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Proceeding Paper

Valorizing Grapefruit (*Citrus x paradisi* Macfad) By-Products: Phenolic Composition and Antioxidant Potential Assessment of Pulp and Waste Extracts †

Kayane Oliveira [1,2], Carla Pereira [1,2], Maria Inês Dias [1,2], Lillian Barros [1,2] and Tânia Pires [1,2,3,*]

[1] Mountain Research Center (CIMO), Polytechnic Institute of Bragança, Santa Apolónia Campus, 5300-253 Bragança, Portugal; kayane@ipb.pt (K.O.); carlap@ipb.pt (C.P.); maria.ines@ipb.pt (M.I.D.); lillian@ipb.pt (L.B.)
[2] Associated Laboratory for Sustainability and Technology in Mountain Regions (SusTEC), Polytechnic Institute of Bragança, Santa Apolónia Campus, 5300-253 Bragança, Portugal
[3] Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Science, University of Vigo, E32004 Ourense, Spain
* Correspondence: tania.pires@ipb.pt
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Abstract: The grapefruit (*Citrus x paradisi* Macfad) is a widely consumed citrus fruit known for its tangy flavor and reddish-pink pulp, commonly used for juice. Despite discarding the peel and mesocarp, they contain valuable antioxidants and bioactive substances.This study assessed grapefruit parts phenolic compounds and antioxidant capacity using three assays. HPLC-DAD/ESI-MS identified phenolic compounds. The mesocarp had the most phenolic compounds (48.4 mg/g), followed by the peel (36.7 mg/g). Peel extract showed the best antioxidant activity. This likely resulted from higher naringenin concentrations, particularly *O*-triglycosyl naringenin and acetyl naringenin. Grapefruit waste and pulp extracts hold promise for diverse industrial applications.

Keywords: phenolic compounds; plant waste; natural antioxidants

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1. Introduction

The grapefruit, scientifically identified as *Citrus* × *paradisi* Macfad, belongs of the citrus fruit family, sharing resemblances with oranges, lemons, and limes [1]. Originating from the Caribbean Islands of Barbados, it boasts yellow-orange flesh with hints of pink and red, offering a tangy flavor profile [2].

Beyond being a source of vitamin C, grapefruit proves abundant in essential nutrients like fiber, minerals, and carbohydrates. Notably, it harbors secondary metabolites endowed with bioactive properties, namely phenolic compounds [3]. These compounds are responsible for the properties of color, taste, and aroma in plant-based foods and also have been linked to a range of health advantages, encompassing notable antioxidant capacity [4].

In grapefruit, phenolic compounds are mainly in the form of flavanones, primarily naringin and narirutin, as well as other glycosides like hesperidin, neohesperidin, didymin, and poncirin, can be highlighted [5]. The glycosylation of these flavanones primarily occurs at position 7 by rutinose or neohesperidose, imparting grapefruit with its distinctive phenolic compound profile [6]. These flavanones are renowned for their diverse therapeutic properties, including anti-inflammatory, anti-hypertensive, lipid-lowering and antioxidant activity [7].

Human cells require antioxidants for protection against the inevitable effects of the respiratory process and oxidative reactions [8]. Recent research has been exploring the

quest for natural alternatives to synthetic antioxidants due to concerns about their potential adverse effects [9]. Studies primarily focus on phenolic compounds of plant origin, which act as free radical scavengers, interrupting chain reactions, and inhibiting metal-catalyzed oxidative processes [10].

As grapefruit is primarily consumed as juice, its mesocarp and peel are regarded as bio-wastes, comprising as much as 50% of the fruit [11]. Nonetheless, these bio-wastes might harbor compounds of significance, as research indicates that in many fruits, a substantial portion of bioactive substances is located in the peel rather than the pulp [12,13].

Based on the provided context, the aim of this study is to identify and quantify phenolic compounds in grapefruit pulp and bio-wastes, as well as to assess the antioxidant activity of different parts of the fruit.

2. Materials and Methods

2.1. Preparation of the Samples

Around one and a half kilograms of grapefruits (*Citrus* × *paradisi* Macfad) were obtained through a single purchase at a local Bragança, Portugal supermarket. These fruits were imported from Spain and represented the Star Ruby variety. Following this purchase, the waste components (peel and mesocarp) were isolated from the pulp, and all parts of the fruit were subsequently frozen, subjected to lyophilization, and placed in a desiccator at room temperature (an average of 25 °C), safeguarded from light, until further analyses.

