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Article

Assessment of Ora-Pro-Nobis (*Pereskia aculeata* Miller) Leaves Shelf-Life in Different Conditions by Using NIR Spectroscopy and Augmented Matrices with Chemometrics

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Ora-pro-nobis (*Pereskia aculeata* Mill) is an unconventional food plant (UFP) rich in proteins, vitamins, fibers, and antioxidants. In this study, ora-pro-nobis leaves were investigated as a proof-of-concept by near-infrared (NIR) spectroscopy concerning the storage time (from collection to 12 days), packaging system: styrofoam-based packaging covered with stretchable polychloride vinyl (PVC) film, and vacuum packaging (nylon/poly), temperature (at room temperature of 20 ºC, and in the refrigerator at 4 ºC), and sanitization condition (washed and without washing). Principal component analysis (PCA) was applied to augmented matrices, showing that unwashed leaves stored in the refrigerator with styrofoam-based packaging covered with PVC film were better preserved over time. Furthermore, it has been suggested that NIR absorptions are related to proteins, carbohydrates, lipids, vitamins, antioxidants, and water from ora-pro-nobis leaves and their physiological reactions over time. By combining NIR spectroscopy and chemometrics, a complete understanding of the shelf life of ora-pro-nobis leaves was achieved.

Keywords: unconventional food plant, PCA, package, temperature, sanitization, storage time

Introduction

Unconventional food plants, known by the acronym UFPs, are increasingly gaining attention in the scientific and social context due to their advantages, such as wide bioavailability, ease of access by the population, low or zero toxic effects, and high nutritional value.[1]

UFPs can be herbs, vegetables, fruits, and flowers that grow spontaneously in nature but are unknown to most people and often confused with weeds.[2] UFPs can also be defined as species that have edible parts (leaves, flowers, stems, fruits, roots, seeds, and inflorescences), which can be included in various food preparations.[3] Numerous species of UFPs are found in abundance and grow involuntarily in flower beds, gardens, orchards, and backyards, whether in the countryside or the city. These plants are easy to cultivate and sustainable. They do not require significant changes in the flora for their production and are adaptable to different soil types and climates.[4]

In Brazil, at least 10% of the native flora, around 4 to 5 thousand species of UFPs, could be part of our daily consumption.[5] However, due to the lack of knowledge by most of the population, several UFPs are ignored.[6]

On the other hand, UFPs can be considered functional foods rich in vitamins, antioxidants, fibers, proteins, and minerals. One example of this situation is the ora-pro-nobis (*Pereskia aculeata* Mill).[7,8]

According to the Brazilian Agricultural Research Corporation (EMBRAPA),[9] the ora-pro-nobis (OPN) belongs to the Cactaceae family, the *Pereskia* genus, the only one in the family that produces leaves and exhibits

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relevant nutritional value in proteins, minerals (zinc, potassium, magnesium, calcium, and iron), fibers and mucilaginous substances bringing health benefits.

The OPN leaves can be used to prepare salads, soups, omelets, and pies, and the leaf flour can serve as an enriching element in the formulation of bread, cakes, and pasta. Furthermore, its mucilage can replace eggs in food preparations, which is especially interesting for consumers with food allergies or dietary restrictions. The OPN fruits can be employed in juices, jellies, mousses, and liqueur production.[7,10] Furthermore, considering its benefits and applicability, the OPN has gained space in Brazil.[11] Several consumers are reluctant due to doubts about its toxicity and eating habits.[12] According to Silva *et al.*,[3] the lack of market space currently observed for UFPs is often due to the marketing system, with a valorization of the conventional plants.

Storage temperature is crucial for the plant's respiratory rate, accelerating or slowing down the metabolism of plants, which continues after harvest. The management and postharvest conservation of OPN can be improved when using cooling and plastic packaging, increasing the shelf life by seven days. Moreover, low temperature (refrigeration) decreases the metabolism of mesophilic microorganisms, which are the most common contaminants.[13] In addition, OPN is depleted in quality attributes, nutraceutical properties, and high perishability due to its high respiratory and transpiration rates, leading to rapid consumption of accumulated carbohydrates reserves and excessive water loss, which contributes to rapid deterioration wilting.[14]

Based on the exposure, it is clear that investigations concerning OPN conservation are essential to improve and turn its commercialization possible. So, this work aims to investigate the OPN conservation in two packaging systems, under different temperatures and over time, using near-infrared spectroscopy (NIRS) and chemometrics.

Experimental

Ora-pro-nobis leaves

Ora-pro-nobis leaves (28 leaves in total) were collected from a property in Campo Mourão, Paraná, Brazil, in June 2021. The harvest time was from 7:30 a.m. to 8:00 a.m. on a day when the temperature was 14 °C.

