Relationship between solar radiation on watercore on apple fruit assessed with MRI

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Abstrat

This work is a preliminary studio of the possibility of assess a relationship between solar radiation and watercore development on apple fruit, during maturation, using a non destructive method such as Magnetic Resonance Imaging (MRI). For such purpose, several low cost solar radiation sensors were designed for the trial and placed at 2 different heights (1.5 and 2.5 m) on 6 adult 'Esperiega' apple trees, in a commercial orchard in Ademuz (Valencia). Sensors were connected along 27 days, during the end of the growth period and start of the fruit maturation process, and radiation measurements of the a-Si sensors were recorded every 1 minute. At the end of this period, fruits from the upper and the lower part of the canopy of each tree were harvested. In all, 152 apples were collected and images with MRI. A Principal Component Analysis, perfomed over the histograms of the images, as well as segmentation methods were performed on the MR images in order to find a pattern involving solar radiation and watercore incidence.

Key words: Non-destructive, non-invasive, segmentation, Esperiega.

1. Introduction

Watercore has been traditionally understood as a physiological disorder affecting apples (*Malus domestica*) in which intercellular spaces are filled with fluid (Faust et al., 1969, Clark et al., 1998a, Clark et al., 1998b) around the core line (Yamada & Teramoto, 2010) Nevertheless, nowadays some varieties that are susceptible to produce it are much demanded in some different parts of the world such as Japan, where the presence of watercore is considered as an indicator of full ripeness (Kasai & Arakawa, 2010). In Spain there are some regions where these apples are very much appreciated, due to their sweet flavour. This is the case of the variety called 'Esperiega', grown in a region of Valencia (Ademuz). They are known as 'Frozen Apples' and might double their price in selected markets.

Despite watercore has been studied by several authors, its physiological origin is not clear. Bowen & Watkins, 1997 reported the relationship between carbohydrate and mineral content in 'Fuji' apples. Some authors have suggested that watercore incidence is related to temperature (Ferguson et al, 1998, Yamada et al., 2005). Yamada et al., 2010 suggested that there exists a relationship between early watercore development and leaf photosynthesis in apple. Anyway, watercore detection has been a hard issue due to the fact that, except in very particular situations (in which affected flesh is close to the skin) external symptoms signalling the presence of watercore are essentially absent in all cultivars (Clark et al, 1998a).

Watercore can appear at different parts of the whole apple volume. This is where the importance of the use of tomography inspection lies in. The application of such methods has increased in agriculture in recent years (Cubero, et al., 2011). One of these methods might be MRI, as it is a non-destructive, non-invasive method that gives an idea of the distribution of watercore in the apple.

MRI is a non destructive, powerful tool for food science. It is based on the association of each spatial region in a sample that has a characteristic nuclear magnetic resonance frequency by means of external linear magnetic gradients (Hills, 1995). Concerning ^{1}H MRI watercore detection in apples, T_2 weighed images provide a good contrast between the watercore affected area and the sound tissue, due to the affected area is filled with water (Wang et al., 1988). The watercored area will be seen brighter than the non affected area in the tomography and thus, there will be differences in the histograms of affected and non affected apples.

The research group LPF_TAGRALIA has focused on the detection of fruit internal breakdown since 1999 (Barreiro et al., 1999), with the latest study dedicated to seed detection in citrus (Barreiro et al., 2008).

The objective of this work is to establish a preliminary methodology in order to find whether there exists any relationship between solar radiation and watercore development in apple trees.

2. Materials and Methods

2.1. Samples

A total of 152 apples, from the variety 'Esperiega', belonging to the same campaign, were harvested from a commercial orchard located in Ademuz (Valencia): 72 apples were picked from the bottom of the tree (apples located at approximately 1.5 m of height) and 80 apples from the top of the tree (apples located at approximately 2.5 m).

2.2. Solar radiation experiments

Solar radiation data were acquired using a low cost system, developed by LPF-TAGRALIA (details are provided in communication P-0350 in this congress). In total, 14 low cost sensors were used. 12 of them were placed on 6 different trees (6 on the top and 6 on the bottom) and two of them were used as reference so they were placed on adedicated support above the tree canopy. According to the solar radiation data measured, the irradiation on the top of the trees was twice the radiation on the bottom. Also, from such data it could be concluded that the ripening season had a very severe radiation index with regard to regular ones (see communication P-0350).

2.3. MRI experiments

All magnetic resonance experiments were carried out on CAI of NMR (UCM) dependencies on a Bruker BIOSPEC 47/40 (Ettlingen, Germany) spectrometer operating at 200 MHz. All experiments were performed under static conditions, with an actively shielded imaging gradient set and a RF volume coil with an inner diameter of 20 cm. The bore of the magnet is horizontal, 147cm long and with 40 cm diameter.

