LWT - Food Science and Technology 142 (2021) 111017

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Wholewheat bread: Effect of gradual water addition during kneading on dough and bread properties

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A R T I C L E I N F O

Keywords:
Wholemeal flour
Rheology
Mixing
^{1}H NMR
Molecular mobility

A B S T R A C T

Owing to the increasing demand for wholewheat flours (WWF) in bread-making, techniques are needed to improve WWF's poor technological properties and the resulting bread quality. We tested the effect of a common bakers' strategy to improve WWF performance: adding part of the water of the recipe during the kneading operation. First, the kneading operating conditions were optimized to perform the gradual water addition. The optimized dough sample was tested in a second trial to make an in-depth investigation of the effect of (i) the amount of water added during kneading and (ii) the total water content on dough molecular mobility (with Low Resolution ^{1}H NMR), dough rheology and bread quality. The gradual addition of water caused significant variations in proton mobility and dynamics; it improved dough rheological properties, but did not affect the bread quality. A higher dough water content (i.e., 60%) than predicted by the Farinograph test significantly improved the bread quality in terms of bread and crumb specific volumes, and bread texture. Combining the gradual water addition with high water content could be a strategy to enhance the quality of WWF doughs and breads, promoting the consumption of fibre-enriched foods.

1. Introduction

Recently, the market demand for healthy foods has grown greatly, since nutritional value has become a determinant quality criterion driving food choices (Schaffer-Lequart et al., 2017). In this context, the use of wholewheat flours (WWFs) in the bread-making process has gained particular attention, since unrefined flours are rich in nutrients such as fibres, vitamins, minerals and oils (Boukid et al., 2018; Hemdane et al., 2016). However, unrefined flours generally display poor bread-making properties which give sticky doughs that are difficult to handle, and end-products with a small loaf volume, coarse and hard texture, nutty odour and bitter/sour taste (Heinio et al., 2016; Hemdane et al., 2016; Boukid et al., 2018; Guerrini et al., 2019). Consequently, the use of WWFs in the bread-making process still represents a challenge for the baking industry and innovative strategies are necessary to improve the bread quality (Guerrini et al., 2019).

Several studies have dealt with the poor technological performance of WWFs, trying to improve dough workability and bread quality. The most common strategies have been focused on treatments of the raw materials (i.e., bran and germ), the sourdough fermentation and modification of the bread recipe, mainly by using improvers (Parenti et al., 2020a, 2020b; Tebben et al., 2018).

A recent interview reported the strategies adopted by bakers using unrefined wheat flour for the bread-making. One of the most common strategies used is the gradual addition of water during kneading (Guerrini et al., 2019), but there are scant research studies on the real effects of the above technique on the WWF dough properties and bread quality (Yang, Guan, Zhang, Li, & Bian, 2019). The key role of water during dough development is well known in the literature; it enables hydration of the flour components and the glass transition of amorphous regions of the starch and amorphous proteins, giving a viscous elastic dough as a result (Cuq et al., 2003; Zhou et al., 2014). Recently, the ^{1}H NMR technique has appeared capable of showing the proton molecular mobility and dynamics in WWF doughs during kneading, with changes in the ^{1}H NMR parameters linked to the molecular phenomena occurring during the dough development (Parenti et al., 2021).

The present study aimed to investigate the effect of gradual water addition during kneading on dough molecular and macroscopic

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https://doi.org/10.1016/j.lwt.2021.111017
Received 10 November 2020; Received in revised form 6 January 2021; Accepted 28 January 2021
Available online 2 February 2021

properties and on bread quality. Two dedicated experiments were performed: (i) an optimization trial of the kneading operating conditions; (ii) an in-depth investigation of the molecular and macroscopic properties of dough and bread as a function of both gradual water addition and total dough water content. Following the approach proposed by Parenti et al. (2021), the molecular properties of the dough were investigated using the ^{1}H NMR technique to gain a molecular insight into the effect of gradual water addition and to relate it to the macroscopic properties of the dough and bread.

2. Material and methods

2.1. Materials

Two batches of a sp. *Triticum aestivum* L., cv. Verna, were used to perform the experimental trials. The wheat was grown in Montespertoli (Florence, Italy) during the 2019–2020 growing season. Wholewheat flour (i.e., extraction rate 98 g/100 g dry kernel, ash content 1.3–1.7 g/100 g dm; Pagani et al., 2014) was obtained using a stone grinding mill and a sieve (two consecutive passages through a 1,100–1,200 μm sieve) at the Molino Paciscopi (Montespertoli, Florence, Italy). The protein content of the wholewheat flour was 11.4 g/100 g d.m. and the Alveograph characterisation showed the following results: $P = 56.00$ mm H_2O, $L = 41.50$ mm, $P/L = 1.36$, $G = 14.30$ mm, $W = 78.00\ 10^{-4}$ J. The mineral water (Sant'Anna, Vinadio, Italy) and fresh brewer's yeast (Lievital, Trecasali, Italy) were purchased at a local market (Florence, Italy).

2.2. The experimental design

Two experimental trials were carried out to investigate the effect of gradual water addition:

(i) an optimization trial for the kneading operating conditions;
(ii) a full factorial trial for the in-depth evaluation of the effects of both gradual water addition and total dough water content.

2.2.1. The optimization trial

The optimization trial (*OT*) was performed using a Box-Behnken design (BBD) and Response Surface Methodology (RSM) to test the effect of the following independent variables on the bread specific volume at three different levels: (i) total water content - W_a (i.e., 56%, 58% and 60% w/flour w); (ii) gradual water addition during kneading - w_a (i.e., 5%, 15%, 25% w/water amount to reach 500 BU in the Farinograph test); (iii) total kneading time - T (i.e., 14 min, 18 min and 22 min); (iv) water addition kneading time - t (i.e., 4 min, 7 min, 10 min). The above lowest water content level (56%) represented the water absorption needed to obtain the Farinograph optimal consistency of 500 BU. Since it was previously reported that WWFs have higher water requirements than the Farinograph prediction (Guerrini et al., 2019; Parenti, Guerrini, & Zanoni, 2020; Zhou et al., 2014), two higher water levels were tested. The amount of water to gradually add during kneading and the time for water addition were selected according to the bakers' indications obtained in a previous study (Guerrini et al., 2019). Finally, the range explored for total kneading time was selected on the basis of preliminary trials. The experimental conditions at the centre point were $W_a = 58\%$, $w_a = 15\%$, $T = 18$ min and $t = 7$ min.

The dough samples were given an alphanumeric code; for example, the code *OT56:5:10:4* identified the dough sample in the optimization trial with $W_a = 56\%$, $w_a = 5\%$, $T = 10$ min and $t = 4$ min. The experimental data from the *OT* were processed in order to find the combination of variables that maximized the specific bread volume.

