

Structural and Nutritional Properties of Pasta from *Triticum monococcum* and *Triticum durum* Species. A Combined ^1H NMR, MRI, and Digestibility Study

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ABSTRACT: The aim of the present study was to characterize the structure of two different types of pasta, namely *Triticum turgidum* ssp. *durum* (cv. Saragolla) and *Triticum monococcum* ssp. *monococcum* (cv. Monlis), under different processing conditions. MRI analysis and NMR spectroscopy (i.e., T_1 and T_2 NMR relaxation times and diffusion parameters) were conducted on pasta, and ^1H NMR spectroscopic analysis of the chemical compounds released by pasta samples during the cooking process was performed. In addition, starch digestibility (enzimatically determined) was also investigated. The NMR results indicated that Saragolla pasta has a more compact structure, ascribed to pasta network and in particular to different technological gluten properties, that mainly determine the lower ability of Monlis pasta in binding water. These results correlate well with the lower rate of starch hydrolysis measured for Monlis pasta compared to Saragolla when both are dried at high temperature.

KEYWORDS: pasta microstructure, MRI, ^1H NMR spectroscopy, T_1/T_2 relaxation times, diffusion, in vitro starch digestibility

INTRODUCTION

Wheat provides a significant amount of carbohydrates and proteins and constitutes a good source of lipids, antioxidants and minerals that, although present in a limited concentration, cover most of the human requirements.¹ Einkorn (*Triticum monococcum* L. ssp. *monococcum*), a diploid hulled wheat with high protein,² carotenoids, tocols,³ and microelements, is a promising candidate for the development of bakery products, baby food and enriched foods.^{5,3} In addition, the French-led Protected Geographical Indication⁶ in Italy led to the development of a new einkorn variety called Monlis. Monlis is characterized by excellent bread-making aptitude, high tocol and carotenoid content and good yielding ability.

The digestibility of starch products is widely studied due to its positive correlation with the glycemic response in humans. Recently, the European Food Safety Authority, via the Panel on Dietetic Products, Nutrition and Allergies, concluded that high postprandial glycemic responses may be detrimental to human physiology. The panel concluded that slowly digestible starch (SDS) in cereal products, compared to rapidly digestible starch (RDS), has a reduced post-prandial glycemic response.⁷ The digestibility of pasta, a very common starchy food, was reported to depend on both intrinsic properties of the wheat ingredients and the processing conditions during extrusion, drying and cooking.^{8,9} For example, extrusion leads to the formation of

starch–protein and starch–lipid complexes,¹⁰ which decreases digestibility. Similarly, the high-temperature drying process leads to highly aggregated proteins that are cross-linked via covalent bonds and disulfide bridges, thereby also decreasing starch digestibility.⁹ Starch gelatinization occurs during cooking, causing swelling of starch granules that in turn leads to an increased enzymatic activity during starch digestion. The efficiency of enzymes also depends on starch hydration^{11,12} and limited amylase affinity and catalytic efficiency have been observed in conditions with low water content.¹³ Finally, inherent characteristics of the wheat, linked to geographical and botanical origins, play important roles, as these characteristics have been shown to be correlated with starch architecture¹¹ and metabolic profile.¹⁴

All of these processes further influence the pasta structure. Nuclear magnetic resonance (NMR) is a widely recognized innovative methodology used in the molecular characterization of food. It is a noninvasive and nondestructive technique capable of probing molecular processes occurring over a wide range of time scales.

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High-resolution NMR spectroscopy can be combined with magnetic resonance imaging (MRI) approaches to characterize the molecular structure as well as the state of water in heterogeneous systems as a food matrix. Specific for the MRI technique, image contrast is governed by spatial variation of the NMR parameters. Depending on the imaging protocol, spin–lattice (T_1) and spin–spin (T_2) relaxation times or self-diffusion coefficient (D) maps can be obtained for a defined sample region. For example, the ^1H relaxation properties of starch samples exhibit a complex multiexponential behavior and show an increase in the more mobile fractions upon gelatinization.^{15,16} The application of NMR was extended to the study of starch in complex systems that contain fat and sucrose in large amount such as cakes,¹⁷ while water diffusion analysis has been applied to pasta,¹⁸ cooked rice,¹⁹ and spaghetti.²⁰ However, while some information is available on the behavior of durum wheat pasta during digestion,⁸ very few data are reported in the literature on einkorn pasta.

The present study aims at investigating the physical, microstructural, and digestibility properties of different types of pasta prepared from *Triticum turgidum* ssp. *durum* (cv. Saragolla) and *Triticum monococcum* ssp. *monococcum* (cv. Monlis) processed at two drying temperatures, 92 and 40 °C. Microstructural characterization was performed by MRI, via analysis of T_1 and T_2 relaxation and diffusion parameters, in combination with NMR T_2 relaxation and free induction decay (FID) analysis. In addition, ^1H NMR analysis of the chemical compounds released by cooking pasta was performed. NMR results correlated well with starch digestibility data, enzymatically determined. Finally, the MRI results were confirmed with Environmental Scanning Electron Microscopy (ESEM) images.

MATERIALS AND METHODS

Samples. The kernels of *T. turgidum* L. ssp. *durum* Desf (cv. Saragolla) and *T. monococcum* L. ssp. *monococcum* (cv. Monlis) were harvested from 100 m² plots that were cropped in 2008–2009 at Sant'Angelo Lodigiano (Po Plain, Italy) following standard agronomic practices. Before milling, the seeds of Monlis (M) were de-hulled with an Otake FC4S thresher (Satake, Japan); de-hulling was not necessary for the free-threshing durum wheat Saragolla (S). Overnight tempering was performed at 14% moisture for M and at 16% for S. The kernels were milled with a Bona 4RB (Bona, Italy) experimental mill, which separates the flour fraction from bran and germ.

Pasta Preparation. Approximately 4 kg samples of M and S flours were processed into short-cut pasta (macaroni) with a P630VR lab pasta maker (Parmasel, Parma, Italy). Dough mixing, performed under vacuum (−60 kPa), lasted 7 min. The pre-extrusion moisture of the dough was 30% for M and 32% for S; the extrusion was performed under 8000 kPa pressure. Pasta drying was carried out at two different temperatures, specifically low (peak temperature 40 °C, 24 h) and high (peak temperature 92 °C, 4 h), in a dark drying cabinet (Braibanti, Padova, Italy) at a constant 75% relative humidity. Samples of S and M pasta at both drying conditions of 92 and 40 °C were obtained. Hereafter, the different S and M pasta species, dried at 40 and 92 °C will be referred to as S40, S92, M40, and M92, respectively.

Cooking Parameters. Pasta samples were cooked in deionized boiling water at a ratio of 1:10. The “al dente” cooking time was determined by observing the disappearance of the white core of the pasta squeezed between two glass plates, according to the AACC 16–50 method (AOAC, 2000).

Chemical Composition of Cooked Pasta. Protein, starch and ash quantifications were performed in duplicate for all types of pasta cooked at 11 and 15 min. Protein quantification was carried out using the Kjeldahl method, as reported in the official method of analysis 976.05.²¹ The total starch content was measured according to method 996.11. Ash content was determined as described in method 923.03.²¹

ESEM Analysis. The microscopic structure of cooked pasta samples was assessed using a TM-1000 Tabletop environmental electronic scanning microscope (Hitachi High-Technology, Tokyo, Japan) at 15 kV accelerating voltage and 28.6 mA emission current. Samples were placed in a metallic support without coating under low vacuum. Images were captured at different magnifications and in triplicate.

