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Effects of Fat and Sugar on Dough and Biscuit Behaviours and their Relationship to Proton Mobility Characterized by TD-NMR

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Abstract

This study aimed to analyse the effect of large variations in fat and sucrose contents of biscuit formulation on dough rheology, biscuit quality and proton mobility. Control dough (full fat and sucrose) and 25 and 50% fat and sucrose-reduced doughs were elaborated. Rheological properties of dough were determined. Dough cooking behaviour and biscuit quality were evaluated. Fat reduction produced an increase in the consistency and elastic properties of the dough. The decrease in sucrose content affected to a lesser extent the rheological properties at room temperature, although it produced significant changes during dough baking. The biscuit quality decreased with fat and sucrose reductions. The reduction of fat or sucrose produced gluten hydration and extra interactions and subsequent cross-linking during baking, and a reduction of sucrose led to the swelling and partial gelatinization of starch during baking. A dipolar reversed eco NMR sequence was applied to determine changes in the amount and mobility of solid and mobile components both in dough and biscuits. TD-NMR results allowed corroborating the proposed hypotheses about the effects on dough and biscuit characteristics as a consequence of reduction of main ingredients, as fat and sucrose. The redistribution of water after baking can be correlated with biscuit factor and breaking force.

Keywords Biscuits · Quality · Rheology · Fat · Sucrose · NMR

Introduction

Short dough biscuits are characterized by high fat–high sugar content and low amount of water, where the role of sugar and fat within the dough are well understood. Sugar plays an important role in the dough properties and in the quality and taste of the baked product. It contributes to reduce dough viscosity and relaxation time, while promoting biscuits spreading and reduction of its thickness and weight. High sugar biscuits are characterized by a highly cohesive dough and crispy texture (Maache-Rezzoug et al. 1998). Fat acts as lubricant on the dough, contributing to the plasticity and dough softening. Further, it plays an important role on texture and flavour and contributes to increase length and reduces biscuit thickness and weight, which are characterized by a friable structure, easy to break (Coultate 1989).

Biscuit quality is evaluated through parameters such as colour, surface cracking pattern, pieces height, diameter and crispness (measured as the force necessary to produce the total break of the structure). Biscuit quality is related to dough viscoelastic properties and it's behaviour during the baking process. In turn, rheological properties depend on the formulation and on ingredient physical characteristics.

The reduction of sugar or fat generates technological troubles in biscuit manufacturing, which could result in the loss of acceptability by consumers. Among the most notorious technological drawbacks, changes on dough rheological properties result in excessive adhesion to work surfaces, and changes

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in baked product shape, colour, density and texture are found. Also, the reduction of fat or sugars generates inconvenience in the productive process and in the product sensory aspect, both visual and in flavour (Maache-Rezzoug et al. 1998).

Time domain nuclear magnetic resonance (TD-NMR) has been successfully applied to food science (van Duynhoven et al. 2010), as the mobility of water in food systems can be determined by studying the protons spin–spin relaxation time (T_2). Different baked products are already studied with NMR, such as bread (Curti et al. 2011) or cakes (Luyts et al. 2013). Assifaoui et al. (2006a, b) studied water mobility in a biscuit dough system and its relation to rheological properties as a function of the water content and temperature. In a previous work (Serial et al. 2016), the effects of fibre addition on dough and biscuit proton mobility was analysed by temperature-dependent TD-NMR experiments in an open system, where water evaporation took place.

Several studies have described and explained the role of the components on the dough and biscuit quality (Maache-Rezzoug et al. 1998; Jacob and Leelavathi 2007; Pareyt and Delcour 2008; Pareyt et al. 2009; Kweon et al. 2014). However, the consequences of significant changes in the proportion of main ingredients such as fat and sucrose have not been extensively studied by TD-NMR. In particular, the amount of solid components is often not determined accurately due to the limitation of NMR equipment to acquire fast decaying signals. In this work, we introduce a mixed magic solid eco sequence, with CPMG acquisition that enables the acquisition of both solid and mobile components. This pulse sequence has been successfully applied for the determination of the fraction of crystalline and amorphous phases in polymer science (Maus et al. 2006). It is hoped that this knowledge will allow a better understanding of the system that can contribute to obtain products with lower contents of these ingredients without significant changes in the sensorial properties. The aim of this work was to study the effect of large variations in fat and sucrose content of biscuit formulation on proton mobility and the water status as new elements to understand the changes in dough rheology and biscuit quality.

Materials and Methods

Dough and Biscuit Preparation

Wheat grains from Baguette 10 cultivar were ground on a four-roller laboratory mill (Agromatic AG AQC 109, Laupen, Switzerland). Flour had the following composition: protein = 8.05% (db), moisture = 14.18%, ash = 0.60% (db) and damaged starch = 9.72%.

Biscuits were prepared according to Serial et al. (2016). The ingredients used in control samples were flour (45%), caster sugar (27%), vegetable shortening (20.2%), powdered skim milk (2.25%), $NaHCO_3$ (0.50%), NaCl (0.42%) and 6% of water. In order to evaluate the effect of fat and sucrose reduction on biscuits formulation, different samples were produced with 50 and 75% of fat (50F and 75F, respectively) and sucrose (50S and 75S, respectively) of control formulation and keeping constant the amount of other ingredients (Table 1).

The dough was manually sheeted to a 0.7 cm thickness and cut in circles of 4.5 cm of diameter and placed on an aluminium sheet. Six pieces were obtained from each batch. The biscuits were baked for 11 min at 180 °C in a forced convection oven (Pauna, Argentina) equipped with a temperature controller. The six biscuit pieces were placed in the centre of the oven and baked. After baking, the biscuits were cooled down to room temperature prior to analysis (~20 °C). The baking tests and associated measurements were replicated three times for each sample.

Dough Testing

Stress Relaxation Test A texture analyser "Instron" Universal Testing machine (INSTRON 3342, USA) with a load cell of 500 N with a 35-mm diameter cylindrical probe was used to conduct the tests. A moulded dough sample of 31-mm diameter and 9-mm height was placed on a flat base directly under a cylindrical plate and compressed 30% (2.7 mm) of its original height with a crosshead speed of 0.5 mm/s. This constant compressive strain was applied to the sample for 120 s (Leung et al. 1983). Sample, base and probe were lubricated with liquid Vaseline to minimize frictional effects. Each test was conducted at room temperature (25 °C) with three replications. Moulded dough was left to rest for an hour at room temperature before the test. Non-linear regression analysis was performed on stress relaxation data to find the constants of the stress relaxation models (SIGMAPLOT 10, Systat Software, Inc., Germany).

Preliminary stress relaxation analyses were conducted to determine the number of the terms of the Maxwell model. Results showed that the model selected as the mechanical analogue for the relaxation behaviour of biscuits dough

Table 1 Dough formulations. Control: full fat and sucrose sample. *F* fat, *S* sucrose. Seventy-five and 50 refer to level of incorporation

Ingredients	Control	75F	50F	75S	50S
Flour (g)	45	45	45	45	45
Caster sugar (g)	27	27	27	20.25	13.5
Vegetable shortening (g)	20.2	15.15	10.1	20.2	20.2
Powdered skim milk (g)	2.25	2.25	2.25	2.25	2.25
$NaHCO_3$ (g)	0.50	0.50	0.50	0.50	0.50
NaCl (g)	0.42	0.42	0.42	0.42	0.42
Water (g)	6	6	6	6	6

included one Maxwell element with a residual spring in parallel (Eq. 1).

$$\sigma_{\downarrow} t = \varepsilon_{\downarrow} 0 * \left(E_{\downarrow} 1 \;\; e^{\uparrow} \left(-t/\tau + E_{\downarrow} 2\right)\right. \tag{1}$$

This equation can be represented by a Maxwell model consisting of two elastic elements (springs) and one viscous element (dashpot). The Maxwell element consists in one spring and one dashpot in series and corresponds to the exponential term. The initial deformation (ε_0) corresponded to 30% of the dough height. τ is the relaxation time ($\tau = \eta/E_1$). Four parameters were informed: E_1 (Maxwell elastic modulus), E_2 (equilibrium modulus at infinite time), the maximum force reached during compression (MF) and the viscous component (η).

