SPECIAL GUEST EDITOR SECTION

Non-Destructive Measurement of the Internal Quality of Citrus Fruits Using a Portable NIR Device

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Abstract

The citrus industry has grown exponentially as a result of increasing demand on its consumption, giving it high standing among other fruit crops. Therefore, the citrus sector seeks rapid, easy, and non-destructive approaches to evaluate in real time and *in situ* the external and internal changes in physical and nutritional quality at any stage of fruit development or storage. In particular, vitamin C is among the most important micronutrients for consumers, but its measurement relies on laborious analytical methodologies.

In this study, a portable near infrared spectroscopy (NIRS) sensor was used in combination with chemometrics to develop robust and accurate models to study the ripeness of several citrus fruits (oranges, lemons, clementines, tangerines, and Tahiti limes) and their vitamin C content. Ascorbic acid, dehydroascorbic acid, and total vitamin C were determined by HILIC-HPLC-UV, while soluble solids and total acidity were evaluated by standard analytical procedures.

Partial least squares regression (PLSR) was used to build regression models which revealed suitable performance regarding the prediction of quality and ripeness parameters in all tested fruits. Models for ascorbic acid, dehydroascorbic acid, total vitamin C, soluble solids, total acidity, and juiciness showed $R_{cv}^2 = 0.77$–0.87, $R_{cv}^2 = 0.29$–0.79, $R_{cv}^2 = 0.77$–0.86, $R_{cv}^2 = 0.75$–0.97, $R_{cv}^2 = 0.24$–0.92, and $R_{cv}^2 = 0.38$–0.75, respectively. Prediction models of oranges and Tahiti limes showed good to excellent performance regarding all tested conditions. The resulting models confirmed that NIRS technology is a time- and cost-effective approach for predicting citrus fruit quality, which can easily be used by the various stakeholders from the citrus industry.

Citrus fruit is one of the world's major fruit crops with global production and international trade growing every year due to the high demand imposed by consumers (1). This need for fresh and processed citrus availability comes mostly from their preferred flavor, delightful taste, affordable economic reach, and consumer awareness of the increasingly recognized health benefits, mostly owing to its vitamin C content (2). Besides being a cofactor of enzymes responsible for the biosynthesis of collagen, vitamin C is also a potent water-soluble antioxidant. However, the variability of vitamin C content and organoleptic properties (e.g.,

sweetness and acidity) in citrus products is greatly influenced by variety, maturity, climate, handling, processing, and storage conditions (2). Moreover, the main quality features that drive consumers to acquire fruit and vegetables are based on external aspects such as appearance, color, size, and absence of blemishes, not necessarily transposed to the internal quality parameters sought by consumers, mostly related to soluble solids content (SSC), titratable acidity (TA), soluble solids to acid (SSC/TA) ratio, and texture, as revised by Arendse and colleagues (3). Therefore, consistent supply of nutritious and pleasant citrus fruits motivates the development of innovative non-destructive tools for field and laboratory measurement as well as sorting and grading based on both external and internal fruit attributes (4).

Numerous non-destructive techniques have been used for the evaluation of internal and external quality characteristics of citrus products. These include near infrared spectroscopy (NIRS) (5), NIRS-based systems such as multispectral and hyperspectral imaging (6), nuclear magnetic resonance imaging (7), and X-ray computed tomography (8). Notwithstanding, NIRS is still one of the most used methods for assessing fresh fruit inner quality (4). In addition, smartphone-based food diagnostic technologies have been emerging and offer significant advantages over traditional platforms in terms of test speed, control, affordability, ease-of-operation, and data management, and require minimal equipment and user involvement (9). These approaches can be divided into two categories: lab-on-smartphone biosensors (involving with fluorescence imaging, colorimetric readers, and electro-analytical platforms) and smartphone optical and spectroscopy (9). Unlike biosensors, in which it is necessary a reagent to trigger the transduction, in smartphone spectroscopy the analysis is performed in a non-invasive manner (9). Also, the entire assembly along with the smartphone can be realized with a total cost of at least five times lower than reference spectrometer platforms (9).

