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Discrimination of *Prunus domestica* L. fruit extracts by FTIR-PCA coupled technique

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Abstract

Plums *(Prunus domestica* L.) are valuable fruits due to their high content of antioxidants, including anthocyanins and anthocyanidins. However, the polyphenol compositions significantly differ for plum varieties. The goal of this study was the evaluation of the similarity/dissimilarity of dry extracts of plum pulp samples from different varieties growing and/or commercialized in Romania, including "Bistriţa", "Stanley", "Renclod", "President" and "Vinete Românești", using the Fourier-transform infrared spectroscopy-principal component analysis (FTIR-PCA) coupled technique. The extracts were obtained from fresh pulp, using ethanol, room temperature, and intermittent stirring for 48 h. Extracts were filtered and dried at moderate temperature in the dark. The dry extract was subjected to FTIR analysis and both wavenumber and intensity of the FTIR specific bands were processed using PCA analysis. The band corresponding to the stretching vibration of the C=O group (flavonoids and organic acids) was identified in all extracts at 1713-1717 cm^{-1}, as well as the those related to the stretching vibrations of the CC aromatic and pyrane skeletons, $\nu^{sk}_{(arC\#C)}$ and ν^{sk}_{pyr}, at 1630-1637 and 1233-1258 cm^{-1}, respectively. Valuable discriminations between plum extract samples obtained from different varieties have been obtained by FTIR-PCA analysis of all wavenumber and intensity parameters. "Bistriţa" samples were grouped in the left side of the PC1 and the other plum samples in the right of this scores plot. Also, other plum varieties were well sub-classified, especially based on the stretching vibrations of the pyrane skeleton and for the carbonyl groups in antioxidant compounds (intensities for the positive side and wavenumbers for the negative side) for the first principal component. The CH and CO bending vibrations or the stretching vibration of the benzene skeleton (wavenumbers or intensities) were important for the positive and negative side of the second principal component, respectively. Moreover, the samples obtained from particular orchards (i.e., "Bistriţa" varieties) were clearly discriminated from the other plum varieties, revealing the quality of these autochthonous fruit varieties.

Keywords: *Prunus domestica* L.; plums; Bistriţa variety; antioxidant activity; polyphenols; anthocyanins; Fourier-transform infrared spectroscopy; multivariate statistical analysis; principal component analysis; discriminant analysis

1. Introduction

Fruits are a very important part of the human diet, they bring both energy intake through the carbohydrates, as well as vitamins (vitamin C, vitamins E, vitamins from the B complex or carotenoids such as provitamin A) and antioxidant polyphenols [1-6]. In our country, the total area cultivated for the production of various fruits, at the level of 2021, was 318,300 ha, according to the latest data updated by FAOSTAT (Food and Agriculture Organization's Corporate Statistical

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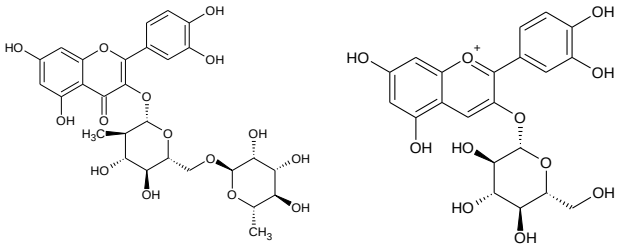
Database) on 24th of March 2023 [7]. Regarding the total fruit production for the same evaluated year, it was 3,060,000 tons. Depending on the cultivated area, plums and similar fruits occupy the second place with 66,730 ha. The production of plums in this period was 807,000 tons, with a productivity of over 12,000 kg/ha [7].

