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An option to shorten baking process

Developing the production process in the bakery industry is a challenge that has been studied for some decades. It is well known that baked products have a short shelf life and that it requires some hours to have freshly baked bread. One of the processes that has been developed and commonly used within this industry in order to prolong shelf life and make it easier for customers to sell freshly baked bread is the freezing process.

Nowadays many of the companies selling freshly baked bread buy the bread dough frozen. They usually finish the process by thawing the dough, proofing it and finally, baking the bread. With this process, supermarkets and other companies can sell freshly baked bread without much effort. At the same time though, the process may sometimes go wrong if customers do not follow all the instructions and maybe skip some of the necessary steps, obtaining bread with low volume and compact crumb. An interesting aspect is to try to shorten the baking process so that the bread industry's customers do not need to go through so many steps in order to get high quality bread. This aspect can serve as competitive advantage when it comes to sales within bread industry.

Different proofing profiles for two different bread doughs (savory and sweet) before freezing were studied in a project carried out at SIK in Gothenburg in order to see how they affected the final texture, volume, microstructure and consumer acceptance of the breads. This study was performed to test the possibility of skipping one of the steps in the process after frozen storage; namely, that of the final proofing step. The results showed that this may be possible for savory bread, but further studies still need to be conducted. Some differences were observed between the breads with different proofing profiles and the consumers also noticed those differences. The different fermentation times also proved to affect the shelf life of the bread, as it was seen that the longest proofing time before freezing had the shortest shelf life. On the other hand, the sweet bread proved to be more challenging, as it is harder to get a strong and homogeneous microstructure when the dough contains a high amount of sugar and fat, resulting in a low volume.

As stated before, great achievements have been made in the bakery industry with regards to savory bread with the aim of developing a process in which the final proofing could be skipped. The next step for researchers would be to develop a recipe for the savory bread that would decrease the differences that resulted from the different proofing profiles, as well as test different mixing techniques for sweet doughs.

Abstract

Within the bakery industry, as well as in all other industries, product development is an important area of research. The freezing process and its effects on the final bread have been studied over the last decades. The main objective of the present study was to verify if bread that has been fully proofed as dough prior to the freezing process has the same quality as bread from doughs that were non-proofed, or partly proofed before freezing, as well as to find out how pre-fermentation affects the porosity and texture of the final bread. Two kinds of bread dough (sweet and rye) mixed in an industrial bakery were proofed at three different profiles (non-proofed, half-proofed and fully proofed), then frozen and stored 2.5 months at a temperature of -20°C. The dough microstructure was studied with a confocal laser scanning microscopy and a computed tomography method. The volume, texture, final appearance and consumer acceptance of the final baked buns were all evaluated.

The results showed that, in the final appearance, the volume of the bread containing rye flour at different proofing profiles was more similar to the volume of the reference sample, which was the non-proofed dough before freezing, than the volume of the sweet breads was. Sweet dough that had been half and fully proofed resulted in a lower volume, even after optimizing the process. Despite the similarities between the buns containing rye flour, a significant difference was also observed between them in the product profile obtained from the sensory analyses, in addition to the specific volume and the compressive stress. Product profiles for the different proofing profiles differed in almost all the tested attributes except in elasticity and flavor. Consumers were also able to distinguish between the products.

Sweet dough showed an uneven gluten network that was not well developed and in some of the dough types showed agglutinated fat. Using 3D-micro-CT images, it was possible to observe some differences in density and areas with no pores. This study reveals that some achievements have been accomplished with regards to savory bread, but there is still a need for more research to be carried out on sweet bread as well as to develop a process in which a final proofing could be avoided in today's bread industry.

The bread baked from dough that was frozen and stored long-term, 4.5 months at -20°C, showed a noticeable volume reduction. Prolonged proofing time was not enough to compensate and obtain a higher volume.

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1. Introduction

Product development is an important aspect of all companies; it helps companies to obtain greater consumer acceptance and to optimize processes, equipment, prices, etc. A great deal has been done within the bakery industry in order to improve and develop the baking process as well as to extend shelf life of baked products. Since these products are known for having a short shelf life, the freezing process has been an important advance for this industry, improving working hours and making it easier for many companies to produce "freshly baked bread". Freezing, however, has some negative effects on the dough and the final bread quality (Yi & Kerr, 2009; Bárcenas & Rosell, 2006; Selomulyo & Zhou, 2007).

Bread quality can be referred to according to its many different characteristics; microbiological, chemical and physical changes such as texture and sensory properties, just to name a few. It can also be classified by other characterizations of breads: consumer based, producer based and product based properties, as they refer to different aspects (Cauvain, 2003). In the last decades, a correlation has been observed between sensory quality, acceptance and the structure and microstructure of the products. Microscopy has for this reason become a good tool for observing the changes caused by different processes on a product like bread (Polaki et al, 2010).

The present study is part of a project where different stakeholders and SIK, Swedish Institute for Food and Biotechnology, study changes on frozen dough and bread. Through this part of the study, the impact of different proofing profiles on the dough and bread structure and microstructure prior to freezing process has been analyzed. A consumer acceptance test was performed to study end users' preferences. A descriptive sensory analysis with some of the bread samples was carried out parallel to this study.

Keywords: Proofing profile, bread structure, microstructure, texture, acceptance

2. Objectives

The main objective of this study was to verify if bread that has been fully proofed as dough prior to the freezing process has the same quality as bread from doughs that were non-proofed, or partly proofed before freezing, as well as to find out how pre-fermentation affects the porosity and texture of the final bread. In order to achieve this, thawed dough as well as freshly baked breads and two day old breads, were analyzed by measuring weight, volume, texture and microstructure. Pictures of the baked buns were also taken in order to study the final appearance. A descriptive sensory analysis was carried out with a trained panel and a minor consumer test was performed with the purpose of analyzing if consumers noticed a difference between the breads that were subjected to different processes.

Two kinds of breads that had been proofed in different ways were tested; the first one contained 19% rye flour and the second one was a sweet bun with 9.3% added sugar. Both varieties were produced in a bakery, frozen, stored 2.5 months at -20°C and then baked.

The key questions in the present study were the following:

- How does the proofing profile affect the texture, volume and appearance of the final bread?

- How are the macrostructure and microstructure of the bread affected by proofing profile?
- Is it necessary to proof the dough after frozen storage in order to achieve a higher quality in the final baked bread?
- How do product profiles differ among the breads baked from fully proofed, half-proofed and non-proofed doughs?
- Do consumers notice a difference between the samples?

3. Background

In addition to being one of the most commonly consumed products in the world, bread is also one of the main products around which a great deal of research in the food industry focuses. Bread quality is related to many properties and concepts such as crumb and crust appearance, physical texture, shortened shelf life due to chemical, microbiological and physical aspects, sensory experience, etc. (Scanlon & Zghal, 2001). Because of its high demand, many improvements on bread have been achieved in the bakery industry in order to satisfy customers' expectations, as well as to develop bread production and preservation (Yi & Kerr, 2009). The achieved innovations have an effect on the structure, which consequently affects the sensory experience (Carr et al, 2006). These deviations are often studied and minimized in order to offer increasingly higher quality.

3.1 Bread ingredients and baking process

All the ingredients and the way of processing them have an impact on the final product. Bread is essentially made out of water, flour, yeast or another razing agent and salt (Scanlon & Zghal, 2001). Wheat flour components are mainly starch (70-80%), lipids (1.1-2.5%), proteins (8-12%), and pentosans (1.5-3%) (Blanchard et al, 2012). Among the flour proteins, gluten constitutes about 80-85%, including glutenins and gliadins that will form the protein network with visco-elastic properties during the baking process (Blanchard et al, 2012).

The different operations during the process affect the dough formation and thereafter the final baked bread. Scanlon and Zghal (2001) mention three important steps to bread making in their study of bread properties: the mixing of the ingredients and development of the dough, the arrangement of the foam structure and the stabilization of the structure.

3.1.1 Mixing and development

Excluding the function of combining all the ingredients, mixing is an important operation given the fact that during this part of the process the structure of the bread is formed, the visco-elastic properties of the gluten are developed and air is incorporated into the dough (Dobraszczyk & Morgenstern, 2003). During the mixing process, the hydrophilic groups in the protein molecules and the starch contained in the flour absorb the water; this step is often called hydration (Scanlon & Zghal, 2001).The visco-elastic gluten network is formed when the glutenin protein molecules, which contain subunits with large elastic polymers with amino acids sequences rich in proline and glutamine, link up to each other until forming long gluten molecules with elastic properties (Figure 1) (Mc Gee, 2004; Shimoni et al, 1996). The resulting network with cross-linked gluten molecules has plastic and elastic properties that allow dough expansion. The plastic characteristics are a consequence of the gliadin proteins present between the glutenins (Mc Gee, 2004). As mentioned before, the incorporation of air is one of the consequences of the mixing process. Air is an important factor related to the volume of the bread and is trapped in the dough while mixing (Scanlon & Zghal, 2001). The starch, constituting about 70% of the wheat flour and responsible for almost half of the dough volume, penetrates the gluten network in which it is encapsulated (Scanlon & Zghal, 2001; Mc Gee, 2004).

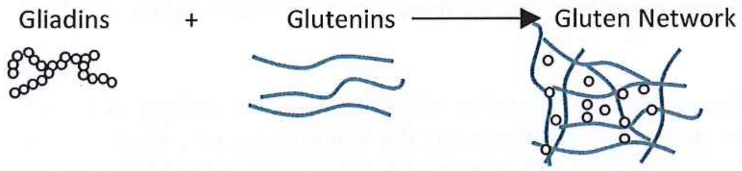

Figure 1. Development of gluten network.

3.1.2 Formation of the bread structure

Santos et al (2008) described bread as an elastic solid foam with a continuous and a discontinuous phase. This foam is formed due to the network of cross-linked gluten molecules already named and starch granules (Santos et al, 2008). When the yeast produces CO_2, the gas cells will expand and the density of the dough will decrease (Scanlon & Zghal, 2001). In order to improve the crumb, bakers usually punch, sheet and shape the bread. This step will control and distribute the bubble sizes in the dough (Dobraszczyk & Morgenstern, 2003; Scanlon & Zghal, 2001). During proofing and baking processes, the growth of gas cells determines the volume, expansion and texture of the final bread. The limit of growth correlates to the stability because of the coalescence of the bubbles which leads to the disclose of gas and collapse (Dobraszczyk & Morgenstern, 2003).

3.1.3 Stabilization of the structure- baking

It is at the baking stage that the final structure is determined. The expansion of the gas bubbles is limited because of an increased viscosity of the gas cell walls and the liquid phase is transformed into a solid one due to thermal conditions (Scanlon & Zghal, 2001). Some polymers in the gluten network aggregate, leading to a more rigid structure as they become more strongly linked (Scanlon & Zghal, 2001). Starch gelatinization and the previously named denaturation of the proteins stimulate the development of the final crumb structure, whereas the high temperatures and dehydration of the surface form the bread crust (Santos et al, 2008).

3.2 Aging and shelf life

Bread and other bakery products are known for having a short shelf life. These products lose their freshness and softness, which is often correlated to lower quality and a decreased consumer acceptance relatively quickly (Curic et al, 2008). The concept used to refer to the aging of bread is staling, and applies to all the changes in bread structure that leads to its decreased quality; it involves recrystallization of amylopectin and a reconnection of the polymers resulting in reduced water mobility parallel to a loss of flavor and aroma (Santos et al, 2008; Curic et al, 2008; Selomulyo & Zhou, 2007). Starch molecules re-associate upon aging and may precipitate leading to a change in consistency (Santos et al, 2008).

In their study about kinetics in staling rate Le Bail et al (2009) showed that bread with crust has a higher staling rate than that without crust. They stated that during storage, the crust tends to catch moisture from the crumb in order to achieve a moisture balance between the two (Le Bail et al, 2009). Another aspect that showed an influence in the staling process was the storage temperature, storing the bread at a freezing temperature accelerates aging (Le Bail et al, 2009).

A number of different techniques have been improved in order to decrease staling in bread. Freezing is one of the most common processes, as it permits the obtainment of a quality very similar to that of fresh bread (Bárcenas & Rosell, 2006). Another option is adding other ingredients to the dough that will help delay the aging of the bread. These ingredients are commonly called bread improvers (Selomulyo & Zhou, 2007).

