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Diversity on Color, Infrared Spectra and Phenolic Profile Correlation in Citrus Fruit Peels

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INTRODUCTION & AIM

Citrus reach an annual worldwide production of more than 130 Mt, but ~40% of such production is lost as peel by-products, which content antioxidant compounds that can be extracted and revalorized as healthy promoting compounds. For the valorization of citrus by-products, it is necessary to characterize their chemical composition. To this end, the differences between **lemon, orange,** & **grapefruit peels** have been evaluated using **CIELab color, UV-Vis-IR spectroscopy, and HPLC-MS/MS.**

METHOD

Freeze-drying & grounding

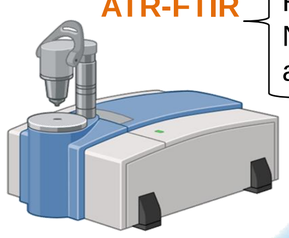
Lemon (*Citrus limon*)

Sweet orange (*Citrus sinensis*)

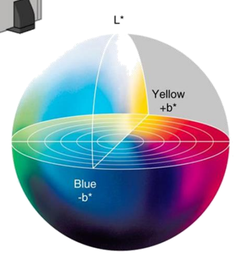
Grapefruit (*Citrus paradisi*)

ATR-FTIR

- Transmittance
- Resolution: 4 cm^{-1}
- Number of accumulations: 32

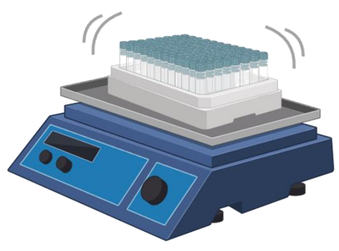


Color by CIELab system



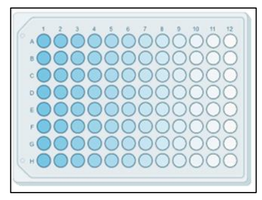
Solid-liquid extraction

0.1 g sample + 10 mL
80% CH_3OH
Shaker: 1 h, 200 rpm



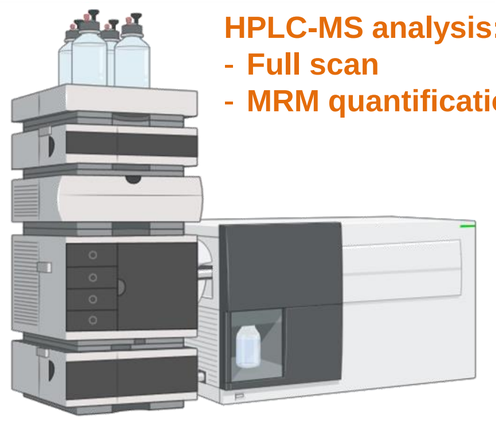
Total phenolic content (TPC) FOLIN test

- Sample volume: 19 µL
- Folin reagent: 29 µL
- Mix-Folin: 192 µL
- Wavelength: 750 nm



Incubation 1 h in darkness

HPLC-MS analysis:
- **Full scan**
- **MRM quantification**



- Stationary phase: C18 column
- Phase A: 0.1% CHOOH
- Phase B: Acetonitrile
- Elution: 0-30 min: 5 – 95% B
  30 - 40 min: 95 - 95%B
  40-45 min: 95 – 10% B

Quantification of:
- Citric acid
- Eriocitrin, Limocitrin-HMG-Glu, Acetyl-naringin, Hesperidin, Rutin, Diosmin, Poncirin, Didymin, Naringin, Narirutin

RESULTS & DISCUSSION

The differences among the FTIR spectra of lemon, orange and grapefruit peels, mainly due to the O-H and C-O stretching and O-H bending enable their discrimination as evidenced in Figure 2.

The small differences observed by ATR-FTIR spectroscopy between the citrus varieties (Figure 1) correspond to notable changes in the chemical composition of the peels, mainly in the nature and quantity of diglycosylated flavonoids, as observed in the HPLC-MS chromatograms (Figure 3).