Plums are the fruits of the species *Prunus domestica* L. (Rosaceae), a species that includes many subspecies and varieties that are cultivated especially in Europe and, of course, in Romania. "Bistriţa" plums are the most famous and cultivated variety in the country, as well as the "Centenary", "Record", "Carpatin" or "Andreea" varieties [8]. The subspecies *domestica*, ssp. *intermedia*, ssp. *damsons*, or ssp. *italica* are also grown in the region, respectively the "Jefferson", "Victoria", "President" or "Stanley" varieties. Anyway, the number of plum varieties is very large [6,9-13]. Among the world's largest producers are China, USA or Spain, but Romania also ranks first in the production of plums. Approximately two-thirds of the plum production is consumed fresh, but the fruits are also processed in the form of compote, juice, frozen or dried. Among the most applied processing methods for plums, it is worth mentioning the obtaining of juices, compotes, respectively drying, the last variant experiencing a significant increase recently [14-16]. Other forms of processing are obtaining paste/purée or sauces.

The energy value of fresh fruit, dried fruit or in the form of compote or juice is 46, 107 and 63-71 kcal/100 g, respectively. The concentrations of total carbohydrates and sugars reach 11.4 and 9.9 g/ 100 g fresh weight (FW). The protein and lipid content is low, but plums have a high content of vitamins A and β-carotene, lutein and zeaxanthin, vitamin C (9.5 mg/100 g), or vitamins K, minerals such as potassium, magnesium, calcium, phosphorus and zinc [6,10-13,15,17].

The classes of antioxidants in fresh plums are represented by hydroxybenzoic, chlorogenic, neochlorogenic acids such as *p*-coumaroylquinic, 3-feruloylquinic, 3-caffeoylquinic acids. Flavonoids and glycosylated flavonoids such as catechin, rutin, quercetin glucoside, quercetin galactoside, quercetin rhamnoside and rosmarinic acid are the most important in plums. Fruit color is mainly provided by anthocyanins and anthocyanidins, especially cyanidin 3-*O*-glucoside (Figure 1) [17-21].



Rutin *Cyanidin 3-glucoside*

Figure 1. Structures of the main flavonoid glycoside and anthocyanin antioxidant compounds in plums

Regarding the aroma compounds in plums, several classes of such compounds have been identified such as ketones, aldehydes, alcohols, esters, lactones and hydrocarbons. Anyway, the composition of flavor compounds depends a lot on the variety, degree of ripening and especially the processing. Aromatic volatile compounds such as 4-hexen-1-ol acetate, hexyl acetate and butyl acetate were identified, but also aldehydes and simple alcohols such as hexanal, 2-hexenal, 1-hexanol, or nonanal. Other volatile compounds were terpinolene, α-terpineol, and many aroma compounds were identified at very low concentration values in fresh fruits [6,12].

The goal of the study was the evaluation of the similarity/dissimilarity of dry extracts of plum pulp samples from different varieties growing and/or commercialized in Romania using the Fourier-transform infrared spectroscopy-principal component analysis (FTIR-PCA) coupled technique.

2. Materials and Method

2.1. Fruit samples and chemicals

Plum samples were collected during the autumn of 2021 and 2022, where washed, the skin and pulp were separated and stored at -20 °C up to the extraction. Eight plum varieties were collected or purchased from the west counties of Romania: "Bistriţa" (from individual orchards of Giroc, Topolovăţu Mic, Timiş county, as well as Măgulicea, Arad county, codes "B"), "Stanley", "President" and "Vinete Româneşti" (Timişoara supermarket, Timiş county, codes "S", "P" and "V"), and "Renclod" (conventional orchard, Caraş Severin county, code "R"). Moreover, "Bistriţa" varieties were coded as "O", while the other plum varieties were coded as "N" in the FTIR-PCA analysis.

Other solvents and chemicals used in this study were ethanol 96% (*v/v*, ChimReactiv, Bucureşti), acetonitrile (HPLC grade, Merck&Co, Inc., Germany), trifluoroacetic acid (TFA, reagent grade, Merck KGaA, Germany), cyanidin·HCl and cyanidin 3-*O*-glucopyranoside·HCl as standard anthocyanins (>97%, PhytoPlan® GmbH, Germany).