The system used in this manuscript (Tellspec, Canada) relies on a three-part system that includes a pocket-sized NIR sensor, a cloud-based patented analysis engine, and a mobile app (10), previously used under different food approaches from macronutrients to allergens and contaminants, giving information on possible fraud, adulteration, and quality of foodstuffs, or even in the quality control of pharmaceutical drugs (11), meat (12), and honey (13). However, there are no available studies regarding its use in citrus fruits. The main goal of this study is to investigate the feasibility of this pocket-sized NIR sensor as an accurate, robust, user-friendly, and cost-effective tool for predicting the ripeness status, organoleptic, and nutritional quality of commercially relevant citrus, supporting the interest of producers, retailers, and consumers.

Experimental

Chemicals

Acetonitrile (HPLC grade) was acquired from Honeywell (Riedel-de Haën, Germany). Deionized water of 0.055 μS/cm was obtained with a Seralpur Pro 90CN from Seral, Ransbach-Baumbach (Germany).

L-ascorbic acid (purity > 99%) and tris-(2-carboxyethyl)-phosphine-hydrochloride (TCEP) were purchased from Carl Roth (Karlsruhe, Germany). Metaphosphoric acid, trifluoroacetic acid (TFA), sodium hydroxide, and phenolphthalein were all analytical grade from Merck, Germany.

Sampling and Sample Preparation

Fruits, without evident injuries or illnesses, were acquired between February and March 2017 in local markets of Porto, Portugal. The sample set comprised 135 samples belonging to various cultivars of the genus Citrus, which included 39 oranges (*Citrus sinensis* L.), 30 lemons (*Citrus limon* L.), 25 clementines (*Citrus × clementina*), 21 tangerines (*Citrus reticula* L.), and 20 Tahiti limes (*Citrus × latifolia*).

Collected fruits were separated in three ripeness stages (unripe, ripe, and overripe) and, at the lab, fruit weight and circumference perimeter were measured, and volume was determined according to the following formula (14):

$$V = \frac{4}{3} \pi \left(\frac{P}{2\pi} \right)^3$$

where V represents fruit volume (mL) and P stands for the fruit circumference perimeter (cm). Juice ratio (J) was also estimated according to:

$$J = \frac{W_j}{W_f} \times 100$$

where W_j represents juice weight (g) and W_f stands for the fruit weight (g).

After morphological measurements, samples were kept refrigerated at 4°C until the following day, when laboratory testing was conducted. Samples were allowed to reach room temperature (20 ± 3°C) before spectra acquisition by portable NIRS sensor and subsequent destructive tests of chemical analysis.

Spectra Acquisition

NIRS spectra of citrus fruits were collected in reflectance mode (log 1/R) using the Tellspec Entreprise sensor (Tellspec Inc., Toronto, Canada) at (20 ± 3°C). The NIRS handheld analyzer incorporates essential components like a reflectance probe, rechargeable batteries, and a Bluetooth module. It is equipped with two integrated tungsten halogen lamps and the reflected light is collected and measured by a single, uncooled InGaAs photodetector (1 mm). The spectrophotometer scans at a non-constant step of 2 nm across a spectral range of 900–1700 nm and presents a signal-to-noise ratio of 5000:1. The device is equipped with a quartz protection lens to prevent damage and dirt accumulation. Daily calibration was performed with Spectralon® white diffuse reflectance standard (99%).

At least 2 scanning spots were selected for each fruit and 16 spectral measurements were made around each spot in 4 separate daily sessions, with the fruit inside a clear plastic bag (polyethylene and polypropylene). A total of 540 spectra were averaged to provide a mean spectrum for each sample.

The spectral pre-treatment procedures, such as standard normal variate (SNV), Savitzky–Golay (SG) smoothing, derivation methods (usually first and second derivative), data normalization, and multiplicative scatter correction (MSC) were applied in order to eliminate the scatter effects in the spectra and improve the accuracy of the models.

