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The use of two-dimensional NMR relaxometry in bread staling: a valuable tool?

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ABSTRACT

Staling of bread was investigated in terms of physico-chemical parameters and one (1D) and two dimensional (2D) ^{1}H Nuclear Magnetic Resonance (NMR) relaxometry. Physico-chemical properties were consistent with those generally reported for bread staling (crumb moisture loss, decrease in frozen water content, formation of amylopectin crystals, crumb hardening). One dimensional ^{1}H NMR investigation suggested the presence of only one T_1 protons population, while T_2 was representative of multiple proton populations, that well related to the observed physico-chemical changes. ^{1}H Two dimensional NMR provided an interesting insight of ^{1}H T_1 dynamics, as it allowed to discriminate the contribution of five protons pools within the ^{1}H T_1 relaxation.

1. Introduction

Bread staling is a phenomenon to which different physico-chemical events contribute, leading to a decreased product acceptability during storage due, primarily, to crumb firming and drying, crust softening and loss of the characteristic fresh bread flavour. Several works dealt with the most important physico-chemical phenomena occurring in staling, but its molecular basis has not yet been completely clarified. Multiple phenomena take place in bread during storage, including starch recrystallization, water migration (macroscopic, from crumb to crust and molecular between starch and gluten), and a decrease in the plasticity of the gluten network (Bosmans et al., 2012; Bosmans, Lagrain, Ooms, Fierens, & Delcour, 2013; Curti, Carini, Tribuzio, & Vittadini, 2014; Fadda, Sanguinetti, Del Caro, Collar, & Piga, 2014; Gray & Bemiller, 2003; Hallberg & Chinachoti, 2002; Vodovotz, Vittadini, & Sachleben, 2002; Sereno, Hill, Mitchell, Scharf, & Farhat, 2007).

Understanding the molecular dynamics that involve starch, gluten and water over storage is, therefore, extremely important to fully elucidate bread staling. To this aim, the potentiality of one dimensional (1D) low resolution ^{1}H NMR in investigating water dynamics and relaxation of different bread components (mainly starch, gluten and water), in relation to product properties, has been highlighted by several studies (Bosmans et al., 2012, 2013; Curti et al., 2014; Curti, Bubici, Carini, Baroni, & Vittadini, 2011; Curti, Carini, Bonacini, Tribuzio, & Vittadini, 2013; Curti, Carini, & Vittadini, 2016; Wang, Choi, & Kerr, 2004). However, due to the complexity of the bread matrix, multiple and partially overlapping protons peaks are observed, making their assignment difficult and complex. The potentiality of two dimensional (2D) NMR (low and high resolution) methods, as a tool to measure relaxation times on two dimensions and improve resolution of peaks with similar 1D relaxation times, has been unravelled by several papers (Furfaro et al., 2009a, 2009b; Hills, Benamira, Marigheto, & Wright, 2004; Marigheto, Venturi, & Hills, 2008; Hills, 2009), and these methods have been applied to different food products (Van Duynhoven, Voda, Witek, & Van As, 2010; Rondeau-Mouro, 2017).

To the authors' best knowledge, no studies dealing with the application of 2D NMR methods on bread are available in the scientific literature. However, some works have been published on cereal-based products. Luyts et al. (2013) investigated cake samples and corresponding model systems (based on cake constituents, i.e. flour, water, sugar, and egg), and reported that 2D measurements allowed to better differentiate the protons populations observable in the 1D experiments, based on their 2D ^{1}H T_1. A more recent work by Serial et al. (2016) focused on the application of 2D measurements in dough and biscuits upon addition of inulin and oat fibre, and allowed to better characterize water mobility and interactions with the added ingredients (fibre) before and after

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cooking of the dough. Kovrlija and Rondeau-Mouro (2017) investigated hydrothermal changes in wheat starch by 2D NMR, describing physico-chemical transformations of starch upon heating.

In light of these promising studies, a first perspective on bread staling molecular dynamics investigated by 2D (T_1–T_2) relaxometry is presented in this work.

2. Materials and methods

2.1. Bread formulation

White bread was produced using the following formulation: 100.0 wheat flour (Molino Seragni s.p.a, Rivolta D'Adda, Italy; moisture 14%, starch 84.3% dry basis, proteins 12.5% dry basis, fibre 2.5% dry basis, ash 0.7% dry basis), 3.0 yeast (AB Mauri Italy S.p.a, Casteggio, PV, Italy), 4.0 sucrose (Coprob S.C.A, Pavia, Italy), 2.0 sodium chloride (Italkali s.p.a., Palermo, Italy), 3.0 sunflower seeds oil (Oleificio Zucchi, Cremona, Italy). The optimal water absorption (58%; g water/100 g flour) was measured with a farinograph (500 Brabender Units, BU) (AACC International Approved Methods of Analysis, Method 54-21 02.Rheological Behavior of Flour by Farinograph, Constant Flour Weight Procedure, 2011).

2.2. Bread production and storage

Bread loaves were produced with a home bread-maker (Backmeister 68511, UNOLD, Germany) using a "basic" program (preheating 17 min; first kneading 5 min; second kneading 13 min; first fermentation 45 min; smoothing 1 min; second fermentation 18 min; smoothing 1 min; third fermentation 45 min; baking 55 min). Bread loaves were cooled to room temperature, placed in polyethylene bags sprinkled with about 4 ml of ethanol, and stored at room temperature. Bread was analyzed fresh (day 0) and after 1, 3, 5 and 7 days of storage. At least three bread productions were carried out on different days for each storage time.