Before packaging, fourteen leaves were washed with tap water and dried on absorbent paper. The other leaves' portions (14 leaves) were packaged without washing.

The ora-pro-nobis leaves presented an average length of 10.1 ± 0.7 cm and an average width of 4.4 ± 0.4 cm.

Packaging of leaves

Two types of packaging were investigated: (*i*) styrofoam-based packaging covered with stretchable polychloride vinyl film (PVC film), grammage 11.25 g m^{-2}, and (*ii*) vacuum packaging with a vacuum packaging machine (maximum vacuum degree of 430 mmHg) using an antibacterial plastic bag (nylon/poly).

A total of three leaves were placed for each packaging system. In addition, one leave was kept as a control, i.e., without packaging.

Storage of ora-pro-nobis leaves

Two storage conditions were investigated: (*i*) at room temperature (20 ºC) and (*ii*) placed in the refrigerator (4 ºC).

Acquisition of NIR spectroscopy data

The NIR spectra were obtained from a portable JDSU microNIR spectrometer (Milpitas, California, USA, sold in 2013). Spectra were recorded over the packaging (on the control leaves directly on the leaves) from 906 to 1600 nm. The spectra were smoothie and transformed through the first derivative by the Savitsky-Golay algorithm,[15] (first-order polynomial and 9 points) using the Matlab software version R2007b.[16]

Samples were monitored for 12 days (immediately after packaging, i.e., time zero, and after 6, 12, 24, 48 h, and every day from 3 to 12 days) to study the effect of the quality of leaves at different systems. Three spectra were acquired at each leave, and the mean was calculated, including the nine spectral replicates from 3 leaves (in each spectral collecting time). Figure 1 summarizes the experimental design.

Principal components analysis (PCA)

Principal component analysis (PCA) is an unsupervised pattern recognition tool known as exploratory data analysis. This tool is used to find trends or similarities in a data set. Thus, collaborating to highlight and extract information from a data set.[17]

PCA removes the correlations between different variables from the data set and transforms them into a new coordinate system named principal component (PC) using an orthogonal transformation. Then, the new coordinates in the data set are searched to retain the largest variances.[18]

Basically, in an **X** matrix (NIR spectrum in this case), each sample is represented by a row vector (mean of the spectra at each time and condition), and each variable has

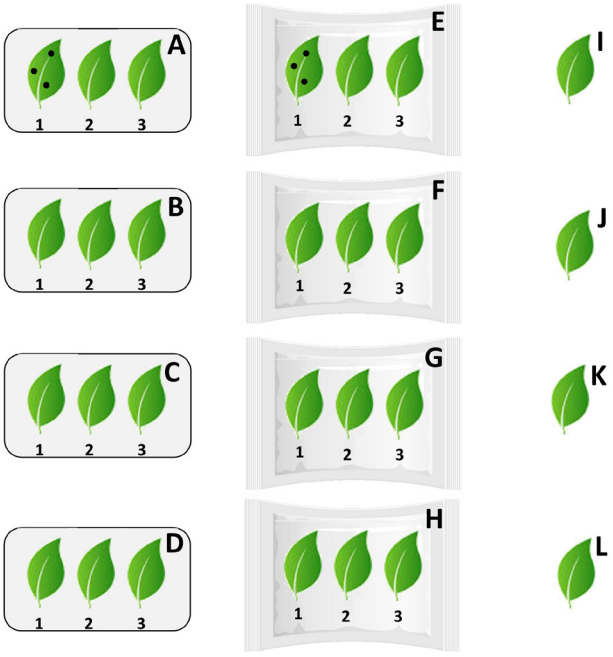

Figure 1. Scheme for experimental design. Styrofoam-based packaging covered with PVC film: washed leaves stored in a refrigerator (**A**); unwashed leaves stored in a refrigerator (**B**); washed leaves stored at room temperature (**C**); unwashed leaves stored at room temperature (**D**). Vacuum packaging: washed leaves stored in a refrigerator (**E**); unwashed leaves stored in a refrigerator (**F**); washed leaves stored at room temperature (**G**); unwashed leaves stored at room temperature (**H**). Without packaging: washed leaves stored in a refrigerator (**I**); unwashed leaves stored in a refrigerator (**J**); washed leaves stored at room temperature (**K**); unwashed leaves stored at room temperature (**L**). Dark points show where spectra were acquired.

a column vector (instrumental NIR results from 906 to 1600 nm). The PCA decomposes the **X** matrix into matrices of scores (**T**) and loadings (**P**), according to equation 1. The step-by-step algorithm is described by Wold *et al*.[19]

$$\mathbf{X} = \mathbf{T}\,\mathbf{P}^{T} \quad (1)$$

In other words, PCA is fundamentally a reduction data method. After the PCA decomposition, the scores and loadings matrices can be used to find the trends or similarities, i.e., with the scores, it is possible to verify trends among samples. In contrast, the loadings indicate which variables are responsible for those similarities observed in the scores.[20] The data were organized in an augmented matrix where the PCA was applied using the Matlab software version R2007b[16] and the PLS-Toolbox 5.2,[21] according to the scheme shown in Figure 2.

