

Effect of frozen storage time on the bread crumb and aging of par-baked bread

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Abstract

The effect of frozen storage time of par-baked bread on the bread crumb and staling of bread obtained after thawing and full baking is described. The moisture content, hardness and retrogradation enthalpy of the amylopectin were determined in the par-baked bread and in the full baked bread after 7, 14, 28 and 42 days of frozen storage at $-25\text{ }^{\circ}\text{C}$. In addition, the effect of frozen storage on the crumb microstructure was analyzed by cryo scanning electron microscopy (Cryo-SEM). The moisture content of both partially and full baked bread decreased with the time of frozen storage. The crumb hardness of the par-baked bread after different periods of frozen storage was kept constant, while that of their full baked counterpart increased with the time of frozen storage. In both types of breads, the enthalpy of amylopectin retrogradation did not vary with the period of frozen storage. The staling, measured as hardness increase and amylopectin retrogradation, increased along the frozen storage. The changes observed on the frozen par-baked bread after thawing were attributed to the damage of bread structures produced by the ice crystallization, and the microstructure study support that conclusion.

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1. Introduction

The partial baking of the bread and its further storage at frozen temperatures is an effective way for retarding the staling process of the baked goods (Bárcenas, Benedito, & Rosell, 2004; Fik & Surowka, 2002; Vulicvic, Abdel-Aal, Mittal, & Lu, 2004). A frozen product requires to strictly keeping the frozen chain during production, transportation and storage, which results very expensive. In the case of bread, the cost is plenty justified due to the savings obtained by reducing the economical losses due to bread staling. Among the traditional methods used for extending the shelf life of the food products during long periods of time, the fro-

zen storage is the unique method that allows obtaining a product with similar characteristics to those of the fresh one in a wide variety of food products. Freezing converts the water present in the foods in a non active compound, and this, together with the low temperatures hinder the microorganism growth and the development of chemical and enzyme reactions responsible of the food spoilage.

In the household, the bread is usually kept frozen, and then thawed and baked just before consumption. However, although it is widely accepted that reheating inverts the staling process, this is not enough to recover all the quality characteristics like the smoothness of the recently baked bread (Hug-Iten, Escher, & Conde-Petit, 2003). In contrast, when the bread is partially baked till the crumb is formed without starting the Maillard reactions and rapidly freezing, it is possible to obtain after an appropriated baking a product with similar characteristics to the

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fresh bread. Additionally, the partially baked bread allows better planning of the production and simplifies the baker's work.

Freezing is a technology that allows keeping almost intact the food properties. Nevertheless, it is widely known that the formation of big ice crystals during the freezing process and the recrystallization phenomenon, that involves changes in the number, size and shape of the ice crystals during the frozen storage, damage the food structure. The ice recrystallization leads to the growth of crystals, being the process driven by surface energy differences (Bevilacqua & Zaritzky, 1982). The crystal growth occurs at constant temperature, but it is accelerated by the temperature fluctuations. The overall result is a decrease in the number of crystals and an increase in their size (Reid, 1983).

In the case of baked goods, the effect of freezing and frozen storage of bread was only studied in the fifties (Pence & Standridge, 1955; Pence, Lubisich, Mecham, & Smith, 1955a; Pence, Standridge, Lubisich, Mecham, & Olcott, 1955b); conversely the frozen doughs have been extensively studied during the last decades (Berglund & Shelton, 1993; Havet, Mankai, & Le Bail, 2000; Le Bail, Grinand, Le Cleach, Martínez, & Quilin, 1999; Ribotta, León, & Añón, 2003; Varriano-Marston, Hsu, & Mahdi, 1980; Wolt & D'Appolonia, 1984). In opposition, the effect of freezing and frozen storage on the partially baked bread has been scarcely described. The aim of this study was to analyze the effect of the frozen storage time on the crumb characteristics and the rate of staling of loaves obtained from partially baked bread stored at frozen temperatures.

