Impact of local hydrothermal treatment during bread baking on soluble amylose, firmness, amylopectin retrogradation and water mobility during bread staling

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Revised: 26 July 2015 / Accepted: 5 August 2015 / Published online: 29 August 2015
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Abstract The impact of hydrothermal processing undergone by bread dough during baking on the degree of starch granule disruption, on leaching of soluble amylose, on water mobility, on firmness and on amylopectin retrogradation during staling has been investigated. Two heating rates during baking have been considered (4.67 and 6.31 °C/min) corresponding respectively to baking temperature of 220 and 240 °C. An increase in firmness and in the amount of retrogradated amylopectin accompanied by a decrease in freezable water has been observed during staling. Although a lower heating rate yielded in larger amount of retrogradated amylopectin retrogradation, it resulted in a lower firmness. Additionally, the amount of soluble amylose and the relaxation times of water measured by Nuclear Magnetic Resonance NMR (T_{20} , T_{21} and T_{22}) decreased during staling. It was demonstrated that the amount of soluble amylose was higher for bread crumb baked at lower heating rate, indicating that an increasing amount of amylose is leached outside the starch granules. This was corresponding to a greater amount of retrogradated amylopectin during staling. Moreover, it was found that the degree of gelatinization differs locally in a same bread slice between the top, the centre and the bottom locations in the crumb. This was attributed to the differences in kinetics of heating, the availability of water during baking and the degree of starch granule disruption during baking. Based on first order kinetic model, it was found

that staling kinetics were faster for samples baked at higher heating rate.

Keywords Staling · Baking conditions · Soluble amylose · Firmness · DSC · NMR

Introduction

Bread staling is a physico-chemical process that involves modifications of the matrix both at the macroscopic and the molecular scales. Concerning the first scale, staling affects crumb firmness, flavor deterioration and crust softening. However, at the molecular scale, this phenomenon affects starch retrogradation and crystallinity, redistribution of water between molecules, decrease in the amount of soluble amylose, changes in the gluten network, interactions between gluten protein and starch granules, etc.

Bread crumb firming develops over several days and is temperature dependant, faster staling being observed at lower temperature. In the literature, the increase in crumb firmness during staling is primarily attributed to a moisture transfer from the crumb to the crust. Most staling models are based on the evolution of the texture during staling. The change in the amount of retrogradated amylopectin which is linked to crumb firming is also considered to monitor bread crumb staling. However, according to Fessas and Schiraldi (1998), crumb firmness might be also related to the structure formed in the course of fermentation and baking, rather than to starch retrogradation and moisture loss. Davidou et al. (1996) reported that bread firming cannot be explained by starch retrogradation alone, or by the moisture content. It might be explained by a difference in the organization of polymers within the amorphous region and the repartition between the amorphous and crystalline regions of bread crumb.

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Starch retrogradation is a temperature and time-dependent phenomenon, which involves reassociation of starch component molecules into a partially crystalline, ordered structure. The crystallization of starch biopolymer is associated to water supplied mainly from the surrounding matrix namely gluten network and starchy matrix contained in the baked crumb. When amylopectine retrogrades (recrystallisation), water is trapped in the crystalline zones; this water switches from free to bound water and enters in the category of non freezable water. According to (Vodovotz et al. 1996), the water being linked to chains of gluten, will be gradually trapped in retrograded amylopectin during storage, resulting in a dehydration of chains of gluten and a hardening of the crumb (Le Bail et al. 2009). Therefore, water is an important factor which interacts with the retrogradation. Other factors were found to influence this phenomenon, namely storage conditions (time and temperature) and heating rate (Le Bail et al. 2009). A relation between baking temperature and retrogradation has been reported by Giovanelli et al. (1997): the higher the baking temperature, the more retrogradation will occur. Moreover, it was demonstrated that in starch gels, the retrogradation depends on the final temperature reached during gelatinization: the higher is the degree of gelatinization, the more retrogradation will occur (Eliasson 1985).

It has been established that during baking of bread dough (for which a relative excess of water is present), starch granules gelatinize and swell. The leaching of amylose polymer occurs once swelling starts and is continuing for prolonged thermal treatment. The leached amylose during baking forms a gel around the ghosts of starch granules which contributes to the firmness of the bread matrix. Therefore, the determination of the amount of soluble amylose can be considered as an indirect parameter linked to crumb texture. Patel et al. (2005) and Seetharaman et al. (2002) observed that the amount of soluble amylose tends to increase with the baking duration. Le-Bail et al. (2011) reported that the amount of soluble amylose increased with increasing duration of the baking plateau at 98 °C using a miniaturized baking system, leading to higher Young's modulus of the crumb at the end of staling. Therefore, baking conditions affect starch properties that contribute to the macroscopic properties of bread.

