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Impact of Water and Flour Components in Dough Investigated through Low-field Nuclear Magnetic Resonance

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Bound and free water within dough strongly affect its rheological behavior and processability, as well as its gluten network. Depending on its total content and on the characteristics of flour constituents, water can be both bound to components and free in the dough. The equilibrium between bound and free water directly impacts the elasticity and extensibility of dough and therefore controls the texture of final products. In this study, Nuclear Magnetic Resonance (NMR) was used to assess the relaxation behavior with the focus on water. The proton spin-spin relaxation measurements were carried out at 20 MHz with a Bruker Minispec mq20 NMR spectrometer (Bruker, Rheinstetten, Germany). The transverse relaxation time in a spin locking field ($T_{1\rho}$) was determined at 25°C following the Carr–Purcell–Meiboom–Gill (CPMG) sequence. The data were analyzed by the continuous distribution model. The dough was prepared with commercial semolina, which was mixed with water in a Brabender Mixer 350 (Brabender® GmbH, Duisburg, Germany) to reach three different levels of water content (30, 50, and 70%, based on semolina weight) and to study the water distribution as a function of the water total content. The dough characterization was carried out also comparing the $T_{1\rho}$ distribution of semolina dough with that of starch-water and gluten-water mixtures and also of pure starch and gluten to assess the role of each flour component. Additionally, the semolina dough sample was compared with a sample prepared with pastry flour, with a lower content of proteins, to investigate the different behavior as a function of the gluten amount. It was found that the dough presents three relaxation processes: one, very fast, is related to crystalline starch, while the other two are characteristic of two water populations, water in intragranular and in extragranular regions of starch, respectively. The comparison with pastry flour showed that the dough prepared with the latter one was less homogenous than the semolina one, with a clear distinction between free and bound water, while the semolina dough seemed to contain several water populations differently bounded, that were responsible for a broader peak at medium water content (around 50%).

1. Introduction

In order to optimise the production process and obtain high quality dough-based products, a deep understanding of water distribution is required. The dynamic properties of water and its distribution within dough are important because they can influence its machinability and rheological behaviour. These properties are fundamental in dough characterization since they influence the design of the production process and the quality of the final product. In recent years, consumers have been placing more emphasis on food wellness and safety, and they are interested in genuine foods with improved sensory characteristics, such as taste and aroma (Fanari et al., 2020a). For this reason, food scientists and nutritionists need robust and non-destructive analytical methods that can effectively give the composition and measure the physicochemical properties and functionality of food matrices. In this perspective, Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful and versatile analytical techniques that can be applied to liquid and solid materials, and it has become increasingly popular in the field of food science for the evaluation and analysis of several foods (Sobolev et al., 2019). Many authors have used NMR spectroscopy to study these parameters with the aim of

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investigating the evolution of starch structure under physical and chemical treatments, under the influence of various levels of ingredients content, and for various botanical origins of starch (Rondeau-Mouro et al., 2015). The non-destructive character, high accuracy, ease of use and reproducibility make NMR a very interesting technique in the food characterization field (He et al., 2020). In addition, NMR spectroscopy is a quantitative technique where, under certain conditions, the area of the NMR signal is directly proportional to the number of nuclei that produce the signal, and a selection of various nuclei can be used for analysis, depending on the nature of the food and on the information one wants to derive (Zhang et al., 2015).

Based on spin-spin T_2 relaxation time measurements, NMR spectroscopy has been shown to provide reliable information on the water and biopolymer mobility, especially in starch-based systems (Hatzakis, 2019). The transition from flour to flour-based products, like pasta or bread, is a complex process in which several transformations take place, including those associated with changes in water distribution. During the kneading stage, flour particles are hydrated, constituents gain mobility, and a continuous cohesive viscoelastic gluten protein network is formed (Fanari et al., 2020b). Starch can absorb up to 46% of the total water in bread dough but, in addition, it acts as an inert filler in the continuous dough protein matrix (Avramenko et al., 2018). During baking, dough undergoes irreversible changes, and water is redistributed.

