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 length 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 2nd/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, naringin, 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](image)

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 Pitești – Mârăcineni. 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 Tătărău, 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>
<thead>
<tr>
<th>Frequency (cm(^{-1}))</th>
<th>Spectral Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500-3200</td>
<td>O-H, N-H stretch, carbohydrates, proteins, alcohols, phenolic compounds</td>
</tr>
<tr>
<td>2960-2950</td>
<td>CH(_3) asym stretching, mainly lipid with a little contribution from protein, carbohydrate, and nucleic acid</td>
</tr>
<tr>
<td>2930-2920</td>
<td>CH(_2) asym stretching, mainly lipid with a little contribution from protein, carbohydrate, and nucleic acid</td>
</tr>
<tr>
<td>2875-2870</td>
<td>CH(_3) sym stretch, mainly protein with a little contribution from lipid, carbohydrate, and nucleic acid</td>
</tr>
<tr>
<td>2860-2840</td>
<td>CH(_2) sym stretching, mainly lipid with a little contribution from protein, carbohydrate, and nucleic acid</td>
</tr>
<tr>
<td>1745-1730</td>
<td>C=O stretch from saturated ester, phospholipid, cholesterol ester, hemicellulose, pectin, lignin, suberin/cutin esters</td>
</tr>
<tr>
<td>1650-1630</td>
<td>C=O stretch (Amide I) from protein, pectin</td>
</tr>
<tr>
<td>1560-1540</td>
<td>C=N and N-H stretch (Amide II), mainly protein</td>
</tr>
<tr>
<td>1455-1440</td>
<td>C-H asym bending of CH(_2) and CH(_3), cell wall polysaccharide, lipid and protein</td>
</tr>
<tr>
<td>1250-1240</td>
<td>C=O stretch from pectic substances, lignin, hemicellulose</td>
</tr>
<tr>
<td>1235-1230</td>
<td>C-O stretch, lignin, xylan</td>
</tr>
<tr>
<td>1170-1105</td>
<td>C-O-C asym and sym stretch of various groups:cutin, cellulose, cell wall polysaccharide</td>
</tr>
<tr>
<td>1045-1020</td>
<td>O-H and C–OH stretch: cell wall polysaccarides (arabinan, cellulose)</td>
</tr>
</tbody>
</table>

Such functional groups and related spectral features in our research 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
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](image)

**Table 2. Some wavenumbers (cm⁻¹) of selected bands in FTIR spectra of the plants studied**

<table>
<thead>
<tr>
<th>leaves</th>
<th>OH</th>
<th>CH₂ asym</th>
<th>CH₂ sym</th>
<th>C=O esters</th>
<th>C=O Amide I</th>
<th>C-O-C</th>
<th>C-OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberry</td>
<td>3357</td>
<td>2915</td>
<td>2848</td>
<td>1732</td>
<td>1631</td>
<td>1162</td>
<td>1031</td>
</tr>
<tr>
<td>Chokeberry</td>
<td>3289</td>
<td>2917</td>
<td>2849</td>
<td>1731</td>
<td>1633</td>
<td>1160</td>
<td>1027</td>
</tr>
<tr>
<td>Strawberry</td>
<td>3269</td>
<td>2916</td>
<td>2848</td>
<td>1732</td>
<td>1635</td>
<td>1145</td>
<td>1047</td>
</tr>
</tbody>
</table>

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 diminished. The peak at 3269 cm⁻¹ is poorly figured. A broad band between 3220 cm⁻¹ and 3260 cm⁻¹ was observed instead of an obvious peak at 3269 cm⁻¹.

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

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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)
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
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