According to the scheme (Figure 2), each matrix represents one storage condition. Moreover, each one was composed with mean spectra in the times zero (immediately after the package), after 6, 12, 24, and 48 h, and every day from 3 to 12 days. In the sequence, it was placed the matrices formed by average spectra at different times for

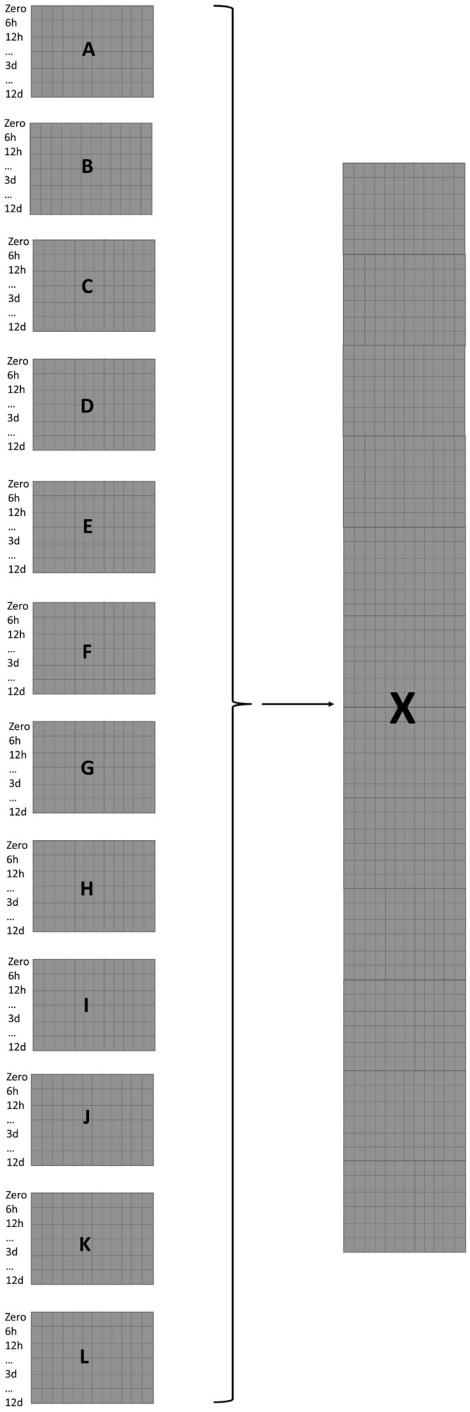

Figure 2. Scheme of the procedure for the augmented matrix (**X**). The matrix formed by average spectra at different times for samples packed in styrofoam-based packaging covered with PVC film: unwashed leaves stored in a refrigerator (**A**); unwashed leaves stored at room temperature (**B**); washed leaves stored in a refrigerator (**C**); washed leaves stored at room temperature (**D**). The matrix formed by mean spectra at a different time for samples in vacuum packaging: unwashed leaves stored in a refrigerator (**E**); unwashed leaves stored at room temperature (**F**); washed leaves stored in a refrigerator (**G**); washed leaves stored at room temperature (**H**). The matrix formed by mean spectra at a different time for samples without packaging: unwashed leaves stored in a refrigerator (**I**); unwashed leaves stored at room temperature (**J**); washed leaves stored in a refrigerator (**K**); washed leaves stored at room temperature (**L**).

samples packed in styrofoam-based packaging covered with PVC film (from **A** to **D**), being: (**A**) when the leaves were unwashed and stored in a refrigerator; (**B**) when the leaves were unwashed, and stored at room temperature; (**C**) the leaves were washed and stored in a refrigerator; and (**D**) the leaves were washed, and stored at room temperature.

In the sequence, it was placed the matrices formed by mean spectra at different times for samples in vacuum packaging (from **E** to **H**) being: (**E**) when the leaves were unwashed and stored in a refrigerator; (**F**) when the leaves were unwashed, and stored at room temperature; (**G**) the leaves were washed and stored in a refrigerator; (**H**) the leaves were washed and stored at room temperature.

