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# RESULTS OBTAINED BY INVESTIGATING PUMPKIN (*Cucurbita maxima* L.) USING FT-IR SPECTROSCOPY

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***Abstract***

*Cucurbita maxima is a plant with a flexible and hanging stem that belongs to the Cucurbitaceae family. The fruit of this species (pumpkin) is characterized by a strong orange peel with distinctive grooves. The Cucurbita maxima L. has a considerable economic importance, beyond this variety huge quantities of seeds and shells can be obtained. Although the shells do not have a final application, they contain large amounts of tocopherols and five times more carotenoids than seeds.*
*Pumpkins are commonly grown for human consumption, for decoration, and also for livestock feed. Because, the pumpkin is cultivated in many countries having very important medical properties are necessary more studies regarding his bioactive compounds and nutritional values. Vibrational spectral techniques, FT-IR, offer several advantages in the context of current research and using this techniques we can identify molecular components in the samples studied. IR spectroscopy is based on the absorption of radiation in the 400-4000 cm$^{-1}$ range which excites molecular vibrations. Our research had the objective to analyze a vibrational spectra of C. maxima pumpkin variety seeds and pulp, using a method which is based on microscopic infrared study (FT-IR).*

***Key words****: pumpkin seed and pulp, FT-Raman (Fourier-transform infrared spectroscopy*).

## INTRODUCTION

*Cucurbita maxima* is an annual herbaceous plant cultivated for its fruit, flowers and seeds. It is an annual plant with a flexible and hanging stem. It has cordiform (heart-shaped) leaves, pentalobulate, large in size with well-marked veins. The flowers are yellow and fleshy, the fruit has a great variation: it can be ovoid or spherical, green or deep orange.
The fruits of this domestic species, pumpkins, (Fig,1), contains inside numerous oval, convex and smooth seeds 2-3 cm long, which in turn contain a white core. There are summer varieties with light-colored fruit and softer seeds, and the autumn varieties that are drier and sweeter, used in confectionery.
The pulp of the fruit is yellow-orange, dense and sweet in taste (Lopez-Anido 2021). Pumpkin is one of the lowest calorie vegetables, instead it is a rich source of antioxidant dietary fiber and vitamins. It is also a rich source of many polyphenolic flavonides, such as alpha and beta carotene, lutein. Carotenes are converted into vitamin A in the body according to needs. Pumpkin also has a high content of fiber, fatty acids and phytosterols (Xiong, 2002). Pumpkin pulp contains many nutritional components, including polysaccharides, carotenoids, minerals, amino acids and active proteins (Zhou et al., 2007). The pulp is a rich source of vitamins: A, B, C, E (Men et al., 2020). Pumpkin seeds content an important source of energy because the values reported to the dry matter is 30-70% protein and 40-50% fat (Winkler et al., 2002; Tepper et al., 2000). In addition, pumpkin seeds are a good source of vitamins and minerals importants for human health: Ca, K, P, Mg and Zn (Fan and Li, 2005).
Pumpkin is a plant that is cultivated and widely found in Romania. There is an increasing interest for many varieties uses of pumpkin because of their high nutritional value, digestive effects and good sensory characteristics (Noelia et al., 2011; Konopacka et al., 2010). The pulp of pumpkin is usually used in soups, pies, and

bakeries while the seeds are used as a snack and pharmaceutical. Therefore, pumpkin has high potential application in the food, cosmetic and pharmaceutical industries. Due to the good acceptance and industrialization of the derived products from pumpkins, more scientific information about the chemical and physicochemical properties of this fruit is important (Quintana et al., 2018).

Figure 1. Pumpkin (*Cucurbita maxima*)

Carotenoids, a complex family of isoprenoids, are relevant in plant foods and agriculture, as nutrients, antioxidants, pigments, and ripening indicators (Sharma et al., 2013; Fiedor and Burda, 2014). They are the main reason for orangered yellow colors of plant tissues, they act as antioxidants, and confer benefits for human health, including provitamin A activity in some cases (Rodriguez-Amaya, 2015).

Fourier transform infrared spectroscopy (FTIR) is a potential alternative for simplifying the analysis of food constituents (Andronie et al., 2021; Keseru et al., 2016; Andronie et al., 2019). Several FTIR-based methods have been developed to discriminate and determine various properties of food matrices (Bassbasi et al., 2014; Biancolillo et al., 2020; Belton et al., 1995; Mellado-Mojica et al., 2016; Anjos et al., 2015, Andronie et al., 2016; Bebu et al., 2020) showcasing an important reduction of chemicals and time consumption, as the main advantage compared to conventional analytical techniques. Moreover, the use of attenuated total reflectance (ATR) devices simplifies the application of FTIR for qualitative and quantitative analysis of materials, including food matrices.

