Influence of the incorporation of fibers in biscuit dough on proton mobility characterized by time domain NMR.

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ABSTRACT

The effect of fiber addition on the distribution and mobility of protons in biscuits is studied by using low resolution time domain Nuclear Magnetic Resonance (TD-NMR). The proportion of flour is reduced in order to incorporate inulin and oat fiber. NMR temperature dependent experiments are carried out in order to gain insight on the processes occurring in biscuit baking. Proton populations were identified measuring spin-spin relaxation times (T_2). The major change in the relaxation profiles upon incorporation of fibers corresponds to mobile water molecules, which appear to be related to dough spreading behavior and biscuit quality. Biscuit samples baked in a commercial oven were studied by two dimensional spin-lattice/spin-spin (T_1-T_2) relaxation maps. The T_1/T_2 ratio is used as an indicator of the population mobility, where changes in the mobility of water in contact with flour components as starch, proteins and pentosans are observed.

Keywords: Low resolution proton nuclear magnetic resonance; T1-T2 correlation maps, biscuit dough, dietary fibers

1. INTRODUCTION

Dietary fiber (DF) has become a very important ingredient as many beneficial effects are related to its ingestion. DF resists digestion and absorption in the human small intestine and undergoes total or partial fermentation in the large intestine (Lunn & Buttriss, 2007), mainly because of the physical properties of enhancing volume, viscosity and water-holding capacity of foods (Jenkins, Wolever, Leeds, Gassull, Haisman, Dilawari, et al., 1978). Health effects are dictated by molecular physicochemical characteristics that determine their solubility in water. Soluble fibers (e.g. oligosaccharides, pectins and β-glucans) increase the gut viscosity and, thus, control the glucose and lipids metabolism (Guillon & Champ, 2000), while insoluble fibers (e.g. cellulose, hemicellulose and lignin) act through their ability to ferment. Fibers health benefits include risk reduction of heart disease, certain types of cancer, obesity, diabetes, lowering blood lipid levels, prevention of constipation, enhanced gastrointestinal immunity and stimulation of beneficial colon micro flora growth (Charalampopoulos, Wang, Pandiella, & Webb, 2002; Topping, 2007).

Despite the well-known health benefits of DF consumption, average intake levels still fall far below recommended ones. Among the factors limiting the addition of fibers to traditional, popular and highly consumed products are the technological troubles in the manufacturing process that may generate upon incorporation of new ingredients to food formulation, inducing loss of consumer acceptance. Modifications made in the formulation impact not only on texture, flavor and quality parameters but also on the vioscoelastic properties of dough, generating technological troubles in the processing.

Sugar-snap biscuits are characterized by a formula with high amount of sugar and fat and very low amount of water, to prevent gluten network development. Water plays a complex role by affecting the nature of interaction between ingredients, determines the conformational state of biopolymers and contributes to dough structuring by modifying its rheological behavior (Eliasson, 1993). Therefore, viscoelastic properties depend strongly on water distribution in the dough (Sai Manohar & Haridas Rao, 2002). Additionally, when the dough is heated during baking, components undergo changes of states. As drying takes place interactions that are governed by the affinity of each one for water change throughout the dough (Chevallier, Colonna, Della Valle, & Lourdin, 2000). The presence of numerous components at diverse physical states makes the study of water distribution very difficult.

Time domain Nuclear Magnetic Resonance (TD-NMR) has been successfully applied to food science (van Duynhoven, Voda, Witek, & Van As, 2010), as the mobility of water in food systems can be determined by studying the protons spin-spin relaxation time (T_2) (Raun, Wang, Chen, Fulcher, Pesheck, & Chakrabarti, 1999). Recently, a study of the the dependence of proton dynamics with temperature of wheat flour dough was presented (Rondeau-Mouro, Cambert, Kovrlija, Musse, Lucas, & Mariette, 2014). Different baked products are already studied with NMR, such as bread (Bosmans, Lagrain, Deleu, Fierens, Hills, & Delcour, 2012; Chen, Long, Ruan, & Labuza, 1997; Wagner, Lucas, Le Ray, & Trystram, 2007; Wang, Choi, & Kerr, 2004) or cakes (Luyts, Wilderjans, Waterschoot, Van