2. Materials and methods

2.1. Breadmaking process

Commercial wheat flour was purchased from local market. Compressed yeast was used as a starter. The bread recipe consisted in wheat flour (8.0 kg), press yeast (2%, flour basis), salt (2%, flour basis) and water (up to optimum consistency of 500 Brabender Units). Ingredients were mixed, rested for 10 min, divided (150 g), kneaded and mechanically sheeted and rolled to obtain bread dough rolls. Dough was proofed at 28 °C and 85% relative humidity for 90 min. Partial baking was performed in an electric oven at 165 °C for 7 min. Par-baked bread was cooled at room temperature till the core center of the loaves reached 40 °C and then placed into a freezer at –35 °C for faster cooling. A part of the baking set, considered as non stored samples, was kept at room temperature for 30 min, then full baked in the same electric oven at 195 °C for 14 min and finally cooled at room temperature for 60 min. Frozen par-baked breads were packed in polypropylene bags and

kept at –25 °C. At different time (7, 14, 28 and 42 days), bread loaves were thawed and baked as was described above. For the aging studies, full baked bread was packed again and stored at 25 °C for 24 h.

2.2. Time–temperature curves during freezing and frozen storage of par-baked bread

A temperature register (Datapaq, Multi-Tracker System) with a thermal barrier and eight thermocouples probes of copper vs. copper–nickel was used for recording the temperature changes during the breadmaking process and storage. Thermocouples were placed into the core center of the par-baked bread and under the surface. The temperature was registered each 30 s and data analyzed by the software of the register (Datapack Limited, UK).

2.3. Moisture and crumb hardness evaluation

For determining moisture content and crumb hardness in the par-baked bread, loaves were taken from the freezer and kept at 25 °C for 2 h, the time required for reaching 20 °C in the core center of the crumb. The moisture content was measured following the standard method (44-15A AACC, 1995) and the crumb hardness analysis was performed in a texturometer TA-XT2i (Stable Microsystems, Surrey, UK). A 2-cm thick slice was compressed with a 25-mm diameter probe up to 50% compression at 100 mm/min speed. Six replicates were analysed.

2.4. Amylopectin retrogradation determination

A differential scanning calorimeter (Perkin–Elmer DSC-7, USA) was used to simulate the oven in a baking process as reported by León, Durán, and Benedito de Barber (1997), although with slight modifications in order to imitate the interrupted breadmaking process (Bárcenas, Haros, Benedito, & Rosell, 2003b). Bread dough samples (18–20 mg) were weighted in stainless steel pans (PE 0319-0218). An empty capsule was used as a reference. After sealing, capsules were heated from 25 to 90 °C, cooled to 40 °C, immediately placed in a freezer at –35 °C for 15 min and then stored at –18 °C for 7, 15, 30 and 42 days. After different frozen storage times, capsules were thawed at 25 °C for 15 min and heated again in the calorimeter from 25 to 110 °C to complete the baking process. Then, three capsules were kept at 25 °C for one hour and then heated in the calorimeter from 25 to 110 °C, in order to determine the possible retrogradation during bread cooling. The rest of the capsules were stored at 4 °C for analyzing the amylopectin retrogradation during bread staling. Samples after 2, 4 and 7 days of storage were scanned in the DSC from 25 to 110 °C. The parameters measured were

the onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c). The enthalpy associated to the amylopectin retrogradation (ΔH_r) expressed in joules per gram of dry sample was estimated by integrating the area under the endothermic peak. Three replicates for each sample were carried out. All the thermal scans were run at 10 °C/min.

2.5. Cryo scanning electron microscopy (cryo-SEM)

A Jeol JSM-5410 scanning electron microscope equipped with a CT-1500 C cryo-unit (Oxford Instruments) was used. The sample was placed on the cryo-specimen holder, and cryo-fixed in slush nitrogen (≤ -210 °C), then transferred to the cryo-unit in the frozen state, where it was fractured, sublimated (15 min at -90 °C) and sputter coated with gold (4 min, 2 mbar). Finally, the sample was transferred to the microscope and observed at 15 kV and -130 °C.

