Can CEST contrast image gluten network?

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Introduction

Non-coeliac gluten sensitivity (NCGS) is a major issue in our occidental countries. NCGS medical causes are unknown. One of the hypothesis to explain NCGS deals with a longer and more complex gluten network leading to a lower digestibility. In this context, a non-invasive analytical method able to image the gluten network in food products is mandatory. However, such method does not exist yet.

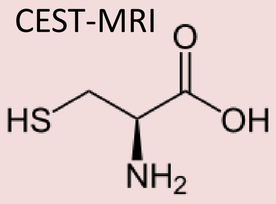
The gluten network is made from the bounding of glutenin and gliadin proteins by the oxidation of thiol moieties to create covalent disulfide bounds. The formation of this network can also be seen as the lost of thiol exchangeable protons.

Chemical Exchange Saturation Transfer (CEST) is an indirect metabolic contrast imaging the exchangeable protons, e.g. ^{1}H from –OH or –NH moieties. To do so, saturation pulses are applied on a large range of offsets and the intensity of the water signal at each offset is mesured to produce a so-called z-spectrum. At the resonance frequency of the exchangeable proton, a lost of water signal is observed and can then be quantified.

Thiol moiety contains exchangeable proton and could be sensitive to CEST contrast. As this contrast has never been applied to this function, we first demonstrate that thiol moiety can be imaged by CEST-MRI and then apply this contrast to dough products.

CEST contrast and thiol moiety

Cystein is used as a molecule model to demonstrate that contrast can be obtained from proton exchange between thiol moieties and water using CEST-MRI



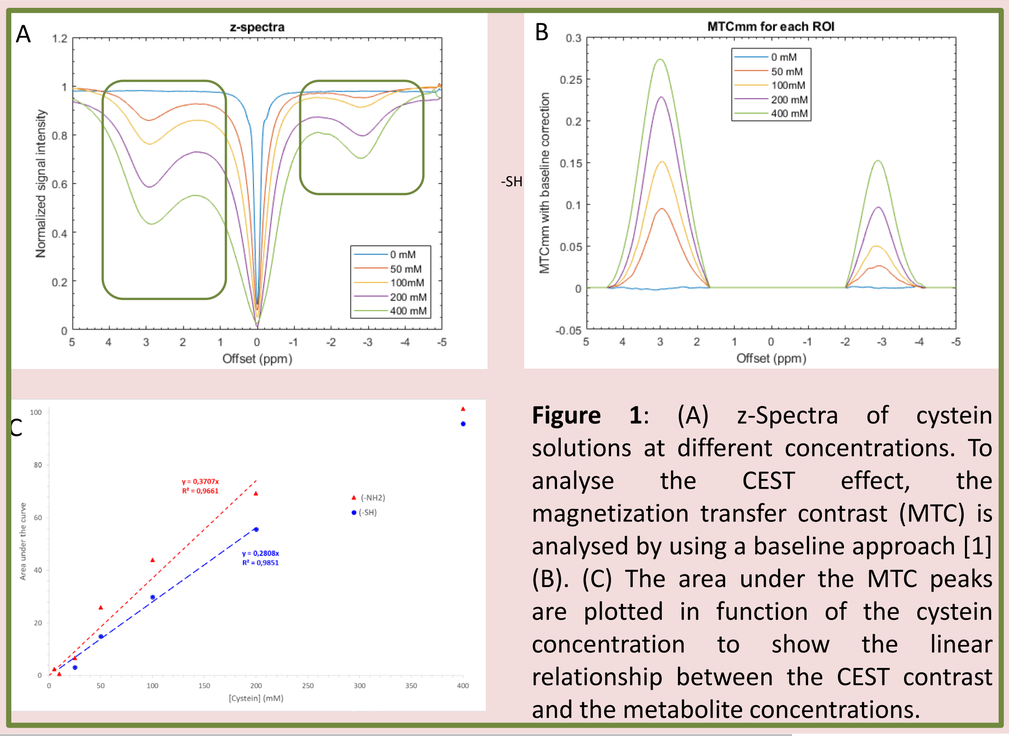


Figure 1: (A) z-Spectra of cystein solutions at different concentrations. To analyse the CEST effect, the magnetization transfer contrast (MTC) is analysed by using a baseline approach [1] (B). (C) The area under the MTC peaks are plotted in function of the cystein concentration to show the linear relationship between the CEST contrast and the metabolite concentrations.

