1H HR MAS NMR metabolomic and non-destructive 2D NMR relaxometry to assess internal quality in apples

Angela Melado-Herreors[a], Pilar Barreiro[a], Palmira Villa-Valverde[b], María-Encarnación Fernández-Valle[b] Eberhard Humpfer[c]

[a] *LPF-TAGRALIA, Technical University of Madrid (UPM), ETSI Agrónomos, Avda. Complutense s/n, Madrid, Spain*
[b] *CAI of NMR and electronic spin. Universidad Complutense de Madrid. Avda. Juan XXIII, nº1, Madrid, Spain.*
[c] *Bruker Bio Spin GmBH. NMR Application.Silberstreifen 76287 Rheinstetten, Germany.*

ABSTRACT

NMR can be considered a multi-scale multidimensional technology in the sense that it provides both spatial insight at macroscopic (MRI) or microscopic level (relaxometry), together with chemical characterization (HR-MAS).

In this study 296 apples (from 4 cultivars) were MRI screened (20 slices per fruit) among which 7 fruits were used for metabolomic study by 1H HR MAS in order to assess various chemical shifts: malic acid, sucrose, glucose, fructose and ethanol. On the first season, tissue samples were taken from the sound and affected apples (near the core, centre and outer part of the mesocarp) belonging to sound and affected locations, while on the second season, tissue samples were focused on the comparison between sound and affected tissue. Beside, MRI and 2D non-destructive relaxometry (on whole fruits, and localized tissue) where performed on 72 and 12 apples respectively in order to compare features at macroscopic (tissue) and microscopic (subcellular) level.

HR MAS shows higher content of α-glucose, β-glucose, malic acid and aromatic compounds in watercore affected tissues from both seasons, while sound tissue reflects higher sucrose. Microscopic (subcellular) degradation of tissue varies according to disorder development and is in good accordance with macroscopic characterization with MRI.

1 Introduction

Watercore is a physiological disorder affecting apples in which intercellular spaces appear filled with fluid around the core line. It can appear at different parts of the whole apple volume and may have different pattern developments: block and radial (Clark et al., 1997; Melado-Herreros et al., 2013). The composition of the apple also suffers variation inter and intra-apple attending to the watercore level of affection.

High Resolution Magic Angle Spinning NMR (HR MAS) allows to work with semi-solid samples by making profit of rapid spinning of the sample (~ 4-6 kHz) at an angle of 54.7º relative to the applied magnetic field, which decreases drastically the effects on line widths of dipolar couplings and chemical shift anisotropy associated to semi-solid samples, while reducing signal broadening due to magnetic susceptibility effects (Sánchez-Pérez et al., 2011). Vermathen et al., (2011), used 1H HR MAS NMR spectroscopy on three different apple cultivars where intra-apple variability of the compounds was found to be significantly lower than the inter-apple variability within one cultivar.

In the present study, 1H HR MAS, combined with non-destructive global and localized 2D relaxometry has been used in order to provide chemical characterization and spatial insight at macroscopic and microscopic level on apples of several cultivars that present different watercore level.

2 Material and Methods

2.1 Samples

For 1H HR MAS experiments, in the first season, 5 apples were selected from a pool of 191 MRI screened fruits (20 slices per fruit). These apples belonged to three different varieties (Ascara-2-, Rebellón-1- and Tempera-1-) which are non commercial cultivars but were chosen for their watercore development susceptibility. In the second season, a commercial cultivar (Verde Doncella) was selected. In this case, 2 apples from a pool of 105 MRI screened apples (20 slices per fruit) were chosen with different watercore affection.

For non-destructive global and localized 2D relaxometry (second season), 12 apples from a pool of 72 MRI screened fruits (20 slices per fruit) were selected, belonging to the cultivar Verde Doncella also with different watercore level affection.

2.2 MR equipment

HR MAS experiments in the first season were performed on a vertical magnet Bruker AMX operating at 500 MHz, placed in the CAI of NMR and electronic spin (Madrid, Spain) dependences. Experiments from the second season were performed on a Bruker 400/54 US PLUS LH D335, located in Bruker BioSpin (Rheinstetten, Germany) dependences.

2D relaxometry experiments were performed on a Bruker BIOSPEC 47/40 (Madrid, Spain) spectrometer operating at 200 MHz (4.7 T) equipped with a actively shielded 26 cm system gradients using a 20 cm volume RF coil.