^1H NMR Relaxation and MRI. Proton NMR T_1 and T_2 relaxation, free induction decay (FID), and MRI experiments were carried out on a Bruker AM 200 WB spectrometer (Bruker, Karlsruhe, Germany) operating at 4.7 T, equipped with a standard microimaging accessory consisting of a ^1H tunable probe-head and a gradient drive unit. Orthogonal field gradient coils were capable of achieving gradients up to 150 G·cm^{−1} with a trigger pulse of 5 μs . Each pasta sample (1 g) was boiled in 100 mL of distilled water for 11, 15, or 20 min. For each time point, the sample was drained in a steel colander and, after removing surface water, inserted into a 10 mm NMR tube. The same protocol was applied to pasta samples with an identical mass boiled in 100% D₂O. Three repetitions were performed for each boiling time. T_1 , T_2 and diffusion MRI experiments were performed in a single experimental session. T_1 and T_2 weighted images were recorded by employing the standard phase/frequency encoding 2D spin–echo sequence. A spectral width of 33 kHz was employed together with an 11 mm field of view (FOV) and a voxel resolution of 86 × 86 × 1000 μm^3 . Selective radiofrequency pulses (1000 Hz bandwidth, Gaussian shaped, truncated at 5% level) were used for slice selection. Other acquisition parameters are reported in the figure captions. T_1 -weighted images were recorded by adopting an echo time of 6.07 ms to minimize the T_2 effect. For each experiment, 15 images (number of averages (NE) = 4) were collected, by varying the repetition time (TR) between 0.05 and 60 s. The two equally spaced echo times were systematically varied in the range of 6–80 ms to collect T_2 -weighted images with a TR = 20 s to eliminate T_1 artifacts. A total of 15 images (NE = 4) were sequentially collected. Diffusion-weighted images were acquired by adopting a modified STEAM sequence specifically tailored for imaging of biological samples characterized by long T_1 and short T_2 relaxations. Seven images (NE = 4) were recorded: a reference image with Z gradient = 0 T·m^{−1} and six images with the Z-diffusion encoding gradients with little delta (δ) of 3 ms in the 0–340 mT·m^{−1} range separated by a big delta (Δ) of 50 ms. All the images were processed on the O2 Workstation (Silicon Graphics, California) with the Paravision software package (Version 2.6, Bruker).

Proton relaxation times and FID analyses were carried out on identical weighed S92 and M92 samples, boiled for 15 min in H₂O or 100% D₂O. The longitudinal magnetization decay (T_1) was measured by the inversion recovery sequence by adopting the following acquisition parameters: inversion recovery time (2τ) from 50 ms to 10 s, NE = 15, NS = 8, TR = 20s. The transversal magnetization decay (T_2) was measured by the common Hahn spin–echo sequence: echo times from 5 μs to 5 s, NE = 45, NS = 4. The CPMG sequence was also employed: 90° = 9 μs , 180° = 19 μs , 180° interpulse delay = 1.1 ms. The CPMG train was varied between 2 and 80 ms. FIDs were determined with one transient in single channel detection mode, 4096 data points, acquisition time = 0.4096 s, dwell time = 100 μs , TR = 30 s. All experiments were carried out in triplicate at 297 K.

Relaxometric and Diffusion Data Analysis. Two or more circular regions of interest (ROIs) of approximately 2 × 10⁶ μm^2 and a ROI on the entire surface were tracked over each T_1 , T_2 and diffusion weighed recorded image. The software returned the maps containing the signal intensity values to be used for the calculation of the T_1 , T_2 and diffusion parameters. Normalized peak intensities of the Fourier-transformed ^1H spectra recorded by using the spin–echo and/or the CPMG pulse sequences were used versus the corresponding time, 2τ , to calculate the T_1 and T_2 relaxation values. All relaxometric data were fitted to a multiexponential function, whose rate constant represented, respectively, the parameters T_1 , T_2 , and D . The experimental data were fitted using the Marquadt–Levenberg algorithm, implemented by the Sigma Plot (Version 1.02) software (Jandel).

^1H NMR Spectroscopy of Boiling Water. Each pasta sample (1 g) was boiled in 100 mL of distilled water for 11 and 15 min. The

boiling water was carefully withdrawn, centrifuged and lyophilized; 15 mg of each sample was then dissolved in phosphate-buffered (pH 7.0) D₂O and inserted into a 5 mm NMR tube (550 μ L) for ¹H NMR experiments. ¹H NMR spectra were recorded on a Bruker DMX 500 spectrometer (Bruker Biospin GmbH Rheinstetten, Karlsruhe, Germany) operating at 11.7 T and equipped with a 5 mm inverse probe and z-gradient. All spectra were acquired in triplicate at 300 K. The spectral width was 6500 Hz, and 32K data points were collected using the standard one-dimensional NOESY pulse sequence.²² We employed a water presaturation scheme with low radiofrequency power during the mixing time (100 ms) and the relaxation delay (1.2 s). An exponential function with a line broadening of 0.3 Hz was applied before Fourier transformation. Processing (phase and spectra baseline correction) was performed with TOPSPIN software (Bruker Biospin). Integrals were normalized to sodium 3-trimethylsilyl propionate (TSP) as a reference.

In Vitro Starch Digestion. Starch digestion of each pasta type was performed *in vitro* following 11 and 15 min of cooking time, modifying the method of Kim et al.²³ Briefly, cooked pasta was minced to mimic the oral food breakdown. Approximately 1 g of minced sample was put into glass vials, each representing a single point of starch digestion. The extent of digestion was assessed at five time points (0, 20, 60, 120, and 150 min). All samples were subjected to a 30 min incubation with pepsin (pepsin from hog stomach, 3348 U·mL⁻¹, Fluka; enzyme:protein ratio 1:30, pH 2.5) to simulate the gastric phase. Afterward, the pH was adjusted to 7.2 and 4 mL of (10 mg·mL⁻¹) pancreatic amylase (Megazyme, Ireland) was added to perform starch digestion. Samples were incubated at 37 °C while being shaken at 170 rpm for 20, 120, and 150 min. The digestion reaction was stopped by ethanol addition. The samples were then centrifuged at 5000 rpm for 10 min at 8 °C and supernatants diluted to 100 mL with 100 mM sodium acetate buffer (pH 4.5). A 100 μ L aliquot was treated with 10 μ L of amyloglucosidase (300 U·mL⁻¹) and incubated at 50 °C for 20 min. Subsequently, 3 mL of glucose oxidase/peroxidase (Megazyme, Ireland) was added, and the samples were incubated for an additional 20 min at 50 °C prior to absorbance detection. The glucose released during digestion was quantified as hydrolyzed starch during the amount of time elapsed, multiplying the absorbance by 0.9. From this value, the rapidly digestible starch (RDS) content was estimated.²⁴ The experiments were performed in triplicate.