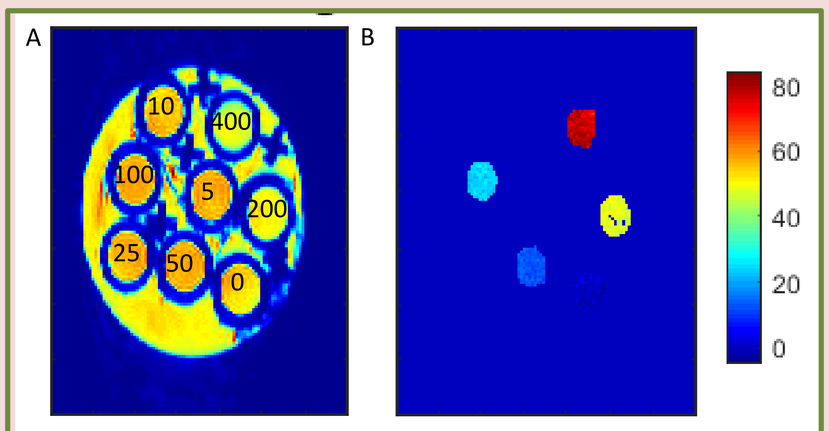


Figure 2: (A) The cystein phantom containing all the concentrations shown in Fig. 1 was imaged by using a classical TurboRARE sequence. (B) The same phantom was imaged by using a CEST contrast. The CEST analysis was performed for offsets between -1.9 to -4.1 ppm corresponding to the thiol moieties. While there is no contrast on the reference image, the different tubes can readily be differentiated from the CEST image.

1. **-SH moieties can be imaged by CEST-MRI**
2. **CEST contrast depends on the metabolite concentration**
3. **CEST for -SH moieties are less sensitive than for –NH_2 groups.**

CEST on dough products

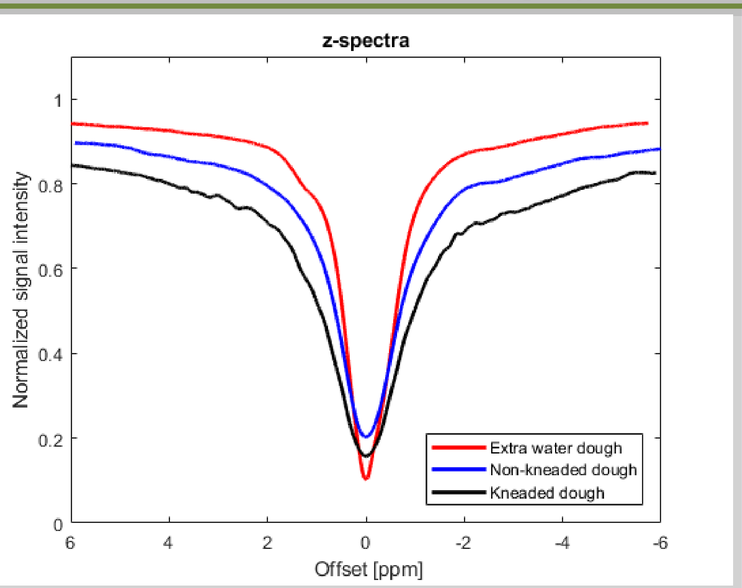


Figure 3: z-Spectra were recorded on different dough preparations. The classical dough preparation (2/1, flour/water, w/w) was either non-kneaded (blue) or kneaded for 5 minutes (black). Another formulation was tested: 1/2, flour/water, w/w (red). The later sample could not be kneaded .

1. The dough z-spectra are significantly wider than for the cystein phantom
2. The z-spectrum broadness is dependent of the sample preparation
3. This change in the z-spectrum envelope could "mask" the CEST-effect from thiol moieties
4. These modifications are most likely due to the presence of a rigid macromolecular network

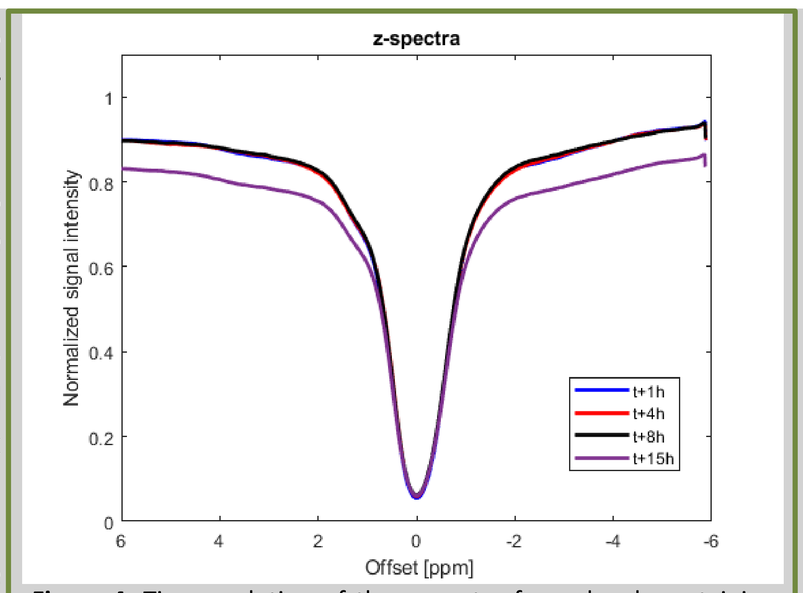


Figure 4: Time evolution of the z-spectra for a dough containing extra water (1/2, flour/water, w/w). z-Spectra were recorded 1h (blue), 4h (red), 8h (black) and 15h (purple) after the sample preparation. While the z-spectra are similar for the first 8h, we can observe a significant change at 15h.