2.3 Experiments

HR MAS experiments were performed on 5 fruits from the first season with different watercore incidence. Several tissue samples were taken from different parts of each apple. For sound apples, only three samples were chosen: one from the core, another one from the center of the mesocarp and the third one corresponding to the outer part of the mesocarp. For affected apples, six tissue samples were studied on each apple: three from the core, two from the center of the mesocarp and one from the outer part of the mesocarp. On apples from the second season three samples of tissue were chosen on the strongly affected apple: one from the core, another one from the middle part of the mesocarp and the third one from the outer part of the mesocarp. On the sound fruit, only one tissue sample was analyzed.

On both cases the methodology followed was the same: as a first step, the rotors were cleaned and tare. Then, the fruit was cut in halves, the first layer of tissue was removed and 45 mg of tissue sample were picked. Samples from the first season were analyzed in the spectrometer without washing them in deuterated water, but samples from the second one were washed in 10 μl of deuterated water. The spectrometer was locked and the temperature was calibrated at spinning conditions.

2D relaxometry experiments were performed on Verde Doncella apples as referred before (various watercore level), and were based on T1/T2 sequence. Two types of relaxometry approaches were used: global and localized. For global relaxometry, an inversion-recovery-CPMG sequence was used. The parameters used were: 64 echoes with an echo time of 8 ms. The T1 dimension was encoded by 64 inversion recovery steps with inversion recovery times logarithmically increased from 5 ms to 15s; the repetition time was of 15s which guarantees the total recovery of the magnetization. For localized relaxometry, an inversion-recovery-CPMG sequence with slice selection was used. The parameters for this acquisition were: 64 echoes with an echo time of 10 ms and 64 inversion recovery steps with inversion recovery times varied from 5 ms to 15s were acquired for each slice; the repetition time was 15s.

2.4 Data analysis

HR-MAS data from the first season were processed with AMIX software (Bruker. Spectra belonging to the second season were analyzed using Spin Works 3.1.7 (Copyright © 2010, Kirk Marat, Univerity of Manitoba). Spectra were displayed and the area of the peaks was computed, using the integration module. This peak is proportional to the number of nuclei that create the signals and therefore, it is only useful when compared with another integral. Principal component analysis was erformed on the extracted peak areas. On the other hand, 2D relaxometry were analyzed by means of Fast Laplace inverse transform (Callagham et al., 2007).

3 Results

According to HR MAS spectra and to 2D relaxometry intra and inter apple differences were found.

3.1 HR-MAS study

HR-MAS study provided several differences inter (Fig. 1) and intra-apple (Fig. 2), depending on the watercore damage level and the area from where the tissue sample was taken. From the visual inspection of the spectra of apples from the first season, it was seen that higher content of water, α-glucose, β-glucose, ethanol and malic acid are found in strongly affected watercore areas. Lower amount of sucrose was found in healthy and light watercore affected tissues.

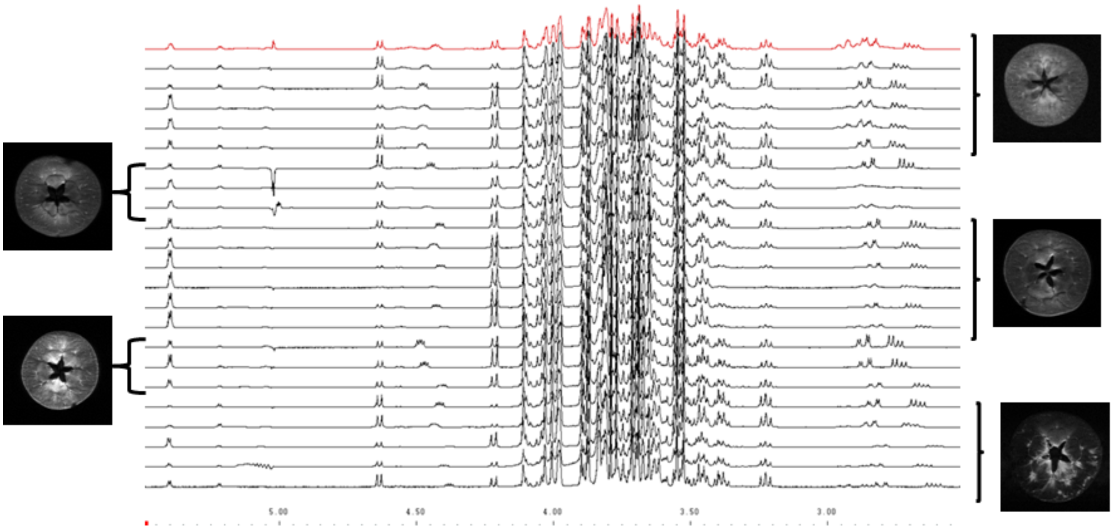


Fig. 1 HR MAS spectra of the first season apples.

