Sensory Evaluation of Bread

Influence of bread preparation, frozen storage temperature and time, and fiber content

Master of Science Thesis in the Master Degree Programme Biotechnology

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Division of Food Science
CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden 2011

Thesis for the Degree of Master of Science

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Front cover: Bread bun with fiber (Ayse Alp)
Printed by Chalmers University of Technology
Göteborg, Sweden 2011

ABSTRACT

Bread as a staple food product is an important part of a balanced diet with its rich starch and complex carbohydrates content and hence widely consumed all over the world. However, fresh bread has a short shelf life due to a number of chemical and physical changes known as staling which is responsible for huge economic losses in the market. One method to prolong shelf-life of bread and keep its freshness is freezing. Freezing transforms the available water in the bread to a less mobile form, minimizing the development of chemical and enzyme reactions. The frozen dough and bread market is still facing many problems regarding adequate control of freezing and thawing rates and keeping the frozen chain during production, transportation and storage.

The influences of bread preparation, frozen storage temperature, fiber content and frozen storage time on the sensory quality of bread have been investigated in this thesis. Two types of commercial frozen dough, one with fiber and one without fiber, were provided by an international bakery group. One part was stored frozen as dough and the other part was frozen as baked bread. Both dough and bread were stored at temperatures of -10°C, -15°C and -20°C for 3.5 months. The sensory quality was evaluated after 2 months and 3.5 months frozen storage by a trained panel performing QDA® (Quantitative Descriptive Analysis). The panel developed and defined 16 attributes related to appearance, flavor, odor, texture by hand and texture by mouth feel.

The findings of this study showed that frozen storage temperature has an important influence on the sensory quality of bread. Highest quality scores were observed with the storage temperature of -20°C but the differences in quality between the storage temperatures of -15°C and -20°C were quite small. Products also lost its quality with prolonged frozen storage especially at -10°C, with decreased scores in freshly baked odor, elasticity and moist, however, with higher scores for compactness, off-odor and off-flavor. Bread with fiber was more compact, less elastic and airy compared to bread without fiber, which might be due to the addition of fiber as whole wheat kernels. To conclude, it is preferable to store dough instead of baked bread at temperature of -20°C with a storage time up to 2 months. As a result the bread will be less compact and contain less off-odor and off-flavor, will be more elastic and contain more moisture and freshly baked odor compared to the frozen stored baked bread.

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1. INTRODUCTION

Bread is an important part of a balanced diet owing to the fact that it is a rich source of complex carbohydrates and starch (FoB, 2007). All over the world, bread is one of the most substantially consumed food products and bread making technology is in all likelihood one of the oldest technologies which has progressed ceaselessly through the years. New materials, ingredients and equipment have been introduced to produce better quality bread while research has generated steady and impressive progress in bread making.

Consumers are becoming more and more conscious of the significance of safe and high quality food products during the last years. Besides, they anticipate food products with acceptable quality and sensory characteristics that supposed to not vary much from the fresh ones. Since new products are developed and introduced to the food market and advance technologies are being used in the production of traditional or conventional food products; for example, application of freezing in bakery products, interest becomes further greater. Because of the importance of guaranteeing the safety of the product and provoking minimum change on the quality of the final food product, ingredients and production technology have to be examined further (Giannou et al., 2003).

In connection with the economic advantage of a centralized production and distribution furthermore the standardization of the quality of food product, frozen bakery goods market has come into prominence recently. Manufacturing of frozen bakery products do not require professional workers and increase the likelihood of providing fresh bread at any time of the day (Matuda et al., 2005). Conversely, conventional bread making is time consuming, requires trained employees and specialized facilities.

Fresh bread has an attractive golden brown crust, a savory roasted aroma, good slicing characteristics, a soft and elastic crumb texture and a moist mouth feel in general (Selomulyo & Zhou, 2007). However, during their storage, due to a number of physical and chemical alterations, the process known as staling, fresh bakery products have a moderately short shelf life. Consequently, during the period of time between baking and consumption, bread gradually loses its freshness together with an increase in crumb hardness and a decrease in flavor and aroma, causing to loss of consumer acceptance (Giannou et al., 2003). Owing to these problems with regard to the complex processes associated with traditional bread making and growing market demands, innovative research for efficient methods is essential to produce high quality bakery products while getting under control undesirable changes and prolonging the shelf life. Alternative new methods have been developed in the past twenty years for the production and preservation of bakery products, including freezing and modified atmosphere technologies (Lorenz & Kulp, 1995).

Freezing process transforms the available water to a less mobile form thus prevents the microorganism growth and the development of chemical and enzyme reactions. For this reason, freezing and frozen storage is one of the most efficient methods to retard the staling of bread. On the other hand, frozen food products require retaining the frozen chain without fail during production, transportation and storage, which means considerably high priced (Bárcenas & Rosell, 2006). Moreover, during frozen storage, prominent temperature fluctuations bring about greater deterioration on dough and bread quality (Phimolsiripol et al., 2008).

This thesis work was performed as a part of a project which is focused on changes in frozen dough and bread. The alterations in the sensory quality of bread with and without fiber during frozen storage were analyzed in this thesis. Another master's thesis which was written by Johanna Eckardt analyzed the changes in microstructure of frozen dough and bread. Also DSC (Differential Scanning Calorimetry) measurements were done by Guo Chen and Jan Swensson at Chalmers University of Technology.

The main objective of this study was to evaluate the effect of bread preparation, frozen storage temperature and time, and fiber content on the sensory quality of bread. The overall objective of this thesis work was to determine the most fresh-like bread sample which was frozen stored at different temperatures during different period of time. Quality evaluation was performed by a trained sensory panel using quantitative descriptive analysis to evaluate the most fresh-like bread sample.

2. BACKGROUND

2.1 Basic ingredients of bread and their role in bread making

The major ingredients in bread making are flour, water, salt, fat and sugars. Leavening agents such as yeast or chemicals can be added to dough. Also improvers, a common term for additives, are frequently used in bread formulations that include stabilizers, emulsifiers, oxidants, gums and enzymes such as exogenous α-amylases, proteases, hydrolases, lipases, and lipoxygenases (Gujral & Singh, 1999).

2.1.1. Flour

Flour which is the most significant ingredient of bread making tempers the particular characteristics of bakery products. When processed into fermented dough, wheat is unique among cereal grains because it contributes a light, palatable, well-risen loaf of bread (Bushuk & Rasper, 1994). Protein, starch and other carbohydrates, ash, fibers, lipids, water and small amounts of vitamins, minerals and enzymes are the main constituents of flour (Charley & Weaver, 1998).

Wheat flour contains of two basic types of protein which are called gliadin and glutenin. Gliadin forms a viscous liquid and is sticky and inelastic. Glutenin forms a rubbery material that is more elastic and tenacious (Singh & MacRitchie, 2001). Gluten which is known as the mixture of gliadin and glutenin gives dough its ability to form thin sheets that will stretch and hold gas. Generally, hard wheat with strong gluten and high-protein content is preferred in breadmaking (Bushuk & Rasper, 1994).

2.1.2 Yeast

The most common yeast used in breadmaking is *Saccharomyces cerevisiae*. Under anaerobic conditions, yeast cells are able to metabolize fermentable sugars such as glucose, fructose, sucrose and maltose. Carbon dioxide is produced as a waste product, which acts as a leavening agent and enhances dough volume. Yeast also can support both gluten network and aromatic compounds production.

Modern bread yeasts are now available all over the world. The most commonly yeast forms used in plant bakeries by far are compressed yeast and cream yeast. Compressed yeast is quite perishable due to its high moisture content around 70%. Active dry and vacuum-packed instant yeasts which are also often used have advantages because of their low moisture level and long shelf-life at room temperature (Charley & Weaver, 1998).

2.1.3 Water

Water which is essential for the formation of dough is responsible for the fluidity. It facilitates the dispersion of yeast cells and is necessary for the dissolution of salt and sugars. Water is also required for the hydrolysis of starch and sucrose. Starch gelatinization occurs during baking with the help of water that also contributes to oven spring. Water is important for the activation of flour enzymes, leads to the development of new bonds between the macromolecules in the flour, and changes the rheological properties of dough. The moisture content and the physicochemical properties of the flour are the main determiners of the amount of added water (Gil et al., 1997).

2.1.4 Sugars

Sugars are usually used by yeast during the early stages of fermentation. Later, by the action of enzymes in the flour, more sugars are released for gas production. In order to increase gas production and to improve the crust color and to sweeten the bread, extra sugar can be added. By retarding pasting of native starch or function as antistaling ingredients preventing starch recrystallization, sugars also act as antiplasticizers (Faridi & Faubion, 1990).

2.1.5 Salt

Salt which is considered as a significant ingredient with a functional role in the production of many bakery products strengthens the gluten, controls the action of yeast and thus controls volume of loaf. It improves flavor and facilitates the action of amylases aiding to sustain a supply of maltose as food for the yeast in dough (Wood, 1989). Salt also prevents the action of flour proteases, which depolymerize proteins of the gluten complex. Yeast dough is sticky and difficult to handle if salt is not added. Salt retards carbon dioxide production by the yeast delaying their fermentation in frozen dough products (Charley & Weaver, 1998).

2.1.6 Lipids

Lipids are used in breadmaking either in the form of fats or oils and are generally referred to as shortening because they tenderize the texture of the finished product. They can improve dough handling, viscoelastic properties, gas retention ability and oven spring; however, they are an optional ingredient in bread (Demiralp et al., 2000). They also affect the mouth feel, flavor, crumb appearance, and crust texture of the final products (Autio & Laurikainen, 1997). Lipids improve product quality by enhancing softness, moistness, and they extend shelf-life because of their antistaling properties. Both endogenous lipids and added fats play an important role during breadmaking and staling of bread (Collar et al., 1998).

2.1.7 Other ingredients

Several ingredients can be added to the dough formulation, usually in small amounts or in special bakery products besides from the ingredients mentioned above in order to improve baking performance and prolong shelf-life. These can either be milk or egg products, flour additives (improvers, maturing agents, enzymes, or vital wheat gluten), dried fruits, spices and herbs, dough conditioners/strengtheners, oxidizing agents, preservatives, and so on (Doerry,1995).

2.2 Bread making methods

There are mainly three methods to produce bakery products. The first method is known as straight dough method where ingredients are mixed in one step. Ingredients used in the dough formulation can vary according to the choice of manufacturer and available equipment. Sponge and dough method is the second method where mixing of ingredients is performed in two steps. During the first step of the process, leavening agent is prepared. Yeast and adequate amount of water and flour are mixed together at the second step. The mixture is allowed to develop for a couple of hours and afterwards it is mixed with the rest of the ingredients. The third method is Chorleywood method where all the ingredients are mixed in an ultrahigh mixer for few minutes (Giannou et al., 2003).

2.3 Processing steps and their role in bread making

Processing steps of bread making including mixing of the ingredients, post mixing operations (kneading, dividing and forming), freezing, thawing, proofing and baking is explained below with their role in bred making.

2.3.1 Mixing

Mixing energy input and type of mixer have important influences on the baked bread quality from frozen dough (Nemeth et al., 1996). Cooling the mixer, delaying the yeast addition and the addition of salt can be performed to ensure proper dough temperature (Dubois & Blockcolsky, 1986). Both gassing power and overall quality of loaves baked from frozen dough are poorer with a final mixing temperature over 20°C as reported by Zounis et al. (2002b). However, better results were found by some authors with high temperatures at the end of mixing and with a reduction of water content (Sahlstrøm et al., 1996). The time of frozen storage is also significant together with the suitable dough temperature after mixing because extended storage has detrimental effects on yeast. Generally, low temperatures after mixing and short resting time result in high quality dough and it shows good stability during frozen storage (Kenny et al., 2001).

Owing to the fact that gluten development is faster in the absence of salt, full dough development can be attained with short mixing times with the delay in the addition of salt. Therefore, lower dough temperature can be assured. Also it is sometimes suggested to delay yeast addition. The yeast is hydrated and becomes active during the first 10 minutes of mixing. Thereby, the degree of fermentation could be reduced by diminishing the time of the yeast in the mixed dough. In the production of frozen dough, since there is a risk of osmotic shock and cell death, yeast is never added at the same time to the salt; although a delay of yeast addition is conventional (Rosell & Gomez, 2007).

2.3.2. Post mixing operations

Post mixing operations include kneading, dividing and forming. In order to minimize the yeast activation, dough is kneaded, divided, shaped and transported to the freezer within a short period of time. The structure of the gas cells and dough development are influenced by all the steps performed in dough making (Autio & Laurikainen, 1997). Dough shape affects the stability and final quality of frozen bakery products. As stated by Havet et al. (2000), slabs and cylinders showed more satisfactory results than round pieces. Gélinas et al. (1995) has also indicated that sheets and cylinders produce breads with higher volume than those obtained from round-shaped dough pieces. Furthermore, intense molding generates heat, leading to enhance fermentation before freezing (Singh & MacRitchie, 2001).

2.3.3 Freezing

Freezing prevents the growth of microorganism and the development of chemical and enzyme reactions by transforming the available water in the foods to a less mobile form. It involves mainly two processes; temperature reduction and phase transition from liquid to solid phase (Gaonkar, 1995). Freezing generally starts at -1^{o}C to -3^{o}C and more of the water becomes frozen in food as the temperature drops. Dough can be frozen by using the available technology which is usually based on product requirements and also cost. The two basic freezing systems which are commercially available are cryogenic method and mechanical refrigeration. Liquid nitrogen or carbon dioxide are used as the cooling agent in cryogenic method and mechanical refrigeration includes blast, plate, spiral, impingent, immersion, and belt or fluidized bed freezers (Giannou et al., 2003).

There are two different approaches to apply freezing temperatures to bread dough; interrupted baking method (pre-baking) and part-baked method (pre-baked). Initially, interrupted baking method was developed to improve the bread quality; however, it led to bread with reduced loaf volume (Inoue & Bushuk, 1992). Subsequently, several studies have been performed to determine the optimum temperature and time for part-baking, concluding that sensory and textural properties of bread manufactured by part-baking process are close to the properties of bread made by conventional method (Fik & Surówka, 2002).

Due to the decreased gas retention and yeast cell viability, freezing and prolonged frozen storage have outstanding influences on dough properties. Havet et al. (2000) pointed out the significant damage in yeast activity and gluten structure owing to the cooling with rapid velocity. The viability of yeast cells decreases at rapid freezing due to the internal ice crystal formation, causing destruction of cell membranes. The gluten structure is disrupted by ice crystal formation and water migration during freezing (Zounis et al., 2002a). Prolonged frozen storage also enhances the crumb firmness of baked breads. The temperature fluctuations should be controlled strictly to prevent water migration and changes in osmotic pressure while the core temperature of the product reaches -20^{o}C (Le Bail et al., 1999). Freezing also leads to minor changes in pigments, flavors and nutritionally important components that may be lost in preparation procedures or deteriorate during frozen storage (Fellows, 1997).

2.3.4 Thawing and proofing

Frozen dough must be thawed under various time–temperature conditions before proofing. Thawing involves the rehydration of the gluten matrix and yeast cells which is essential for best performance of the dough (Kulp et al., 1995). The thawing process can be applied at a certain temperature; however, a step by step temperature increase is more favorable during thawing. This is explained by the fact that condensation happens on the dough surface, as dough is colder than the surrounding air particularly when there is a large temperature difference between the dough surface and the surrounding air, resulting in spotting and blistering of the crust. This effect is prevented by a stepwise increase in temperature. Moreover, an increase in the temperature of the outer regions of the dough is caused by rapid thawing; on the other hand, the centre of the dough still remains frozen (Kenny et al., 2001). It was found by Sharadanant and Khan (2003) that the freezing and thawing processes lead to an increase in the amount of liquid released. The addition of salt, sugars or hydrocolloids to the dough formulation reduces this side effect.

Before baking, thawed dough should be proofed in order to the action of yeast, which is needed for dough maturing or ripening. Optimum rheological properties for instance optimum balance of extensibility and elasticity as well as good machinability is obtained by properly matured dough which gives bread with desirable volume and crumb characteristics. Alcohol and carbon dioxide are produced by the fermentation of yeast during dough maturing. Alcohol affects the colloidal nature of the flour proteins and changes the interfacial tension within the dough. In additional, carbon dioxide partly dissolves in the aqueous phase of the dough and forms weakly ionizable carbonic acid, leading to a decrease in the pH. Crumb structure and volume of bread are determined by gas cells stabilization and gas retention. Gas cell structure dramatically influences frozen storage stability in the case of frozen dough. Dough containing a large number of small bubbles with a narrow size distribution and thick walls is desirable (Autio & Laurikainen, 1997). Proofing time needed for frozen-thawed dough is inevitably longer than that for conventional dough due to the lower dough temperature and the loss of dough gas retention power and yeast activity (Kulp et al., 1995).

2.3.5 Baking

Baking which is the last part of the breadmaking procedure results in a series of physical, chemical and biological alterations such as evaporation of water, formation of porous structure, volume expansion, protein denaturation, starch gelatinization, crust formation, browning reactions, protein crosslinking and rupture of gas cells (Sablani et al., 2002). Optimization of oven operating condition is needed in order to reduce energy consumption and to improve product quality. Controlled constant temperature in the oven is the usual industrial practice to accomplish optimal baking. The time and temperature of the baking process significantly influence the quality and shelf life of baked product. The quality of wheat affects the bread volume which is an important measurement for baking properties. Protein and lipid contents have also influences on baking properties (Mondal & Datta, 2008).

Gas production and evaporation in company with the change in rheological properties lead to the loss of gas retention. Increased temperature also encourages the formation of protein cross links, causing setting of loaf during baking. The studies of Singh (2005) using SE-HPLC confirmed that polymeric proteins tend to decrease while low molecular weight proteins tend to increase during bread baking. Due to aggregation or cross-linking decrease in protein solubility was also observed with time of baking.

Baking alters the sensory properties of foods, improves palatability and extends the range of tastes, aromas and textures. Enzymes and microorganisms are also destroyed during baking (Fellows, 1997). The flavor of bread is mainly formed during fermentation and baking processes. Several alcohols are formed during fermentation; on the other hand, much of these alcohols are lost during baking. The development of the crust and browning reactions which is primarily the result of a Maillard type browning reaction rather than caramelization (DeMan, 1990) during baking contributes to the formation of bread flavor. The baking process generally does not differ much from the conventional baking in case of frozen dough products.

2.4 Properties of dough and bread in general

A series of changing procedure known as gelatinization which occurs above 65^{o}C in the cell membranes affect the physical properties of wheat flour during baking, enhancing dough viscosity and impairing the extensibility of the dough. An amorphous structure in final bread is caused by gelatinized starch (Primo-Martin et al., 2006). A continuous protein phase and a discontinuous non-gelatinized starch phase in the outer crust of bread was observed by confocal scanning laser microscopy of the structure. Conversely, the crumb and inner crust represented a gelatinized starch network with respect to a protein network. Crispness is also determined by the protein phase of the outer crust. Starch gelatinization can be considered as a critical factor which influences the baking time and the extent of gelatinization in the bread crumb, it may be an indicator of minimum baking index (Mondal & Datta, 2008).

Crust formation is a restricting factor, which limits the expansion of the dough (Zhang et al., 2007). It was suggested by Zanoni et al. (1994) that crust could inhibit water vapor flow from pore to the dough surface. Also crust browning which takes place when the temperature is greater than 110^{o}C demonstrated an experimental correlation with weight loss during baking and with oven temperature. Other authors also reported that development of crust browning depends on oven temperature and has a correlation with the weight loss during baking (Wahlby & Skjoldebrand, 2002).

Surface color due to the crust browning may be considered as a critical index of baking (Zanoni et al., 1995). The Maillard reaction that leads to the development of color and aroma in the bread crust could be associated with the formation of toxic compounds for instance carcinogenic acrylamide. Up to 10 g/kg acrylamide formation was reported by Brathen & Knutsen (2005) who examined the influence of baking time and temperature on the formation of acrylamide in bread. Ahrné et al. (2007) also showed the effect of crust temperature and water content on acrylamide formation in bread crust. Their results indicated that relatively lower amount of acrylamide was determined in high temperature baking and low water content. However, that was not acceptable from a consumer standpoint because bread was too dark and other sensory attributes of the bread was not acceptable. Thus they recommended steam and falling temperature baking which may produce bread with an acceptable crust color and significantly decreased acrylamide content.

Bread dough which is a viscoelastic material shows an intermediate rheological behavior between a viscous liquid and an elastic solid. Thanks to this rheological behavior, it can retain the gas produced during the fermentation stage, contributing an aerated crumb bread structure. Both dough machinability and textural characteristics of the bread are influenced by the viscoelastic network to a great extent (Rosell & Foegeding, 2007).

Rheological properties of dough which are crucial for both product quality and process efficiency can be related to specific volume and textural attributes of breads. Water content strongly affects dough rheology and thus dough machinability. Incorporation of less water in the formula of frozen bakery products in order to minimize free water in the dough is recommended. This is because of the fact that free water induces water migration and ice crystal development, which can be damaging for yeast cells and gluten protein, resulting in decreased gas retention (Perron et al., 1999). Since chilled water slows down yeast activity and accelerates freezing of dough pieces, it can also be preferred.

The hydrophobic interactions play an important role in maintaining the gluten network integrity. Those interactions become weak when the temperature decreases. Thus, prolonged frozen storage of the dough could yield a steady deterioration of the gluten network. The presence of ice crystals formed during freezing could also rupture of the gluten structure, and therefore, the rate of freezing has an important effect on the frozen dough quality (Havet et al., 2000). The gluten weakening leads to an increase in the proofing time, a reduction in the oven spring and the dough resistance to stress conditions, resulting in baked breads with a flat surface, crumbs with a coarse texture, and large and non-uniform air cells (Gélinas et al., 1996) Therefore, the production of frozen dough requires flour with superior quality than that used in conventional bread making processes.

Concerning fibers, they can bring about gluten diluting effect and an interruption of the starch–gluten matrix therefore cause a decrease in final bread volume. However, water binding capacity of solubilized fibers and interaction with cross-linking materials influence starch gelatinization. Early starch gelatinization leads to insufficient dough development but after cooling in baking, moderate and consistent starch gelatinization promotes stability (Polaki et al., 2010). Addition of dietary fibers can impede both gelatinization and retrogradation kinetics, depending on the ratio of insoluble to soluble fibers since insoluble fibers can hinder the amylopectin retrogradation kinetics (Santos et al., 2008).

If fibers are not incorporated into the dough or if not the appropriate fiber is added, bread crumb deteriorates more quickly during freezing. Basically, fibers interrupt gluten matrix in the dough and thus decrease the quality of dough and bread, but the adverse effects depend on fiber characteristics. Also fibers have protective effects on the yeast activity which is followed by the effects on the final proof (Filipović et al., 2008).

2.5 Staling of bread

Bakery products generally have a very short shelf life and the quality of bakery products mainly depends on the period of time between baking and consumption. Bread staling which usually means the loss of bread freshness, is associated with an increase in crumb hardness and flavor changes during storage, causing a loss of consumer acceptance (Hebeda et al., 1990). The hardening of the crumb is a complex phenomenon and influenced by multiple mechanisms. Factors affecting the staling of bread crumb mainly involve starch retrogradation. On the other hand, it is not the only factor related to bread staling. Baik and Chinachoti (2000) stated that both the hardening of the crumb and the softening of the crust involves in significant alterations in bread texture. The migration of water from the crumb to the crust which is promoted by the higher water activity of the crumb according to the crust also has a significant role in bread staling (Eliasson & Larsson, 1993) since it leads to a plasticizing effect on the crumb structure (Hug-Iten et al., 2003). Ronda et al. (2011) also pointed out that moisture content of the crumb of 7-days frozen fully baked (FB) and partly baked (PB) breads decreased with storage at 4^{0}C. On the other hand, moisture content of the crust increased (Figure 1).

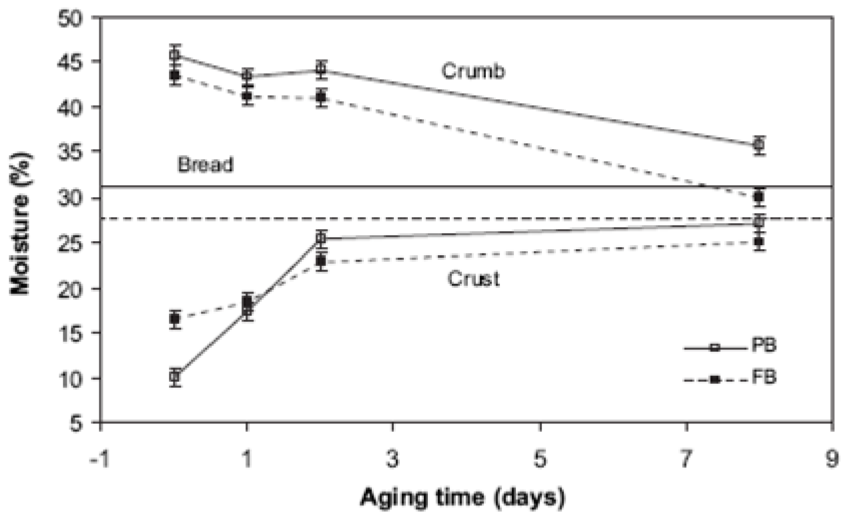

Figure 1 Effect of bread storage at 4°C on crust and crumb water of 7-days frozen fully baked (FB) and partly baked (PB) breads (Ronda et al., 2011)

The physical alterations associating with the retrogradation of starch suggested as the primary reason of bread staling, since starch is the major constituent in the bread crumb (Seow & Teo, 1996). Starch retrogradation which is a temperature and time dependent phenomenon, involves reassociation of starch component molecules into a partially crystalline, ordered structure, leading to partial crystallization of both amylose and amylopectin. It was suggested by Ribotta and Bail (2007) that although amylose retrogradation occurs during first hour after baking, amylopectin retrogradation is the major cause related to bread firming. This was explained by the fact that pure amylose crystallizes in water within hours nevertheless several days is required for amylopectin crystallization in water (Miles et al., 1985). A nucleation-limited growth process taking place above the glass transition temperature in a mobile, viscoelastic, fringed-micelle network was suggested as a reason of the slow crystallization of amylopectin (Roos, 1995).

Hydrogen bond formation occurring between the continuous protein matrix and the discontinuous fragments of starch granules through the displacement of the intermediate water molecules is also promoted during staling. Redistribution of water occurs because of the diffusion of water molecules toward the neighboring sites (Davidou et al., 1996). It was also stated by Gray and BeMiller (2003) that the nature of the gluten network changes due to the shifting of water distribution from gluten to amylopectin while the amylopectin retrogradation occurs. Consequently, mobility of water plays an important role in amylopectin recrystallization and the formation of hydrogen bonds between gluten and starch, which are responsible for bread staling.

In order to prolong the shelf life of the bread products by slowing down the staling process in the stored bread, many studies have been performed to develop different additives and enzymes. In wheat breads, different emulsifiers like sodium/calcium stearoyl lactylate, mono/diglycerides (Stampfli & Nerste, 1995); hydrocolloids such as carboxy methylcellulose, guar gum, alginate, and xanthan (Rosell et al., 2001); different α-amylases, hemicellulases and lipases (Haros et al., 2002) are used extensively as antistaling agents to retard the bread staling. Some of the changes which take place during staling can also be ameliorated by reheating to temperatures around 50-70^{o}C (Eliasson & Larsson, 1993).

2.6 Frozen bakery products

Since fresh bread has a short shelf life, problems with the preservation of bakery products together with the accelerating market demands and the complications with the traditional bread making procedure pioneered the development of new technologies in bakery industry (Carr et al., 2006). Starting in the late 19th century, the use of low temperatures like refrigeration and freezing in the food industry has become progressively popular in the bread making industry since it prolongs the shelf-life of bakery products, delays the proofing-baking phase, and while saving on equipment and labor costs, it enables to produce freshly baked products (Kenny et al., 1999). In order to minimize economic losses related to the staling of bread which reduces consumer acceptance, the application of low-temperature technology to bakery products seems to be a quite reasonable technique (Hebeda et al. 1990). Furthermore, the increased consumer demand of convenience, health, and quality are responded by the application of low temperatures to bakery products (Rosell, 2010).

Low and freezing temperatures can be applied to bakery products at different points of the process (Figure 2). In spite of the different methods, frozen dough, par-baked bread and frozen bread are the most popular applications of frozen technology.