Chemical Analysis

Immediately after acquiring the NIRS spectra, the fruit was cut into pieces and each spot was separately squeezed by an

electric juicer (Philips Viva Collection, HR 1832, The Netherlands). The juices (N = 282) were divided in two portions, one for direct determination of SSC and TA, and another portion to be stored at −80°C until vitamin C quantification.

Total soluble solids contents (° Brix) was measured by a handheld digital refractometer (VWR, type A Cat No. 635–0722, Belgian), while TA was estimated by titrimetry based on official methods of analysis (15) and results were reported in citric acid equivalents (g per 100 mL of juice).

The total vitamin C content, alongside with the individual fractions of ascorbic acid (AA) and dehydroascorbic acid (DHA) were determined according to Barros and co-workers (16) by hydrophilic interaction liquid chromatography (HILIC). Briefly, defrosted samples were diluted with a metaphosphoric acid solution (1:5, 6.25% m/v) and vortexed. For AA determination, 500 μL of acetonitrile was added to diluted samples (500 μL), centrifuged (10 min, 13 300 rpm, 4°C), and the supernatant was injected in the HPLC system. Regarding total vitamin C, diluted samples (400 μL) were mixed with a TCEP aqueous solution (100 μL, 10 mM), vortexed and incubated for 1 h at 40°C. Afterward, 500 μL of acetonitrile was added to the previous solution, which was then centrifuged (10 min, 13 300 rpm, 4°C) and the supernatant was injected in the HPLC system. The DHA content was estimated by subtracting the amount of AA to the total vitamin C content as described by (17). Chromatographic analysis was accomplished with a JASCO HPLC system equipped with an auto-sampler (Jasco AS-950, Japan), an oven (ECOMEco2000, Czech Republic), and a photo-diode array detector (Jasco MD—4010, Japan). Data were analyzed using Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France). The chromatographic separation was achieved by HILIC using a YMC-Triart Diol-HILIC column (15 cm × 3 mm; 3 μm, YMC, Japan) and acetonitrile–water–trifluoroacetic acid (90:9.9:0.1, v/v) was used as mobile phase at a flow rate of 0.80 mL/min, operating at constant temperature (23°C). The compounds were identified by chromatographic comparison with authentic standards at 268 nm. Quantification was based on the UV signal response using six-level calibration curves and results were expressed in milligram of AA per 100 mL of juice.

Statistical Analysis

Pearson's correlation was established, at a 5% significance level, between morphological and chemical data.

Partial least squares regression can simultaneously evaluate the effect of more than one X-random variables on Y-target variable (18). Therefore, PLSR was used to investigate the relationships between morphological and chemical features (Y-vector) and NIRS spectra (X-matrix) in terms of the prediction of Y-target variable from X-random variables. Five-fold cross-validation (CV) was used to test the predictive significance of the regression. Root mean square error and the coefficient of determination of cross-validation (RMSECV and R^2CV) were used to evaluate the performance of the PLSR models.

Principal component analysis (PCA) was applied to cluster samples according to the type of citrus fruits.

Linear discrimant analysis (LDA) was used as a supervised learner to train models for fruit type calibration. The number of latent variables used were 2 with tolerance set to 0.001.

The data analysis was performed in Tellspec proprietary framework for machine learning model development.

Results and Discussion

Morphology and Chemical Composition

The citrus fruits with higher worldwide commercial relevance are oranges, lemons, limes, grapefruit, and tangerines (1). In the current study, morphological data of these fresh citrus at different ripeness stages, are presented in Figure 1. Average weight and volume of collected fruits are within those reported in the literature (19), although our range is slightly wider, tentatively addressed to include a wider range of commercial maturation stages. In fruits and vegetables, size, mass, volume, and density attributes are usually correlated, with volume and mass together determining fruit density (19). Differences in density have also been utilized for quality inspection of citrus (19), thus it is of utmost importance to include morphological information in mathematical modelling to predict the quality of fruits.