3.2.1 Freezing possibilities

Frozen storage has been a common process within the bakery industry for many years. As previously mentioned, this step helps delay staling and extend shelf life due to the fact that microorganisms are not able to grow at such low temperatures and low water activity; it also delays the chemical and enzyme reactions (Bárcenas & Rosell, 2006). Apart from the quality aspects of the bread, freezing is a popular process and often used because it reduces the labor hours, the long processes and the knowledge required to bake fresh bread (Yi & Kerr, 2009; Naito et al, 2004).

In the bread industry, the freezing options often used are unproofed frozen doughs, pre-proofed frozen doughs, partially baked products and baked frozen bread (Meziani et al, 2011; Curic et al, 2008; Bárcenas & Rosell, 2006: Gabric et al, 2011).

Unfermented frozen doughs need both time for thawing and proofing prior to the baking process, as well as competent staff with some baking knowledge. This method is often used for sweet buns and pizza dough etc. (Le-Bail et al, 2010; Meziani et al, 2012a). What is often referred to as pre-fermented frozen dough or partially fermented dough is a process in which the dough ingredients are mixed and left to proof; the fermentation is then interrupted by lowering the temperature and storing the dough frozen (Le-Bail et al, 2010). Partially baked products, also called Bake Off Technology (BOT), is thought to be leading the bakery market. The production process followed is as the fresh bread mixing, resting, dough shaping, proofing, and baked. The difference is that the baking process is interrupted when the crumb is formed but no Maillard reaction has started, at this point the bread is rapidly frozen (Bárcenas & Rosell, 2006).

3.2.1.1 Negative effects of freezing storage

Even if freezing processes have helped to increase the shelf life of bakery products, it is also well known that the structure of the bread is damaged as a result of storage at low temperatures. When the dough is subjected to temperatures below zero, free water leaks and forms ice crystals that may grow and damage the gluten network during storage (Bárcenas & Rosell, 2006; Naito et al, 2004; Yi & Kerr, 2009; Meziani et al, 2012b). These ice crystals may also affect the yeast membrane leading to a decreased viability (Naito et al, 2004; Yi &

Kerr, 2009). A third effect that has been observed is crust flaking, mainly in BOT which may be related to the large difference of temperatures (freezer to oven) (Curic et al, 2008). All the above mentioned effects result in different texture, pore size density, volume and consumer acceptance than the expected ones. In some studies the focus has been the effect of long-term storage (Giannou & Tzia, 2007; Bárcenas & Rosell, 2006), others focus on the temperature rates (Mezani et al, 2011; Mezani et al, 2012a; Le-Bail et al, 2010), and others on freezing-thawing cycles and the effect of these on the crumb, gluten fibres and bubble sizes (Naito et al, 2004; Lucas et al, 2010).

The behaviour of pre-proofed doughs was investigated in a study conducted by Lucas et al (2010). The authors mentioned that doughs that have been subjected to proofing before freezing showed a deformation, an increased volume of large bubbles, a decrease of specific volume and height, and a densification of the bottom part of the dough leading to a larger collapse of the dough. The reason behind all these aspects seems to be the compression of the gases contained in the dough during cooling leading to a rupture of the dough between the air bubbles, resulting in their coalescence (Lucas et al, 2010). The same study showed that the greatest collapse was observed in the doughs that had the longest fermentation time previous to freezing and were thawed before baking (Lucas et al, 2010).

3.2.2 Dough improvers

In order to decrease the damage caused by freezing and in order to delay retrogradation and increase shelf life, different dough improvers are used in the bakery industry. Improvers is a term used for all the additives used in doughs to help stabilize, emulsify, decrease oxidation, strengthen the dough, etc (Selomulyo & Zhou, 2007). The most common improvers are emulsifiers, hydrocolloids, ascorbic acid, and even fats and dietary fibers (Santos et al, 2008; Selomulyo & Zhou, 2007).

Dietary fiber has shown to both increase the nutritional value of bread and to optimize freezing. Santos et al (2008) presented in their study that bread baked with fiber delayed and lowered amylopectin retrogradation. Different types of dietary fiber were evaluated in a later study and showed an effect on pore size distribution, gluten network and microstructure of the tested breads producing changes in the sensory attributes (Polaki et al, 2010).

Emulsifiers act as improvers as they interact with the starch and prevent the migration of water between gluten and starch. Surfactants may also act together with the lipids contained in the dough reducing the surface tension in the bubbles (Selomulyo & Zhou, 2007). The most common emulsifiers are diacetyl tartaric acid esters of mono and diglycerides (DATEM) and sucrose esters (Selomulyo & Zhou, 2007). DATEM is known as a dough strengthener as it causes aggregation of the gluten proteins resulting in a much stronger network. It also acts as a crumb softener as it interacts with both the amylose and amylopectin molecules and reduces water migration (Selomulyo & Zhou, 2007). Sucrose esters interact with proteins and avoid flocculation due to the hydrophilic head of the emulsifiers, which will interact with the protein surface and the lipophilic tale, which then react with the non-polar side of the amino acids (Selomulyo & Zhou, 2007).

Hydrocolloids are known for stabilizing emulsions and foams. In baking processes it is seen that hydrocolloids form complexes with the starch polymers. They also influence the melting, gelatinization and retrogradation of starch (Selomulyo & Zhou, 2007).

Ascorbic acid helps to decrease oxidation. It also works as a strengthener due to the creation of disulphide bonds in the gluten network (Selomulyo & Zhou, 2007). Lipids help during freezing storage as they protect bio-membranes from damage on account of the low temperatures during freezing (Naito et al, 2004).

3.3 Other ingredients

3.3.1 Rye flour

Rye is one of the most commonly used cereals now a days in the northern part of Europe and the second most used in the bread industry (Michalska et al, 2008; Rakha et al, 2010). This cereal is known for having the highest amount of dietary fiber (DF) among the different cereals. The principal components of DF in rye are arabionoxylan, fructan and β-glucan (Andersson et al, 2010; Rakha et al, 2010). The DF content, as well as the antioxidants present in rye flour, are some of the reasons why breads baked with rye flour are considered to be healthier than white wheat bread (Michalska, 2008). Some studies have shown that the milling extraction rate and the amount of whole grain present in the dough, as well as the kind of bread that is produced, affect the fiber and antioxidant content in the final bread. Crisp breads, for instance, showed a much higher amount than soft breads (Andersson et al, 2010; Rakha, 2010). The reason why soft breads show a lower fiber content is because in order to develop a strong gluten network, a large amount of refined wheat flour needs to be added (Rakha et al, 2010).

Besides the health aspects, it has been noticed that rye flour has an effect on baking processes. The amount of rye in the dough directly influences the Maillard reaction during the baking process. This effect is a consequence of variation in the amount of carbohydrate due to changes in amylase activity, which in turn affect the reaction with the proteins (Michalska et al, 2008). Another aspect is that rye has an important effect on staling and starch retrogradation of bread as it contains a great amount of arabinoxylan in the cell walls, which has a high water-holding capacity (Rakha et al 2010).

3.3.2 Sugar

Freezing storage of sweet breads and pastries has long been used by industries. These kinds of products contain large amounts of sugar and also butter or other oils and alcohols that protect the bio-membranes and have a strong influence on sensory experience (Naito et al, 2004; Meziani et al, 2012c). On the other hand, Huang et al (2008) present baking of frozen sweet dough as a challenge. Freezing processes, as mentioned before, affect the final structure and quality of the bread. When sugar is present in the dough, the osmotic pressure increases and the yeast cells may dehydrate more quickly, decreasing the gas production ability and leading to a lower final volume (Huang et al, 2008; Meziani, 2012a). Meziani et al (2012c) also mentioned in their study that sugar increases the development of yeast before freezing. Another result reported as a consequence of having sugar in bread dough is that the rheological characteristics changed, increasing the final rigidity of the bread due to the formation of ice crystals (Meziani, 2011).

However, it has been reported that sweet dough requires a longer mixing process than white bread dough in order to develop the matrix of gluten as well as the porous structure of the dough (Calderón-Dominguez et al, 2003; Tlapale-Valdivia et al, 2010).

3.4 Microscopy

There is a strong relation between the macrostructure and the microstructure of food products, as well as with their sensory attributes (Polaki et al, 2010). Different kinds of microscopy techniques have therefore been a useful tool to analyze the structures of different products (Polaki et al, 2010). Microscopy has been used in the bakery industry to examine dough and bread microstructures and the physical changes that take place during the preparation and storage processes (Peighambardoust et al, 2010). Different studies have shown that microscopy techniques provide accurate images of the different components in the microstructure. For example, it is useful to study the starch granules and the protein network in bread dough (Peighambardoust et al 2006; Peighambardoust et al, 2010; Polaki et al, 2010). Confocal laser scanning microscopy (CLSM) is one of the most commonly used techniques.

3.4.1 CLSM

The confocal laser scanning microscope (CLSM) allows the analysis of dynamic conditions and the studied samples may be bulk samples, which permits the obtainment of information about the microstructure of the product at different depths (Lorén et al, 2007). CLSM is a fluorescent microscope that works because of the fluorescent properties of some atoms and molecules that absorb the light in a certain wavelength and then release it after some time. As most food products are not fluorescent it is necessary to stain them so that they can be visible in the microscope (Lorén et al, 2007). CLSM can be equipped with a temperature table that makes it possible to control and follow all the changes in the structure of the samples at changing temperatures; at a freezing stage or during heating (Lorén et al, 2007).

3.5 3D Micro-CT

During the 1970s the computed tomography method (CT) was developed in order to analyze, with X-ray, the internal structure of different objects without destroying them. Later on, this technology was further developed, making it possible to detect microscales, called micro-CT or μCT (Ho et al, 2013). In this technique, the X-rays are sent through the studied objects and, depending on the density and atomic number, they will be more or less absorbed, resulting in projected images. These images are the reconstruction of virtual slices of the object from different angles providing information of the internal structure of the analyzed object (Ho et al, 2013).

3.6 Sensory analyses and consumer attitude

The organoleptic perception of bread is of great importance when regarding consumer acceptance of bread and their attitude towards buying a certain product or not (Carr et al, 2006). Sensory analysis has therefore often been used by researchers as a tool to measure quality and acceptance (Carr et al, 2006; Polaki et al, 2010; Curic et al, 2008). Lassoued et al (2008) allude to the importance of bakery industries in evaluating sensory perception, but that is not often taken into account because it is time consuming to have a group of panelists that meets for several training sessions (Lassoued et al, 2008). Since a correlation has been shown between the sensory evaluations and instrumental methods, many companies choose to rely on instrumental measurements (Lassoued et al, 2008). There is still, however, a need to take into consideration organoleptic characteristics and how individuals perceive them.

The sensory analyses that are often used to evaluate bread quality are descriptive analysis and consumer acceptance (Heenan et al 2008).

3.6.1 Descriptive analysis

The objectives of a descriptive sensory analysis may be to obtain a product profile or to identify changes in a product. The product profile is a description of all the characteristics of the evaluated product and the determination of which sensory attributes that are related to acceptance (Lawless & Heymann, 2010). Usually, a trained panel with 8 to 12 panelists meets for training sessions where the different evaluating terms are chosen and described in order for them to all refer to the same aspect for the same attribute; the training sessions are often two hours long. After training, there is a final assessment in which all the chosen attributes are evaluated for further analysis. Data from these analyses is also used to interpret the results obtained from consumer hedonic tests (Lawless & Heymann, 2010).

3.6.2 Consumer acceptance test

Hedonic tests are commonly used for analyzing consumer acceptance. Hedonic tests show preferences and make it easy to obtain information about the degree of likeability or dislikeability by calculating mean and standard deviation. The scale used for this test is a 7- or 9-point scale ranging from "dislike extremely" to "like extremely" (Lawless & Heymann, 2010). An example if this scale is shown in Figure 2.

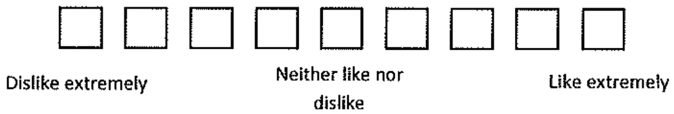

Figure 2. 9-point hedonic scale used for consumer acceptance tests.

4 Materials and Methods

4.1 Experiment overview

Quantitative methods were applied throughout the present study. The analyses of dough and bread were carried out by measuring weight, volume, texture and microstructure; photos were also taken. A product profile was performed by a trained panel for the bread containing rye flour and a hedonic consumer test was also carried out for these bread samples. Figure 3 shows the organization and the various steps included in the study. For some of the analyses, letters were used in order to identify the samples; these are showed in table 1.

