Detection of Astringent and Deastringent Persimmon Fruits using Hyperspectral Imaging Technology

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Abstract

Persimmon fruit cv. 'Rojo Brillante' is an astringent cultivar due to its content of soluble tannins. Traditionally, the consumption of this cultivar has been only possible when the astringency has been naturally removed before harvest, when fruit is overripe and the manipulation is very delicate. In recent years, new postharvest treatments, which allow astringency removal while preserving high flesh firmness, have been developed. Among them, the most widely used in commercial settings is based on exposing fruits to high CO_2 concentrations for 24 h–36 h. This method promotes anaerobic respiration in the fruit, giving rise to an accumulation of acetaldehyde and insolubilizing tannins at the end of the treatment. The effectiveness of this treatment is controlled by means of methods that are destructive, time-consuming and only a few samples per batch can be analysed. For this reason, the objective of this work is to study the application of the hyperspectral imaging technology in the detection of astringent and deastringent fruits non-destructively. A total of 300 fruits were used and exposed to CO_2 during different times in order to obtain fruit with different content of soluble tannins. The hyperspectral images of the fruits were acquired using a VIS-NIR hyperspectral system, which covers the spectral range 450-1040 nm. A reference analysis of soluble tannins was performed in order to find out if the fruits were astringent or deastringent. The spectral information of the two thirds of the fruits was used to build the classification models by means of partial least squares (PLS) and support vector machine (SVM) discriminant analysis methods. The remaining third was used to validate the models as test set. As result, 92.6 % astringent and 84.4 % deastringent fruits were classified correctly using the SVM method. This shows the great potential of hyperspectral imaging technology to detect astringent and deastringent fruits in industrial setups.

1. Introduction

Currently, Spain is the first producer of persimmon fruit in Europe and the third producer in the world, after China and South Korea (FAOSTAT, 2016). During the last ten years, the harvested area has increased from 2,000 to 14,000 ha, located mainly in the Mediterranean region, and the production has been augmented from 33 to 310 thousand tons (FAOSTAT, 2016). The main variety cultivated is 'Rojo Brillante', which is astringent until the fruit is over-ripe and the texture is too soft. The main reason for this increase in its production is the application of new postharvest techniques that allow obtaining fruit with a crispy texture like an apple and sweet taste without astringency. One of the most effective astringency removal methods is to apply high concentrations of CO_2 (95-98 %), holding the fruit in airtight chambers for 24 h at 20 °C and 90 % relative humidity (Salvador et al., 2008). As the consequence of these anaerobic conditions, soluble tannins (ST) which are responsible of astringency are polymerised by acetaldehyde to form an insoluble compound that is not astringent. This is the current astringency removal method adopted by the industry to commercialise 'Rojo Brillante' persimmon fruit with crispy texture.

The control of the effectiveness of this method is usually assessed by measuring the content of ST that remains in the flesh using analytical techniques, considering the fruit is totally non-astringent when the content of soluble tannins is less than 0.04 % (Tessmer et al., 2016). However, this is a slow and destructive analytical method and not the whole batch is either controlled. The alternative method also employed is based on the reaction of ST with $FeCl_3$, leading to a blue staining; the intensity of the staining observed after a slice of the flesh gets in contact with foils soaked in $FeCl_3$ solution depends on its content level of ST. Although this method is faster and easier than the analytical determination of ST, it is also destructive and subjective and therefore it is necessary to search for new rapid, reliable and non-destructive techniques.

Hyperspectral imaging is a promising optical technique for quality inspection of agricultural and food products integrating the main advantages of spectroscopy and imaging. Hyperspectral imaging can simultaneously acquire spectral and spatial information both and detect spectral features in regions of the electromagnetic spectrum such us the ultraviolet, NIR or infrared regions (Lorente et al., 2012). Their use has been widely studied to control the quality of fruit and vegetables during postharvest. Regarding to persimmon fruit and astringency, previous studies have been conducted to predict the content of ST or assess the astringency in different varieties using spectroscopy (Zhang et al., 2013; Altieri et al., 2017; Cortés et al., 2017) and hyperspectral imaging (Munera et al., 2017b & 2017c). Most of the studies report useful prediction models but they are not precise for ST prediction in fruits with low content because their limit of detection is above 0.10 % ST and this value does not guarantee a non-astringency sensory value.