2.2.2. The full factorial trial

The full factorial trial (*FFT)* tested the effects of the following variables on the dough ^{1}H NMR molecular mobility and rheology and on the bread quality: (i) gradual water addition during kneading - w_a; (ii) total water content - W_a. The water addition kneading time and the total kneading time were set according to the *OT* results.

The above two variables were tested at two levels:

- $w_a = 5\%$ w/water amount to reach 500 BU (i.e., the *5%*w_a level) vs $w_a = 0\%$ (i.e., the *0%*w_a level);
- $W_a = 56\%$ w/flour w, which was the water amount to reach the Farinograph optimal consistency of 500 BU (i.e., the W_a*56%* level) vs $W_a = 60\%$ w/flour w, which was the optimized water amount from the RSM trial (the W_a*60%* level as shown in section 3.1 of the Results).

Furthermore, the ^{1}H NMR Relaxometry technique was used to monitor the molecular kinetics of the WWF doughs during kneading, in order to show the effect of the variable w_a and its interactions with the kneading time T (w_a*T) and the total water content W_a (w_a*W_a).

The dough samples were given an alphanumeric code; for example, the code *FFT56:5* identified the dough samples in the full factorial trial with $W_a = 56\%$ and $w_a = 5\%$.

2.3. Bread-making

The dough samples were prepared in 500 g batches. The basic formulation was: flour (310 g), fresh brewer's yeast (13 g); the amount of water was selected according to the above experimental design. Kneading was performed using Kitchen Aid Professional Mixer (5KSM185PS, KitchenAid, St. Joseph, Michigan, USA) operating with a dough hook (model KSM35CDH) at 110 rpm; then, each dough was placed in a bread making machine (Pain doré, Moulinex, Ecully, France) to perform the proofing step for 90 min at room conditions (T = 22 ± 2 °C, relative humidity = 50%), and baked at 150 °C for 50 min. Three replicates of the whole breadmaking process were performed in the *FFT*.

2.4. Measurement methods

2.4.1. ^{1}H NMR measurements

Proton molecular mobility was investigated with a low-resolution (20 MHz) ^{1}H NMR spectrometer (the MiniSpec, Bruker Biospin, Milan, Italy) operating at 25.0 ± 0.1 °C. ^{1}H Free Induction Decay (FID) and ^{1}H T_2 Carr-Purcell-Meiboom-Gill (CPMG) experiments were performed to investigate the fastest and the most slowly relaxing protons observable in the time frame of the experimental window. The dough samples were prepared in the same conditions as the bread-making process without the addition of yeast.

The samples were analysed every 3 min during the kneading step for a total of 8 kneading periods as described in Parenti et al. (2021). Due to the time required for the acquisition of the ^{1}H NMR signals, to the short time interval between the selected kneading times, and in order to analyse samples within a maximum of 1 min after kneading (to avoid different resting times), two batches of dough had to be prepared for each replicate. Hence the experiments required the preparation of a total of 2 dough samples x 4 replicates x 4 treatments (2 water levels x 2 water addition levels) = 32 batches of dough. This operating approach inevitably introduced variability in the data-set since each dough replicate did not come from the same batch. Furthermore, bread dough is a complex food matrix characterised by a high inherent variability. For the above reasons and since scant scientific data was reported in the literature on ^{1}H NMR mobility and dynamics during wheat dough kneading (Kim & Cornillon, 2001; Parenti et al., 2021; Sangpring et al., 2017), all the significant differences among dough samples as a function of the experimental variables were discussed in the Results. FID signals were acquired using a single 90° pulse, followed by a dwell time of 7 μs and recycle delay of 1 s, a 0.5 ms acquisition window, 32 scans and 900 data points. Six ^{1}H FID replicates were acquired for each sample treatment. A two-component model (exponential and Gaussian) (Russell, 1983) was used to fit the curves in order to obtain quantitative information about the proton relaxation time and percentage of protons belonging to the more rigid and more mobile proton populations which were measurable within the FID experimental time frame (7–500 μs).

^{1}H T_2 (transverse relaxation time) was obtained using a CPMG pulse sequence with a recycle delay of 1 s, an interpulse spacing of 0.04 ms, 2500 data points and 32 scans. A high number of scans were applied to enhance the signal-to-noise ratio. As described in Parenti et al. (2021), just one ^{1}H T_2 curve was acquired for each dough replicate, for a total of four replicates. The ^{1}H T_2 curves were analysed as quasi-continuous distributions of relaxation times using UPEN software (Alma Mater Studiorum, Bologna, Italy). ^{1}H T_2 CPMG relaxation decays were also fitted with a discrete exponential model (Sigmaplot, v.6, Systat Software Inc., USA) to obtain relaxation times and proton population abundances.

Pop"X″ abbreviations were used to identify the relative abundances of ^{1}H NMR proton populations; similarly, the relaxation times were identified as $T_{''X''}$.

2.4.2. Dough rheology

The Brabender Farinograph test was performed following the standard method (AACC 54–21.02) in order to determine the amount of water necessary to obtain dough samples with a reference consistency of 500 BU. Three replicates were performed.

The Alveograph test was performed following the standard method (AACC 54–30.02) with some modifications in order to measure the rheological properties of the dough with the same characteristics as the samples prepared for the bread-making trials. The kneading step was performed using a Kitchen Aid Professional Mixer (5KSM185PS, KitchenAid, St. Joseph, Michigan, USA); then the dough samples were put in the Alveograph chamber, and the standard protocol was adopted. The dough tenacity - P (mm H_2O), defined as the maximum overpressure; dough extensibility - L (mm), the average bubble length at rupture; swelling index - G (mm), the square root of the volume of air necessary to inflate the dough bubble until it ruptures; flour strength - W (10^{-4} J), the energy required to inflate the dough bubble to the point of rupture; and ratio between dough tenacity and extensibility - P/L were measured (Bordes et al., 2008). Three replicates were performed.

2.4.3. Bread quality

The following parameters were determined to evaluate the bread quality: bread volume (L), bread specific volume (L/kg), crumb specific volume (L/kg), crumb and crust moisture contents (g/100 g) (expressed as the ratio between crumb or crust moisture and dough moisture since different water amounts were used to prepare the dough), and Texture Profile Analysis (hardness - N, cohesiveness, chewiness - N*mm, springiness - mm). The above measurements were performed according to Parenti, Guerrini, Cavallini, et al. (2020). Three measure replicates and three treatment replicates were performed for each of the above parameters.

