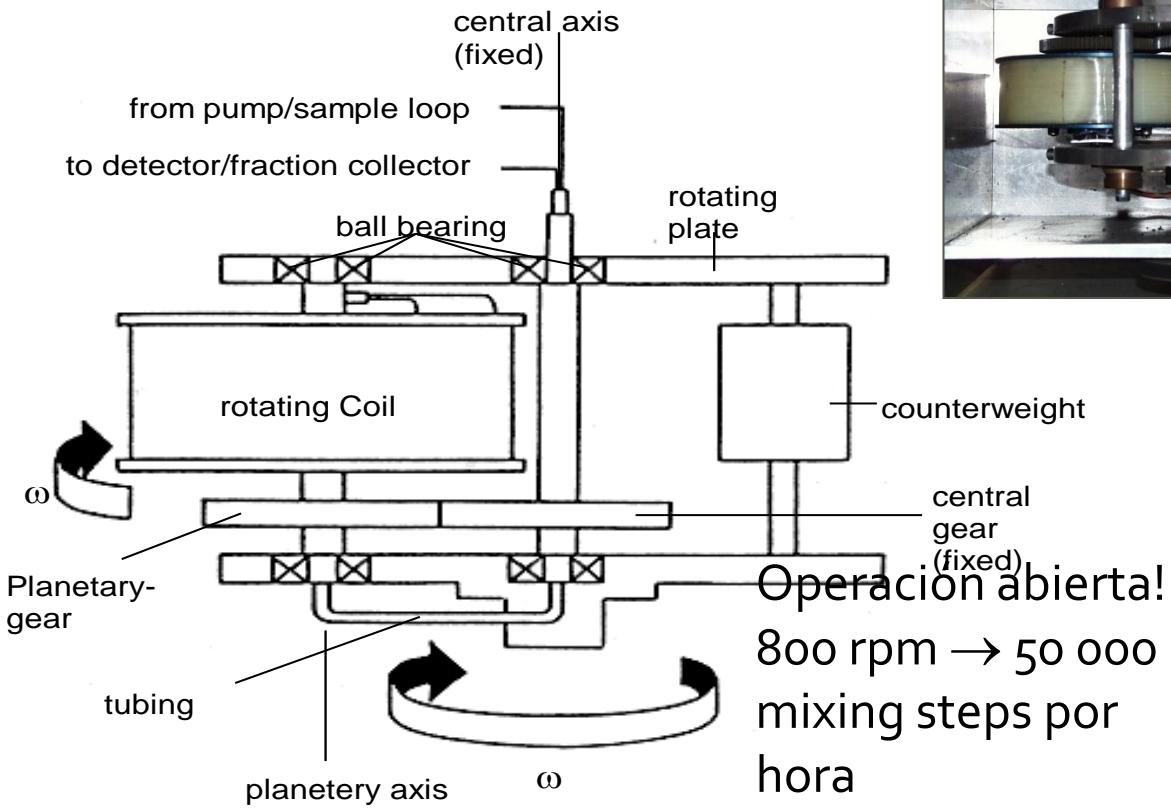
Losing Weight and Getting Healthy Just Got Easier

An advertisement for Maqui Berry Select. It features the product packaging (a bottle and a box) and text highlighting its benefits for weight loss and health. The background is dark purple with white and yellow text.

# Aislamiento de productos naturales por medio de la cromatografía en contracorriente



# Instrumentación en la HSCCC



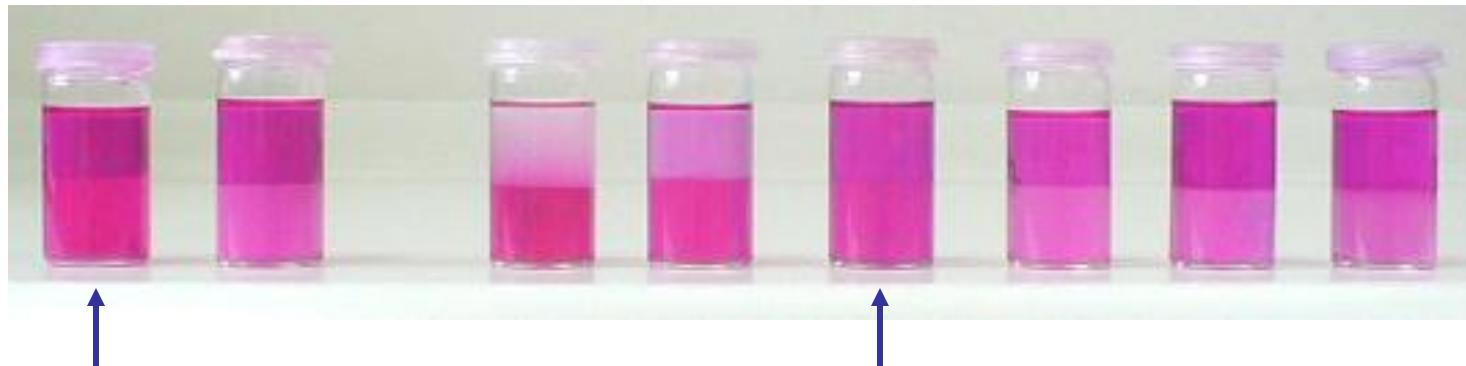


# Selección del Sistema de Solventes en HSCCC

- 2-3 mg de muestra
- Se disuelve en varios sistemas solventes bifásicos, agitar, reposar
- Análisis de las 2 fases (HPLC, TLC, GC, UV-Vis, etc.)
- Determinación del coeficiente de partición (**requerido  $K = 0.5$  a  $1$** )

$$K = c \text{ (fase estacionaria)} / c \text{ (fase móvil)}$$

- $K < 0.2 \Rightarrow$  elución muy rápida, poca separación
- $K > 1.5 \Rightarrow$  separación con una duración muy larga,



# CCC de pigmentos naturales

- **Carotenoides**

n-Hexano/CH<sub>2</sub>Cl<sub>2</sub>/acetonitrilo (30 : 11 : 18, v/v/v), tail-to-head

- **Pigmentos relacionados con la clorofila**

n-Hexano/metanol (2 : 1, v/v), head-to-tail

- **Antocianos**

MTBE/n-butanol/acetonitrilo/agua (2 : 2 : 1 : 5, v/v/v/v, acidificado con 0.1% TFA), head-to-tail

- **Betalainas**

Etanol/acetonitrilo/solución saturada de sulfato de amonio /agua (1 : 0.5 : 1.2 : 1, v/v/v/v), tail-to-head