2.3. Physico-chemical properties

2.3.1. Moisture content

Moisture content (MC) (%, g water/100 g sample) of crumb (from loaf centre) was determined by weight loss by oven drying (NSV 9035, ISCO, Milan, Italy) at 105 °C to constant weight. At least triplicate samples of crumb were analyzed for each bread loaf.

2.3.2. Texture

Bread crumb texture was measured using a TA.XTPlus Texture Analyzer (Stable Micro Systems, Goldalming, UK). At least six cubic portions (8 cm^3) of crumb were extracted from the central slices of each bread loaf and compressed with a TPA test (force = 0.05 N, 40% deformation, cylindrical probe – P/35 Dia Cylinder Aluminium). Crumb texture was described in terms of Hardness (maximum height of the first compression peak), Cohesiveness (ratio of the areas of the second to the first compression peak) and Springiness (ratio of the length of the second to the first compression peak).

2.3.3. Thermal analysis

A Q100 Differential Scanning Calorimeter (TA Instruments, New Castle, DE, USA) was used to measure thermal properties of breads. The instrument was calibrated with indium (melting point: 156.6 °C, melting enthalpy: 28.71 J/g) and mercury (melting point: −38.83 °C, melting enthalpy: 11.44 J/g). About 4 g of crumb from loaf centre were compressed with a 2.5 kg weight to obtain a flat and compact specimen to maximize heat transfer during the experiment. Compressed bread crumb samples (5–10 mg) were taken and placed in hermetic stainless steel pans (Perkin Elmer, Waltham, MA, USA) and heated from −80 °C to 130 °C at 5 °C/min. DSC data were analyzed with a Universal Analysis Software (v. 3.9A, TA Instruments, New Castle, DE, USA) to measure the melting enthalpy of ice (from −20 to +15 °C) and retrograded amylopectin (50–80 °C). Frozen water content (FW, at the given experimental conditions) was calculated, as previously described (Curti et al., 2014).

2.3.4. Statistical analysis

A one-way-analysis of variance (ANOVA) (followed by least significant difference test, LSD) at $p \leq 0.05$ was carried out to verify significant differences in the evaluated parameters during storage (IBM SPSS v.20, New York, USA).

2.3.5. Nuclear Magnetic Resonance (^1H NMR)

^1H mobility was investigated with a low resolution NMR spectrometer (20 MHz, the miniSpec, Bruker Biospin, Milano, Italy) operating at 25.0 ± 0.1 °C. About 1.5 g of bread crumb (taken from the bread loaf centre) were placed into a 10 mm NMR tube, compressed, sealed with Parafilm® (to prevent moisture loss during the NMR experiment) and analyzed. For all experiments the recycle delay was 3 s ($\geq 5\ T_1$), and 90° and 180° pulse lengths were adjusted for each sample.

2.3.5.1. 1D relaxometry

^1H longitudinal relaxation time ($^1H\ T_1$) experiment was carried out with an Inversion Recovery sequence, with a log-spaced interpulse ranging from 0.1 ms to 4000 ms depending on the sample and 20 data points.

^1H transverse relaxation time ($^1H\ T_2$) experiment was carried out with a CPMG pulse sequence, with an interpulse spacing of 0.04 ms, 32 scans and 4000 data points.

Curves were analyzed as quasi-continuous distributions of relaxation times using the UPENWin software (Alma Mater Studiorum, Bologna, Italy). Default values for all UPEN parameters were used with the exception of one parameter (LoXtrap), that was set to 1 to avoid extrapolation of times shorter than the first experimental point.

$^1H\ T_1$ and T_2 CPMG relaxation decays were also fit with a discrete exponential model (Sigmaplot, v.6, Systat Software Inc. USA).

2.3.5.2. 2D relaxometry

T_1–T_2 data were acquired with a ^1H bi-dimensional T_1–T_2 experiment (Bruker BioSpin GmbH, Rheinstetten; Germany) kindly provided by Bruker for tests purposes. The pulse sequence consists of an Inversion – Recovery (180 degrees pulse – delay t_i – 90 degrees pulse) followed by a CPMG train (tau – 180 degrees pulse – tau), whose echoes intensities are recorded. The pulse sequence is reported in Fig. 1, where the 90 degree pulse is represented as a white rectangle, the 180 degree pulses are represented as grey rectangles; T_E is the echo time and the signal acquired for every CPMG echo is illustrated as open circles. The CPMG echo train typically has from few to tens of thousands of points.

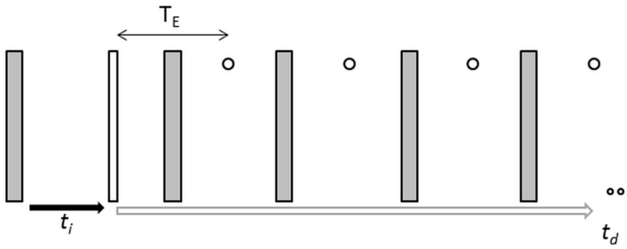

Fig. 1. ^1H bi-dimensional T_1–T_2 experiment sequence: 90° pulse (white rectangle); 180° pulse (grey rectangle); echo time (T_E); delay time between 90° and 180 pulses (t_i); signal acquired for each CPMG echo (open circles).