Assessing the near infrared spectroscopy tool to explore phytochemical profile of *Nothofagus antarctica* (ñire) leaves

Ma. Gabriela Mattera[1], Marianela Batistelli[2], Pablo Peri[3], Alejandro Martinez-Meier[4]

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Introduction

Plants have evolved to detect adverse environmental conditions and generate diverse biochemical, morphological and physiological responses in order to grow and survive at a specific environment (Ahanger et al. 2017; Hossain et al. 2018). Plant tolerance to severe abiotic stresses (e.g., heat and drought) involves different strategies ranging from avoidance mechanisms to active protecting actions against damage (Estravis-Barcala et al. 2020). Signaling pathways that involve the reactive oxygen molecules (ROS) production and the activation of antioxidant response are recognized as protective mechanisms. In this regard, several studies have shown how the plant secondary metabolisms evolved in order to play a specific physiological and ecological role for adapting to the growing environment (reviewed by Holopainen et al. 2018). Understanding how plants modulate their responses to different stress factors could help to elucidate and predict if plants can cope with heat and drought, the two major abiotic stressors agents in the context of global climate change. Species that currently grow in areas with ecological conditions similar to the changes forecasted (e.g., species that grow at the most xeric end of its distribution) could hold the adaptive capacity to survive and even colonize new areas in future scenarios (Krutovsky 2012). *Nothofagus antarctica*, a native species of Patagonian template forest, could represent a clear example of this, since it shows the greatest ecological range of the South American Nothofagus genus (Donoso et al. 2006), growing from valley bottoms, steep slopes with shallow soils , floodplain environments and post-fire scrub towards the most xeric limit of the Patagonian forests (Veblen et al. 1996). In addition, this species develops different morphological variants as the result of locally adapted ecotypes (Vidal et al. 2004), an expression of plasticity or a combination of both factors (Steinke et al. 2008). This study aimed at assessing the ability of near infrared spectroscopy (NIRs) tools to distinguish the phytochemical profile (including the production of antioxidants such as phenolic compounds) in leaves of two *N. antarctica* populations.

Material and Methods

N. antarctica leaves were harvested during summer of 2021 in two populations apart by 1º latitude (~180 Km distantly), which grow with different thermic and hydric regimens. Within each population, 20 trees were considered, maintaining a minimum distance of 50 m between sampled trees in order to prevent collecting closely related individuals. Among each tree, leaves from different stratum were collected (< 1.5m, 1.5-3.0m, and >3.0m). The harvested leaves were suddenly saved in liquid nitrogen until freeze-dried treatment was carried out. NIR spectrum for each sample was acquired from the both sides of five freeze-dried leaves, without damage, by using a MPA FT-NIR (Bruker, Germany) equipped with an integrating sphere and controlled by OPUS 7.5 software. This equipment converts the reflectance measurements into absorbance, allowing the acquisition of full absorbance spectra in the range from 9000 to 3900 cm-1 with a resolution of 8 cm-1. Each spectrum consisted of 32 averaged scans. Then, a spectral data array for each sample was built using three types of pre-processing methodologies: No Data Preprocessing, Min-Max-Normalization and First Derivative (Conzen 2014). An exploratory analysis for assessing spectrum distribution, identifying outliers and detecting association was carried out by Principal Component Analysis (PCA) using the software Matlab version 7.0 (The Mathworks, Natick, Massachussets, USA, 2007).

1 IFAB (INTA EEA Bariloche –CONICET). Contacto: mattera.gabriela@inta.gob.ar.
2 IFAB (INTA EEA Bariloche –CONICET). Contacto: batistelli.marianela@inta.gob.ar.
3 INTA EEA Santa Cruz and UN Patagonia Austral (CONICET).
4 IFAB (INTA EEA Bariloche –CONICET); INTA EEA Bariloche.

The same procedures were done for the spectral range corresponding to the C-H molecule signals (related to phenolic nature) (Cozzolino 2008).

Results

A characteristic NIR full spectrum of *N. antarctica* leaves is shown in Figure 1. Data analysis showed differences among the used pre-processing methodologies for the explained variance by each principal component (Table 1). The third component was more significant for the First Derivate pre-processing in comparison with the others. Figure 2 shows PCA loading and score plots of three principal components of the spectra of *N. antarctica* leaves for each pre-processing methodology. Differentiation among the scores of each population was observed, which was higher when considering the First Derivate pre-processing data array. Similar results were found when it was considered only the spectral range of C-H of phenolic compounds. PCA score plot considering the first derivate pre-processing in the spectral range of C-H is similar to Figure 2b.