Dynamic Rheological Measurements A controlled stress rheometer RHEOPLUS/32 (Anton Paar, Germany) was used in the oscillatory tests. The rheometer was equipped with a 25-mm parallel plate measuring geometry. Measurements were done according to Blanco Canalis et al. (2017).

The linear viscoelastic region was determined by a strain sweep at a frequency of 1 Hz. This was determined to be up to 0.1%, and a target strain of 0.05% was used in all the experiments. Frequency sweep was performed in the range from 0.01 to 20 Hz at 25 °C. A dough sample was obtained by a cylindrical biscuit cutter and placed in the lower plate. The upper plate was lowered until the final gap (2.0 mm), and the excess of dough protruding from the edge of the plate was carefully trimmed. Dehydration was prevented by adding low-viscosity silicone around the plate edges. The sample was rested for 5 min before measurement was conducted.

Temperature scans were performed to investigate the main transformations in the dough undergoing heating. After equilibrating at the initial temperature (25 °C) for 5 min, the sample was heated at a rate of 4 °C/min to the final temperature of 100 °C. During heating, the sample was sheared at a 10 Hz frequency and a strain of 0.05%. A static force of 500 mN (optimum determined in preliminary test) was applied to allow the upper plate to be in contact with sample during the temperature scan.

A fresh sample was loaded for each measurement. Results were expressed in terms of the storage modulus (G'), loss modulus (G'') and tan δ (G''/G'). Two fresh samples of dough were measured and dough elaborated at least by duplicate to ensure reliable results.

Dynamic Diameter Measurements In order to monitor changes on biscuit dimensions during baking, biscuit videos were taken during baking using a digital camera (Sony DSC-W320, Japan). Measurements were done according to Blanco Canalis et al. (2017). The AVI files obtained were analysed by ImageJ software (National Institutes Health, USA) to obtain biscuit diameter, thickness and spread rate at 30-s intervals after calibration of the image using the metal reference. Two parameters were determined from these profiles: maximum diameter of biscuit pieces reached during baking (MD) and biscuit set time (ST). Biscuit set time was defined as the time at which the biscuit reaches its maximum spread during baking. Four biscuits at each cooking batch and three batches were measured to ensure reliable results. The presented values of MD and ST are the average of all biscuits measured of each sample.

Biscuit Evaluation

Biscuit Factor Six biscuits were obtained by batch and four biscuits (the most homogeneous ones) were selected to determine biscuit factor. To quantify biscuit quality, the term biscuit factor (BF) was introduced as the ratio between the width and thickness of four biscuits taken at random (Serial et al. 2016). Biscuits were elaborated by triplicate to ensure reliable results.

Biscuit Texture The breaking strength was measured with the triple beam snap technique using Instron Universal Testing machine (INSTRON 3342, USA) with a load cell of 500 N. The base gap of the two support beams was adjusted to 36 mm. Each biscuit was centred on the base and the travel distance of the blade was 35 mm; pre-test and speeds were 0.5 mm/s. The average value of four replicates from three independent biscuit production batches was performed. The maximum force to produce the total break of the biscuit was determined and informed as Hardness (H).

Biscuit surface colour in terms of brightness (L^*), redness (a^*) and yellowness (b^*) was measured using a spectrophotometer (Minolta CM-500 d series, Japan). The whiteness values of biscuits were calculated for four biscuits per batch as described by Hsu et al. (2003) as follows (Eq. 2):

$$\mathrm{WV} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \tag{2}$$

Biscuit Surface Characteristics Biscuit surface was analysed by the degree of surface cracking according to Blanco Canalis et al. (2017). Colour images were converted to 8-bits 256 grey level images. Images were segmented and analysed. A circle single field of view (FOV) of 4-mm diameter was evaluated for each image. This FOV captured the majority of the biscuit surface and was taken from the centre of the biscuit. Images were segmented and binarized. Pixels with a grey level higher than the threshold value will be associated to the crack regions, while the remaining pixels to the biscuit surface. The biscuit feature chosen was crack area to total area ratio (the degree of surface cracking) and the fractal dimension (D), which characterize the biscuit surface irregularity, by the texture complexity. The average value of four replicates from three independent biscuit production batches was informed.

NMR Measurements

Proton relaxation studies were carried out on a Bruker minispec mq20 spectrometer operating at a frequency of 20 MHz for 1H. Three formulations were studied: control (full fat and sucrose), 50% fat reduction (50F) and 50% sucrose reduction (50S) in raw and cooked state.

Measurements were performed at 30 °C, where the sample temperature was controlled with a BVT3000 unit (Bruker Corporation) capable of stabilizing the sample temperature with a precision of 0.1 °C. A combined sequence that consists of an initial pulsed magic-sandwich echo followed by a Carr-Purcell-Meiboom-Gill sequence (MSE-CPMG) (Maus et al. 2006) was used. The initial MSE refocuses dipolar interactions of solid-like components, while the CPMG sequence (Carr and Purcell 1954; Meiboom and Gill 1958) was used to measure protons with higher mobility in elastomers, that is, longer transverse relaxation (T_2) values. The pulse width of the 90° and the 180° pulses were 2.62 and 5.18 μs, respectively; 128 scans were collected and averaged with a recycle delay of 3 s. One point every 2 μs was acquired from 0 to 55 μs for the MSE sequence, and 15,000 echoes were acquired with an echo time of 100 μs for the CPMG sequence. The signal decay, $S(t)$, was normalized to each system weight (m) and may be expressed as (Le grand et al. 2007) (Eq. 3)

$$\frac{S(t)}{m} = A\exp\left(-\frac{t^2}{T_{2,S}^2}\right) + \sum_{k=1}^{m} P_k\exp\left(-\frac{t}{T_{2,k}}\right) \qquad (3)$$

where A represents the population of solid components and P_k is the contribution to the signal intensity for each of the relaxation times probed by the CPMG pulse sequence. Even though a continuous distribution of P_k values is obtained, these systems can be characterized by three main populations in biscuit dough, as described by Assifaoui et al. (2006a), which are denoted as C, D and E. The deconvolution procedure is detailed in the Supplementary Material.

Additionally, two-dimensional relaxation maps were recorded as a function of the spin–lattice and spin–spin relaxation times (denoted as T_1–T_2 maps). Measurements were performed at room temperature for the three formulations each of which was analysed in raw and cooked state. The T_1 dimension was acquired with 32 inversion recovery steps, with logarithmically spaced time intervals sufficient to reach equilibrium.

Relaxation time distributions were obtained from the signal decays by applying a numerical algorithm that solves one- and two-dimensional Fredholm integrals of the first kind (Song et al. 2002). In order to quantify the proton populations, the relaxation time distributions were deconvoluted into Gaussian peaks with characteristic mean relaxation times. In this way, the quantity of protons from a certain population is represented by the area of the corresponding Gaussian function. All analyses were done in triplicate and coefficients of variation were less than 10%.