2.6. Statistical analysis

In order to assess significant differences among samples, it was performed a multiple comparison analysis of samples using the program Statgraphics Plus 5.1 (Statistical Graphics Corporation, UK). Fisher's least significant differences (LSD) test was used to describe means with 95% confidence.

3. Results and discussion

3.1. Time–temperature curves of freezing and frozen storage of the par-baked bread

The time–temperature changes of the par-baked bread during freezing are showed in Fig. 1. In the crumb and the crust were distinguished the three characteristics stages obtained during a food freezing. Firstly, when the loaves enter into the freezer it was observed the bread cooling till reaching the initial freezing temperature. In

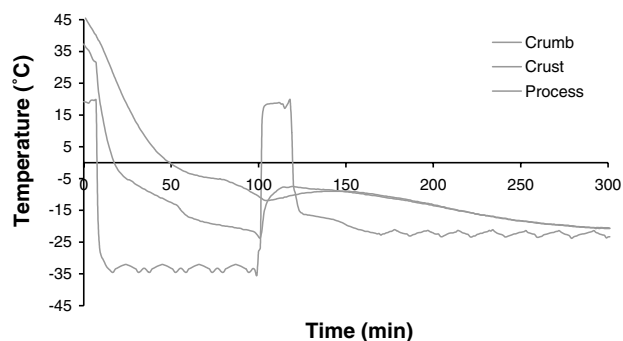


Fig. 1. Temperature–time curves during freezing and frozen storage of par-baked bread.

this stage, the curve showed a pronounced slope that describes a rapid cooling. The freezing of the crumb center started at -5 °C with a minor slope due to the heat used to transform the water from liquid to solid state. It was observed a further increase in the slope when the temperature reached -8 °C in the crumb center, corresponding to the cooling after freezing. During this third stage, the temperature decreased till reaching the freezer temperature. The time–temperature curve and the freezing temperature were similar to that obtained by Hamdami, Monteau, and Le Bail (2004). The rapid increase in the crust temperature (from -23.7 to -7.5 °C in 16 min) during the last stage corresponded to the time required for packaging; that change was not observed in the crumb center, which temperature was still decreasing due to the difference of temperature between the core center (-11 °C) and the crust (-23.7 °C). After packaging, the loaves were stored at -25 °C obtaining a steady decrease in the crust temperature but not in the crumb till both reached equilibrium at -9 °C. From this point, crust and crumb followed the same tendency showing continuous decrease till arrive at -22.5 °C. During storage, the loaves temperature fluctuated between -20 and -22.5 °C.

3.2. Effect of time of frozen storage on the moisture content of both the partially and full baked bread

The moisture content of the par-baked bread and the full baked bread is showed in Table 1. The moisture content of the par-baked bread was higher than that of the full baked bread, because during the full baking some water is evaporated (Leuschner, O'Callaghan, & Arendt, 1997). The moisture content of the par-baked bread significantly ($P < 0.05$) decreased during frozen storage. Vulicevic et al. (2004) found that the moisture content of a formulated par-baked bread stored at frozen temperatures was one of the quality attributes mostly affected during frozen storage. This can be attributed to a reduction of the water retention capacity of the bread constituents. Similar effects have been described during freezing and frozen storage of bread dough systems. Seguchi, Nikaidoo, and Morimoto (2003) found that

Table 1

Effect of the time of frozen storage at -25 °C on the moisture content of the par-baked bread and the full baked bread

Time (days)	Moisture content (%)	
	Par-baked bread	Full baked bread
0	41.40 ± 0.03h	36.21 ± 0.01c
7	41.03 ± 0.07f	36.22 ± 0.07c
14	41.03 ± 0.02f	36.50 ± 0.07d
28	41.22 ± 0.03g	35.77 ± 0.08b
42	40.81 ± 0.04e	35.33 ± 0.05a

