THE EFFECTS OF ADDITIVES ON DOUGH RHEOLOGICAL PROPERTIES OF PRE-PROOFED FROZEN DOUGH AND BAKING QUALITY OF BREAD STICKS

By

JUNTANEE URIYAPONGSON

Bachelor of Science
Kasetsart University
Bangkok, Thailand
1983

Master of Science
North Dakota State University
Fargo, North Dakota
1993

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2002

THE EFFECTS OF ADDITIVES ON DOUGH RHEOLOGICAL PROPERTIES OF PRE-PROOFED FROZEN DOUGH AND BAKING QUALITY OF BREAD STICKS

Thesis Approved:

Patricia Rayas-Duarte

Thesis Adviser

Stanley E. Williams

Willie Mayfield

Gerald H. Brusewitz

Timothy A. Pettitt

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to my advisor, Dr. Patricia Rayas for her support, excellent supervision, guidance, and wonderful friendship. I am also thankful to my committee members, Dr. Stanley E. Gilliland, Dr. Gerald H. Brusewitz, Dr. William McGlynn and former committee member Dr. Lowell Satterlee, who retired before my thesis completed, for their support and suggestions on my research work, and to Dr. Carla L. Goad for her assistance and suggestion in statistical analysis of my data.

I am also thankful to David Moe, pilot plant manager for his assistance and suggestion for the trouble shooting of equipment and instrument problems, to Phoebe Doss for SEM assistance, to Frank McKee for his support and giving me food industry experience, to Dr. Inteaz Alli for his support and giving me experience in Quality Assurance in food industries, his caress, and been very good friend, to Dr. Mahendra Kavdia for technical assistance in computer work and my friends for their good companies and different life during my study.

I would like to thank my excellent and wonderful husband (Dr. Suthipong Uriyapongson), my daughter (Natnaree Uriyapongson) and my son (Tanayu Uriyapongson) for their love, patience and understanding through all these years of my study and also to my mom and dad for their love and support.

Finally, I would like to thank the Royal Thai Government, and Dr. Patricia Rayas for financial support and Oklahoma Food and Agricultural Products Research and Technology Center, and Khon Kaen University for supporting during these years of study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

Chapter III

Chapter IV

Chapter V

NOMENCLATURE

H_{mD}	Maximum height of doughs, mm
V_T	Total gas production or total volume, mL
H_{mG}	Maximum height of gas production, mm
V_R	Retention volume of gas, mL
T_x	Time of beginning of gas release, min
mL	Milliliter
A	Area under the curve of micro-extensibility
E	Extensibility using micro-extensibility test
Rmax	Resistance to extension using micro-extensibility test
Rmax/E	Ratio of resistance to extension and extensibility using micro-extensibility test
g	Gram
G'	Storage modulus, Pa
G"	Loss modulus, Pa
G*	Complex modulus, Pa
η^*	Complex viscosity, Pa.s
rh	Relative humidity
r	Correlation coefficient

CHAPTER I

INTRODUCTION AND OBJECTIVES

Introduction

One of the major concerns of the manufacturing industry, including the baking industry, is the shortage of labor and skilled technicians. The bake-off section in supermarkets and most pizza franchises stores use frozen dough bread products, which allows them to produce freshly baked products with a minimum of processing and capital investment. The frozen dough is prepared at a central bakery or frozen dough manufacturing facility and delivered frozen to supermarkets and food service institutions. The frozen dough market requires a frozen shelf life of 3 to 6 months and this has been a challenge for the industry. (Reed and Nagodawithana 1991). Among the challenges encountered is a significant deterioration of the overall quality of the final product as the frozen storage exceeds 3 months (Nakagawa 1997). Longer frozen dough shelf life means a reduction in product waste from a variety of factors including staling, mold growth, and loss of quality (Cauvain and Young 2000). To maintain its profitability, a manufacturing bakery would like to extend the shelf life of their frozen dough products by minimizing loss of quality.

Individual dough pieces are fermented at around 40°C and 85% relative humidity to obtain a desirable gas production by yeast, affecting dough height and structure (Cauvain and Young 2000). Fermentation or proofing times are optimized to keep production time as short as possible to provide desirable product characteristics and increase product through put. During the fermentation step air cells are evenly

dispersed through out the dough. The air cells are precursors of the texture and flavor that result in the delicate balance of aroma and structure of freshly baked bread. Two types of frozen dough process are available in industrial bakeries: 1) pre-proofed frozen dough, and 2) unproofed or unfermented frozen dough. The pre-proofed frozen dough is defined as the dough that has been proofed and then frozen as compared to unproofed frozen dough that are frozen prior to proofing (Nakagawa 1997).

Pre-proofed frozen dough offers many advantages to manufactures, supermarkets, bakery stores and consumers. Advantages for the manufacturer are to remain competitive in the marketplace by increasing sales with just-in-time production and the control of process to ensure high quality for customers. The advantages for bakers are a reduction of production time due to the elimination of mixing proofing time. The quick bake off provides consistency of quality minimizing product loss without skilled workers, thus reducing the cost of production. Currently, one of the challenges of frozen dough is the reduction of volume due to insufficient oven spring after and the formation of brown spots and blisters in the bread crust after long periods of frozen dough storage. However, no reports are found in the literature addressing the deterioration of crust quality of bread.

Among the theories explaining the reduction of quality of frozen dough are the decrease in gassing power by of the loss of yeast viability during the freezing stage, and the loss of dough strength due to changes in the rheological properties of the gluten network (Inoue and Bushuk 1991). The addition of additives could protect gluten matrix to form regular and uniform pore sheets from freezing damage (Kenny et al 1999 and Sahlstrøm et al 1999). This dissertation addresses the gas production and retention

and the rheological changes of pre-proofed frozen dough containing additives. The rheological tests performed in this study used full formula dough, including yeast, yielding a more complex system but closer to the problem in commercial production.

Objectives

The objectives of this study were to:

1. Determine the effects of a commercial dough conditioner (CDC), methylcellulose (MC), and a mix of CDC and MC on maximum dough height, total gas volume and retention volume of frozen dough and baking quality of bread sticks.
2. Investigate the effects of glutathione and dead yeast (heat treated) on the rheological properties of dough using dynamic rheometer and micro-extensibility and baking quality evaluation.
3. Study changes of dough and bread crumb structure using scanning electron microscopy.

LITURATURE CITED

Cauvain, S. P., and Young, L. S. 2000. The contribution of water during processing, baking, cooling and freezing. Pages 72-94 in: Bakery Food Manufacture and Quality, S. P. Cauvain and L. S. Young ed. Blackie Academic & Professional, London, UK.

Inoue, Y., and Bushuk, W. 1991. Studies on frozen doughs. I. Effects of frozen storage and freeze-thaw cycles on baking and rheological properties. Cereal Chem. 68:627-631.

Kenny, S., Wehrle, K., Dennehy, T., and Arendt, E. K. 1999. Correlation between empirical and fundamental rheology measurements and baking performance of frozen bread dough. Cereal Chem. 76:421-425.

Nakagawa, M. 1997. Pre-proofed frozen dough technology. http:/aabe.org/libra. preproofed_frzn.html.

Reed, G., and Nagodawithana, T. W. 1991. Use of yeast in baking. Pages 315-368 in: Yeast Technology, 2nd eds. Van Nostrand Reinhold/AVI: New York.

Sahlstrøm, S., Nielsen, A. O., Færgestad, E. M., Lea, P., Park, W. J., and Ellekjær, M. R. 1999. Effect of dough processing conditions and DATEM on Norwegian hearth bread prepared from frozen dough. Cereal Chem. 76:38-44.

CHAPTER II

LITERATURE REVIEW

Frozen dough is widely used for bread production. The frozen dough allows short baking process for retail outlets with freshly baked flavor and aroma any time for the consumer. The quality of bread made from frozen dough depends on formulation and processing conditions. Shelf life of frozen dough is affected by the gradual deterioration of the quality of bread during the frozen storage of the dough. As the storage time increases the grain gets coarser, texture gets firmer and the loaf volume decreases. Two possible factors have been identified for the loss of the baking quality: 1) decrease in gassing power due to decreasing yeast activity and yeast viability and 2) gradual lost of dough strength and diminished gas-holding properties (Inoue and Bushuk 1991). Many of the factors that reduced yeast activity or damaged gluten network resulting in poor baking performance might influence the dough either independently or synergistically.

Hosomi et al (1992) suggested three approaches to improve the frozen dough quality. The first approach was improving gassing power by new yeast strains resistant to freeze damage. The second approach was the use of storage bulk yeast for pre-fermentation dough before freezing as described by several researchers (Lorenz and Bechtel 1964; Kline and Sugihara 1968; Lorenz 1974; Hsu et al 1979a,b, Tanaka et al 1980). The third approach was the use of additives and dough ingredients. Räsänen et al (1997a) suggested that to achieve good baking quality of frozen dough, a proper balance between dough elasticity (gas holding capacity), porosity (intact gluten

network) and gassing power (yeast viability) needed to be established. This chapter involves the discussion of factors that cause the deterioration of frozen dough quality.

I. Decreasing in Gassing Power

The decrease in gassing power of frozen dough is due to yeast activity and viability, dough formulation and processing conditions such as freezing rate, freezing temperature, frozen storage time, freeze-thaw cycles and fermentation before and after freezing.

I.1. Effect of Yeast

Flour and yeast are the two ingredients identified as main source of variation in baking properties. Variation in yeast performance may be due to poor processing or to the perishable nature of compressed yeast. Uniformity of yeast is by far the most important criteria of quality for bakers (Reed and Nagodawithana 1991). Age and source of yeast are also important in yeast performance. Kline and Sugihara (1968) reported that yeast from two different sources had different frozen stability for frozen dough. Storing yeast at 1.7°C significantly improved frozen dough stability by increasing the lag period of yeast when incorporated into the dough. No contamination of mold or bacterial was observed when yeast was stored at 1.7°C up to seven weeks. Kline and Sugihara (1968) concluded that selection of commercial yeast sources and yeast storage temperature of 1.7°C might help in preserving yeast for frozen dough.

Reed and Peppler (1973) defined three main functions of yeast:1) produces the leavening gas to expand the dough; 2) affects the rheological properties of the dough; and 3) contributes to the typical fermentation flavor of yeast-raised products. Leavening gas or gassing power is one of the important factors in baked goods quality in both frozen and fresh dough. Leavening action of yeast containing 30% $NaHCO_3$ was 350 ml of CO_2 per hour per 100 g dough (Reed and Peppler 1973). Release of gas by baking powder is fast during baking and once it has been released there is no further leavening action. In comparison, the formation of leavening gas from yeast is sustained for longer time than baking powder if sufficient fermentable sugar is available (Reed and Nagodawithana 1991).

There are two methods of determining fermentation activity of baker's yeast (Reed and Nagodawithana 1991). The first method is an actual baking with the measuring of volume of baked bread and/or volume of proof dough at a set proof time or at a set of proof height required to attain in the pan. The second method is measuring the amount of CO_2 produced in a given time period of the bulk yeast or yeasted dough. The gassing test of bulk yeast such as determination of CO_2 using titration, volumetric determination or by the measurement of the pressure have the disadvantage of neglecting the effect of osmotic pressure on yeast fermentation activity in a dough. Thus the method to determine gassing power of baker's yeast by the test of yeasted dough volume is preferred. However, for measuring yeast fermentation activity dough should contain 6% sugar to supply sufficient available fermentable sugar (Shogren et al., 1977). Several instruments have been developed for measuring CO_2 production. Some designs measure CO_2 in pressure cups equipped

with pressure gauges with simultaneous determination of 12 samples in individual channels (Rubenthaler et al., 1980). Commercial instruments for the automatic recording of CO_2 are the Swedish SJA Fermentograph, risograph and rheofermentometer. Rheofermentometer measures gas produced in the dough and escaped from the dough. Dough volume and dough expansion are recorded by a manometer and valve system containing soda lime. Pressure measurements are taken directly from fermentation chamber for total gas production and from absorption bottle for absorbed CO_2 given off the dough (Shuey 1975).

Even though bulk yeast cells are cryoresistant (Bruinsma and Giesenchlag 1984), eight times freeze-thaw cycles and long frozen storage (130 days) have little effect in CO_2 production of bulk yeasts (Neyreneuf and Van Der Plaat 1991). The effect of directly freezing bulk yeast is different from yeast in a dough mass (Hsu et al 1979a; Wolt and D'Appolonia 1984a) in which CO_2 production is reduced throughout frozen storage (Neyreneuf and Van Der Plaat 1991).

I.1.1. Effect of Yeast Type

There are 3 types of baker's yeast available in the market for use in baked products: 1) cream yeast, containing about 18% solids, 2) compressed yeast (CY), containing about 30% solids, and 3) active dry yeast (ADY), containing about 92% solids. ADY is available in 3 forms: regular active dry yeast (ADY), instant active dry yeast (IADY), and protected active dry yeast (PADY) coming from different processing stages (Reed and Nagodawithana 1991). CY requires filtration using rotary vacuum filter for addition of concentration, extrusion and cutting into 1-pound cake.

Production of cream yeast is similar to CY but the process is stopped before dewatering and extrusion. Thus the cream yeast contains more liquid and can be shipped to bakeries in liquid pumpable form in tank trucks. It has slight advantage in stability compared to CY due to the elimination of warming up period during mixing and extrusion. ADY is dried in continuous belt driers and can be used in dough as rehydrated in warm water (35°-40°C). It is also available in ground form in aluminum foil pouches (N_2 flushed) which can be added directly into dry ingredients if the dough water is warm (hot tap water 45°-55°C). IADY is dried in fluid bed drier and always packaged under vacuum or in an inert atmosphere to prevent loss of activity. It can be used for baking by direct addition to flour or dry ingredients. PADY is produced with the addition of a 0.1% antioxidant to the press cake before drying. PADY is suitable for used in premixes of dry ingredients. All of these yeast types have different composition, shelf life and fermentation activity in various types of doughs as shown in Table I. The yeast types have been studied for suitable uses in frozen and traditional dough products for many years. However, various reports of using different yeast types in frozen dough have contrasting results and still are controversial. This is due in part to the complexity of the changes in molecular structure, variation of formula and processing conditions of frozen dough studies conducted by different investigators.

Contradictory results in the performance of different yeast types in frozen dough are reported. Zaehringer et al (1951) and Merritt (1960) suggested that ADY might be superior to CY in maintaining shelf life in frozen dough due to the longer lag period of ADY. The dough from ADY had longer proof times than the dough from

CY. Longer proof times of ADY release more reducing agents in to the dough compared to CY (Kline and Sugihara 1968). El-Hady et al (1996) showed that total gas production of CY decreased (4%) more than that of IADY (1.8%) in frozen dough after12 weeks of storage.

However, the above reports are different from Wolt and D'Appolonia (1984b). They found that the gassing power for ADY on dry basis is only slightly lower than that of IADY and CY. They also found that fresh CY had a lower percentage of dead yeast cells than either ADY or IADY. Dead yeast cells are believed to release glutathione (GSH), a reducing agent, to the dough. The fresh CY contained 4.9% dead cells and no detectable amounts of GSH were found. ADY and IADY contained higher dead yeast cells (13.0 and 18.6%) than that of CY due to the dry process itself. Wolt and D'Appolonia also reported that fresh CY had slightly better proof-time stability than ADY and IADY over a 20 weeks of frozen storage. Neyreneuf and van der Plaat (1991) confirmed that dried yeast with fluidized bed drying (IADY) from original compressed yeast gave lower loaf volume than the original compressed yeast. The results might be due to the structure and functional integrity of the yeast cytoplasmic membrane (van Dam 1986) and increase the sensitivity of dry yeasts to freezing (Kline and Sugihara 1968, Javes 1971 and Wolt and D'Appolonia 1984b).

Gelinas et al (1994) reported that fresh cream yeast and fresh compressed (CY) from 16 commercial yeast batches had similar gassing power in nonfrozen dough using the Risograph instrument. Variation in gassing power was found between yeast batches and within supplier and types. Both yeasts had also similar gassing power

after storage at 4°C up to three weeks. When both yeasts were compared fresh and after storage at 4°C for three weeks, the relative freeze-thaw tolerance of non pre-fermented dough did not change.

I.1.2. Effect of Yeast Strain

There were two yeast strains of *Saccharomyces cerevisiae* used in the production of baker's compressed yeast in the United States until early of 1970s. Since then, the baking industry has required a dry yeast strain with improved fermentation activity and improved performance in high sugar dough and yeast-leavened frozen dough (Reed and Nagodawithana 1991). Some new strains that meet these requirements have been available. However, acceptance by the baking industry has been slow, partly because of the high cost in production and distribution of several strains by yeast manufactures. The specific yeast strains for specific use and production has been described in patents and publications such as the production of instant dry yeast by Langejan and Khoudokormoff (1976) and by Jacobson and Trivedi (1987); osmotolerant yeasts by Legman and Margalith (1983); frozen dough leavening by Sasaki and Oshima (1987), Hino et al (1987) and Oda et al (1986).

Hosomi et al (1992) reported that improving gassing power by new yeast strains resistant to freeze damage is one approach of the possible solution of improving frozen dough quality. The development of suitable yeast strains for the food industry has been made based on traditional methods of hybridization or mutation (Reed and Nagodawithana, 1991). Genetic engineering techniques have been used in obtaining new yeast strains. It is difficult to explain particular detail properties of genes for the

industrial strains but the usefulness of particular strain depends on the growing conditions (Reed and Nagodawithana 1991).

Oda et al (1986) selected 11 yeast strains with higher trehalose concentrations than commercial baker's yeast from 300 *S. cerevisiae.* Trehalose was reported as a cryoprotective agent in yeast cells (Oda et al 1986, Uno et al 1986, and van der Plaat 1988, Neyreneuf and van der Plaat 1991). These yeast strains performed well in sweet dough (30% sugar) after 7 days of frozen storage. However, the proper selections of yeast strains for frozen dough include yeast resistance to freezing and a selection of yeast with improved stability during frozen storage.

Wada et al (1999) developed IADY with freezing and drying tolerance for manufacturing frozen dough. The yeast activity and baking properties from this yeast had little effect on freezing, thawing and frozen storage of frozen dough. Takano et al (1999) produced new polyploid baker's yeast with resistance to long-term frozen storage in both low-sugar and high-sugar doughs. Tanghe et al (2000) and Dijck et al (2000) introduced different mutants using industrial yeast strains that improved freeze resistance during fermentation.

I.2. Effect of Dough Formulation

The loss of baking quality of frozen dough can be limited to a certain degree by adjustments in formulation (Lorenz 1974, Marston 1978) such as type of yeast (Kline and Sugihara 1968, Hino et al 1987, Neyreneuf and van der Plaat 1991), yeast level Neyreneuf and van der Plaat (1991), type of shortening and level (Lorenz 1974,

Marston 1978, Inoue et al 1995), type of flour (Neyreneuf and van der Plaat 1991), oxidizing agents (Lorez 1974, Hsu et al 1979a, Inoue and Bushuk 1991), other additives (Nonami et al 1984, Noll 2000) and processing condition (Merritt 1960, Lorenz 1974).

Due to the reduction of gassing power of the dough during frozen storage caused by decreasing yeast viability, adding more yeast in the frozen dough formula is one way to provide more gassing power and adequate stability of frozen dough. Neyreneuf and van der Plaat (1991) reported that adding 50% more yeast from the regular level (3-4%) to 6% (flour basis) maintained satisfactory bread volume made from frozen dough when subjected to prolong frozen storage up to 90 days of frozen storage. This increased yeast level was necessary and had apparently no negative effects on taste and flavor of the bread (Inoue et al 1995).

Sugar is one of the important ingredients that affect gassing power of baker's yeast. High level of sugar in frozen dough minimized free water content and minimized ice crystallization formed in the dough (Hsu et al 1979a). Reed and Nagodawithana 1991 gathered information and reported that yeast fermentable sugars are gluclose, fructose, sucrose, maltose, raffinose, glucodifructose and glutafructosans, polysaccharides composed of fructose and glucose. Fermentable sugars by baker's yeast are monosaccharides and disaccharides. Only some polysaccharides are fermentable. The rate of CO_2 production in dough from yeast is related to the sugar type. Glucose is fermented faster than fructose, maltose and sucrose (Tang et al 1972). Readily fermentable sugars in wheat flour were reported between 1 and 2%

(Friedemann et al., 1967) and no more than 1% (D'Appolonia et al 1971, Reed and Peppler 1973). Additional fermentable sugar (maltose) is available as soon as dough is mixed by the action of α- and β-amylases on damaged starch. Sucrose added in dough is hydrolyzed by yeast sucrase (invertase) to constituent monosaccharides.

I.3. Effect of Processing Condition

I.3. 1. Effect of Fermentation

Many researchers reported that fermentation prior to freezing caused reduction in bread volume (Merritt 1960, Kline and Sugihara 1968, Lorenz 1974). Hsu et al (1979a, 1979b) suggested a severe damage of yeast when it was activated prior to freezing. Currently, there is no satisfactory explanation of the mechanism of this deleterious effect (Reed and Nagodawithana 1991). The degree of resistance to fermentation prior to freezing varies in different yeast strains. However, stability of yeast during frozen storage is one of the evaluations of strain selection.

Räsänen et al (1997b) reported that 25 min prefermentation had no effect on the amount of liquid phase on fresh dough but it had a trend to increase on frozen dough stored at 7 and 14 days. Proofed dough had higher water content than unproofed dough and it was proposed that moisture was absorbed during fermentation period in the proof cabinet (Czuchajowska et al 1989). The fermented dough showed higher liquid phase than unfermented dough resulting from water separation of gluten polymers during their extension (Räsänen et al 1997b). Räsänen et al (1997b) showed that shorter pre-fermentation time (25 vs. 40 min) and addition of commercial dough

conditioner (S-kimo containing wheat flour, gluten, glucose, ascorbic acid, and diacetyl tartaric acid ester of mono-diglycerides or DATEM) improved frozen dough quality.

I.3.2. Effect of Mixing Condition

Delaying yeast and salt addition during dough mixing step improves the stability of frozen dough. Delaying yeast incorporation during mixing minimized gas production before freezing and cause reduction of dough strength (Dubois and Blockcolsky (1986), Evenson (1987), and Neyreneuf and van der Plaat (1991). Mixing time has been reported to play an important role on dough and bread volume resulting from well developed gluten network (Rouillé et al 2000).

I.3.3. The Effect of Temperature

The rate of yeast fermentation affected by the temperature during fermentation in the proofer and early phase of baking. Oven spring during baking occurs rapidly due to function of additional CO_2 formation by yeast, expansion of gases (CO_2 and water vapor) and the driving out of dissolved CO_2 and alcohol. The specific contribution of yeast on oven spring has not been clarified (Reed and Nagodawithana 1991).

Van Uden (1971) reported that vegetable cells of baker's yeast are quickly killed at temperature exceeding 50°C. He showed that 95% cells were killed in 18 minutes at 50°C and in 6 minutes at 52°C. Garver et al (1966) reported that temperature of dough affected the maximum fermentation rate and the time period to

reach that rate. A 25% increase of fermentation rate was obtained when temperature was raised from 29°C to 33.5 °C.

I.3.4. Effect of Other Dough Processing Condition

The effect of sheeting-molding conditions and dough shape of frozen dough processing were studied by Gélinas et al (1995). These authors reported that sheeting-molding conditions had no significant effect on the frozen dough stability. The shape of the dough is also important. A ball shaped frozen dough shape produced lower bread volume than cylinder shape at 20 weeks of frozen storage at –18°C.

I.3.5. Effect of Freezing Rate and Temperature

There are two basic commercial freezing systems for frozen dough production: 1) cryogenic process using liquid nitrogen, and 2) mechanic refrigeration using air blast (El-Hady et al 1996). Ice crystal formed during freezing results in microstructural changes in frozen food. Large ice crystals are formed with slow freezing processes while a relative large number of small ice crystals are formed with rapid freezing. A rapid freezing rate provides more uniform ice crystals throughout the frozen materials that lead to a higher quality of frozen products (Reid 1990).

The freezing rate and storage temperature affect gassing activity of yeast. Yeasts can be killed by a fast freezing rate (Mazur and Schmidt 1968). Increasing freezing rate from 0.05 to 0.5°C/min reduced yeast activity (Lamb and Bender 1977). However the effect of freezing rate on dough stability was lower compared to the final

freezing temperature (Hsu et al 1979b). The levels of yeast damage varied at different temperature. Hsu et al (1979a) reported that slow dough freezing at -20°C was better than at -40°C. The same authors also showed that lower storage temperature than initial freezing temperature made frozen dough less stable. Weakening of dough with increased proofing time occurred after one week of frozen storage at -20°C (Inoue and Bushuk 1991). Dough frozen at high air velocity (3m/sec) at -20°C and after one-week storage gave higher yeast activity and bread quality compared to high (3m/sec) at -30°C and low (1m/sec) air velocity at -20°C (El-Hady et al 1996). Contrasting results reported by Havet et al (2000) who found that high air velocity (3m/sec) decreased baking performance. The same authors studied yeast activity and damage of gluten network associated with decreased baking performance of frozen dough at three different freezing rates (air speed 1, 2 and 3m/sec). Their results showed that there was a constant decreased in specific volume of frozen dough with increasing freezing rate (9% decreased at air speed 3m/sec compared to 2m/sec). They also concluded that freezing rate had a synergistic effect on both yeast activity and dough rheology and subsequent loaf volume.

I.3.6. Effect of Frozen Storage and Freeze Thaw Cycles

Godkin and Cathcart (1949) reported that bulk yeast could be frozen and thawed without loss or with minimal loss of fermenting activity. The commercial compressed yeast could be stored at 4°C up to 6 weeks without significant loss of gassing power (Wolt and D'Appolonia 1984a). Duration of frozen storage is also

important for frozen dough properties (Kline & Sugihara 1968, Meric et al 1997, Le Bail et al 1996b, and 1999). Both frozen storage and freeze thaw cycles affect extensibility and maximum resistance to extension (Rmax) properties of dough. Wolt and D'Appolonia (1984a) showed a reduction of the extensibility of yeasted and nonyeasted dough with frozen storage.

Extensigraph analysis reported by Inoue et al (1994) showed that Rmax decreased significantly after one day, at 70 days frozen storage and three freeze-thaw cycles. The authors found an increase in dough extensibility only at 70 days of frozen storage. They also reported a strong negative relationship between extensibility and gassing power ($r \geq -0.95$). The factors involved in weakening the dough might be related to differences in reducing sugars, protein solubility and changes in high molecular weight gluten oligomers of the doughs shown in electrophoretic patterns. They suggested that low reducing sugars content of 3T-F cycles dough resulted from the fermentation occurred during the repeated freezing and thawing. The changes in structure of gluten protein by repeated thawing and freezing were observed as increased protein solubility. In contrast, Kline and Sugihara (1968) suggested that the weakening of frozen storage of dough was partly caused by releasing reducing substances from dead yeast cells.

Compared to unfrozen dough, the loaf volume of bread made from frozen dough decreased after one and seven days of storage (El-Hady et al 1996). The rheological dough behavior changed with storage time but the most rapid changes were between the unfrozen dough and frozen dough after one day of storage. Lower bread volume of frozen dough was due to a decrease in gas production. Results of

Risograph analysis showed that the total gas production was reduced by 33.4% for the frozen dough after four weeks storage at -20 °C and 49.7% for the dough subjected to three freeze-thaw cycles (El-Hady et al 1996). They demonstrated that the most rapid change in rheological behavior was between fresh and one day frozen storage dough. Frozen dough stored up to 4 weeks could produce acceptable bread.

Brummer et al (1993), and Räsänen et al (1995, 1997a) reported that one day frozen dough provided similar bread quality to fresh or non-frozen dough. In contrast, El-Hady et al (1996) and Inoue et al (1994) reported that bread volume from one day frozen dough significantly reduced compared to fresh dough. Räsänen et al (1997a) showed that loaf volumes of frozen dough decreased after seven days of frozen storage. Dough frozen for up to 30 days showed similar fermentation properties to seven days frozen storage using maturograph but peak height slightly dropped. Dough stored for 90 days had an increased final proof time to near 100 min due to a decrease yeast viability observed by the release of CO_2 produced. Yeast viability decreased as the frozen storage time increased (Kline and Sugihara 1968, Inoue et al 1994).

Many reports confirmed that freeze-thaw resistance of yeast was partly related to the presence of trehalose, a non-reducing disaccharide of glucose with cryoprotective properties (Oda et al 1986, Uno 1986, and van der Plaat 1988). Neyreneuf and van der Plaat (1991) also supported that the high trehalose content (17%) in yeast imparted a resistance to freezing. Freeze-thaw damage was caused mainly due to fermentation of the dough before freezing. Fluctuation of freezing temperature and prolonged thawing were harmful to yeast. However, the dough with

high trehalose content caused moderate damage in yeast by pre-fermentation (Dunas 1991).

Temperature fluctuations during storage and storage time were important factors influencing yeast activity (Le Bail et al 1999) and rheology of frozen dough (Berland 1993). Le Bail et al (1999) found that fluctuation in temperature during frozen storage resulted in significant differences in bread volume. The small temperature fluctuations (±0.4°C) caused 6.7% reduction of dough volume after 37 days of frozen storage. Large temperature fluctuations of freezer by exposing to room temperature reduced 48% of dough volume after 37 days of storage. These authors suggested that a formation of ice crystals during temperature fluctuation of frozen storage affected either yeast activity or gas retention of the dough.

Laaksonen and Roos (2000) studied glass transitions occurring in frozen dough at sub-zero temperature. The glass transition of frozen dough occurred below -30°C. Thus, common freezer temperatures (-20°C) would not maintain the glassy state in dough during frozen storage. Therefore, at the storage temperature above -30°C, the dough structure is an unsteady state where the rate ice crystals formation can induce changes.

II. Loss of Dough Strength

The loss of dough strength or dough weakening and diminished gas-holding properties are due to changes in rheological properties of the thawed dough. The changes in rheological properties of thawed dough were caused by many factors including disruption of the gluten network due to ice crystal damage of the three-

dimensional network (Varriano-Marston et al 1980), and releasing of glutathione, reducing substances from dead yeast cells (Kline and Sugihara 1968).

The loss of dough strength has been studied by dough extensibility measurements of large deformations, small deformation rheological analysis with dynamic rheometer, protein solubility, and protein composition using SDS-PAGE. Major changes in frozen doughs are related to the releasing of reducing agents from dead yeast cells which weaken the gluten network resulting in poor gas retention and longer proof time (Kline and Sughiara 1968, Hsu et al 1979a, b). The rheological changes of dough are associated with an altered relaxed stage of film formed by the gluten matrix. The addition or excess of reducing reagent such as glutathione in the dough interfered with gluten disulfide formation (Eliasson 1990). Some investigators suggested that the weakening of gluten network was due to ice crystals formation and not due to reducing agents from dead yeast cells (Varriano-Marston et al 1980, Wolt and D'Appolonia 1984a, and Autio and Sinda 1992).

II.1. Effect of Water

Water is an important component and plays a significant role in yeast activity and in the control dough temperature in frozen dough. The ratio of water to flour and other ingredients is important in dough processing and rheological properties of the dough. The optimum water level of dough is different for each flour type, and dough formula, such as for conventional bread and frozen dough bread.

Freezing separates water from dough as a result of ice crystal formation below 0°C. As water is removed and formed ice, the frozen food forms an unfrozen phase by

freeze-concentration of solutes (Franks 1985, Blanshard and Franks 1987, Roos and Karel 1991a, b, c, and Goff 1992). The maximum formation of ice crystal is controlled by the glass transition of the unfrozen phase (Levine and Slade 1988, Roos 1998). As the glass transition controls rates of recrystallization of ice and diffusion-controlled reactions, the glass transition of frozen dough and its components such as starch and gluten may affect the stability of frozen dough (Levine and Slade 1988).

The formation of ice crystals in yeast and gluten network during freezing and frozen storage and its effect on the quality of baked products has been reported (Kline and Sugihara 1968, Varriano-Marston et al 1980, Burglund et al 1991). Berglund et al (1991) indicated that after 24 weeks of frozen storage there was less free water distributed throughout the frozen dough and more ruptured gluten network causing poor gas retention and reduced loaf volume.

A reduction of 2% water in the frozen dough formula from the optimum water of normal bread dough recipes improved bread quality (Lorenz 1974, Brummer et al 1993; El-Hady et al 1996). The bread made from frozen dough with reduced water content had higher loaf volumes and better porosity than those of optimum water content. The optimum water content for frozen dough was lower than fresh dough and unique for different flour types (Räsänen et al 1997a). Räsänen et al (1997a) also reported that fermentation properties using maturograph test of the dough with optimum water content showed decrease of CO_2 production during the first week of frozen storage and remained essentially at the same level at two weeks of storage. But the dough with reduced water content showed a significant decrease of CO_2 production at two weeks of storage. The authors concluded that a reduction of water content by

2% from the optimum level increased elasticity and rigidity of frozen dough but decreased slightly loaf volume. The dough with reduced water showed a decreased porosity of frozen dough compared to those with optimum water. The decrease porosity was due to thicker walls around the air bubbles and smaller number of large cells. More elastic dough with thick walls was required for withstanding freezing and frozen storage compared to viscous and fragile dough. However, the effect of decreased water addition in frozen dough on dough peak height was unique for different flour types. El-Hady et al (1996) suggested that the effect of lower water addition could be related to the amount of freezable water and not to the effect of ice in yeast cells and gluten network.

Eliasson and Larsson (1993) described a method for phase-separation of flour dough by ultracentrifugation. Dough was separated into two phases, a water-swelled protein phase (gluten) and a liquid phase (solubles and dispersed starch granules). This simple technique was useful to relate water in the dough phase and dough rheological measurements. The separation of the two phases was obtained when the water content of dough was high enough to show a gluten phase (Larsson and Eliasson 1996a). Räsänen et al (1997b) studied the amount of liquid phase of prefermented frozen dough and showed that frozen storage increased the amount of liquid phase and decrease storage modulus of water-flour mixtures. The most significant change occurred during the first week of frozen storage might be due to the negative effect of ice crystal formation. They also reported that reduced water content of the dough showed a smaller liquid phase and high rigidity (G') after frozen storage. Räsänen et al (1995, 1997a) reported that shorter pre-fermentation time and reduced water content

of the frozen dough prevented physical changes in pore structure. Small pore sizes and thick walls of air cells of this dough could withstand freezing and retained their shape during thawing. However, there was no correlation between the amount of liquid phase and total water content of the dough. The phase separation appeared to be related to the rheological properties of the dough. The more viscous dough gave better separation and more liquid phase (Räsänen et al 1997b). The same authors showed that autoradiography with tritiated (^{3}H) labeled water was a valuable method to analyze the changes in the distribution of macroscopic water in frozen dough. The autoradiographs showed distribution of small air bubbles and pore size in the dough and had a good correlation with baking results.

Other methods are used for testing water distribution in the dough includes scanning electron microscope using cryo-stage (Gan et al 1990) and freezable water using a differential scanning calorimeter (DSC) (Lu and Grant 1999a). The latter authors indicated that the amount of freezable water changed at the initial freezing and subsequent frozen storage of dough. The rate of the change of the amount of freezable water varied in wheat cultivars and it was influenced by protein quality and quantity. A large increase in the amount of freezable water occurred in the dough from initial freezing up to 8 weeks storage and began to decline slowly until 16 weeks of frozen storage. The highest protein content wheat showed an amount of freezable water up to 16 weeks. They explained that high water binding in high protein dough was continuously liberated from the gluten structure as the frozen storage progressed.