# Tumbo o curuba...

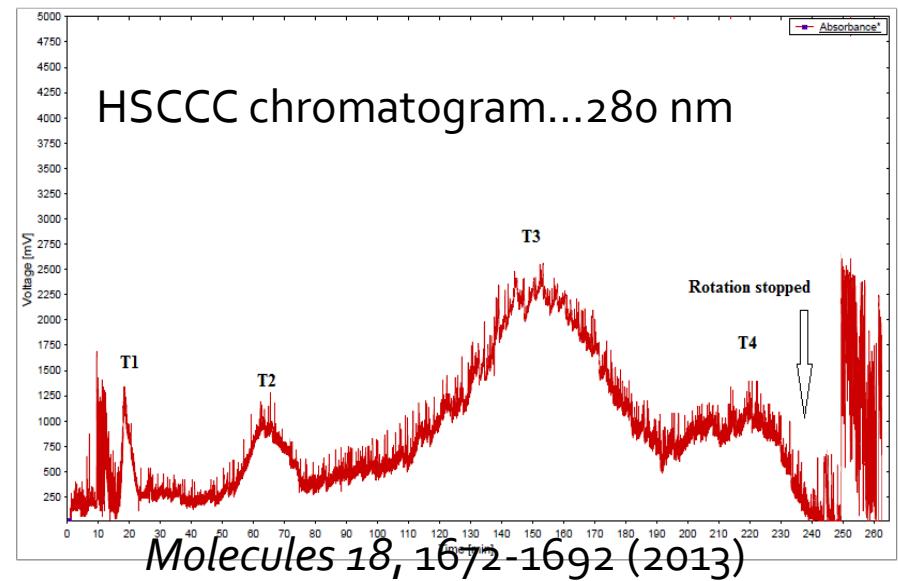
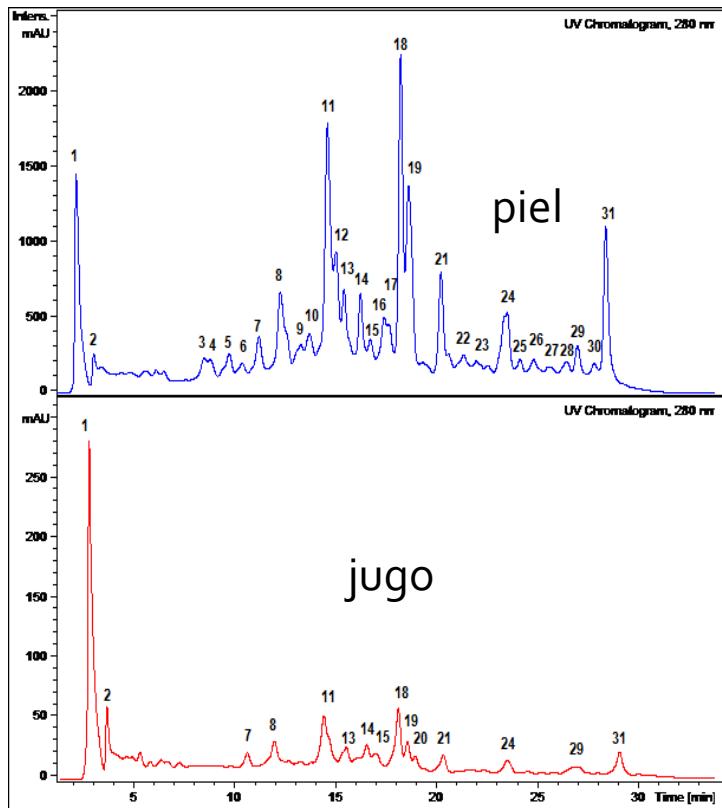


Una fruta enredadera de los Andes

Se usa mucho para jugo y  
por propiedades sedantes de las hojas,  
porque no comercializarlo  
como jugo dietario saludable?

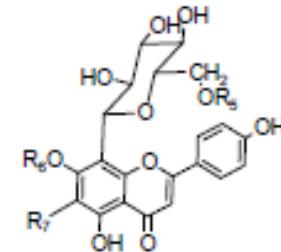
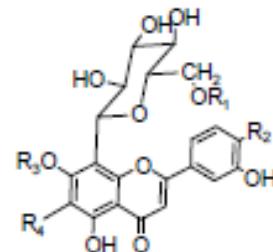


## Arriba piel: abajo pulpa (280 nm)



Five compounds were isolated by HSCCC, including 11: schaftoside, 13: vicenin II, 16: vitexin, 19: orientin and 31: acetyl-methyl-orientin

# Flavonoides glicosidados (full MS and MS-MS)

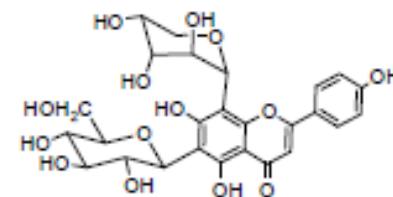


	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>		R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>
3	H	OH	Glucose	H		1	H	Glucose
5 *	H	OH	H	Glucose		9 *	H	Arabinose
8	H	OH	H	Pentose		13 *	H	Arabinose
10	H	OMe	H	Glucose		16 *	H	Glucose
18	Ac	OH	Glucose	H		20	Ac	H
19*	H	OH	H	H		27	Ac	H
21	H	OMe	H	H				
24	Ac	OH	H	H				
31*	Ac	OMe	H	H				

Molecules 18, 1672-1692 (2013)

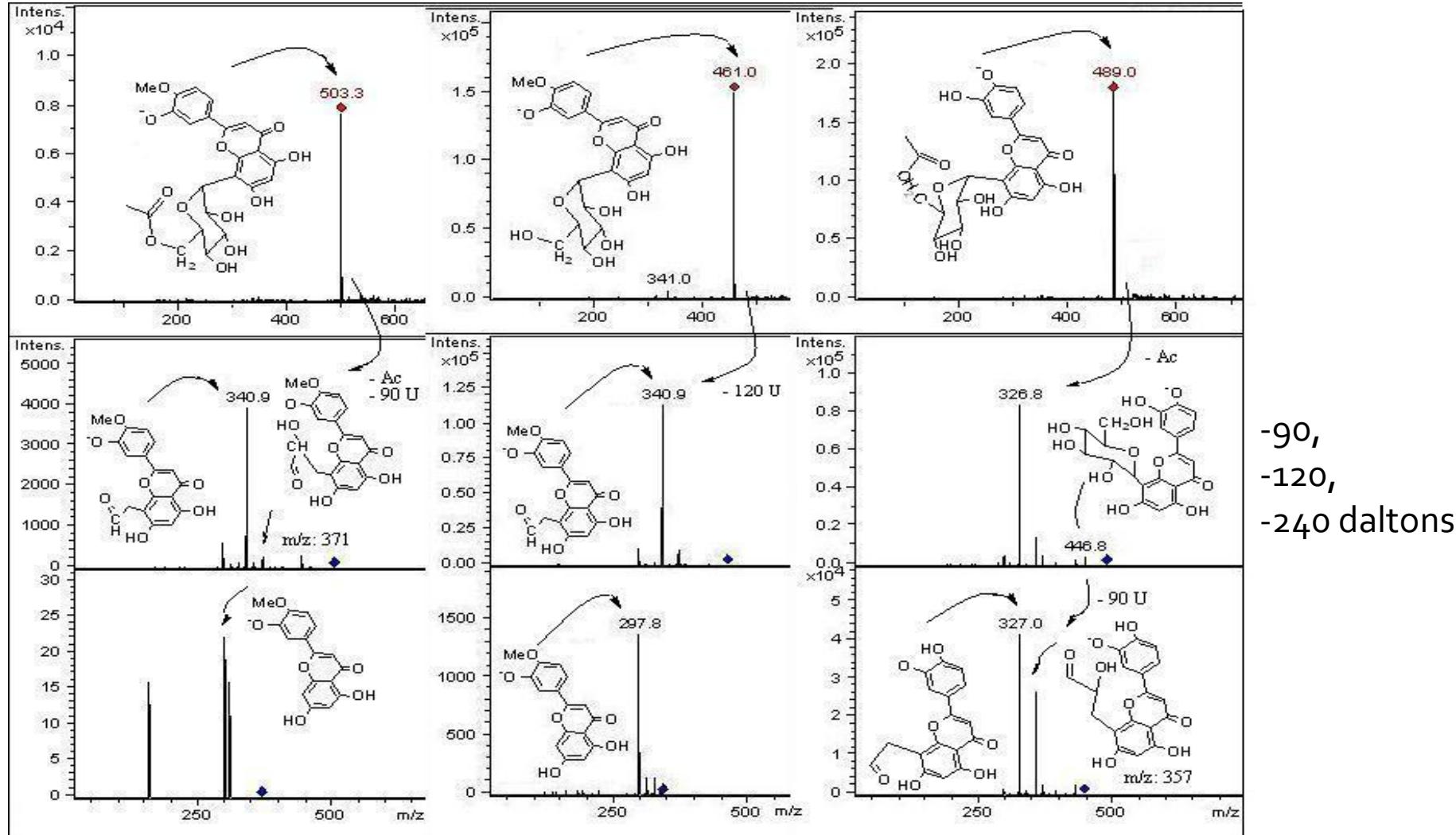
LA PIEL

TIENE MUCHOS C-GLICOSIDOS  
CON PROPIEDADES SEDANTES Y ANTIOXIDANTES!

