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Daily Freshness Decay of Minimally Processed Apples using Vis/NIR Multispectral Imaging: Preliminary Tests

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In recent years, a substantial increase in the consumption of fresh-cut, or minimally processed, fruit and vegetables has been occurred. Changes in human life styles in fact often lead the consumers towards ease of use and healthy products. Monitoring the quality decay of fresh-cut products is necessary to control the freshness level during the entire production chain. Hence fresh-cut fruit and vegetable sector could be greatly helped by new analytical methods that are no-destructive and could be integrated into the production chain.
The objective of this preliminary study was to test vis/NIR multispectral imaging to qualitatively assess the freshness decay throughout the day (12 hours) of fresh-cut apple slices.
Three Cripps Pink apples were bought at a large retail supermarket. Three samples were obtained by cutting a slice from an apple, transversally with respect to the stem-calyx axis. Only half slice portion was treated to simulate a ready-to-eat product, by dipping it for 5 minutes into an aqueous solution of 2% (w/v) L-ascorbic acid, while the other half was left unprocessed as a control portion. The apples were monitored every sixty minutes for twelve consecutive hours through the use of an 18-channel imaging system (430-970 nm, VideometerLab, by Videometer A/S, Denmark).
A colour correction was performed on each acquisition through a normalization with respect to a multicolour reference acquired together with each sample, in order to take into account the stability of measuring conditions. Each image was segmented by using the k-means algorithm on the RGB picture and the apple flesh, i.e. the only part of interest, was separated from the background, the peel and the fruit core. After these pretreatments, all the acquisitions of the day for each slice were put together in a single dataset, unfolded in a two dimensional matrix, and the Principal Component Analysis (PCA) was applied. False color scores images were finally recomposed in order to highlight significant differences developed in a single apple slice.
The results are encouraging. For all the samples the analysis distinguished between the treated portion of the slice and the unprocessed one, in particular with PC2 scores surface. Furthermore a clear time evolution along hours is shown by the apple slices for both the processed and unprocessed portions. This preliminary study demonstrated the applicability of multispectral imaging as a rapid and non-destructive approach for monitoring the freshness decay throughout the hours of minimally processed apple slices.

1. Introduction

In recent decades, there has been a substantial increase in the consumption of fresh-cut or minimally processed fruit and vegetables. The international Fresh-cut Produce Association (IFPA, 1999) defines fresh-cut products as “any fruit or vegetable or combination thereof that has been physically altered from its original form, but remains in a fresh state”. The growth in the ready to use vegetable industry is due to: (i) their ease of use, in fact changes in human life styles have led consumers to move towards ready-to-eat products and (ii) nutritional properties indeed it is known as source of vitamins, minerals, fiber and antioxidants (Kader, 2008).

The technological treatments extend the shelf-life of the most processed foods. Instead, ready-to-eat products are characterized by a shelf-life shorter than that of the original unprocessed raw material, as shown by Guerzoni et al. (1996). In fact the sequence of operations necessary to produce a fresh-cut product (i.e. washing, trimming, peeling and/or cutting) promotes the biochemical and microbial instability of the product itself. These foods are often subjected to rapid loss of colour, organic acids, vitamins and other compounds that determine flavour and nutritional value.
Monitoring the quality decay of fresh-cut products is necessary to control the freshness level during the entire production chain. Hence fresh-cut fruit and vegetable sector could be greatly helped by new analytical methods that are accurate, rapid and could be integrated into the production chain (Beghi et al., 2013). The applicability of optical methods, i.e. vis/NIR and NIR spectroscopy, in agro-food sector has widely be proven. Hyperspectral and multispectral imaging system represent now new possibilities to be adopted.
The research presented in this work represents a preliminary study on minimally-processed Cripps Pink apples. The objective was to test vis/NIR multispectral imaging to qualitatively assess the freshness decay throughout the day (12 hours) of fresh-cut apple slices.

2. Materials and methods

2.1 The sampling

Some fresh Cripps Pink apples were bought at a large retail supermarket and three samples, i.e. three minimally-processed apple slices, were prepared according to the following procedure.