II.2. Effect of Flour Quality

Marston (1978) recommended a medium to strong gluten flour for frozen dough products. High-quality protein flour is more important than protein quantity and more critical for frozen dough (Wolt and D'Appolonia 1984a,b, and Inoue and Bushuk 1992). Inoue and Bushuk (1992) reported that overly strong wheat flour, unsuitable for conventional bread making, performed better than strong flour in frozen dough. The extra strength was needed to maintain high oven spring during baking even after losing dough strength during freezing and frozen storage. Strong flour had a small decrease in loaf volume. Frozen dough showed a sharp decrease in maximum resistance after initial freezing (one day of frozen storage) and gradually decreased during frozen storage. However, the rate of decrease of maximum dough resistance using extensigraph depended on flour strength. The gassing power of frozen dough was similar to nonfrozen control dough during the first two weeks of frozen storage but significantly decreased after six weeks (Inoue and Bushuk 1992). Räsänen et al (1997a) found a large change in peak height of dough (using maturograph) during the first week of frozen storage and remained almost constant up to 2 weeks. The changes in rheological properties supported the baking performance. High deterioration in baking quality occurred during the first week of frozen storage and the percentage of change in loaf volume of frozen dough varied in different flours. The authors indicated that freeze stability of flours could not be predicted according to traditional flour analysis such as protein content, ash content, falling number, wet gluten, farinograph and extensigraph analysis of fresh dough. Their earlier work (Räsänen et

al 1995) showed that flour with a small ratio of water solubles to wet gluten was more resistant to changes of freezing and thawing. This ratio had a low correlation with loaf volumes of partly fermented frozen dough. The same authors concluded that the baking quality of frozen partly pre-fermented doughs was more dependent on process conditions than on flour properties. Some flour types may require more than 2% water reduction to improve the quality of frozen dough. Thus, flour types relate to the amount of water added in the dough formula and affect frozen dough properties.

Lu and Grant (1999b) reported that the exchange of fractionated starch, water soluble, gliadin and glutenin components of strong flour into weak flour resulted in better baking quality of frozen dough. The gliadin and starch fraction improved frozen dough quality but not as much as glutenin while minimal contribution of water-soluble fractions was observed.

Perron et al (1999) demonstrated that the baking quality of 16 weeks frozen dough improved by blending base flour with various cultivars up to 50% to 75% levels. The evaluations of the performance of specific wheat cultivars blends included loaf volume, loaf appearance, crumb structure and proofing requirements. They also concluded that it was difficult to relate the inherent mixing dough strength of various cultivars to frozen-dough baking quality.

II.3. Effect of Glutathione

Glutathione is a disulfide reducing agent released from dead yeast cells (Kline and Sugihara 1968). In fresh wheat dough, glutathione reacts as a reducing agent and is able to breakdown gluten network, rupturing disulfide cross-links in gluten by

SH/SS interchange. Berland and Launay (1995) demonstrated that small concentrations of glutathione (15 or 30 ppm) had no detectable effects on dough rheological properties using dynamic tests with control stress rheometer. Glutathione at higher concentrations (50 to 150 ppm) decreased storage (G') and loss (G") moduli and produced weak dough. They explained that low concentrations of glutathione added to the fresh dough reduced some disulfide bonds but the mean molecular weight of glutenins would not be sufficiently reduced and no significant change in dough structure had occurred. In theory, higher concentrations of glutathione added would reduce the size of glutenins and affect dough rheological properties by modifying its structure. However, the role of glutathione on the baking performance of frozen dough has been studied by various investigators but there is no agreement on its effect.

Wolt and D'Appolonia (1984a) demonstrated that the leaching of glutathione was not responsible for the rheological changes of dough during frozen storage. Autio and Sinda (1992) reported that the rheological changes in frozen and thawed dough did not relate to reducing substances from dead yeast. They showed that addition of dead yeast (0.17 and 0.33% of dough) did not affect relaxation time of the doughs after freezing and thawing, but the addition of 100 ppm reduced glutathione substantially decreased relaxation time of the doughs. They suggested that glutathione caused a reduction in gluten.

II.4. Effect of Starch Characteristics

Gluten protein and starch control the rheology of fresh dough (Medcalf 1968). Lindahl and Eliasson (1986) showed the effect of gelatinized starches from different

wheat species on the rheological properties of dough. He and Hoseney (1991a, 1992) showed that isolated gluten-water dough and dough made from flours of different baking quality had different rheological properties. They concluded that the differences in rheological properties of gluten-water dough and flour dough were caused by starch-gluten interactions and these interactions might be responsible for the differences in baking quality. Petrofsky and Hoseney (1995) confirmed the earlier reports that the dough made from starches isolated from different wheat cultivars mixed with a constant-gluten rate gave significant differences in rheological properties.

Freezing and thawing of frozen dough caused a decrease G' modulus or elastic behavior, increase tan δ of frozen dough and delayed starch gelatinization (Autio and Sinda 1992). The authors suggested that these processes might involve the loss of polymer cross-linking, weakening of the gluten network and separation of starch granules from the gluten network as reported by Berglund et al (1991). Wolt and D'Appolonia (1984b) found that the starch characteristics in bread crumbs changed with frozen storage. The amount of soluble starch extracted from bread crumb and both amylose and amylopectin content in the soluble starch decreased as frozen storage time increased.

The gelatinization temperature of starch depends on crystallinity in the granule, total moisture content and moisture distribution (Levine and Slade 1990). Berglund et al (1991) suggested that freeze-thaw cycles drew water out from gluten matrix. Less water associated with the gluten matrix and starch resulted in more free water separated and pooled into large ice crystals. The increased onset temperature of starch

gelatinization during freezing and thawing might be associated with less water in starch, a delay in the diffusion of water into starch granules or the increased crystallinity of starch granules. The mentioned factors can cause rheological changes in frozen dough (Autio and Sinda 1992).

II.5. Effect of Other Dough Additives

Additives such as dough improvers containing oxidants, surface active agents, enzymes, etc, can offset deterioration of frozen dough quality after several weeks of frozen storage. Kline and Sugihara (1968) reported that bromate improved loaf volume of frozen dough after five weeks of storage but longer proofing time was required. However, bromate decreases gassing power of yeast. The bromate levels of 20-30 ppm offered the best combination of proofing time and bread volume. ADY might be more susceptible to the effect of bromate compared to CY (Kline and Sugihara 1968).

Addition of surface-active agents such as sodium stearoyl-2-lactylate (SSL), diacetyl tartaric acid ester of monoglycerides (Marston 1978, Varriano-Marston et al 1980, Davis 1981; Wolt and D'Appolonia 1984b), and oxidants (Lorenze and Bechtel 1965, Varriano-Marston et al 1980, Wolt and D'Appolonia 1984a) improved finished products made with frozen doughs. The use of oxidants such as ascorbic acid (AA) in combination with enzymes (α-amylase with hemicellulase activity) in frozen dough affected the sulfhydryl groups of gluten protein and improved quality of frozen dough bread (De Stefanis 1995, Faisy and Neyreneuf, 1996, and Rouille et al 2000). El-Hady et al (1999) showed that frozen dough contained AA alone or AA with potassium

bromate or SSL gave higher gas production and higher dough height during frozen storage than those without AA. Maximum resistance to extension of frozen dough containing AA alone or AA with potassium bromate was greater than those of unfrozen dough. The authors concluded that the use of AA or AA with potassium bromate or with SSL improved baking and rheological properties of frozen dough and provided acceptable volume of bread for up to 3 weeks. Rouille et al (2000) reported that AA and 7 days frozen storage time did not have a significant effect on the specific volume of bread. However, AA significantly increased specific volume of bread as increase mixing time and speed of mixing. These authors concluded that the effect of AA on frozen dough depended on the flour and type of mixer.

Commercial dough additives have been used to improve the baking quality of frozen dough. Räsänen et al (1997b) showed that the commercial dough conditioner (S-kimo) composed of wheat flour, gluten, glucose, AA, and diacetyl tartaric acid ester of mono-diglycerides (DATEM), affected the rheological properties and the amount of liquid phase of frozen dough. The S-kimo with shorter prefermentation time (25 min) improved the water distribution of the prefermented doughs. The dough contained small ice crystals and no large water patches in thawed dough shown by autoradiographs after frozen storage. S-kimo improved dough-mixing properties and its capacity to bind water. When the water binding properties of dough increased, the amount of free water in the number of ice crystals decreased.

Nonami et al (1984) reported that egg yolk improved the overall quality of frozen dough bread. Wakamatu et al (1983) found that the gelation of low-density lipoprotein (LDL) solution from egg yolk containing 1-10% NaCl was inhibited at –

20°C. LDL-water-NaCl complex increased the proportion of unfrozen water. Addition of egg yolk alone, sugar ester and sugar ester plus egg yolk decreased freeze damage to frozen dough (Hosomi et al 1992). These additives improved oven spring and gave higher loaf volume up to three weeks of frozen storage due to a lower decrease in gassing power and increase gas retention of the dough compared to control. Yeast cells were partially protected from damage during freezing and frozen storage, while dough membranes were stronger by increasing surface membrane tension resulting in less gas leakage.

In summary, the frozen dough quality could be preserved with managing and optimizing the process, levels of water, yeast and use of additives.

TABLE I

Composition, Shelf Life and Fermentation Activity of Commercial Yeast Products[1]

			Active Dry Yeast		
	Compressed Yeast	**Cream Yeast**	**Regular**	**Protected**	**Instant**
Moisture, %	67.0-72.0	82	7.5-8.3	4.5-6.5	4.5-6.0
Protein, dry basis, %	60	60	38-48	40-42	39-41.5
Shelf life					
Refrigerated (2°-4.5°C)	3-4wk	3-4wk	6 mo[a] 1 yr[b]	9 mo[a]	1yr plus[b]
Room temp (21°C)	perishable	perishable	3 mo[a] 1 yr[b]	6 mo[a]	1 yr[b]
Fermentation activity[c]					
in regular doughs[d]	24.5-26.1	-	15.8-17.4	15.8-7.4	17.9-20.5
in sweet doughs[e]	10.9-12.5	-	9.2-10.0	9.2-10.0	9.2-10.0
in lean doughs[f]	25.9-28.8	-	13.6-14.3	13.6-14.3	20.5-21.9

[1] Source: From Sanderson et al 1983 and Trivedi et al 1989.
[a] In drum or bags, not packaged under vacuum or inner atmosphere.
[b] Packaged under vacuum or inner atmosphere.
[c] In mM CO_2 produced per g of yeast solids per hr.
[d] 4-12% sugar added.
[e] 15-25% sugar added.
[f] No sugar added.

LITERATURE CITED

Autio, K., and Sinda, E. 1992. Frozen doughs: Rheological changes and yeast viability. Cereal Chem. 69:409-413.

Berglund, P. T.; Shelton, D. R., and Freeman, T. P. 1991. Frozen bread dough ultrastructure as affected by duration of frozen storage and freeze-thaw cycles. Cereal Chem. 68:105-107.

Berland, S. 1993. Etude rhéologique et calorimétrique de l'influence de traitements de congélation-décongélation sur les propriétés des pâtes de farine de blé Répercussion en technologie boulangère. Thèse de Doctorat, Université Paris 11, ENSIA-Massy. France.

Berland, S., and Launay, B. 1995. Rheological properties of wheat flour dough in steady and dynamic shear: effect of water content and some additives. Cereal Chem. 72:48-52.

Blanshard, J. M. V., and Franks, F. 1987. Ice crystallization and its control in frozen-food systems. Pages 51-65 in: Food Structure and Behavior. J. M. V. Blanshard, and P. Lillford, eds. Academic Press: London.

Bruemmer, J. M., Neumann, H., and Morgenstern, G. 1993. Freezing dough pieces for wholemeal wheat rolls. Tiefkuehlung von Teiglingen fuer Weizenvollkornbroetchen. Getreide, Mehl Und Brot. 47:24-28.

Bruinsma, B. L., and Giesenschlag, J. 1984. Frozen dough performance. Compressed yeast-instant dry yeast. Baker's Dig. 58:6-7, 11.

Czuchajowska, Z., Pomeranz, Y, and Jeffers, H. C. 1989. Water activity and moisture content of dough and bread. Cereal Chem. 66:128-132.

D'Appolonia, B. L., Gilles, K. A., Osman, E. M., and Pomeranz, Y. 1971. Carbohydrates. In Wheat Chemistry and Technology. Y. Pomeranz, 2nd. ed. American Association of Cereal Chemists: St. Paul, MN.

Davis, E. W. 1981. Shelf-life studies on frozen doughs. Bakers' Dig. 55(3):12-13, 16.

De Stefanis, V. A. 1995. Functional role of microingredients in frozen dough. Pages 91-117 in: Frozen and Refrigerated Doughs and Batters. K. Kulp, K. Lorenz, and J.-M. Brümmer, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

Dijck, P.-van, Gorwa, M. F., Lemaire, K., Teunissen, A., Versele, M., Colombo, S., Dumortier, F., Pingsheng, Ma., Tanghe, A., Loiez, A., and Thevelein, J. M. 2000. Characterization of a new set of mutants deficient in fermentation-induced loss of stress resistance for use in frozen dough applications. International J. of Food Microbiology. 55(1-3):187-192.

Dubois, D. K., and Blockcolsky, D. 1986. Frozen bread dough. Effect of dough mixing and thawing methods. AIB Tech. Bull. Vol. VIII, No. 6. American Institute of Baking, Manhattan, KS.

Dunas, F. E. T. 1991. Yeast in frozen bread doughs. Dissertation Abstracts International, -C. 52(4)535:ISBN 91-628-0256-9.

El-Hady, E. A., El-Samahy, S. K., Seibel, W., and Brümmer, J. M. 1996. Changes in gas production and retention in non-prefermented frozen wheat doughs. Cereal Chem. 73:472-477.

El-Hady, E. A., El-Samahy, S. K., and Brümmer, J. M. 1999. Effect of oxidants sodium-stearoyl-2-lactylate and their mixtures on rheological and baking properties of nonprefermented frozen doughs. Lebensmittel Wissenschaft and Technologie. 32(7):446-454.

Eliasson, A. C. 1990. Adsorption of wheat proteins on wheat starch granules. Cereal Chem. 67:366-372.

Eliasson, A. C., and Larsson, K. 1993. Physicochemical behavior of the components of wheat flour. In: Cereals in breadmaking. Marcel Dekker: New York.

Evenson, M. 1987. New developments in frozen dough technology. Pages 85-89 in: Proc. 63rd Annual Meeting of the American Society of Baking Engineers. The Society: Chicago, IL.

Faisy, F., and Neyreneuf, O. 1996. Performance d'une association enzymatique "Glucose oxydase-Hémicellulases" pour remplacer l'acide ascorbique en panification. Industrie des Céréales. Avril. Mai-Juin:4-12.

Franks, F. 1985. Complex aqueous systems at subzero temperatures. Pages 497-509 in Properties of Water in Food, D. Simatos and J. L. Multon, eds. Marunus Nijhoff Publishers:Netherlands.

Friedemann, T. E., Witt, N. F., and Neighbors, B. W. 1967. Determination of starch and soluble carbohydrates. I. Development of method for grains, stock feeds, cereal foods, fruits and vegetables. J. Assoc. Office. Anal. Chem. 50:945.

Gan, Z, Angold, R. E., Williams. M. R., Ellis, P. R., Vaughan. J. G., and Galliard, T. 1990. The microstructure and gas retention of bread dough. Journal of Cereal Science 12(1):15-24.

Garver, J. C., Navarine, I, and Swanson, A. M. 1966. Factors influencing the activation of baker's yeast. Cereal Sci. Today. 11:410-418.

Gélinas, P., Fiset, G., LeDuy, A., and Goulet, J. 1989. Effect of growth conditions and trehalose content on cryotolerance of bakers' yeast in frozen doughs. Appl Environ Microbiol. 55:2453-2459.

Gélinas, P., Lagimoniere, M., and Dubord, C. 1993. Baker's yeast sampling and frozen

dough stability. Cereal Chem. 70:219-225.

Gélinas, P., Lagimoniere, M., and Rodrigue, N. 1994. Performance of cream or compressed yeast in frozen and nonfrozen doughs. Cereal Chem. 71:183-186.

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

Godkin, W. J., and Cathcart, W. H. 1949. Fermentation activity and survival of yeast in frozen fermented and unfermented doughs. Food Technol. 3:139-146.

Goff, H. D. 1992. Low-temperature stability and the glassy state in frozen foods. Food Research International. 25(4):317-25.

Havet, M., Mankai, M., and Le Bail, A. 2000. Influence of the freezing condition on the baking performances of French frozen dough. J. of Food Engineering. 45(3):139-145.

He, H., and Hoseney, R. C. 1991a. Gluten, a theory of how it controls bread making quality. Chap. 1 in: Gluten Proteins 1990. W. Bushuk and R. Tkachuk eds. Am. Assoc. Cereal Chem. St. Paul, MN.

He, H., and Hoseney, R. C. 1991b. Differences in gas retention, protein solubility, and rheological properties between flours of different baking quality. Cereal Chem.68:526-530.

He, H., and Hoseney, R. C. 1992. Factors controlling gas retention in nonheated doughs. Cereal Chem. 69:1-6.

Hino, A., Takano, H., and Tanaka, Y. 1987. New freeze-tolerant yeast for frozen dough preparations. Cereal Chem. 64:269-275.

Hoseney, R. C., Zeleznak, K., and Lai, G. S. 1986. Wheat gluten: a glassy polymer. Cereal Chem. 63:285-286.

Hosomi, K., Nishio, K., and Matsumoto, H. 1992. Studies on frozen dough baking. I. Effects of egg yolk and sugar ester. Cereal Chem. 69:89-92.

Hsu, K. H., Hoseney, R. C., and Seib, P. A. 1979a. Frozen dough. I. Factors affecting stability of yeasted doughs. Cereal Chem. 56:419-424.

Hsu, K. H., Hoseney, R. C., and Seib, P. A. 1979b. Frozen dough. II. Effects of freezing and storing conditions on the stability of yeasted doughs. Cereal Chem. 56:424-426.

Inoue, Y., and Bushuk, W. 1991. Studies on frozen doughs. I. Effects of frozen storage and freeze-thaw cycles on baking and rheological properties. Cereal Chem. 68:627-631.

Inoue, Y., and Bushuk, W. 1992. Studies on frozen doughs. II. Flour quality requirements for bread production from frozen dough. Cereal Chem. 69:423-428.

Inoue, Y., Sapirstein, H. D., Takayanagi, S., and Bushuk, W. 1994. Studies on frozen doughs. III. Some factors involved in dough weakening during frozen storage and thaw-freeze cycles. Cereal Chem. 71:118-121.

Inoue, Y., Sapirstein, H. D, and Bushuk, W. 1995. Studies on frozen doughs. IV. Effect of shortening systems on baking and rheological properties. Cereal Chem. 72:221-225, 228.

Jacobson, G. K., and Trivedi, N. B., inventors; 1987. Improved yeast strains, method of production and use in baking.

Javes, R. 1971. The ingredients and the processes. Effect on shelf life of frozen, unbaked yeast-leavened dough. Baker's Dig. 45(2):56-59.

Kline, L., and Sugihara, T. F. 1968. Frozen bread doughs. I. Prepared by the straight dough method. Baker's Dig. 42(5):44-50.

Laaksonen, T. J., and Roos, Y. H. 2000. Thermal, dynamic-mechanical and dielectric

analysis of phase and state transitions of frozen wheat doughs. J. of Cereal Sci. 32(3):281-292.

Lamb, J. and Bender, L.D. 1977. Freezing without killing-The priority for research. Baking Ind. J. 10(1):19.

Langejan, A., and Khoudokormoff, B. 1976. High protein active dry baker's yeast. U.S. Patent 3,993,782,11-23.

Larsson, H., and Eliasson, A. C. 1996a. Phase separation of wheat flour dough studied by ultracentrifugation and stress relaxation. I. Influence of water content. Cereal Chem. 73:18-24.

Larsson, H., and Eliasson, A. C. 1996b. Phase separation of wheat flour dough studied by ultracentrifugation and stress relaxation. I. Influence of mixing time, ascorbic acid, and lipids. Cereal Chem. 73:25-31.

Le Bail, A., Harvet, M., Pasco, M., and Chourot, J. M. 1996a. Application of freezing rate expressions and gassing power to frozen bread dough. Pages 9-13 in: Proceedings of The ASME Congress. Vol. 34. HTD/BED: Atlanta.

Le Bail, A., Pasco, M., Meric, L., and Cahagnier, B. 1996b. Influence of freezing rate on yeast activity in frozen bread dough. In: The 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., and Quilin, E. 1999. Influence of storage conditions on frozen French bread dough. J. of Food Engineering. 39:289-291.

Legman, R., and Margalith, P. Z. 1983. Interspecific protoplast fusion of *S. cerevisiae* and *S. mellis*. Eur. J. Appl. Microbiol. Biotechnol. 18:320-322.

Levine, H., and Slade, L. 1988. Water as plasticizer: physicochemical aspects of low-moisture polymeric systems. Pages 79-86 in: Water Science Reviews. Vol 3. Water dynamics. F Franks, eds. Cambridge University Press: New York.

Levine, H., and Slade, L. 1990. Influences of the glassy and rubbery states. Pages 157-331 in: Dough Rheology and Baked Product Texture: Theory and practice. H. Faridi, and J. M. Faubion, eds. Van Nostrand Reinhold/AVI: New York.

Lindahl, L., and Eliasson, A. C. 1986. Effects of wheat proteins on the viscoelastic properties of starch gels. J. Sci. Food Agric. 37:1125-1132.

Lorenz, K., and Bechtel, W. G. 1965. Frozen bread dough. Baker's Dig.39(4):53-59.

Lorenz, K. 1974. Frozen dough. Bakers' Dig. 48(2):14-15, 18-19, 22, 30.

Lu, W., and Grant, L. A. 1999a. Role of flour fractions in breadmaking quality of frozen dough. Cereal Chem. 76:663-667.

Lu, W., and Grant, L. A. 1999b. Effects of prolonged storage at freezing temperatures on starch and baking quality of frozen doughs. Cereal Chem. 76:656-662.

Marston, P. E. 1978. Frozen dough for breadmaking. Bakers' Dig. 52(5):18-20, 37.

Mazur, P., and Schmidt, J. J. 1968. Interactions of cooling velocity, temperature, and warming velocity on the survival of frozen and thawed yeast. Cryobiology 5:1-17.

Medcalf, D. G. 1968. Wheat starch properties and their effect on bread baking quality. Baker's Dig. 42(4):48-50, 52, 65.

Meric, L., Lambert, G. S, Neyreneuf, O., and Richard, M. D. 1995. Cryoresistance of baker's yeast Saccharomyces cerevisiae in frozen dough: contribution of cellular trehalose. Cereal Chem. 72:609-615.

Meric, L., Lambert-Guilois, S., Neyreneuf, O., and Richard-Molard, D. 1997. Cryoresistance of Baker's yeast *Saccharomyces cerevisiae* in frozen dough:

contribution of cellular trehalose. Cereal Chem. 72:609-615.

Merritt, P. P. 1960. The effect of preparation on the stability of frozen unbaked, yeast leavened doughs. Baker's Dig. 34(4):57-61.

Neyreneuf, O., and van der Plaat, J. B. 1991. Preparation of frozen French bread dough with improved stability. Cereal Chem. 68:60-66.

Nonami, Y., Saito, M., Shikano, K., Modeki, F., and Suzuki, A. 1984. Effects of chicken egg yolk on the ultrastructure and loaf volume of white bread. Nippon Shokuhin Hogyo Gakkaishi. 31:570-576.

Oda, Y., Uno, K., and Ohta, S. 1986. Selection of yeasts for breadmaking by the frozen-dough method. Appl Environ Microbiol. 52(4):941-943.

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

Petrofsky, K. E., and Hoseney, R C. 1995. Rheological properties of dough made with starch and gluten from several cereal sources. Cereal Chem. 72:53-58.

Räsänen, J., Harkonen, H., and Autio, K. 1995. Freeze-thaw stability of prefermented frozen lean wheat doughs: effect of flour quality and fermentation time. Cereal Chem. 72:637-42.

Räsänen, J., Laurikainen, T., and Autio, K. 1997a. Fermentation stability and pore size distribution of frozen prefermented lean wheat doughs. Cereal Chem. 74:56-62.

Räsänen, J., Blanshard, J. M. V., Siitari Kauppi, M., and Autio, K. 1997b. Water distribution in frozen lean wheat doughs. Cereal Chem. 74:806-13.

Reed, G., and Peppler, H. J. 1973. Use of yeast in baking. Pages 103-164 in: Yeast Technology. AVI Publishing Co.: Westport, Conn.

Reed, G., and Nagodawithana, T. W. 1991. Use of yeast in baking. Pages315-368 in: Yeast Technology, 2nd eds. Van Nostrand Reinhold/AVI: New York.

Reid, D. S. 1990. Optimizing the quality of frozen foods. Food Technology. 44:78-82.

Roos, Y. H. and Karel, M. 1991a. Phase transitions of amorphous sucrose and frozen sucrose solutions. J. of Food Sci. 56:266-267.

Roos, Y. H. and Karel, M. 1991b. Nonequilibrium ice formation in carbohydrate solutions. Cryo-Letters. 12:367-376.

Roos, Y. H. and Karel, M. 1991c. Amorphous state and delayed ice formation in sucrose solution. International Journal of Food Sci and Tech. 26:553-566.

Roos, Y. H. 1998. Role of water in phase transition phenomena in foods. Pages 57-86 in: Phase/State Transitions in Foods, M. A. Rao and R. W. Hartel, eds. Marcel Dekker: New York.

Rouillé, J., Le-Bail, A., and Corcoux, P. 2000. Influence of formulation and mixing conditions on breadmaking qualities of French frozen dough. J. of Food Engineering 43(4):197-203.

Rubenthaler, G. L., Finney, P. L., Dermaray, D. E., Finney, K. F. 1980. Gasograph: Design construction and reproducibility of a 12 channel gas recording instrument. Cereal Chem.57:212-216.

Sanderson, G. W., Reed, G., Bruinsma, B., and Cooper, E. J. 1983. Yeast fermentation in bread making. Am. Inst. Baking, Res. Dept. Tech. Bull. 5(12).

Sasaki, T., and Oshima, Y. 1987. Induction and characterization of artificial diploids from the haploid yeast *Torulaspora delbrueckii*. Eur. J. Appl. Microbiol. Biotechnol. 53(7):1504-1511.

Shogren, M. D., Finney, K. F., and Rubenthaler, G. L. 1977. Note on the determination of gas production. Cereal Chem. 54:665-668.

Shuey, W. C. 1975. Practical instruments for rheological measurements on wheat products. Cereal Chem. 52:42r-81r.

Takano, H., Shima, J., Iyo, C., Mori, K., Suzuki, Y., Nakajima, R., and Watanabe, H. 1999. Frozen dough-resistant and high-sugar dough-resistant, practical bakers' yeast. European Patent Application.

Tanaka, Y., Kawakami, K., and Takano, H. 1980. Effect of pre-storage on freeze-tolerance of compressed bakers' yeast in frozen dough. Report-of-the-National-Food-Research-Institute-[Shokuryo-Kenkyusho-Kenkyu-Hokoku]. 36:64-71.

Tang, R. T., Robert, J., Robinson, J., and Hurley, W. C. 1972. Quantitative changes in various sugar concentrations during bread making. Baker's Dig. 46(4):48-55.

Tanghe, A., Teunissen, A., Dijck, P.-van, and Thevelein. J. M. 2000. Identification of genes responsible for improved cryoresistance in fermenting yeast cells. International J. of Food Microbiology. 55(1-3):259-262.

Trivedi, N., Hauser, J., Nagodawithana, T., and Reed, G. 1989. Update on baker's yeast. Am. Inst. Baking Technol. Bull. 11(2):1-5.

Uno, K., Oda, Y., Shigenori, O. 1986. Freeze resistant dough and novel microorganisms for use. European patent application no. 86302275.2.

van Dam, H. 1986. The biotechnology of baker's yeast: old or newbusiness?. Pages 117-131 in: Chemistry and Physics of baking. J. M.. Blanshard , P. J. Frazier, and T. Galliard, ed. Royal Society of Chemistry, London, England.

van der Plaat, J. B. 1988. Baker's yeast in frozen dough. State of the art. Pages 110-129 in: Cereal Science and Technology in Sweden. N. G. Asp, ed. University of Lund, Lund, Sweden.

Van Uden, N. 1971. Kinetics and energetics of yeast growth. In: The Yeasts, Vol. 2, A. H. Rose and J. S. Harrison, ed. Academic Press: NewYork.

Varriano-Marston, E., and Hsu, K. H, and Mahdi, J. 1980. Rheological and structural changes in frozen dough. Bakers' Dig. 54(1):32-34, 41.

Wada, Y., Hitokoto, S., Hamada, K., Ando, M., and Suzuki, Y., inventors; 1999. Instant dry yeast for use in frozen dough-baking process. European Patent Application.

Wakamatu, T., Sato, Y., and Saito, Y. 1983. On sodium chloride action in gelation process of low-density lipoprotein (LDL) from hen egg yolk. J. Food Sci. 48:507-512, 516.

Wolt, M. J., and D' Appolonia, B. L. 1984a. Factors involved in the stability of frozen dough. I. The influence of yeast reducing compounds on frozen-dough stability. Cereal Chem. 61:209-212.

Wolt, M. J., and D' Appolonia, B. L. 1984b. Factors involved in the stability of frozen dough. II. The effects of yeast type, flour type, and dough additives on frozen-dough stability. Cereal Chem.61:213-221.

Zaehringer, M. V., and Mayfield, H. L., and Odland, L. M. 1951. The effect of certain variations in fat, yeast, and liquid on the frozen storage of yeast doughs. Food Research. 16:353-359.

CHAPTER III

THE EFFECTS OF A DOUGH CONDITIONER AND METHYLCELLULOSE ON DOUGH AND BAKING PROPERTIES OF PRE-PROOFED FROZEN DOUGH

J. Uriyapongson, and P. Rayas-Duarte

ABSTRACT

The effects of 1.5% commercial dough conditioner (CDC), 1% methylcellulose (MC) and a combination of 1.5% CDC and 1% MC on fresh and frozen dough (1 day to 12 weeks of frozen storage) were studied using two commercial flours (hard red spring wheat, HRS, and hard red winter wheat, HRW). Baking quality and dough behavior was measured using a Rheofermentometer.

Freezing decreased specific volume of all doughs when comparing fresh vs 1 day of frozen storage. Addition of MC and CDC+MC significantly increased specific volume in both flours. Addition of MC improved crust score of bread sticks of fresh and frozen dough for HRS flour and improved crumb firmness in both flours. The reduction in crumb firmness with MC in both flours was 6.6 to 44.5% at 4 to 12 weeks.

The control dough from both flours showed rapid reduction of maximum dough height, and gas production and retention at 1 day and after 4 weeks frozen storage. The time of gas release (T_x) for both flours increased as the frozen storage increased. CDC+MC improved gas production and retention slightly from 1 day up to

4 weeks of storage and then significantly at 12 weeks ($P < 0.01$) in HRW flour. Maximum dough height was improved with CDC+MC for both flours. Gas release start time was increased or delayed by 19.9% in HRS and 18.5% in HRW dough from fresh to 1 day frozen storage due to the effect of freezing. No increase in gas release start time was observed with the addition of CDC and MC. Addition of CDC delayed beginning of gas permeability in dough frozen for 1 day by 6.9 and 16.9% for HRS and HRW flour respectively. Addition of MC to HRS flour delayed the onset time of gas permeability by 32.7%. Baking scores and Rheofermentometer parameters showed linear correlation ($r \geq 0.623$).

INTRODUCTION

Frozen dough is widely used for in-store bakery bread production due to the benefits of providing fresh baked products and reducing labor costs. However, the overall quality of baked goods deteriorates gradually with increased storage time of frozen dough. Processing and formulations that include additives are suggested to extend the dough shelf life during freezing, thawing, and frozen storage. Among the additives used to improve frozen dough quality are bromate (Kline and Sugihara 1968), sodium stearoyl-2-lactylate (SSL) and diacetyl tartaric acid ester of monoglycerides (Marston 1978, Varriano-Marston et al 1980, Davis 1981; Wolt and D'Appolonia 1984b). Improvement of frozen dough quality has been achieved with oxidants (Lorenze and Bechtel 1965, Varriano-Marston et al 1980, Wolt and D'Appolonia 1984a), combination ascorbic acid (AA) with α-amylase (De Stefanis 1995, Faisy and Neyreneuf 1996, Rouille et al 2000), AA alone or in combination with potassium bromate or SSL (El-Hady et al 1999). Other additives used include egg yolk (Nonami et al 1984), sugar ester and their combination (Hosomi et al 1992), honey (Addo 1997), and wheat fiber (Noll 2000).

Gas production, gas retention and dough development are important aspects of fermentation (Bloksma 1990a,b). The decrease in gas production of yeast in frozen dough and gas retention due to the loss of dough strength affect the baking quality during frozen storage (Inoue and Bushuk 1991). The method of testing yeasted dough systems is more suitable compared to testing the bulk yeast that lacks the effect of osmotic pressure on yeast fermentation activity in a dough system. Example of instruments developed for measuring CO_2 production and gas retention of the dough

with automatic recording of gas evaluation are the fermentograph, risograph and rheofermentometer. Differences in dough quality due to protein content, flour treatment with additives and mixing processes were studied as changes in dough rise, gas formation and gas retention with a rheofermentometer (Czuchajowska and Pomeranz 1993a).

Freezing and thawing of frozen dough causes weakening of gluten network and separation of starch granules from gluten network. The amount of water associated with gluten matrix and starch decreased resulting in more free water separated and pooled into large ice crystals (Berglund et al 1991). Methylcellulose (MC) has ampholytic properties, with affinity for both aqueous and non-aqueous phases in dough system due to the presence of methoxyl groups at hydroxyl cites of cellulose (Bell 1990). These groups produce a water-soluble polymer, with affinity to the non-polar or lipid phase of dough. Bell (1990) reported improvement in dough strength, bread structure and bread softness with the addition of MC to frozen dough. The high water binding capacity of MC resulted in an interaction with water during frozen storage.

The objectives of this study were to evaluate the effect of MC, a commercial dough conditioner (CDC) and the combination of CDC and MC (CDC+MC) on total gas production and gas retention of frozen dough and the relationship of these parameters to the baking quality of bread sticks.

MATERIALS AND METHODS

Flour and Additives

Two commercial flours, hard red spring (HRS) wheat (Dakota Mill & Grain Co, Grandforks, ND) lot 30024, 1998 and hard red winter (HRW) high gluten wheat (Shawnee Milling, Shawnee, OK) lot 24-01-00, 1998 were used. Flour moisture, protein, ash, and Farinograph analyses were made according to Approved Methods 44-15A, 46-11A, 08-01, 54-21 respectively (AACC 1995). A commercial dough conditioner (CDC) NB "SL-67" (Caravan Products Co. Inc, Totowa, NJ) containing dextrose, diacetyl tartaric acid ester of mono-diglycerides (DATEM), ascorbic acid, potassium iodate, azodicarbonamide (ADA) was used. Three treatments tested were: 1) 1.5% CDC, 2) 1 % methylcellulose (MC, The Dow Chemical Co., Midland, MI), and 3) a mix of 1.5% CDC and 1% MC. A control for each flour, with no additives was also tested.

Yeast

Compressed baker's yeast (Fleischmann's Yeast Ltd., Fenton, MO) delivered to a commercial bakery was used within 7 days of arrival. The yeast was stored at 4°C. Shelf life of compressed yeast stored at 2-4.5°C is 3-4 weeks (Trivedi et al 1989, Reed and Nagodawithana, 1991). The gas production of the compressed yeast used in this experiment was tested with a full dough formula using a rheofermentometer. No significant differences were found in gas production ($P < 0.01$) of compressed yeast stored at 4°C for one and eight days.

Dough Formulation

The formula for the control dough on a baker's percent basis included 100% flour, 2.1% yeast, 1.5% salt, 4.5% shortening, 4% sugar, 50 ppm ascorbic acid, and 0.25% malted wheat flour (flour basis). The water absorption for the control HRS and HRW flour dough was 58.5 and 59.6%, respectively, using the farinograph (APPENDIX A and B). The water absorption used in this control dough formula was 56 and 57.5% (2.5 and 2.1% reduction from the farinograph optimum water absorption) for the HRS and HRW flour, respectively. Reductions of 1.1, 0 and 1.1% water absorption from the controls for the samples containing MC, CDC and CDC+MC respectively were used in the dough formula. These values were experimentally determined in preliminary tests to obtain optimum bread sticks quality in terms of loaf volume, crust and crumb characteristics at fresh and 1 day frozen dough.

Dough Mixing

Two independent batches of dough (800 g of flour) were used for each treatment. A Hobart mixer equipped with a water bath (Isotemp 1028P, Fisher Scientific, Inc., Pittsburgh, PA) at 5°C was used. Yeast and salt were added after 5 and 9 min of mixing, respectively, with total mixing time of 11 min. The delayed addition of yeast and salt during mixing was used as recommended by Dubois and Blockcolsky (1986), Evenson (1988), and Neyreneuf and van der Plaat (1991). Final dough temperature was 13-15°C.