In bread dough (with around 50% w.w. of water), two main proton populations at $T_2(1)$ = 2-5 ms and $T_2(2)$ = 10-40 ms were found. These populations have been attributed by Engelsen et al. (2001) to water in association with gluten ($T_2(1)$, corresponding to about 35% of the entire population), and starch ($T_2(2)$, corresponding to about 65% of the entire population). Serial et al. (2016), instead, assigned the first population to water in the intra-granular regions and the second population to water located in the extra-granular regions of starch. During the hydration process of dough, part of water diffuses into the starch granules, producing a reversible swelling, while the remaining water molecules interact by hydrogen bonding with the exposed hydroxyl groups of amylose and amylopectin (Rondeau-Mouro et al., 2015). The hydration process occurs, at first, in the amorphous regions, and later in the crystalline regions. Consequently, the hydrogen bonds in the crystalline regions are wicker compared to those in the amorphous regions, and water is not homogeneously distributed in starch. This could be the reason why two different mobilities of starch-associated water are possible. Moreover, Sun et al. (2020) demonstrated that a correlation exists between NMR water peak areas and rheological parameters η, G′, and G″. Assifaoui et al. (2006) found that an increase in the water content induces lower G' and this increase the population $T_2(2)$, without changing the $T_2(1)$ one, suggesting that the added water remained outside of starch granules, increasing the volume of the continuous phase and decreasing the dough viscosity. Considering this, NMR has the potential to be a very interesting method in dough characterization from the perspective of process optimization. Despite all these positive characteristics and the growing number of publications that involve NMR and foods, this technique is rarely used in this area (Spyros & Dais, 2012). The main reasons are probably its high cost, the relatively low sensitivity, and the lack of NMR expertise by many food scientists.

The aim of this article is to characterize the water populations in the dough by means of NMR measurements. With this purpose, gluten-water and starch-water mixtures, together with pure starch and gluten, were characterized to assess the role of each flour component. To study the water distribution in the dough, samples with different water content were also analyzed. Additionally, the semolina dough sample was compared with a sample prepared with pastry flour, with different properties, to investigate the different response as a function of the gluten amount. This characterization could rise the potentiality of NMR as a tool for the analysis of multicomponent systems, such as dough, and offers good opportunities to advance the field of food science.

2. Materials and Methods

To study the water effect, commercial semolina (CS) was mixed with distilled water to reach three different levels of water content (30, 50, and 70%, based on semolina weight). The pastry flour (PF) dough was prepared with a water content of 50%, based on semolina weight.

The dough samples were prepared by mixing 300 g of commercial semolina with distilled water in a Brabender Measuring Mixer 350 (Brabender® GmbH, Duisburg, Germany) for 5 min, using a rotational mixing speed of 20 rpm. The value of 20 rpm was chosen to prevent occurrence of structure breaks in the dough structure. In Table 1 the properties of the semolina and pastry flour used in the work are reported.

Starch-water and gluten-water mixtures were prepared by mixing wheat pure gluten/starch with an amount of 50 % of distilled water based on pure starch/gluten weight. Wheat pure gluten (GP) and wheat starch powder (SP) were obtained from Minerva (Minerva Handelsgesellschaft m.b.H., Wermsdorf, Germany). The same ingredients, as they are, were used for the characterization of pure starch and pure gluten. Table 2 reports the sample compositions and their identification acronym. For each sample, three replicate measurements were performed, and the mean value was taken into account for the study. Nuclear Magnetic Resonance (NMR)