Means followed by the same letter were not significantly different ($P < 0.05$).

freezing and frozen storage of the bread dough increase the amount of free water molecules. In addition, Sharanant and Khan (2003) stated that the water retention capacity of the dough decreases with the time of frozen storage, due to the physical damage promoted by the ice recrystallization on the gluten network. Additionally, the amount of freezable water, namely the fraction of free water that is not bound to gluten during dough formation, increases with the time of frozen storage due to the damage produced on the gluten network by the freezing and frozen storage (Bhattacharya, Langstaff, & Berzonsky, 2003; Lu & Grant, 1999).

3.3. Effect of the time of frozen storage on the hardness of both partially and full baked bread

The crumb hardness was evaluated on the par-baked bread after different time of frozen storage and also on the full baked bread and after 24 h of aging at 25 °C (Fig. 2). The crumb hardness of the par-baked breads were significantly ($P < 0.05$) lower than that of the full baked counterpart, which was expected because the significantly ($P < 0.05$) higher moisture content of the par-baked bread. The inverse relationship between the hardness and the moisture content has been previously reported (He & Hosney, 1990; Rogers, Zeleznak, Lai, & Hosney, 1988). Schiraldi and Fessas (2001) proposed that water acts as a plasticizer in the bread; the decrease in the moisture content favors the formation of hydrogen bonds among the starch polymers or between the starch and the proteins yielding greater hardness.

In Fig. 2 is also observed that the hardness of the par-baked bread was almost constant (not significant differences, $P < 0.05$, were found) during the period of frozen storage. Similar results were observed for Irish brown soda bread (Leuschner et al., 1997). This behavior indicates that at frozen temperatures, the phenomena involved in the hardening were not present or they occurred in a very slow rate.

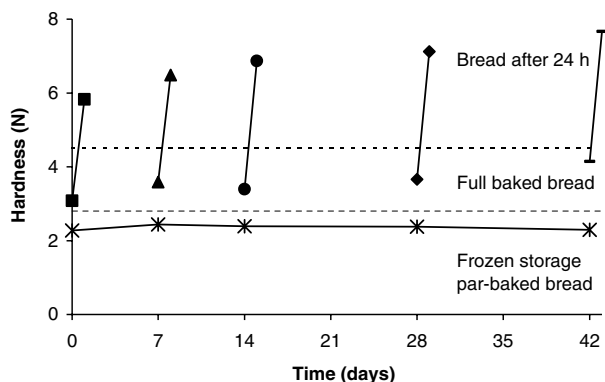


Fig. 2. Effect of frozen storage time at -25 °C on the hardness of the par-baked bread after partially baking, freezing and frozen storage (*) and the full baked counterparts (■, ▲, ●, ◆, –) including their hardness after 24 h of staling at 25 °C.

However, the initial hardness of the full baked bread showed a significant ($P < 0.05$) increase with the time of frozen storage. A similar increase in the hardness was observed in formulated breads obtained with interrupted baking process and frozen storage (Fik & Surowka, 2002; Vulicevic et al., 2004). The increase in the initial hardness as a consequence of the frozen storage, indicates that the damage of the bread constituents produced during frozen storage might produce some effects during the full baking or the posterior cooling that favor the hardening. The amylose recrystallization plays an important role in the initial crumb hardness and in the first stages of aging (Hug-Iten et al., 2003; Kim & D'Appolonia, 1977; Zobel & Kulp, 1996), because the formation of amylose network brings about the bread hardening. A microscopic study revealed that during baking there is a phase separation between amylose and amylopectin leading to an accumulation of amylose in the center of the starch granules (Hug-Iten, Handschin, Conde-Petit, & Escher, 1999). Conversely, the damage produced on the starch granules by the ice recrystallization, which increases with the time of frozen storage, would allow leaching of intracellular amylose, increasing the interaction between the inter and intragranular amylose and the formation of a network of amylose that bring about an increase in the crumb hardness.