For the sensory analyses, three proofing processes were conducted (see "Rye dough" in figure 3): non-proofed before freezing, which was thawed and proofed during 45 min and used as reference; half-proofed before freezing, which was baked directly when removed from freezer; and fully proofed before freezing, which was also baked directly from the freezer. For the other analyses such as volume and texture, 6 different proofing processes were carried out (Figure 3): those three already mentioned as well as non-proofed before freezing, baked directly from the freezer; half-proofed before freezing, proofed a second time before baked; and fully proofed, which is thawed and baked.

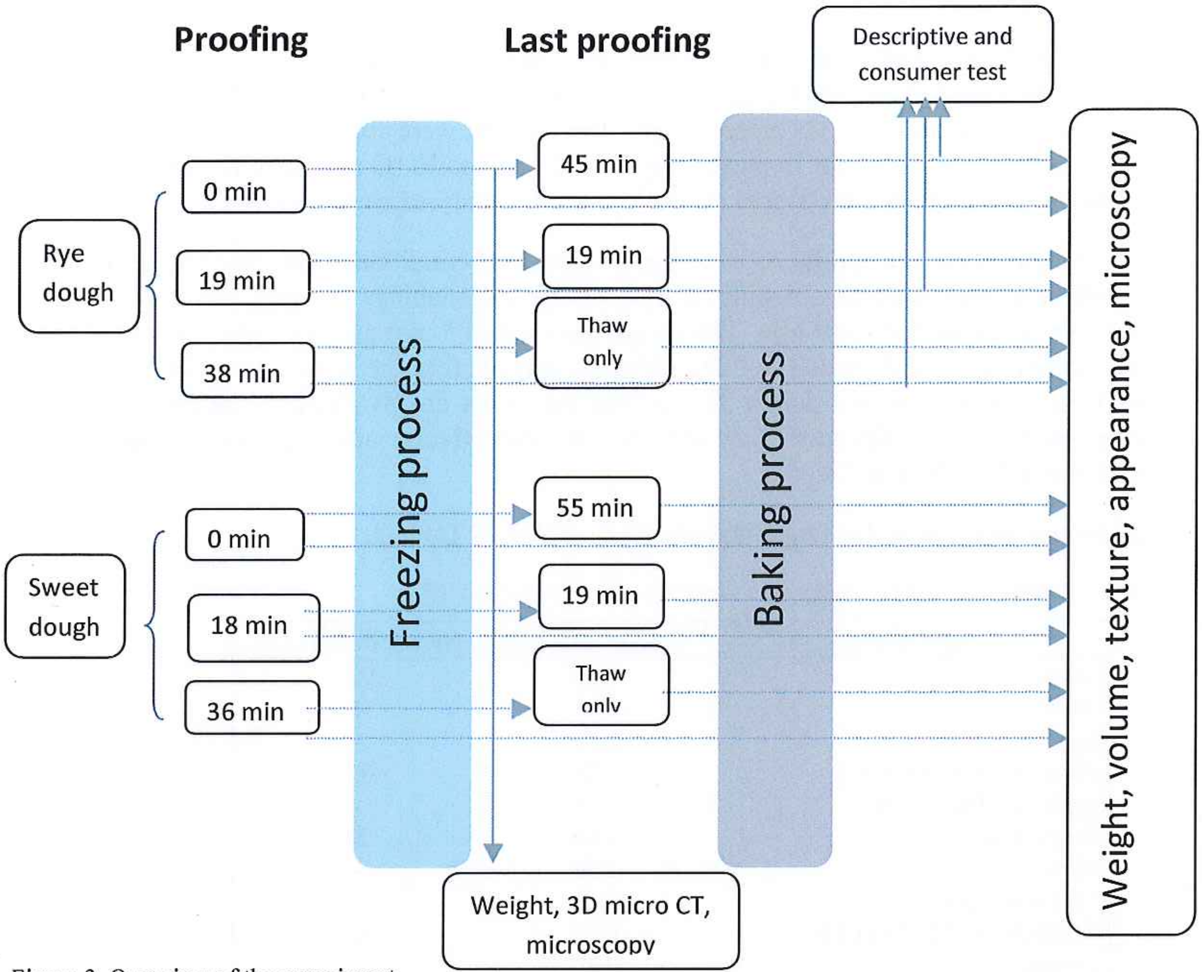

Figure 3. Overview of the experiment.

Table 1. Scheme for identifying the samples during the study and their processes.

Rye		Sweet	
Sample	Process	Sample	Process
1 A	Non-proofed before freezing, frozen, thawed, proofed, baked (reference)	2 A	Non-proofed before freezing, frozen, thawed, proofed, baked (reference)
1 B	Half-proofed before freezing, frozen, baked	2 B	Half-proofed before freezing, frozen, baked
1 C	Fully proofed before freezing, frozen, baked	2 C	Fully proofed before freezing, frozen, baked
1 D	Non-proofed before freezing, frozen, baked	2 D	Non-proofed before freezing, frozen, baked
1 E	Half-proofed before freezing, frozen, thawed, final proofing, baked	2 E	Half-proofed before freezing, frozen, thawed, final proofing, baked
1 F	Fully proofed before freezing, frozen, thawed, baked	2 F	Fully proofed before freezing, frozen, thawed, baked

4.2 Dough preparation, proofing and freezing

The dough for two different types of bread was prepared at Fazer, Umeå, 2.5 months before the study was carried out. One kind of dough contained 19% rye flour and the other one was a sweet dough containing 9.3% added sugar. The doughs were formed into small buns, 60 g, frozen on trays for 24 h, put in plastic bags and boxes at -30 °C and then transported to SIK, Gothenburg, were they were stored at -20 °C until the analyses were performed.

Both dough types were prepared in common industrial conditions following the same steps: ingredient scaling, kneading, resting, dough scaling and shaping. The ingredients were mixed for 2 min and kneaded for 9 min. The dough then rested 5 min and was later cut and shaped. The formed buns were treated in three different ways before the freezing process: one group was fully proofed (the rye dough 38 min and the sweet dough 36 min), another group was partly proofed or half-proofed (19 and 18 min respectively) and the third group was not proofed at all but frozen directly.

The information about the dough ingredients is shown in Table 2.

Table 2. Bread recipe used to prepare the samples for further study at SIK.

Ingredients	Rye bread (kg)	Sweet bread (kg)
Water	15.5	9.5
Yeast	1.15	1
Salt	0.47	-
Panolux (fat with E471)	1.25	0.8
Rye flour (fine sieved)	9.3	-
Wheat flour	18.6	24
Syrup	1.55	-
Freeze powder	0.2	-
Emulsifier E472 (DATEM)	0.09	0.1
Promoat	0.65	-
Sugar	-	4
Bread Improver- Freeze stability	-	1.13
Rapeseed oil	-	2.4

4.3 Final proofing and baking process

In order to optimize proofing and baking processes one trial was carried out for the bread containing rye while four trials were needed to optimize the sweet bread. The temperature curve was analyzed for half- and non-proofed doughs during baking in one of the trials. The dough was treated as follows depending on the proofing profile of each sample:

4.3.1 Bread containing rye flour

Six samples of non pre-fermented dough were taken out from the freezer, put on trays and thawed for 45 min. This step was followed by a final proofing in a proofing chamber (Sveba Dahlen, Sweden) for 45 min at 38°C and 80% RH (relative humidity). After 5 min for conditioning, the samples were baked for 15 min with 20 sec of steam (Sveba Dahlen, S400 oven, Sweden); the initial temperature was 230 °C and was then lowered to 200 °C; this bread was used as reference. Another six samples of non pre-fermented dough were withdrawn from the freezer and after just 5 min baked with the same conditions as those previously named.

Half-proofed dough was also treated in two different ways, the first 6 samples were baked 5 min after being taken out of the freezer, with the same oven conditions as the reference; the other 6 samples were thawed and proofed in the chamber for 19 min at 38 °C and 80% RH. After this step, the samples were baked as the others.

Fully proofed dough was taken out of the freezer and baked after 5 min, as well as thawed and baked as mentioned. The amount of bread was 6 samples for each treatment.

4.3.2 Sweet bread

The sweet bread dough had also the same amount of treatments as the rye-containing bread. The difference was that the reference was thawed 45 min and proofed for 55 min in the proofing chamber; after 5 min for conditioning, the bread was baked at 200°C for 10 min; no steam was used for it. All the other samples were treated in the same way as the rye bread at: 230°C at the beginning and then decreased to 200°C, but with a different steam treatment, namely, 5 sec steam at the beginning and a spray after 3 min. The baking time was 13 min for the samples coming directly from the freezer, 12 min for the thawed ones and 11 min for half-proofed dough that was proofed in the chamber for 19 min before baking. In order to follow the temperature changes in the bread, the reference and half-proofed doughs were measured during the baking process using a thermo element (Datapaq, 9000). The surface of the dough, as well as the center part were assessed.

4.4 Mass and volume measurements

The bread samples that were withdrawn from the freezer for proofing and baking were weighed using a ±0.01 g precision scale while frozen. The baked samples were weighed once more 1 hour after baking. At that same point, the volume of the buns was measured by rapeseed displacement according to the AACC Method 10-05 (2001). Three out of six samples were kept in plastic bags for 2 days at room temperature; their mass and volume were measured again with the same methods.

Some dough samples, of both those containing rye flour and the sweet one, were kept in the freezer and baked two months later. Their volume was measured in order to quantify the effect of the freezing storage time on the final volume.

4.5 Texture

The bread texture was measured one hour after baking using a texture analyzer (Instron, 5542) with a cylindrical specimen of 20 mm diameter, a compressive strain of 40%, an extension of 1.7 mm/sec and a compressive load of 0.01N. The bread slices were approximately 1.5 cm thick. Three samples of each treatment were kept in plastic bags room temperature and their texture was measured after two days with the same procedure as mentioned above.

4.6 Macroscopic structure

In order to analyze the macroscopic structure of the bread crumb, bread slices (1.5 cm) were scanned with a PC scanner (Canon N1240U). Photographs of the vertical slices were taken with a system camera (Nikon D70).

4.7 Microscopic structure

The microstructural analyses were carried out both on the frozen dough and on baked rye and sweet breads. A confocal laser scanning microscope (CLSM) was used, utilizing a Leica TCS SP2 (Germany) instrument. The frozen dough was prepared in a cryochamber (Leica CM 1900, Germany) at -15°C, sliced into 40 µm thick samples and put on objectives. The samples were air-fixed in formaldehyde for 1.5 hours and then stained. Acriflavin was used to stain the starch granules applying a 488 nm wavelength laser, Texas Red for protein with a 594 nm wavelength laser and Bodipy for fat also using a 488 nm wavelength laser.

The analyses of the baked bread were completed with images taken with a light microscope (LM) as it was difficult in some cases to see the structure of the gelatinized starch with the CLSM. The LM used was a Nikon Microphot-FXA (Japan) and 10x, 20x and 40x objectives. The bread samples were sliced in a cryochamber into 10 µm and fixed and stained with Light green in acetic acid for the protein, osmium vapor for fat and Lugol's iodine in order to stain the starch granules.

4.7.1 3D-Micro-CT

The tests used for analyzing the structure of the different doughs were taken with an x-ray tomography equipment (GE | phoenix V | xm 240, Germany) at SLU, in Uppsala. The studied samples were both rye and sweet bread dough with three different processes: non-proofed, half-proofed and fully proofed before freezing. The samples were thawed about one hour before the analyses. The resolution of the images taken was 45µm. These images were obtained during two cycles, a fast one of 3 min and a longer one of about 21 min. The pictures were analyzed with FIJI, ImageJ 1.47 (USA).

4.8 Sensory analysis

The sensory analysis carried out for this study was a descriptive test for the bread containing rye flour and a consumer test for both kinds. For this part of the study three proofing profiles of each kind of bread were tested: a non-proofed dough before freezing that was thawed, proofed and baked as the reference; a sample that was half-proofed before freezing and baked directly from the freezer; and a fully proofed sample that also was baked directly from the freezer.

4.8.1 Descriptive test

A quantitative descriptive analysis (QDA) was performed in order to get a product profile of frozen dough and bread. The main test was preceded by two training sessions with 6 assessors, 5 women and 1 man, to identify and evaluate the sensory attributes of the samples. These were related to finger texture, mouth sensation and flavor, in total, 9 properties.

During both the training sessions and the main test, the panelists were provided with a bun on a white paper plate that was coded with a 3-digit number. The samples were at room temperature and analyzed with red light in order to mask the color of the buns. A 100 mm line scale was used to score the studied properties; the scale was labeled with "little" at 10 mm and "much" at 90 mm. Two replicates were evaluated during the main test.

