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Nitrogen fertilization affects Fourier Transform Infrared spectra (FTIR) in *Physalis* L. species

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ABSTRACT

Assessing the influence of fertilization on medicinal plants in conjunction with spectral analysis may indicate alterations in the chemical profile of the species in response to nitrogen fertilization. The objective consisted in characterizing by fractions of plants of *Physalis peruviana* and *Physalis angulata*, known by cape gooseberry and camapú, respectively, in different nitrogen doses, by means of analysis of Attenuated Total Reflectance in the Infrared with Fourier Transform (ATR-FTIR). The experiment was carried out in individual pots with completely randomized substrate design with 5 repetitions, using four doses of N (0, 200, 400 and 600 Kg ha^{-1}). The fractions of leaves, stems and roots of both species were characterized by ATR-FTIR spectroscopy. The specific compounds related to the functional groups present in the fractions of the analyzed species, such as cellulose, pectin and phenolic compounds, are induced as a function of nitrogen fertilization, altering the common absorption peaks. The study offers a precise means to identify functional groups present in the species of the genus and with possible pharmacological use.

1. Introduction

The genus *Physalis* (Solanaceae) has more than 100 species that are characterized by the presence of a calyx, enveloping and protecting the fruit against herbivores and weathering (Silva et al., 2013). Among the species, *Physalis peruviana* L., native to the Andes region, has been incorporated into small fruit crops, with high productive potential for subtropical regions (Moura et al., 2016; Trevisani, et al., 2016). The specie *Physalis angulata* L. is native to Brazil and is known for its medicinal use and potential for commercialization (Lorenzi and Matos, 2008).

Due to the great potential of the genus, research is conducted with the species mainly for the identification and evaluation of substances that have medicinal potential (Chang et al., 2016; Sisley et al., 2017), but few are related to their cultivation and management. In this line of research, the works carried out by Rodrigues et al. (2014) and Moura et al. (2016) stand out for *P. peruviana*. For *P. angulata*, the studies developed by Cruz et al. (2015) and Leite et al. (2017). However, more studies are needed for the establishment of commercial crops of these species, especially related to mineral nutrition, either for the production of fruits or phytopharmaceuticals.

In natural or cultivated environments, plants are subject to nutritional stress, due to the lack or excess of nutrients, as in the Brazilian semi-arid, one of the regions of occurrence of *P. angulata* and which, according to Freitas et al. (2011), has soils with low nitrogen levels. Among the macronutrients, nitrogen is one of the most required for the growth of plants and, in addition, its availability influences the concentrations of secondary compounds (Ibrahim et al., 2011). Plants of the genus *Physalis* present phenolic compounds (mainly physalins), flavonoids and antioxidant activities, these are substances that play important roles in the protection and prevention of different diseases (Olivares-Tenorio et al., 2016; Carniel et al., 2016).

Reconciling studies of mineral nutrition in medicinal plants, such as those of the genus *Physalis*, with infrared analysis may indicate the variability of the chemical profile in response to the fertilization used. In this sense, infrared spectroscopy with Fourier Transform (FTIR) offers a fast and non-destructive way to obtain a biochemical fingerprint of the samples, where the main functional groups and connections can be identified, providing structural information about the chemical compounds present (Palacio et al., 2014).

There are no literary studies available related to the analysis of infrared spectra on the influence of mineral nutrition in the fractions of

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Fig. 1. *Physalis angulata*, also known as camapú (left); *Physalis peruviana*, also known as cape gooseberry (right).

leaves, stems and roots of the genus *Physalis*. In view of the above, the present work has the objective of characterizing different fractions by means of ATR-FTIR of plants of *P. angulata* and *P. peruviana* under different nitrogen doses.

2. Material and methods

2.1. Obtaining plant material

The study was realized at the Higher Technical School of Agricultural Engineering, University of Valladolid, Palencia Campus, Spain, during the period from February to June 2017. *Physalis angulata* and *Physalis peruviana* plants were grown in a greenhouse under natural photoperiod, with opening the zenith window when the temperature exceeded 22 °C (Fig. 1). The pots were maintained at field capacity throughout the experimental trial.