Statistical Analysis. Statistical analysis for relaxation and diffusion data was performed by the Prism program Version 4 (GraphPad Software Inc., La Jolla, CA, USA) and the Sigma Plot software (Jandel). The results were compared by one-way analysis of variance (ANOVA). Tukey-Kramer multiple comparison test was used to determine significant differences between samples. $P < 0.05$ was accepted as the level for statistical significance.

RESULTS AND DISCUSSION

Chemical Composition of Cooked Pasta. Chemical and nutritional compositions of M and S pasta samples dried at 40 and 92 °C after 11 and 15 min cooking times were determined, and the results are reported in Table 1. In all tested conditions, S and M samples exhibited significantly different ($p < 0.001$) protein contents, which was not affected by the cooking time. The total starch in cooked pasta was quantified and the results suggest that for S pasta starch content increased significantly ($P < 0.001$) with cooking time. Although a significantly higher ($p < 0.001$) ash content was found in S (mean value of all S samples = 0.60%) compared to M (mean = 0.29%), both these figures fall into the typical range for plain flour.

ESEM Analysis. ESEM experiments were carried out for all pasta samples. Representative results from M92 and S92 are displayed in Figure 1. A different structural matrix for the two pasta types: S (Figure 1A) and M (Figure 1B) at each processing phase can be observed. Dried S (a) and M (b) both showed the presence of partially gelatinized starch. It is well

Table 1. Chemical and Nutritional Compositions of Monlis (M) and Saragolla (S) Pasta Types, Dried at 40 and 92 °C, at Cooking Times of 11 and 15 min^a

sample	protein (%)	ash (%)	total starch (%)	rapidly digestible starch (RDS) (%)
Cooking Time = 11 min				
M92	15.22 ^{b,c,d#} (±0.02)	0.25 ^{b,c,d#} (±0.02)	72.69 ^{b,c#} (±0.68)	28.27 ^{b#,A#} (±1.30)
S92	14.43 ^{a,c#} (±0.05)	0.55 ^{a,c,d#,B*} (±0.04)	71.09 ^{a,c#,d*} (±0.13)	36.41 ^{a,c,d#} (±0.39)
M40	15.11 ^{a#,b,d#} (±0.06)	0.36 ^{a,b,d#,C*} (±0.01)	74.23 ^{a,b,d#} (±0.38)	30.79 ^{b#,C#} (±2.77)
S40	14.45 ^{a,c#,D*} (±0.03)	0.67 ^{a,b,c#,D#} (±0.01)	72.15 ^{b#,c#} (±0.25)	30.22 ^{b#,C#} (±0.21)
Cooking Time = 15 min				
M92	15.22 ^{b,c,d#} (±0.02)	0.26 ^{b,d#,c*} (±0.01)	73.39 ^{b#} (±0.08)	33.08 ^{b,c#} (±0.01)
S92	14.49 ^{a,c#} (±0.01)	0.65 ^{a,c#,d*} (±0.01)	72.11 ^{a,c#,B#} (±0.55)	36.52 ^{a,c#} (±1.70)
M40	15.12 ^{a,b,d#} (±0.05)	0.32 ^{a#,b,d#} (±0.02)	74.11 ^{b,d#} (±0.54)	36.00 ^{a#} (±1.10)
S40	14.52 ^{a,c#} (±0.03)	0.54 ^{a,c#,b*} (±0.07)	72.83 ^{c#,D*} (±0.23)	34.98 (±1.60)

^aStandard deviations are given in parentheses. Different lowercase letters (a, b, c, d) indicate significant differences of the types of pasta within each boiling time. Different capital letters (A, B, C, D) indicate significant differences of the same type of pasta at the different boiling times. Symbols: * $P < 0.05$, # $P < 0.001$.

known that the gelatinization process during pasta preparation (in particular, during kneading–extrusion and drying) can cause a transition of the starch structure from crystalline to amorphous.²⁵ This phenomenon was more evident at the high drying temperature (92 °C) and in S (a) compared to M (b). The different matrix characteristics were preserved after cooking. At 11 min, in S pasta, some defects were apparent, as observed by the big inhomogeneity in the matrix (see arrow, (c)). These defects were probably introduced during the mixing and/or forming steps and can be ascribed to not completely hydrated and mixed dough and/or to the extrusion process. The relevance of structuring of pasta components at each processing step has been widely reported in the literature.²⁶ Release of starch degradation products was found to be slower in pasta than in other cereal products mainly due to pasta compact structure resulting from the extrusion process.²⁷ The relationship between mixing time and structural homogeneity, in relation to fine gluten structure formation was investigated as well.²⁸ In the present study in-homogeneities partially disappeared with overcooking, giving rise to holes endowed with a regular roundish shape of lower dimension (not shown). Moreover, at 11 min the compact structure of the kneading matrix displayed a cluster-like structure of durum wheat as well as swollen but not disrupted starch granules embedded on a protein matrix. These features show up in the cross section ESEM image displayed in e). In contrast, several cracks were easily detectable on the external surface of the M92 pasta sample (d) as well as in the cross section image (f) most likely due to the lower technological gluten properties. At 15 min, integral starch granules were localized in the internal matrix and were clearly observable in S (g). Zones of gelatinized starch filaments and fibrils close to the cracks were detectable in M (h), suggesting microstructural changes of the inner layer.

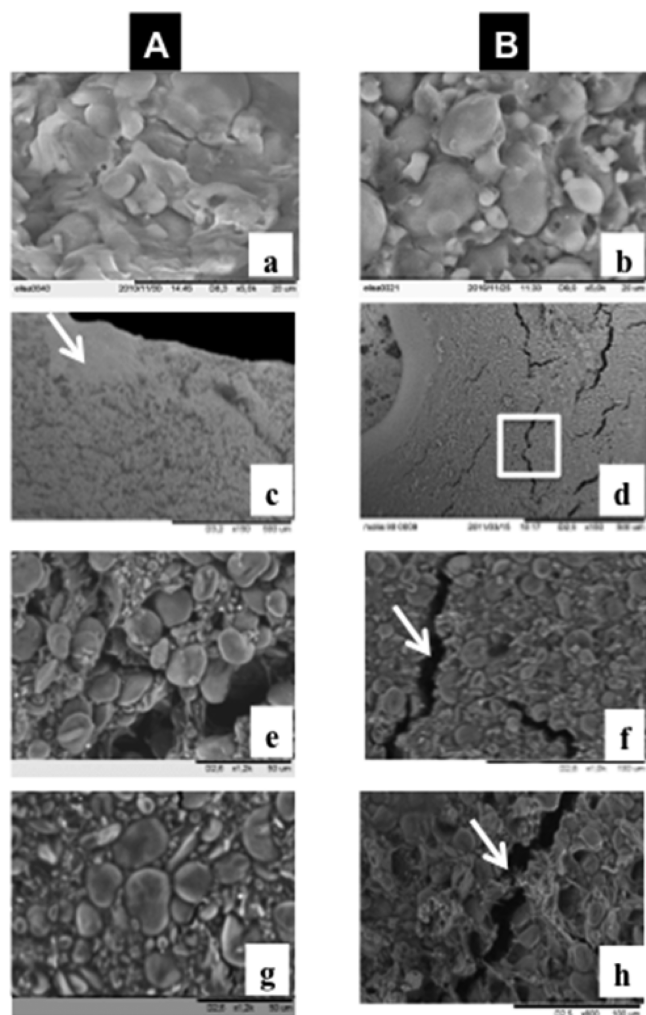


Figure 1. Coupled ESEM images of (A) S92 and (B) M92 pasta samples. S92 (a) and M92 (b) dried pasta sections, scale bar 20 μm . For 11 min cooking: surface sections of S92 (c) and M92 (d), scale bar 500 μm . A big inhomogeneity is shown in (c) (see arrow); gelatinized cracks are visible in (d) (see square). Cross sections of S92 (e), scale bar 50 μm , and M92 (f), scale bar 100 μm . For 15 min cooking: cross sections of S92 (g), scale bar 50 μm , and M92 (h), scale bar 100 μm . Internal cracks are shown (see arrows).