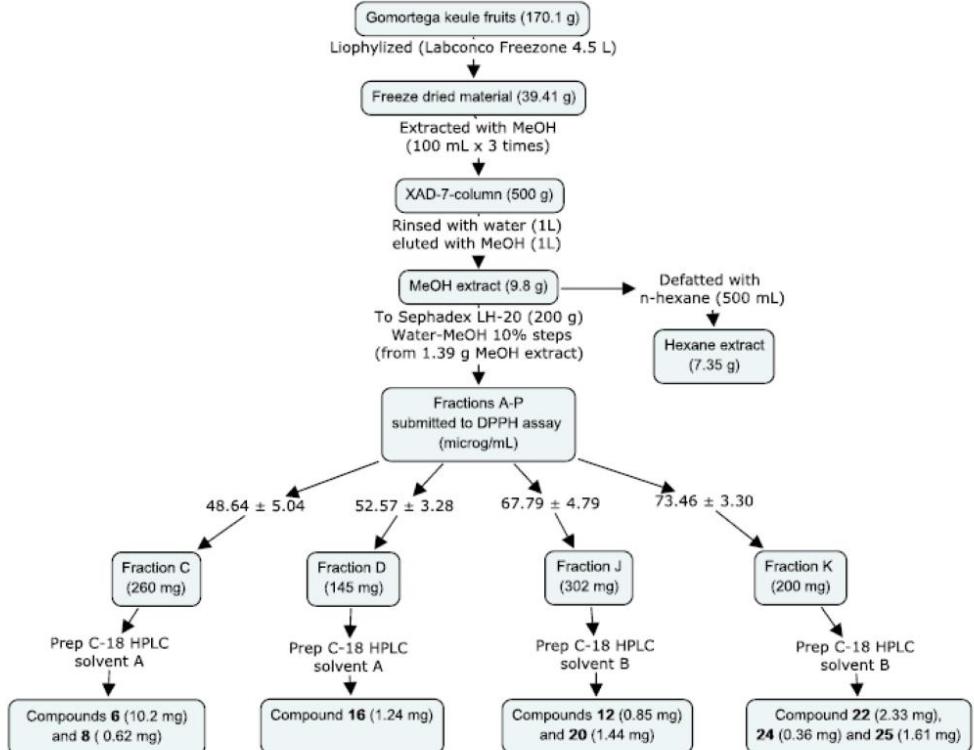
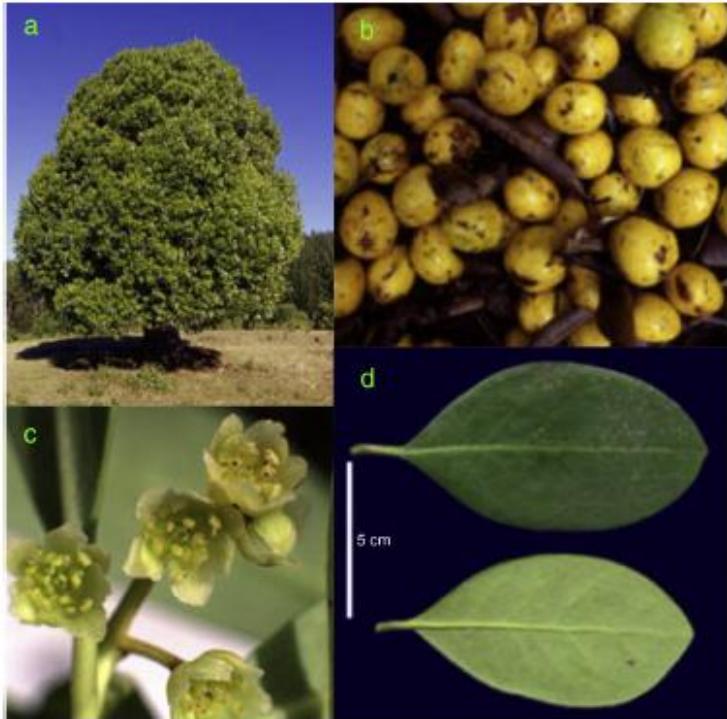


11 \*

# Algunos aislados por HSCCC y 40 detectados trampa iónica Son c-glycosidos! Propiedades venotónicas y sedantes!



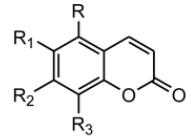
# Queule, un árbol único con frutas comestibles



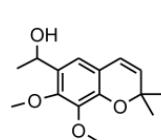
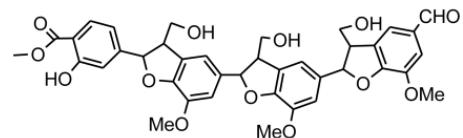
Las gomortegáceas (*Gomortegaceae*) son una familia de angiospermas del orden laurales. Tiene un único género *gomortega* y una única especie *Gomortega keule*.

# Huellas dactilares

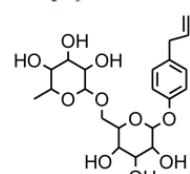
## Coumarinas, flavonoides, ácidos grasos ...



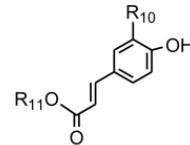
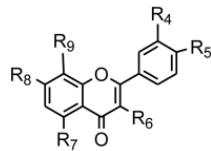
	R	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
8	H	OCH <sub>3</sub>	OGlu	H
9	H	OCH <sub>3</sub>	OCH <sub>3</sub>	OH
10	Prenyl	OCH <sub>3</sub>	OCH <sub>3</sub>	Prenyl
11	CH <sub>3</sub>	CH <sub>3</sub>	OAc	OAc
12	H	OCH <sub>3</sub>	OH	H
20	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H
22	H	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>
24	H	OCH <sub>3</sub>	OCH <sub>3</sub>	OH
25	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>



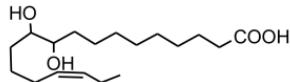
**4 Orthochromene A**



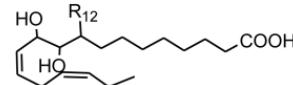
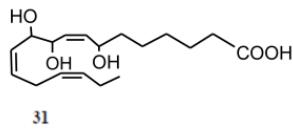
**15 Lusitanicoside**



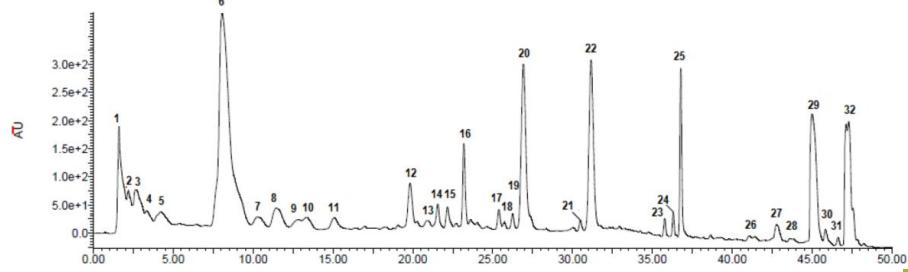
R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>	R <sub>11</sub>
16 OH	OH	O-Rut	OH	OH	H	6 OH	QA
17 H	OH	O-Rut	OH	OH	H	7 OCH <sub>3</sub>	Glu
18 OCH <sub>3</sub>	OCH <sub>3</sub>	H	O-Glu	OCH <sub>3</sub>	H		
19 OCH <sub>3</sub>	OH	O-Rut	OH	OH	H		
28 H	OH	OH	O-Glu	O-Prenyl			



**30 10(S),11(R)-dihydroxy-15(Z)-octadecaenoic acid**



**29 R<sub>12</sub> = OH**  
**32 R<sub>12</sub> = H**



Tiene muchos compuestos interesantes!