The capability of this technique for the determination of carotenoids in food matrices has been demonstrated in tomatoes, with good performance in lycopene quantification (De Nardo et al., 2009). However, there are no many previous studies of either FTIR-ATR or other MIR-based techniques for the evaluation of carotenoids in pumpkin or squash pulp, two of the richest sources of these compounds among fresh produce.

The pulps of pumpkins having a high percentage of carbohydrate and the seeds are characterized by a high amount of oil and protein.

The Fourier-transform infrared spectra in combination with chemometrics and fatty acid composition was used to analyze pumpkin seed oil blended into extra virgin olive oil (Rohman et al., 2013).

The purpose of this study was to analize the minerals and determination of total polyphenols in pumpkin powder and also to study functional groups of the pumpkin seed using FT-IR spectroscopy.

## MATERIALS AND METHODS

***Experimental for determination of total polyphenols in pumpkin***

The pumpkin powder was obtained by drying the pumpkin pulp at room temperature for 7 days.

The principle of the method for the determination of total polyphenols in ingredients of plant origin such as pumpkin powder is that in a basic medium and in the presence of phenols, the mixture of phosphotungstic and phosphomolybdic acids is reduced to blue oxides of tungsten and molybdenum with maximum absorption of 765 nm.

In order to obtain pumpkin pulp extract, 40 ml of methanol (extraction solution) was added to 5 grams of pumpkin powder, mixed with the laboratory blender, and then centrifuged for 5 minutes at 10,000 rpm. 5 ml of distilled water, 1.5 ml of sodium carbonate solution (10%), 0.5 ml of sample extract and 0.5 ml of Folin-Ciocalteu solution were added into a 15 ml vial. The samples were left for 45 minutes in the dark at room temperature and then measure at a wavelength of 765 nm against a blank solution (ethanol). The measurements are compared with a gallic acid calibration curve (25, 50, 100, 250 ppm) and the results are expressed in gallic acid equivalent.

For the determination of total polyphenols we used a Lambda 25 Ultraviolet-Visible Molecular Absorption Spectrophotometer, Perking Elmer.

***Experimental for determination of minerals***

The preparation of the samples consisted of weighing 0.2 grams of powder obtained from pumpkin seeds and placed in a Berzelius beaker. A volume of 9 ml of 65% $HNO_3$ was added to the weighed sample and placed on a sand bath for mineralization. The sample was boiled in the sand bath for about an hour, after which the glass was removed from the sand bath and allowed to cool. After cooling, 2 ml of 30% hydrogen peroxide ($H_2O_2$) were added, each glass containing the mineralized sample for complete oxidation of the organic matter and left on the sand bath for about 20 minutes. The resulting solution was filtered into 25 ml volumetric flasks, flushed with ultrapure water and transferred to plastic bottles. The samples were then analyzed with an inductively coupled plasma-optical emission spectrometer.

The Perkin Elmer inductive coupled plasma mass spectrometer, ELAN DRC II type, with quadrupole and equipped with a reaction cell for the elimination of interferences, with a mass range of 3-240 m/z, resolution below 1 amu (IU-36, Serial Q1970307H), was used to determine metals.. The device is calibrated using standard calibration solutions prepared from stock solution (Multi-element Calibration Standard 3, PerkinElmer).

***Experimental for FT-IR spectroscopy***

The seeds samples of pumpkin were crushed using a commercial blender obtaining a powder that was used on the same day.

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.

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 seeds) to 200 mg KBr. Then the mixture was condensed in 15 mm dies at a pressure equal to 10 t till 2 min (Crișan et al., 2019). All spectra were acquired over 256 scans. The spectral data were analysed using Origin 6.0 software (Figure 2).

***Statistical analysis***

The IBM SPSS v.19.0 for windows, was used for statistical analysis. Basic statistics, was implemented in order to emphasize the arithmetic mean (X) ± standard deviations (SD) of the content of the total polyphenols and minerals in the pumpkin powed. The mean concentration of minerals was compared using One-Sample T-test with the standard values reported by USDA (The U.S. Department of Agriculture) relative to different categories of pumpkin seeds: dried pumpkin seeds respectively pumpkin seeds, roasted without salt. Differences of the means were considered to be significant when p-value < 0.05 (Paunovic et al., 2019).

## RESULTS AND DISCUSSIONS

***Polyphenols results***

The result obtained after the determination of the total polyphenols in the pumpkin powder of pulp is presented in table 1 and it can be seen that it does not exceed the maximum allowed limits, being 480 mg/kg.