Preparation of Fresh and Frozen Dough Bread Sticks

For the baking test, the cool dough was sheeted using a noodle machine (H. F. Kejenteraan SDN. BHD, Co., Johor, Malaysia) equipped with 7x22 cm (width x length) stainless steel rolls. The sheet of dough was folded and laminated 5 times to obtain 6 layers and 9 mm thickness. Rectangular bread sticks (160x25x9 mm, length x width x height) of 35±0.5 g were proofed at 30°C and 85% relative humidity for 55 min (Fermentation Cabinet model 505-11. National Manuf., Lincoln NE). The dough used in the rheofermentometer analysis was obtained from 150g dough samples which was laminated by sheeting as described above and shaped into a 10 cm diameter disc and proofed. All the rectangular and round samples were frozen in air blast freezer at -30°C and stored in closed plastic bags in a freezer at -20°C.

Baking Test

The samples for 0 day treatment were freshly baked to obtain baked breadsticks with no freezing. Pre-proofed frozen dough bread sticks were thawed in baking trays, covered with plastic at room temperature (25°C) for 1 hr before baking. Bread sticks were baked at 260°C for 5.5 min. Baked bread sticks were cooled on racks for 20 min and their volume (rapeseed displacement) and weight recorded. The crust of bread sticks was scored using a scale of 0 to 10, with 10 as the most desirable and without defects. The bread sticks were kept in 3 hr sealed plastic bags for crumb firmness test.

Measurement of Crumb Firmness

Three 1-cm slices were obtained from the center of each bread stick. On each slice, two firmness measurement of the crumb were recorded with a total of 6 measurements for one bread stick. Firmness was measured using a TA-XT2 Texture Analyzer (Texture Technologies Corp., New York) with a perspex flatted end cylindrical of 6 mm diameter. Pre-test, test, and post-test speeds were 4.0, 1.0, and 1.0 mm/sec, respectively, and trigger force was 10g. The puncture distance was set at 25% compression dept as described in AACC Standard Method 74-09 (AACC, 1995).

Measurement of Gas Production and Dough Behavior

Changes in dough rise, gas production and gas retention were determined using a rheofermentometer (Chopin S.A., Villeneuve la Garenne, France). A frozen dough sample (150 g) was removed from the freezer and placed directly into the instrument fermentation vat. The test for fresh dough (0 day) sample was placed directly into the fermentation vat after sheeting and rounding. The test used stress weight of 2000 g, 25°C and a 5 hr protocol. Maximum height of dough (H_{mD}) in mm, total gas production or total volume (V_T) in cc/g, maximum height of gas production (H_{mG}) in mm, retention volume of gas (V_R) in cc/g, and time at which gas permeability started from the dough (T_x) in hr, were recorded. Example of calculation for these parameters is shown in APPENDIX C.

Statistical Evaluation

Statistical analyses were performed using a mixed model with Statistical Application Systems software, SAS version 8.2 (SAS Institute Inc., Cary, NC). Mean differences were obtained using a mixed procedure and least significant difference (LSD). Relationships were established using Pearson's correlation coefficient (r). The baking test and rheofermentometer tests were done in duplicate batches for each flour. The number of total observations for specific volume, crust score and rheofermentometer test was 128 while those for crumb firmness was 1536.

RESULTS AND DISCUSSION

Analytical and Dough Properties of Flours

The proximate analysis and farinograph properties using are summarized in Table I. HRS flour had higher protein than HRW (13.5 and 10.2% respectively). Farinograph parameters show that HRS flour had 9.1 times longer peak time (17.3 vs 1.9 min) and 1.5 times higher stability to mixing (18.9 vs 12.7 min) compared to HRW. These parameters were typical of each flour type and agree with overall stronger gluten of HRS compared to HRW flour.

Baking Results

Specific Volume

There was a significant interaction ($P < 0.01$) of flour type, additives and frozen storage time on the means of specific volume of the bread sticks. When fresh and frozen control dough were compared, bread sticks made with HRS had higher specific volume than HRW at 0 day and 1 day of frozen storage ($P < 0.05$). However, there were no significant differences in specific volume between both flours at longer time of frozen storage (after 1day and up to 12 weeks).

Specific Volume of HRS-Bread Sticks (Table II, APPENDIX D-a). Specific volume of fresh (not frozen) bread sticks made from HRS flour did not change by the addition of MC, CDC and CDC+MC (Table II). The control dough showed a significant decrease in specific volume at 1 day (18.8% reduction) and 1 week (38.3 % reduction) of frozen storage ($P < 0.01$) and no significant differences from 1 up to 8 weeks of frozen storage. These results agreed with those reported by El-Hady et al (1996) who reported a decrease in loaf volume of bread made from frozen dough after one and seven days of frozen storage. The addition of CDC in dough did not improve the specific volume of bread sticks in fresh and frozen dough. The addition of CDC+MC improved the specific volume at 1 day, 1, 2 and 12 weeks of frozen storage (6.4, 15.8, 6.8, and 17.9 % increase, respectively) compared to the control. The addition of MC gave the highest specific volume of bread sticks from 1 day up to 8 weeks of frozen storage ($P < 0.01$). The increase of specific volume compared to the control ranged from 35.9 to 7.2% for 1 day to 12 weeks.

Specific Volume of HRW-Bread Sticks (Table II, APPENDIX D-b). Specific volume of control bread sticks decreased significantly ($P < 0.01$) from fresh

to 1 day frozen storage dough. After one day of frozen storage, the specific volume of control (no additives) of bread sticks significantly decreased (18.6%) compared to the fresh (no frozen storage). The specific volume of the control bread sticks remained unchanged from 1 day up to 3 weeks of frozen storage ($P < 0.05$). A significant decrease of control bread sticks specific volume was observed at 4, 8 and 12 weeks of frozen storage. Similar patterns of reduction of bread sticks with addition of CDC, MC and CDC+MC were observed for specific volume. The addition of CDC did not improve the specific volume of bread sticks. The addition of MC and CDC+MC improved the specific volumes of bread sticks at 1 day (20.8%, and 16.7% increase, respectively) and 1 week (10.1% and 13.2% increase, respectively) of frozen storage.

The reduction of specific volume of bread sticks in the control dough of both flours after freezing agreed with Inoue and Bushuk (1992b). They reported that the bread volume gradually decreased as the frozen storage time increased. The rate of the reduction appeared to relate to flour strength. Our results also agree with Räsänen et al (1997a) showing deterioration of baking quality during the first weeks of frozen storage followed by a slower decrease afterwards. The rate of deterioration of frozen dough appeared to be dependent on protein quality and freeze stability of the dough they formed.

Crust Score

There was a significant interaction ($P < 0.01$) of flour type, additives and frozen storage time of crust score of frozen dough bread sticks. A significant decrease of crust score of the control bread sticks was observed for frozen dough at 4 and 3 weeks for HRS and HRW flour, respectively ($P < 0.01$, Table II, APPENDIX E).

HRS flour showed similar crust score for all treatments up to 3 weeks of frozen storage except for CDC+MC which showed a significant decrease. The addition of MC showed the highest crust score and CDC and CDC+MC had lower scores compared to the control. Samples made with HRW flour, overall showed a continue decrease in crust score as the storage time increased. The low crust scores at 8 and 12 weeks reflected crust paler than the control.

Crumb Firmness.

Crumb firmness of bread sticks is shown in Fig. 1 and APPENDIX F. There was a significant interaction of flour type and frozen storage time, and additives and frozen storage time ($P < 0.01$). There was no significance difference in crumb firmness at fresh and 1 day frozen for both flours (Fig. 1). The control dough of HRS and HRW gave similar crumb firmness across all the storage time in this study. The crumb firmness of HRS control dough significantly increased from 4 to 12 weeks of frozen storage. In contrast, in HRW control dough the onset of firmness increased at 8 weeks of storage ($P < 0.01$).

The increase of crumb firmness as a function of frozen storage time is reported in Fig. 1. Overall, the two flours showed similar patterns with no change in firmness during the first 4 weeks of frozen storage followed by 2 to 3 times increase in firmness at 8 and 12 weeks. Compared to the control bread sticks, the MC-1% treatment showed no increase with HRW and a slower rate of increasing in firmness with HRS at 12 weeks. The firmness values of HRS flour, compared to the control, showed a reduction of 44.5, 6.6 and 33.0% at 4, 8 and 12 weeks of storage, respectively ($P<0.01$). Compared to the control, bread sticks firmness of HRW containing CDC

showed a decrease in firmness of 35.9 and 42.5 % at 8 and 12 weeks, respectively. The combination of CDC+MC also slowed the firmness of bread sticks made with HRW when stored at 8 and 12 weeks (37.7 and 10.2 % decrease, respectively, compared to be control). The high water binding capacity and thermal gelation properties of MC and CDC+MC impact stability to the dough emulsion during freezing and are barriers for moisture loss during baking (Bell 1990, Anonymous 1996).

HRS and HRW flour showed marked differences in the farinograph properties (Appendix A and B). However, similar baking attributes (specific volume, crust score and crumb firmness) of these control samples were observed after 1 day of frozen storage. These results agree with Wolt and D'Appolonia (1984b) who reported that high protein content and gluten strength did not indicate superior frozen dough performance in extended storage.

Rheofermentometer Parameters

The rheofermentometer has been reported to be suitable for the evaluation of the gas production and gas retention of fresh dough (Czuchajowska and Pomeranz 1993a,b) and frozen dough (El-Hady 1996). These results reputely were similar to the risograph (El-Hady 1996). There was a significant interaction ($P < 0.05$) among flour type, additives and frozen storage time of all parameters of the profiles of gas production (H_{mG}, V_T, V_R, T_x,) and dough development (H_{mD}). An example of dough development and gas production profiles of HRS-control dough of fresh and frozen at 1 day, 1, 8 and 12 weeks are shown in Fig. 2. The patterns of both dough

development (Fig. 2a) and gas production (Fig. 2b) significantly decreased ($P < 0.05$) after one day freezing and decreased as frozen storage time increased (1 to 12 weeks). The time of maximum height (T_1) and beginning of gas release (T_x) (Fig. 2b) are shown for fresh dough and 1 day frozen storage samples. No gas release was detected in the 5 hour test with frozen dough after 1 day of frozen storage. HRW-control dough had similar pattern of reduction of dough development and gas production profile with frozen storage time (pattern not shown) to those of HRS-control dough.

Rheofermentometer Parameters of Control Dough

There was a significant reduction ($P < 0.05$) of H_{mG}, H_{mD}, V_T, V_R of fresh dough vs 1 day frozen of the control–HRS dough (Fig. 3, Table III, APPENDIX G and H). These parameters remained unchanged for 4 weeks of frozen storage. The reduction of rheofermentometer parameters agrees with the decrease of specific volume of bread sticks made from control-HRS dough. Small but significant correlations coefficients ($P < 0.001$) between rheofermentometer parameters and specific volume, crust score and crumb firmness were observed (Table VII). The correlation coefficients (r) ranged from 0.52 to 0.71 (Table IV, example of the plots in Appendix I). Control-HRW dough showed similar trend as the control-HRS dough with a r range from 0.49 to 0.64 ($P < 0.001$, Table IV). Correlation coefficient values were overall higher for the HRS flour than HRW (as Table IV shows).

HRS and HRW showed similar pattern of reduced rheological properties (H_{mG}, V_T, V_R H_{mD}) from 1 day up to 4 weeks and a significant decrease at 8 and 12 weeks (Fig 3 and APPENDIX H, J and K). The time when the gas release was detected (T_x) in the control dough of HRS and HRW flour (Table V) increased significantly at 1 day

of frozen storage (average 19.2%), and maintained similar values for up to 4 weeks. No gas release (T_x absent) was observed at 8 and 12 weeks. This evidence shows that freezing even for one day decreased the CO_2 produced by yeast. The results were similar to the report by Räsänen et al (1997a) who found that an increase frozen storage time (90 days) of preproofed frozen dough resulted in increased final proof time. Yeast viability decreased with longer frozen storage times were also reported by Kline and Sugihara (1968) and Inoue et al (1994). The T_x results were negatively correlated ($P<0.001$) to V_T and V_R values of control dough in both flours. The average *r* values from both flours were -0.68 (Tx vs. V_T) and –0.65 (Tx vs. V_R) for HRS and HRW, respectively (Table VI).

The reduction of total gas produced (V_T, 17.6% and 7.4% for HRS and HRW respectively, Table III) at the initial freezing of frozen dough may be caused by cell injury of yeast subjected to freezing. The amount of dead yeast cells increased during the freezing stage resulting in a reduction of yeast activity and gas production. Wolt and D'Appolonia (1984a) reported that the amount of dead yeast cells increased from 4.9 to 11.4% and gassing power using pressuremeter decreased for fresh and 2 weeks of frozen storage, respectively. The comparison of fresh vs. 1 day frozen dough showed an increase of Tx (19.9 and 18.5% for HRS and HRW flour, respectively) and decrease retention volume (15.5% and 6.9% in HRS and HRW flour, respectively) suggested a reduction of yeast activity and dough rheological properties. This could be due to oxidation/reduction and changes of gluten proteins when yeast cells were injured and glutathione was released. The range of glutathione found in a similar study was 0 to 2.08 mg/g of dry yeast at fresh and 2 weeks of frozen storage (Wolt and

D'Appolonia 1984a). These authors also reported that the amount of dead yeast cells increased from fresh dough to 2 weeks of frozen storage, was similar from 2 to 4 weeks and increased again at 6 weeks. Their results agree with our results in that V_T and V_R values remained similar up to 4 weeks and continued to reduce. Compared to the control, V_T and V_R values decreased 61.3 and 59.0% at 8 weeks and 46.3 and 44.6% at 12 weeks for HRS and HRW respectively. At 12 weeks V_T and V_R decreased an average at 80.8 and 95.2% for both process. The reduction of V_T and V_R at 8 and 12 weeks (Table III) might be caused by a reduction of gas production from an increase of dead yeast cells and glutathione in frozen dough. as reported by Wolt and D'Appolonia (1984a) and Neyreneuf and Van Der Plaat (1991). Long frozen storage periods caused ice crystals that may cause damage in the gluten network and separation of starch granule due to pooled ice crystals (Berglund et al 1991).

Our results agree with the report of El-Hady et al (1996) that the loaf volume of bread made from frozen dough decreased and the rheological dough behavior changed rapidly between fresh and 1 day frozen storage dough and maintained similar values up to 4 weeks of frozen storage. El-Hady et al (1996) concluded that lower bread volume of frozen dough was due to a decrease in gas production and not a decrease in gas retention. However, our results showed that lower bread volume of frozen dough was due to a decrease in gas production and gas retention ($P < 0.05$, Table III).

As discussed above, freezing and frozen storage time increased T_x which is related to the reduction of V_T and V_R, and baking performance of the dough, thus increasing proof time. Although the bread sticks were preproofed, additional volume

can be achieved during the resting period after complete thawing time at room temperature. The volume could also be boosted during the oven spring at the beginning of baking. The longer the frozen storage time, the less viable yeast remained in the frozen dough, and longer rest period time is required to further increase the product volume.

Rheofermentometer Parameters of the Dough with Additives

Overall the additives did not improve or only very slightly affected (at 1 day or 1 week) the rheological properties H_{mG}, V_T, and V_R, of HRS-dough (APPENDIX G and Table III). In HRW-dough, CDC and CDC+MC slightly increased all these parameters (range 1.3 to 10.8%) from fresh up to 2 weeks for CDC and 4 weeks frozen storage for CDC+MC but significantly increased at 12 weeks of frozen storage ($P < 0.05$ and 0.01 respectively). The increase of H_{mG}, V_T, and V_R with CDC at 12 weeks of frozen storage was 60.9, 408.0, and 416.5%, respectively, while the increase of the same parameters with CDC+MC was 151.8, 650.0, and 670.6%, respectively. CDC and MC alone did not improve H_{mD} compared to the control (without additives) in both flours (Fig. 3). The combination of CDC and MC yielded higher H_{mD} values during frozen storage for up to 3 and 4 weeks for the dough made from HRS and HRW flour ($P < 0.05$), respectively. CDC+MC also contributed to the maintenance of yeast viability and gluten properties as seen by the increase of H_{mD}, V_T, and V_R. CDC+MC increased V_T, and V_R at 1 week in HRS and 3 and 4 week frozen in HRW flour. The increase of H_{mD}, V_T, and V_R values was related to the improvement of baking quality of the frozen dough compared to the control as observed by the correlation coefficients (r) range $|0.41$ to $0.90|$, ($P < 0.001$, Table VI).

CDC reduced T_x of dough made with HRS flour at 1 day of frozen storage but reduced at 1 day up to 3 weeks of frozen storage for HRW flour ($P < 0.01$, Table V). MC gave a significant reduction of T_x in HRS flour at 1 day, 2 and 4 weeks frozen ($P < 0.01$). This evidence supported the baking performance of bread sticks with the addition of MC and CDC. The reduction of T_x with CDC supported the slight increase of specific volume of bread sticks with the addition of CDC in HRW flour at 1 day and 1 week of frozen storage. The reduction of T_x (Table V) also supported the increase of specific volume of bread sticks with the addition of MC in HRS flour at 1 day and up to 12 weeks of frozen storage (Table II). There was a negative correlation between T_x and specific volume of bread sticks, $r = -0.683$ ($P < 0.001$, Table VI).

MC reacts as stabilizer to inhibit the growth of ice crystal in frozen foods (Anonymous 1996). The addition of MC in frozen dough may reduce gluten damage due to the formation of ice crystals. CDC contains the surfactant or dough strengthening agent DATEM that may prevent deterioration of rheological properties of frozen dough. Wolt and D'Appolonia (1984b) reported that DATEM inhibited the reduction in resistance to extension and extensibility of frozen dough. DATEM was also reported to decrease the deterioration effects due to freezing and frozen storage and to improve bread volume (Marston 1978, Varriano-Marston et al 1980, Davis 1981, Sahlstrøm et al 1999).

The reaction of CDC in Tx of frozen dough was different in HRS and HRW flour. This result agreed with Wolt and D'Appolonia (1984b) who showed that DATEM had different effect on proof time in different type of flours. There is no apparent explanation for this different effect on frozen storage of the dough.

CONCLUSIONS

MC, CDC and MC + CDC affected the baking quality and rheological properties of frozen dough. These additives showed different effects on the two type of flours. HRS dough containing MC showed improvement in the crust score and specific volume up to 12 weeks of frozen storage time. HRW dough did not show improvement of color crust with any treatment. The specific volume of HRW bread sticks improved for a short frozen storage (1 day to 1 week) with MC and CDC. MC maintained soft crumb bread up to 8 and 12 weeks in HRS and HRW flour, respectively. Overall, the combination of CDC+MC appeared to delay the damage to yeast and gluten proteins from freezing as observed from an improvement in total gas volume, retention volume and maximum dough height for HRW flour with the higher values observed at 3 weeks of frozen storage. The addition of MC to HRS flour reduced the time of gas released by the yeast and this could be translated into shorter rest time after freezing and before baking. Significant correlations of baking properties and rheofermentometer parameters suggest that the latter one can be used when quantitative differences of additives used in frozen dough need to be evaluated for yeast and dough strength and stability. The rheofermentometer offers numerical evaluation of changes in dough rise, gas formation and gas retention.

TABLE I

Chemical Composition and Farinograph Properties of Hard Red Spring(HRS) and Hard Red Winter (HRW) wheat flour

Evaluation	HRS	HRW
Moisture (%)[a]	13.6	12.9
Ash (%)[a]	0.58	0.5
Protein (%)[a]	13.5	10.2
Farinograph		
Absorption (%)	58.5[b]	58.4[c]
Peak time (min)	17.3[b]	1.9[c]
Stability (min)	18.9[b]	12.7[c]

[a] Values on 14% mb.

[b] In APPENDIX A.

[c] In APPENDIX B.

TABLE II
Baking Score of Bread Sticks Made from Hard Red Spring (HRS) and Hard Red Winter (HRW) Flour[a]

	Frozen Storage Time	HRS		HRW	
		Crust Score	Specific Volume (cc/g)	Crust Score	Specific Volume (cc/g)
Control	0 day	10.0 ± 0.9	4.1 ± 0.3	9.3 ± 0.0	3.6 ± 0.4
	1 day	10.0 ± 0.5	3.3 ± 0.3	9.8 ± 0.0	2.9 ± 0.1
	1 week	9.5 ± 0.6	2.8 ± 0.2	9.5 ± 0.6	2.9 ± 0.2
	2 weeks	9.8 ± 0.9	2.8 ± 0.2	9.3 ± 0.5	3.1 ± 0.1
	3 weeks	9.5 ± 1.1	2.7 ± 0.1	9.0 ± 0.5	2.8 ± 0.1
	4 weeks	8.8 ± 0.9	2.6 ± 0.2	8.8 ± 1.5	2.6 ± 0.1
	8 weeks	6.5 ± 1.1	2.5 ± 0.1	8.0 ± 4.4	2.4 ± 0.1
	12 weeks	5.0 ± 0.6	2.1 ± 0.2	5.5 ± 1.1	2.3 ± 0.1
CDC, 1.5%	0 day	10.0 ± 0.0	3.9 ± 0.7	10.0 ± 0.0	3.5 ± 0.2
	1 day	10.0 ± 0.0	3.4 ± 0.0	10.0 ± 0.0	3.2 ± 0.4
	1 week	10.0 ± 0.6	3.0 ± 0.1	9.5 ± 0.0	3.1 ± 0.1
	2 weeks	10.0 ± 0.5	2.8 ± 0.1	9.8 ± 0.0	2.9 ± 0.0
	3 weeks	10.0 ± 2.4	2.9 ± 0.1	7.5 ± 0.0	2.7 ± 0.1
	4 weeks	6.3 ± 0.9	2.8 ± 0.1	7.3 ± 2.9	2.5 ± 0.3
	8 weeks	6.3 ± 0.5	1.8 ± 0.1	7.3 ± 1.5	2.3 ± 0.1
	12 weeks	4.3 ± 0.0	2.1 ± 0.0	5.0 ± 1.5	2.0 ± 0.3
CDC+MC, 1.5+1%	0 day	9.3 ± 0.5	3.9 ± 0.6	9.8 ± 0.9	3.8 ± 0.3
	1 day	10.0 ± 0.6	3.5 ± 0.3	9.5 ± 0.0	3.4 ± 0.1
	1 week	9.3 ± 1.7	3.2 ± 0.4	7.5 ± 0.9	3.3 ± 0.1
	2 weeks	9.8 ± 0.8	3.0 ± 0.1	7.0 ± 0.5	2.8 ± 0.3
	3 weeks	8.3 ± 0.5	2.8 ± 0.0	8.8 ± 0.9	2.8 ± 0.2
	4 weeks	6.0 ± 0.5	2.7 ± 0.2	9.6 ± 2.4	2.4 ± 0.2
	8 weeks	5.8 ± 0.9	2.6 ± 0.1	4.3 ± 2.2	2.5 ± 0.4
	12 weeks	3.8 ± 0.5	2.5 ± 0.1	4.3 ± 2.5	1.9 ± 0.1
MC, 1%	0 day	10.0 ± 0.6	4.2 ± 0.7	9.5 ± 0.0	3.7 ± 0.0
	1 day	10.0 ± 1.1	4.5 ± 0.2	9.0 ± 0.0	3.5 ± 0.3
	1 week	10.0 ± 1.7	3.6 ± 0.1	8.5 ± 0.0	3.2 ± 0.2
	2 weeks	10.0 ± 1.5	3.3 ± 0.3	8.8 ± 0.0	2.9 ± 0.2
	3 weeks	9.0 ± 1.7	3.0 ± 0.3	8.5 ± 0.0	3.0 ± 0.1
	4 weeks	9.5 ± 5.0	2.9 ± 0.2	5.8 ± 0.6	2.6 ± 0.3
	8 weeks	8.9 ± 2.9	3.0 ± 0.4	4.8 ± 1.0	2.4 ± 0.1
	12 weeks	8.8 ± 0.0	2.3 ± 0.1	5.0 ± 0.5	2.1 ± 0.1

[a] Mean ± standard deviation, n = 4.

TABLE III

Retention Volume and Total Volume Using Rheofermentometer for Dough Samples Made from Hard Red Spring (HRS) and Hard Red Winter (HRW) Flour[a]

	Frozen Storage Time	HRS V_R[b] (mL)	HRS V_T[c] (mL)	HRW V_R (mL)	HRW V_T (mL)
Control	0 day	1101 ± 79	1171 ± 84	905 ± 4	934 ± 6
	1 day	930 ± 103	965 ± 142	842 ± 29	864 ± 38
	1 week	872 ± 21	879 ± 31	841 ± 1	856 ± 2
	2 weeks	863 ± 36	877 ± 37	814 ± 9	835 ± 7
	3 weeks	836 ± 11	848 ± 12	781 ± 4	795 ± 4
	4 weeks	754 ± 31	764 ± 39	709 ± 26	716 ± 26
	8 weeks	452 ± 150	454 ± 152	501 ± 19	503 ± 20
	12 weeks	217 ± 98	220 ± 96	43 ± 24	44 ± 25
CDC[d], 1.5%	0 day	1016 ± 94	1044 ± 112	904 ± 85	933 ± 100
	1 day	958 ± 37	976 ± 41	853 ± 31	888 ± 35
	1 week	822 ± 40	835 ± 52	888 ± 79	925 ± 92
	2 weeks	842 ± 75	850 ± 83	865 ± 65	897 ± 85
	3 weeks	711 ± 24	702 ± 45	737 ± 11	746 ± 11
	4 weeks	638 ± 12	641 ± 9	603 ± 82	612 ± 83
	8 weeks	328 ± 96	330 ± 95	362 ± 18	365 ± 18
	12 weeks	144 ± 11	146 ± 11	220 ± 47	224 ± 47
CDC+MC[d], 1.5+1%	0 day	938 ± 19	944 ± 20	924 ± 50	949 ± 51
	1 day	929 ± 31	942 ± 23	905 ± 5	922 ± 1
	1 week	965 ± 29	984 ± 31	892 ± 9	904 ± 10
	2 weeks	876 ± 36	881 ± 38	857 ± 23	864 ± 24
	3 weeks	749 ± 83	751 ± 84	899 ± 29	903 ± 28
	4 weeks	547 ± 111	549 ± 111	785 ± 11	789 ± 13
	8 weeks	397 ± 167	399 ± 167	407 ± 41	409 ± 43
	12 weeks	77 ± 29	84 ± 21	328 ± 28	330 ± 18
MC[d], 1%	0 day	939 ± 171	965 ± 203	857 ± 152	861 ± 153
	1 day	896 ± 45	960 ± 14	826 ± 37	841 ± 37
	1 week	831 ± 6	837 ± 9	868 ± 69	884 ± 72
	2 weeks	892 ± 21	924 ± 50	750 ± 50	759 ± 46
	3 weeks	746 ± 17	759 ± 4	775 ± 52	783 ± 52
	4 weeks	769 ± 71	795 ± 89	671 ± 106	675 ± 108
	8 weeks	432 ± 105	436 ± 105	287 ± 14	290 ± 13
	12 weeks	77 ± 29	248 ± 57	103 ± 19	105 ± 19

[a] Mean ± standard deviation, each value is a mean from 2 measurements. Analysis was done in two independent batches with two subsamples per batch.

[b] V_R = Retention volume.

[c] V_T = Total volume.

[d] CDC = Comercial Dough Conditioner, MC = Methylcellulose.

TABLE IV

Correlation Coefficient (*r*) Between Baking Results and Rheological Dough Behavior using Rheofermentometer[a] of Hard Red Spring (HRS) and Hard Red Winter (HRW) Flour[b]

Flour	Parameter	Treatment	V_R (mL)	V_T (mL)	H_{mG} (mm)	H_{mD} (mm)
HRS	Specific Volume	Control	0.666	0.706	0.521	0.609
		CDC[c]	0.758	0.759	0.805	0.724
		MC[c]	0.540	0.552	0.449	0.470
		CDC+MC[c]	0.514	0.517	0.428	0.575
	Crust Score	Control	0.653	0.637	0.603	0.606
		CDC	0.717	0.705	0.699	0.690
		MC	0.501	0.521	0.510	0.419
		CDC+MC	0.851	0.854	0.897	0.799
	Crumb Firmness	Control	-0.789	-0.761	-0.822	-0.778
		CDC	-0.869	-0.854	-0.873	-0.893
		MC	-0.705	-0.654	-0.643	-0.473
		CDC+MC	-0.882	-0.881	-0.825	-0.870
HRW	Specific Volume	Control	0.530	0.546	0.490	0.639
		CDC	0.791	0.794	0.789	0.712
		MC	0.533	0.534	0.494	0.551
		CDC+MC	0.493	0.514	0.472	0.409
	Crust Score	Control	0.750	0.749	0.731	0.646
		CDC	0.683	0.688	0.690	0.647
		MC	0.306	0.306	0.303	0.317
		CDC+MC	0.464	0.474	0.444	0.429
	Crumb Firmness	Control	-0.731	-0.736	-0.633	-0.728
		CDC	-0.928	-0.920	-0.915	-0.882
		MC	-0.646	-0.649	-0.639	-0.621
		CDC+MC	-0.826	-0.826	-0.727	-0.656

[a] Rheofermentometer parameters: H_{mD} = Maximum height of dough development, H_{mG} = Maximum height of gas production, V_R = Retention volume, V_T = Total volume T_X = Beginning time of gas release.

[b] Statistical analysis was performed separately for each type of flour and additives, n = 16. All correlation coefficients were significant at $P < 0.001$.

[c] CDC = Comercial Dough Conditioner, MC = Methylcellulose.

TABLE V

Time of Beginning of gas Release Using Rheofermentometer for Dough Samples Made from Hard Red Spring (HRS) and Hard Red Winter (HRW) Flour[a]

	Frozen Storage time	HRS Flour T_x[b] (hr)	HRW Flour T_x (hr)
Control	**0 day**	3.51 ± 0.19	3.38 ± 0.06
	1 day	4.21 ± 0.05	4.00 ± 0.00
	1 week	4.25 ± 0.11	4.17 ± 0.00
	2 weeks	4.42 ± 0.23	3.92 ± 0.12
	3 weeks	4.38 ± 0.17	4.13 ± 0.05
	4 weeks	4.58 ± 0.35	4.46 ± 0.00
	8 weeks	NR[c]	NR
	12 weeks	NR	NR
CDC[d], 1.5%	**0 day**	3.97 ± 0.63	3.79 ± 0.41
	1 day	3.92 ± 0.35	3.33 ± 0.24
	1 week	4.36 ± 0.43	3.28 ± 0.43
	2 weeks	4.42 ± 0.35	3.40 ± 0.60
	3 weeks	5.00 ± 0.10	4.50 ± 0.00
	4 weeks	NR	4.54 ± 0.05
	8 weeks	NR	NR
	12 weeks	NR	NR
CDC+MC[d], 1.5+1%	**0 day**	4.75 ± 0.15	3.84 ± 0.23
	1 day	4.58 ± 0.24	4.04 ± 0.05
	1 week	4.21 ± 0.29	4.83 ± 0.00
	2 weeks	5.00 ± 0.25	4.84 ± 0.12
	3 weeks	5.00 ± 0.20	4.86 ± 0.15
	4 weeks	NR	NR
	8 weeks	NR	NR
	12 weeks	NR	NR
MC[d], 1%	**0 day**	3.72 ± 0.07	4.67 ± 0.12
	1 day	2.84 ± 0.54	4.50 ± 0.00
	1 week	4.46 ± 0.05	4.00 ± 0.00
	2 weeks	3.91 ± 0.83	4.42 ± 0.58
	3 weeks	4.34 ± 0.47	4.63 ± 0.17
	4 weeks	3.86 ± 0.00	NR
	8 weeks	NR	NR
	12 weeks	NR	NR

[a] Mean ± standard deviation, each value is a mean from 2 measurements. Analysis was done in two independent batches with two subsamples per batch.

[b] T_X = Beginning time of gas release.

[c] NR = No data of T_x recorded.

[d] CDC = Comercial Dough Conditioner, MC = Methylcellulose.

TABLE VI

Correlation Coefficient (r) Between Baking Results and Rheological Dough Properties Using Rheofermentometer[a]

	Tx	Crust score	Specific volume	Crumb firmness
Baking Results				
Crust score		1	0.697	-0.801
Specific volume			1	-0.748
Crumb score				1
Rheological Dough Behavior[b]				
H_{mD}	-0.424	0.754	0.736	-0.818
H_{mG}	-0.564	0.826	0.742	-0.840
V_R	-0.651	0.831	0.772	-0.888
V_T	-0.68	0.852	0.788	-0.891
Tx	1	-0.648	-0.683	0.623

[a] Statistical analysis was performed for all treatments, n = 64. All correlation coefficients were significant at $P < 0.001$.

[b] Rheofermentometer parameters: H_{mD} = Maximum height of dough development, H_{mG} = Maximum height of gas production, V_R = Retention volume, V_T = Total volume, T_X = Beginning time of gas release.

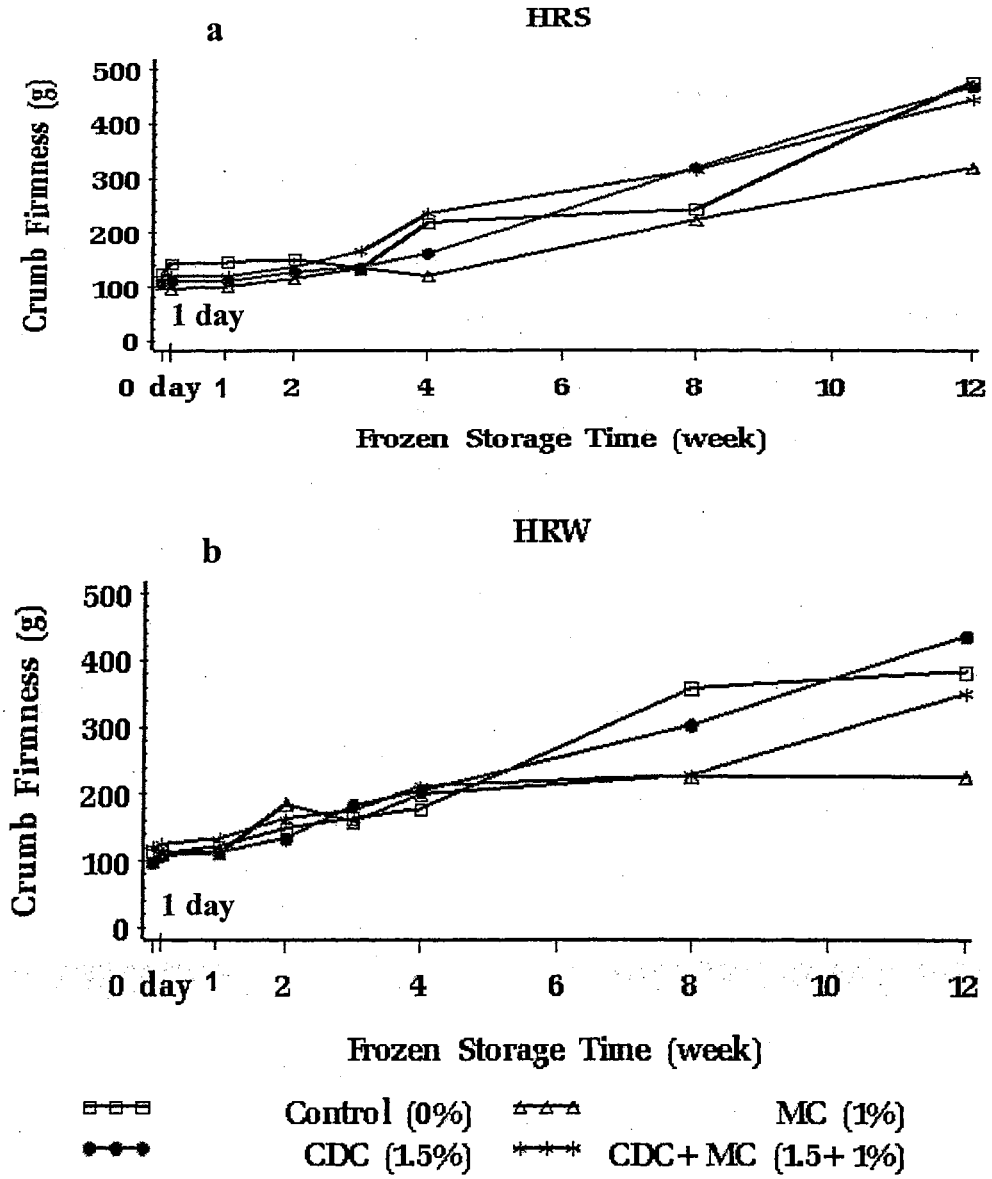


Fig. 1. Crumb firmness as a function of frozen storage time of bread sticks made from hard red spring (HRS) flour (**a**) and hard red winter (HRW) flour (**b**).

