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ANALYSIS OF LEAVES USING FTIR SPECTROSCOPY AND PRINCIPAL COMPONENT ANALYSIS DISCRIMINATION OF DIFFERENT PLANT SAMPLES

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Abstract
The present study was conducted to characterize various bioactive phytoconstituents from blueberry, chokeberry and strawberry leaves using FTIR spectroscopy. Attenuated total reflectance (ATR) spectra of plant leaves display complex absorption features related to organic constituents of leaf surfaces. The spectra can be recorded rapidly without special sample preparation. This paper explores sources of ATR spectral variation in leaves, including compositional, positional variations.

Keywords: blueberry, chokeberry, strawberry, FTIR-ATR, PCA, spectral study.

1. INTRODUCTION

Three types of leaves has been studied: **strawberry**, **chokeberry** and **blueberry**. Strawberry *(Fragaria x ananassa* Duchnese) is a species which forms large, trifoliate, long petiolate, toothed, shiny or pubescent leaves on the stolons; with stipels that vary in shape, size and color. The blueberry bush (*Vaccinium corymbosum* L., 1753) has fairly large leaves in size, about 5 – 7 cm in lenght and 2.3 – 3.5 cm in width, of elliptical or lanceolate shape, coriaceae, short petiolate. The upper surface of leaves is green, more darker than the lower side. Chokeberry (*Aronia melanocarpa* (Michx.) forms leaves in the 2 nd/3rd part of the plant, occuring in April. Aronia leaves are oblong, elliptical or ovale, 3 – 6 cm long, green, shiny, glabrous, sharp or obtuse, with acuminate tips. Leaf margins has a slightly serrate aspect, the leaf petiole having 0.6 cm size or more less (Hoza et al., 2011).

Plants are the good sources for the discovery of pharmaceutical compounds and medicines which are used to cure ailments with no side effects compared with synthetic drugs. They have many secondary metabolites which confer specific characteristics and properties to plants.

Each FTIR spectrum of a compound can express a unique “fingerprint”, which allows FTIR spectroscopy to be used in the classification of different samples or identification of the unknown samples. (De Luca et al., 2011, Topală et al., 2017, Topală and Tătaru, 2018).

The analyses of the leaves have been performed at large extent with the view to studying essential constituents, the secondary metabolites, the physics and chemical interactions between them and identifying healthy species from virused (Bunaciu et al., 2011, Topală et al., 2017).

Phenolic compounds such as quercetin, rutin, narigin, catechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents (Dai and Mumper, 2010). Medicinal plants

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are known to produce diverse substances possessing antioxidant properties having ability to protect the human body against cellular oxidation. Recent research suggested that diets rich in polyphenolic compounds and flavonoids are associated with longer life expectancy and found effective in many health-related properties, such as anticancer, antiviral and anti-inflammatory activities.

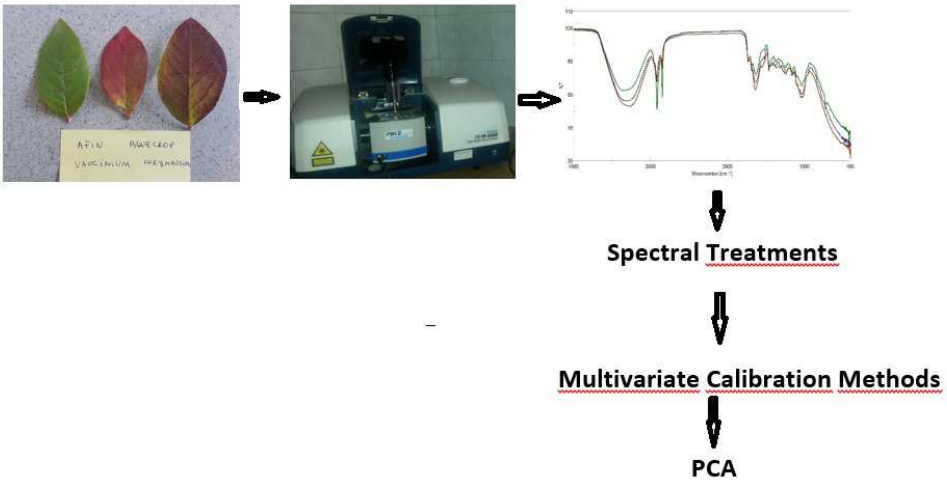

Figure 1. A method for rapid analysis of authentication of leaves sample

2. MATERIALS AND METHODS

Materials

Blueberry, chokeberry and strawberry leaves (Figure 1) were harvested from the experimental field from the Research Institute for Fruit Growing Pitesti – Mărăcineni.

