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Vitamin C distribution in acerola fruit by near infrared hyperspectral imaging

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The study aims at developing a methodology for qualitative mapping of nutraceutical compounds in fruit by near infrared hyperspectral imaging (NIR-HSI), focusing on vitamin C mapping in acerola (*Malpighia emarginata* D.C.), a Brazilian super-fruit characterised by its high content of ascorbic acid. Despite the fact that the spectral approach has often been applied to agricultural crops, research on acerola is very limited. So far, it is known that ascorbic acid decreases in acerola during ripening, but there is no information about its distribution in the fruit from green to red ripe maturity stages. Towards this aim, hyperspectral images of ten sliced acerola, picked at three maturity stages, were acquired using a SisuChema NIR-HSI system. On the pre-processed images, combined in a comprehensive matrix, principal component analysis was computed to select relevant components for classical least square (CLS) regression. CLS allowed distribution maps of ascorbic acid to be obtained (non-negativity, LOF=1.9%), using, as reference spectra, acerola juices enriched with 0% and 5% of vitamin C powder. The pixels correlated with 5%-enriched juice showed a reduction from 29% to 6.5% according to colour changes, confirming a vitamin C decrease along the ripening stages. These results demonstrated the reliability of NIR-HSI for the evaluation of vitamin C distribution inside the different acerola areas. The presented approach presents the basis for qualitative mapping of nutraceutical compounds in fruits.

Keywords: acerola, vitamin C, near infrared hyperspectral imaging (NIR-HSI), classical least square (CLS)

Introduction

Acerola (*Malpighia emarginata* D.C.) is a round-shaped fruit, with diameter varying from 3 cm to 6 cm and a very thin protective peel that quickly ripens and encases its fleshy and succulent pulp. At the initial ripening stage, the fruit has a full green colour, changing to yellow-reddish and finally to red or purple when completely ripened, depending on the genotype. The colour of the fruits is not only a sign of pigment changes, but is also linked to complex biochemical changes occurring during ripening, which involve all its main compounds. Indeed, acerola is rich in many nutrients, such as protein, carotenes, thiamine, riboflavin, niacin, proteins, calcium and phosphorus, but its main appealing feature is related to its high vitamin C content.¹

Vitamin C is the generic term for all compounds exhibiting the biological activity of L-ascorbic acid,² including ascorbic acid and dehydroascorbic acid, and is one of the most important nutritional quality factors in many horticultural crops. Vitamin C biological activity is relevant in the development and maintenance of the human body's health status.³ An intake of 100–200 mg per day has been suggested, since stress in modern life is known to increase the requirement for antioxidant compounds.²

Vitamin C is a six-carbon keto-lactone, a strong reducing agent, which serves as an antioxidant and as a cofactor in hydroxylation reactions. Hydrogen donation from ascorbic acid is thought to be primarily responsible for the antioxidant prop-



erties attributed to ascorbic acid. Vitamin C quantification in preparations and juices is traditionally carried out, according to AOAC 1968, by volumetric titration of the sample with a water solution of DCPIP (2,6-dichlorophenolindophenol) as the redox titrant and colour indicator.⁴ Although the chemical analysis is easy to setup, the sample preparation and the costs in terms of time and reagents are drawbacks that call for a non-destructive and accurate method such as near infrared (NIR) spectroscopy. Indeed, the NIR spectrum includes abundant information on hydroxyl (O–H), amino (N–H) and C–H's vibration absorption, so, associated with chemometric techniques, has been demonstrated to be a very powerful tool for routinely studying ascorbic acid.⁵ Thus, selection of specific regions of the spectra that could be attributed to vitamin C is advised in order to obtain relevant information for the topic. Moreover, the implementation of NIR-HSI systems permits the integration of spatial information to spectral absorption, which is a suitable approach for fruit quality evaluations.

Despite the fact that the spectral approach is often applied to agricultural crops, research on acerola is very limited and, until now, only the authors have studied the quality characteristics using non-destructive techniques.^{6,7} Therefore, there is strong novelty in this work that aims to develop a methodology for qualitative mapping of vitamin C in fruit using NIR-HSI. In particular, the distribution in acerola from green to red-ripe maturity stages is presented as a case study. This basic knowledge could be of great interest for both growers and the fruit industry, considering the economic importance of acerola due to its high vitamin C content. Moreover, the idea of developing a distribution map of a nutraceutical compound in a tropical fruit is completely new.