Finally, it was placed the matrices formed by mean spectra at different times for samples without packaging (from **I** to **L**) being: (**I**) the leaves unwashed and stored in a refrigerator; (**J**) the leaves unwashed and stored at room temperature; (**K**) the leaves washed and stored in a refrigerator; (**L**) the leaves washed and stored at room temperature. By concatenating all those matrices, it was formed the hypermatrix named **X**.

Results and Discussion

The preprocessed NIR spectra of all ora-pro-nobis leaves are shown in Figure 3. In contrast, the raw NIR spectra for the leaves under different conditions are shown in the Supplementary Information (SI) section (Figure S1). Concerning the raw NIR spectra, it is crucial to highlight that the signal changes as time increases. So, there are storage conditions when the difference is not expressive. On the other hand, there are storage conditions where the difference over time is significant. Furthermore, it is also in the SI section (Figure S2), the plot for raw NIR spectra for unwashed leaves stored at room temperature and in styrofoam-based packaging covered with PVC film. By regarding the plot with all spectra (3 spectra *per* leaf, i.e., 9 spectra *per* sample in this packaging system) it is possible to observe that there are repeatability and robustness in the spectra acquiring and in the proposition.

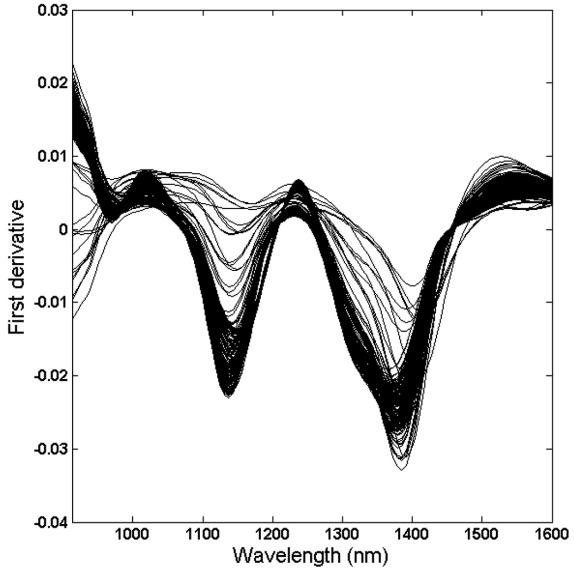

Figure 3. Preprocessed NIR spectra of ora-pro-nobis leaves under different packaging systems over time.

Regarding Figure 3, the preprocessing was done through the Savitsky-Golay algorithm,[15] based on the first derivative (9 points and first-order polynomial). The first derivative corrects for baseline shifts. Furthermore, derivatives may enhance the resolution of overlapping signal features, emphasizing minor structural differences among very similar signal profiles. However, to overcome noise enhancement (a natural consequence of the derivation of noisy signals), the Savitzky-Golay algorithm is integrated with a smoothing step performed by polynomial interpolation with a moving window through the signal. In this way, an important point that does not influence itself result interpretation is represented by choice of the smoothing parameters (polynomial degree and the data-point size of the moving window). The extent of smoothing increases with decreasing the polynomial degree and increasing the window size.[22]

Furthermore, the smoothing level can be refined by varying the window size (odd integer values are admitted). Nonetheless, there is no rule, and the best compromise depends on the total number of signal points, signal resolution, and the signal-to-noise ratio. Although, a 2nd-degree polynomial is suggested to be selected only in the case of elevated noise levels.[22]

Figure 3 was obtained from augmented matrices, defined as concatenation between two or more matrices of bilinear data from different systems, which share some or all of their compounds in a third direction, representing the qualitative or quantitative difference between samples. Thus, simultaneously analyzing different mixtures of the same compounds under different conditions (in this case, the packaging system) is a bright, reliable way to extract information about the systems' individualities.[23,24]

The interpretation of the augmented matrix was performed through PCA, with mean-center preprocessing, and valuable information was recorded from the scores and loadings of PC1 (Figure 4) and scores (Figure 4) and loadings (Figure 5) for PCs 2 and 3, respectively. Spectra related to the common order of the augmented data set by columns are considered invariant,[24] and the scores allow different profiles for each matrix.

