

Discriminant analysis of Mediterranean pine nuts (*Pinus pinea* L.) from Chilean plantations by near infrared spectroscopy (NIRS)

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a b s t r a c t

Pinus pinea L. is one of the most important nut species in the world given the high nutritional and culinary value of its seed, the pine nuts, with increasing demand. The objective of this study was to assess the potential of visible and near infrared spectroscopy (VIS þ NIRS) to analyse different sample presentations and to discriminate geographical origins of Mediterranean pine nut grown in Chile. Pine nuts were collected from 76 adult trees in three growth macrozones previously defined for stone pine in Chile. Original spectroscopic data were obtained by means of a Foss NIRSystems 6500 SYII spectrophotometer using a transport module. Reflectance was employed in the wavelength range of 400e2500 nm. The best means of sampling for the pine nuts used in this study were also studied. After analysing the spectro-scopic data, discriminant models were obtained by means of discriminant partial least square (DPLS) with all samples. For the three macrozones previously identified, 87.8% of samples was correctly clas-sified in the cross validation stage with the best model for pine nuts spectra analysis, obtained with shelled intact pine nuts. Results indicate that NIRS technology is capable of differentiating between pine nut samples of different geographical origins with errors ranging between 12.2 and 9.2%, and demon-strate the potential of VIS þ NIRS technology as a rapid and accurate method for predicting the geographical origin of Mediterranean pine nuts.

1. Introduction

Stone pine (*Pinus pinea* L.) is a well-known species mainly for the economic use of its edible pine nuts. It is a native species from the European Mediterranean basin, where there are over 700,000 ha of forests; 490,000 ha are located in Spain, 175,000 in Portugal, 46,000 in Italy, 43,000 in Turkey, 21,000 in Tunisia, 13,000 in France and 12,000 in Lebanon, and smaller areas in Morocco (3000 ha), Israel (2000 ha) and Greece (1500 ha). It is also present in other countries of the southern hemisphere, including New Zealand, Australia and Chile in small plantations (Loewe, 2015). Over the last century, there has been an important expansion of the stone pine plantations towards the southern and eastern areas of the European Mediterranean, as well as an increase of planted areas in countries out of its original distribution, aimed mainly at pine nut production (Boutheina, Hedi, & Balandier, 2013). Stone pine nuts are among the most important dried fruit species in the world (Fady, Fineschi, & Vendramin, 2004), with an increasing demand and high prices (Barranco and Ortuño, 2003; INC, 2014).

The species was introduced in Chile by Europeans, and since the beginning of the 20th century has been used as a forest tree in coastal sand dunes stabilization programs (Albert, 1909) and was later included in afforestation and rural development programs (Loewe & Delard, 2012). Currently, there are over 500 ha of plantations established in Chile in different regions, settings and management conditions (Loewe, 2015; Loewe, Delard, & Álvarez, 2014, pp. 3e6), and new plantations are increasing. Since the 1990's, several studies on the species were developed to assess its potential as a productive fruit forest species for the Mediterranean areas of Chile, confirming its adaptation expressed through a superior growth and fruiting to the one presented in the main distribution

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http://dx.doi.org/10.1016/j.foodcont.2016.09.012

areas across the world (Loewe, Delard, González, Mutke, & Fuentes, 2012, 2014, Loewe, Balzarini, Álvarez, Delard & Navarro-Cerrillo, 2016; Loewe & González, 2003). Three differentiated growth macrozones were defined for the species in Chile according to growth patterns (Loewe, Delard, Balzarini, Álvarez, & Navarro-Cerrillo, 2015).

P. pinea populations show a low provenance differentiation, high phenotypic plasticity, tolerance to poor soils and to adverse climatic conditions, and strong sensitivity to intra- and interspecific competition (Boutheina et al., 2013; Mutke, González-Martínez, Viana, Gordo, & Gil, 2008; Sánchez-Gómez, Cano, Cervera, & Aranda, 2009, pp. 2e7; Thuiller et al., 2003), being interpreted as low genetic variability (Sánchez-Gómez et al., 2009, pp. 2e7; Vendramin et al., 2008). In fact, several authors have reported limited genetic variability between provenances (Evaristo, Costa, Baeta, & Pais, 2002; Fallour, Fady, & Lefevre, 1997; Gordo, Mutke, & Gil, 2007; Gómez, Aguiriano, Alía, & Bueno, 2002). Contrarily, Alvarez, Toledo, Abellanas and Martin (2004) reported a high degree of variation in populations of Andalucia using mega gametophyte storage proteins as markers, even higher than in maritime pine (*P. pinaster*) from Iberian Peninsula populations, suggesting that this technique could detect genetic diversity of the species.

Pine nuts have high protein, unsaturated fatty acids and fiber content (Salas-Salvadó, Ros Rahola, & Sabaté Casellos, 2005), low molecular weight carbohydrates (Ruiz-Aceituno, Ramos, Martínez-Castro, & Sanz, 2012), vitamins (folic acid, niacin, tocopherols, B6 and B2), minerals and low sodium content, plus phytoesterols and polyphenols (Blomhoff, Carlsen, Frost Andersen, & Jacobs, 2006) as well as inositols (Ruiz-Aceituno, Rodríguez-Sánchez, Sanz, Sanz, & Ramos, 2014), being a highly nutritional seed (Segura, Javierre, Lizarraga, & Ros, 2006). Stone pine nuts are particularly interesting given its linoleic and linolenic acids content (Costa & Evaristo, 2008).

The mechanism for tracking certified material from the forest to the final product through the chain of custody certification (Wingate & Mcfarlane, 2005) is a very desirable development given the long-lasting taste alteration caused by pine nuts from other pines, called dysgeusia, and also referred as *Pine Mouth Syndrome*. It is an unpleasant bitter, metallic taste disturbance originated by the consumption of pine nuts from other pine species, especially from Chinese pine nuts (*P. koraiensis*) which often is commercialized mixed with seeds of others pine species (Mutke, Gordo, Khouja, & Fady, 2013), some of them even non edible, like *P. armandii* and *P. massoniana* (Destaillats, Cruz-Hernández, Giuffrida, & Dionisi, 2010; EC, 2011; Handy, Timme, Jacob, & Deeds, 2013), and as well caused by allergy to *P. pinea* nuts (García-Menaya et al., 2000). In spite of this and the increasing number of companies willing to be included in forest certification schemes worldwide, stone pine nuts are difficult to be traced. For this reason, it is of interest developing an objective analytical method useful to verify the regional species origin of pine nuts, ensuring the traceability and making possible the implementation of a chain of custody certification, to clearly distinguishing it from other species pine nuts.