2.5. Data processing

2.5.1. Response Surface Methodology (RSM) and full factorial trial

In the *OT*, bread quality in terms of the bread specific volume was estimated through application of a Box-Behnken design (BBD) based on RSM. The second-order model resulted the most appropriate. The results were examined using R software; the RSM was estimated by partial least square (PLS) for the 28 runs of the BBD design. From the response variable (Y_1 = bread specific volume), model summary and lack-of-fit tests were performed considering linear, quadratic and cubic models. The quadratic model proved to be the most accurate. The bread-making conditions that maximized the bread specific volume were considered optimal.

In the *FFT*, the data were analysed with two-way ANOVA to assess significant differences ($p < 0.05$) resulting from the tested variables (i.e., addition of water, w_a, and total water content, W_a, and their interaction, w_a*W_a).

2.5.2. ^{1}H NMR modelling

In the molecular kinetics of WWF dough kneading, ^{1}H NMR data also tested the effect of the kneading time (T) on the proton mobility. A three-way ANOVA was used to assess significant differences resulting from the addition of water (w_a), total water content (W_a), kneading time (T) and their interactions (w_a*T, w_a*W_a, $T*W_a$, w_a*W_a*T). In order to predict the ^{1}H NMR parameters of the dough samples during the kneading time as a function of total water content W_a (56% vs 60%) and gradual water addition w_a (*5%* vs *0%*), the experimental data were fitted with a linear model in function of the continuous variable T, and the two categorical variables W_a and w_a, and with a second-order model in function of the continuous variable T.

The data were analysed using R software. A three-way ANOVA was performed in order to assess significant differences ($p < 0.05$) resulting from the tested variables (T, W_a, and w_a) and their interactions ($T*W_a$, $T*w_a$, $W*w_a$, $T*W_a*w_a$). The not significant terms ($p > 0.05$) were removed from the model as suggested by Dunn and Smyth (2018). Following this, the model was further checked with the ANOVA model.

The ^{1}H NMR results were also analysed at the optimum kneading time as a function of both variables W_a and w_a. A two-way ANOVA was used to assess significant differences due to the main effect of each factor (W_a and w_a) and due to their interaction (W_a*w_a).

3. Results

3.1. The optimization trial (OT)

The *OT* tested the operating conditions to optimize bread quality when performing gradual water addition during kneading. The second-order model was the most appropriate for fitting the data; R^2 and adjusted R^2 showed high values, 0.771 and 0.638, respectively. The p value of the effectiveness of the model was 9.174 10^{-5}, and the lack-of-fit of the model was not significant; hence, RSM sufficiently estimated the bread specific volume when testing the four selected variables.

The first-order ($p = 0.048$) and second-order ($p = 0.040$) coefficient of W_a significantly influenced the bread specific volume, and W_a significantly interacted with the other variables. The bread specific volume was optimized by combining $W_a > 58\%$ with $w_a < 7.5\%$ (w/water amount to reach 500 BU). Very similar trends were observed for W_a*T and W_a*t; optimization of the bread specific volume was achieved by combining $W_a > 58\%$ with T in the range of 18–22 min, and $W_a > 58\%$ with t in the range of 3–8 min.

The variable w_a showed a significant effect of the second-order coefficient ($p = 0.020$): the bread specific volume was maximized when w_a was close to 5% (w/water amount to reach 500 BU). The interaction w_a*T ($p = 0.008$) showed that, almost independently from T, a w_a value of around 5% significantly increased the bread specific volume. Therefore, the bread specific volume was found to be optimized by the combination of variables in the *OT60:5:20:5* dough samples, that is, with $W_a = 60\%$, $w_a = 5\%$, $T = 20$ min and $t = 5$ min.

3.2. Full factorial trial (FFT)

3.2.1. ^{1}H NMR kinetics during kneading

The ^{1}H FID experiment curves fitting detected the presence of the more rigid population A relaxing in the range of 15.24–15.80 μs while the more mobile population B relaxed in the 349.39–371.76 μs range. The ^{1}H T_2 distributions of the relaxation times showed the presence of four populations identified as populations C, D, E, and F, from the least to the most mobile proton population, respectively. These populations relaxed in the range of 0.22–0.50 ms, 3.22–4.09 ms, 10.03–15.06 ms and 41.84–55.25 ms for populations C, D, E and F, respectively. The relative abundance of population A (popA) + population B (popB) gave 100% of the FID proton signal, whereas the relative abundance of populations C (popC), D (popD), E (popE), and F (popF) gave 100% of the CPMG signal. The prevalent [1]H FID proton population was popA, representing 78.23–80.26% of the total observed protons, whereas popB encompassed 19.83–22.12% of the total protons. In the [1]H T_2 time-frame window, popE was found to be the most abundant, encompassing more than 50% of the total detected protons (51.86–57.11%), followed by popD (25.38–30.36%), popC (8.26–10.54%) and popF (5.40–14.60%). Since the relaxation time of populations B and C overlapped, these proton populations were considered to represent the same protons; hence, only population C was discussed as belonging to the CPMG experiment signal.

The results of the [1]H NMR kinetics obtained during kneading here only focused on the effect of w_a and its interactions with variables T (w_a*T) and W_a (w_a*W_a), since the main effect of T and W_a has already been discussed in Parenti et al. (2021).

Considering the FID signal, popA was significantly ($p = 0.033$) affected by the main effect of the variable w_a. The parameter was significantly lower in both *FFT56:5* and *FFT60:5* compared to *FFT56:0* and *FFT60:0* (79.70% vs 79.75% and 79.06% vs 79.25%) (Fig. 1a). T_A was significantly affected by w_a ($p = 0.001$), w_a*T ($p = 0.003$) and w_a*W_a ($p = 8.140\ 10^{-9}$). The interaction w_a*T showed that w_a determined the highest increase in T_A during kneading, as revealed by the steeper slope that characterized the trends of *FFT56:5* compared to *FFT56:0* and *FFT60:5* compared to *FFT60:0* (Fig. 2b). The interaction w_a*W_a revealed that w_a caused a higher T_A value in *FFT56:5* compared to *FFT56:0* (15.59 μs vs 15.47 μs), as shown by the higher intercept in the former than the latter (Fig. 1b). On the other hand, *FFT60:5* showed a lower T_A value compared to *FFT60:0* (15.47 μs vs 15.50 μs) as demonstrated by the smaller intercept in the former than the latter (Fig. 1b). However, in 60% water-content dough this difference concerned the first five kneading times (3, 6, 9, 12, 15 min), whereas in the last 3 kneading times (18, 21, 24 min) samples almost overlapped, showing similar values of T_A.

Considering the CPMG signal, w_a ($p = 1.800\ 10^{-4}$) and w_a*T ($p = 0.003$) had a significant effect on popC. The interaction w_a*T showed that during kneading popC followed a different trend in the control samples compared to the treated samples. In *FFT56:5-FFT60:5* samples, popC remained almost unchanged as shown by the slope approx. = 0 (Fig. 3a). Conversely, the parameter grew in *FFT56:0-FFT60:0* samples, as shown by the positive slope of these sample trend (Fig. 3a). T_{2C} was not significantly affected by the gradual water addition (Fig. 3a).