To avoid problems caused by the effect of water on FTIR spectra, all samples were dried at 45°C for 72 h, ground into fine powder in an agate mortar. The samples were crushed and were subject to extraction with various solvents, the extracts being the basis of some other research.

Analysis of leaves by Attenuated Total Reflectance Mid-Infrared (FT-MIR) Spectroscopy

The ATR-FTIR spectra were recorder in a range between 4000-400 cm^{-1} using a FTIR Jasco 6300 spectrometer, detector TGS, apodization Cosine. An ATR accessory equipped with a diamond crystal (Pike Technologies) was used for sampling. Each leaves sample (fresh and dry) was put directly on the surface of the diamond ATR.

The spectral data were processed with JASCO Spectra Manager II software. Each sample of leaves, without any preparation, were scanned at 4 cm^{-1} resolution, accumulation: 100 scans. All spectral determinations were run in triplicate and values were averaged Background reference spectra were recording using air after every sample to minimize the interference due to carbon dioxide and water vapor in the atmosphere. Between measurements, the ATR crystal was carefully cleaned using acetone then dried with a soft tissue.

Data Analysis

Infrared Spectra were exported from Spectra Manager, in ASCII (dx) format, into the Unscrambler Software (Edition X 10.3, Camo. Oslo Norway) for chemometric analysis. Spectra were pre-processed using the second-derivative transformation, the Savitzky-Golay derivation. The use of spectra derivatives with Savitzky-Golay algorithm as a chemometric pre-processing technique is widely reported in most classification based on FTIR spectroscopy (Chatfield and Colins, 1980; Jolliffe, 1986; Topală and Tătaru, 2018). The principal component analysis (PCA) model was

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developed using cross validation. PCA was performed both on the entire spectral range (4000 to 400 cm^{-1}), and on the MIR 'fingerprint' (1750 to 700 cm^{-1}).

3. RESULTS AND DISCUSSIONS

FT-MIR spectral fingerprinting of leaves samples

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds.
Leaves are complex assemblages of organic compounds and it might be expected that they would display distinctive spectral features in the thermal infrared energy range (FTIR; 4000–400 cm^{-1}). Fundamental vibration modes of various molecular functional groups produce characteristic spectral absorption features that can serve to 'fingerprint' many compounds (Silverstein & Webster, 1998).
The peaks in mid-IR spectrum from the vibration in different functional groups appear in characteristic frequencies of IR (Table 1).

Table 1. Some general bands assignments of MIR spectrum of plants based on literature (Kumar et al., 2016, Turek-Kaya and Huck, 2017)

Frequency (cm^{-1})	Spectral Assignments
3500- 3200	O-H, N-H stretch, carbohydrates, proteins, alcohols, phenolic compounds
2960-2950	CH_3 asym stretching, mainly lipid with a little contribution from protein, carbohydrate, and nucleic acid
2930-2920	CH_2 asym stretching, mainly lipid with a little contribution from protein, carbohydrate, and nucleic acid
2875-2870	CH_3 sym stretch, mainly protein with a little contribution from lipid, carbohydrate, and nucleic acid
2860-2840	CH_2 sym stretching, mainly lipid with a little contribution from protein, carbohydrate, and nucleic acid
1745-1730	C=O stretch from saturated ester, phospholipid, cholesterol ester, hemicellulose, pectin, lignin, suberin/cutin esters
1650-1630	C=O stretch (Amide I) from protein, pectin
1560-1540	C=N and N-H stretch (Amide II), mainly protein
1455-1440	C-H asym bending of CH_2 and CH_3, cell wall polysaccharide, lipid and protein
1250-1240	C=O stretch from pectic substances, lignin, hemicellulose
1235-1230	C-O stretch, lignin, xylan
1170-1105	C-O-C asym and sym stretch of various groups:cutin, cellulose, cell wall polysaccharide
1045-1020	O–H and C–OH stretch: cell wall polysaccarides (arabinan, cellulose)