2.2. Identification and Quantification of Phenolic Compounds

The identification and quantification of phenolic compounds were carried out using hydroethanolic extracts. To prepare these extracts, a maceration extraction technique was employed. One gram of each grapefruit part—pulp, peel, and mesocarp—was accurately weighed, and 30 mL of an 80:20 (*v*/*v*) ethanol/water solution was added. The mixture was continuously stirred at room temperature for one hour (at 150 rpm). Subsequently, the supernatant was filtered through Whatman No. 4 filter paper, and this process was repeated, with the sample undergoing another 30 mL extraction with the same solvent. The filtrate was then subjected to rotary evaporation at 40 °C to remove the alcoholic fraction, while the aqueous fraction was frozen and freeze-dried for subsequent analyses.

Phenolic compounds were identified and quantified using a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA), following the procedure previously described by Bessada et al., 2016 [10]. Detection was carried out using a DAD (with preference wavelength of 280 nm) and a mass spectrometer (LTQ XL mass spectrometer, Thermo Finnigan, San Jose, CA, USA). Quantification of all compounds was based on a 7-level calibration curve of naringenin standard ([12.5–800] μg/mL): $y = 18{,}433x + 78{,}903$, with $R^2 \geq 0.999$. Results were expressed in milligrams per gram of extract.

2.3. Evaluation of Antioxidant Activity

A selection of assays was performed to assess the antioxidant activity of the extracts, aiming to gauge their effectiveness through various mechanisms. In order to determine the IC_{50} values, grapefruit pulp and waste extracts were reconstituted in ethanol (2.5 mg/mL) and subsequently diluted. Following the procedure described by Barros et al., 2014 [14], the inhibition of lipid peroxidation in porcine brain homogenates was assessed by measuring the reduction in thiobarbituric acid reactive substances (TBARS). The oxidative hemolysis inhibition assay (OxHLIA), as described by Lockowandt et al., 2019 [15], was performed using sheep blood samples, with results expressed as the inhibitory concentration (IC_{50} value, μg/mL) capable of delaying Δt hemolysis by 60 min. Additionally, the capacity to scavenge the organic radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was evaluated following the methodology described by Cardeñosa et al., 2019 [16]. Trolox was used as the positive control for all assays.

2.4. Statistical Analysis

The quantitative results were presented as the mean ± standard deviation. Statistical analysis of the data was conducted employing analysis of variance (ANOVA), accompanied by either Tukey's Honestly Significant Difference (HSD) test or the t-student test, contingent on the nature of the comparisons. A significance level of *p*-value = 0.05 was adopted for these evaluations. RStudio software (Team RStudio: Integrated Development for R. RStudio, PBC, Boston, MA, USA) was utilized for this analytical process.

3. Results

3.1. Phenolic Compounds

The profile of phenolic compounds present in the extract obtained from the pulp, peel, and mesocarp of *Citrus x paradisi* Macfad is presented in Table 1. The identification and quantification of compounds were performed using HPLC-DAD/ESI-MS. Peak 3 ([M-H]$^-$ at *m*/*z* 579) was validated as naringenin through a comparative analysis of retention time, absorption spectrum, and mass against the lab's standard compound. The remaining four peaks were tentatively assigned as naringenin derivatives: peak 1 ([M-H]$^-$ at *m*/*z* 741), 2 ([M-H]$^-$ at *m*/*z* 579), 4 ([M-H]$^-$ at *m*/*z* 621), and 5 ([M-H]$^-$ at *m*/*z* 593), corresponding to naringenin *O*-triglycoside, narirutin, acetyl naringenin, and poncirin, respectively. Peaks 1 and 4 were absent in the pulp.

The mesocarp emerged as the predominant part in terms of phenolic content (48.4 mg/g of extract), followed by the peel (36.7 mg/g). The statistical analysis highlighted significant differences in both total compound quantification and individual levels among all extracts.

The literature suggests the presence of over 40 phenolic compounds in *Citrus x paradisi* Macfad [17–19]. Nonetheless, this study reinforces naringin and its derivatives as the principal flavonoids in grapefruit. Another inquiry into grapefruit juice affirmed analogous flavanones, yet the phenolic content in the pulp was notably lower than observed here, likely due to the distinction in sample types (grapefruit pulp juice vs. grapefruit pulp extract) [20].

This study underscores naringenin and naringin as the predominant phenolic compounds in grapefruit extracts, contributing to the well-recognized bitter taste and diverse bioactive traits of grapefruit.

Table 1. Quantification (mg/g of extract) and tentative identification ^ of phenolic compounds present in the grapefruit (*Citrus x paradisi* Macfad) pulp and waste extracts.