In this context, the objective of this work was to study the application of hyperspectral imaging to classify correctly the fruits as astringent (A) and de-astringent (DA) using the value 0.04 % ST content as threshold.

2. Materials and Methods

Fruit samples and experimental design

In this work, 100 persimmon fruits (*Diospyros kaki* cv. 'Rojo Brillante') were harvested in L'Alcudia (Valencia, Spain) at commercial maturity during three consecutive weeks. A total of 300 fruits with apparently similar size and colour and without any defects or bruises were analysed. In order to obtain different levels of soluble tannins content, 90 fruits where not treated, being considered as control. The rest of the fruits were introduced in a container and exposed to air containing 95 % CO_2 at 20 ºC and 90 % relative humidity during 12 h (90 fruits) and 24 h (120 fruits).

Hyperspectral imaging acquisition

The hyperspectral imaging system consisted of an industrial camera (CoolSNAP ES, Photometrics, AZ, USA), coupled to two liquid-crystal tuneable filters (Varispec VIS-07 and NIR-07, Cambridge Research & Instrumentation, Inc., MA, USA). The camera was configured to acquire images with a size of 1392x1040 pixels and a spatial resolution of 0.14 mm/pixel at 60 different wavelengths every 10 nm, in the working spectral range of 450 - 1040 nm. The scene was illuminated by indirect light from twelve halogen spotlights (37 W) (Eurostar IR Halogen MR16. Ushio America, Inc., CA, USA) evenly distributed in a circle inside a hemispherical aluminium diffuser. The inner surface of the aluminium diffuser was painted white with a rough texture to maximise its reflectivity and to minimise directional reflections. The fruits were introduced manually into a fruit holder, with the stem-calyx axis lying horizontal. The images were acquired using customised software developed at IVIA, obtaining a total of 300 images.

Reference analyses

The ST content in each fruit was analysed by spectrophotometry by the Folin-Denis method (Taira, 1995), as described by Arnal & De Rio (2004), expressing the results as % of fresh weight. The analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test, was conducted using the software Statgraphics (Manugistics Corp., Rockville, USA) in order to determine significant differences in ST content in fruits.

Image processing and data analysis

All images were imported into MATLAB R2017a (The MathWorks, Inc. MA, USA) to be pre-processed using the customised toolbox HYPER-Tools (Mobaraki, N. & Amigo, J.M., 2018). The image processing started with the correction of the relative reflectance by using white and black references. Then, the corrected images were clipped and compressed to reduce the computation time. A binary mask was applied to remove properly the background and leaves of calyx. The average reflectance spectrum was determined by averaging the relative reflectance spectra of the whole area of each fruit. Finally, the spectra were randomly partitioned into two sets: two thirds of the spectra were used to calibrate the models (training set) while the remaining third was used for independent validation (test set). Second derivative (2D) using Savitzky-Golay smoothing (3 points smoothing window, second order polynomial) was applied to remove physical phenomena in the spectra as additive and multiplicative effects and to improve the subsequent classification models (Rinnan et al., 2009). Then, each pre-treated spectrum was normalised using mean centre.

Partial Least Squares (PLS) and Support Vector Machine (SVM) discriminant analysis models were built to classify the fruits as astringent or deastringent. PLS discriminant analysis is a variant of PLS regression in which the independent variable is categorical, expressing the class membership of the samples. It is performed in order to sharpen the separation between groups of observations by maximising the covariance between the spectra and the independent variable so that a maximum separation among classes is obtained (Lorente et al., 2012). SVM is a machine learning technique based on kernels proposed by Cortes and Vapnik (1995). The SVM are linear classifiers, in which data are separated by a hyperplane defined by a number of support vectors. These support vectors are part of the training set and are used to define the boundaries of the two classes. The models were calibrated using the pre-processed spectra of the training set and validated using the samples of the test set.