An alternative explanation for the hardness increase is consequence of the growth of the ice crystals during the frozen storage. Those crystals could damage the protein network formed by the denatured proteins, which are responsible of the crumb structure and that damage might increase due to the thawing and baking. Yamauchi et al. (1999) observed an increase in the crumb hardness of the bread obtained from frozen dough and they ascribed that effect to the degradation of the crumb structure promoted by freezing. In fact, Kou and Chinachoti (1991) showed that crumb compression produces a mechanical damage and this becomes evident by an increase in the crumb hardness. Some effect has also been attributed to the elasto-plastic changes due to the modification of starch structure (Fik & Surowka, 2002).

Since the alteration produced by the ice crystallization on the structural constituents of bread occurred during frozen storage, it should be expected that the frozen par-baked bread would have a similar behavior concerning the hardness to the fresh bread. However, it should be stressed that the moisture content of the par-baked bread was higher than that of the full baked bread. This difference could be decisive for the occurrence of the amylose crystallization phenomena or for becoming evident the mechanical damage due to the plasticizer role of the water on the food systems.

Regarding the staling, the crumb hardness considerably increased along the 24 h storage at 25 °C (Fig. 2).

The bread hardening is a complex process that although has been widely studied (Chinachoti & Vodovotz, 2001; D'Appolonia & Morand, 1981; Kulp & Ponte, 1981; Maga, 1975; Zobel & Kulp, 1996), is still not completely understood. The rapid increase of the crumb hardness can be attributed to the amylopectin recrystallization (Hug-Iten et al., 2003; Schoch & French, 1947), the formation of complexes between starch and proteins (Martin, Zeleznak, & Hoseneý, 1991), the water redistribution among the bread constituents (He & Hoseneý, 1990), and other phenomena that easily occur in this baked product during storage.

The hardness increase of the full baked bread during aging significantly ($P < 0.05$) augmented with the period of frozen storage of the par-baked bread, for instance the bread from 42 days stored par-baked had a hardness increase of 3.53 N in front of 2.75 N for the bread from non stored par-baked bread. This finding agrees with previous results of Bárcenas et al. (2004) for par-baked bread added with hydrocolloids.

3.4. Effect of the frozen storage period on the retrogradation enthalpy of amylopectin in both the partially and full baked bread

The retrogradation enthalpy of the amylopectin was measured in the par-baked bread after 7, 15, 30 and 42 days of frozen storage at $-25\text{ }^{\circ}\text{C}$. For aging studies, the retrogradation enthalpy was also determined in the full baked bread after 2, 4 and 7 days of storage at $4\text{ }^{\circ}\text{C}$, this temperature was selected for accelerating the staling process. In Fig. 3 can be observed that the par-baked bread did not show a retrogradation peak at any time of frozen storage. Likely, if all the water present in the system was in solid state, there are little possibilities that the starch polymers can interact among them, forming the crystalline structures associated to the retrogradation phenomena.

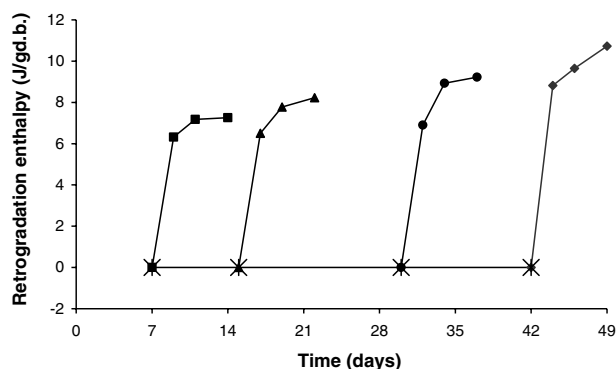


Fig. 3. Effect of frozen storage time at $-25\text{ }^{\circ}\text{C}$ on the retrogradation enthalpy of the par-baked bread after partially baking, freezing and frozen storage (*) and the full baked counterparts (■, ▲, ●, ◆) with their behavior during storage at $25\text{ }^{\circ}\text{C}$.