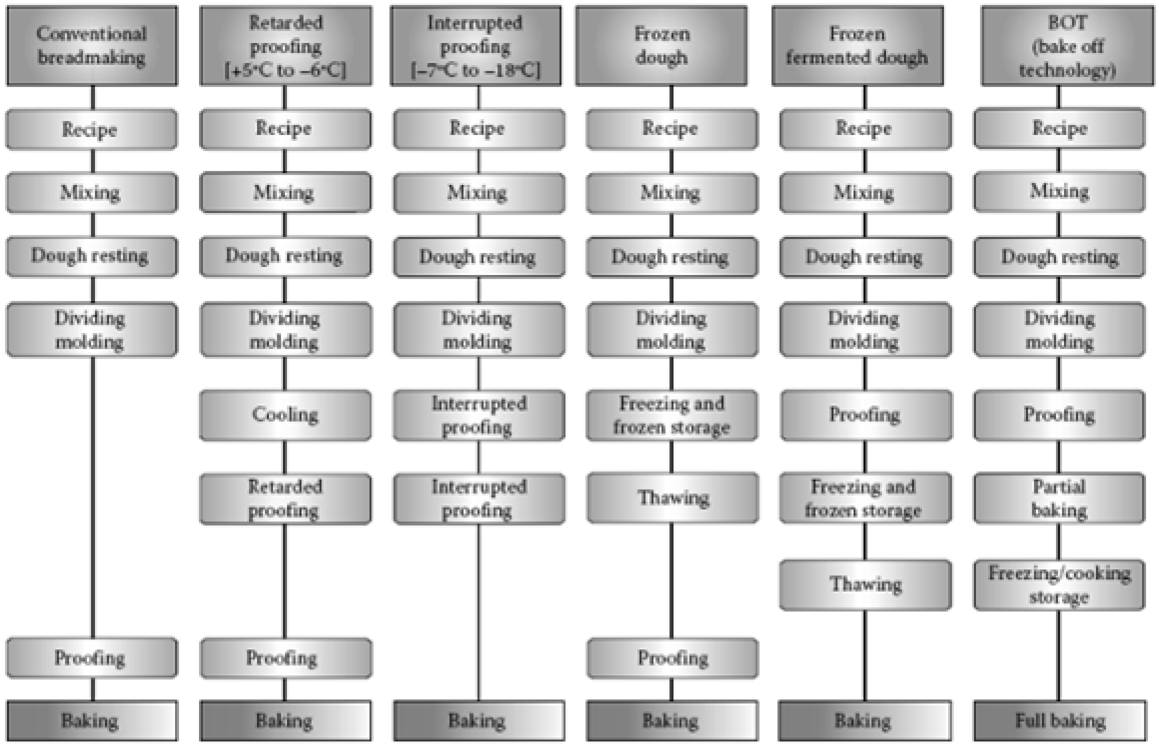

Figure 2 Methods of bread making with the application of low temperatures (Rosell, 2010)

Even though frozen dough technology requires extra cost for freezing, transportation, and frozen storage, also some instruction and knowledge for accomplishing the product in the bakery, it is considered as an alternative to conventional or traditional bread making, allowing large-scale centralized production, storage, and distribution in the frozen state, and proofing and baking in retailers or bakeries. The use of frozen dough is quite feasible due to the low volume of the unfermented dough, which is considerably convenient when storage is concerned (Giannou & Tzia, 2007). On the other hand, when energy is considered, this kind of product is expensive (Carr et al., 2006).

Besides the advantages, the loss of quality during storage and transportation is a significant problem with frozen bakery goods (Stauffer, 1993). It was indicated by Vetter (1979) that the baked products from frozen dough had low volume and coarse texture, and shorter shelf life. However, recently, these problems have been handled by prolonging the shelf life of the frozen dough up to six months, and generally it is only shortened because of failures in the cold chain during transportation or temperature fluctuations during storage (Rosell &

Gomez, 2007). According to Phimolsiripol et al. (2006) under extreme fluctuating temperature conditions, structural damage and diminished CO_2 production of dough occur after 30 days of frozen storage.

Not only fluctuating temperature conditions but also the selection of appropriate flour has a considerable influence on the quality of frozen bakery products. Wheat flour for production of frozen bread and dough should have greater strength than that used in conventional bread making (Bhattacharya et al., 2003). Studies showed that wheat varieties with strong gluten content give the best results (Lu & Grant, 1999a; Boehm et al., 2004). It should also be considered when it comes to the selection of proper flour that protein quality and gluten strength are more important than the amount of proteins (Inoue & Bushuk, 1992) The glutenin fractions have a key role in the quality of the frozen dough, followed by the fraction of gliadins and the starch (Lu & Grant, 1999b).

2.7 Effects of freezing and frozen storage on dough and bread quality

Various problems emerge from the application of freezing temperatures to bread dough, including progressive loss of the dough strength; decreased CO_2 retention capacity and longer fermentation time; decreased yeast activity; lowered loaf volume; and deterioration in the texture of the final product (Selomulyo & Zhou, 2007). In this section, influences of freezing and also frozen storage on bread dough and quality will be explained.

2.7.1 Dough strength and structure

Frozen stored dough has a reduced strength, which leads to a decrease in loaf volume, giving a low-quality product. The release of reducing substances from yeast, the reduction of gluten cross-linking raised by ice recrystallization and the water redistribution promoted by an alteration in the water binding capacity of dough constituents may lead to the loss of dough strength (Selomulyo & Zhou, 2007).

Hsu et al. (1979) stated in their study that during freezing, the release of reducing substances, such as glutathione from yeast reduces the strength of the dough by breaking down disulphide bonds in the gluten proteins which is an important factor in characterizing the rheology of gluten.

On the other hand, Varriano-Marston et al. (1980) reported that ice crystallization plays a role in the weakening of the gluten protein network, consequently weakening the dough and elongating the proofing time. It was also approved that the longer proofing time was in consequence of the demolition of yeast during freezing, concluding decreased gas production. Same researchers indicated further that the recrystallization process may also severely change the structural components of the dough, particularly protein.

Berglund et al. (1991) observed a disrupted gluten matrix due to the development of ice crystals in non-fermented dough stored for 6 months which was less continuous, more cleaved and separated from starch granules. This gluten matrix retains gas inadequately and therefore leading a decreased loaf volume and increased proof time of frozen dough. By the generation of large ice clusters during recrystallization, the starch granules were additionally damaged, further promoting the decreased ability of the gluten to retain gas during proofing.

However, damaged starch linearly increases the water absorption capacity of flour (Tipples, 1969). This suggestion was further favored by the indications of Lu & Grant (1999a) that the amount of freezable water in frozen dough increases with frozen storage time. Bot (2003) further reported that at a frozen storage temperature of -15^{o}C, the increase in the amount of freezable water was higher than at -25^{o}C. Bhattacharya et al. (2003) attributed that effect to the deterioration of gluten network resulting in a migration of water and an increase in the amount of freezable water.

2.7.2 Yeast viability and gas production

Yeast viability is greatly affected by the freezing and frozen storage temperatures. Frozen dough manufacturing and frozen storage lead to important losses in the number of viable yeast cells. According to the findings of Ribotta et al. (2003a), after 3 months of frozen storage, roughly half of the original yeast cells became unviable.

It was suggested that freezing the yeast in a dough system shows an increased susceptibility to cell damage with respect to the freezing of yeast directly since in the dough system, yeast is under osmotic stress (Lorenz & Kulp, 1995). During freezing and frozen storage, due to the decreased number of viable yeast cells, a reducing compound such as glutathione is released, destroying the disulphide bonds among proteins and causing to a weakening effect on the gluten.

The ability of yeast to produce CO_2 is determined by the strain, the number of yeast cells, the cell activity, and the amount of fermentable sugars. Especially, the freezing and thawing rate, frozen storage temperature and duration, and freeze-thaw cycles further influence the gassing power of yeast. Fast freezing rates decreases the gassing power (El-Hady et al., 1996). Rapid freezing also concluded with a much higher sensitivity to storage duration than slow freezing. Le Bail et al. (1996) obtained the maximum yeast activity with a slow freezing rate of -0.19^{o}C/min. Yi and Kerr (2009a) reported that dough samples stored at -20^{0}C showed maximum gassing power, particularly at slow freezing rates. It was also indicated by the same researches that gas production decreased with longer frozen storage periods.

During freezing and frozen storage, even with controlled temperature, rapid processing and the use of freeze tolerant yeast, some losses in the fermentative capacity are inevitable. For that reason, it is suggested to increase the amount of yeast between 30% and 100% in the formulation of frozen dough. On the other hand, the performance of frozen dough can be improved with the application of freeze tolerant yeast allowing the increase of the fermentation period before freezing (Takano et al., 2002b) which further increases the volume of baked bread from frozen dough when freeze tolerant yeasts are used (Takano et al., 2002a).

The different strains of *Saccharomyces cerevisiae* show different sensitivities to freezing damage, probably with regard to their cytoplasm content (Manzoni et al., 1995). Murakami et al. (1996) indicated in their findings that due to the effect of lipid content and its chemical structure on the fluidity of the plasma membrane, they might have an influence on the freeze tolerance of the yeast. Presently, there are special yeast strains which have a quite good performance in frozen dough thanks to their freeze tolerance ability. Furthermore, the freeze tolerance can be improved by directed mutagenesis of *Saccharomyces cerevisiae* (Codón et al., 2003). The identification of the yeast genes responsible for freeze tolerance introduces a new approach for developing freeze tolerant yeasts (Tanghe et al., 2000).

2.7.4 Bread quality

The most important quality parameters in foods in general are appearance, taste, flavor, texture and nutrition. In the case of bread, the key attributes are flavor and texture for the consumer; however, the overall perception of bread is influenced significantly by the freshness, color, texture and biting properties besides the flavor attributes (Heiniö, 2006).

The amount of liquid which is released from the frozen dough during thawing substantially influences the bread making properties; for instance, bread height and specific volume. Corresponding to the studies of Seguchi et al. (2003), the liquid from thawed bread dough was separated by centrifugation and concluded that the amount of liquid exuded from the dough was increased by freezing-and-thawing cycle. It was further stated by the same authors that there was a drastic adverse correlation between the amount of centrifuged liquid and bread making properties. This phenomenon, due to the freezing and following thawing, is known as dough syruping that is caused by diminished water binding capacity of the dough. Gys et al. (2003) indicated that the loss of water retention capacity of the dough and the following increase in dough syruping was led by the degradation of cell wall polysaccharide arabinoxylan by endogenous xylanases. If dough is prepared by using flour from partially debranned grain, it shows decreased xylanase activity levels by 60% compared to flours made from whole kernels and respectively hindered the solubilization and degradation of arabinoxylans (Gys et al., 2004).

Deterioration of bread quality is also highly influenced by the frozen storage temperature of dough. The quality loss includes the alterations in dough rheology as mentioned previously in consequence of water mobilization from the hydrated gluten to the ice phase during storage (Bot & de Bruijne, 2003). During baking, the gluten rehydration does not take place and excess water may migrate to the starch paste, therefore influencing the yield stress of the starch paste and disrupting the baking performance of the dough. This is particularly accurate at temperatures not far below the glass transition temperature (T'_g) of the dough

(Laaksonen & Roos, 2000). Frozen dough is expected to be moderately stable with storage time, if it is stored well below T'_g (Slade et al., 1989).

The size of the resulting ice crystals has a significant influence on the overall quality of bread; particularly the texture as far as freezing is concerned. In order to determine the size of ice crystals, the rate of freezing should be considered as the main factor. More rapid freezing rates and lower storage temperatures result in development of smaller ice crystals, which give a better product quality (Mallett, 1994). On the other hand, it was showed that slow rates (-2^{0}C/min) provide higher yeast survival levels and bread scores (Gélinas et al., 1995). This can be explained by the fact that ice is formed outside the yeast cell at relatively slow freezing rates and cause a relative increase in external solute concentration and therefore hyperosmotic conditions which lead to a relative dehydration. Conversely, high freezing rates cause to the development of small ice crystals inside yeast cells, leading to the disintegration of cell membranes if recrystallization into larger crystals take place particularly during prolonged frozen storage and slow thawing at low temperatures (Lu & Grant, 1999b). Thus, the rate of freezing should be optimized properly in order to maintain the bread quality.

Storage conditions such as total storage time, stacking arrangement of product containers within the freezer unit, type of freezer unit, base storage temperature, temperature fluctuations, freeze–thaw cycles, permeability and integrity of packaging materials, packaging atmosphere, and light exposure are crucial for preserving frozen bread quality (Kennedy, 2000). A decrease in the quality of frozen bread is observed with prolonged storage time. Lipids, carbohydrates and proteins in the bread may be oxidized, causing rancidity and off-flavors. Enzymatic degradation or structural changes may also affect the taste and textural properties. Very low temperatures do not guarantee a better product quality because chemical reactions can still continue at very low temperatures over extended storage times. Especially lipids are susceptible to degradation and may undergo autooxidation over time. These changes are responsible for many off-flavors not only in bread but also in all foods (Mallett, 1994; Kennedy, 2000). During frozen storage, changes in the structure and arrangement of amylose and amylopectin molecules which will be reflected during starch gelatinization and retrogradation can be stimulated by the redistribution of water and ice recrystallization in dough as mentioned previously. Dough exhibits prominent degree of starch retrogradation with the longer frozen storage times. Besides, bread made from frozen dough exhibits faster starch retrogradation on low temperature (4^{o}C) storage with respect to bread made from non-frozen dough, contributing an increase in bread firmness (Ribotta et al., 2003b).

Conditions of fluctuating temperature can result in some loss of bread quality. For example, partial thawing may cause some loss of sensory quality and shelf-life with increased temperature (Mallett, 1994). During transportation of frozen bread in refrigerated trucks or in cases of freezer malfunction at retail stores, undesirable conditions can take place.

Frequent opening and closing of the freezer also causes temperature fluctuations that can conversely influence quality over a period of time. Conditions of fluctuating temperature may also occur in the consumer's home freezer or during transport by the consumer from the retail store to the home. Due to the problems that cause deteriorations in quality, the frozen storage temperature should be kept optimally and temperature fluctuations should be controlled strictly.

As a consequence, obtaining a bakery product from frozen dough that has similar characteristics to freshly made bread is a complicated issue due to the modification of the final structure by various parameters. Several technical modifications including the isolation of freeze-resistant yeasts, addition of improvers such as emulsifiers and water-binding agents, addition of wheat proteins, modification of dough composition, use of heat stable enzymes, optimization of mixing, freezing and freeze-thaw cycles have been introduced to improve frozen dough (Selomulyo & Zhou, 2007).

2.8 Sensory quality

"Food quality" has a plenty of meanings in the food industry according to the different professionals. It is the nutritional value of the food according to the nutritionists; on the other hand, it refers to the safety of the food to microbiologists. It also indicates the stability of the food to chemists. However, consumers are the major arbiters of food quality due to their purchase and/or non-purchase of the food. From this point of view, the importance of sensory quality of the food product must certainly be considered.

Sensory quality defines food quality as "the acceptance of the perceived characteristics of a product by consumers who are the regular users of the product category or those who comprise the market segment" (Cardello, 1995). According to this definition, sensory characteristics of the food product are the most outstanding and important variables determining both the acceptability and perceived quality. From this standpoint, the characteristics related to appearance, aroma, flavor and texture which are given below with a couple of examples can be considered as the most important quality parameters (Meilgaard et al., 1999).

- Appearance characteristics
  - Color (uniformity, intensity)
  - Size and shape (dimensions, geometry)
  - Surface texture (shine, smooth/rough)
- Aroma characteristics
  - Olfactory sensations (chocolate, vanilla)
  - Nasal feeling factors (cool, pungent)
- Flavor characteristics
  - Olfactory sensations (fruity, rancid)
  - Taste sensations (salty, sweet, bitter, sour)

- Oral feeling factors (metallic, astringent, heat, burn)

- Texture characteristics
  - Mechanical parameters (hardness, viscosity, elasticity)
  - Geometrical parameters (grainy, foamy)
  - Fat/moisture parameters (oily, juicy, moist, dry, wet)

2.8.1 Sensory characteristics of bread

Bakery products represent attractive sensory characteristics especially when they are freshly baked. Dough ingredients, processing methods and duration of fermentation and baking highly influence sensory characteristics. Most of the ingredients in the dough mixture produce flavor compounds and the addition of other ingredients such as salt, sugar, fat or milk usually contribute to the flavor of bakery products (Schieberle, 1996).

Freshly baked bread presents a golden brown crust, a creamy white crumb, and an attractive aroma and flavor which is a result of the combination with alcoholic fermentation and caramelization of sugars in the crust. The level of yeast in the recipe also contributes to bread flavor. On the other hand, during baking, when heat reactions, such as the Maillard reaction and caramelization, occur, the most significant flavor compounds are formed. Maillard browning which is responsible for the formation of a flavored brown crust and very important for the flavor perception of baked products involves the reaction of reducing sugars with amino groups (Cauvain, 1998). The flavor of bread crumb is influenced by enzymatic and possible fermentation reactions; however, the flavor of bread crust is affected by heat reactions (Kirchhoff & Schieberle, 2001). Texture which is the response of the tactile senses to physical stimuli is related to crispy and crackly characteristics. Bread should show soft, tender, smooth and moist mouth feel; however, crumb should be elastic and cohesive and show decreased adhesiveness (Giannou & Tzia, 2006).

On the other hand, most especially, softness, which is a characteristic of fresh bread, decreases rapidly during storage. As mentioned previously, when bread is taken out from the oven, a number of changes also called as staling, start, leading to deterioration of the bread quality. By the changes in taste as well as in texture, the consumer can perceive these quality deteriorations easily. The typical fresh bread aroma is lost and a “stale” flavor develops over time. With respect to the textural changes in bread, the crumb becomes dry and hard; on the other hand, the crust becomes soft and leathery. During staling of bread, some of the flavor compounds evaporate; however, others may form complexes with starch, because of the fact that some of the bread aroma can be recovered by means of reheating (Gellynck et al., 2009).

2.8.2 Sensory characterization

The main objective of sensory analysis is to measure food characteristics and the influences of those attributes on food acceptance by utilizing human perception. Sensory evaluation uses all senses such as sight, smell, taste, touch and hearing in determination of food products. Obviously, appearance, odor, taste, and mouth feel of a food product have enormous effects on food preference. Hence, sensory characterization is essential to comprehending the characteristics of food and food acceptance. The area of sensory science has been growing and new techniques are developing in order to obtain better data and generate better results.

The goal of a particular sensory assessment must be decided in advance, since that objective helps to determine the types of data which are needed to make specific test choices. The question of what results are important to the company or researcher is a key question in deciding what types of tests and thus what type of panelists are needed. Samples to be used in sensory analysis must be carefully chosen and prepared to assure reproducibility of results and the suitability of the information collected to the objective of the analysis. Conditions of preparation, handling, and presentation to panelists should be carefully documented to enable results to be reproduced in subsequent tests (Chambers IV et al., 2006).

In this study, a descriptive test method was decided to be used in order to obtain detailed description of the aroma, flavor and texture characteristics of the samples therefore general introductory information about descriptive tests are given below.

2.8.2.1 Descriptive tests

The “descriptive analysis” technique of sensory evaluation identifies, describes and quantifies sensory qualities of a given food product such as visual, textural, auditory, olfactory and gustatory. Descriptive analysis test includes eight general steps: collecting panelists, developing and defining descriptive terms, training of the panel in using the scale, evaluating the performance of panel, performing test, analyzing data, evaluating results and communicating the results.

Descriptive tests requires a panel of five to ten trained assessors who can completely describe and measure detailed sensory characteristics of the food product, and who are able to communicate their perceptions accurately and exactly. Descriptive panelists may be recruited from internal and external sources and training usually requires several training sessions per product.

Results of a descriptive test include an entire description of the sensory characteristics of a product which is helpful for understanding sensory acceptability, examining how formulation or process alterations influence sensory properties, associating instrumental measurements to sensory data, determining the critical attributes significant to quality control or shelf-life studies. There are different sorts of descriptive analysis, including the “flavor profile” method, the “quantitative descriptive analysis” method and the “spectrum” method. Many sensory departments develop their own adapted methods of descriptive analysis, generating fundamentally the same type of information (Gillette, 1994). Here in this study, quantitative descriptive analysis method was performed to evaluate the samples. For this reason, only quantitative descriptive analysis is mentioned below.

2.8.2.1.1 Quantitative descriptive analysis

Quantitative Descriptive Analysis® (QDA®) was developed by Dr. Herbert Stone, Dr. Joel Sidel and their colleagues at the University of California Davis (Lopetcharat & McDaniel, 2005). Today, as one of the most significant tools to examine problems associated with flavor, appearance, and texture, also a way to guide product development efforts, QDA® has attained extensive acceptance (Marsili, 2006). In order to determine the appropriate terms and procedures, this method uses statistical analysis to a great extent.

A panel of five to eight trained assessors analyzes perceived appearance, aroma, flavor and texture characteristics of a food product and develops appropriate attributes specific to the product. Panelists, who are provided with samples for training, develop and define the common terminology with the help of a panel leader who arranges meetings, leads discussions, obtains the consensus of the panel, and communicates the results of to the users. The panel is trained to use the intensity scale to score the product. Members of the panel evaluate all test samples individually in separate booths. Appearance, odor, flavor, texture and mouth feel attributes of the product can be evaluated by the panel during evaluation. Panelists enter the data into a computer, or the score sheets are collected individually from the panelists as they are completed, and the data are entered for computation (Meilgaard et al., 1999).

The results from a QDA test are statistically analyzed and usually represented graphically as histograms or in the form of a “spider plot” for interpretation. Common statistical methods such as analysis of variance, multivariate analysis, one-, two- or multiway analysis of variance, paired comparison tests can be used to analyze data from descriptive analysis panels (Chambers IV et al., 2006).

3. MATERIALS, METHODS AND METHODOLOGICAL CONSIDERATIONS

3.1 Frozen dough samples and experimental design

Two different frozen dough types, with fiber and without fiber, were provided by Lantmännen Unibake which is an international bakery group expert in fresh and frozen bakery products in Denmark. Samples were transported from Denmark to SIK Gothenburg in a vehicle with freezing function. Ingredients and nutritional information are given in Table 1 and 2 respectively.

Table 1 Ingredients of bread with fiber and bread without fiber[1]

Bread with fiber	**Bread without fiber**
Wheat flour	Wheat flour
Water	Water
Wheat kernels	Yeast
Yeast	Vegetable oil
Sugar	Sugar
Vegetable oil	Skimmed milk powder
Skimmed milk powder	Wheat gluten
Wheat gluten	Iodized salt (0.8 g per 100 g)
Iodized salt (0.8 g per 100 g)	Stabilizers (E412, E466)
Stabilizers (E412, E466)	Dextrose
Dextrose	Emulsifier (vegetable E472e)
Emulsifier (vegetable E472e)	Flour treatment agent (E300)
Flour treatment agent (E300)	Enzymes
Enzymes	

Table 2 Nutritional information per 100 g[1]

	Bread with fiber	**Bread without fiber**
Energy (kJ/ kcal)	1250/300	1200/280
Protein (g)	11	10
Carbohydrate (g)	48	45
Fat (g)	6	6.5

[1] taken from the website http://www.lantmannen-unibake.com (Access: 18 April 2011)

Figure 3 shows how the samples were organized after they were delivered. One part was baked in the bakery at SIK and stored at three different temperatures, -20°C, -15°C and -10°C, as baked bread. The other part was stored directly as raw dough at the stated temperatures. Samples were stored for 2 and 3.5 months counted from the arrival at SIK. After each storage time the frozen stored bread was thawed under controlled conditions and the dough was proofed and baked by using the same methods as for the samples baked before storage.

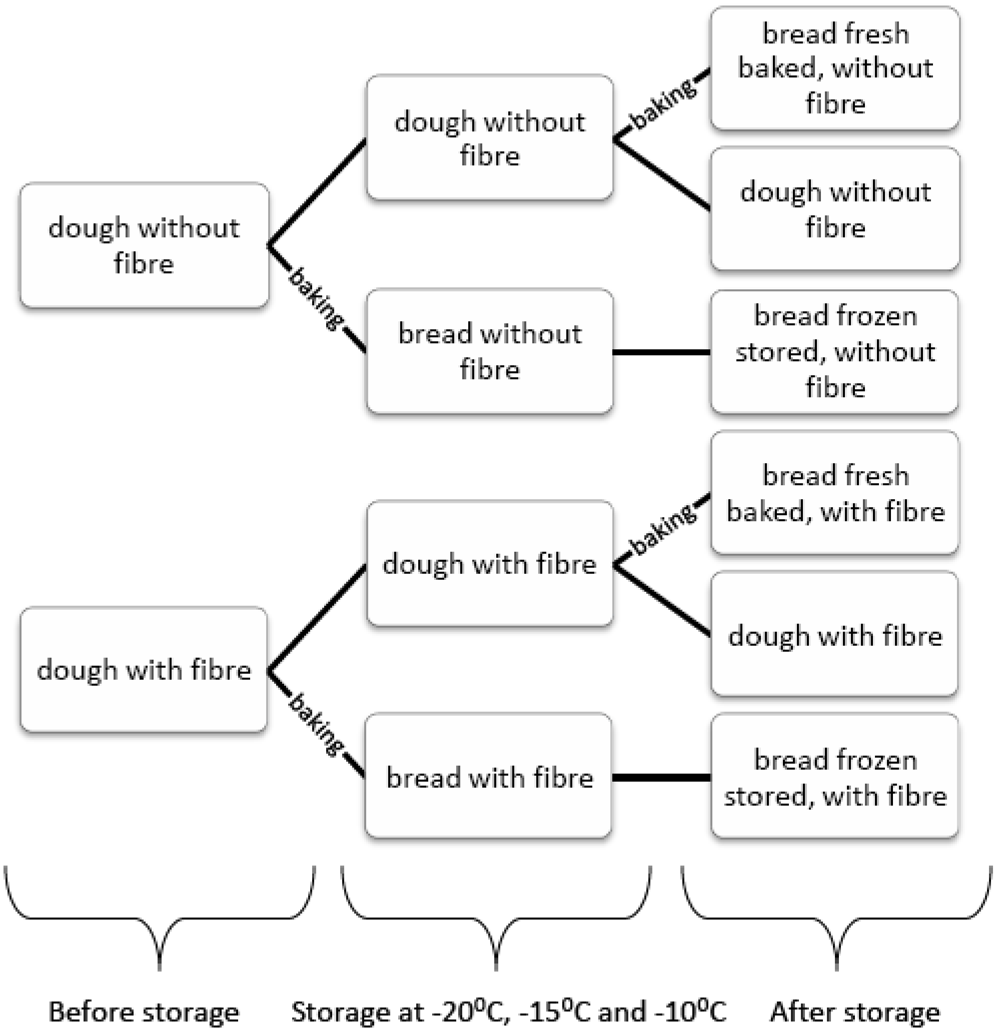

Figure 3 Chart of sample organization

Figure 4 Frozen dough samples without fiber and with fiber respectively

3.2 Frozen storage regimes

Samples were provided and transported as raw frozen dough by Lantmännen Unibake in boxes of 130 (dough with fiber) and 150 (dough without fiber) pieces per carton box wrapped in a plastic bag.

Samples, which were baked at SIK were put into plastic bags of 9 to 12 pieces and closed with a staple and labelled. All baked samples were frozen at –20°C in a walk-in freezer which was placed in a refrigerated room. The freezer was equipped with a fan, so that there was air convection.

After the baked bread had been frozen at –20°C, it was divided into the different freezing temperatures of -10^{o}C, -15^{o}C and -20^{o}C. Loggers were placed to each freezer in order to measure the temperature and the relative humidity inside the freezers and also to observe the temperature fluctuations. The temperature and the relative humidity were recorded four times a day. The charts of temperature and relative humidity fluctuations can be seen in Appendix A.

The cartons of the raw dough samples wrapped in plastic were also divided into the different freezers with the stated temperatures and stored in this way of packaging.

3.3 Thawing, proofing and baking

The frozen dough samples were withdrawn from the freezer; 12 samples were put on one tray with baking paper and covered with a plastic. They were thawed 75 minutes at room temperature. Thereafter they were put into a proofing chamber and proofed for 50 minutes at 35°C and 75 % RH. Afterwards the plastic was taken off and the samples were baked for 10 minutes at 190°C in an air oven. The trays were automatically rotated horizontally during the baking time. When only a few samples were baked, the oven was filled with other type of dough pieces to simulate a full oven with equal baking conditions. After baking all samples were taken off the tray, put on a wooden table and cooled for one hour at room temperature and for another one hour at controlled temperature of 24°C. Trays were covered with aluminum foil during transportation from the bakery to the chamber at 24^{o}C owing to prevent aroma migration. For dough without fibre and dough with fibre the same procedure was used.

After storage, frozen stored bread was withdrawn from the freezer, taken out of the bag and thawed for 130 minutes at controlled temperature of 24°C. During thawing they were placed on a tray and covered with a plastic. Tests showed that the core temperature reached the ambient temperature after that time.