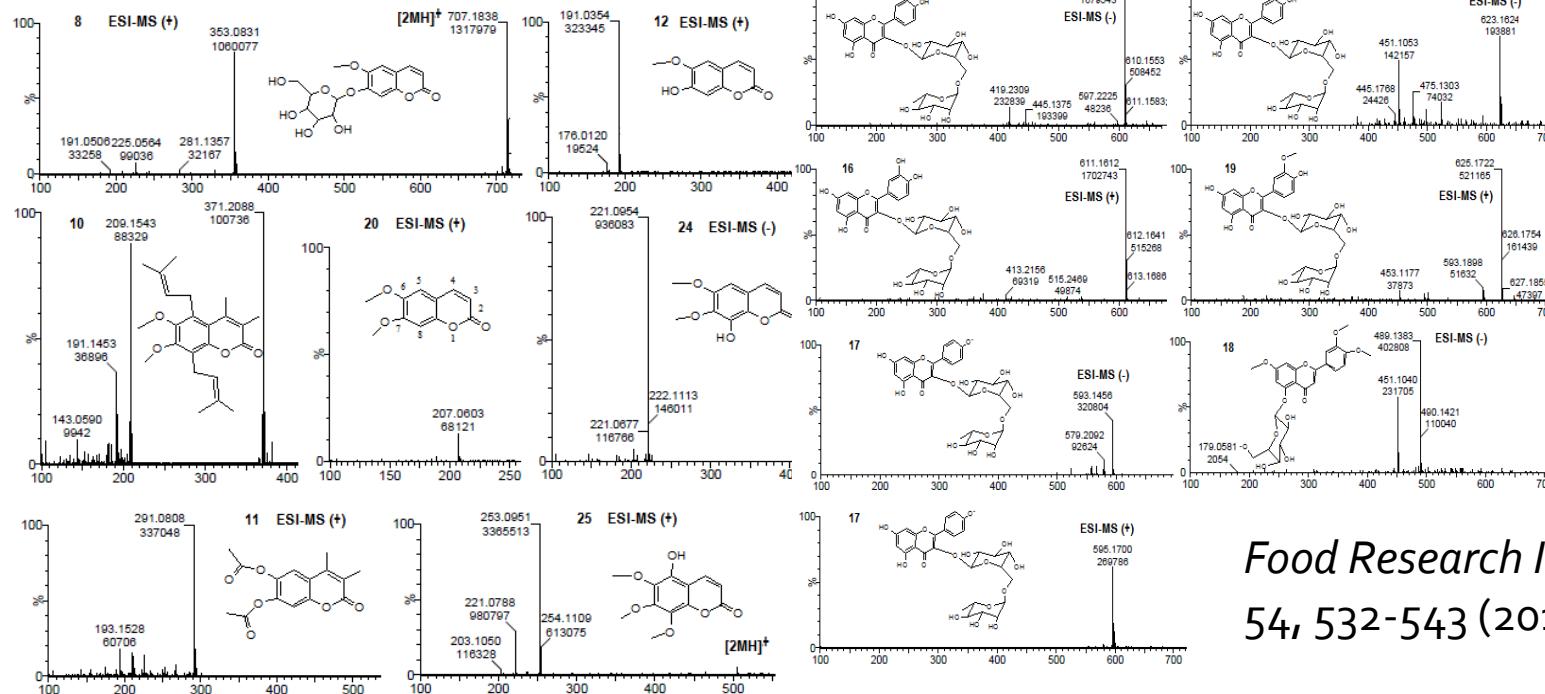
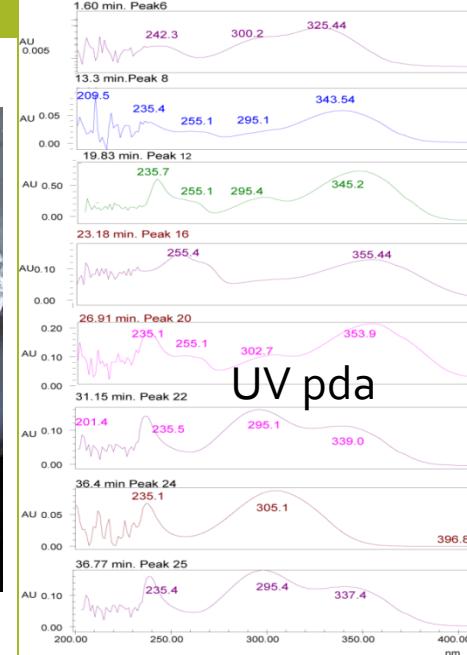
## Cumarinas

## Flavonoides, ácidos fenólicos

Oxilipinas, ácidos grasos dietarios!

# Utilizando unToF

MASAS DE ALTA RESOLUCIÓN



Food Research International  
54, 532-543 (2013).



# Que utilidad puede tener un extracto de queule?

- Las cumarinas, Junto con la troxerutina se emplea para el tratamiento del síndrome varicoso: flebitis, trombosis, tromboflebitis, congestión linfática, linfangitis; hemorroides, edema posoperatorio y postraumático.
- Los taninos y flavonas son antioxidantes, antiinflamatorios y anticancerígenos

