A comparison of cuticular wax composition in fruit of three olive cultivars

Clara Diarte [1], Agustí Romero [2], Jordi Graell [1], Markus Riederer [3], Isabel Lara [1, ✍ (lara@quimica.udl.cat)]

[1] Universitat de Lleida, Unitat de Postcollita-XaRTA, AGROTÈCNIO, Lleida, Spain
[2] IRTA-Mas de Bover, Programme on Oliviculture, Ealiotechnic and Nuts, Constantí, Spain
[3] Universität Würzburg, Julius-von-Sachs Institut für Biowissenschaften und Botanischer Garten, Würzburg, Germany

INTRODUCTION AND EXPERIMENTAL

Olive (*Olea europaea* L.) growing has outstanding economic relevance in Spain, the country being the main olive oil producer and exporter in the world. In spite of the relevance of fruit skin properties for overall quality, water loss and susceptibility to mechanical damage, rots and infestations, very few published studies have addressed the cuticle composition of the intact olive fruit.

Fruit samples of the cultivars 'Arbequina', 'Argudell' and 'Sevillenca' were harvested at the black stage from the experimental orchards at the IRTA-Mas de Bover research center located in Constantí (41°09'N; 1°12'E), Spain. Water permeance of whole fruit was determined as in Huang et al. (2017). Cuticular membranes were isolated enzymatically from skin disks excised from the fruit (Fig. 1), and cuticular waxes extracted in chloroform, analysed (GC-FID) and identified (GC-MS). All procedures were adapted from those described elsewhere (Belge et al., 2014). In order to visualize pores, cracks or defects on the fruit surface, samples at the green stage were submitted to the toluidine blue (TB) test (Tanaka et al., 2004).

Fig. 1. Cuticular membranes isolated from olive fruit picked at the black stage.

RESULTS AND CONCLUSIONS

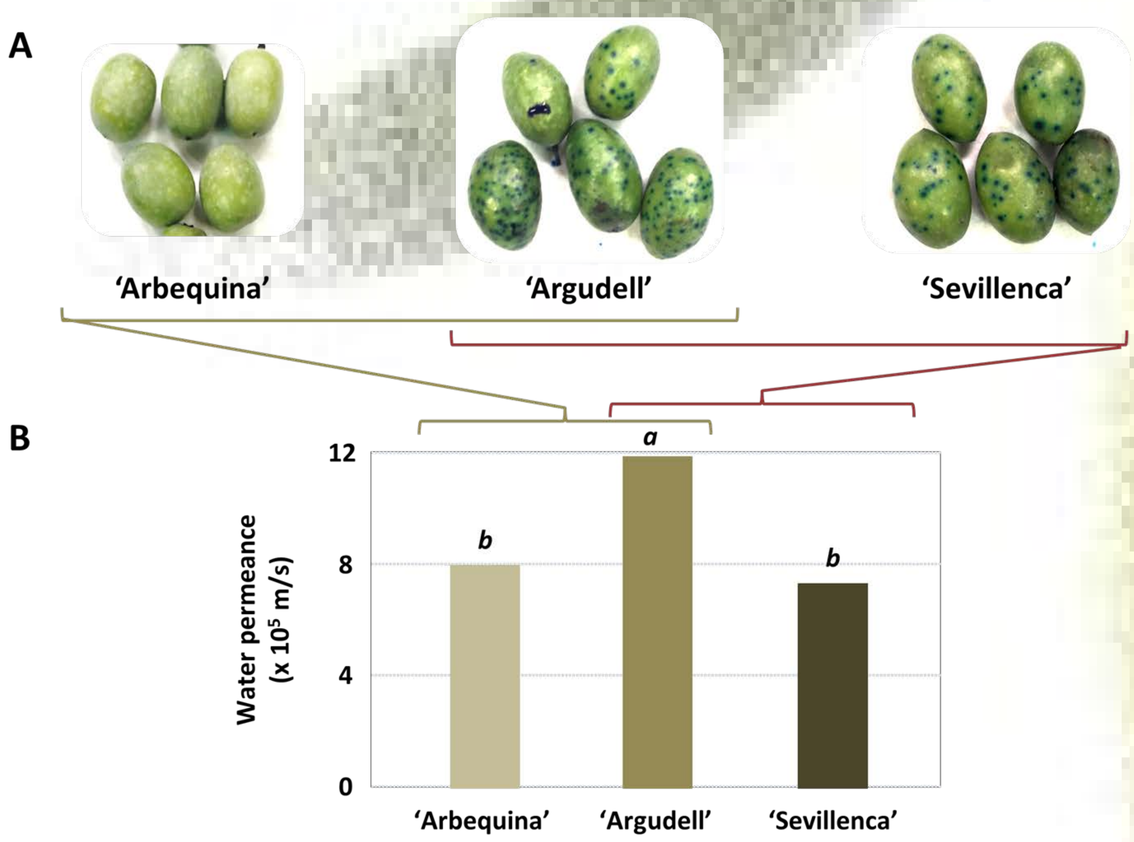

Fig. 2. **(A) Toluidine blue (TB) staining and (B) water permeance of 'Arbequina', 'Argudell' and 'Sevillenca' olive fruit picked at the black stage.** Permeance values correspond to means of 12 replicates. Different letters on bars represent significant differences at $P \leq 0.05$.

