Application Note

Time Domain NMR in R&D: studying the properties of plant-based meat analogue burgers during "cooking process"

Introduction

Meat analogue products based on plant protein sources have been widely considered a rich replacement for conventional meat. The main reason for the increased interest in meat analogues is public awareness of a healthier diet and, above all, a sustainable environment (less greenhouse gas emissions and depletion of natural resources). However, mimicking the sensory texture and mouthfeel attributes is still a key challenge in this topic. Therefore, adequate instrumental techniques are required to better understand and characterise plant-based meat matrices and consequently correlate them with sensory analysis.

Time Domain NMR (TD-NMR)

Time-domain NMR (TD-NMR) is a versatile technique for measuring food sample composition and, more broadly, quality control in a range of industries. It has also been successfully used to investigate water and fat distribution in animal meat products for many decades and has recently been applied to plant-based meat (PBM). [1,2] Furthermore, TD-NMR is a non-destructive tool and benefits from excellent repeatability, high accuracy, and reliability.

Relaxometry Analysis in Food Science

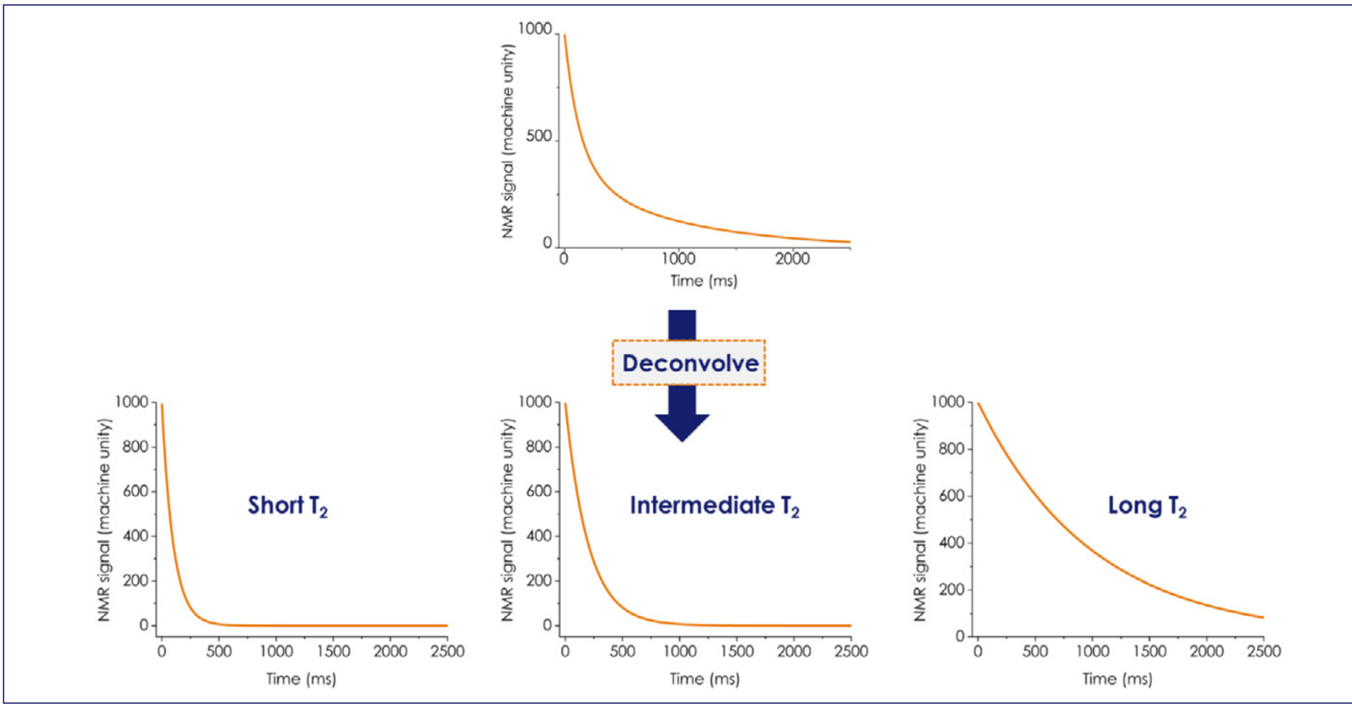
Most of the TD-NMR research on food samples is based on relaxometry methods, which are widely used to analyse the composition and distribution of water and fat. T_2 (transverse relaxation time) is the primary relaxation parameter used in food analysis. It is commonly measured using the Carr-Purcell-Meiboom-Gill (CPMG) sequence, which relies on a 90° pulse followed by a train of 180° pulses to generate a train of echoes.