Dough samples were measured before cooking. Once baked, the biscuits were left at room temperature and subsequently analysed. In order to avoid moisture changes, a small portion of the biscuit samples were placed inside 10-mm-diameter NMR tubes and then sealed with parafilm. A closed Teflon sample holder was used for dough samples.

Differential Scanning Calorimetry (DSC)

Thermal analyses were performed with a DSC823e Calorimeter, and the thermograms were evaluated by STARe Default DB V9.00 software (Mettler Toledo, Switzerland). Dough and biscuit samples were ground and approximately 2 g were packaged in filter paper cartridges and defatted by stirring in hexane (1:10, solids/solvent ratio) overnight. After 24 h, the cartridges were recovered and placed in an oven at 40 °C for 24 h. Defatted samples (~20 mg) of biscuits and dough were accurately weighed into 100 mg DSC pans, and distilled water was added with a Hamilton microsyringe to achieve solid/water ratio of 1:2. Pans were hermetically sealed and allowed to rest for at least 12 h at room temperature to equilibrate the starch–water mixture before heating in the DSC. Samples were heated in the calorimeter from 30 to 120 °C at 10 °C/min (Slade et al. 1996). Onset temperatures (To) and the enthalpy of starch gelatinization (ΔHg) were obtained of thermograms and informed. Analysis was done by triplicate to ensure reliable results.

Statistical Analysis

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

Results and Discussion

Effects of Fat and Sucrose Reduction on Dough Rheology

Biscuit dough are known as "short dough" since they are characterized by low elasticity and high viscosity systems. Stress relaxation uniaxial compression can describe the ability of a material to relax an imposed stress under conditions of constant strain. In a stress relaxation test, the sample is given an instantaneous strain and the stress required to maintain the deformation is observed as function of time at constant temperature (~25 °C). Dough relaxation parameters are shown in

Table 2. Fat reduction significantly increased E_1, E_2, σ_0 and η ($p < 0.05$). E_1 increased about 2.4 and 5.3 times and σ_0 2.2 and 4.7 times when fat was reduced to 75F and 50F, respectively. The elastic moduli are parameters for quantifying the stiffness of a material (Peleg 1987); the higher the elastic modulus, the stiffer the samples. Sucrose reduction significantly decreased most of parameters (except $E_{2,}$ which was not modified): E_1, the imposed initial stress and viscous module, indicating a softer dough, which can be related to the relative reduction of the solid content, the increase of relative fat content and the increase of water availability on dough formulation.

Oscillatory tests in the linear viscoelastic region were carried out to obtain information about dough structure (Fig. 1). Frequency sweep showed differences in both elastic and viscous components. Storage (G') and loss (G'') moduli increased with frequency, G' values were always higher than G'' values, indicating the prevalence of elastic properties over the viscous ones. The evolution of both moduli was similar in all samples: G' increased steadily with frequency, while G'' showed a higher increase at higher frequencies. The general profile showed a strong decrease of tan δ between 0.01 Hz and around 0.9 Hz, which is related to a higher increase of G' over G'', indicating the system shift toward elastic properties at lower frequencies. At a higher frequency, tan δ increased in all samples, denoting a more viscous behaviour.

Biscuit dough showed a progressive increase of the modules when fat was reduced, reaching significant differences at the highest reduction value ($p < 0.05$), which was 3.4 (G') and 4.2 (G'') times related to the control sample values (at 1 Hz of frequency, Table 2). On the other hand, sucrose reduction at 50% produced slight reductions for both G' and G'' at 25 °C; however, these changes were not significant ($p > 0.05$). Fat reduction progressively increased the tan δ (G'/G'') values which represents a more viscous than elastic behaviour. Contrary, the sucrose reduction decreased the tan δ values ($p < 0.05$) (Fig. 1, Table 2).

Fat reduction produced a more consistent dough. These observations are in agreement with reports from other authors, who also noticed that biscuit dough becomes harder when its formulation contains lower fat levels (Maache-Rezzoug et al. 1998; Sudha et al. 2007; Pareyt et al. 2009).

In order to evaluate dough viscoelastic behaviour during heating, temperature sweeps from 25 to 100 °C of all samples were carried out. Figure 2 shows G' and G' during temperature sweeps. A continuous decrease of both G' and G'' with heating was observed with an inflection around 50–60 °C, from which the slope declined. In this temperature range, some changes are produced: Fat melts and sucrose completely dissolves. The progressive dissolution of sucrose yields an additional sucrose–water solvent phase. Delcour and Hoseney (2010) estimated that as sucrose dissolves in water, each gram of sucrose per gram water results in about 0.66 mL greater aqueous volume.

Fat reduction increased both G' and G'' compared to the control during the whole temperature sweep (only 50F sample was statistically different, $p < 0.05$), although the curve shapes were similar. However, when sucrose was reduced, some changes were noticed: Until 50–60 °C, the curve shapes and values were similar to control, but for higher temperatures, the value G' was higher in 50S sample, indicating higher elasticity when sucrose was reduced ($p < 0.05$).

The maximum of tan δ indicated the highest viscous to elastic behaviour due to fat melting and sugar solubilization, with a maximum between 50 and 70 °C. Fat reduction increased the tan δ values between 25 and 50 °C, indicating higher viscous to elastic behaviour in this temperature range. However, the maximum value of the tan δ of 50F sample (0.68 ± 0.01) was significantly lower than the control (0.72 ± 0.03) ($p < 0.05$), and no differences were found in 75F (0.70 ± 0.01) (see Table S1 of the Supplementary Material).

On the other hand, sucrose reduction progressively decreased the tan δ values during the whole temperature sweep, suggesting lower viscous to elastic behaviour. The maximum values of the tan δ of 75S (0.65 ± 0.01) and 50S (0.59 ± 0.01) were statistically lower ($p < 0.05$) than the control. Regarding to the temperature at which the maximum tan δ value occurs, only 50S was significantly lower ($p < 0.05$) compared to control sample, showing a faster increase of viscous to elastic behaviour (see Table S1 of the Supplementary Material).

Table 2 Effects of fat and sugar reduction on rheological properties of the dough samples. Stress relaxation behaviour: *E1* elastic component 1, *E2* elastic component 2, *MF* maximum force, η viscous component. Elastic (G') and viscous (G'') modules and tan δ at 25 °C. *C* (Control): full fat and sucrose sample. *F* fat, *S* sucrose. Seventy-five and 50 refer to level of incorporation

Sample	E_1 (Pa)	E_2 (Pa)	MF (*N*)	η (Pa s^{-1})	G' (kPa)	G'' (kPa)	tan δ
C	35.1^{c}	16.3^{c}	302^{c}	336^{c}	142.8^{b}	56.9^{bc}	0.399^{c}
75F	84.0^{b}	28.3^{b}	654^{b}	644^{b}	154.3^{b}	66.3^{b}	0.429^{b}
50F	187.3^{a}	49.9^{a}	1429^{a}	1303^{a}	392.7^{a}	191.7^{a}	0.488^{a}
75S	26.2^{d}	18.2^{c}	281^{d}	230^{d}	130.2^{b}	48.5^{c}	0.372^{d}
50S	23.6^{d}	15.2^{c}	246^{d}	218^{d}	122.5^{b}	43.4^{c}	0.355^{e}

Values in the same column with common letter are not significantly different ($p > 0.05$)