PopD was significantly affected by w_a ($p = 1.136\ 10^{-4}$) and w_a*T ($p = 0.002$). In the first five kneading intervals (3, 6, 9, 12, 15 min), w_a significantly increased popD in both *FFT56:5* and *FFT60:5* compared to *FFT56:0* and *FFT60:0*, respectively. From the sixth point (18 min) up to the end of the process (24 min), *FFT56:5* almost overlapped with *FFT56:0* and *FFT60:5* with *FFT60:0* (Fig. 2b). T_{2D} was significantly affected by w_a*W_a ($p = 0.046$). *FFT56:5* showed a lower value of the parameter compared to *FFT56:0* (3.45 ms vs 3.51 ms), whereas *FFT60:5* was not significantly different from *FFT60:0* (3.60 ms vs 3.57 ms). In Fig. 3b this effect is revealed by the lower intercept of *FFT56:5* compared to *FFT56:0*, and by the same value of the *FFT60:5* and *FFT60:0* intercepts.

PopE was significantly affected by w_a ($p = 1.247\ 10^{-4}$), w_a*T ($p = 0.0148$) and w_a*T^2 ($p = 0.049$). The interactions w_a*T and w_a*T^2 showed that during kneading popE followed a different trend in the treated samples compared to the control samples. In *FFT56:0* and *FFT60:0* popE grew following a parabolic curve and reached a maximum at approx. 18 min and 21 min, respectively. Similarly, *FFT56:5* and *FFT60:5* showed a parabolic increase in popE, but characterized by a lower positive slope compared to *FFT56:0* and *FFT60:0*, respectively. The maximum values of popE in the above samples were similar to the control samples, but they reached them at longer kneading times: *FFT56:5* at 21 min and *FFT60:5* at 24 min of kneading (Fig. 2c). T_{2E} showed a significant effect of w_a ($p = 0.004$) and w_a*W_a ($p = 0.0144$). In *FFT56:5* the parameter was significantly lower than in *FFT56:0*, whereas in *FFT60:5* T_{2E} was not significantly different from that of *FFT60:0* (Fig. 3c).

PopF was significantly affected by w_a ($p = 5.981\ 10^{-5}$) and w_a*T ($p = 0.004$). At the beginning of kneading, w_a reduced popF, whereas at the end the samples with and without the gradual addition of water showed more similar values to their respective control (Fig. 2d). T_{2F} was not significantly affected by w_a (Fig. 3d).

3.2.2. [1]H NMR molecular mobility of dough samples at the optimum kneading time

The effect of W_a and w_a on [1]H NMR mobility and dynamic was tested on the dough samples kneaded to the optimum kneading time (see 3.1 paragraph, Table 1). Both W_a and w_a variables significantly affected the [1]H NMR parameters, whereas their interaction did not show significant effects. W_a significantly affected popA, popD, popE, and popF, and T_{2C} and T_{2E}. Compared to the 60% water-content doughs (*FFT60:0-FFT60:5*), the 56% water-content doughs (*FFT56:0-FFT56:5*) were characterized by higher popA and popD. Conversely, the *FFT60:0-FFT60:5* samples showed significantly higher popE and popF compared to *FFT56:0-FFT56:5*. W_a also affected T_{2C} and T_{2E}. T_{2C} was significantly higher in the 56% water-content samples (*FFT56:0-FFT56:5*) than the 60% water-content dough samples (*FFT60:0-FFT60:5*), whereas the

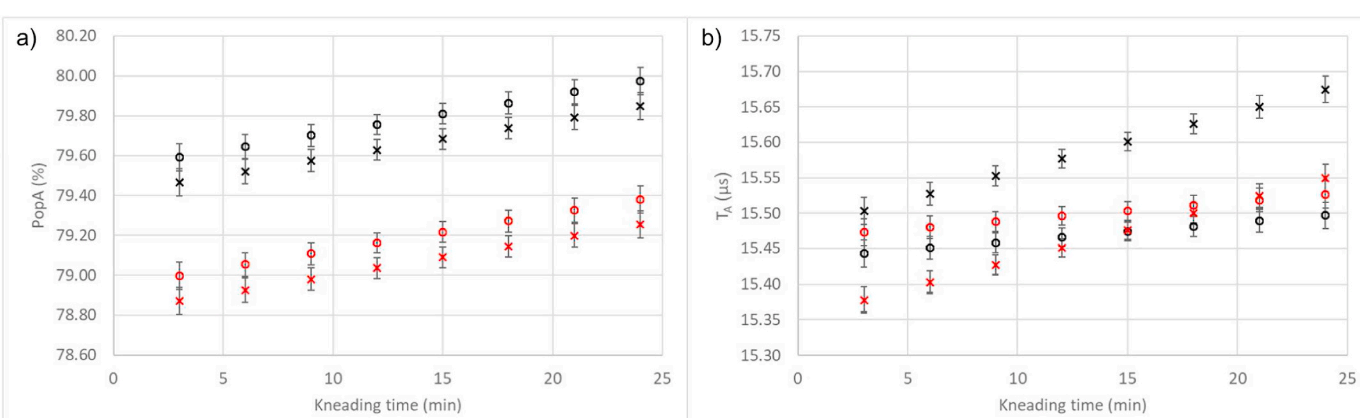

Fig. 1. Kinetic models of [1]H NMR proton populations in WWF doughs during kneading obtained from single-pulse Free Induction Decay (FID) experimental data: a) relative abundance of population A (popA, %) and b) relaxation time of population A (T_A, μs). The symbol "o" represents *FFT56:0* (control WWF dough at 56% water content), "o" *FFT60:0* (control WWF dough at 60% water content), "x" *FFT56:5* (WWF dough at 56% water content with gradual 5% water addition during kneading) and "x" *FFT60:5* (WWF dough at 60% water content with gradual 5% water addition during kneading). The black bars represent the 95% confidence interval of the model.