The intensities of these echoes decay over time, describing the T_2 decay. The other relaxation time is the longitudinal one (T_1), which is commonly measured by the Inversion Recovery (IR) sequence and combined with CPMG in the 2D methods (IR-CPMG) to obtain the T_1-T_2 correlation.

In food samples, the T_2 decay is generally described by multi-exponential components related to fat and water proton* content. The T_2 value may range from microseconds to seconds depending on the type of fat, water content, and the environment in which the water protons are confined. In a typical food sample, the water protons correspond to more than one T_2 component, representing distinct "classes" of proton-bearing water populations, which have much faster T_2 relaxation than free water.[3] The longest T_2 relaxation time is assigned to a fraction with the highest level of proton mobility, while the shortest one represents the water protons strongly bound to other ingredients or confined within the food matrix.

1] F. Nasrollahzadeh et al., *Food Hydrocoll.*, 2024, **151**, 109829. [2] F. K.G. Schreuders et al., *Food Hydrocoll.*, 2020, **101**, 105562.
* "Proton" is commonly used to describe a hydrogen (^{1}H) nucleus

Scheme 1 shows an example of T_2 decay for a multi-component sample.



Scheme 1 Multiexponential transverse relaxation (T_2) decay.

Relaxometry analysis of meat/meat analogues

TD-NMR has been widely used in the food processing industry to investigate water-protein interactions. This has provided insights into several macroscopic properties, such as water-holding capacity (WHC), which describes the capability of the protein to retain the water inside the pores, fat and water content, and textural properties changes during cooking and storage stages.[3]

- Studies have indicated that a water proton T_2 value decreases as a function of protein concentration, indicating a lower degree of freedom and stronger interactions with other ingredients.
- It has been found that there is a correlation between the T_2 relaxation decay and WHC of different types of meat, which affects the textural properties (juiciness/tenderness) and quality of the final product. [3]
- T_2 has been used to investigate the effect of the cooking process, showing that higher cooking temperature promotes the migration of bound water within the fibrous structure, as evidenced by longer T_2 relaxation decay.[4]
- 2D TD-NMR experiments have also been useful for studying food [5]. For example, they can better distinguish fat and water signals in food matrices.

^{1}H T_2 distribution from 15 to 75 °C

This study investigated the water and fat distribution of two soya-based burgers under heating conditions (15 - 75°C) using MQC-R and a ^{1}H 18 mm liquid variable temperature probe. Nutrition information per 100 g of each burger is compiled in Table 1. The raw CPMG (T_2) decays were acquired on both samples and then processed using a1D inverse Laplace transformation (ILT).

Figure 1 shows samples **A** and **B**'s T_2 distribution time (1D-ILT) as the temperature increases from 15˚C to 75˚C. For sample **A**, containing 19% fat content, the number of components and the relative area increased by raising the temperature. In addition, the T_2 relaxation time shifts gradually to longer values at higher temperatures. Those changes may be related to structural transformation with the heating, as they are primarily caused by physico-chemical events, such as fat crystal melting (and maybe separation from water), shrinkage, and fluid expulsion.

Table 1 Nutrition information per 100g.

Samples	PBM burger A	PBM burger B
Fat (g)	19 (rapeseed and coconut oil)	6 (rapeseed, sunflower and coconut oil)
Protein (g)	17 (pea and rice)	18 (pea and wheat)
Salt (g)	0.7	1.1
Carbohydrate (g)	3.5	8.4

[3] H. C. Bertram et al., *Meat Sci.* **2002**, 60, 279-285.