Other researchers have studied the effect of baking process on staling kinetics. For example, Le-Bail et al. (2009) reported that a rapid baking (6 min to reach 98 °C) resulted in a faster staling. Le-Bail et al. (2011) investigated the impact of the duration of the plateau of baking on staling kinetics and reported that low temperature and a long baking plateau (at around 98 °C) induced a decrease in crumb firmness in the bread.

To assess changes in water mobility of the bread crumb, multiple proton Nuclear Magnetic Resonance (NMR) techniques can be used. These techniques are most sensitive to changes in water dynamics. Many authors have studied states

of water in bread during staling (Curti et al. 2011) by proton FID, T_2 and T_1 relaxation time. The spin–spin relaxation time T_2 is a function of the spin species and the chemical and physical environments surrounding the spins (Yi et al. 2009). Others used ^1H NMR to study changes in water during staling of bread baked from frozen dough of different freezing rates and storage temperature (Yi and Kerr 2009). Chen et al. (1997) found three fractions of water in fresh bread crumb, one with a peak T_2 at 8–12 ms, one at 280–320 ms, and one at 2.0–2.6 ms, which they attributed to regions of differing water mobility. The component at 8–12 ms was attributed to water strongly associated with starch and gluten through hydrogen bonding. Engelsen et al. (2001) found three components in bread that were measured during the baking process.

This study was undertaken to assess the impact of the heating rate during baking on bread crumb staling. Bread had a format of sandwich bread (10 × 10 cm section). Two heating rates have been considered. The impact of local hydrothermal processing on water content, soluble amylose, water mobility, firmness and starch retrogradation has been considered.

Materials and methods

Bread preparation

Wheat flour was supplied by Girardeau (Boussay, France) and characterized by 12.2 % moisture content (dry basis) and 60 % water absorption at 500 BU (Brabender Unit). The recipe is composed of 60 % water, 2 % salt (Sifto, table salt, Canada), 2 % dry milk powder (Canada), 2 % sugar (Iantic, natural granulated sugar, Canada), 2 % sunflower oil (Unico, Inc., Ontario, Canada), 2 % dried yeast (Fleischmann's instant yeast, Canada) and 0.01 % ascorbic acid. All ingredients were mixed in a spiral mixer (Hobart, Canada) for 4 min at speed 1 followed by 8 min at speed 2. Water and yeast were added firstly, followed by the rest of ingredients. After mixing, dough reached a temperature of 20–23 °C. Dough pieces of 770 g each were rested for 15 min before being divided and molded manually. Then, they were rolled and shaped manually. After that, they were introduced in the mould (10 cm × 10 cm × 20 cm). Proofing was carried out in a fermentation cabinet (Hobart, Canada) at 35 °C, 90 % RH during 70 min.

When fermentation was completed, the moulds were installed in a revolving tray oven (model LCT4.12, LC bakery Equipment Service, Ltd., Brantford, Ontario) to be baked without injection of steam. In order to characterize the temperature at bread core during baking, histories of time-temperature were saved using data logger (Scorpion 2 Data Logging Measurement System, Reading Thermal System). After baking, breads were withdrawn from the oven,

decapsulated out of the pan and left at ambient temperature for 2 h. After that, fresh pan breads were sliced into 12.5 mm thick slices with an electric slicing machine. Bread slices with intact crust were putted in hermetic plastic boxes (5 cm × 10 cm × 20 cm) and stored at 15 °C for 9 days. Each box contained four slices which will be used for further analysis. In this study, bread staling was studied at local scale by considering three distinct zones of the bread crumb (Fig. 1), named: the crumb centre (CC), the top crumb (TC) and the bottom crumb (BC). To prepare samples for analysis, bread slices were allowed to equilibrate at room temperature for 1 h (still in the sealed box). Then, crumb samples taken from the three zones were grounded using a coffee grinder (Black and Decker CBG100S, Smartgrind coffee grinder), to obtain homogenized samples with fine particles. These samples were used to measure moisture content, water activity, soluble amylose, DSC and NMR analysis. However, for firmness analysis, intact bread crumb slices were used.