The collection and analysis of the obtained data was done with the program FIZZ 2.47 B in order to determine the significant differences at 95%. The scores of each attribute were compared by Tukey test at $P \leq 0.05$.

4.8.2 Consumer acceptance test

A minor consumer test was conducted in order to analyze consumer acceptance and liking. 15 tasters tried the same three samples used for the descriptive test (bread containing rye flour) one hour after baking. The scored attributes were appearance, texture and overall liking. Each consumer had a survey with a 9-point hedonic scale, where they could grade the attributes and write some comments if necessary. The bread samples were served in paper dishes and marked with a 3-digit code. The samples were served in a different order for each taster.

4.9 Data analysis

The results obtained were analyzed with Microsoft Excel (2007), except for the descriptive sensory analyses that were processed with FIZZ 2.47 B. The program included in the tomography, Phoenix V, was used for the images obtained in the micro-CT.

T-test was calculated to examine how big the significant difference was between the reference and the other samples.

5 Results and Discussion

5.1 Mass and volume

5.1.1 Bread containing rye flour

After baking, both the mass and volume of the bread containing rye flour showed to be affected by the proofing profile of the dough (Appendix 1). The final volume of the buns with different proofing profiles (half and fully proofed) showed a significant difference when compared to the reference sample (non-proofed before freezing). The only treatment that did not follow this trend was the half-proofed dough that was thawed and proofed 19 min before baking (E); the resulting volume of these breads did not differ much from the reference.

With regards to mass loss, the only significant difference was observed in the fully proofed dough that was baked directly after being withdrawn from the freezer, which showed a much lower loss, a mean of 2.68 g. The other treatments showed a more homogeneous result, 5.91 to 6.41 g. The bread's final center temperature could not be the cause of this result as it was around 98°C for all samples, with the greatest varying difference being 0.6 °C.

The specific volume showed a large standard deviation for all the studied samples as shown in Figure 4. The specific volume showed the same result as the volume (ml), namely that breads baked from doughs that were proofed before freezing had the lowest volume. As can also be observed in the figure below, sample E (rye) which had been half-proofed before freezing and was subjected to a final proofing before baking had almost the same specific volume as the reference (sample A) which was not proofed before freezing but thawed and proofed before baking. The results obtained from samples B had a higher specific volume than fully proofed samples (C).

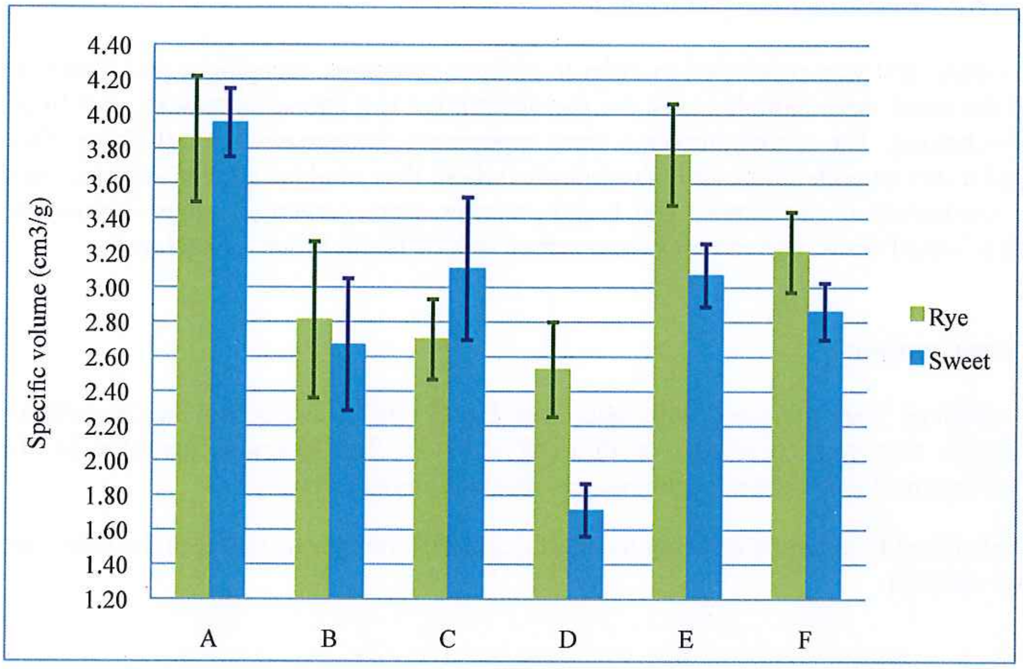

Figure 4. Specific volume of bread containing rye flour (green) and sweet bread (blue). The letters indicate the different processes. A- non-proofed dough before freezing, thawed and proofed; B- half-proofed dough, baked directly from freezer; C- fully proofed dough before freezing process, baked directly; D- non-proofed dough, baked directly from freezer; E- half-proofed dough, thawed and proofed 19 min after freezing before baking process; F- fully proofed dough, thawed before baking.

5.1.2 Sweet bread

The volume of the sweet breads was greatly affected by the proofing profile before freezing, as can be seen in figure 5, even after an improved baking process. In order to understand this phenomenon, the heat transfer during baking was measured and analyzed (data not shown), but it, unfortunately, did not provide any new information.

As it also can be seen in figure 4, the specific volume of half and fully proofed breads (B, C) was significantly lower than the reference.

In this study, as well as was seen in the study of Lucas et al (2010), the bread doughs that were subjected to proofing before freezing showed a decrease in specific volume and height (Appendix 1) in comparison to those doughs frozen directly after formed.

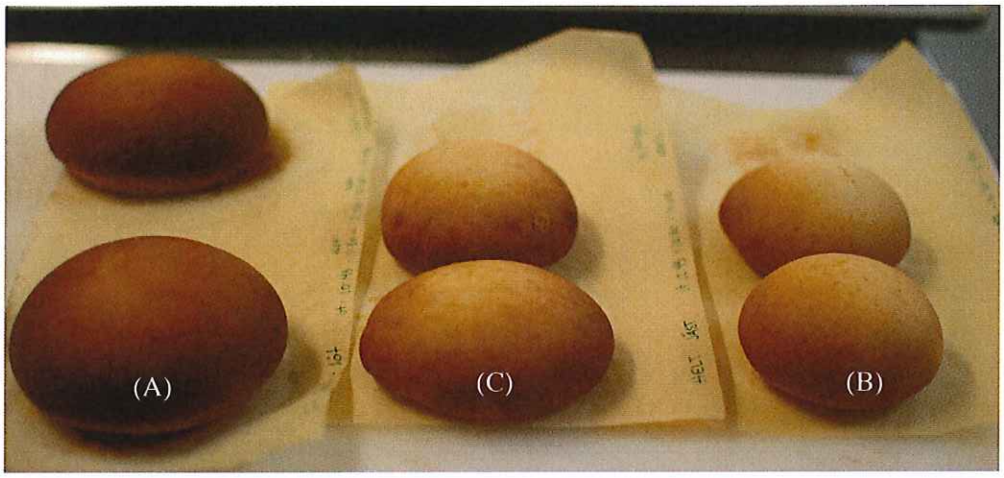

Figure 5. Final appearance of the sweet buns. A-Reference, non-proofed dough before freezing, thawed and proofed; B- half-proofed dough, baked directly from freezer; C- fully proofed dough before freezing process, baked directly.

5.1.3 Volume measured after four months storage in freezer

Two months after the initial measurements, both types of bread were baked and analyzed again to evaluate their final volume. The results showed a noticeable volume reduction as a result of two months storage in -20°C and, as was seen in the initial study, the standard deviation was also large in this case. In order to see if the volume reduction could be fixed by applying a larger final proofing before baking, an additional 10 and 20 min were tested, though not providing any positive results. Figure 6 shows the results of specific volume measurements of the final bread baked from dough that was stored 4.5 months. The figure shows the difference between the doughs stored 2.5 months and those stored 4.5 months. It also shows that prolonged proofing time in the dough containing rye flour was not enough to compensate for the loss of volume. With regards to the sweet bread, a minor improvement in volume can be seen when prolonging the final proofing by 10 min, but also a deterioration when adding 20 min. The samples shown in figure 6 were treated in the same way as the reference sample (A), which means that the dough was not proofed before freezing, thawed and proofed in a proofing chamber for 45 and 55 min for rye and sweet bread respectively. Other proofing profiles were also analyzed, none of these maintained their volume after 2 months of extra frozen storage (data not shown).

The obtained results show that, as is mentioned by other researchers, long-term frozen storage affects negatively the final bread (Giannou & Tzia, 2007; Bárcenas & Rosell, 2006). The temperature curve in the present study was analyzed and showed a small variation between -19 and -22°C.

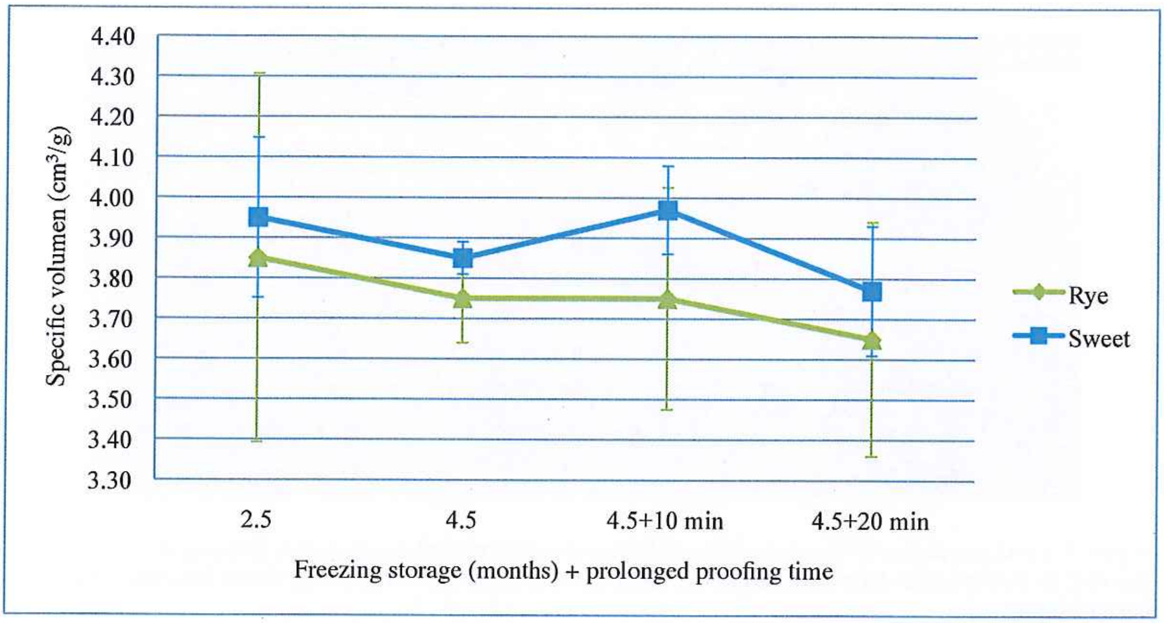

Figure 6. Specific volume of rye and sweet bread baked from dough that has been stored 2.5 and 4.5 months. The dough that had a prolonged proofing time with 10 and 20 min in order to compensate the volume reduction can be seen.

5.2 Texture

The texture of fresh and two day old bread was measured using a texture analyzer. This method showed a clear picture of how stiffer the crumb of the bread had become after two days, as both Young's modulus and compressive stress result in a much higher value when measuring a stiff structure.

5.2.1 Rye bread

Bread samples containing rye flour proved to have a longer shelf life than sweet ones as they showed a lower compressive stress when measuring texture after 2 days. Figure 7 shows both the mean and standard deviation of how the different proofing profiles affect the final texture of both kinds of bread. The dough that was not proofed before freezing, thawed and proofed for 45 min in a proofing chamber before baked (sample A), and which was used as a reference, showed the lowest modulus (data not shown) and compressive stress (figure 7). It was also seen that the greatest deterioration over time was experienced by fully proofed bread, both in the bread that was baked directly from the freezer and the dough that was thawed before baking. If we were to only analyze freshly baked bread, we could say that the half-proofed dough baked directly from the freezer results in a harder crumb. This treatment showed to be the least affected by time (two days at room temperature), with just a 0.05 MPa difference. These samples also had a higher standard deviation in comparison to the other analyzed breads.

5.2.2 Sweet bread

For the sweet bread, the same procedure was used for measuring the texture as the one used with rye bread. In this case the bread showed a much tauter texture both the fresh and the two days old samples, compared to the samples containing rye flour (Figure 7). The modulus measurements reach 0.6 MPa for the two days old fully fermented dough that has been thawed before baking.