The complexity of a PCA model depends on the purpose for which the PCA results are used. A discussion proposed by

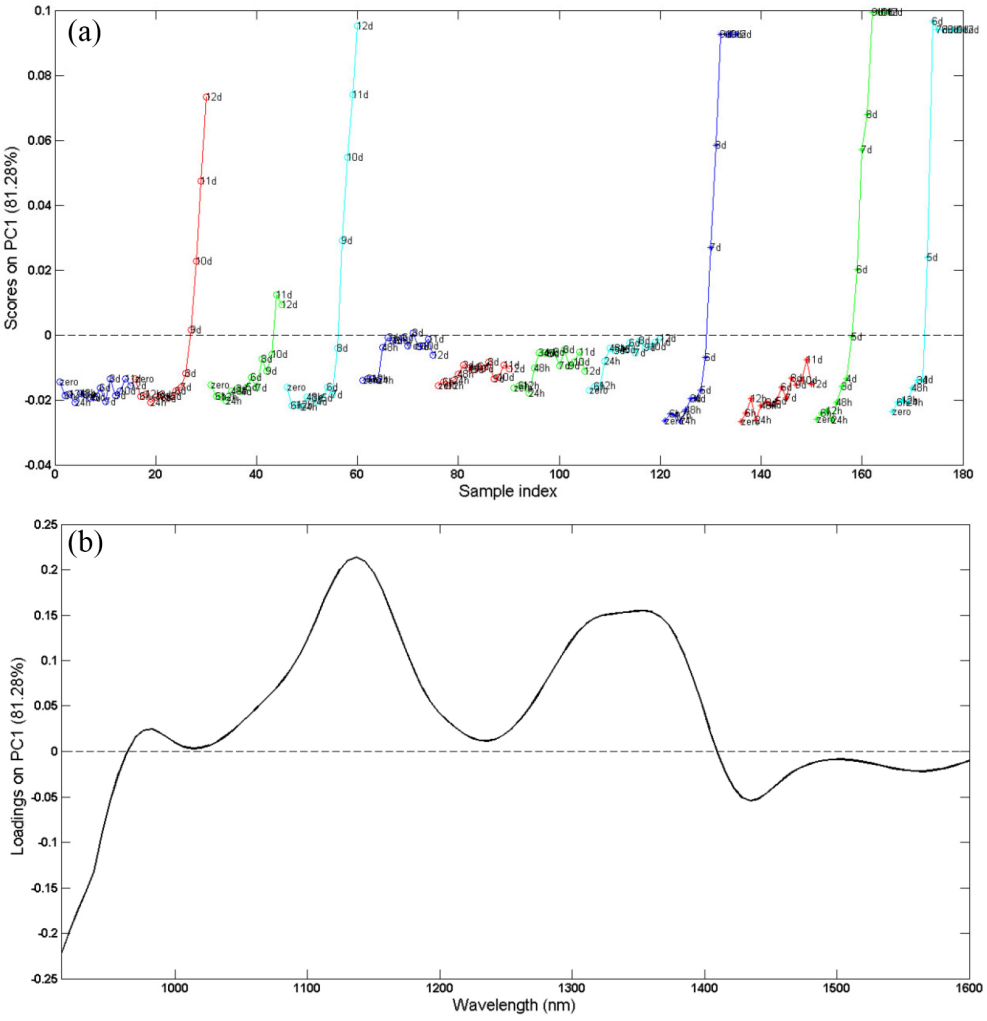

Figure 4. Scores (a) and loadings (b) on PC1. (o) Styrofoam-based packaging covered with PVC film; (•) vacuum packaging; (∗) without packaging. Blue: unwashed leaves stored in a refrigerator, red: unwashed leaves stored at room temperature, green: washed leaves stored in a refrigerator, cyan: washed leaves stored at room temperature.

Rodionova *et al.*[18] shows that considering a data set obtained from a single population, usually the data fits one of the following cases: (*i*) noise or fully random data (in this case, any number of PCs may be considered optimal); (*ii*) structure + noise (so only the PCs explaining the structural part are important); (*iii*) structure + noise + outliers.

On the other hand, sometimes, a data set consists of individual measurements associated with different populations (that meet this research): (*i*) spectra obtained in different experimental rounds (e.g., the same instrument, but different days); (*ii*) a case of two or more mixed populations results in structural and noise parts: structure 1 + noise 1 + structure 2 + noise 2 + ... + structure *n* + noise *n*. Moreover, the structural parts can also overlap, which means they contain shared information, which is common for both groups. In such cases, selecting the optimal number of components is not straightforward.[18]

In this way, considering the discussion, it is possible to suggest that the main information on PC1 concerns leaf dryness (the naked eye could observe that). So, the unwashed leaves stored in a refrigerator with styrofoam-based packaging covered with PVC film and the leaves stored with vacuum packaging (unwashed and washed, stored in a refrigerator or at room temperature) were the conditions where the leaf dryness was not observed.

Regarding the scores in PC2, it is possible to observe a general decrease over the storage time. However, there is an increase in some cases after a specific time, mainly with unwashed leaves stored at room temperature and in the refrigerator and washed leaves stored in the refrigerator with styrofoam-based packaging covered with PVC film. This fact can also be observed with unpackaged and unwashed leaves stored in the refrigerator and with washed leaves stored at room temperature and in the refrigerator. This effect is slight for leaves stored under vacuum packaging and at room temperature.