1. Evolution of the z-spectrum at t+15h is characteristic of the presence of a strong macromolecular network
2. Asymmetry due to –OH moieties from sugar observable on the z-spectra (~+1 ppm)
3. Asymmetry between -2 to -4 ppm is most likely due to thiol moiety exchange and/or macromolecule NOE transfer

Simulation of CEST-spectra

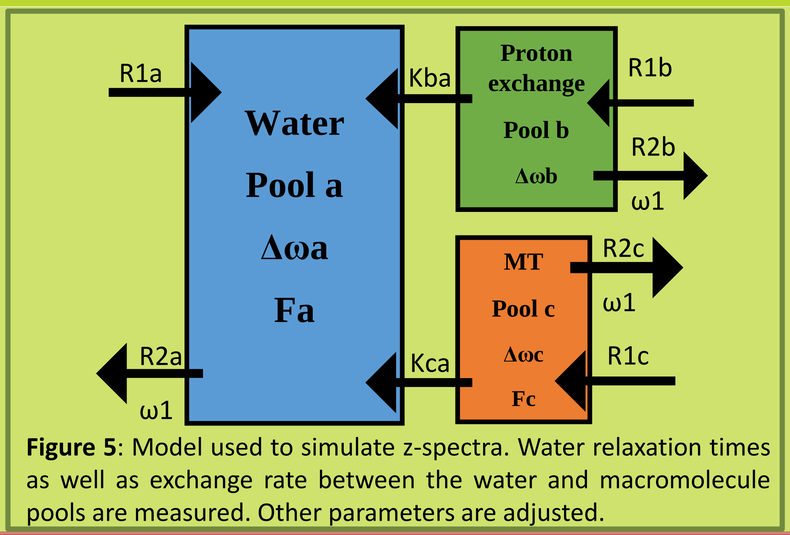


Figure 5: Model used to simulate z-spectra. Water relaxation times as well as exchange rate between the water and macromolecule pools are measured. Other parameters are adjusted.

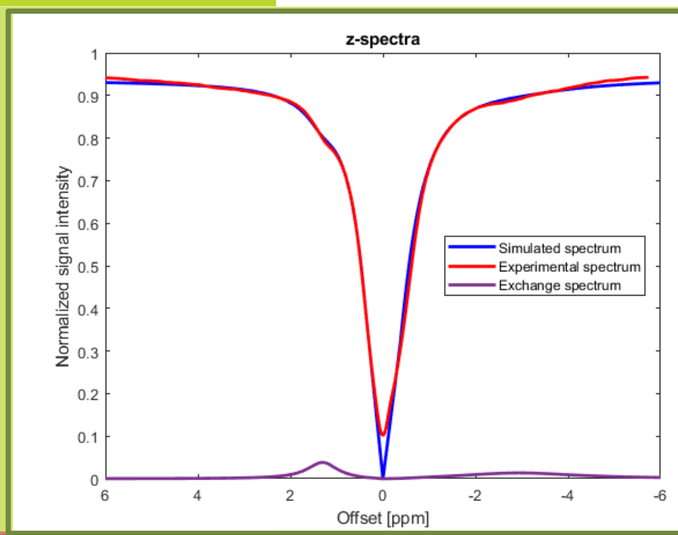


Figure 6: z-Spectrum obtained from a dough prepared with 1/2, flour/water , w/w (red). A manual adjustment of the experimental z-spectrum is represented in blue. From the model, the exchange between the exchangeable protons and water can readily be quantified (purple). From this exchange-only spectrum, the thiol moieties can be imaged.

1. Fitting of experimental data to a model could help to retrieve thiol-CEST in dough products
2. Chemical exchange from thiol moieties is spread over a wide range of offsets, decreasing the method sensitivity
3. Fitting the data allows separating CEST from other macromolecular exchanges which can appear at upfield frequencies

Conclusions

- CEST-MRI can be used to contrast thiol moieties
- CEST-MRI on dough products leads to wide z-spectrum envelope due to the presence of macromolecules. Exchange between water and macromolecules by other mechanisms than chemical exchange overlaps in the z-spectrum
- To extract CEST effect, a 3-pool model can be used to fit the data (work in progress)

(1) T. Jin, P. Wang, X. Zong, S.-G. Kim, *Magn. Reson. Med.* 2013, 69:760.

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