

DEVELOPMENT OF A CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ANALYSIS OF EIGHT CAROTENOIDS AND TWO VITAMINS IN FOOD SAMPLES

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Background

The health promoting properties of carotenoids, namely against cancer and cardiovascular diseases, besides their provitamin A activity, have enhanced the scientific interest on these compounds. In the view of the great number of compounds of this family, the need of developing methods for the simultaneous analysis of the most common compounds of this group is unquestionably required.

Fruits in general and exotic fruits in particular are known to be good sources of carotenoids.

Therefore in the present work, mango (*Mangifera indica* L.) and papaya (*Carica papaya* L.) (Figure 1) were the selected matrices to develop an extraction procedure and a chromatographic method to determine simultaneously eight carotenoids and two vitamins (A and E).

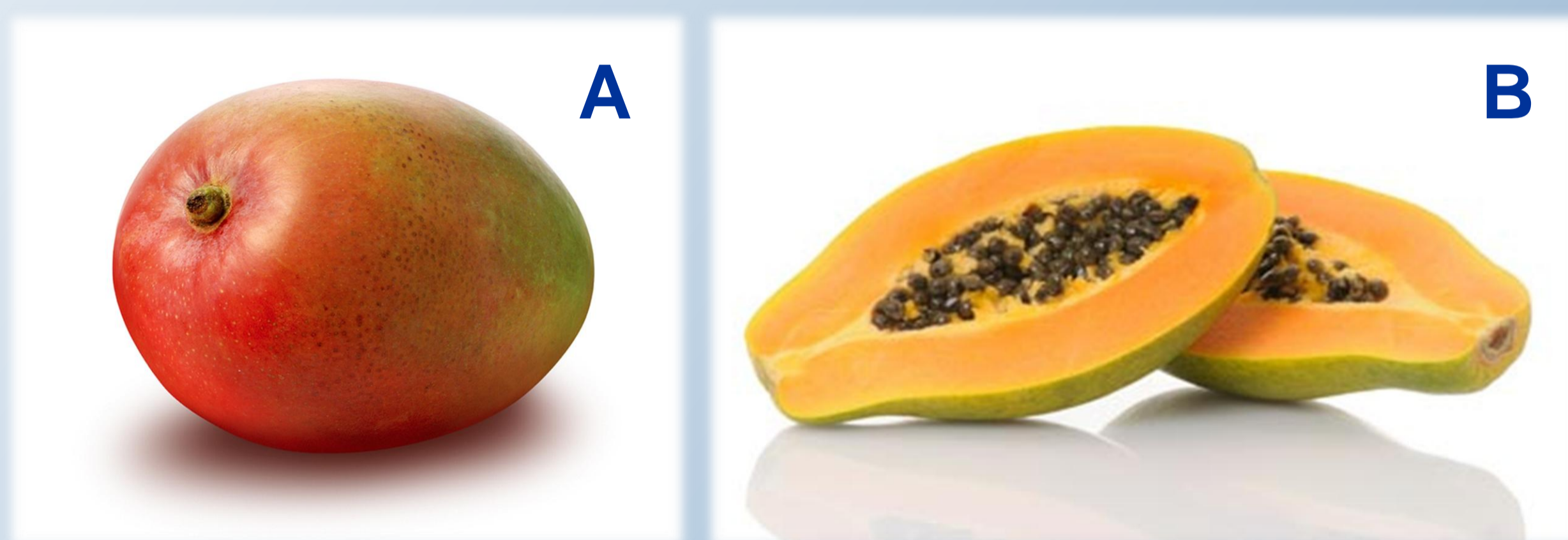


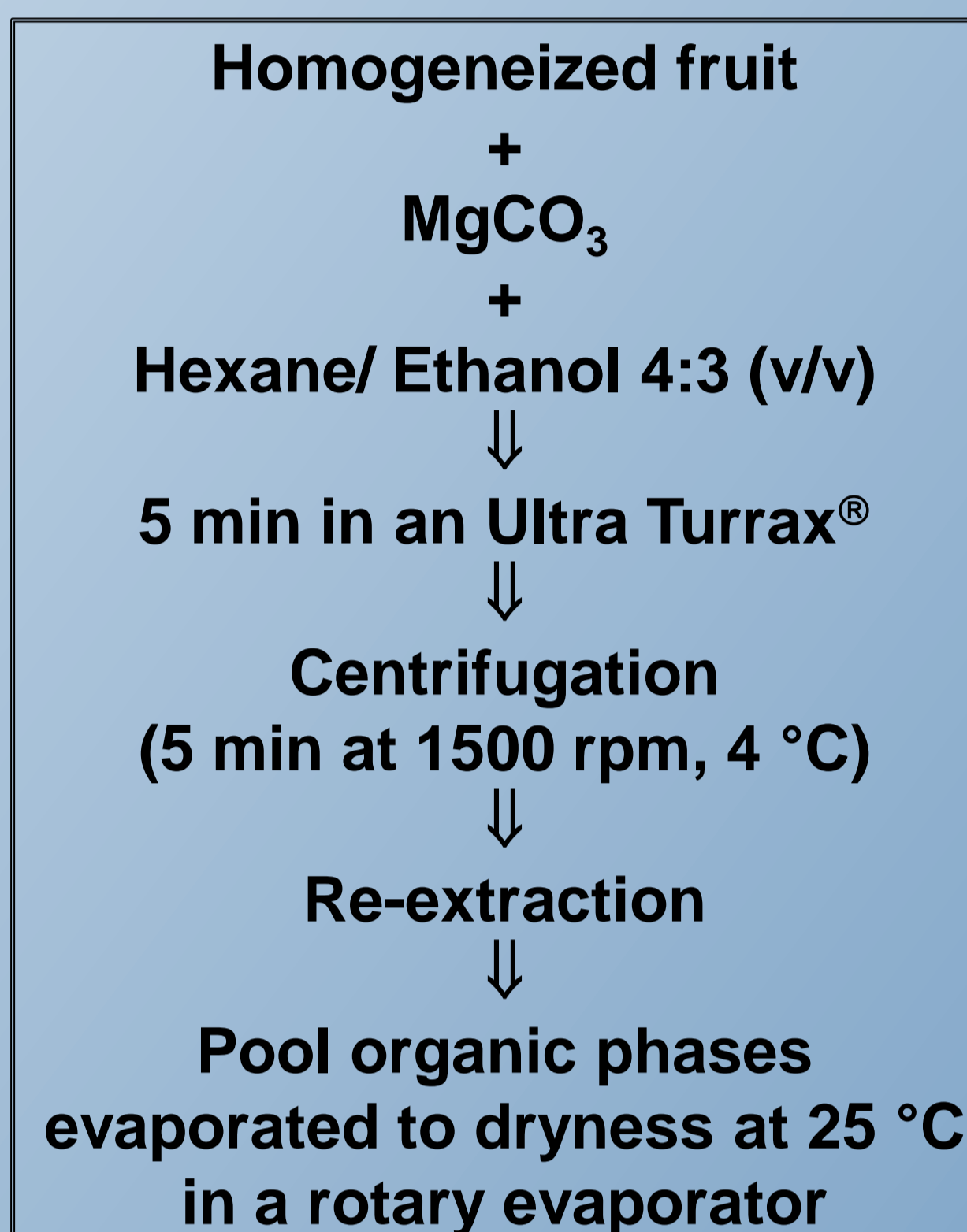
Figure 1. Selected matrices for carotenoids analyses. A – Mango (*Mangifera indica* L.); B - Papaya (*Carica papaya* L.)

The selected carotenoids were α -carotene, β -carotene, β -cryptoxanthin, lutein, lycopene, neoxanthin, violaxanthin and zeaxanthin. The aim is to further apply this method to determine carotenoids in traditional foods from Black Sea Area Countries in the frame of the European Project BaSeFood (Sustainable exploitation of bioactive components from the Black Sea Area traditional foods).