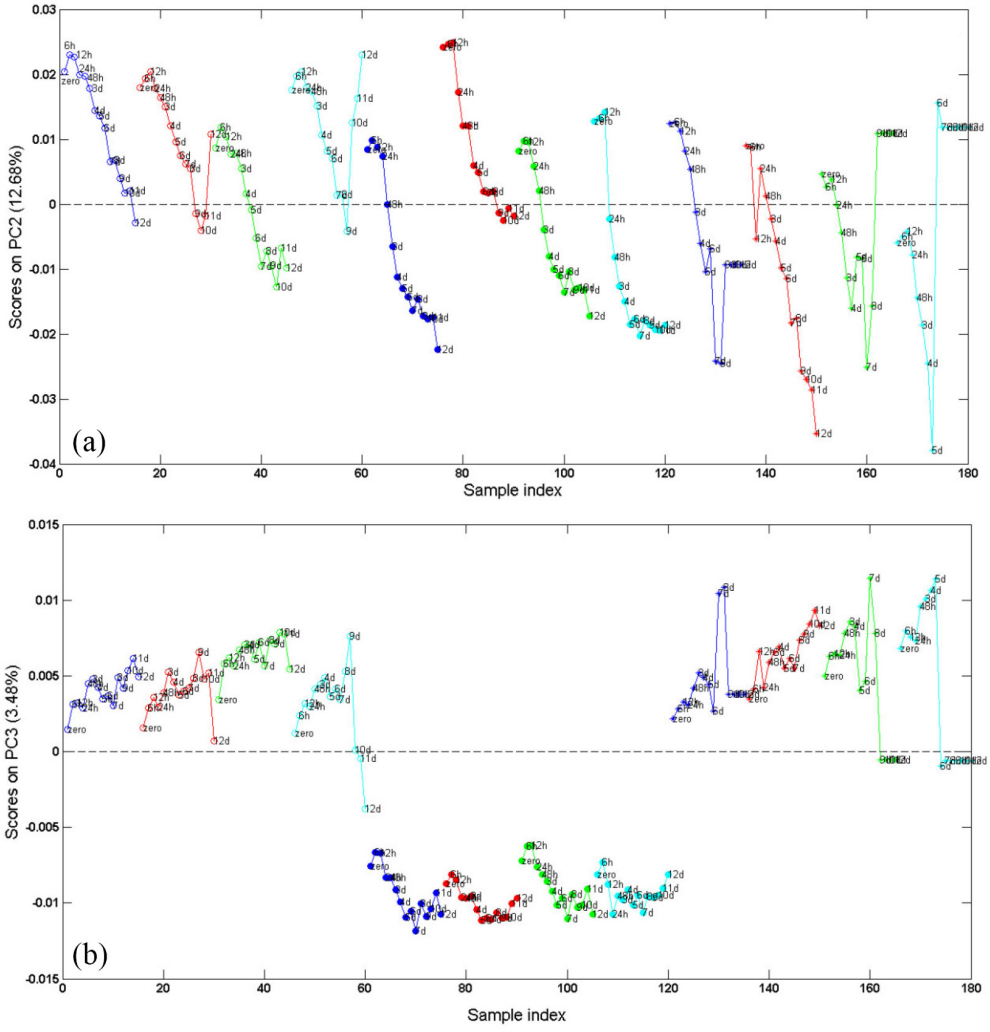

Figure 5. Scores on PC2 (a) and PC3 (b). (o) Styrofoam-based packaging covered with PVC film; (•) vacuum packaging; (∗) without packaging. Blue: unwashed leaves stored in a refrigerator, red: unwashed leaves stored at room temperature, green: washed leaves stored in a refrigerator, cyan: washed leaves stored at room temperature.

The study of postharvest physiology of plants involves metabolic processes and changes in different parts of plants, from the moment they are harvested to complete senescence. Deterioration processes result from complex changes in physiological conditions, such as the depletion of reserves by the respiration process, wilting due to excessive loss of water through transpiration, and stem occlusion after cutting, which obstructs the conducting vessels, causing air embolism and deposition of chemical substances.[14,25] These processes can explain the scores decrease over time and, in many cases, an increase in the scores plot after a determined time.