Haesendonck, Brijs, Courtin, et al., 2013). Assifaoui et al. (A. Assifaoui, D. Champion, E. Chiotelli, & A. Verel, 2006; Ali Assifaoui, Dominique Champion, Eleni Chiotelli, & Aliette Verel, 2006) studied water mobility in a biscuit dough system and its relation to rheological properties as a function of the water content and temperature. They found four proton populations with different mobility in a biscuit dough with 19.4 % (w.b.) of water at 25 ºC. The first one obtained with a Free Induction Decay (FID) sequence at T_2 ~ 11 μs, was attributed to non-flexible protons associated with the oil crystalline phase, gluten and starch. The rest of the populations were measured with a Carr-Purcell-Meiboom-Gill (CPMG) sequence at $T_2(1)$ ~ 2 ms, $T_2(2)$ ~ 12 ms and $T_2(3)$ ~ 105 ms and correspond intra-starch granule water, water in interaction with starch and sucrose and apolar phase (fat phase), respectively. However, no studies have involved the influence of temperature on the proton mobility in biscuit dough for low water content (< 10 %).

In one dimensional (1D) NMR the signal is recorded as a function of one time variable; most ^{1}H NMR studies of food products only measure T_2. NMR relaxometry has made an important advance with the advent of a fast algorithm for two dimensional inverse Laplace inversion, being the signal recorded as a function of two time variables, T_1 and T_2, where T_1 is the spin-lattice relaxation time (Y.-Q. Song, 2009; Y. Q. Song, Venkataramanan, Hurlimann, Flaum, Frulla, & Straley, 2002). The advantage of this procedure is that T_1 and T_2 are measured simultaneously and the two dimensional (2D) spectra, showing peaks characterized by their intrinsic T_1 and T_2 values contain more information than conventional one dimensional relaxation time spectra. However, assignment of the proton populations can be a difficult task. Peak assignment is far from straightforward, but in spite of this, T_1-T_2 spectra are an important tool to evaluate the evolution of proton populations as the system undergoes physico-chemical changes. ^{1}H NMR has been applied to study the mobility of water and biopolymers in alimentary products and by the study of the T_1-T_2 spectra the multi-component proton populations were identified. Luyts et al. (Luyts, et al., 2013) studied egg-water and flour-water model systems and cake crumb samples to examine proton populations in pound cake, being able to differentiate the two most mobile populations from the 1D profile into three and two different proton populations in the 2D profile. Hills et al. (B. Hills, Benamira, Marigheto, & Wright, 2004) applied the technique to three classes of food: egg, cellular tissue and food hydrocolloids presenting experimental tools that might be useful in peak assignment and show the potential of this technique for quality control in food area.

The aim of this work is to relate the dough behavior during baking and biscuit quality to proton mobility upon addition of inulin and oat fiber. An open system is used in which water evaporation from the dough takes place during temperature dependent NMR experiments. Changes in relaxation times of different proton populations were related to the expansion observed in real biscuit samples during the baking process in an industrial oven. Additionally, two dimensional experiments correlating T_1 and T_2 are used to characterize the changes in the mobility of the different components of the system for dough and biscuits upon addition of inulin and oat fiber.

2. EXPERIMENTAL SECTION

2.1 Doug preparation.

Standard biscuit dough (SBD) was elaborated with regular ingredients, given by hard wheat flour (45 g), sugar (27 g), margarine (20.2 g), water (8.5 g or 8.2 % of the total dough), milk powder (2.25 g), salt (0.42 g) and $NaHCO_3$ (0.50 g). The procedure named Micro method III, described by Finney et al. (Finney, Morris, & Yamazaki, 1950) with modifications incorporated by the CYMMYT (International Center for corn and wheat improvement) was used (León, 1996). The procedure seeks to avoid formation of gluten in the dough. Flour is incorporated at the end of the preparation to prevent gluten formation during whipping of the dough (Kim & Cornillon). During mixing lipids act as a lubricant, competing with water to cover the surface of the flour, preventing the formation of excessive gluten in the sample (Goldstein & Seetharaman, 2011). The preparation protocol used in making the biscuit dough was: sugar, milk, salt and $NaHCO_3$ were mixed in a first step. Then butter was added and beaten for two minutes with an electric mixer. Afterwards water was added to the dough and the mixture was beaten for one more minute. Finally flour was added and two minutes of beating with the electric mixer were carried out. In this study and for analyzing a system closer to the standards used in industry fat was not previously melted and sugar was not dissolved before being added to the dough.