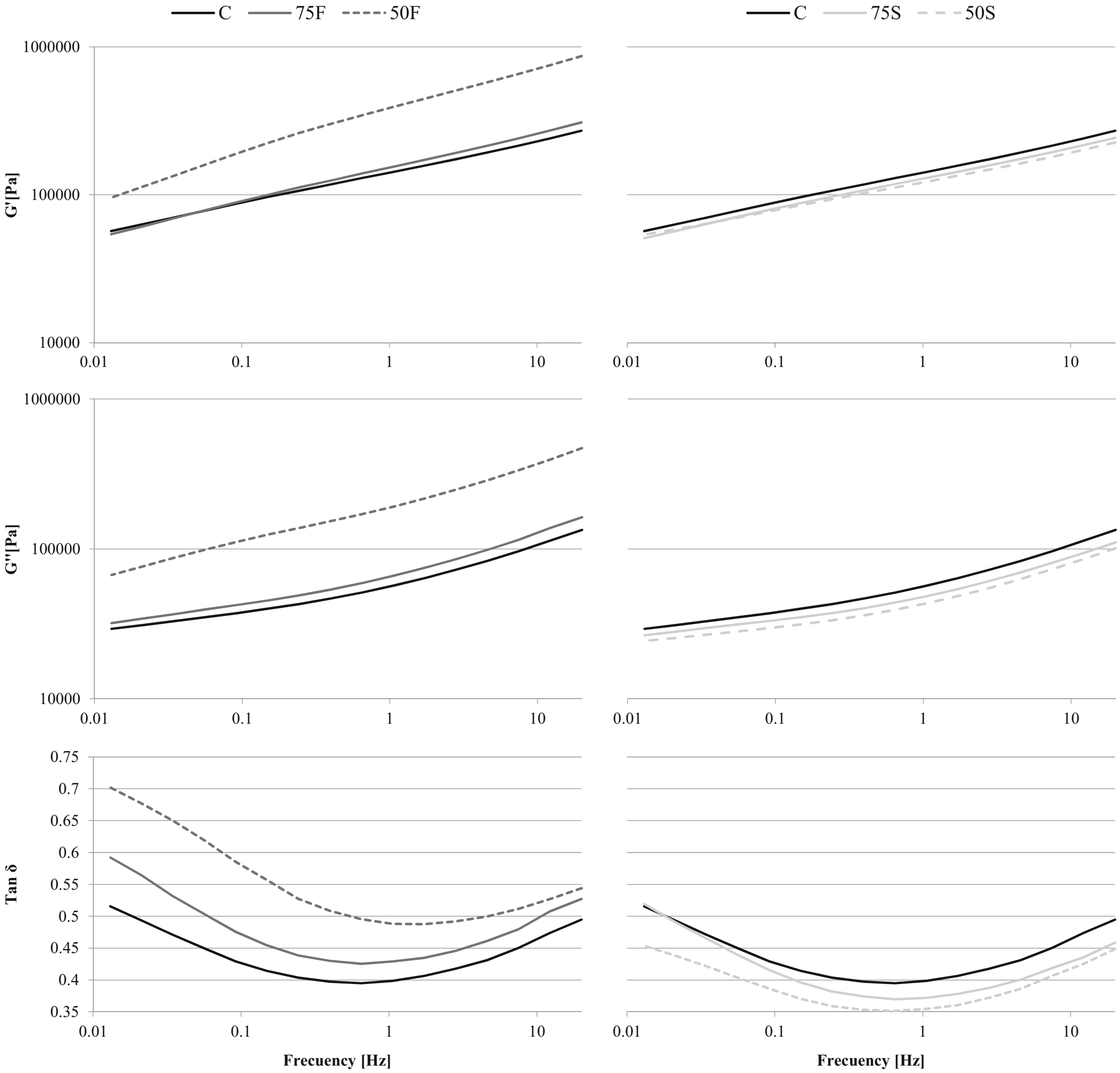

Fig. 1 Effects of fat and sugar reduction on biscuit dough rheology as function of frequency. Elastic (G') and viscous (G'') modules and tan δ at temperature = 25 °C. *C* (Control): full fat and sucrose sample. *F* fat, *S* sucrose. Seventy-five and 50 refer to level of incorporation.

Effects of Fat and Sucrose Reduction on Dynamic Diameter Measurements During Baking

Table 3 shows the biscuit spreading parameters during baking. It is known that longer set times are related with better biscuit quality (Miller and Hoseney 1997; Blanco Canalis et al. 2017).

Fat reduction affected the dough maximum diameter; however, it did not change the set time. The low MD can probably be related to a decrease in the system fluidity when fat melts during heating.

Sucrose reduction produced an important decrease on dough radial expansion capacity during baking, resulting the lowest MD and ST values for 50S.

Changes in the rheological behaviour and in the dynamic diameter measurements during baking could be related to the protein partial hydration and the formation of protein–protein interactions due to a greater availability of water in the system when both fat and sucrose were reduced. Simultaneously, since fat acts as a lubricant in the dough, it is expectable that a reduction in their relative content results in an increase of the dough consistency and the opposite effect when fat increase.

Effects of Fat and Sucrose Reduction on Biscuit Quality

Biscuits with large diameter, tender bite and uniform surface cracking are considered to be superior (Pareyt and Delcour 2008; Delcour and Hoseney 2010).

Biscuit factor (BF) values ranged between 3.4 (50S) and 6.7 (control) (Table 3). Fat and sucrose reduction produced thicker and smaller diameter biscuits. The effect of sucrose reduction was higher than fat reduction, reaching 50% diameter reduction over control samples. The effect of sucrose and