MRI Analysis. Figure 2 shows T_1 - and T_2 -weighted xy transversal spin-echo MRI images collected from S92 (A) and M92 (B) pasta samples after 11 and 15 min of cooking. At 11 min, a central dark region throughout the whole sample was observable in the T_1 -weighted images of both S92 (a) and M92 (b) pasta samples, ascribable to the so-called “al dente” pasta texture, namely to regions that were not completely cooked. In addition, in-homogeneities were present in the T_2 -weighted image displayed in (c) (see arrow) and (d) and were ascribed to processing dough defects that were also observed by ESEM (Figure 1). A dimension of about 400 μm was estimated from Figure 1c, which should also be detectable by the MRI technique. A more homogeneous pasta reticulate, with an increased signal-to-noise ratio (SNR) could be observed by lengthening the cooking time to 15 min in S92 (e) and M92 (f). It is clear from both the images that there was disappearance of the central dark region observed at 11 min. As observed in ESEM images, inhomogeneities changed their

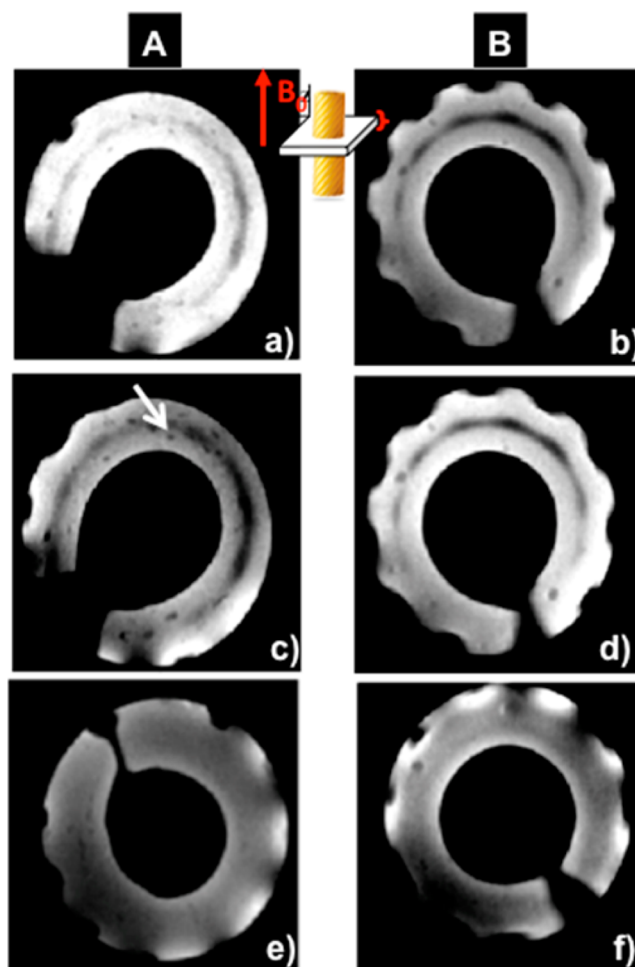


Figure 2. Coupled MRI transversal xy spin echo images of (A) S92 and (B) M92 pasta samples. The sketch shows the xy imaging plane cutting the pasta sample that is orthogonally placed in the NMR tube. All images were recorded with 128×128 matrix, $\text{FOV} = 11 \text{ mm} \times 11 \text{ mm}$, voxel resolution = $86 \times 86 \times 1000 \mu\text{m}^3$, $\text{NE} = 4$, $\text{RG} = 4$, and are displayed at the same gray levels for comparison. For 11 min cooking: S92 (a) and M92 (b) T_1 ($\text{TR} = 1 \text{ s}$) weighed images. A central dark region, ascribable to the “al dente” texture, is visible in both the images. S92 (c) and M92 (d) T_2 ($\text{TR} = 10 \text{ ms}$) weighed images. A magnification of the defects attributed to the processing phases is shown (indicated by the arrow in (c)). For 15 min cooking: S92 (e) and M92 (f) T_1 weighed image ($\text{TR} = 1 \text{ s}$). The central dark region due to the pasta matrix not being uniformly cooked disappeared with overcooking.

shape and reduced with overcooking at dimensions below MRI resolution.

T_1 MRI Relaxation. The T_1 mean data values ($\pm \text{SD}$) and signal percentages for the different pasta types (11, 15, and 20 min) are reported in Table 2. T_1 magnetization decay resulted in a single exponential for all pasta types at 11 min, with significantly shorter values for S pasta compared to M pasta ($P < 0.05$), suggesting, even after short cooking time, S pasta network endowed with a greater rigidity than M. All pasta types resulted in significantly different T_1 values ($P < 0.05$) on the basis of Tukey’s multiple comparison test. In particular, M92 exhibited the longest T_1 value and the greatest variability (SD). This latter could be ascribed to the presence of not uniformly cooked pasta regions as highlighted also by the T_1 - and T_2 -weighted images (see Figure 2). At 15 min, two relaxation

Table 2. Proton Longitudinal Relaxation Time Values (T_1 , in s) and Signal Percentages of Monlis (M) and Saragolla (S) Pasta Types, Dried at 40 and 92 °C and at Cooking Times of 11, 15, and 20 min^a

cooking time (min)		sample			
		M92	M40	S92	S40
11		1.02 ^{d*,B#}	0.91 ^{d*,B#}	0.85 ^{B#}	0.76 ^{a,b*,B#}
		(±0.13)	(±0.09)	(±0.07)	(±0.04)
		100%	100%	100%	100%
15	short	0.76 ^{b#,d*,A#}	0.47 ^{a,c#,d*,A#}	0.71 ^{b#,A#}	0.63 ^{b,a*,A#}
		(±0.04)	(±0.02)	(±0.05)	(±0.08)
	long	1.50 ^{D#}	1.61	1.23 ^{D#}	1.27 ^{D#}
		(±0.56)	(±0.58)	(±0.24)	(±0.52)
		82.56%	67.78%	15.75%	81.70%
		(±10.0)	(±28.2)	(±1.78)	(±19.6)
20	short	1.28 ^{b,d*}		1.03 ^{a*}	0.97 ^{a*}
		(±0.12)		(±0.19)	(±0.04)
	long	2.43 ^{C#}	1.33	2.74 ^{C#}	1.99 ^{C#}
		(±0.56)	(±0.20)	(±0.74)	(±0.03)
		52.44%	100%	30.13%	18.22%
		(±7.22)		(±4.30)	(±8.19)

^aStandard deviations are given in parentheses. Different lowercase letters (a, b, c, d) indicate significant differences of the types of pasta within each boiling time. Different capital letters (A, B, C, D) indicate significant differences of the same type of pasta at the different boiling times. Symbols: * $P < 0.05$, # $P < 0.001$.

components could be identified for all pasta types: a short component (significantly shorter than at 11 min, $p < 0.001$) and a long component with different signal percentages (approximately 70–80% in M and 15% in S92). Both short and long components of T_1 values lengthened after 20 min of cooking, with the short component of S92 being significantly shorter ($P < 0.05$) than M92. Upon lengthening the cooking time, the S92 pasta type maintained its compact network.

