Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CHARACTERIZATION OF FOREST TREE SEEDS USING VIBRATIONAL SPECTROSCOPY

Luisa ANDRONIE[1], Ioana POP[1], Florica MATEI[1], Iulia COROIAN[1], Adina TRUȚĂ[1], Aurelia COROIAN[2], Ștefania ANDRONIE[2]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Forestry and Cadastre, 3-5 Calea Mănăştur Street, Cluj-Napoca, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, 3-5 Calea Mănăştur Street, Cluj-Napoca, Romania

Corresponding author email: andronie_luisa@yahoo.com

Abstract

The biological activity and the pharmaceutical properties of plants are strongly dependent on their structure. In the current study, the FT-IR spectroscopy technique was applied to detect molecular components in forest tree seeds samples. We used FTIR (Fourier transform infrared) spectroscopy because this tehnique gives information on specific functional chemical groups present in organic matter. However, the whole spectrum may also contain information that relates to more general chemical or biological properties. The FT-IR results revealed the presence of different bio-active compounds in pine such as flavonoids, tannins, sugars, acids, proanthocyanidins, carotenoids, citric metabolites and others. The comparison of the antioxidant activity of the studied forest tree emphasized a hierarchy of the antioxidant capacity in pineseeds and beechseeds. The purpose of the study was to investigate the antioxidant capacity and nutritional value of pineseeds and beechseeds. Antioxidant capacity was evaluated with photochemical assay as well as humidity, protein, fibre, lipid and carbohydrate content. The highest antioxidant capacity was observed in beechseeds 1292.79 ± 5.71 in comparation whit pineseeds 1.78 ± 0.39 (µg/mg equivalent ascorbic acid).
These results can provide useful information providing a research interest for the identification of new molecular compounds from forest tree seeds.

Key words*: pineseed (Pinus sylvestris), beechseed (Fagus sylvatica), FT-IR spectroscopy (Fourier transform infrared), antioxidants.*

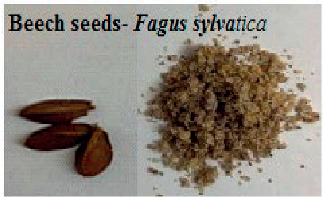
INTRODUCTION

Conifers, including Scots pine (*Pinus sylvestris* L.), are a major source of wood products for forest industry.
The various species of pine are distributed throughout the northern hemisphere, found in Asia, Europe and North America, both in the wild (in coniferous forests) and in pine plantations that exist in several countries. The main pine seed producing countries are China, North Korea, Russia, Pakistan, Afghanistan, Italy, Spain, Portugal, Turkey. (Sharashkin and Gold, 2004).
This type of seeds contains a large amount of vitamin A, as well as lutein, an essential antioxidant for preventing macular degeneration. The significant magnesium content in pine seeds makes them ideal, through consumption, for eliminating fatigue and stress.
Pinolenic acid is a polyunsaturated fatty acid found exclusively in pine nut oil. It may help lower LDL (bad) cholesterol levels in the blood (Lee et al., 2004). Selective increase in pinolenic acid (all-cis-5, 9, 12-18:3) in Korean pine (*Pinus koraiensis*) nut oil by crystallization and its effect on LDL-receptor activity.
Beech (*Fagus sylvatica* L.) species are largely represented in the European, Asia and North America flora and various parts of the tree are used in traditional medicine.
The non-extractable components in bark include polysaccharides (cellulose, hemicellulose and pectic substances), phenolic polymers (lignin and high-molecular-weight tannins) and cross-linked polyesters (cutin and suberin (El-Bahy, 2005).
Some of the most important health benefits of beech include its ability to stimulate hair growth, protect the immune system, improve the health of the skin, boost newborn health, reduce respiratory distress, prevent infections, relieve pain, detoxify the kidneys, and protect against chronic diseases (Takala et al., 2023).