Sample **B**, with lower fat content (6%), shows a different T_2 distribution profile than **A**, indicating a distinct structure and water/lipid partitioning behaviours under similar heating conditions. In general, T_{22} and T_{23} in sample **B** are shorter than in **A** (Figure 2), suggesting a tighter structure and less water mobility in the protein matrix of burger **B**. This statement is supported in the following section with the 2D experiment.

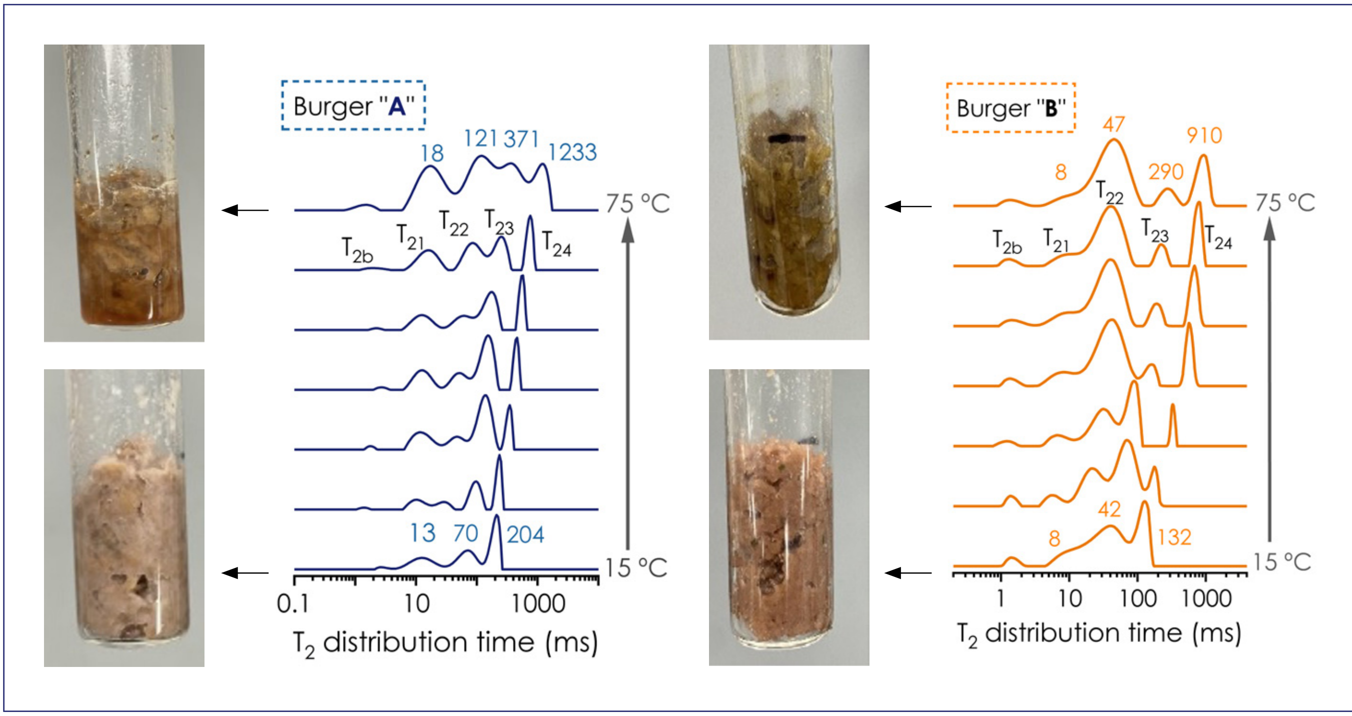


Figure 1 T_2 distribution in PBM burgers **A** (left) and **B** (right) from 15 to 75 °C.

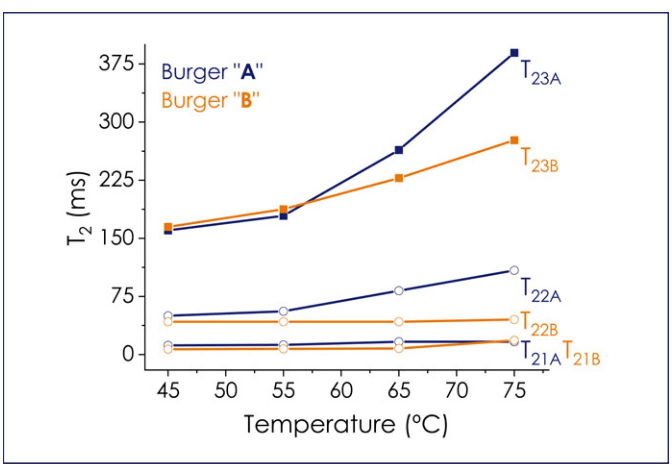


Figure 2 T_2 values over the heating process for both PBM burgers **A** (blue) and **B** (orange).