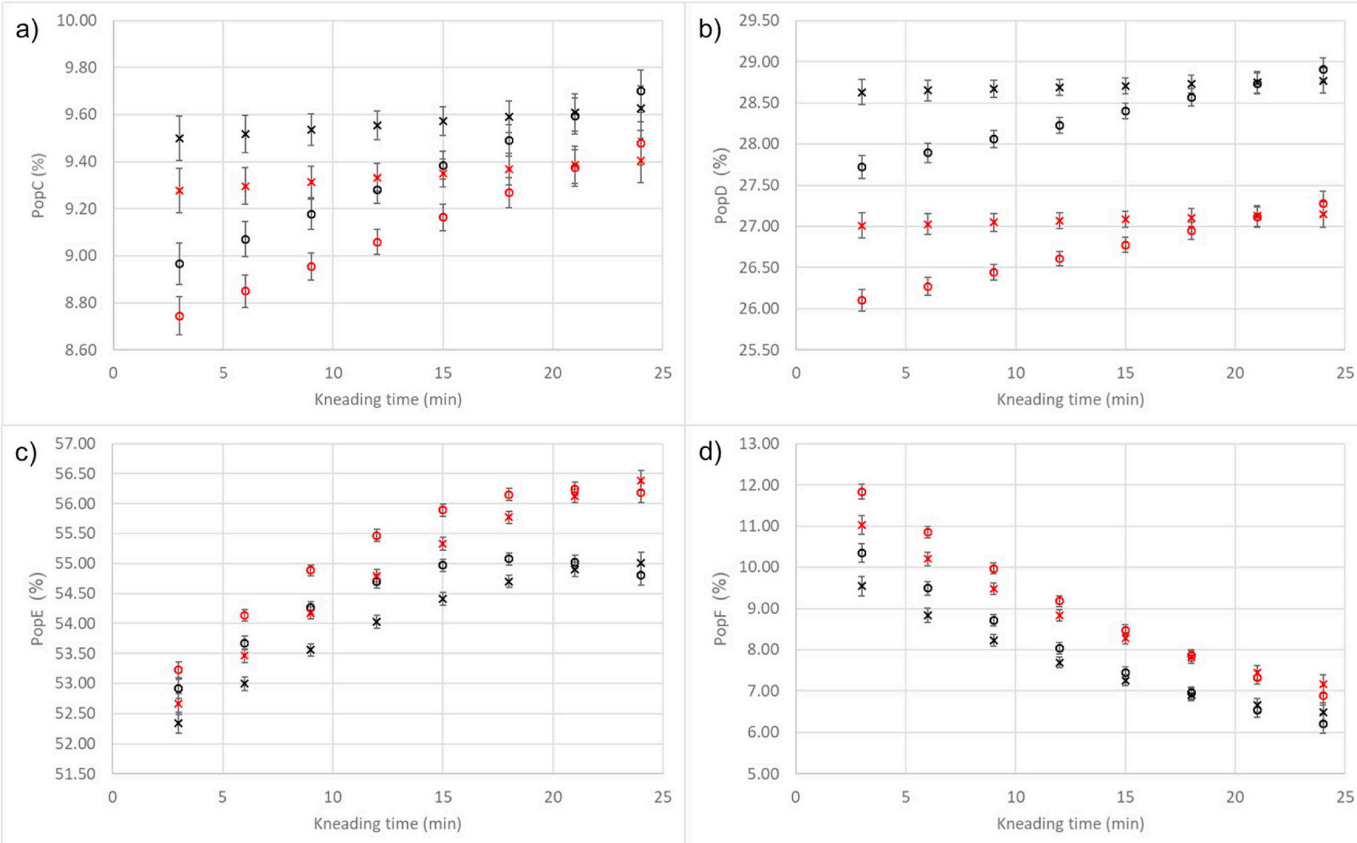

Fig. 2. Kinetic models of [1]H NMR proton populations in WWF doughs during kneading obtained from CPMG pulse sequence experimental data: a) relative abundance of population C (popC, %), b) relative abundance of population D (popD, %), c) relative abundance of population E (popE, %) and d) relative abundance of population F (popF, %). The symbol "o" represents *FFT56:0* (control WWF dough at 56% water content), "o" *FFT60:0* (control WWF dough at 60% water content), "x" *FFT56:5* (WWF dough at 56% water content with gradual addition of 5% water during kneading) and "x" *FFT60:5* (WWF dough at 60% water content with gradual addition of 5% water during kneading). The black bars represent the 95% confidence interval of the model.

opposite result was obtained for T_{2E}.

The variable w_a significantly affected popA, popF, and T_A. The *FFT56:5-FFT60:5* samples were characterized by a significantly lower popA and popF, and by a significantly higher T_A compared to the *FFT56:0-FFT56:5*.

3.2.3. Dough rheology

The Alveograph results are shown in Table 1. Both variables W_a and w_a showed a significant main effect on the dough rheological properties.

All the Alveograph parameters were significantly affected by W_a. The *FFT60:5-FFT60:0* samples showed a decrease in both dough tenacity - *P* of approx. 36% and flour strength - *W* of approx. 30% compared to the *FFT56:5-FFT56:0* samples. Conversely, the *FFT60:5-FFT60:0* samples were characterized by an increase in both extensibility - *L* of approx. 22% and swelling index - *G* of approx. 11% compared to the *FFT56:5-FFT56:0* samples. The *P/L* value in the *FFT60:5-FFT60:0* samples was approx. 50% lower compared to the *FFT56:5-FFT56:0* samples.

It was interesting that the effects of the variable w_a on *L* and *G* were, although relatively smaller, similar to those of the variable W_a. The variable w_a significantly increased *L* (by approx. 11%) and *G* (by approx. 5%), whereas it decreased *P/L* (by approx. 10%). The effect observed on *W* was the opposite to what was obtained in the *FFT60:5-FFT60:0* dough samples, since w_a caused a significant increase (by approx. 7%) in the flour strength. *P* was not significantly affected by the variable w_a.

3.2.4. Bread quality

The bread quality results are shown in Table 2. W_a caused a significant main effect on bread quality, and the interaction W_a*w_a significantly impacted crumb moisture (expressed as the crumb moisture - dough moisture ratio). On the other hand, w_a did not display significant effects. The variable W_a significantly impacted the bread specific volume, the crumb specific volume and the bread crumb texture.

The *FFT60:5-FFT60:0* samples showed a significantly higher specific volume (3.15 ± 0.04 L/kg) compared to the *FFT56:5-FFT56:0* (2.99 ± 0.07 L/kg) samples. The same trend could be outlined for crumb specific volume, since the parameter showed a value of 3.24 ± 0.10 L/kg in the *FFT60:5-FFT60:0* samples, which resulted significantly higher than that of the *FFT56:5-FFT56:0* samples, 3.07 ± 0.17 L/kg.

Regarding the crumb texture, the higher the total water content, the better the bread quality. A significant enhancement of crumb cohesiveness from 0.264 ± 0.029 to 0.309 ± 0.012 was obtained when the level of water was increased from 56% to 60%. Similarly, the *FFT60:5-FFT60:0* samples showed significantly increased springiness, from 0.752 ± 0.036 mm to 0.793 ± 0.024 mm.

The effect of the interaction W_a*w_a on crumb moisture - dough moisture ratio showed that the gradual addition of water had a different impact on the parameter as a function of total water content. At the lowest water content, a significant increase of the parameter was obtained in *FFT56:5* compared to *FFT56:0* (95.95 ± 0.50 vs 95.08 ± 0.50) whereas no significant differences were observed at the highest water content between *FFT60:0* and *FFT60:5* (96.19 ± 0.50 vs 95.40 ± 0.50).

