Asparagine in plums detected by CEST–MRI

Short communication

Asparagine in plums detected by CEST – MRI

Rachele Podda[a,b], Daniela Delli Castelli[a], Giuseppe Digilio[a], Maria Lodovica Gullino[b] and Silvio Aime[a]

[a]Department of Molecular Biotechnology and Health Sciences, Molecular Imaging and Preclinical Centers, University of Torino, Torino, Italy

[b]Center of competence for the innovation in the agro-environmental sector (AGROINNOVA). University of Torino, Via Leonardo da Vinci, 44, 10095 Grugliasco, Torino, Italy

Highlights

- CEST-MRI technique is applied for the first time in fruits
- pH in plums can be monitorated during the ripening process
- Saturation transfer of amide protons from Asn can be followed by means of CEST-MRI experiments at low magnetic field.

Abstract:

Magnetic Resonance Imaging (MRI) relies on the topological distribution of the intense water NMR signal and may be used to report about changes in the internal structures of fruits associated to ripening, storing, pathogen infection. Herein the use of CEST- MRI (Chemical Exchange Saturation Transfer) is introduced to show that in addition to structural information, insights into the presence in the fruits of specific chemicals may be gained, Asparagine is present in plums at relatively high concentration (≈ 10 – 20 mM) and owns two amide protons (at 2.1 and 2.8 ppm down field from water) in slow exchange with water protons. By irradiating the amide resonances with a proper rf-field it is possible to transfer saturated magnetization to the “bulk” water signal. The attained change in signal intensity reflects the extent of prototropic exchange between amide and water protons that is modulated by the local pH.

1.Introduction

Magnetic resonance imaging is extensively used because it can provide images of internal structures with a good compromise between spatial and temporal resolution. Its wide range parameters linked to water and nondestructive evaluation of various internal quality factors lead to innovative studies in food chemistry [1]. Preliminary studies applied to fruits were focused on T_2, T_1, diffusion coefficient assessment, for internal quality assessment during storage and ripening process studies [2]. In damaged tissues, a contrast is immediately visible in the MR final image.

In this communication we report preliminary results obtained by applying the emerging procedure named CEST-MRI [3]. It relies on the detection of molecules containing mobile protons in slow exchange with water. Fruits may contain many molecules that respond to this requisite, namely aminoacids, peptides, saccharides. CEST-MRI is an encoding frequency procedure that, in principle, allows selecting the contrasting effect due to a specific molecule as the transfer of saturated magnetization (ST) is associated to the irradiation of the mobile proton absorption of the molecule of interest. As a first proof of concept of this approach the resonance of the amide moiety of asparagine (Asn) have been exploited as this amino-acid is known to occur at relatively high concentration in plums [4]

2. Results and discussion

Asparagine contains two water exchangeable amide protons that can be exploited for MRI via the CEST effect. To assess the level of asparagine in plum pulp and to assess whether the pulp contained significant sources of exchangeable protons in addition to those of asparagine, the metabolite profile of plum pulp has been acquired by high resolution ^{1}H-NMR spectroscopy at 14T (corresponding to 600 MHz proton Larmor frequency, Figure 1). The most intense signals in such ^{1}H-NMR spectrum are due to sugars (contributing to the spectral region 4.2-3.2 ppm and 5.4-5-2 ppm), which are known to be very abundant in plum juice under the form of both monosaccharides, disaccharides (mainly sucrose) and fructo-oligosaccharides (FOS) [5,6]. In addition to the saccharide resonances, the resonance of Asn H_β (2.96 and 2.86 ppm) and Asn side chain amide protons (7.60 and 6.88 ppm) are clearly detectable. No other significant sources of exchangeable amide protons with chemical shifts close to the amide resonances were detected. A concentration of Asn of 17.0±0.5 mM could be determined by integrating the Asn H_β resonances with respect to that of reference 60 mM TSP. The concentration of Asn did not change significantly in plums from harvest up to 14-days ripening time (full ripening).