The chemical composition of citrus fruits that includes the sugar content (SSC, ° Brix), acidity (TA), and vitamin C is detailed in Figure 2. Soluble solids content of tested fruits varies from 5.8° Brix (unripe lemon) to 19.9° Brix (ripe tangerine), while TA ranges between 0.3 (ripe clementine) and 8.0% (or g/100 mL, unripe lime). Citrus quality is dependent on several intrinsic and extrinsic factors, such as cultivar or rootstock, harvest period, climate, fruit thinning, hormones, irrigation, and mineral nutrition (2). Therefore, a wide range of both parameters was predictable, although regarding ripe fruits average values are within those described in international standards (20, 21).

Vitamin C is often recognized as the major antioxidant in citrus. Like sweetness and acidity, vitamin C content in fruits can be affected by numerous features such as postharvest treatment, storage conditions, as well as oxidative stress. In our
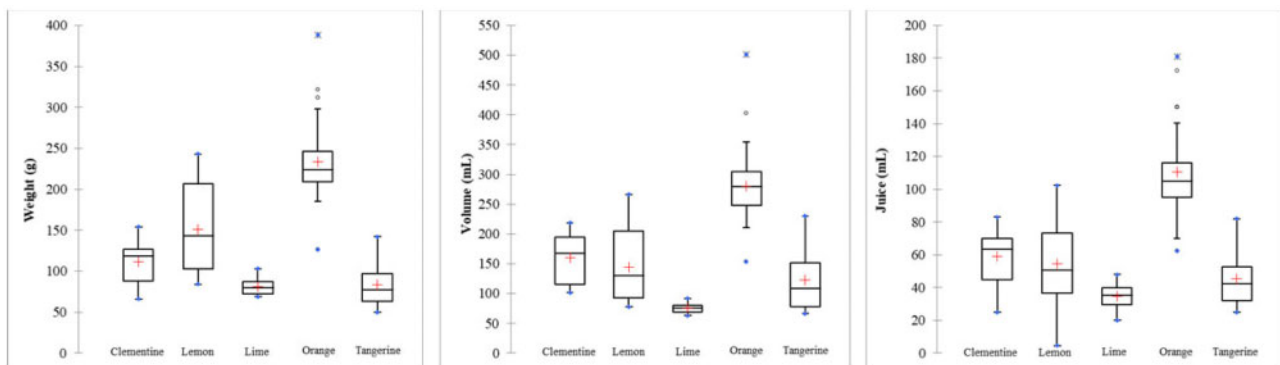