Generally, packaging based on styrofoam covered with PVC film suggests more remarkable preservation over time. The unwashed leaves stored in the refrigerator with styrofoam-based packaging covered with PVC film seem to be better preserved over time, reaching the side of the negative scores after 11 days of storage. On the other hand, with vacuum packaging systems, only unwashed leaves stored at room temperature seem to be preserved, reaching eight days, while in other cases, it is suggested that the leaves were preserved only for a few hours. In addition, for unpacked leaves, it is also suggested that the preservation occurred only for a few hours in all cases.

Plants and vegetables are minimally processed before reaching the market. However, minimal processing, such as sanitization, can lead to problems preserving quality since exposed surfaces trigger physiological reactions, favoring oxidation and, consequently, browning.[26]

In PC3, the pattern observed in PC2 is also shown. On this PC, it is possible to see a differentiation between the storage of the leaves in the vacuum pack on the negative side of the PC3 and the leaves stored with Styrofoam-based packaging covered with PVC film and without packaging on the positive side of this PC. Thus, it is possible to assign information about the type of packaging used for

this PC. Furthermore, although PC2 presents 12.68% of the explained variance and PC3 only 3.48%, it has been reported in previous studies[27-29] that the principal information of a data set is sometimes not present in the PC that presents the highest explained variance.

To explain the observations obtained by the scores, the loadings plot (Figure 6) shows the important spectral regions.

The positive PC2 loadings correspond to the regions from 970 to 1070 nm (H_2O absorption in the third overtone region), 1160 to 1210 nm (CH, CH_2, and CH_3 absorption in the second overtone region), and 1340 to 1450 nm (CH, CH_2, CH_3, $CONH_2$, ArOH, ROH, and H_2O absorptions in the second overtone region).[30] Moreover, specific absorptions of the O–H stretch band in water (979, 1200, 1408, and 1453 nm) can also be attributed to these positive loadings.[31]

The negative side of PC2 loadings is related to the ranges from 900 to 970 nm (CH, CH_2, CH_3, ArOH, and ROH absorption in the third overtone region), 1070 to 1160 nm (RNH_2, and ArCH absorption in the third overtone region), 1210 to 1340 nm, and 1450 to 1600 nm (CONHR, and RNH_2 absorption in the second overtone region, and ArCH absorption in the first overtone region).[30] Furthermore, the region above 1450 nm is strongly correlated to protein absorption (1570 nm N–H (2ν) of -CONHR, 1463 nm N–H (2ν) of -$CONH_2$, 1471 N–H (2ν) of -CONHR, 1483 nm N–H (2ν) of -$CONH_2$, 1490 N–H (2ν) of -CONHR, 1492 –H (2ν) of $ArNH_2$, 1500 nm N–H (2ν) of -NH_2, 1510 nm N–H (2ν) of -$CONH_2$, 1520 nm N–H (2ν) of -$CONH_2$, 1530 N–H (2ν) of RNH_2, 1529-1600 nm N–H (2ν) stretch from secondary amides in proteins.[31]

The loadings on PC2 can be related to the chemical composition of ora-pro-nobis leaves. According to a previous study,[7] the chemical composition of 100 g of OPN leaves contains: energy 26 kcal, protein 2 g, lipids 0.4 g, carbohydrates 5 g, fibers 0.9 g, calcium 79 mg, phosphor 32 mg, iron 3.6 mg, retinol 250 mcg, vitamins B1, B2, and C of 0.02 mg, 0.10 mg, and 23 mg, respectively, niacin 0.50 mg. Furthermore, a total phenolic content value of 60.09 mg gallic acid equivalent (GAE) g^{-1} was achieved with OPN leaves extracts by pressurized liquid extraction (10 MPa, 4 mL min^{-1}, 15 min, 110 ºC, ethanol). For an antioxidant activity with DPPH IC_{50} (the effective concentration to reduce 50% the antioxidant activity of the radical DPPH, compared to a blank solution) and FRAP (ferric reducing antioxidant power) under the same extraction conditions, the results were 1.64 mg mL^{-1} and 0.17 $mmol_{TE}$ g^{-1}.[8]

Regarding antioxidants, they can be divided into two chemical groups: amines and phenolics. Antioxidant amines can be subdivided into five categories by common chemical types: (*i*) secondary diarylamines: phenyl naphthylamines, substituted diphenylamines, and *para*-phenylenediamines; (*ii*) ketone-amine condensates; (*iii*) aldehyde-amine condensates; (*iv*) alkyl aryl secondary amines; and (*v*) primary arylamines.[32]

Likewise, phenolic antioxidants can also be subdivided into basic chemical groups: (*i*) hindered phenols; (*ii*) hindered bisphenols; (*iii*) hindered thiobisphenols: and (*iv*) polyhydroxy phenols.[32]

Based on PC2 loadings, it is possible to suggest that the absorptions are related to proteins, carbohydrates, lipids, vitamins, antioxidants, and water from OPN leaves and their physiological reactions over time.

