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Proceeding Paper

Analysis of Polyphenol Content and Antioxidant Capacity of Hybrid Mandarin Peel †

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Abstract: Mandarin cultivars (*Citrus reticulata*) represent 22% of the total number of citrus fruit crops. Mandarin peels are an abundant source of natural flavonoids and other antioxidants. To determine the polyphenol content and antioxidant capacity of hybrid mandarin peel, 33 samples of hybrid mandarins (Clemenvilla, Nadorcott and Ortanique), from the province of Valencia (Spain), were selected. Fresh mandarin peel extracts were prepared by ultrasound-assisted extraction (400 W, 80% v/v duty cycle, 40 °C) for 30 min, employing ethanol 50% (v/v) as the solvent in a 1:10 (w/v) solid–liquid ratio. C18 cartridges (200 mg) were employed for the solid phase extraction clean-up process, and an ultra-performance liquid chromatography system, coupled with a quadrupole time-of-flight mass spectrometer, was used to identify, and quantify the polyphenols. Clemenvilla and Ortanique showed the highest antioxidant capacity using DPPH and TEAC, respectively. For these three hybrids, the main polyphenol present in the samples was hesperidin, which was higher in the Nadorcott peel (72 ± 7.0 μg/g). Moreover, narirutin was higher in Ortanique and Nadorcott (33 ± 6.3 and 31.8 ± 6.8 μg/g, respectively), and rutin was higher in Clemenvilla samples (7.3 ± 3.8 μg/g). The results suggest that mandarin peels are an important source of polyphenol compounds with a high antioxidant capacity.

Keywords: mandarin peel; polyphenols; hesperidin; antioxidant capacity

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1. Introduction

Citrus fruit is one of the principal crops worldwide, with mandarin cultivars (*Citrus reticulata*) representing 22% of the total number of citrus fruit crops [1]. These fruits are well accepted by consumers because of their sweet flavors and easy peeling [2]. Mandarin fruit residues (peel, seeds and pulp) are usually discarded, without regard for their potential nutritional and commercial value. Mandarin peels make up approximately 35–40% of the weight of the fruit [3], and they are an abundant source of natural flavonoids [4] and other antioxidants.

This study addressed the characterization of polyphenols from three varieties of hybrid mandarin peel (Clemenvilla, Nadorcott and Ortanique) that are not widely studied. Ultrasound-assisted extraction (UAE), a solid phase extraction (SPE) clean-up process and an ultra-performance liquid chromatography system, coupled with a quadrupole time-of-flight mass spectrometer (UPLC-QTOF-MS/MS), were employed to determine, and quantify the polyphenols from the hybrid mandarin peel.

2. Materials and Methods

2.1. Plant Materials and Extraction Method

Thirty-three lots of hybrid mandarins (n = 10 Clemenvilla, n = 10 Nadorcott and n = 13 Ortanique), procured by citrus farmers in the province of Valencia (Spain), were selected. The number of samples for each lot was 15–20 mandarins. Fresh mandarin peel extracts were prepared according to the method reported by Anticona et al. [5], using UAE. Firstly, 6 g of peels were placed in a beaker glass, with ethanol–water (50:50, v/v) as the solvent, in a solid–liquid ratio of 1:10 (w/v). The extraction was assisted by an ultrasonic processor QSONICA Q500 (Newtown, CT, USA), under the following conditions: 400 W, 80% v/v duty cycle, 40 °C, for 30 min. The extracts were centrifuged (4000 r.p.m., 4 °C, 5 min) and the supernatants were filtered by a membrane filter, Whatman no. 1, with a pore size of 11 µm (Whatman International Ltd., Maidstone, UK), and were collected to be stored at −20 °C in dark conditions, until use. The procedure is described in Figure 1.

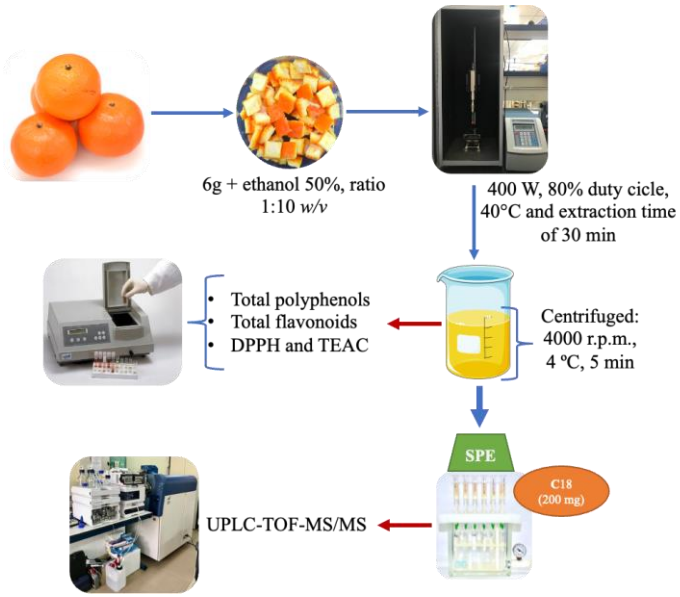

Figure 1. Mandarin peel extraction and polyphenol analysis.