Histories of time-temperature were averaged and temperature profiles were determined from two series of baking; two bakings were performed at 220 °C and two others at 240 °C, during 25 min. The heating rate was calculated in the linear region of rapidly increasing temperature between 45 and 85 °C. So that, breads baked at 240 °C have the highest heating rate (6.31 °C/min) while breads baked at 220 °C have the lowest heating rate (4.67 °C/min).

Moisture content and water activity

Moisture content expressed in g/g w.b. (wet basis), was determined using a Moisture Analyser Ohaus-MB45 (Ohaus Corporation, Parsippany, NJ). 1 g of ground sample was dried at 130 °C for 4 min. Water activity was measured using Aqua Lab Series 4TE water activity meter (Decagon Devices, Inc., Pullman, WA, USA). Mean values from 5 replicate measurements were reported.

Soluble amylose

Soluble amylose was determined according to AACC method 61–03 (AACC 1999a). 100 mg of ground bread crumb were

Fig. 1 Defines three locations in the slice of pan bread: the top, the centre and the bottom

weighed in 100 mL volumetric flasks. Then, 1 mL of 95 % ethanol and 9 mL of 0.1 N NaOH were added, containers were shaken, and kept for 2 h at ambient temperature (21 °C). Then, distilled water was added to reach 100 mL and the container was capped and inverted until thoroughly mixed.

An aliquot (5 mL) was transferred into a volumetric flask containing about 50 mL of distilled water, 1 mL of 1 N acetic acid and 2 mL of iodine solution, and mixed. Distilled water was added to 100 mL and the flask was capped and inverted to thoroughly mix. A blank was prepared using 5 mL of 0.09 N NaOH. Samples were allowed to stand for 20 min before the absorbance at 620 nm was measured using CM 3500-d spectrophotometer (Konica Minolta Sensing Inc., Mahwah, NJ, USA).

A calibration was done with a blank and with a reference sample (Corn starch, 25 % amylose (dwb), Argo, Best Food, CPC International Inc., NJ U.S.A.). Three determinations were done for each crumb sample at each storage time.

Firmness

An Instron universal testing machine (TA-XT2, Texture Analyser, USA) was used to measure the firmness of bread crumb during storage according to the method described by AACC 74–09 (AACC 1999b). A 6-mm cylinder probe compressed samples to 25 % of their original height with pre-test speed of 1.0 mm/s, test speed of 1.7 mm/s, and post-test speed of 10 mm/s and maximum deformation 40 %. Measurements were carried out on the three zones of the slice bread (the centre, the top and the bottom). All analyses were conducted with six repetitions for each crumb sample at each storage time.

Enthalpies of transition

10 mg of ground breadcrumb samples were weighed and sealed in Alodined hermetic sample pans and lids. Samples and reference pans were placed inside a Differential Scanning Calorimeter (TA Q1000, TA Instruments, New Castle, DE). The experimental procedure entailed cooling of the sample from 25 °C to –40 °C at a rate of 5 °C/min. The sample was then held isothermally for 5 min and heated at the rate of 5 °C/min up to 100 °C. Enthalpies of transition (melting of ice at 0 °C and melting of retrograded amylopectin at 55 °C) were estimated by integration of each peak found in the thermogram and were expressed in J/g dry matter of sample. The amount of freezable water (FW) was calculated by dividing the peak at about 0 °C by the specific latent heat of fusion of water (334 J/g). Measurements were performed in duplicate for each crumb sample and for each storage duration.

NMR measurements

Proton relaxation measurements were carried out using a Bruker MQ20 20 MHz NMR analyzer (Bruker, Milton, ON, CA). Approximately 0.5 g of ground breadcrumb samples was placed into glass tubes (18 cm height and 8 mm diameter). The NMR tubes with samples were closed with a cap to limit moisture loss. The transverse relaxation (T_2) time in the ms time domain was measured using the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence, which consists of a 90° pulse followed by a train of 180° pulses to refocus the NMR signal. The 90–180° pulse spacing was set to 0.25 ms. Transverse relaxation curves were analyzed with a Contin routine using minispec software Version 2.3 (Bruker, Milton, ON, CA). This routine calculates a distribution of T_2 that best describe the data. The relaxation time (T_2) was used to monitor the mobility of water during storage. All measurements were made at 25 °C and carried out for a total of 12 NMR determinations for each crumb sample at each storage time.

