Determination of the origin of carobs using FTIR and Chemometrics: preliminary results

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1. Introduction

Carob tree (*Ceratonia siliqua L.*) has been widely grown in Mediterranean region for years. According to FAO (Food Agriculture Organization) the countries with the highest carob production in 2014 were Spain, Italy, Portugal, Morocco, Turkey, Greece, Cyprus and Lebanon. The main components of carob tree are the pods and the seeds. The seeds (about 10% of the fruit), are industrially used to produce locust bean gum (LBG) as a thickener and food stabilizer or flavoring. The carob pods, contain high amounts of carbohydrates, polyphenolic and antioxidant compounds and low amounts of insoluble dietary fibers, proteins, minerals and lipids. Nowadays, the main use of carob pods is for animal feed. For humans, it is mostly used as a cocoa substitute due to its low price and as a caffeine free product [1–3].

In Cyprus, carob tree is widely known as "teratsia". According to macroscopic observations on carob pods it is believed that there are three cultivars of carob tree: *Tylliria*, *Koumpota* and *Kountourka*. In the old days it was characterized as the "black gold of Cyprus", since it was the product with the largest agricultural exports and an important source of income. A number of traditional carob products are produced in Cyprus: carob syrup (charoupomelo), carob powder and pastelli [4].

Fourier transform infrared (FTIR) spectroscopy has been widely used in the food and drug industry because it is simple (requiring minimum sample preparation), rapid, low-cost and non-destructive. Chemometrics is the science of extracting molecular relevant information from complex multidimensional data by using multivariate analysis techniques [5].

The powerful combination of FTIR and chemometrics has been successfully applied in many research areas in food and beverages (Table 1). According to our knowledge, only Alabdi et al. used FTIR and chemometrics techniques, Hierarchical Cluster Analysis (HCA), Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA), in order to discriminate and classify samples of pods and seeds from four Moroccan regions [6].

The goal of the present work was the application of FTIR and Chemometrics as a methodology in order to differentiate the origin of carobs as well their type using 16 carob cultivars from 7 Mediterranean countries (Cyprus, Greece, Italy, Spain, Turkey, Jordan and Palestine).

Table 1: Applications of FTIR and chemometrics in food and beverages.

Method	Wavelength (cm^{-1})	Target	Product	Reference
FT-MIR with HCA, PCA and PLS-DA	4000-650	Discrimination/ Classification	Carobs	[6]
FTIR with PCA and CA	4000-700	Discrimination/ Classification	Coffee	[7]
FT-MIR/ATR	1800-900	Discrimination	Greek red wines	[8]
FTIR with PCA, CA, LDA and CART	1900-750	Authenticity	Cyprus traditional wine "Commandaria"	[9]
FTIR/ATR with PCA, PLS and kNN	1850-880	Detection of Sugar Adulterants	Apple Juice	[10]
FTIR/ATR with PLS	1800-900	Prediction of total phenolic and flavonoid contents and antioxidant capacity	Moscatel dessert wines	[11]

FT-MIR: Fourier transform mid-infrared
HCA: Hierarchical cluster analysis
PLS-DA: Partial least squares-Discriminant analysis
PCA: Partial component analysis
CA: Cluster analysis
ATR: Attenuated total reflection
LDA: Linear discriminant analysis
CART: Classification and regression trees
kNN: k-Nearest neighbors

2. Experimental part

Carob pods (flesh and seed) from Cyprus and six other countries (Greece, Italy, Spain, Turkey, Jordan and Palestine) were used (Table 2). The seed was grounded in the Laboratory mill 3100, while the flesh was grounded in blender Cuisine 4200 magimix. The FTIR analysis was performed both in the flesh and the seed. The transmittance spectra were obtained under controlled environmental conditions on a Jasco FT/IR-6100 spectrophotometer in two different ways: a) as a KBr pellet and b) with small sample placement on ATR on a ZnSe. The spectra recorded in the wavelength region of 400-4000 cm^{-1} with 128 scans and a 16 cm^{-1} resolution. A background was collected before each sample was analyzed and then subtracted from the sample spectra prior to further analysis. The first- and second- derivative were applied to the recorded transmittance spectra. The spectra recorded by the use of KBr tablets, gave better discrimination and were therefore used for further chemometric analysis. The chemometric analysis of spectroscopic data was performed with SIMCA software (version 13.0, Umetrics, Sweden). PCA and CA chemometric techniques were used for the classification of the samples.