MRI screening was made in 2D T2-weighed Rapid Acquisition with Relaxation Enhancement (RARE) sequences, targeted at obtaining all the images for a visual inspection. Coronal images (x-z plane) were obtained from apples placed with their central axis along the y-axis of the magnet. The MRI sequence parameters were: recovery time (TR) 511 ms, echo time (TE) 15 ms. The Field of View (FOV) and the slice thickness used were 8 × 8 cm and 3 mm respectively. Images were collected with 256 × 128 acquisition matrix sizes. The total acquisition time was 2 minutes 2 seconds and 20 slices were obtained for each apple. All Images were acquired in 16 bits.

From MR images, the eight central slices of each apple were chosen for their classification, by four specialists, into three classes, depending on their apparently watercore level: 1-sound apples (27 apples), 2-light watercore (71 apples) and 3-strong watercore apples (54 apples) (Fig.1). Number of apples and their category and position belonging are provided in Table 1.

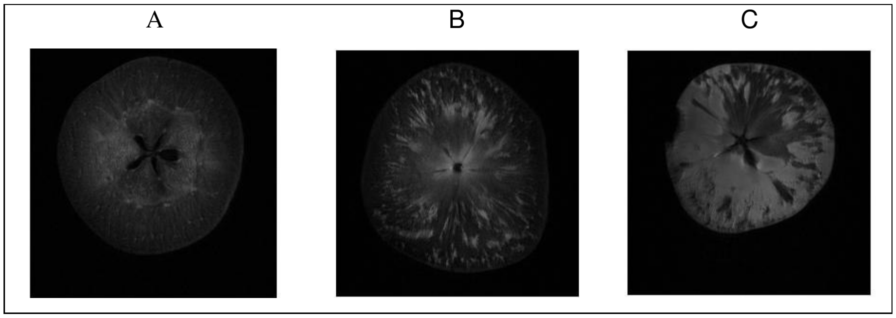


FIGURE 1: Central slices of MR images belonging to the three groups, classified by the experts. A. Sound apple. B. Light watercored apple. C. Strong watercored apple

TABLE 1. Number of apples related to the category, according to the four experts and the position on the tree

	Sound apple	Light watercore	Strong watercore	TOTAL
Top	19	33	28	80
Bottom	8	38	26	72
TOTAL	27	71	54	152

2.4. Image processing and data analysis

For image processing, the software Matlab 7.0 was used. Two image processing methods were used: 1) Principal Component Analysis (PCA) applied to 8 bit image histograms (without background automatically removed), and 2) affected watercore tissue segregated based on 16 bit histogram data. As indicated, PCA was performed on the histograms of the images as reported by the authors in a previous research (Melado et al., 2010), in order to assess any relationship between the PCs and the position of the apple on the tree and thus, the solar radiation received by the fruits. As a second step, a segmentation method, based on the histogram of the image, was applied to the affected apples in order to be able to evaluate a pattern on the watercore development related to the solar radiation absorbed by the fruit.

For **PCA**, images were converted into 8 bits in order to decrease the image processing time. Then, every image was pre-processed in order to avoid the background noise. For such purpose and to be sure that the only thing analyzed was the apple tissue (the *region of interest*, ROI), the background was eliminated by applying a segmentation using Otsu method (Otsu, 1969). Then the normalized histogram of the whole apple was obtained, using the 20 slices of the tomography. PCA was calculated and 6 Principal Componentes (PC) were chosen as they captured 99.29% of the explained variance.

For the **segmentation** of sound and watercore affected tissue, a method based on the gray level of the 16 bit histograms was used. The central slice of each apple was obtained, and then, two manually selected segmentation levels were chosen on the histogram of each image, trying to segregate the background of the image (which corresponds to the lowest grey levels), from the sound tissue and the damaged tissue (the one that presents the highest grey levels). Then, the percentage of affected area of each apple was computed. This second procedure was considered as a reference, together with a visual classification of the level of affection made by 4 experts.

3. Results

From the PCA performed on 8 bit histogram data, no differences between apples exposed to high solar radiation (those from the top of the tree) and apples exposed to lower solar radiation (those located on the bottom) are appreciated. Fig. 2 shows PC1 (with 65.84% of explained variance) against PC2 (25.54% of explained variance). Apples that were supposed to be exposed to higher solar radiation levels appear mixed with apples exposed to lower radiation levels. This can be due to an unusual solar radiation incidence in such campaign, which is not typical of that region in the ripening season (details are provided in communication P-0350 in this congress). What is indeed clear is that PC2 above -0.01 reports either light or strong watercore, that is to say PC2 is related with the existence of damaged tissue, though light damage also reports high PC1 values (generally above 0.05), in spite of strong watercore which seems associated with low PC1 levels (<-0.05)

It is interesting to state that though there is no linear relationship among PC1 and PC2, a quadratic behaviour is found. We might speculate that light damage refers to a different pattern and evolution that that of deeply affected apples, while sound apples stay near the origin of coordinates.