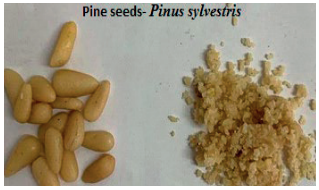
The beech acorn contains Omega 6 and Omega 3 which have been shown to have medicinal properties. Nuts of beech and pine are tasty nutrient-dense nuts produced in the fall by beech and pine trees (*Fagus* s., *Pinus* s.).
The biological activity and the pharmaceutical properties of plants are strongly dependent on their structure.
In the last year, FT-IR spectroscopy has been introduced as a very efficient and nondestructive analytical tool for the reliable way to determine the functional groups of tree components.
IR (infrared) spectroscopy is one such method which may be applied to dried samples and gives information on the broad chemical groups present depending upon the absorption peaks present. However, the whole spectrum may contain information that may relate to more general physical, chemical or biological properties. NIR (Near infrared reflectance) spectroscopy has been used extensively to assess changes during litter decomposition (McLellan et al., 1991), composting (Ben Dor et al., 1997) and for the prediction of soil (Couillard et al., 1997) and peat properties (HaÈmaÈlaÈinen and Albano, 1992; McTiernan et al., 1998).
FTIR is widely used in quantitative and qualitative analysis of wood because of its capacity to give information on the presence of functional groups, on composition and on some specific structural features (Esteves et al., 2013).
Fourier Transform Infrared Spectroscopy (FT-IR) techniques were used by Feria-Reyes et al., (2023) to evaluate the tannin content in the bark of five pine species.
Also, FT-IR spectroscopy has also been used to study the chemical and biological properties of peat in pine forests (Chapman et al., 2003).
The use of the FT-IR technique has also been reported for the analysis of food matrices (Andronie et al. 2016, Bebu et al. 2020), but literature mentions lots of works that involve the use of this technique and its great potential to be used in a large variety of other research fields (Andronie et. al. 2019; Keseru et al., 2016; Bebu et al., 2019, Andronie et al., 2022).
In this work, we employed FT-IR spectroscopy to characterize the spectra of the pine seeds *(Pinus sylvestris*) and beech seeds (*Fagus sylvatica*). Also, we analyzed the antioxidant capacity of the two types of seeds.

MATERIALS AND METHODS

In this research, we analyzed two different varieties of forest trees seeds (pine - *Pinus sylvestris* var. *hamata*) and beech *Fagus sylvatica tree* from the forests of Romania) according to the analytical information obtained from dried seeds by means of Fourier transform infrared spectroscopy (FT-IR). One common varieties of seeds from forest tress plants, pine seed *(Pinus sylvestris*), beech seed (*Fagus sylvatica*) was bought from a naturist store. The seeds samples were crushed using a commercial blender (Figure 1).



a)



b)

Figure 1. The forest trees seeds: beech - *Fagus sylvatica* (a) and pine - *Pinus sylvestris* (b)

Moisture determination
The determination of moisture content was evaluated by the air-oven method, according to the methodology previously described by Kumar and Balasubrahmanyam (1986) with slightly modifications. The weighted sample was introduced into a vial containing lid and conditioned at 105 °C for at least 45 minutes until a constant mass was obtained and therefore cooled in air-oven. Then 5-10 g of sample were spread uniformly on the entire surface of the vial and weighted with an accuracy of 0.001 g.
The humidity content was expressed in percentages.

FT-IR spectroscopy
The sample was prepared using calcinated potassium bromide as a matrix material and was mixed at a proportion of 3 mg of the sample (powder of pine seeds and beech seeds)

to 200 mg KBr. Then the mixture was condensed in 15 mm dies at a pressure equal to 10 t till 2 min. Same procedure was applied for all samples (Crișan et al., 2019)

Measurements were carried out on the infrared scale of 350-4000 cm^{-1} and a spectral resolution was set at 4 cm^{-1} using a Jasco FT-IR-4100 spectrophotometer (Oklahoma City, OK United States) using KBr pellet technique. All spectra were acquired over 256 scans.

The spectral data were analysed using Origin 6.0 software (Figures 2 and 3).

Measurements were carried out on infrared scale of 500-4000 cm^{-1}.

Photochemical assay

The ACL (Antioxidant Capacity of the Liposoluble Compounds) method previously described by (Hegedus et al., 2010), was used for estimating the antioxidant capacity. Photochem® equipment (Analytik Jena AG, Jena, Germany) was used for measuring the antioxidant capacity.

The hydrophilic and lipophilic radical-scavenging activities were measured using the ACL kits, provided by the manufacturer.

