

Chania, 07/07/2025



Molecular (DNA) analysis of olive leaf samples and olive oil to identify their genetic relation.

BioCoS received 3 samples from Oreino EVOO:

i) Batch of Leaf Samples 1, ii) Batch of Leaf Samples 2 and iii) Olive oil bottle 100mL

Analysis Methodology

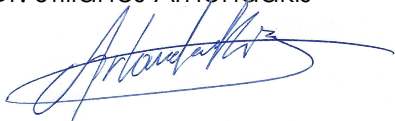
The total DNA was isolated separately for each of the two batches (leaf samples) using the NucleoSpin Food (Machery-Nagel), employing a pooling method; approximately 10 leaves were pooled together per batch. Regarding the olive oil the DNA was isolated utilizing a different extraction method dedicated to fatty matrices. For all the samples (olive leaves and olive oil) a real-time PCR analysis coupled with High Resolution Melting profiling using BioCoS's proprietary genetic markers was carried out.

Results

Regarding the two batches (leaf samples), it is evident that they share the same genetic profile, with negligible differences in the melting temperature that can be attributed to the expected technical variability. In order to identify their genetic identity, BioCoS utilized reference samples of cv. Koroneiki and cv. Mastoidis (Tsunati). From the HRM analysis using BioCoS's proprietary SNP marker Olea-1, it is clear that the two batches display an intermediate genetic profile of cv. Koroneiki and cv. Mastoidis (Figure 1). This can be related to the varietal composition of the orchard that possibly has both varieties, supported further by the sample collection form indicating the presence of a polinator variety, or it is due to potential inoculation of some of the sampled trees. The aforementioned claim is also corroborated by the morphological variability of the leaves, represented in the two batches. Regarding the olive oil, its genetic profile was assessed utilizing a second BioCoS's proprietary SNP, Olea-2, and as depicted in Figure 2, it shares high genetic similarity with the leaves, however it cannot be considered identical, probably due to the different percentage of its varietal content.

On behalf of BioCoS PC

Dr. Stilianos Arhondakis



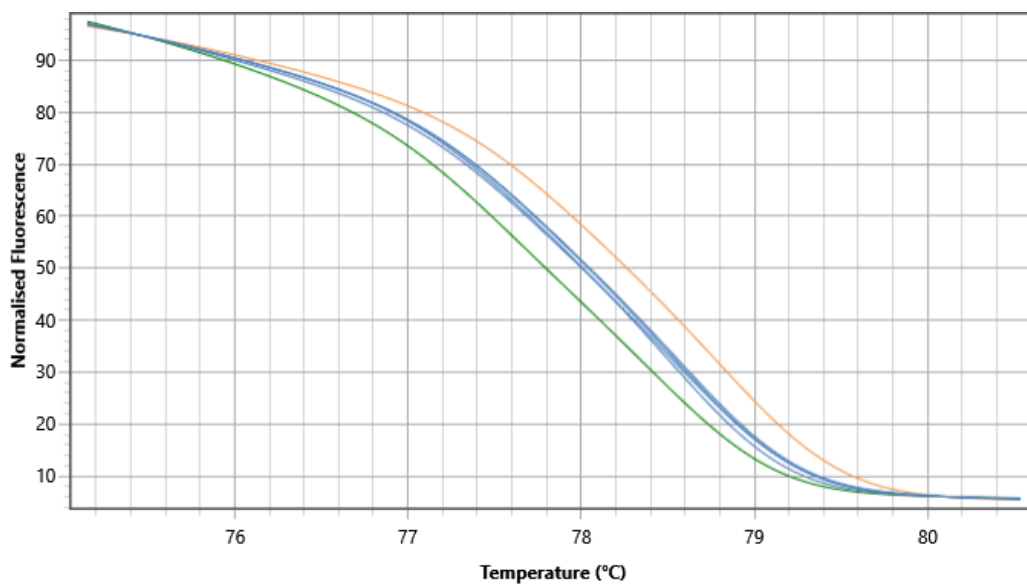


Figure 1: HRM Curve Analysis using Olea-1 SNP marker. Line Colours: Green; reference sample of cv. Mastoidis, Orange; reference sample of cv. Koroneiki, Blue; Batches of olive leaf samples.

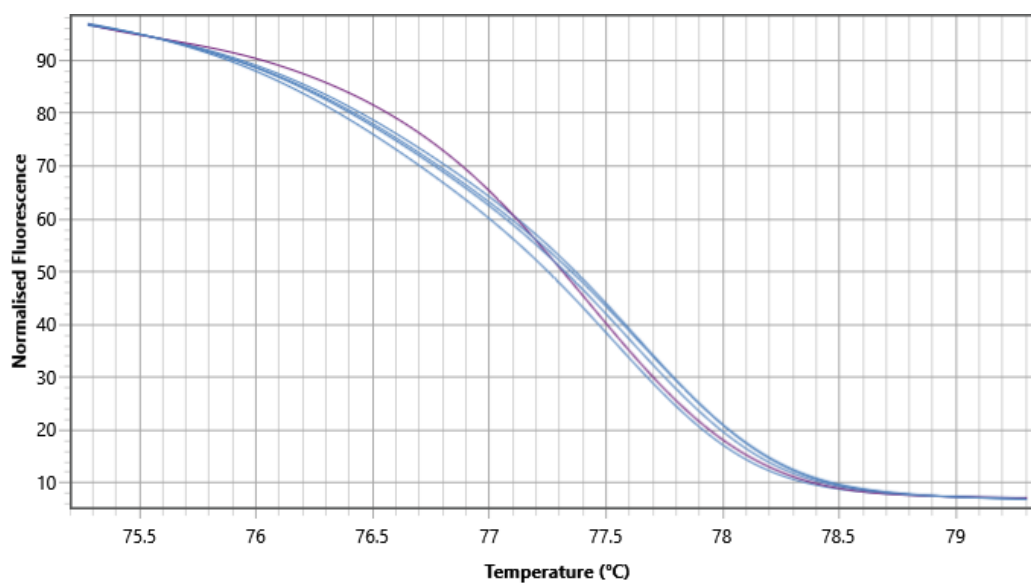


Figure 2: HRM Curve Analysis using Olea-2 SNP marker. Line Colours: Blue; Batches of olive leaf samples, Purple; Olive oil sample.

Note: This method of analysis has not yet been accredited and therefore the results cannot be used in the case of legal disputes. Results are limited to the samples provided by the company and BioCoS does not hold any responsibility to other lot numbers. The results are confidential between the involved parties.