Paper ID:

Development of new molecular tools to assess argan oil authenticity: detection of olive oil as a potential adulterant

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Abstract

Argan oil is a traditional product obtained from the argan tree (*Argania spinosa* L.), which is endemic only to Morocco. Both cosmetic and food grade argan oil are commercialized worldwide, attaining very high prices in the international market. For that reason, argan oil is very prone to be adulterated, in particular with cheaper vegetable oils. Therefore, it is important to develop methodologies that can be used in control and inspection programs in order to guarantee argan oil authenticity and quality. In particular, there is the need for methodologies that allow the accurate identification of vegetable oils illegally added to argan oil. The present work aims at developing novel approaches based on DNA markers to detect the presence of adulterants, using olive oil as case study. A novel olive-specific PCR assay was developed, enabling the clear detection of 1% (w/w) of olive oil in argan oil. Subsequently, a real-time PCR assay with EvaGreen dye was developed for quantitative analysis in a dynamic range of 1-50%, with acceptable correlation coefficient and PCR efficiency considering the type of food matrix. Both, qualitative and quantitative PCR assays can provide simple, fast and high-throughput tools to detect the presence of adulterant oils in argan oil.

Materials and methods

Sample preparation



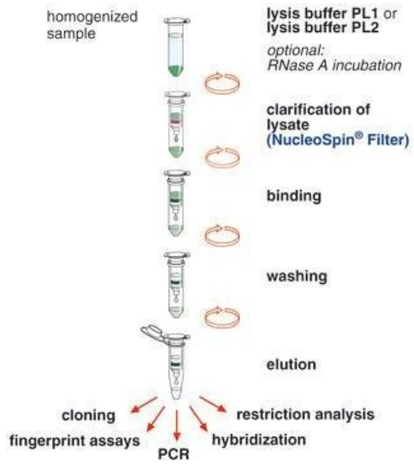


Samples of authentic argan oil were acquired from a producing cooperative in Morocco, while olive oil samples were obtained from local stores in Portugal.

Binary model mixtures were prepared with the addition of known amounts of olive oil in argan oil in the proportions of 50, 25, 5, 1% (w/w), followed by a pre-concentration by centrifugation.

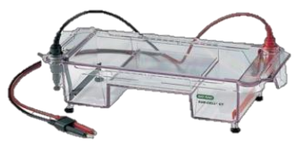
DNA extraction

- ✓ Nucleospin Plant kit, protocol B
- ✓ DNA concentration and purity by UV-spectrophotometry.



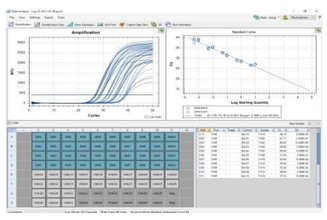
Qualitative PCR

- ✓ In silico analysis to design of *Olea europaea* L. and *A. spinosa* L. specific primers targeting the chloroplastidial matK gene and the ITS2 region, respectively.
- ✓ Species-specific PCR assays to produce amplicons of 109 and 117 bp for olive and argan, respectively.







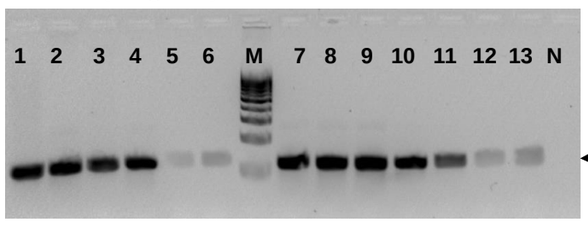


Real-time PCR

- ✓ Normalised quantitative method ΔCq with EvaGreen dye.
- ✓ Quantification of olive oil in argan oil.

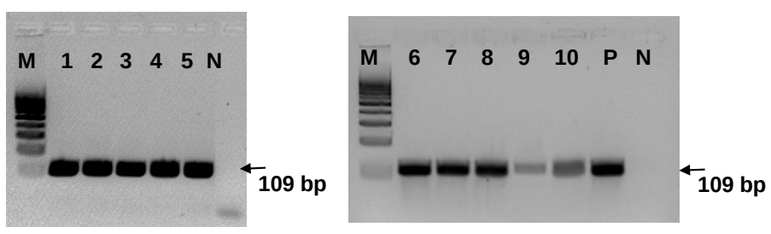
Results and discussion

- ➢ An argan-specific PCR assay was successfully developed with sensitivity down to 0.01 pg of DNA (Fig. 1).
- ➢ An olive-specific PCR assay was successfully developed with sensitivity down to 0.01 pg of DNA and 1% of olive oil in argan oil (Fig. 2).



