



# Primary and Secondary Metabolites Content in Eight Varieties of Blood Oranges grafted onto *Citrus macrophylla*

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## Background

Blood orange has a higher content of bioactive compounds than white orange, conferring additional benefits for human health (Morales *et al.*, 2021). In Europe the cultivation of blood oranges is increasing due to their excellent organoleptic and nutraceutical properties, which give this fruit functional status (Forner *et al.*, 2023). In Spain, its cultivation is scarce and not widespread, however, consumer interest is increasing substantially due to its numerous benefits for human health (Legua *et al.*, 2021). For this reason, it is a necessary and timely characterization and comparison between the main varieties of blood orange consumed in Spain (one of the main oranges producing and exporting countries in the world) versus the most widespread and consumed varieties internationally.

### Aim of the study

The main objective is the nutritional characterization of varieties of blood oranges, which were grown under the same environmental conditions. The content of primary metabolites was characterized by <sup>1</sup>H NMR and secondary metabolites by HPLC-ESI-DAD-MS<sup>n</sup> of eight varieties of blood orange grafted onto *Citrus macrophylla* and cultivated under the environmental conditions of southeastern Spain.

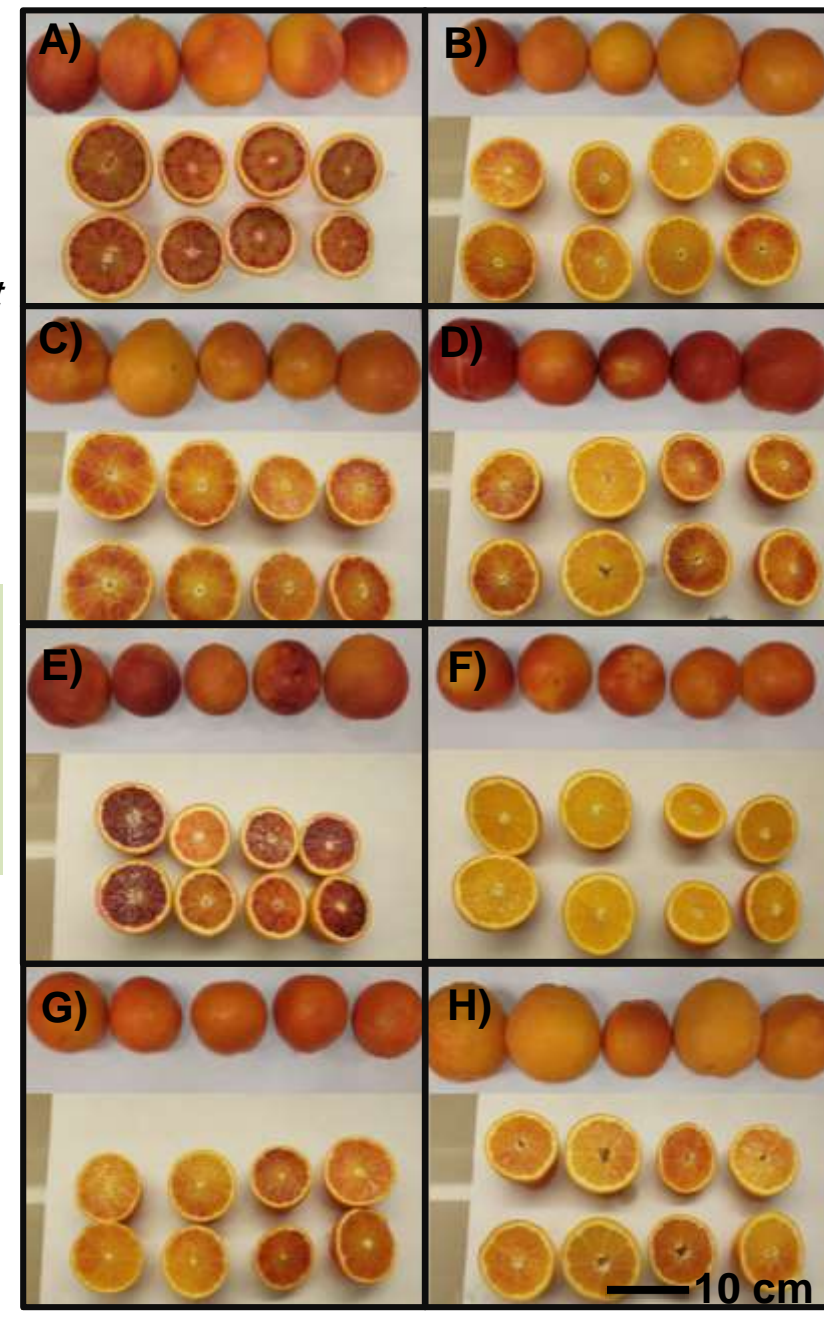
## Material & Methods

The “Sanguinelli” (A), “Tarocco Sant’Alfio” (B), “Tarocco Dalmuso” (C), “Tarocco Rosso” (D), “Tarocco Gallo” (E), “Tarocco Scirè” (F), “Tarocco Meli” (G) and “Moro” (H) (*Citrus sinensis* (L.) cv. “Tarocco” and cv. “Moro”, respectively) varieties were collected from plants grafted on *Citrus macrophylla* and grown in an experimental farm located in Orihuela, Alicante (Spain) (38.06733781,-0.98229272). This farm had an EC of 0.44 dS m<sup>-1</sup> (20°C), pH of 7.25, temperature of 12°C and relative humidity (RH) of 58% at harvest time.

In Spain, due to environmental conditions, the stage of commercial consumption of blood oranges runs from January to March. Therefore, the samples considered in this study were harvested in January 2023. In this way, the fruit was harvested manually at the physiological maturity stage, with the aim of ensuring similarity to commercial standards, it was immediately transported to the laboratory starting its analysis that same day.

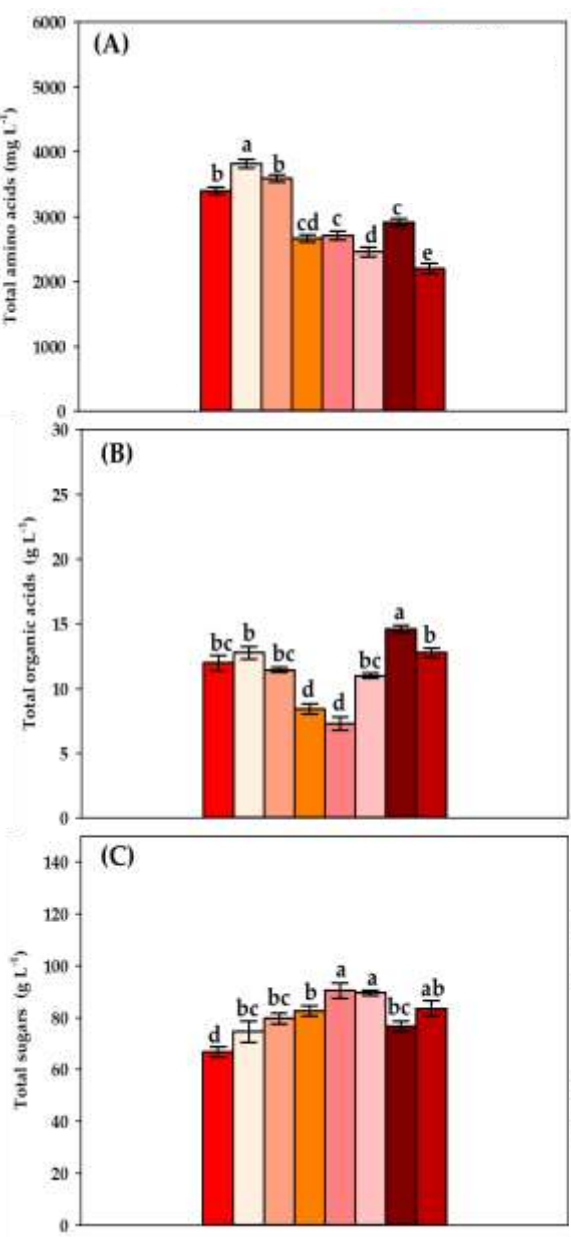


Then, for each variety of blood orange, the juice of 25 pieces of fruit was obtained. Previously, the 25 fruit pieces were divided into six replicas (*n* = 6) for metabolomics analysis (15 mL/replica), which were stored at - 80 °C. Data are the mean ± SE. Different letters indicate statistically significant differences (ANOVA, HSD Tukey test; *p* < 0.05).