In the case of full baked bread neither appeared a retrogradation peak at 1 h after the full baking. The same result was obtained in the bread made by straight bread-making process (Czuchajowska & Pomeranz, 1989; Hug-Iten et al., 2003). The absence of retrogradation peaks in the recently baked bread does not agree with the crumb hardness results, because full baked bread had higher crumb hardness than the par-baked bread (Fig. 2). Although the existence of a cause-effect relationship between the starch retrogradation and crumb hardness have been continuously questioned (Baik & Chinachoti, 2000; Dragsdorf & Varriano-Marston, 1980; Ghiasi, Hoseneý, Zeleznak, & Rogers, 1984; Hallberg & Chinachoti, 2002; Martin & Hoseneý, 1991; Sahlström & Braten, 1997), it has been admitted that both phenomena can occur simultaneously (Gray & Bemiller, 2003). In the present study, it is worthy to highlight some causes for explaining the difference between the amylopectin retrogradation and crumb hardness in both the partially and full baked bread. The highest hardness observed in the full baked bread in comparison to par-baked bread was likely due to the loss of moisture content during the second baking. In opposition, there was not any water loss in the assessment of the retrogradation enthalpy since the capsules were hermetically sealed. In fact, in order to confirm the last statement capsules were weighted after each thermal scan (results not shown) showing a constant weight. Thus the capsules containing the simulated par-baked bread and the ones containing the simulated full baked bread had the same water content available for the amylopectin retrogradation. Levine and Slade (1990) stated the importance of the water content for the amylopectin crystallization. They proposed the necessity of a minimum amount of water for the polymer chains plasticization, in order to have the required mobility for the crystallization process and for the water incorporation into the structure of the crystalline networks. In addition, concerning the amylose crystallization during the bread cooling, it is possible that the amylose had already retrograded before the DSC analysis, because the amylose retrogradation is very rapid. The amylose crystals only melted at $150\text{ }^{\circ}\text{C}$ (Eberstein, Hoepcke, Konieczny-Janda, & Stute, 1980), thus they could not be detected at the scanning temperatures used in this study ($25\text{--}110\text{ }^{\circ}\text{C}$).

In Fig. 3 can be also observed that samples from all the different frozen times presented a rapid increase in the retrogradation enthalpy after 2 days storage at $4\text{ }^{\circ}\text{C}$, and a more slowly one beyond that time. This increase was expected because the temperature used for storage was optimum for the amylopectin recrystallization (Slade & Levine, 1987). The increase in the amylopectin retrogradation during aging of bread has been previously reported (Baik & Chinachoti, 2000; Bárcenas, Haros, & Rosell, 2003a; León et al., 1997; Ribotta et al., 2003; Rogers et al., 1988; Xie, Dowell, & Sun,

2004), and, although it is recognized that is not the unique phenomena during staling, it is the main responsible of it (Baik & Chinachoti, 2000; Gray & Bemiller, 2003; Krog, Olesen, Toenaes, & Joensson, 1988; Slade & Levine, 1991; Xie et al., 2004; Zobel & Kulp, 1996). On the other hand, the retrogradation enthalpy of the amylopectin at 24 hours storage increased with the enhancement of the frozen storage time, being the enthalpy increase of 0.94 J/g db in the samples from 7 days frozen storage and 1.9 J/g db in the samples from 42 days of frozen storage. This results support the hypothesis that frozen storage during a prolonged period had high recrystallization and in consequence, great damage on the structural constituents of the crumb. It is possible that the damage also promotes an increase in the amount of free molecules of water and in turn the retrogradation enthalpy, because it has been reported that there is a positive relationship between the retrogradation enthalpy of the starch and the moisture content of the bread (Chinachoti & Steinberg, 1986; León et al., 1997; Rogers et al., 1988). In addition, the interaction among the starch polymers might be facilitated in the damaged structure leading to greater amylopectin recrystallization.