was used to measure the proton spin-spin relaxation at 20 MHz with a Bruker Minispec mq20 NMR spectrometer (Bruker, Rheinstetten, Germany). Approximately 1 g of sample was placed in a disposable glass test tube (Ø = 10 mm). The tube was sealed with a stopper to prevent moisture loss during the measurement. The transverse relaxation time in a spin locking field ($T_{1\rho}$) was determined at 25°C following the Carr–Purcell–Meiboom–Gill (CPMG) sequence for each sample (Meiboom and Gill, 1958). The 90–180° pulse separation (τ) was 0.25 ms.The number of data points for fitting was 2,000, while the recycle delay was set to 5 s. The data were analyzed by the continuous distribution model, performing inverse Laplace transform of the data using a Matlab script (Iari-Gabriel, 2020). The dough characterization was carried out by comparing the $T_{1\rho}$ distribution of each sample to assess the impact of each flour component on it. In particular, intensity peaks in the continuous distribution were analyzed with regard to dimension and position, using ORIGIN (v.9.0 PRO, OriginLab Corporation, USA).

Table 1: Properties of flours used in this study, percentage are based on 100 g

	Proteins (%)	Carbohydrates (%)	Fats (%)
Commercial Semolina (CS)	12.0	69.0 (0.8 of sugars)	0.8
Pastry Flour (PF)	10.0	71.0 (0.4 of sugars)	1.0

Table 2: Samples composition and water percentage amount based on flour or starch/gluten powder weight

	Sample	Water (%)	Acronym
with water	Commercial semolina	30.0	CS30
		50.0	CS50
		70.0	CS70
	Pastry flour	50.0	PF50
	Pure Gluten	50.0	GP50
	Pure Starch	50.0	SP50
no water	Pure Gluten	0	GP
	Pure Starch	0	SP

3. Results

Table 3 reports the data related to the intensity peaks identified in the continuous distribution for each sample. The peak parameters here considered are the position (center, starting and ending relaxation time), the height, and the area. Additionally, the total area under the distribution curve is reported (column 7). It should be noticed that most of the samples showed two peaks (CS50, CS70, PF50, SP50), while other samples (CS30, GP, SP) only one peak, and only one sample (GP50) three peaks in the continuous distribution.

Table 3: Peak parameters for flours used in the study; percentages are based on flour or starch/gluten powder weight

Sample	T_2 peak time (ms)	T_2 peak beginning time (ms)	T_2 peak ending time (ms)	T_2 peak Height	T_2 peak area	Total curve area
CS30	2.984	0.521	17.073	1.610	8.100	8.434
CS50	1.067	0.231	1.177	0.444	0.296	106.800
	17.073	1.177	109.750	2.337	101.301	106.800
CS70	5.337	1.485	15.199	2.420	23.353	72.874
	27.186	15.199	77.426	1.465	49.396	72.874
PF50	0.368	0.115	1.322	0.366	0.194	64.857
	9.545	1.322	54.622	3.500	64.092	64.857
GP50	0.020	0.010	0.163	0.508	0.042	68.408
	0.521	0.163	2.105	0.324	0.291	68.408
	15.199	2.984	61.359	3.252	68.074	68.408
SP50	1.177	0.291	1.485	1.031	1.013	45.482
	7.564	1.485	27.186	3.454	44.264	45.482
GP	0.521	0.145	2.364	0.278	0.266	4.992
SP	0.933	0.259	3.351	0.611	0.854	0.940

To identify the different populations shown by the dough samples, the continuous distribution of pastry flour dough, semolina dough, starch-water, and gluten-water mixtures, all with a water content of 50%, were compared. Figure 1 shows this comparison. Starch-water mixture exhibited two populations. The first one, $T_{1\rho}$ (1) (peak at 1 ms), can be observed also in the semolina dough sample, where the peak is not easily visible due to the overlapping with the other more pronounced peak, relative to the second population. The second population, identifiable for SP50, can be observed also in the other samples, where the peak center is shifted to higher relaxation times. In particular, it seems that the position of this peak might be connected to the gluten content, and in turn, to the protein amount in the dough, since it shifts to higher relaxation times at higher protein contents. Pastry flour (around 10% of proteins) dough has a peak at 10 ms, while semolina one (around 12% of proteins) dough shows a high intensity relaxation at around 17 ms, similar to that one shown by gluten-water mixture (identified at 15 ms). This fact suggests that gluten-water interactions are weaker if compared to starch-water interactions. However, it seems that a high gluten content in the dough leads to stronger networks with a higher capacity to incorporate starch which, in turn, can absorb more water molecules. Additionally, GP50 and PF50 showed a low intensity peak at around 0.5 ms. Following the population attributions discussed in the introduction section (Serial et al. 2016), the relaxation range around 1 ms can be attributed to water in the intragranular regions of starch, inside the gluten network, where the starch granules are embedded. On the other hand, the population weakly bounded that was found with relaxation times around 10-20 ms, is attributable to bulk water in the extra granular regions of starch. After the previous statements, it is reasonable to think that semolina dough has a higher amount of less mobile water compared to pastry flour dough, since its peak is broader and with higher intensity in the regions with lower $T_{1\rho}$, shown in Figure 1.