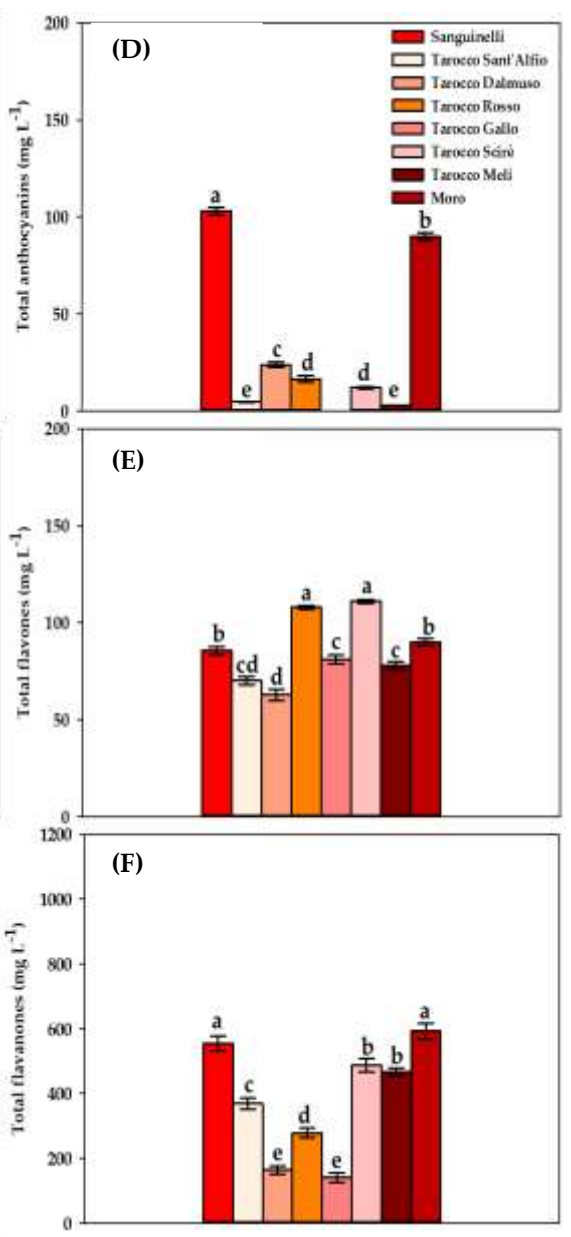
## Results 1: Primary metabolites

The amino acids present in the juice has proven that amino acids are responsible for the quality of the taste of food (Susan *et al.*, 1981). In this study, the total amount of essential amino acids varied from 3821.61 to 2256.88 mg L<sup>-1</sup> in “Tarocco Sant’Alfio” and “Moro”, respectively (Figure A). The overall quality of the juice, organic acids and sugars are considered one of the most important and determining factors (Butelli *et al.*, 2021). In general, statistically significant differences (*p* < 0.05) in total organic acid and sugar content were found between the varieties (Figure B and C). Our results were similar to those obtained Cebadera-Miranda *et al.*, (2019) and Sicari *et al.*, (2016).



## Results 2: Secondary Metabolites

Foods that contain it among their composition high levels of phenolic compounds obtain a higher added value on the market (Habibi *et al.*, 2022). Total anthocyanin content was higher in “Sanguinelli” and “Moro” than in “Tarocco Rosso” (Figure D), as previously reported by Butelli *et al.*, (2021). “Tarocco Gallo” and “Tarocco Scirè” obtained the highest total concentrations of flavones and “Sanguinelli” and “Moro” the highest total concentrations of flavanones (Figure E and F). Our results were similar to those obtained by Cebadera-Miranda *et al.*, (2019) and Morales *et al.*, (2021).



## Conclusions and perspectives

1. The highest content of amino acids, organic acids and sugars were found in “Tarocco Sant’Alfio”, and “Tarocco Dalmuso”, “Tarocco Meli” and “Moro”, and “Tarocco Meli” and “Tarocco Scirè”, respectively, being the most important varieties in terms of nutritional content.
2. “Moro” and “Sanguinelli” juice obtained the highest content of phenolic compounds, being especially interesting for consumers who demand fruits with a high content of bioactive compounds. In fact, “Moro” juice had the highest levels of total flavones and flavanones, and “Sanguinelli” in total anthocyanins.
3. The knowledge generated in this work can be used for the agri-food industry by identifying some of the most interesting varieties of blood oranges from the nutritional point of view.