Material and methods

Data acquisition

Ten "Junko", the most commercialised cultivar of acerola, were harvested at three maturity stages based on skin colour: four immature-green, two intermediate-yellow and four red-ripe acerola. The hyperspectral images of acerola were acquired with a SisuChema NIR-HSI system (SPECIM Spectral Imaging Ltd, Finland) working in the spectral range 900–2500 nm ($11000\text{--}4000\text{ cm}^{-1}$) with spectral sampling equal to 4 nm and spectral resolution of 6 nm, equipped with a 50 mm lens with minimum pixel size of $150\text{ }\mu\text{m}$ (Figure 1). Since acerola is a three-lobed drupe, the fruit was cut so as to remove two of the lobes and the two related kernels. Each lobe sampled for the analysis was dabbed gently with a piece of absorbent material in order to dry it and avoid direct light reflection.

A PerkinElmer Frontier Fourier transform (FT)-NIR spectrometer equipped with a reflectance accessory (spectral range $4000\text{--}12000\text{ cm}^{-1}$; 64 scans; spectral resolution 8 cm^{-1}) was used to acquire the spectra of enriched acerola juice; vitamin C powder (anhydrous, purity $\geq 99\%$, Sigma Aldrich, Steinheim, Germany) was added to the nectar in steps of 0.5%, up to 5% w/v.⁸ Working with fruit juice, instead of water,

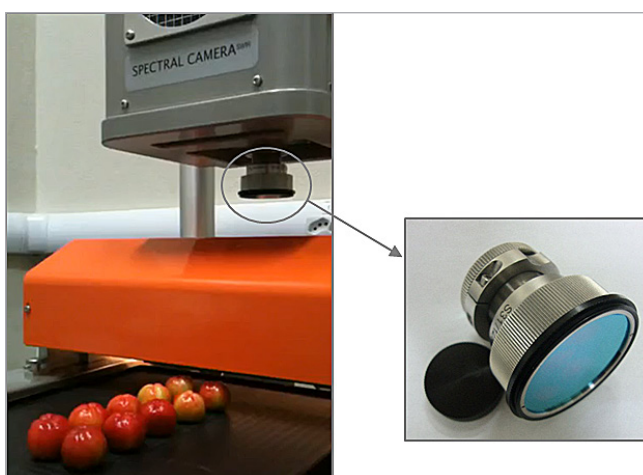


Figure 1. SisuChema NIR-HSI system during the acquisition of acerola samples and a detail of the 50 mm lens.

allowed the fruit composition to be mimicked, reducing the variability between the reference spectra and the chemical images.

Data pre-processing

The NIR-HSI spectra were first transformed from reflectance to the pseudo-absorbance scale [$\text{Log}(1/R)$], then the images were masked for background removal. As the two systems had different spectral ranges and resolution, a strategy was needed to merge the information. Therefore, a resampling was carried out to overcome the different linearity of the spectra. *A priori* variables selection between 7200 cm^{-1} and 6700 cm^{-1} was carried out, focusing on the most characteristic vitamin C absorbance.⁵ NIR-HSI and FT-NIR spectra were pre-processed with standard normal variate (SNV) and first derivative (Savitzky–Golay algorithm, 11 points, second degree polynomial) to enhance the spectral trend.

Data analysis

On the pre-processed data, a principal component analysis (PCA) was carried out, reducing the dimensionality of the data and underlining the importance of the different components in explaining the variance of the system.⁹ Using the explorative information coming from PCA, a classical least square (CLS) analysis was performed to obtain distribution maps of vitamin C in acerola fruits. CLS is a very useful method in hyperspectral analysis due to its simple chemical interpretation.^{10,11} It allows distribution maps to be obtained for each reference spectrum used to describe the system under study. Thus, the only requirements of CLS are reference spectra, describing the compounds of interest in the system, and the assumption that any mixture spectrum can be described as a linear combination of the reference spectra.¹² In our study, the two spectra of acerola juices enriched with 0% and 5% of vitamin C powder were used as references; these spectra were considered as the absence and the presence of vitamin C, respectively.

When CLS is applied, it is possible to set a threshold on the enhanced correlation maps to highlight the percentage of pixels that have a correlation with the reference spectrum greater than a selected value. Nevertheless, setting this threshold is not an easy task and to our knowledge no objective method has been reported so far.¹³ For this reason, in the present study, pixel attribution to each reference spectrum lays the basis only on the non-negativity of the concentration, avoiding a thresholding approach.

The above-mentioned data analyses were performed using Hypertool (<http://www.models.life.ku.dk/HYPERTools>) working under the Matlab environment (v. 2014b, The MathWorks).