- A sample was obtained from a apple by cutting a 18 mm thick slice from the middle part of the fruit, transversally with respect to the stem-calyx axis. The slices were cut by using an automatic slicer in order to get parallel-faces slices and standardize the operation.
- For each sample, only half slice portion was treated (see the scheme in Figure 1) to simulate a ready-to-eat product, by dipping it for 5 minutes into an aqueous solution of 2% (w/v) L-ascorbic acid, while the other half was left unprocessed as a control portion (Luo et al. 1996, Gil et al. 1998, Soliva-Fortuny 2002).
- As removed from the dipping, the slices were first drained for three minutes, any excess liquid was then gently absorbed with paper towels, and a final drying air flow at room temperature (20° C) was applied for other three minutes. After that, the samples were ready to be measured and the experiment began, approximately 15 minutes after the cutting operation.

The experiment consisted in monitoring the apple slices by taking a picture with a multispectral imaging system every sixty minutes for twelve consecutive hours. A total of 36 multispectral images were therefore acquired on the three slices.

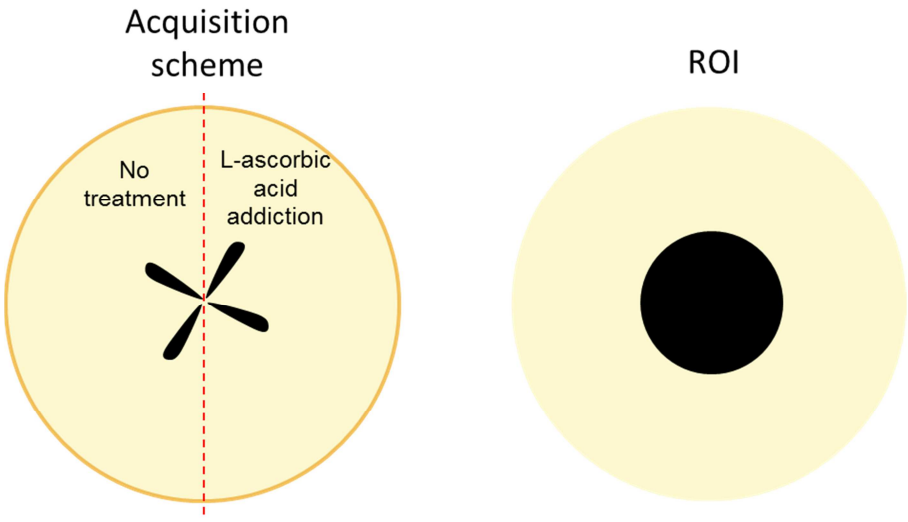

Figure 1: Scheme of an apple slice (on the left) with labels indicating the L-ascorbic acid treated portion and the unprocessed one. The apple slice is composed of the flesh, the core and the peel. Final identification of the region of interest ROI (on the right), after the masking procedures.

2.2 The multispectral imaging system

The multispectral camera used is the commercial imaging system VideometerLab, by Videometer A/S (Denmark), that acquires multispectral images in up to 18 different wavelengths ranging from 430 to 970 nm, i.e. 430, 450, 470, 505, 565, 590, 630,645, 660, 700, 850, 870, 890, 910, 920, 940, 950, 970 nm.

The VideometerLab system is characterized by a standardized easy-to-use acquisition setup, realized thanks to the interposition of an integrating sphere with a diameter of 420 mm between the camera and the sample, for light diffusion purposes. The camera is located on the top of the sphere looking downwards through it, while the sample has to be placed at the bottom inside the sphere, resulting exactly aligned under the camera focus. When performing an acquisition, a sequence of radiant powers at the 18 different wavelengths is obtained by strobing successively among the high power LED light sources, located into the sphere. At the same time, the monochrome high resolution CCD camera takes an image of the sample at each wavelength. In this way, an acquisition finally results in a multispectral image of dimensionality W x H x λ = 1280 x 960 x 18 (where W and H refer to the spatial width and height, and λ to the spectral wavelength).

This system ensures reproducibility of acquired images with regard to the spatial focus and the spectral calibration. The diffuse illumination conditions into the sphere are in fact standardized. Furthermore, the spectral calibration is automatically optimized for each individual channel with simple adjustments of the light intensity for each wavelength band, resulting in an improved signal-to-noise ratio.

2.3 Data Processing

The data processing was carried out using the MATLAB software, in the R2014a release equipped with the Image Processing Toolbox and the Statistics Toolbox (The Mathworks Inc., Natick, USA). All the software developed for the data processing is freely available upon request.