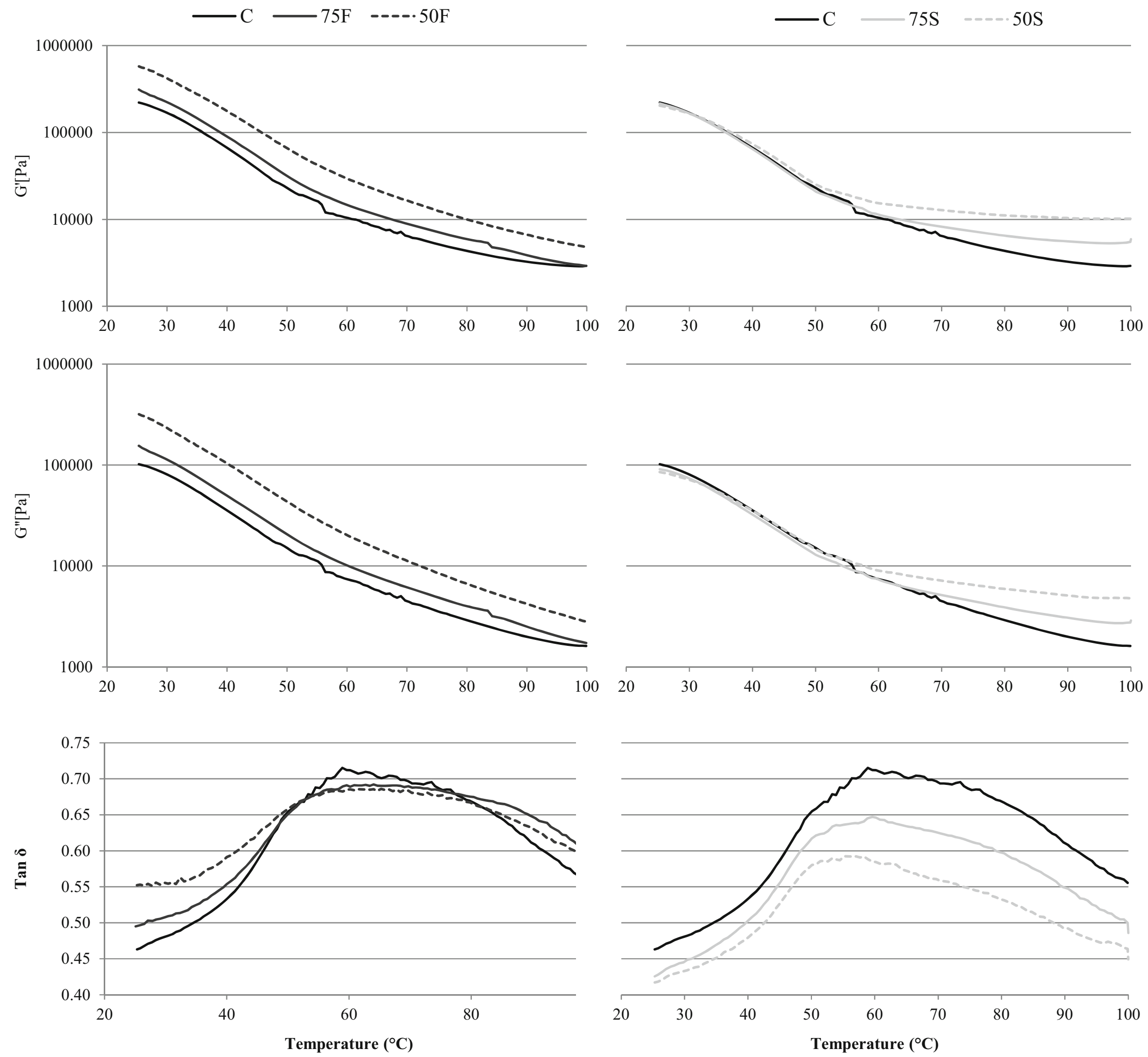

Fig. 2 Effects of fat and sugar reduction on biscuit dough rheology as function of temperature. Elastic (G') and viscous (G'') modules and tan δ at frequency = 10 Hz. *C* (Control): full fat and sucrose sample. *F* fat, *S* sucrose. Seventy-five and 50 refer to level of incorporation.

Table 3 Effects of fat and sugar reduction on biscuit spreading behaviour and biscuit quality parameters. *MD* maximum diameter reached during baking, *ST* set time, *BF* biscuit factor, *%R* % reduction in biscuit factor over control, *WV* whiteness value, *AF* area fraction, *D* fractal dimension, *H* breaking force. *C* (Control): full fat and sucrose sample. *F* fat, *S* sucrose. Seventy-five and 50 refer to level of incorporation

Sample	MD (cm)	ST (min)	BF	*%R*	WV	AF	*D*	*H* (N)
C	6.11^{a}	5.33^{a}	6.7^{a}	–	56.4^{b}	7.3^{ab}	1.43^{a}	66.5^{c}
75F	6.10^{a}	5.00^{a}	5.4^{b}	20	56.7^{b}	8.9^{a}	1.45^{a}	106.0^{b}
50F	5.58^{b}	5.00^{a}	4.6^{c}	31	58.5^{ab}	6.7^{ab}	1.38^{ab}	145.5^{a1}
75S	5.73^{b}	4.00^{b}	4.6^{c}	32	59.0^{ab}	2.9^{c}	1.25^{b}	58.9^{d}
50S	5.20^{c}	4.00^{b}	3.4^{d}	50	61.2^{a}	0.3^{d}	1.01^{c}	26.2^{e}

Values in the same column with common letter are not significantly different ($p > 0.05$)

fat reduction on BF was related to a decrease in the diameter but also an increase in the piece thickness. Similar results were found by Pareyt et al. (2009) and Sudha et al. (2007), who showed positive and negative correlation between fat content and biscuit diameter and height, respectively.

These results are expectable, since both fat and sucrose reductions affected the dough viscosity properties. Fat also influences the air incorporation during dough mixing. The amount of air incorporated affects the viscosity of the system (Jacob and Leelavathi 2007), which itself affects the biscuit spreading. Thus, lower levels of fat produced higher dough viscosities and G' and G'' values and more viscous to elastic behaviour at room temperature and during baking that control dough which resulted in smaller biscuit diameter.

Sucrose, on the other hand, affects the viscosity by contributing to the aqueous phase, and its reduction resulted in more solid-like dough behaviour and higher dough viscosities and G' and G'' values at high temperatures during baking.

As was previously mentioned, two simultaneous effects are produced: protein partial hydration and protein–protein interactions due to a greater availability of water in the system when both fat and sucrose were reduced and the change of system fluidity as consequence of the modifications in fat proportion.

Three bending point assay evaluates the maximum force necessary to produce the total structure break (Gaines 1994). Biscuit average breaking force (H) showed differences between samples (Table 3). Biscuit hardness decreased with sucrose reduction, an expectable result because of the sucrose crispiness role. Similar results were found in other studies (Gallagher et al. 2003; Pareyt et al. 2009). Fat reduction increased (2.2 times the control) the biscuit hardness. It is known the role of fat as softener, since the lubricating effect and making it tenderer. The opposite result was found with fat increasing by other authors in previous works (Pareyt et al. 2009; Sudha et al. 2007). They also noticed that the biscuit porosity increased when more fat was present in the formulation.

Another important quality parameter is a high degree and uniform cracking pattern. The cracking pattern occurs during baking by sucrose recrystallization in the biscuit surface (Hoseney 1994). Higher area fraction (AF) and fractal dimension (D) means higher degree of cracking and surface roughness (Table 3). Fat reduction did not affect AF and D values. Sucrose reduction reduced drastically the AF ($P < 0.05$), reaching the lowest value in 50S (4.5% of control). Other authors also found that sucrose reduction in biscuit dough results in a very smooth surface (Pareyt et al. 2009). Fractal dimension values showed the same trend than AF: no differences for fat reduction and a decrease for sucrose reduction, although the lowest value represented the 70.6% of control (50S). Lower D values are related to less complex and rough surface. The results were expected, since sucrose has a very important role in texture, providing crispiness to the biscuit; therefore, sucrose reduction results in a smoother surface.

The biscuit's surface colour is, together with texture and taste, a very important element for the initial acceptability by consumers. The whiteness value (WV) mathematically combines lightness and yellow-blue into a single term and has been used to compare colour in this study. The higher the whiteness value, the whiter the sample. Whiteness value ranged between 56.4 (C) and 61.2 (50S); however, only 50S (Table 3) was statistically different, indicating that the caramelization process and Maillard reaction (which produce surface browning) during baking are less intense in biscuits made with sucrose reduction.

Effect of Fat and Sugar Reduction on Water Mobility and Thermal Properties

NMR measurements were carried out for C (control sample, full fat and sucrose), 50F (50% fat reduction) and 50S (50% sucrose reduction) to study the water distribution within the dough and the biscuits. Figure 3 shows the scheme of the data acquired for control dough using the MSE-CPMG sequence, normalized by the sample weight, typically of 200 mg. A similar protocol was recently implemented for the study of hydrothermal changes in wheat starch, where the initial signal decay is monitored after a 90° pulse (FID (free induction decay)) (Kovrlija and Rondeau-Mouro 2017; Rondeau-Mouro et al. 2015). The advantage of the MSE is that the initial receiver dead time is circumvented by the use of an echo, and the typical loss of the initial c.a. 12 μs of signal is recovered. The data analysis in our work differs from that of Kovrlija and Rondeau-Mouro (2017), where a numerical inversion of all the data is carried out (Rondeau-Mouro et al. 2016). Here, we apply a numerical inversion of the data acquired by the CPMG sequence (Song et al. 2002) and subtract this result to the entire data set. The result is a Gaussian function indicated by A in Fig. 3, which arises from the solid components of the sample (see Supplementary Material for a detailed deconvolution description). For all systems studied in this work, a single Gaussian function described the solid part. We have previously described this population by measuring the FID, where for the inhomogeneous magnetic fields in low field spectrometers, the populations with longer relaxation times appear shorter (T_2*) and were assigned to a population B (Serial et al. 2016). This population is no longer observed with the current experimental approach. For the sake of clarity, we will keep the notation for the populations with longer relaxation times, which are acquired with the CPMG sequence. The distribution of relaxation times is shown in the Fig. 3 inset and is modelled by three Gassian functions. Population C corresponds to bound water that interacts weakly with CH protons from starch (intragranular) and gluten; intergranular water which interacts with sucrose and starch and gluten protons which are assigned to population D and a population with a long relaxation time corresponding to fat and dissolved sucrose is represented by population E (Assifaoui et al. 2006a, b; Serial et al. 2016).