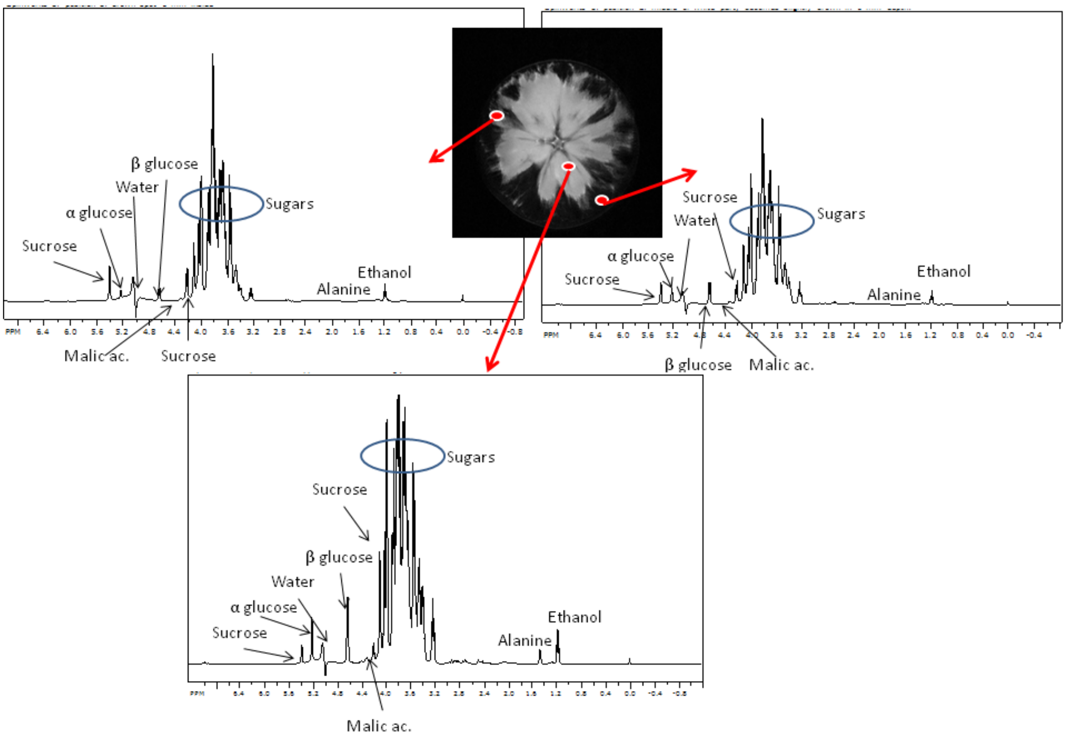


Fig. 2 HR MAS spectra of different parts of a watercore affected apple. Several variations in sugars and malic acid are found depending on the area of the apple.

The area of the peaks was used as a quantitative validation to the qualitative spectra observed in the previous season. Only the relative integral size is meaningful. Thus, global area between 0.5ppm and 10.0 ppm was taken as reference. A principal component analysis was performed on such quantitative data and the percentage of variance with respect to the total area of the peaks was computed (Fig. 3).