2.2. Chemical Analysis Methods

The total polyphenol (TP) and total flavonoid (TF) contents were determined according to the methods described by Anticona et al. [5]. For TP, 3 mL of anhydrous sodium carbonate (Na_2CO_3) solution (2%, w/v) and 100 µL of Folin–Ciocalteau reagent (1:1, v/v) were added to an aliquot of 100 µL of diluted sample. The mixture was incubated for 1 h at room temperature. The absorbance was measured at 765 nm using a UV/Vis Lambda 2 spectrophotometer (Perkin Elmer, Waltham, MA, USA). The results were expressed as mg of gallic acid equivalent (GAE)/100 g fresh weight (FW) of peel. The TF determination was carried out by mixing 100 µL of appropriately diluted samples with 1088 mL of ethanol (30%, v/v). Further, 48 µL of sodium nitrite ($NaNO_2$) solution (0.5 mol/L) was added and the mix was vortexed. After 5 min of reaction, 48 µL of aluminum chloride hexahydrate ($AlCl_3.6H_2O$) (0.3 mol/L) was added. The mixture was vortexed and allowed to react for 5 min at room temperature. Then, 320 µL of sodium hydroxide (NaOH) (1 mol/L) was added and the mixture was vortexed again. The absorbance was measured at 510 nm, and the results were expressed as mg of catechin equivalents (CE)/100 g fresh weight (FW) of peel.

To determine the antioxidant capacity, the 2,2-diphenyl-1-picrylhydryzyl (DPPH) radical scavenging assay was applied, according to the method described by Anticona et al. [5]. The DPPH-colored radical was used to measure the initial absorbance at 515 nm. The reaction was started by adding 50 µL of sample, in a suitable dilution, to 1.45 mL of DPPH

radical (0.06 mM). After being incubated for 30 min at room temperature, the final absorbance was measured. In the case of the Trolox equivalent antioxidant capacity (TEAC) assay, the method described by Zulueta et al. [6] was employed, with modifications for the final reaction tested. Following this, 25 mL of ABTS radical (ABTS•+) (7 mM) was prepared with 440 µL of potassium persulphate $K_2S_2O_8$ (140 mM) and allowed to stand in darkness at room temperature for 12–16 h. The solution was diluted with ethanol until an absorbance of 0.70 ± 0.02 was reached at 734 nm and 30 °C. The absorbance of 2 mL of formed ABTS•+ was recorded as the initial absorbance, and 100 µL of appropriately diluted samples were added. The mixture was incubated for 3 min and the final absorbance was measured. In both assays (DPPH and TEAC), the percentage of inhibition (% I) was calculated using the following formula (Equation (1)):

$$\% \, I = [(A0 - A1)/A0] \times 100 \quad (1)$$

where A0 is the absorbance of the control and A1 is the absorbance in the presence of the sample. The results were expressed as mM Trolox equivalent (mM TE).

2.3. Chromatographic Analysis

Before the chromatographic analysis, 5 mL of sample was placed on C18 cartridges (200 mg) for the SPE clean-up process, according to the method described by Gonzales et al. [7], with some modifications. A UPLC-QTOF-MS/MS was used to identify and quantify the main polyphenols in the samples.

The UPLC-QTOF-MS/MS analysis was performed on an LC SCIEX system equipped with a ACQUITY UPLC C18 column, 50 × 2.1 mm, 1.7 µm (Waters, Milford, MA, USA), applying the following elution binary gradient at a flow rate of 0.4 mL/min: 0–5 min, isocratic 70% A (water/formic acid, 99.9/0.1 [*v*/*v*]), 30% B (methanol/formic acid, 99.9/0.1 [*v*/*v*]); 5–12 min, linear from 30 to 95% B; 12–18 min, isocratic 95% B; 18–18.5 min, linear from 5 to 70% A; 18.5–25 min, isocratic 70% A. The injection volume was 5 µL. The compounds were detected from m/z 100 to 950 in negative ion mode in a transfer time of 100 ms. Automated calibration was performed using an external calibrant delivery system. The MS used an information-dependent acquisition method with the survey scan type (TOF-MS) and the dependent scan type (product ion) using −30 V of the collision energy. Data were qualitatively evaluated using the PeakViewTM software. Relative quantification was performed using Multiquant 3.0.3 software.