Peak	Rt (min)	λmax (nm)	[M-H]$^-$ (*m*/*z*)	MS^2 (*m*/*z*)	Tentative Identification Identification	Quantification (mg/g of Extract)		
						Pulp	Peel	Mesocarp
1	12.05	286	741	MS^2:579 (100) MS^3:271 (100)	Naringenin *O*-triglicosyl	tr.	1.07 ± 0.02 *	0.80 ± 0.01 *
2	19.12	284	579	MS^2:271 (100)	Naringinin	1.00 ± 0.05 ^{c}	5.1 ± 0.3 ^{b}	6.7 ± 0.4 ^{a}
3	20.36	284	579	MS2:459 (34), 271 (100)	Naringenin	9.2 ± 0.3 ^{c}	26.6 ± 0.4 ^{b}	35.6 ± 1.37 ^{a}
4	24.85	284	621	MS^2:501 (12), 579 (76), 601 (5), 271 (100)	Acetyl Naringenin	tr.	2.55 ± 0.04 *	1.415 ± 0.009 *
5	31.21	284	593	MS^2:387 (12), 327 (15), 309 (5), 285 (98)	Poncirine	0.099 ± 0.006 ^{c}	1.50 ± 0.03 ^{b}	3.903 ± 0.003 ^{a}

Total	10.3 ± 0.7 [a]	36.8 ± 1 [b]	48 ± 1 [a]

Only the majority of the phenolic composition (>0.5 mg/g of extract) was identified and quantified. Results are presented as mean ± standard deviation. tr: traces. The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test, and in each row, different letters indicate significant differences. * Indicates statistically significant differences obtained by a *t*-student test ($p < 0.05$). Calibration curve of naringenin ($y = 18{,}433x + 78{,}903$).

3.2.1. Antioxidant Activity

The results of antioxidant activity are presented in Table 2 and displayed as IC_{50} values (representing the sample concentration that provides 50% of antioxidant activity). Therefore, the peel extract showed the best result in all the performed methodologies (TBARS, DPPH, and OxHLIA). However, no significant difference was detected when comparing the peel with the mesocarp in the DPPH assay; otherwise, the pulp showed lower antioxidant activity in all the tested assays.

The hydroethanolic extracts from the mesocarp and peel of grapefruit in this study showed higher antioxidant activity compared to a study conducted by Agudelo et al., 2017 [21], where the most remarkable outcome was observed in the TBARS assay with a freeze-dried liquidized grapefruit specimen (IC_{50} of 1.65 mg/mL). This concentration was notably higher than that obtained in the present study (IC_{50} of 0.181 mg/mL). This disparity underscores our extract's capacity to inhibit lipid peroxidation by 50% at notably lower concentrations, thus highlighting its superior antioxidant activity.

The stronger antioxidant activity of the peel compared to the other extracts may be related to the high concentration of naringenin and its derivatives, particularly *O*-triglycosyl naringenin and acetyl naringenin, phenolic compounds that are found in higher amounts in the peel (Table 1) and have demonstrated antioxidant activity as previously described by other authors [20,22]. Based on the literature and the findings of this study, grapefruit peel extracts exhibit a promising antioxidant capacity with potential applications in the food industry.

Table 2. Antioxidant activity of extracts from grapefruit (*Citrus x paradisi* Macfad) pulp and waste, evaluated using TBARS, DPPH, and OxHLIA assays.

	IC_{50} (mg/mL)		
	TBARS	**DPPH**	**OxHLIA (60 min)**
Pulp	1.33 ± 0.05 [a]	12.9 ± 0.8 [a]	128 ± 7 [a]
Peel	0.181 ± 0.003 [c]	6.9 ± 0.7 [b]	26 ± 1 [c]
Mesocarp	0.8 ± 0.2 [b]	8.2 ± 0.3 [b]	43 ± 3 [b]
Trolox	N/A	N/A	21.8 ± 0.2

Results are presented as mean ± standard deviation. N/A: not applicable. The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test, and in each row, different letters indicate significant differences ($p < 0.05$).

4. Discussion

In summary, the grapefruit pulp and bio-wastes contain significant amounts of phenolic compounds, with naringenin being the predominant compound in all parts. The mesocarp has the highest total phenolic content at 48 mg/g of extract, followed by the peel at 36.8 mg/g of extract.

All three parts of the grapefruit (pulp, mesocarp, and peel) exhibited antioxidant activity in the different assays (TBARS, DPPH, and OxHLIA), with the peel standing out for its superior antioxidant capacity across all methods. The remarkable antioxidant capability demonstrated by the peel may be attributed to the presence of *O*-triglycosyl naringenin and acetyl naringenin, compounds with proven antioxidant activity, found in higher quantities in this part of the fruit.

For future research, the goal is to achieve a comprehensive identification and quantification of all phenolic compounds in grapefruit, without limiting the focus to the major ones. Additionally, there is an intention to utilize the obtained extract in the formulation of a final product, aiming to assess the inherent antioxidant capacity of the product itself.

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