Such functional groups and related spectral features in our redearch include hydroxyl (OH) in alcohols and acids, carbonyl (C=O) in esters, ketones, aldehydes and acids, and methyl (CH_3) and methylene (CH_2) in alkanes (Figure 2). The spectrum displays aliphatic features for CH_2 bands at 2916 cm^{-1} and 2849 cm^{-1} likely owing to greater wax thickness. Main ester bands are at 1732 cm^{-1} (for C=O) and at 1145 cm^{-1} (for C-O). Polysaccharides feature displays a narrower band at around 1030 cm^{-1}. In mid-IR spectra, amide I and amide II modes are attributed to proteins.
Table 2 presents principal bands assignments and the spectra of leaves investigated.
Analysing the spectra of these three samples, similar spectral features were generally obtained. No visual differences were observed between the MIR spectra of leaves samples analysed. It was

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observed that water absorption peaks dominate the spectrum. Chemometric analysis allow the differentiation of these leaves.

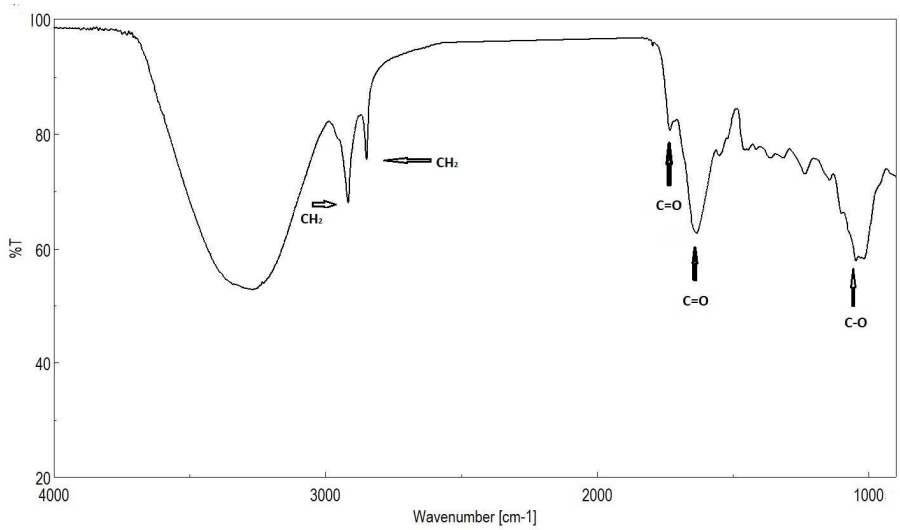

Figure 2. ATR –MIR Spectra for strawberries sample at room temperature. Main attributions

Table 2. Some wavenumbers (cm^{-1}) of selected bands in FTIR spectra of the plants studied

leaves	Frequency (cm^{-1}) of groups						
	OH	CH_2 asym	CH_2 sym	C=O esters	C=O Amide I	C-O-C	C-OH
Blueberry	3357	2915	2848	1732	1631	1162	1031
Chokeberry	3289	2917	2849	1731	1633	1160	1027
Strawberry	3269	2916	2848	1732	1635	1145	1047

Figure 3 presents a comparative spectrum of strawberry fresh and dry leaves. It is noticed that the water absorption band in dry leaf is much diminuated. The peak at 3269 cm^{-1} is poorly figured.
A broad band between 3220 cm^{-1} and 3260 cm^{-1} was observed instead of a obvious peak at 3269 cm^{-1}.