3.5. Influence of the frozen storage time on the microstructure of the bread from par-baked bread

The microstructure of the bread obtained from par-baked frozen bread without storage (Fig. 4(b)) and with 42 days storage at -25°C (Fig. 4(c)) was compared with the microstructure of the bread obtained by conventional breadmaking process (Fig. 4(a)).

The appearance of the gas cell wall of the bread from par-baked frozen bread without storage was similar to that of the bread from conventional process. In both cases it was possible to differentiate two phases, the continuous phase formed by the elastic protein network of denatured gluten and lixiviated starch polymers and the discontinuous phase composed by clearly distinguished starch granules embedded in the continuous

phase (Gray & Bemiller, 2003). In this complex structure some cavities were also present. Rojas, Rosell, Benedito de Barber, Pérez-Munuera, and Lluch (2000) described similar structures in the fresh bread obtained by conventional process. Instead, in the gas cell walls of the bread from par-baked bread after prolonged frozen storage (Fig. 4(c)) it was observed a disordered structure, without distinguishing the crumb components. In addition the microstructure showed a dry, compact and hair-like cracked aspect. The appearance of this bread could confirm the presence of ice crystal that damage the crumb structure, mainly the protein matrix, bringing about the rupture of the denatured gluten network and the subsequent release of the bound water, and also some damage and deformed starch granules.

The effect of the freezing on the microstructure of bread dough has been previously studied (Berglund, Shelton, & Freeman, 1991; Ribotta, Pérez, León, & Añón, 2004; Varriano-Marston et al., 1980), concluding that this process mainly damages the protein matrix. Similar results were obtained by Naito et al. (2004) in the bread from frozen doughs. Berglund et al. (1991) also observed an internal damage in the starch granules as a consequence of the freezing and they suggested that the damaged starch granules could remove water from the protein matrix.

4. Conclusions

The increase in the time of frozen storage of the par-baked bread leads to a decrease in the quality of the resulting full baked bread, namely a loss in the moisture content and an increase of the crumb hardness. In addition, long times of frozen storage seems to be associated to greater aging rates. The microstructure results from cryo-SEM might indicate that the physical damage undergone by the crumb constituents of the par-baked bread during frozen storage caused by the progressive growing of the ice crystals. This damage seems to be the main responsible of the quality loss and the greater speed rate of aging.

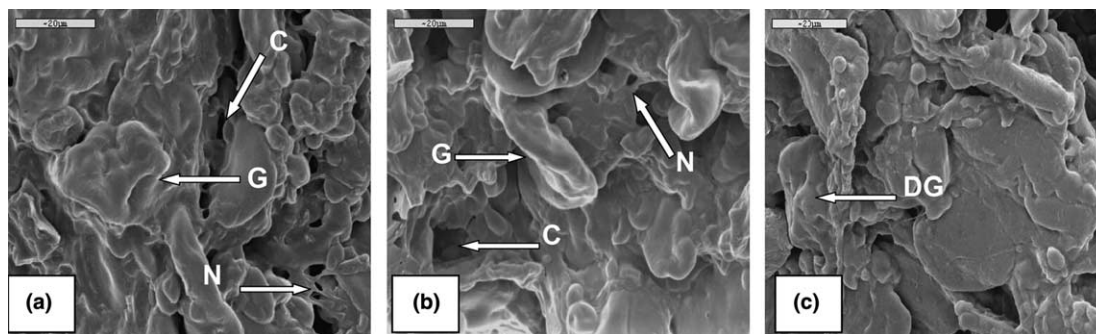


Fig. 4. Cryo-SEM micrographs of gas cell walls (1500X) of bread from conventional breadmaking process (a), bread crumb from par-baked bread after freezing, thawing and baking (b), and crumb bread from par-baked frozen bread after 42 days of frozen storage (c). The arrows show starch granules (G), cavities (C), protein network (N), and damaged granules (DG).

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