Qualitative analysis by NIRS chemometric techniques has been successfully used to distinguish the origin of agricultural and forest products with common chemical characteristics (Roberts, Workman, & Revves, 2007; Shenk and Westerhaus, 1995b; Shenk, Workman, & Westerhaus, 2010; Williams and Norris, 2001), and is gaining strong acceptance in food science and technology, with many different applications. In the forest sector, the first results concerning NIRS spectra and wood chemical properties were presented 20 years ago, showing the suitability of this technique for the quantitative assessment of the physical, mechanical, and chemical properties of wood, i.e. longitudinal modulus of elasticity, lignin and cellulose content, wood density even in *P. pinea*-, showing significant variation between trees (Fernandes et al., 2013), moisture, wood age (Schimleck et al., 2000; Via et al., 2008), pulp yield (Labbé, Droppelmann, Balocchi, & Peredo, 2013) and other wood parameters (Tsuchikawa, 2007). This technique has also been applied to identify wood species of different genera, wood samples from species of the same genus, and different origins of the same species (Brunner, Eugster, Trenka, & Berganminstrotz, 1996; Tsuchikawa, Inoue, Noma, & Hayashi, 2003), as well as to discriminate geographical origin of cork planks and stoppers (Prades, Gomez-Sanchez, Garcia-Olmo, & Gonzalez-Adrados, 2012) and to assess their quality (Prades, Gómez, García & González, 2013). In agriculture, NIRS has been used to discriminate fruits of different productive areas, breeds or genotypes and even of protected geographical indication (Lin, & Yin, 2009). It has also been applied to predict the content of some minerals in leaves of different species, including *P. pinea* (Petisco et al., 2005, Petisco, García-Criado, Mediavilla, Vázquez De Aldana, & Zabalgogeazcoa, 2006) and to assess seed vigor in *P. patula* (Tigabu & Oden, 2003, 2004), as well as seed moisture content (Torbjörn & Geladi, 2003), seed water interactions (Torbjörn, 2003) and seed sources (Tigabu, Oden, & Lindgren, 2005) in *P. sylvestris*. Moreover, NIRS has been used to determine *Quercus ilex* subsp. ballota acorn chemical quality, with significant differences in fruit chemical composition between different populations being found (Valero et al., 2012). Regarding Mediterranean pine nuts, and to the best of our knowledge, qualitative analysis by NIRS has not been reported.

The general objective of this study of *P. pinea* was to provide a first approach to the use of NIRS to analyse chemical composition of nuts. Specific objectives were (1) investigate the impact of sample presentation on the accuracy of NIRS discriminant models; and (2) investigate the potential use of NIRS in predicting seed-quality traceability in Chile. Hence, we conducted a two-step sequential study to assess the use of NIRS to identify the origin of *P. pinea* nuts, aiming at evaluating the suitability of NIRS technology for authenticating geographical origins of Mediterranean pine nuts in Chile, and to determine the best sample presentation to perform the analyses.

2. Material and methods

2.1. Experimental material

The Chilean Forest Institute (INFOR) has made a long-term, large-scale national research effort on gathering stone pine data in Chile, covering an area between Coquimbo (30.819817^{0}S) and Araucanía (38.990393^{0}S) regions. Inventories of stone pine populations were performed including regular plantations, windbreaks, groves and isolated trees established for commercial and environmental purposes along the country, and three macrozones (MZs) named North, Dry Coast and South were identified according to height and DBH growth rates (Loewe et al., 2015). These MZs present a high variability of climatic features. Average annual and maximum annual temperatures decrease from north to south; on the other hand, the minimum annual average temperature is the lowest in the Dry Coast MZ. Rainfall increases from north to south, whereas hydric indices diminish, suggesting lowest water stress in the South MZ (Table 1).

From the existing plantations in Chile, we selected superior trees (ST) for cone production by applying the individual valuation method (Ipinza, 1998) given the high environmental and tree age variability. We examined 3349 stone pine trees distributed in 122 sites aged 8 years and up (e.g. considered to be in Chile the beginning of the reproductive age) from which we selected 76 trees identified to be of superior cone production. Trees were characterized according to DBH (diameter at 1.3 m height), total height,

Table 1
Climatic characterization of stone pine growth macrozones in Chile.

Variable	North MZn ¼ 48	Dry Coast MZn ¼ 27	South MZn ¼ 54	All sites n ¼ 129
Annual average temperature (^{0}C)	14.1 (11.8; 16.7)	13.6 (12.5; 15,1)	13.2 (11.0; 14.2)	13.6 (11.0; 16.7)
Annual max average temperature (^{0}C)	21.9 (17.6; 25.1)	21.0 (17.9; 23.0)	19.8 (16.3; 22.5)	20.8 (16.3; 25.1)
Annual min average temperature (^{0}C)	7.5 (5.5; 9.9)	7.0 (5.5; 7.7)	7.5 (5.3; 9.2)	7.4 (5.3; 9.9)
Annual total rainfall (mm)	383.7 (116.2; 1260.1)	648.3 (416.3; 816.0)	1047.0 (523.6; 1666.9)	716.7 (116.2; 1666.9)
Annual hydric index (mm)	-995.4 (-1349.7; -107.3)	-743.7 (-914.4; -474.2)	-244.1 (-736.4; e 332.9)	-628.2 (-1349.7; 332.9)
Annual thermal oscillation (^{0}C)	14.3 (9.2; 17.3)	14.0 (10.7; 17.0)	12.3 (8.2; 15.1)	13.4 (8.2; 17.3)

Values in parentheses correspond to minimum and maximum values recorded per variable. Hydric index was calculated as accumulated rainfall minus potential evapotranspiration in the period. The number of sites where selected stone pine trees are located is indicated as *n*. Climatic data were obtained from Chilean National Environmental Information System (www.inia.cl, www.dga.cl).

crown length and diameter, and productive fruit variables (cone number). Three cones were harvested in winter 2013 from each selected tree, distributed along the three macrozones: North (34), Dry Coast (12) and South (30) (Fig. 1).