Argan DNA:
1 – Leaves
2 – Seeds
3 – Oil (food) 50 mL
4 – Oil (cosmetic) 50 mL
5 – Oil (food) 1 mL
6 – Oil (cosmetic) 1 mL
M – DNA ladder
7 – 10 ng
8 – 1 ng
9 – 0.1 ng
10 – 0.01 ng
11 – 1 pg
12 – 0.1 pg
13 – 0.01 pg
N – negative control

Fig. 1. Agarose gel electrophoresis of qualitative PCR products targeting the ITS2 region of *A. spinosa*.



Olive DNA:
1 – 10 ng
2 – 1 ng
3 – 0.1 ng
4 – 0.01 ng
5 – 1 pg
N – negative control
Olive oil in argan
6 – 50%
7 – 25%
8 – 10%
9 – 5%
10 – 1%
P – positive control

Fig. 2. Agarose gel electrophoresis of qualitative PCR products targeting the matK gene of *O. europaea*.

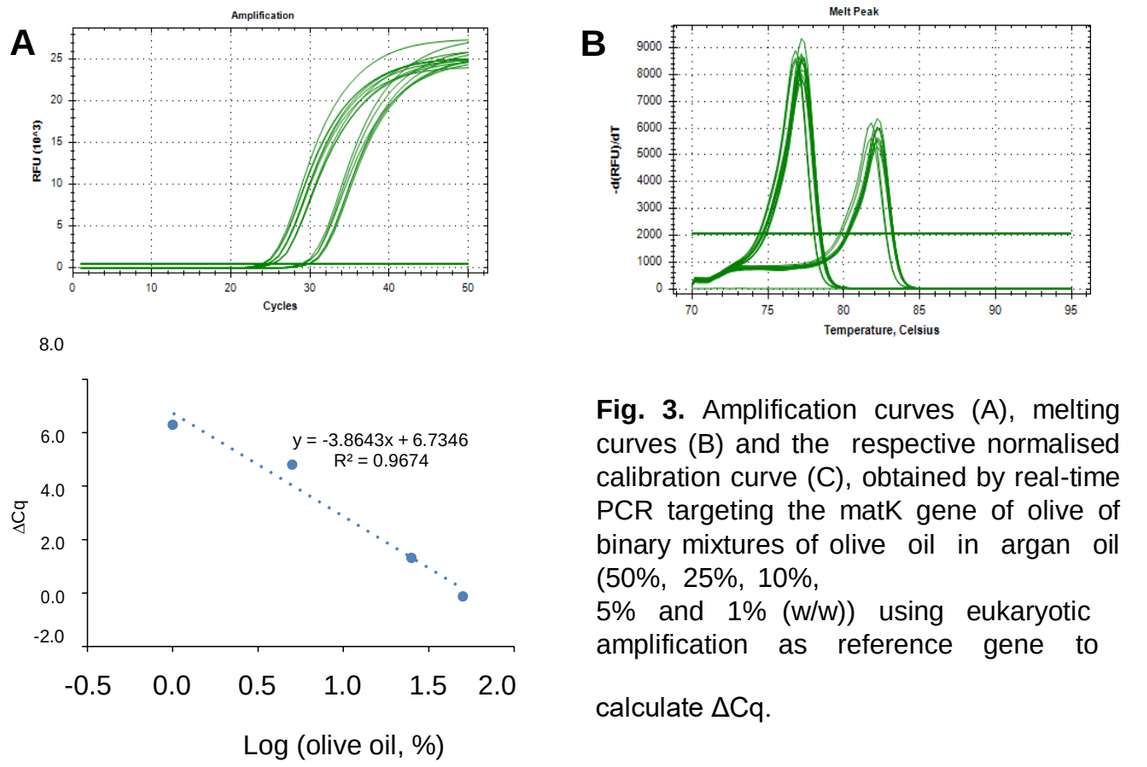


Fig. 3. Amplification curves (A), melting curves (B) and the respective normalised calibration curve (C), obtained by real-time PCR targeting the matK gene of olive of binary mixtures of olive oil in argan oil (50%, 25%, 10%, 5% and 1% (w/w)) using eukaryotic amplification as reference gene to calculate ΔCq.

- ➢ A normalised real-time PCR assay was successfully developed targeting the matK gene of olive and applied to binary oil mixtures (Fig. 3).
- ➢ The assay confirmed the limit of detection of 1% of olive oil, in a dynamic range of 1-50%, with acceptable correlation coefficient and PCR efficiency (81.1%), considering the type of food matrix (Fig. 3C).

conclusion:

It's can be concluded, that it is possible to extract trace amount of amplifiable DNA from vegetable oils a simple protocol based mostly on the use of the Nucleospin Plant kit
Based on the results obtained in this work, the new PCR method is proven to be a straightforward and effective tool for the detection of olive oil in argan oil.

Acknowledgments

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