A. B.

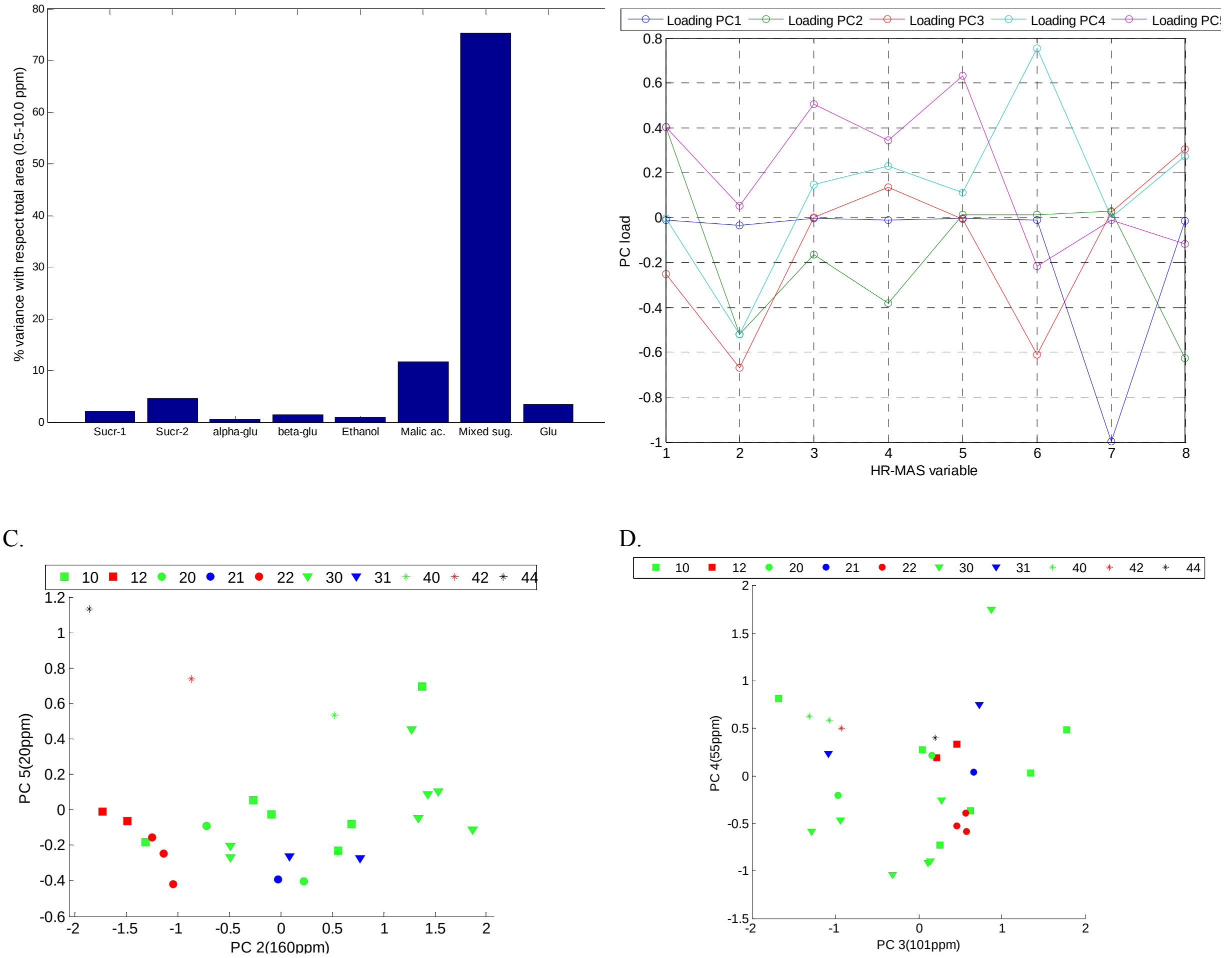


Fig. 3 A. % of the variance respect to the total area of the peaks (0.5-10.0 ppm). B. Loadings from the PCA. C. PC2 against PC5. Differences on type of damage and cultivar are shown, the first digit indicates variety while the second refers to warercore leve. D. PC3 against PC4.

3.2 2D relaxometry

From the relaxometry, as watercore is due to the water movement from the intracellular (vacuole V to cytoplasm, C) to the intercellular spaces, the higher differences were seen in water distribution, which can be found around 10^0 and 10^{-1} values (Hernández-Sánchez et al., 2007). Fig 4 shows the different global 2D relaxometry correlation spectra of 2 apples with distinct watercore affection. On the healthy apple, water at several microscopic structures can be addressed (vacuole and cytoplasm), however for affected tissue compartiments are degraded and cannot be isolated.

In order to be able to study each area of the apple separately, localized relaxometry has been applied on 7 different regions of each of the apples. Again, and in agreement with MRI and the HR MAS results, intra-apple differences are found depending on the area of study (Fig. 5). In this case, all the areas corresponding to water and sugars of the sound apple appear separately. However, on the affected apple, these peaks appear merged, which means the presence of free water and the degradation of internal microstructure.