2.2. Sample preparation

The training set used in this study included four different levels of pine nut processing: in-shell pine nuts (IS), whole shelled pine nuts (SH), and humid (HF) and dried pine nut flour (DF) (Fig. 1S). In-shell pine nuts were obtained directly from the harvested cones, and stored properly in dry conditions (4 ^{0}C), protected from direct sunlight until analysis. Shelled whole pine nuts were obtained by manually cracking in-shell pine nuts, and separated from the external shell and from the inner seed coat. Immediately after the spectra were taken, pine nuts (including embryos) were ground in a blade mill (Moulinex AD56 42) until a homogeneous powder was obtained, constituting the humid flour preparation. Later, this flour was weighed and dried in a forced-air drier at 45 ^{0}C for 48 h, homogenized in a Waring Blender (LB20E, Waring Products, New Hartford, CT, USA), and sieved (1-mm mesh), to obtain a fine homogeneous flour. Samples were stored at 4 ^{0}C until NIRS analysis.

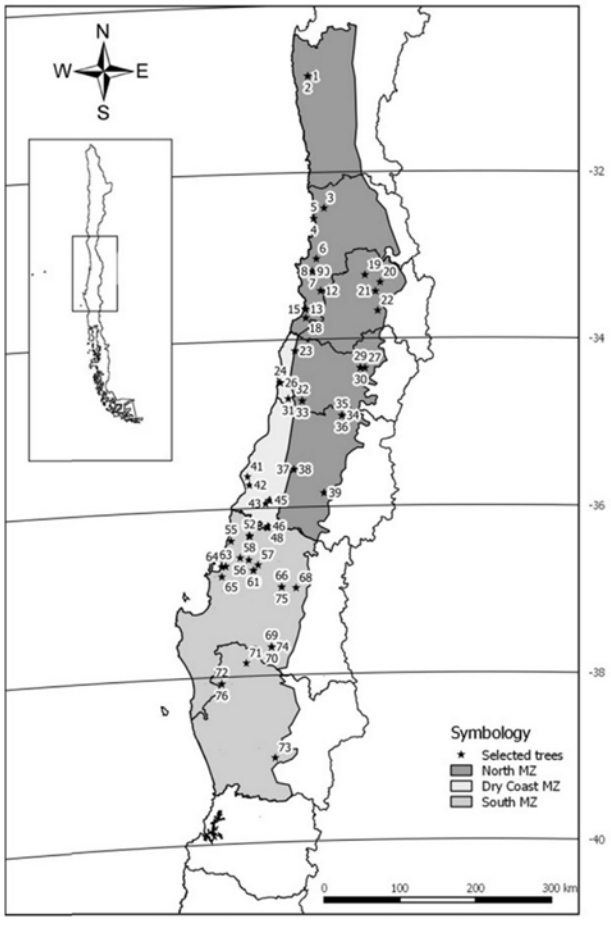


Fig. 1. Distribution of selected stone pine trees in Chile by macrozones.

2.3. Instrumentation and collection of spectra

The samples were scanned by reflectance using a circular cup in a transport module connected to a Foss-NIRSystems 6500 spectrophotometer equipped with auto gain detectors: one for 400e1098 nm (known as the VIS region) and another for 1100e2500 nm (known as the NIR region). Reflectance spectra were collected every 2 nm from 400 to 2500 nm. The absorbance data were obtained and stored as log (1/R) where R is the reflectance. Data were stored using WinISI II software version 1.5 (Infrasoft International, Port Matilda, PA, USA). A reference spectrum was recorded before analysing each sample. Three spectra for each tree were taken in the case of whole pine nuts, both in-shell and shelled, and two in the case of flour samples. These spectra were averaged to obtain the mean spectrum that was used to determine relationships with geographical origin.

2.4. Qualitative chemometric analysis

The training set of pine nut samples were qualitatively analysed using Matlab R2016a (The Mathworks, MA). The total population of spectra was divided randomly and proportionally to geographical origin into a calibration training set.

The origin of samples was determined by using classification models developed by discriminant partial least square (DPLS) (Kowalski, 1984). Briefly, DPLS correlates spectral variations (X) with defined classes (Y), and maximizes the covariance between the two types of variables, for developing a classification model (Massart et al., 1997; Vandenginste, Buydens, De Jong, Lewi, & Smeyers-Verbeke, 1998). An unknown sample was classified according to the value predicted by the DPLS model, which ideally should be close to the values used to codify the class.

Before developing the classification models, the spectral data were subjected to different signal pre-treatments. Scatter

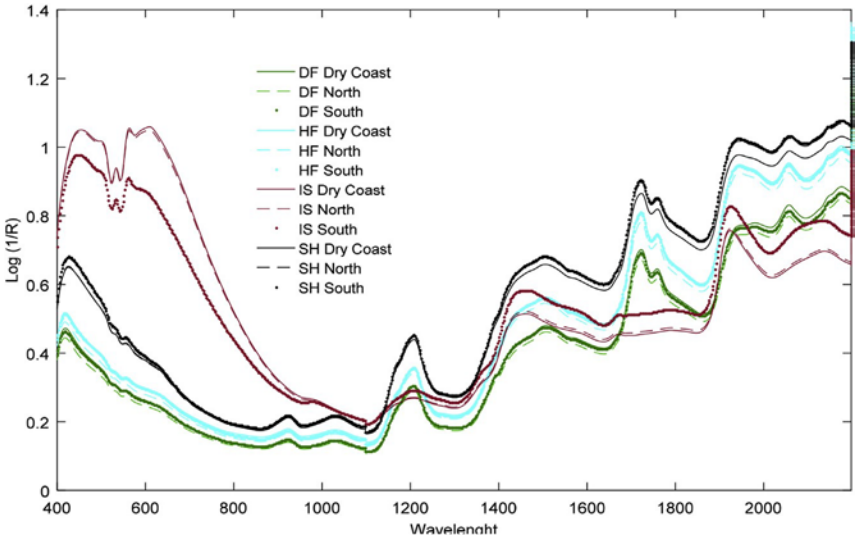


Fig. 2. Mean spectra of the three macrozones obtained for in-shell (IS), shelled pine nuts (SH), humid (HF) and dried (DF) pine nut flour samples.

correction was accomplished using the standard normal variate and detrend transformation (SNV þ DT) algorithm (Barnes, Dhanoa, & Lister, 1989). The spectra were transformed using different ranges of the 400e2500 nm spectrum and different combinations of derivative math treatments applied to the spectral data. Matlab derivative math treatments are denoted by a four-digit notation (a, b, c, d), where *a* is the derivative order, *b* is the derivative gap, *c* is the smoothing segment, and *d* is the second smoothing segment (Shenk and Westerhaus, 1995a). Moreover, the influence of different wavelength regions was evaluated, and various qualitative models were applied successively on the full spectrum (VIS þ NIR; 400e2500 nm), the NIR region (NIR; 1100e2500 nm), and the 400e2200 region, as this is the region recommended by the manufacturer of the optic fiber probe to obtain spectra with a suitable signal-to-noise ratio.

