On the potential of a portable nano FT-NIR for predicting total phenol, flavonoid and oleuropein content of dried olive mill leaves: A preliminary study

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Introduction

Olive mill leaves are usually of poorer quality compared to those freshly cut and dried, due to inadequate handling, tissue damage, delays in sampling, transport, and drying. Even so, within the frame of circular economy the interest in their exploitation in the field of feeds is growing as they are still expected to contain bioactive phenols. Thus, it is desirable to be able to screen them after drying, with regards to the content in total or individual (oleuropein) phenols and decide on further use. To do so various techniques are required, which makes evaluation costly and time-consuming. An alternative would be the use of infrared (IR) spectroscopy combined with predictive models, and desirable to measure on site of drying. Even though portable IR spectrometers are available in the NIR region, there is no available information for such purpose. A single publication regards the use of a benchtop spectrometer, and plant material carefully collected and handled [1].

Aim

To examine whether a portable nano FT-NIR instrument using an optical fiber in the reflection mode could provide prediction models for olive mill dried leaves regarding the total phenol, total flavonoid and oleuropein content

Materials and methods

Olive leaves collected from 5 different olive mills located in the region of Lakonia (**Fig.1A**) were transferred to EAS and dried in an oven at two different temperatures. The leaves were ground, sieved and a portion was extracted according to pharmacopoeia [2] for the determination of **total phenol (TP), total flavonoid (TFLA) and oleuropein (OLE) content**s [3,4]. Whereas a portion of the powder was used to record NIR spectra (1350-2500 nm) with a **NANOQ-2.5 FT-NIR** (OCEAN INSIGHT, Orlando, FL), coupled to a light source **HL-2000-HP-FHSA**, and **a NANOQ-RPROBE-600-VIS-NIR** optical fiber suitable for reflectance measurements. The color was determined with a MiniScan XE Plus (Hunter Associates Lab Inc., Reston, VA, USA) chromatometer, model 4500L and residual moisture with a Kern & Sohn GmbH (Albstadt, Germany) infrared moisture analyzer (**Fig. 1B**).
Spectra were treated with Spectragryph (Minitab 16software for optical spectroscopy Version 1.2.16d (Oberstdorf, Germany) and .2.1 (State College, PA).