Another interesting result, visible in Figure 1, is the very rapid relaxation (0.01 ms) that characterizes all the samples, with the exception of the GP50 one, for which the intensity of this relaxation is very weak. This fact may suggest that this relaxation is linked to crystalline starch (very rigid structure). Figure 2 shows the continuous distribution of relaxation times of pure gluten and pure starch (without water addition). As it can be seen, both samples showed the very intense and rapid relaxation at around 0.01 ms. These results confirm the attribution of this relaxation to a very rigid structure that, in this case, could be either gluten or starch crystalline phases. Moreover, the other relaxation times, 0.5 ms for GP and 1 ms for SP, can be related to the water content in the powders.

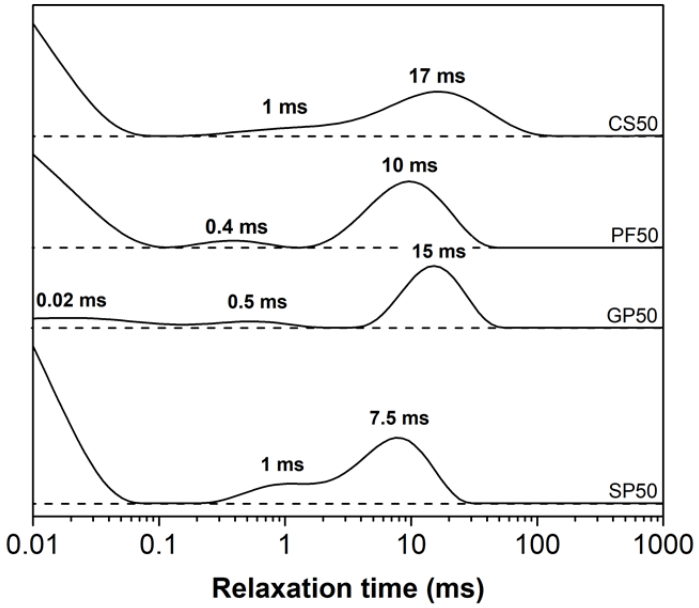

Figure 1: Continuous distribution of spin-spin relaxation times ($T_{1\rho}$, obtained from CPMG experiment) of gluten-water and starch-water mixtures, commercial semolina dough, and pastry flour dough, at a water content of 50%

The water content impact was evaluated by comparing the relaxation time distribution of CS30, CS50, and CS70, shown in Figure 3. At low water content, if the relaxation of about 0.01 ms is not considered, the dough shows just one population (1-8 ms) with a peak in intensity at around 3 ms. As the water content increases first to 50% and after to 70%, this peak becomes broader and gradually splits into two peaks. For CS50 a very

pronounced peak at around 17 ms can be found, and a small partial peak at around 1 ms which is, for the most part, overlapped with the other one. A further increase of the water content leads to the formation of two distinct peaks, as it is visible in Figure 3 for the CS70 sample. This sample showed a population with a relaxation time of 5 ms and another one with 27 ms. The results confirmed the assumptions previously made about the populations. An increase of the water content in the system leads to a reduction of less mobile water, since the gluten network allows the incorporation of more water. At the same time the bulk water content increases with the mobility of water molecules. This is likely due to the difficulties of the network to interact with such an amount of water.