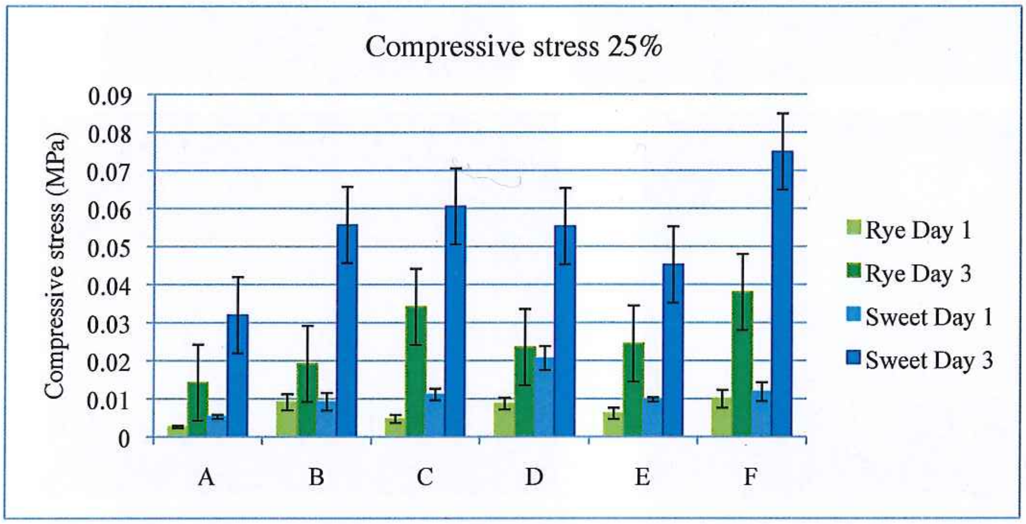

Figure 7. Texture of fresh and two day old bread. A- non-proofed dough before freezing, thawed and proofed; B- half-proofed dough, baked directly from freezer; C- fully proofed dough before freezing process, baked directly; D- non-proofed dough, baked directly from freezer; E- half-proofed dough, thawed and proofed 19 min after freezing before baking process; F- fully proofed dough, thawed before baking.

5.3 Macroscopic structure

5.3.1 Rye bread

The macrostructure of the bread containing rye flour was analyzed using both a scanner and photographs. It can be seen in these figures that the porosity of the bread is highly affected by the proofing profile and the final processes before baking. Figure 8 shows a selection of the scanned bread slices. The reference sample that was not proofed before freezing, thawed and proofed when withdrawn from freezer showed more homogeneous pores in the crumb. The fully proofed dough, on the other hand, had a very heterogeneous result; some of the samples presented large bubbles that collapsed under the crust creating a large area of air, while other samples had smaller bubbles. In almost all the analyzed cases, the larger bubbles were found under the crust (See figure 8, sample (C, F)). One can also observe that fully proofed bread slices did not obtain the desired volume; this effect is clearly seen when compared to the reference. It may be a consequence of the collapsed dough and the reason why some breads had large bubbles under the crust. This aspect is in agreement with previous studies in which the authors found that collapse was greater when the dough was subjected to a longer fermentation prior to freezing (Lucas et al, 2010).

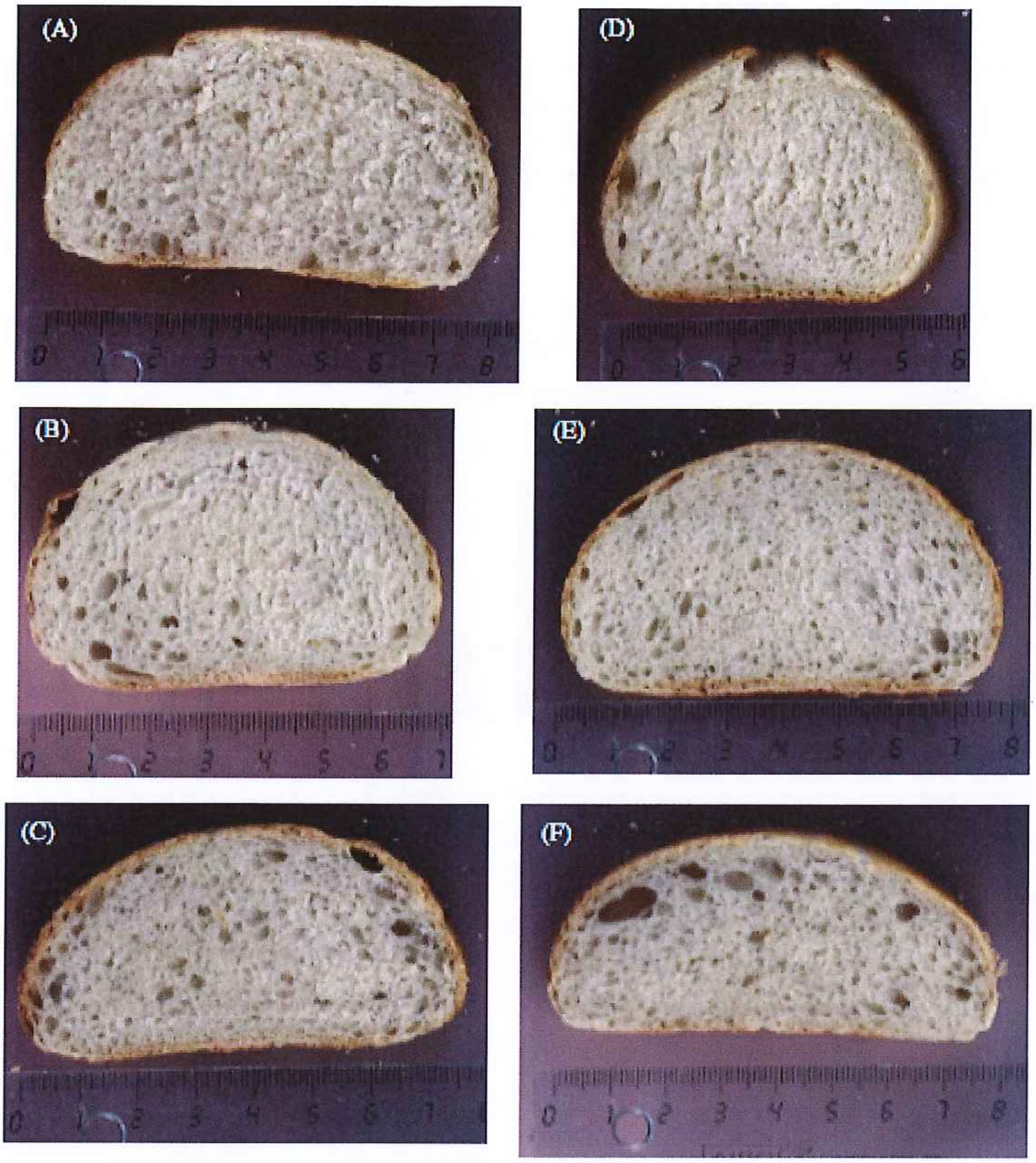

Figure 8. Scanned bread containing rye flour. A, D- non-proofed before freezing; B, E- half-proofed, 19 min, before freezing; C, F – fully proofed, 38 min, before freezing; B, C, D- baked directly when withdrawn from freezer; A- thawed and proofed for 45 min; E- thawed and proofed for 19 min; F thawed and baked.

5.3.2 Sweet bread

As was mentioned in section 5.1.2, sweet bread buns showed with very poor volume. Samples that were subjected to proofing before freezing ended up smaller, with a compact crumb and triangular form. The final sweet buns differ from those containing rye flour in the bubbles, as the rye bread showed much larger air bubbles in half-proofed and fully proofed doughs than the non-proofed sample, while in the sweet samples this difference was not noticeable. Both the triangular forms and the homogeneous crumb can be seen in figure 9.

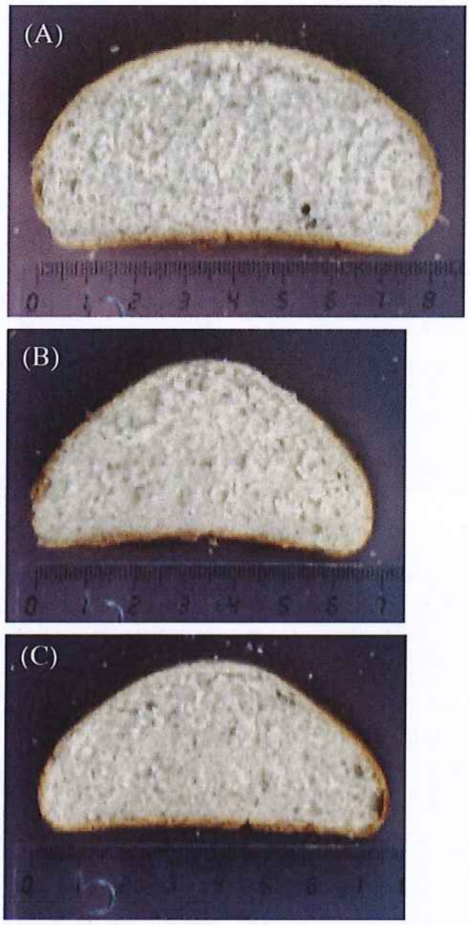

Figure 9. Scanned sweet bread slices. A- reference, non-proofed dough, thawed and proofed before baked; B- half-proofed dough before freezing, baked directly from the freezer; C- fully proofed dough before freezing, baked directly.

5.4 Microscopic structure

5.4.1 Dough

The microstructure of the dough, analyzed by CLSM, showed very clearly the starch granules and the protein network. The microstructure of rye (1) and sweet (2) dough proofed at different conditions (A-C) are shown in figure 10. The protein is shown in red color and the starch granules in green The results showed a homogeneous gluten protein network in the dough containing rye flour while the sweet dough showed a more irregular and not very developed network.

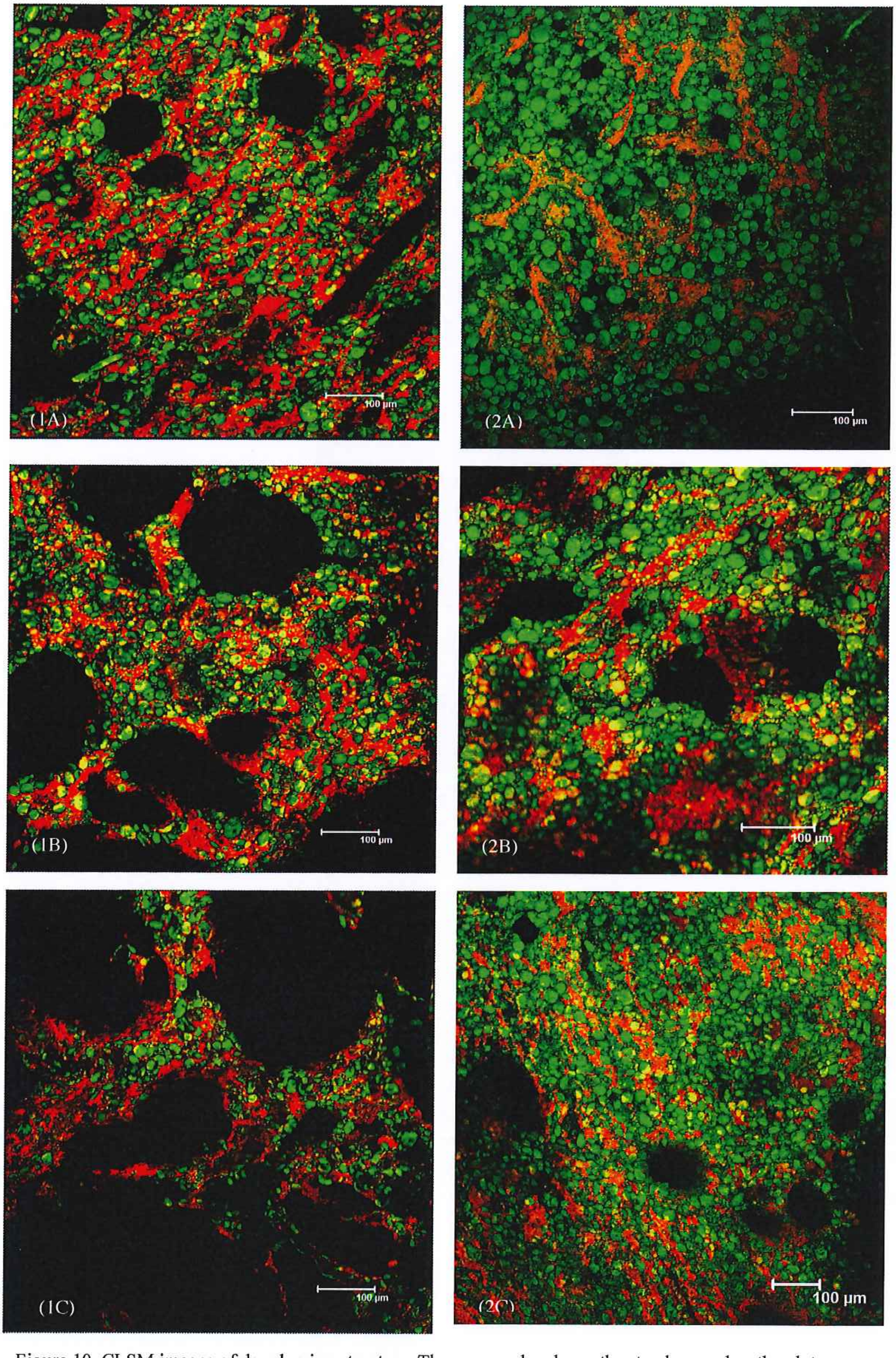

Figure 10. CLSM images of dough microstructure. The green color shows the starch granules; the gluten network is shown in red. 1-Dough containing rye flour; 2- sweet dough; A- reference, non-proofed dough; B-half-proofed dough before freezing; C- fully proofed dough before freezing.