Figure 4a shows the area of each population for control 50F and 50S dough samples. Upon reduction of fat or sugar, an increase per gram was observed in the solid component signal (A), with $T_2 \sim 16$ μs. Upon fat reduction, population E decreased as expected. Population D, corresponding to mobile water, increased significantly upon reduction of sugar, consistent with the softer dough observed in rheological experiments.

Upon cooking, Population D lost its intensity, partially due to water evaporation and to its structuration with the solid components (see Fig. 4b). The redistribution of water can be correlated with biscuit quality parameters. A great increase in population A was observed for all formulations upon baking, indicating a strong bounding of water molecules to the solid components of the systems, which undergo cross-linking. Figure 5a shows that population A increases in the same

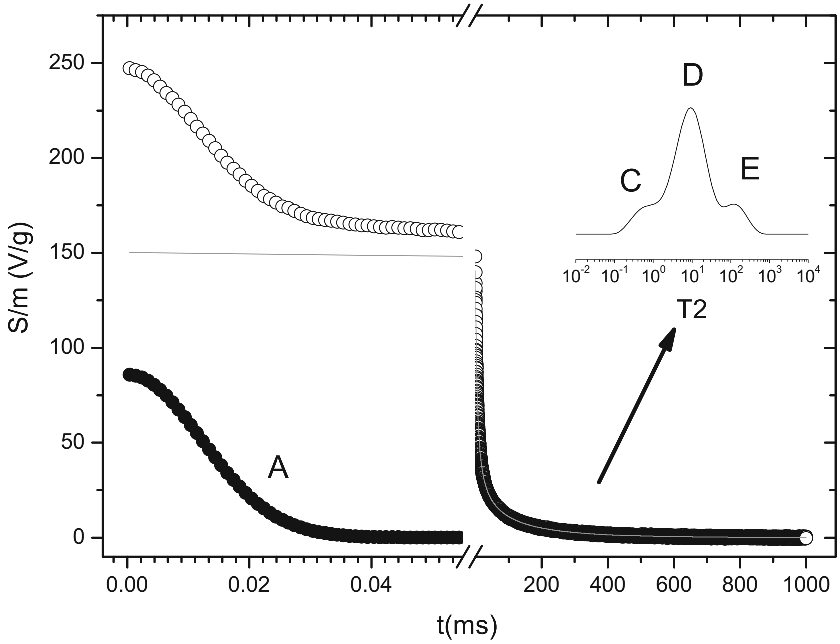

Fig. 3 Normalized NMR signal (open symbols) for control dough using the MSE-CPMG sequence. The initial MSE ($t < 100$ μs) refocuses dipolar interactions of solid-like components, while the CPMG sequence ($t > 100$ μs) measures protons with higher mobility. The inset shows the numerical inversion of the CPMG data where different relaxation times corresponding to water in contact with starch (C), mobile water (D) and fat and dissolved sucrose (E) are observed. Subtraction of this fitting to the data provides information on solid components (A: filled cirles)

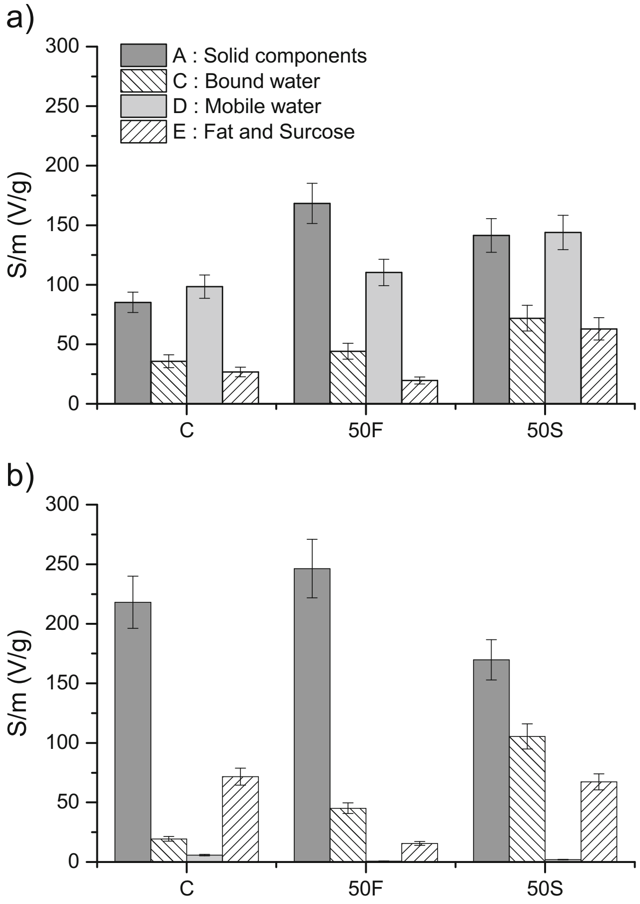

Fig. 4 Populations A (solid components), C, D and E determined by NMR for full fat and sucrose sample (C), 50% fat reduced sample (50F) and 50% reduced sucrose sample (50S) for dough formulations (**a**) and for the corresponding biscuits (**b**)

manner as the breaking force (H), being the formulation with sugar reduction the softer biscuit, while the formulation with fat reduction the hardest one.

The most interesting behaviour was showed by population C, corresponding to water slightly bounded with solid components, which presented relaxation times in the order of 600 μs. The main binding mechanism for this type of water is hydrogen bonding, which seems to affect the overall system mobility. Control formulation had the lowest amount of this type of water and presented the highest biscuit factor (BF). On the other hand, sugar reduction had the largest component C, with the lowest BF as shown in Fig. 5b and Table 3. The inverse relation between population C is also observed in Table 3 with respect to the maximum diameter (MD) and the set temperature (ST) for instance.