Table 1. Total polyphenols

| Sample (grams of pumpkin powder) | \Extraction volume (ml) | \Result mg /kg GAE equivalent |
|---|---|---|
| 5 ± 0.11 | 40 | 480 ± 0.02 |

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

***Mineral results***

Following the determination of the minerals in the pumpkin powder of pulp, it was observed that the analyzed samples did not contain heavy metals (cadmium, cobalt, nickel = <LDS).

The unit of measurement of the samples is mg/kg and the results obtained are presented in tables 2. The values presented in tables 2 reveal that the pumpkin core has a high content of calcium (Ca)-1048.1 and potassium (K)-1045.3 mg/kg. Also important is the concentration of magnesium (Mg)-718.2 mg/kg. The content of the other minerals can be seen in the tables presented.

***Statistical analysis for the minerals content***

ANOVA analysis was followed by Tukey's test in order to perform multiple comparisons regarding the minerals content in the pumpkin powder and the Turkey test revealed significant differences between the mean amount of minerals contained.

Table 2. The results obtained for minerals

| | | | |
|---|---|---|---|
| **Ca (mg/kg)** 1048.1±0.8 | **K (mg/kg)** 1045.3±0.82 | **Mg (mg/kg)** 718.2±0.78 | **Fe (mg/kg)** 96.8±0.34 |
| **Na (mg/kg)** 88.9±0.37 | **Al (mg/kg)** 17±0.3 | **Cu (mg/kg)** 15±0.8 | **Zn (mg/kg)** 9.3±0.36 |
| **Sr (mg/kg)** 4±0.3 | **Pb (mg/kg)** 0.6±0.1 | **Co (mg/kg)** <LOD | **Ni (mg/kg)** <LOD |
| **Cr (mg/kg)** <LOD | **Cd (mg/kg)** <LOD | **Mn (mg/kg)** <LOD | **Ba (mg/kg)** <LOD |

*LOD= below the detection limit of the device

The average amount of Ca, Fe contained in the pumpkin powder of pulp is statistically significantly higher than the reference values specified by USDA for dried pumpkin seeds respectively for pumpkin seeds, roasted without salt.

The average amount of K, Zn recorded in the pumpkin powder of pulp is statistically significantly lower than the reference values specified by USDA for dried seed or roasted seeds.

The average amount of Na determined in the pumpkin powder is statistically significantly higher than in the case of dried pumpkin seeds respectively statistically significantly lower than in the case of roasted pumpkin seeds without salt.

***FT-IR analysis***

We used FT-IR spectroscopy to evaluating the functional groups as part of a compound in chemical analysis. In the food industries, this technique it was also used as a monitoring quality control because of its rapid screening and quantification of chemical components in samples.

Most chemical bond components have vibrational movements in the medium infrared spectrum (4000-350 $cm^{-1}$), such as lipids, proteins, carbohydrates and nucleic acid molecules.

The FT-IR spectrum was used to identify the functional groups of the active components present in the sample, based on the peak's values in the region of IR radiation. The presence of carotenoids, amides of proteins was reported, according to FT-IR analysis.

As can be seen in Figure 2, the FTIR spectra of pumpkins *(Cucurbita maxima* L.) of seeds were evaluated in frequency of 4000-500 $cm^{-1}$.

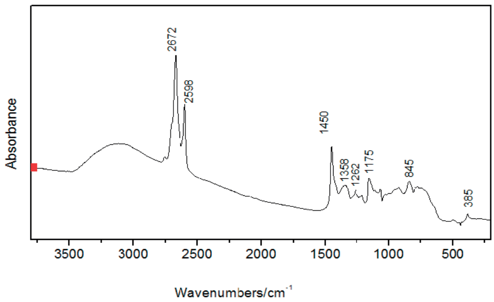


Figure 2. FT-IR spectra of pumpkin (*Cucurbita maxima*) seeds

Generally, a frequency at 3450-3250 $cm^{-1}$ is hydroxyl groups (O-H) from carbohydrate or other compounds such as carboxyl acid and ketone. The absorption around 3120-3030 $cm^{-1}$ is associated with (N-H) groups from amide. The bands at 1105-1010 $cm^{-1}$ is associated with lipids (Indrianingsih et al. 2019). The range of frequency at 2950-2850 $cm^{-1}$ is related to CH absorptions.

The region of approximately 1450 $cm^{-1}$ is associated with vibrations of antisymmetric deformation of $CH_3$ groups (change in HCH angles) and $CH_2$ groups (scissor vibrations) (Berezin et al., 2005; Schluckeer et al., 2003).

## CONCLUSIONS

Based on the obtained results, it can be concluded that the FT-IR spectroscopy is a reliable instrumental technique for the determination of mean components in seeds of plants.

The results of this study will be used for the preparation of new products and the extraction of microcomponents with technological functionality.

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