Figure 1. Morphological data of fresh citrus fruits (orange, N = 39; lemon, N = 30; clementine, N = 25; tangerine, N = 21; lime, N = 20).
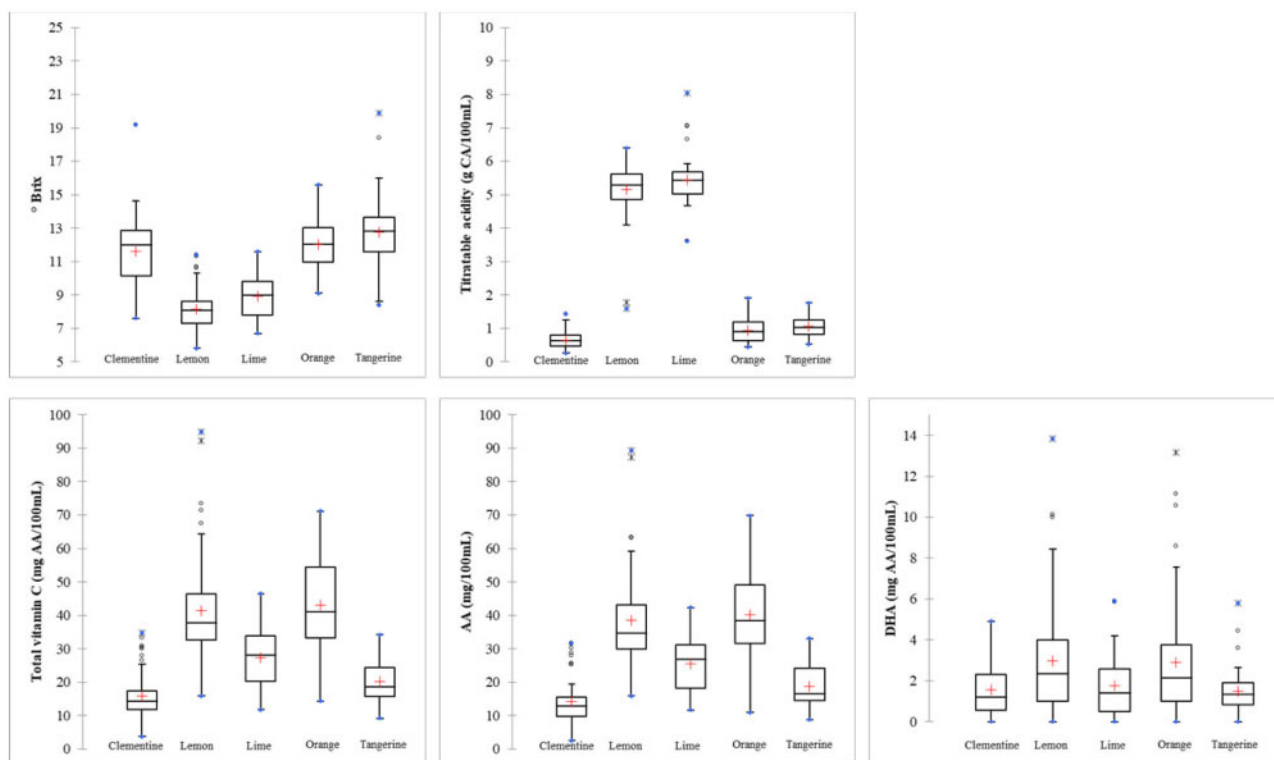
Figure 2. Chemical composition of fresh citrus fruits scanned spots (orange, n = 84; lemon, n = 66; clementine, n = 50; tangerine, n = 42; lime, n = 40). AA = ascorbic acid; CA = citric acid; DHA = dehydroascorbic acid.

study, total vitamin C varied from 3.7 (unripe clementine) to 94.9 mg/100 mL (overripe lemon, Figure 2), which is in accordance with reported data (22, 23).

In citrus, vitamin C is composed mainly by its reduced form, AA, and minority by its first oxidation product, DHA, which seems to possess equivalent biological activity to AA (22). As it can be observed in Figure 2, DHA comprises, on average, 6.4% (in limes) to 9.8% (in clementines) of total vitamin C, as anticipated. Nevertheless, the current study intended to ascertain whether the NIRS measurements would be able or not to distinguish between both oxidation fractions of this hydrophilic vitamin.

Prediction of Fruit Quality from NIRS Spectra

Spectral data in the NIR region represent absorption bands that are related to overtones and combinations of fundamental vibrations mainly due to –CH, –NH, –OH functional groups (24). However, in order to accomplish adequate calibration and robust prediction models, the NIR spectral data often require preprocessing treatments to improve signal-to-noise ratio and correct light-scattering effects that arise due to non-homogenous distribution of sample particles, particle size distribution, and morphology (24). Multiplicative scatter correction and SNV are two popular NIR spectra pre-processing methods that reduce spectral distortions due to scattering (24, 25). However, when scattering effects are removed, the chemical signal may also be reduced. Therefore, the use of derivatives such as SG derivatives (26), is an alternative for correcting the effect of overlapping peaks and removing spectral base line offset and baseline slope (24). The original dataset of NIR spectra and the result of applying a combination of SNV and SG derivatives are shown in

Figure 3. In Figure 3A, well-defined absorption bands are detected at 970 nm and 1470 nm attributed to the second and first overtones of OH stretching, respectively (4). This observation was expected considering the hydration degree of citrus fruits (80–90%) (2), leading to those characteristic water bands. Citrus fruits are also known by high carbohydrate content (9–11%) (2) which can be corroborated by the absorption band at around 1200 nm that corresponds to the second overtones of CH and CH_2 stretching and at around 1370 nm regarding CH combination vibrations (4, 25). By applying SNV or MSC and second-derivative SG (Figure 3B and C) the effect of light scattering is reduced, and the signal is enhanced.