3.4 Sensory evaluation

The sensory analysis was conducted in a sensory laboratory at SIK- The Swedish Institute for Food and Biotechnology, Göteborg, Sweden. A quantitative descriptive analysis (QDA) was performed for assessing the sensory characteristics of frozen dough and bread. The sensory analysis was preceded by four training sessions at which a panel of 7 assessors consisting of 6 women and 1 man was trained to identify and evaluate sensory characteristics of the products. They were also trained in the use of 100 mm line scale. 16 attributes related to appearance, flavor, aroma and texture were developed and evaluated by trained assessors. Each assessor was provided with one bun on a paper plate which was coded with a 3-digit random number for the evaluation. Sample evaluations were performed at room temperature under normal lightening conditions. The attributes were evaluated on a 100 mm line scale labeled “little” at 10 mm and “much” at 90 mm. All samples were assessed in random order in two replicates. The data collection was done by using a computer system, Biosystems FIZZ 2.46 B and analyzed statistically.

Figure 5 Sensory evaluation booth in the sensory laboratory at SIK

3.5 Statistical data analysis

ANOVA was performed using the statistical program of FIZZ 2.46 B to determine the significant differences between samples at 95% confidence interval. For each attribute, panel scores were averaged and compared by Tukey test at $P< 0.05$. Further, a pairwise comparison analysis was performed to observe the significant differences between the samples stored for 2 months and 3.5 months.

Partial least squared (PLS) regression was also performed by statistical programme SIMCA-P+ from Umetrics to relate the sensory attributes to the effect of frozen storage temperature and time.

4. RESULTS AND DISCUSSION

4.1 Sensory characteristics of bread samples

The sensory attributes developed and defined by the panel members are given in Table 3 below.

Table 3 Sensory attributes and definitions used for the sensory evaluation

Sensory attributes	**Definitions**
1. Appearance	
Even shape	The degree of even form of the bun (round /uneven)
Air bubbles	The degree of spotted/speckled surface because of small air bubbles
Crumpled crust	The degree of crumpled/wrinkled surface of the bun
2. Odor	
Total odor	Intensity of odor, no matter what it smells
Freshly baked odor	Smell of fresh bread
Yeasty odor	Smell of yeast
Off-odor	Smell of something other than “regular” smell of bread
3. Flavor	
Total flavor	Intensity of flavor, no matter what it tastes
Yeast flavor	Flavor of yeast
Off-flavor	Taste of something other than “regular” flavor of the bread
4. Texture by finger	
Cracking	The amount of crust cracks when pressure is applied
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
5. 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 5-10 seconds

Figure 6 shows crumpled crust characteristic and Figure 7 shows air bubbles on the surface of the samples.

Figure 6 Crumpled crust on the surface of the samples

Figure 7 Air bubbles on the surface of the samples

4.2 Sensory evaluation after 2 months frozen storage

In this section, influence of bread preparation, frozen storage temperature and fiber content on sensory quality of both freshly baked bread and frozen stored bread after 2 months storage are reported. The results are given in Appendix B. Significant differences in sensory attributes are shown with “✦” mark on the spider plots.

4.2.1 Influence of bread preparation on sensory quality of bread

No differences was detected between freshly baked bread and frozen stored bread with a storage temperature of -20°C while significant differences were generally detected in air bubbles, elasticity, freshly baked odor, airy and moist attributes between freshly baked bread and frozen stored bread with a frozen storage temperature of -10°C.

Freshly baked and frozen bread samples with fiber stored at -10°C were compared after 2 months storage and significant differences were observed in air bubbles, springiness, elasticity, freshly baked odor, airy and moist characteristics (Figure 8). On the other hand, at -20°C, there was no significant difference between those samples (Figure 9).

Frozen stored bread had lower scores in elasticity and moist but higher scores in compactness compared to freshly baked bread. Basically, that might be explained by the dehydration of water during baking and frozen storage. Besides the microstructure results of Eckardt (2011) which indicated that frozen stored breads showed big amount of gaps in the microstructure since water might have separated from the gluten matrix or from the starch fraction during storage due to recrystallization. Moreover, samples probably lost more water due to the high mobility of water at relatively high storage temperature of -10°C.

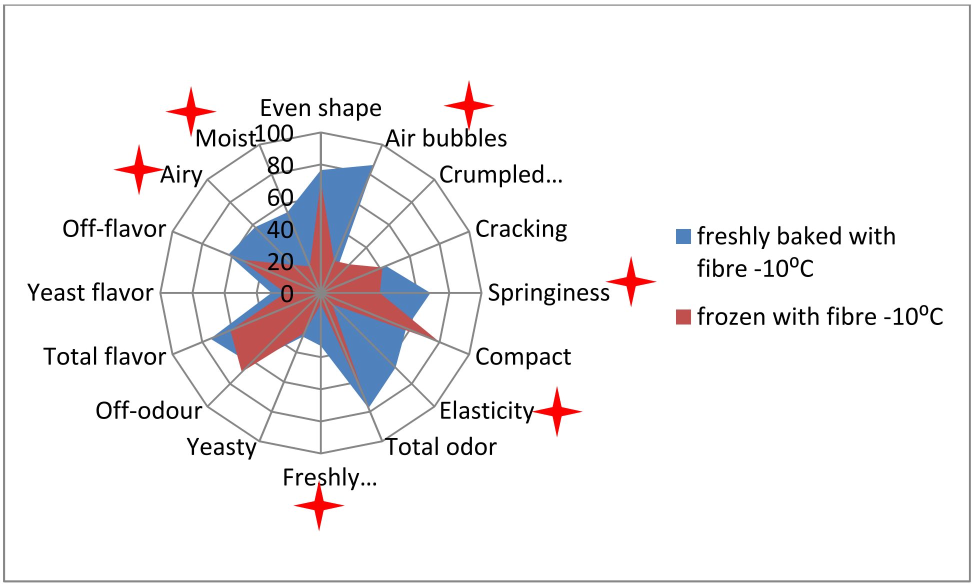

Figure 8 Spider plot of freshly baked and frozen with fiber at -10°C after 2 months storage

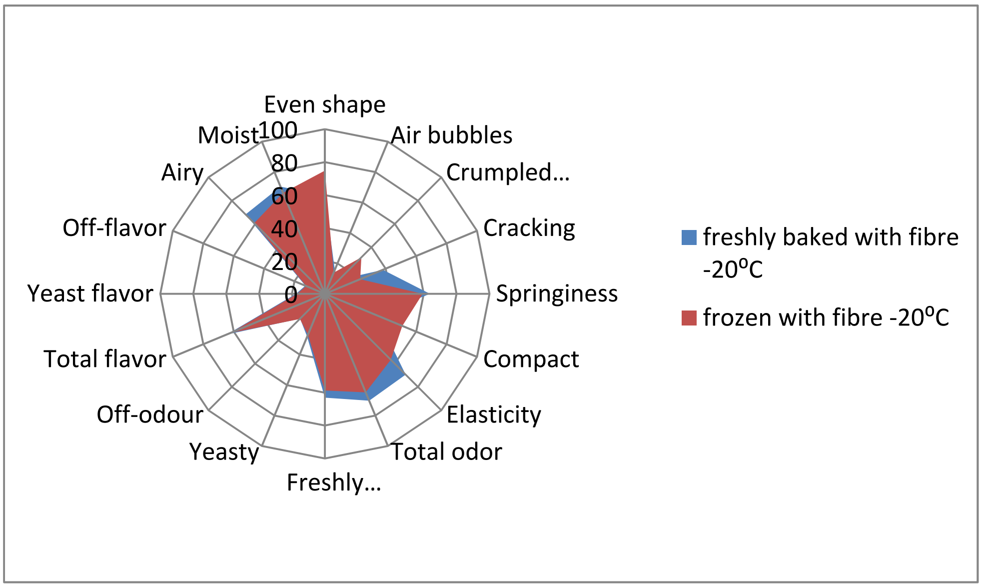

Figure 9 Spider plot of freshly baked and frozen with fiber -20°C after 2 months storage

Significant differences were found in air bubbles, crumpled crust, compactness, elasticity, freshly baked odor, off-odor, off-flavor, airy, moist attributes between freshly baked and frozen stored bread without fiber at -10°C after 2 months storage (Figure 10); however, there was no significant difference between those samples when they were stored at -20°C (Figure 11).

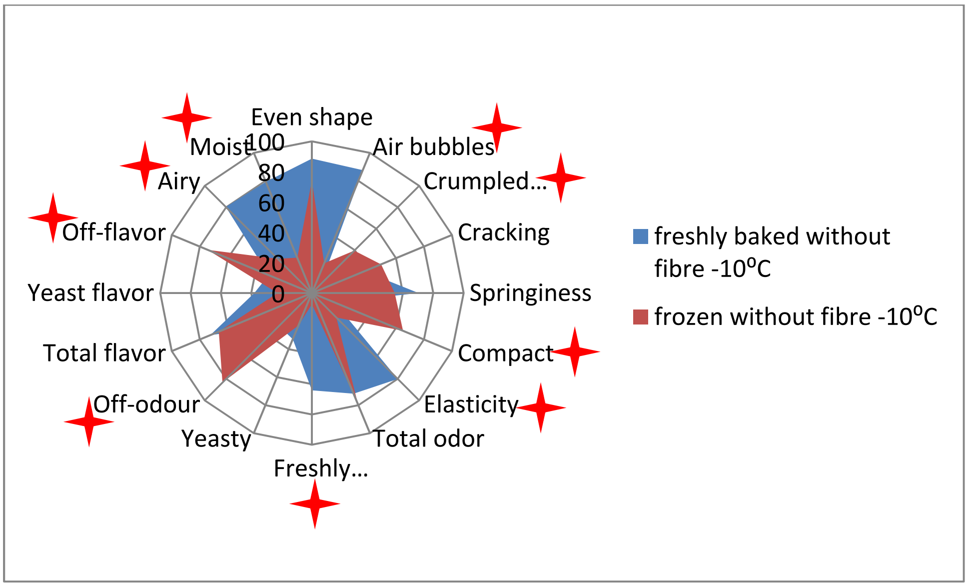

Figure 10 Spider plot of freshly baked and frozen without fiber -10°C after 2 months storage

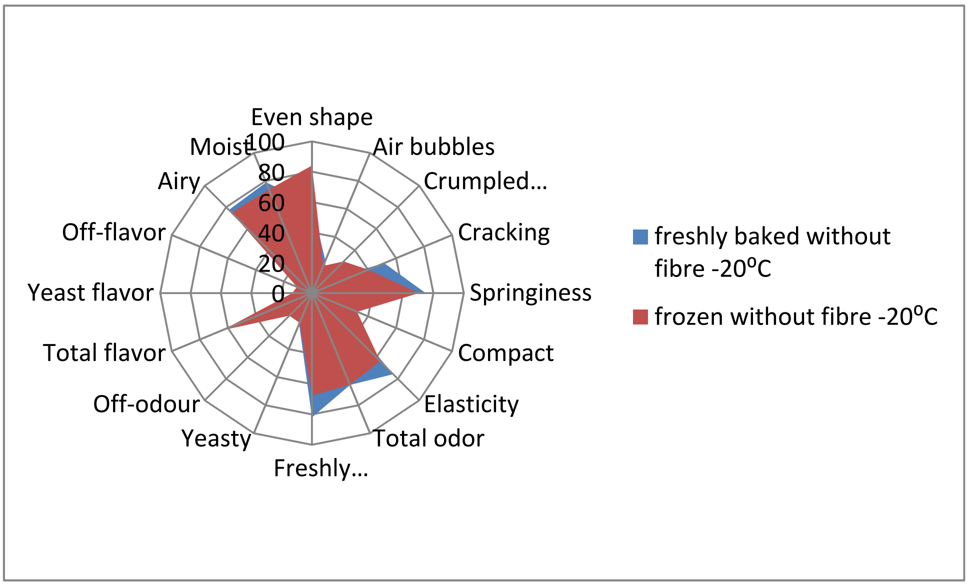

Figure 11 Spider plot of freshly baked and frozen without fiber -20°C after 2 months storage

Consequently, according to the sensory evaluation results of breads after 2 months frozen storage, it can be stated that with a decrease in frozen storage temperature from -10°C to -20°C, significant differences could be minimized between freshly baked and frozen bread samples. Furthermore, only crumpled crust and moist attributes of the bread samples showed significant differences at a frozen storage temperature of -15°C ($P<0.05$).

4.2.2 Influence of the frozen storage temperature on sensory quality of bread

It can be stated that frozen storage temperature has a considerable influence on sensory quality of bread since a change in frozen storage temperature caused significant differences in sensory attribute scores of bread samples.

Freshly baked bread with fibers showed a significant difference in compactness between the frozen storage temperatures of -10°C and -20°C. Furthermore, bread samples stored at -10°C were significantly different from -15°C and -20°C in air bubbles, freshly baked odor, off-odor, total flavor and off-flavor as seen in Figure 12. However, there were no significant differences between freshly baked bread samples stored at -15°C and -20°C which could be related to the freezer conditions. The temperature of the freezer of -20°C (mean: -19.0°C, max: -15.3°C, min: -20.4°C) was fluctuating more compared to the freezer of -15°C (mean: -16.1°C, max: -12.5°C, min: -17.6°C). The mean temperature of -10°C freezer was -8.2°C.

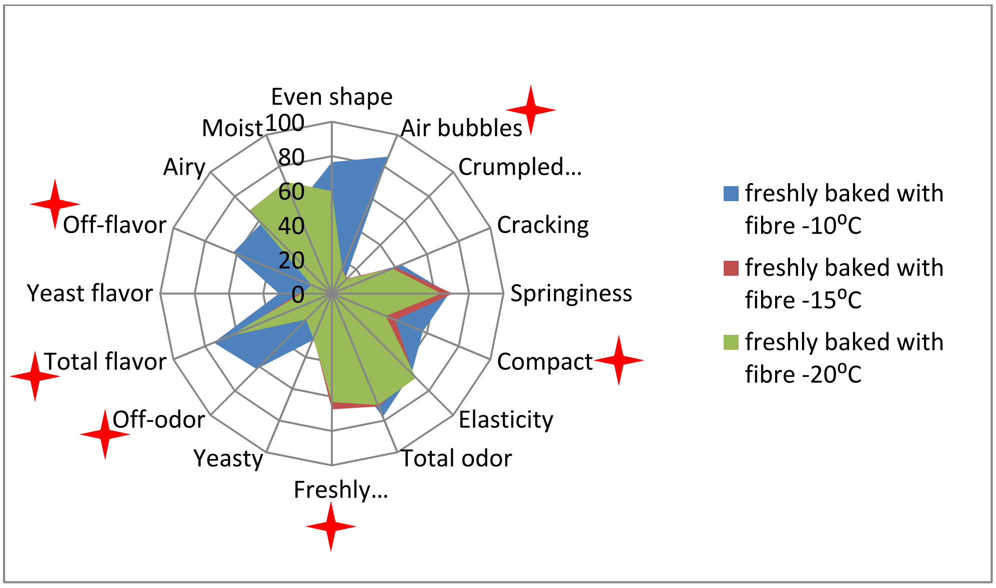

Figure 12 Spider plot of freshly baked bread with fiber at different temperatures after 2 months storage

As shown in Figure 13, frozen bread with fiber stored at -10°C was significantly different from -15°C and -20°C regarding springiness, compactness, elasticity, freshly baked odor, off-odor, and off-flavor. Moreover, there was significant difference between the samples stored at -10°C and -20°C in airy attribute. Moist attribute was significantly different between those three samples at P<0.05 confidence level.

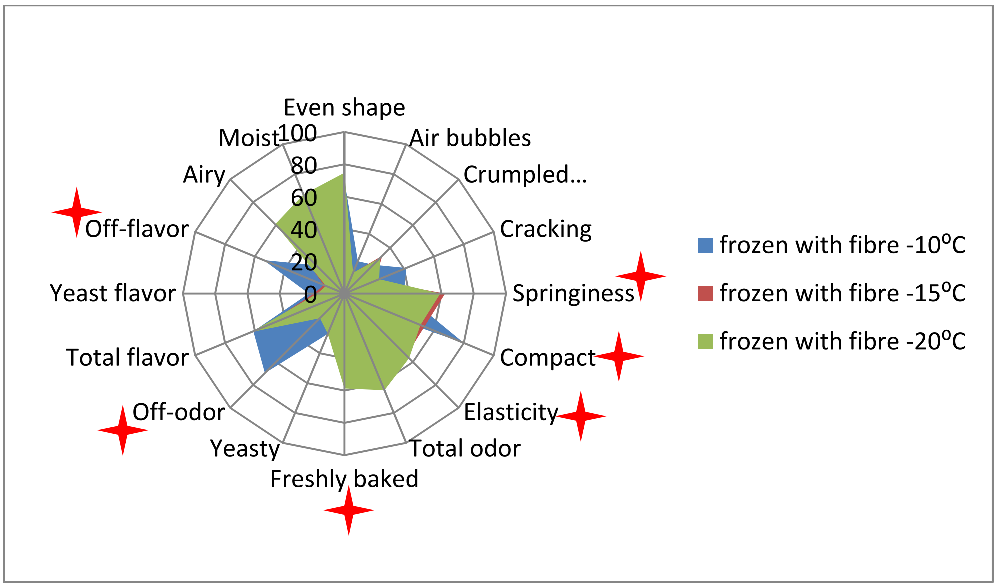

Figure 13 Spider plot of frozen bread with fiber at different temperatures after 2 months storage

The effect of different frozen storage temperatures on the sensory attributes of freshly baked bread without fiber is shown in Figure 14. Samples stored at -10°C were significantly different from the samples stored at -15°C and -20°C in even shape, air bubbles and yeast flavor. Furthermore, samples stored at -10°C were significantly different from the samples stored at -20°C in off-flavor but not significantly different from the samples stored at -15°C. No significant differences could be observed between the bread samples stored at -15°C and -20°C.

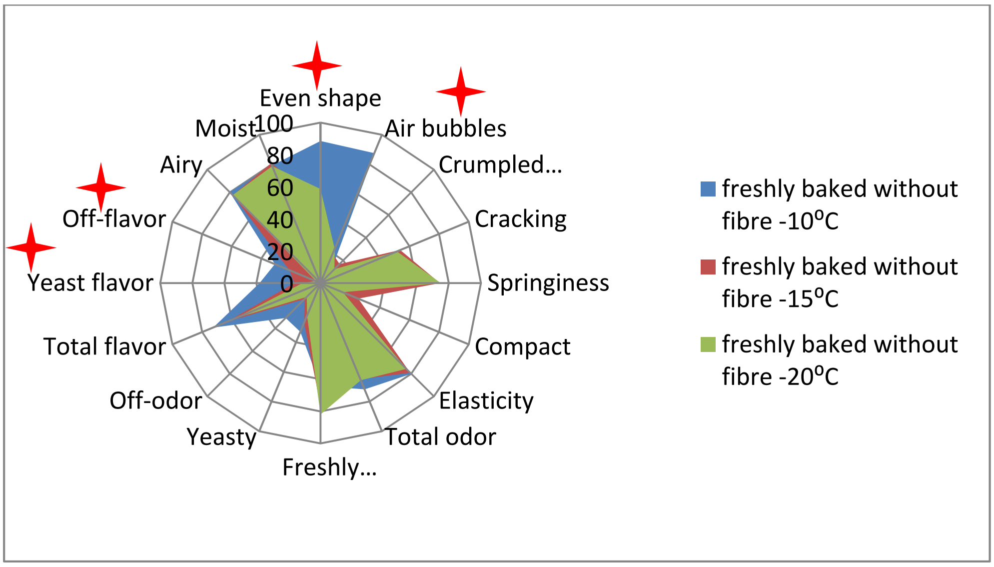

Figure 14 Spider plot of freshly baked bread without fiber at different temperatures after 2 months storage

According to Figure 15 below, frozen bread without fiber stored at -10°C was significantly different from samples stored at -15°C and -20°C with respect to compactness, elasticity, freshly baked odor, off-odor, off-flavor, airy and moist attributes.

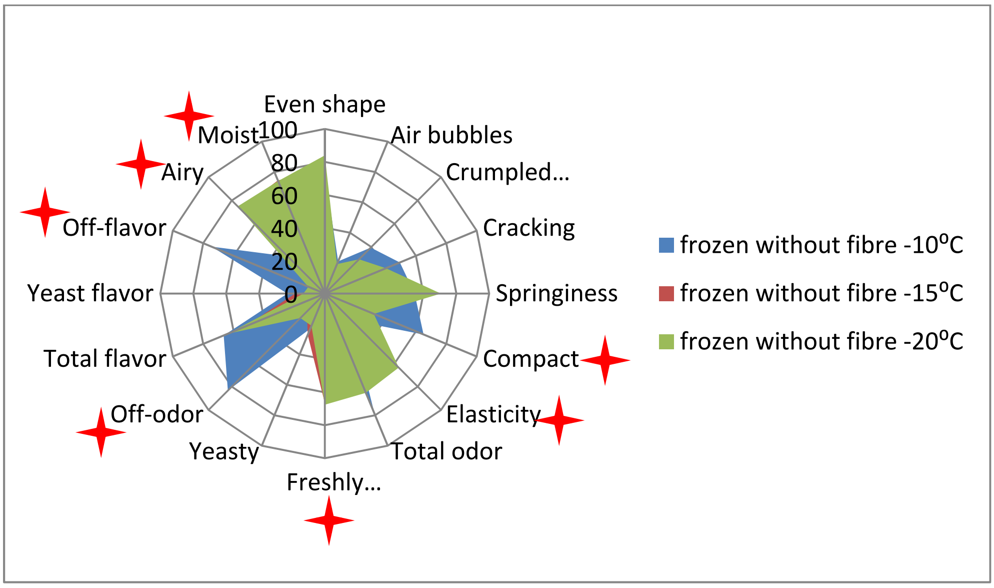

Figure 15 Spider plot of frozen bread without fiber at different temperatures after 2 months storage

4.2.3 Influence of the fiber content on sensory quality of bread

No effect of fibers on the sensory characteristics of the bread samples stored at -20°C could be observed. However, there were significant differences between freshly baked breads stored at -10°C with fiber and without fiber. No differences were detected between frozen breads with and without fiber at -10°C. Nevertheless it is important to keep in mind that fiber was added as whole wheat kernels.

There were no significant differences between freshly baked bread stored at -20°C with and without fiber regarding all sensory attributes (Figure 16). Similarly, for the frozen bread stored at -20°C, no differences were detected in the presence of fibers (Figure 17).

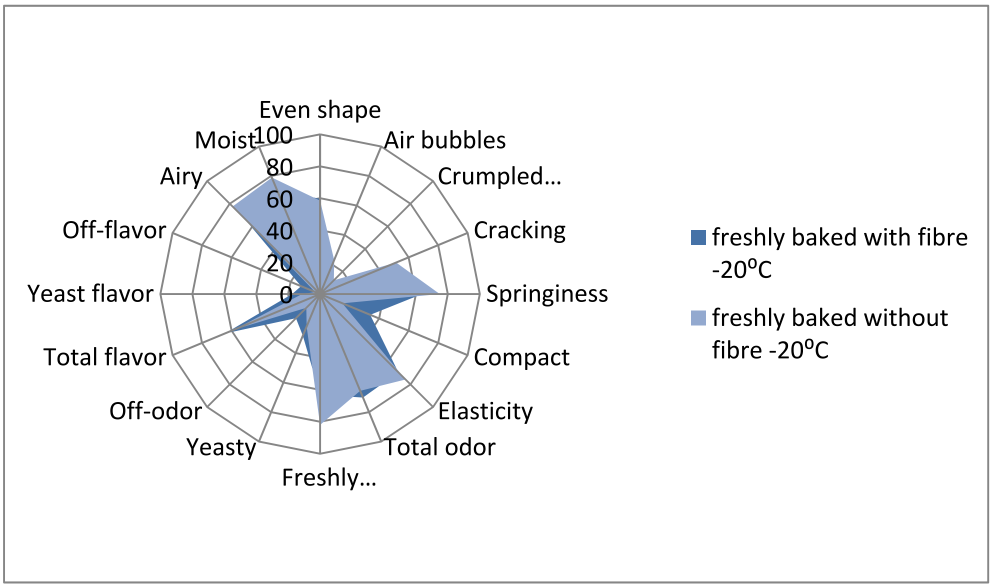

Figure 16 Spider plot of freshly baked bread stored at -20°C with and without fiber after 2 months storage

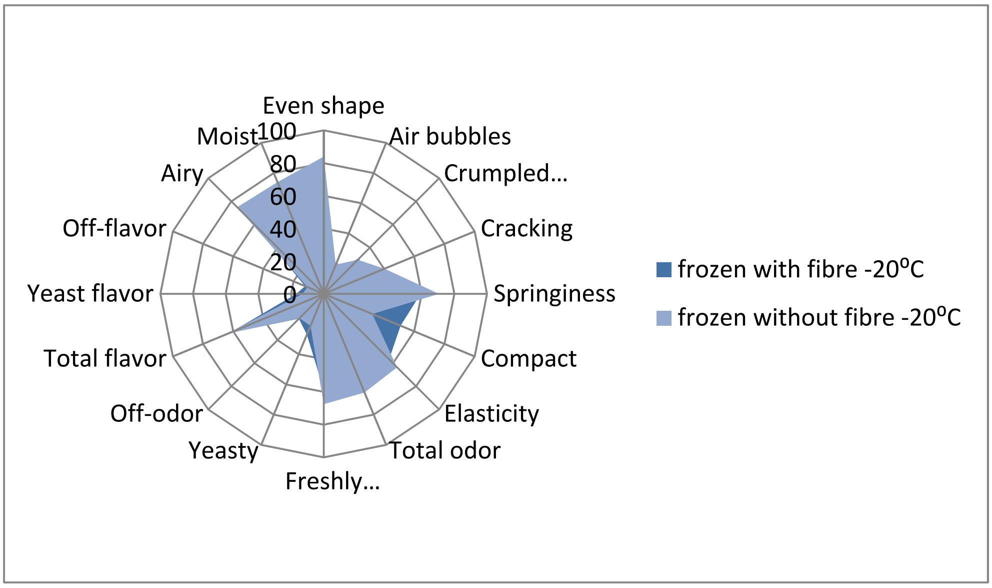

Figure 17 Spider plot of frozen bread stored at -20°C with and without fiber after 2 months storage

Concerning the freshly baked bread with and without fiber stored at -10°C, significant differences were observed in compactness, freshly baked odor, off-odor, off-flavor, airy and moist sensory attributes (Figure 18). On the other hand, there were no significant differences between frozen breads stored at -10°C with respect to their fiber content (Figure 19).

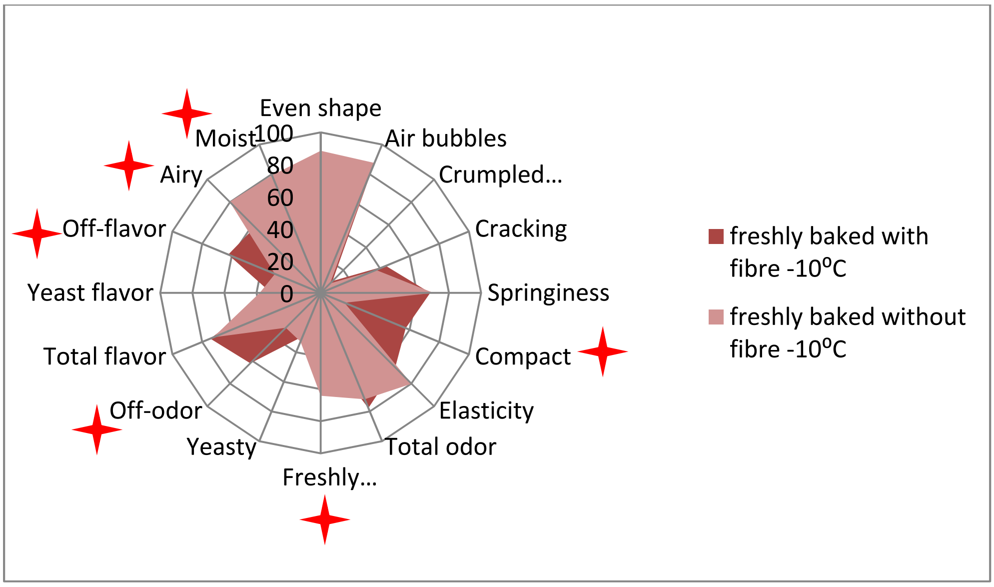

Figure 18 Spider plot of freshly baked bread stored at -10°C with and without fiber after 2 months storage

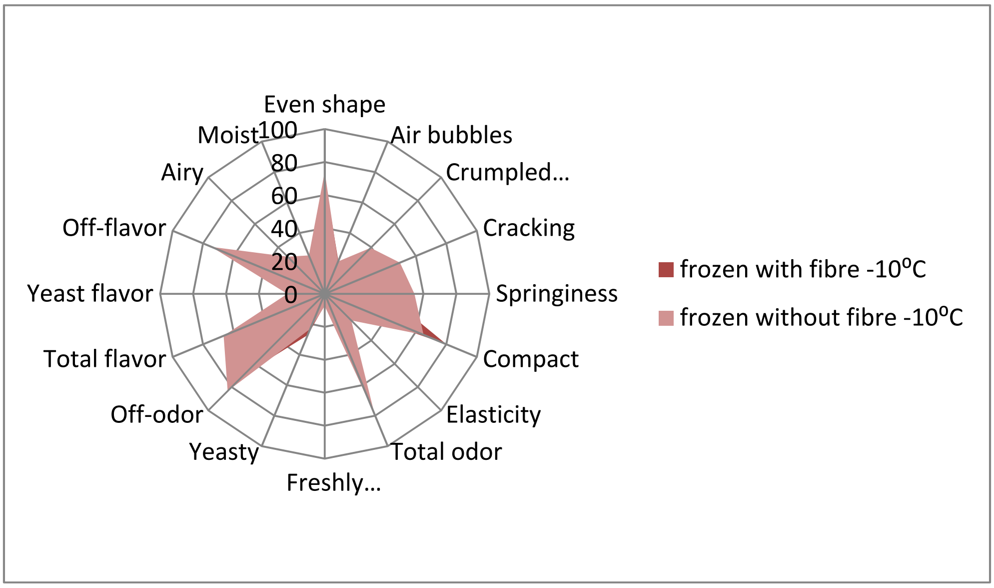

Figure 19 Spider plot of frozen bread stored at -10°C with and without fiber after 2 months storage

4.3 Sensory evaluation after 3.5 months frozen storage

In this section, influence of bread preparation, frozen storage temperature and fiber content on sensory quality of bread samples were evaluated after 3.5 months frozen storage. Sensory scores are given in Appendix C.