Crust moisture - dough moisture ratio, bread hardness, and bread chewiness were not significantly impacted by the tested variables.

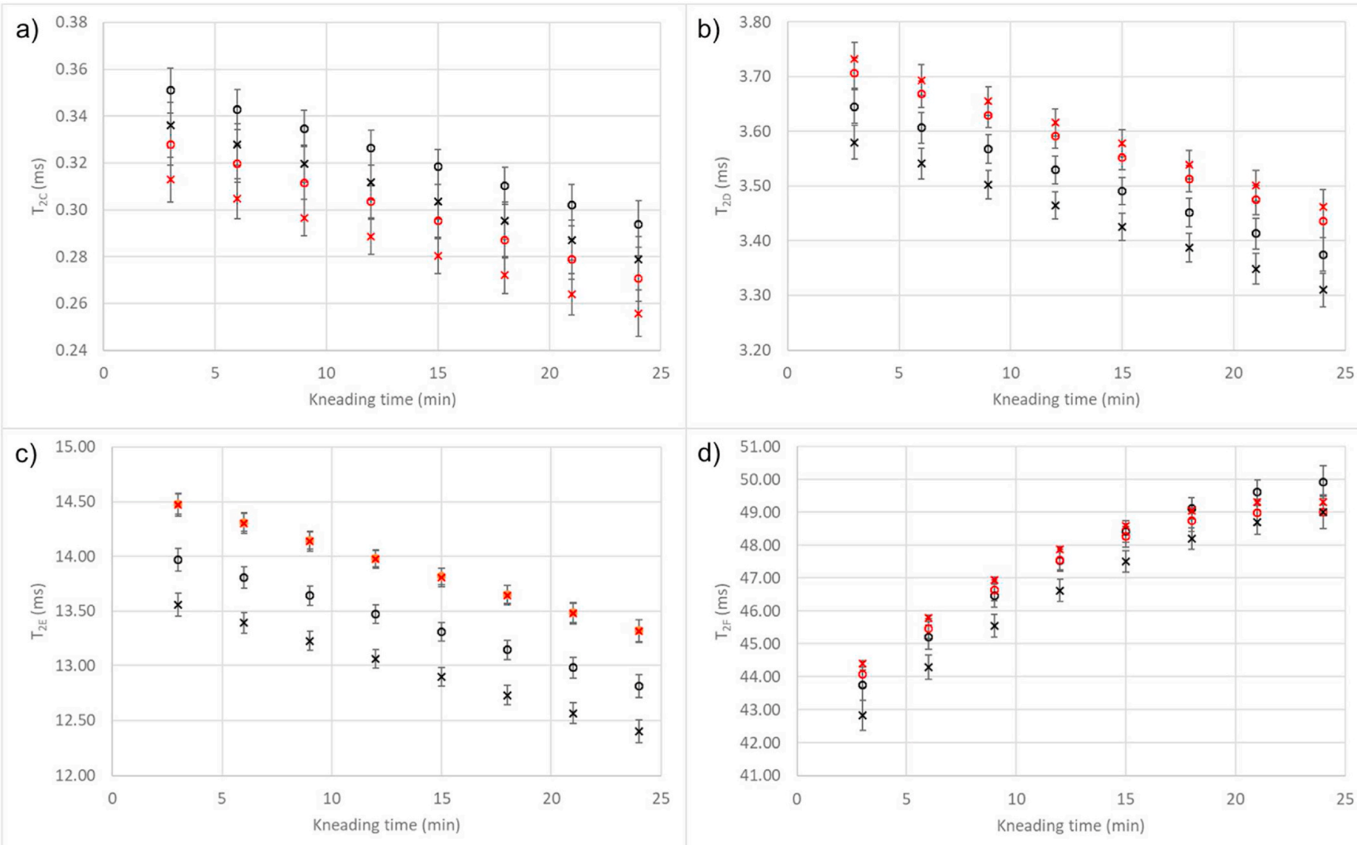

Fig. 3. Kinetic models of ^{1}H NMR proton populations in WWF doughs during kneading obtained from CPMG pulse sequence experimental data: a) relaxation time of population C (T_{2C} ms), b) relaxation time of population D (T_{2D}, ms), c) relaxation time of population E (T_{2E}, ms) and d) relaxation time of population F (T_{2F}, ms). The symbol "o" represents *FFT56:0* (control WWF dough at 56% water content), "o" *FFT60:0* (control WWF dough at 60% water content), "x" *FFT56:5* (WWF dough at 56% water content with gradual 5% water addition during kneading) and "x" *FFT60:5* (WWF dough at 60% water content with gradual 5% water addition during kneading). The black bars represent the confidence interval of the model.

Table 1
^{1}H NMR characterization at optimum kneading time (20 min) and Alveograph parameters of *FFT* dough samples.

^{1}H NMR parameters	Samples				SE	p W_a	p w_a	p W_a*w_a
	FFT60:5	FFT60:0	FFT56:5	FFT56:0				
***popA* (%)**	79.18^{ax}	79.35^{ay}	79.73^{bx}	80.06^{by}	0.25	***	*	ns
T_A (μs)	15.53^{x}	15.50^{y}	15.61^{x}	15.47^{y}	7.45 10^{-5}	ns	*	ns
***popC* (%)**	9.17	9.44	9.51	9.75	0.37	ns	ns	ns
T_{2C} (ms)	0.27^{a}	0.24^{a}	0.28^{b}	0.33^{b}	0.04	*	ns	ns
***popD* (%)**	26.72^{a}	27.05^{a}	29.07^{b}	28.94^{b}	0.37	***	ns	ns
T_{2D} (ms)	3.41	3.42	3.37	3.46	0.09	ns	ns	ns
***popE* (%)**	56.39^{a}	56.18^{a}	54.78^{b}	55.14^{b}	0.37	***	ns	ns
T_{2E} (ms)	13.43^{a}	13.46^{a}	12.53^{b}	12.85^{b}	0.27	***	ns	ns
***popF* (%)**	7.73^{a}	7.32^{a}	6.65^{b}	6.18^{b}	0.49	***	ns	ns
T_{2F} (ms)	48.13^{x}	48.79^{y}	48.05^{x}	51.10^{y}	1.45	ns	*	ns
Alveograph parameters	**Samples**				***SE***	***p W_a***	***p w_a***	***p W_a*w_a***
	FFT60:5	***FFT60:0***	***FFT56:5***	***FFT56:0***				
***P* (mm H_2O)**	35.50^{a}	36.25^{a}	56.00^{b}	56.00^{b}	1.89	***	ns	ns
***L* (mm)**	56.00^{ax}	51.25^{ay}	46.50^{bx}	41.50^{by}	0.77	***	**	ns
P/L	0.63^{ax}	0.71^{ay}	1.22^{bx}	1.36^{by}	0.08	***	*	ns
***G* (mm)**	16.65^{ax}	15.98^{ay}	15.15^{bx}	14.30^{by}	0.32	***	**	ns
***W* (10^{-4} J)**	58.50^{ax}	55.50^{ay}	85.00^{bx}	78.00^{by}	2.77	***	*	ns