The plants were produced from seeds obtained from matrices of *P. angulata* and *P. peruviana* cultivated in the Horto Florestal Experimental Unity, belonging to the State University of Feira de Santana, Brazil. The seeds were sown in a polypropylene seedbed with commercial substrate and kept on heated benches. 30 days after the emergence of the seedlings, when they reached two pairs of true leaves, the transplant was performed for individual pots with 65% commercial substrate, 30% soil and 5% washed sand. The chemical characteristics of the substrate were, in $g\,m^{-3}$: NO_3-N = 84; NH_4-N = 60; K_2O = 288; SO_3 = 18.5; MgO = 27.75; P_2O_5 = 164.88; Fe = 8.58; Mn = 3.17; CaO = 2.50; Mo = 2.43; Cu = 1.94; Zn = 0.98; B = 0.46; and pH 6.0.

The design used was completely random, with two species – *Physalis angulata* and *Physalis peruviana*, 4 treatments (0, 200, 400 and 600 $kg\,ha^{-1}$ of N) and 5 repetitions. The doses were defined according to the recommendation of 400 $kg\,ha^{-1}$ for the tomato crop in Spain according to the Ministry of Agriculture and Fisheries, Food and Environment (Mompó and García, 2010), using calcium ammonium nitrate to supply the needs of N.

2.2. Sample preparation and FTIR measurements

The culture was completed 60 days after the transplant and three plants were used per treatment for the analyzes, defined at random. The samples were separated into different fractions (leaves, stems and roots), brought to the oven at 60 °C until reaching a constant weight, following the analogous procedure described by Cruz et al. (2015) and then weighed on an analytical balance. To obtain a 1 mm powder, the samples were crushed in an ultracentrifugal mill and homogenized (Sanchez-Sastre, 2016).

The prepared samples were analyzed and characterized by infrared attenuated total reflectance spectroscopy with Fourier transform (ATR-FTIR) using ThermoNicolet iS50 spectrophotometer (ThermoFissher Scientific, Walthanm, MA, USA). The spectra were recorded in the medium infrared range (4000–400 cm^{-1}) at a spectral resolution of 4 cm^{-1}, with 32 scans per sample (Carrión-Prieto et al., 2017).

2.3. Statistics

The vibratory data were analyzed with the software SIGMAPLOT 11.0 (Systat Software Inc., Chicago, USA), focusing on the region of the fingerprint (1900–800 cm^{-1}). The comparison was made in relation to the FTIR spectral peaks and analysis of the corresponding functional groups. The data were correlated with the presence of spectral peaks characteristic of the species analyzed in different levels of fertilization. The dry mass data have been subjected to the analysis of variance and regression, adjusting the equations of the evaluated characteristics, as dependent variables of the nitrogen concentrations (Leite et al., 2017).

3. Results and discussion

3.1. Spectroscopic analysis

The different infrared spectra are presented in Fig. 2, where the absorption peaks of the different functional groups are observed for the species analyzed in the control treatment (0 $Kg\,ha^{-1}$ of N). The highest absorption peaks in leaves, stems and roots, occurred at wavelengths between 3500 and 3000 cm^{-1}, corresponding to the absorption due to the stretching of the OH bands (Jones, 2012), at 2920 cm^{-1} and 2850 cm^{-1}, attributed to the presence of polysaccharides, lipids and carbohydrates (CH stretch) (Cao et al., 2017), and in the range of 1900–800 cm^{-1}, which is the fingerprint region, in the which occurs most of the variations of infrared absorption (Carrión-Prieto et al., 2017).

The common peaks observed in the spectra of both species are the fingerprints and the exclusive peaks represent the presence of specific compounds that can be induced due to environmental stress and seasonal changes (Kumar et al., 2016), as well as under the amount of nutrients supplied. Variations in absorbance were observed in the fingerprint range for the different amounts of N and between the fractions of *P. angulata* and *P. peruviana*, showing the influence of nitrogen fertilization on the functional groups present (Fig. 3).