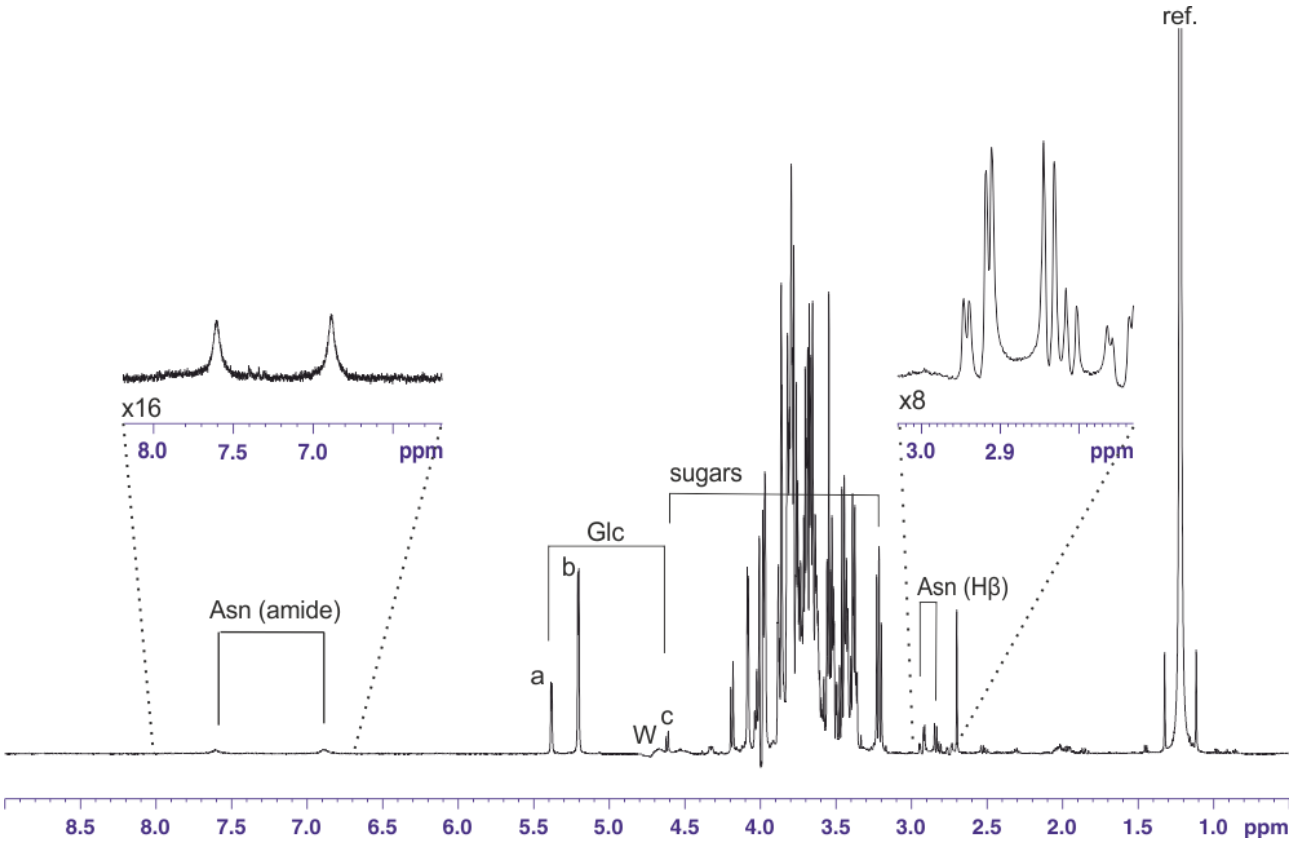


Figure 1. ^{1}H-NMR spectrum at 600 MHz of plum juice at 7-days post harvest (1:10 dilution with dH_2O, H_2O/D_2O 95/5 v/v, pH 4.5, 25 °C). Water suppression was achieved by excitation sculpting with gradients to avoid saturation transfer of exchangeable protons. The resonances of asparagine amide and Hβ are magnified in the inserts. **a:** anomeric proton resonance of sucrose/FOS; **b:** anomeric proton resonance of α-D-glucose, **c:** anomeric proton resonance of β-D-glucose; **R:** reference *tert*-butanol; **w:** residual water signal.