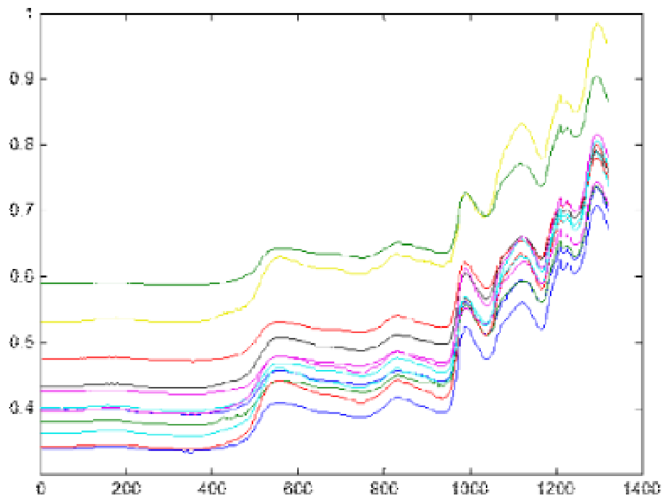

Figure 1. A characteristic NIR fingerprint of a subset of N. antarctica leaf samples of both populations.

Table 1. Explained variances (%) of PCA analysis for each preprocessing methodology

Pre-processing	PC1	PC2	PC3
Non Data Pre-processing	99.87	0.12	0.005
Min-Max-Normalization	98.75	1.22	0.030
First Derivate	96.03	2.53	1.490

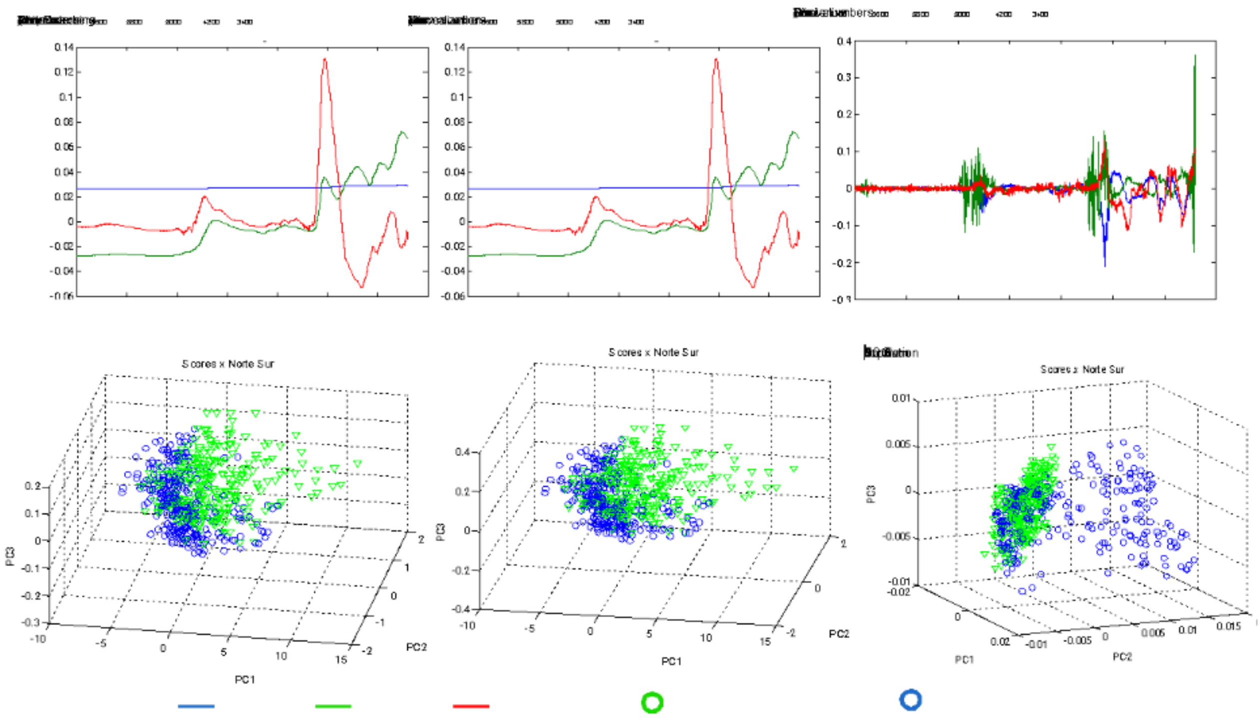

Figure 2. PCA loadings (a) and scores (b) of the three principal components of N. antarctica spectra from two populations (northern vs southern) using three different preprocessing methodologies.

Discussion

Near infrared spectroscopy methodology was useful for assessing the *N. antarctica* phytochemical profile in different populations. This non-destructive technique has the advantages of being economic, eco-friendly and time-consuming efficient (Batistelli et al. 2018, Machado et al. 2018). In this study, the pre-processing of data array was highly relevant to improve the differentiation capability. In particular, the First Derivate allowed emphasizing the differentiation between northern and southern populations due to the higher relevance of the third component in comparison with the other methodologies. In regard with this, First Derivate pre-processing accentuates the spectrum signals (Conzen 2014) which is directly related to the quantity of phytochemical compounds. In the case of spectral range that includes phenolic compounds, the studied populations showed differential performances; thus, diverse physiological responses could be taking place regarding the growing environmental conditions. These findings are highly relevant in order to understand the eco-physiological performance of this species to climate change.

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