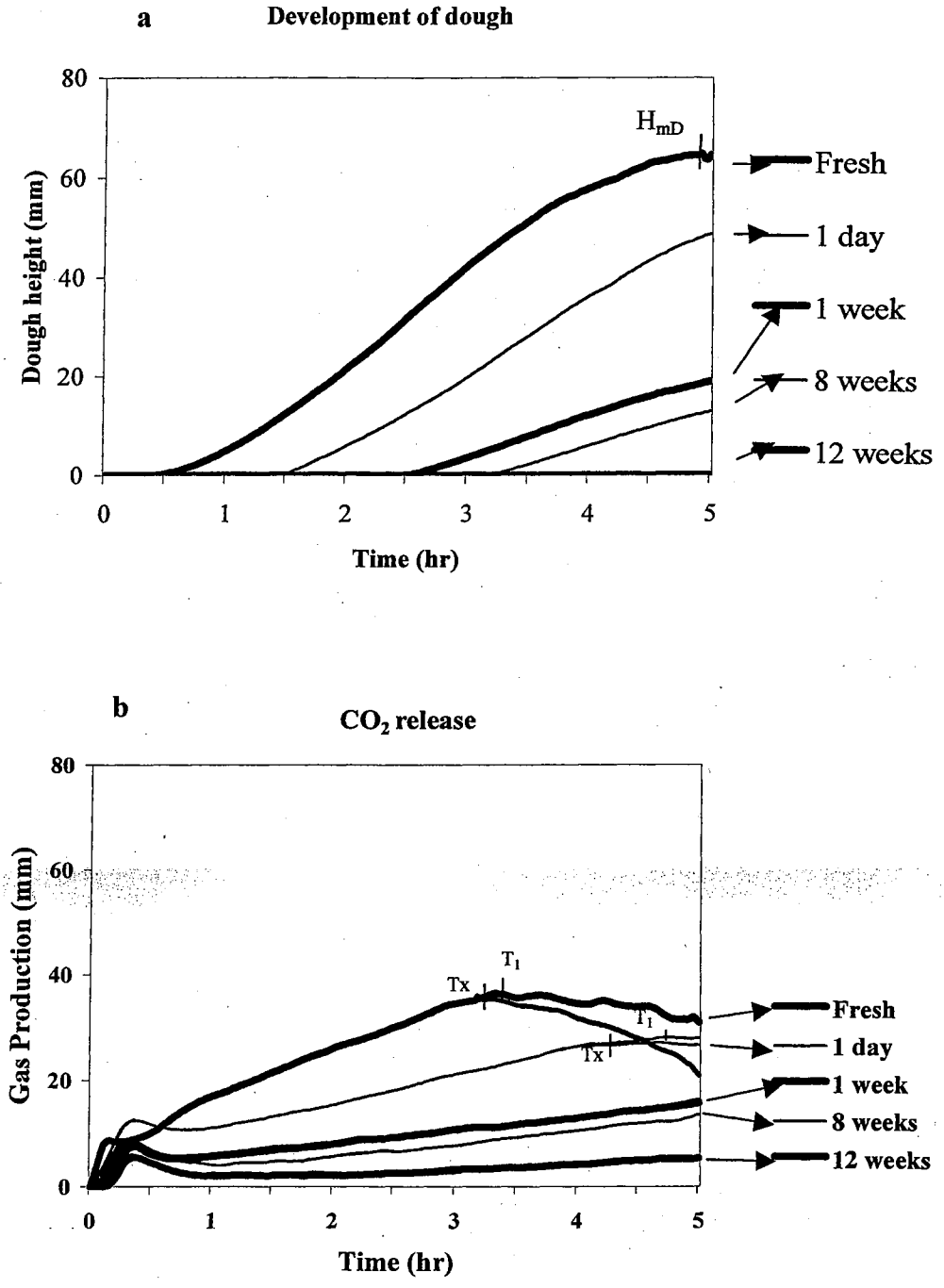


Fig. 2. Pattern of dough development (**a**) and gas release (**b**) in fresh and frozen dough of hard red spring (HRS) flour as a function of time. H_{mD} = maximum dough height, T_1 = time of maximum height of gas, and T_x = time of beginning of gas release.

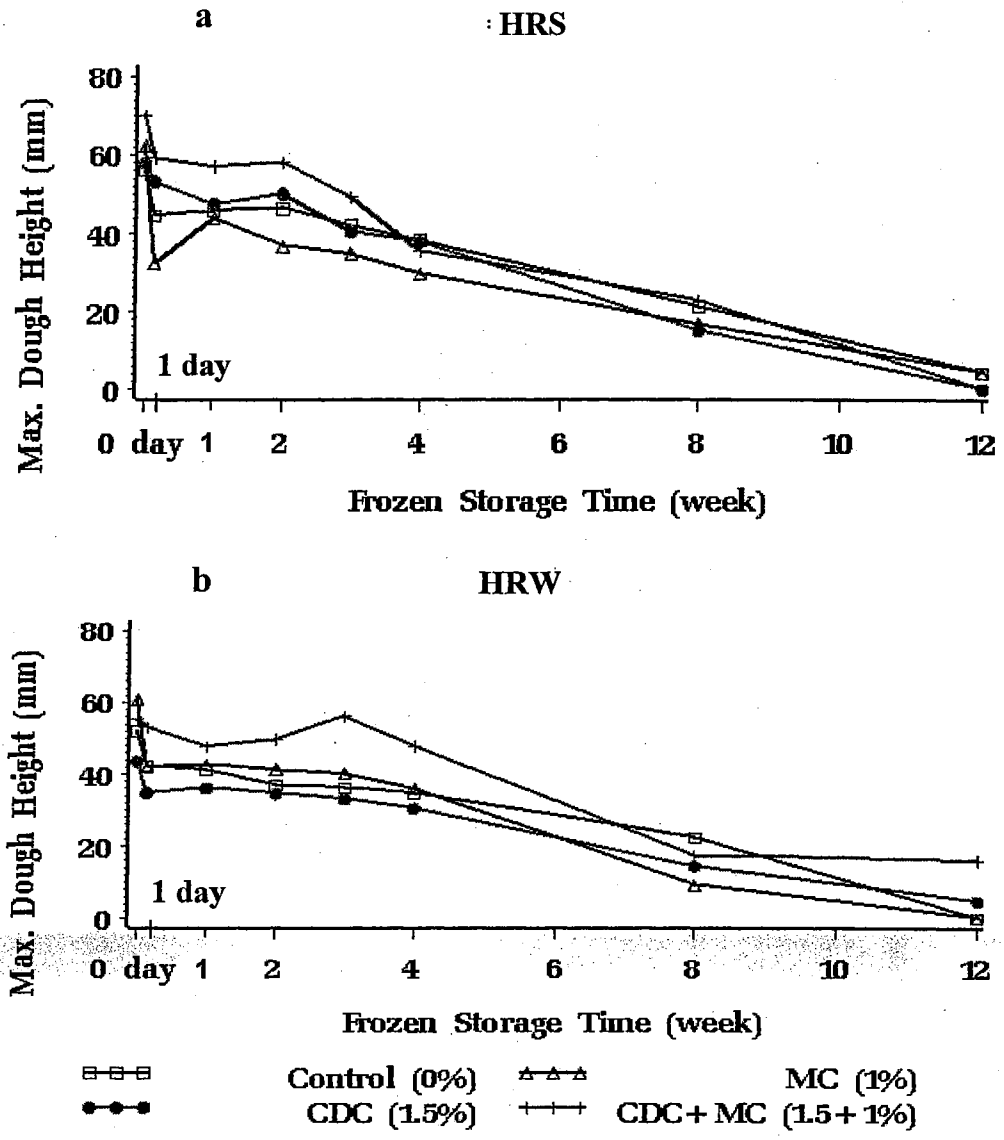


Fig. 3. Maximum height of dough development (H_{mD}) as a function of frozen storage time of bread sticks made from hard red spring (HRS) flour (**a**) and hard red winter (HRW) flour (**b**).

LITERATURE CITED

Addo, A. 1997. Effects of honey type and level on the baking properties of frozen wheat flour doughs. Cereal Foods World. 42:36-40.

American Association of Cereal Chemists. 1995. Approved Methods of the AACC, 9 th ed. Method 08-01, final approval April 1961, revised October 1976 and October 1981, rviewed October 1994; Method 44-15A, approved October 1975, revised October 1981 and October 1994; Method 46-11A, approval October 1976, revised October 1982 and September 1985, reviewed October 1994; and Method 54-21, final approval November 1995. Method 74-09, final approval November 1995, reviewed October 1996. The Association: St. Paul, MN.

Anonymous, 1996. A food technologist's guide to Methocel food grade gums. Page 4-15. The Dow Chemical Co.

Bell, D. A. 1990. Methylcellulose as a structure enhancer in bread making. Cereal Foods World. 35:1001-1006.

Berglund, P. T., Shelton, D. R., and Freeman, T. P. 1991. Frozen bread dough ultrastructure as affected by duration of frozen storage and freeze-thaw cycles. Cereal Chem. 68:105-107.

Bloksma, A. H. 1990a. Rheology of bread-making process. Cereal Foods World. 35:228-236.

Bloksma, A. H. 1990b. Dough structure, dough rheology and baking quality. Cereal Foods World. 35:237-244.

Czuchajowska, Z and Pomeranz, Y. 1993a. Gas formation and gas retention. I. The system and methodology. Cereal Foods World. 38:499-503.

Czuchajowska, Z and Pomeranz, Y. 1993b. Gas formation and gas retention. II. Role of vital gluten during baking of bread from low-protein or fiber-enriched flour. Cereal Foods World. 38:504-511.

Davis, E. W. 1981. Shelf-life studies on frozen doughs. Bakers' Dig. 55(3):12-13, 16.

De Stefanis, V. A. 1995. Functional role of microingredients in frozen dough. Pages 91-117 in: Frozen and Refrigerated Doughs and Batters. K. Kulp, K. Lorenz, and J.-M. Brümmer, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

Dubois, D. K., and Blockcolsky, D. 1986. Frozen bread dough. Effect of dough mixing and thawing methods. Am. Inst. Baking Technol. Bull. 8(6):1-7.

El-Hady, E. A., El-Samahy, S. K., Seibel, W., and Brümmer, J. M. 1996. Changes in gas production and retention in non-prefermented frozen wheat doughs. Cereal Chem. 73:472-477.

El-Hady, E. A., El-Samahy, S. K., and Bruemmer, J.-M. 1999. Effect of oxidants sodium-stearoyl-2-lactylate and their mixtures on rheological and baking properties of nonprefermented frozen doughs. Lebensmittel Wissenschaft and Technologie. 32(7):446-454.

Evenson, M. 1988. New developments in frozen dough technology. Pages 85-89 in: Proc. Annu. Mtg. Am. Soc. Bakery Eng., 63rd. The Society: Chicago.

Faisy, F., and Neyreneuf, O. 1996. Performance d'une association enzymatique "Glucose oxydase-Hémicellulases" pour remplacer l'acide ascorbique en panification. Industrie des Céréales. Avril. Mai-Juin:4-12.

Hosomi, K., Nishio, K., and Matsumoto, H. 1992. Studies on frozen dough baking. I. Effects of egg yolk and sugar ester. Cereal Chem. 69:89-92.

Inoue, Y., and Bushuk, W. 1991. Studies on frozen doughs. I. Effects of frozen storage and freeze-thaw cycles on baking and rheological properties. Cereal Chem. 68:627-631.

Inoue, Y., and Bushuk, W. 1992b. Studies on frozen doughs. II. Flour quality requirements for bread production from frozen dough. Cereal Chem. 69:423-428.

Inoue, Y., Sapirstein, H. D., Takayanagi, S., and Bushuk, W. 1994. Studies on frozen doughs. III. Some factors involved in dough weakening during frozen storage and thaw-freeze cycles. Cereal Chem. 71:118-121.

Kline, L., and Sugihara, T. F. 1968. Frozen bread doughs. I. Prepared by the straight dough method. Baker's Dig. 42(5):44-50.

Lorenz, K., and Bechtel, W. G. 1965. Frozen bread dough. Baker's Dig.39(4):53-59.

Marston, P. E. 1978. Frozen dough for breadmaking. Bakers' Dig. 52(5):18-20, 37.

Neyreneuf, O., and van der Plaat. J. B. 1991. Preparation of frozen French bread dough with improved stability. Cereal Chem.68:60-66.

Noll, B. 2000. Functional application of wheat fibre in frozen dough products. European Food & Drink Review, Spring, 53-56.

Nonami, Y., Saito, M., Shikano, K., Modeki, F., and Suzuki, A. 1984. Effects of chicken egg yolk on the ultrastructure and loaf volume of white bread. Nippon Shokuhin Hogyo Gakkaishi. 31:570-576.

Räsänen, J., Laurikainen, T., and Autio, K. 1997a. Fermentation stability and pore size distribution of frozen prefermented lean wheat doughs. Cereal Chem. 74:56-62.

Räsänen, J., Blanshard, J. M. V., Siitari-Kauppi, M., and Autio, K. 1997b. Water distribution in frozen lean wheat doughs. Cereal Chem. 74:806-813.

Reed, G., and Nagodawithana, T. W. 1991. Use of yeast in baking. Pages315-368 in: Yeast Technology, 2nd eds. Van Nostrand Reinhold/AVI: New York.

Rouille, J., Le-Bail, A., and Corcoux, P. 2000. Influence of formulation and mixing conditions on breadmaking qualities of French frozen dough. J. of Food Engineering 43(4):197-203.

Sahlstrom, S., Nielsen, A. O., Faergestad, E. M., Lea, P., Park, W. J., Ellekjaer, M. R. 1999. Effect of dough processing conditions and DATEM on Norwegian heart bread prepared from frozen dough. Cereal Chem. 76:38-44.

Trivedi, N., Hauser, J., Nagodawithana, W. T., and Reed, J. 1989. Update on baker's yeast. Am. Inst. Baking, Res. Dept. tech. Bull. 11(2).

Varriano-Marston, E., and Hsu, K. H, and Mahdi, J. 1980. Rheological and structural changes in frozen dough. Bakers' Dig. 54(1):32-34, 41.

Wakamatu, T., Sato, Y., and Saito, Y. 1983. On sodium chloride action in gelation process of low-density lipoprotein (LDL) from hen egg yolk. J. Food Sci. 48:507-512, 516.

Wolt, M. J., and D' Appolonia, B. L. 1984a. Factors involved in the stability of frozen dough. I. The influence of yeast reducing compounds on frozen-dough stability. Cereal Chem. 61:209-212.

Wolt, M. J., and D' Appolonia, B. L. 1984b. Factors involved in the stability of frozen dough. II. The effects of yeast type, flour type, and dough additives on frozen-dough stability. Cereal Chem.61:213-221.

CHAPTER IV

EFFECT OF GLUTATHIONE ON FUNDAMENTAL AND EMPIRICAL DOUGH BEHAVIOR OF PRE-PROOFED FROZEN DOUGH

J. Uriyapongson, and P. Rayas-Duarte

ABSTRACT

Effects of glutathione on fresh and pre-proofed frozen dough properties were investigated using dynamic stress rheometry and micro-extensibility with addition of three levels of reduced glutathione (GSH, 80, 160 and 240 ppm) and six storage times (0 and 1 day, 2, 4, 6 and 8 weeks of frozen storage). Three relaxation times (1, 13 and 26 min) after loading the dough in the rheometer were used to determine storage (G'), loss (G"), and complex (G*) moduli and complex viscosity (η*). Better correlations for G' (r = 0.678 and 0.622 at frequency 0.05, and 10 Hz, respectively) and G" (r = 0.699, and 0.690 at frequency 0.05, and 10 Hz, respectively) with micro-extensibility area were observed at 26 min relaxation time compared to 1 and 13 min. The addition of three levels of GSH to fresh dough reduced G' (by 16.4 to 55.9 %) and G" (by 13.7 to 52.2%). Freezing and frozen storage caused increase in G', G", G* and η*. The addition of all levels of GSH reduced dough strength indicated by the reduction in maximum to resistance (Rmax) and the ratio of maximum to resistance to extensibility (Rmax/E). The reduction in Rmax at all relaxation times was ranged from 16.2 to 59.4%. An increase in extensibility was observed with 240 ppm GSH at all frozen storage and rest period times.

Phase separation using ultracentrifugation was used for analysis of liquid and solid phases for water distribution in the dough. Addition of GSH caused an increase of liquid phase (30.6-35.3%) in fresh dough and an increase of 10.3-20.7% in frozen dough after one day frozen storage. A reduction in the water content of the solid phase of frozen dough was observed at one day and 8 weeks of frozen storage. Negative correlations of water content in the solid phase with dough extensibility and area using micro-extensibility test were found (r = -0.594 and -0.563 respectively, $P < 0.001$). This inverse relationship indicates the importance of water distribution and water mobility to the rheological properties of frozen dough.

INTRODUCTION

The baking quality of wheat flour depends largely on the quantity and quality of gluten proteins, particularly insoluble glutenin and gliadin fractions (Hoseney et al 1969a, b). The dough developed during mixing forms a gluten network that when baked it forms the structure of baked products. Thiol and disulfide groups in dough are related to rheological properties of the dough and baking quality (Bloksma 1975). Glutathione is a tripeptide that can be present in flour in the free reduced (GSH), free oxidized form (GSSG) or mixed disulphide (PSSG) (Ewart 1988). Glutathione has a reactive sulfhydryl of the cysteine side chain that serves as nuecleophile, a reductant and a scavenger of free radicals. The reaction of glutathione as a reductant in gluten network results in the formation of glutathione disulfide (Dong and Hoseney 1995). Disulfide-sulfhydryl interchange occurred in flour-water dough and mixing promoted the reaction of disulfide groups and GSH (Sullivan 1968). Both GSSH and GSH increase extensibility of dough, but the increase by GSSH is less than GSH. The loss of dough strength of frozen dough is caused by changes in the rheological properties of the dough and gluten network (Inoue and Bushuk 1991). Glutathione inside yeast cells is released to the dough matrix when the yeast dies due to freezing damage, weakening the gluten network in frozen dough (Kline and Sugihara 1968, and Autio and Sinda 1992).

Dough is a viscoelastic material (Hibberd and Parker 1975). The empirical rheological tests such as extensigraph, farinograph, and alveograph are performed with large deformation designed to evaluate the processing properties of the material. In

contrast, fundamental rheological tests use small deformations of the dough (Edwards et al 1999). Examples of these tests are creep and creep recovery (Hibberd and Parker 1979) and dynamic oscillation tests reported in dough (Abdelrahman and Spies 1986, Faubion and Hoseney 1990, Amemiya and Menjivar 1992).

Reports that frozen storage and freeze thaw cycles affect the strength of the dough are found in the literature (Inoue et al 1994). The resistance to extension (Rmax) of frozen dough was reported to decrease after 1 day and 70 days of frozen storage and three thaw-freeze (3T-F) cycles (Inoue et al 1994). These authors also reported a strong negative relationship between extensibility and gassing power ($r \geq -0.95$). This suggests that the dough lost yeast viability and extensibility.

The dynamic tests provide valuable insight for the relationship between chemical and rheological properties of dough (Abdelrahman and Spies 1986). The dynamic tests provide well-defined rheological parameters, such as the storage (G') and loss moduli (G"), and viscous counterparts (η" and η') conducted in the linear region of food materials (Hibberd and Parker 1975). Dynamic rheological test have been used to determine the properties of dough after the incorporation of additives (Miller and Hoseney 1999a, b, Hahn and Grosch 1998, Berland and Launay 1995, Wei-Dong and Hoseney 1995).

Moisture content affects the dynamic behavior of dough (Berland and Launay 1995). Separation of layers or phases from frozen dough using ultracentrifugation was used to study the relationship between the amount of water in each phase and the rheological properties of fresh and frozen dough. (Eliasson and Larsson 1993). Dough samples yielded four phase separations formed by a liquid phase (low molecular

weight, water soluble compounds), gel layer (starch and soluble protein), gluten phase (polymeric protein) and starch phase (Eliasson and Larsson 1996a). Eliasson and Larsson proposed that the phase separation depended on the water content. Phase separation were also studied as a function of mixing time, ascorbic acid and lipids, which are known to affect the baking behavior of wheat cultivars (Eliasson and Larsson 1996b). Phase separation was cultivar-dependent and a linear increase of water incorporation to the gluten phase was observed with an increase in mixing time. Lecithin impaired the phase separation while the addition of ascorbic acid improved it (Eliasson and Larsson 1996b). Räsänen et al (1997b) studied water distribution in frozen lean dough. The amount of liquid phase of nonyeasted frozen dough increased with frozen storage time and the most significant increase occurred during the first week of frozen storage. Frozen storage decreased the storage modulus of the dough but no correlations were found between rheological properties and amount of liquid phase. This suggests that the factors that affect both water holding capacity of the dough components and elastic behavior of the dough take place during frozen storage.

Rheological tests of nonyeasted doughs have been reported but few studies had been reported for yeasted dough. The study of yeast-fermented dough posses more challenges due to its complexity and the transient nature of the dough physical properties with time . Two publications on small deformation test of yeasted dough are reported in the literature (Kaufmann and Kuhn 1994, Räsänen et al 1997b). Since the gluten structure and rheological properties of yeasted and nonyeasted doughs is different, the effects of freezing are also different (Inoue and Bushuk 1991, Räsänen et

al 1997b). Despite its challenges, the rheological properties of yeasted frozen dough will illustrate practical relations to the bread making of frozen dough.

The objective of this study was to characterize the rheological properties of pre-proofed, yeasted frozen dough by dynamic rheology with a series of rest time periods after loading the dough on the rheometer. The effect of glutathione on the rheological properties of pre-proofed frozen dough was also characterized by empirical and fundamental rheological tests. The relationship of phase separation using ultracentrifugation with the rheological peopreties was also assessed.

MATERIALS AND METHODS

Commercial hard red spring (HRS) flour (Dakota Mill & Grain Co., Grand Forks, ND) with the composition of 13.5% moisture, 13.5% protein, and 0.58% ash (14% mb) was used in this study. Flour moisture, protein, and ash analyses were made according to Approved Methods 44-15A, 46-13, 08-01 respectively (AACC 1995). Four levels of reduced glutathione (GSH) (Sigma-Aldrich, St. Louis, MO) 0, 80, 160 and 240 ppm were added to the dough. Rheological properties and phase separation of the four treatments were studied at 0, 1 day, 2, 4, 6 and 8 weeks of frozen storage.

Dough Formulation and Preparation

Control full formula dough for bread stick was optimized as described in Chapter III. The dough formula expressed as baker's percentage was 100% HRS flour, 5% compressed baker's yeast (Fleischmann's Yeast Ltd., Fenton, MO), 1.5% salt, 4% shortening, 6% sugar, 50 ppm ascorbic acid, and 0.25% malted wheat flour

(flour basis). Optimum bake water absorption was 0.5% lower than Farinograph absorption (58.5%, in APPENDIX-A) determined with standard method 54-21 (AACC, 1995). The 5% yeast used in this study is higher than the standard formula for freshly baked process as suggested Neyreneuf and van der Plaat (1991). The 50% more yeast was sufficient to maintain satisfactory bread volumes of frozen dough when prolonged storage without negative effects on taste and flavor (Neyreneuf and van der Plaat 1991).

Two independent batches of 800 g flour each were mixed in a Hobart mixer equipped with a circulating water bath at 5°C. Yeast and salt were added after 5 and 9 min of mixing with a total mixing time of 11 min. The final dough temperature averaged 15°C. The dough was sheeted to a 10 mm thickness, cut into160x27x10 mm (LxWxH), and standardized to a weight of 40±0.5 g as describer earlier (Chapter IV). The bread stick strips were proofed at 30°C and 85% relative humidity for 40 minutes (Fermentation Cabinet model 505-11. National Manuf., Lincoln, NE). The proofing time was reduced to 40 min compared to 55 min in Chapter III and conventional bread as suggested by Räsänen et al (1997b). Fresh dough represented 0 day storage time was tested immediately for all dough analyses. The samples for frozen dough analysis were

frozen in a blast freezer at –30°C for 30 min and stored in zip lock plastic bags in a chest freezer at –20°C.

Phase Separation Test

Phase separation was determined using a modified method based on the procedure of Larsson and Eliasson (1996a). Briefly, fresh and frozen dough were thawed out for 1.5 hr and a 20 g sample was centrifuged at 100,000xg for 2 hr at 25°C (XL-700 Ultracentrifuge, Beckman Instruments Inc, Palo Alto, CA). The clear phase and yellow gel (scrapped off from the top of the solid dough layer) were collected and their weight recorded. The remaining pellet was removed from the tube and its weight recorded. Both layers were dried at 135°C, 2 hr (AACC method 44-19, 1995) and the percent moisture was calculated (Räsänen et al 1997b). All samples were analyzed in two independent batches of dough processed in different day of storage time.

Rheological Properties of the Dough

Dynamic Mechanical Test

Rheological properties of fresh and frozen dough were determined using a dynamic stress rheometer Rheolist AR 1000 (TA Instruments, New Castle, DE) equipped with parallel plates and at 25°C. A 4 cm diameter-crosshatch geometry parallel plate and a 9-cm diameter sand paper (3M, no. 150) glued to the base plate were used to minimize slippage of the dough sample. To prevent drying, a solvent trap was used filled with water on the upper geometry and a clear plastic cover. The exposed edges of the dough samples were also coated with mineral oil as recommended by Edwards et al (1999). Edwards et al reported that mineral oil coated dough samples did not dry for up to a 30 min test. The 5±0.5g samples of fresh and frozen dough was manually rounded and flatten between the sand paper and upper

plate. The gap was set at 2000 μm. Three strain sweep-experiments were carried out to identify the linear region before performing the frequency sweep test at 0.1% strain. Storage (G'), loss (G"), and complex (G*) moduli and complex viscosity (η*) were recorded. The frequency sweep test was performed from 0.01 to 15 Hz. After loading the dough sample onto the rheometer, analysis of fresh and frozen dough were recorded at three resting or relaxation time periods (1, 13 and 26 min).

Empirical Rheological Tests

Micro-extensibility of Dough Using Texture Analyzer

A modified method based on the texture analyzer application method and Suchy et al (2000) was used. The frozen dough was thawed out for 1.5 hr in zip lock plastic bags at room temperature (25°C). About 15 g dough was shaped as an oval and placed over the Teflon-coated block containing thin channels as per manufacturer's instructions. The dough was pressed tightly by the upper half of the Teflon-coated block, clamped and excess dough extruded out from the block removed. Dough exposed to air was coated with mineral oil. The dough block was cut into 10 uniform strips (0.75-1.0 g). The dough strips were rested in the block for 3 min before the test. The first dough strip was removed from the block by carefully sliding the upper block and picked up with a thin spatula. The remaining dough strips were left in the block covered with the upper block to prevent drying. Seven dough strips were tested immediately after removal from the block by positioning it across the Kieffer rig holder. The instrument was used in tensile test with a pre-test speed of 2.0 mm/s, test speed of 3.3 mm/s and post-test speed of 10.0 mm/s, distance of 110 mm and a trigger

force of 5g. The parameters recorded (APPENDIX L) were extensibility as the distance from start to the maximum force (E), area under the curve (A), maximum resistance to extension (Rmax), resistance to extension at a distance of 20 mm (R20mm), and the Rmax/E ratio. After the measurement of the dough at relaxing time 0 min, the same dough was reshaped into an oblong shape and rested in the zip lock plastic bag for 45 and 90 min before cut into strips and retested.

Statistical Analysis

Statistical analyses were performed using a mixed model (PROC MIXED) with Statistical Application Systems software, SAS version 8.2 (SAS Institute Inc., Cary, NC). Sample differences were obtained using a mixed procedure and least significant differences (LSD). Relationships between parameters (small deformation dynamic and micro-extensibility tests) were established using Pearson's correlation coefficient (*r*) with PROC CORR.

RESULTS AND DISCUSSION

Phase Separation

Amount of Liquid Phase in Dough.

The amount water in the phases separated by ultracentrifugation as a function of storage time is shown in Table I and APPENDIX M. Overall, higher values of liquid phase are obtained from the dough containing GSH at 0 and 1 day of storage when compared to the control (Table I). The liquid phase included gelatinous layer

containing water and soluble material, such as soluble proteins, hydrolyzed lipid, sodium chloride and soluble carbohydrate.

Overall, the addition of GSH resulted in an increased liquid phase (range 30.6-35.3%) of the fresh dough compared to control. Comparing dough at 0 vs. 1 day frozen, the control (no addition of GSH) and 80 ppm GSH showed 15.8 and 10.3% increase of liquid phase. Freezing the dough for one day resulted in an increased liquid phase compared to the 0 day, not frozen samples. An increase of 22.8 and 18.6% of liquid phase was observed at 1 day and 2 weeks of frozen storage, respectively, compared to the control at 0 day. There was no significant change in the amount of liquid phase of frozen dough with GSH 160 and 240 ppm up to 6 weeks of frozen storage. However, a trend of reduced amounts of liquid phase was observed at 8 weeks of frozen storage, except with 80 ppm GSH. Sublimation of water usually occurs during the frozen storage of foods. The bread stick dough showed sublimation at 8 weeks of frozen storage recorded as the presence of ice crystals outside the product and captured inside the plastic bag. The freezing and the presence of GSH might have decrease the water holding capacity of the gluten proteins and allowed the mobility of water to increase. Water was sublimed outside the bread sticks resulting in a reduction of water content in solid phase and liquid phase in some treatments at 8 weeks frozen storage.

More liquid phase was obtained after the addition of GSH due to the relaxing effect by the exchange of sulfhydryl/disulfide bonds between GSH and gluten proteins. The highest level of GSH (240 ppm) gave the highest amount of liquid phase compared to all other treatments at 6 ad 8 weeks of storage (Table I). This

suggests a synergestic effect of GSH and frozen storage that resulted in a reduction of the capacity of the gluten matrix to hold the water as tightly as in fresh dough.

Lower molecular weight proteins could be present in the liquid phase. This suggests that the reduction of the gluten proteins occur due to the cleaving of interchain disulfide bonds resulting in the depolymerization of gluten proteins and decreased molecular weight (Yoshida et al 1980). The freezing and frozen storage also enhanced the loss of water from polymeric proteins. As more water is pulled away from the proteins, as recorded by the increased amount of liquid phase after freezing and frozen storage, the growth of ice crystals in the system will rupture the yeast cells (Räsänen et al 1997b). These authors also showed that the freezing process had a stronger effect on the amount of liquid phase formed in yeasted dough than frozen storage time.

Amount of Solid Phase.

The three levels of GSH tested (80, 160 and 240 ppm) reduced the amount of solid phase of the fresh and frozen dough ($P < 0.05$, 0.01 and 0.01 respectively, Table I and APPENDIX M). However, similar amounts of solid phase were observed among 80, 160 and 240 ppm GSH levels. The solid phase is made mainly of protein, starch and water; a reduction is due mainly to the loss of water, as described in the following section, and perhaps some low molecular weight components including proteins.

Amount of Water in Solid Phase.

There was no significant difference in the amount of water in the solid phase of all fresh doughs. Table I shows a trend to reducing the amount of water present in the solid phase when comparing 0 day (not stored) to the 1 day frozen dough (11.87% decrease) with 240 ppm GSH (Table I and APPENDIX M). These results confirmed that freezing reduced the amount of water in the solid phase. The weakening of the gluten network caused a lower water holding capacity of the polymeric proteins, which have exchanged disulfide bonds with glutathione (GSSP) and thus reduced its molecular weight and spectial arrangement in the system. The amount of water in the solid phase remained unchanged up to 6 weeks of frozen storage when a reduction was recorded ($P<0.05$).

Micro-extensibility

Maximum Resistance to Extension (Rmax)

Rmax of 0 day dough (not stored) with no relaxation time (0 min), first extension testing, was similar for the control and 80 and 160 ppm GSH while a reduction of 39.4% was observed with 240 ppm GSH (Fig. 1a). In contrast, after 45 and 90 min of relaxation time, all three levels of GSH (80, 160, and 240 ppm) produced lower Rmax compared to the control: 32.5, 37.6, and 59.4% at 45 min (Fig. 1b) and 32.5, 19.2, and 47.9% at 90 min, respectively (Fig. 1c). Trend of lower Rmax in the presence of GSH was observed for all three levels and three rest period times ($P<0.01$). These results also agree with Kuninori and Sullivan (1968) who found that GSH affected the rheological properties of the dough caused by sulfhydryl-disulfide

interchange. These results agree with the report by Hahn and Grosch (1998) who found that the addition of GSH to flour/water dough (100 nmole/g flour) decreased the Rmax and increased extensibility of the fresh dough made from DNS flour.

There was no significant change in Rmax in the control sample tested at 0 min relaxation time when comparing 0 day (not stored) vs. 1 day frozen stored. These observations contrast to a marked dropped (~16-18% reduction) of Rmax after one day of frozen storage of doughs made from four types (strong and weak) of commercial Canadian wheat (Inoue and Bushuk 1992). The loss of dough strength was explained by the initial freezing alone while prolonged frozen storage gradually decreased Rmax depending on flour strength (Inoue and Bushuk 1992). In this study with HRS flour, no reduction in Rmax as observed during frozen storage of the control dough until after 6 weeks of frozen storage (0 min relaxation time). However, our results were similar to the report by Inoue and Bushuk (1994) in that there was no change in Rmax at 1 and 7 day frozen storage but was significantly reduced at 70 days. Inoue and Bushuk reported a negative correlation ($r > -0.9$) of the yeast/dough gassing power and dough extensibility.

Extensibility (E)

No significant differences in extensibility were observed in doughs tested without relaxation time (0 min) up to 6 weeks of frozen storage with all the GSH levels (Fig. 2a). Comparing one day to 6 and 8 weeks of storage, a small but significant increase of extensibility was recorded ($P < 0.05$). Only 240 ppm GSH showed a significant increase in extensibility starting at 6 weeks of frozen storage,

when tested at 45 and 90 min relaxation time (Fig. 2b, c). Overall, 240 ppm GSH also showed an increased extensibility at each time period of frozen storage except at 0 min rest period time

The addition of GSH in this study supported the weakening of the dough during freezing and frozen storage as suggested by Kline and Sugihara (1968). The level of dough increments of extensibility during frozen storage was found flour dependent (Inoue and Bushuk 1992).

Deformation Energy Area (A)

Compared to the control, the addition of GSH to the dough caused an increase of deformation energy area in the first 2 weeks of frozen storage and then the curves were inverted (Fig 3a b c). At 4 weeks of frozen storage the trend changed to lower areas from samples containing GSH and higher areas for the control. This trend was for the most part consistent in the three rest period tests. This suggests that this parameter could be detecting a true change in the structure of the dough. The cross over may indicate the overall work of the test showing signs of toughening of the dough at 4 weeks of storage, that other parameters did not detect. If these observations were an artifact of the test they will not have correlation with any other tests.

Resistance to Extension at 20 mm (R20mm)

R20mm values of control vs. GSH containing dough were similar except for higher values at 160 ppm GSH at 0, 1 and 2 weeks, 0 min rest period and 0 and 1 day 90 minute rest period (Fig 4a b c). The lowest value of R20 was with the dough containing 240 ppm GSH of at all relaxation times and frozen storage times. The

overall trend of the curves of R20mm and Rmax was different. While Rmax showed the control dough with higher values, R20mm was a band of tracings with a spread between 10 and 20g, with no clear trend.

Ratio of Resistance to Extension and Extensibility (Rmax/E ratio)

This ratio is used to describe the shape of the extensibility curve (Rasper and Preston 1991). Overall the three measurements showed higher values for the control samples, except at 0 days, 0 min rest time and 8 weeks of storage (Fig 5a, b, c). GSH addition at 80 and 160 ppm gave overall similar Rmax/E ratios, while 240 ppm showed the lowest ratios. Lower Rmax/E ratio describes loss of dough strength due to lower resistance to extension or more extensible dough.