Reducing the number of physical and chemical variables is beneficial to explain the variance of the dataset. Thus, a PCA was performed and the two-dimensional score plot explaining 78% of total variance is shown in Figure 4A. Overall, there is a satisfactory discrimination of fruit type mostly by PC1 except for clementines, which seems to overlap all remaining citrus. In addition, the contribution of spectral points that allow the distinction of citrus fruits was evaluated by the loadings from PCA (Figure 4B). The absorbance band at 1400 nm, at both PCs, was the largest contributor to dataset variance, followed by the absorbance band around 1230 nm and 1170 nm. Even after spectral data pre-treatment, significant differences between citrus fruits can only be verified after an integrated analytical approach combining NIRS and chemical data.

Prior to building a calibration model by PLSR, spectral ranges between 900–950 nm and 1630–1700 nm were removed considering that they were dominated by noise. The outcomes of the PLSR models for NIRS regarding all citrus fruits are detailed in Table 1. Predictive models encompassed single latent variables and, in general, resulted in low RMSECV together with high R^2_{cv}
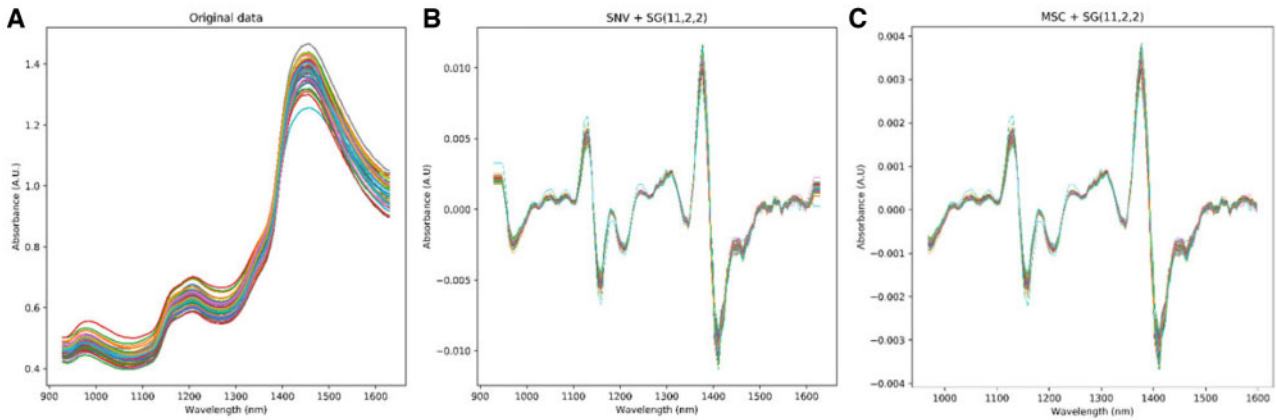
Figure 3. Near infrared spectra without data pre-treatment (A), after SNV plus SG smoothing (B), and after MSC plus SG smoothing (C; 11 wavelengths gap size, second-order polynomial, and second-order derivative). MSC = multiplicative scatter correction; SG = Savitzky–Golay; SNV = standard normal variate.
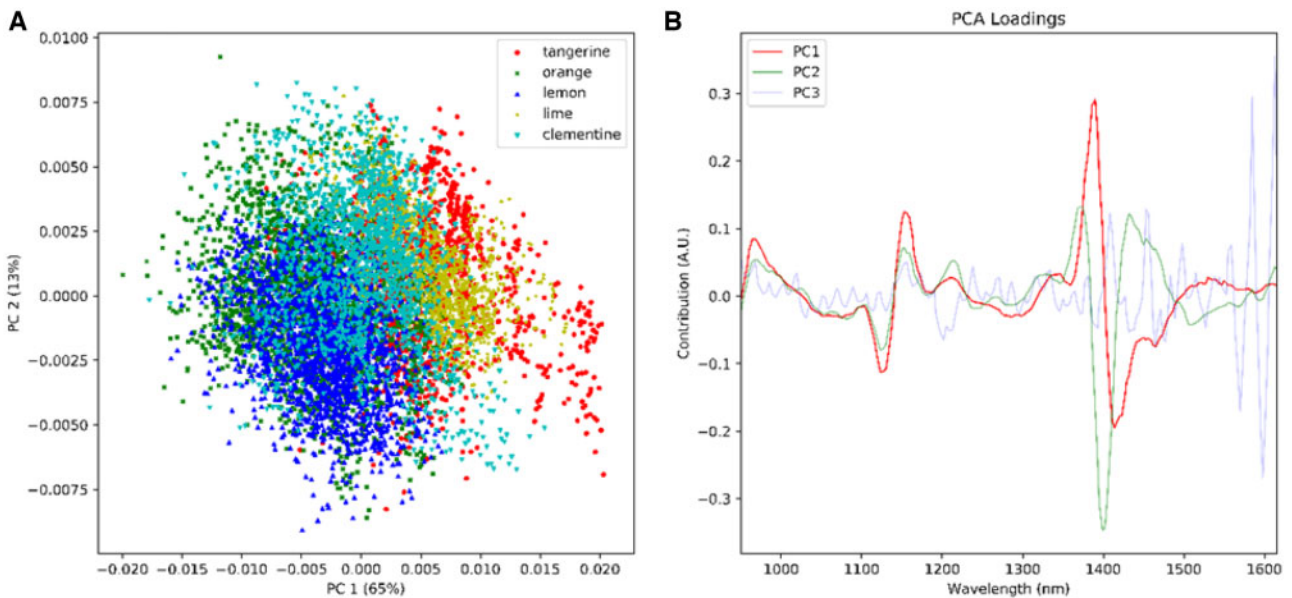