Table 2: Carob cultivars.

Country	Cultivars	Type
Cyprus	3 (*Tylliria, Koumpota, Kountourka*)	flesh and seed
Greece	3 (*Imera, Imera, Unknown*)	flesh and seed
Italy	4 (*Raexmosa, Giubiliana, Saccarata, Unknown*)	flesh and seed
Spain	3 (*Negra, Rojal, Metalafera*)	flesh and seed
Turkey	1 (*Fleshy*)	flesh and seed
Jordan	1 (*Unknown*)	flesh and seed
Palestine	1 (*Unknown*)	flesh and seed

3. Results and discussion

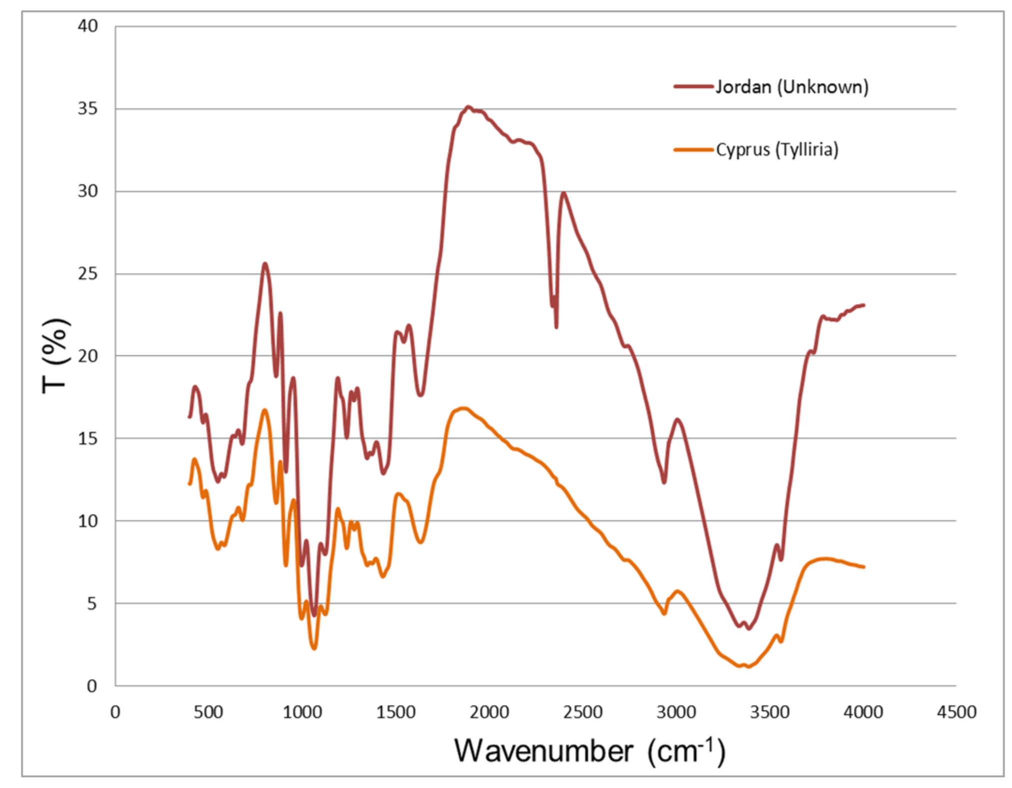

Figure 1: Carob flesh FTIR spectra obtained by transmittance readings employing KBr pellets.