2.2 Ingredient variation.

In order to study the influence of dietary fibers on water mobility, two different fiber types were used, Inulin (IN) and Oat fiber (OF). The sample preparation and all ingredients correspond to the SBD formula, except for hard wheat flour where 33 g instead of 45 g were used. Inulin replaced dough (IND) had 12 additional grams of IN of polymerization level greater than 23 (Orafti HP), whereas in Oat fiber replaced biscuit dough (OFD) 12 g of OF (Canadian Harvest 200 β-glucan) were incorporated. In both cases this represented 11.60 % of fiber in the total mass. All samples were heated up to 80 ºC monitoring the process by NMR relaxation measurements for several temperatures.

2.3 NMR Measurements.

Proton relaxation measurements were performed on a Bruker minispec mq20 spectrometer operating at a frequency of 20 MHz for ^{1}H, with a dead time of 18 µs. A BVT3000 temperature control unit (Bruker Corporation) capable of stabilizing the sample temperature with a precision of 0.1 °C was used for all the experiments. The analyses were done by inspection of the signals obtained with two typical NMR sequences, Free Induction Decay (FID) and Carr-Purcell-Meiboom-Gill (CPMG) (Carr & Purcell, 1954; Meiboom & Gill, 1958). Spin-spin relaxation times T_2^* were measured with a FID experiment and one point every 2 µs was acquired for a time span ranging from 18 µs to 1 ms. For the CPMG sequence, used to determinate T_2 times greater than 100 µs, 15000 echoes were acquired with an echo time of 100 µs. For both sequences, the pulse widths of the 90º and the 180º pulse were 2.62 µs and 5.18 µs respectively. 128 scans were collected and averaged with a recycle delay of 3 s. All analysis were done in triplicate and coefficients of variation were less than 10%.

A Teflon homemade sample holder that generates a convective flow of N_2 gas in the sample was built and placed inside a 10 mm NMR tube in which a hole was punched in the bottom in order to allow temperature regulation by nitrogen flow. The purpose of the sample holder design is to mimic the usual baking process in a convection oven and allow the exit of moisture from the sample. Measurements were carried out in a temperature range of 30 ºC to 80 ºC and changes were carried out at a rate of 3 °C/min. A waiting period of 10 minutes was introduced to render the stabilization of the system before each measurement. Both, FID and CPMG signal amplitudes were corrected for temperature effects using the signal obtained from a sample of extra virgin olive oil whose FID signal was recorded as a function of temperature in the range of 30-110ºC. The use of this type of oil is standard in NMR industry because it presents a single liquid phase for the whole temperature range. In this way, changes in the signal amplitude due to temperature variation of the Boltzmann distribution of the population levels and different performance of the receiver coil are accounted for.

2D-NMR proton relaxation measurements, T_1-T_2, were performed at room temperature for the three dough formulas (SBD, OFD and IND) each of which was analyzed in raw and cooked state. Each sample was analyzed three times to confirm the reproducibility of the measurements. Samples of dough were measured immediately before baking; while the biscuits were left outside the oven to cool to room temperature and then were kept in airtight containers to avoid further changes in moisture. All samples were sealed during the 2D NMR measurements, biscuit samples were extracted from the center of each biscuit. The T_1 dimension was acquired with 32 inversion recovery steps, with logarithmically spaced time intervals sufficient to reach equilibrium. Samples were placed inside the 10 mm NMR tube, covered with a Teflon cap and sealed with a paraffin film in order to prevent moisture changes in the system, for the cooked sample biscuit crumbs were placed in the NMR tube. Results were compared to those acquired at high magnetic fields, which were obtained with a Bruker Avance II spectrometer operating at 300 MHz. A proton dedicated Doty DSI-703 probe with 5 µs dead time was used. Samples were placed in a 5 mm outer diameter sample holder.

For both FID and CPMG measurements, relaxation curves were fitted to a continuous distribution of exponential decay functions, which are determined with an inverse Laplace transform algorithm (Provencher, 1982; Tikhonov & Arsenin, 1977) (a Matlab software provided by the Victoria University of Wellington, New Zealand was used). The result is a relaxation time distribution which can be characterized by different Gaussian functions, where the areas represent the population of each component and mean T_2 value represents the component mobility.