Figure 4. Principal component analysis score plots (A) and loadings (B) of physical and chemical parameters determined in fresh citrus fruits.

Table 1. Results of PLSR between chemical parameters and juice ratio (X-variables) and spectral data (Y-variables) for different citrus fruits

	Orange		Tangerine		Clementine		Lime		Lemon	
	R^{2a}	RMSE[b]	R^2	RMSE	R^2	RMSE	R^2	RMSE	R^2	RMSE
Juice ratio	0.726	3.788	0.580	4.870	0.382	4.780	0.746	2.292	0.422	6.121
°Brix	0.829	0.651	0.970	0.381	0.959	0.396	0.927	0.324	0.754	0.605
TA[c]	0.768	0.162	0.919	0.088	0.508	0.100	0.744	0.245	0.319	0.691
Vitamin C	0.766	6.495	0.816	2.769	0.772	3.920	0.864	1.497	0.845	2.873
AA[d]	0.775	6.276	0.868	2.360	0.767	3.714	0.785	1.606	0.769	3.524
DHA[e]	0.620	0.754	0.289	1.022	0.787	0.666	0.577	0.630	0.606	0.880

[a] R^2 = Coefficient of determination.
[b] RMSE = Root mean square error.
[c] TA = Titratable acidity.
[d] AA = Ascorbic acid.
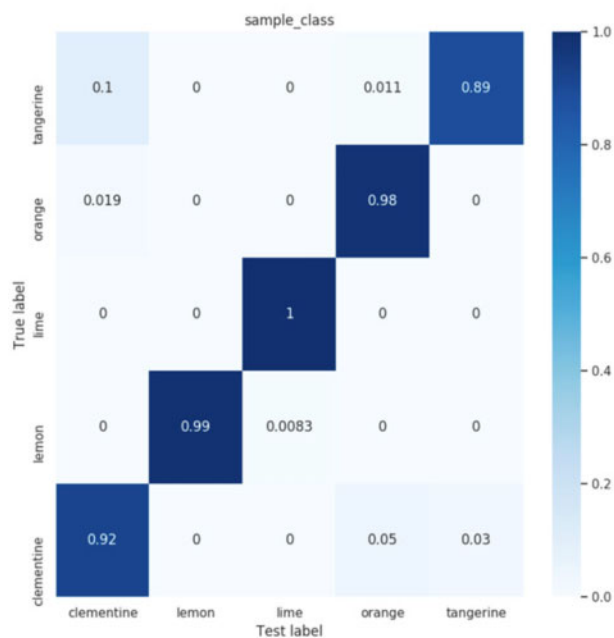[e] DHA = Dehydroascorbic acid.