When the dough was rested for 45 and 90 min, the addition of GSH showed a reduction in Rmax/E ranging from 34-95% (Fig. 5b,c) in fresh dough. This suggests that GSH disrupted the gluten network and caused reduction in viscoelastic properties of the dough even for fresh dough. When the dough was frozen for one day, the Rmax/E ratio decreased 9.9 and 34.3% for the samples containing 80 and 160 ppm GSH. At 0 min rest time, the comparison of 0 vs. 1 day frozen dough reduced the Rmax/E ratio of the dough containing 80 and 160 ppm GSH (Fig. 5a). However, no changes of Rmax/E ratio were observed with GSH at 240 ppm for the three resting times during storage. For no rest time (Fig.5a), addition of GSH at all levels gave lower Rmax/E ratio than frozen control dough and remained unchanged up to 4 weeks at 6 and 8 weeks frozen storage, Rmax/E ratio of control and dough with all levels of GSH decreased ($P < 0.05$ and < 0.01 respectively). Rmax/E ratio of the dough with all levels of GSH remained lower than the control and unchanged at all relaxation times

and frozen storage until 6 weeks. At 8 weeks of frozen storage, the control frozen dough had Rmax/E ratio similar to the dough containing GSH at 0 and 45 min rest time and 80 ppm GSH at 90 min rest time. Similar Rmax/E ratio of control dough and 80 ppm GSH dough at 8 weeks of storage suggests similar viscoelastic properties. This could be due to an increase of GSH in the control dough originated by the release of GSH from dead yeast cells when frozen damage from

Ice formation occurred in the dough (Kline and Sugihara 1968).

Correlation

Significant negative correlation was observed between deformation energy area (A) and water in solid phase (r = -0.5943, $P < 0.001$, in Table II). A negative correlation was also found between extensibility (E) and amount of water in solid phase (r = -0.563, $P < 0.001$, in Table II). Despite the low values of X, these results support the importance of managing the mobility in the frozen dough system.

Viscoelastic Properties Using Rheometer

Effect of Relaxation Time and Frequency

Literature reports on the requirement of resting of dough before oscillatory testing ranges from 1 min (Lindahl and Eliasson 1992) to 1 hr to obtain rheological values with lower variation due to sample handling (Edwards et al 1999). Methods have included resting the dough after mixing, before loading and after loading on the rheometer. In our experiment, three relaxation times (1, 13 and 26 min) were given to the dough after loading onto the rheometer (APPENDIX N-AC.). There was a

significant interaction between G', G", G* and η* with GSH levels, frozen storage time and relaxation time ($P<0.001$). Overall, using 1 minute relaxation time, the dough showed similar G', G", G* and η* values as a function of frequency for the control, dough with different frozen storage times (Fig. 6 top, and APPENDIX N-V top) and GSH levels (Fig. 7 top, and APPENDIX W-AC). When the dough was tested after 13 and 26 min rest, overall, higher values of G', G", G* and η* were observed for frozen dough except for fresh (0 day) dough (APPENDIX N-AC, middle and bottom, respectively). Our results did not agree with Lindborg et al (1997) who reported that the maximum viscosity of fresh dough increased with relaxation time. However, our results showed that relaxation time did not affect G', G", G* and η* of fresh dough but for frozen dough these parameters increased as the relaxation time increased.

Generally, G' significantly increased in the high frequency rang of 1 to 15 Hz, except for G' at 80 and 240 ppm GSH at 13 min relaxation time (APPENDIX N-b and O-b, middle) which was independent of frequency. As the GSH levels increased, the elastic behavior was more independent of frequency, as observed by lower slope (flatter curves). The elastic (G') and viscous (G") behavior appeared to be independent of frequency at 0.02 up to 1Hz. At 5 Hz a small inflection of G', G", and G* was observed. Compared to the elastic behavior (G') numerical value, the viscous behavior or G" module was about 0.5 times lower at low frequency (0.02-1 Hz) and about 3 times higher at relatively high frequency (5-15 Hz). The values are clustered together at low frequency up to 1 Hz except for the moduli of 80 and 240 ppm GSH at 8 weeks of frozen storage. The G* had similar values to G' at all frequencies and

relaxation times. The complex viscosity (η*) showed opposite direction to G', G", and G*. The values of all treatments were clustered at 5 to 15 Hz.

The relaxation time at 26 min showed better correlation (Table III) of G', G", G* and η* with deformation energy area of micro-extensibility test at both low frequency (0.05 Hz) and high frequency (10 Hz) (r = 0.62-0.69, $P < 0.001$) compared to those at 1 min relaxation time (r = 0.38-0.52, $P < 0.001$).

The patterns of the viscous and elastic behavior are broadened when the dough is rested for 13 and 26 min. A trend to higher values was observed and this might be explained by removing the structure stress of the dough caused by the manipulation during loading. No difference in the values of G', G", G* and η* with the relaxation times of 13 and 26 min were observed. These results agreed with Edwards et al (1999) who reported no change in of G' or tan δ from 10 to 30 min at 2 Hz with non yeast-durum wheat dough. One min resting time did not allow sufficient structural relaxation time of the dough compared to 13 and 26 min resting time (Dreese et al 1988).

The G', G", G*, and η* of the fresh dough were more consistence but varied for frozen dough depended on frequency and relaxation time (Appendix N-U). At high frequency from 5 up to 15 Hz with 13 and 26 min relaxation times showed distinct differences among the frozen dough.

Effect of Frozen Storage Time

For fresh dough, the control sample (without addition of GSH) showed similar G'and G* to the dough containing 80 ppm GSH but higher than the dough with 160

and 240 ppm GSH (Fig. 8). For fresh dough, no differences of G" was observed between GSH containing samples and the control. In frozen dough at 1 day, the control dough had significantly higher G', G" and G* than all the dough with GSH ($P < 0.05$, Fig. 8). The increase of frozen storage time from 1 day up to 4 weeks did not increase these values but remained similar to 1 day frozen dough. An upward trend for G' and G" was observed in all samples from 4 to 8 weeks.

These results do not agree with Autio and Sinda (1992) and Räsänen et al (1997b) who showed that G' decreased during frozen storage of nonyeasted dough. No clear trend of G' in different flour types was observed in yeasted frozen dough (Räsänen et al 1997b). A trend of an increase in G' in one flour out of four types in the results of Räsänen et al (1997b) is similar to our results. The differences might be due to flour protein quality and quantity, and glutathione naturally present in the flour.

Freezing and frozen storage affected ice crystal growth from free water in frozen doughs observed as dark pores by autoradiography (Räsänen et al 997b) and dark patches by low temperature scanning electron microscopy (Berglund et al 1991). The formation of ice crystals implies a dehydration of the gluten network changing the original hydration of the polymers. Water acts as a plasticizer and changes would affect the rigidity of the system. Räsänen et al (1997b) reported that the dough with higher rigidity (higher G') gave smaller loaf volume which agreed with this report (data shown in Chapter V). The increase of G' was supported by more solid-like behavior, an increase in the amount of liquid phase and decrease of water content of solid phase reported earlier.

Effect of Glutathione (GSH)

The addition of GSH in fresh and frozen dough reduced G', G", G* and η* (Fig. 8, 9, and APPENDIX V-AC).). The addition of 80, 160 and 240 ppm GSH to fresh dough reduced G' by 16.4, 30.8 and 55.9 %, respectively and reduced G" by 13.7, 23.0 and 52.2% respectively. At 8 weeks of frozen storage, G' and G* increased with the addition of 80 and 160 ppm GSH.

Bloksma (1972, 1975) and Jones et al (1974) concluded that only small fraction of disulfide groups were rheological effective on the dough. They also reported that the addition of GSH in the fresh dough changed the rheological properties of the dough and the dough became softer in contrast. But Berland and Launay (1995) found that the addition of 15 and 30 ppm GSH had no rheological effects while 50 and 150 ppm GSH decreased G', G" and |η*|. They explained that the addition of 15-30 ppm GSH to the dough caused some reduction of disulfide bonds but did not sufficient to reduce the average molecular weight of glutenins. On the other hand, with addition of GSH at 50-150 ppm, the size reduction of glutenins modifying its structure leaded to a change in theological value. Large variation of the theological properties can also cause by the handling of the dough during the test. The structure of yeast- fermented frozen dough can be more susceptible to handling compared to non-yeasted dough.

The presence of glutathione in dough caused a reduction in G' and dough become softer because the viscoelastic properties of the dough are primarily related to the continuous protein phase (Wei-Dong and Hoseney 1995). The reduction of average molecular weight of gluten protein might have occurred due to the increase of

sulfhydryl-disulfide interchange during mixing (Wei-Dong and Hoseney 1995). The reduction of G' and G" caused by the addition of GSH was supported by our results of phase separation. The increase of GSH reduced the molecules of gluten protein resulting in molecular shifts, high amounts of liquid phase in the dough and less solid-like behavior (decreased G') compared to the control. The G" increased as the levels of GSH increased supported by the reduction of the amount of solid phase of the dough. The change in these rheological values of the dough due to high levels of GSH and longer frozen storage time modified the balance between the viscous and elastic behavior (Berland and Launay 1995).

Correlation

The linear correlation coefficients (Table IV) of G', G", G* and η* with the amount of liquid phase were low but significant $P < 0.05$ to 0.001 ($r = -0.473$, -0.379, -0.462 and –0.453 respectively). They were also low for water content in solid phase ($r = -0.461$, -0.489, -0.470 and -0.388respectively). Räsänen et al (1997b) reported lower correlation between G' and the amount of liquid phase with prefermented frozen dough compared with water-flour mixtures.

Kenny et al (1999) reported that the resistance to extension using extensigraph and complex modulus using stress rheometer of fresh and frozen unyeasted doughs were positively correlated with loaf volume ($r = 0.86$ and 0.64, $P < 0.01$). Our results showed significant correlation of G', G", G* and η* with specific volume. However, with the measurement at low frequency (0.05 vs. 10 Hz) and longer relaxation time (1 vs 26 min) the correlation increased (Table III). Rheological viscoelastic properties

and micro-extensibility parameters had also positive correlations (r = 0.67-0.70, Table III). Similar correlations of viscoelastic properties with extensigraph parameters (r = 0.64) were reported by Kenny et al (1999).

CONCLUSION

Using dynamic rheology, the reduction of the elastic and viscous behavior of the dough due to GSH was found to range from 14 to 56%. Allowing the dough to rest for 26 min in the rheometer improved the detection of rheological differences. The strength of the dough containing GSH measured after 45 and 90 min relaxation showed good correlation with the elastic and viscous moduli of the dough. The selection of 45 min relaxation time would be sufficient for routine micro-extensibility testing of frozen dough. The addition of GSH yielded dough with half its original strength and more extensible. Changes in dough rheological properties during freezing are related to the water distribution in the dough. Negative correlation coefficients were obtained for liquid and solid phase separation with dynamic rheometry and micro-extensibility parameters (r = -0.4 and –0.6, respectively). Thus determination of the changes in rheological properties of frozen dough could be done using phase separation, dynamic rheometry and micro-extensibility with proper selection of resting time.

TABLE I

Measurement of Phase Separation of Dough using Ultracentrifugation[a]

Frozen storage time (weeks)	Liquid phase (%)	Solid phase (%)	Water in liquid phase (%)	Water in solid phase (%)
Control				
0	11.85	86.14	73.41	35.06
1	13.72	85.59	73.00	35.84
2	16.84	82.17	72.69	33.02
4	14.55	83.31	75.14	32.31
6	14.93	84.34	74.13	33.60
8	13.39	84.67	76.64	30.50
GSH[b], 80 ppm				
0	15.47	83.66	73.25	34.82
1	15.14	81.93	72.71	33.95
2	17.96	78.90	73.03	33.71
4	18.37	80.24	72.70	34.96
6	15.47	83.67	71.58	33.35
8	15.74	86.01	71.37	33.81
GSH, 160 ppm				
0	16.04	84.69	71.75	36.53
1	16.56	80.40	73.08	34.04
2	15.91	82.54	71.46	33.28
4	16.35	82.52	71.44	34.34
6	17.70	82.29	72.35	32.93
8	15.05	82.80	67.97	32.23
GSH, 240 ppm				
0	15.71	82.20	72.49	38.40
1	15.98	82.90	71.41	33.84
2	16.47	82.22	73.27	33.68
4	19.94	78.52	72.91	32.64
6	19.25	79.70	73.23	31.57
8	16.92	82.59	72.19	33.69

[a] Mean ± standard deviation, each values is a mean of 4 measurements. Analysis was done in two independent batches with two subsamples per batches.

[b] GSH = glutathione reduced form.

TABLE II

Correlation Coefficient of the Dough Rheological Properites Using Micro-extensibility and Ultracentrifugation[a]

	A	E	R_{max}	F_2	R_{max}/E
Liquid phase (%)	-0.0966	0.0816	-0.4028	-0.1990	-0.3441
Water in liquid phase (%)	0.2071	0.1855	0.2481	0.2783	0.1936
Water in solid phase (%)	-0.5943 ***	-0.5630 ***	-0.1576	0.0231	0.0760

[a] Duplicate analysis of dough sample, n = 48.

[b] *** = Significant at $P < 0.001$.

TABLE III

Correlation Coefficient of the Dough Rheological Properties Using Rheometer and Micro-extensibility[a]

	R_{max}	E	A	F_2	$R_{max/E}$
0.05 Hz, relaxation time 1 min[b]					
G'[a]	0.46689***[c]	0.28811*	0.51889***	0.22892	0.21696***
G''[a]	0.44872***	0.25515	0.45398***	0.24564	0.22164***
G*[a]	0.49086***	0.29154*	0.48639***	0.22046	0.24419***
η*[a]	0.46963***	0.29129*	0.51437***	0.21749	0.22012***
0.05 Hz, relaxation time 13 min					
G'	0.05617	0.42558***	0.33510*	-0.09413	-0.0738 *
G''	0.01954	0.43901***	0.31141*	-0.12746	-0.1022 ***
G*	0.49184***	0.28783*	0.55073***	0.21378	0.2219***
η*	0.05265	0.42743***	0.32941*	-0.10918	-0.0773 *
0.05 Hz, relaxation time 26 min					
G'	0.34319*	0.38226	0.67817***	0.18240	0.0669*
G''	0.28129	0.42698***	0.69891***	0.14737	0.0039
G*	0.33344*	0.38647***	0.67942***	0.17660	0.0579
η*	0.3495*	0.39288***	0.68972***	0.18544	0.07176*
10 Hz, relaxation time 1 min					
G'	0.49362***	0.08095	0.38369***	0.35466*	0.31012***
G''	0.42645***	0.15173	0.44079***	0.32482*	0.22706***
G*	0.50716***	0.09033	0.40469***	0.31876*	0.30692***
η*	0.48513***	0.1085	0.39855***	0.32142*	0.29354***
10 Hz, relaxation time 13 min					
G'	0.30837*	0.36313*	0.4145***	0.06132	0.11898***
G''	0.30837*	0.36313*	0.4145***	0.06132	0.11898***
G*	0.30837*	0.36313*	0.4145***	0.06132	0.11898***
η*	0.30837*	0.36313*	0.4145***	0.06132	0.11898***
10 Hz, relaxation time 26 min					
G'	0.37283***	0.38376***	0.62212***	0.14599	0.10094***
G''	0.25487	0.46405***	0.69024***	0.08420	-0.0248
G*	0.35259*	0.39852***	0.63264***	0.13262	0.08111***
η*	0.3711***	0.40131***	0.64221***	0.14203	0.09669***

[a] Duplicate analysis of dough sample, n = 48.

[b] Frequency (Hz) and dough resting time (min) using Rheometer.

[c] *, *** = Significant at $P < 0.05$, and 0.001 respectively.

[d] G' = storage modulus, G'' = loss modulus, G* = complexs modulus, η* = complex viscosity.

TABLE IV

Correlation Coefficient of Dough Rheological Properties Using Rheometer and Phase Separation Using Ultracentrifugation[a]

	G'[c]	G''	G*	η*
10 Hz, relaxation time 1 min				
Liquid phase (%)	-0.473 ***[b]	-0.379 *	-0.462 ***	-0.453 ***
Water in liquid phase (%)	0.377 ***	0.341 *	0.371 ***	0.375 ***
Water in solid phase (%)	-0.243	-0.319 *	-0.272	-0.263
10 Hz, relaxation time 26 min				
Liquid phase (%)	-0.391	-0.280	-0.375	-0.331
Water in liquid phase (%)	0.271	0.223	0.271	0.181
Water in solid phase (%)	-0.461 *	-0.489 *	-0.471 *	-0.388

[a] Duplicate analysis of dough sample, n = 48.

[b] *,**, *** = Significant at $P < 0.05$, 0.01 and 0.001 respectively.

[c] G' = storage modulus, G'' = loss modulus, G* = complexs modulus, η* = complex viscosity.

TABLE V

Correlation Coefficient of the Dough Rheological Properites Using Rheometer and Baking Quality Parameter of Bread Sticks[a]

	G'[d]	G''	G*	η*
At 10 Hz, relaxation time 1 min[b]				
Specific volume	-0.339 *[c]	-0.424 **	-0.356 *	-0.345 *
Crust score	0.388 **	0.282	0.361 *	0.385 **
Crumb score	0.278	0.155	0.233	0.256
Crumb firmness	-0.040	0.078	-0.012	-0.018
At 0.05 Hz, relaxation time 1 min				
Specific volume	-0.515 ***	-0.483 ***	-0.505 ***	-0.499 ***
Crust score	0.326 *	0.297 *	0.335 *	0.340 *
Crumb score	0.105	0.089	0.116	0.105
At 0.05 Hz, relaxation time 26 min				
Specific volume	-0.647 ***	-0.694 ***	-0.652 ***	-0.657 ***
Crust score	0.203	0.135	0.193	0.189
Crumb score	-0.090	-0.165	-0.102	-0.105

[a] Duplicate analysis of dough sample, n = 48.

[b] Correlation with rheological properties at that frequency (Hz) and at that relaxation time (min) using Rheometer.

[c] *, **, *** = Significant at $P < 0.05$, 0.01, and 0.001 respectively.

[d] G' = storage modulus, G'' = loss modulus, G* = complexs modulus, η* = complex viscosity.

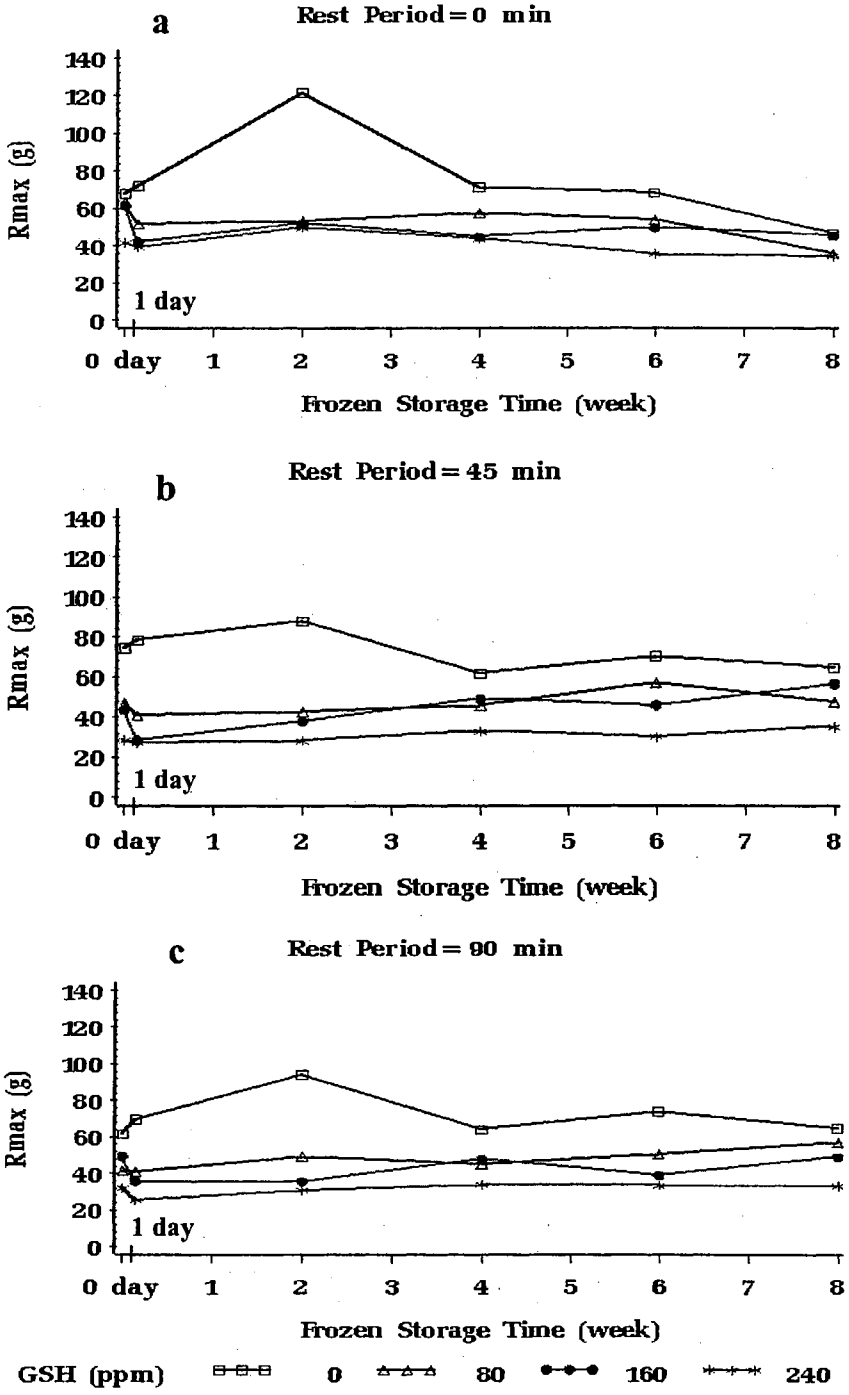


Fig. 1. Maximum resistance to extension (Rmax) of hard red spring flour-dough containing glutathione as a function of frozen storage time.
The measurements were done at rest period time: **a**) 0 min, **b**) 45 min, and **c**) 90 min.

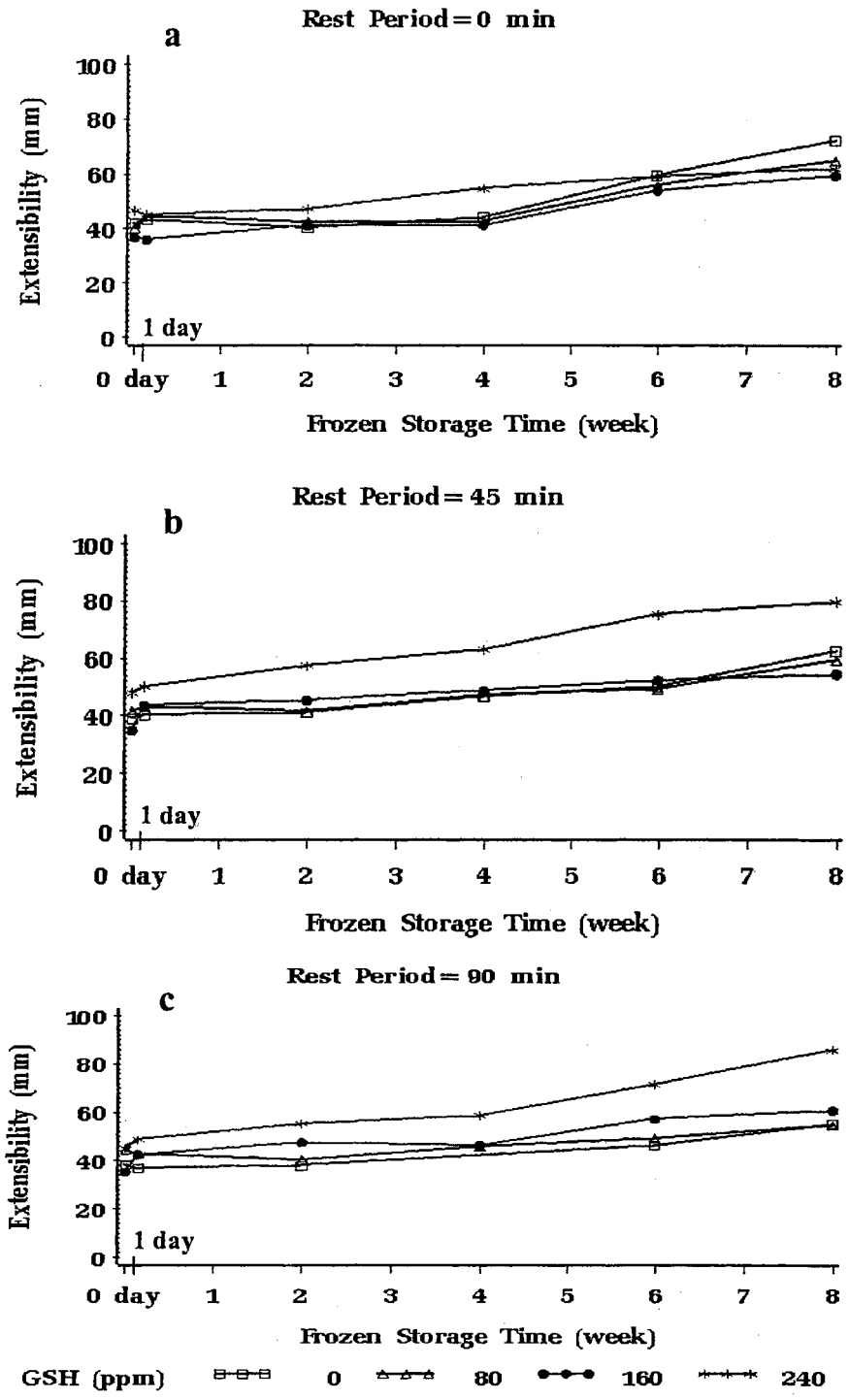


Fig. 2. Extensibity (E) of hard red spring flour- dough containing glutathione as a function of frozen storage time.The measurements were done at rest period time: **a**) 0 min, **b**) 45 min, and **c**) 90 min.

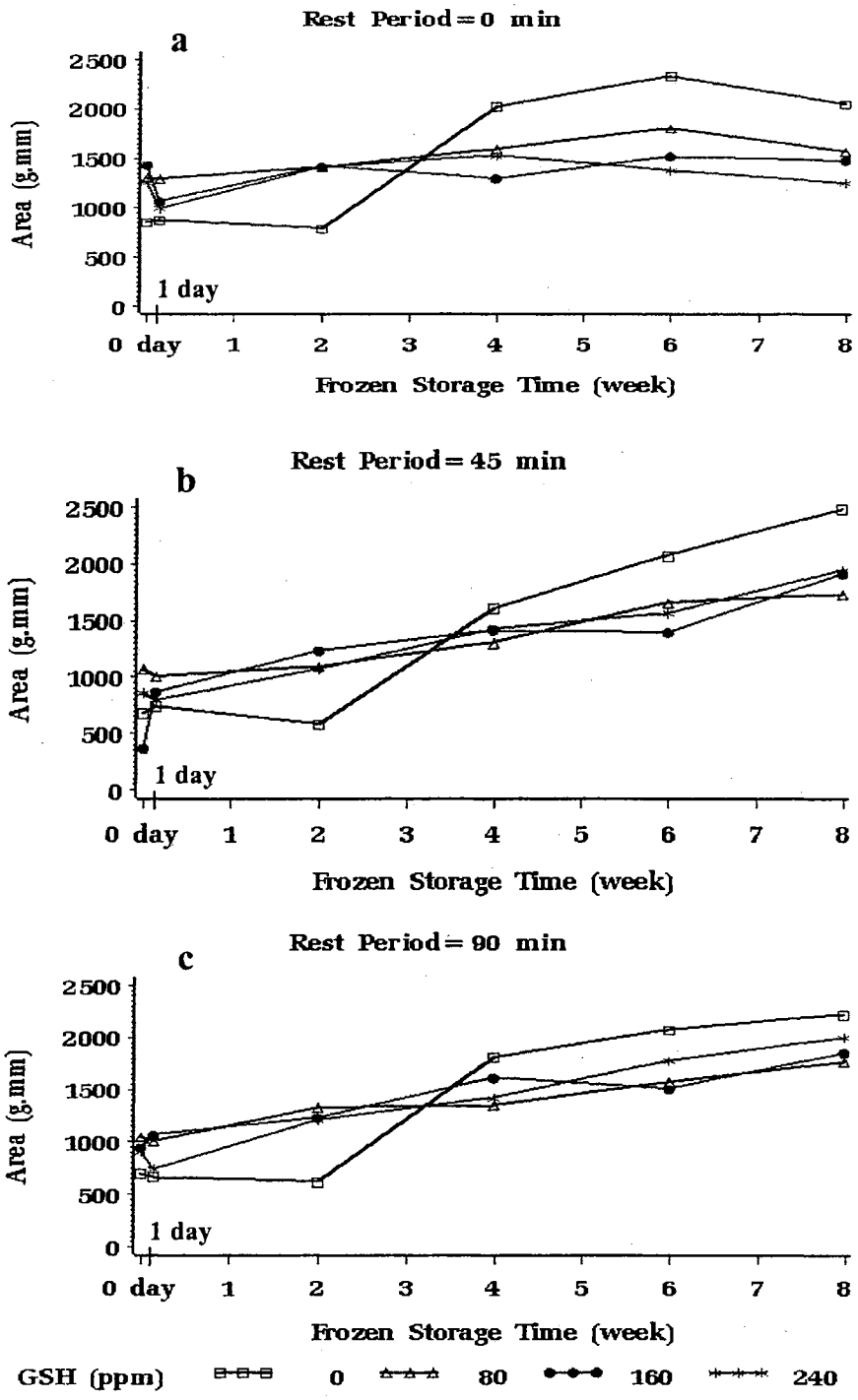


Fig. 3. Area under the curves(A) of HRS-dough made from flour with addition of different amount of glutathione at fresh and different frozen storage time. The measurement was done at: **a)** 0 min, **b)** 45 min, and **c)** 90 min of resting time of the dough.

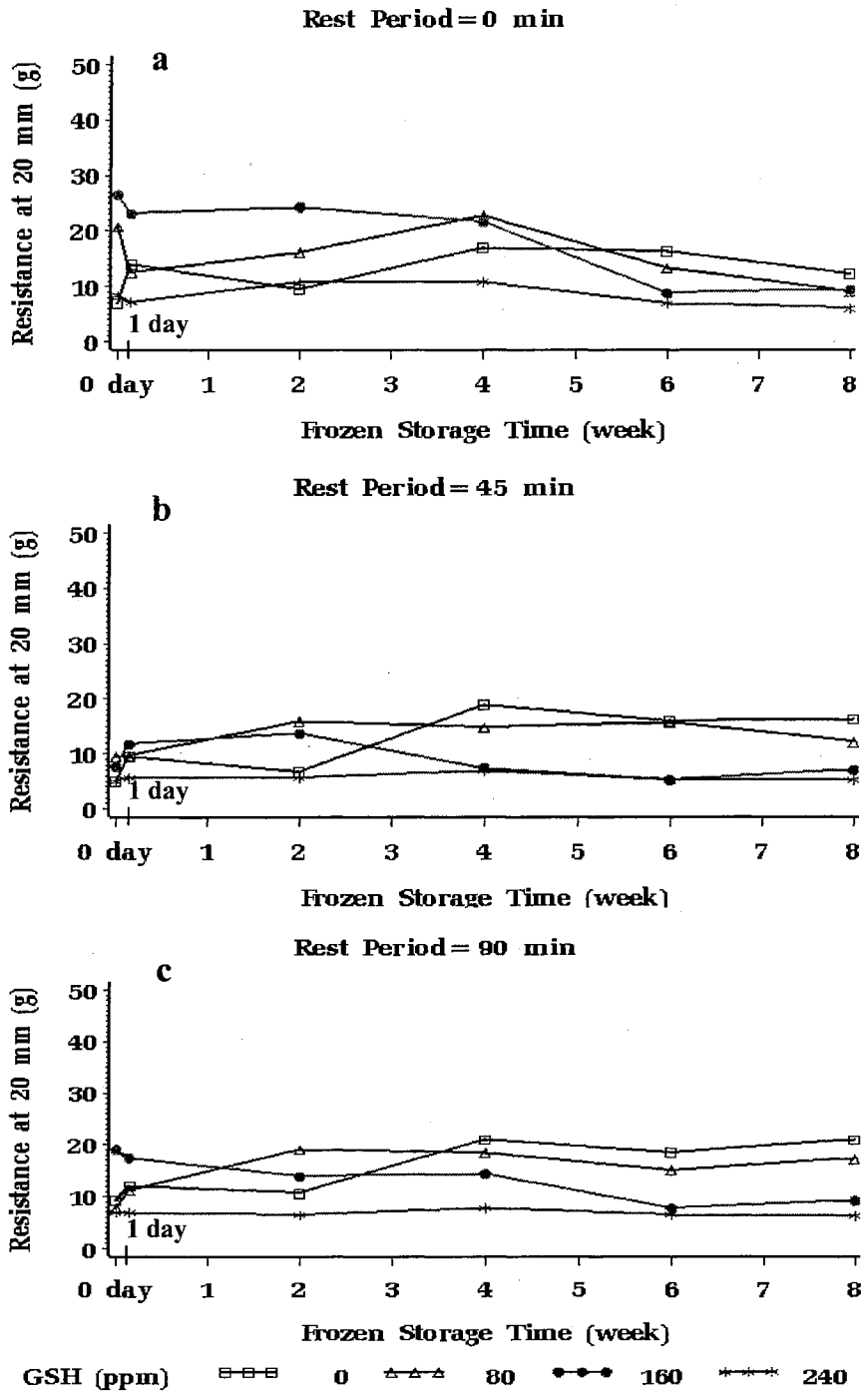


Fig. 4. Resistance to extension at the distance of 20 mm (R20mm) of HRS-dough made from flour with addition of different amount of glutathione at fresh and different frozen storage time. The measurement was done at: **a)** 0 min, **b)** 45 min, and **c)** 90 min of resting time of the dough.

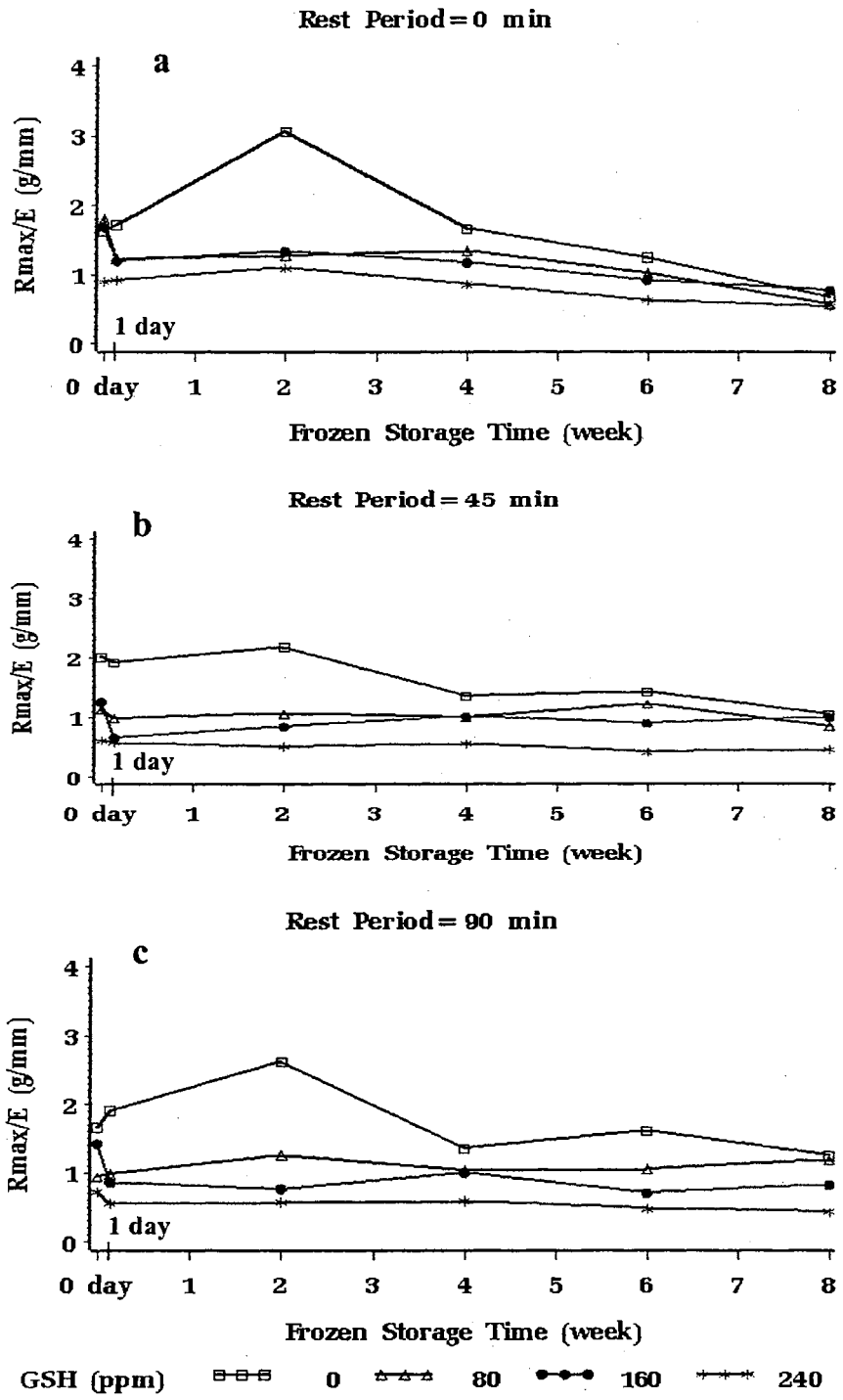


Fig. 5. Viscoelastic ratio(Rmax/E) of HRS-dough made from flour with addition of different amount of glutathione at fresh and different frozen storage time The measurement was done at: **a)** 0 min, **b)** 45 min, and **c)** 90 min of resting time of the dough.