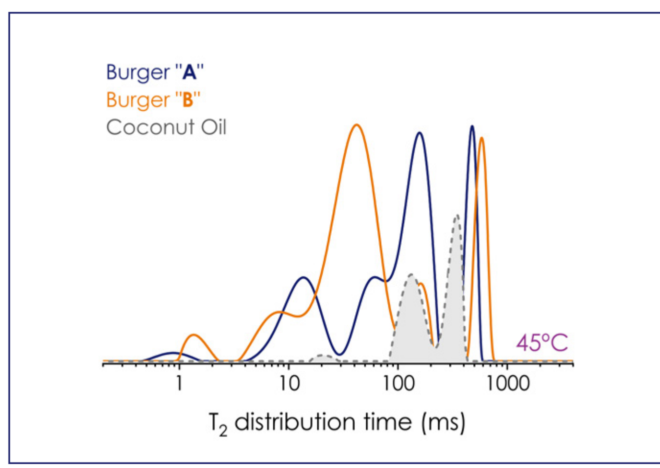


Figure 3 T_2 distribution in PBM burgers **A** (blue) and **B** (orange) and coconut oil (grey) at 45 °C.

To better visualise which component may be affected by the lipids content, the CPMG (T_2) decay of coconut oil was acquired at 45°C. As shown in Figure 3, components T_{23} and T_{24} are more likely to represent not only water but also the lipid's protons, reinforced in the 2D maps. However, T_{21} and T_{22} are more closely associated with water protons. According to the literature, T_{2b} has also been assigned to the protein-bound protons [1,2]. Previous studies have also reported four components [2] for a blend of different plant proteins and five components for plant-based analogues [1].

T_1-T_2 maps from 15 to 75°C

IR-CPMG experiments were conducted and processed using the NMR ProLab software by Green Imaging, a straightforward approach to obtain the T_1-T_2 maps using the 2D ILT processing method.

T_1-T_2 maps agree with the 1D-T_2 distribution but have more detailed information.

[4] H. Wang et al., *Food Hydrocoll.* **2022**, 128, 107540. [5] J. P. C. M Peters et al., *Food Hydrocoll.*, 2017, **65**, 144-156.

2D maps (Figure 4) show that the T_{22} component for the PBM **A** is a combination of T_2 of two components, while in the PBM **B**, the T_{22} seems to overlap three components. This shows that T_1-T_2 maps are helpful in studying more complex systems where multiple components overlap.

The diagonal line is associated with a T_1/T_2 ratio equal to 1. As the signals go to the left of this line, the T_1/T_2 ratio becomes higher. The higher T_1/T_2 for PBM **B** at 75 °C indicates that the mobility of water protons is more restricted in PBM **B**. This suggests that water has weaker mobility in PBM **B**, i.e., its structure is more rigid, supporting the T_2 distribution data.