Figure 5. Confusion matrix showing the results of the linear discriminant analysis model for fruit type classification.

as desired (27). Only four exceptions did not provide satisfactory performances ($R^2_{cv} < 0.5$), which were DHA for tangerines, juice ratio for clementines, tangerines and lemons, and TA for lemons (Table 1). Regarding juice ratio, there is a considerable variability of fruit total weight (i.e., flavedo, albedo, carpel, seeds, and juice) at the different ripeness states of clementines, tangerines, and lemons (Figure 1), thus affecting the accurate estimation of juice ratio. Considering that AA comprises about 90% of total vitamin C, similar R^2_{cv} were already expected. On the contrary, DHA constitutes a minor portion of total vitamin C and, despite all preventive measures taken, it can easily be formed during juice extraction, thus explaining the less satisfactory performance of DHA models (Table 1). Finally, TA of lemons showed a greater variance in comparison to the remaining fruits (Figure 1) likely due to ripeness range, which then was mirrored in the models. Regarding fruit type classification, linear discriminant analysis achieved a 95.6% precision for all fruit types. The model seems to have less confidence classifying tangerines and clementines correctly as it can be seen in Figure 5. Besides these two fruits, the remaining 3 species showed precision higher than 98%.

Throughout the years, several methods have been developed for the determination of vitamin C in fruits, including spectrophotometry, electrophoresis, titration, and the most selective, accurate, and precise approach—HPLC (28). Despite the advantages of the chromatographic determination of total vitamin C and its bioactive fractions, this is still a destructive methodology, thus making further uses of tested samples unfeasible. Therefore, NIRS has been increasingly used to quantify the content of ascorbic acid in several fruits and vegetables (29–32). In comparison to our study, Xia and colleagues (29) obtained similar coefficients of determination ($R^2_{cv} = 0.7765$) for predicting the content of total vitamin C in oranges (assessed by titration) after NIR spectral pre-processing by MSC. In bell peppers, total vitamin C (also quantified by titration) showed a similar range of coefficients of determination ($R^2_{cv} = 0.64$–0.78) when NIR spectra was collected in reflectance mode (30). On the other hand,

Malegori and co-workers (31) did not obtain suitable PLSR models for either total vitamin C (assessed by titration) or TA in acerola ($R^2_{cv} < 0.42$). As verified, the handheld device not only allows the assessment of TA, °Brix, and total vitamin C in citrus, but it also enables the discrimination between AA and DHA, while preserving the fruits integrity. As far as we are aware, this is the first study that shows the potential of combining the chromatographic measurement of vitamin C with NIRS to build mathematical models to predict the individual contribution of vitamin C fractions.

In conclusion, this work demonstrated, for the first time, the fitting performance of NIRS portable sensors at predicting multiple chemical components in citrus fruits, including vitamin C fractions. Moreover, machine learning algorithms and data pre-treatments were very useful for both regression and classification assignments. In this way, these devices constitute a cost-effective, easy to use, accurate, and environmental-friendly tool to monitor the internal quality of citrus fruits, whose affordability make it an option for retailers or even consumers.

Competing interests

Diogo B. Gonçalves, Rafael Queirós, Mark Bloore, Zoltán Kovács, and Isabel Hoffmann declare themselves to be representatives of Tellspec, LTD (London, UK).

The authors declare they have no actual or potential competing financial interests.

Acknowledgments

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