It should be noted that when the frozen dough was stored for 3.5 months, the color of the resultant bread was dark and not uniform in color, as shown in Figure 20. This may be due to the increased leaching of amylose and degraded dextrins, which in turn contribute to Maillard reactions. Water redistribution and availability may also play a role in color darkening and non-uniformity. A faster freezing rate and low dough storage temperature has resulted in dark, not uniform bread color in previous studies (Yi & Kerr, 2009b).

Figure 20 Darker and less uniform color of freshly baked bread with fiber stored at -10°C freezer for 3.5 months

4.3.1 Influence of the bread preparation on sensory quality of bread

Sensory evaluation revealed the differences between frozen stored breads and freshly baked breads. Mostly texture attributes for instance cracking, springiness, compactness, elasticity and moist characteristics showed significant differences between frozen stored breads and freshly baked breads.

After 3.5 months frozen storage at -20°C, there were no significant differences between freshly baked bread and frozen bread without fiber at a confidence level 95% (Figure 21) similarly to previous results after 2 months frozen storage. On the other hand, when the same bread samples were stored at -10°C, there were significant differences regarding air bubbles, crumpled crust, springiness, compactness, elasticity, freshly baked odor, yeast flavor and moist (Figure 22).

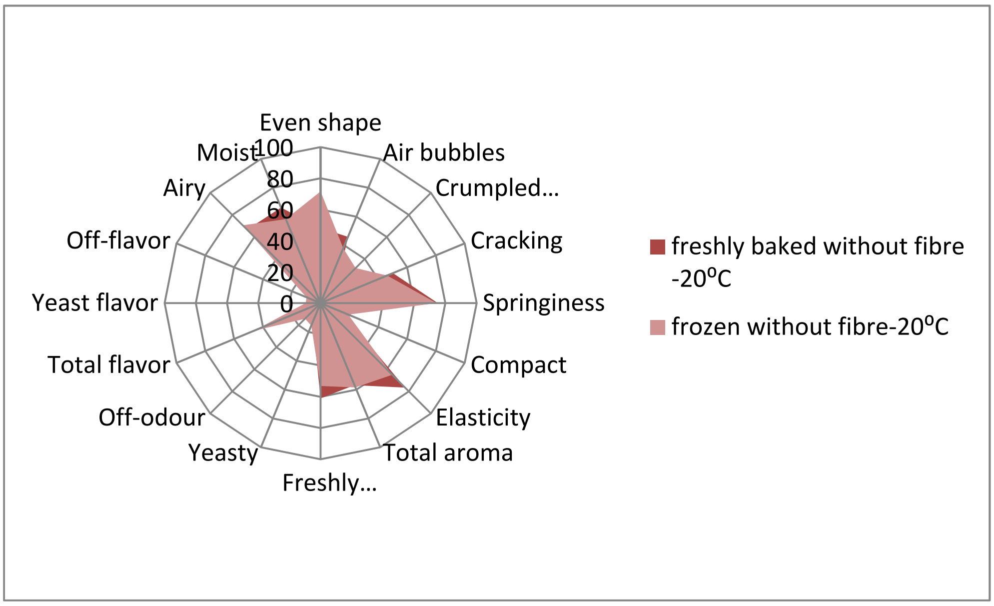

Figure 21 Spider plot of freshly baked and frozen without fiber -20°C after 3.5 months storage

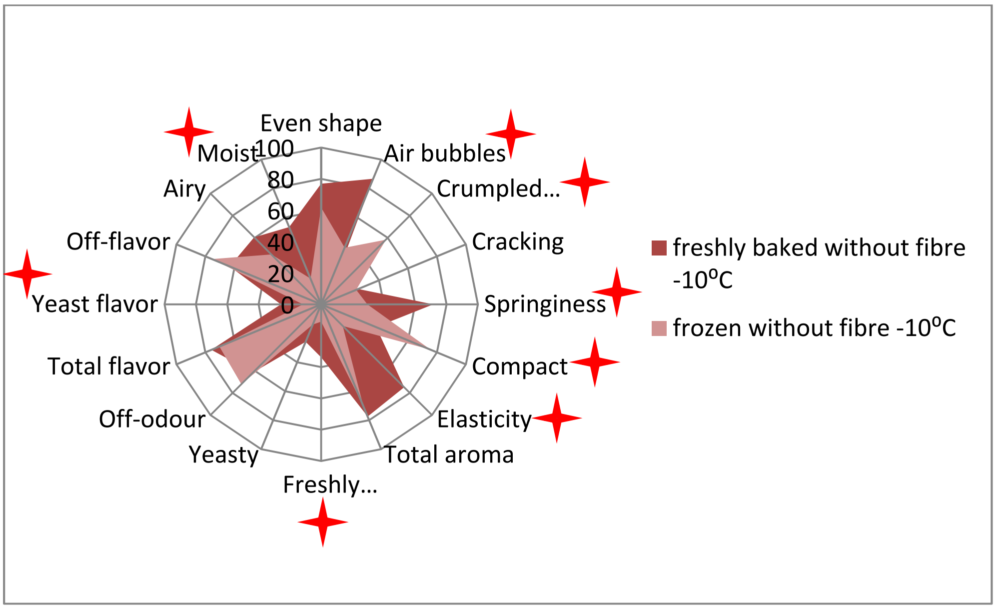

Figure 22 Spider plot of freshly baked and frozen without fiber -10°C after 3.5 months storage

Significant differences (P<0.05) were detected after 3.5 months storage at -10°C between freshly baked bread and frozen bread with fiber concerning air bubbles, crumpled crust, springiness, elasticity, total flavor, airy and moist (Figure 23). Furthermore, there were significant differences at -20°C between freshly baked bread and frozen bread with fiber in even shape, crumpled crust, cracking and elasticity (Figure 24).

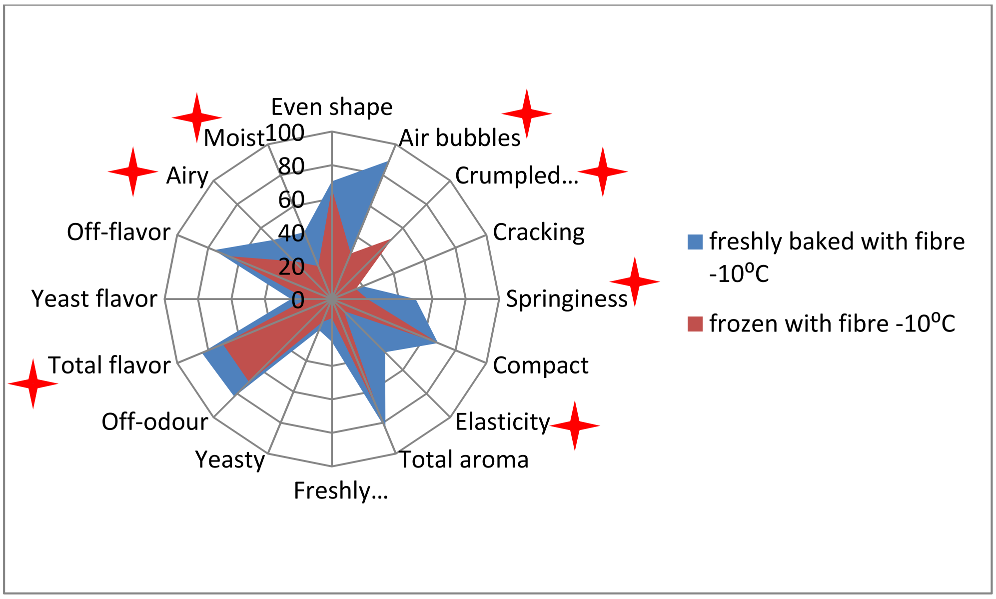

Figure 23 Spider plot of freshly baked and frozen with fiber -10°C after 3.5 months storage

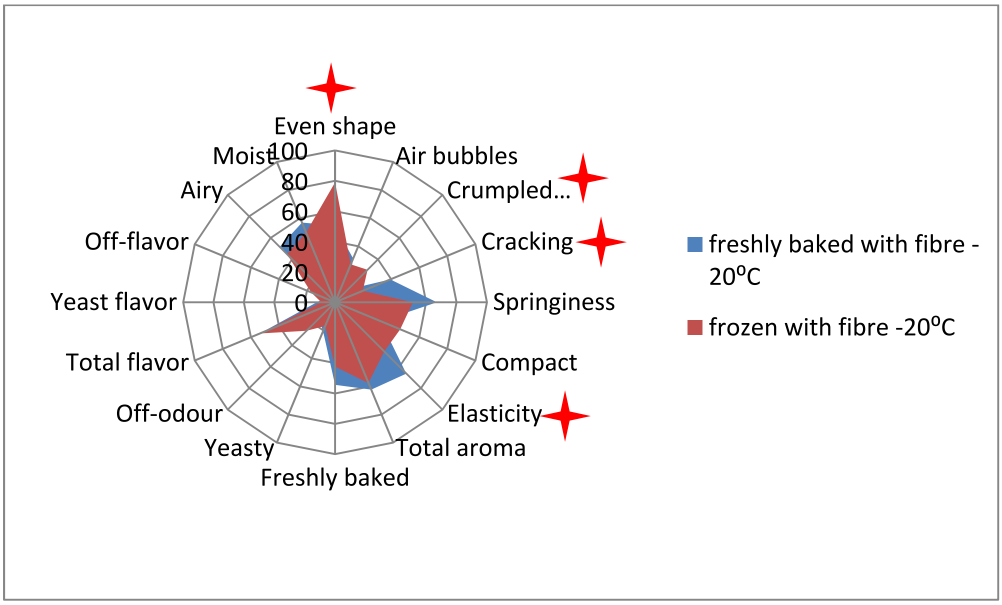

Figure 24 Spider plot of freshly baked and frozen with fiber -20°C after 3.5 months storage

4.3.2 Influence of the frozen storage temperature on sensory quality of bread

In general, the sensory characteristics related to odor, flavor and texture were significantly influenced by different frozen storage temperatures.

The results of three different frozen storage temperature on freshly baked bread with fiber was evaluated in the spider plot below (Figure 25). Significant differences were observed between samples stored at -10°C and -15°C regarding air bubbles, compactness, elasticity, total odor, freshly baked odor, off-odor, total flavor and off-flavor. Likewise, there were significant differences between bread samples stored at -10°C and -20°C in air bubbles, cracking, compactness, elasticity, total odor, freshly baked odor, off- odor, total flavor and off- flavor. On the other hand, comparing the samples stored at-15°C and -20°C, no significant differences were detected probably due to the described temperature fluctuations in freezers.

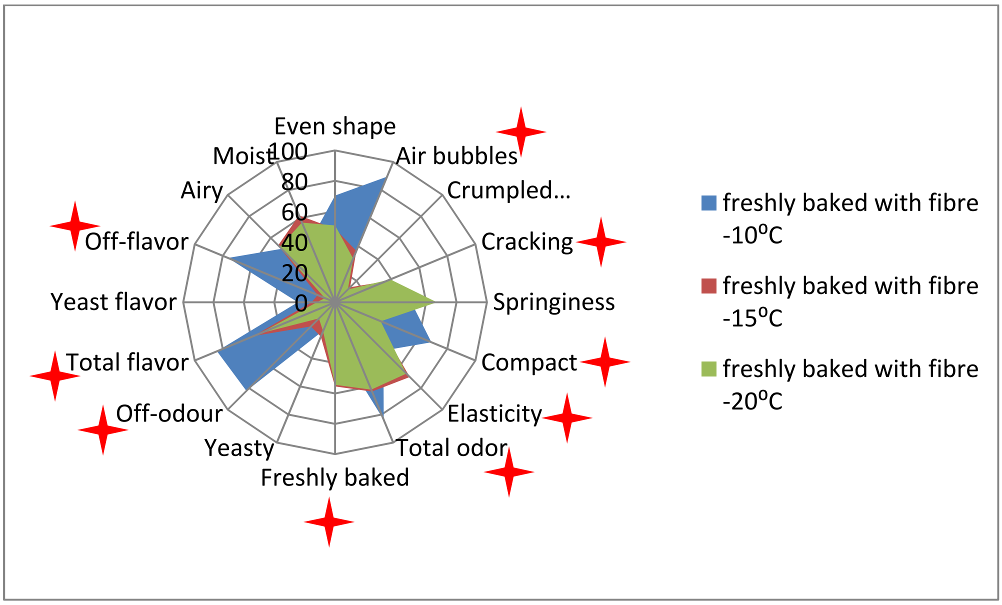

Figure 25 Spider plot of freshly baked with fiber at different temperatures after 3.5 months storage

There were significant differences at a confidence level of 95% between freshly baked bread without fiber stored at -10°C and -15°C regarding even shape, air bubbles, cracking, total odor, freshly baked odor, off-odor, total flavor, off-flavor. However, the only significant difference was observed in total flavor between the same bread samples stored at -15°C and -20°C. Moreover, even shape, air bubbles, cracking, compactness, total odor, freshly baked odor, off-odor, total flavor, yeast flavor and off-flavor attributes showed significant differences between frozen storage at -10°C and -20°C (Figure 26).

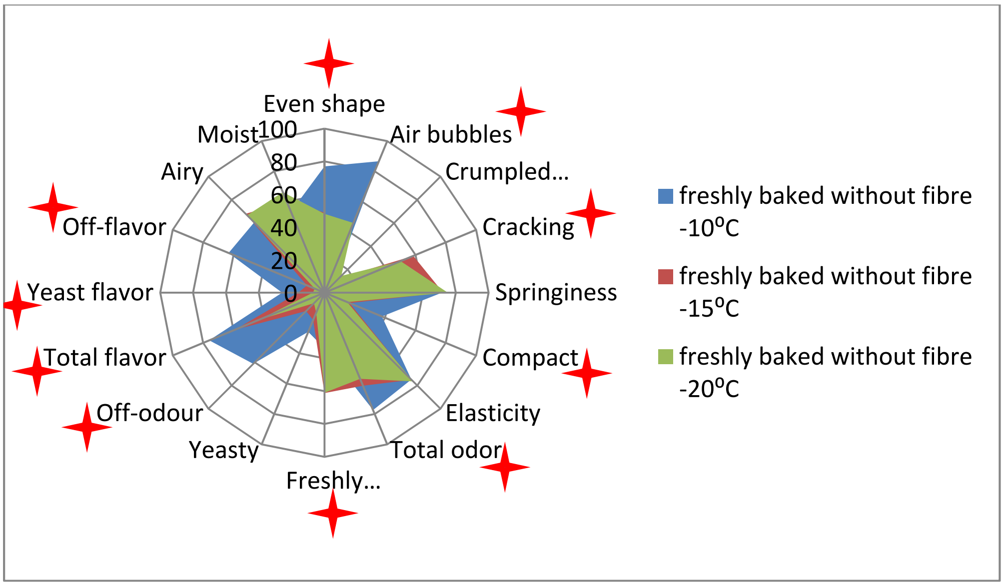

Figure 26 Spider plot of freshly baked without fiber at different temperatures after 3.5 months storage

According to the spider plot below (Figure 27), significant differences were observed between the storage of frozen bread with fiber at -10°C and -15°C in crumpled crust, springiness, elasticity, total odor, freshly baked odor, off-odor, total flavor, off-flavor and moist. Similarly, same bread samples stored at -10°C and -20°C differed in crumpled crust, springiness, compactness, elasticity, total odor, freshly baked odor, off-odor, total flavor, off-flavor and moist. However, no significant differences were detected between the storage temperatures of -15°C and -20°C.

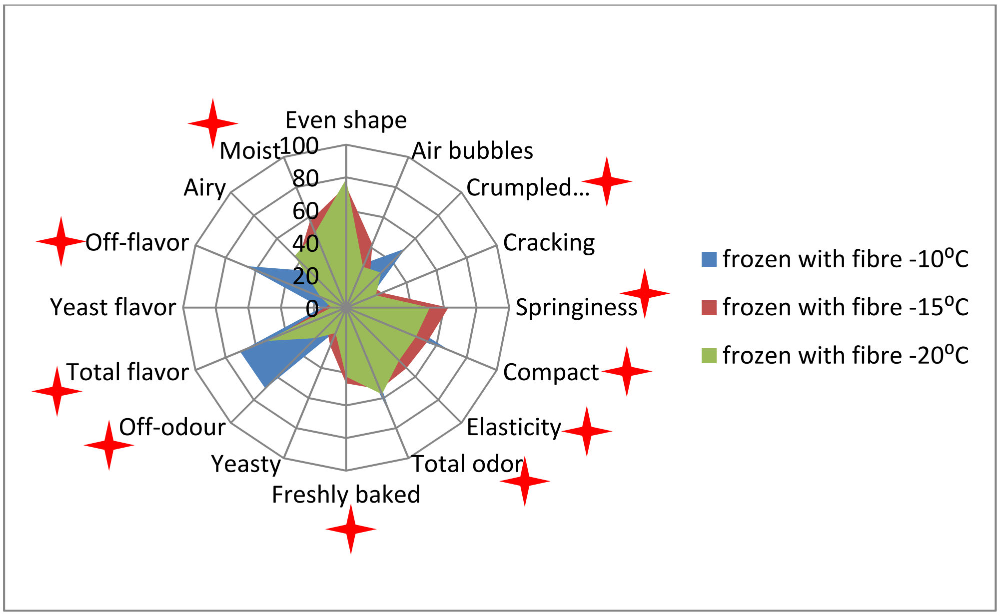

Figure 27 Spider plot of frozen bread with fiber at different temperatures after 3.5 months storage

As demonstrated in the spider plot below (Figure 28), crumpled crust, springiness, compactness, elasticity, total odor, freshly baked odor, off-odor, total flavor, off-flavor, airy and moist attributes of frozen bread without fiber significantly differed between frozen storage at -10°C and -15°C. Besides there were no significant differences between the bread samples stored at -15°C and -20°C. However, significant differences were detected between frozen storage at -10°C and -20°C regarding crumpled crust, cracking, springiness, compactness, freshly baked odor, off-odor, total flavor, off-flavor, airy and moist.

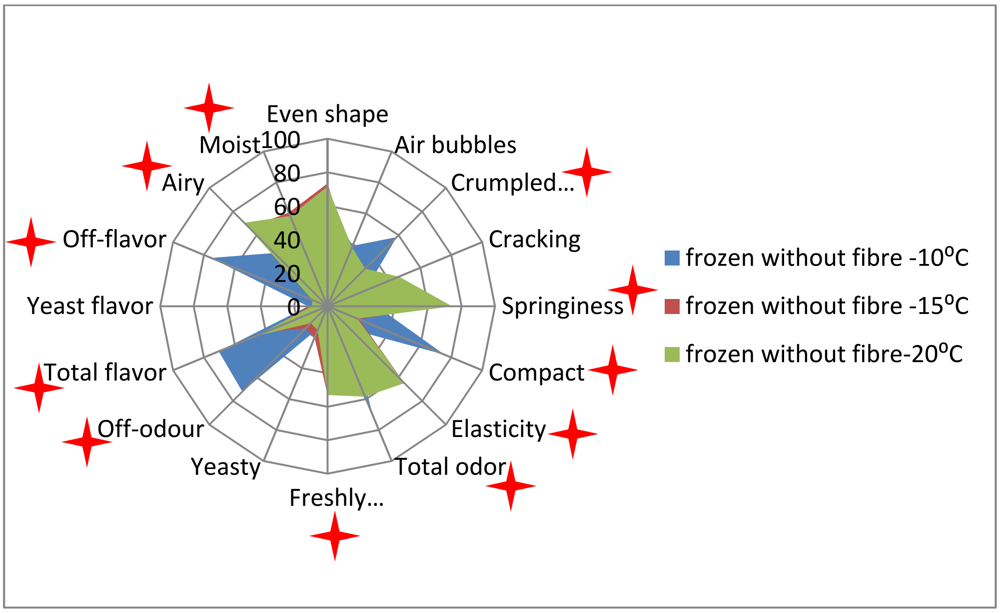

Figure 28 Spider plot of frozen bread without fiber at different temperatures after 3.5 months storage

4.3.3 Influence of the fiber content on sensory quality of bread

According to the sensory results after 3.5 months frozen storage, fibers had negative effects mainly on texture by finger attributes of bread samples.

No significant differences were observed between freshly baked breads with and without fiber stored at -20°C for 3.5 months as shown in Figure 29. However, for the frozen breads with and without fiber, there were significant differences in cracking, springiness, compactness, elasticity and airy characteristics (Figure 30).

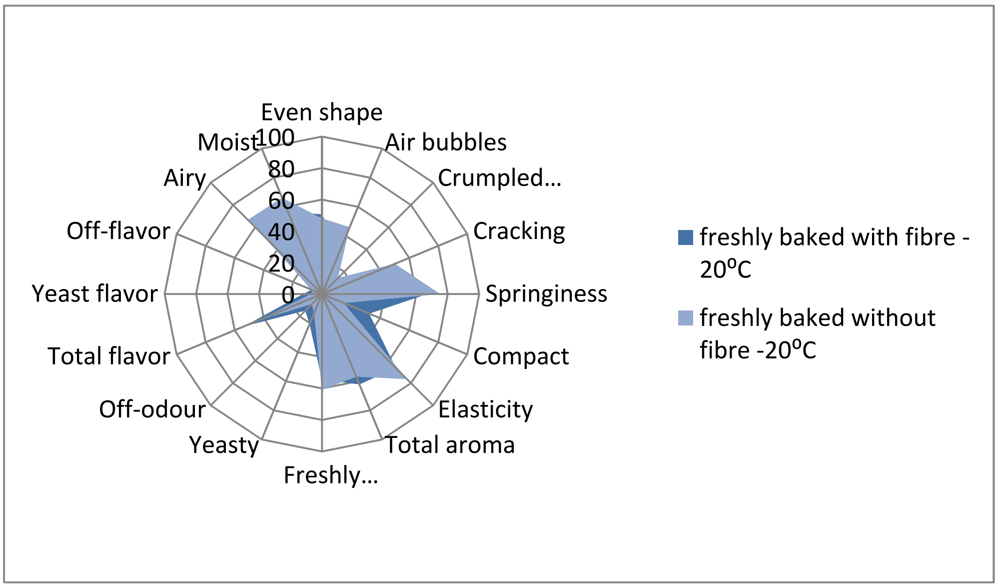

Figure 29 Spider plot of freshly baked with and without fiber -20°C after 3.5 months storage

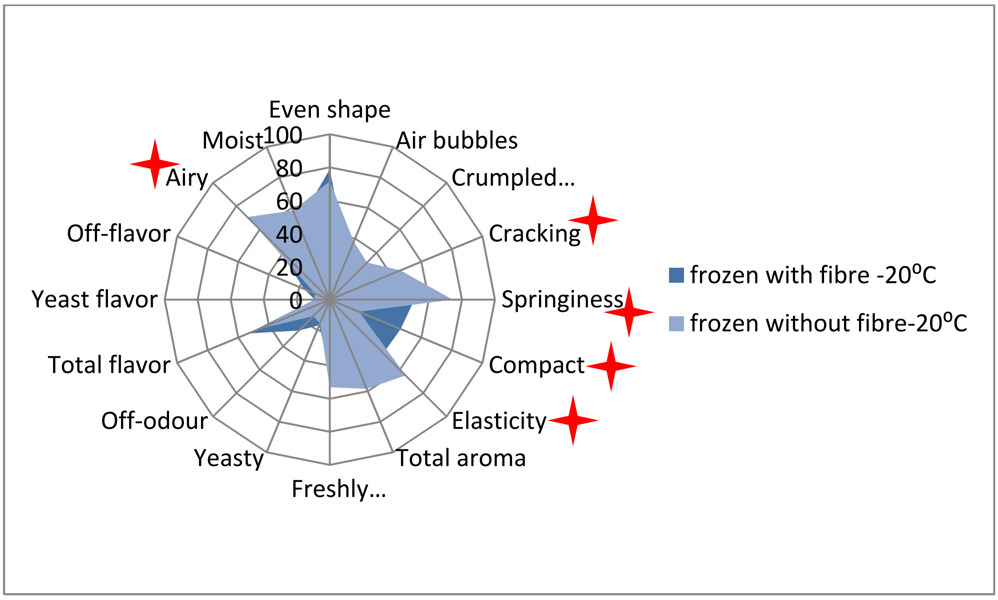

Figure 30 Spider plot of frozen with and without fiber -20°C after 3.5 months storage

In the spider plot below (Figure 31), freshly baked bread with and without fiber stored at -10°C were compared and significant differences were detected in compactness and elasticity characteristics at a confidence level of 95%. On the other hand, there were no significant differences between frozen bread with fiber and without fiber stored at -10°C as shown in Figure 32.

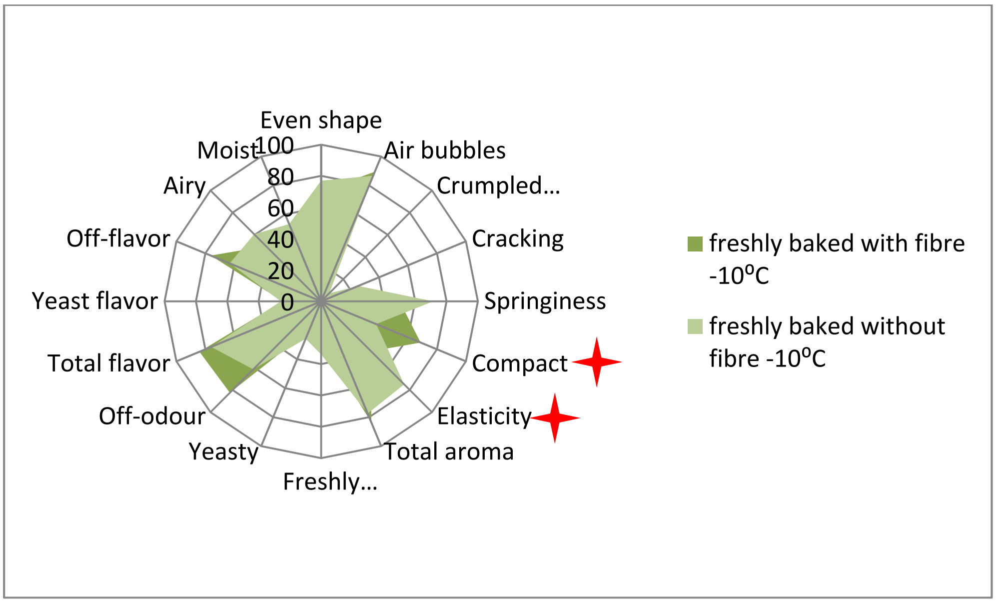

Figure 31 Spider plot of freshly baked with and without fiber -10°C after 3.5 months storage

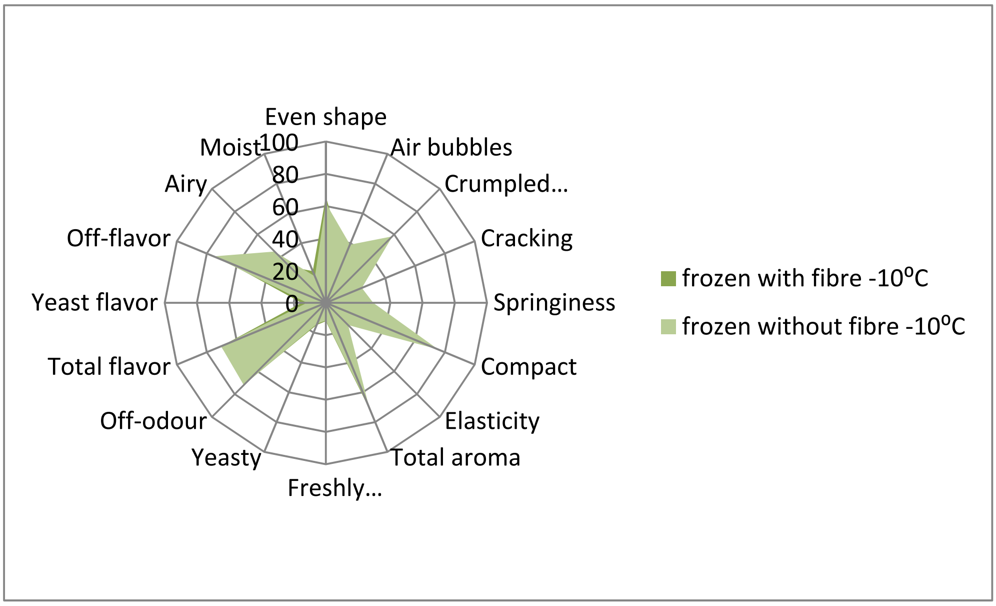

Figure 32 Spider plot of frozen bread with and without fiber -10°C after 3.5 months storage

4.4 Comparison of sensory results of bread after 2 months and 3.5 months frozen storage

In this section, the sensory characteristics after 2 months and 3.5 months frozen storage of the bread samples are compared. All sensory attributes are examined one by one. Sensory evaluation scores and Tukey pairwise comparison test P-values are given in Appendix B, C and D respectively.