3. Results and Discussion

Reference analysis

The application of the CO_2 treatment during different time resulted in fruit with a wide range of values of soluble tannins between 0.91 % in untreated fruits and 0.02 % in fruits treated during 24 h (Table 1). In the three harvests it was observed the reduction of ST related to the duration of the treatment being the results statistically different.

The value of ST in which 'Rojo Brillante' persimmon fruit is considered non-astringent was evaluated by Tesmeer et al. (2016) using a sensory panel. This cultivar was qualified as non-astringent when the value of ST was 0.04 %. However, the fruits were in the over-ripened stage and with a soft texture. Since no studies have been performed in fruits treated with CO_2, the value obtained by Tessmer et al. (2016) was considered the threshold value to classify the fruit in this work. Thus, all untreated fruits were considered as astringent, 59 fruits treated during 12 hours were considered as astringent and 31 as deastringent. As the ST content in fruits treated during 24 h was no higher than 0.03 % these fruits were directly considered as deastringent.

Table 1. Soluble tannins content at each treatment duration and harvest, and number of astringent and de-astringent fruit. Different letters in the same column indicates significant differences between treatment duration ($p\text{-value}<0.05$), according to Tukey's (HSD) test.

Treatment	Soluble tannins						Class	
	Harvest 1		Harvest 2		Harvest 3			
	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD	#A	#DA
Untreated	0.69 ^{a}	0.16	0.61 ^{a}	0.08	0.91 ^{a}	0.13	90	0
12 h	0.09 ^{b}	0.08	0.11 ^{b}	0.09	0.37 ^{b}	0.18	59	31
24 h	0.02 ^{c}	0.01	0.03 ^{c}	0.02	0.03 ^{c}	0.00	0	120

Spectral analysis

The mean spectrum of astringent and deastringent fruits followed a very similar pattern (see Fig. 1). The use of second derivative transformation allowed separating overlapped peaks and therefore more differences could be visualised, especially in the NIR region, between 700 nm and 800 nm, and between 900 nm and 1040 nm. These differences are mainly related to the water absorption peak, located at 970 nm (Lu and Peng, 2006), carbohydrates at 710 nm and 920 nm (Siedliska et al., 2018) and phenolic compounds, located at 790 nm and between 940 nm and 1000 nm (Siedliska et al., 2018; Noypitak el al., 2015). All these slight differences are associated to the effect of deastringency treatment. As Salvador et al (2007) pointed out, after treatment with CO_2, soluble solids levels drop significantly. This reduction is related to the loss of astringency, since the measurement of soluble solids includes the soluble tannins that cause astringency and, following CO_2 treatment, these tannins become insoluble (Arnal and Del Río, 2003). Furthermore, although deastringent treatment does not affect the firmness of the fruit in early stages of ripeness, the softening of flesh is possible in following stages.

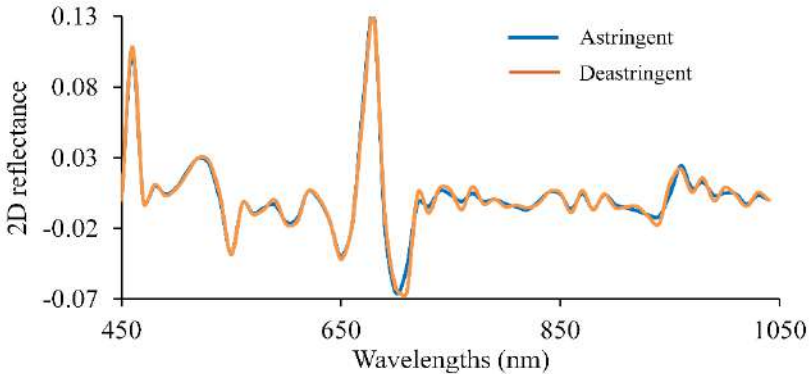

Figure 1. Mean spectra of astringent and deastringent fruits.