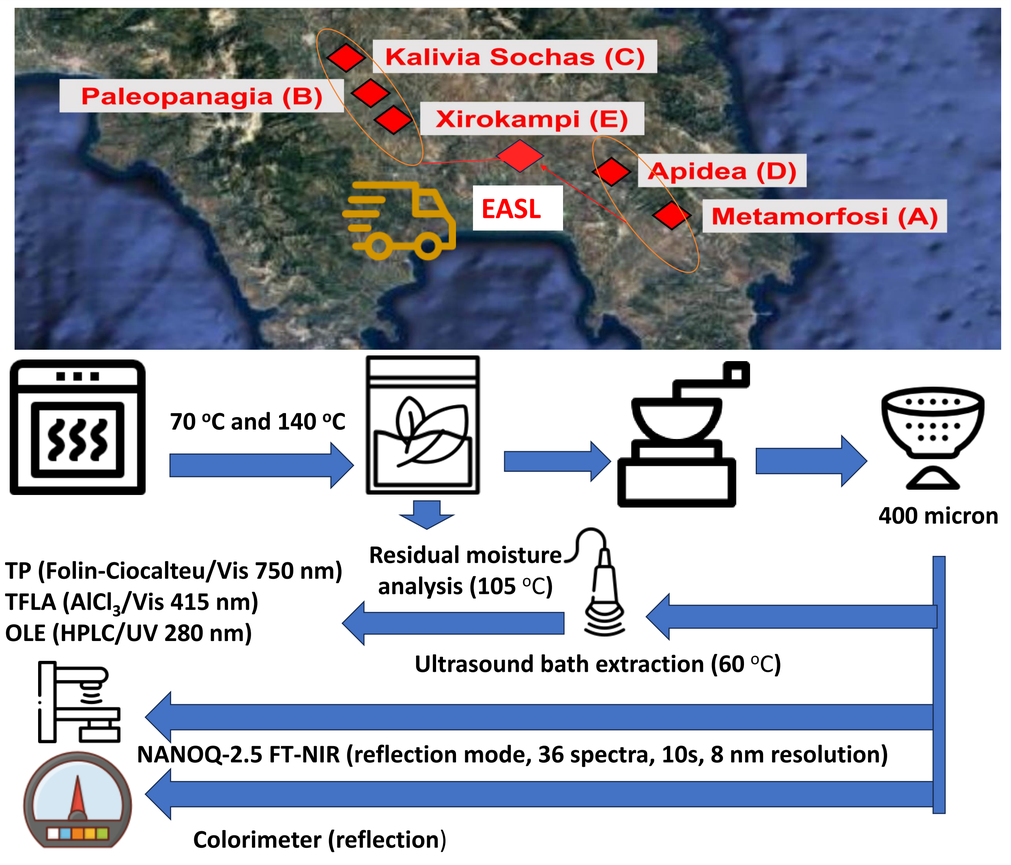

Fig.1 A) sampling, B) experimental part

Results and Discussion

Olive leaf characterization:
The **residual moisture** was low despite the difference in the temperature used and in the range 2.4-6.6% w/w, weigh below the level of 10% recommended by the pharmacopoeia [2]. Regarding the **color parameters** of the dried samples, those dried at 70 °C presented L values in the range of **53.8 - 62.9** suggesting that they were brighter than those dried at 140 °C (**L= 25.6 - 31.5**). The samples dried at the two temperatures presented positive **a** values with those measured for the leaves dried at the lower temperature being slightly higher (**2.9-7.2 vs 2.9-5.0**) but with significantly higher **b** values (**30.5-38.5 vs 10.2-17.8**). Consequently, the first group of powdered leaves were **ochre-like**, whereas the second **brown**. Obviously, these leaves were expected to be of low quality, considering that the corresponding parameters for a freshly collected and dried olive leaf were **L=37.1**, **a=-5.0** and **b= 18.0**, with negative a value suggesting the presence of green pigments. The **TP content** range was wide, namely **14-57 g GAE/kg** (gallic acid equivalents), with average 31.5 g/kg, less for **TFLA, 1.7-5.5 g QUE/kg** (quercetin equivalents), with average 3.7 g/kg and very wide for **OLE** (**0.4-56.8 g/kg**), with average 12.3 g/kg. The wide range of values appeared to be extended so that the material was interesting to be examined with the nano FT-NIR and chemometrics .

FT-NIR spectroscopy:
The recorded spectra per sample were treated with the software to remove spikes related to noise, followed by 10-point smoothing with Savitsky-Golay algorithm, before applying multiplicative scatter correction (MSC) and averaged. Mean centering was also examined, and except for the original spectrum the 2[d] derivative was also used. In **Fig 2A** and **B** are given representative spectra and their second derivative for powder leaf samples dried at 70 and 140 °C respectively.

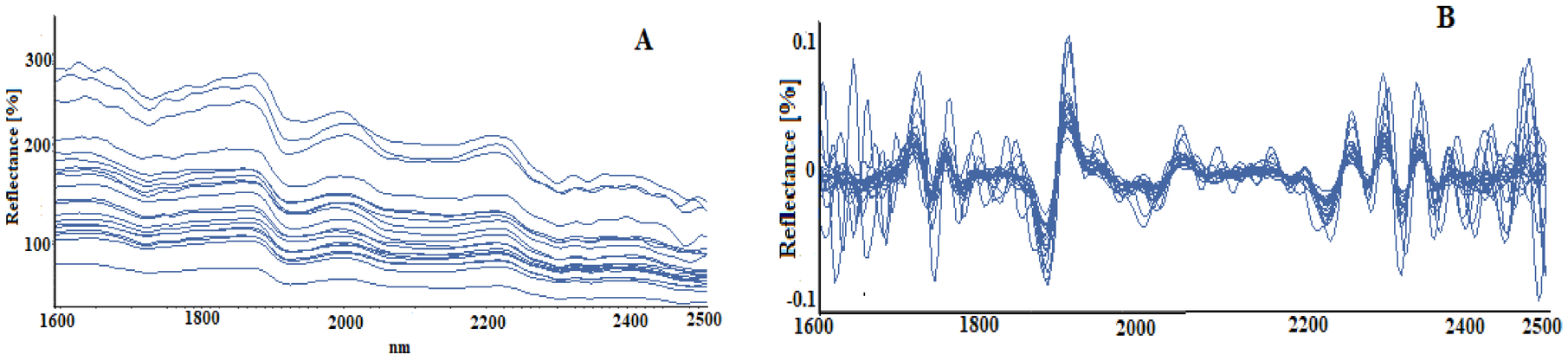

Figure 2. FT-NIR spectra (1600-2500 nm) of dried powdered leaf samples after preprocessing (**A**) and after calculation of their 2[d] derivative (**B**)

Except for quantitative differences, qualitative ones were observed in the spectral region 1600-1700 nm.
After different trials, **the best results** for the specific **dataset were obtained via selecting the region 1600-2500nm (also proposed by [1]) and the batch of preprocessed original spectra** instead of their 2[nd] derivative.
The data from ANOVA and those characterizing the models upon selection and validation are given in **Table 1**. The corresponding response plots are given in **Fig 3**, while some quantitative findings are given in **Table 2.**