The peaks in the region of the phenolic rings (Carpita et al., 2001) were observed in the different fractions of the species analyzed. In the leaves of *P. angulata* the deficiency of N promoted a greater absorbance for this structural group (Fig. 3a). According to Godoy et al. (1997), plants adapted to poor nutrient environments, such as *P. angulata*, have low levels of nitrogen and high content of phenolic substances in the leaves, which is an important characteristic for commercial crops for phytopharmaceutical production. Increases in phenols under nitrogen deficient conditions were also reported by Ibrahim et al. (2011), being a behavior different from that observed for the species *P. peruviana*, where an increase in the content of the phenolic compounds is suggested with the supply of N (Fig. 3b).

The low absorbance values at the wavelengths of 1339 and

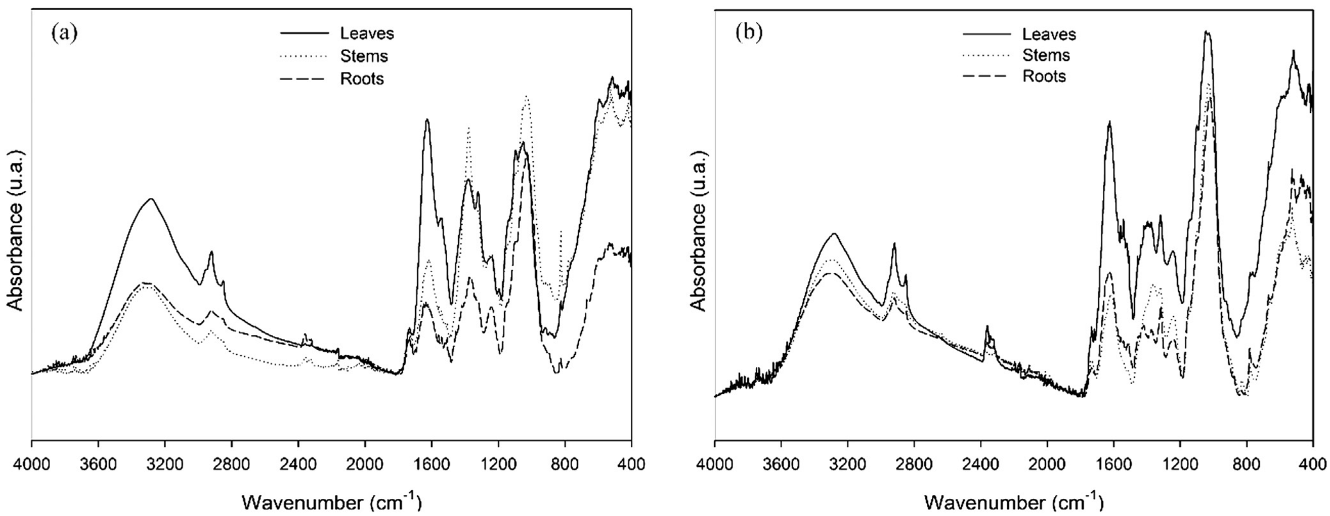

Fig. 2. Infrared spectra with Fourier transform by species and different parts of plants of the genus *Physalis*, with correction of the baseline: (a) *Physalis angulata* and (b) *Physalis peruviana*.

1143 cm^{-1} were observed in the condition -N (without nitrogen supply) in the stems of *P. angulata*, and in 1370, 1342 and 1318 cm^{-1} for the fractions of leaves and stems of *P. peruviana*, which evidences a lower amount of cellulose (Rubio-Díaz et al., 2012), on the other hand, the highest peaks at 1145 and 1104–1100 cm^{-1} for both species correspond to the increase of pectin (Fig. 3c and d) (Coimbra et al., 1999). Changes with FTIR spectroscopy for cellulose and pectin were also observed by Fernandes et al. (2013) when analyzing grapevine cells (*Vitis vinifera*) under an N deficiency, affirming that these compounds are the main ones to be affected by mineral stress.