^{1}H NMR parameters: relative abundance of population A (popA, %), population C (popC, %), population D (popD, %), population E (popE, %) and population F (popF, %); relaxation time of population A (T_A, μs), population C (T_{2C}, ms), population D (T_{2D}, ms), population E (T_{2E}, ms) and population F (T_{2F}, ms). Alveograph parameters: dough tenacity (*P*, mm H_2O), dough extensibility (*L*, mm), swelling index (*G*, mm), flour strength (*W*, 10^{-4} J) and ratio between dough tenacity and extensibility (*P/L*). Data are expressed as mean ± *SE*. *p W_a*, *p w_a* and *p W_a*w_a* represent effects of the tested factors: W_a refers to total dough water, w_a to the addition of 5% water (w/water amount to reach 500 BU in the Farinograph test) after 5 min of kneading, and W_a*w_a represents the interaction between these factors. *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. "ns" indicates no significant differences at $p < 0.05$. Means in a row with different superscripts are significantly different ($p < 0.05$). Specifically, "a" and "b" refer to the main effect of W_a, whereas "x" and "y" refer to the main effect of w_a.

Table 2
Quality characteristics of *FFT* bread samples.

Parameter	Samples				SE	p W_a	p w_a	p W_a*w_a
	FFT60:5	FFT60:0	FFT56:5	FFT56:0				
Bread specific volume (L/kg)	3.15^a	3.14^a	3.00^b	2.98^b	0.06	**	ns	ns
Crumb specific volume (L/kg)	3.32^a	3.16^a	3.12^b	3.02^b	0.13	*	ns	ns
Crumb moisture/dough moisture	95.40	96.19	95.95	95.08	0.50	ns	ns	*
Crust moisture/dough moisture	50.70	51.24	49.57	49.36	1.44	ns	ns	ns
Hardness (N)	2.917	3.870	4.250	4.697	1.673	ns	ns	ns
Cohesiveness	0.318^a	0.300^a	0.271^b	0.258^b	0.023	*	ns	ns
Springiness (mm)	0.781^a	0.805^a	0.767^b	0.738^b	0.031	*	ns	ns
Chewiness (N mm)	1.136	0.933	0.883	0.911	0.418	ns	ns	ns

Data are expressed as mean ± *SE*. *p* W_a, *p* w_a and *p* W_a*w_a represent effects of the tested factors: W_a refers to total dough water, w_a to the addition of 5% water (w/water amount to reach 500 BU in the Farinograph test) after 5 min of kneading, and W_a*w_a represents the interaction between these factors. *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. "ns" indicates no significant differences at $p < 0.05$. Means in a row with different superscripts are significantly different ($p < 0.05$). Specifically, "a" and "b" refer to the main effect of W_a.

4. Discussion

In the bread-making process, generally all the ingredients are added together before kneading (Zhou et al., 2014). However, Guerrini et al. (2019) found that bakers using WWF gradually add water during kneading. We investigated the effect of this practice coupled with the effect of the total water content.

The variable w_a significantly affected the ^{1}H NMR molecular kinetics of the WWF dough during kneading. The relaxation times of populations C and F were not significantly affected by w_a (Fig. 3a and d). This means that the mobility of the protons of popC, assigned to some CH protons of amorphous starch and CH protons of gluten in the sheets with little contact with the confined water (Bosmans et al., 2012), and those of popF, attributed to the "free" water in the dough system (Li et al., 2015.; Lu & Seetharaman, 2013; Parenti et al., 2021; Wang et al., 2017), was not affected by the lower availability of water in the first minutes of kneading.

The interaction w_a*T had a significant effect on the relative abundances of all the CPMG populations (popC, popD, popE and popF) (Fig. 2), which include the protons of flour constituents located in different structural environments and the protons of water bound to the flour constituents. The variable w_a acted as a "destabilization factor" on these population abundances, since in the first minutes of kneading when the gradual water addition was performed, they showed different kinetics compared to the control samples obtained without the gradual water addition. Indeed, as a function of the gradual water addition, at the beginning of kneading CPMG populations were different to the control sample, but as kneading proceeded the differences decreased (Fig. 2). PopE was the dominant CPMG population, corresponding to starch extra-granular water and water in the gluten matrix (including mobile protons of water in exchange with hydroxyl protons of starch on the granule surface, and water protons surrounding the sheets in exchange with gluten protons) (Bosmans et al., 2012). The significant increase in popE during kneading was interpreted as the progressive hydration of the gluten proteins by the most mobile water protons, followed by the development of the gluten matrix (Parenti et al., 2021). In the w_a samples, popE showed a lower rise than in the control samples (Fig. 2c): the lower initial availability of water seemed to have slowed down the gluten hydration and the subsequent protein cross-linking, making this population achieve the same values as the control samples at longer kneading times. PopC and popD were higher in the w_a samples than in the control doughs at the beginning of kneading, and remained almost unchanged during processing, whereas in the control samples these parameters linearly increased (Fig. 2a–b). The above results may reveal that the molecular domains involved in popC (i.e., some CH protons of amorphous starch and CH protons of gluten in the sheets with little contact with the confined water) and popD (i.e., hydroxyl protons of intra-granular water and starch, but also some CH protons of gluten and exchanging protons of confined water and gluten) (Bosmans et al., 2012) were more hydrated at the beginning of kneading and reached the final hydration faster in the w_a samples than in the control samples.

PopF, attributed to "free" water (Li et al., 2015; Lu & Seetharaman, 2013; Parenti et al., 2021; Wang et al., 2017), was lower than the control doughs during kneading; although the difference was higher in the first minutes of processing, it was also significant at the optimum kneading time (Fig. 2d).