Detection of astringent and deastringent fruits

Due to the importance of detecting as many astringent fruits as possible, the priority of the classification in this work was to try to maximise the correct classification of these fruits by the model. The PLS model was calibrated using 14 latent variables, and the internal validation of the model using a single 10-fold cross validation classified correctly 85.0 % of the samples. The SVM model was calibrated using 89 support vectors, and 89.0 % of the samples were correctly classified. In this case the model using SVM was the more accurate in classifying astringent fruits and also deastringent fruits.

The external validation or classification of the test set is shown in Figure 2. The class for each fruit was predicted by introducing the mean spectrum measured into the previously built models. The result was visualised in the fruit surface showing the fruit coloured in blue if the mean value was assigned by the model to astringent or orange if it was assigned to deastringent class. SVM was the more accurate model to classify astringent fruits and also deastringent fruits of the test set, classifying 92.2 % of astringent and 84.4 % of deastringent fruits correctly.

Table 2. Results of calibration of classification models.

Model	#LV/#SV	Correct classification (%)					
		Calibration			Cross Validation		
		A	DA	Total	A	DA	Total
PLS	14	90.7	88.3	89.5	86.0	84.0	85.0
SVM	89	98.1	95.8	97.0	90.7	87.2	89.0

#LV = number of latent variables; #SV = number of support vectors; A = astringent; DA= deastringent

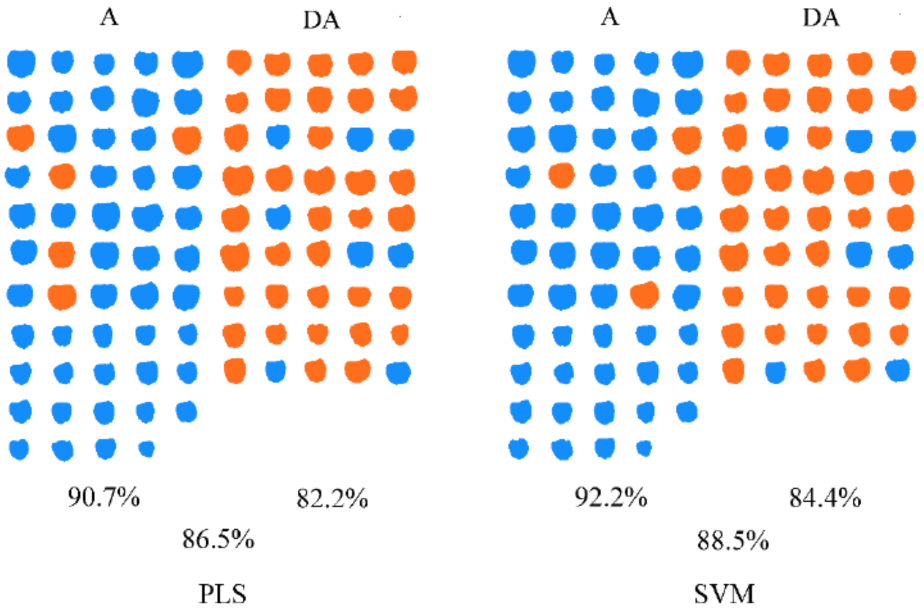

Figure 2. Classification of astringent and deastringent fruits of test set using PLS and SVM models.

4. Conclusions

This work demonstrates the capability of hyperspectral imaging detecting astringent and deastringent 'Rojo Brillante' persimmon fruits. The more accurate classification was performed using the SVM model obtaining a correct classification of 92.6 % astringent and 84.4 % deastringent fruits. Thus, this technique may have potential as a tool for rapid and non-destructive control of effectiveness of the astringency removal treatment applied to 'Rojo Brillante' persimmon fruit, allowing the detection of fruit that still is astringent before it is marketed.

Nevertheless, the results of this study should be confirmed on a larger sample set of fruits grown in different areas and harvested at different stages of ripeness before they can be implemented in an in-line system. Furthermore, in order to establish a practical tool in industry, an optimal reduction of the spectral information is necessary to speed up this classification.

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