Next, CEST-MR images were acquired on a scanner working at a magnetic field of 1T. First, a phantom of six tubes containing 17 mM Asn (*i.e.* the concentration measured in the plum pulp) and at different pH values was assessed by CEST-MRI, as it is well-known that the exchange rate of amide protons with bulk water is base-catalyzed and rapidly increases with pH. Therefore, at the magnetic field of 1T, the pH dependent modulation of the exchange rate can significantly affect the saturation transfer efficiency. The dependency of ST on pH has been assessed within the pH interval 3.3–5.5, to include pH conditions of plum pulp juice after harvest (pH 3.3 for the cultivar considered) and at full ripening (pH 4.5). As expected (Figure 2), high ST% (35%) can be obtained from the solutions at the more acidic pH 3.3 (irradiation frequency offset at 2.1 ppm from the water resonance), while the CEST effect steadily drops to 10% at pH 5.5.

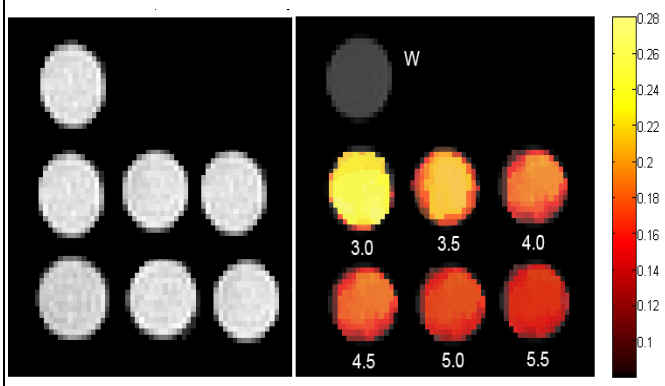
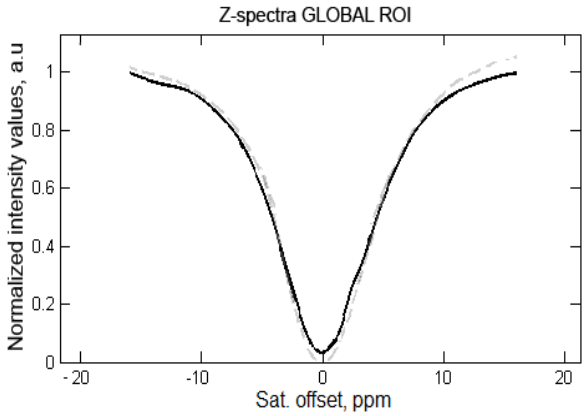
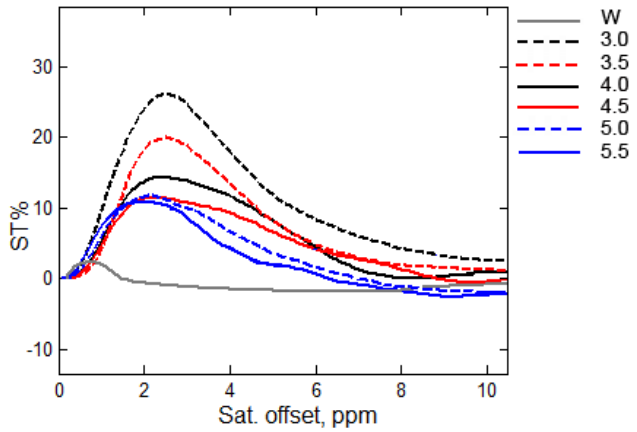
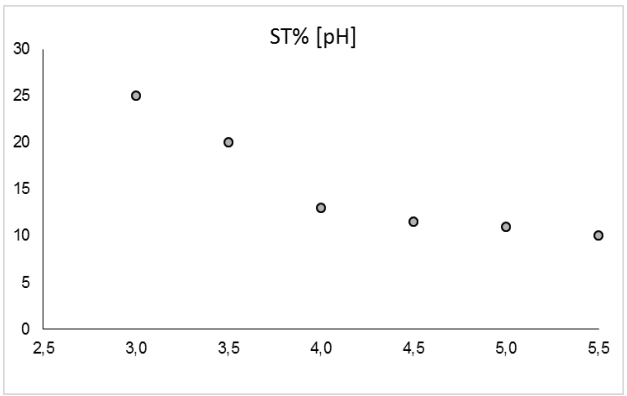
	A	B
	 W 3.0 3.5 4.0 4.5 5.0 5.5 0.28 0.26 0.24 0.22 0.2 0.18 0.16 0.14 0.12 0.1	 Z-spectra GLOBAL ROI Normalized intensity values, a.u Sat. offset, ppm
	C	D
	 W 3.0 3.5 4.0 4.5 5.0 5.5 ST% Sat. offset, ppm	 ST% [pH]