The mixing time for both types of bread was 2 min, and 9 min for kneading, which may explain the poor protein network seen with the CLSM in the sweet samples. It should also be mentioned that the sweet samples contained much more fat than those containing rye, which decreases the percentage of protein in the dough. At the same time, though, these images show no homogeneous network, which as described by Mc Gee (2004), has elastic characteristics and allows a good development of the dough and, later on, results in a bread with a desirable volume. This corroborates with what some studies mention regarding a longer mixing time needed for the development of the gluten network in sweet doughs (Calderón-Dominguez et al, 2003; Tlapale-Valdivia et al, 2010).

The homogeneous and well-developed protein network in rye bread can once again be seen when analyzing fat and protein in the CLSM images as well as a poor, interrupted gluten network in the sweet bread. Figure 11 shows an example of a half-proofed sweet dough (left) compared to a half-proofed dough containing rye flour (right). The protein phase is shown in red and the fat phase is shown in green.

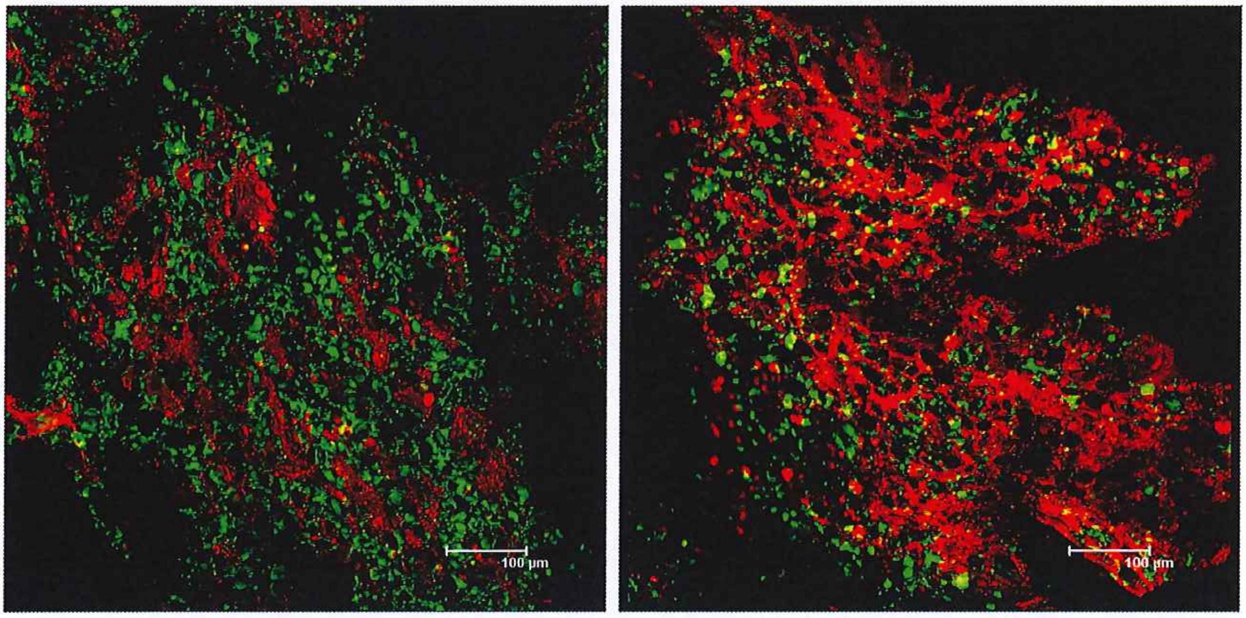

Figure 11. CLSM images of half-proofed dough. The image in the left shows a sweet dough, to the right can be seen a dough with rye flour. Fat is shown in green and protein in red.

5.4.2 Bread

The microstructure of the final bread was analyzed by two different techniques, CLSM and LM. Sweet buns presented a non-continuous protein network similar to the one seen in the analyzed doughs. In all cases, swollen starch granules could be seen. These did not present the round uniform structure that they showed when the doughs were analyzed, but rather a more elongated and slightly bigger shape. When referring to the lipid phase, in both techniques it was observed that, apart from having a greater amount of fat, in some cases, the fat appears to be agglutinated as can be seen in figure 12; the LM image (left) represents the protein in turquoise and the fat in brown; the CLSM image (right) shows the protein in red and the fat in green. These images show the gluten network and the fat phase in sweet buns with CLSM and LM.

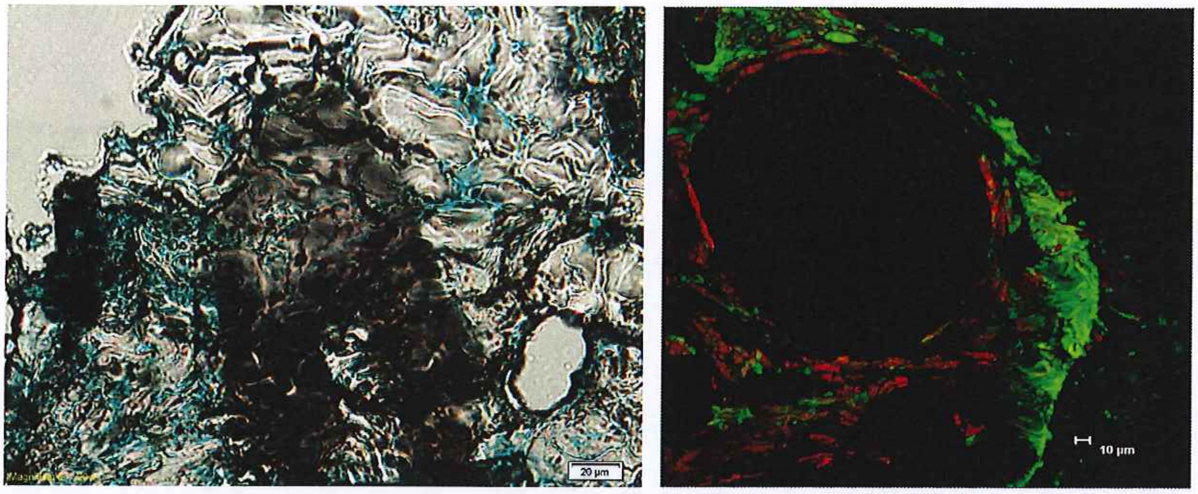

Figure 12. Microstructure of protein and fat phases in sweet bread. The image on the left was taken using an LM technique; the brown stains show the lipid phase and the turquoise is the gluten network. The image on the right was taken with CLSM; red is the protein network, green shows fat.

Fibers were observed in some of the micrographs analyzed, both for sweet and rye buns. Bread containing rye flour had a more homogeneous lipid phase. In these breads fat droplets (shown with arrows in figure 13) spread through the protein network and the starch granules could be seen. It was not possible, in some of the analyzed breads, to differentiate the starch and fat phases as the iodine solution used for staining starch hid the brown color of the stained fat.

Figure 13 shows LM micrographs of bread containing rye flour prepared by the tree different proofing processes A-C. Images to the left show the fat in brown, protein in turquoise and uncolored starch granules. The images to the right show the colored starch granules in purple.

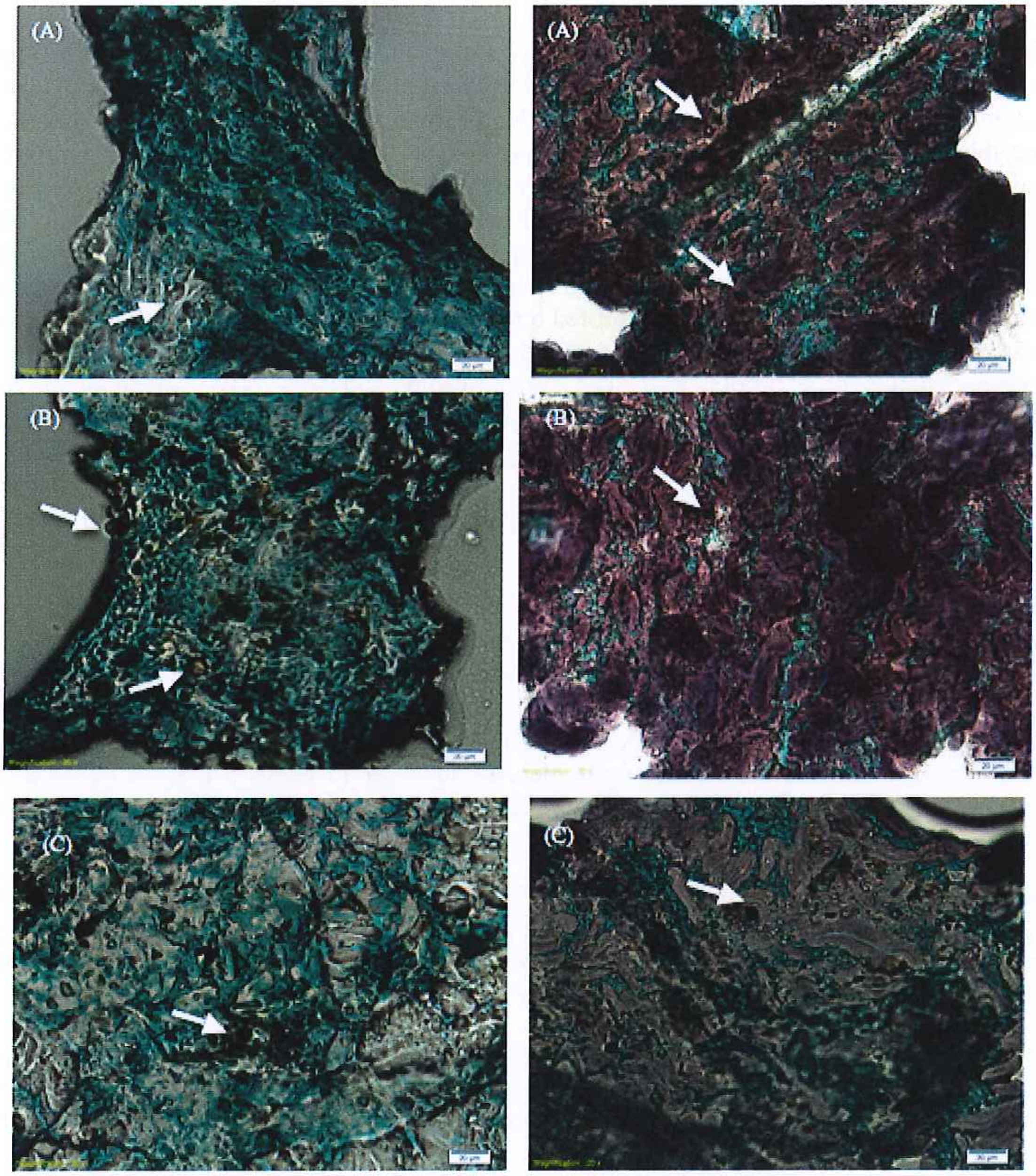

Figure 13. LM micrographs of rye bread. Brown stains show fat; turquoise shows gluten network; purple shows starch. A- non-proofed before freezing, B- half-proofed, C- fully proofed. The arrows are pointing to some (of many) fat droplets.