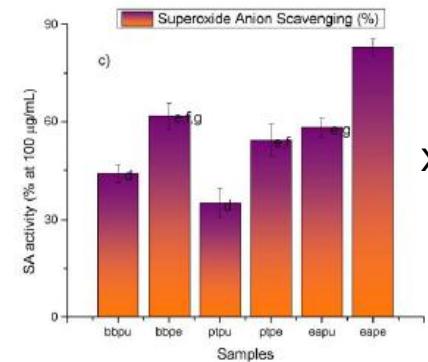
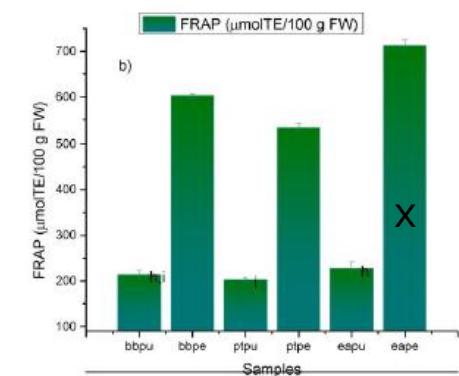
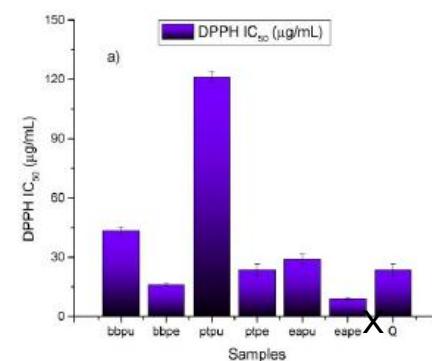
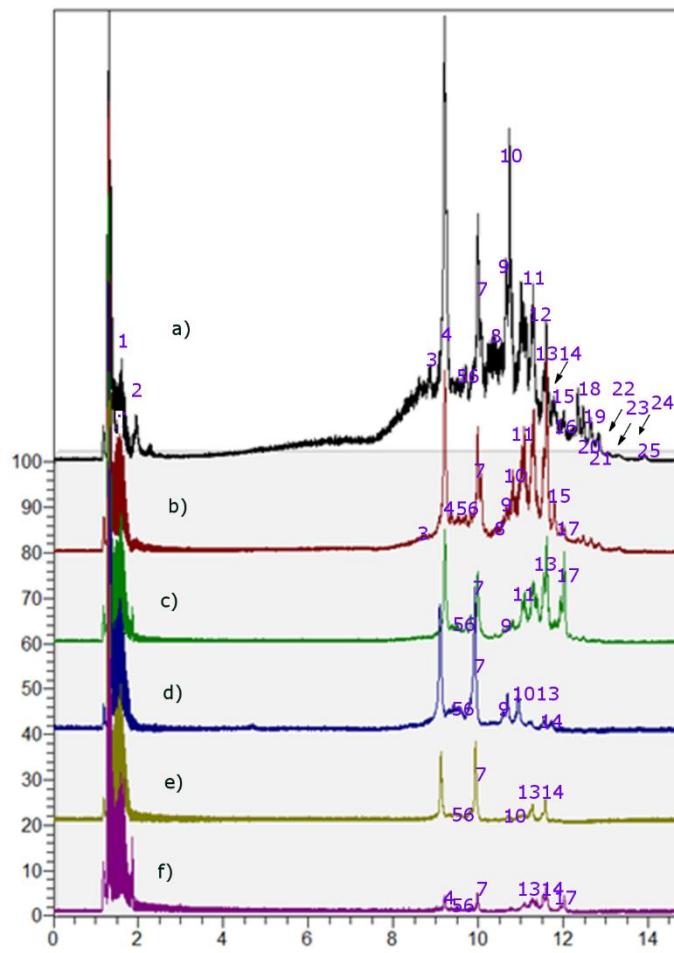




# Pera del desierto (pera de pascua, de Toconao)



Cromatogramas de a) piel pera pascua, b) piel Beurre Bosc  
 c) pulpa Beurre Bosc pulp d) pulpa Packam's triumph pulp e)  
 piel Packam's triumph f) pulpa pera pascua



La cascara es la mas antioxidante



# Identificación de fenólicos en peras de Pascua con LC-PDA-HR-OT-ESI-MS (orbitrap).



Peak #	Uv max	Tentative identification	molecular formula	Retention time	Theoretical mass	Measured mass	Accuracy (Dppm)	Other ions			Molecules 2015, 20						
								(quinic acid C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> )	(quinic acid C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> )	(quinic acid C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> ) a)	(quinic acid C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> ) b)	(Quercetin C <sub>15</sub> H <sub>9</sub> O <sub>7</sub> ) d)	(Quercetin C <sub>15</sub> H <sub>9</sub> O <sub>7</sub> ) e)	C <sub>28</sub> H <sub>31</sub> O <sub>16</sub>	C <sub>30</sub> H <sub>23</sub> O <sub>12</sub>	C <sub>12</sub> H <sub>11</sub> O <sub>7</sub>	C <sub>22</sub> H <sub>21</sub> O <sub>12</sub>
1	325	caffeyl-glucose	C <sub>15</sub> H <sub>17</sub> O <sub>9</sub> <sup>-</sup>	1.79	341.0878	341.0872	1.75	191.0555	(quinic acid C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> )	13	254, 354	Isorhamnetin-3-O-2'	C <sub>28</sub> H <sub>31</sub> O <sub>16</sub>	11.22	623.1618	6	
2	330	Unknown quinic acid derivative		1.82		426.9632	-0.5079	191.0193	(quinic acid C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> )	14	279	rhamnosyl)glucose	A-type procyanidin dimer C <sub>30</sub> H <sub>23</sub> O <sub>12</sub> <sup>-</sup>	11.24	575.11950	5	
3	236, 326	3-O-caffeylquinic acid (3-CQA)*	C <sub>16</sub> H <sub>17</sub> O <sub>9</sub> <sup>-</sup>	8.63	353.0878	353.0878	0	707.1827 [2M-H] <sup>-</sup>	191.0556	(quinic acid C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> )	15		p-coumaroyl malate	C <sub>12</sub> H <sub>11</sub> O <sub>7</sub>	11.26	279.0510	2
									191.0556	(quinic acid C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> )	16	254, 354	Isorhamnetin-3-O-galactose	C <sub>22</sub> H <sub>21</sub> O <sub>12</sub> <sup>-</sup>	11.49	477.1038	4
4	236, 329	4-O-caffeylquinic acid (4-CQA)	C <sub>16</sub> H <sub>17</sub> O <sub>9</sub> <sup>-</sup>	9.12	353.0878	353.0876	0.566	191.0555	(quinic acid C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> ) a)	17	236, 329	di-O-caffeylquinic acid isomer (di-CQA)	C <sub>25</sub> H <sub>23</sub> O <sub>12</sub> <sup>-</sup>	11.56	515.1195	5	
5	280	(+)-catechin*	C <sub>15</sub> H <sub>13</sub> O <sub>6</sub> <sup>-</sup>	9.14	289.07176	289.0715	2.7937			18	254, 354	Isorhamnetin-3-O-glucose*	C <sub>22</sub> H <sub>21</sub> O <sub>12</sub> <sup>-</sup>	11.57	477.1038	4	
6	279	B-type procyanidin dimer	C <sub>30</sub> H <sub>25</sub> O <sub>12</sub> <sup>-</sup>	9.16	577.13515	577.1349	1.4471	289.0713 (monomer)		19	236, 329	di-O-caffeylquinic acid isomer(di-CQA)	C <sub>25</sub> H <sub>23</sub> O <sub>12</sub> <sup>-</sup>	11.74	515.1195	5	
7	280	Gallocatechin-3-O-glucose	C <sub>21</sub> H <sub>21</sub> O <sub>13</sub> <sup>-</sup>	9.64	481.0987	481.0985	1.6903			20	254, 354	isorhamnetin-3-O-(6'-acetyl)-glucoside	C <sub>24</sub> H <sub>23</sub> O <sub>13</sub> <sup>-</sup>	11.90	519.1144	5	
8	236, 329	5-O-caffeylquinic acid (5-CQA)*	C <sub>16</sub> H <sub>17</sub> O <sub>9</sub> <sup>-</sup>	9.87	353.0878	353.0876	0.566	707.1826 [2M-H] <sup>-</sup> 191.0551 (quinic acid C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> ) b)		21	254, 354	isorhamnetin-3-O-(6'-acetyl)-galactoside	C <sub>24</sub> H <sub>23</sub> O <sub>13</sub> <sup>-</sup>	11.97	519.1144	5	
9	280	B-type procyanidin dimer monohexose	C <sub>36</sub> H <sub>36</sub> O <sub>27</sub> <sup>-</sup>	10.59	740.6608	739.1668	0.49			22	254, 350	3-acetyl-3,5,4'-tryhydroxy-7methoxy-flavone	C <sub>18</sub> H <sub>13</sub> O <sub>7</sub> <sup>-</sup>	12.43	341.0667	3	
10	240, 325	Feruloyl quinic acid (3-FQA)	C <sub>17</sub> H <sub>19</sub> O <sub>9</sub> <sup>-</sup>	10.65	367.1034	367.1028	1.2398	c)		23		A-type procyanidin dimer	C <sub>30</sub> H <sub>23</sub> O <sub>12</sub> <sup>-</sup>	12.60	575.11950	5	
11	254, 354	Rutin*	C <sub>27</sub> H <sub>29</sub> O <sub>16</sub> <sup>-</sup>	10.77	609.1461	609.1455		301.0344	(quercetin C <sub>15</sub> H <sub>9</sub> O <sub>7</sub> ) d)	24	240, 312	3-p-Coumaroylquinic acid	C <sub>18</sub> H <sub>9</sub> O <sub>7</sub> <sup>-</sup>	13.29	337.09289	3	
12	254, 354	Quercetin-3-O-glucose*	C <sub>21</sub> H <sub>19</sub> O <sub>12</sub> <sup>-</sup>	10.97	463.0882	463.0876	1.1091	301.0353	(Quercetin C <sub>15</sub> H <sub>9</sub> O <sub>7</sub> ) e)	25	254, 347	Kaempferol-3-O-glucose	C <sub>21</sub> H <sub>19</sub> O <sub>11</sub> <sup>-</sup>	13.31	447.0721	4	