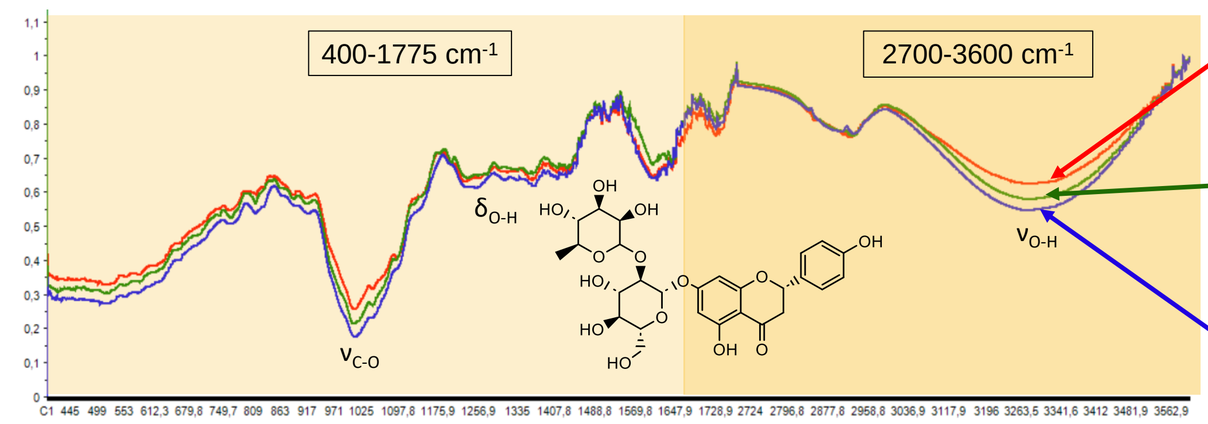


Figure 1. ATR-FTIR average spectra (N=5) of lemon, orange, and grapefruit freeze-dried peels.

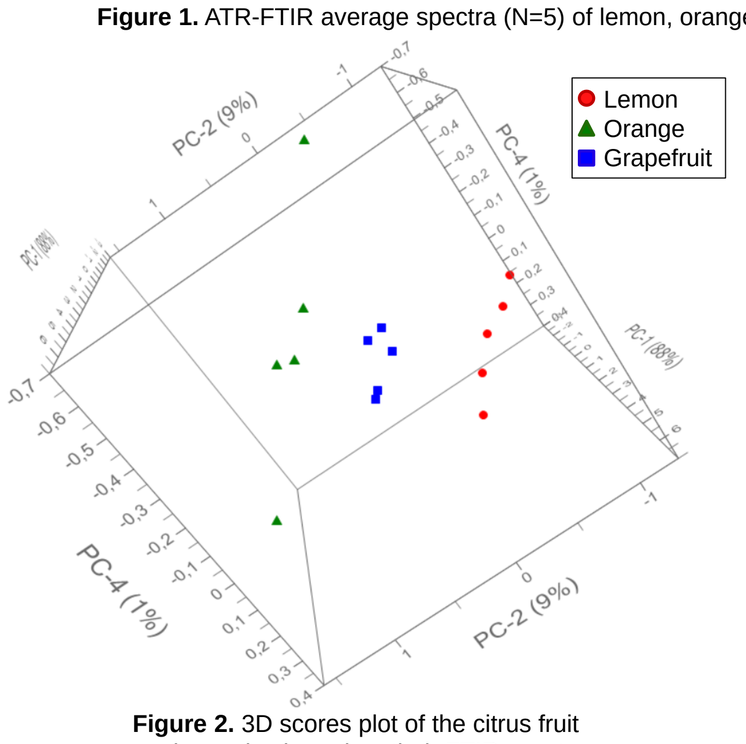


Figure 2. 3D scores plot of the citrus fruit peel samples based on their FTIR spectra.

The PCA calculated from the combination of all determined variables (L*, a* and b* of the CIELab system, TPC, selected FTIR absorbance bands, and the content of individual compounds), shows a clear physicochemical differentiation of the peel of the three varieties (Figure 4). The most influential factors in the discrimination are the b* color parameter and the content of naringin, narirutin, eriocitrin, hesperidin, didymin, and citric acid.