2.4 Dough and biscuit quality parameters.

Changes in the diameter of biscuit dough pieces prepared for the different formulations was monitored in situ in a forced convection oven (Pauna, Argentina) equipped with a temperature controller. Circular samples of 0.7 cm thickness and 4.5 cm diameter were placed on an aluminum foil. K-type thermocouples were placed on the biscuit sheet (oven temperature) and inside the dough. Baking was performed for 11 min at 180 ºC and temperatures were recorded every 10 s with a TES-1307 data logger (Electrical Electronic Corp., Taiwan). From pictures taken in the oven two parameters were informed: maximum diameter during heating (MD) and set temperature (ST) define as the temperature at which stops spreading. Width/thickness factor (W/TF). Six biscuits were obtained by batch and 4 samples (the most homogeneous ones) were selected to determine the ratio between the width and thickness (León, 1996). Biscuits were elaborated by triplicate to ensure reliable results.

Thermal dough behavior was measured by TGA (Thermo-gravimetric Analysis). Samples (~10 mg) were heated in aluminum pans from 25 ºC to 120 ºC, using a heating profile of 4 ºC/min. and run in triplicate. Mass loss was determined as the difference between initial and final weight and expressed as a percentage.

To quantify biscuit quality, the width/thickness of four samples was measured, according to (León, 1996). The biscuit texture was measured with the triple beam snap technique using ‘Instron’ Universal Testing machine (INSTRON 3342, USA) with a load cell of 500 N. The base gap of the two support beams was adjusted to 36 mm. Each cookie was centered on the base and the travel distance of the blade was 35 mm, pre-test and speeds were 0.5

mm/s. The parameter informed is the hardness (N) required to produce the total break of the biscuit structure.

2.5 Statistical analysis.

The data obtained were statistically treated by variance analysis, while means were compared by Fisher’s LSD test at a significance level of 0.05. These tests were carried out using INFOSTAT statistical software (Universidad Nacional de Córdoba, Argentina).

3. RESULTS AND DISCUSSION

3.1. Mobility determined by FID experiments

The relaxation time distribution of the FIDs showed two proton populations in the temperature dependent experiments. A population (A) with short relaxation times (TA = 59 µs) which has previously been associated to rigid CH protons groups of starch and gluten, that are in little contact with water, while the second population (B with TB = 1.65 ms) was assigned to protons of water and those components in high interaction with water which represent the mobile part of the signal, as shown in Fig. 1 (A. Assifaoui, D. Champion, E. Chiotelli, & A. Verel, 2006; Bosmans, Lagrain, Deleu, Fierens, Hills, & Delcour, 2012; Luyts, et al., 2013).

As the dough temperature increases, many simultaneous physico-chemical events take place, where the most notorious are the melting of solid fat crystals and the evaporation of water. The decrease of water content can be directly assigned to a decrease in the FID intensity, which has a variation of 13% over the measured temperature span. By fitting the

FID relaxation time distributions it can be observed (see Fig. 1a) that the area corresponding to population A gradually increased, while the mobile component (B) decreases with temperature, as expected. This behavior could be accounted for by considering that as the system becomes dryer there is less contact of starch and gluten molecules with water, therefore part of the protons in population B became less mobile and increase the amount of protons in population A. In the next section it can be observed that 50 ºC is the temperature where the most noticeable effects in the system mobility take place. The associated relaxation times (see Fig. 1b) remain practically constant for all measured temperatures, indicating that the FID analysis is not a suitable for monitoring changes in the water dynamics of the different components of the dough. The loss of mass was corroborated with TGA measurements of biscuit dough as a function of temperature, where a total weight loss of approximately 12% was also observed (data not shown).

3.2. Mobility determined by CPMG experiments

Information of water dynamics can be obtained by the application of a CPMG sequence. Assifaoui et al. (A. Assifaoui, D. Champion, E. Chiotelli, & A. Verel, 2006) showed that for biscuit dough three main populations are obtained corresponding to water that interacts weakly with CH protons from starch and gluten, intergranular water which interacts with sucrose and starch and gluten protons, and a population with a long relaxation time corresponding to palm oil. In the present work we use a similar formulation for the dough preparation with the use of margarine instead of oil. Margarine at ambient temperature presents two distinct relaxation times due to the presence of fat crystals in the sample, as temperature increases and melting occurs a single population is expected (Luyts, et al., 2013) (see Fig. S1 of the Supplementary Material).