• Molecules 2016, 21(1), 92



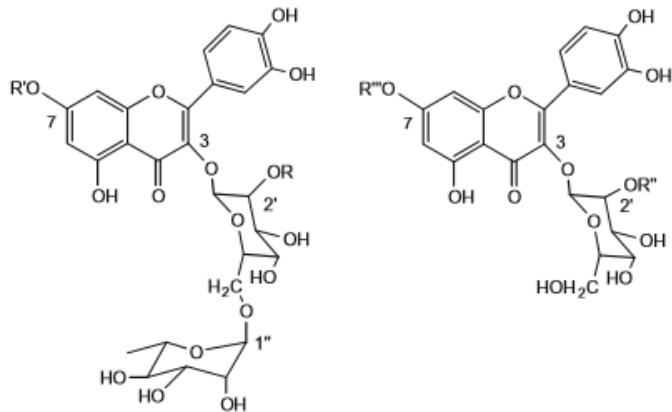
# Papaya chilena

- Sabrosa y pequeña, Tiene papaina una enzima endopeptidaza, pero nunca se había estudiado los fenólicos del fruto popular!



Se cultiva en La Serena

# Compuestos detectados y aislados



- 10. R =  $\alpha$ -Rhamnose, R' = Hexose
- 12. R'' = hexose, R''' = H
- 14. R = Coumaric acid, R' = Hexose
- 15. R = H, R' = Hexose
- 16. R = Caffeic acid, R' = H
- 17. R =  $\alpha$ -Rhamnose R' = H
- 18. R = Coumaric acid, R' = H
- 20. R = R' = H

- 12. R'' = hexose, R''' = H
- 19. R'' = R''' = hexose.

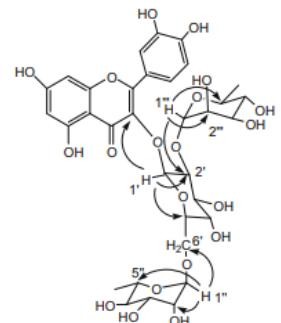
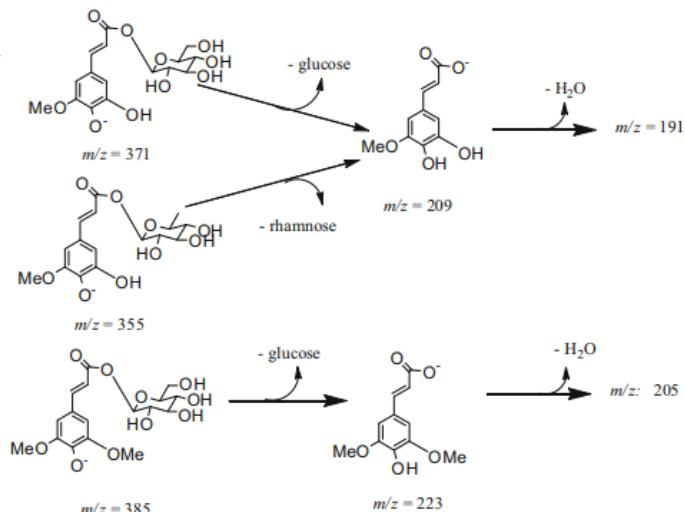


Fig. 3. Important HMBC correlations for Manghaslin (peak 17).



Manghaslin, y rutin fueron aislados

# Cromatograma y relaciones biosintéticas

M.J. Simings et al. / Food Chemistry 115 (2009) 775–79

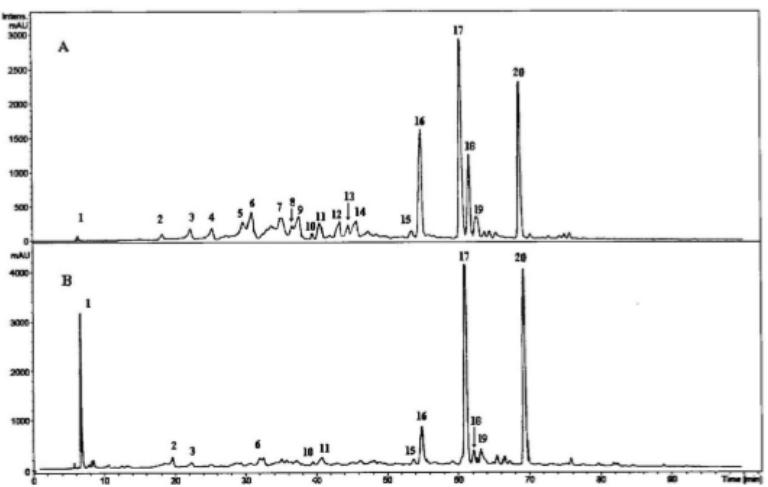
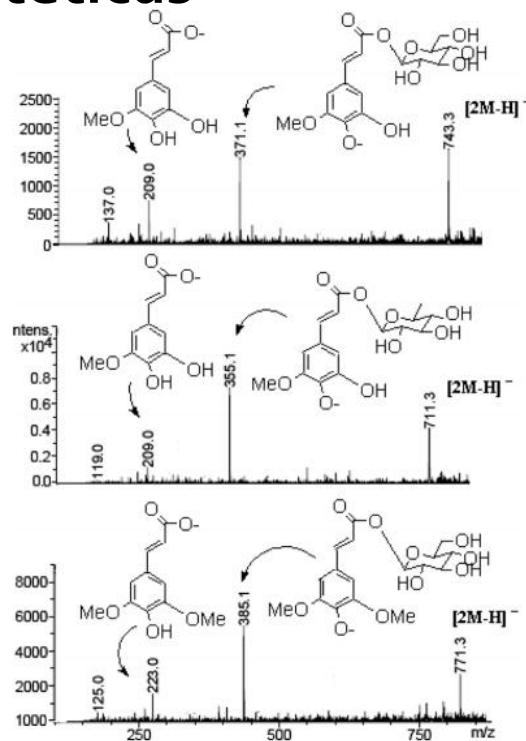
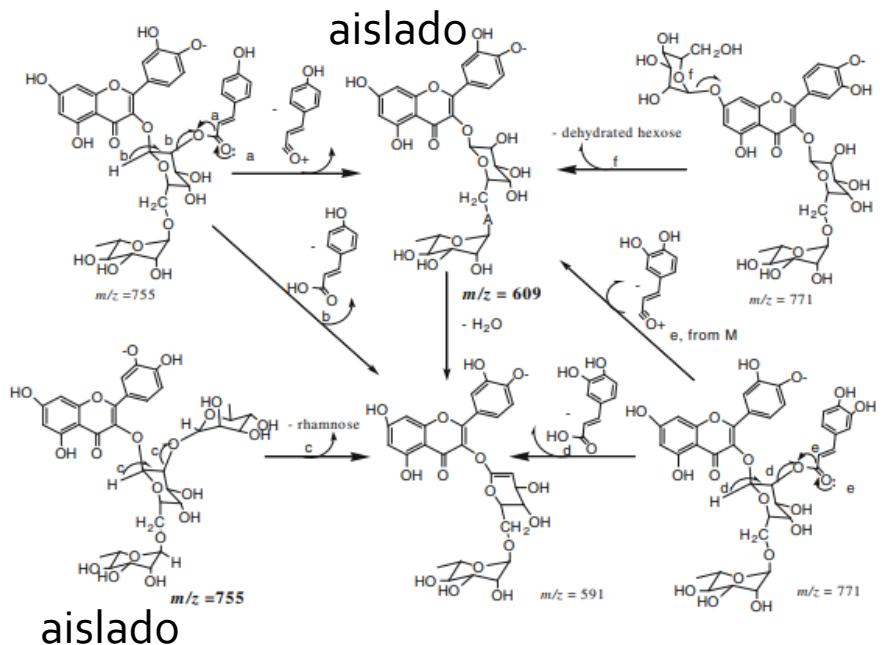


