# OPTIMIZATION OF A DNA PURIFICATION PROTOCOL BY MICROSCALE SOLID PHASE EXTRACTION (µSPE) AND MICROFLUIDICS FOR FOOD PRODUCTS ON A WASHABLE AND REUSABLE DEVICE

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## Introduction

Olive oil is a product of added value and one of the most at risk of food fraud, therefore efficient analytical tools for food authenticity are especially important in this sector. DNA is an interesting target analyte that could contribute to the detection of fraud in olive oil. DNA extraction is critical step in the analytical procedure, due to the low DNA content and its high degree of fragmentation, in consequence, the main objective of this work was to improve DNA extraction and purification.

Miniaturized devices for nucleic acid analysis present several advantages when compared with traditional methods, such as the smaller volumes required, allowing the use of less quantities of reagents and reducing the costs as well as improving the performance of the system by allowing faster and more sensitive analysis[1]. DNA extraction and purification are critical steps for the success of DNA analysis to obtain sufficiently concentrated DNA with high quality, therefore several microfluidic devices designed for this purpose have been developed[1,2,3,4]. Microscale solid phased extraction (µSPE) is one of the most attractive methods to be adapted to microchips, having the advantage of putting in contact a higher volume of initial binding material with the solid phase and recover the DNA in a lower volume during the elution phase. This feature allows to concentrate the DNA when minute amounts of these molecules are present in the sample, such as olive oil.

At the International Iberian Nanotechnology Laboratory (INL) a washable and reusable DNA purification system, designed to contain a commercial disposable silica membrane, was previously fabricated and optimized using a standard DNA solution[5]. In the present work, the protocol was adapted for olive oil samples and a pre-treatment study was performed for these complex food samples. The efficiency of the DNA purification approach was determined by estimating the DNA yield, and purity was also evaluated.

## Materials & Methods

### INL DEVICE


### FOOD SAMPLES

Extra Virgin Olive Oil (EVOO)

Olive Oil Composed of refined and virgin olive oils (OOC)

### PROCEDURE

1st Pre-treatment study of EVOO and OOC with NucleoSpin®

2nd DNA purification of EVOO with INL device & NucleoSpin®

Spiked with 100µg/ml of low molecular weight salmon sperm DNA (Sigma®)

### PRE-TREATMENT STUDY

- **Method I**: NucleoSpin® Food (Macherey-Nagel) used following manufacturer´s instructions
- **Method II**: Starting amount of 5ml. Lysis buffer was proportionally added and 1 mL of the lysis aqueous phase was used in further steps.
- **Method III**: Based on *Costa et al.*[6] and *Dooley et al.*[7]. Initial amount:50ml. First concentration step: 257300xg for 1h. Second concentration: 21380xg for 1h in 2 ml tubes.
- **Method IV**: With a starting amount of 5 ml divided in three tubes, method III was followed from the second concentration step: 21380xg for 1h

### DNA PURIFICATION PROTOCOL

PRE-TREATMENT (Concentration + Lysis) › BINDING › WASHING › ELUTION (NucleoSpin® & INL device) › QUANTIFICATION (•UV-VIS Spectrophotometry •Fluorescent Spectrometry)

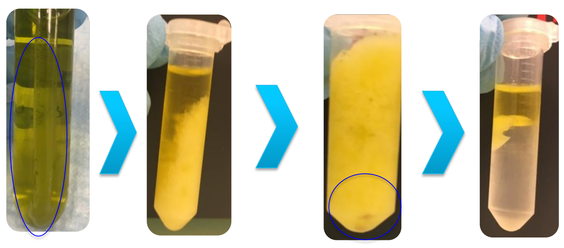
For INL Device, the method described by *Carvalho et al.*[5] was used.