2.2. Obtaining of plum extracts

Classical extraction of the pulp plum samples was achieved. The extraction was performed in a sealed flask of 100 mL, using 5.26±0.14 g pulp sample and 25 mL of ethanol 96%. The extraction was performed for 48 h in the dark, with intermittent stirring. The extract was then filtered, washed with 1-2 mL of ethanol and completed to 25 mL with the same solvent. Approximately 0.89±0.12 g of dry extract was obtained after complete removal of the solvent under moderate temperature in the dark. The samples were subjected to HPLC and FTIR analyses.

2.3. High pressure liquid chromatography (HPLC) analysis

An amount of 0.36±0.05 g of dry extract was completely dissolved in 0.5 mL aqueous solution containing 0.4% TFA. The solution was filtered through PTFE syringe filter (0.22 μm, diameter of 25mm, Teknokroma, Barcelona, Spain) and analyzed using an analytical HPLC (PU-2080Plus Intelligent pump, LG-2080-04 gradient unit, LG-2080-54 4-Line degasing module, UV-2070Plus Intelligent Detector, and LC-NetII/ADC interface, Jasco, Easton, MD, USA). The anthocyanins and anthocyanidins were quantified in extracts and in the fresh samples using calibration curves for the standard compounds. A Nucleosil 100 C18 column (250 mm length, 4 mm internal diameter and 5 μm particle diameter) and an A/B of distilled water 0.4% TFA / acetonitrile 0.4% TFA gradient system (15% B for 0-6 min, 15-22% B for 6-20 min, 22-35% B for 20-35 min, 35-15% B for 35-40 min, 15% B for 5 min) were used. The wavelength and the solvent flow were set at 525 nm and 1 mL/min, respectively. A sample volume of 20 μL was injected into the HPLC system. The acquisition and handling of the HPLC data were performed using a Jasco ChromPass Chromatography Data System, ver. 1.7.403.1.

2.4. Fourier-transform infrared spectroscopy (FTIR) analysis

FTIR analysis was performed for dry extracts using a Bruker Vertex 70 (Bruker Optik GmbH, Ettlingen, Germany), equipped with a single-reflection Platinum diamond attenuated total reflectance ATR module. The scanning range was set at 4000-400 cm^{-1}, a resolution of 4 cm^{-1}, and 64 scans for both extracts and baseline. The OPUS ver. 7.2 acquisition and handling software had used. All determinations were performed as triplicates and results were presented as mean ± standard deviation (SD).

2.5. Principal component analysis (PCA)

PCA was performed using both wavenumbers and intensities of the FTIR bands corresponding to the significant stretching and bending vibrations, which were identified in all plum extract samples. Also, the wavenumbers and intensities as subsets were used in the PCA analysis and discrimination of the plum samples. The *Principal Components & Classification Analysis* module from the Statistica 7.1 (StatSoft), with centered and normalized values was used. PCA was based on correlations and the variances were determined using the relation SS/(n-1).

3. Results and Discussion

3.1. Chromatographic and infrared spectroscopic analyses of plum extracts

The liquid chromatography of plum extracts revealed a content of cyanidin 3-*O*-glucoside and cyanidin in the ranges of 0.16-0.94 and 0.05-2.70 mg/kg FW, respectively. However, the higher content of cyanidin 3-*O*-glucoside was obtained for "Bistriţa" - Măgulicea samples at 0.94 mg/kg FW. On the other hand, samples of "Bistriţa" variety – Măgulicea had the highest content of cyanidin of 2.70 mg/kg FW. Generally, the "Bistriţa" varieties were more concentrated in anthocyanins and anthocyanidins than the commercialized plums. Also, the anthocyanidins were more concentrated in comparison with the anthocyanins in almost all samples. This can be due to the partial hydrolysis of anthocyanins in the presence of TFA (solubilization and analysis) and/or during the extraction and concentration/drying. The similarity of samples was not significantly affected by this process, as can be

observed from FTIR-PCA analysis (see the next section).