As expected, with prolonged frozen storage, bread samples significantly lost their quality; scores for freshly baked odor, elasticity and moist decreased but scores for compactness, off-odor and off-flavor increased in general.

Sensory results for even shape characteristic are shown in Appendix E, Figure E1. Freshly baked bread without fiber stored at -10°C had the highest score in even shape at the end of 2 months storage. However, frozen bread with fiber stored at -20°C had the highest score after 3.5 months storage. Freshly baked bread with fiber at -15°C and without fiber at -15°C and -20°C showed lower scores which were not significantly different at a confidence level 95% in even shape both after 2 months and 3.5 months storage. Furthermore, there were no significant differences between 2 months and 3.5 months frozen stored bread samples according to the Tukey pairwise comparison test at a confidence level of 95% ($P<0.05$).

Sensory results concerning air bubbles are shown in Appendix E, Figure E2. Freshly baked samples with and without fiber stored at -10°C showed air bubbles on the surface of the bun; on the other hand, other samples did not have significant amount of air bubbles on the surface. With prolonged frozen storage from 2 months to 3.5 months, the number of air bubbles on the surface of bread samples increased. Freshly baked bread with fiber -15°C and -20°C, freshly baked without fiber -20°C, frozen with fiber -15°C and -20°C, frozen without fiber -10°C, -15°C and -20°C showed significant differences in air bubbles between 2 months and 3.5 months frozen storage. It is obvious that this sensory attribute was influenced by prolonged storage considerably.

Frozen bread without fiber stored at -10°C had the highest score in crumpled crust which was not significantly different from the other frozen stored bread samples both after 2 months and 3.5 months frozen storage. Freshly baked bread with fiber stored at -10°C showed a significant decrease in crumpled crust with prolonged frozen storage. However, sensory scores of frozen bread with and without fiber stored at -10°C increased significantly. Thus, there was not a general trend between 2 months and 3.5 months frozen stored breads owing to the fact that some bread samples showed a decrease but some of them showed an increase concerning the crumpled crust scores (Figure 33). It can also be stated that frozen storage temperature -10°C led to the development of crumpled crust in breads especially in frozen breads with respect to frozen storage temperature of -15°C and -20°C. The wrinkles on the crust might be due to the dehydration during freezing and also storage in the frozen state, causing shriveling of the samples. This can be explained by the diffusion of water from the crumb to the crust and also sublimation from the crust to the surrounding environment. Thus, the different kinetics of those processes, a lower rate of diffusion and a greater loss of water by sublimation, could explain the formation of crumpled crust structure (Ronda et al., 2011).

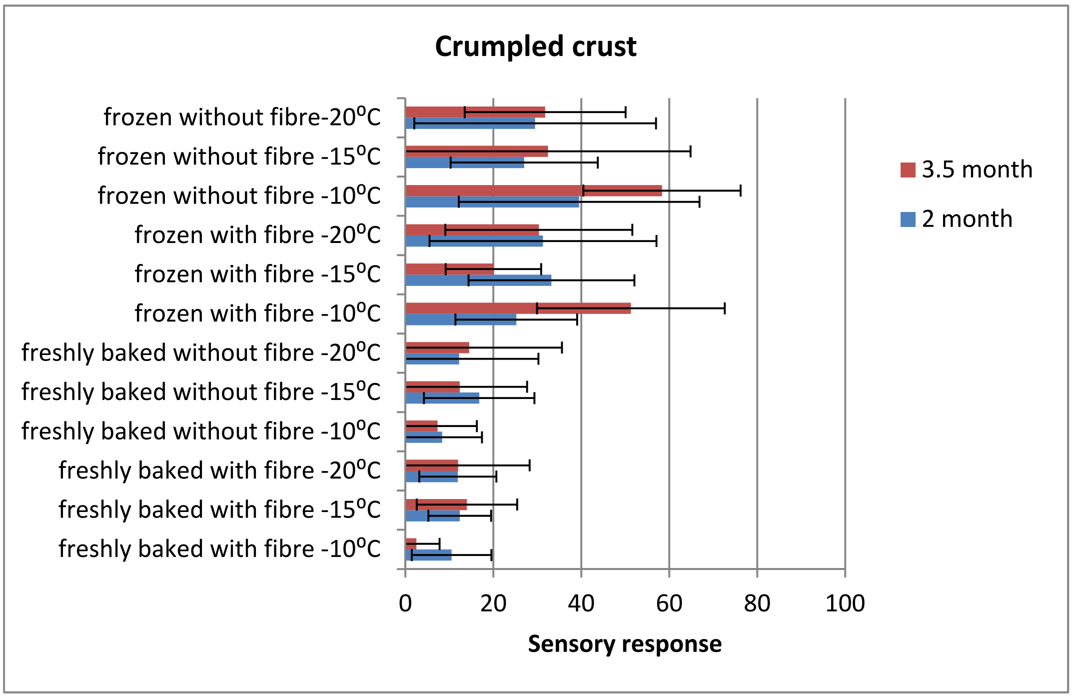

Figure 33 Sensory responses to crumpled crust after 2 months and 3.5 months storage

Freshly baked bread without fiber at -15°C had the highest score in crust cracking after 2 months and 3.5 months storage as shown in Appendix E, Figure E3. However, frozen stored breads with fiber generally showed low cracking scores. Freshly baked bread with fiber -10°C and frozen bread without fiber -10°C showed significant differences in cracking with prolonged frozen storage from 2 months and 3.5 months.

The nutritional value, the quality and the taste of bread are mostly related to concerns with crust. From this point of view, several process parameters such as air humidity during proofing and chilling, temperature of the sample when it enters the freezer, baking and storage conditions such as temperature fluctuations influence the issues with crust. The differences in water content between the crust and the crumb also a large concentration of water—ice under the crust might be the prospective causes of crust cracking (Le Bail et al., 2005).

The sensory scores for springiness as shown in Appendix E, Figure E4 were rather similar among the samples after 2 months storage; however, frozen bread with fiber stored at -10°C showed a significant difference in springiness with respect to other bread samples, except frozen bread without fiber stored at -10°C. Furthermore, the differences regarding sensory scores between the samples increased with prolonged frozen storage. Significant differences were detected at a confidence level of 95% between 2 months and 3.5 months frozen storage concerning springiness in freshly baked bread with fiber at -10°C and frozen bread without fiber at -10°C. It can also be noted that frozen stored bread samples were less springy than freshly baked samples and bread samples without fiber were springy than those with fiber.

The most compact bread sample was frozen bread with fiber stored at -10°C. This bread sample was not significantly different from freshly baked bread with fiber at -10°C and frozen bread without fiber at -10°C after 2 months storage. Generally compactness of the bread samples decreased with a lower frozen storage temperature and frozen stored breads were more compact than freshly baked breads as seen in Figure 34 due to the fact that a freezing-thawing cycle after final baking provokes relatively harder and more compact crumb (Poinot et al., 2008). Also fibers as wheat kernel caused a more compact bread structure. According to those generalized conclusions, freshly baked bread without fiber at -20°C is the least compact bread sample both after 2 months and 3.5 months frozen storage. Only one significant difference was detected at a confidence level of 95% in freshly baked bread without fiber stored at -10°C between 2 months and 3.5 months storage. Besides, compactness of the bread samples usually increased with prolonged frozen storage at -10°C; on the other hand, decreased at -15°C and -20°C. It was also reported by Chen and Swensson (2011) that the amount of freezable water was higher in samples stored at high temperatures like -10°C. This fact together with the contribution of higher storage temperatures to the mobility of water results in aging of samples in other words firming.

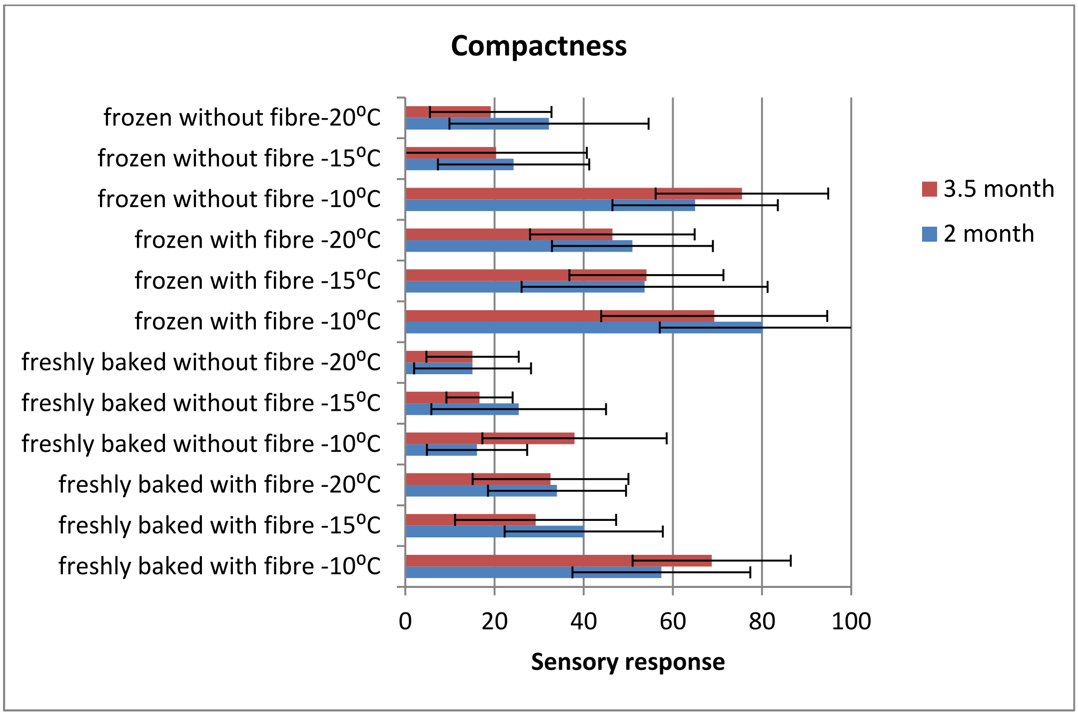

Figure 34 Sensory responses to compactness after 2 months and 3.5 months storage

Elasticity and compactness of bread samples can be related to each other as the more compact means the least elastic bread sample. Frozen bread with fiber -10°C which was indicated as the most compact bread sample was also the least elastic sample after 2 months storage. In addition, there was a wide difference in elasticity scores of frozen samples between -10°C and -15°C, both with and without fiber; however, the difference between -15°C and -20°C was negligible. Furthermore, fresh samples did not show significant differences in elasticity with changing freezing temperatures and fiber content.

Freshly baked bread with fiber stored at -10°C showed significant difference between 2 months and 3.5 months frozen storage concerning elasticity (Figure 35). However, there were no significant differences in other bread samples. Elasticity of samples decreased with prolonged frozen storage as a general rule but an increase in elasticity was determined in some samples stored at -15°C and -20°C, which is not significant. Moreover, freshly baked bread without fiber was more elastic than other breads and it seems fibers reduced the elasticity of bread samples. It was reported by Kenny et al. (1999) and Ribotta et al. (2007) that freezing process reduces the elasticity of wheat dough. This could be explained by ice crystal formation which further causes damage on the gluten network, leading to a reduction in gluten cross-linking, decreasing elasticity.

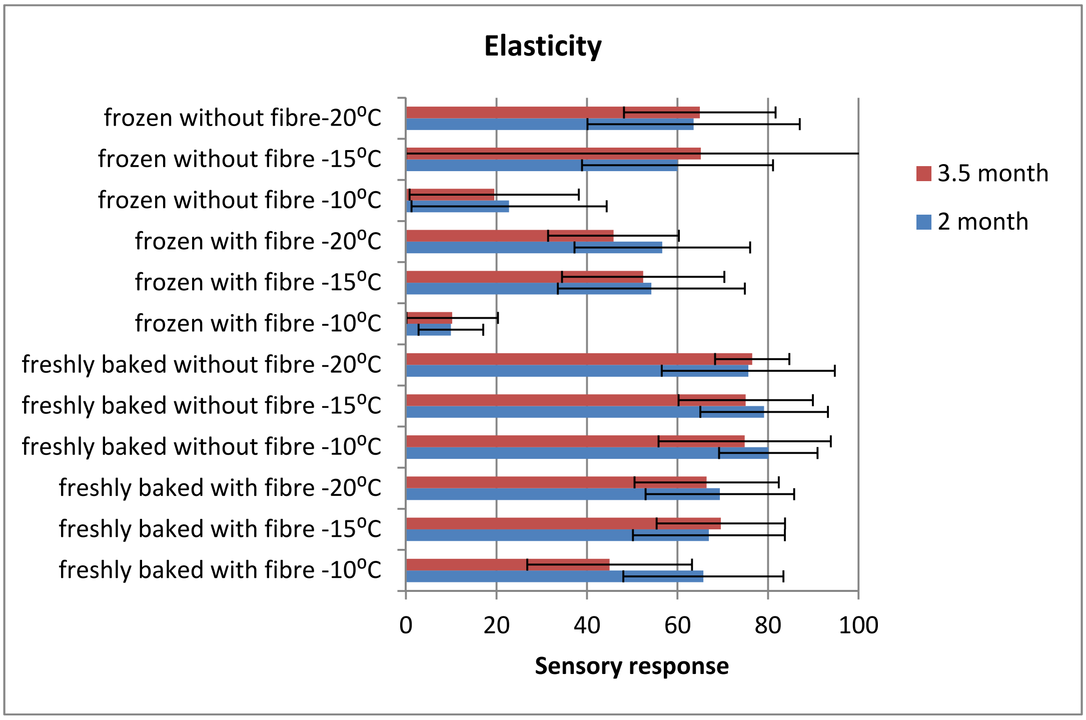

Figure 35 Sensory responses to elasticity after 2 months and 3.5 months storage

According to the sensory scores of total odor as seen in Appendix E, Figure E5, there were no such wide differences in total odor between the all bread samples stored for 2 months. However, frozen bread with fiber stored at -15°C was significantly different from frozen bread without fiber stored at -10°C. Sensory scores of total odor reduced with prolonged frozen storage but there were relatively small differences. However, frozen bread without fiber stored at -10°C and -15°C showed significant differences between 2 months and 3.5 months frozen storage in total odor. Conversely, there were no significant differences at 95% confidence interval between other bread samples regarding total odor. Moreover, freshly baked breads usually had higher scores in total odor than frozen breads.

Freshly baked odor as an important indicator of the most fresh-like bread sample increased with decreased frozen storage temperature as shown in Figure 36. Frozen bread samples both with fiber and without fiber showed a wide difference in freshly baked odor between the storage temperatures of -10°C and -15°C. However, there were not significant differences between -15°C and -20°C. Both frozen bread with fiber and without fiber stored at -10°C had the lowest scores and were not significantly different from each other. On the other hand, freshly baked without fiber -20°C had the highest score which was not significantly different from frozen bread without fiber -15°C and -20°C. Freshly baked odor of samples decreased after 3.5 months frozen storage when compared with 2 months storage. However, there were significant differences regarding freshly baked without fiber -10°C, -15°C and -20°C, frozen with fiber -20°C, frozen without fiber -15°C and -20°C between 2 months and 3.5 months frozen storage. No significant differences were detected in other bread samples. Therefore, it can be pointed out that freshly baked bread without fiber was significantly influenced by prolonged storage from 2 months to 3.5 months; although, it had a better sensory quality than other bread samples after 2 months storage.

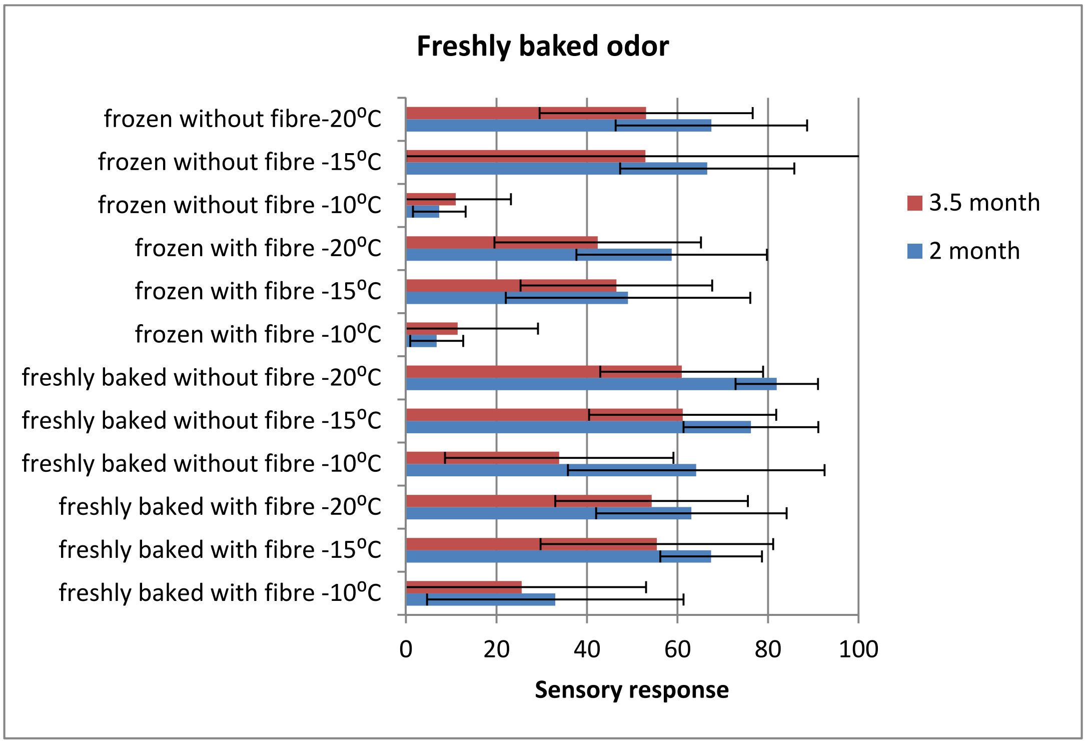

Figure 36 Sensory responses to freshly baked odor after 2 months and 3.5 months storage

No significant differences were detected at a confidence level of 95% between all bread samples in yeasty odor both after 2 months and 3.5 months frozen storage as shown in Appendix E, Figure E6. However, yeasty odor showed a decrease with prolonged frozen storage. This can be explained by the fact that yeast activity is reduced by freezing and frozen storage.

Off-odor increased with an increase in frozen storage temperature, especially at temperature of -10°C as seen in Figure 37. There were no significant differences between the bread samples stored at -10°C, except freshly baked without fiber -10°C which generated less off-odor compared to other bread samples stored at -10°C. Furthermore, there were no significant differences between -15°C and -20°C for all bread samples.

Off-odor of freshly baked bread with fiber and without fiber stored at -10°C significantly increased with prolonged storage. Conversely, some bread samples showed a decrease in off-odor that was not significantly different between 2 months and 3.5 months frozen storage. Generally, frozen storage of bread samples at high temperatures such as -10°C stimulated the development of off-odor with extended frozen storage time.

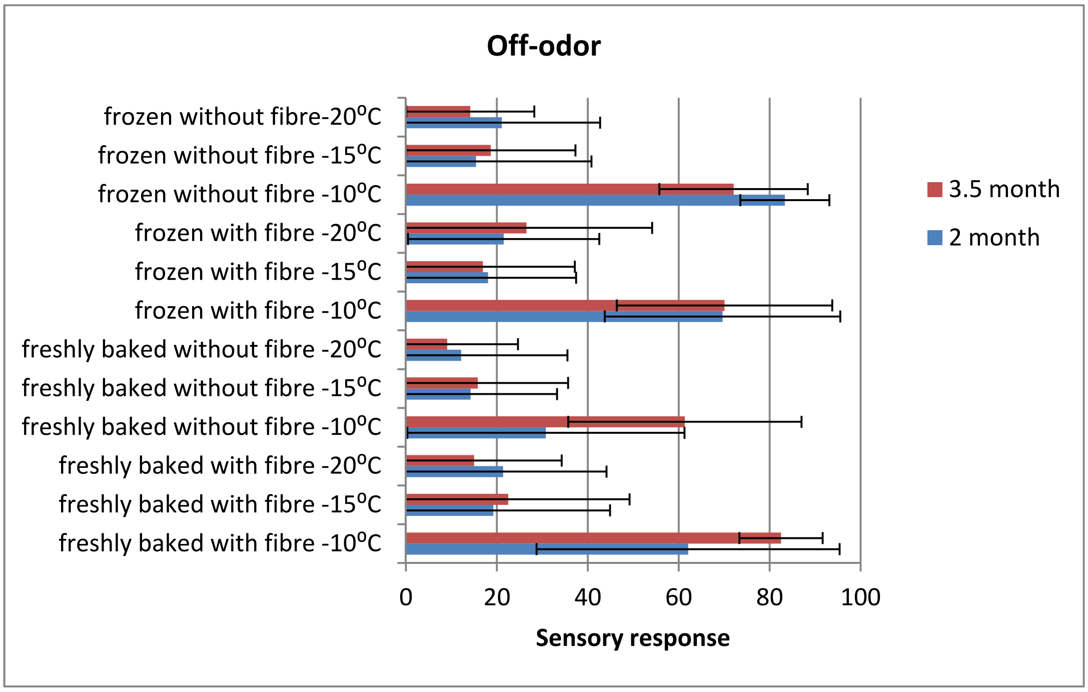

Figure 37 Sensory responses to off-odor after 2 months and 3.5 months storage

According to the sensory results related to total flavor as seen in Appendix E, Figure E7, both after 2 months and 3.5 months storage, freshly baked with fiber at -10°C were indicated by the panel members as the highest in total flavor. Otherwise, it seems that there was not a general trend in total flavor between the bread samples depending to the temperature, fiber content and freshly baked or frozen bread.

Total flavor of bread samples decreased in most cases but some samples showed an increase in total flavor with extended frozen storage period and there was a significant increase in freshly baked bread with fiber stored at -10°C. It might be due to the yeast which can influence the amount of Maillard volatile compounds and compounds resulting from lipid oxidation, affecting the sensory properties of bread. Yeast activity is supposed to be higher at -10°C, contributing to the formation of Maillard precursors that lead to the release of Maillard volatile compounds. Furthermore, Frasse et al. (1992) indicated that higher yeast activity stimulates a reduction in lipoxygenase activity, leading to a decrease in the formation of volatile compounds from lipid oxidation which causes unpleasant flavor in breads. Thus, increased quantity of Maillard volatile compounds and decreased amount of volatile compounds from lipid oxidation could be a reason of significant increase in total flavor of freshly baked bread with fiber -10°C. On the other hand, freshly baked bread without fiber -20°C, frozen bread without fiber -15°C and -20°C showed a significant decrease in total flavor between 2 months and 3.5 months frozen storage.

Freshly baked bread without fiber stored at -10°C had the most yeasty flavor but it was not significantly different from freshly baked bread with fiber stored at -10°C after 2 months storage. Yeasty flavor decreases with decreased frozen storage temperature as seen in Appendix E, Figure E8. With extended period of frozen storage from 2 months to 3.5 months, yeast flavor reduced. It might be due to the decreased yeast activity since it was indicated by several researches that yeast activity is reduced by freezing and frozen storage. However, only one significant difference was determined between 2 months and 3.5 months frozen storage concerning yeasty flavor in frozen bread without fiber stored at -15°C.

In Figure 38, it is seen that bread samples stored at -10°C had the highest scores in off-flavor. The off-flavor content of the bread samples decreased considerably with a decrease in temperature from -10°C to -15°C; on the other hand, significant differences were not observed between the samples stored at -15°C and -20°C. Any effect associated with the fiber content could not be observed in off-flavor. Besides only freshly baked with and without fiber stored at -10°C showed significant differences with prolonged frozen storage.

Off-flavor is a complex character and the panel indicated different descriptions of the off-flavor such as bitter, vinegar and pungent. This explains the variance in sensory response as seen in Figure 38.

The results indicated increased off-flavor in frozen stored bread samples. Novotni et al. (2011) stated that frozen dough could be kept for 22 days at -18°C without significant changes in oxidative stability; however, during frozen storage of fully baked bread, oxidative stability decreased. Furthermore, it was indicated that bread aroma is negatively affected by volatile compounds that result from lipid oxidation (Poinot et al., 2008). Hence, frozen bread samples show decreased oxidative stability during frozen storage, inducing the development of volatile compounds due to lipid oxidation which cause unpleasant odor and flavor in frozen bread samples.

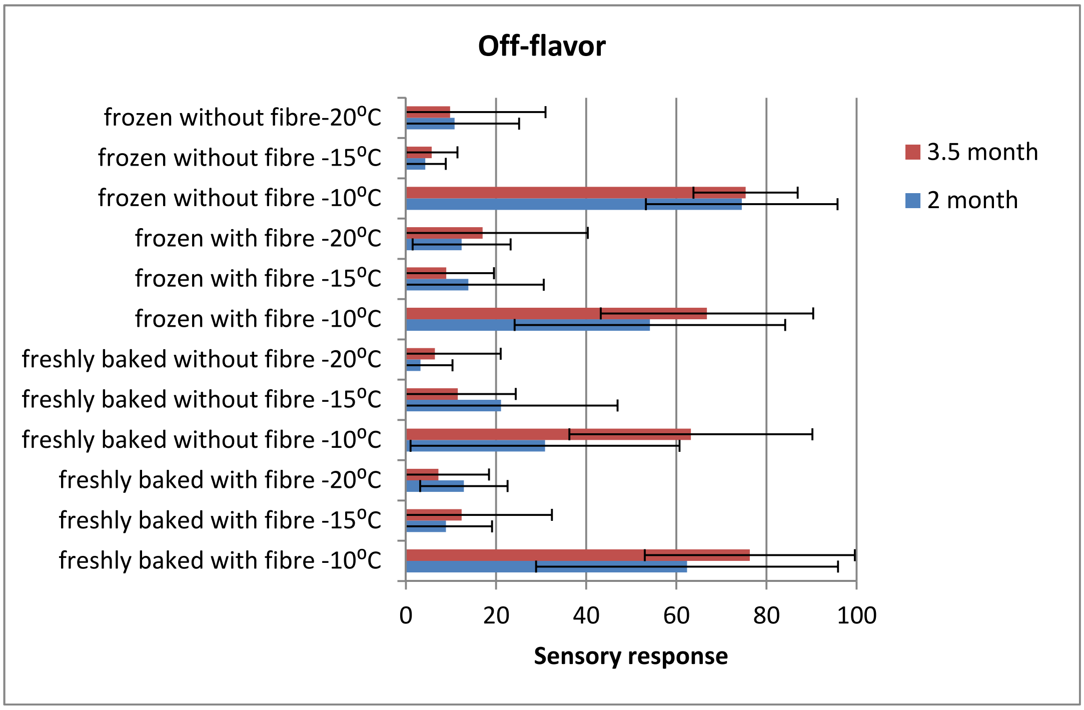

Figure 38 Sensory responses to off-flavor after 2 months and 3.5 months storage

Sensory results given in Appendix E, Figure E9 show that the least airy bread sample was frozen bread with fiber stored at -10°C. Generally, airy attribute increased with a decrease in storage temperature, except for freshly baked bread without fiber. Bread samples without fibers were more airy than those with fibers and also freshly baked samples were more airy than frozen bread samples. The airy characteristic decreased with extended frozen storage, except for frozen bread with and without fiber stored at -10°C which showed a negligible increase. On the other hand, there were significant differences regarding airy between 2 months and 3.5 months frozen storage in freshly baked bread with fiber -20°C and without fiber -10°C.