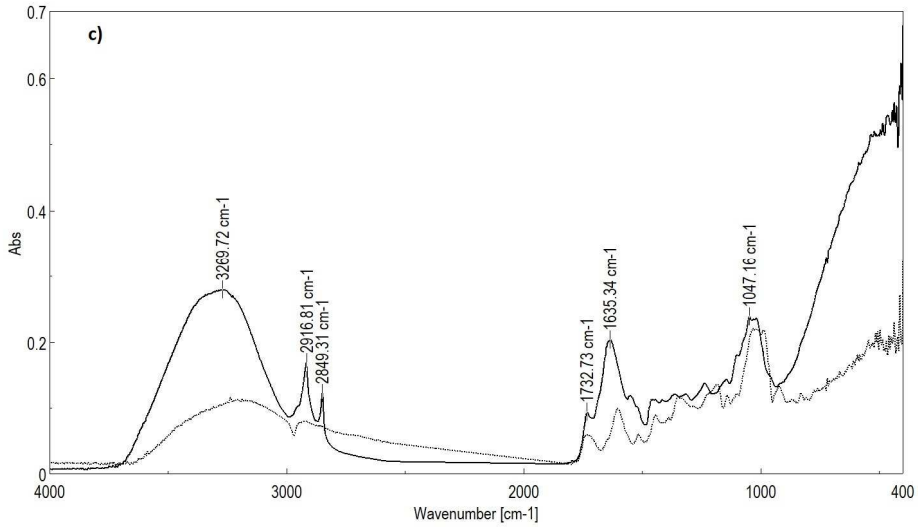

Figure 3. Overlayed ATR-FTIR spectra of strawberries (with dotted line spectrum of dry leaf)

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The chemometric analysis. Principal Component Analysis (PCA)

Principal component analysis (PCA) is one of the most common multivariate techniques. The purpose of this method is to decompose the data matrix and concentrate the source of variability in the data into the first few PCs**.** The scatter plots of PC1 (variability; 53%) x PC2 (variability; 26%) and PC3 (variability; 12%) are shown in figures 4 and 5, respectively. The first three principal components (PCs) represent 91% of the total variance. The scatter plot of PC1 against PC2 shows differentiation between these three type of leaves. The strawberries leaves (c) are in left of the graph and are separated from the rest blueberries (a) in the positive right quadrate and chokeberries (b) leaves- in the negative right quadrate.

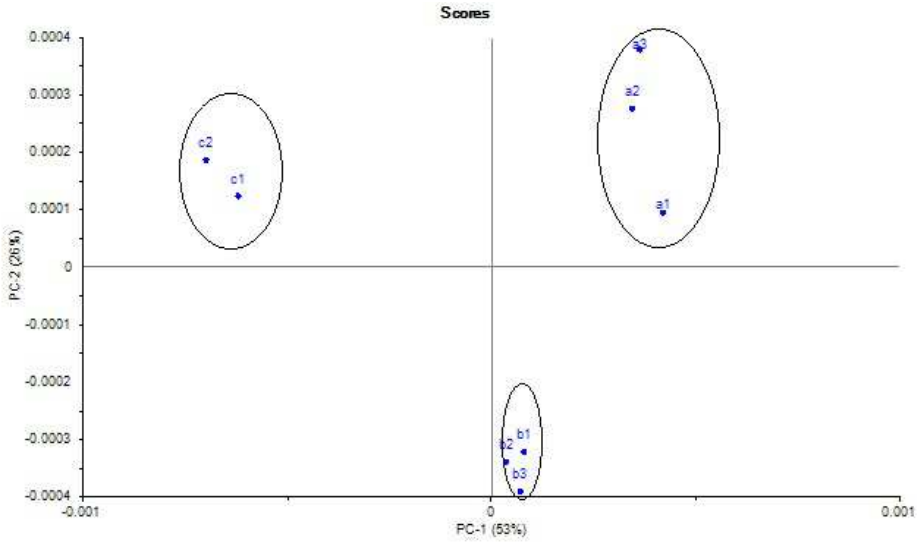

Figure 4. PCA score plots (PC1 x PC2) for leaves investigated (a- blueberry, b- chokeberry, c- strawberry)