FTIR analysis of plum extracts and the main anthocyanin and chlorogenic acid standard compounds reveals some important bands corresponding to stretching and bending vibrations of the specific bonds and groups in these antioxidants. The significant region is 1750-700 cm^{-1}, but the region of 3300-2800 cm^{-1} is also relevant (Figure 2). The comparison of plum extracts reveals small differences between the wavenumbers of the significant FTIR bands, which cannot be easier accounted. Also, their intensities are also difficult to evaluate. For example, the stretching vibrations of the OH and CH groups have wavenumber values in narrow ranges of 3293.2-3293.7 and 2926.9-2930.0 cm^{-1}, respectively (Table 1).

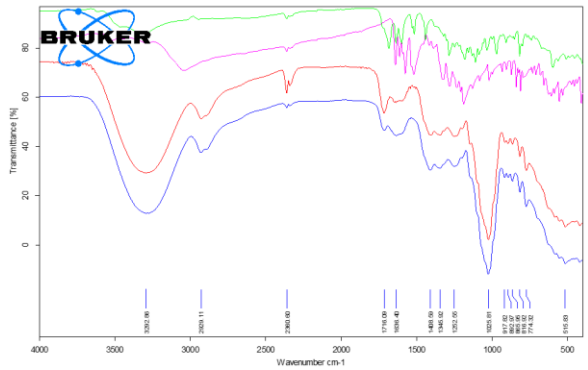


Figure 2. Superimposed FTIR spectra for the "Bistriţa" varieties collected from Topolovăţu Mic and Măgulicea regions (blue and red), in comparison with the standard cyanidin and chlorogenic acid (pink and green)

Table 1. Band assignments from the FTIR analysis of the representative plum extracts

FTIR assignment*	**Wavelength, cm^{-1}** (Mean ± SD)		
	B-Tm**	***B-M*****	***P*****
$\nu_{(O-H)}$	3293.2(±0.5)	3293.3(±0.3)	3292.7(±0.0)
$\nu^{as}_{(C-H)}$	2929.7(±0.8)	2926.9(±0.2)	2930.0(±0.5)
$\nu_{(C=O)}$	1716.2(±0.2)	1716.6(±0.0)	1716.5(±0.0)
$\nu^{sk}_{(arC\#C)}$	1636.4(±0.0)	1636.7(±0.1)	1636.2(±0.0)
$\delta_{(phO-H)}$	1408.6(±0.0)	1406.3(±0.1)	1407.5(±0.0)
$\nu_{(C-O)}$	1345.8(±0.2)	1341.0(±0.1)	1340.8(±0.0)
ν^{sk}_{pyr}	1252.4(±0.2)	1251.1(±0.0)	1254.2(±0.8)
$\nu_{(C-O-C)}$	1025.8(±0.0)	1025.7(±0.1)	1026.9(±0.0)
$\delta_{(arC-H/C-O)}$	917.8(±0.0)	915.8(±0.1)	921.3(±0.0)
	866.0(±0.1)	865.6(±0.0)	867.0(±0.0)
	816.9(±0.0)	816.7(±0.0)	817.5(±0.1)
	774.3(±0.0)	773.7(±0.0)	773.9(±0.0)

* *ν* – stretching vibration, *δ* – bending vibration, *as* – asymmetric, *sk* – skeleton, *ar* – aromatic moiety, *ph* – phenolic, *pyr* – pyrane ring
** B-Tm, "Bistriţa" variety (Topolovăţu Mic), B-M, "Bistriţa" variety (Măgulicea), P - "President" variety