In Figure 39, it can be seen that frozen bread both with and without fiber stored at -10°C were quite dry according to the other bread samples. In general, freshly baked samples were more airy than frozen bread samples and samples without fiber than with fiber. Except for frozen bread with fiber stored at -10°C and -15°C, moisture content of bread samples decreased with extended frozen storage time. This probably is due to a reduction in water retention capacity of the bread constituents (Bárcenas & Rosell, 2006). Freshly baked without fiber -10°C, -15°C and -20°C and frozen with fiber -20°C showed significant differences between 2 months and 3.5 months frozen storage regarding moist characteristic. It can be stated that freshly baked bread without fiber is more influenced by frozen storage time when moisture content is considered.

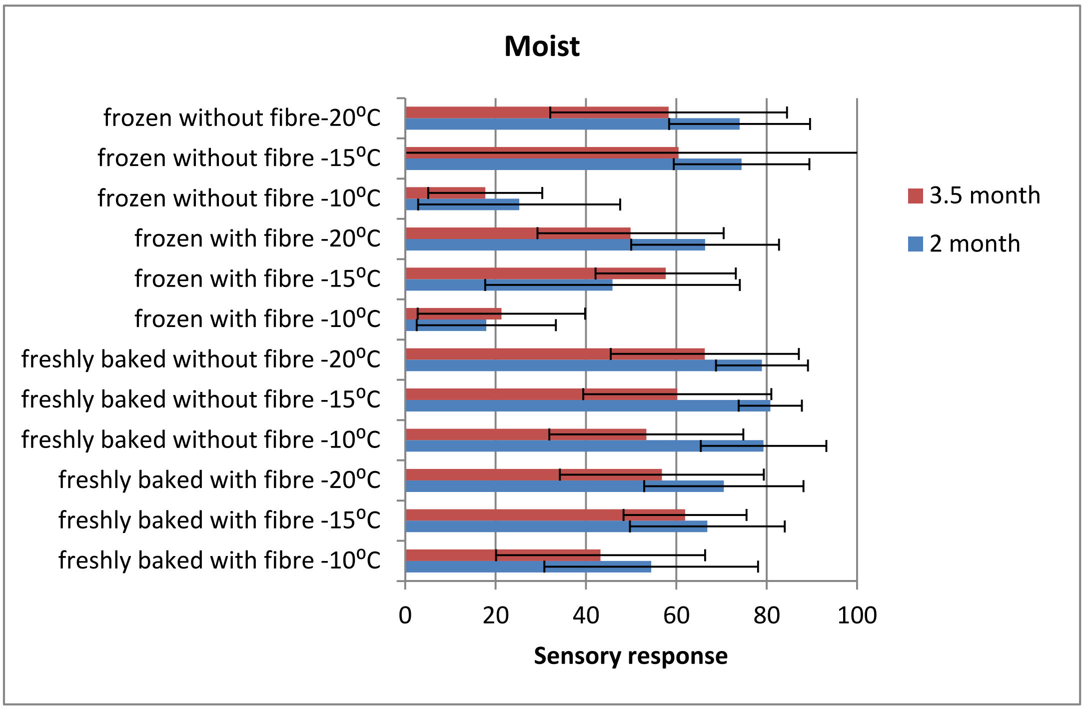

Figure 39 Sensory responses to moist after 2 months and 3.5 months storage

4.5 Multivariate analysis of all sensory attributes and their relation to frozen storage temperature and time

To improve our understanding further, a PLS model was applied as shown in Figure 40. Crumpled crust was the most significant sensory variable related to the frozen stored bread. Besides off-flavor, off-odor, compactness and even shape characteristics were significant sensory attributes of importance for the frozen stored bread. On the other hand, freshly baked bread can be described by cracking, elasticity, airy, springiness, moist and freshly baked odor characteristics. Frozen storage temperature mostly has influence on off-flavor, off-odor, total odor, total flavor, yeast flavor and air bubbles characteristics. Furthermore, frozen storage temperature was more influential than storage time on the sensory characteristics of the bread samples.

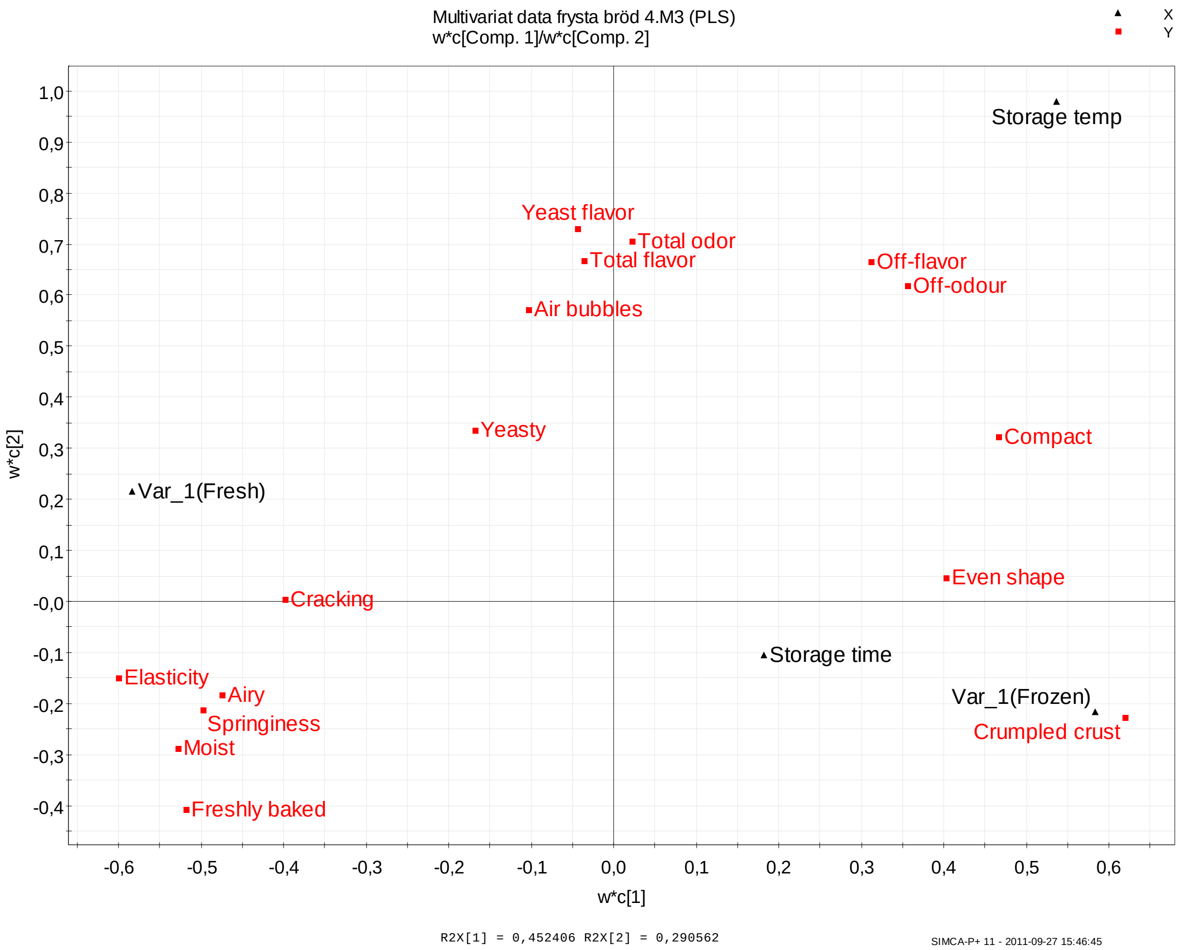

Figure 40 Partial least square (PLS) regression for sensory attributes of freshly baked and frozen stored breads with storage temperature and storage time

5. CONCLUSIONS

This thesis demonstrated that bread samples significantly lost their sensory quality with prolonged frozen storage. Stored bread samples had less freshly baked odor, elasticity, and moist texture but became more compact and developed off-odors and off-flavors.

Highest sensory quality was maintained with the storage temperature of -20°C. However, there was not a big difference in sensory quality between the storage temperatures of -15°C and -20°C. A storage temperature of -10°C resulted in significant defects in quality which might be due to the high mobility of water, causing recrystallization. Those samples showed high scores in compactness, off-odor and off-flavor but low scores in elasticity, freshly baked odor and moist sensory characteristics.

Frozen stored breads could mostly be characterized by a crumpled crust and they were quite compact and dry. Quality defects observed in frozen stored bread could be caused by a migration of water. Freshly baked breads were generally elastic and moist and also had high scores in freshly baked odor but the air bubbles on the surface with the storage temperature of -10°C was clearly detected.

Regarding fibers, bread with fiber was less elastic and airy but more compact compared to bread without fiber. Therefore, it could not be stated that fiber as whole wheat kernels has a positive influence on sensory quality of bread.

According to the results of the sensory analysis performed in this thesis, it can be suggested to store dough instead of baked bread at -20ºC. This recommendation was also supported by the results of DSC (Differential Scanning Calorimetry) of water and ice dynamics done by Chen and Swensson at Chalmers University of Technology and microstructure results carried out by Eckardt at SIK within this project.

6. FUTURE OUTLOOK

It is clear that there are still many potential lines of research that should be followed to minimize the adverse effects of dough and bread freezing. The next investigations will be performed with the same dough and bread after 6 months frozen storage to observe the further influences of frozen storage time on sensory quality of bread samples.

Further investigations which focus on freeze tolerant yeast strains to improve the freeze tolerance by directed mutagenesis can be studied. Also yeast genes responsible for freeze tolerance can be identified for developing freeze tolerant yeasts. Production and processing parameters such as freezing rate, thawing rate and proofing time should be optimized and different combinations of those parameters can be investigated more to obtain a better quality product. Besides that the effect of lower temperatures such as -25°C and -30°C can be tested; however, higher energy consumption should be taken into account when freezing and frozen storage are considered in order to achieve a reasonable product. On the other hand, temperature fluctuations should be strictly controlled without failures in the cold chain during transportation and storage.

Moreover the influence of fiber which is fine dispersed in the product can be analyzed. Positive nutritional effect of fibers would make it worthwhile. Also with respect to the improvers such as different types of enzymes and emulsifiers, the influences of different combinations and amounts on sensory quality of bread can be tested and evaluated. Conflicting results found in the literature on the effects of some improvers indicate the need for further investigations. Also the amount of different ingredients such as salt, sugar and fat should be adjusted properly to achieve bread with better sensory quality. Further research and analysis should be conducted to better understand the issues and mechanisms involved. Different wheat varieties with strong gluten content can be tested and evaluated in frozen dough and bread because of the fact that wheat flour for production of frozen dough and bread should have greater strength than that used in conventional bread making.

Since the main aim of this thesis was to study the sensory quality, determinants of the quality perception of bread, optimization of sensory quality attributes and description of the overall quality should be investigated more and further tests should be done because we are working with human perceptions.

ACKNOWLEDGEMENTS

First of all, I would like to thank to Maud Langton and Annika Åström for giving me a great opportunity to work at SIK as a diploma student. It was really a great experience for me. Special thanks to Annika Åström who supervised me during this thesis work, helped me in evaluating results and reviewed the thesis. I also would like to say my big thanks to Susanne Ekman who showed me everything in the sensory laboratory, helped me a lot during sensory analysis and interpreting and reviewing of the results.

Furhermore, thanks to Camilla Öhgren for her assistance during this work. Also I would like to thank to Johanna Eckardt for her great collaboration in baking and preparation of samples. Besides great thanks to all other people who are involved in this project, for instance Daniel Johansson who helped me with multivariate data analysis and Emma Holtz who introduced me to the bakery.

In addition, I would like to thank to my supervisor Prof. Dr. Ulf Svanberg for the assessment of this thesis.

As an end big thanks to my wonderful family. I always feel your love and support in my heart during my study abroad.

REFERENCES

Ahrné, L., Andersson, C., Floberg, P., Rosen, J., Lingnert, H. (2007). Effect of crust temperature and water content on acrylamide formation during baking of white bread: steam and falling temperature baking. *LWT – Food Science and Technology* 40 (10): 1708–1715.

Autio, K. & Laurikainen, T. (1997). Relationship between flour/dough microstructure and dough handling and baking properties. *Trends in Food Science and Technology* 8: 181–185.

Baik, M. Y. & Chinachoti, P. (2000). Moisture redistribution and phase transitions during bread staling. *Cereal Chemistry* 77: 484-488.

Bárcenas, M. E. & Rosell, C. M. (2006). Effect of frozen storage time on the bread crumb and aging of par-baked bread. *Food Chemistry* 95: 438-445.

Berglund, P., Shelton, D., Freeman, T. (1991). Frozen bread dough ultra-structure as affected by duration of frozen storage and freeze–thaw cycles. *Cereal Chemistry* 68: 105–107.

Bhattacharya, M., Langstaff, T. M., Berzonsky, W. A. (2003). Effect of frozen storage and freeze–thaw cycles on the rheological and baking properties of frozen doughs. *Food Research International* 36: 365–372.

Boehm, D. J., Berzonsky, W. A., Bhattacharya, M. (2004). Influence of nitrogen fertilizer treatments on spring wheat (*Triticum aestivum L.*) flour characteristics and effect on fresh and frozen dough quality. *Cereal Chemistry* 81: 51–54.

Bot, A. (2003). Differential canning calorimetric study on the effects of frozen storage on gluten and dough. *Cereal Chemistry* 80: 366–370.

Bot, A., de Bruijne, D. W. (2003). Osmotic properties of gluten. *Cereal Chemistry* 80: 404–408.

Brathen, E., Knutsen, S. H. (2005). Effect of temperature and time on the formation of acrylamide in starch based and cereal model system, flat breads and bread. *Food Chemistry* 92 (4): 693–700.

Bushuk, W., & Rasper, V. F. (1994). Wheat-Production, Properties and Quality, pp. 25-27. Chapman and Hall.

Cardello, A. V. (1995). Food quality: relativity, context and consumer expectations. *Food Quality and Preference* 6 (3): 163-170.

Carr, L. G., Rodas, M. A. B., Della Torre, J. C. M., Tadini, C. C. (2006). Physical, textural and sensory characteristics of 7-day frozen part-baked French bread. *LWT-Food Science and Technology* 39: 540-547.

Cauvain, S. (1998). Improving the control of staling in frozen bakery products. *Food Science Technology* 9: 56–61.

Chambers IV, E., McGraw, S., Smiley, K. (2006). Sensory Analysis of Frozen Foods. In: Handbook of Frozen Food Processing and Packaging, pp. 561-576. CRC Press.

Charley, H. & Weaver, C. (1998). Foods–A Scientific Approach, pp. 164–167, 202–205, 224–225. Prentice-Hall.

Chen, G. and Swensson, J. (2011). Water and ice dynamics in frozen bread and dough.

Codón, A. C., Rincón, A. M., Moreno-Mateos, M. A., Delgado-Jarana, J., Rey, M., Limón, C., Rosado, I. V., Cubero, B., Penate, X., Castrejón, F., Benítez, T. (2003). New *Saccharomyces Cerevisiae* baker's yeast displaying enhanced resistance to freezing. *J. Agric. Food Chem.* 51: 483–491.

Collar, C., Armero, E., Martínez, J. (1998). Lipid binding of formula bread doughs: relationships with dough and bread technological performance. *Lebensmittel Wissenschaft und Technologie* 207: 110–121.

Czuchajowska, Z. & Pomeranz, Y. (1989). Differential scanning calorimetry, water activity, and moisture contents in crumb center and near crust zones of bread during storage. *Cereal Chemistry* 66 (4): 305–309.

Davidou, S., Le Meste, M., Debever, E., Bekaert, D. (1996). A contribution to the study of staling of white bread: effect of water and hydrocolloid. *Food Hydrocolloids* 10: 375–383.

DeMan, J. M. (1990). Principles of Food Chemistry, pp. 281–282, 320–322. Chapman and Hall.

Demiralp, H., Çelik, S., Köksel, H. (2000). Effects of oxidizing agents and defatting on the electrophoretic patterns of flour proteins during dough mixing. *European Food Research and Technology* 211: 322–325.

Doerry, W. (1995). Baking Technology. Vol. I. Breadmaking. Kansas City: The American Institute of Baking, pp. 5–17, 20–51; Vol. II. Controlled Baking, pp. 108–126.

Dubois, D. K., Blockcolsky, D. (1986). Frozen bread dough, effect of dough mixing and thawing methods. *Technical Bulletin of the American Institute of Baking* 8: 1–7.

Eckardt, J. (2011). The microstructure of dough and bread—effect of fiber, freezing temperature and storage time.

El-Hady, E., El-Samahy, S., Seibel, W., Brummer, J. (1996). Changes in gas production and retention in non-prefermented frozen wheat doughs. *Cereal Chemistry* 73: 472–477.

Eliasson, A. C. & Larsson, K. (1993). Bread. In: Eliasson, A.C., Larsson, K. (Eds.), Cereals in Breadmaking. A Molecular Colloidal Approach, pp. 325-363. Marcel Dekker, New York.

Fik, M. & Surówka, K. (2002). Effect of prebaking and frozen storage on the sensory quality and instrumental texture of bread. *Journal of the Science of Food and Agriculture* 82: 1268-1275.

Faridi, H. & Faubion, J. M. (1990). Dough Rheology and Baked Product Texture, pp. 252–268. AVI Books.

Fellows, P. (1997). Food Processing Technology–Principles and Practice, pp. 348–352, 418–440. Woodhead Publishing.

Filipović, J., Popov, S., Filipović, N. (2008). The behavior of different fibers at bread dough freezing. *Chemical Industry & Chemical Engineering Quarterly* 14 (4): 257−259.

FoB, (2007). European Bread Market. Retrieved 9 June 2011, from http://www.bakersfederation.org.uk/who_we_are.aspx.

Frasse, P., Lambert, S., Levesque, C., Melcion, D., Richard-Molard, D., Chiron, H. (1992). The influence of fermentation on volatile compounds in French bread crumb. *Lebensmittel-Wissenschaft und-Technologie* 25: 66–70.

Gaonkar, A. G. (1995). Food Processing – Recent Developments, pp.227–240. Elsevier Science.

Gellynck, X., Kuhne, B., Van Bockstaele, F., Van de Walle, D., Dewettinck, K. (2009). Consumer perception of bread quality. *Appetite* 53: 16–23.

Gélinas, P., Deaudelin, I., Grenier, M. (1995). Frozen dough: effects of dough shape, water content, and sheeting-molding conditions. *Cereal Foods World* 40: 124–126.

Gélinas, P., McKinnon, C. M., Lukow, O. M., Townley-Smith, F. (1996). Rapid evaluation of frozen and fresh dough involving stress conditions. *Cereal Chemistry 73*: 767–769.

Giannou, V., Kessoglou, V., Tzia, C. (2003). Quality and safety characteristics of bread made from frozen dough. *Trends in Food Science & Technology* 14: 99-108.

Giannou, V. & Tzia, C. (2006). Quality and Safety of Frozen Bakery Products. In: Handbook of Frozen Food Processing and Packaging, p. 495. CRC Press, Taylor & Francis Group, Boca Raton, FL.

Giannou, V. & Tzia, C. (2007). Frozen dough bread: quality and textural behavior during prolonged storage-prediction of final product characteristics. *Journal of Food Engineering* 79: 929-934.

Gil, M. J., Callejo, M. J., Rodríquez, G. (1997). Effect of water content and storage time on white pan bread quality: instrument evaluation. *Lebensmittel-Wissenschaft und Technologie* 205: 268–273.

Gillette, M. (1994). Sensory Analysis. In: Source Book of Flavors, 2nd Ed. pp. 817-838. Chapman & Hall, New York.

Gray, J. A. & BeMiller, J. N. (2003). Bread staling: Molecular basis and control. *Comprehensive Reviews in Food Science & Food Safety* 2: 1-21.

Gujral, H. S. & Singh, N. (1999). Effect of additives on dough development, gaseous release and bread making properties. *Food Research International* 32: 691–697.

Gys, W., Courtin, C.M., Delcour, J. A. (2003). Refrigerated dough syruping in relation to the arabinoxylan population. *Journal of Agricultural and Food Chemistry* 51: 4119–4125.

Gys, W., Courtin, C. M., Delcour, J. A. (2004). Reduction of xylanase activity in flour by debranning retards syruping in refrigerated doughs. *Journal of Cereal Science* 39: 371–377.

Haros, M., Rosell, C. M., Benedito, C. (2002). Effect of different carbohydrases on fresh bread texture and bread staling. *European Food Research Technology* 215: 425–430.

Havet, M., Mankai, M., Le Bail, A. (2000). Influence of the freezing conditions on the baking performances of French frozen dough. *Journal of Food Engineering* 45: 139–145.

Hebeda, R. E., Bowles, L. K., Teague, W. M. (1990). Developments in enzymes for retarding staling of baked goods. *Cereal Foods World* 35: 453–457.

Heiniö , R. L. (2006). Sensory Attributes of Bakery Products. In Y. H. Hui (Ed.), Bakery Products: Science and Technology, pp. 285–298. Ames: Blackwell Publishing.

Heyman, H. & Lawless, H. T. (1998). Introduction to Applications. In: Sensory Evaluation of Food: Principles and Practices, pp. 17–24. New York: Chapman and Hall.

Hsu, K., Hoseney, R., Seib, S. (1979). Frozen dough. I. Factors affecting the stability of yeasted doughs. *Cereal Chemistry* 56: 419–424.

Hug-Iten, S., Escher, F., Conde-Petit, B. (2003). Staling of bread: Role of amylose and amylopectin and influence of starch-degrading enzymes. *Cereal Chemistry* 80: 654-661.

Inoue, Y. & Bushuk, W. (1992). Studies on frozen dough. II. Flour quality requirements for bread production from frozen dough. *Cereal Chemistry* 69(4): 423–428.

Kennedy, C. J. (2000). Managing Frozen Foods. Boca Raton, FL: CRC Press.

Kenny,S., Wehlre, K., Dennehy, T., Arendt, K. E. (1999). Correlations between empirical and fundamental rheology measurements and baking performance of frozen bread dough. *Cereal Chemistry* 76 (3): 421–425.

Kenny, S., Grau, H., Arendt, E. K. (2001). Use of response surface methodology to investigate the effects of processing conditions on frozen dough quality and stability. *Eur. Food Res. Techno.* 213: 323–328.

Kirchhoff, E. & Schieberle, P. (2001). Determination of key aroma compounds in the crumb of a three-stage sourdough rye bread by stable isotope dilution assays and sensory studies. *Journal of Agriculture and Food Chemistry* 49: 4304–4311.

Kulp, K., Lorenz, K., Brúmmer, J. (1995). Frozen and Refrigerated Doughs and Batters, pp. 1–3, 93, 148. Minnesota: American Association of Cereal Chemists.

Laaksonen, T .J. & Roos, Y. H. (2000). Thermal, dynamic-mechanical, and dielectric analysis of phase and state transitions of frozen wheat doughs. *Journal of Cereal Science* 32: 281–292.

Le Bail, A., Pasco, M., Meric, L., Cahagnier, B. (1996). Influence of the freezing rate on yeast activity in frozen bread dough. In: Proceedings of the 10th International Cereal and Bread Congress, Porto Carras, Greece, 9–12 June.

Le Bail, A., Grinand, C., Le Cleach, S., Martinez, S., Quilin, E. (1999). Influence of storage conditions on frozen french bread dough. *Journal of Food Engineering* 39: 289–291.

Le Bail, A., Monteau, J.Y., Margerie, F., Lucas, T., Chargelegue, A., Reverdy, Y. (2005). Impact of selected process parameters on crust flaking of frozen partly baked bread. *Journal of Food Engineering* 69: 503-509.

Lopetcharat, K. & McDaniel, M. (2005). Sensory Analysis of Foods. In: Methods of Analysis of Food Components and Additives, CRC Press.

Lorenz, K. & Kulp, K. (1995). Freezing of Doughs for the Production of Breads and Rolls. In: Kulp, K., Lorenz, K., Brúmmer, J. (Eds.), Frozen and Refrigerated Doughs and Batters. American Association of Cereal Chemists Inc., pp. 135–149. St. Paul, Minnesota, USA.

Lu, W. & Grant, L. A. (1999a). Effects of prolonged storage at freezing temperatures on starch and baking quality of frozen doughs. *Cereal Chemistry* 76: 656–662.

Lu, W. & Grant, L. A. (1999b). Role of flour fractions in breadmaking quality of frozen dough. *Cereal Chemistry* 76: 663–667.

Mallett, C. P. (1994). Frozen Food Technology. London, UK: Blackie Academic and Professional.

Mandala, I., Polaki, A., Yanniotis, S. (2009). Influence of frozen storage on bread enriched with different ingredients. *Journal of Food Engineering* 92: 137-145.

Manzoni, M., Popoli, A., Cavazzoni, V. (1995). Effect of freezing on some *Saccharomyces Cerevisiae* strains for breadmaking. *Microbiol. Alim. Nutr.* 13: 141–148.

Marsili, R. T. (2006). Comparing Sensory and Analytical Chemistry Flavor Analysis. In: Sensory-Directed Flavor Analysis, Marsili Consulting Group, Rockford, IL, USA.

Matuda, T. G., Parra, D. F., Lugao, A. B., Tadini, C. C. (2005). Influence of vegetable shortening and emulsifiers on the unfrozen water content and textural properties of frozen French bread dough. *Lebensmittel-Wissenschaft und –Technologie* 38: 275–280.

Meilgaard, M. C., Civille, G. V. and Carr, B. T. (1999). Descriptive Analysis Techniques, 3rd Ed. In: Sensory Evaluation Techniques, CRC Press LLC, Boca Raton, Florida.

Miles, M. J., Morris, V. J., Orford, P. D., Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research* 135: 271-281.

Mondal A. & Datta A.K. (2008). Bread baking – A review. *Journal of Food Engineering*, 86: 465-474.

Murakami, Y., Yokoigawa, K., Kawai, F., Kawai, H. (1996). Lipid composition of commercial bakers' yeasts having different freeze-tolerance in frozen dough. *Biosci. Biotechnol. Biochem. 60*: 1874–1876.

Nemeth, L. J., Paulley, F. G., Preston, K. R. (1996). Effects of ingredients and processing conditions on the frozen dough bread quality of a Canada Western Red Spring wheat flour during prolonged storage. *Food Res. Int.* 29: 609–616.

Novotni, D., Ćurić D., Galić, K., Škevin D., Neđeral, S., Kraljić, K., Gabrić, D., Ježek, D. (2011). Influence of frozen storage and packaging on oxidative stability and texture of bread produced by different processes. *LWT-Food Science and Technology* 44: 643-649.

O'Brien, C. M., Mueller, A., Scannell, A. G. M., Arendt, E. K. (2003). Evaluation of the effects of fat replacers on the quality of wheat bread. *Journal of Food Engineering* 56 (2–3): 265–267.

Perron, C. E., Lukow, O. M., Bushuk, W., Townley-Smith, F. (1999). The blending potential of diverse wheat cultivars in a frozen dough system. *Cereal Foods World* 44 (9): 667–672.

Phimolsiripol, Y., Siripatrawan, U., Tulyathan, V., Cleland, D. J. (2008). Effects of freezing and temperature fluctuations during frozen storage on frozen dough and bread quality. *Journal of Food Engineering* 84: 48–56.

Primo-Martin, C., Pijpekamp, A. V. D., Vliet, T. V., Jongh, H. H. J., Plijter, J. J., Hamer, R. J. (2006). The role of the gluten network in the crispness of bread crust. *Journal of Cereal Science* 43 (3): 342–352.

Poinot, P., Arvisenet, G., Grua-Priol, J., Colas, D., Fillonneau, C., Le Bail, A., Prost, C. (2008). Influence of formulation and process on the aromatic profile and physical characteristics of bread. *Journal of Cereal Science* 48: 686-697.

Polaki, A., Xasapis, P., Fasseas, C., Yanniotis, S., Mandala, I. (2010). Fiber and hydrocolloid content affect the microstructural and sensory characteristics of fresh and frozen stored bread. *Journal of Food Engineering* 97: 1-7.