Fig. 1. HPLC-DAD chromatogram of mountain papaya fruit MeOH extract at 320 nm (A) and 254 nm (B).



# Frutilla fresa blanca chilena



Fragaria chiloensis var chiloensis  
Es una antecesora de la frutilla común



Fig. 1 (A) White strawberry culture in Constitución, Región del Biobío, Chile. (B) Picking white strawberry. (C) *Fragaria chiloensis* var. *chiloensis* L. *chiloensis* (white strawberry) fruit. (D) Flowering *Fragaria chiloensis* var. *chiloensis* L. plant growing at the Andean slopes of Chilean A-C: Rudy Montenegro, B: Cristina Theodores.

# Comparamos tres: la blanca, la patagónica y la ananassa

Tentative identification of phenolic compounds in strawberry fruits by HPLC-DAD, LC-MS and LC-MS/MS data

Peak #	Rt (min)	$\lambda$ max (nm)	MW	[M+H] <sup>+</sup> or [M-H] <sup>-</sup>	+/- ions	[2M-H] <sup>-</sup>	MS/MS ions	Tentative identification	Species	F. ch subf.
1	2.9	236, 256	784	783	-		481, 301, 275	Ellagitannin	Chi, Pa	E
2	2.9	235, 315	354	353	-	707	295, 175, 111	p-Coumaroyl-glucoside	Cha	
3	4.0	235, 350	784	783	-		481, 301, 275	Ellagitannin	chi, Pa	E
4	8.5	234, 279	1154	1153	-		865, 577, 407, 289	Procyanidin tetramer	pa	B
5	10.3	237, 278	1156	1155	-		865, 577, 407, 289	Procyanidin tetramer	Cha	
6	10.8	237, 278	1154	1153	-		865, 695, 577, 407, 289, 287	Procyanidin tetramer	pa	B
7	11.4	275, 512	448	449	+		287	Cyanidin-3-O glucoside	chi, Pa, Cha	D
8	12.9	235, 350	934	933	-		915, 633, 465, 451, 301	ellagitannin	pa	
9	13.7	234, 278	1186	1185	-		863, 633, 577, 431, 268	Procyanidin tetramer	Chi	
10	13.8	234, 254	934	933	-		897, 635, 631, 479, 451, 301	ellagitannin	pa	
11	13.9	266, 504	432	433	+		271	Pelargonidin-3-O glucoside	chi, Pa, Cha	D
12	14.6	239, 278	290	289	-	579	245, 221, 149, 123	Catechin	chi	B
13	14.7	237, 278	1154	1153	-		865, 561, 289	Procyanidin tetramer	Chi	
14	14.9	266, 504	578	579	+		433, 271	Pelargonidin-3-O rutinoside	Chi	
15	15.4	234, 278	1155	1154	-		849, 575, 289	Procyanidin tetramer	Chi	
16	16.5	275, 354	292	291	-	583	247, 191	Unknown	Chi	
17	16.9	235, 278	1152	1151	-		865, 577, 407, 289	Procyanidin tetramer	Chi	E
18	17.0	255, 349	934	933	-		633, 451, 301	ellagitannin	pa	
19	18.2	247, 348	936	935	-		633, 451, 301	ellagitannin	pa	
20	18.5	276, 512	534	535	+		287, 449	Cyanidin-malonyl-glucoside	chi, Pa	D
21	19.1	234, 279	866	865	-		785, 577, 469	Procyanidin trimer	Chi	C
22	20.9	266, 504	518	519	+		271, 433	Pelargonidin-malonyl- glucoside	chi, Pa,	D
23	21.2	236, 255	934	935	-		633, 451, 301	ellagitannin	pa	
24	22.1	234, 255	936	935	-		301	ellagitannin	Pa, Cha	B, e
25	23.0	236, 255	936	935	-		451, 301	ellagitannin	Pa, Cha	E
26	24.5	235, 250	936	935	-		633, 301	ellagitannin	Chi	C, E
27	25.8	255, 356	434	433	-	867	301, 300, 257, 229	Ellagic acid pentoside	chi, Pa, Cha	E
28	26.7	255, 358	448	447	-	895	357, 300, 257	Ellagic acid rhamnoside	chi, Pa, Cha	B, E
29	27.0	235, 254	936	935	-	1871	301, 245	ellagitannin	Chi	
30	28.1	255, 362	302	301	-		257, 229, 185	Ellagic acid	chi, Pa, Cha	C, E
31	29.8	254, 352	464	463	-	927	300, 271, 179, 151	Quercetin hexoside	Chi	B
32	30.5	254, 351	478	477	-	955	301, 179, 151	Quercetin glucuronide	chi, Pa, Cha	B, E
33	31.3	254, 351	436	435	-	871	303, 285, 179, 151	Quercetin pentoside	chi, Pa	B, C
34	33.1	235, 349	934	933	-		451, 301	ellagitannin	chi, Pa	B, C
35	33.8	254, 351	434	433	-	867	300, 179, 151	Quercetin pentoside	Chi	B
36	35.0	254, 346	462	461	-	923	285, 257, 163	Kaempferol glucuronide	chi, Pa	B, C
37	35.6	254, 355	492	491	-	983	315, 145, 177	Methyl-quercetin-glucuronide	Chi	B
38	40.2	254, 346	594	593	-	1187	447, 285, 255	Kaempferol coumaroyl-hexoside	chi, Pa, Cha	B
39	40.5	254, 349	594	593	-	1187	447, 284, 255	Kaempferol coumaroyl-hexoside	chi, Pa, Cha	B
40	40.8	242, 286	1138	1137	-		487, 797, 469	Unknown	pa	

Chi: *Fragaria chiloensis* ssp. *chiloensis* f. *chiloensis*, Pa: *Fragaria chiloensis* ssp. *chiloensis* f. *patagonica* and Cha: *Fragaria x ananassa* cv. Chandler.