The change of the T_2 distributions of standard biscuit dough as a function of the temperature is shown in Fig. 2a, where populations with low mobility are denoted as C, mobile water as D and margarine as E and E'. Luyts et al.(Luyts, et al., 2013) have also associated signal from CH groups of dissolved sucrose to population E. For the lowest temperature measured (T = 30 ºC) four populations are distinguished, where the two with longer T_2 correspond to margarine as mentioned before. As temperature increases (T = 50 ºC shown in Fig. 2b) the first notorious process observed is the melting of the fat. At this temperature the relaxation time associated to D remains constant while opposite behaviors are observed for C and E. Fat increases (E population) its mobility upon melting of the crystal phase, with a corresponding increase in the relaxation time. On the other hand, C population slightly decreases its mobility upon increasing temperature. This population corresponds to water associated to flour components having strong affinity for water, as starch, proteins and pentosans.

At high temperatures the relaxation profiles undergo a remarkable change. Figure 2c corresponds to a temperature of 65 °C, where a portion of the free water has evaporated. Population C decreases in intensity and relaxation times. The most probable scenario is that a fraction of CH groups corresponding to starch, proteins, pentosans are no longer in contact with water and thus contribute to the intensity of population A, which increases with increasing temperature as shown in Fig. 1a. Additionally, sugar concentration may progressively increase due to increased sugar solubility and reduced amount of water with increasing temperature. The most remarkable observation is the broad distribution of D at high temperatures, which can be described by the presence of two distributions for all samples investigated in this work. A population with increasing relaxation time (D’) is present and the temperature at which this effect takes place changes for different dough formulations as shown in next section.

Figure 3a shows the mean value of the relaxation times obtained for SBD as a function of temperature. Longer relaxation times, TE, corresponding to fat, are observed to have the same behavior as of pure margarine. Bound water and CH groups from flour components, TC, present a decrease in mobility upon a temperature raise. The most mobile water content, TD, remains mainly constant until T_S = 50 ºC is reached, afterwards two components are observed, one that does not present a considerable change in relaxation time and the new population, named TD', that has an increasing mobility with temperature. The intensity of this last population is low, as shown in Fig. 3b. The population assigned to D decays at approximately a constant rate upon water evaporation, whereas the intensity of the fat population (E) can be seen to increase for temperatures above 40 ºC. This can be related to the melting of fat crystals, proton signals that contributed to population A for temperatures below ~ 35 ºC are present in population E when temperature increases (See Fig. S1). For temperatures above 50 ºC the intensity of the distribution remains mainly constant. As the system becomes more fluid, hydrophilic components of the flour are able to incorporate more water giving rise to an increase in the area of C until 50 ºC is reached, afterwards this area decreases with temperature as described previously. This is in agreement with the behavior observed for the population of the FID (Fig 1b) where the solid components (A) increase more noticeably after 50 ºC.

Figure 2d shows the data for samples extracted from the center of biscuits baked in an industrial oven, and measured at 30 ºC. The main component corresponds to E, where margarine presents a single population with the same relaxation time (TE = 80 ms) as population E in the dough sample at the corresponding temperature (Fig. 2a). Population D presents a very low intensity with a single population that slightly shifts towards lower relaxation values (from TD = 6.6 ms to TD = 5.6 ms), indicating a reduction in mobility of the components with mobile water. On the other hand, the less mobile components undergo a reduction in relaxation rates of approximately 70 % upon baking (from TC = 0.8 ms to TC = 0.23 ms).

3.3. Incorporation of fibers.

The effect of incorporation of fibers in the different dough formulations during the baking process was studied. No noticeable effect was observed in the FID measurements (results not shown), indicating that for the used amount of fibers the most rigid parts of the different systems do not show an appreciable change as determined by NMR. Additionally, the same decrease in the overall signal intensity was recorded for all samples as a function of temperature, indicating that fibers did not retain a substantial excess of water during the baking process.

The relaxation times measured with the CPMG sequence proved to be more sensible to the incorporation of fibers. It must be noted that for the low percentage of added fibers no substantial difference was observed in the 1D relaxation profiles at ambient temperature for any of the studied formulas. Figure 4 shows the evolution of the relaxation times with temperature for IND and OFD, which appear over imposed on the data for the SBD. The incorporation of inulin does not show an appreciable change within the experimental error; with perhaps a slight decrease in T_s of around 3 °C. Surprisingly, the incorporation of oat fibers renders the same T_2 distribution as SBD at ambient temperature. Nevertheless, T_s clearly rises in nearly 10 °C with respect to the other formulations. The true nature of the origin of population TD’ has not yet been established and is subject of on-going studies. The OF incorporation increases the split temperature of the most mobile water content (TD) to TD’ so retarding the increasing mobility of this water fraction. This effect can be related to the measurements of the spread rate for the different formulations determined by optical methods inside the oven.