The qualitative analysis methods were validated by studying the percentage of samples that were classified correctly or incorrectly (Massart et al., 1997). A total of 76 samples were used to calibrate and full cross validate each model, with 34 belonging to the North MZ, 12 to the Dry Coast MZ and 30 to the South MZ.

Classification errors corresponding to the proportion of incorrectly classified samples (1-accuracy) were calculated according to the equation: Classification error ¼ (FP þ FN)/(TP þ TN þ FP þ FN) Considered rates correspond to: TP: True Positive, FP: False Positive, TN: True Negative, FN: False Negative.

3. Results

3.1. Spectral analysis according to sample presentation

Fig. 2 shows the mean spectra obtained for the four different sample presentations in the visible and NIR regions by macrozone. The mean spectrum of the whole pine nuts (SH) is very close to the mean spectrum of the two different flour samples (HF and DF). Main differences were found between the IS spectra and the other sample types in the visible region and over 1900 nm.

In-shell pine nuts spectra show differences among South MZ and the other two, mainly from 400 to 800 nm (visible range) and 1100 nm up. Shelled whole pine nuts spectra show a slightly different pattern for samples from the Dry Coast MZ from 1100 nm and up. The two flour samples present no differences among MZ, with a clear superposition of spectra. In the visible region (400e1100 nm), the differences between the mean spectra show the same patterns for all type samples, with the exception of in-shell pine nut, given its darker colour. Absorption peaks were observed at 550; 1200; 1500; 1720; 1760; 1940 and 2350 nm. The bonds in the main compounds of pine nuts were identified in the absorption peaks corresponding to fatty acids (1200 nm) and to proteins (1520 nm).

3.2. Discriminant models

Models were developed for the entire spectral range (400e2500 nm) and using only the NIRS region (1100e2500 nm). Although the results were similar, the best models were obtained in the 400 to 2500 nm region for all cases. With just one exception, the best pre-treatment was (SNV þ DT), and the best mathematical treatment was the second derivative. The average classification errors in cross validation were 21% for IS, and with the most processed samples (HF and DF) 14%. SH showed a 12.2% error, being the best preparation for discriminating among the three macrozones.

Fig. 3 shows the two-dimensional distribution of the spectral populations of pine nuts in the best-selected model at the country scale, as well as the previously defined separation into three growth macrozones; the best models for each case are described in detail in Table 2. In all cases, an overlap of the Dry Coast MZ with the other two is evident, which is expected given its size and intermediate location.

Fig. 4 shows the predicted *vs* the actual MZ origin for the preparation of SH sample, the one that obtained the best results in terms of classification error.

The small-sized Dry Coast MZ is located at an intermediate position between the North and South MZs, with intermediate climate characteristics (Table 1), with the exception of annual minimum average temperature, which is 0.5 ^{0}C lower than in the other two macrozones. Considering this, we assigned Dry Coast MZ trees to either the North or South enlarged MZs. This reassignment was done following as criteria an influential geographical feature, the Maule River, which determines the limit between these two MZs in the area where they meet. The two new built macrozones were named as North' and South', and each contain 38 samples (Fig. 5).

Fig. 6 shows the two-dimensional distribution of the different samples of spectral populations of pine nuts in the best-selected model at the country scale, as well as the separation between the two new stone pine macrozones, with the best models being