The extractions for ACL measurements were also carried out according to the manufacturer's protocol using Kit and methanol.

For these measurements we used 1 g of extract of the sample with 20 ml of methanol, then centrifuge the sample for 5 minutes (10,000 rpm).

The equipment calibration and measurement of samples were based on the inhibition of free radicals. The values of antioxidant capacity were achieved by establishing measurement curves that were compared to the measurement curves obtained for the standard solution.

The results were expressed in ascorbic acid equivalents (µg/mg). Data was calculated automatically using a software program called PCL soft.

Statistical analysis

The IBM SPSS v.19.0 for Windows, was used for statistical analysis. Basic statistics were implemented in order to emphasize the arithmetic mean (X) ± standard deviations (SD) of the content of humidity and antioxidant capacity of the studied forest seeds.

The mean concentration of humidity and antioxidant capacity were compared across the various forest plants using ANOVA followed by Tukey's test. Differences of the means were considered to be significant when p-value < 0.05 (Paunovic et al., 2019). ANOVA analysis was followed by Tukey's test in order to perform multiple comparisons regarding the antioxidant capacity of considered forest tree seeds.

RESULTS AND DISCUSSIONS

FT-IR analysis

The FT-IR spectra were used to identify the functional groups of the macronutrients bades on the IR absorption in typical spectral regions.

The characteristics of pine and beech seeds were the bands obtained from proteins, lipids and carbohydrates and these were clearly highlighted in the spectra obtained by the FT-IR technique.

Carbohydrates possess characteristic IR absorptions between 1200 and 750 cm relevant to coupling and combination of stretching and deformation or vibrational modes of individual bonds in the molecular skeleton.

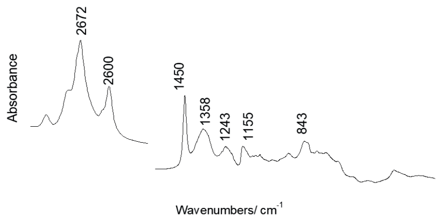


Figure 2. FT- IR spectrum of pine seeds (*Pinus sylvestris*)

The absorption bands for rocking of CH_2 may be found at 721 cm^{-1}.

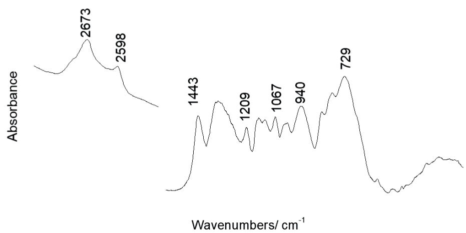


Figure 3. FT- IR spectrum of beech seeds (*Fagus sylvatica*)

The nutritional quality of the studied

The nutritional values for 100 g of two types of forest plants and the characteristic differences among them were present in Table 1.

Table 1. Nutritional parameters of the investigate seeds (100 g/sample)

Samples	Calories (kcal)	Lipids (g)	Protein (g)	Carbohydrates (g)	Fiber (g)
Pine	673±3.08	68.40±0.89	13.7±0.73	13.1±0.69	3.7± 0.89
Beech	619±2.05	51±1.39	17.21±1.24	20.59±0.34	11.5± 0.48

Note: *The mean difference at significant p< 0.05.

In pine seeds was identified the highest concentration in lipids and calories.

The beech seeds have the highest carbohydrates and fibers content in comparison with their content in pine. In addition, the beech showed an content of protein in comparison with pine.

Statistical analysis for the humidity content and antioxidant capacity

The test revealed significant differences between the mean amounts of calories, lipids and protein contained respectively in *Pinus sylvestris* and *Fagus sylvatica* (Table 1). Also, the amount of fiber contained in beech is significantly higher than that reported in pine seeds.

The Turkey test revealed significant differences between the mean amount of antioxidants contained respectively in the two categories of forest trees considered. The results of Turkey‘s test are displayed in Table 2.

Table 2. The content of humidity and antioxidant capacity in tested seeds

Samples	Humidity	Antioxidant Capacity (μg/mg equivalent ascorbic acid)
Pine (Pinus sylvestris)	0.39± 0.04	1.78 ± 0.08
Beech (*Fagus sylvatica*)	0.51 ± 0.07	1292.79 ± 5.71

Note: *The mean difference at significant p< 0.05.