5.5 Micro-CT

The images taken with the x-ray tomography equipment were analyzed with the Fiji image program. All the images were processed with ICA-color. Figure 14 shows rye (1) and sweet (2) dough prepared with three different proofing processes (A-C). In this figure, it possible to see the difference in the density of the doughs. The density differences due to proofing profiles were also noticeable represented by brightness. Rye bread showed a well-developed porosity, which can be seen even in the non-proofed dough. The sweet bread, on the other hand showed a much higher density. The non-proofed sample showed areas without pores and some uneven bubbles. Another aspect to analyze is that the half-proofed sample does not seem to be less dense than the non-proofed one. The pores are much more homogeneous than those in the dough analyzed for non-proofed bread, but if it is compared with rye bread, it does not show to be as proofed as the rye dough. Scanlon and Zghal (2001) mentioned in their study that a result for the expansion of the gas cells in the dough during proofing there is a decrease in the dough density, which is in agreement with the images of the rye dough in which one can see that density decreases when the proofing profile increases. On the other hand, the gas cells of the sweet dough do not seem to expand much even when referred to the fully proofed dough.

When referring to the fully proofed samples, the sweet dough shows an area that is much lighter than the other. When compared to the corresponding rye dough, this does not show a complete proofing. As was reported by other authors, the mixing process is very important for developing the porosity as air is incorporated to the dough (Scanlon & Zghal, 2001). In the analyzed samples, the higher density and the lack of porosity may explain the short mixing time which, as mentioned before, must increase in order to develop a good network and porosity taking into account the ingredients present in the sweet dough. (Calderón-Dominguez et al, 2003; Tlapale-Valdivia et al, 2010).

Authors like Dobraszczyk and Morgenstern (2003) and Scanlon and Zghal (2001) mentioned the correlation between the proofing profile and the bubble sizes as well as their coalecence and collapse. The results from the micro-CT analyses show some collapse in the fully proofed samples on account of the gas release between the pores.

In figure 14, sample (2A), there is an arrow pointing to an area with no pores. The analysis of non-proofed sweet dough was repeated and two replicates were tested in order to verify this phenomenon. During the second test a few more dense areas were seen, but they were not as noticeable as in the first test.

Images of vertical cuts are shown in appendix 3, where it possible to see the porosity, collapse and density differences of both types of dough.

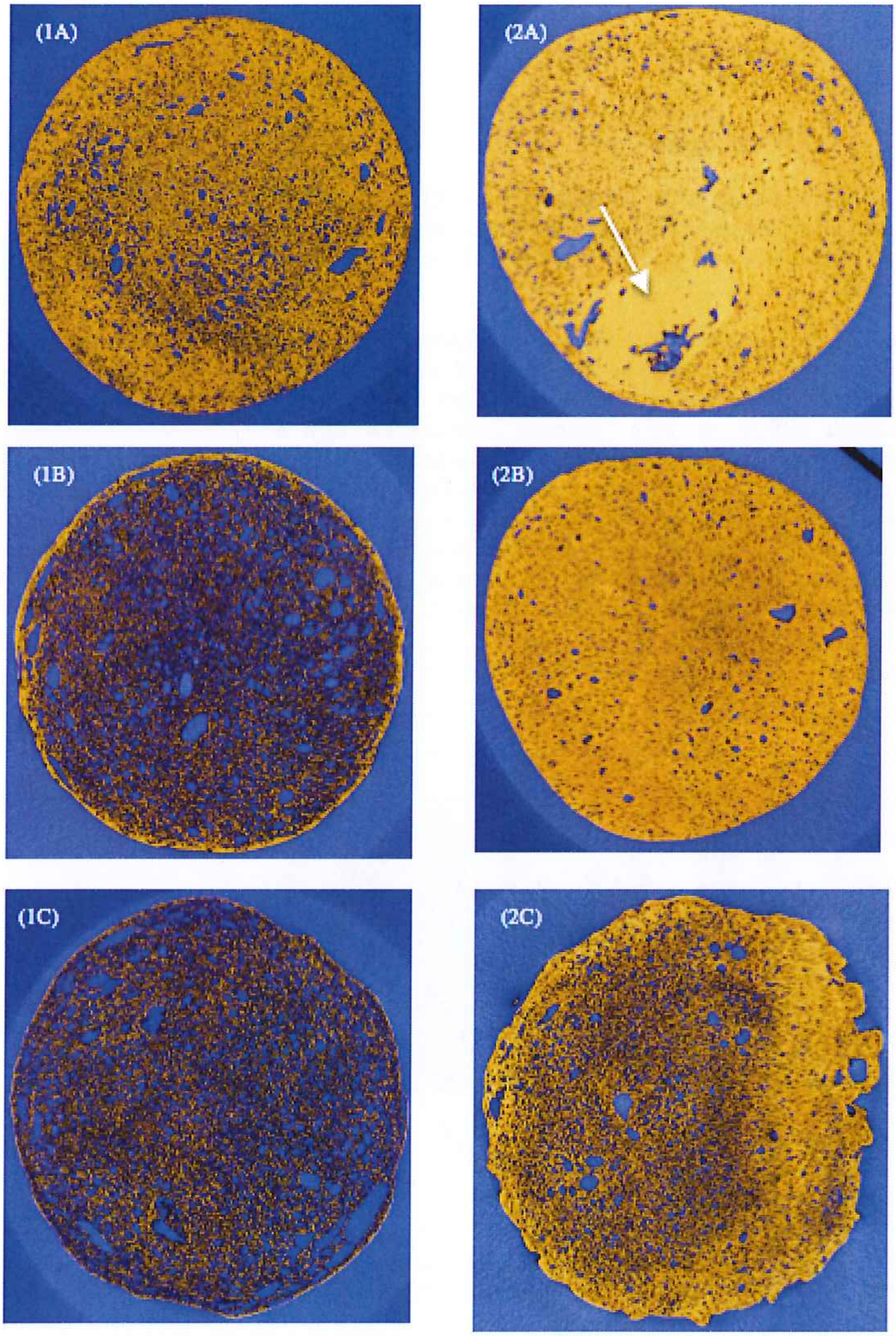

Figure 14. Micro- CT images of thawed bread doughs. 1-rye, 2- sweet. A- non-proofed before freezing, B- half-proofed, C- fully proofed. The arrow points to an area with no pores developed.

5.6 Sensory evaluations

5.6.1 Descriptive test

During the training sessions, the trained panel determined the sensory attributes that were to be scored later during the main session for the evaluation the rye buns. These concepts can be seen in appendix 4. All the assessors agreed on the definitions before the main test was conducted.

The information obtained from the main evaluation showed that the reference sample differs significantly from the half and fully proofed samples. At the same time the differences between half and fully proofed breads were not significant in most of the cases. The most pronounced differences were observed in properties such as springiness, compactness, airiness and moisture where the reference had significantly better quality (Figure 15). Another aspect the panelists commented on was that the fully proofed bread showed an irregular result as some of the buns showed to have big bubbles under the crust and a collapsed crumb at the bottom. This aspect then affected other characteristics such as springiness. The characteristic "elasticity" did not differ much from sample to sample as the other characteristics, which can be seen in the similar scores received by the panel.

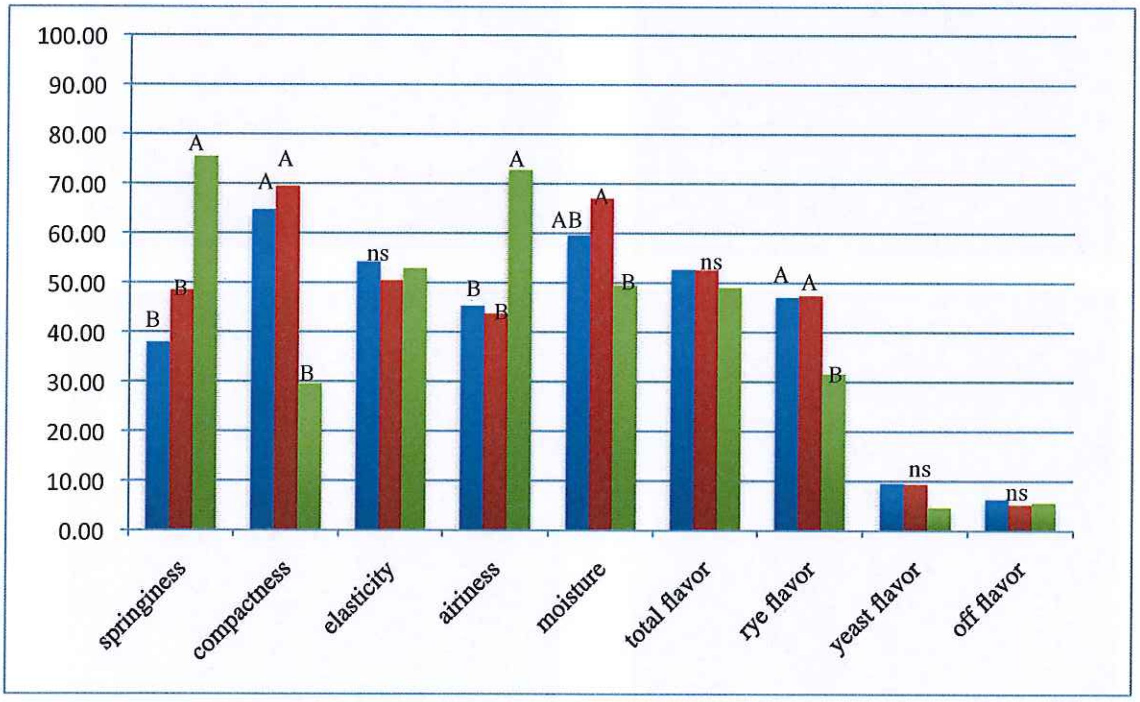

Figure 15. Sensory attributes of bread buns containing rye flour scored by trained panelists (%). Significant difference is seen between A and B; ns- no significant difference. Reference- blue, half-proofed- red, fully proofed- green.

5.6.2 Consumer test

Three attributes were evaluated in the consumer test: appearance, texture and overall liking. The tasters were asked to comment on those characteristics. The obtained comments referred to the color of the crust, the elasticity of the bread, volume and flavor. The objective in conducting this test was to analyze if consumers noticed a difference between half-proofed and fully proofed samples. In bread containing rye flour, a significant difference was observed in the overall liking between the samples, which indicates that consumers do notice their difference (Figure 16). Some tasters mentioned that fully proofed bread was experienced as "doughy", which is in agreement with the results obtained from the descriptive test done by trained panelists.

Another aspect noticed is that there was not a significant difference in texture between the reference and the half-proofed samples (p=0.88), the difference between the reference while the bread that had been fully proofed before freezing had a significant difference, higher than 95% (p=0.01).

The data obtained with the sensory analyses was in agreement with the results obtained from the texture analyzer, both the descriptive test and the consumer acceptance test. It is clear that the reference (not fermented before freezing, thawed and proofed in a proofing chamber) received the highest scores from consumers, and showed the highest springiness and the lowest compactness in the obtained product profile (see section 5.2).

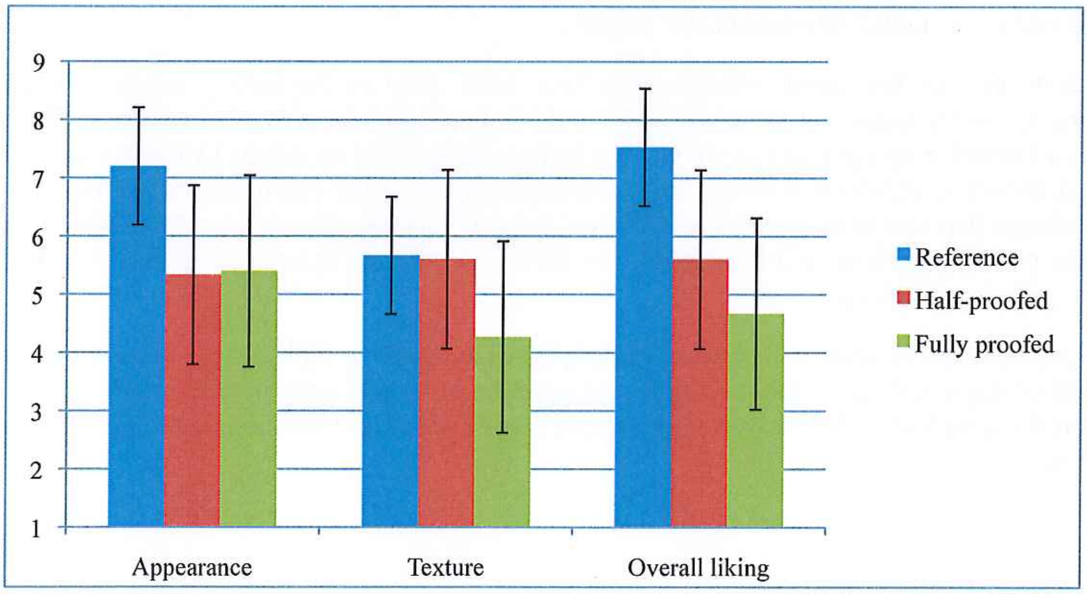

Figure 16. Mean and standard deviation obtained in a consumer tests using a 9-point hedonic scale. The scored attributes were appearance, texture and overall liking.