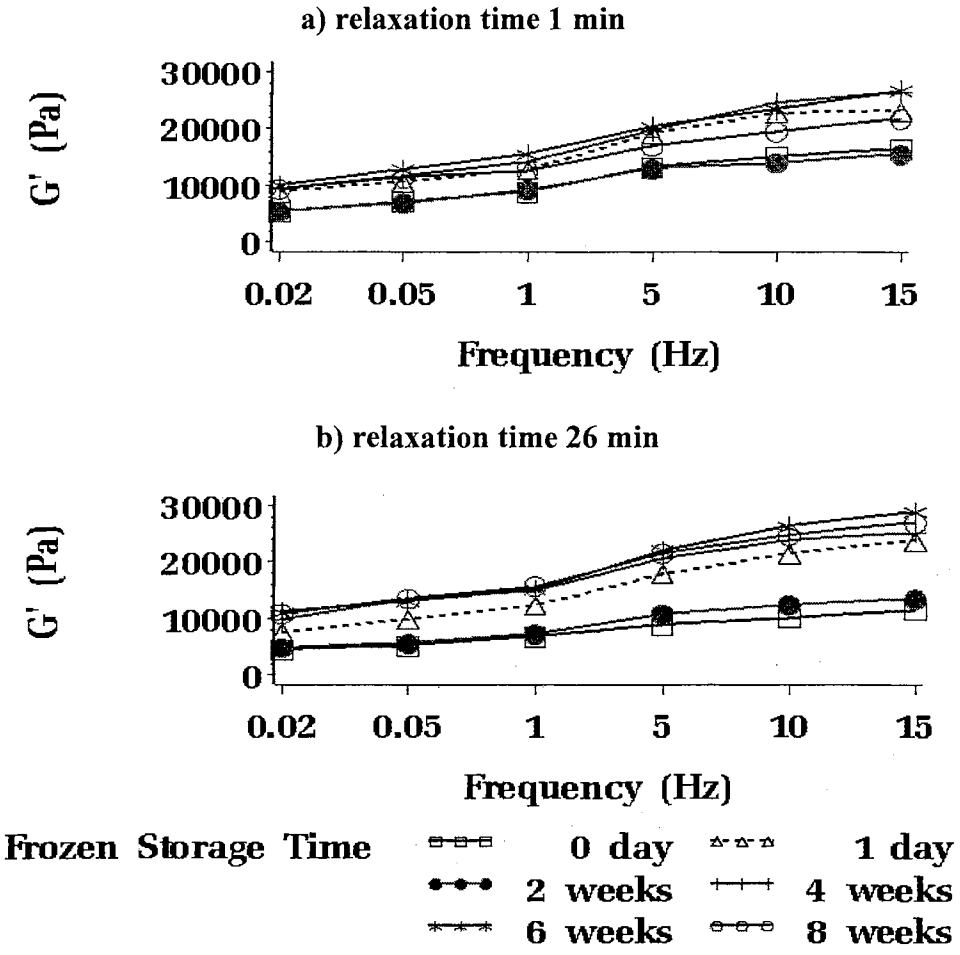


Fig. 6. Storage modulus (G') as a function of frequency of control dough at relaxtion time **a**) 1 min, **b**) 26 min.

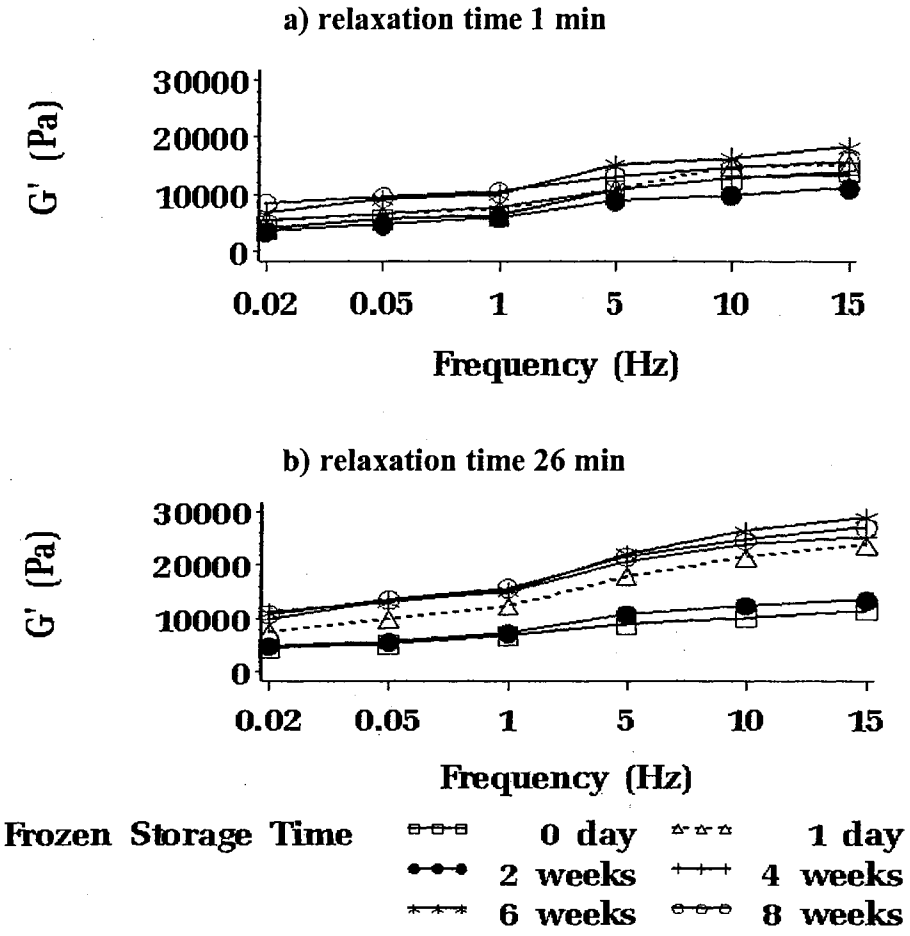


Fig. 7. Storage modulus (G') as a function of frequency of dough with 80 ppm GSH at relaxation time of **a)** 1 min, **b)** 26 min.

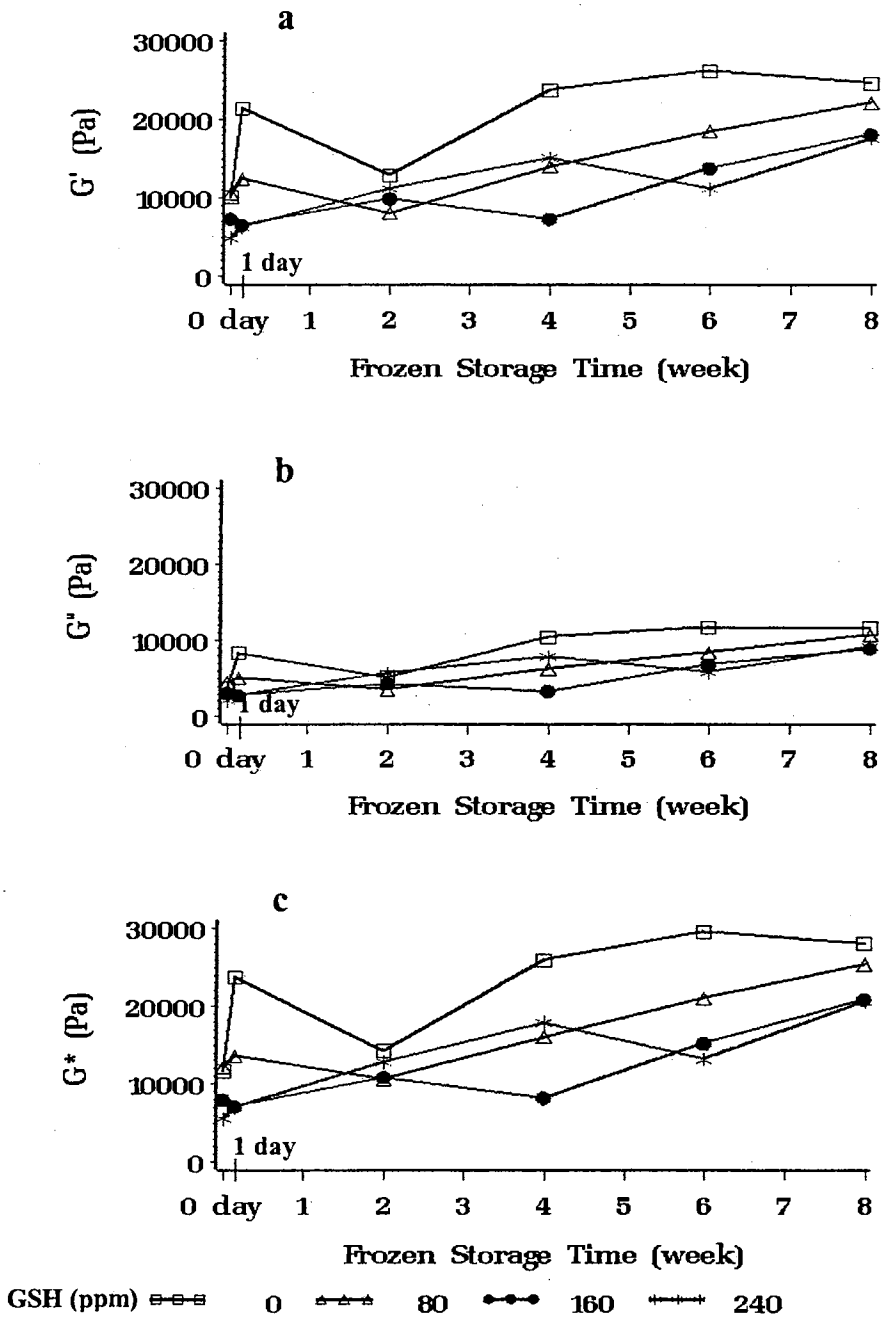


Fig. 8. Storage modulus, G' (**a**), loss modulus, G" (**b**), and complex modulus, G* (**c**) as a function of frozen storage time (0 and 1 day, 2, 4, 6, and 8 weeks). The measurement were used at frequency 10 Hz, and relaxation time 26 min.

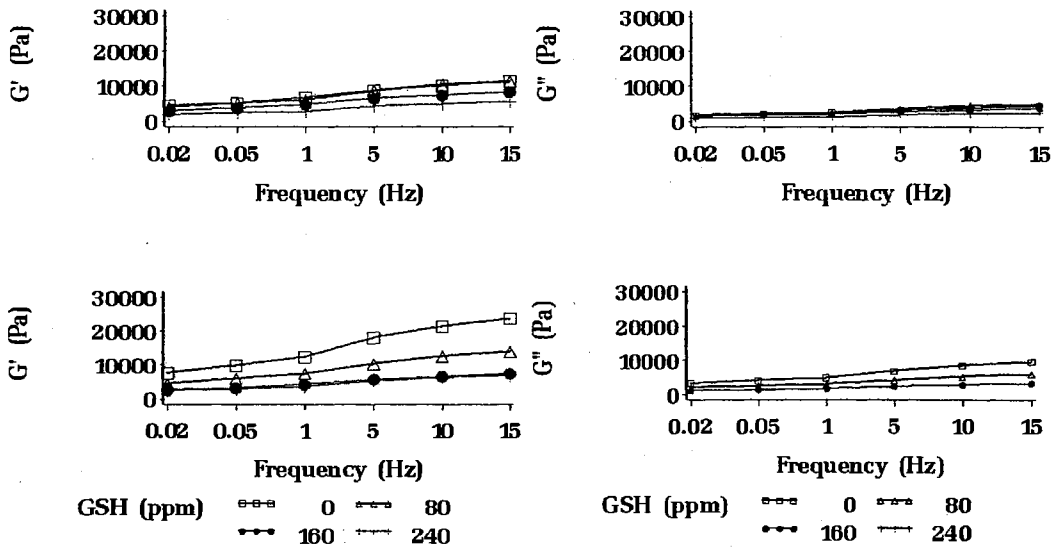


Fig. 9. Storage modulus (G') as a function of frequency of fresh dough and frozen dough with 0, 80, 160 and 240 ppm glutathione (GSH).

LITERATURE CITED

Abdelrahman, A., and Spies, R. 1986. Dynamic rheological studies of dough systems. Pages 87-103 in: foundamental of dough rheology, H. Faridi and J. M.. Faubion eds. Am. Assoc. Cereal Chem: St. Paul, MN.

Amemiya, J. I., and Menjivar, J. A. 1992. Comparison of small and large deformation measurements to characterize the rheology of wheat flour doughs. J. Food Eng. 16:91-108.

American Association of Cereal Chemists. 1995. Approved Methods of the AACC, 9 th ed. Method 08-01, final approval April 1961, revised October 1976 and October 1981, rviewed October 1994; Method 44-15A, approved October 1975, revised October 1981 and October 1994; Method 46-11A, approval October 1976, revised October 1982 and September 1985, reviewed October 1994; Method 44-19, final approval April 1961, revised October 1975, review October 1982 and1994; Method 54-21, final approval November 1995. Method 74-09, final approval November 1995, reviewed October 1996. The Association: St. Paul, MN.

Autio, K., and Sinda, E. 1992. Frozen doughs: Rheological changes and yeast viability. Cereal Chem. 69:409-413.

Berglund, P. T.; Shelton, D. R., and Freeman, T. P. 1991. Frozen bread dough ultrastructure as affected by duration of frozen storage and freeze-thaw cycles. Cereal Chem. 68:105-107.

Berland, S. 1995. Rheological properties of wheat flour doughs in steady and dynamic shear: effect of water content and some additives. Cereal Chem. 73 (1):48-52.

Berland, S., and Launay, B. 1995. Rheological properties of wheat flour dough in steady and dynamic shear: effect of water content and some additives. Cereal Chem. 72:48-52.

Bloksma, A. H. 1972. The relation between the thiol disulfide contents of dough and its rheological properties. Cereal Chem. 49:104-117.

Bloksma, A. H. 1975. Thiol and disulfide groups in dough rheology. Cereal Chem 52: 170r-183r.

Bloksma, A. H. 1990a. Rheology of bread-making process. Cereal Foods World. 35:228-236.

Bloksma, A. H. 1990b. Dough structure, dough rheology and baking quality. Cereal Foods World. 35:237-244.

Dreese, P. C., Faubion, J. M., and Hoseney, R. C. 1988. Dynamic rheological properties of flour, gluten, and gluten-starch doughs. II. Effect of various processing and ingredient changes. Cereal Chem 65:354-359.

Edwards, S.C. 1999. Mercury contamination of the eel (Anguilla anguilla) and roach (Rutilus urtilus) in East Anglia, UK. Env.Mon.Ass. 55 (3): 371-387.

Eliasson, A. C., and Larsson, K. 1993. Physicochemical behavior of the components of wheat flour. In: Cereals in breadmaking. Marcel Dekker: New York.

Ewart, J.A.D., 1988. Studies on disulfide bonds in gltenin. Cereal Chem. 65 (2):95-100.

Hibberd, G. E., and Parker, N. S. 1975. Dynamic viscoelastic behaviour of wheat flour doughs, Paert 4. Non-linear behaviour. Rheot. Acta 14:151-157.

Hibberd, G.E 1975. Dynamic viscoelastic behavior of wheat flour doughs. IV. Non-linear behaviour. Rheo. Acta. 14 (2):151-157.

Hoseney , R.C., 1969b. Functional (breadmaking) and biochemical properties of wheat flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation. Cereal Chem. 46 (2):126-135.

Hoseney, R.C. 1969a. Functional (breadmaking) and biochemical properties for wheat flour components.II Role of water solubles. Cereal Chem. 46 (2):117-25.

Inoue, Y., and Bushuk, W. 1991. Studies on frozen doughs. I. Effects of frozen storage and freeze-thaw cycles on baking and rheological properties. Cereal Chem. 68:627-631.

Inoue, Y., and Bushuk, W. 1992. Studies on frozen doughs. II. Flour quality requirements for bread production from frozen dough. Cereal Chem. 69:423-428.

Inoue, Y., Sapirstein, H. D., Takayanagi, S., and Bushuk, W. 1994. Studies on frozen doughs. III. Some factors involved in dough weakening during frozen storage and thaw-freeze cycles. Cereal Chem. 71:118-121.

Jones, I. K., Phillips, J. W., and Hird, F. J. R. 1974. The estimation of rheologically important thiol and disulphide groups in dough. J. Sci. Food Agric. 25:1-10.

Kaufmann, B and Kuhn., M. 1994. Rheologie von Wiezenteigen mit Hefe. Getreide Mehl und Brot 48:50-52.

Kenny, S., Wehrle, K., Dennehy, T, and Arendt E. K. 1999. Correlations between empirical and fundamental rheology measurements and baking performance of frozen bread dough. Cereal Chem. 76:421-425.

Kline, L., and Sugihara, T. F. 1968. Frozen bread doughs. I. Prepared by the straight dough method. Baker's Dig. 42(5):44-50.

Kuninori, T. 1968. Disulphide-sulphydryl interchange studies of wheat flour. II. Reaction of glutathione. 45 (5): 486-95.

Larsson, H. 1996. Phase separation of wheat flour dough studied by ultracentrifugation and stress relaxation II. Influence of mixing time, ascorbic acid, and lipid. Cereal Chem. 73 (1):25-31.

Larsson, H. 1996. Phase separation of wheat flour dough studied by ultracentrifugation and stress relaxation I. Influence of water content. Cereal Chem. 73 (1):18-24.

Lindahl, L and Eliassson, A.C. 1992. Influence of added enzymes on the rheological properties of a wheat flour dough. Cereal Chem. 1992. Cereal Chem. 69 (5):524-546.

Lindborg, K.M. 1997. Time-resolved shear viscosity of wheat flour doughs-effect of mixing, shear rate, and resting on the viscosity of doughs of different flour. Cereal Chem. 74 (1):49-55.

MacRitchie, F. 1976. The liquid phase of dough and its role in baking. Cereal Chem. 53:318-326.

Miller, K. A., and Hoseney, R. C. 1999a. Effect of oxidation on the dynamic rheological properties of wheat flour-water doughs. Cereal Chem. 76:100-104.

Miller, K. A., and Hoseney, R. C. 1999b. Dynamic rheological properties of wheat starch-gluten doughs. Cereal Chem. 76:105-109.

Neyreneuf, O., and van der Plaat. J. B. 1991. Preparation of frozen French bread dough with improved stability. Cereal Chem.68:60-66.

Räsänen, J., Blanshard, JMV, Siitari Kauppi M., and Autio, K. 1997b. Water distribution in frozen lean wheat doughs. Cereal Chem. 74:806-13.

Räsänen, J., Blanshard, JMV, Siitari Kauppi M., and Autio, K. 1997b. Water distribution in frozen lean wheat doughs. Cereal Chem. 74:806-913.

Räsänen, J., Laurikainen, T., and Autio, K. 1997a. Fermentation stability and pore size distribution of frozen prefermented lean wheat doughs. Cereal Chem. 74:56-62.

Rasper, V. F., and Preston, K. R. 1991. The Extensigraph Hanbook. American Association of Cereal Chemists: St. Paul.

Spies, R. 1989. Application of rheology in the bread industry. In: Dough Rheology and Baked Product Texture. H. Faridi and J. M. Faubion, eds. Van Nostrand Rein hold. New York.

Suchy, J., Lukow, O. M., and Ingelin, M. E. 2000. Dough microextensibility method using a 2-g mixograph and a texture analyzer. Cereal Chem. 77:39-43.

Sullivan, F.E. 1968. Refining of oils and fats. J. Amer. Oil. Chem. Soc. 45 (10)

Sullivan, R. 1968. Method for recovery of viruss from milk and milk products. J. Dai. Sci. 51 (11):1748-1751.

Surrnacka-Szczesniak, A. 1988. Applying rheology to dough and baked goods. Cereal Foods World. 33:841.

Wei-Dong. and Hoseney, R.C. 1995. Effects of certain breadmaking oxidants and reducing agents on dough rheological properties. Cereal Chem. 72 (1):58-64

Yoshida, M., Hamauzu, Z., and Yonezama, D. 1980. An activity of disulfide groups of gliadin and glutenein. Agric. Biol. Chem. 44:657-661.

CHAPTER V

BAKING PERFORMANCE AND DOUGH BEHAVIOR OF PRE-PROOFED FROZEN DOUGH CONTAINING GLUTATHIONE AND HEAT-TREATED YEAST

J. Uriyapongson, and P. Rayas-Duarte

ABSTRACT

The effect of reduced glutathione (GSH) and heat-treated yeast on pre-proofed frozen dough was studied using three levels of GSH (80, 160 and 240 ppm) and two levels of heat-treated yeast (5 and 10%). Changes in dough behavior and baking quality of fresh and frozen dough were investigated using micro-extensibility test, baking scores and scanning electron microscopy.

Specific volume of freshly baked bread sticks was reduced with the addition of 160 and 240 ppm of GSH, and 5 and 10% heat-treated yeast ($P<0.01$). Reduction in specific volume (34.6%) of the control occurred at 4 weeks of frozen storage. Bread sticks made with heat-treated yeast in both flours showed large brown blisters and pale background crust while the crust of bread sticks made with the addition of GSH contained many small brown spots. The control breadstick dough stored for 20 weeks showed similar crust defects to those made from dough containing heat-treated yeast. Bread sticks with pale background crust were seen with the addition of heat-treated yeast. Results were similar for 0 and 1 day frozen dough as described by an increase of lightness value (L*) and a reduction of red (+a*), yellow (+b*), and C* chromaticity

values. The reduction of +a* was 93.5 to 97.8 % and +b* was 16.6 to 60.8% with the addition of heat-treated yeast. A higher reduction in +a* and +b* was observed with 5% heat-treated yeast compared to 10%. All parameters from the crust color evaluation showed high correlation with specific volume of bread sticks (r ranged from -0.93 to 0.87, $P<0.001$). Starch gelatinization in bread sticks made from frozen dough dropped at 8 weeks of frozen storage (12.3 to 31.1% reduction, $P<0.001$).

Frozen dough samples showed small pores and thick cell walls after frozen storage and this increased with the addition of GSH. The presence of GSH caused thickening of the cell walls producing a rough grain structure as the GSH concentration increased. A trend of lower resistance to extension (Rmax) values with the addition of GSH and increased Rmax values with addition of heat-treated yeast was observed for the doughs. However, the dough with GSH and heat-treated yeast showed an increase in extensibility (E) and area (A) but a reduction in Rmax/E ratio. Increasing the rest period of the dough improved E and Rmax/E ratio of the dough containing heat-treated yeast.

INTRODUCTION

Yeast is one of the most important factors that control the shelf life of frozen dough products. The yeast's capacity of producing CO_2 is reduced during frozen storage thus affecting the quality of the final product (Kline and Sugihara 1968, Inoue et al 1994, and Räsänen et al 1997a). There are contrasting results in the literature on the performance of the different types of yeast used (cream, compressed, and active dry), formula and processing conditions. El-Hady et al (1996) reported that a higher reduction of total gas production in frozen dough made with compressed yeast (CY) than with instant active dry yeast (IADY) was reported. However, gassing power of IADY was found only slightly higher than CY and the percentage of heat-treated yeast cells higher for ADY or IADY than for fresh CY (Wolt and D'Appolonia 1984b). During a period of 20 weeks of storage, fresh compressed yeast appeared to produce slightly better proof-time stability than ADY and IADY (Wolt and D'Appolonia 1984b). The release of reduced glutathione (GSH) from heat-treated yeast cells is associated with the reduction of gluten proteins and deterioration of quality (Wolt and D'Appolonia 1984b). The cytoplasm membrane structure and integrity of different yeast types, and their sensitivity as a result of production processes might influence their performance in frozen dough products (Kline and Sugihara 1968, Javes 1971, Wolt and D'Appolonia 1984b, and Neyreneuf and van der Plaat 1991).

Cream yeast offers advantages over compressed yeast including pump-ability, better dispersion during dough mixing, and standardization of solid contents for gassing activity (Van Horn 1989). Cream yeast is obtained with the same process as in compressed yeast except that it does not include a dewatering step yielding lower

solids than the latter one (18 and 30%, respectively) (Van Horn 1989). While the gassing power of cream and compressed yeast is similar in nonfrozen dough when tested using a Risograph, differences in gassing power and freeze tolerance varied in these products depending on the manufacturing (Gelinas et al 1993, 1994).

The viability and activity of yeast influenced by different factors including quantity used (Neyreneuf and van der Plaat 1991), type of shortening and levels (Lorenz 1974, Marston 1978, Inoue et al 1995), oxidizing agents (Lorenz 1974, Inoue and Bushuk 1991), and other additives in the formula ((Nonami et al 1984, Noll 2000). Other important factors are processing conditions (Dubois and Blockcolsky 1986, Neyreneuf and van der Plaat 1991, Gélinas et al 1995, and Rouille et al 2000), freezing rate and temperature (Mazur and Schmidt 1968, Marston 1978, Hsu et al 1979a, b, Reid 1990, Inoue and Bushuk 1991, El-Hady et al 1996, Le Bail et al 1999, Havet et al 2000, and Laaksonen and Roos 2000), fermentation before freezing (Merritt 1960, Kline and Sugihara 1968, Lorenz 1974, Räsänen et al 1997b), and frozen storage and freeze-thaw (Kline & Sugihara 1968, Wolt and D'Appolonia 1984a, Berglund et al 1991, Inoue et al 1994, El-Hady et al 1996, Räsänen et al 1997a, Meric et al 1997, Le Bail et al 1996b, 1999).

Reduced glutathione (λ-glutamyl-cysteinylglycine, GSH) is an important tripeptide that protects cell integrity from oxidative stresses in practically all the cells (Havel et al 1999). In dough systems, GSH reduces the gluten proteins resulting in a decrease in polymer cross-linking and weakening of the three-dimensional network. The freeze-thaw process of dough affects the starch by changing the water distribution and causing separation of starch granules from the gluten network. As a result, the

elastic behavior of the dough decreased (decrease of storage modulus, G', and tan delta) delaying the starch gelatinization (Autio and Sinda 1992). Large ice crystals could be formed during the freeze-thaw cycles when the water holding capacity of the gluten proteins shifts, allowing the growth of pooled water (Berglund et al 1991). A change in the water diffusion rate in the starch granules and a possible re-arranging of molecules could result in an increase in crystallinity thereby delaying starch gelatinization (Levine and Slade 1990).

Scores of flavor and aroma of bread made from frozen dough were comparable to fresh bread after up to four weeks of dough frozen storage (El-Hady et al 1996). However, loaf volume decreased after 1 day and 1 week of storage compared to fresh loaves due to a reduction in gas production and modification of rheological properties of the dough (El-Hady et al 1996). In contrast, other authors reported that the bread quality measured as volume, appearance, crumb and grain structure, and crust color of 1 day and 1 week were similar to fresh bread (Räsänen et al 1995, 1997a). A number of methods have been developed to quantitate bread structure in addition to the subjective score assignment (Moss 1974, Bechtel et al 1978, Varriano-Marston 1980, Junge et al 1981, Fretzdorff et al 1982, Gan et al 1990, Berglund et al 1991, Sapirstein et al 1994, Räsänen et al 1995, 1997a and b, Hayman et al 1998b, and Ishida et al 2001). This area will continue to evolve until a rapid, relatively inexpensive and reliable method is applicable for various baked products.

The appearance of crust is an important factor of quality. Small white spots and blisters on the crust occurred when the dough surfaces lose water during a holding period at 40°F, also known as retarding step (Cauvain and Young 2000). White crust

spots were formed during the retarding step when excess moisture condensed on the surface of the dough pieces. The water droplets reduced the local concentration of sugars that take part in the Maillard browning reaction (Cauvain 1998).

The objective of this study was to determine the effect of glutathione and heat-treated yeast in the crust and overall quality of bread sticks.

MATERIALS AND METHODS

Two types of flour, hard red spring (Dakota Mill & Grain Co., Grand Forks, ND) and hard red winter wheat (Shawnee Milling, Shawnee OK), four levels of reduced glutathione (0, 80 160 and 120 ppm) and six frozen dough storage times (fresh or 0, 1 day, 2, 4, 6, and 8 weeks) were used. Flour moisture, protein, ash, and farinograph analysis were made using approved methods (AACC 1995), 44-15A, 46-11A, 08-01, and 54-21, respectively. Compressed baker's yeast (Fleischmann's Yeast Ltd., Fenton, MO) was used within a week of delivery from the distributor.

Heat-treated yeast was prepared by heating an aqueous yeast suspension (25%) at 50°C for 18 min based on the method of Van Uden (1971) that reported 95% dead cells after heating for 18 min at 50°C. Survival yeast was determined using pour plate method with acidified Potato Dextrose Agar. Duplicate plating of serial dilution using 1% sterile peptone buffer was used and the samples were incubated at room temperature (25°C) for 7 days before yeast colony counting. Control dough samples containing 5% compressed yeast were compared to two treatments containing 5 and 10% heat-treated yeast at 0 and 1 day frozen storage.

Preparation of Frozen and Fresh Dough

Control full formula dough for bread sticks was optimized as described earlier (Chapter IV). The formula consisted of 100% HRS or HRW flour, 5% compressed yeast, at 1.5% salt, 4% shortening, 6% sugar, 50 ppm ascorbic acid, 0.25% malted wheat flour, flour basis. Bake absorption of 65 and 57.6% for HRS and HRW flour, respectively was optimized from the farinograph water absorption. Two independent batches of 800 g flour each were mixed in a Hobart mixer equipped with a circulating water bath at 5°C. Yeast and salt were added after 5 and 9 min of mixing with a total mixing time of 11 min. The final dough temperature averaged 15°C. The dough was sheeted to a 10 mm thickness, cut into160x27x10 mm (LxWxH), and standardized to a weight of 40±0.5 g as describer earlier (Chapter IV). The bread stick strips were proofed at 30°C and 85% relative humidity for 40 minutes (Fermentation Cabinet model 505-11. National Manuf., Lincoln, NE). Fresh samples, 0 day, were baked immediately while samples to be frozen and stored, were frozen in a blast freezer at –30°C for 30 min and stored in zip lock plastic bags at -20°C.

Baking Test

Pre-proofed frozen dough bread sticks were thawed out for 1.5 hr at room temperature (~25°C). Samples were baked at 260°C for 5.5 min as described in Chapter IV. Volume (rapeseed displacement) and weight were recorded after cooling for 30 min. Bread sticks crust and crumb scores were determined using a scale of 0 to 10, with 10 being the most desirable. Two crust scores were used: 1) crust color and

2) absence of brown spots. A crust color score of 10 represented the most desirable golden brown color. The absence of brown spots score was based in the number of brown areas and blisters. A score of 10 was the most desirable and reflected absence of brown blisters. Crumb score factors included fine or coarse grain, cell wall thickness and distribution, color, and softness to touch.

Crumb firmness was evaluated using a Texture Analyzer TA-XT2 (Texture Technologies Corp., New York) equipped with a 6 mm diameter perspex cylindrical probe. Three 1-cm slices were obtained from the center of bread sticks and analyzed for two firmness measurements per slice, with a total of six observations per bread stick. A trigger force of 10 g and pre-test, test and post-test speeds of 4.0, 1.0 and 1.0 mm/sec, respectively, were used. A 25% compression test was used as described in approved method 74-09 (AACC 1995).

Color Measurements

Crust color of bread sticks was measured in a Minolta spectrophotometer CM-3500d (Minolta Co. Ltd, Osaka, Japan) using 8 mm target mask. Two bread sticks per treatment batch and four measurements on each bread stick were performed. Measurements using two color spaces, L*a*b* and L*C*h were recorded. The color maps determined lightness (L*), chromaticity coordinates of red-green (a*) and yellow-blue (b*), chroma (C*) and hue angle (h) (Anonymous 1998).

Scanning Electron Microscopy (SEM)

Samples of frozen dough and baked bread sticks were freeze dried and analyzed in a SEM model JXM 6400 (JEOL Ltd., Tokyo, Japan) at accelerating voltage of 10 kV. Briefly the sample preparation consisted in mounting the samples on specimen stubs with silver paint (Fullman Inc., Latham, NY) and coating under vacuum with gold-palladium at approximately 200 Å/min.

Micro-extensibility Test

A modified method of Suchy et al (2000) and the manufacturer's application was used to determine micro-extensibility using a Texture Analyzer TA-XT2 as described in Chapter IV. A tensile test mode with the following settings was used: trigger force of 5 g and pre-test, test, and post-test speed at 2.0, 3.3 and 10.0 mm/s, respectively. The parameters measured were the maximum resistance to extension (Rmax, g), extensibility measured as the distance until the dough ruptures (E, mm), area under the curve (A, mm^2), resistance to extension at 20 mm (F2, g), and viscoelastic ratio (Rmax/E). Full formula dough samples were mixed (800 g batches) and a subsample of 20 g was used for the micro-extensibility tests. Samples were formed into strips as manufacturer's procedure. Three measurements of the same dough sample were recorded at 0, 45, and 90 min rest. After the dough was tested at 0 min, immediately after mixing with no resting time, the samples were reshaped into strips, stored in zip lock plastic bags and re-tested after 45 and 90 min resting time. A total of 665 observations were recorded.

Gelatinized Starch

Gelatinized starch on bread sticks was determined by a modified method of Chiang and Johnson (1977). Briefly, totally gelatinized starch was prepared by adding 1N NaOH (1 mL) to a dispersed sample (6.67 mg bread sample/mL water, 3 mL aliquot), followed by a 5 min reaction and neutralization with 1N HCl (1 mL). Partially and totally gelatinized samples were digested with glucoamylase (Rhizopus glucoamylase, Sigma-Aldrich, St. Louis, MO) in acetate buffer (0.15 N and pH 4.5) for 30 min at 40°C. Two mL of 25% trichloroacetic acid was added to stop the reaction and samples were centrifuged (16,000 x g, 5 min). Supernatant aliquots (0.5 mL) were mixed with 1 mL of *o*-Toluidine reagent (Sigma-Aldrich Co., St. Louis, MO.), boiled for 10 min and cooled. Five mL of glacial acetic acid were added and absorbance measured at 630 nm. The percent gelatinization was calculated as the ratio of A_{630} of partially vs totally gelatinized sample.

RESULTS AND DISCUSSION

Addition of GSH

Specific Volume

A significant interaction of GSH levels and frozen storage time from specific volume of bread sticks was observed ($P<0.001$). The specific volume of freshly baked bread sticks, 0 day frozen storage, was only affected by 160 ppm of GSH, showing a

15.5% reduction of specific volume ($P<0.01$, Table I, APPENDIX AD). No differences in specific volume were observed in samples from 0 and 1 day frozen storage. The reduction of specific volume of the control (0% GSH) occurred significantly at 4 weeks of frozen storage (34.6% reduction). However, a reduction of specific volume was observed at 2 and 4 weeks of frozen storage ($P<0.001$) with the samples containing 80 (20.3 and 23.5% reduction, respectively) and 240 ppm of GSH (15.1 and 28.9% reduction, respectively).