The significance was determined at $p < 0.05$.

The results were expressed or plotted as the mean values ± standard deviation.

Relationships among antioxidant capacity and humidity content of pine and beech seeds are presented in Table 3. We obtained a strong significant positive correlation at $p<0.05$ level between antioxidant capacity and humidity content of considered seeds.

Table 3. Linear correlation coefficients between antioxidant capacity and humidity content of tested seeds

Samples	Antioxidant Capacity (μg/mg equivalent ascorbic acid) - *Pinus sylvestris*	Antioxidant Capacity (μg/mg equivalent ascorbic acid) - *Fagus sylvatica*)
Humidity - pine *(Pinus sylvestris)* %	0.62*	-
Humidity - beech (*Fagus sylvatica*) %	-	0.93*

Note: *Correlation is significant at p< 0.05

CONCLUSIONS

The data revealed that the beech have the highest antioxidant capacity when compared with pine samples. Beech samples from natural shop exhibit antioxidant capacity and more investigations should be performed in order to describe the complex interactions between antioxidants and the human body. It is promising to observe that molecular compounds, such as those in pine and beech seeds, may provide a potential interest in food and pharmaceutical research.

The comparison of the antioxidant activity of the studied forest tree seeds emphasized a hierarchy of the antioxidant capacity in pine seeds and beech seeds.

The resulted data revealed that the beech seeds have the highest antioxidant capacity. We obtained a strong significant positive correlation at $p<0.05$ level between antioxidant capacity and humidity content of considered seeds.FT-IR spectroscopy could provide quantitative information concerning the functional groups of treem components

REFERENCES

Andronie, L., Coroian, A., Miresan,V., Pop, I., Raducu, C., Rotaru, A., OLAR L. (2016). Results obtained by investigating saffron using FT-IR spectroscopy. *Bulletin UASVM Animal Science and Biotechnologies*, 73(2). 238-239.

Andronie, L., Holonec, L., Pop, I., Truta, A. M., Odagiu, A., Salagean, T., Sobolu, R., Coroian, A., Balta, I., & Șuba, E. E. (2019). Antioxidant Capacity of Several Romanian Forest Fruits (*Rosa canina* L., *Prunus spinosa* L., *Vaccinium vitis-idaea* L. and *Cornus mas* L.*). Notulae Botanicae Horti Agrobotanic,* Cluj-Napoca, 47(4), 1178-1184.

Andronie, L., Pop, I., Matei, F., Coroian, A., Rotaru, A., Sobolu. R., Maties, A., Roman, V., Bebu, A. (2022). Results obtained by investigating pumpkin (*Cucurbita maxima* L.) using ft-ir spectroscopy, *Scientific Papers. Series B, Horticulture*. Vol. LXVI, No. 2, Bucuresti.

Bebu, A., Andronie, L., Odagiu, A. (2019). Vibtational study for citrus fruits using Fourier Transform Infrared, *Scientific Papers, Series B, Horticulture*, Volume: LXIII, Issue: 1 Pages: 565-568.

Bebu, A., Andronie, L., Maties, A., Svetlana Micle, S., Darjan, S., Culcear, O. (2020). Comparative analysis between mushrooms *Lactus piperatus* and *Agaricus bisporus* (champignon) using FT-IR spectroscopy, *Scientific Papers. Series B, Horticulture*. Vol. LXIV, No. 1, 633-637.

BenDor, E., Inbar, Y., Chen, Y. (1997). The reflectance spectra of organic matter in the visible near-infrared and short wave infrared region (400± 2500 nm) during a controlled decomposition process. *Remote Sensing of Environment* , 61, 115.

Chapman, S.J., Campbell, C.D., Fraser, A.R., Puri, G. (2001). FTIR spectroscopy of peat in and bordering Scots pine woodland: relationship with chemical and biological properties, *Soil Biology & Biochemistry*, 33, 1193-1200.

Couillard, A., Turgeon, A.J., Shenk, J.S., Westerhaus, M.O. (1997). Near infrared refectance spectroscopy for analysis of turf soil profile. *Crop Science,* 37(5) 1554-1559.