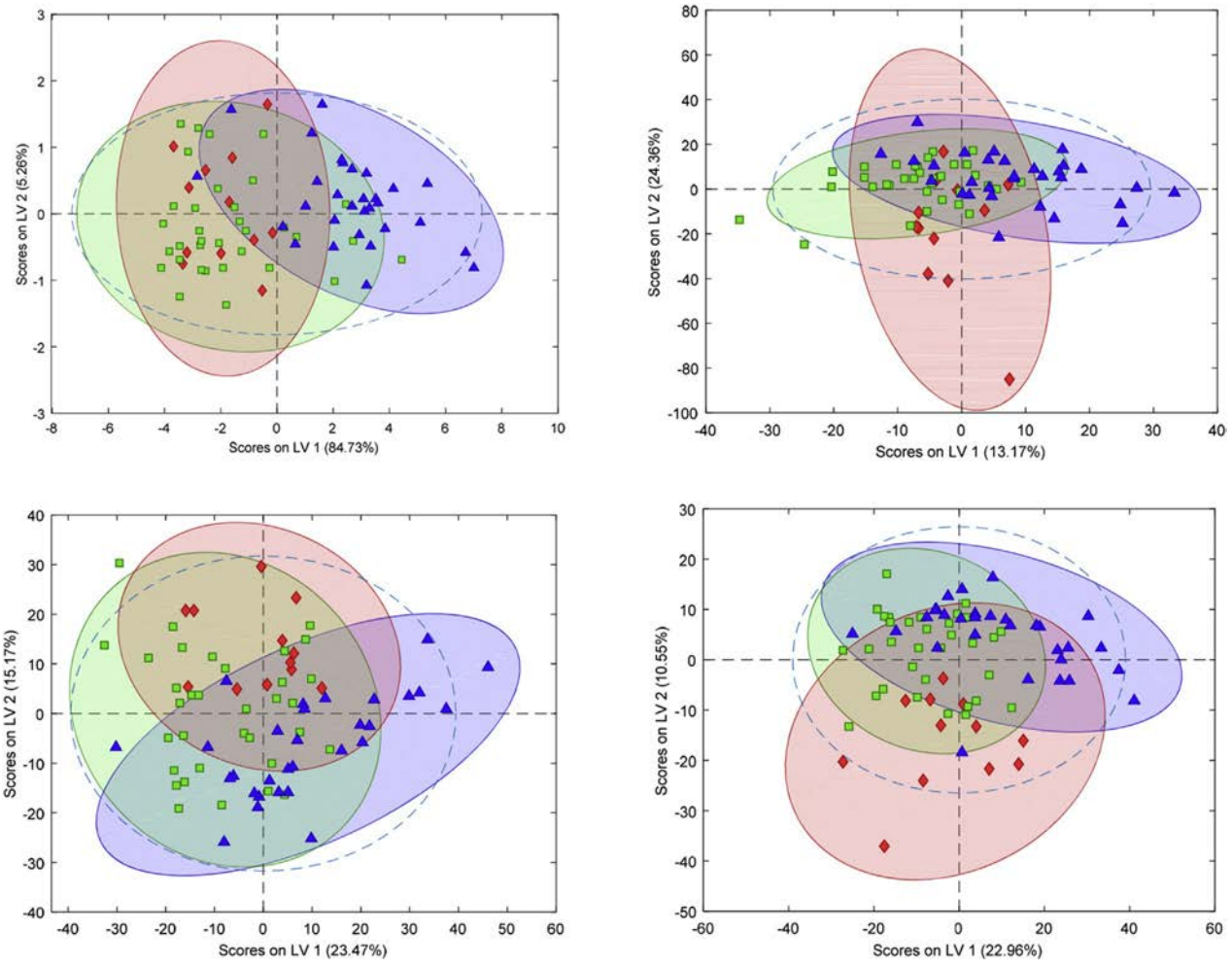


Fig. 3. Discriminant PLS plot of spectra of pine nuts from three Chilean growth macrozones in the selected models for in-shell pine nuts (IS) (top left), shelled whole pine nuts (SH) (top right), humid pine nut flour (HF) (bottom left) and dried pine nut flour (DF) (bottom right). Red diamonds: Dry Coast MZ, Green squares: South MZ, Blue triangles: South MZ; dotted line: 95% confidence level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

described in Table 3. As predicted, classification errors decreased in most cases.

3.3. Country-scale models to discriminate between pine nuts from Chilean macrozones

The best classification model at a country scale considering three macrozones was obtained with shelled pine nuts (SH), with 87.8% of the samples being correctly classified, followed by humid and dried flour (DF) samples, each with 86.0%. In a second analysis, when discriminating between the two macrozones, the best sample type was HF or DF indistinctly, with 90.8% of samples correctly classified in both cases. Fig. 7 evidences that only two samples belonging to the North' MZ were misclassified by the discriminant model in the cross validation.

At the most important wavelength for the MZ discrimination, the coefficients of the discriminant models on the visible region (Fig. 8) were close to zero. In the NIR region, the most important absorption bands with the highest variability for pine nuts specifically were bands 1200; 1500; 1720; 1760; 1940 and 2350 nm.

Table 2
Models for the classification of different pine nut presentations for three Chilean growth macrozones (cross validation results); spectral range 400e2500 nm.

Sample type	In-shell (IS)		Shelled pine nuts (SH)		Humid flour (HF)		Dried flour (DF)	
Geographical origin	Number of misclassified samples	Partial classification error (%)	Number of misclassified samples	Partial classification error (%)	Number of misclassified samples	Partial classification error (%)	Number of misclassified samples	Partial classification error (%)
North MZ	15/34	21.1	7/34	18.4	5/34	18.4	8/34	19.7
Dry Coast MZ	5/12	28.9	2/12	6.5	6/12	7.9	5/12	14.5
South MZ	4/30	13.1	5/30	11.8	5/30	15.8	3/30	7.9
Total Classification error (%)		21.0		12.2		14.0		14.0
Best Model	1441		0011		0011		1441	
Latent variables	12		9		10		13	

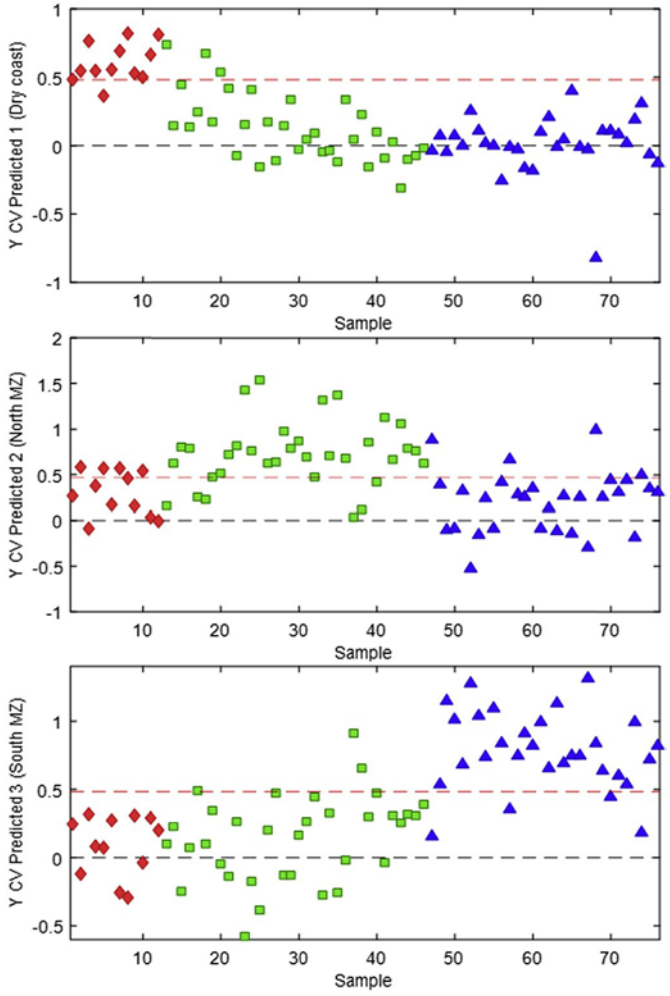


Fig. 4. Predicted vs measured cases in cross validation for shelled pine nut (SH) by macrozone (red: Dry Coast MZ, green: North MZ, blue: South MZ). A value of 0.5 distinguishes between classes (ordinate axis). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