The N doses also caused changes in the absorbance of the spectra for the roots of the two species, with different behaviors between 1500 and 1200 cm^{-1}. Other bands were highlighted at 826 and 829 cm^{-1}, due to the stretching of the aromatic ring (Namiesnik et al., 2014) (Fig. 3e and f). The functional cell region of cellulose, hemicellulose and lignin in the roots showed higher peaks (1374, 1318 and 1242 cm^{-1}) when 400 kg ha^{-1} of N was supplied in *P. angulata*, and, for *P. peruviana*, the absorbance peaks in 1373, 1317 and 1238 cm^{-1} were higher when supplying 600 kg ha^{-1} of N. The peaks related to the compounds and phenolic rings for this fraction underwent a greater influence of nitrogen fertilization in plants of *P. angulata*.

Phenolic compounds were found in several species of the genus *Physalis* (Medina-Medrano et al., 2015; Silva et al., 2016; Abreu et al., 2017), hence, for a better characterization, the absorption spectrum of these substances was enlarged in Fig. 4. The peaks observed in the 1730 cm^{-1} band of the fractions evaluated may be associated with the presence of phospholipids or due to the absorption of estercarbonyl (C=O), which are basic structures of the phenolic compounds (Cobaleda-Velasco et al., 2017a). A reduction in absorbance was also observed for this functional group in leaves of *P. angulata* with the increase of nitrogen fertilization, being a different behavior from that observed in the same fraction of *P. peruviana* (Fig. 4a and b). Bertoncelli et al. (2016) verified a decrease in the concentration of total phenolic compounds in fruits of *Physalis pubescens* and *Physalis peruviana* with the increase of nitrogen in the fertilization.

3.2. Physalins and antioxidant capacity

The physalins, one of the characteristic phenolic compounds of the genus *Physalis*, are easily detected by infrared spectroscopy by virtue of the different oxygenated functions present in the group, limiting the carbonylated absorptions of the ketones, enones, lactones (α and γ) whose bands can occur between regions of 1790–1650 cm^{-1} (Tomassini et al., 2000). Stretches related to physalins were observed in all fractions of *P. angulata* and *P. peruviana* (Fig. 4). The most accentuated variations according to the fertilization occurred in the leaves of both species (4a and 4b), agreeing with Ertürk et al. (2017), which affirm that the leaves of *P. peruviana* present a greater quantity of phenolic compounds in relation to the other fractions of the plant.

The fractions of the stems of *P. angulata* and *P. peruviana* did not present large variations of absorbance depending on the doses of N (Fig. 4c and d). The spectra of the roots of *P. peruviana* showed a similar behavior (Fig. 4e and f). According to Cobaleda-Velasco et al. (2017b), the roots of *P. angulata* do not present a significant quantity or diversity of phenolic compounds, however, it is possible to observe that there was an increase in the absorption in 1733 cm^{-1} when supplying the fertilization recommended for the tomato, being able to indicate an increase in the production of these compounds (Fig. 4e).

The antioxidant capacity of both species is correlated with the peaks in the 1630 cm^{-1} region, probably as a function of the flavonoid compound (Moţ et al., 2011) (Fig. 4). Higher absorbance values were observed in leaves of *P. angulata* without nitrogen fertilization, indicating higher concentrations of flavonoids, followed by absorbance values for the stem and root. Abreu et al. (2017) evaluated *P. angulata* plants cultivated in different periods of the year, found a higher content of total flavonoids in the fraction of the leaves, which was justified in function of the aerial part of being exposed to luminosity. The species *P. peruviana* presented a similar behavior, although its antioxidant capacity is reduced under deficient conditions of nitrogen.

3.3. Dry matter content

However, the total amount of the phenolic compounds produced can be reduced indirectly with the excess of nitrogen fertilization, since there is a tendency to reduce the dry matter of the fractions for both species, especially in the leaves, the main responsible organ of the production of the physalin compound (Fig. 5). The amount of dry matter for all the fractions of *P. angulata*, and leaves and stems of *P. peruviana* presented a quadratic behavior, similar to Leite et al. (2017) when evaluating the growth of *P. angulata* under nitrogen doses. For the dry matter of the roots of *P. peruviana* a linear regression was observed.