Statistical analysis

The obtained data were treated by ANOVA variance analysis using Statgraphics plus 5.1 software. Fisher's LSD test was used to compare means at the 5 % significant level.

Results and discussions

Moisture content and water activity

Figures 2a, b show that the crumb moisture content and water activity decreased during storage, as a result of water migration from the crumb to the crust. Moreover, the moisture content depends on the baking conditions. For example, the moisture content of the bottom crumb samples baked at 220 °C was higher (37.66 %) than that of crumb samples baked at 240 °C (38.68 %) at the end of storage. Therefore, baking at higher heating rate contributes to the dehydration of the bread crumb, leading to lower moisture content and water activity. However, when baking at 220 °C, the bread crumb contained more total water which decreased significantly during storage. This findings are in accordance with results reported by Patel et al. (2005); breads baked at lower heating rate lost less moisture following storage compared to those baked at higher heating rate.

Regarding the different zones of the slice of bread, Fig. 1a shows that the bottom and the top crumb samples contained less water than the centre location, whatever heating rate was. These observations can be explained by the difference in heating rate distributed during baking between these three zones. In fact, because the top (or upper) location of the bread crumb was exposed to higher heat flux than the bottom and the

centre, this resulted in a higher dehydration. Moreover, during storage, the moisture content in the top and the bottom locations changed more than the centre location, due the presence of crusts surrounding the crumb (top crust and bottom crust). Crust has much lower water content than crumb and moisture tends to diffuse toward it during storage. Therefore, the effect of different heating rates on the final moisture contents of crumb samples near crust zone has been pointed out. These observations are in agreement with regards to the evolution of water activity during storage.

Firmness

An increase in crumb firmness was observed for all samples during storage, as shown by Fig. 3. However, some differences occurred depending on baking conditions which affect the structure of the matrix of each zone. The firmness was the highest in the bottom location of the slice and the lowest in the top location, in comparison with the centre location. In addition, the top location presented higher firmness than the centre location. This can be explained by the impact of heating conditions which is higher in the top location than in the centre location, leading to a relatively harder matrix. Although, heating rate affects the cellular structure of the matrix, in our case, there were no significant difference in structure between bread baked at 220 and 240 °C. Therefore, apart the structure, another factor can explain bread crumb firmness, namely moisture loss during storage. It was observed that the higher heating rate resulted in higher moisture loss. As a result, the firmness was found to be higher for samples baked at 240 °C than for those baked at 220 °C during staling. This is in agreement with results reported by Patel et al. (2005) who showed that a faster heating rate contributes to a harder crumb because of the prolonged duration of the baking plateau at 98 °C.

Other factors can explain the increase in firmness during staling, namely the degree of starch gelatinization during baking and the evolution of retrograded amylopectin during storage. In fact, when starch granules are more gelatinized, the matrix will be less ordered. In the case of the centre and the top locations in the crumb, where starch granules are supposed to be fully gelatinized and disrupted thanks to a prolonged hydrothermal treatment in comparison to centre location, a higher amount of soluble amylose and a lower firmness at the end of baking and during storage was observed.

Soluble amylose

Figure 4 shows that the amount of soluble amylose decreased significantly after two days of staling for all samples and continue to decrease slightly throughout the storage period. This trend has been observed by previous authors and can be explained by the fact that during storage, the starch biopolymers undergo reorganization

Fig. 2 Evolution of moisture content (g/g w.b.) (a) and a_w (b) of the top, centre and bottom crumbs of breads baked at 220 and 240 °C during storage at 15 °C

resulting in the formation of amylopectine crystallites which are insoluble, contributing to the decrease in starch solubility.

It was mentioned previously that the amount of soluble amylose may be related to the crumb firmness. Regarding our results, it seems that the crumb of the bottom location in

Fig. 3 Effect of storage at 15 °C on firmness of the top, centre and bottom crumbs of breads baked at 220 and 240 °C during 25 min

Fig. 4 Effect of storage at 15 °C on the amount of soluble amylose of the top, centre and bottom crumbs of breads baked at 220 and 240 °C during 25 min

the slice had the lower quantity of soluble amylose and had the higher firmness. However, the top and centre crumbs had the higher soluble amylose and the lower firmness. These results are in agreement with the hypothesis concerning the relation between firmness and the amount of soluble amylose: it appears that a higher amount of soluble amylose results in a lower firmness. Nevertheless, there is still some controversy on this relation in the existing literature. Some specific interactions may also interfere in particular with the gluten network and its organization and also with parameters such as heating rate, crust amount etc.