At first sight, different regions are visible in an image, corresponding to different objects (see the slice scheme in Figure 1): there is the sample (i.e. the apple slice), a multicolour reference (the use of which will be discussed later), the background, etc. Furthermore, with regard to the sample, only the apple flesh represents the real region of interest (ROI), which is influenced by the freshness decay of the product, i.e. the investigated phenomenon.

In addition to all this, the possible presence of problematic pixels must be considered. These can be dead pixels due to a malfunction of the CCD sensor in the camera, or saturated pixels that could be caused by any specular reflections related to wet points or, in general, to liquid drops in the field of view.

All the pixels in an image that refer to areas that have nothing to do with the investigated phenomena, including the peel and the fruit core areas, can significantly affect the results and must therefore be removed, as explained by Vidal and Amigo (2012).

This task, commonly known as a problem of image segmentation, was realized starting from the RGB images, by defining a series of masks to identify the different regions, i.e. objects. A mask related to a certain object is intended to be a logical index matrix of the same size of a single-wavelength image, containing a logical 1 (true) where the object is present, and a logical 0 (false) otherwise. These masks could then be applied successively up to identify the apple flesh data into each of the multispectral cube images, this data reshaped in 2-dimensional matrices, and chemometric processing could be finally carried out.

Specific algorithms were developed to automatically segment all the acquired images.

First of all, considering an acquisition, any possible presence of dead or saturated pixels in each wavelength image was taken into account by isolating them with a first sparse mask. The multicolour reference was then simply masked using built-in MATLAB functionalities, since this region has regular geometric rectangular shape and since it always has the same fixed position for all the acquisitions. Once this region was removed, it was possible to separate the apple flesh from the background and the peel by applying the k-means algorithm on the RGB image data on a segmentation with 2 clusters. Apple flesh in fact is very different from both the background and the peel in terms of colour, and this difference is clearly identified by the k-means algorithm. Finally, for each acquisition a circular disk with a fixed diameter was placed in the center of the slice in order to remove the fruit core area from the apple flesh. This wanted to simulate the action of the automatic machine that mechanically removes the core when producing ready-to-eat apples.

The final result of the masking procedures is schematically shown in Figure 1, on the right.

Amigo (2010) explained how the lighting conditions are a critical aspect in multispectral imaging. In general, there is a need to assure that the changes in the images do come from the samples modifications caused by the investigated phenomena, and not from any changes in the illumination, in the instrument response or, in general, in the measuring conditions.

As explained above, the used camera system intends to reduce the negative effects related to these issues, by adopting an integrating sphere and giving a pre-set acquisition setup. To further take into accounts this

aspects, at each acquisition (i) a dark zero-light image was taken, and (ii) a home-made colour standard reference was inserted into the camera field of view and acquired together with the sample.
First of all, each acquisition was referred to its zero level, by subtracting the corresponding dark image to every of the 18 wavelengths images. This wanted to reduce any possible bias due to the environmental noise, i.e. any light draughts entering the integrating sphere, and the electronic noise of the instrument.
A colour correction was then applied to each acquisition, as described in detail by Vidal et al. (2011) and by Tian et al. (2002). This is based on the home-made standard realized with 8 coloured plastic patches: gray, dark, yellow, red, orange, green, blue and white. The scale of colours was expressly defined to be varied, in order to possibly have a broad spectral response. In practice, the correction procedure consists of two steps. In the first step, only the colour standards data images (identified with the dedicated masks) are considered. Starting from one of these taken as a reference, an intensity linear transformation is determined for each acquisition, in order to make the correspondent colour standard as similar as possible to the reference one. The linear operator is calculated through a least squares minimization of the residuals, and in general it is specific for each acquisition. In the second step, the determined corrective transformations are applied to the flesh data images, so that they can be compered.
The PCA (Principal Component Analysis) is one of the most useful and widespread exploratory techniques within the framework of Food Science. Its ability to extract the main sources of variability and qualitatively study distribution of elements on an image makes PCA a versatile and essential tool. The adaptation of the bilinear PCA model to multi- and hyper-cubes comes with a previous step of unfolding the 3-dimension matrix into a 2-dimiension matrix. This procedure is widely explained by Vidal et al. (2011), Amigo et al. (2008) and Amigo et al. (2013).
In order to investigate all the acquisitions at the same time, all the multispectral images were put together in a single dataset, mean centering pre-treatment applied, and a single PCA model was built. Due to computational issues, this was possible only after a reduction to 75% of the spatial sizes of the images, by using a bicubic interpolation algorithm together with an anti-aliasing filtering, to limit the impact of aliasing on the reduced images. After the application of PCA, the final step is the re-folding of the principal component scores matrix to obtain the so-called scores images or surfaces. The scores surfaces explain the variability of the pixels, making PCA an ideal technique for qualitative analysis with no need for any other external information.