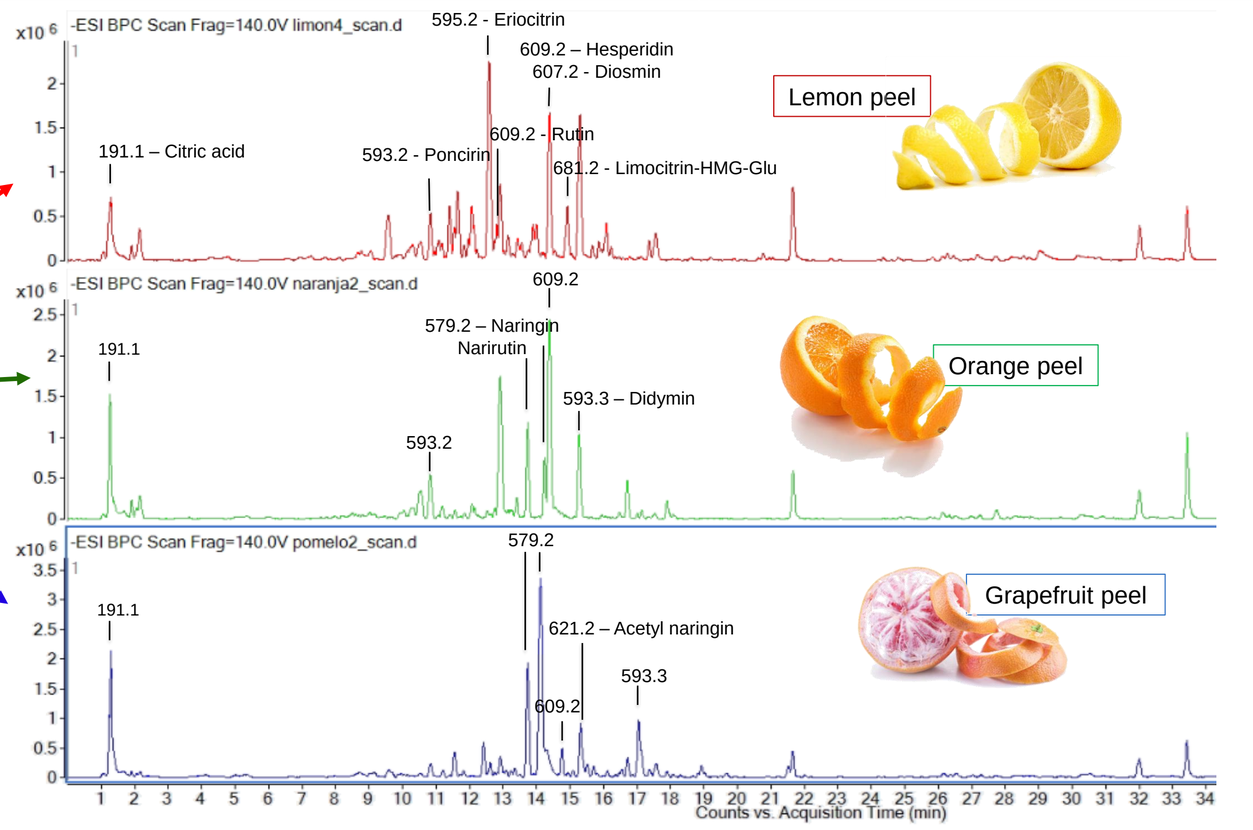


Figure 3. Full scan chromatograms of lemon, orange, and grapefruit peel by HPLC-MS (ESI-QqQ)

Table 1. Measurement of the variables with the greatest difference among citrus species peels. Concentration expressed as g/kg dw. Average ± sd (N=5). Different letters indicate significant differences (p<0.01)

Variable	Lemon	Orange	Grapefruit
b*	23 ± 2^{c}	45 ± 2^{a}	39 ± 2^{b}
[Naringin]	0.017 ± 0.005^{b}	0.81± 0.03^{b}	11.3 ± 0.4^{a}
[Narirutin]	0.25 ± 0.03^{c}	3.6 ± 0.1^{b}	13.1 ± 0.4^{a}
[Eriocitrin]	5.1 ± 0.5^{a}	0.08 ± 0.01^{b}	0.10 ± 0.01^{b}
[Hesperidin]	5.5 ± 0.1^{b}	8.9 ± 0.2^{a}	0.31 ± 0.03^{c}
[Didymin]	1.1 ± 0.1^{b}	1.15 ± 0.05^{b}	3.75 ± 0.08^{a}
[Citric acid]	7.9 ± 0.6^{a}	0.10 ± 0.01^{c}	3.7 ± 0.10^{b}

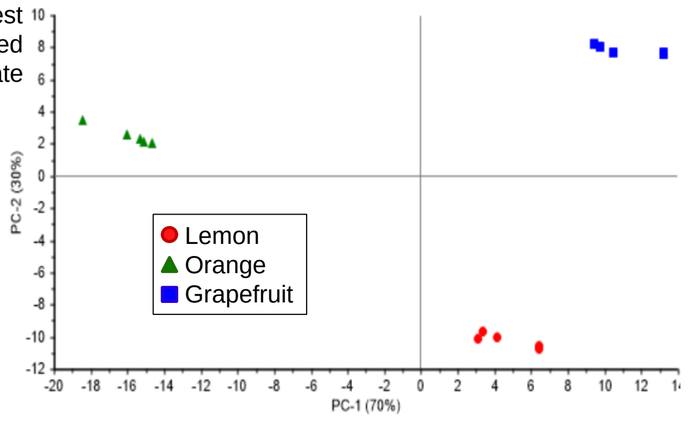


Figure 4. 2D scores plot of the citrus peels based on the combination of all variables (CIELab color, TPC, FTIR absorbance, and content of individual compounds).

ACKNOWLEDGEMENTS

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CONCLUSION

The color differences observed in the CIELab system of **lemon, orange** and **grapefruit peels** correlate with differences in **chemical composition** through their characterization by **UV-Vis and FTIR spectroscopy**, and **HPLC-MS/MS,** mainly in the content of major flavonoids, such as narirutin, naringin, eriocitrin, hesperidin, didymin and citric acid.

Lemon peel contains a greater variety of flavonoids in its chemical composition.

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