3. Results and Discussion

3.1. Bioactive Compounds

The TP and TF contents varied according to the hybrid mandarin variety employed ($p < 0.05$). The TP content was higher in the 'Ortanique' samples compared with the 'Clemenvilla' and 'Nadorcott' peels (Table 1). These results differ to the values obtained by Safdar et al. [3], who obtained values that ranged from 2439 to 3248 mg GAE/100 g in mandarin peel powder treated using UAE. In addition, Nipornram et al. [8] obtained 14,899 mg GAE/100 g of TPC in the peel powder of *C. reticulata* Blanco cv. Sainampueng. These differences are due to the structures of the samples analyzed, because in our study, fresh peels were employed. In this line, Londoño-Londoño et al. [9] observed greater differences between the TP content of the fresh peel and peel powder of *C. reticulata* samples obtained using UAE.

Flavonoids are the principal bioactive compounds in citrus peel [10]. The 'Clemenvilla' samples had the highest values of TF content compared with 'Nadorcott' and 'Ortanique' (Table 1). Ho and Lin [11] obtained a total of 790 mg CE/100 g of *C. reticulata* peel powder extract, showing that the principal differences in the concentration of TF are due to the structural characteristics of the samples analyzed.

Table 1. Total polyphenol and flavonoid contents determined in hybrid mandarin peels.

Bioactive Compound	Clemenvilla	Nadorcott	Ortanique
TP (mg GAE/100 g FW ± SD)	828.4 ± 95.8 [a]	724.2 ± 43.0 [a]	1155.2 ± 171.3 [b]
TF (mg CE/100 g FW ± SD)	89.6 ± 15.5 [a]	70.9 ± 12.5 [a]	71.7 ± 17.8 [a]

[a,b]: different letters in the same row indicate that there are statistically significant differences ($p < 0.05$) between the values of each variety. TP: total polyphenols; GAE: gallic acid equivalent; FW: fresh weight; SD: standard deviation; TF: total flavonoids; CE: catechin equivalent.

3.2. Antioxidant Capacity

DDPH and TEAC assays were employed to assess the antioxidant capacity of the hybrid mandarin peels. There are useful methods that can be applied to determine the antioxidant capacity of fruit samples, and it is recommended to employ two or more methods [12]. As can be observed in Figure 2, the 'Clemenvilla' and 'Ortanique' extracts showed the highest antioxidant capacity, using DPPH (14 ± 3.8 mmol Trolox/100 g) and TEAC (32 ± 3.8 mmol Trolox/100 g) assays, respectively. In addition, the values of mmol TE/100 g obtained by DPPH were lower than the values obtained by the TEAC assay. This is similar to the antioxidant capacity results of whole 'Murcott' mandarin samples observed in a study by Gironés-Vilaplana et al. [13], using DPPH (2.5 mmol TE/100 g) and TEAC (6.47 mmol TE/100 g). The different values of total antioxidant capacity obtained by the assays employed reflect the difference in the ability of bioactive compounds to reduce the DPPH and ABTS radicals in these types of in vitro assays. The main difference is that the DPPH assay is more sensitive to hydrophobic compounds, while the TEAC assay is more sensitive to hydrophilic antioxidants, such as polyphenols [14]. In this sense, the mmol TE values in the samples assessed by the TEAC assay were in the same order as the mg GAE values (TP), as follows: 'Ortanique' > 'Clemenvilla' > 'Nadorcott'. The results obtained by the TEAC assay were higher than the values determined by Montero-Calderon et al. [15] (3.97 ± 0.15 mmol TE/100 g) in samples of orange peel treated using UAE (400 W, 30 min, 50% ethanol). Additionally, M'hiri et al. [16] showed lower TEAC values of orange peel extracts using ultrasound.