La frutilla blanca tiene menos cantidad de antocianinas que la frutilla normal (Chandler, roja) pero tiene mas cantidad de acido elágico, un compuesto no pigmentado y por eso posee alta actividad antioxidante!

Journal of Food Composition and Analysis 23 (2010) 545–553



Original Article

Determination of phenolic composition and antioxidant activity in fruits, rhizomes and leaves of the white strawberry (*Fragaria chiloensis* spp. *chiloensis* form *chiloensis*) using HPLC-DAD-ESI-MS and free radical quenching techniques

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M.J. Simirgiotis, G. Schmeda-Hirschmann/Journal of Food Composition and Analysis 23 (2010) 545–553

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**Table 2**

Free radical-scavenging activity measured by the DPPH\* bleaching test, superoxide anion scavenging activity (SA) total anthocyanin (TA), total flavonoid (TF) and total phenolic content (TP) of MeOH extracts of the fruits, leaves and rhizomes of the white strawberry *Fragaria chiloensis* spp. *chiloensis* f. *chiloensis*.

Extract	Yield (%)	TP	TF	TA	SA (%)	DPPH*
Fruits	1.72	2.72 ± 0.01	2.00 ± 0.01	43.6 ± 0.01	79.3 ± 0.76	38.7 ± 0.80
Leaves	15.32	1.99 ± 0. 01	0.83 ± 0.01	0.00	67.60 ± 1.01	49.40 ± 0.81
Rhizomes	9.03	1.45 ± 0. 02	0.55 ± 0.00	0.00	55.0 ± 1.50	64.8 ± 1.54

All measurements are expressed as mean ± SD (n = 3). Yield expressed as g/100 g dry weight. Superoxide anion scavenging activity (SA) is presented as percent inhibition evaluated at 50 µg/mL. Antiradical DPPH\* activity is expressed as IC<sub>50</sub> in µg/mL. TA expressed as mg cyanidin 3-glucoside equivalents/100 g dry weight, TF expressed as g quercetin equivalents/100 g dry weight, TP as g gallic acid equivalents/100 g dry weight. All values in the same column are significantly different (P < 0.05).



# Fitoestrógenos y menopausia



- La menopausia produce trastornos en la mujer. En promedio este cambio se produce en el entorno de los 51 años.
- Síntomas, Sofocos, descalcificación, falta del deseo sexual, mal humor, depresión, calores, insomnio, sequedad vaginal, caída del cabello y problemas de peso, entre otros.
- **fitoestrógenos**, responsables de que las mujeres asiáticas tuvieran un porcentaje menor de enfermedades, que pueden ser consecuencia de la menopausia, como cáncer de colon, mama y útero, diabetes, sobrepeso, problemas cardíacos, osteoporosis, etc.
- Por comer derivados de soya!

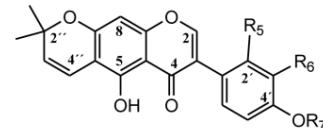
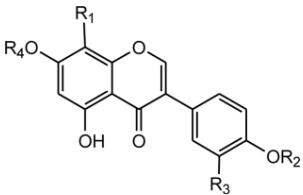
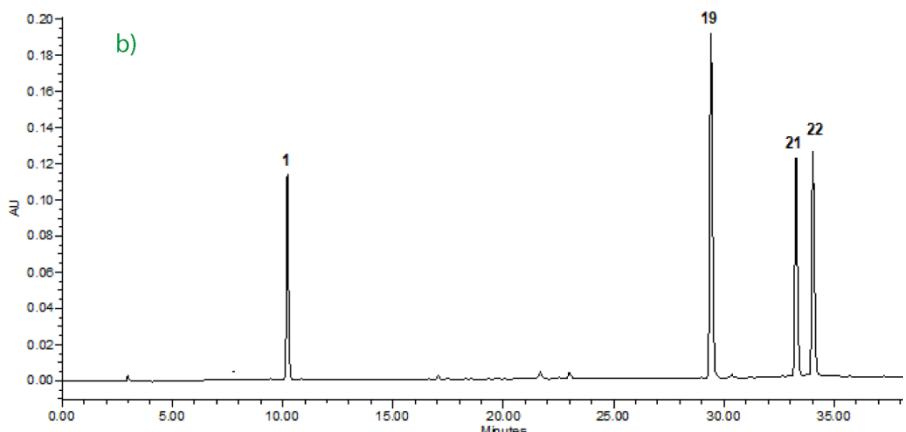
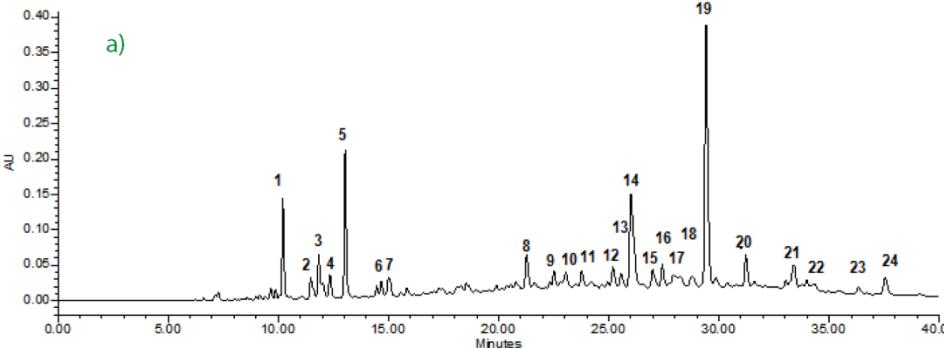
# *Azorella madreporica*, planta del desierto lleno de isoflavonas fitoestrogénicas



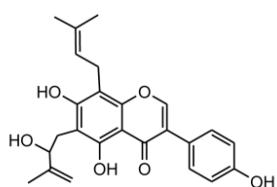
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# Análisis de HPLC PDA-ESI-ToF-MS

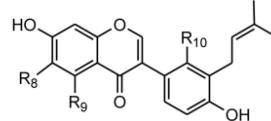
## Aislamiento



	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>
1*	Glu	H	H	H	10	H	Prenyl
4	Glu	Ac	H	H	19*	H	H
5	Ac-Glu	Ac	H	Glu	21*	OH	Prenyl
6	Ac-Glu	H	H	H	22*	H	Ac
7	Glu	Ac	H	H			
9	H	H	H	H			
11	H	H	OH	H			
13	CH <sub>3</sub>	H	H	H			
14	Prenyl	H	H	H			
15	DiMeall	H	H	H			



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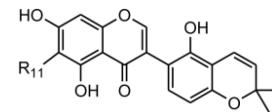


R<sub>8</sub> R<sub>9</sub> R<sub>10</sub>

17 H H H

18 Prenyl OH OH

23 H OH OH



R<sub>11</sub>

20 C<sub>5</sub>H<sub>10</sub>OH

24 H

Tiene muchos isoflavonas! Puede ser bueno para crear nutracéuticos!

# Conclusiones

- Al ser frutas nativas con mucho componente endémico hay posibilidades de encontrar **compuestos novedosos y propiedades nuevas o interesantes** de las frutas, hojas, etc, para realizar medicamentos cosméticos o suplementos dietarios! porque hay muchas especies únicas que pueden producir compuestos con un interés biológico, comercial y para la salud.



# Gracias por escuchar



Agradecimientos a Gabriel Vargas Arana, Giovanna Babilonia Ríos,  
Instituto de Investigación de  
La Amazonia Peruana