Samples baked at 220 °C (slow heating rate) had higher soluble amylose than those baked at 240 °C. This can be explained by the fact that in the case of a slow heating rate, starch granules are exposed to longer exposure at temperatures above the onset temperature of starch gelatinization. Consequently, they are supposed to experience an increase in disorder and a more pronounced disruption. In turn, this leads to the formation of amylose associations or amylose gel like structure inside (intragranular) and outside (intergranular) the granules which contributes to an increase in the amount of soluble amylose. This suggests that in the top and the centre location, intergranular amylose associations maybe dominant. On the opposite, it seems that for the bottom location, amylose associations are more in the form of intragranular aggregates.

As reported by some authors, the degree of gelatinization depends on moisture content and heating rate during baking. According to Patel and Seetharaman (2010), the gelatinization process is governed by moisture content for lower heating rate and by heating rate for higher heating rate. Based on these statements, some explanations of our results can be proposed. In fact, during baking, granule swelling and amylose leaching appear to be more pronounced for the top centre locations. Then a limitation due to loss of moisture in the top location contributes to a reduction of the extent of gelatinization. However, in the bottom location of the bread, the heating is more intense during baking, contributing to a limitation of the gelatinization (less starch disruption). These hypotheses can explain the higher amount of soluble amylose in the top and the centre location and the lower amount of soluble amylose in the bottom location.

Enthalpies of transition

In order to study the effect of heating rate on staling, the melting enthalpy of retrograded amylopectin (ΔH) and the amount of freezable water (FW) were assessed. It was widely known that during storage, amylopectin polymers, which undergo partial gelatinized during the baking process, tend to recrystallize with the incorporation of water in the amylopectin crystallites (Gray and Bemiller 2003). The retrogradation of amylopectin involves the reordering of the amylose

branched chains in short-term ageing and a slower reorganization of amylopectin after longer periods. This supposes that depending on the degree of gelatinization, affected by heating rate and baking process, the retrogradation of amylopectin will be different.

Results showed that FW decreased greatly after 2 days of storage and remained constant during the following days (Fig. 5a). This decrease was due, in the first hand, to a loss of moisture by migration from the crumb to the crust, and, in the other hand, to the incorporation of water into the starch crystalline structure (Hallberg and Chinachoti 2002; Ribotta and Le Bail 2007).

Although, Fig. 5a shows a significant difference between the top, centre and bottom locations of the same slice regarding the amount of FW. No significant difference of this parameter was observed in function of the baking conditions. However, it seems that the FW of samples baked with lower heating rate tend to be higher than that of samples baked with higher heating rate.

The evolution of the enthalpy of melting of retrograded amylopectin was also measured during storage. Figure 5b shows that the amylopectin endotherm of each sample increased with storage time and remained constant after 4 days of staling.

Fig. 5 Effect of storage at 15 °C on the amount of FW (a) and the enthalpy of melting of retrograded amylopectin (b) of the top, centre and bottom crumbs of breads baked at 220 and 240 °C during 25 min

Overall, as reported previously, baking at lower heating rate and in the presence of higher moisture environment resulted in greater dispersion of amylose and amylopectin (Patel and Seetharaman 2010). This will result in higher retrogradation of amylopectin, as suggested by Eliasson (1985). These authors demonstrated that for starch gels, the higher is the degree of gelatinization, the more retrogradation will occur. Moreover, it was reported that retrogradation is very sensitive to the water content in starch gels. It increased sharply in starch gels with increasing moisture content (Zelevnak and Hosenev 1986). Regarding our results, it seems that during staling, the amylopectin retrogradation is higher for breads baked at lower heating rate than for breads baked at higher heating rate. This suggests that more energy was needed for melting of the crystalline structure of starch in breads baked at lower heating rate than in breads baked at higher heating rate. These results are in agreement with the results of the soluble amylose; samples baked at 220 °C contain more soluble amylose due to the higher degree of gelatinization, resulting in a higher retrogradation enthalpy.

Figure 5b shows that amylopectin retrogradation is higher for crumb of the centre and the top locations, after two days of storage, than that in the bottom location whatever baking conditions were. These results can be explained essentially by the differences in heating rate and moisture content; besides, it can be pointed out that they are consistent with the results of the amount of soluble amylose. In fact, more soluble amylose was found in the centre and top locations, and as a result, more retrogradation was detected in these zones.