Figure 2. CEST-MRI of asparagine solutions at different pH (3.3, 3.5, 4.0, 4.5, 5.0, 5.5), 1T. **A)** CEST-MR images of 17 mM Asn (irradiation offset at 2.1 ppm with respect to the water resonance). Pixels showing ST% above 10% (noise threshold) are represented in false color scale, else they are shown in gray **B)** Z-spectrum of 17 mM Asn (pH 3.3), water reference in gray line . By increasing pH, the amide protons exchange faster thus approaching the coalescence, resulting in the decrease of saturation transfer efficiency. **C)** ST% Asn 17 mM (with water reference, W). **D)** Plot of ST% (from images in B) versus pH.

On this basis, CEST-MR images of the whole plum were acquired (Figure 3). The irradiating rf offset was at 2.1 ppm and the images were acquired at 7 and 14 days after harvest. Conventional T_2-weighted MR images were also acquired to visualize the internal structure and texture of the fruits. At day 7 post harvest, a very high ST% (25%) was clearly detected all over the fruit. Conversely, at day 14, the ST% is reduced to 15%. The reasons for the observed behaviour may rely on different causes, namely changes in Asn concentration, change in pH or change in T_1 of water protons. Through the acquisition of the high resolution ^{1}H-NMR spectrum and T1 measurements of the plum's pulp of these samples it assessed that Asn concentration and T_1 of water did not change with ripening. Conversly, the pH increased from 3.3 to 4.7. Thus we conclude that the change in pH is the main determinant for the observed change in CEST-MR images taken at day 7 and 14 after harvest.