The same observations can be done for the stretching vibration of the carbonyl group in flavonoids and hydroxycinnamic/benzoic acids (1716.2-1716.6 cm^{-1}), stretching vibration of the aromatic C#C bond in the benzene skeleton at 1636.2-1636.7 cm^{-1} (anthocyanins, flavonoids, acids having benzene skeleton etc.), or stretching vibration of the C-O-C group at 1025.8-1026.9 cm^{-1}. On the other hand, some bending vibrations have values in a wider range, such as in the case of those for the phenolic OH groups (1406.3-1408.6 cm^{-1}) or aromatic C-H and C-O groups from the 773-922 cm^{-1} region (Table 1). As a consequence, FTIR parameters (wavenumbers and intensities) were used as input in the PCA analysis for the evaluation of the similarity/dissimilarity between plum varieties.

3.2. Discrimination of plum varieties by FTIR-PCA coupling technique

An attempt was made to identify some FTIR parameters that would allow the discrimination of plum extracts from various varieties, using multivariate PCA analysis. Both the parameters corresponding to the characteristic wavenumbers and the band intensities were used. Generally, the band intensities from 1711 and 1258 cm^{-1} are important for the discrimination of plum samples on the positive side of the PC_1, while the wavenumbers at 3272 and 1711 cm^{-1} influence the classification through the negative side of the PC_1 (Figure 3). Moreover, the intensities and wavenumbers at lower values are important for the discrimination through the PC_2 (e.g., bands from the 818 and 772 cm^{-1}). A clear discrimination of the samples were obtained for plum varieties obtained from individual orchards ("Bistriţa" varieties, blue in Figure 4a), in comparison with the other plum varieties (red in Figure 4b). Also, duplicate samples are well grouped in both classes (e.g., "S" – "Stanley", "R" – "Renclod", "P" - "President" and "V" – "Vinete Românești" varieties, Figure 4a and b). The first three PCs explain 80.16% of the variance of the FTIR data, being sufficient for the discrimination (Figure 5).

Very good classifications were obtained if only FTIR wavenumbers or intensities were used as input variables for the PCA analysis. Thus, the "Bistriţa" samples are located in the right side of the PC_2 versus PC_1 scores plot when only wavenumbers were used as input variables (12 variables).

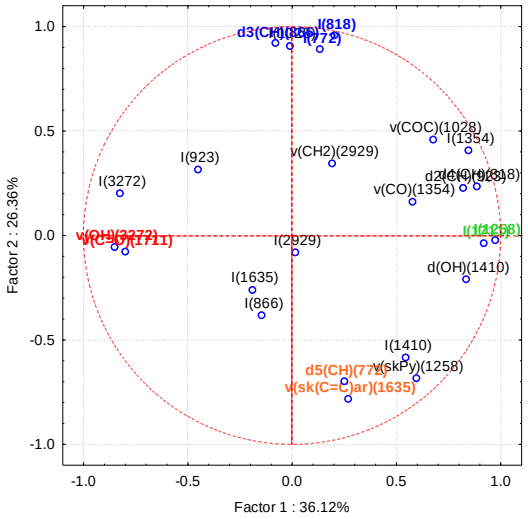


Figure 3. PC_2 *versus* PC_1 loadings plot from the PCA analysis of plum extracts using both FTIR wavenumers and intensities as input variables