## Results

### 1st PRE-TREATMENT

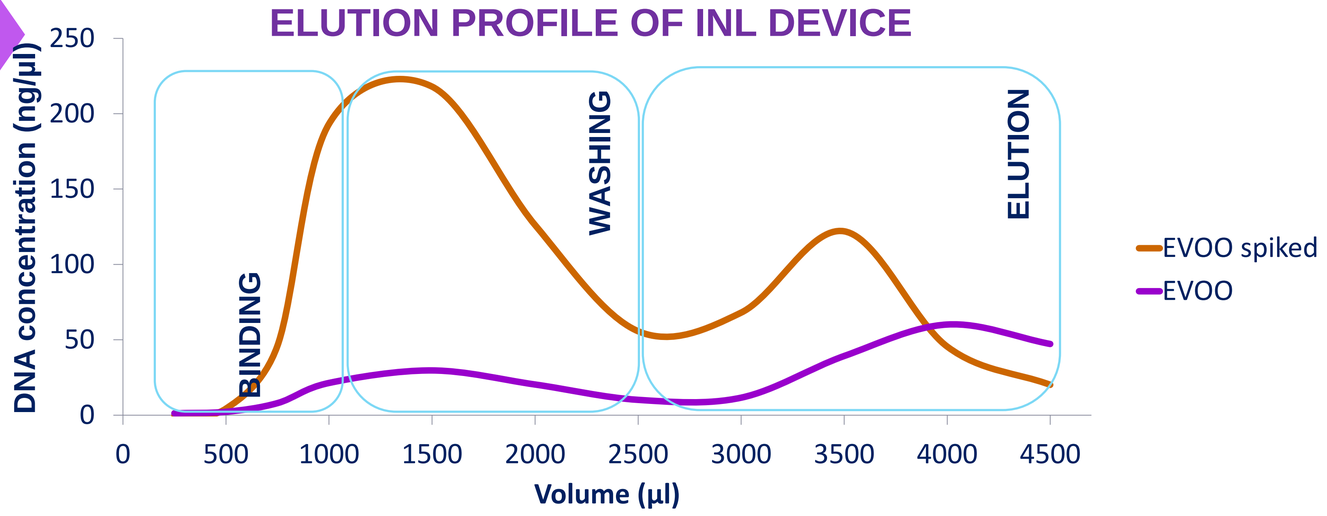
DNA detected (ng/µl)

| Sample | Method I | Method II | Method III | Method IV |
|---|---|---|---|---|
| EVOO | <2 | <2 | 8.1 | <2 |
| OOC | <2 | <2 | 7.9 | <2 |

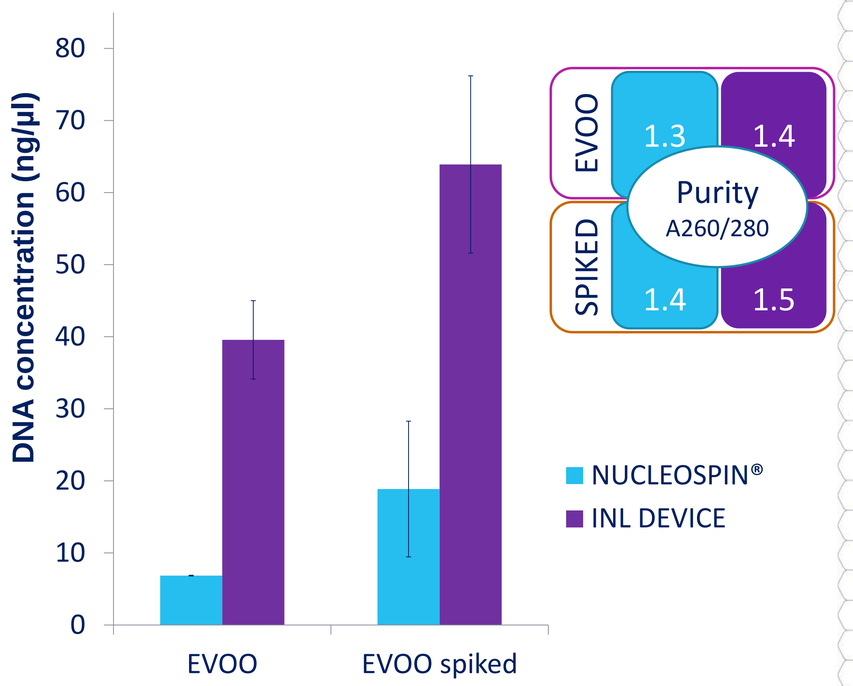


Pellets obtained after centrifugation following method III

### 2nd ELUTION PROFILE OF INL DEVICE




### TOTAL DNA CONCENTRATION




| DNA eluted (µg) | NUCLEOSPIN® | | INL DEVICE | |
|---|---|---|---|---|
| EVOO | 1.4 | 200 ml | 79.1 | 2000 ml |
| EVOO spiked | 3.8 | | 127.8 | |

## Conclusion

- A pre-treatment method adequate for DNA isolation from Olive Oil, a complex matrix with minute amounts of DNA, was developed.
- The microfluidic device was more efficient than NucleoSpin® for olive oil samples. It had more surface for DNA binding and major yield due to the larger membrane and the higher eluted volume.
- Future work: evaluate DNA purity with PCR

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