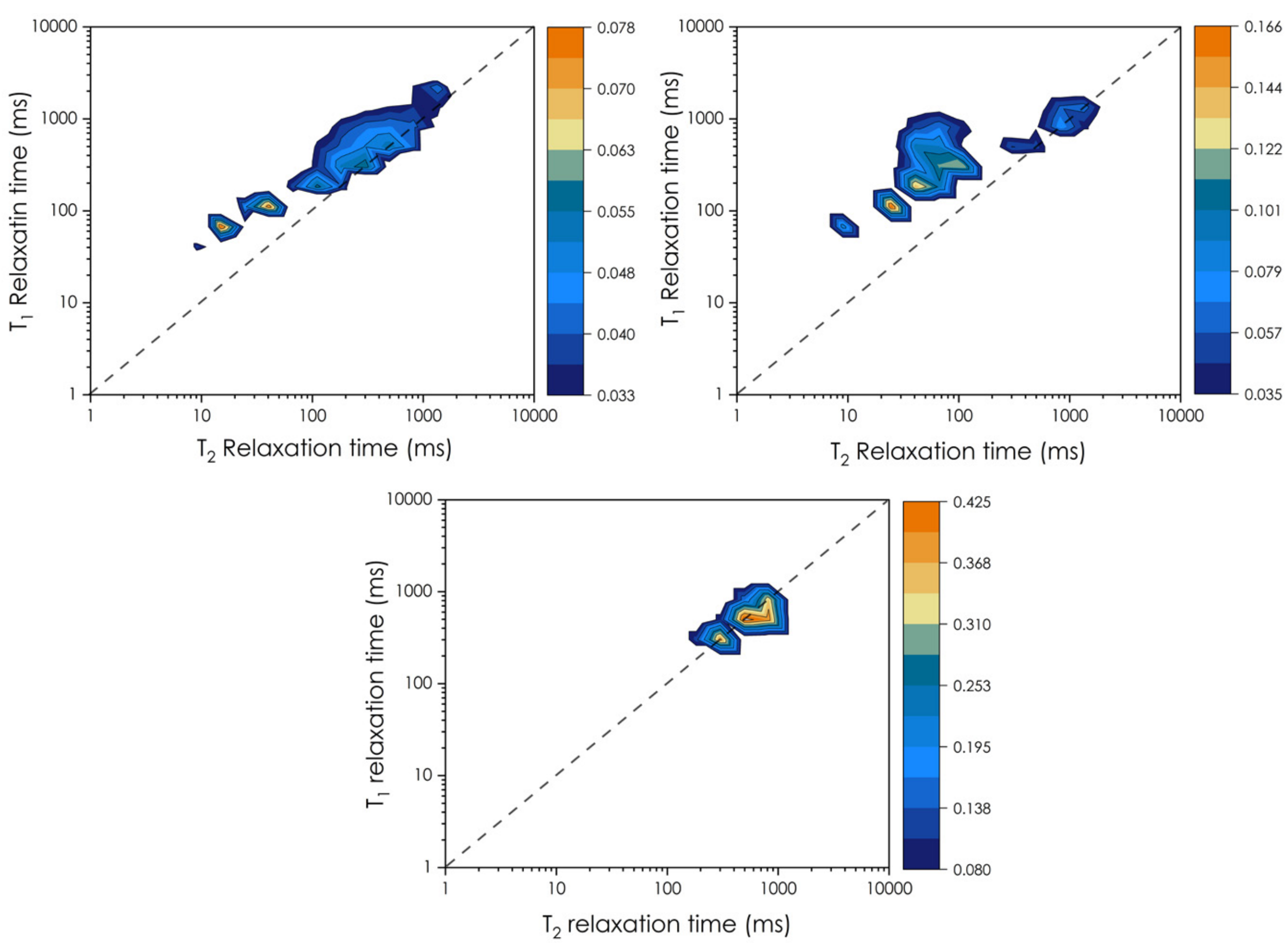


Figure 4 T_1-T_2 maps at 75 °C for PBM **A** (on the top left), **B** (on the top right), and coconut oil (on the bottom).

Summary

- This study demonstrates the potential of TD-NMR to explore the structural changes during simulation of the “cooking process” of plant-based meat analogue burgers, which has the potential to help improve the sensory experience of plant-based products.
- 2D correlation maps have been shown to be a powerful additional technique, particularly for R&D. MQC-R offers NMR ProLab software from Green Imaging that simplifies acquisition and processing of data from various TD-NMR 1- and 2-D experiments.
- Time domain NMR is a straightforward, non-destructive and non-invasive method that can provide insight into the structure of meat and plant-based meat analogues.
- NMR’s accessibility and reproducibility make it an excellent analytical technology tool for product development and increasing customer satisfaction by simulating meat taste and flavour.
- Minimal sample preparation and no hazardous chemicals are required for TD-NMR analysis.

If you have any questions about this application note, please contact our experts: **magres@oxinst.com**

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