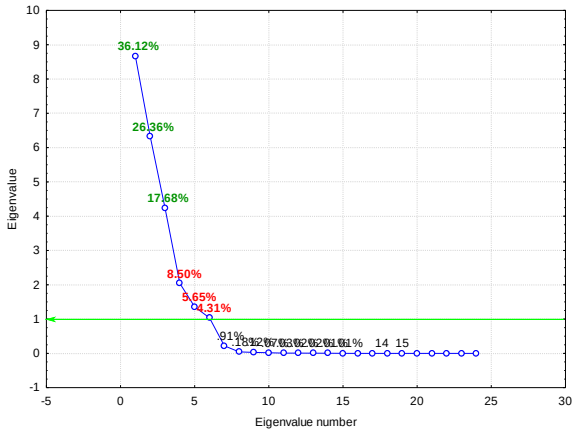
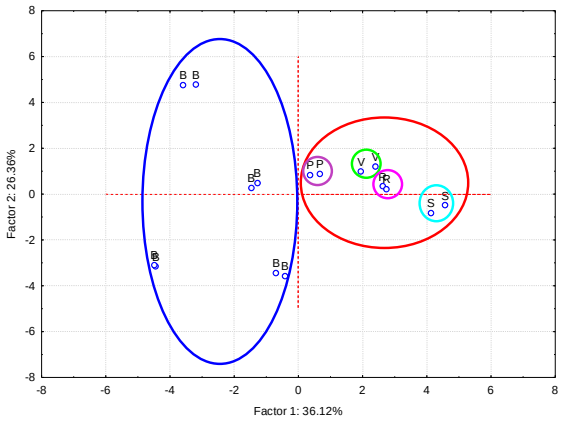
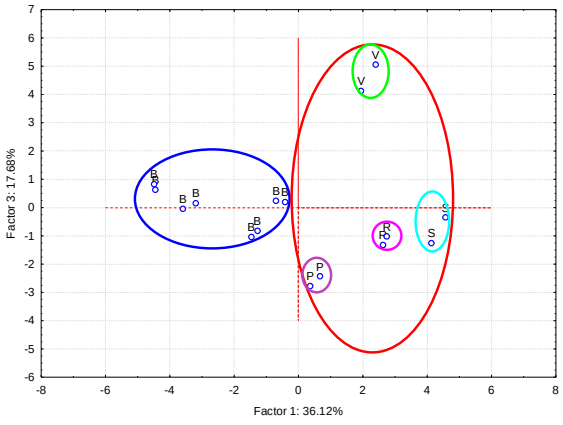


Figure 5. Eigenvalues for all principal components (24 variables) from the PCA analysis of plum extracts using both FTIR wavenumers and intensities as input variables; variables in green explain 80.16% of the variance of the data

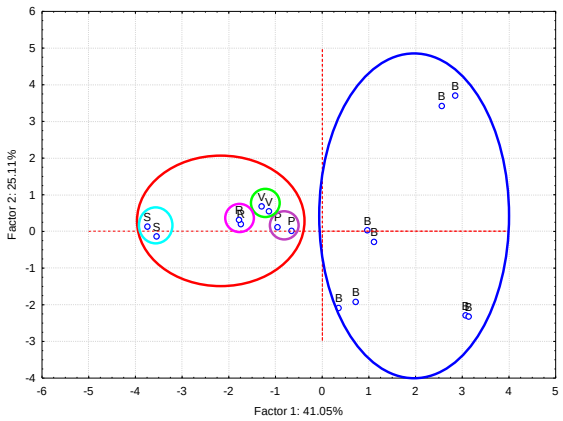


(a)

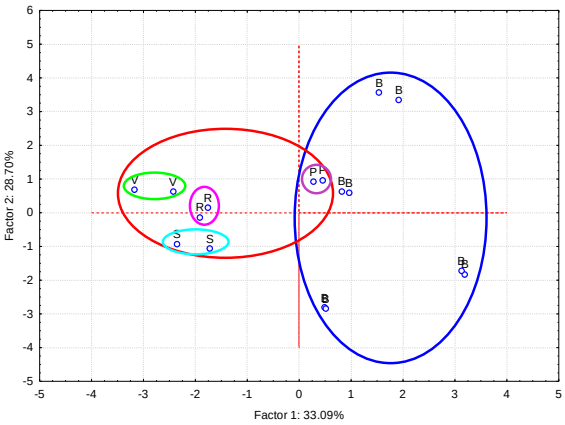


(b)

Figure 4. (a) PC_2 *versus* PC_1 and (b) PC_3 *versus* PC_1 scores plots from the PCA analysis of plum extracts using both FTIR wavenumers and intensities as input variables



(a)