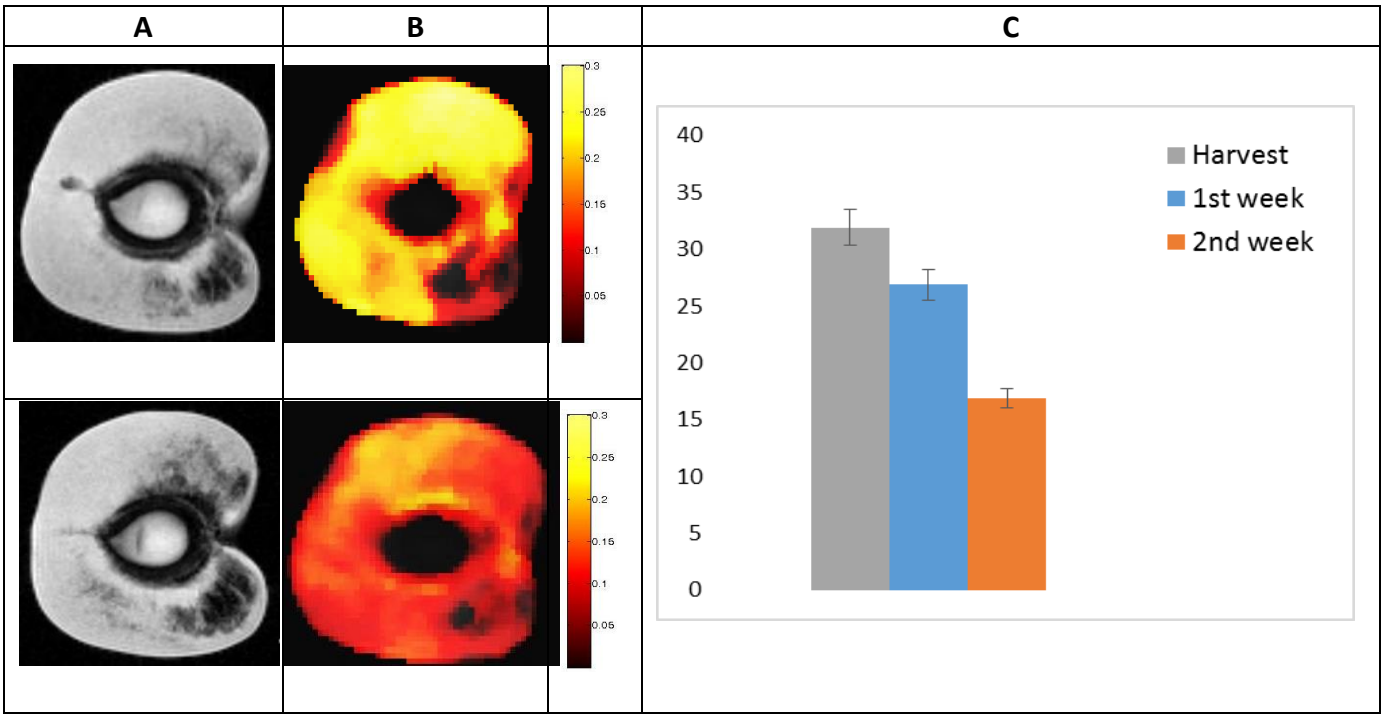


Fig. 3 : plum fruit T_1-weighted images (A) and ST maps (B) at different chemical shift in thermal scale, from acquired CEST spectra after 7 (left column) and 14 days (right column) post harvest. ST maps were calculated at Δppm values of amide protons for Asparagine from bulk water (4.7 ppm) . Mean values of ST% for the whole plum study (C)

3. Conclusions

This is the first time that CEST-MRI technique has been used to food MRI research area. The herein reported results show that CEST-MRI may be highly informative on the chemical composition of a fruit. Actually the detection of ST% arising from sugar exchanging protons (5.0 – 5.5 ppm) may provide important insights into the ripening process. This task has not been possible to be addressed on the MRI 1T scanner used to this work.

4. Materials and Methods Section

4.1 Fruit imaging

Samples from *Prunus domestica* cv. TC Sun Angeleno were supplied by Sanifrutta fruit firm, Saluzzo, Piedmont region. Healthy plums with an average diameter of 5 cm were chosen for experiments. MRI and CEST scans were performed using an ICON Avance M2 (Bruker) with a NdFeBmagnet (Aspect). Operative magnetic field B_0 was 1 T. T_1- and T_2-weighted plum images were acquired for preliminar observation before CEST experiments. Fast low angle shot (FLASH) and Rapid acquisition with Refocused echo (RARE) methods was used and for T1- and T2-

weighted images, respectively. FLASH parameters were set with echo time 5 s, repetition time 184.5 s, 4 averages pulse angle 30 degrees. RARE parameters were set with repetition time 6 s, effective echo time 12.5 ms, rare factor 24. Geometry parameters were fixed for both sequences as follows: 'field of view 50×50mm/slice thickness 2 mm/matrix 128*128px/ voxel size 0.3 mm^3'. Temperature was mantained at 6°C to prevent consistent tissue destruction and perform scans for 20 days.

All target compounds in this study has small Δω offsets ranging from about 1 (hydroxyls group of sugars) to 6 ppm (some amine groups). Spillover effect was minimized with low saturation intensities. CEST effects was visualized by means of super-imposed map on a T_1 weighted image with no magnetization transfer (MT) pulse. CEST MRI scans were performed using RARE method, which parameters were defined as described above; MT module was active and impulse was a block pulse set in lenght to 3 s and amplitude 3uT. Other setups were an echo time of 12 s, repetition time 10 s, one number of repetitions, refocusing flip angle 180 deg., rare factor 24 and one average. In plane geometry was modified for single axial slices, 4 mm thickness, FOV 50×50 mm, matrix 64×64 px, voxel size 1.2 mm^3.

4.2 ^{1}H-NMR spectroscopy.

All ^{1}H-NMR spectra were acquired on a BrukerAvance III spectrometer operating at 14.1 T, corresponding to a proton Larmor frequency of 600 MHz, equipped with an inverse Z-gradient 5 mm double resonance inverse probe. The temperature was controlled within ±0.1 K through the BTO2000 VTU system. ^{1}H-NMR spectra aimed at assessing the presence of exchangeable protons were carried out by diluting the plump juice 1:10 with dH_2O without pH adjustment. An aliquot of 540 μL of the diluted sample were added with 50 μL of D_2O for field/frequency lock and 1 μL of *tert*-butanol as a secondary chemical shift reference. ^{1}H-NMR spectra were acquired with a pulse sequence performing water suppression by excitation sculpting with gradients [7] to minimize saturation transfer from water to exchangeable protons (Bruker pulprog zgesgp). Acquisition parameters included 3 s relaxation delay, 64 K complex data points, 16 ppm spectral window, 128 scans, and 3 ms selective pulse length for water suppression. To quantify the metabolite levels and to obtain metabolic profiles, the plum juice was diluted 1:10 with 100 mM phosphoric buffer pH 3.3 (final pH checked and eventually adjusted to to 3.35±0.05 pH units). A volume of 540 μL juice was added with 60 μL of D_2O containing reference *tert*-butanol to yield a final concentration of 60±0.05 mM. ^{1}H-NMR spectra were acquired with the one-dimensional noesy pulse sequence with pre-irradiation of the water signal for water suppression. Acquisition parameters included 5 s relaxation delay, 128 scans, 4 dummy scans, 20.5 ppm spectral window, 64 K complex data points,