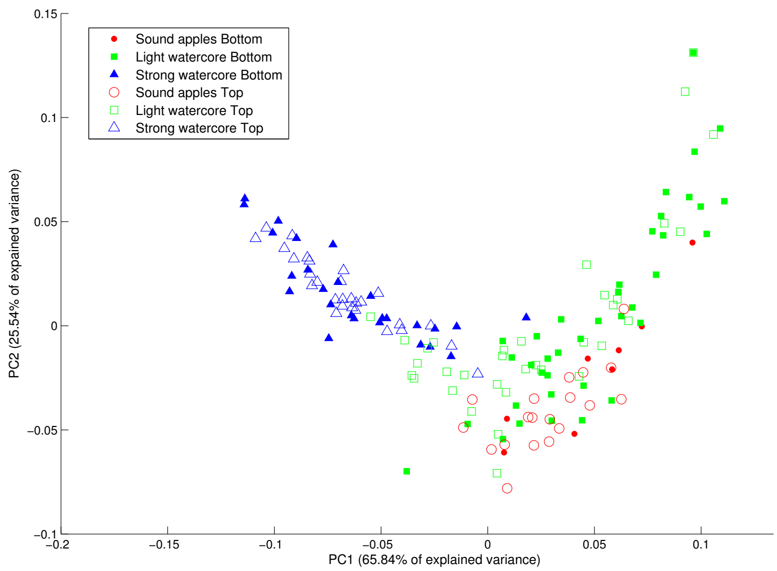


FIGURE 2: PC1 (65.84% captured variance) versus PC2 (25.54% captured variance)

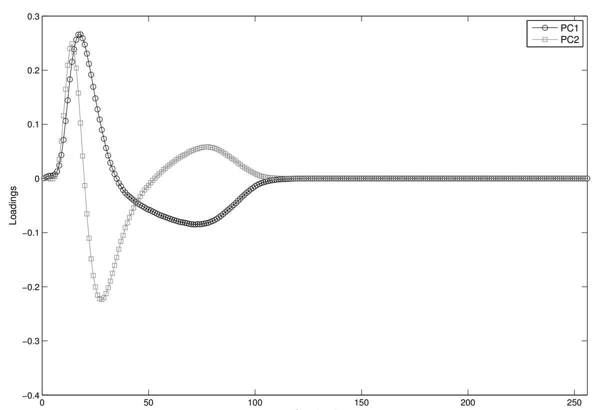
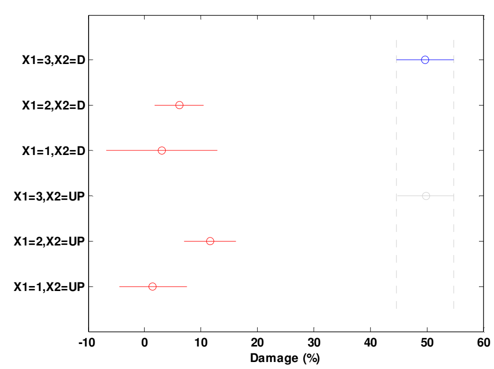


FIGURE 3. PC Loadings versus grey level for PC1 and PC2.



FGURE 4. Multiple comparison of means for damage level (1 2 3, sound, light, and strong) and position on the tree (Down –D-, up –UP-)

Figure 3 plots the PC Loadings versus grey level of the 8 bit image histograms, where PC1 shows to be related to the histogram shape while PC2 refers to the first derivative of the histograms.

ANOVA performed on the percentage of affected tissue according to segmentation (Figure 4) indicates a very significant effect regarding to visual categorization (49.8%$\pm$1.8%, 8.9 $\pm$1.5%, and 2.3%+2.7%, the high variability for sound may refer to visually misclassify fruits). No significant differences were found for the position of fruits on the tree, or the interaction among both factors (damage*position). However the multiple-comparison of means clearly indicates that the level of affected area for light watercore class in the upper side of the tree (11.6% $\pm$ 2.3%) was significantly higher than that of light affected down-side apples (6.2% $\pm$ 2.1%).

TABLE 2. 2-Way ANOVA

	Sum of Squares	**Degrees of Freedom**	**Mean Squares**	**F**	**Prob>F**
Damage	62467.7	2	31233.80	185.46	0
Position	212.5	1	212.49	0.30	0.5854
Damage*Position	332.5	2	166.23	0.99	0.3752
Error	24588.6	146	168.41		
Total	90284.5	151			

4. Conclusions

Unsupervised multidimensional analysis (PCA) did not show general differences between apples exposed to higher rates of solar radiation. Neither a supervised segmentation method allowed a significant identification of apples that were exposed to higher rates of solar radiation, even though the level of affected tissue for strong damage is similar in apples from the upper and lower part of the trees, slightly affected apples had a higher amount of affected tissue in the upper position (11.6% $\pm$ 2.3%) than in the shadow (6.2% $\pm$ 2.1%).

An interesting outcome of unsupervised multidimensional analysis is that two different patterns of watercore are found, with light damaged affected apples never developing into strong damage. It might be speculated that two different processes might be acting that result in a similar defect though with significant different spatial location.

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