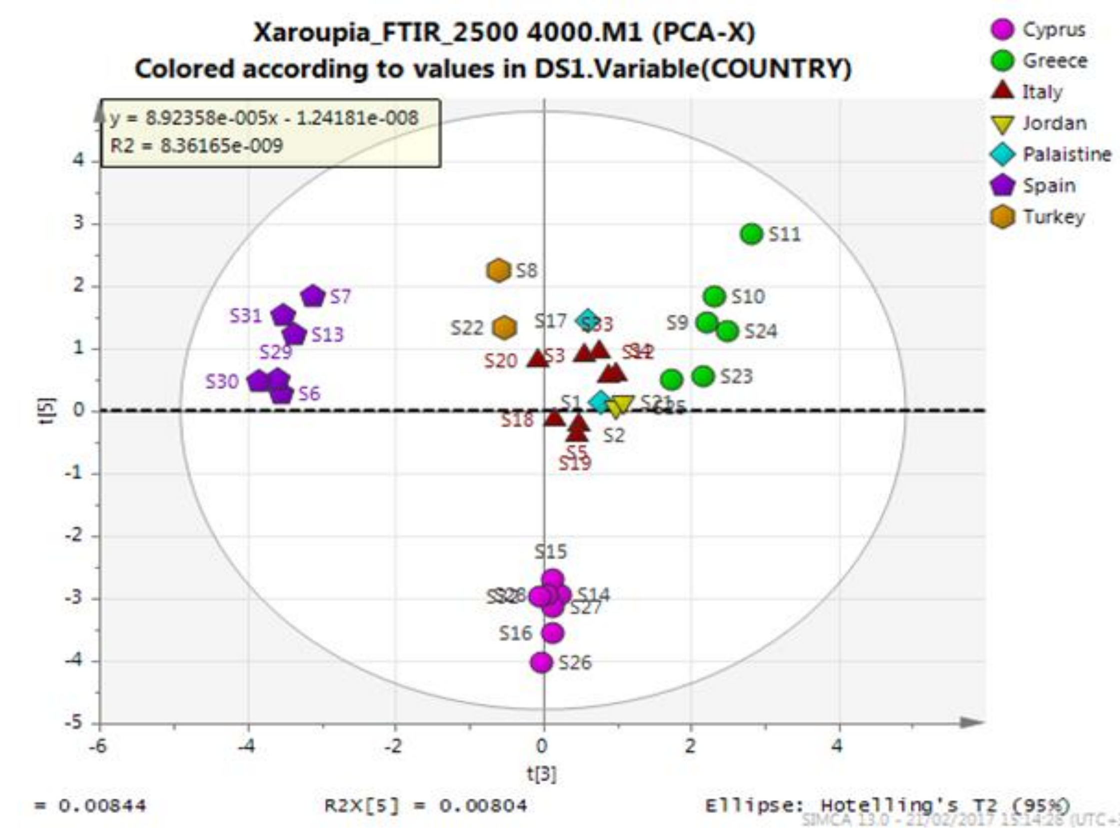

Figure 2: PCA scatter plot of FTIR spectra (2500-4000 cm^{-1}).

Figure 2 shows the PCA results (PC3 vs. PC5 score plot) of FTIR spectra (KBr, transmission) in the wavelength range of 2500-4000 cm^{-1}. In this case, there was clearly differentiation between the carob samples depending on the country of origin. Four separate groups can be identified: a) carobs from Cyprus (which was very well formed), b) carobs from Spain, c) carobs from Greece and d) carobs from Italy, Jordan and Palestine. Some small degree of separation between the samples in the last group was suggested in the hyperplane. The samples from Turkey were slightly distinguished from the last group.