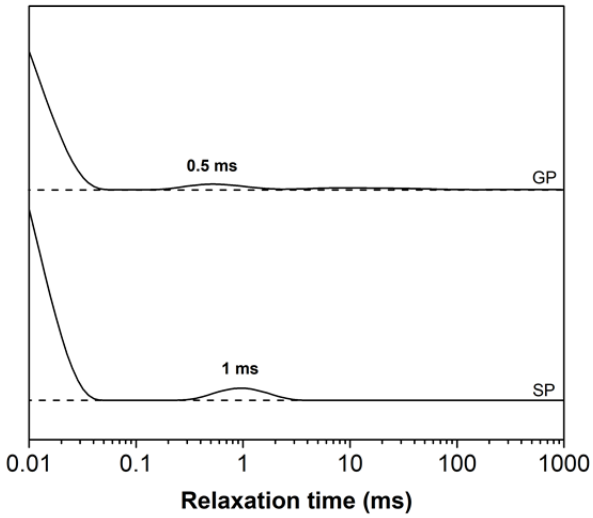

Figure 2: Continuous distribution of spin-spin relaxation times ($T_{1\rho}$, obtained from CPMG experiment) of pure starch and pure gluten samples without any water addition

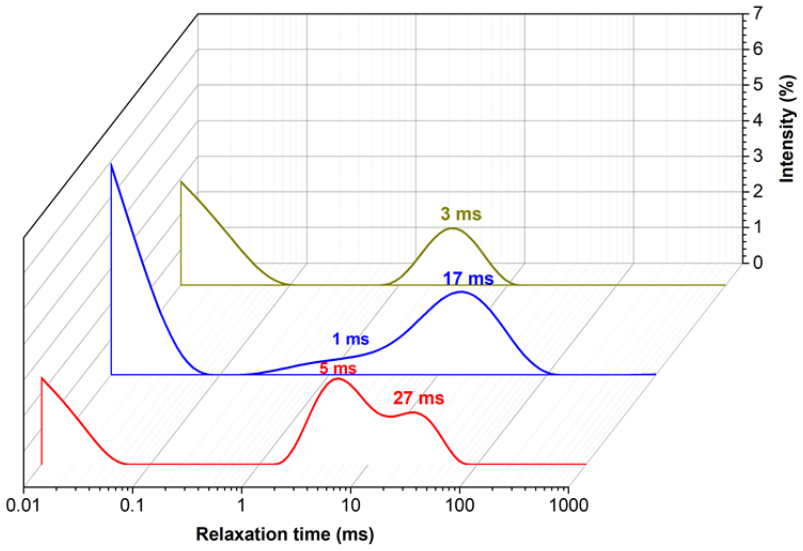

Figure 3: Continuous distribution of spin-spin relaxation times ($T_{1\rho}$, obtained from CPMG experiment) of CS30 (dark yellow line), CS50 (blue line), and CS70 (red line)

4. Conclusions

The aim of this article was to study the water populations in the dough with NMR in order to investigate the potentiality of this technique in food characterization. In particular, the role of each flour component and the impact of water content were investigated. The results show the presence of two main populations: population 1, with $T_{1\rho}$ (1)=1-3 ms, and population 2, with $T_{1\rho}$ (1)=10-30 ms. The first population consists of water bound in

the intra-granular regions of starch inside the gluten network, while the second one refers to water in the network but not absorbed by the starch granules, or to bulk water outside the network, depending on the mobility. As a general statement, the higher the water content, the more mobile the latter one is. Gluten amount in the dough positively influences the water binding capacity because the more is the gluten in the dough and more extended will be the network (Fanari et al., 2019). Talking about the technique evaluation, the proposed analysis is promising even in view of the current accessibility to instrumentation based on low-field NMR analysis, that could be even exploited for online monitoring and control processes. Anyway, the ability to discriminate between different populations has to be improved. A future work development could be the performance of detailed analysis on flour characteristics, like grain size distribution, or protein and starch characterization, to include in the study also the impact of these parameters.