6 Conclusion

The goal of investigating the impact of proofing profile on bread's final volume, texture, structure, microstructure, appearance and acceptance through this study was accomplished.

Significant differences were observed in the product profiles acquired in the descriptive test in almost all the tested attributes except for elasticity, total flavor and off flavor. Consumers were able to distinguish the products in appearance and overall liking.

Proofing profile showed to negatively affect the volume, specific volume and texture of the final bread, probably because of the collapse of the proofed dough in the freezer in correlation to the growth and coalescence of the gas cells in the proofed dough.

Sweet dough did not show a developed gluten network and fat was agglutinated in some of the analyzed samples. In contrast, the rye bread dough, had a homogeneous protein matrix and the lipid phase was even distributed in the structure as small droplets. In the 3D-micro-CT images, it was possible to observe a decreasing density with a longer proofing profile in the dough containing rye flour, while the sweet dough showed areas with no pores as well as differences in the density of the proofed dough samples, probably caused by a poor mixing process.

7. Further studies

As a result of the large amounts of data produced by the many different analyses carried out during this study, it would be interesting to perform a multivariate analysis in order to better understand the correlation between different processing conditions and properties of the doughs and buns studied throughout this project.

This study reveals that great achievements have been done in the bakery industry when referring to savory bread, but more research would be needed in the area of sweet bread. With the aim of developing a process in which the final proofing could be skipped when baking the bread at clients' companies, it would be interesting to examine the yeast vitality and viability in the dough that has been proofed and frozen, how ice crystals develop in the dough with different proofing profiles and how these may affect the microstructure, the gluten network, and the yeast's membrane.

More studies could be done with mixing techniques when working with doughs that have high amounts of sugar and fat, combining time and speed in order to incorporate the right amount of air in the dough as well as to develop a strong gluten network and a homogeny porosity in the dough.

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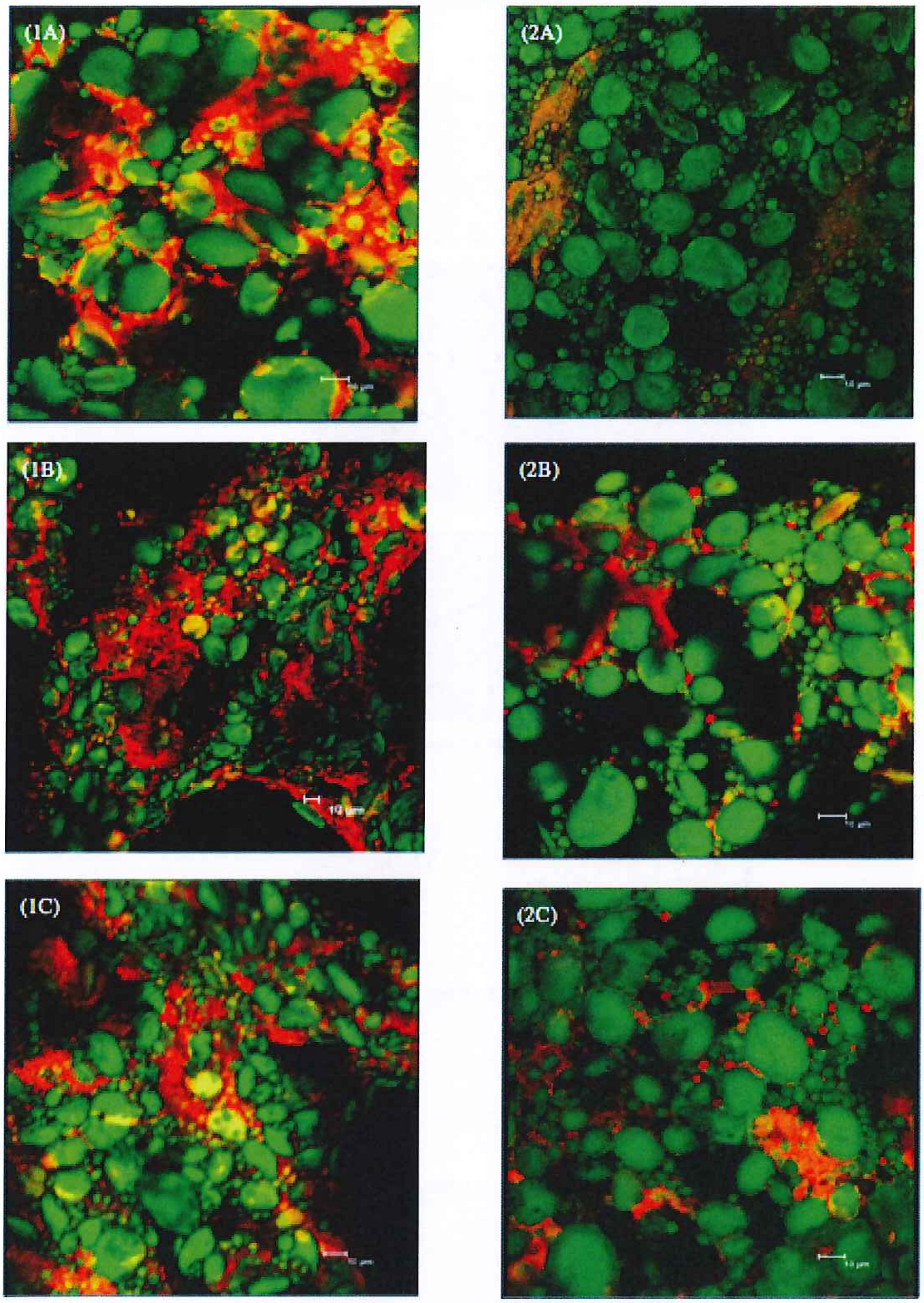
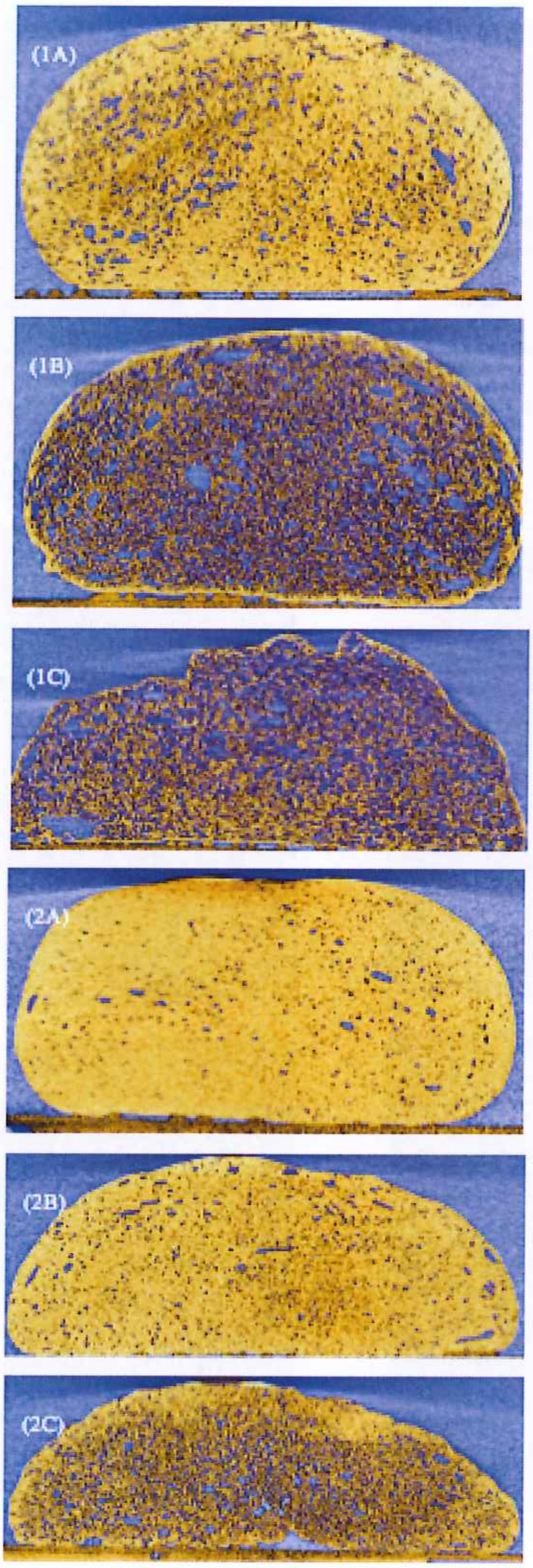
Appendix

Appendix 1. Mean and standard deviation of weight before and after baking, volume, diameter and height of the baked buns. w0- dough weight when withdrawn from freezer; w1- bread weight one hour after baking; w2- bread weight 2 days after baked; 1-Rye; 2-Sweet; A- non-proofed dough before freezing, thawed and proofed; B- half-proofed dough, baked directly from freezer; C- fully proofed dough before freezing process, baked directly; D- non-proofed dough, baked directly from freezer; E- half-proofed dough, thawed and proofed 19 min after freezing before baking process; F- fully proofed dough, thawed before baking.

	w0 (g)	sd	w1 (g)	sd	w2 (g)	sd	v (cm3)	sd	diameter (mm)	sd	height (mm)	sd
1A	58.55	1.60	52.16	1.46	51.03	1.64	200.83	16.86	80.37	5.93	44.33	1.16
1B	59.49	1.93	53.58	3.16	53.59	3.76	150.00	21.68	75.58	3.59	36.97	5.53
1C	57.87	2.53	55.19	3.01	53.66	3.85	149.17	17.44	78.16	4.12	39.84	6.07
1D	59.51	1.17	53.10	1.23	53.10	1.66	134.17	15.30	66.48	1.11	45.10	2.41
1E	59.88	0.89	53.73	0.79	53.51	0.24	202.50	17.54	78.21	2.70	44.38	1.94
1F	58.25	1.17	51.75	1.29	50.60	1.31	165.83	12.81	80.01	3.93	37.49	2.85
2A	61.28	0.38	56.32	0.52	55.29	0.68	222.50	11.73	82.63	1.52	42.11	1.76
2B	59.84	1.17	54.90	0.92	53.88	1.37	146.67	22.29	67.86	1.63	36.73	1.77
2C	59.62	0.77	54.77	0.77	53.77	1.25	170.00	21.21	76.11	1.93	35.19	1.63
2D	61.58	0.80	58.39	0.82	57.94	0.83	100.00	8.37	61.46	1.96	35.14	1.80
2E	59.38	0.16	54.36	0.24	52.73	0.62	166.00	9.83	70.16	2.60	38.76	1.67
2F	59.59	1.15	53.60	1.26	52.33	0.67	153.33	8.16	75.60	8.16	42.96	0.67



Appendix 2. Dough microstructure, 10 μm. The green color shows the starch granules, the gluten network is shown in red. 1-Dough containing rye flour; 2- sweet dough; A- reference, non-proofed dough; B- half-proofed dough before freezing; C- fully proofed dough before freezing.



Appendix 3. Micro- CT images of thawed bread doughs, vertical cut. 1-rye, 2- sweet. A- non-proofed before freezing, B- half proofed, C- fully proofed.

Appendix 4. Table with the sensory attributes used for analyzing bread buns containing rye flour during the descriptive test.

Sensory attributes	Definitions
1. Texture by finger	
Springiness	How fast the bun returns to its shape after pressure
Compactness	How airy or dense is the bun when pressure is applied
Elasticity	How elastic is the bun when it is broken
2. Texture by mouth feel	
Airy	The degree of air in the bun-assessed after first or second chew
Moist	The degree of moisture in the bun – assessed after chewing for 5 seconds.
3. Flavor	
Total flavor	Intensity of flavor, no matter what it tastes
Rye flavor	Intensity of rye flavor
Yeast flavor	Flavor of yeast
Off-flavor	Taste of something other than "regular" flavor of the bread