It is believed that plants growing under low nitrogen levels conditions contain more compounds of secondary metabolites than plants that grow in an environment with high levels of this nutrient (Ibrahim et al., 2011). What can explained by the hypothesis of the carbon:nutrient balance, when nutritional limitation (in this case nitrogen) has a more adverse effect on growth than photosynthesis, and there is accumulation of carbohydrates that are not used for biomass production

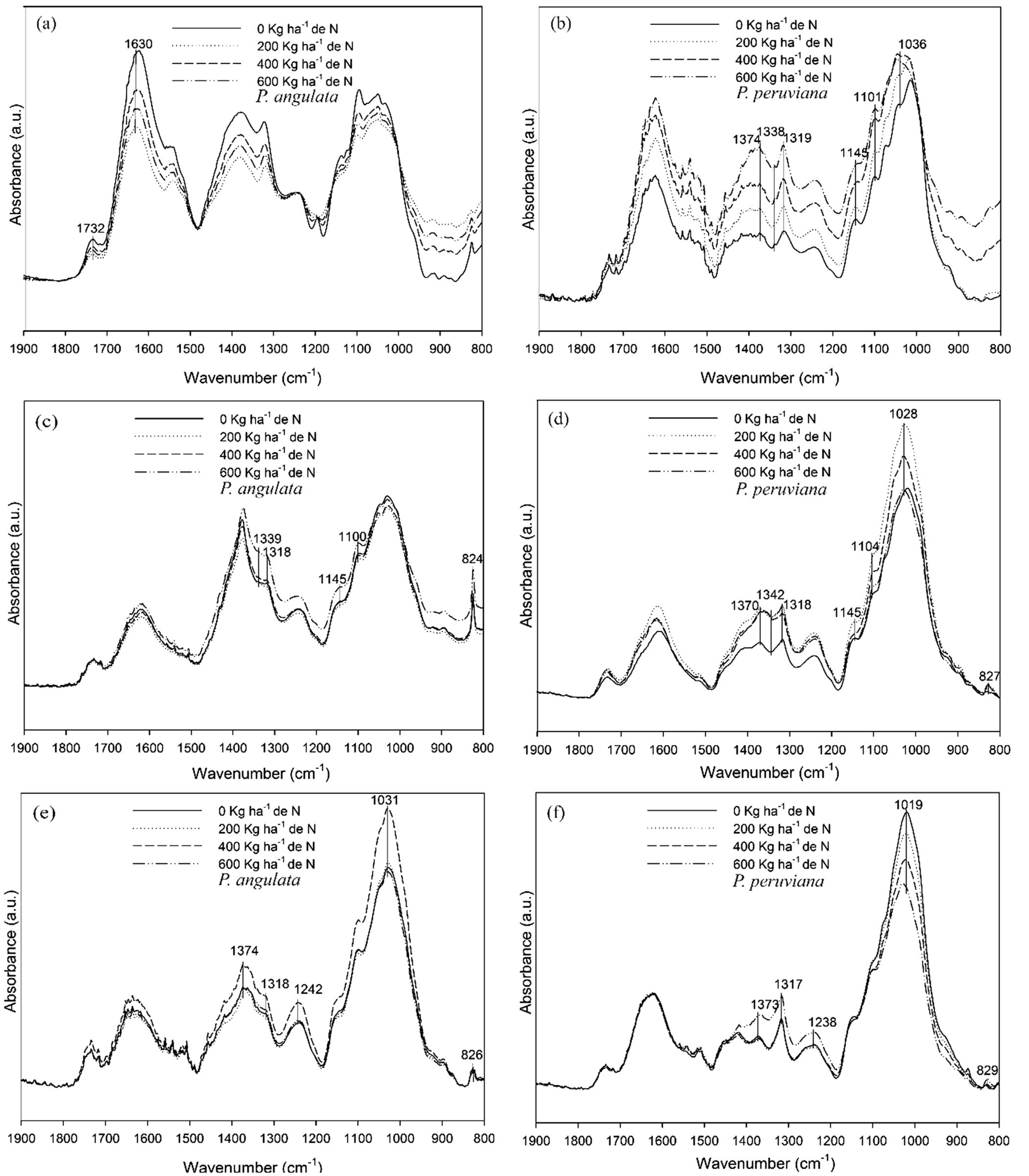

Fig. 3. Infrared spectra with Fourier transform by species and different parts of plants of the genus *Physalis*, in the fingerprint region: (a, b) leaves; (c, d) stems and (e, f) roots.