In addition, it is clear that after 3 days of staling, the amount of retrograded amylopectin in the top location of the crumb was lower than that in the bottom and the centre locations of the crumb. This can be explained by the fact that during storage, as the crumb from the top location loses water, due to the water transfer from the top crumb to the top crust, less water became available for retrogradation resulting in lower retrograded amylopectin at the end of storage.

Comparing Fig. 4 and 5b, the amylopectin retrogradation seems to not correlate with the increase of firmness during staling. This observation was supported by some authors (Rogers et al. 1988; Seetharaman et al. 2002). They reported

that the increase in bread firming was strongly related to the presence of lipids, delaying staling and moisture content. However, many researchers reported that the retrogradation of amylopectin is strongly related to the increase in bread firmness during storage (Le-Bail et al. 2011). They explained this phenomenon by the fact that amylopectine crystallites trapped progressively the freezable water during storage, resulting in a dehydration of the matrix and an increase of the rigidity of the gluten network and of the amylose gel formed during baking; this results globally in an increase of the Young's modulus of the crumb.

A first order kinetic model was used to fit the kinetics behavior of amylopectin retrogradation during staling as given by equation (1):

$$\Delta H(t) = \Delta H_{\infty} + (\Delta H_0 - \Delta H_{\infty}) \cdot \exp(-t/\tau) \quad (1)$$

ΔH_0 , ΔH_{∞} , and τ correspond to the initial enthalpy of retrograded amylopectin, the final enthalpy of retrograded amylopectin and the time constant, respectively (t = time, same unit as τ).

Table 1 gives parameters of the first order model used for fitting the evolution of the melting enthalpy of retrograded amylopectin. It shows that the enthalpy of retrograded amylopectin at the end of staling for samples baked with lower heating rate ($\Delta H_{\infty} = 4.79$ J/g), was significantly higher than that of samples baked with higher heating rate ($\Delta H_{\infty} = 4.04$ J/g). A correlation between these results and those found for the amount of FW can be suggested. In fact, samples baked at lower heating rate show more retrograded amylopectin because the amount of FW was higher.

Regarding the time constant τ , Table 1 shows that the value of τ for samples baked at lower heating rate was higher than for those baked at higher heating rate. So that, baking at lower heating rate resulted in a slower staling rate. Moreover, results showed that the value of τ of the top and centre locations was lower than that of the bottom samples, whatever baking conditions were. This supposes that the crumb from the top and centre locations exhibit a faster staling than the crumb from the bottom location, due to the higher heating and the more pronounced extent of gelatinization in these two zones.

Table 1 Parameters of the 1st order kinetic model used to fit the evolution of the enthalpy of melting of the retrograded amylopectin of bread crumb during staling (with ΔH_0 = initial enthalpy of retrograded

amylopectin, ΔH_{∞} = enthalpy of retrograded amylopectin at the end of storage and τ = time constant)

	220 °C			240 °C		
	ΔH_0 (J/g _{dm})	ΔH_{∞} (J/g _{dm})	τ (days)	ΔH_0 (J/g _{dm})	ΔH_{∞} (J/g _{dm})	τ (days)
Top	0	3.48 ^c	1.14 ^c	0	2.85 ^f	0.87 ^f
Bottom	0	4.31 ^b	2.56 ^a	0	3.84 ^d	2.39 ^b
Centre	0	4.79 ^a	1.75 ^c	0	4.04 ^c	1.34 ^d

Means followed by different letters in a column are significantly different at $p < 0.05$. (d.m. refers to dry matter)

◀ **Fig. 6** **a** Effect of storage on the area and the peak temperature of the endothermic enthalpy (expressed in J/g dry matter) of retrograded amylopectin in bread crumb baked at 240 °C. **b** represents a multipeak endotherm after 9 days of storage

Further results (data not shown), showed that the peak temperature of the endotherm differ between samples baked at 220 and 240 °C and between the centre, the top and the bottom locations. In fact, for samples baked at 240 °C, a shift of the

Fig. 7 Effect of storage on relaxation times (**a**, **b** and **c** corresponds to T_{20} , T_{21} and T_{22} , respectively) of bread crumbs baked at 220 and 240 °C during 25 min

peak of the calorimetric thermogram to higher temperatures was observed (57.32, 58.80 and 60.23 °C, corresponding to the centre, bottom and top locations respectively, measured after 9 days of storage). However, for samples baked at 220 °C, no significant difference in the peak temperature was observed between the centre, the bottom and the top locations, measured at the same day (56.72, 56.79 and 56.82 °C, respectively) as shown in Fig. 5b.