Table 1 Data from ANOVA, PLSR model selection and validation process

	No components	%variance explained	*F*	*P*	R^2	PRESS
TP	2	99.9	13.67	0.000	0.62	1671
TFLA	3	99.9	10.46	0.000	0.66	19.43
OLE	2	99.8	9.24	0.002	0.52	3180

Table 2 Actual, fitted and cross-validated values for TP, TFLA, OLE based on PLSR models

	TP			TFLA			OLE		
	Actual	Fitted	Cross-V.	Actual	Fitted	Cross-V.	Actual	Fitted	Cross-V.
Min	14.4	19.4	17.5	1.7	2.1	2.5	0.4	-2.7	-6.3
Max	57.4	57.3	62.6	5.5	5.7	6.8	56.8	40.1	47.9
Aver.	31.4	31.4	31.5	3.7	3.7	3.7	12.3	12.3	12.3

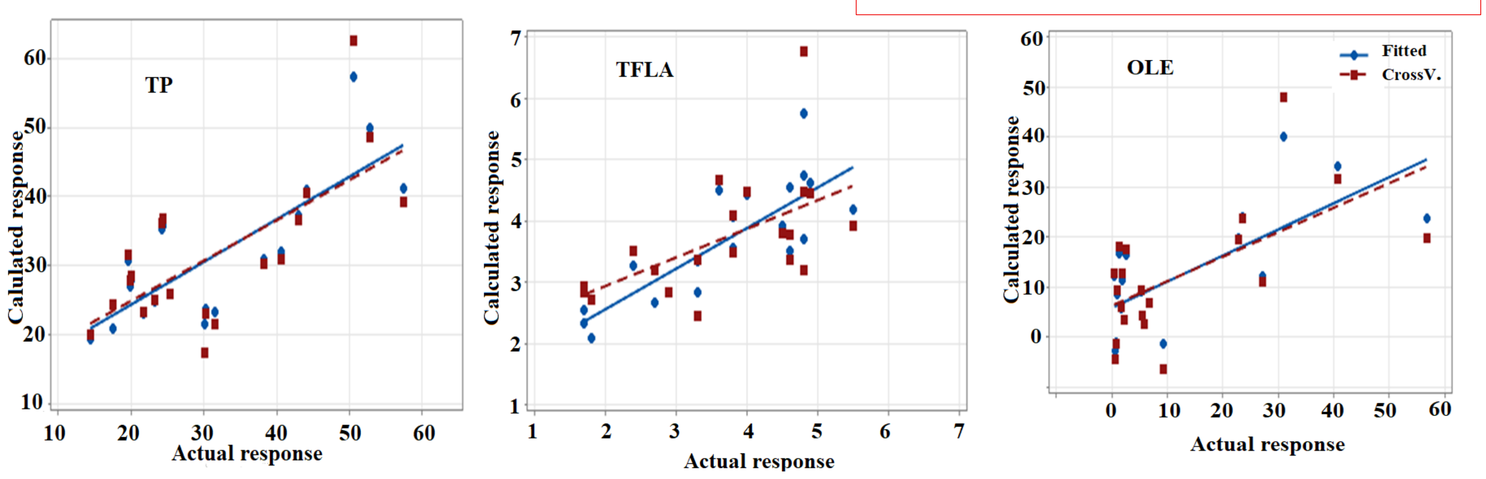

Figure 3 PLSR correlation plots for estimating **TP**, **TFLA**, and **OLE** (before --- and after --- cross-validation)

Observations

- Three statistically significant models explaining almost 100% of the total variance were obtained, with an R^2 value in the range 0.52 to 0.66, the lowest one found for OLE (Table 1).
- The models for TP and TFLA fitted well the data compared to the one for OLE (Table 2), but after cross-validation, the models for TP and TF overestimated both the min and max values, whereas the opposite was observed for OLE. Thus, for a too low content of OLE (0.4 g/kg) a negative value was predicted. The observed behavior for OLE model could possibly related to the fact that 15 out of 20 samples presented very close experimental values between 0.4 and ~10 g/kg (unbalanced population).
- The findings were poorer to those reported by Can et al. [1]. The discrepancies could probably be related to the reasons discussed in the introduction.
- Despite the need for further refining of the models and the inclusion of more samples, considering the leaf quality, the findings for screening purposes are rather promising taking into account that the portable FT-NIR can be used on-site.

References: [1] Can et al. 2018, J. Food Meas. Characterization, 12:2747–2757, [2] Eur. Pharmacopoeia 2005, [3] Papoti et al., 2018, *Foods*, *7(12), 197*; [4] Cvek et al. 2007, *Phytochem Anal, 18(5), 451,* [5] Method COI/T.20/Doc. No 29; N.

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