3. Results and discussion

The PCA resulted in three most significant PCs explaining together almost all of the total variance equal to 98.21% (Figure 3). The PC2 scores surface, with the 22.14% of the variance explained, clearly reveals an evolution in the hours for all the three samples, as shown in Figure 2. The three apple slices are arranged in the figure in the column-wise order (see the vertical axis labels), while their time evolution is shown along the horizontal direction (see the horizontal axis labels).

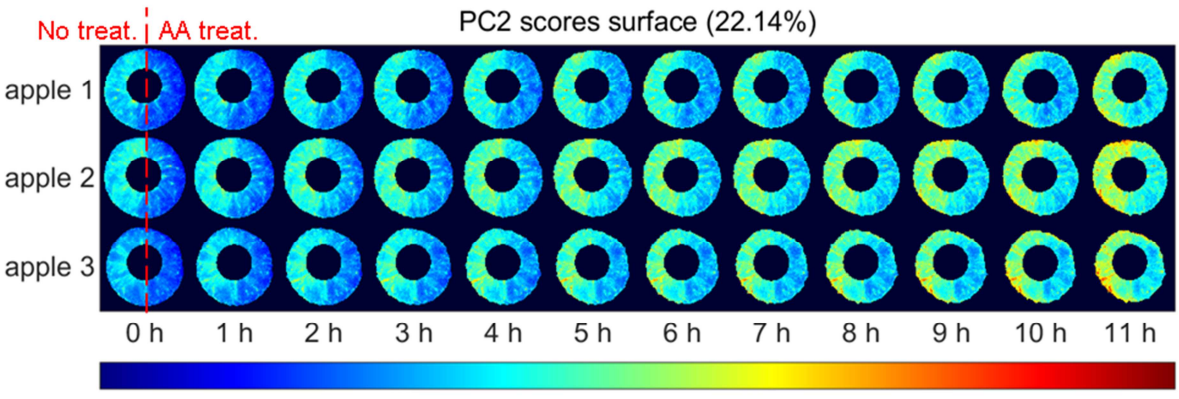

Figure 2: PC2 scores surface coming from the PCA. The 3 apple slices monitored are presented in the column-wise order, while the row-wise order shows the evolution of these samples along the hours during the day. An indication for the L-ascorbic acid (AA) treated portion and the unprocessed one is given in the first acquisitions.

The dark colours are widely distributed in the samples at the first acquisitions, i.e. in the left part of the figure. They can be associated with good levels of freshness, since they are characteristic of acquisitions realized on freshly cut slices. The dark colours tend to consistently disappear over the time. They are progressively replaced by clearer colours, which therefore can be referred to an increase of the degree of apples senescence, mainly related with the fluid loss. The clearest colours become predominant in the last acquisitions in the right part of the figure, meaning that the freshness degree of the product decreased during the 12-hour long experiment.

Furthermore, the same PC2 scores surface also highlights the difference between the right half of a slice, which was treated with the L-ascorbic acid, and the no-treated left part. For all the slices and for all the acquisitions, the treated region is always characterized by a darker colour, in comparison to the no-treated part. This is visible since the first measure at the beginning of the experiment. Only some minutes after the cutting operation, when the first acquisition is realized, are indeed sufficient to promote the start of the browning oxidation processes in the no-treated region, and this is detected by the multispectral analysis. In general, both the portions in a slice evolve in an analogous way along the hours, as explained above, but the degradation of the treated part is slower and slighter than the untreated regions. The effect of the L-ascorbic acid therefore is evident, at least from an optical point of view, in reducing the process of browning of the samples. This is an important aspect since the appearance of the fresh-cut products is critical for the consumer's opinion.