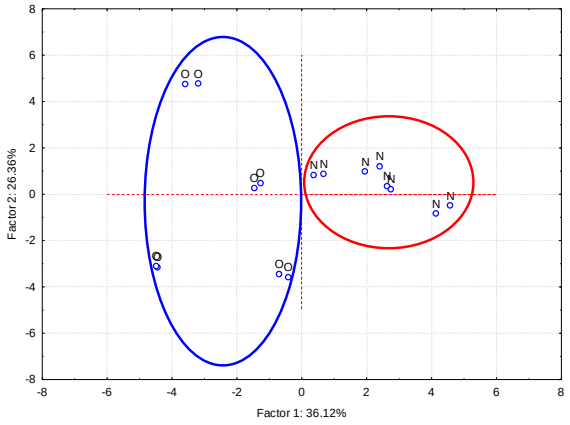


(b)

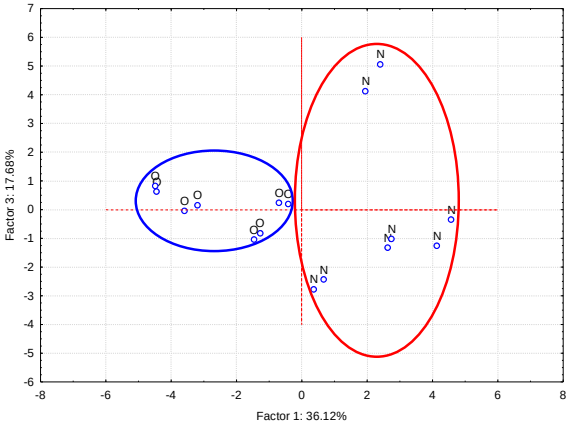
Figure 6. PC_2 *versus* PC_1 scores plots from the PCA analysis of plum extracts using only FTIR wavenumers (a) or intensities (b) as input variables

In this case, the first two PCs explain 66.16% from the variance of the data (Figure 6a). The classification is similar if only FTIR intensities were used as input variables for the PCA analysis (12 variables). However, there are small interferences for the "President". The explained variance is also close to the value above (Figure 6b).

Moreover, the discrimination based on the source of production (from private orchards, marked with "O", or from supermarkets/orchards of some producers, marked with "N") was also successful. When using all considered FTIR parameters (wavenumbers and intensities) and cultivars as samples, a representation of PC_2 *versus* PC_1 scores plot shown a clear discrimination of these two classes (Figure 7a).



(a)



(b)

Figure 7. (a) PC_2 *versus* PC_1 and (b) PC_3 *versus* PC_1 scores plots from the PCA analysis of plum extracts using both FTIR wavenumers and intensities as input variables and samples codes as "O" – for plum varieties collected from the individual orchards ("Bistriţa") and "N" for plum varieties purchased from supermarket or from conventional orchards

A homogeneous distribution of the parameters was observed, with a significant influence for PC_1 of the parameters corresponding to the range 1030-700 cm^{-1} (not presented). The discrimination was similar also for PC_3 versus PC_1 scores plot (Figure 7b). In both scores plots, "Bistriţa" varieties are located in the left of these representations.

4. Conclusion

Valuable discriminations between plum extract samples obtained from different varieties have been obtained by FTIR-PCA analysis of all wavenumber and intensity parameters. "Bistriţa" samples were clearly grouped against the other plum samples, which agreed with the differences observed by liquid chromatographic analysis. Moreover, commercialized plum varieties were also sub-classified, especially based on the stretching vibrations of the pyrane skeleton and for the carbonyl groups in antioxidant compounds. The CH and CO bending vibrations or the stretching vibration of the benzene skeleton (wavenumbers or intensities) were important for the discrimination of plum varieties. By far, the samples obtained from particular orchards (i.e., "Bistriţa" varieties) were clearly discriminated from the other plum varieties, revealing the quality of these autochthonous fruit varieties.

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