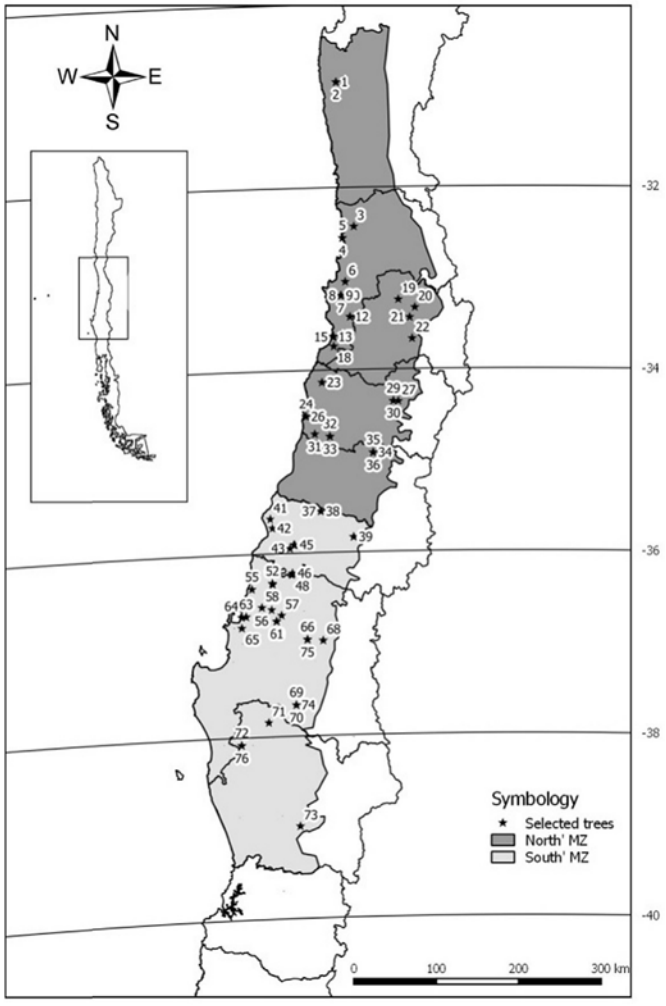


Fig. 5. Distribution of selected stone pine trees in Chile according to two newly defined macrozones, named as North' and South'.

4. Discussion

Given the precision, versatility and simultaneity characteristics of NIRS technique, it has been widely used to assess fruit quality expressed as different components and attributes (sugar and soluble solids content, acidity, firmness, etc.) (Lin and Yin, 2009), including several dried fruits. In this work, NIRS was used to segregate different geographic origins of *P. pinea* in Chile.

NIRS has been applied in hazelnut to detect kernel flaws (Moscetti et al., 2013; Pannico et al., 2015) and contaminated kernels (Kalkan, Beriat, Yardimci, & Pearson, 2011), and to authenticate Protected Designation of Origin (PDO) fruits. In almonds, it has been applied to detect fungal infection (Liang, Slaughter, Ortega-Beltran, & Michailides, 2015), seed damage (Nakariyakul, 2014) and to assess bitterness (Borràs, Amigo, Van Den Berg, Boqué, & Busto, 2014). In walnut, NIRS has been used to monitor kernel quality changes (Jensen, Sorensen, Engelsen, & Bertelsen, 2001); in shea nut, to characterize fat profiles and to segregate geographic origins (Davrieux et al., 2010); in peanut, to determine protein and amino acids (Wang, Wang, Liu, & Du, 2012), moisture content (Govindarajan, Kandala, & Subbiah, 2009) and oil acid value (Rao et al., 2009); and in *Quercus ilex* acorn, to assess chemical quality (Valero et al., 2012).

Absorption spectra were characterized for the first time for Mediterranean pine nuts, being the assignments of absorption bands and functional grouping to its main compounds not been reported. The coefficients of the discriminant models on the visible region close to zero confirm that the spectral variability observed in the colour does not contribute to the discriminant power of the models. We initially assumed it to be similar to other nuts, based on the similar patterns described for several dry fruits such as peanut (Govindarajan et al., 2009), walnut (Jensen et al., 2001), shea nut (Davrieux et al., 2010) and others; our results confirm this hypothesis since a correspondence was observed. In particular, we found records for other dry fruits in 6 out of the 7 observed peaks: 550 nm for hazelnut, 1200 nm for peanut, almond and shea nut; 1720 nm for peanut, almond, hazelnut and shea nut; 1760 nm for peanut and almond; 1940 nm for hazelnut and shea nut; and 2350 nm for peanut, hazelnut and shea nut. No coincidence was found for the band 1500 nm. In fact, according to the literature, over 950e1650 nm the peanut spectra ranges contain information on the relative proportions of CeH bonds (usually from fats and oil), OeH bonds -found in water- and NeH bonds found in protein (Wang et al., 2012). In particular, it was concluded that protein and amino acid contents of peanuts can be determined through NIRS. For peanut oil, bands associated with CH stretching vibration (Rao et al., 2009) were reported. Govindarajan et al. (2009) reported