Results and discussion

FT-NIR data from enriched juices showed variation when ascorbic acid powder was added in concentrations above 2.5%, suggesting the suitability of this technique for the qualitative evaluation of vitamin C distribution by chemometric methods. In particular, the band from 7200 cm^{-1} to 6700 cm^{-1} was influenced by increasing concentration of vitamin C; the visual observation is confirmed by Liu *et al.*,⁵ who referred to the $7200\text{--}6700\text{ cm}^{-1}$ range which is highly affected by vitamin C absorption, with a broad band around 7000 cm^{-1} . Besides, ascorbic acid, as a diprotic acid, can form four kinds of intermolecular hydrogen bond (OH and C=O) and the OHs connected with C3 and C2 have lower vibrational frequencies because of the conjugate action of the double bond and carbonyl. After removal of the background, spectra pre-processing and selection of the most informative region of the NIR spectrum, the 3D data cube was ready to be analysed. PCA was performed on the NIR-HSI data to explore the possible clustering of the pixels related to vitamin C along the ripening stages of acerola. The scree-graph of the eigenvalues highlighted that

the first two components explained the variance of the system, whereas the remaining variability was ascribable to noise and suggested that, in our system, the vitamin C mapping could be described as two-component system. Therefore, two NIR spectra were chosen (0% and 5% of vitamin C powder addition) as the reference input for the CLS algorithm, which performed direct regressions of each spectrum (associated to each pixel) onto the two reference spectra, respectively. The CLS model, constrained with non-negativity, described the system well; the Lack of Fit (LOF) being 1.9%. In Figure 2, on the left, the second distribution map (juice with 5% added vitamin C) obtained by CLS is presented. To be thorough, the spectra of one acerola are presented in Figure 2 (right); highlighting in red the spectra of the pixels classified by CLS as strongly correlated with the juice with 5% added vitamin C.

Relating the pixels recognised as correlated with vitamin C (5%-enriched juice) with the total pixels constituting each fruit (Table 1), a decrease of ascorbic acid related pixels was seen along with fruit colour changes from green to red. The mean percentage of pixels attributed to 5%-enriched juice varied from 28.8% to 8.5% for green and red berries, respectively (Table 1), denoting a reduction of more than 18 percentage points. These reductions along the colour changes are confirmed by Vendramini and Trugo¹⁴ and De Assis *et al.*¹ who found a vitamin C decrease by titration of acerola pulp.

Conclusions

The CLS approach allowed the development of distribution maps for qualitative evaluation of the vitamin C distribution inside the different areas of the fruit in an understandable way and without the need for further modelling. The NIR-HSI images showed a reduction in vitamin C-related pixels during ripening, which started in the inner core in immature-green

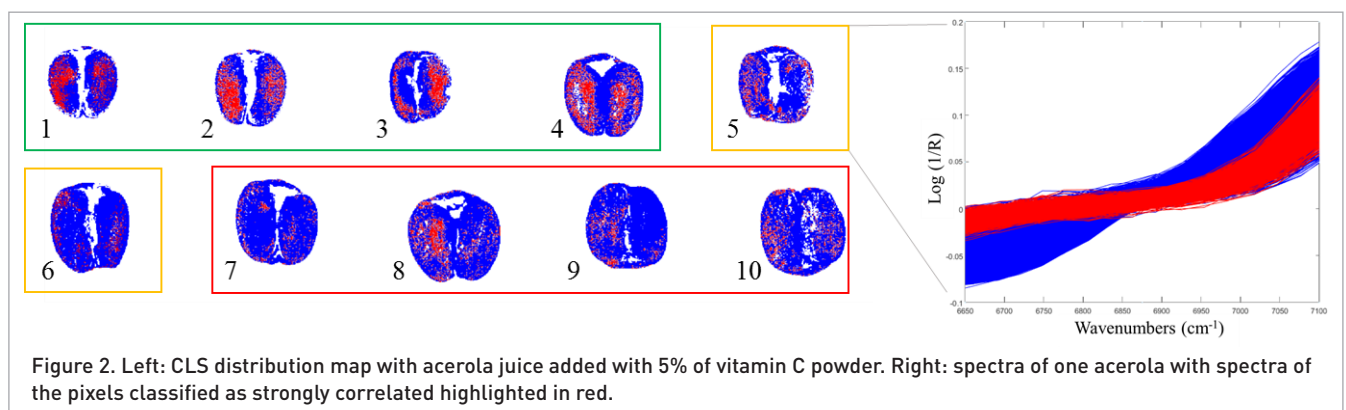


Figure 2. Left: CLS distribution map with acerola juice added with 5% of vitamin C powder. Right: spectra of one acerola with spectra of the pixels classified as strongly correlated highlighted in red.

Table 1. Pixels correlated with juice enriched in vitamin C (5%) expressed as percentage of the total pixels of each acerola fruit analysed.

Ripening stage	Green				Yellow		Red			
Fruit	1	2	3	4	5	6	7	8	9	10
Vitamin C (%)	27.8	28.8	24.1	26.1	15.7	15.1	8.5	6.4	7.6	10.8

fruit and spread throughout the red-ripe acerola fruit. The approach presented here offers a basis for qualitative mapping of nutraceutical compounds in fruits; thus providing scientific knowledge of vegetables' physiology which can be useful for industry.

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