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References

Assifaoui A., Champion D., Chiotelli E., Verel A., 2006, Rheological behaviour of biscuit dough in relation to water mobility, International Journal of Food Science and Technology, 41(2), 124-128.

Avramenko N. A., Tyler R. T., Scanlon M. G., Hucl P., Nickerson M. T., 2018, The chemistry of bread making: The role of salt to ensure optimal functionality of its constituents, Food Reviews International, 34(3), 204-225.

Engelsen S. B., Jensen M. K., Pedersen H. T., Nørgaard L., Munck L., 2001, NMR-baking and multivariate prediction of instrumental texture parameters in bread, Journal of Cereal Science, 33(1), 59-69.

Fanari F., Desogus F., Scano E. A., Carboni G., Grosso M., 2019, The rheological properties of semolina doughs: Influence of the relative amount of ingredients, Chemical Engineering Transactions, 76, 703-708.

Fanari F., Desogus F., Scano E. A., Carboni G., Grosso M., 2020a, The Effect of the Relative Amount of Ingredients on the Rheological Properties of Semolina Doughs, Sustainability, 12(7), 2705.

Fanari F., Carboni G., Grosso M., Desogus F, 2020b, Thermal Properties of Semolina Doughs with Different Relative Amount of Ingredients, Sustainability, 12(6), 2235.

Hatzakis E., 2019, Nuclear Magnetic Resonance (NMR) Spectroscopy in Food Science: A Comprehensive Review, Comprehensive Reviews in Food Science and Food Safety, 18(1), 189-220.

He Y., Bai X., Xiao Q., Liu F., Zhou L., Zhang C., 2020, Detection of adulteration in food based on nondestructive analysis techniques: a review, Critical Reviews in Food Science and Nutrition, 1-21.

Iari-Gabriel M, 2020, rilt (https://www.mathworks.com/matlabcentral/fileexchange/6523-rilt), MATLAB Central File Exchange, Retrieved December 29, 2020.

Meiboom S., Gill D., 1958, Modified spin-echo method for measuring nuclear relaxation times, Review of scientific instruments, 29(8), 688-691.

Rondeau-Mouro C., Cambert M., Kovrlija R., Musse M., Lucas T., Mariette, F., 2015, Temperature-Associated Proton Dynamics in Wheat Starch-Based Model Systems and Wheat Flour Dough Evaluated by NMR, Food and Bioprocess Technology, 8(4), 777-790.

Serial M. R., Blanco Canalis M. S., Carpinella M., Valentinuzzi M. C., León A. E., Ribotta P. D., Acosta R. H., 2016, Influence of the incorporation of fibers in biscuit dough on proton mobility characterized by time domain NMR, Food Chemistry, 192, 950-957.

Sobolev A. P., Thomas F., Donarski J., Ingallina C., Circi S., Cesare Marincola F., Capitani D., Mannina L., 2019, Use of NMR applications to tackle future food fraud issues, Trends in Food Science and Technology, 91, 347-353.

Spyros A., Dais P., 2012, Introduction, Chapter In: Belton P. S. and Wood R. (Ed.), NMR Spectroscopy in Food Analysis, The Royal Society of Chemistry, Cambridge, UK, 1-4.

Sun Y., Zhang M., Chen H., 2020, LF-NMR intelligent evaluation of rheology and printability for 3D printing of cookie dough pretreated by microwave, LWT - Food Science and Technology, 132, 109752, 1-8.

Zhang H., Zhang L., Sun X., Xie S., 2015, Applications of Low-Field Pulsed Nuclear Magnetic Resonance Technique in Lipid and Food, Chapter In: Rahman A. u. and Choudhary M. I. (Ed.), Applications of NMR Spectroscopy, Vol 1, Bentham Science Publishers, Sharjah, U.A.E, 3-56.