Phenolic profile of fruit industry byproducts determined by HPLC-DAD-MS/MS

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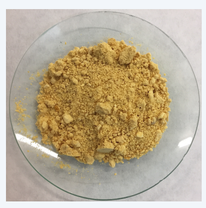
Introduction

The fruit sector produces large amounts of wastes during processing, which are an important source of high quantities of dietary polyphenols. The bioconversion of fruit by-products into new functional and clean label ingredients/additives represents a sustainable approach with great potential of application for the food sector[1]. The aim of this work was the exhaustive characterization of different extracts yielded from by-products of orange and lemon juice extraction (mix of peel, pomace, and seeds) by the identification and quantification of the polyphenols recognized by their antioxidant properties and so their potential health benefits.

Materials and Methods

1) CITRUS BYPRODUCTS

Orange byproduct



Lemon byproduct

Figure 1. Fruit byproducts lyophilized obtained from the orange (left) and lemon (right) fruits.

2) SAMPLE PREPARATION

Extraction
- 5g
- 50 mL EtOH
- 30 min

Centrifugation
- 11952 g
- 15 min
- 15 ºC

Filtration
- 0.22 um

Figure 2. Extraction of phenolic compounds and sample preparation

3) LC-PDA-ESI-MS/MS ANALYSIS

Table 1. LC-PDA-ESI-MS/MS work conditions

LC Conditions		
Equipment	LC - PDA Thermo Fisher Scientific	
Column	Kinetex® 5 µm EVO C18 100 Å (150 x 3 mm)	
Column temp.	30 ºC	
Flow rate	0.6 mL min^{-1}	
Injection vol.	20 µL	
Separation gradient		
Time (min)	% H2O/Acetic acid (0.1%, v/v)	% MEOH/Acetic acid (0.1%, v/v)
0	95	5
3	90	10
10	80	20
18	70	30
25	30	70
33	0	100
40	0	100
41	95	5
46	95	5

Figure 3. LC-PDA-ESI-MS/MS

Detectors Conditions	
PDA	
Equipment	ACCELA PDA Detector
Scan Range (nm)	200–400
Acquisition (nm)	205, 278, 300 and 360
MS/MS	
Equipment	TSQ Quantum Access MAX
Ionization mode	ESI negative and positive mode
Spray voltage	2500 V
Vaporizer temp.	340 ºC
SIM m/z	249
Collision energy (CE)	-30 eV
Nebuliser Gas	Nitrogen

Results and Discussion

1) LC-PDA-ESI-MS/MS

Orange by-product
278 nm

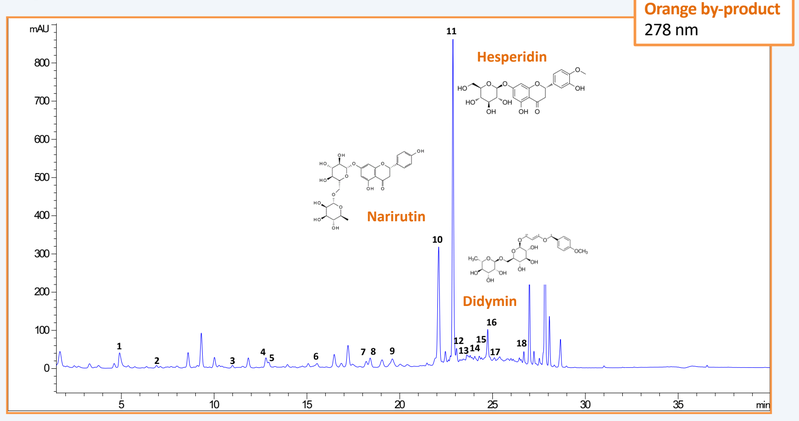


Figure 4. Chromatogram of orange by-product extract acquired at 278 nm

Lemon by-product
278 nm

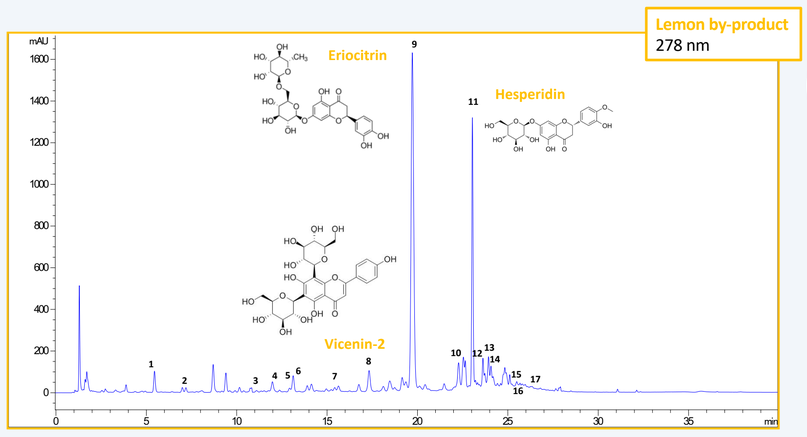


Figure 5. Chromatogram of lemon by-product extract acquired at 278 nm

Table 2. Main phenolic compounds identified in orange byproduct extract identified by LC-PDA-MS/MS

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^{-1}$ (m/z)	MS/MS (m/z)	Molecular Formula	Structural Class	Tentative identification
					//		
10	20.56	290; 330	579	271	$C_{27}H_{32}O_{14}$	Flavanone glycosides	Naringenin-7-O-rutinoside (Narirutin)
11	21.36	290; 355	609	301	$C_{28}H_{34}O_{15}$	Flavanone glycosides	Hesperetin-7-O-rutinoside (Hesperidin)
16	23.19	285; 330	593	285	$C_{28}H_{34}O_{14}$	Flavonols glycosides	Isosakuranetin-7-O-rutinoside (Didymin)
					//		

Table 3. Main phenolic compounds identified in orange byproduct extract identified by LC-PDA-MS/MS

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^{-1}$ (m/z)	MS/MS (m/z)	Molecular Formula	Structural class	Tentative identification
					//		
8	15.68	270; 340	593	473	$C_{26}H_{28}O_{14}$	Flavone glycosides	Apigenin-6,8-di-C-glucoside (Vicenin-2)
9	18	285; 330	595	449	$C_{27}H_{32}O_{15}$	Flavanone glycosides	Eriodyctiol-O-rutinoside (Eriocitrin)
11	21.36	290; 355	609	301	$C_{28}H_{34}O_{15}$	Flavanone glycosides	Hesperetin-7-O-rutinoside (Hesperidin)
					//		

- Orange by-product was that with a high number of polyphenols, while lemon extract was that with high concentrations;
- The main compounds present in the orange by-product were Naringenin-7-O-rutinoside, Hesperetin-7-O-rutinoside, Isosakuranetin-7-O-rutinoside;
- The lemon extract was characterized by the presence of Apigenin-6,8-di-C-glucoside, Eriodyctiol-O-rutinoside, Hesperetin-7-O-rutinoside;
- The polyphenolic profile of the by-products was similar to those described by other authors for the respective fruit juices. However, the concentration could be up to 10-fold higher according to the variety of the fruit [2, 3].

Conclusion

The results highlight that the origin of the extract affects its composition, and therefore the characterization of these profiles is mandatory for food application. These fruits by-products may be a low-cost source of polyphenols that can be used as food ingredients/additives minimizing their environmental impact.

References

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