Figure 6a provides the thermograms of the crumb from the bottom location baked at 240 °C measured after 2, 4, 7 and 9 days of storage. It shows that during storage, the peak of the endothermic enthalpy decreases and the area increases. After 9 days of storage, a multipeak endotherm was observed as shown in Fig. 6b. This can be explained by the lower content of water present in the sample at the end of staling. It is known that, at low water content, less water is absorbed in the amorphous parts and only a fraction of crystallites within the granule is cooperatively disrupted, giving rise to a first endotherm. Redistribution of water in the granules then occurs and the remaining crystallites melt at a higher temperature, which in turn would correspond to the presence of the second peak (Jacobs et al. 1998).

NMR relaxometry

NMR was used to investigate the water relaxation characteristics of stored breads, baked with two heating rates, and to determine relaxation times. The understanding of water distribution, at the molecular scale in bread crumb is of great interest to study staling kinetics as affected by baking conditions. Three distinct regions of T_2 distributions were observed for all samples, named T_{20} , T_{21} , T_{22} . After 2 h following the baking, T_{20} ranged from 0.8 to 1.6 ms, T_{21} varied between 7.5 to 10.2 ms, and T_{22} ranged from 80 to 100 ms. According to Engelsen et al. (2001), the shortest T_2 component was attributed to water associated with gluten, the next shortest component attributed to water associated with starch and pentosans, and the longest component attributed to diffusive exchange of water between starch and protein fractions. Moreover, our values of relaxation times were in the same order of magnitude with those found in literature. For example, Assifaoui et al. (2006) reported three CPMG populations ($T_{21} = 2$ ms, $T_{22} = 12$ ms, $T_{22} = 105$ ms) in the biscuit dough containing sugar and oil. They attributed the first population of intra-granular protons (inside starch granules), the second one, the most sensitive to water content variations, corresponded to inter-granular water, especially in interaction and competition with sucrose and starch. The last population is associated with apolar protons corresponding to the fat fraction present in the biscuit formula. Therefore, the presence of fats and sugars in the pan bread formula influences water mobility.

Figure 7a, b and c show the evolution of transverse relaxation times T_2 during storage for the top, bottom and centre locations for crumbs of bread baked at 220 and 240 °C. They showed that T_2 values decreased over storage time for all samples, indicating an overall decrease in water mobility and an increase in water binding. It is expected that the decrease in water mobility is due to the incorporation of water molecules into starch crystalline structure that develops upon storage as reported by Chen et al. (1997). Moreover, the crumb from the centre location with higher moisture content had higher T_2 values, reflecting higher water mobility, whatever baking conditions were. These samples presented a smaller decrease in T_2 values over storage time and had higher values than the bottom and the top locations of the crumb of the bread slice. These results confirmed that the relaxation time increased with the moisture content indicating increased mobility of protons, as reported by Assifaoui et al. (2006). According to these authors, the increased mobility of protons can be related to an increase of free volume, in which starch granules are free to move and to the proton population likely associated with water.

Conclusion

In this paper, the impact of two heating rates on macroscopic and microscopic properties of bread crumb during staling has been investigated. Moisture content, water activity, firmness, retrogradation of amylopectin, relaxation of protons and soluble amylose were found to be affected by heating and staling. It was demonstrated that the modifications of starch organization by heat effects during baking can be responsible of increasing firmness. In fact, lower heating rate causes more dispersion of amylose and amylopectin and lead to greater amylopectin retrogradation and contributes to higher amount of soluble amylose and lower firmness. Finally, using a first order kinetic model, staling kinetics were found to be faster for samples baked with higher heating rate. Further studies performed on the starch isolated from the bread crumb can clarify some points by determining pasting and thermal properties and X-ray patterns after iodine exposure.

Acknowledgments This work was supported by the government of Tunisia (PhD grant of E. Besbes), the Scientific Council of Oniris (PhD grant support) and the University 'Nantes, Angers, Le Mans' (LUNAM). The authors acknowledge the collaboration of the University of Guelph, Department of Food Science, to carry out this research.