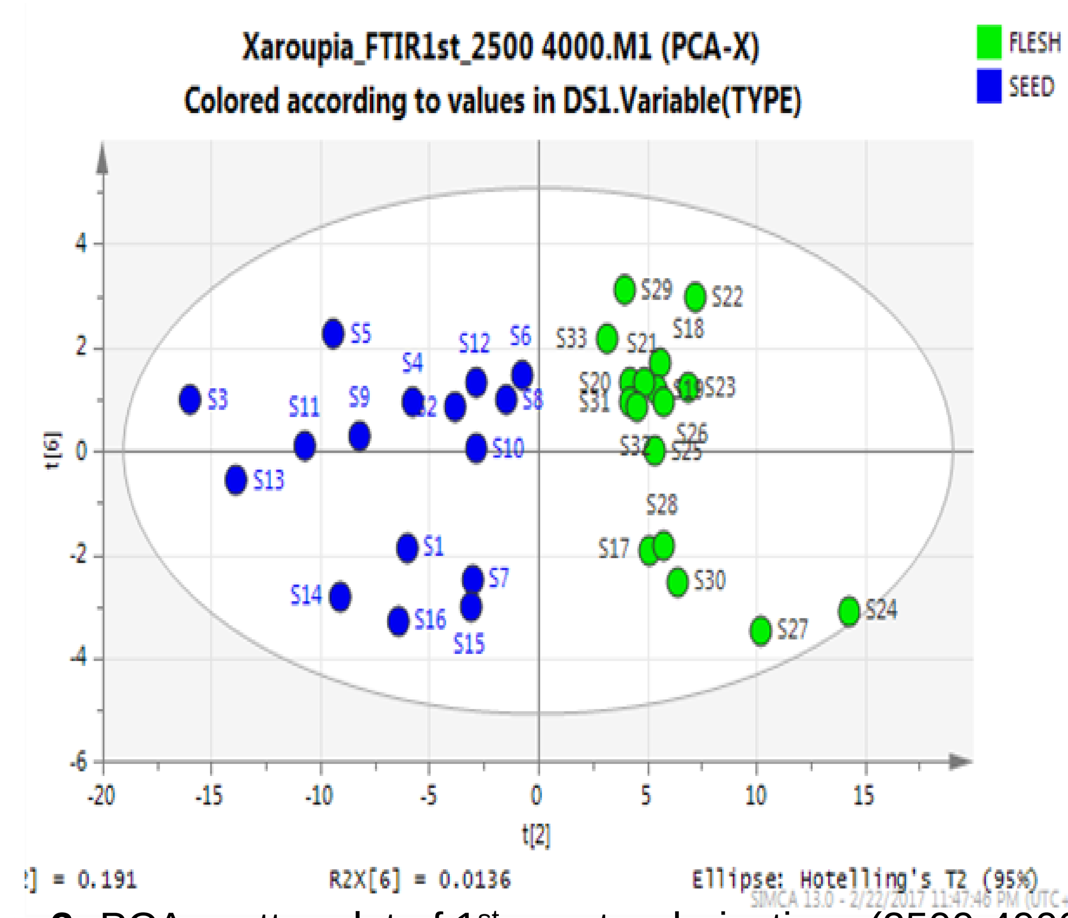

Figure 3: PCA scatter plot of 1[st] spectra derivatives (2500-4000 cm^{-1}).

Figure 3 shows the PCA results (PC2 vs. PC6 score plot) of the data obtained from the application of the first derivative to the recorded spectra in the wavelength range 2500-4000 cm^{-1}. It is observed that the carob samples differentiated according to their type. The separation on the basis of the type of the samples is readily apparent from the plot showing the two groups: a) samples of carob flesh and b) samples of carob seed.

4. Conclusions

The results show that the carob samples could be separated into distinct groups depending on their origin and type. The use of appropriate algorithm must give groups of samples (e.g. as dendrogram) with confidence level greater than 85%. The uncertainty of the method is of great importance for the development of the models that may differentiate carobs of different origin. Therefore, to build such models, much larger sample sets comprising carobs from many years and harvests from different countries would be needed.

5. References

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