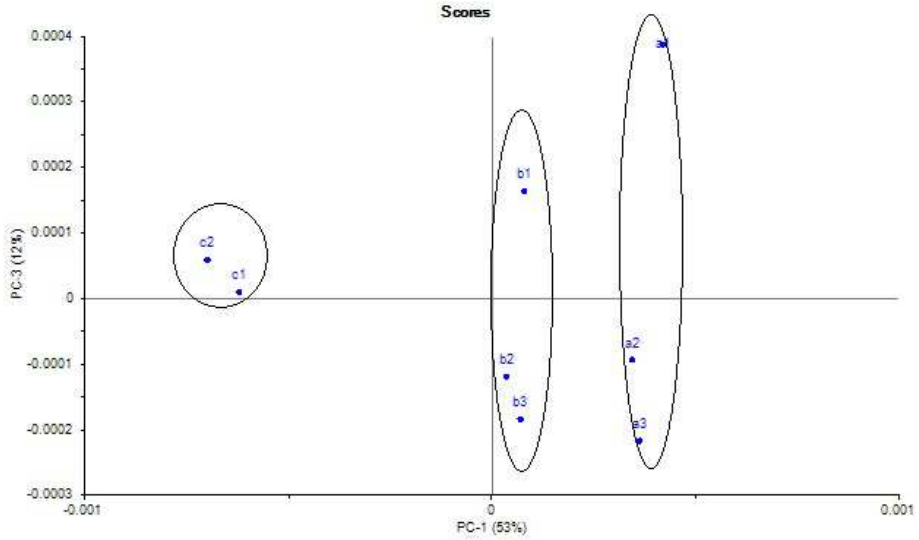

Figure 5. PCA score plots (PC1 x PC3) for leaves (a- blueberry, b- chokeberry, c- strawberry)

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4. CONCLUSION

FTIR spectroscopy provided more information through the fingerprints region of plants, rendering the technique direct and simple. To summarize, a combined FT-IR and PCA analysis is a powerful method for discrimination plant leaves.

5. REFERENCES

Bunaciu, A., Aboul-Enein, H.I., Fleschin S. (2011). Recent Applications of Fourier Transform Infrared Spectrophotometry in Herbal Medicine Analysis, Applied Spectroscopy Reviews, 46, 251–260.

Chatfield, C., Colins, A.J. (1980). Introduction to Multivariante Analysis, Chapman &Hall, London.

Dai, J., Mumper, R.J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 15(10), 7313-7352.

De Luca, M., Terouzi, W., Ioele, G., Kzaiber, F., Oussama, A., Oliverio, F., Tauler, R., Ragno, G. (2011). Food Chem., 124, 1113.

Hoza, D., Velcea, M., Turza, F. (2011) Cultivarea arbuştilor fructiferi [Cultivation of fruit trees], Ed. Rentrop si Straton.

Jolliffe, I.T., Principal Component Analysis. 1986. Springer-Verlag, New York, 1st Ed.

Kumar, S. Lahlali, R. Liu, X. Karunakaran, C. (2016). Infrared spectroscopy combined with imaging: A new developing analytical tool in health and plant science. *J Appl. Spectrosc. Rev.* 51, 466–473.

Silverstein RM, Webster FX. (1998). Spectrometric identification of organic compounds, 6th edn. New York, NY, USA: John Wiley & Sons, 71–143.

Topală, C.M., Tătaru, L.D. (2018). Rapid Method for the Discrimination of Romanian Wines Based on Mid-Infrared Spectroscopy and Chemometrics, *Rev. Chim.* (Bucharest), 2, 469-473.

Topală, C.M., Tătaru, L.D., Buciumeanu, E.C., Guță, I.C. (2017). FTIR spectra of grapevines (*Vitis vinifera L.*) in the presence of virus infections", *Acta Hortic.* 213-318 DOI 0.17660/ActaHortic.2017.1188.41

Turek-Kaya, S., Huck, C.W. (2017). A Review of Mid-Infrared and Near-Infrared Imaging: Principles, Concepts and Applications in Plant Tissue Analysis. Molecules, 22, 168.

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