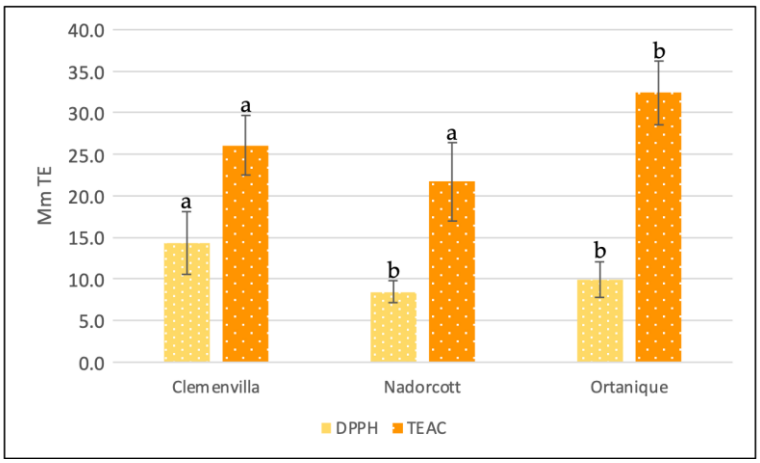

Figure 2. Differences in antioxidant capacity assessed by DPPH and TEAC assays in hybrid mandarin peels. a,b: different letters in the same color indicate that there are statistically significant differences ($p < 0.05$) between the values. TE: Trolox equivalent.

3.3. Identification and Quantification of Polyphenols by Ultra-Performance Liquid Chromatography System Coupled with a Quadrupole Time-of-Flight Mass Spectrometer Analysis

The principal polyphenol composition of mandarin peel extracts can be observed in Table 2. Fayek et al. [17] indicated that UPLC-QTOF-MS/MS is a useful technique to analyze the phenolic composition in citrus peels. The main polyphenol present in the hybrids

was hesperidin, which was higher in the 'Nadorcott' peel (72 ± 7.0 µg/g). According to this, Nipornram et al. [8] and Hayat et al. [18] determined that hesperidin is one of the major compounds in mandarin peel. However, in their study, Zhao et al. [19] observed that nobiletin is the main polyphenol, followed by hesperidin. A slightly higher concentration of hesperidin from mandarin peel extract was reported by Safdar et al. [3] (84.41 µg/g). In second position, in regards to the amount detected, is narirutin in 'Ortanique' and 'Nadorcott' (33 ± 6.3 and 31.8 ± 6.8 µg/g, respectively), and rutin in 'Clemenvilla' (7.3 ± 3.8 µg/g). In the case of narirutin amounts, a notable difference was observed in Clemenvilla samples, in which the amount was lower than in the 'Nadorcott' and 'Ortanique' peels. Further studies are necessary to explain these differences. Lower concentrations of rutin (1.0 µg/g) were obtained by Zhao et al. [19] in clementine peel extracts. In relation to ferulic acid and 4-hidroxibenzoic acid, 'Clemenvilla' and 'Nadorcott' exhibit high concentrations. However, higher concentrations of ferulic acid were observed by Safdar et al. (3) in 'Kinnow' mandarin peels (42.56 µg/g).

Table 2. Polyphenol compounds identified and quantified in hybrid mandarin peels by UPLC-QTOF-MS/MS.

Compound	Molecular Formula	[M-H]$^-$ *m*/*z* (−)	Clemenvilla	Nadorcott	Ortanique
4-hidroxibenzoic acid	$C_7H_6O_3$	137.02442	4.1 ± 10.7 ^{a}	1.9 ± 0.8 ^{b}	2.1 ± 1.2 ^{b}
Rutin	$C_{27}H_{30}O_{16}$	609.14611	7.3 ± 3.8 ^{a}	5.6 ± 3.3 ^{ab}	6.9 ± 2.2 ^{ab}
Ferulic acid	$C_{10}H_{10}O_4$	193.05063	1.5 ± 0.7 ^{a}	6.8 ± 0.9 ^{b}	2.2 ± 0.5 ^{c}
Narirutin	$C_{27}H_{32}O_{14}$	579.17193	4.3 ± 2.6 ^{a}	31.8 ± 6.8 ^{b}	33.1 ± 6.3 ^{b}
Hesperidin	$C_{28}H_{34}O_{15}$	609.18249	63.7 ± 10.7 ^{a}	72.3 ± 7.0 ^{b}	63.7 ± 6.8 ^{a}

Concentrations are expressed in µg/g FW of peel. $^{a–c}$: different letters in the same row indicate that there are statistically significant differences ($p < 0.05$) between the values of each variety.

4. Conclusions

The results suggest that there are significant differences in the contents of TP and TF, and the antioxidant capacity, according to the varieties analyzed. Finally, hesperidin is the major phenolic compound in hybrid mandarin peels, and narirutin and rutin were identified and quantified in the samples analyzed. The analyzed mandarin peels are an important source of polyphenol compounds with a high antioxidant capacity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/Foods2021-11100/s1. Poster: Analysis of Polyphenol Content and Antioxidant Capacity of Hybrid Mandarin Peel.; Video: Analysis of Polyphenol Content and Antioxidant Capacity of Hybrid Mandarin Peel.

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