Table 1. **Compositional properties of cuticular membranes isolated from 'Arbequina', 'Argudell' and 'Sevillenca' olive fruit picked at the black stage.**

	Cultivar		
Parameter	**'Arbequina'**	**'Argudell'**	**'Sevillenca'**
Fruit surface (cm^2) [1]	6.5 b	9.7 a	10.4 a
Cuticle yield (mg cm^{-2}) [2]	2.5 a	2.7 a	2.5 a
Wax content (mg cm^{-2})	0.46 b	0.46 b	0.54 a
Wax (% over total cuticle)	18.4 b	17.0 b	22.5 a
ACL acyclic waxes [3]	24.6 a	24.0 a	24.4 a
Ratio acyclic to cyclic waxes	0.26	0.21	0.35
Ratio wax to cutin	0.49	0.45	0.64

Different letters within a row represent significant differences at $P \leq 0.05$. Wax content data are means of three replicates.
[1] Fruit surface corresponds to the average of 12 individual fruit. Surface area was calculated assuming a spherical shape, with the radius taken as the mean of the polar and the equatorial radii of the fruit.
[2] Cuticle was isolated from roughly 100 cm^2 of fruit skin. Yield values are the mean of two replicates.
[3] Weighed average chain length of acyclic wax compounds.

Table 2. **Composition (% over total waxes) of cuticular waxes in 'Arbequina', 'Argudell' and 'Sevillenca' olive fruit picked at the black stage.**

	Cultivar		
Compound type	**'Arbequina'**	**'Argudell'**	**'Sevillenca'**
Triterpenes (%)	67.6	66.4	57.6
Fatty acids (%)	9.6	8.1	15.1
Alcohols (%)	5.8	3.4	3.0
n-Alkanes (%)	2.3	3.0	2.4
Sterols (%)	0.8	1.6	1.5
Unidentified (%)	13.9	17.5	20.4

Cuticular waxes were recovered in chloroform extracts. Values represent means of three replicates.

The TB test revealed SURFACE DIFFERENCES among the three cultivars assessed (Fig. 2A). 'Argudell' and 'Sevillenca' showed discontinuities on fruit surface, while 'Arbequina' did not. However, while 'Argudell' fruit displayed the highest WATER PERMEANCE values, consistent with the presence of surface pores, those for 'Arbequina' and 'Sevillenca' fruit were similar despite the differences revealed by the TB test (Fig. 2B).

Cuticular waxes were extracted and analysed. TOTAL WAX CONTENT was similar in 'Arbequina' and 'Argudell' in spite of significant differences in water permeance (Table 1). In contrast, wax coverage was significantly higher in 'Sevillenca' fruit, even though water permeance was similar to that in 'Arbequina' samples (Fig. 2B).

Detailed compositional analysis of cuticular waxes showed that 'Sevillenca' fruit displayed the highest RATIO OF ACYCLIC TO CYCLIC compounds in comparison with 'Arbequina' and 'Argudell'. Wax analyses showed that these ratio differences arose from lower triterpene and higher fatty acid percentages in 'Sevillenca' cuticles as compared to the other two cultivars (Table 2). The weighted AVERAGE CHAIN LENGTH (ACL) of acyclic wax components was similar for all three cultivars, and thus apparently not related to differences in water permeance in these samples.

Data suggest that water permeance of olive fruit may be modulated by different cuticle-related factors, none of which appear to have a major role on this trait by itself. These would include:

- **Presence of surface discontinuities**
- **Total wax coverage**
- **Ratio of acyclic to cyclic waxes, the former potentially providing more efficient barriers against water loss**
- **Wax to cutin ratio**

REFERENCES

- Belge B, Llovera M, Comabella E, Graell J, Lara I. 2014. Fruit cuticle composition of a melting and a nonmelting peach cultivar. J. Agric. Food Chem. 62*:* 3488-3495 (DOI: 10.1021/jf5003528).
- Huang H, Burghardt M, Schuster AC, Leide J, Lara I, Riederer M. 2017. Chemical composition and water permeability of fruit and leaf cuticles of *Olea europaea* L. J. Agric. Food Chem. 65:8790-8797 (DOI: 10.1021/acs.jafc.7b03049).
- Tanaka T, Tanaka H, Machida C, Watanabe M, Machida Y. 2004. A new method for rapid visualization of defects in leaf cuticle reveals five intrinsic patterns of surface defects in *Arabidopsis*. Plant J. 37:139-146 (DOI:10.1046/j.1365-313X.2003.01946.x).

ACKNOWLEDGEMENTS

This work was funded through grants AGL2015-64235-R (MINECO/FEDER, Spain), and TR265 (Universitat de Lleida, Spain). C. Diarte is the recipient of a predoctoral scholarship granted by the Universitat de Lleida.