The time evolution of the samples is slightly visible also in the PC1 scores surface, while the difference between the treated and no-treated portions is not so evident, although PC1 explains the 72.33% of the variance (data not shown). Similar considerations apply to PC3 scores surface, but this is associated with only the 3.74% of explained variance.

The PCA loadings plot for the first three PCs is shown in Figure 3. As stated above, the time evolution of the samples is described in particular by PC2. Its loadings plot indicates some positive and negative peaks at certain wavelengths, in the first visible range 430-470 nm and for the NIR wavelengths greater than 850 nm, while for the wavelengths between this two ranges the curve gets close to the zero. Therefore the senescence progress is mainly described by three filters on the blue region, which are sensitive to the colour, but also by filters in the near infrared range that are crucial to detect changes in the samples along the time. Further investigations to these qualitative considerations should be carried out for a possible choice and reduction in the filters number.

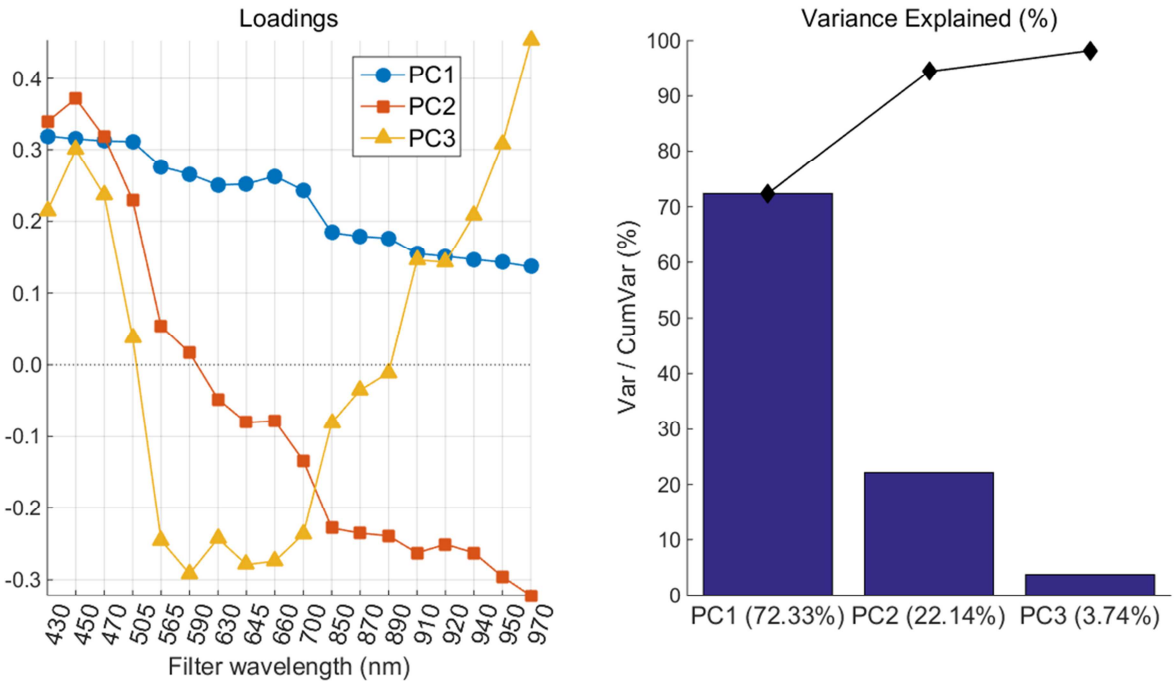

Figure 3:PCA loadings for the first three PCs as a function of camera filter wavelengths (on the left), and percentage of the total variance explained by the same PCs (on the right).

4. Conclusions

This preliminary study demonstrated the applicability of multispectral imaging as a rapid and non-destructive approach for monitoring the freshness decay throughout the hours of minimally processed apple slices.
In particular, PCA demonstrated to be an effective technique for a simple and qualitative evaluation of the freshness decay along the time of ready-to-eat apples.
Further research is necessary, first of all to increase the number of the samples in order to validate the results. Other data processing techniques and chemometrics analysis should be applied and evaluate, e.g. a possible variable selection to reduce the number of filters could be carried out. Quantitative techniques should be considered in order to have an objective evaluation of the freshness decay.
The study should then be applied to industrial minimally processed apple slices and also the presence of plastic package should be considered, in order to test this technology directly in operative conditions.

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