Crișan, I., Vidican, R., Olar, L., Stoian, V., Morea, A., Ștefan, R. (2019). Screening for Changes on Iris germanica L. Rhizomes Following Inoculation with Arbuscular Mycorrhiza Using Fourier Transform Infrared Spectroscopy. *Agronomy*, 9:815. doi:10.3390/agronomy9120815.

E-Bahy, G.M.S. (2005). FTIR and Raman spectroscopic study of Fenugreek (*Trigonella foenum graecum* L.) seeds, *Journal of Applied Spectroscopy*, Vol. 72, No. 1.

Esteves, B., Marques, A., Domingos1, I,. Pereira, H. (2013). Chemical changes of heart treated pine and eucalypt wood monitored by FTIR, Maderas. *Ciencia y tecnología,* 15(2): 245-258.

Feria-Reyes, R., Ramírez-Cruz, S.O., Ruiz-Aquino, F., Robledo-Taboada, L.H., Sánchez-Medina, M.A., Mijangos-Ricárdez, O.F., Gabriel-Parra, R., Suárez-Mota, M.E., Puc-Kauil, R., Porcallo-Vargas, J. (2023).Pine Bark as a Potential Source of Condensed Tannin: Analysis through Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX). *Forests*, 14(7):1433.

HaÈmaÈlaÈinen, M., Albano, C. (1992). Principal variations in the chemical composition of peat: predictive peat scales based on multivariate strategies. *Journal of Chemometrics,* 6, 41-56.

Hegedus, A., Engel, R., Abrankó, L., Balogh, E., Blázovics, A., Hermán, R., Halász, J., Ercisli, S., Pedryc, A., Sefanovits Banyai, E. (2010). Antioxidant and Antiradical Capacities in Apricot (*Prunus armeniaca* L.) Fruits: Variations from Genotypes, Years, and Analytical Methods. *Journal of Food Science*,75(9):722-730.

Jin-Won, L., Kwang-Won, L., Seog-Won L., In-Hwan, K., Chul, R. (2004). Selective increase in pinolenic acid (all-cis-5,9,12-18:3) in Korean pine nut oil by crystallization and its effect on LDL-receptor activity. *Lipids*, 39(4):383-7.

Kesezu, A., Andronie, L., Maniutiu, D., Pop, I., Rotaru, A. (2016). Characterization *Momordica charantia* using FT-IR spectroscopy**.** *Bulletin UASVM Holticulture and Forestry*, 73(2).

Kumar, K.R., Balasubrahmanyam, N. (1986). Moisture sorption and the applicability of the Brunauer-Emmett-Teller equation for some dry food products. *Journal of Stored Products Research* 22(4):205-209.

McLellan, T.M., Aber, J.D., Martin, M.E., Melillo, J.M., Nadelhoffer, K.J. (1991). Determination of nitrogen, lignin, and cellulose content of decomposing leaf material by near-infrared reŻectance spectroscopy. *Canadian Journal of Forest Research*, 21, 1684-1688.

McTiernan, K.B., Garnett, M.H., Mauquoy, D., Ineson, P., Couteaux, M.M. (1998). Use of near-infrared re¯ectance spectroscopy (NIRS) in palaeoecological studies of peat. *Holocene*, 8, 729-740.

Paunovic, D., Kalusevic, A., Petrovic, T., Urosevic, T., Djinovic, D., Nedovic, V., Popovic-Djordjevic, J. (2019). Assessment of Chemical and Antioxidant Properties of Fresh and Dried Rosehip (*Rosa canina* L.) *Notulae Botanicae Horti Agrobotanici,* Cluj-Napoca 47(1)108-113.

Sharashkin, L., Gold, M. (2004). Pine nuts: species, products, markets, and potential for U.S. production. In: Northern Nut Growers Association 95th Annual Report. Proceeding for the 95th annual meeting, Columbia, Missouri, August 16-19.

Takala, R., Ramji, D.P., Choy, E.(2023). The Beneficial Effects of Pine Nuts and Its Major Fatty Acid, Pinolenic Acid, on Inflammation and Metabolic Perturbations in Inflammatory Disorders. *Int J Mol Sci*. Jan 6;24(2):1171**.**