In heterogeneous systems, relaxation is affected by the interior structure as a result of water-macromolecules interaction. Therefore, not only translation and rotation of water molecules affect relaxation, but also a diffusive and chemical exchange process between water molecules and biopolymers strongly contributes to relaxation values. Starch, the most abundant biopolymer in pasta systems, together with proteins, plays an important role in these processes.²⁹ As a consequence relative populations of the components cannot necessarily be taken as accurately reflecting the number of spin in a particular environment.³⁰ Only under the slow diffusion limit ($a^2\Delta R_2/D \ll 1$, where a is the size of the environment and $R_2 = 1/T_2$) NMR signals will be characterized by several components signature of different local environments, each with its own relaxation time.³¹ Multiexponential relaxation behavior for water has been consistently reported in starch–water systems because of slow diffusive exchange and this regimen is also suggested by combining ESEM (30 μm for granule size) and diffusion (in the order of 10^{-10}) results in the current study. The distribution and dynamic state of water, following swelling and gelatinization, was previously studied in rice starch granules.³² By the analysis of the longitudinal magnetization decay curve during the swelling process the authors identified two components, and the short T_1 value was ascribed to water–starch interaction. After gelatinization, only one T_1 value associated with water in the gel state was observed. A similar trend could be identified in the present study by the T_1 analysis (Table 2). Two components were identified even when the cooking time was extended to 20 min,

with the exception of M40 whose relaxation was fitted to a single exponential. Indeed, we hypothesize that, upon lengthening the cooking time, the pasta matrix mobility can be enhanced by an increase in the gelatinization processes, thereby causing a progressive passage from a inhomogeneous network toward a more uniform carbohydrate gel phase. However, gelatinization appeared to take place to a greater extent in M pasta type, generally showing a longer T_1 relaxation value and particularly in M40 after 20 min of cooking. The different behavior of M, compared to S, might be ascribed to the different protein reticulate and, at the same time, this feature might be at the basis of a greater accessibility of starch granules to the enzymatic attack in M compared to S (see below).

MRI Diffusion. At 11 min, for all types of pasta, water diffusion resulted in a single exponential. A significantly higher ($p < 0.05$) diffusion value for M40 with respect to S pasta type was calculated (Table 3). In contrast, two components could be identified at 15 min, with the short component of M92 reaching a D value close to free water ($2.22 \times 10^{-9} \text{ m}^2 \text{ s}^{-2}$, $r^2 = 0.98$). Both M92 short and long components attained significantly higher values with respect to S92 ($P < 0.05$), suggesting greater translational mobility. The diffusion data at 20 min resulted in a single exponential with D values far from those found for pure water and significantly lower ($P < 0.05$) than the short components calculated at 15 min.

T_1 relaxation and diffusion parameters indicated that the S pasta endowed a more compact structure than M pasta at all cooking times. The different components identified might be related to the starch gelatinization process occurring during cooking.

As pointed out, gelatinization, by far the most common and impactful transformation in starch (pasting, baking), results from the input of heat in the presence of water. Water enters the granule and plasticizes the amorphous component of the starch. The lengthening of the cooking time is known to cause an increase in the degree of gelatinization through swelling of starch granules and rupture and ultimately produces the gel

Table 3. Water Diffusion Data (D , in $\times 10^6 \text{ m}^2 \cdot \text{s}^{-1}$) and Signal Percentages of Monlis (M) and Saragolla (S) Pasta Types, Dried at 40 and 92 °C at Cooking Times of 11, 15, and 20 min^a

		sample					
		M92	M40	S92	S40		
cooking time (min)		11	3.51 ^{B#} (±0.70) 100%	4.51 ^{d,c,g,B#} (±0.49) 100%	3.07 ^{b,g,B#} (±0.88) 100%	3.00 ^{b,g,B#} (±0.19) 100%	
		15	short	17 ^{b,c,d,g,A#} (±5.56) 21.10%	7.48 ^{a,g,A#} (±1.77) 11.89%	8.08 ^{a,g,A#} (±1.33) 36.95%	9.08 ^{a,g,A#} (±1.14) 20.54%
				long	3.53 ^{c,d,g,D#} (±0.02) 78.89% (±0.03)	3.21 ^{D#} (±0.05) 88.10% (±0.06)	2.56 ^{a,g,D#} (±0.04) 63.04% (±0.08)
20					4.16 ^{d,g,C#} (±0.03) 100%	4.46 ^{d,g,C#} (±0.03) 100%	4.31 ^{d,g,C#} (±0.01) 100%

^aStandard deviations are given in parentheses. Different lowercase letters (a, b, c, d) indicate significant differences of the types of pasta within each boiling time. Different capital letters (A, B, C, D) indicate significant differences of the same type of pasta at different boiling times. Symbols: * $P < 0.05$, # $P < 0.001$.

state. To observe the starch through the complete process of gelatinization, the cooking time for the pasta was extended up to 20 min, well beyond the usually adopted cooking time. Indeed, the gelatinization process is strongly affected by many parameters, including botanical species, source, presence and amount of solutes (such as sugars) that lead to changes in many functional properties such as viscosity, solubility and digestibility. At the same time, functional changes are expected to be determined by structural modifications of the pasta network

that can be monitored by MRI, throughout an estimation of rotational (T_1) and translational (D) mobility, mainly of water. The enhanced rotational and translational mobility of water, probed by T_1 relaxation and diffusion data, indicated a greater network mobility of M compared to S, taking into account exchange effects. In other words, the constituents of M (protein bodies, filaments, and fibrils) are less effective at restricting water movement. Water mobility significantly increased at 15 min, while further differences were not observed at 20 min. This result suggests similar interactions between water and macromolecules at 15 and 20 min but not unrestricted diffusion, which would be observed if there was complete disruption of the starch granule. In fact, self-diffusion constants (D) were in the order of $10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$, significantly slower than that of free water. At the same time, the root-mean-square distance in one-dimensional diffusion, $(2D\Delta)^{1/2}$ was approximately 6–7 μm . This distance is considerably below the mean granule size of approximately 30 μm calculated by ESEM analysis (Figure 1e,g), thus confining diffusion pathway within the granule. On the other hand, if the internal granule structure was ruptured by gelatinization, water would display diffusion parameters similar to those in a normal three-dimensional pathway. We can therefore conclude that hindered diffusion within the starch gels takes place as a consequence of incomplete gelatinization.