(Hattas et al., 2017; Scoginfs, 2018) and can converted to secondary metabolites without nitrogen, as observed in this research.

Nitrogen is one of the mineral elements that most absorbed by plants and the excess or lack of this nutrient can affect the growth and biomass accumulation. This is because nitrogen is an essential structural constituent of proteins, nucleic acids, chlorophyll and some hormones, and its application as fertilizer is a strategy to increase crop performance (Ata-Ul-Karim et al., 2016; Saud et al., 2017). The biomass reduction under deficiency or nitrogen excess was reported in other Solanaceae crops, such as *Lycopersicon esculentum* (Badr et al., 2016) and *Solanum melongena* (Souza et al., 2017).

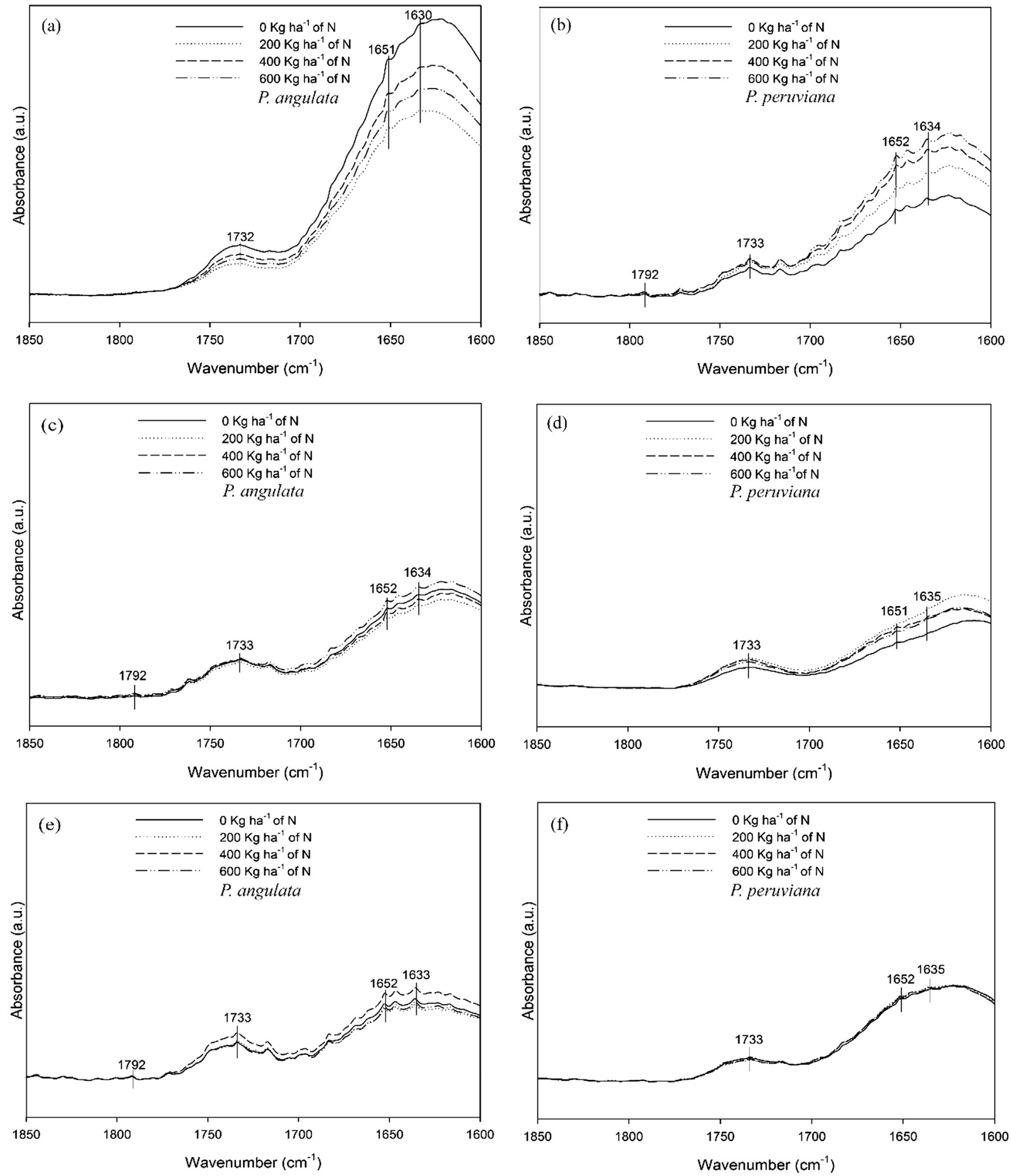

Fig. 4. Infrared spectra with Fourier transform for different parts of plants of the genus *Physalis*, in the region of the phenolic compounds: (a, b) leaves; (c, d) stems and (e, f) roots.

4. Conclusions