4 Discussion

4.1 On HR MAS

Vermathen et al., (2011) report an extensive study (90 spectra) on 13 compounds identified by means of HR-MAS in several apple cultivars. In this study greater inter-fruit differences are found compared to intra-fruit samples in most varieties (Gloden and Rubens) except for Braeburn, which typically suffers from internal breakdown. In our study, apple samples from disorder affected tissues have shown to have significant differences with regard to sound tissues of the same apple. Vermathen et al., (2011) also report very strong inverse correlation between sucrose and α and βglucose which is in good accordance with our results.

4.2 On 2D relaxometry

Non-destructive global and localized 2D relaxometry has been obtained. This technique has only been previously reported at a destructive level, since it has always been applied to fruit tissue samples (Hernández-Sánchez et al., 2007, Marighetto et al., 2008) and not to whole fruits. Therefore it becomes feasible to preform non-destructive macroscopic and microscopic monitoring of disorder development.

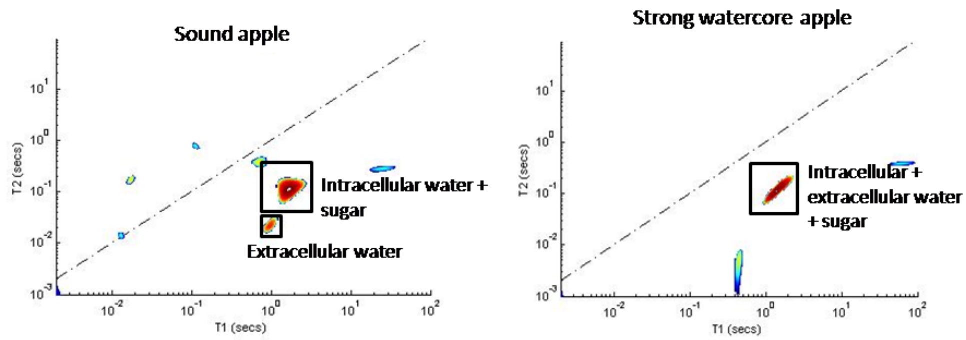
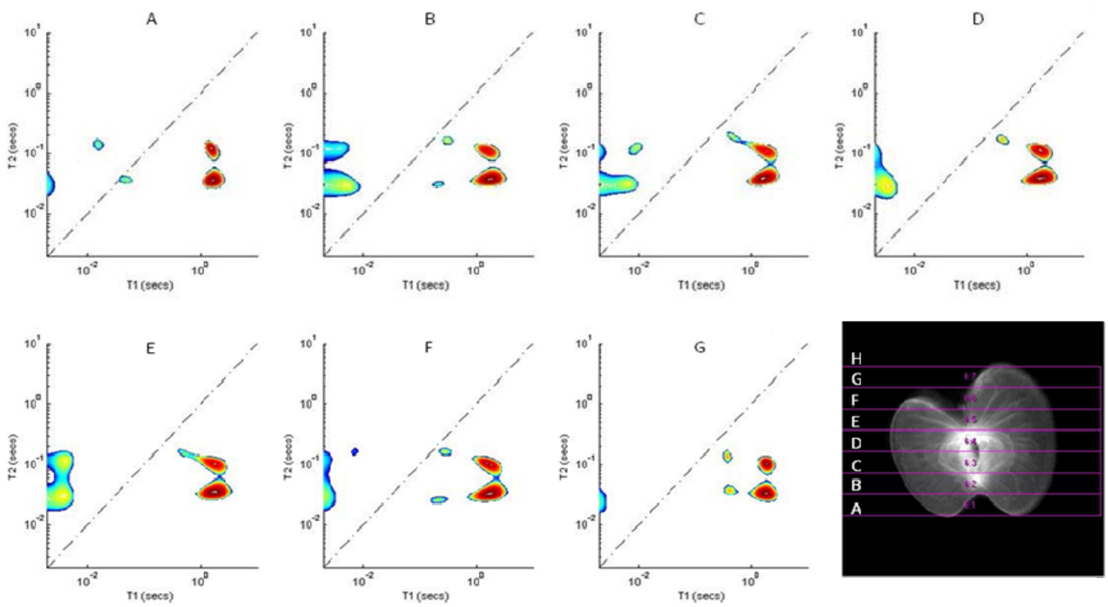


Fig. 4 Global 2D relaxometry spectra for 4 apples with different watercore affection level.