Ribotta, P. D., Leon, A. E., Anon, M. C. (2003a). Effects of yeast freezing in frozen dough. *Cereal Chemistry* 80: 454–458.

Ribotta, P. D., Leon, A. E., Anon, M. C. (2003b). Effect of freezing and frozen storage on the gelatinization and retrogradation of amylopectin in dough baked in a differential scanning calorimeter. *Food Research International* 36: 357–363.

Ribotta, P. & Bail, A. (2007). Thermo-physical assessment of bread during staling. *LWT – Food Science and Technology* 40 (5): 879–884.

Ronda, F. & Roos, Y. H. (2008). Gelatinization and freeze-concentration effects on recrystallization in corn and potato starch gels. *Carbohydrate Research* 343: 901-911.

Ronda, F., Caballero, P. A., Quilez, J., Roos, Y. H. (2011). Staling of frozen partly and fully baked breads. Study of the combined effect of amylopectin recrystallization and water content on bread firmness. *Journal of Cereal Science* 53: 97-103.

Roos, Y. H. (1995). Phase Transitions in Foods. Academic Press, San Diego, CA.

Rosell, C. M., Rojas, J. A., Benedito de Barber, C. (2001). Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocolloids* 15: 75–81.

Rosell, C. M. & Foegeding, A. (2007). Interaction of hydroxypropylmethylcellulose with gluten proteins: Small deformation properties during thermal treatment. *Food Hydrocoll.* 21: 1092–1100.

Rosell, C. M. & Gómez, M. (2007). Frozen dough and partially baked bread: An update. *Food Reviews International* 23 (3): 303-319.

Rosell, C.M. (2010). Innovation in Food Engineering: New Techniques and Products. CRC Press, p. 60, 63, 64.

Sablani, S. S., Baik, O., Marcotte, M. (2002). Neutral networks for predicting thermal conductivity of bakery products. *Journal of Food Engineering* 52: 299-304.

Sahlstrøm, S., Nielsen, A. O., Faergestad, E. M., Lea, P., Park, W. J., Ellekjaer, M. R. (1996). Effect of dough processing conditions and DATEM on Norwegian hearth bread prepared from frozen dough. *Cereal Chemistry* 76: 38–44.

Santos, E., Rosell, C. M., Collar, C. (2008). Gelatinization and retrogradation kinetics of high-fiber wheat flour blends: a calorimetric approach. *Cereal Chemistry* 85 (4): 455–463.

Schieberle, P. (1996). Intense aroma compounds— useful tool to monitor the influence of processing and storage on bread aroma. *Advances in Food Science* 18: 237-244.

Seguchi, M., Nikaidoo, S., Morimoto, N. (2003). Centrifuged liquid and breadmaking properties of frozen-and-thawed bread dough. *Cereal Chemistry* 80: 264–268.

Selomulyo, V. O. & Zhou, W. (2007). Frozen bread dough: effects of frozen storage and dough improvers. *Journal of Cereal Science* 45: 1-17.

Seow, C. C., & Teo, C. H. (1996). Staling of starch based products: a comparative study by firmness and pulsed NMR measurements. *Starch* 48: 90–93.

Sharadanant, R. & Khan, K. (2003). Effect of hydrophilic gums on the quality of frozen dough. I. Dough quality. *Cereal Chemistry* 80: 764–772.

Singh, H. & MacRitchie, F. (2001). Application of polymer science to properties of gluten. *Journal of Cereal Science* 33: 231–243.

Singh, H. (2005). A study of changes in wheat protein during bread baking using SE-HPLC. *Food Chemistry* 90 (1–2): 247–250.

Slade, L., Levine, H., Finley, J.W. (1989). Protein-Water Interactions. Water as a Plasticizer of Gluten and Other Proteins. In: Philips, R. D., Finley, J. W. (Eds.), Protein Quality and the Effects of Processing, pp. 9–124. Marcel Dekker, New York.

Stampfli, L., & Nerste, B. (1995). Emulsifiers in bread making. *Food Chemistry* 52: 353–360.

Stauffer, C. E. (1993). Frozen Dough Production. In B. S. Kamel & C. E. Stauffer (Eds.), Advances in Baking Technology, pp. 88–106. New York: VCH Publishers.

Takano, H., Ishida, N., Koizumi, M., Kano, H. (2002a). Imaging of the fermentation process of bread dough and the grain structure of baked breads by magnetic resonance imaging. *J. Food Sci.* 67: 244–250.

Takano, H., Naito, S., Ishida, N., Koizumi, M., Kano, H. (2002b). Fermentation process and grain structure of baked breads from frozen dough using freeze-tolerant yeasts. *J. Food Sci.* 67: 2725–2733.

Tanghe, A., Teunissen, A., Van Dijck, P., Thevelein, J. M. (2000). Identification of genes responsible for improved cryoresistance in fermenting yeast cells. *Int. J. Food Microbiol.* 55: 259–262.

Tipples, K. H. (1969). The relation of starch damage to the baking performance of flour. *Baker's Digest* 43: 28.

Varriano-Marston, E., Hsu, K. H., Mahdi, J. (1980). Rheological and structural changes in frozen dough. *Baker's Digest* 54: 32–34.

Vetter, J. L. (1979). Frozen unbaked bread dough: past, present, future. *Cereal Foods World* 24: 42–43.

Wahlby, U. & Skjoldebrand, C. (2002). Reheating characteristics of crust formed on buns, and crust formation. *Journal of Food Engineering* 53(2): 177–184.

Wang, J., Rosell, C. M., de Barber, C. B. (2002). Effect of the addition of different fibers on wheat dough performance and bread quality. *Food Chemistry* 79: 221–226.

Wood, B. J. B. (1989). Microbiology of Fermented Foods. Vol. 1, p. 176. Chapman and Hall.

Yi, J. & Kerr, W. L. (2009a). Combined effects of freezing rate, storage temperature and time on bread dough and baking properties. *LTW-Food Science and Technology* 42: 1474-1483.

Yi, J. & Kerr, W. L. (2009b). Combined effects of dough freezing and storage conditions on bread quality factors. *Journal of Food Engineering* 93: 495-501.

Zanoni, B., Pierucci, S., Peri, C. (1994). Study of bread baking process – II. Mathematical modeling. *Journal of Food Engineering* 23 (3): 321–336.

Zanoni, B., Peri, C., Bruno, D. (1995). Modelling of browning kinetics of bread crust during baking. *Lebensmittel-Wissenschaft und-Technologie* 28 (6): 604–609.

Zhang, L., Lucas, T., Doursat, C., Flick, D., Wagner, M. (2007). Effects of crust constraints on bread expansion and CO_2 release. *Journal of Food Engineering* 80 (4): 1302–1311.

Zounis, S., Quail, K. J., Wootton, M., Dickson, M. R. (2002a). Studying frozen dough structure using low-temperature scanning electron microscopy. *Journal of Cereal Science* 35: 135–147.

Zounis, S., Quail, K. J., Wootton, M., Dickson, M. R. (2002b). Effect of final dough temperature on the microstructure of frozen bread dough. *Journal of Cereal Science* 36: 135–146.

APPENDIX

A. Temperature and humidity fluctuations in freezers

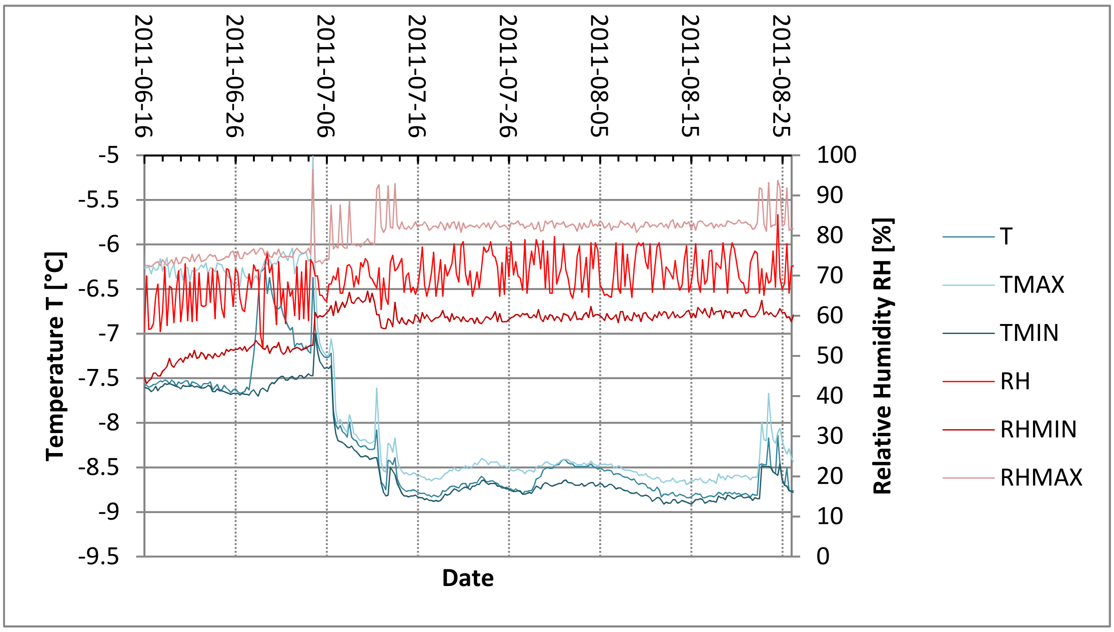

Figure A1. Temperature and humidity fluctuations at -10°C freezer

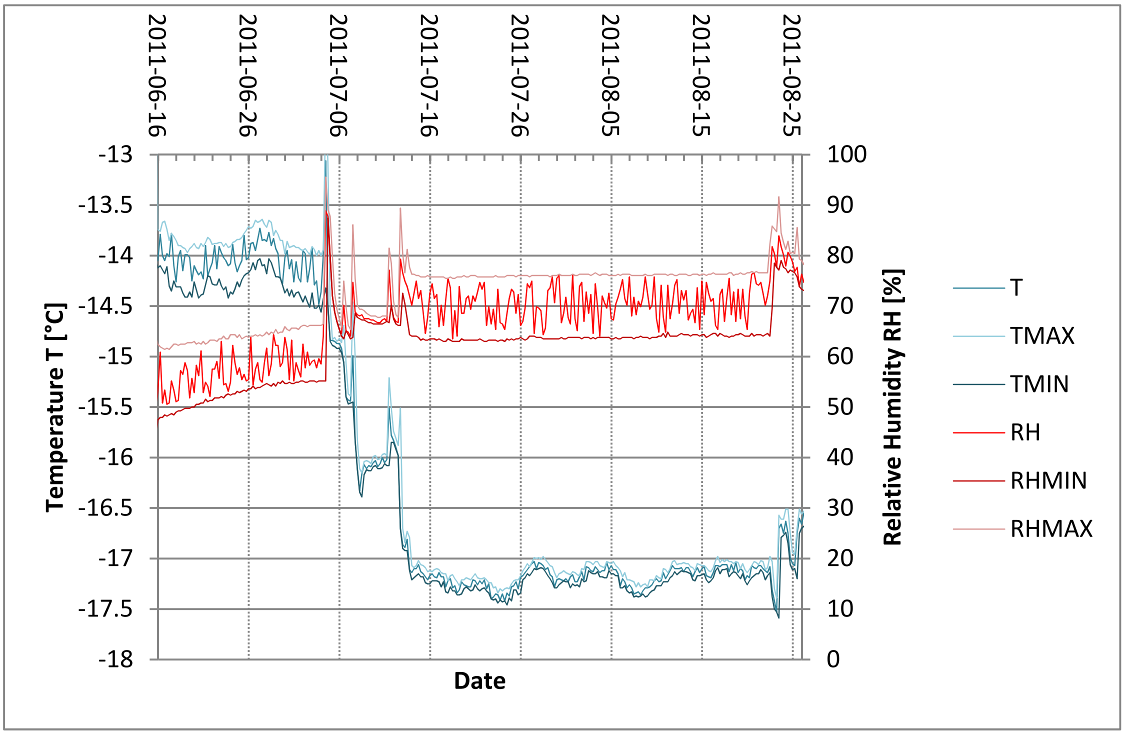

Figure A2. Temperature and humidity fluctuations at -15°C freezer

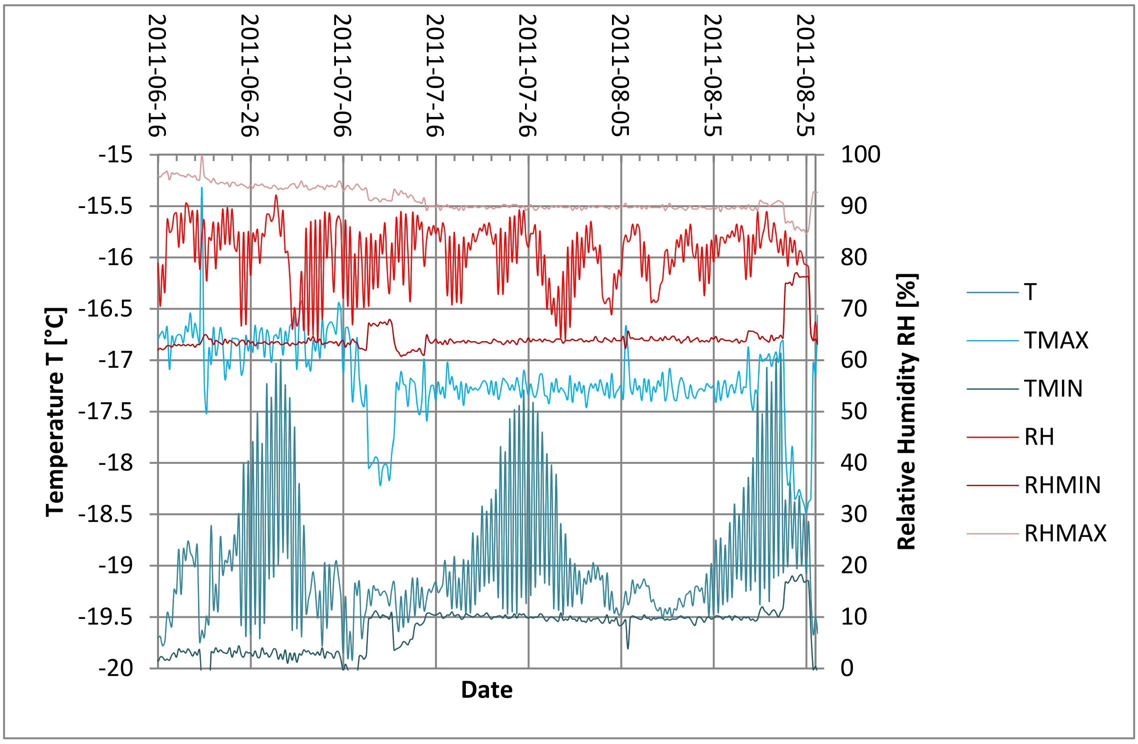

Figure A3. Temperature and humidity fluctuations at -20°C freezer

B. Sensory evaluation results after 2 month frozen storage

Table B1. Sensory evaluation results of appearance attributes

Sample	Even shape	Air bubbles	Crumpled crust
Freshly baked with fiber -10^{o}C	76.57±21.95abc	86.29±7.57a	10.50±9.07de
Freshly baked with fiber -15^{o}C	51.14±24.95c	15.86±14.35b	12.36±7.12cde
Freshly baked with fiber -20^{o}C	59.64±25.71bc	15.64±11.89b	11.93±8.77cde
Freshly baked without fiber -10^{o}C	88.57±6.17a	87.64±9.11a	8.36±9.07e
Freshly baked without fiber -15^{o}C	51.79±27.34c	19.79±13.31b	16.79±12.56bcde
Freshly baked without fiber -20^{o}C	58.57±25.59bc	23.00±16.66b	12.21±18.04cde
Frozen with fiber -10^{o}C	69.86±18.79abc	21.64±13.85b	25.21±13.85abcde
Frozen with fiber -15^{o}C	66.21±25.95abc	13.86±8.88b	33.21±18.87ab
Frozen with fiber -20^{o}C	74.79±17.59abc	13.93±10.10b	31.29±25.82abc
Frozen without fiber -10^{o}C	74.14±17.53abc	21.21±14.23b	39.50±27.36a
Frozen without fiber -15^{o}C	74.50±13.35abc	15.21±8.80b	27.00±16.73abcde
Frozen without fiber-20^{o}C	84.00±9.73ab	19.36±14.10b	29.50±27.48abcd

Mean value ± standard deviation

The difference between levels with same letter is not significant (P<0.05)

Table B2. Sensory evaluation results of texture by finger attributes

Sample	Cracking	Springiness	Compactness	Elasticity
Freshly baked with fiber -10^{o}C	43.93±24.50ab	68.29±13.65a	57.43±19.94abc	65.71±17.69ab
Freshly baked with fiber -15^{o}C	40.00±24.80abc	70.50±12.38a	40.00±17.73cde	66.93±16.78ab
Freshly baked with fiber -20^{o}C	38.14±21.68abc	63.79±17.05a	34.00±15.48def	69.36±16.42ab
Freshly baked without fiber -10^{o}C	37.36±15.24abc	70.29±28.57a	16.07±11.26f	80.07±10.88a
Freshly baked without fiber -15^{o}C	53.71±19.69a	74.36±19.45a	25.43±19.59ef	79.14±14.09a
Freshly baked without fiber -20^{o}C	51.57±21.58a	74.93±20.98a	15.07±13.11f	75.64±19.13ab
Frozen with fiber -10^{o}C	41.50±36.06abc	36.71±27.57b	80.14±23.06a	9.93±7.15c
Frozen with fiber -15^{o}C	20.36±13.85c	62.29±22.92a	53.64±27.59bcd	54.21±20.66b
Frozen with fiber -20^{o}C	23.21±21.41bc	59.36±21.19a	50.93±18.04bcd	56.64±19.40b
Frozen without fiber -10^{o}C	49.43±28.13a	54.57±27.59ab	65.00±18.55ab	22.79±21.56c
Frozen without fiber -15^{o}C	35.71±21.58abc	70.79±19.18a	24.29±16.97ef	60.00±21.10ab
Frozen without fiber-20^{o}C	40.29±23.15abc	70.57±13.85a	32.21±22.34def	63.57±23.45ab

Mean value ± standard deviation
The difference between levels with same letter is not significant (P<0.05)

Table B3. Sensory evaluation results of odor attributes

Sample	Total odor	Freshly baked	Yeasty odor	Off-odor
Freshly baked with fiber -10^{o}C	77.64±13.15ab	33.00±28.33d	29.43±20.97a	62.07±33.34a
Freshly baked with fiber -15^{o}C	71.07±11.98ab	67.43±11.23abc	27.64±21.68a	19.21±25.69b
Freshly baked with fiber -20^{o}C	70.21±12.52ab	63.07±21.05bc	28.21±23.45a	21.36±22.77b
Freshly baked without fiber -10^{o}C	71.79±17.34ab	64.14±28.36abc	32.93±24.60a	30.79±30.48b
Freshly baked without fiber -15^{o}C	64.86±15.89ab	76.21±14.89ab	26.86±23.42a	14.21±19.02b
Freshly baked without fiber -20^{o}C	65.57±18.49ab	81.93±9.11a	22.00±21.52a	12.14±23.39b
Frozen with fiber -10^{o}C	74.93±18.51ab	6.79±5.86e	27.00±28.75a	69.64±25.90a
Frozen with fiber -15^{o}C	62.21±19.64b	49.07±27.01cd	27.14±21.66a	18.07±19.36b
Frozen with fiber -20^{o}C	64.79±15.80ab	58.71±21.07bc	26.93±23.54a	21.50±21.04b
Frozen without fiber -10^{o}C	79.21±10.32a	7.36±5.83e	23.07±19.79a	83.36±9.80a
Frozen without fiber -15^{o}C	64.36±19.22ab	66.57±19.24abc	27.14±22.14a	15.43±25.39b
Frozen without fiber-20^{o}C	65.29±15.78ab	67.50±21.16abc	21.21±22.53a	21.07±21.63b

Mean value ± standard deviation
The difference between levels with same letter is not significant (P<0.05)

Table B4. Sensory evaluation results of flavor attributes

Sample	Total flavor	Yeast flavor	Off-flavor
Freshly baked with fiber -10ºC	74.50±21.38a	30.86±28.94ab	62.36±33.50a
Freshly baked with fiber -15ºC	61.57±20.84ab	19.21±17.12bc	8.86±10.24c
Freshly baked with fiber -20ºC	64.43±22.55ab	17.57±14.52bc	12.86±9.70bc
Freshly baked without fiber -10ºC	72.50±17.09ab	38.00±28.21a	30.86±29.85b
Freshly baked without fiber -15ºC	63.07±15.36ab	16.36±15.26bc	21.07±25.37bc
Freshly baked without fiber -20ºC	57.86±25.38ab	11.29±11.85c	3.21±7.13c
Frozen with fiber -10ºC	61.00±25.57ab	22.14±25.69abc	54.14±30.02a
Frozen with fiber -15ºC	55.36±22.90ab	19.00±14.32bc	13.86±16.71bc
Frozen with fiber -20ºC	62.14±19.67ab	16.86±17.44bc	12.36±10.87bc
Frozen without fiber -10ºC	66.50±26.18ab	22.71±22.40abc	74.50±21.25a
Frozen without fiber -15ºC	53.07±21.72b	18.07±13.01bc	4.29±4.55c
Frozen without fiber-20ºC	63.86±20.08ab	13.00±16.65c	10.79±14.30bc

Mean value ± standard deviation
The difference between levels with same letter is not significant (P<0.05)

Table B5. Sensory evaluation results of texture by mouth feel attributes

Sample	Airy	Moist
Freshly baked with fiber -10ºC	58.00±25.04bc	54.43±23.65bc
Freshly baked with fiber -15ºC	64.64±18.27abc	66.86±17.13ab
Freshly baked with fiber -20ºC	68.21±21.04ab	70.50±17.64ab
Freshly baked without fiber -10ºC	80.21±15.01a	79.29±13.89a
Freshly baked without fiber -15ºC	77.36±15.56ab	80.79±7.00a
Freshly baked without fiber -20ºC	77.50±17.72ab	78.93±10.18a
Frozen with fiber -10ºC	24.79±17.48d	17.93±15.40d
Frozen with fiber -15ºC	43.93±30.42cd	45.86±28.18c
Frozen with fiber -20ºC	60.71±21.85abc	66.36±16.35ab
Frozen without fiber -10ºC	31.93±28.27d	25.21±22.36d
Frozen without fiber -15ºC	72.71±20.92ab	74.43±15.01a
Frozen without fiber-20ºC	74.93±14.08ab	74.00±15.59a

Mean value ± standard deviation
The difference between levels with same letter is not significant (P<0.05)

C. Sensory evaluation results after 3.5 month frozen storage

Table C1. Sensory evaluation results of appearance attributes

Sample	Even shape	Air bubbles	Crumpled crust
Freshly baked with fiber -10°C	70.36±31.12abcd	89.50±11.63a	2.50±5.29e
Freshly baked with fiber -15°C	48.36±25.96d	36.64±16.59bc	14.00±11.42cde
Freshly baked with fiber -20°C	50.64±22.69bcd	31.14±15.92bc	12.00±16.25e
Freshly baked without fiber -10°C	76.93±19.56a	86.71±10.30a	7.29±8.95e
Freshly baked without fiber -15°C	48.86±29.42cd	31.07±18.77bc	12.3±15.32de
Freshly baked without fiber -20°C	48.07±24.33d	46.00±16.90b	14.50±21.08bcde
Frozen with fiber -10°C	66.00±13.36abcd	29.43±13.85bc	51.29±21.34a
Frozen with fiber -15°C	74.79±12.87ab	41.93±14.66bc	20.00±10.86bcde
Frozen with fiber -20°C	78.93±10.80a	27.07±10.19c	30.36±21.26bcd
Frozen without fiber -10°C	61.79±14.59abcd	39.07±16.79bc	58.36±17.87a
Frozen without fiber -15°C	73.43±15.34abc	33.86±18.19bc	32.43±14.73b
Frozen without fiber-20°C	71.57±21.51abcd	38.00±19.86bc	31.79±18.31bc

Mean value ± standard deviation
The difference between levels with same letter is not significant (P<0.05)

Table C2. Sensory evaluation results of texture by finger attributes

Sample	Cracking	Springiness	Compactness	Elasticity
Freshly baked with fiber -10°C	19,93±18,71ef	50,14±26,04bc	68,71±17,73ab	45,00±18,21c
Freshly baked with fiber -15°C	35,50±23,91bcde	60,14±18,25ab	29,21±18,06def	69,57±14,19ab
Freshly baked with fiber -20°C	39,43±20,38bcd	67,00±12,43ab	32,57±17,47cdef	66,43±15,94ab
Freshly baked without fiber -10°C	25,64±18,92def	71,50±22,27ab	37,93±20,66cde	74,86±19,04a
Freshly baked without fiber -15°C	58,57±21,59a	72,29±19,50ab	16,64±7,43ef	75,07±14,85a
Freshly baked without fiber -20°C	50,21±23,19ab	75,14±12,27a	15,07±10,34f	76,50±8,22a
Frozen with fiber -10°C	15,50±13,35f	21,29±12,72d	69,29±25,34a	10,21±10,12d
Frozen with fiber -15°C	23,86±19,13def	62,79±16,48ab	54,07±17,26abc	52,43±17,94bc
Frozen with fiber -20°C	19,79±26,36ef	51,29±19,99bc	46,43±18,45bcd	45,86±14,44c
Frozen without fiber -10°C	24,21±19,20def	29,43±25,52cd	75,50±19,35a	19,50±18,71d
Frozen without fiber -15°C	30,07±23,43cdef	65,93±21,05ab	20,36±13,09ef	65,14±15,32ab
Frozen without fiber-20°C	46,21±20,73abc	74,36±11,65a	19,14±13,63ef	64,93±16,74ab

Mean value ± standard deviation
The difference between levels with same letter is not significant (P<0.05)

Table C3. Sensory evaluation results of odor attributes

Sample	Total odor	Freshly baked	Yeasty odor	Off-odor
Freshly baked with fiber -10°C	83,79±9,11a	25,57±27,49cd	20,07±20,10a	82,50±9,16a
Freshly baked with fiber -15°C	63,64±15,57bcde	55,43±25,70a	24,43±17,28a	22,50±26,70b
Freshly baked with fiber -20°C	62,21±16,20cde	54,29±21,28a	20,57±13,34a	15,00±19,26b
Freshly baked without fiber -10°C	77,43±9,92ab	33,86±25,25bc	25,86±18,14a	61,36±25,67a
Freshly baked without fiber -15°C	61,36±17,02cde	61,14±20,67a	18,00±14,31a	15,79±19,87b
Freshly baked without fiber -20°C	56,86±18,99de	60,93±18,00a	13,14±13,68a	9,07±15,56b
Frozen with fiber -10°C	74,21±15,47abc	11,43±17,74d	14,93±17,86a	70,07±23,71a
Frozen with fiber -15°C	54,21±21,25e	46,50±21,17ab	25,43±16,28a	16,93±20,21b
Frozen with fiber -20°C	57,50±12,81de	42,36±22,82abc	16,86±10,07a	26,50±27,66b
Frozen without fiber -10°C	70,29±13,09abcd	11,00±12,18d	13,93±14,27a	72,07±16,32a
Frozen without fiber -15°C	54,79±14,37e	52,93±23,35ab	20,29±13,74a	18,64±19,72b
Frozen without fiber-20°C	58,50±16,72de	53,07±23,55ab	15,57±10,73a	14,14±14,08b

Mean value ± standard deviation
The difference between levels with same letter is not significant (P<0.05)

Table C4. Sensory evaluation results of flavor attributes

Sample	Total flavor	Yeast flavor	Off-flavor
Freshly baked with fiber -10°C	84,07±8,83a	24,50±16,34ab	76,29±23,30a
Freshly baked with fiber -15°C	58,14±15,46cd	15,43±11,30abc	12,36±20,03b
Freshly baked with fiber -20°C	53,64±19,40de	12,00±15,32bc	7,21±11,20b
Freshly baked without fiber -10°C	75,93±11,33ab	25,79±15,49a	63,21±26,96a
Freshly baked without fiber -15°C	59,00±12,97cd	15,93±13,03abc	11,50±12,85b
Freshly baked without fiber -20°C	43,50±17,02e	7,07±9,64c	6,43±14,61b
Frozen with fiber -10°C	70,43±14,21bc	15,21±15,70abc	66,79±23,56a
Frozen with fiber -15°C	47,00±17,21de	13,00±11,54abc	8,93±10,59b
Frozen with fiber -20°C	53,57±16,82de	9,07±7,32c	17,00±23,36b
Frozen without fiber -10°C	70,36±16,44bc	11,71±15,79bc	75,36±11,57a
Frozen without fiber -15°C	40,64±20,35e	11,36±9,21bc	5,71±8,36b
Frozen without fiber-20°C	46,36±15,45de	9,50±11,43c	9,79±21,19b