During the baking process biscuit diameter increases linearly during the first minutes until reaching a maximum value and then remains fixed or slightly shrinks (Abboud, 1985). From pictures taken in the oven two parameters were informed: maximum diameter during heating (MD) and set temperature (ST) define as the temperature at which spreading stops (Table 1).

Sample profiles followed the same trend although different parameter values were found. OF significantly decreased MD and ST during baking. It is known that dietary fiber retains more water than wheat flour, which in turn may affect the dough viscosity. If dough viscosity increases, a decrease in the maximum diameter is expected. ST is related to set time (when biscuit stops spreading) and this parameter determines biscuit quality, therefore higher ST result in wider biscuits pieces (Miller & Hoseney, 1997; Miller, Hoseney, & Morris, 1997).

On the other hand, inulin increases MD and ST despite enhancing the system viscosity. Inulin MD showed the highest value although no statistical differences with SBD were found. ST was significantly higher than control dough. These changes during baking produce significant differences on biscuit quality. It is generally accepted that a good biscuit formulation produces tender final products with large diameter and with uniform surface-cracking pattern. The results show that IN increases 36 % the biscuit W/TF (width/thickness factor), and OF decreases 12 % this factor compared to SBD biscuit. Inulin biscuits also presented the more tender texture (54.62±4.21 N, 52% of control sample), while oat fiber biscuits were significantly harder (123.05±9.27 N, 23% over control). These results indicate a difference on biscuit quality with IN and with OF incorporation.

As previously described, the incorporation of OF increases the split temperature of the most mobile water content (TD to TD’). It is suggested that the delay in the increasing mobility of this water fraction is responsible for a decrease in the lubrication capacity, needed for the effective expansion of the dough during the baking period. Therefore the extra lubrication factor is available when the baking process is nearly finished and the dough is no longer able to expand.

The improving effect of inulin may be related to the extra lubrication factor as described above and the particular conformation of inulin molecules that enclose great amount of water by developing bond area may reduce starch swelling and decrease dough consistency (Peressini & Sensidoni, 2009).

3.4. 2D experiments.

In this section we use 2D relaxation maps to evaluate water mobility of dough and in biscuits, which are baked in a commercial oven as described in Section 2.4. Figure 5a shows the T_1-T_2 spectra belonging to the SBD sample, where the $T_1 = T_2$ diagonal, which corresponds to isotropic liquid dynamics, is shown as a guideline. The fat populations (E) with the highest mobility, are located in the $T_1 = 1.6T_2$ diagonal and are resolved as three different contributions. Population D, corresponding to mobile water molecules, can also be resolved as three different populations that present a common value of T_1 = 60 ms, with the following relaxation ratios: $T_1 = 4T_2$, $T_1 = 6.7T_2$ and $T_1 = 24T_2$. Finally, water molecules related to C fall next to the diagonal $T_1 = 70T_2$, even when the spin-lattice relaxation time is higher: T_1 = 70 ms. A minor change is observed for OFD in population C (Fig. 5c), where the T_1 /T_2 ratio decreases with respect to SBD and IND. These particular values are dependent on the magnetic field on which the experiment is carried out. For instance, at

300 MHz it was observed (see Fig. S2) that T_1 shifts to higher values for all the populations. On the other hand, T_2 of population E increases with the magnetic field while those for populations C and D decrease, giving rise to a completely different set of T_1/T_2 ratios. The decrease in T_2 is due to strong internal field gradients created by magnetic susceptibility differences that arise due to changes in the sample composition (B. P. Hills, 2006).

For the biscuit samples (Figs. 5d-f) populations D are not visualized in the 2D experiment, however, a projection in the T_2 axis renders two minor populations. A comparison of the 1D experiment with this projection is shown in Fig. S3. Populations C and E shift towards higher T_1 values. We center our attention on population C. A considerable shift in the T_1/T_2 ratio is present, in Figs. 5d-f the diagonal $T_1 = 600\ T_2$ line is drawn. For the SBD (Fig. 5d) population C shifts to $T_1 = 700\ T_2$, for the IND (Fig. 5e) to $T_1 = 630\ T_2$ whereas for the OFD (Fig. 5f) population C shifts to $T_1 = 520\ T_2$. The drastic reduction in the values of T_2 is most probably due to the high interaction of water with hydroxil groups of starch, proteins or penthosans. It has previously been determined that there is no exchange of water between different environments and this type of water (Luyts, et al., 2013). For this highly bonded water molecules, relaxation arises not only due to quenched molecular rotations and displacements, but also from chemical exchange between water and hydroxyl groups. Additionally, the area of population C of both IN and OF biscuits is two-fold higher than SB. This indicates that in the final state there is an increase in water with low mobility in contact with hydrophilic components due the presence of fibers.