The specific compounds related to the functional groups present in the roots, stems and leaves are induced as a function of nitrogen fertilization, altering the common absorption peaks. Deficiency in nitrogen can increase the amount of phenolic compounds and their antioxidant capacity in plants of *Physalis angulata*, since there is no positive response to nitrogen fertilization for these characteristics. Low levels of nitrogen can reduce the production of phenolic compounds and their antioxidant capacity in *Physalis peruviana* plants.

The study by FTIR analysis offers accurate means to identify functional groups present in *Physalis* species with a possible

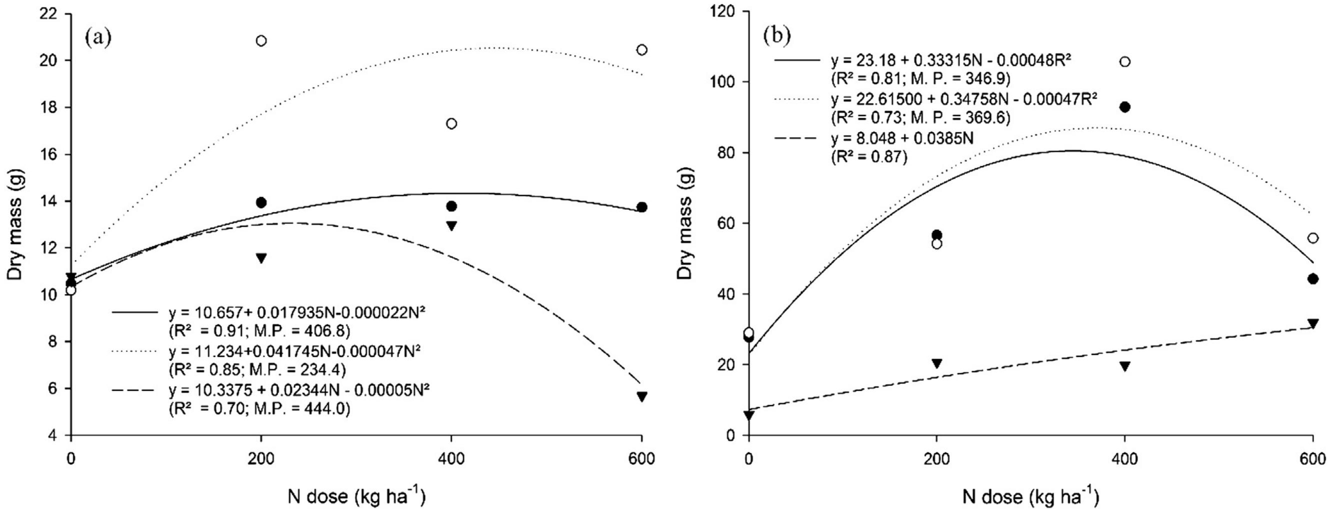

Fig. 5. Dry mass in leaves (●), stems (○) and roots (▾) of *Physalis angulata* (a) and *Physalis peruviana* (b).

pharmacological use. Supplementary nitrogen fertilization is not recommended for induction of phytopharmaceuticals from plants of *Physalis angulata*, while the supply of nitrogen is an important inducer of phytochemical compounds in plants of *Physalis peruviana*.

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