The PC3 loadings can be highlighted by differentiating samples regarding the packaging type. While the region from 900 to 1200 nm presents similar behavior with PC2 loadings, the same information regarding the samples over

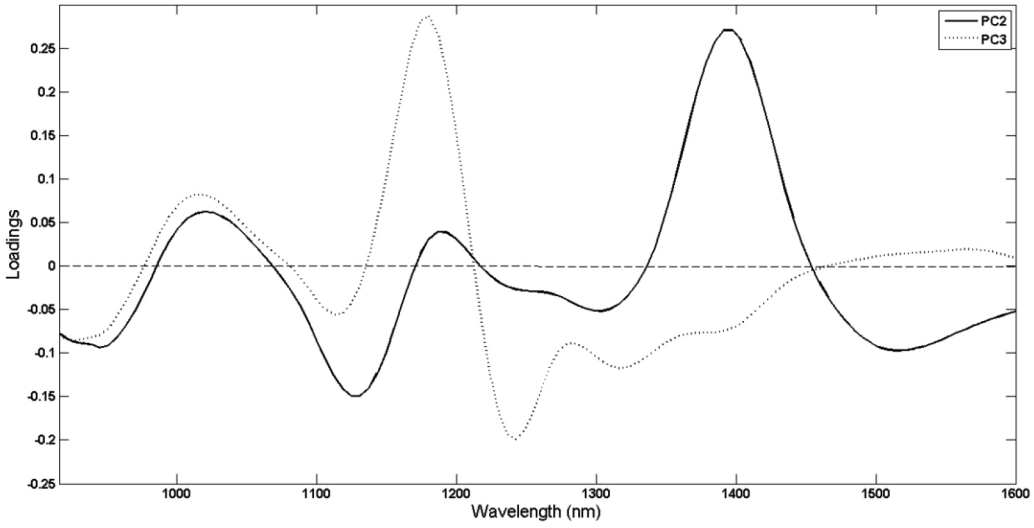

Figure 6. Loadings on PC2 and PC3.

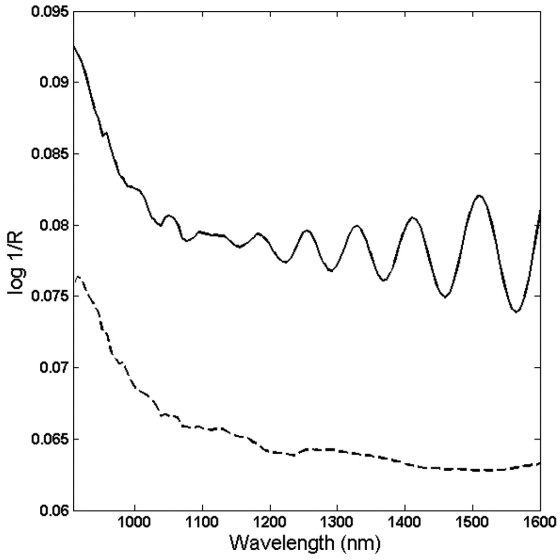

Figure 7. Packaging spectra. (—) PVC film; (---) vacuum packaging.

time is also present in PC3. On the other hand, comparing the loadings plot above 1200 nm with the packaging spectra (Figure 7), the distinction between the two package types is clear from 1200 to 1600 nm.

Conclusions

In this proof-of-concept study, ora-pro-nobis leaves were investigated for storage time, packaging system, temperature, and sanitization condition by near-infrared spectroscopy. The PCA was applied in the augmented matrices condition, showing informative plots, demonstrating the relationship between the samples by the scores plot and a complete understanding when combining it with the loadings plot.

The results suggested that unwashed leaves stored in the refrigerator with styrofoam-based packaging covered with PVC film were more preserved over time. Furthermore, it was suggested that NIR absorptions are related to proteins, carbohydrates, lipids, vitamins, antioxidants, and water of the ora-pro-nobis leaves and their physiological reactions over time, and also by the leaf dryness.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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Author Contributions

Fernanda Lansa Furlan was responsible for conceptualization, data curation, formal analysis, investigation, resources, software, validation, visualization, writing original draft; Makoto Matsushita for conceptualization, funding acquisition, investigation, project administration, writing-review and editing; Aline Coqueiro for conceptualization, data curation, formal analysis, investigation, resources, software, validation, visualization, writing-review and editing; Paulo Henrique Março for conceptualization, data curation, investigation, resources, software, writing-review and editing; Patrícia Valderrama for conceptualization, data curation, formal analysis, funding acquisition, investigation, project administration, resources, software, validation, visualization, writing-review and editing.

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