10 ms mixing time, and 25 Hz bandwidth of the water suppression pulse. The levels of asparagines was evaluated by comparing the area of the Asn Hβ signal to that of reference TSP. Metabolite signals were analyzed by the AMIX 3.9.2 software package implemented with the Bruker BBIOREFCODE 2.0.0 database and the BioMagResBank metabolomics database [8].

4.4 CEST analysis.

Raw MRI data were processedby means of custom-made software program implemented in MATLAB (the Mathworks Inc., Natick, MA, USA) [9] .Z-spectra and saturation transfer (ST) maps were corrected by means of interpolation method [10].

Acknowledgements

This work was partially carried out with the support of the CRC foundation for scientific innovation (Cassa di Risparmio di Cuneo), Cuneo, Italy. One of the authors (RachelePodda) undertook this work under the support of the ‘‘Center Of Exellence In Preclinical Imaging (CEIP)’’, insideBioindustry Park del Canavese, CollerettoGiacosa, Ivrea, Italy.

References

[1] Mariette F., Collewet G., Davenel A., Lucas T. and Musse M. (2012) Quantitative MRI in Food Science & Food Engineering. Encyclopedia of Magnetic Resonance.

[2] Hall L.D., Evans S.D. and Nott K.P (1998) Measurement of textural changes of food by MRI relaxometry. Magnetic Resonance Imaging, Vol. 16, 485–492.

[3]. Van Zijl P. C. M and Yadav N. N. (2011) Chemical exchange saturation transfer (CEST): What is in a name and what isn't? Magnetic Resonance in Medicine. Volume 65, 927–948.

[4] Ogasanović (2007) Amino acids content in the fruit of some plum cultivars and hybrids. VIII International Symposium on Plum and Prune Genetics, Breeding and Pomology, International Society for Horticultural Science. Acta Horticulturae, 734.

[5] Kosmal M., Milala J., Kołodziejczyk K., Markowski J., Zbrzeźniak M., Renard M.G.C. (2013) Dietary fiber and cell wall polysaccharides from plum (*Prunus domestica* L.) fruit, juice and pomace: Comparison of composition and functional properties for three plum varieties. FoodResearch International, Vol. 54 , 1787–1794.

[6] Cortacero R.S., Segura-Carretero A., Cruces-Blanco C., Hernainz-Bermudez de Castro M., Fernandez-Gutierrez A.(2004) Analysis of carbohydrates in beverages by capillary electrophoresis with precolumnderivatization and UV detection. Food Chemistry, Vol. 87, 471–476.

[7] Wu, P.S.C.; Otting, G. (2005) Rapid pulse length determination in high-resolution NMR. Journal of Magnetic Resonance, Vol. 176, 115–119

[8] Markley JL, Anderson ME, Cui Q, Eghbalnia HR, Lewis IA, Hegeman A, Li J, Schulte C, Sussman M, Westler WM, Ulrich EL, Zolnai Z (2007) New Bioinformatics Resources For Metabolomics. Pac Symp. Biocomput., 157-168.

[9] Chu V., Hamarneh G. (2006): MATLAB-ITK Interface for Medical Image Filtering, Segmentation, and Registration, Proc. of SPIE, Vol. 6144, 61443T-7

[10] J. Stancanello, E. Terreno, D. DelliCastelli, C. Cabella, F. Uggeri and S. Aime 2008: Development and validation of a smoothing-splines-based correction method for improving the analysis of CEST-MR images Contrast Media Mol. Imaging, 3 136–149.