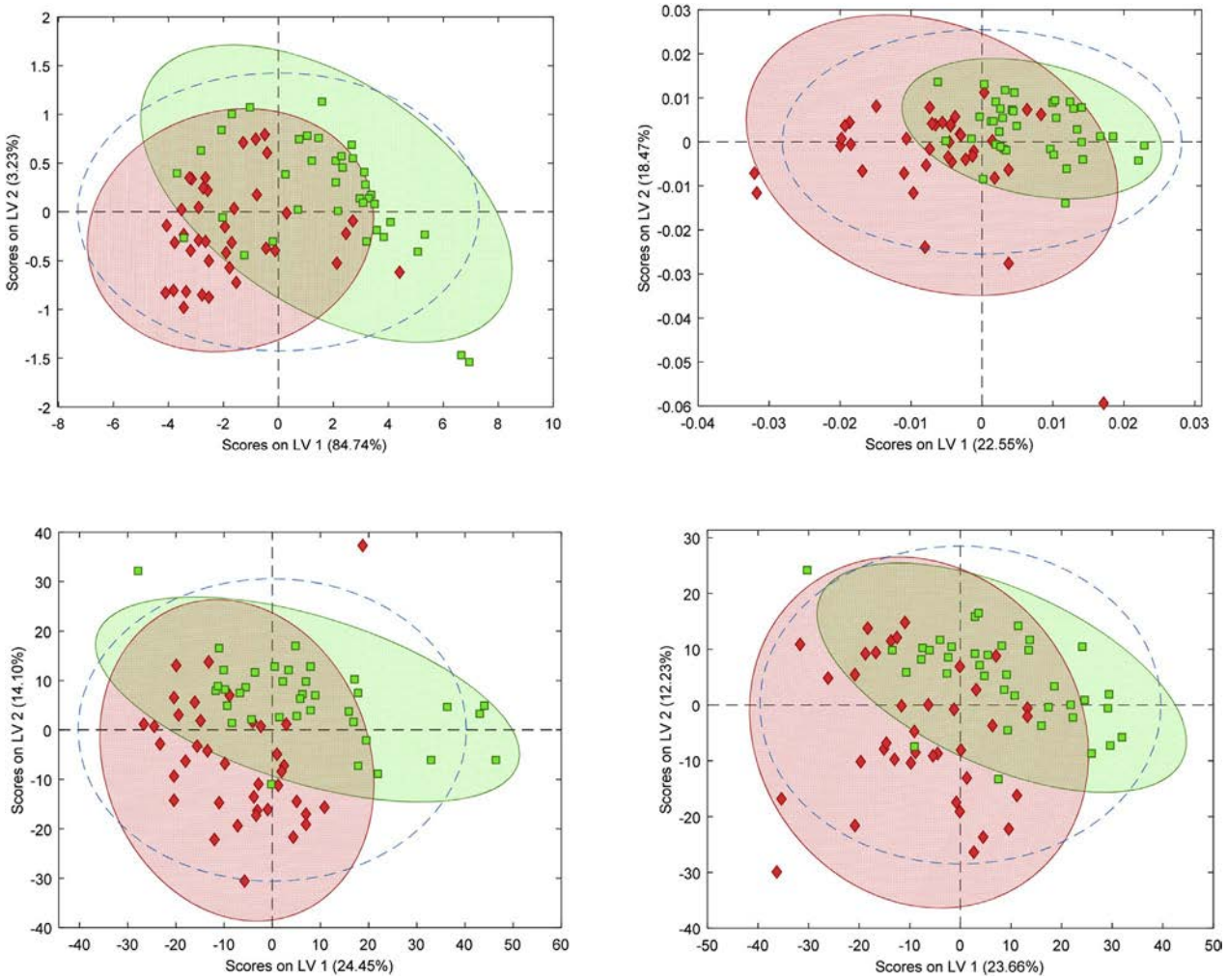


Fig. 6. Discriminant PLS plot of spectra of pine nuts from two macrozones in the selected models for in-shell pine nuts (IS) (top left), shelled whole pine nuts (SH) (top right), humid pine nut flour (HF) (bottom left) and dried pine nut flour (DF) (bottom right). Red diamonds: North' MZ, Green squares: South' MZ, dotted line: 95% confidence level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

peaks at 1033; 1159 and 1358 nm for whole peanut kernels. For almonds, Liang et al. (2015) reported bands associated with lipid absorbance and with cellulose. Nakariyakul (2014) described peaks at 710, 930 and 1120 nm, being absorbance peaks at 930 and 1120 nm attributed to oil.

For hazelnuts, Moscetti, Radicetti, Monarca, Cecchini, and Massantini (2014) reported several complex bands because contain information of various molecular vibration and generic functional groups, some of them associated with fatty acid content, unsaturated fatty acids with *cis* double bonds, protein acid and ester stretching vibrations, and others to polysaccharides, carbohydrates and lipids. Moscetti et al. (2013) identified bands important for classifying hazelnut flaws, with 1250e1650 nm being the predominant band, related to oil oxidation. According to Pannico et al. (2015), the raw adsorption spectra of hazelnut kernels showed peaks related to OeH and CeH tones (i.e. carbohydrates) and to double C bond stretching and deformation band of lipids. Davrieux et al. (2010) characterized shea nut fat profile, describing wavelength peaks corresponding to absorption bands of chemical bonds specific to fat, such as CeH, OeH, CH2 and C]C. Most important bands for discriminating the origin of pine nuts grown in Chile relate to protein (1200 and 1940 nm), lipids (1500; 1720; 1760 and 2350 nm), and polysaccharides and carbohydrates (2350 nm).

The results obtained for pine nut classification according to

Table 3
Models for the classification of pine nuts in Chile by two enlarged macrozones (cross validation results); spectral range: 400e2500 nm.

Sample type	In-shell (IS)	Shelled pine nuts (SH)	Humid flour (HF)	Dried flour (DF)
Geographical origin	Number of misclassified samples	Number of misclassified samples	Number of misclassified samples	Number of misclassified samples
North' MZ	6/38	7/38	2/38	4/38
South' MZ	8/38	7/38	5/38	3/38
Total Classification error (%)	18.9	18.4	9.2	9.2
Best Model	1441	2105	1441	2105
Latent variables	8	8	8	8

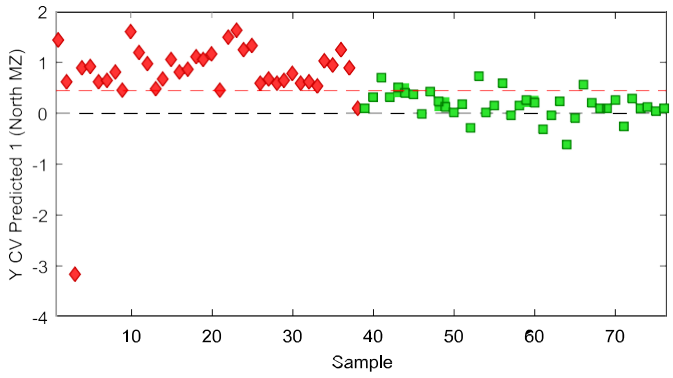


Fig. 7. Predicted vs measured cases in cross validation for humid flour (HF) in two macrozones (red diamonds: North' MZ, green squares: South' MZ). A value of 0.5 distinguishes between both classes (ordinate axis). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Chilean geographical origins, considering the three macrozones previously described, showed a classification error of 12.2% for whole shelled pine nuts. The chemical composition of pine nuts grown in the three MZs is similar, differed only in oleic acid (higher in the South MZ) and potassium (higher in the Dry Coast MZ). Potassium, as well as other minerals, protein and moisture, were determined with NIRS in alfalfa (González-Martín, Hernández-Hierro, & González-Cabrera, 2007), and oleic acid was found useful to segregate peanut cultivars (Shin, Craft, Pegg, Phillips, & Eitenmiller, 2010) and to differentiate shea nut populations according to geographical origin (Davrieux et al., 2010).