Crust Score

Crust scores for freshly baked samples were similar for control and GSH (Table I, APPENDIX AE). Comparison of freshly baked bread sticks with 1 day frozen storage samples, showed a significant decrease in crust score for all the samples containing GSH (Table I). The crust scores of bread sticks samples made from frozen dough seemed to remain constant for the duration of the frozen storage times of this study. The scores for control and 80 ppm GSH were similar and the score for 160 and 240 ppm GSH were lower than the former two ($P<0.001$, Fig. 1, APPENDIX AE). Crust and crumb scores showed significant positive correlation ($r=0.7488$, $P<0.001$, Table III).

Crumb Score

When bread sticks were freshly baked (0 day frozen storage time), no significant differences in crumb scores were found between the control and most of the

GSH levels, except for 240 ppm that showed lower scores (Table I, APPENDIX AF). These bread sticks (240 ppm GSH) had coarse texture and darker grain (Fig. 2a). Freezing the control samples for one day did not affect the crumb score, but reduced the score of the dough with addition of GSH, especially 160 and 240 ppm GSH ($P<0.001$, Table I). GSH containing breadsticks at these two levels produced coarser grain with larger gas holes than the control and 80 ppm GSH (Fig. 2 and 3). Control breadsticks compared during the frozen storage times showed comparable crumb score up to 4 weeks and a decrease in quality at 6 and 8 weeks ($P<0.05$ and 0.01, respectively). Large gas holes are formed when several small holes coalesce into few larger ones. This coalescence favored with the addition of 160 and 240 ppm GSH, indicates a fundamental change in the gluten matrix fibers, such as more susceptible and weak fibers that do not hold the gas produced during baking.

Objective measurements of crumb firmness recorded using the Texture Analyzer showed an increase in crumb firmness only with the addition of 240 ppm GSH and after 4 weeks of storage ($P<0.001$, Table II, APPENDIX AG). These observations matched the subjective observations (by touching) of crumb firmness on bread sticks. A negative correlation of crumb firmness with specific volume and crumb score was found during the frozen storage ($r= -0.6276$ and -0.7498, respectively, $P<0.001$, Table III).

Gelatinized Starch

Significant differences in gelatinized starch were only observed in bread stick samples made from frozen dough stored for 8 weeks (Table II, APPENDIX AH).

Compared to the freshly baked control samples, a range of 12.3 to 31.1 % reduction of gelatinized starch was observed at 8 weeks ($P<0.001$). Comparing the control samples with the GSH-containing samples, there was an overall trend to higher values in the latter samples. This trend of high values of gelatinized starch could be due to depolymerization of gluten network caused by GSH, thus more will be the water available for starch gelatinization. The reduction in gelatinized starch after 8 weeks might be caused due to loss of water from the bread sticks, observed as ice crystals formed inside the plastic bag and surrounding the bread sticks (sublimation).

Addition of Heat-treated Yeast

Specific Volume

The addition of 5 and 10% heat-treated yeast reduced the specific volume of bread sticks made with both flours, reduction range 34.7-45.3% in fresh dough ($P<0.001$, Table IV, APPENDIX AI). Bread sticks made with 10% heat-treated yeast contained higher survival yeast (APPENDIX AJ), showed higher specific volume compared to 5%, with both flours. The additional of 5 and 10% heat-treated yeast at 1 day frozen showed a similar reduction of specific volumes of bread sticks to fresh (0 day of frozen storage) in both flours. Specific volumes of bread sticks seemed not to be affected by 1 day frozen storage, except for the control with HRW flour (Table IV). A similar trend was observed in specific volume when GSH was added, however, the specific volumes of the bread sticks were higher than the ones containing 5 and 10% heat-treated yeast (Table I vs. IV).

Crumb Score

Bread sticks containing 5 and 10% heat-treated yeast produced dense grain structures (Fig. 4) with large gas holes and hollow structures between the crumb and crust. These bread sticks were not scored since all of them would have received the same low score of zero.

Crust Color Score

Color scores were given to the crust disregarding brown spots (evaluated in the following section) with a scale of 0 to 10. A score of 10 was given to desirable golden brown surface and the scores decreased as discoloration appeared, resembling powdery unbaked dough. Both flours gave similar scores (Table IV, APPENDIX AK). Control samples scores were 10 and the score decreased with the addition of 5 and 10% heat-treated yeast at both 0 and 1day of frozen storage. Average decrease in crust color score was 73.7 and 53.5% for 5 and 10%, respectively, for HRS flour and 76% and 44.7% for HRW flour. A significant correlation of specific volume with color score was observed ($r = 0.8161$, $P < 0.001$, Table V). Insufficient yeast to produce reducing sugars and a shift to basic pH inhibited the Maillard reaction resulting in pale crust. The rate of Maillard reaction is dependent of sugar structure, pH, temperature, and absence or presence of metal ions (Whistler and Daniel 1985). Sucrose gives less Maillard browning than glucose, fructose and maltose (Maillard 1912). The dough with 5 and 10% heat-treated yeast has a low percentage of viable yeasts and less invertase to convert reducing sugar (fructose and glucose) from sucrose present in the

dough formula. Some crust browning color was observed in the breadsticks made with 10% heat-treated yeast compared with less browning in the 5% heat-treated yeast, suggesting the survival of viable yeast and thus invertase activity to enrich the dough with more reacting reducing sugars.

The Maillard reaction is reduced at pH higher than 6 (Ellis 1959). The pH of yeasted straight dough ranges from 4.8 to 5.5 and is obtained when CO_2 and ethanol dissolved into the dough (Reed and Peppler 1973, Reed and Nagodawithana 1991). The pH after 1.5 hr of thawing of the control dough was 5.7 and with heat-treated yeast addition averaged 5.8 for both flours. Thus, pH did not account for the dramatic differences in color since it is still less then 6.

Crust Score Based on the Absence of Brown Spots

Large brown blisters on the crust, separating the crust from crumb by hollow structures were observed when heat-treated yeast was added to the dough for bread sticks (Fig. 5). This effect was similar for both types of flours at 0 and 1 day of storage. These blisters were more pronounced with the 10% heat-treated yeast (Table IV, APPENDIX AL).

Blisters can be formed by an accumulation of water vapor from the moisture in the dough, trapped by the differential drying stage of the crust. A minimum amount of ethanol, CO_2 and organic compounds is expected since the dough crumb was heavy and flat or "dead." In the interface of the crust and the upper region of the crumb the water vapor might have increased further the temperature resulting in enhanced

Maillard reaction or even caramelization. Maillard reaction is affected by both pH and temperature, while caramelization is mostly temperature dependant (Whistler and Daniel 1985). Caramelization might occur from the sucrose in the dough formula and localized high temperature.

Contributing to the larger blisters observed when 10% heat-treated yeast was added, compared to the 5%, is the increased content of hydroquinone and amino acid from heat-treated yeast. Hydroquinones are aromatic compounds that contribute to the Maillard reaction. They require an alkaline environment to drive the reaction to form melanin and aromatic compounds that contribute to the color and flavor of baked products (Kohama et al 1990). Hydroquinone is present in yeast at 160 μg/kg of yeast (Kohama et al 1990). Thus, more browning activity due to localized high temperature occurred at the area where more gas or steam was accumulated between crumb and crust.

Color – Spectrophotometer Measurements

L*a*b* color space. A significant interaction of the type of flour, level of heat-treated yeast, and freezing was observed with L*a*b* ($P<0.001$). Lightness values (L*) increased compared to the control when heat-treated yeast was added to both HRS and HRW wheat flour (Fig. 6). The increase of L* was similar for 0 and 1 day storage of frozen dough. The red chromaticity (+a*) was similar for the control sample at 0 and 1 day freezing for HRS flour (Fig. 7a) and decreased 93.5 and 84.1% with the addition of 5 and 10% heat-treated yeast, respectively. The addition of 5 and

10% heat-treated yeast caused a reduction of +a* of 97.3 and 54.8%, respectively in HRW flour (Fig. 7b). When the samples were compared at 0 and 1 day freezing, the +a* value of the bread sticks samples decreased 96.2 and 95.4% with the addition of 5 and 10% heat-treated yeast to HRS flour, respectively. The same comparison for HRW flour yielded a decrease of 97.8 and 75.4% +a* values. This indicates that lower reduction of red chromaticity when 10% heat-treated yeast was added could be due to viable yeast that was able to produce about 10% more browning. Breadsticks made with HRW flour seemed to have more favorable conditions for browning when the 10% heat-treated yeast was present; 30% more red chromaticity than HRS. Differences in the amount of sugars in the flour and the rate of reducing sugar formed by the yeast could explain these observations.

The yellow chromaticity (+b* value) of bread sticks showed similar trend to a* values for both flours (Fig. 8). No significant differences of 0 vs 1 day frozen dough were observed for HRS flour while a 16.8% reduction of +b* value was obtained for HRW flour. After the addition of 5 and 10% heat-treated yeast, the +b* value decreased 47.9 and 37.2%, respectively in HRS and 53.1 and 16.6% respectively in HRW flour at 0 day. The reduction of +b* value at 1 day frozen storage of the dough samples with 5 and 10% heat-treated yeast in HRS flour were 60.6 and 51.8%, and 60.8 and 33.4% in HRW flour, respectively.

L*C*h* color space. This color space showed similar results to the L*a*b*. The difference between these two color spaces is the cylindrical coordinates used in L*C*h* versus the rectangular coordinates used in L*a*b* (Anonymous, 1998). No

differences in chroma values (C*) were observed for HRS flour at 0 and 1 day of frozen storage (Fig. 9), while C* decreased 19.7% for HRW flour. A reduction range of 2.2 to 63.0% of C* reflects a shift in the color map from the red to the gray direction. C* values for HRS decreased 51.0 and 40.6%, when 5 and 10% heat-treated yeast were added. The comparison of C* values at 0 vs 1 day storage gave a reduction of C* of 62.9 and 54.7%, respectively. The addition of 5 and 10% heat-treated yeast decreased C* value by 56 and 20% respectively, while the comparison of 0 vs 1 day of frozen storage yielded a decrease of 63 and 37%, respectively.

The hue angle (h*) expressed in degrees is defined starting at +a* axis (red) with a value of 0° and +b* (red) with a value of 90° (Anonymous 1998). Higher h* values were observed for the samples with heat-treated yeast addition compared to control, suggesting a shift from red towards yellow (Fig. 10). No change in hue angle was observed when the dough of both flours was frozen for one day. All the parameters from both color spaces showed correlation with specific volume of bread sticks (r ranged from -0.94 to 0.87, $P < 0.001$, Table V). A negative correlation was found between specific volume and L* value ($r = -0.9321$, $P < 0.001$) and crust color score ($r = -0.8953$, $P < 0.001$) of bread sticks. The score of brown spots present in breads sticks also had a negative correlation with L* and h* values ($r = -0.5748$ and -0.5067, respectively, $P < 0.001$, Table V). There were significant positive correlations between specific volume with crust color score, a*, b* and C* (r range 0.81 - 0.94). The positive correlation of crust color and specific volume is due to the residual yeast that improved these parameters; larger amounts of residual viable yeast would be present in the 10% vs. 5% addition.

Bread and Dough Structure with SEM

Effect of Heat-treated Yeast

Examples of typical bread stick photographs of control, and addition of 5 and 10% heat-treated yeast are shown in Fig. 4 and 5. The uniform grain distribution of the crumb from control sample contrasts with a collapsed grain structure with elongated voids mainly in the interface of the top layers of grain and crust. Crumb structure with fine, uniform cells and thin walls are desirable in this type of product. The grain with heat-treated yeast has also a wet or uncooked appearance due to the lack of CO_2 gas and expansion of the grain during baking. The brown blisters contrast with the pale general background of the crust and they were formed in both flours (Fig. 5). A closer look at the grain (Fig. 4) shows that both flours formed acceptable grain with the control and similar defects with the addition of heat-treated yeast. A typical view of the crust of control breadsticks at 1 and 20 weeks of storage (Fig. 1a) shows that the storage alone can cause similar blisters as the ones observed when heat-treated yeast is added (Fig. 5).

Effect of GSH

Typical scanning electron micrographs of freeze-dried dough are shown in Fig. 11. Micrographs at 1000X magnification showed the gluten matrix covering the starch granule structures. Overall, the large lenticular starch granules seemed to be surrounded by the smaller round starch granules. The micrographs showed very

similar structures for the control sample at 0 and 6 weeks frozen storage (Fig. 11a, b) and at 80 ppm GSH at 0 and 1 day frozen storage (Fig. 11c, d). Except that the 1 day frozen storage sample started to show separation of the gluten covering and void areas formed with some stretched fibrils. At lower magnification (25x vs. 1000x in Fig. 12 vs. 11) globular structures or "gas cells" are observed and some of them collapsed. As the control dough is stored, the structure changes from larger oblong globules or cells with large ruptures to smaller more round globules with more ruptures and smaller holes compared to the control (Fig. 12). This difference in dough structure suggests that the gluten film is easier to disrupt in the sample with longer frozen storage time, 6 weeks vs. 1 day. The presence of 80 and 160 ppm GSH caused thickening of the cell walls producing a rough grain structure (Fig. 13b, c) and increased smaller holes when the sample was stored for 1 day (Fig. 13b, d).

Frozen dough samples showed smaller pores and thicker cell walls compared to fresh dough (0 day) (Fig. 12). A dough lacking viable yeast cells will not have the gas pressure to enlarge the small air bubbles introduced during mixing and expand them as CO_2 is produced in a normal grain cell structure, that results in an airy light crumb of bread sticks. It will also lack the distribution of the pores and the pressure by the force of the expansion of small pores into large ones during baking (Ishida et al 2001). Coarse and not uniform crumb grain in bread sticks might be caused when the crush of large pores destroys some grain walls (Fig. 14).

SEM micrographs showed a reduction of pore size in control sample as the freezing storage increased from 0 to 6 weeks (Fig. 12). The control sample showed large pore sizes and a range of hole sizes from large to small. As the frozen storage

progressed, the pores and holes were smaller compared to the control (Fig. 12). Some areas of the dough looked lacking pores, with essentially very thick walls producing coarse crumb. These areas might have been a result of a combination of lack of yeast activity, ice crystal formation followed by the disruption of yeast cell wall, and disturbance of the gluten sheets.

The dough structure affected crumb structure of bread sticks. The dough with thin gluten walls of fresh control dough provided uniform and fine elongated crumb (Fig. 14a). The small and deep pores with thick gluten walls of frozen dough provided coarser crumb (Fig. 14b, c, and d) compared to those observed in the control fresh dough (Fig. 14a). Thus, the coarse structures of bread crumb increased as the frozen storage time increased. When GSH was added to the dough, small pores with thick walls produced coarse and round grains (Fig. 15). Larger and coarser crumb structures were obtained as GSH increased from 80 to 240 ppm in fresh dough (Fig. 15b-c). Freezing appeared to enlarge the grain size and produce tears of the crumb with the frozen dough containing GSH (Fig. 16). The tears in crumb grain appeared more prominent in the bread containing 240 ppm GSH (Fig. 16d).

Micro-extensibility

Effect of Heat-treated Yeast

There was a significant interaction of all the micro-extensibility parameters and the flour type, level of yeast, freezing time and resting time of the dough ($P<0.001$). The control dough of HRS flour had higher resistance to extension (Rmax) than HRW (Fig. 17 and 18). HRS flour at 0 day had similar values of Rmax for control and

dough with heat-treated yeast at all rest periods (Fig. 17). However, the HRS dough containing heat-treated yeast showed a reduction of Rmax after freezing compared to the control ($P < 0.01$). In contrast, at 0 day HRW dough containing heat-treated yeast increased Rmax compared to the control at all rest times with the highest values observed with the addition of 5% heat-treated yeast (Fig. 18). Freezing the dough did not significantly affect Rmax and resistance to extension at 20 mm (R20mm) in control HRS and HRW dough (Fig. 17-20). These observations agreed with the report of Kenny et al (1999) but contrast with the results of Inoue and Bushuk (1991) and Inoue et al (1995) who reported a decrease in Rmax after freezing the dough.

The extensibility (E) of the control dough made with HRS flour (Fig. 21) was higher than the HRW (Fig. 22). Extensibility has been related to the genetic control of molecular weight distribution of polymeric proteins in wheat (Verbruggen et al 2001). Dough made with HRS and HRW containing 5 and 10% heat-treated yeast had higher E than the control at 0 and 1 day frozen ($P < 0.05$ and 0.01, respectively). Similar values of E were observed for 0 and 1 day frozen for most of the rest times except for 0 min in both flours containing heat-treated yeast showing high E values. A possible shift in molecular weight distribution is possible by sulfhydryl-disulfide interchange by GSH. The slackening of dough is related to the presence of GSH contained in flour and yeast (Ponte et al 1960). Wheat flour contains about 1.4 to 2.4 microequivalents of reduced GSH (Kuninori et al 1968). About 2 mg GSH per g yeast was found to leach out at rehydration temperature of 30 to 40°C (Ponte et al 1960) and 1.27 mg GSH per g yeast at rehydration temperature of 50°C. (Kuninori et al 1968). Yeast thionic acid reducing enzyme catalyzes the dough-slackening reaction by reducing

disulfide bonds, thus causing additional extensibility of the dough (Black et al 1960). By adding heat-treated yeast both GSH and thionic acid reducing enzyme has increased extensibility of the dough.

Control dough (0 day) made with HRS flour had higher values of A than the control HRW flour at all resting times (Fig. 23 and 24). At 0 day, the addition of heat-treated yeast yielded similar Rmax/E ratio compared to the control at 0 min rest time and reduced ratio at 45 and 90 min rest time for both flours (Fig. 25 and 26). When the dough was frozen (1 day) the Rmax/E ratio of 5 and 10% heat-treated yeast was reduced compared to control (Fig. 25 and 26). Rmax/E ratio of 5 and 10% heat-treated yeast increased in both flours as the rest period increased.

Overall, dough made with HRS flour had higher Rmax, E, A and Rmax/E ratios than HRW flour dough. Rest period and freezing affected the rheological properties of dough from both flours. The overall addition of 5 and 10% heat-treated yeast reduced Rmax in HRS except in HRW and reduced Rmax/E ratio in both flours. E and A increased in both flours.

Effect of GSH

A direct comparison of the addition of heat-treated yeast and GSH to the control dough from HRS flour (0 and 1 day of frozen storage) on the micro-extensibility properties is reported in Figs. 27 to 30. Rmax of the control dough at 0 day was similar in all treatments except for 240 ppm GSH which shows lower values (Fig. 27). When the dough was frozen, lower Rmax values were obtained with heat-treated yeast (except 90 min rest period) and GSH. Addition of 240 ppm GSH in the

dough showed the lowest Rmax in fresh and 1 day frozen samples, compared to the control. This implies that the reducing action of GSH affects more drastically the polymeric proteins when subjected to freezing than when adding the heat-treated yeast or GSH without freezing.

For the most part, similar values of E were obtained with control dough and the treatments, including 0 vs. 1 day frozen, except for 5 and 10% heat-treated yeast at 0 min rest period which had higher E (Fig. 28). With this exception, we can generalize that the dough extensibility seemed not affected by the GSH but affected by heat-treated yeast and freezing treatment. These observations suggest residual enzymatic activity in the heat-treated yeast preparation that survived the heat treatment.

The values of A were consistently higher for the 5 and 10% heat-treated yeast treatment than the control and the GSH treatments at both 0 and 1 day of frozen storage (Fig. 29, and APPENDIX AM). The Rmax/E ratio was significantly reduced with the addition of 5 and 10% heat-treated yeast at 1 day frozen at 0 min rest period time compared to the control and GSH-containing dough (Fig. 30). Freezing significantly reduced Rmax/E ratio ($P < 0.01$) of the dough with heat-treated yeast (Fig. 30a) and when the dough was rested and tested again the Rmax/E ratio increased (Fig. 30b, c). In the dough with GSH, Rmax/E ratio was not affected by the resting period but was affected slightly by freezing.

Values of area A had a negative correlation with specific volume and crust color score with the 5 and 10% heat-treated yeast (r = -0.8338 and -0.8012, respectively, $P < 0.001$, Table VI). A and E also showed significant correlation with color space values (r range 0.68-0.90, Table VII). E and Rmax/E also had significant

correlations with specific volume (r = -0.6437 and 0.5730, respectively) and crust color (r = -0.7346 and 0.6879, respectively, Table VI). But there was no correlation of baking quality with Rmax (Table VI and VII).

The highest correlation of dough containing GSH was observed with the area A and specific volume (r = -0.7694, $P < 0.001$, Table VI). The next high correlation was observed with Rmax/E ratio and crust and crumb score and crumb firmness (r = 0.5946, 0.5893 and -0.5095, respectively). Extensibility E showed significant negative correlations with specific volume and crumb score and positive correlation with crumb firmness (r = -0.5310, -0.5113 and 0.5532, respectively, Table VI). In contrast to heat-treated yeast, GSH showed significant correlations of Rmax with crust and crumb score and crumb firmness (r = 0.5377, 0.4413, and -0.2964, respectively, Table VI).

CONCLUSIONS

The defects of frozen dough bread sticks containing heat-treated yeast and GSH differed in the magnitude of brown defects and hollow structures formed under the crust, development of pale crust and coarse grain with thicker pore walls. There is no doubt that managing the freezing rate of frozen dough is a critical step in the processing, determining crucial phenomena in the system including the disruption of yeast cells allowing them to release GSH. However, heat-treated yeast and GSH combined with freezing damage could explain the majority of the defects. The blisters can be explained by damage to the yeast cells alone. Thus, by preventing heat-treated yeast cells during processing, baking processors will avoid crust blisters.

Both heat-treated yeast and GSH caused an exchange of sulfhydryl-disulfide interchange resulting in an increased dough slackening. Heat-treated yeast in the dough reduced fermentation activity and reduced the browning reaction resulting in discoloration of the crust. By extending the resting of the frozen dough would some yeast fermentation activity will be recovered as well as an improvement in rheological properties. However, if high levels of GSH or enzyme activity depolymerizes the gluten network and changed its molecular structure, then the dough would not recover during the rest time.

TABLE I

Baking Score of Bread Sticks Made from Hard Red Spring (HRS) Flour[a]

	Frozen Storage Time	Specific Volume (cc/g)	Crust Score	Crumb Score
Control	**Fresh**	4.2 ± 0.1	10.0 ± 0.0	10.0 ± 0.0
	1 day	4.0 ± 0.1	9.6 ± 0.3	9.9 ± 0.3
	2 weeks	3.9 ± 0.0	9.8 ± 0.3	8.9 ± 1.3
	4 weeks	2.8 ± 0.1	9.0 ± 0.7	9.0 ± 0.0
	6 weeks	3.0 ± 0.2	8.3 ± 0.3	8.3 ± 0.4
	8 weeks	2.8 ± 0.1	9.0 ± 0.0	8.0 ± 0.0
GSH[b], 80 ppm	**Fresh**	4.2 ± 0.1	10.0 ± 0.0	10.0 ± 0.0
	1 day	4.1 ± 0.1	8.5 ± 0.6	9.0 ± 0.6
	2 weeks	3.4 ± 0.2	8.1 ± 1.3	8.0 ± 0.7
	4 weeks	3.3 ± 0.1	8.4 ± 1.1	8.5 ± 0.6
	6 weeks	3.0 ± 0.1	7.9 ± 0.6	8.3 ± 0.3
	8 weeks	3.2 ± 0.1	7.6 ± 1.9	6.8 ± 0.5
GSH, 160 ppm	**Fresh**	3.6 ± 0.2	9.5 ± 0.6	9.9 ± 0.3
	1 day	3.8 ± 0.1	5.8 ± 1.0	7.3 ± 0.3
	2 weeks	3.5 ± 0.2	6.4 ± 1.3	7.1 ± 0.3
	4 weeks	3.7 ± 0.2	5.3 ± 1.0	7.5 ± 0.0
	6 weeks	3.1 ± 0.1	5.0 ± 1.2	5.3 ± 0.5
	8 weeks	2.8 ± 0.1	5.5 ± 0.6	4.8 ± 1.0
GSH, 240 ppm	**Fresh**	4.3 ± 0.3	9.1 ± 0.3	9.3 ± 0.3
	1 day	4.5 ± 0.2	5.0 ± 0.0	8.6 ± 0.5
	2 weeks	3.7 ± 0.2	6.9 ± 0.3	7.0 ± 0.0
	4 weeks	3.1 ± 0.1	6.5 ± 0.6	5.3 ± 0.5
	6 weeks	3.3 ± 0.2	5.4 ± 1.6	3.0 ± 1.2
	8 weeks	3.2 ± 0.1	5.8 ± 1.3	3.3 ± 1.5

[a] Mean ± standard deviation, each values is a mean of 4 measurements. Analysis was done in two independent batches with two subsamples per batches.

[b] GSH = glutathione reduced form.

TABLE II

Measurement of Crumb Firmness and Gelatinized Starch for Bread Sticks Made from Hard Red Spring (HRS) Flour[a]

	Frozen Storage Time	Crumb Firmness (g)	Gelatinized Starch (%)
Control	**Fresh**	76.1 ± 12.8	85.1 ± 3.8
	1 day	72.6 ± 14.9	87.3 ± 9.8
	2 weeks	87.4 ± 26.4	94.2 ± 2.0
	4 weeks	130.8 ± 33.9	86.5 ± 3.4
	6 weeks	146.8 ± 23.1	77.1 ± 9.7
	8 weeks	109.5 ± 14.5	71.3 ± 9.8
GSH[b], 80 ppm	**Fresh**	76.7 ± 14.8	89.8 ± 7.6
	1 day	82.5 ± 10.0	89.6 ± 5.0
	2 weeks	117.1 ± 19.4	94.4 ± 1.9
	4 weeks	110.3 ± 21.8	91.8 ± 3.2
	6 weeks	152.1 ± 21.9	84.0 ± 6.2
	8 weeks	132.9 ± 58.7	61.9 ± 14.9
GSH, 160 ppm	**Fresh**	77.0 ± 6.1	95.0 ± 2.4
	1 day	98.8 ± 33.6	95.4 ± 1.2
	2 weeks	135.8 ± 21.4	92.0 ± 2.3
	4 weeks	117.7 ± 24.1	88.9 ± 1.5
	6 weeks	141.7 ± 28.2	89.6 ± 2.9
	8 weeks	165.0 ± 43.7	73.4 ± 9.6
GSH, 240 ppm	**Fresh**	76.2 ± 10.8	93.7 ± 2.4
	1 day	100.9 ± 16.7	90.9 ± 4.5
	2 weeks	126.8 ± 30.1	92.9 ± 3.8
	4 weeks	191.3 ± 56.2	87.7 ± 3.2
	6 weeks	216.2 ± 80.9	87.0 ± 6.4
	8 weeks	188.3 ± 45.8	82.2 ± 3.9

[a] Mean ± standard deviation, each values is a mean of 4 measurements. Analysis was done in two independent batches with two subsamples per batches.

[b] GSH = glutathione reduced form.

Table III
Correlation Coefficient (*r*) of Baking Parameters of Bread Sticks Made from Frozen Dough Containing Glutathione[a]

	Specific volume	Crust score	Crumb score	Crumb firmness
Specific volume	1	0.213	0.487 ***[b]	-0.628 ***
Crust score		1	0.749 ***	-0.473 ***
Crumb score			1	-0.750 ***
Crumb firmness				1

[a] Correlation coefficient analysis of n = 48.

[b] *** = Significant at $P < 0.001$.

TABLE IV

Baking Scores of Bread Sticks Made from Non-frozen and Frozen Dough, with and without Addition of Heat-treated Yeast Using Hard Red Spring (HRS) and Hard Red Winter (HRW) Flour[a]

	Heat-treated yeast (%)	Frozen storage (day)	Specific volume (cc/g)	Crust score	Absence of brown spots score
HRS	**0**	**0**	4.2 ± 0.1	10.0 ± 0.0	10.0 ± 0.0
		1	4.0 ± 0.1	9.8 ± 0.3	9.6 ± 0.3
	5	**0**	2.3 ± 0.1	3.2 ± 1.1	5.8 ± 2.7
		1	2.5 ± 0.2	2.0 ± 0.0	7.0 ± 3.2
	10	**0**	2.7 ± 0.2	5.2 ± 2.0	3.6 ± 2.9
		1	2.9 ± 0.3	4.0 ± 1.4	6.0 ± 2.8
HRW	**0**	**0**	4.3 ± 0.1	10.0 ± 0.0	10.0 ± 0.0
		1	3.0 ± 0.0	9.4 ± 0.3	9.3 ± 0.3
	5	**0**	2.4 ± 0.2	2.4 ± 1.3	5.6 ± 3.3
		1	2.4 ± 0.2	2.3 ± 1.8	5.1 ± 2.5
	10	**0**	2.8 ± 0.1	5.6 ± 2.4	2.0 ± 0.0
		1	3.0 ± 0.2	5.1 ± 2.0	3.6 ± 3.4

[a] Mean ± standard deviation, each values is a mean of 4 measurements. Analysis was done in two independent batches with two subsamples per batches.

Table V

Correlation Coefficient (*r*) of Crust Color, Specific Volume, and Crust Scores of Bread Sticks Made with Frozen Dough Containing Heat-treated Yeast[a]

	Specific volume	Crust color score	Absence of brown spots score
Color measurement[c]			
L*	-0.932 *** [b]	-0.895 ***	-0.575 ***
a*	0.941 ***	0.872 ***	0.542 ***
b*	0.870 ***	0.882 ***	0.425 *
C*	0.886 ***	0.884 ***	0.444 *
h	-0.922 ***	-0.887 ***	-0.507 ***
Specific volume	1.000 ***	0.816 ***	0.521 ***
Crust color score		1.000 ***	0.644 ***
Brown spots score			1.000

[a] Correlation coefficient analysis of n = 24.

[b] *, *** = Significant at $P < 0.05$ and 0.001 respectively.

[c] Color measurement using spectrophotometer; L* = Lightness, +a* = red, +b* = yellow, C* = chroma value, and h = hue angle value.

Table VI

Correlation Coefficient (*r*) of Dough Properties Using Micro-extensibility and Baking Properties of Bread Sticks Made with Frozen Dough

	Rmax[d]	E[e]	A[f]	R20mm[g]	Rmax/E[h]
Heat-treated yeast addition					
Specific volume	-0.155	-0.644 ***[c]	-0.834 ***	-0.463 *	0.573 ***
Crust color score	-0.135	-0.735 ***	-0.801 ***	-0.195	0.688 ***
Brown spot score	0.065	-0.629 ***	-0.639 ***	-0.084	0.631 ***
GSH addition[b]					
Specific volume	-0.059	-0.531 ***	-0.769 ***	-0.193	0.203
Crust score	0.538 ***	-0.274	-0.186	0.233	0.595 ***
Crumb score	0.441 **	-0.511 ***	-0.367 *	0.329 *	0.589 ***
Crumb firmness	-0.296 *	0.553 ***	0.498 ***	-0.239	-0.510 ***

[a] 0, 5 and 10% heat-treated yeast, n = 24.

[b] 0, 80, 160, and 240 ppm glutathione, n = 48.

[c] *, **, *** = Significant at $P < 0.05$, 0.01, and 0.001 respectively.

[d] Rmax = Resistance to extention at maximum

[e] E = extensibility

[f] A = Area

[g] R20mm = Resistance to extension at 20 mm

[h] Rmax/E = Ratio of resistant to extension at maximum and extensibility.

TableI VII

Correlation Coefficient (*r*) of Dough Properties Using Micro-extensibility and Crust Color of Bread Sticks Containing Heat-treated Yeast[a]

	Rmax[c]	E[d]	A[e]	R20[f]	Rmax/E[g]
Color measurement[h]					
L*	0.149	0.741 ***[b]	0.900 ***	0.427 *	-0.671 ***
+a*	-0.109	-0.707 ***	-0.860 ***	-0.438 *	0.659 ***
+b*	-0.163	-0.686 ***	-0.795 ***	-0.351	0.618 ***
C*	-0.153	-0.693 ***	-0.808 ***	-0.367	0.627 ***
h	0.144	0.703 ***	0.846 ***	0.403	-0.647 ***

[a] Correlation coefficient analysis of n = 24.

[b] *, *** = Significant at $P < 0.05$ and 0.001 respectively.

[c] Rmax = Resistance to extention at maximum

[d] E = extensibility

[e] A = Area

[f] R20mm = Resistance to extension at 20 mm

[g] Rmax/E = Ratio of resistant to extension at maximum and extensibility.

[h] Color measurement using spectrophotometer; L* = Lightness, +a* = red, +b* = yellow, C* = chroma value, and h = hue angle value.

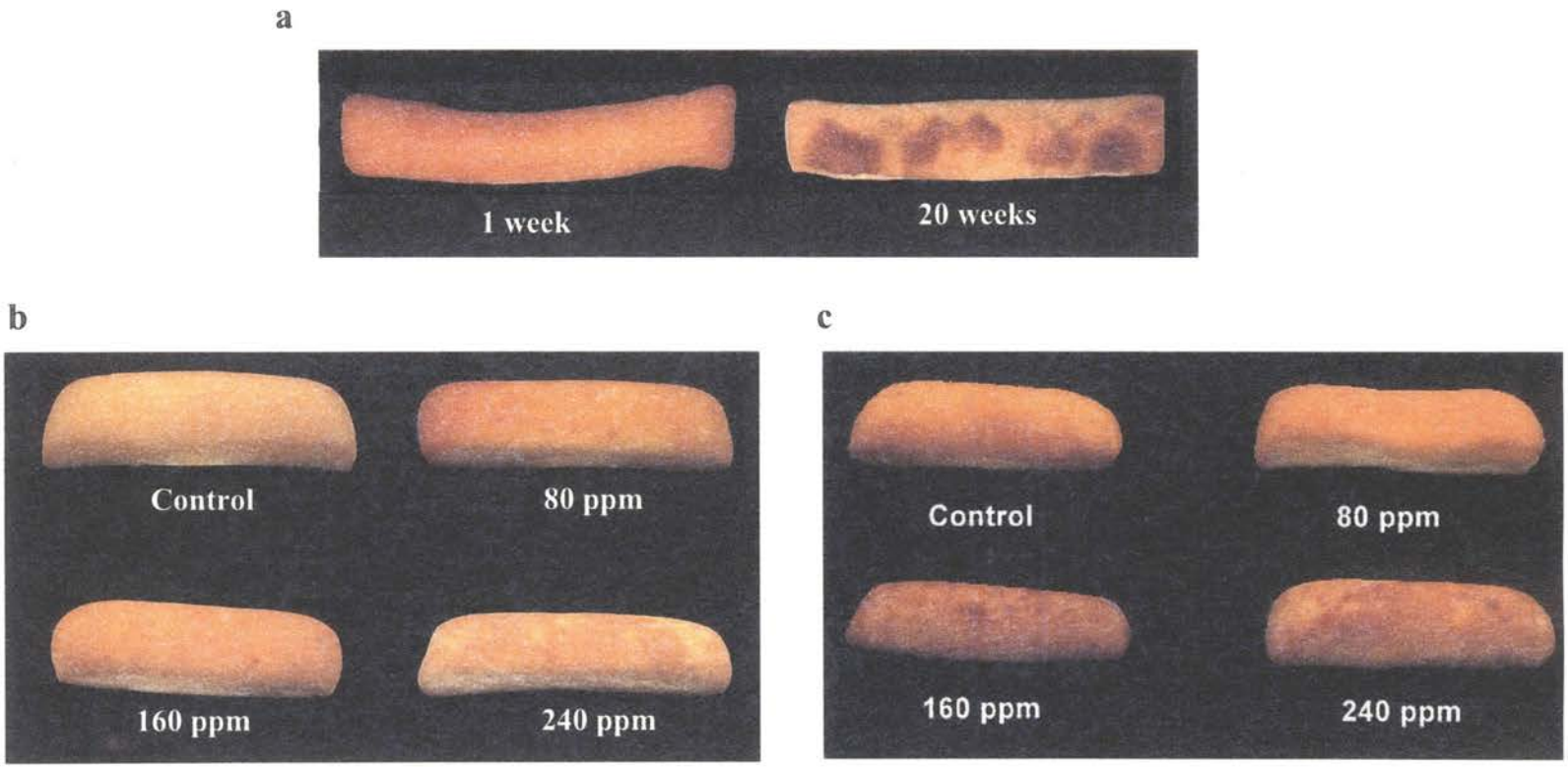


Fig. 1. Bread sticks with hard red spring flour: **a)** a typical of crust from control breads at 1 week and 20 weeks of frozen storage, Crust from dough containing 0, 80, 160 and 240 ppm glutathione (GSH) frozen stored for **b**) 0 day (fresh), **c)** 1 day.