Figure 6 shows the two-dimensional maps corresponding to the different samples. With the T_2 vs T_1 spectra, it is possible to study the evolution of the proton populations as the system undergoes physico-chemical changes. It must be mentioned that the peak assignment requires an entire knowledge of the involved system; authors had made an important advance in a previous work identifying the proton populations in the two-dimensional maps (Serial et al. 2016). The $T_1 = T_2$ line is drawn in all graphs and indicates the position where isotropic liquids would appear; thus populations with higher mobility (E) were located near this diagonal. The $T_1 = 70\ T_2$ line is included as a guideline. Figures 6a, c, e corresponds to control, 50F and 50S dough, respectively. Figure 4b, d, f corresponds to control, 50F and 50S biscuits, respectively. Populations denoted as C and D (with lower mobility) were represented by a T_1 values between 60 to 70 ms. As previously described (Serial et al. 2016), population D is not resolved for

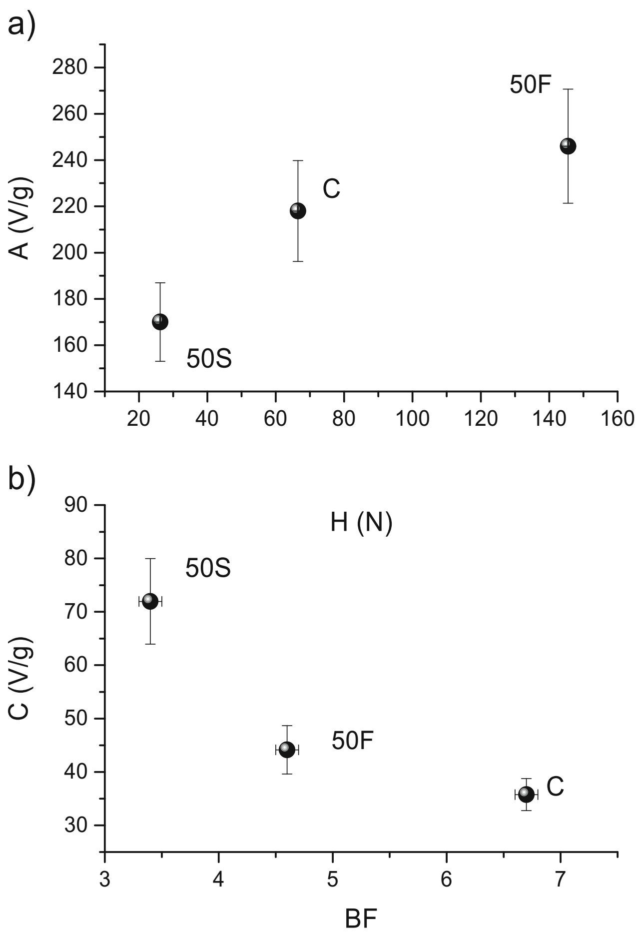

Fig. 5 Populations A (solid components) and C (bound water) determined by NMR for biscuits with full fat and sucrose sample (C), 50% fat reduced sample (50F) and 50% reduced sucrose sample (50S). **a** NMR fraction (A) as a function of biscuit hardness (H). **b** Bound water component in biscuits (C) as a function of biscuit factor (BF)

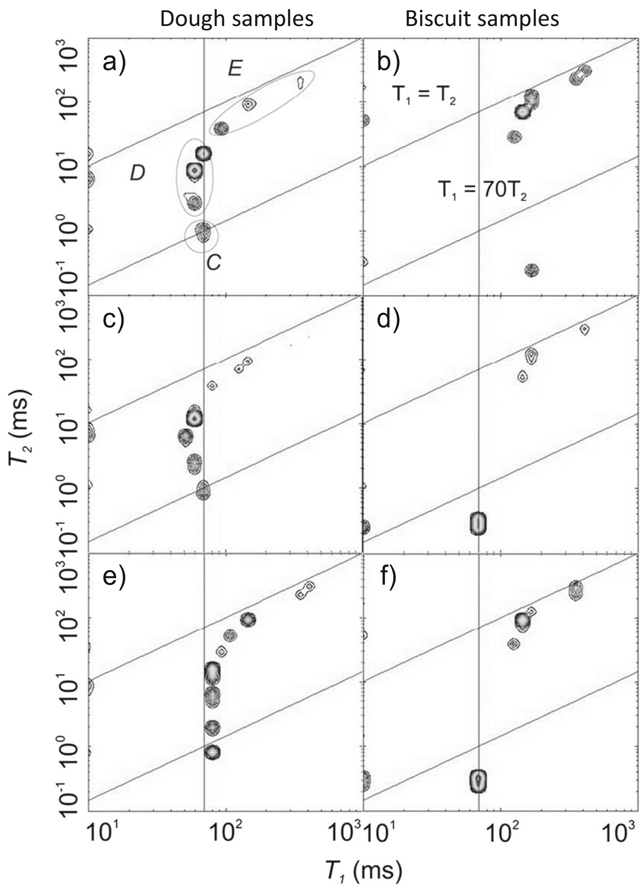

Fig. 6 T_2 vs T_1 spectra belonging to control, 50F and 50S dough samples (a, c and e), and to control, 50F and 50S biscuit samples (b, d and f). C: Proton population corresponding to bound water and starch. D: Proton population corresponding to mobile water in interaction with starch and sucrose. E: Proton population corresponding to fat

biscuit samples indicating that most of the free water is evaporated or structured during cooking.

The main features for the dough samples were very similar, with a slight decrease in the longitudinal relaxation times of the mobile components (D) upon reduction of fat (Fig. 6b), probably due to a decrease in the lubrication of the system, while an overall increase in T_1 was observed upon reduction of sucrose. The main differences in relaxation times among the different formulations were observed in the biscuit samples. We have previously associated the shift of component C to the $T_1 = 600\ T_2$ to the high interaction of water with hydrophilic components of flours as hydroxyl groups of starch, proteins or pentosans. Formulations with a reduction of fat or sucrose showed a very intense signal corresponding to population C, where a reduction in the longitudinal relaxation time indicated the low mobility of this population, which in turn is associated with the rheological changes observed for this dough samples.

Differential Scanning Calorimetry (DSC)

When the starch granules are heated to a certain temperature in the presence of water, the native structure of the granules is distorted, producing an irreversible transition from an ordered to a disordered structure and the release of amylose into the medium. This process is called gelatinization. Biscuit system has low water content, not enough to produce complete starch gelatinization. However, starch granules are able to swell during biscuit baking. In order to corroborate whether or not some starch gelatinization occurred in the different samples during baking, DSC tests were carried out with dough and biscuit samples with the highest levels of reduction of both components. The dough and biscuit samples were defatted to prevent fat interfering with the measurement. Defatted dough and biscuit samples plus water (1:2, solid/water ratio) were analysed. Thermograms obtained from dough and biscuit samples showed a curve with a shoulder corresponding to the gelatinization (results not shown) between 60 and 80 °C approximately. Table 4 shows the onset temperatures (To) and the enthalpies (ΔHg) of starch gelatinization of defatted dough

Table 4 Effects of fat and sugar reduction on starch gelatinization onset temperatures (To) and gelatinization enthalpies (ΔHg). *C* (Control): full fat and sucrose sample. *F* fat, *S* sucrose. Fifty refers to level of incorporation

	To (°C)	(ΔHg (mJ/mg)
C biscuit	65.70 ± 0.80^{a}	2.21 ± 0.19^{a}
C dough	65.93 ± 0.25^{a}	2.22 ± 0.13^{a}
50F biscuit	66.81 ± 0.66^{m}	2.93 ± 0.32^{m}
50F dough	65.68 ± 0.28^{m}	3.39 ± 0.34^{m}
50S biscuit	67.45 ± 1.22^{t}	2.41 ± 0.53^{s}
50S dough	63.72 ± 0.32^{s}	4.10 ± 0.66^{t}

Values in the same column at each formulation with common letter are not significantly different ($p > 0.05$)

and biscuits. The enthalpy and the onset temperature of the starch gelatinization of the 50S biscuit were lower and higher than the values of the 50S dough, respectively, indicating that some partial gelatinization (~59% of gelatinization degree) happened during baking in this sample. It is suggested that the reduction of the proportion of sucrose leaves a higher amount of free water available for the starch during baking. The full recipe (control dough) and fat reduction did not have a significant effect ($p > 0.05$) on the thermal parameters of the biscuits when compared to the respective dough samples.