Accordingly, considering that oleic acid should be at the base of the classification ability of the models, we regrouped trees in two MZs, obtaining lower errors, which amounted to 9.2% for pine nut flour, humid or dried. In fact, the comparison of the model errors for 3 and 2 macrozones (Tables 2 and 3 respectively) showed a decrease in total errors for IS, HF and DF (2.1; 4.8 and 4.8% respectively), but an increase of 6.2% in the case of SH. Our results are in line with results from Tigabu (2003), who identified *Pinus sylvestris* seed sources with 71, 93 and 100% accuracy; but of lower precision than results reported by Prades et al. (2012) when discriminating geographical origin of cork planks and stoppers from the most representative cork-producing areas in the world, with 98% of the international cork plank samples and 95% of the Spanish being correctly classified in the calibration and validation stages. This difference could be explained by the exotic origin of stone pine in Chile. The technique use for discriminating cultivation areas is in agreement with findings of Lin and Yin (2009) and with Fu, Ying, Zhou, and Xu (2007), who pointed out that even fruits of the same variety planted in different areas display distinguishable qualities.

Other factors that influence discrimination relate to the environmental conditions where stone pine grows. It is difficult to explain exhaustively the results obtained for geographical origin discrimination, but they are most likely due to a combination of factors that require further investigation. In fact, Stellacci et al. (2012) indicated that growth under different conditions might affect discrimination due to differences in water and nutrient availability and temperature regimes leading to variation in seed composition.

For sample typology, the best-tested sample with the NIRS technique in the analysis with three macrozones was shelled pine nuts (SH), but the error with this sample increased when considering two wider macrozones. This can be attributed to the large and heterogeneous kernel sizes since whole shelled pine nuts sit in only 1 to 2 layers in the measuring cell of the NIRS instrument, generating air gaps between kernels. The size of these air gaps varies from one filling to another, yielding variable transmission or absorbance values for the same sample when emptied and refilled, as reported by Govindarajan et al. (2009) in peanuts. Even if results in flour can be better than in whole kernels, given the relevance of shelled whole pine nut commercialization, SH presentation would be the easiest to work with sample because it is cost and time-effective. Accordingly, we suggest broadening sample size in the future to improve predictions with this practical presentation, found to be satisfactory for several whole grains and seeds (Williams & Sobering, 1993), almonds (Borràs et al., 2014) and even whole apples (Paz, Sánchez, Pérez-Marín, Guerrero, & Garrido-Varo, 2009).

We also suggest examining pine nut quality and origin of a greater number of samples as part of the traceability process using this analytical approach, including pine nuts from other species, regions and countries to compare and improve our results in future research.

5. Conclusions

NIRS measurements were employed for identifying geographical origins of *P. pinea* nuts according to environmental variables

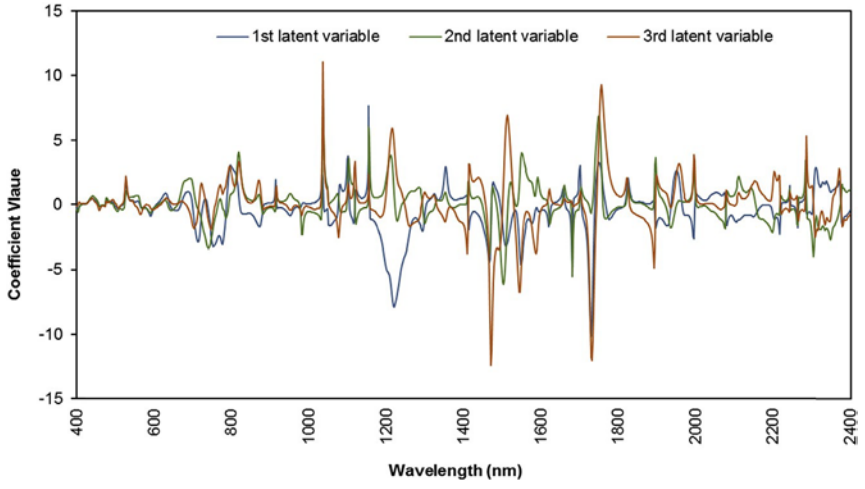


Fig. 8. Weights of the bands for the visible and NIR region of the models obtained for pine nut dried flour at a Chilean scale with two macrozones (derivative 2, 10, 5, and 1 with an error of 9.2%).

and nut quality. Different sample presentations of pine nuts (in-shell and shelled whole pine nuts, and humid and dried pine nut flour) were tested, and multivariate classification models at the country scale were developed using a training set composed of pine nuts harvested from different macrozones where stone pine is grown in Chile. The results indicate that NIRS technology is capable of differentiating between pine nut samples of different geographical origins with errors ranging between 12.2 and 9.2%. The best sample for geographical discrimination among the three Chilean macrozones previously described was shelled pine nuts.

Considering the relevance of the shelled pine nut market, and the different results that this presentation can show, sample size should be increased to improve predictions with this easy to work presentation, which has been found to be satisfactory for several whole grains, seeds and fruits, including dried fruits.

Accordingly, the main application of this technique aims to improve traceability, which is essential for controlling quality and origin in the pine nut industry, and is relevant considering the increasing number of allergy cases reported for pine nuts mostly from other pine species.

Implementing this technique at an industrial scale will require the use of training sets tailored to a specific aim, as well as deeper knowledge of the chemical and structural characteristics related to the spectral ranges on which classification models rely. These results should be enriched by developing models that are more robust by including pine nuts of other geographical origins, collected during several years.

Acknowledgments

This work was supported by FONDEF, CONICYT, Chilean Ministry of Education (grant code D11I1134). NIR data were obtained using NIRS hardware and software provided by the NIR/MIR Spectroscopy Unit of the SCAI at the University of Cordoba, Campus de Excelencia CEIA3 (Spain).

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