T_2 Relaxation Analysis. Different from longitudinal relaxation, transverse relaxation is sensitive to dipole–dipole interactions. Thus, T_2 -weighted images are effective at highlighting sample inhomogeneities (Figure 2c,d). However, even if image contrast clearly pointed to the presence of regions endowed with different mobility, all T_2 decays calculated from MRI data were fitted to a single exponential. The calculated T_2 values (13–20 ms range, data not shown) lengthened when the cooking time increased but were shorter than bulk water, therefore reflecting the interactions between water and macromolecules. It is well known that water molecules with a near isotropic motion are characterized by a T_1/T_2 ratio value near unity and T_2 values that are low compared to those of bulk

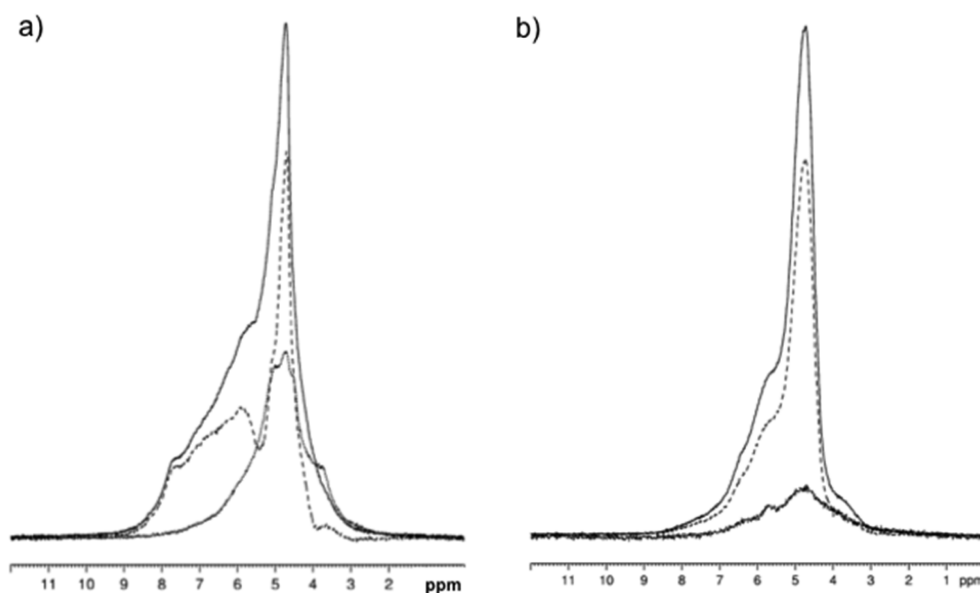


Figure 3. 200 MHz ^1H NMR 1D spectra recorded from identically weighing S92 (a) and M92 (b) pasta samples cooked for 15 min in H_2O (continuous line) and D_2O (dotted line). The calculated difference spectra are also displayed (dashed line).

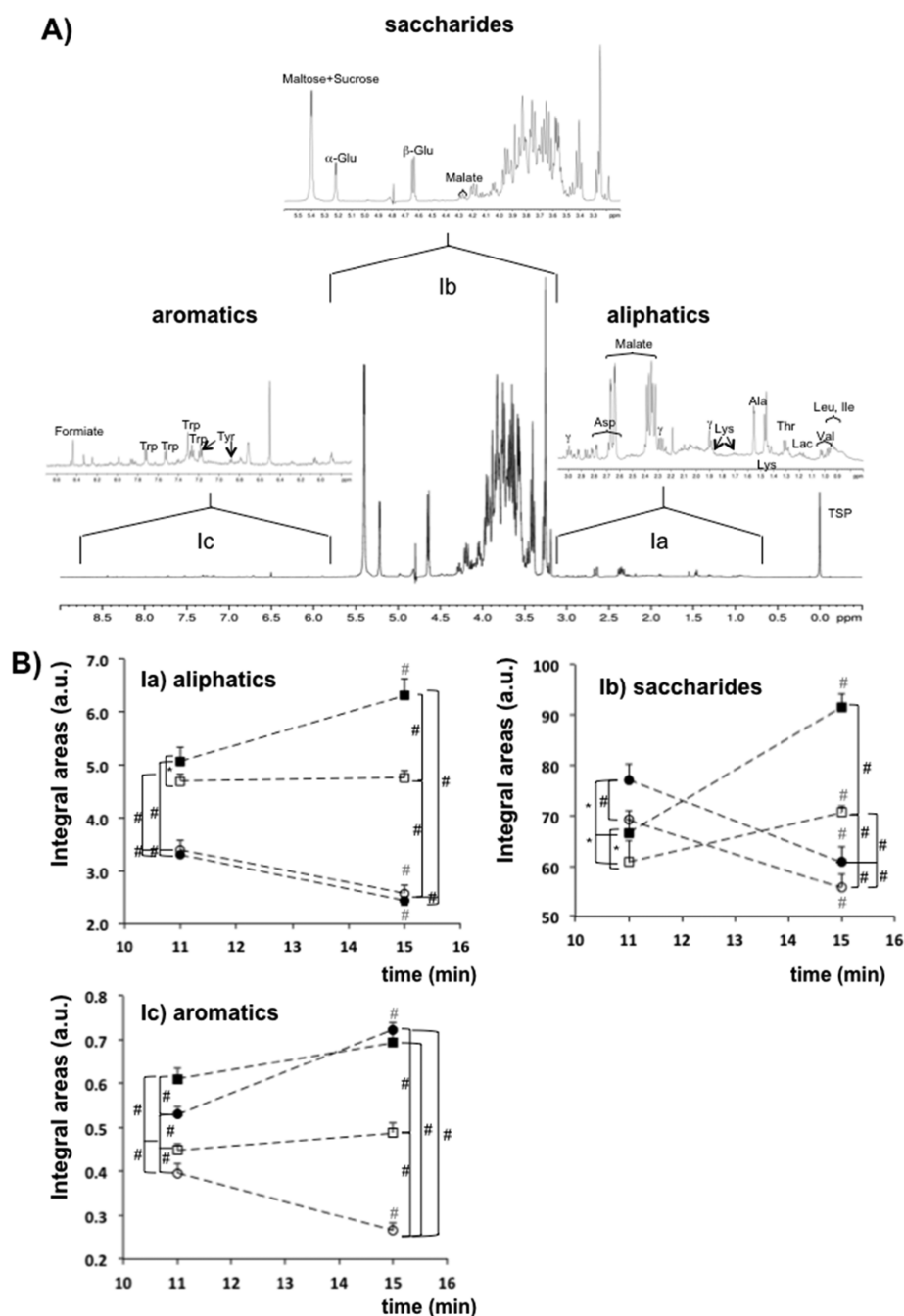


Figure 4. (A) 500 MHz 1D ^1H NMR spectrum recorded from the cooking water of a S92 sample at 15 min of cooking time. The integrated spectral regions are shown into brackets: Ia, aliphatics (1–3 ppm); Ib, saccharides (3–5.6 ppm); and Ic, aromatics (5.8–8 ppm). The regions are zoomed-in in the insets with labeling of the main assigned compounds. (B) Plots of the integral areas (arbitrary units) versus the cooking time (11–15 min) of the three integrated spectral regions (Ia–Ic): M92 (■), M40 (□), S92 (●), and S40 (○). Results are expressed as mean $\pm$ SD. Significant differences of types of pasta at the same cooking time are indicated by brackets; significant differences at different cooking times are displayed in gray. Symbols: * $P < 0.05$, # $P < 0.001$. Dotted straight lines are drawn to guide the eye.