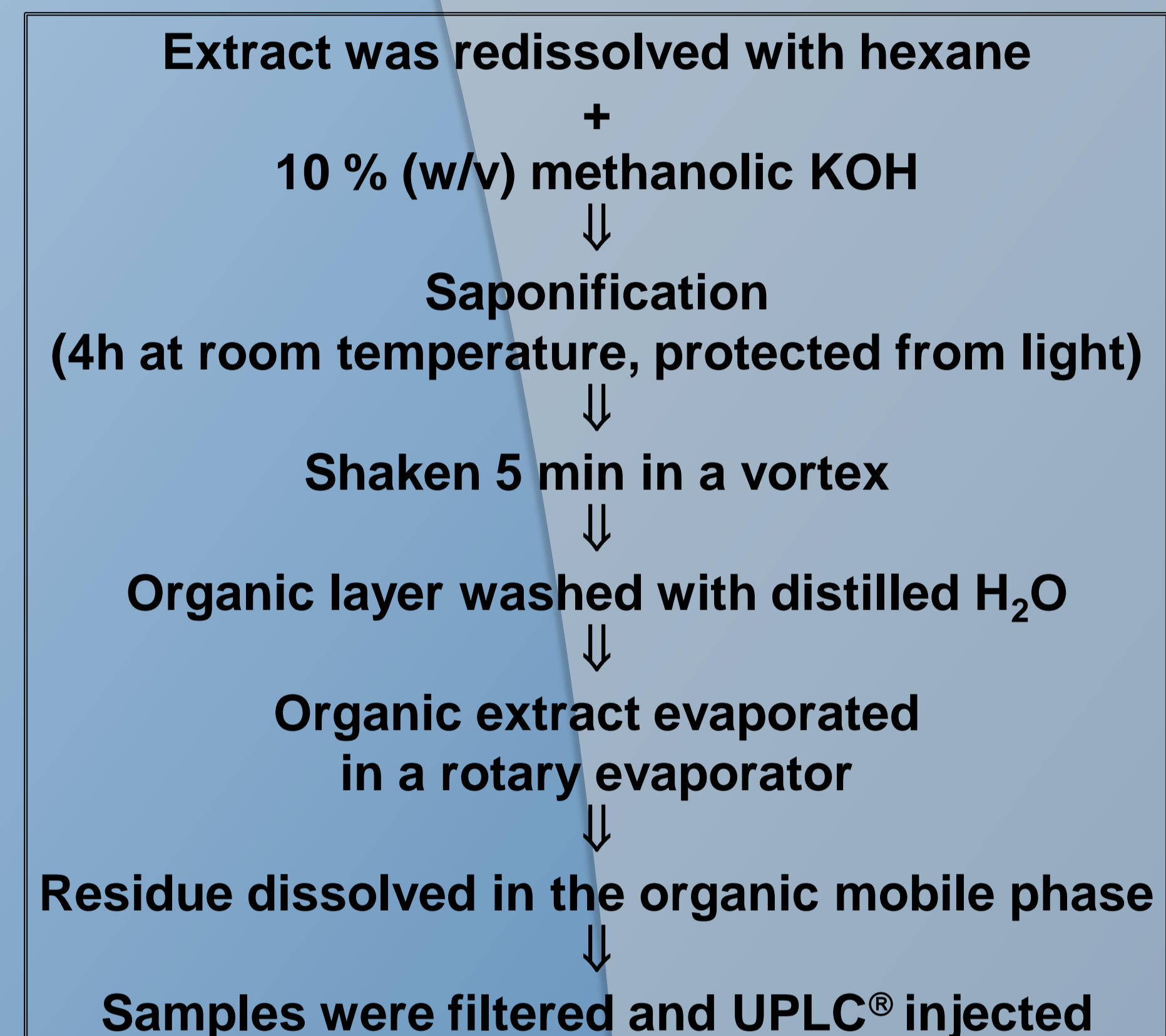
Materials and methods

Preliminary assays were performed in order to establish optimal extraction conditions. Two extraction procedures were optimised: with and without saponification with KOH.

Extraction of carotenoids



Saponification



Chromatographic conditions

Table 1- Conditions of the UPLC[®] method.