PopA, assigned to the protons of crystalline starch, amorphous starch and gluten not in contact with water (Bosmans et al., 2012), showed a significantly lower value in the w_a samples than in the control samples (Fig. 1a). A lower water availability at the beginning of kneading may have reduced the absorption of water by starch granule, decreasing the extent of the hydration of the amorphous starch regions. The interaction w_a*W_a showed a significant effect on T_A, T_{2D}, and T_{2E} (Fig. 1b; Fig. 3b and c). At the lower water content, w_a is able to produce a higher mobility in the starch fraction (i.e., higher T_A in 56% water content samples), leading to the hypothesis that the lower the water availability at the beginning of kneading, the weaker the interaction of the water with starch granules. Conversely, the decrease of T_{2D} and T_{2E} may reveal a higher strength of the protons bound to the flour constituents when the gradual water addition is performed, disclosing that the affinity of these proton populations for water molecules increased in the presence of low water availability at the beginning of kneading. Yang et al. (2019) studied different water addition methods in noodle doughs (67% of water content on total wet basis): the water-holding capacity increased when water was added several times during kneading as proven by the reduction of the CPMG relaxation times (Yang et al., 2019). Results of T_{2D} and T_{2E} in lower water-content doughs were consistent with Yang et al. (2019), whereas the other CPMG relaxation times were not significantly affected. Different results may arise from different experimental conditions such as raw material, total moisture content of the dough and the water addition method.

The ^{1}H NMR results at the optimum kneading time further confirmed the above interpretation (Table 1). PopF appeared significantly reduced (approx. 1.73%) by w_a, showing that this technique is able to improve the water-affinity of the flour constituents. At the optimum kneading time, the w_a doughs were characterized by a significantly lower popA and popF, and by a higher T_A. The main effect of W_a at the optimum kneading time was consistent with Parenti et al. (2021) (Table 1). The 56% water-content doughs were characterized by a higher popA, popD, and T_{2C}, whereas the 60% water-content doughs showed greater popE, popF and T_{2E}.

The dough rheology was significantly impacted by W_a and w_a (Table 1). WWF doughs are generally difficult to work since they are characterized by poor rheological properties: high tenacity and stickiness, low extensibility (Guerrini et al., 2019; Parenti et al., 2019). Adding a higher water amount than that predicted by the Farinograph greatly improved dough extensibility (*L*), dough swelling index (*G*) and reduced dough tenacity (*P*), but negatively affected the flour strength

(*W*). The rheological effects observed at the highest water level could be explained by the ^1H NMR data: the greater hydration of the gluten proteins (popE), producing a better gluten network development, and the higher amount of “free” water (popF), acting as a plasticizer in the WWF doughs.

The variable w_a produced a significant improvement in the dough extensibility (*L*), swelling index (*G*), *P/L* value and flour strength (W_a). The molecular insight revealed by the ^1H NMR technique showed that w_a reduced popF, which could reveal an increase in the dough water-affinity. Yang et al. (2019) showed that adding the water bit by bit during kneading significantly increased the degree of protein polymerization and improved the dough elasticity.

Therefore, both w_a and W_a significantly improved WWF dough workability as shown by Alveograph parameters. Furthermore, since an improvement of WWF dough stickiness could be empirically assessed in doughs at the highest water content and in doughs prepared with the gradual water addition, further investigations on this issue by means of the standard method would be interesting (Chen & Hoseney, 1995; Tietze et al., 2016).

The variable w_a did not significantly impact bread quality; a significant interaction with W_a (W_a*w_a) was observed on crumb moisture - dough moisture ratio, which showed a higher value in the lowest water-content doughs with the gradual water addition compared to the control. Bread quality was significantly improved by the highest water content, which caused the greatest bread and crumb specific volume and the best crumb texture in terms of cohesiveness and springiness. The ^1H NMR parameters of the highest water-content doughs showed greater hydration of the gluten proteins and a higher amount of “free” water, which is known to act as a plasticizer agent in dough. Therefore, consistently with the RSM and ^1H NMR results and with the literature, an appropriate hydration of WWF significantly increased the dough extensibility, and swelling index, and reduced the dough tenacity and *P/L* ratio; despite the reduction in flour strength, these rheological changes significantly improved the bread quality (Hemdane et al., 2016; Schmiele et al., 2012). Indeed, WWF doughs at the highest water-content showed the greatest bread and crumb specific volume, and the highest springiness and cohesiveness values, which correspond to a significant improvement of bread quality (Zhou et al., 2014). The higher water requirement is probably connected to the presence of fibres which compete with the starch and gluten fractions, altering the water redistribution in the dough (Curti et al., 2013; Khalid et al., 2017). This result probably emphasised the fact that the proper technological evaluation of unrefined flours requires different quality tests compared to refined flours (Parenti, Guerrini, & Zanoni, 2020). Furthermore, the different results for the water absorption value obtained in the Farinograph test compared to that obtained by the Optimization trial showed that kneading conditions as kneading speed, kneading geometry, sample mass, temperature, bread formula etc., may significantly change the flour water requirements. Therefore, the standard reference of dough consistency (500 BU) may not correspond to the water amount able to give the highest bread quality.

The ^1H NMR technique showed that doughs subjected to gradual water addition were characterized by a higher water-affinity that may be related to the significantly improved rheological properties. Probably, these improvements were too small to produce a positive effect on the bread quality but resulted in better WWF dough workability (Guerrini et al., 2019).

5. Conclusions

The gradual water addition significantly changed the proton mobility and dynamics in the dough during kneading, showing that the availability of water in the first minutes of dough processing can significantly change the physical-chemical characteristics of WWF doughs. The ^1H NMR results showed the lowest relative amount of “free” water, enhancing the dough’s water-affinity. Although no significant effects were obtained on bread quality, the gradual water addition significantly improved the dough workability (i.e., higher dough elasticity, swelling index and flour strength), which can account for the widespread use of this practice among bakers.

The WWFs had higher water requirements than the Farinograph value. The higher the dough water content, the better the dough rheology (i.e., lower dough tenacity, higher dough extensibility and swelling index), except for the decrease in the flour strength. Indeed, the ^1H NMR results revealed the greatest hydration of the gluten proteins and the highest amount of “free” water acting as a plasticizer in the dough system. A dough water content of 60% significantly improved the bread quality since the highest bread and crumb specific volumes and the highest crumb cohesiveness and springiness values were obtained.

Therefore, both the gradual water addition and the highest dough water content allowed to improve WWF dough workability, which still represents a bread-making issue due to the poor WWF technological quality and elevated dough stickiness. Combining the gradual water addition during kneading with the inclusion of higher water amount than the Farinograph value could be an interesting strategy to improve the bread-making performance of WWFs. These results encourage further investigations on empirical bread-making practices specifically adopted when using WWFs, in order to give insights into the effects of these techniques on the quality of WWF doughs and breads, promoting their implementation and the consumption of fibre-enriched breads.

CRediT authorship contribution statement

Ottavia Parenti: Conceptualization, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing. **Eleonora Carini:** Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision. **Mia Marchini:** Investigation, Data curation, Writing - original draft, Writing - review & editing. **Maria Grazia Tuccio:** Investigation, Data curation, Writing - original draft, Writing - review & editing. **Lorenzo Guerrini:** Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision. **Bruno Zanoni:** Conceptualization, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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