water were ascribed to an interaction of water molecules with starch.³² In the present study, the T_2 values calculated from T_2 -weighted images reflected the presence of a water-biopolymer interactions, mainly by starch. Unfortunately, the echo time adopted by the spin–echo MRI method ($TE = 6.07$ ms) prevented the determination of fast T_2 relaxation mechanisms. Therefore, the relaxation mechanism was further investigated by spectroscopic analysis. The dynamics were monitored on a short time scale (10–1000 μs) using the spin–echo sequence and on a longer time scale (2–80 ms) using the CPMG pulse

sequence. The study focused on S92 and M92 samples at 15 min of cooking time. Two main relaxation time components resulted from the spin–echo analysis; the long component (M92, 26.35 ± 6.15 ms; S92, 20.19 ± 3.95 ms) with a greater percentage signal (approximately 80%) in both pasta types. By contrast, the short component (M92, 6.00 ± 0.7 ms; S92, 1.99 ± 0.99 ms) was significantly shorter for S92 ($P < 0.01$), thereby suggesting a more rigid structure for this latter. Intriguingly, although one might expect the solid part of the FID of the spin–echo decay to have a significant Gaussian component, all

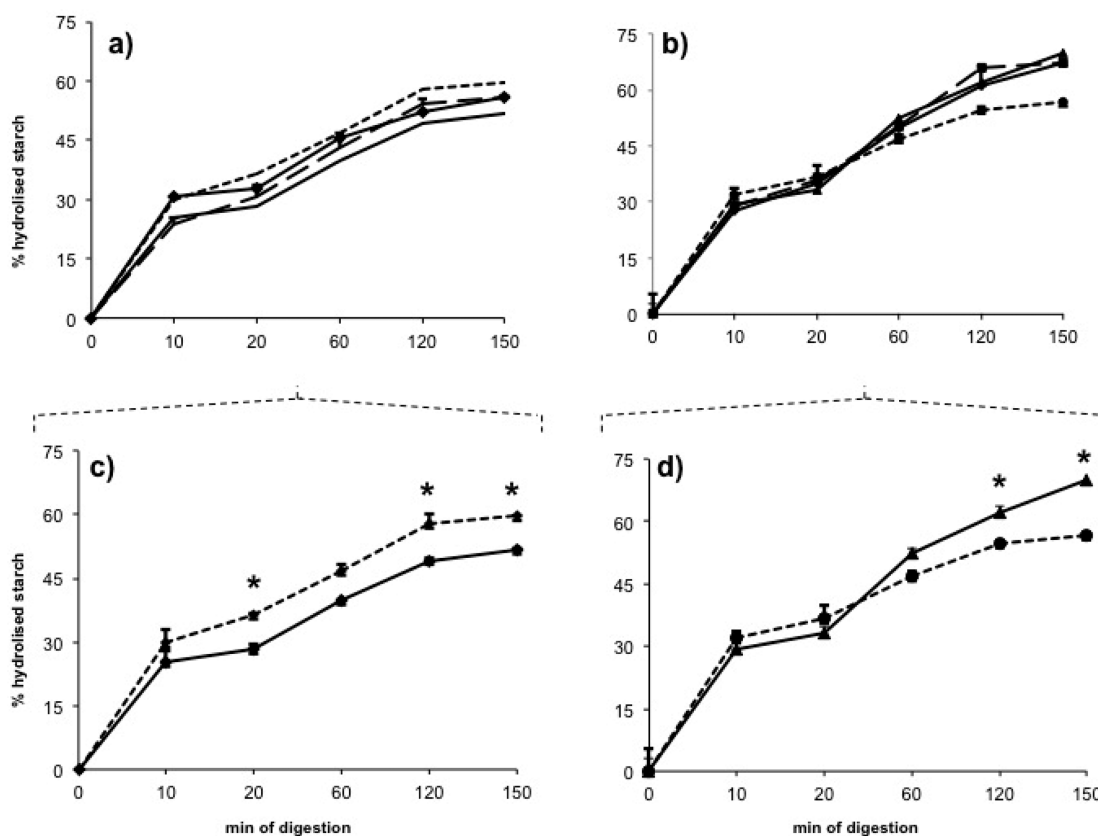


Figure 5. (a) Digestion curves of M92 (▲), S92 (●), M40 (■), and S40 (◆) samples after 11 min of cooking time. Abscissa: 0 (no digestion), 10, 20, 60, 120, and 150 indicate the time points of digestion (in min; mean of triplicate analysis for each time point). (b) Digestion curves of the four species of pasta at 15 min (mean of triplicate analysis $\pm$ SD). Abscissa and symbols as in (a). (c) Zoom-in of the starch digestibility curves, to better enhance the differences, of the S92 and M92 samples shown in (a); significant differences ($p < 0.05$) at time points 20, 120, and 150 min. (d) Zoom-in of the starch digestibility curves of S92 and M92 shown in (b); significant differences ($p < 0.05$) at time points 120 and 150 min.

data were well represented as a biexponential decay with no evidence of a solid-like dipolar component, independently of the cooking time, in agreement with the results obtained by Tang et al. on fully swollen native potato starch granules at 295 K.³³

Pasta is an extremely complex matrix. It includes carbohydrates, proteins and fiber elements that cannot be analyzed individually. Therefore, the structural network as a whole was further investigated by the comparison of the FIDs recorded from M92 and S92 pasta samples of identical weight cooked for 15 min in either H₂O or D₂O (100%). After D₂O exchange, the NMR signal of pasta samples mainly originates from the nonexchanging protons, i.e., those of CH groups involved in the H-bonded network that will contribute to the rigid component of the decay.

Figure 3 shows the 1D ¹H NMR spectra from S92 (a) and M92 (b) samples in H₂O (continuous line) and D₂O (dotted line) and the difference spectra (dashed line). The spectral signals of these latter originate from water and exchangeable protons mainly starch, and in a lower amount from proteins (see Table 1), while a contribution of fats could be almost excluded, due to the absence of the wheat germ in both pasta types. A dry matter percentage of 92.33 ± 0.001 and $93.25 \pm 0.001\%$ was calculated for raw M92 and S92, respectively, while measuring the sample weight at both the end of cooking and raw a ratio of 2.69 ± 0.08 and 2.28 ± 0.09 on dry basis for S92 and M92 was calculated. Based on these data, from the normalized spectral areas, the amount of water and mobile

protons was calculated as 58 ± 0.5 and $41 \pm 0.7\%$ of the cooked weight on dry basis for M92 and S92 respectively. The results enabled us to conclude that, during the cooking process, water is absorbed by starch of both pasta types in the same way. However, this water is then distributed in the pasta matrix differently, essentially owing to the lower ability of M92 matrix in binding water and this was ascribed to the lower technological gluten properties of M92 pasta, with respect to S92 type.

¹H NMR Spectroscopy of Cooking Water. The pasta–water interaction was also elucidated by high-resolution ¹H NMR spectroscopy. Data were acquired from samples of the water in which the pasta was boiled (11 and 15 min) to identify and quantify the chemical compounds released by the different types of pasta that were calculated from the spectra via integral areas.