Mean value ± standard deviation
The difference between levels with same letter is not significant (P<0.05)

Table C5. Sensory evaluation results of texture by mouth feel attributes

Sample	Airy	Moist
Freshly baked with fiber -10ºC	49,86±21,24cde	43,21±23,13b
Freshly baked with fiber -15ºC	53,00±19,57bcde	61,93±13,61ab
Freshly baked with fiber -20ºC	51,57±18,30bcde	56,79±22,56ab
Freshly baked without fiber -10ºC	60,50±24,91abcd	53,36±21,47ab
Freshly baked without fiber -15ºC	67,86±19,42ab	60,21±20,81ab
Freshly baked without fiber -20ºC	66,64±20,72abc	66,29±20,80a
Frozen with fiber -10ºC	31,79±20,73f	21,29±18,52c
Frozen with fiber -15ºC	41,57±23,16ef	57,64±15,51ab
Frozen with fiber -20ºC	44,71±22,91def	49,86±20,60ab
Frozen without fiber -10ºC	45,00±28,44def	17,71±12,63c
Frozen without fiber -15ºC	66,86±20,52abc	60,50±21,61ab
Frozen without fiber-20ºC	70,57±17,18a	58,29±26,21ab

Mean value ± standard deviation
The difference between levels with same letter is not significant (P<0.05)

D. Tukey pairwise comparison test results

Table D1. P-values* from t-test of appearance attributes

Samples	Even shape	Air bubbles	Crumpled crust
freshly baked with fiber -10ºC	0,600	0,382	0,003
freshly baked with fiber -15ºC	0,738	0,013	0,691
freshly baked with fiber -20ºC	0,391	0,002	0,989
freshly baked without fiber -10ºC	0,064	0,791	0,784
freshly baked without fiber -15ºC	0,798	0,077	0,381
freshly baked without fiber -20ºC	0,251	0,006	0,772
frozen with fiber -10ºC	0,490	0,152	0,002
frozen with fiber -15ºC	0,227	0,000	0,069
frozen with fiber -20ºC	0,405	0,005	0,917
frozen without fiber -10ºC	0,063	0,005	0,033
frozen without fiber -15ºC	0,842	0,004	0,351
frozen without fiber-20ºC	0,096	0,008	0,762

* P<0.05 is significantly different (Tukey pairwise comparison test)

Table D2. P-values* from t-test of texture by finger attributes

Samples	Cracking	Springiness	Compactness	Elasticity
freshly baked with fiber -10ºC	0,001	0,044	0,091	0,000
freshly baked with fiber -15ºC	0,498	0,073	0,152	0,652
freshly baked with fiber -20ºC	0,809	0,517	0,833	0,508
freshly baked without fiber -10ºC	0,115	0,913	0,003	0,349
freshly baked without fiber -15ºC	0,548	0,743	0,112	0,482
freshly baked without fiber -20ºC	0,780	0,970	1,000	0,824
frozen with fiber -10ºC	0,008	0,053	0,240	0,923
frozen with fiber -15ºC	0,581	0,920	0,949	0,720
frozen with fiber -20ºC	0,340	0,246	0,508	0,056
frozen without fiber -10ºC	0,005	0,023	0,122	0,691
frozen without fiber -15ºC	0,434	0,597	0,469	0,485
frozen without fiber-20ºC	0,267	0,464	0,088	0,881

* P<0.05 is significantly different (Tukey pairwise comparison test)

Table D3. P-values* from t-test of odor attributes

Samples	Total odor	Freshly baked	Yeasty odor	Off-odour
freshly baked with fiber -10ºC	0,174	0,179	0,210	0,032
freshly baked with fiber -15ºC	0,162	0,178	0,599	0,668
freshly baked with fiber -20ºC	0,130	0,310	0,178	0,284
freshly baked without fiber -10ºC	0,216	0,009	0,215	0,005
freshly baked without fiber -15ºC	0,597	0,043	0,167	0,830
freshly baked without fiber -20ºC	0,178	0,001	0,058	0,439
frozen with fiber -10ºC	0,882	0,332	0,218	0,960
frozen with fiber -15ºC	0,235	0,734	0,806	0,888
frozen with fiber -20ºC	0,129	0,017	0,095	0,313
frozen without fiber -10ºC	0,021	0,229	0,226	0,054
frozen without fiber -15ºC	0,040	0,034	0,200	0,614
frozen without fiber-20ºC	0,270	0,026	0,324	0,202

* P<0.05 is significantly different (Tukey pairwise comparison test)

Table D4. P-values* from t-test of flavor attributes

Samples	Total flavor	Yeast flavor	Off-flavor
freshly baked with fiber -10^{o}C	0,023	0,509	0,021
freshly baked with fiber -15^{o}C	0,523	0,478	0,516
freshly baked with fiber -20^{o}C	0,058	0,283	0,149
freshly baked without fiber -10^{o}C	0,341	0,099	0,001
freshly baked without fiber -15^{o}C	0,328	0,926	0,278
freshly baked without fiber -20^{o}C	0,025	0,134	0,449
frozen with fiber -10^{o}C	0,150	0,401	0,143
frozen with fiber -15^{o}C	0,224	0,254	0,236
frozen with fiber -20^{o}C	0,082	0,117	0,442
frozen without fiber -10^{o}C	0,564	0,093	0,873
frozen without fiber -15^{o}C	0,048	0,039	0,511
frozen without fiber-20^{o}C	0,017	0,513	0,881

* $P<0.05$ is significantly different (Tukey pairwise comparison test)

Table D5. P-values* from t-test of texture by mouth feel attributes

Samples	Airy	Moist
freshly baked with fiber -10^{o}C	0,334	0,218
freshly baked with fiber -15^{o}C	0,137	0,466
freshly baked with fiber -20^{o}C	0,023	0,119
freshly baked without fiber -10^{o}C	0,011	0,005
freshly baked without fiber -15^{o}C	0,180	0,007
freshly baked without fiber -20^{o}C	0,090	0,042
frozen with fiber -10^{o}C	0,249	0,362
frozen with fiber -15^{o}C	0,792	0,178
frozen with fiber -20^{o}C	0,071	0,040
frozen without fiber -10^{o}C	0,111	0,099
frozen without fiber -15^{o}C	0,521	0,071
frozen without fiber-20^{o}C	0,351	0,056

* $P<0.05$ is significantly different (Tukey pairwise comparison test)

E. Comparison of sensory evaluation results after 2 months and 3.5 months frozen storage

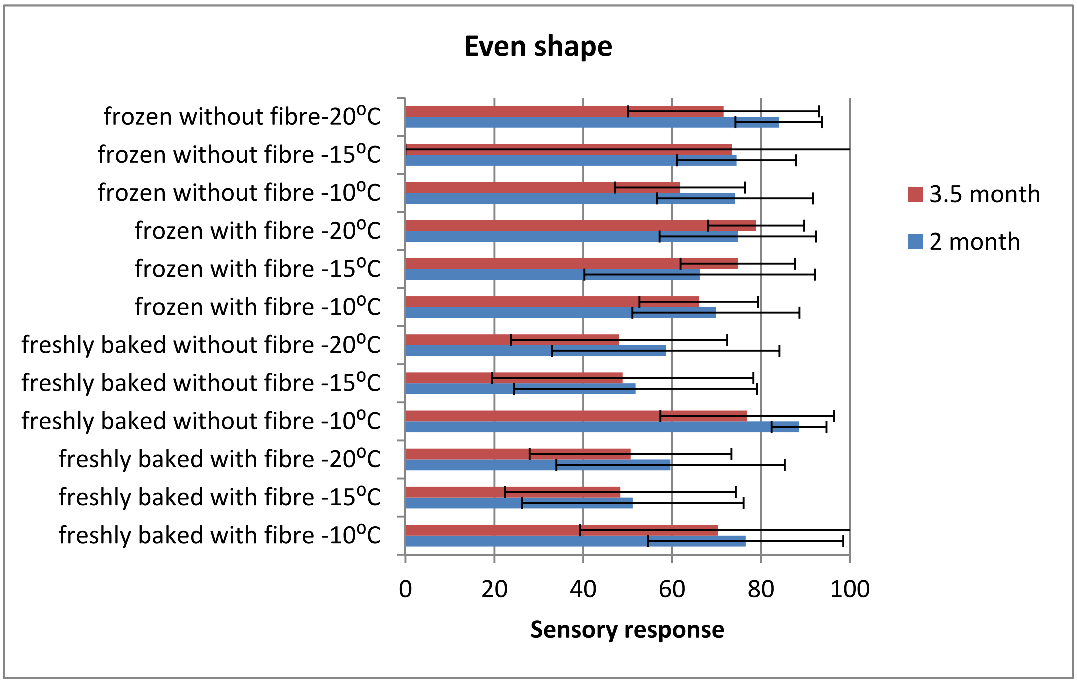

Figure E1. Sensory responses to even shape after 2 months and 3.5 months storage

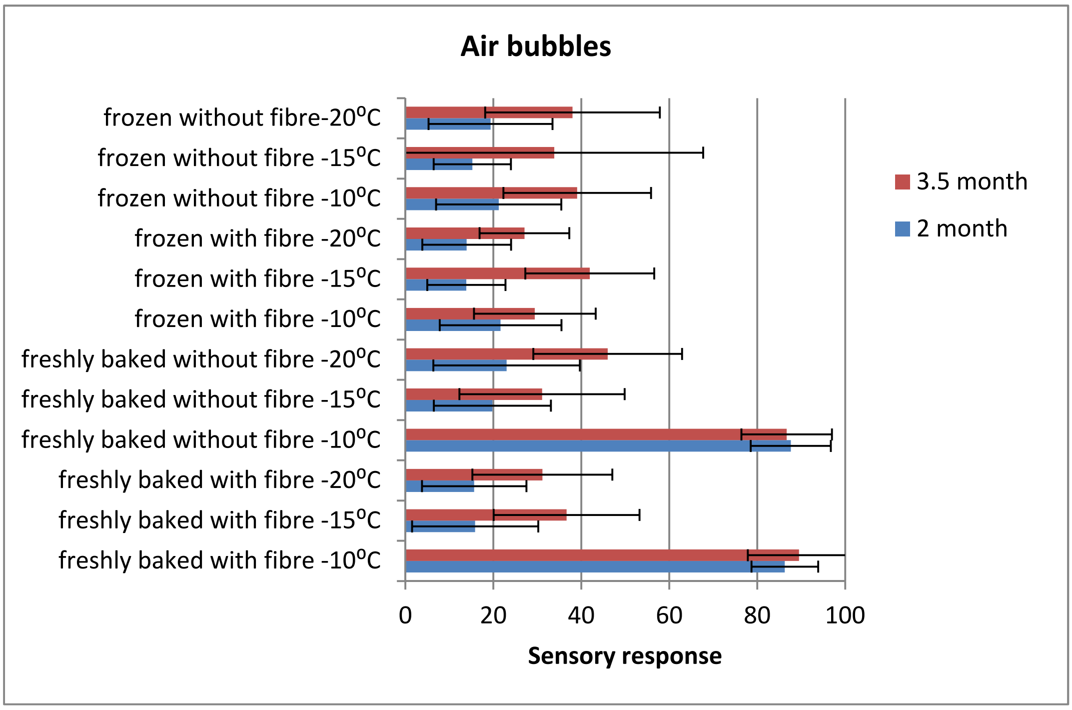

Figure E2. Sensory responses to air bubbles after 2 months and 3.5 months storage

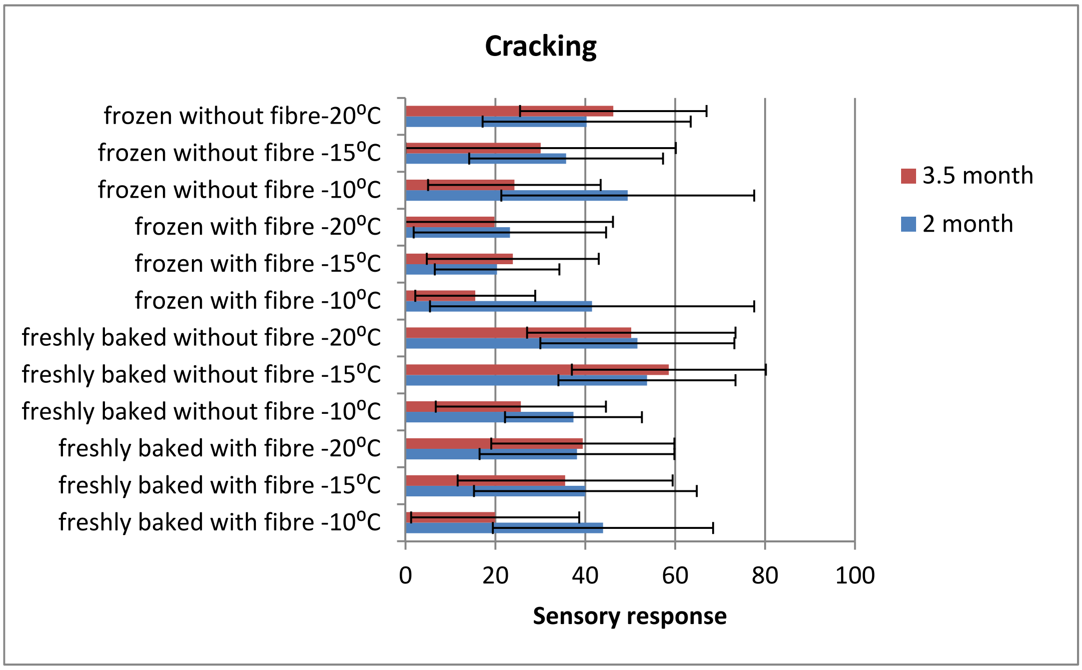

Figure E3. Sensory responses to cracking after 2 months and 3.5 months storage

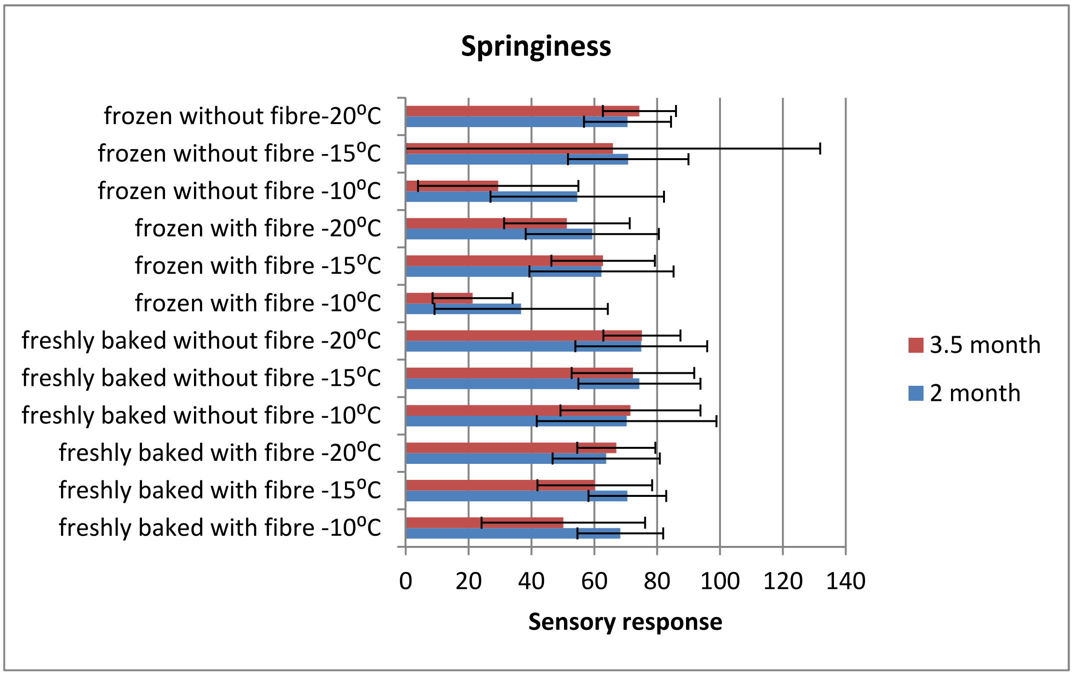

Figure E4. Sensory responses to springiness after 2 months and 3.5 months storage

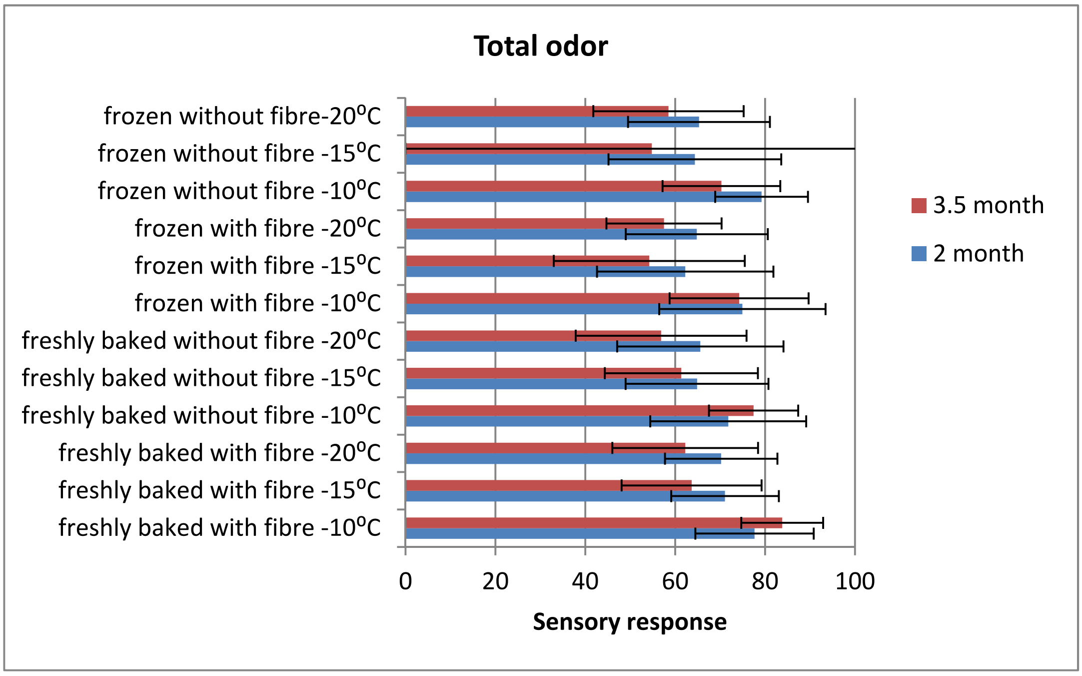

Figure E5. Sensory responses to total odor after 2 months and 3.5 months storage

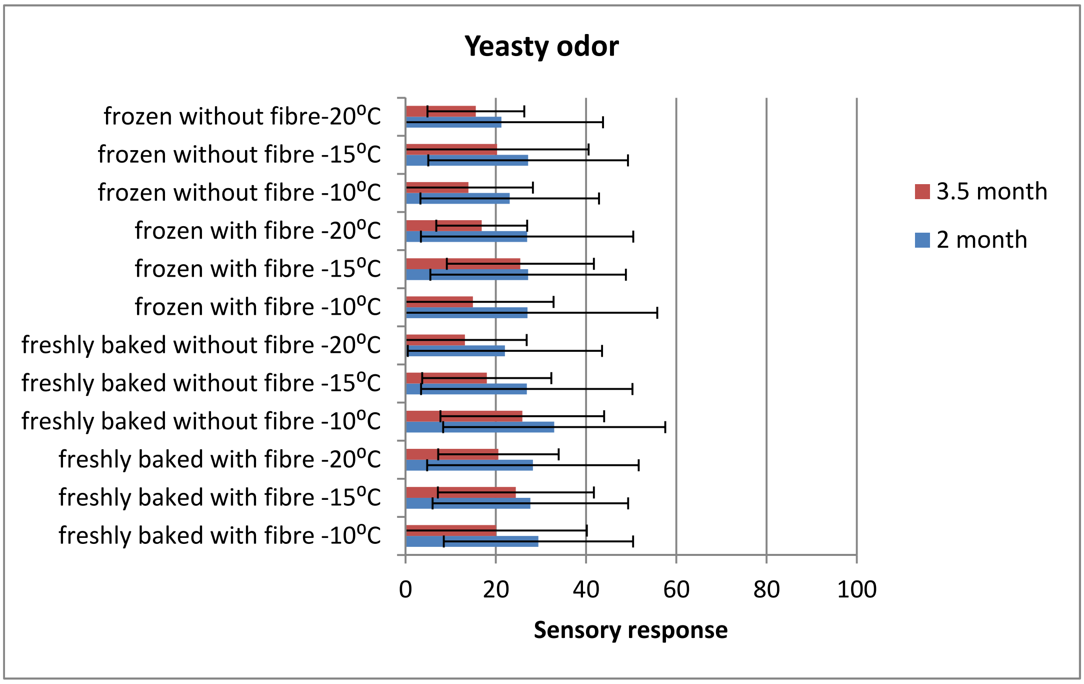

Figure E6. Sensory responses to yeasty odor after 2 months and 3.5 months storage

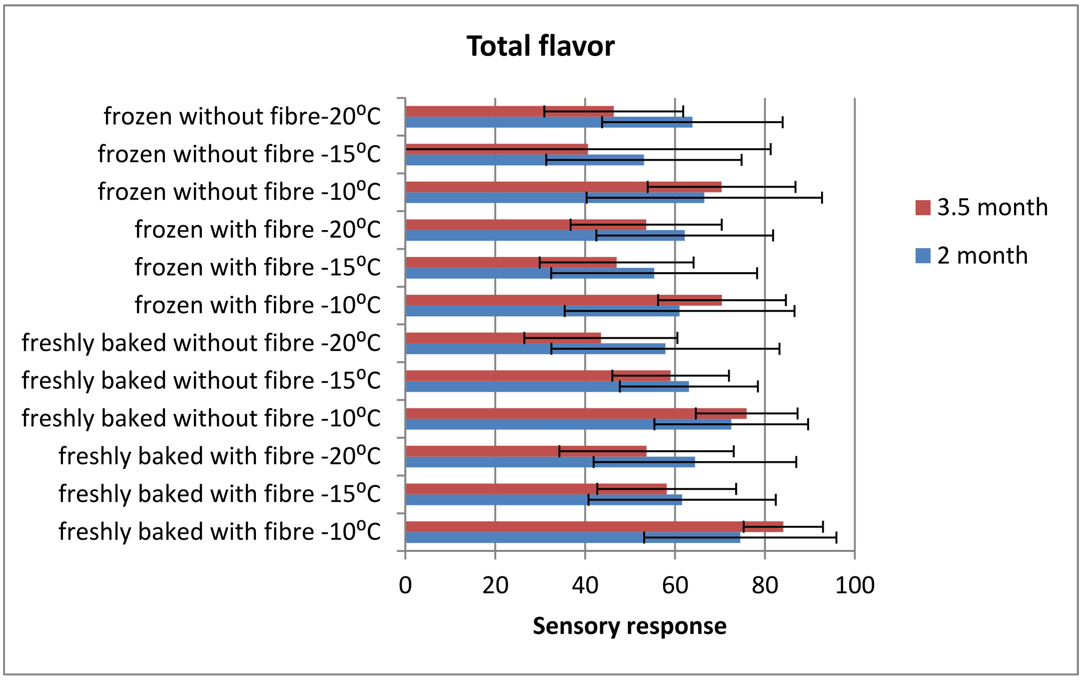

Figure E7. Sensory responses to total flavor after 2 months and 3.5 months storage

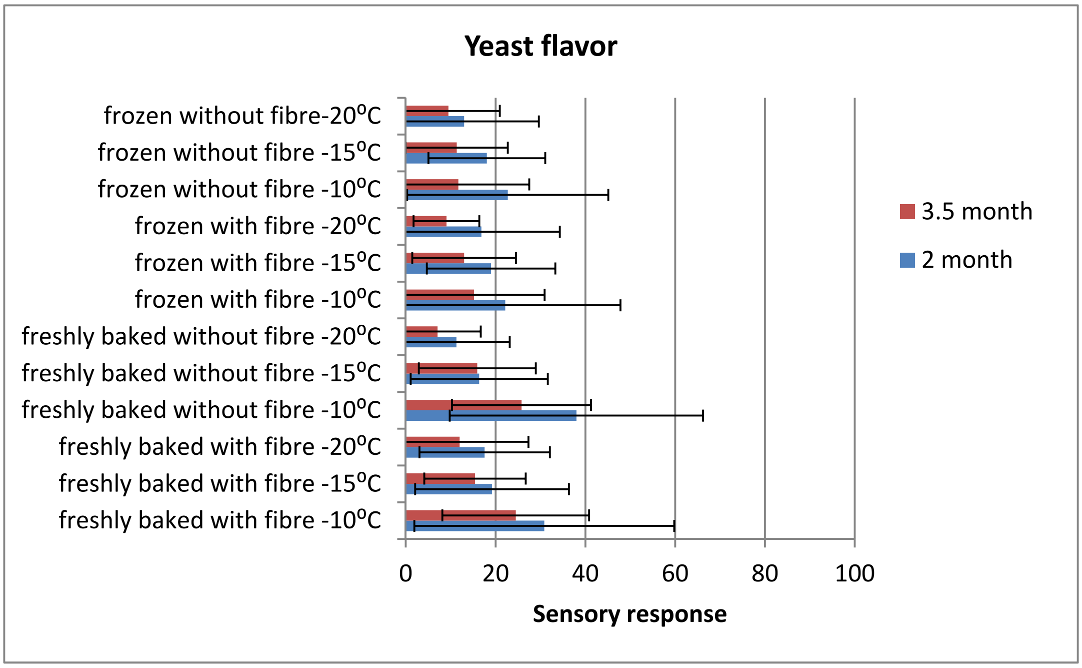

Figure E8. Sensory responses to yeast flavor after 2 months and 3.5 months storage

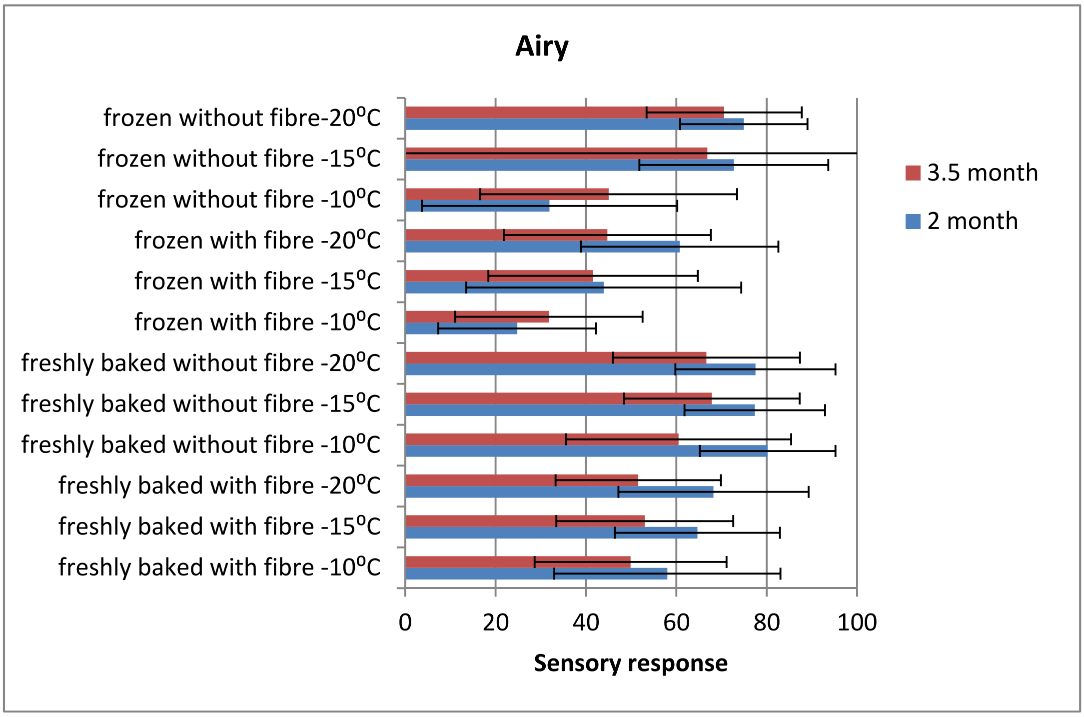

Figure E9. Sensory responses to elasticity after 2 months and 3.5 months storage