4. CONCLUSIONS

Temperature dependent TD-NMR experiments carried out in an open system, where water evaporation takes place; allow obtaining relevant information on water mobility in biscuit dough at a microscopic level. 1D relaxation profiles of biscuit dough show that the water mobility is not significantly affected upon addition of inulin and oat fiber. As temperature increases, the most mobile water population splits into two populations with different T2 values, where the splitting temperature is slightly decreases for IND and significantly increased for OFD. It is suggested that the phase with high mobility, together with fat, provides the necessary lubrication for the dough effective expansion during baking. This information can be related with biscuit quality parameters such as dough and biscuit spreading capacity. Inulin dough expands to a similar maximum diameter during baking and produces a higher width/thickness ratio after baking than SBD. On the other hand, OFD ceases its expansion during baking at a lower temperature than SBD, rendering a biscuit with smaller width/thickness ratio, and consequently harder in texture. This is consistent with our hypothesis that OFD does not have enough lubrication during baking to be able to fully expand as indicated by the splitting of population D at a higher temperature. 2D measurements carried out in biscuits show that the relaxation times of water in contact with hydrophilic components are affected by the presence of dietary fibers. Further studies are necessary to understand the relation of changes in T1/T2 ratios and amplitude of population C with the biscuit quality. In summary, we believe that temperature dependent low field TD-NMR and 2D NMR may offer added value to the development of new biscuit formulations, providing significant information on the baking dynamics and final product in relation with the biscuit quality.

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Figure 1: Evolution of (a) FID signal intensity (circles) and (b) associated relaxation times as a function of the dough temperature. Population A (filled circles), with a relaxation time TA represents solid components in the dough. Population B (squares), with relaxation time TB represents mobile components. A variation of 13% of FID intensity is observed consistent with water evaporations. For temperature above 50 ºC population A grows in intensity at expense of population B, denoting the onset for the baking stage.

Figure 2: Relaxation time distributions obtained with an ILT at three different temperatures of standard biscuit dough: (a) 30 ºC, (b) 50 ºC and (c) 65 ºC. Each population is characterized by a Gaussian distribution. Population C corresponds to water in contact with solid structures, D to mobile water. E and E' mainly to margarine. d) Biscuit baked in a commercial oven.

Figure 3: Evolution of (a) relaxation times for water in contact with solid components (TC) mobile water (TD) and margarine (TE) and (b) corresponding population areas (C, D and E) as a function of the sample temperature for SBD. The temperature variation rate was 3 ºC /min. At 50 ºC population D splits in two distinct populations denoted by D, D' with relaxation times TD, TD'.

Figure 4: Evolution of (a) relaxation times (TC, TD, TD' and TE) as a function of temperature for (a) IND and (b) OFD formulations (•) over imposed to the SBD data (o).The temperature variation rate was 3 ºC /min. In OFD the temperatures at which population D splits in two distinct ones shifts to 60 ºC.

Figure 5. T_2 vs. T_1 spectra belonging to the SBD, IND, OFD samples (a-c) and their corresponding biscuit samples baked in a commercial oven (d-f). The full line corresponds to the T1 = T2 diagonal, while the dashed line (a-c) represents $T_1 = 70\ T_2$ and (d-f) $T_1 = 600\ T_2$. Population corresponding to margarine (E) splits into three discernible populations that appear mainly in the $T_1 = 1.6T_2$ diagonal. Mobile water (D) presents a single of $T_1 = 60$ ms, with the relaxation ratios: $T_1 = 4T_2$, $T_1 = 6.7T_2$ and $T_1 = 24T_2$. Water in contact with solid components has a short relaxation time ratio of $T_1/\ T_2 = 70$. For the biscuits, population D is not observed as most of the free water has evaporated and population's C shift to lower ratios as T_2 relaxation is enhanced by a greater relaxation with OH groups through hydrogen bonding.