References

- AACC (1999a) AACC method 61–03: amylose content of milled rice. Approved Methods of the American Association of Cereal Chemists: 1–4

- AACC (1999b) AACC method 74–09.1: Measurement of bread firmness by universal Testing machine. Approved methods of the American Association of Cereal Chemists: 1–4
- Assifaoui A, Champion D, Chiotelli E, Verel A (2006) Characterization of water mobility in biscuit dough using a low-field ^1H NMR technique. *Carbohydr Polym* 64:197–204
- Chen PL, Long Z, Ruan R, Labuza TP (1997) Nuclear magnetic resonance studies of water mobility in bread during storage. *LWT* 30: 178–183
- Curti E, Bubici S, Carini E, Baroni S, Vittadini E (2011) Water molecular dynamics during staling by nuclear magnetic resonance. *LWT Food Sci Technol* 44:854–859
- Davidou S, Le Meste M, Debever E, Bekaert D (1996) A contribution to the study of staling of white bread: effect of water and hydrocolloid. *Food Hydrocoll* 10:375–383
- Eliasson AC (1985) Retrogradation of starch as measured by differential scanning calorimetry. In Hill, Munck (Eds.), *New approaches to Researches on Cereal Carbohydrates* Elsevier Science, Amsterdam
- Engelsen SB, Jensen MK, Pedersen HT, Norgaard L, Munck L (2001) NMR-baking and multivariate prediction of instrumental texture parameters in bread. *J Cereal Sci* 33:59–69
- Fessas D, Schiraldi A (1998) Texture and staling of wheat bread crumb: effects of water extractable proteins and 'pentosans'. *Thermochimica Acta*: 17–26
- Giovanelli G, Peri C, Borri V (1997) Effects of baking temperature on crumb staling kinetics. *Cereal Chem* 74:710–714
- Gray JA, Bemiller JN (2003) Bread staling : molecular basis and control. *Compr Rev Food Sci Food Saf* 2:1–21
- Hallberg L, Chinachoti P (2002) A fresh perspective on staling: the significance of starch recrystallisation on the firming of bread. *J Food Sci* 67:1092–1096
- Jacobs H, Mischenko N, Koch MHJ, Eerlingen RC, Delcour JA, Reynaers H (1998) Evaluation of the impact of annealing on gelatinisation at intermediate water content of wheat and potato starches: a differential scanning calorimetry and small angle X-ray scattering study. *Carbohydr Res* 306:1–10
- Le Bail A, Boumali K, Jury V, Ben-Aissa F, Zuniga R (2009) Impact of baking kinetics on staling rate and mechanical properties of bread crumb and degazed bread crumb. *J Cereal Sci* 50:235–240
- Le-Bail A, Agrane S, Queveau D (2011) Impact of the baking duration on bread staling kinetics. *Food Biopro Technol*. doi:[10.1007/s11947-011-0634-3](https://doi.org/10.1007/s11947-011-0634-3)
- Patel BK, Seetharaman K (2010) Effect of heating rate at different moisture contents on starch retrogradation and starch-water interactions during gelatinization. *Starch* 62:538–546
- Patel BK, Waniska RD, Seetharaman K (2005) Impact of different baking processes on bread firmness and starch properties in breadcrumb. *J Cereal Sci* 42:173–184
- Ribotta PD, Le Bail A (2007) Thermo-physical assessment of bread during staling. *Lebensmittel-Wissenschaft und-Technologie* 40:879–884
- Rogers DE, Zeleznak KJ, Lai CS, Hosney RC (1988) Effect of native lipids, shortening, and bread moisture on bread firming. *Cereal Chem* 65:398–401
- Seetharaman K, Chinnapha N, Waniska RD, White P (2002) Changes in textural, pasting and thermal properties of wheat buns and tortillas during storage. *J Cereal Sci* 35:215–223
- Vodovotz Y, Hallberg L, Chinachoti P (1996) Effect of aging and drying on thermomechanical properties of white bread as characterized by dynamical mechanical analysis (DMA) and differential scanning calorimetry (DSC). *Cereal Chem* 73:264–270
- Yi J, Kerr WL (2009) Combined effects of dough freezing and storage conditions on bread quality factors. *J Food Eng* 93:495–501
- Yi J, Johnson JW, Kerr WL (2009) Properties of bread made from frozen dough containing waxy wheat flour. *J Cereal Sci* 50:364–369
- Zeleznak KJ, Hosney RC (1986) The role of water in retrogradation of wheat starch gels and bread crumb. *Cereal Chem* 63:407–411