Biscuits are characterized by high fat and sugar levels and low water levels (< 5%). In the dough formulations, sugar is the major ingredient after flour, and sucrose is the most common sugar used in baking. Sugars provide sweetness and are responsible of the structural features of the biscuits. Sucrose in sugar snap doughs partially dissolves since the high ratio sugar/water is present and then recrystallizes or forms an amorphous glass after baking, which strongly affects the texture of the baked biscuit (Manley 2000). According to Slade and Levine (1994), sugars are plasticizers of the biopolymers of wheat flour, but concentrated aqueous sugar solutions, as it is found in biscuit doughs, act as antiplasticizers. As a result, gluten development during dough mixing and starch gelatinizationduring baking is delayed or prevented (Kweon et al. 2014).

During baking, the progressive dissolution of sucrose yields an additional sucrose-water solvent phase. Thus, higher sucrose levels usually increase the fluidity of the dough and improve the spread rate and lead to a higher biscuit diameter (Pareyt et al. 2009; Kweon et al. 2014).

Because of high levels of sugar and insufficient water, most of the starch granules do not gelatinize. However, starch granules are able to swell during baking. They usually retain their identity and remain intact and act as “filler” in the matrix provided by the other materials present supporting the biscuit’s structure (Pareyt and Delcour 2008). Chevallier et al. (2000) showed that parts of the granules are only partially birefringent and that others are slightly swollen after baking. Biscuits from recipes with higher water and/or lower sugar levels may show some starch gelatinization (Delcour and Hoseney 2010). When some degree of gelatinization occurs, the granules that gelatinize first will take (most of) the available water (Ghiasi et al. 1983) and consequently limit the available water for other granules to gelatinize. When some of the starch is gelatinized, more water can be held in the biscuit during baking.

In our study, sucrose reduction produced a softer dough as was confirmed by stress relaxation uniaxial compression test at room temperature, which can be related to the reduction of the solid content on dough formulation and to the relative increase of fat content. Oscillatory test showed only slight changes ($p > 0.05$) at room temperature. However, at higher temperatures (> 60 °C), the sucrose reduction increased the dough consistency (higher G' and G'' than control dough), as was showed by the temperature sweep tests. Sucrose reduction progressively increased the elastic to viscous behaviour, as was shown by the decrease of the tan δ values during the whole temperature sweep. This is consistent with the partial gelatinization happened during baking in this sample, as shown by DSC, and the increase in T_1 for the mobile component (D) determined by NMR (Fig. 6) which suggests that sucrose reduction allows a greater amount of water available for the system. In the same way, Pareyt et al. (2009) suggested that the reduction of sucrose produced faster rates of gluten hydration and subsequent cross-linking during baking. The increase in the consistency of the dough during baking, which stops dough spreading and negatively affected the biscuit quality parameters, can be explained by the development of interactions between the gluten proteins and by the partial gelatinization of the starch.

At the dough stage, fat is a crucial structural component. Higher fat levels reduced dough consistency and elasticity as was demonstrated by stress relaxation uniaxial compression and oscillatory tests. Fat has high lubricant effect and competes with water during mixing to cover the surface of the flour, preventing or retarding the formation of gluten in the dough (Manley 2000) and affecting gluten entanglement interactions (Pareyt et al. 2010). In other study, Pareyt et al. (2010) described that fat does not completely prevent gluten network formation because it melts during baking. It is suggested that a reduction of fat in the system allows not only the initial formation of gluten during mixing but also the formation of extra interactions during baking due to less capacity of this phase to form a barrier for gluten–gluten interactions and reactions.

Additionally, higher fat levels generally increase air incorporation, especially when a creaming stage is used, leading to lower biscuit dough density and hardness (Maache-Rezzoug et al. 1998; Pareyt et al. 2009b).

Fat reduction not only increased both G' and G'' compared to the control dough during the whole temperature sweep but also produced higher viscous to elastic behaviour between 25 and 50 °C, as was shown by the temperature sweep tests. The changes in dough fluidity reduced dough maximum diameter during baking and produced thicker and smaller diameter biscuits and increased biscuit hardness.

The increment of the G' and G'' moduli was in agreement with the NMR results; by comparing the mobile to solid ratios, it turns out that the 50F dough formulation presented a lower value respect to the control formulation, whereas no appreciable change was observed for the 50S dough formulation. The two-dimensional maps of dough samples showed a decrease in the longitudinal relaxation times of the mobile components (D) upon reduction of fat, probably due to a decrease in the lubrication of the system.

When temperature rises during baking, fat melts and sucrose completely dissolves, resulting in an increment of dough fluidity. The reduction of fat allowed the gluten hydration, with a subsequent cross-linking during baking that decreased the expansion of the gas cells during baking, since less air is incorporated (Pareyt et al. 2009). A lower capacity to cover the surface of the flour particles and a reduction of the lubricant effect during mixing, together with a higher gluten development and lower expansion of gas during baking, increased elastic and viscous properties of the dough samples. Consequently, dough spreading stops and negatively affected the biscuit quality parameters.

The increase of biscuit hardness as consequence of fat reduction can be explained by the loss of lubricating effect, the decrease of biscuit porosity and density (Baltsavias et al. 1999), and the increase of biscuit cell wall strength due to the extra gluten polymerization (Pareyt et al. 2010). Note that the breaking force (H) follows the same trend as the amount of the solid phase in biscuits (NMR population A), being 50 F the largest values and 50 S the lowest ones.

Two-dimensional maps of dough samples (Fig. 6) showed a decrease in the longitudinal relaxation times of the mobile components (D) upon reduction of fat, while an overall increase in T_1 was observed upon reduction of sucrose. Regarding to biscuit samples, reduction of fat or sucrose showed the low mobility of population C, which changes from $T_2 \sim 600$ μs in dough to $T_2 \sim 200$ μs in biscuits. On the other hand, longitudinal relaxation increases for component C in the control sample upon cooking. These results agree to the formation of extra interactions during baking due to less capacity of the fat phase (when fat was reduced) to form a barrier for gluten–gluten interactions and reactions. Regarding sucrose reduction, faster rates of gluten hydration and subsequent cross-linking during baking and higher water availability to be taken by starch granules, or some of them, and gelatinize when sucrose were reduced. These processes can explain the decrease of intergranular water interacting with sucrose and starch and gluten protons (population D) associated with an increase of bound water (population C) interacting with CH protons from starch and gluten.

Conclusions

Fat reduction increased consistency and elastic properties of the dough at room temperature and during heating. The decrease in sucrose content affected to a lesser extent the rheological properties at room temperature, although it produced significant changes during dough heating, increasing the consistency and decreasing the spreadability. The changes in dough rheological properties produced a decrease of the biscuit quality, as biscuit diameter and hardness. It was found that a reduction of fat or sucrose produced the gluten hydration and extra interactions and subsequent cross-linking during baking, and a reduction of sucrose led to the swelling and partial gelatinization of starch during baking.

TD-NMR results allowed corroborating the proposed hypotheses about the effects on dough and biscuit characteristics as a consequence of reduction of main ingredients. The use of a dipolar refocused acquisition, combined with multipulse acquisition, enables an accurate determination of the different components; in particular, solid components are determined without the usual hampering due to receiver dead times. The mixed magic solid eco-sequence, with CPMG acquisition, introduced in this study, enabled the acquisition of both solid and mobile components. The results allowed to correlate the hardness of biscuits with the amount of solid components and the spreading properties of dough with the fraction of bounded water. As an explanation of this last behaviour, it was proposed that the degree of hydrogen bonding created between this fraction of water and the solid components diminishes the amount of water available to effectively act as a lubricant during the cooking process.

This work is an important effort to explain such a complex systems as short dough by different methodologies with the hope to gain knowledge and prediction capacity to explain the changes in the behaviour and final product quality

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