Instrument	ACQUITY™, Ultra Performance Liquid Chromatograph, (Waters) with an auto-sampler binary solvent manager and a PDA detector
Pre-Column	ACQUITY BEH, C18 VanGuard Pre-column (Waters)
Column	ACQUITY UPLC [®] BEH C18 reverse phase column (Waters)
Column temperature	20 °C
Injection volume	10 μ L
Mobile phase	(A) ultrapure water; (B) acetonitrile/ methanol (with 0.05 M ammonium acetate)/ dichloromethane (75:20:5)
Runtime	22 min
λ	292 nm (α -tocopherol); 325 nm (retinol); 450 nm (carotenoids)

Results

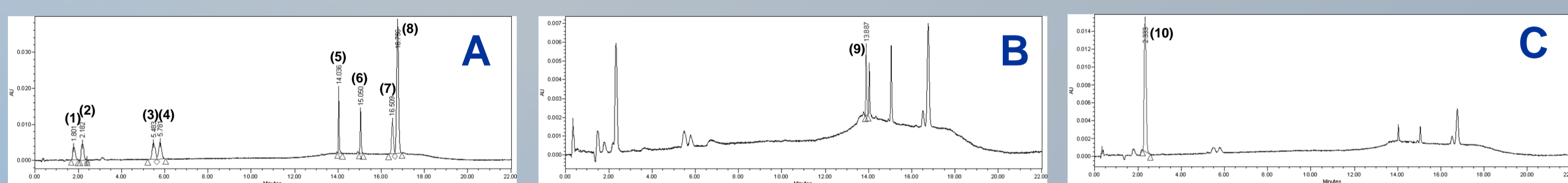


Figure 2: Chromatogram of a standard mixture of carotenoids and two vitamins. A: at 450 nm (1) neoxanthin; (2) all-*trans*-violaxanthin, (3) zeaxanthin, (4) lutein, (5) β -cryptoxanthin; (6) lycopene. (7) α -carotene and (8) β -carotene; B- (9) α -tocopherol at 295 nm; C- (10) retinol at 325 nm.

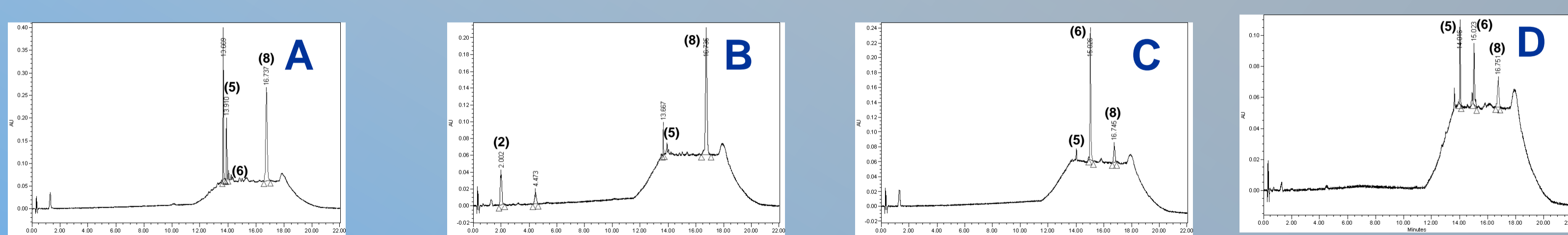


Figure 3: Chromatograms of samples. (A) Mango without saponification; (B) Mango with saponification; (C) Papaya without saponification; (D) Papaya with saponification.

Acknowledgments

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Discussion

Mango (*Mangifera indica* L.)

- Major carotenoid - β -carotene
- Without saponification – it was better to quantify α -tocopherol, β -cryptoxanthin, lycopene and β -carotene
- With saponification - it was possible to identify *trans*-violaxanthin

Papaya (*Carica papaya* L.)

- Major carotenoid - Lycopene
- Without saponification – procedure more suitable to quantify lycopene and β -carotene.
- With saponification – it was better to quantify β -cryptoxanthin

Conclusion

Depending on the fruit and on the carotenoid studied, saponification might be needed. Therefore it is recommended to use both extraction procedures. The chromatographic method allows obtaining excellent separation of the ten compounds in just 22 min.