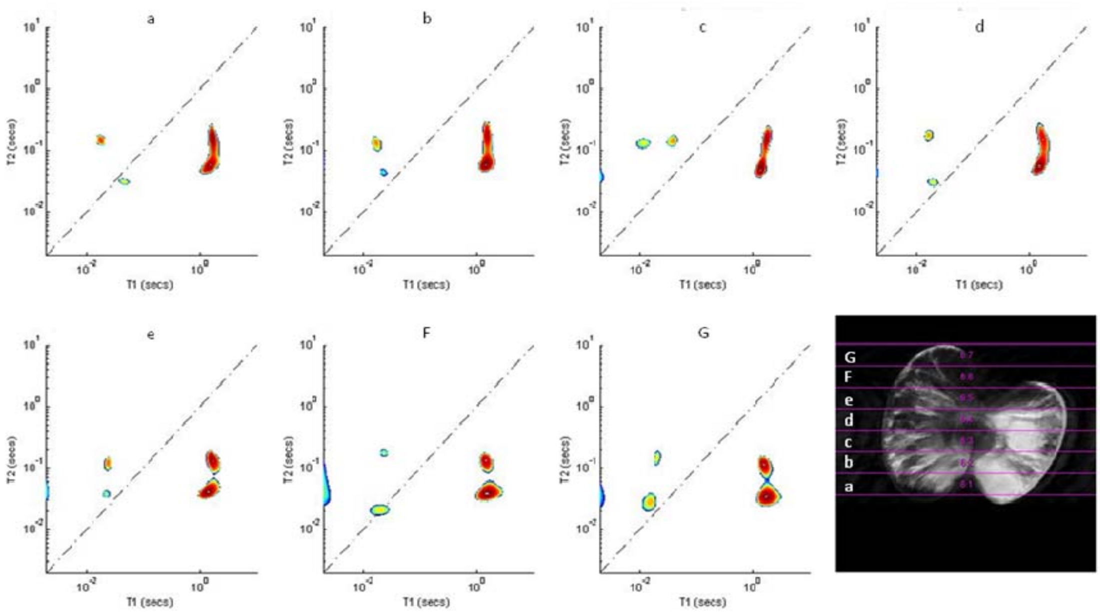


Fig. 5 2D localized relaxometry spectra for 7 regions for two different apples: a sound apple (top) and an affected apple (bottom)

5 Conclusions

HR MAS shows higher content of α-glucose, β-glucose, malic acid and aromatic compounds for the tissues affected by watercore on apples from both seasons, while sound tissue reflects higher sucrose. Microscopic (subcellular) degradation of tissue, evaluated with non-destructive global and localized 2D relaxometry, varies according to disorder development and is in good accordance with macroscopic characterization with MRI.

Acknowledgements

This publication has been produced with the financial support of the European Union (project FP7-226783 - InsideFood). The opinions expressed in this document do by no means reflect the official opinion of the European Union or its representatives. The authors would like to pay tribute to Brian Hills, admired pioneer NMR scientist that has past-away. This work would have been impossible without him.

References

Callaghan, P.T., C.H. Arns, C.H., Galvosas, P., Hunter, M.W., Qiao, Y., Washburn, K.W., 2007. Recent Fourier and Laplace perspectives for multidimensional NMR in porous media. Magnetic Resonance Imaging 25(4): 441-444.

Clark, C.J., Hockings, P.D., Joyce, D.C., Mazucco, R.A., 1997. Application of magnetic resonance imaging to pre- and post-harvest studies of fruits and vegetables. Post. Biol. and Tech. 11(1), 1-21.

Hernández-Sánchez, N., Hills, B., Barreiro, P., Marigheto, N., 2007. An NMR study on internal browning in pears. Post. Biol. and Techn. 44(3), 260-270.

Marigheto, N., Venturi, L., Hills, B., 2007. Two-dimensional NMR relaxation studies of apple quality. Post. Biol. And Tech. 48, 331-340.

Melado-Herreros, A., Muñoz-García, M-A., Blanco, A., Val, J., Fernandez-Valle, M-E., Barreiro, P., 2012. Relationship between solar radiation on watercore on Apple fruit assessed with MRI. Poster. International Conference of Agricultural Engineering (CIGR-AgEng2012).

Sanchez-Perez, E.M., 2011. Thesis. Aplicaciones de la resonancia magnética nuclear al estudio del perfil metabólico en tomates.

Vermathen, M., Marzorati, M., Baumgartner, D., Good, C., Vermathen, P., 2011. Investigation of different apple cultivars by High Resolution Magic Angle Spinning NMR. A feasibility study. J. Agric. Food Chem., 59, 12784-12793.