Figure 4A shows the ¹H NMR spectrum recorded from a S92 cooked water sample at 15 min. The resonance assignments of the main signals were made with an internal database of standard compounds and by the aid of two-dimensional experiments (TOCSY and HSQC). All samples were found to contain the same chemical compounds: amino and organic acids (malate and formate) and saccharides (α - and β -glucose, sucrose, and maltose). However, these components were in different amounts depending on the duration of the cooking time. The plots of the calculated integrals are displayed in Figure 4B. A clear separation of M with respect to S species could be observed in both aliphatic (organic and amino acids)

region (a): M showed a greater amount of released compounds than S, independent of the drying temperature and cooking time. An opposite trend was observed for M and S pasta as concerns saccharides, whose release, between 11 and 15 min, was found to significantly ($P < 0.001$) increase and decrease, respectively. The behavior of the compounds investigated by NMR spectroscopy (i.e., amino acids, saccharides, and low-molecular-weight molecules released in water during the cooking process) can be viewed as complementary to that involved in the digestibility analysis, where the chemical components retained in the pasta matrix (i.e., those not released into the water during the cooking process) contribute to the measurement. Demonstrating this phenomenon, when increasing the duration of the cooking time, M92 was found to release the most compounds into the cooking water, in agreement with the more mobile matrix that was suggested with relaxation and diffusion data (Tables 2 and 3).

In conclusion, our results enabled us to establish a correlation between matrix structures and low-molecular-weight compounds released into water while boiling (i.e., water-soluble compounds). Our data suggest a greater release of compounds from matrices with higher mobility, as observed for M. Accordingly, a lower tendency to release compounds with a longer cooking time was found for S, which is inherently poorer in proteins and exhibited significantly shorter values for T_1 (Tables 1 and 2).

Starch Digestibility Analysis. The effect of cooking time on S and M pasta both low and high temperature dried on starch digestibility was assessed. Starch digestibility was measured by detecting saccharides released during *in vitro* starch digestion. Figure 5a shows the kinetics of starch hydrolysis (digestion curves) for the four pasta types cooked for 11 min. The trend of starch hydrolysis appeared to be similar for each sample. However, M92 pasta seemed to be less digested than the analogous durum (S92), as shown by the lower hydrolyzed starch value at each time point (Figure 5c). Specifically, a significant difference between the two curves was observed at 20 min of starch digestion ($P < 0.05$), corresponding to the rapidly digestible starch (RDS) fraction. This fraction is nutritionally important because it correlates with high postprandial blood glucose levels. The presence of higher amounts of readily digestible sugars and starches contributes to a high glycemic index.⁷

The degree of starch hydrolysis of pasta cooked for 15 min (Figure 5b) increased for M92, M40, and S40 compared to pasta cooked for 11 min. However, no significant differences ($P > 0.05$) were detected during the first 20 min of digestion, suggesting that RDS did not increase as a consequence of increasing the duration of the cooking time. Nevertheless, a significant difference between M92 and S92 pasta (Figure 5d) at 120 and 150 min was detected. In particular, the digestibility did not change for S92 between 120 and 150 min of digestion, as shown by the hydrolyzed starch values at these time points. These results suggest that starch hydrolysis was likely not enhanced from overcooking, but more likely that there was a synergic effect of the high temperature drying process and the presence of the strong protein network.⁹ As a result, there was a delay in the accessibility of amylolytic enzymes to the starch. On the other hand, the starch hydrolysis drastically increased between 120 and 150 min of digestion in M92 pasta. NMR data in general suggested a less compact structure of M with respect to S. In particular, as already pointed out, M and S saccharide release showed an opposite trend at 11 versus 15 min.

These results are in agreement with the NMR, MRI and ESEM analyses where an increase in the cooking time corresponded to a rise in starch gelatinization of the inner part of M pasta (Figure 1h). By contrast, ESEM analysis did not show relevant differences of the internal microstructure in S pasta (Figure 1e), suggesting that starch gelatinization was limited. Additionally, NMR data suggested a reduced water mobility of S92 with respect to M92 pasta, mainly ascribable to the different ability of the gluten proteins to bind water (see Figure 3). At the same time, diffusion data suggested that water movement was restricted to the inner part of the starch granule.

Currently, durum wheat semolina is the preferred ingredient for the production of high quality pasta due to its color, flavor and cooking quality. Its structure is complex, with starch granules incorporated in a protein matrix. In particular, it is believed that gluten protein aids in the formation of network surrounding the starch granules, thereby acting as a barrier to starch digestion.³⁴ This can explain why S92 pasta was not more susceptible to amylolytic digestion at a prolonged cooking time. ESEM analysis also confirmed this result, showing that at 15 min of cooking, starch granules were partially gelatinized and entrapped in the protein network (Figure 1g). MRI analysis also indicated that S pasta is endowed with a more packed structure ascribable to the protein reticulate; M pasta was characterized by water with greater rotational and translational mobility. In addition, this is the reason that for M92, starch hydrolysis increased with increasing incubation time with digestive enzymes, while this was not observed for S92. Water availability in the food matrix is important for amylase activity: the more the water diffuses into the system, the higher is the catalytic efficiency.¹³

Analysis of the cooking water by NMR spectroscopy showed that a higher release of compounds into the water was found from the network with higher mobility. With respect to S, at 11 min, M pasta demonstrated a high leakage of aliphatic compounds from the pasta matrix, while the leakage of polysaccharides required at least 15 min, in agreement with the starch digestibility data. The present study highlighted significant differences in starch digestion of pasta made with *T. monococcum* (cv. Monlis), compared to the commonly used durum wheat (cv. Saragolla) dried at 92 °C cooked to “al dente” texture. These results suggest a synergic effect between wheat genotype and processing. As reported in the literature, drying is essential for maintaining the pasta structure, especially during overcooking. The protein network of LT (55 °C) dried and then cooked pasta was found partially disrupted. On the contrary, the protein network was found preserved after VHT (100 °C) together with a lower extent of starch swelling.³⁵ High drying levels could play an effect on microstructure by enhancing protein cross-link thereby highlighting the protective role played by proteins, limiting water absorption by granules.³⁶ In agreement with literature data, in this study, NMR relaxation and diffusion results suggested a considerable effect of drying temperature mainly with overcooking as well. Moreover the release of compounds in the aliphatic region (Figure 4B) differed significantly ($p < 0.001$) for M and S pasta species at both 11 and 15 min cooking times.

NMR analysis suggested that S pasta has a more compact structure, ascribable to different network properties. More generally, this study demonstrates that investigating the food structure aids to understand its behavior during gastro-intestinal digestion. For this purpose, we propose NMR, in conjunction with enzymatic digestibility methods as valid methodologies for

estimating how starch may behave during cooking and subsequently during its degradation in the human body.

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Notes

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ABBREVIATIONS USED

MRI, magnetic resonance imaging; ESEM, environmental scanning electron microscopy; NMR, nuclear magnetic resonance; T_1 , spin-lattice relaxation time; T_2 , spin-spin relaxation time; ROI, region of interest; TE, echo time; D , diffusion; FID, free induction decay; AQ, acquisition time; RG, receiver gain; M, Monlis; S, Saragolla; RDS, rapidly digestible starch; LT, low temperature; VHT, very high temperature

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