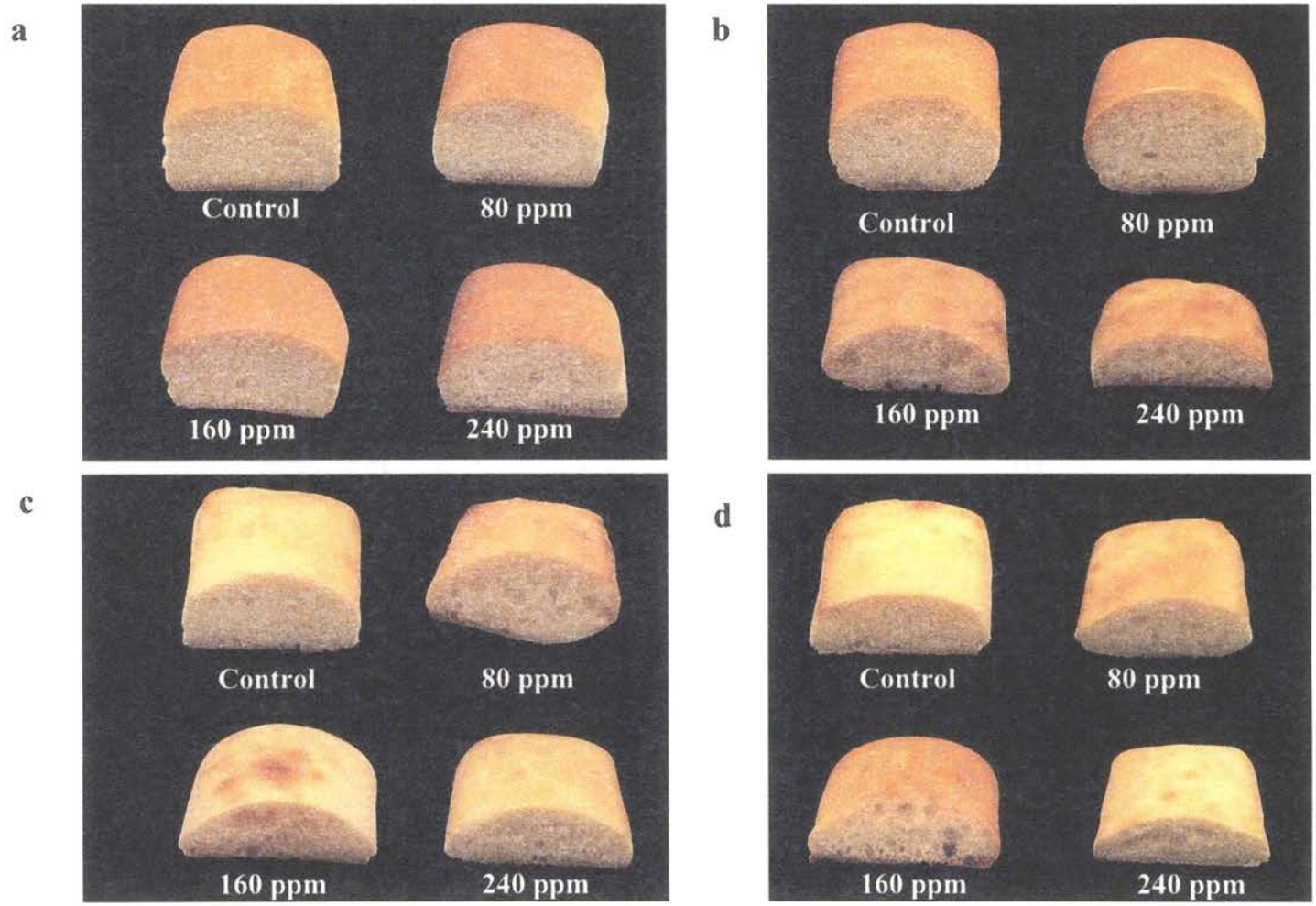


Fig. 2. Bread sticks made from hard red spring flour containing 0, 80, 160 and 240 ppm glutathione (GSH) at different frozen storage times: **a)** 0 day (fresh dough), **b)** 1 day, **c)** 2 weeks, and **d)** 4 weeks.

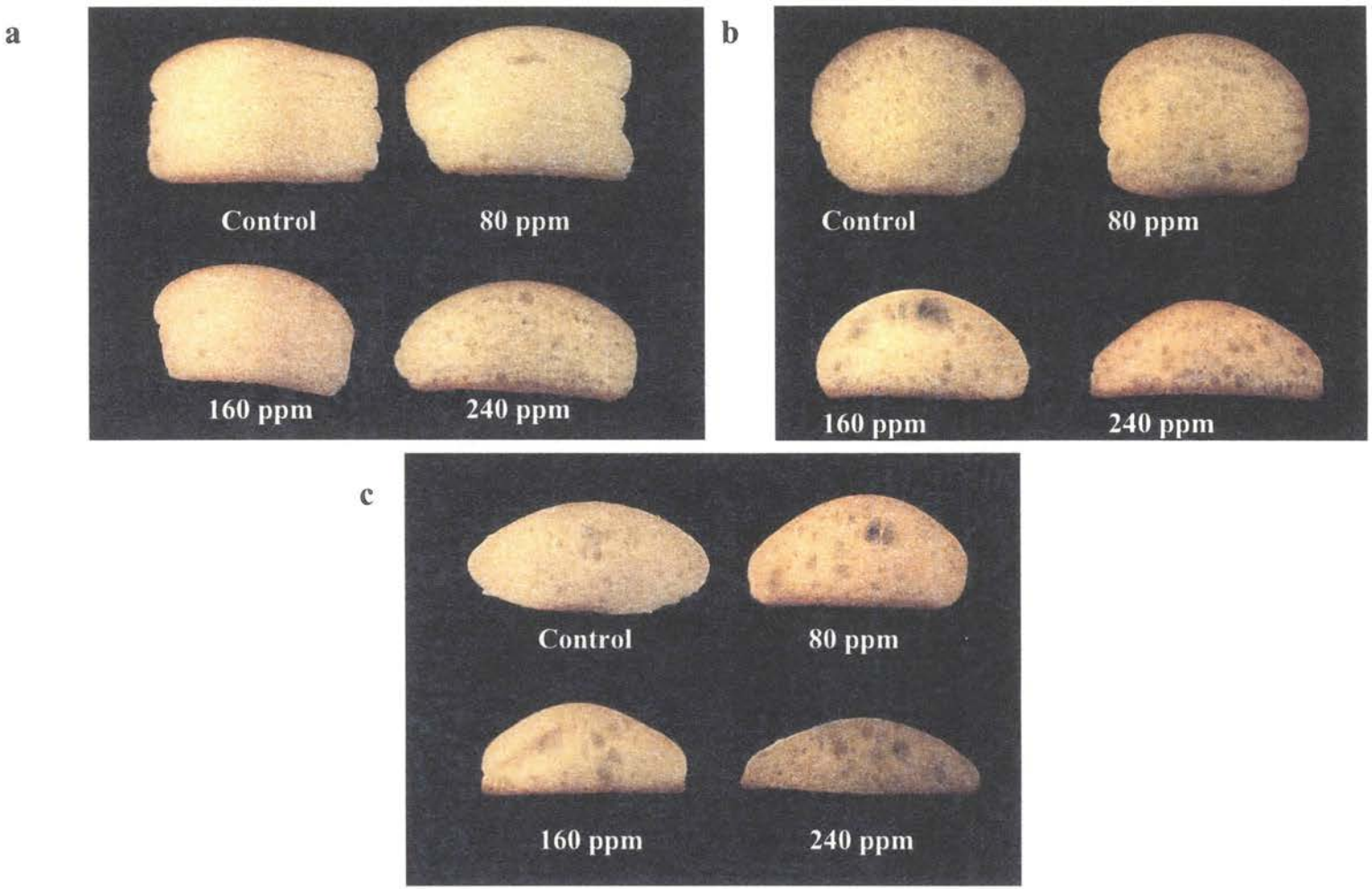


Fig. 3. Crumb of bread sticks made from hard red spring flour containing 0, 80, 160 and 240 ppm Glutathione (GSH) at different frozen storage times: **a)** 0 day (fresh dough), **b)** 1 day, and **c)** 8 weeks

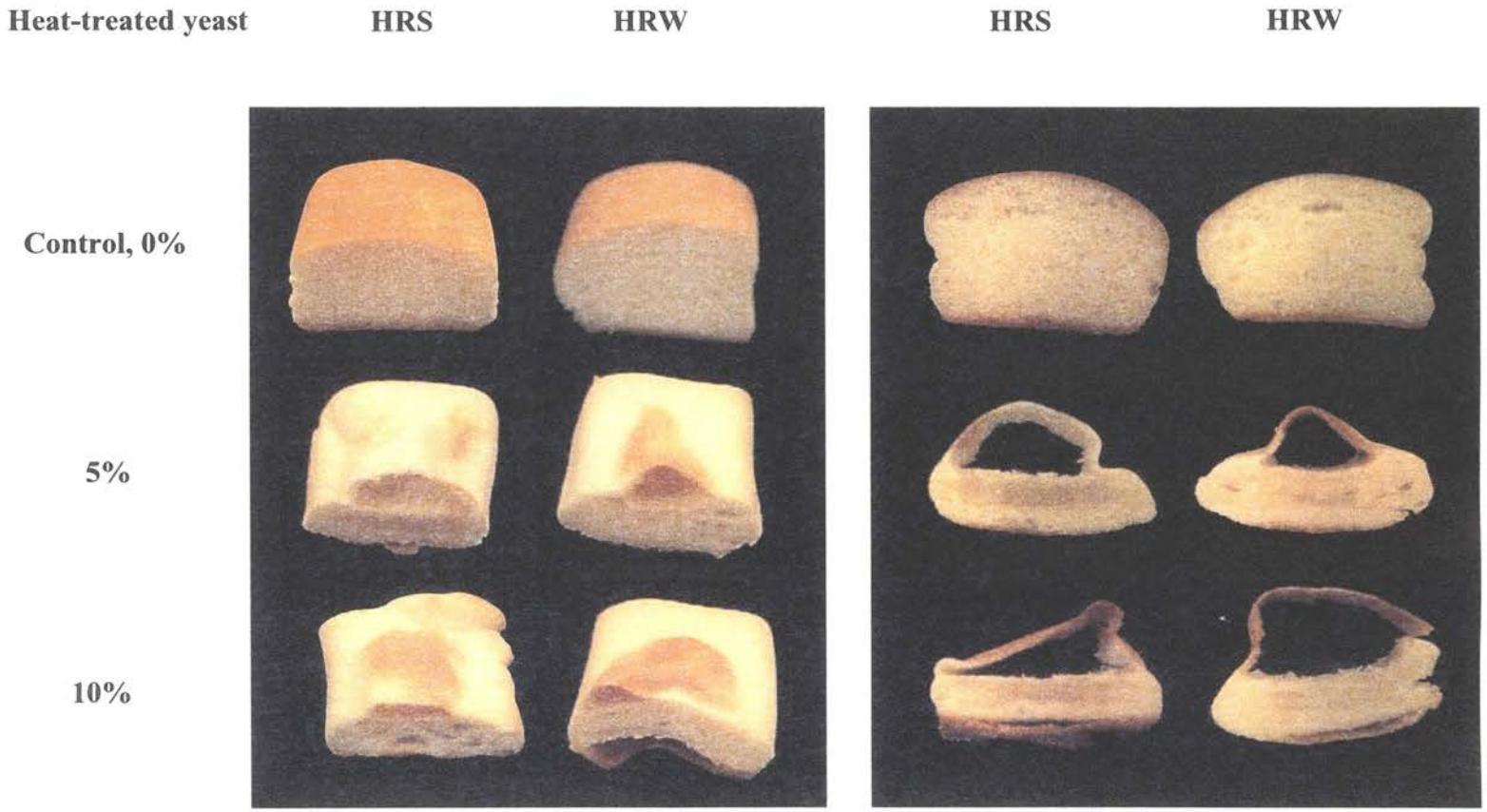


Fig. 4. Bread sticks from fresh dough made from hard red spring (HRS) and hard red winter (HRW) wheat flours. Dough containing 0, 5 and 10% heat-treated yeast.

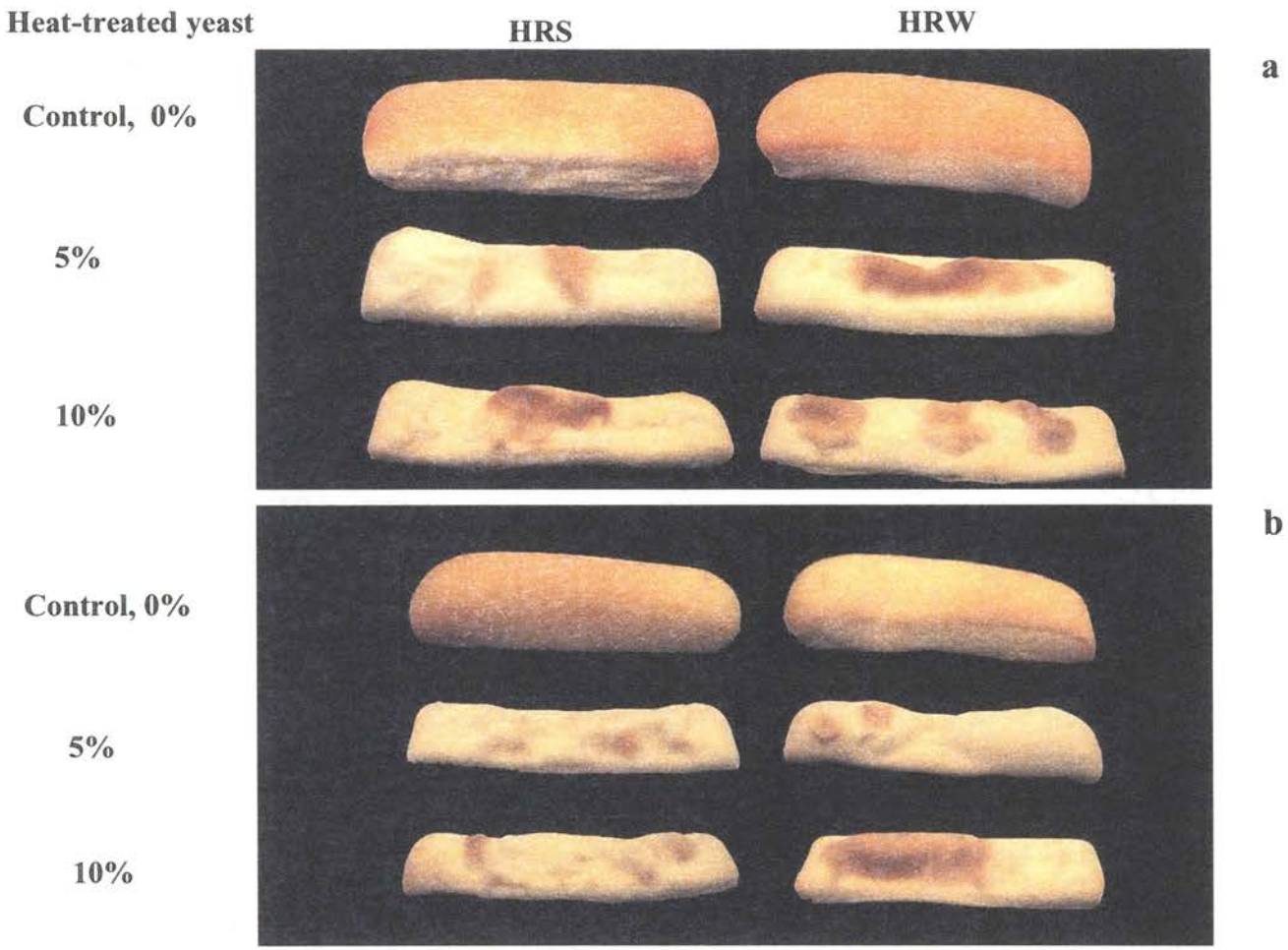


Fig. 5. Bread sticks made from hard red spring (HRS) and hard red winter (HRW) wheat flours, dough containing 5 and 10% heat-treated yeast at: **a)** fresh, and **b)** 1 day frozen.

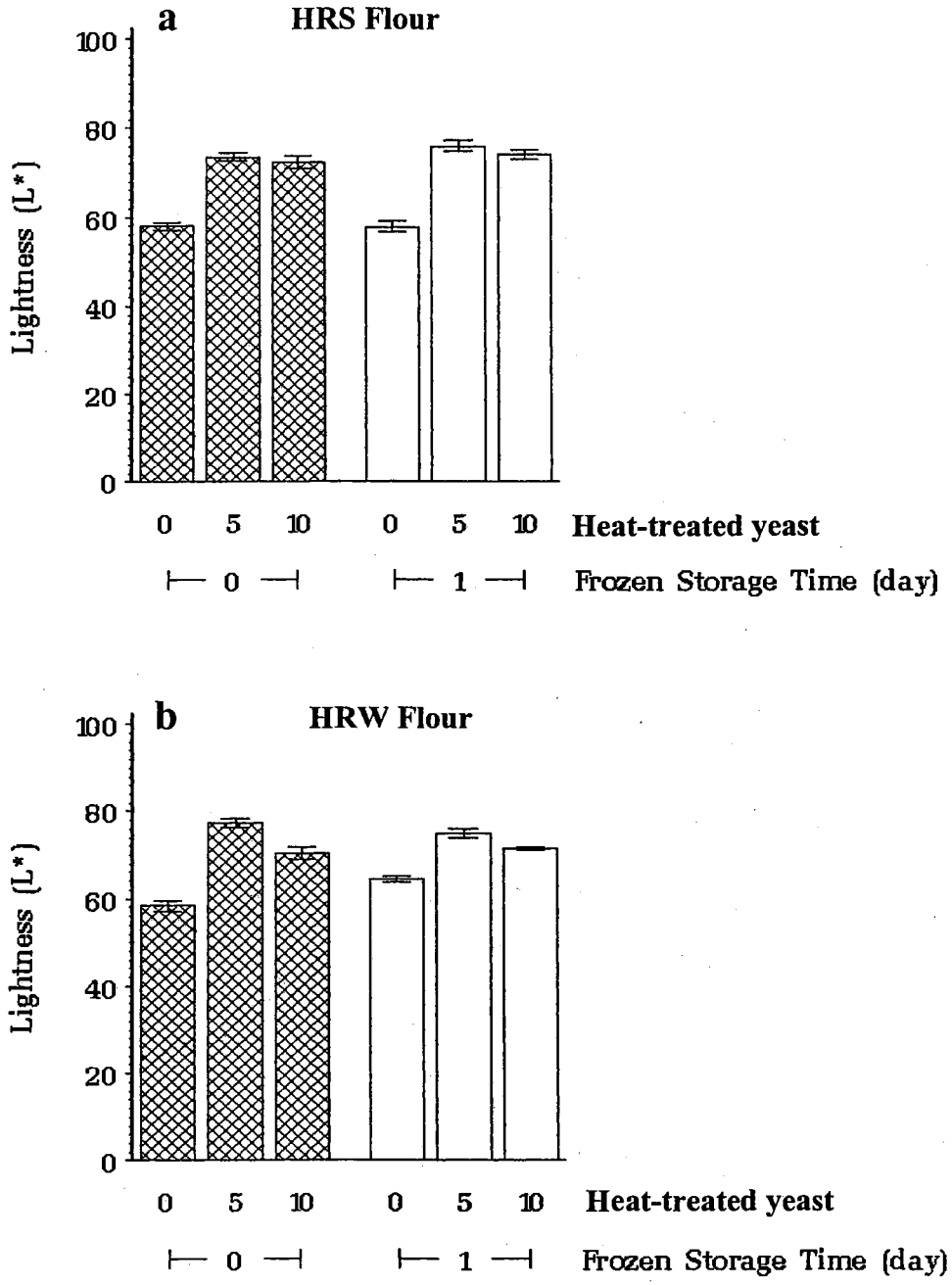


Fig. 6. Lightness (L*) value of bread sticks crust as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Hard red spring (HRS) (**a**) and hard red winter (HRW) flour **(b).** Bar = standard deviation, n = 16.

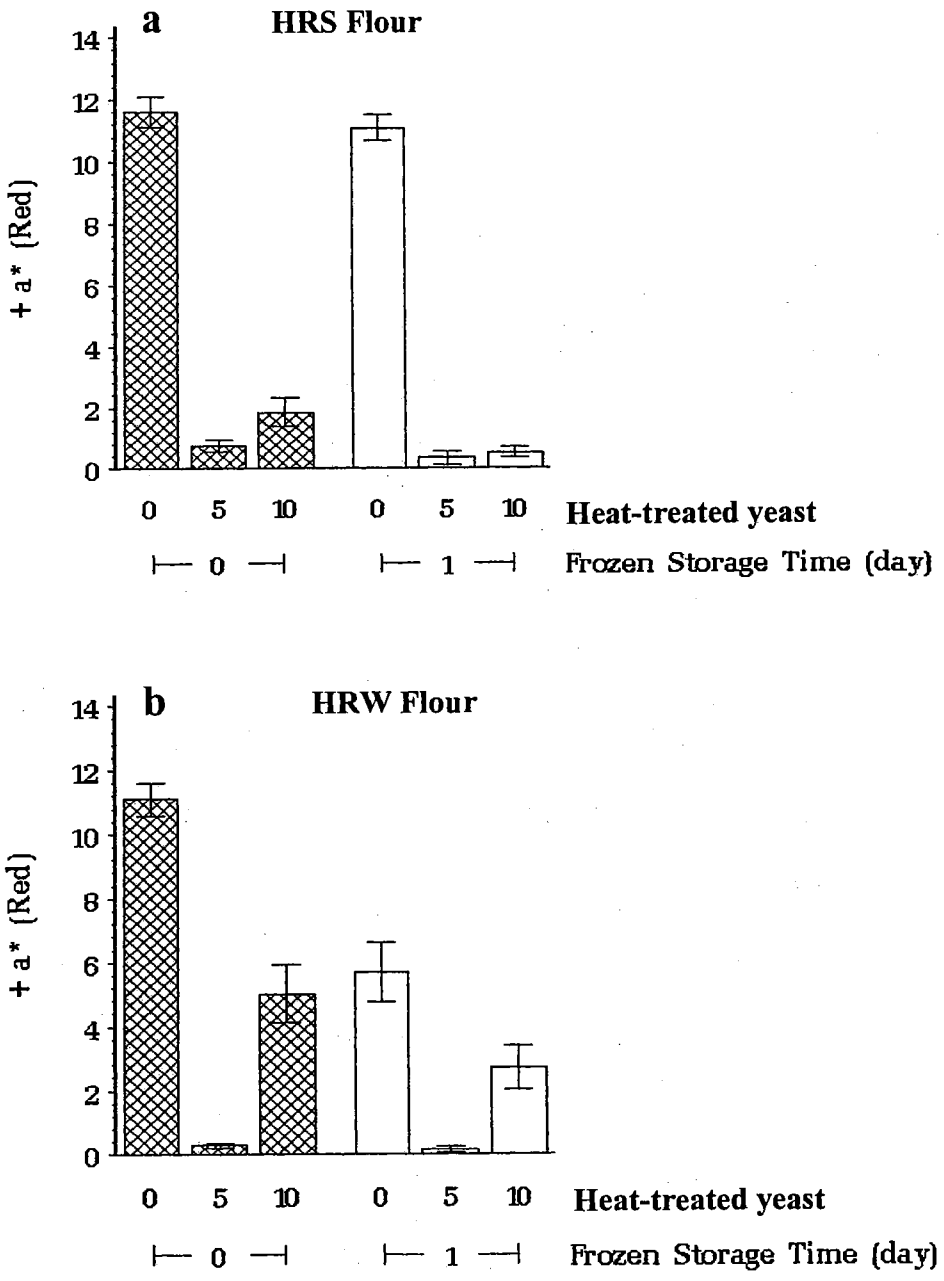


Fig. 7. Red (+a*) value of bread sticks crust as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Hard red spring (HRS) (**a**) and hard red winter (HRW) flour (**b**). Bar = standard deviation, n = 16.

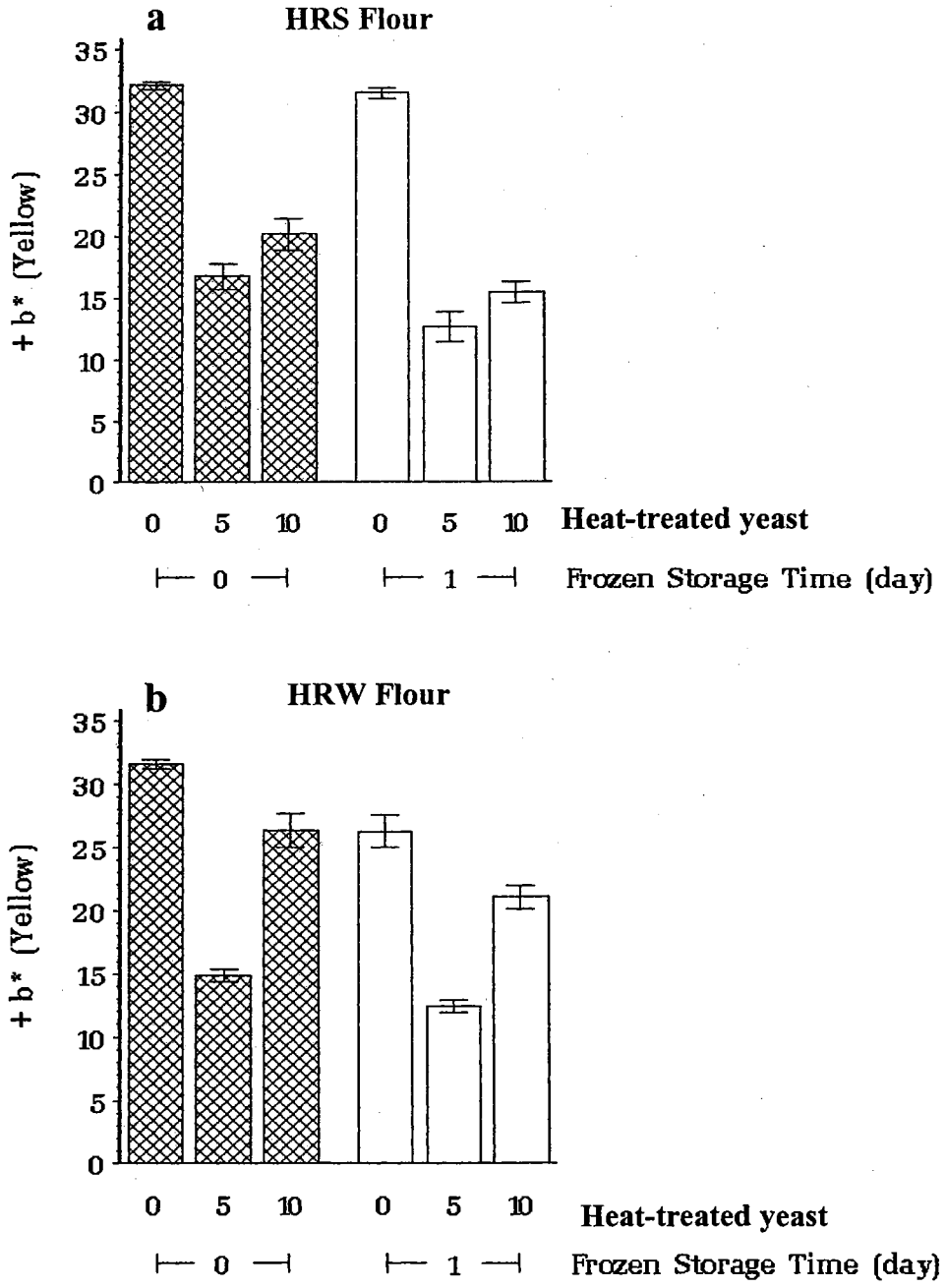


Fig. 8. Yellow (+b*) value of bread sticks crust as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Hard red spring (HRS) (**a**) and hard red winter (HRW) flour (**b**). Bar = standard deviation, n = 16.

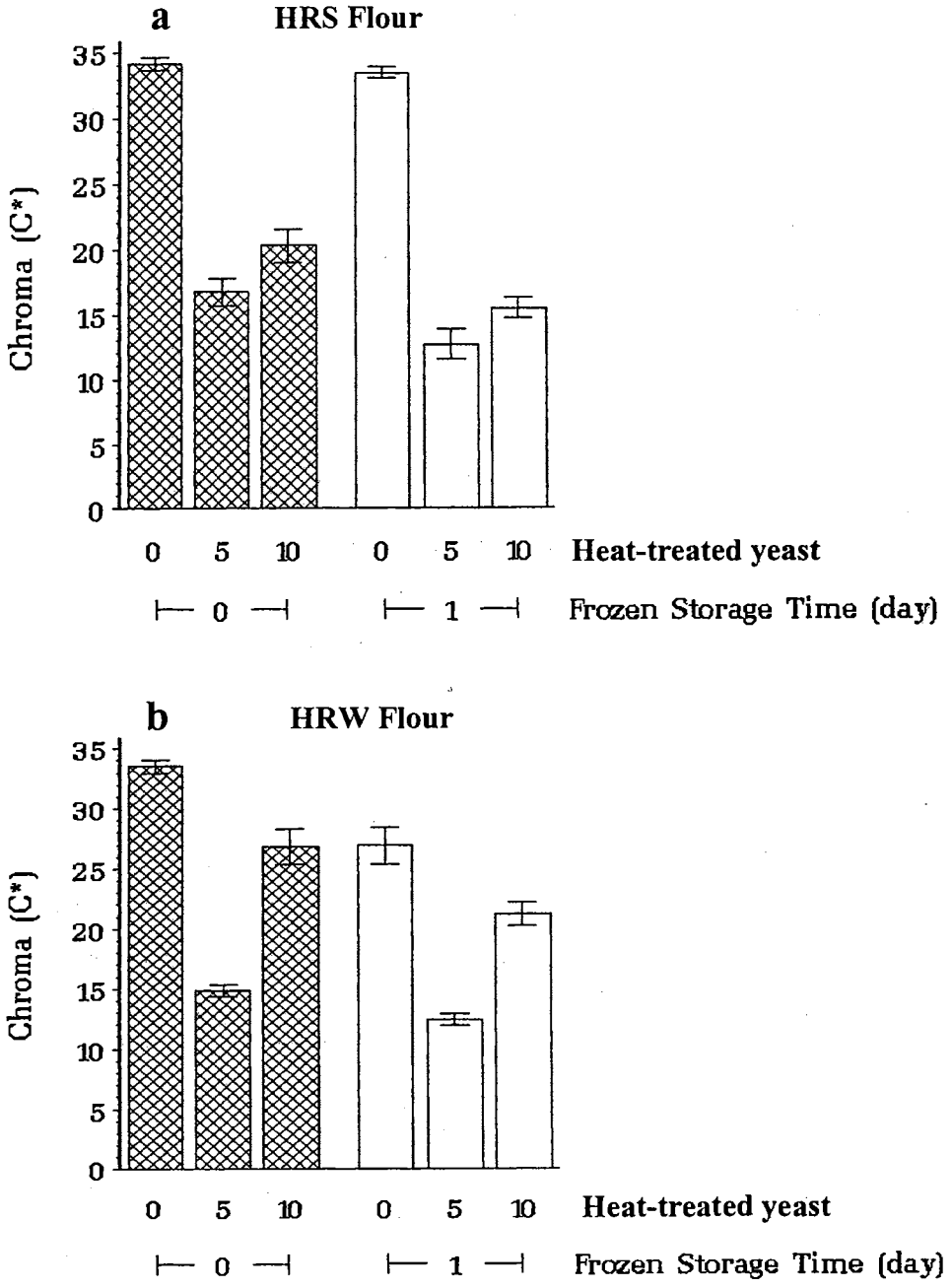


Fig. 9. Croma (+c*) value of bread sticks crust as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Hard red spring (HRS) (**a**) and hard red winter (HRW) flour (**b**). Bar = standard deviation, n = 16.

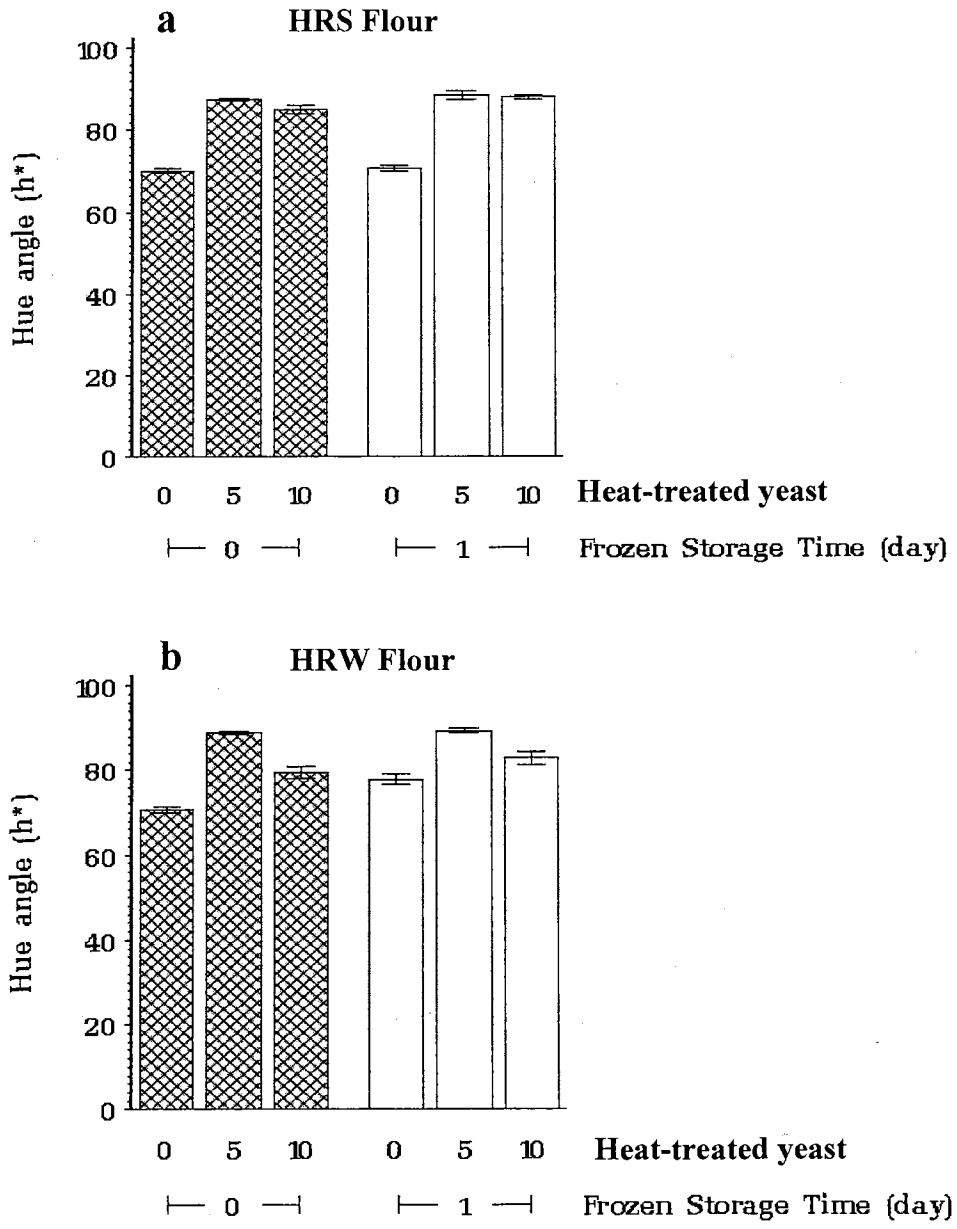


Fig. 10. Hue angle (+h*) value of bread sticks crust as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Hard red spring (HRS) (**a**) and hard red winter (HRW) flour (**b**). Bar = standard deviation, n = 16.

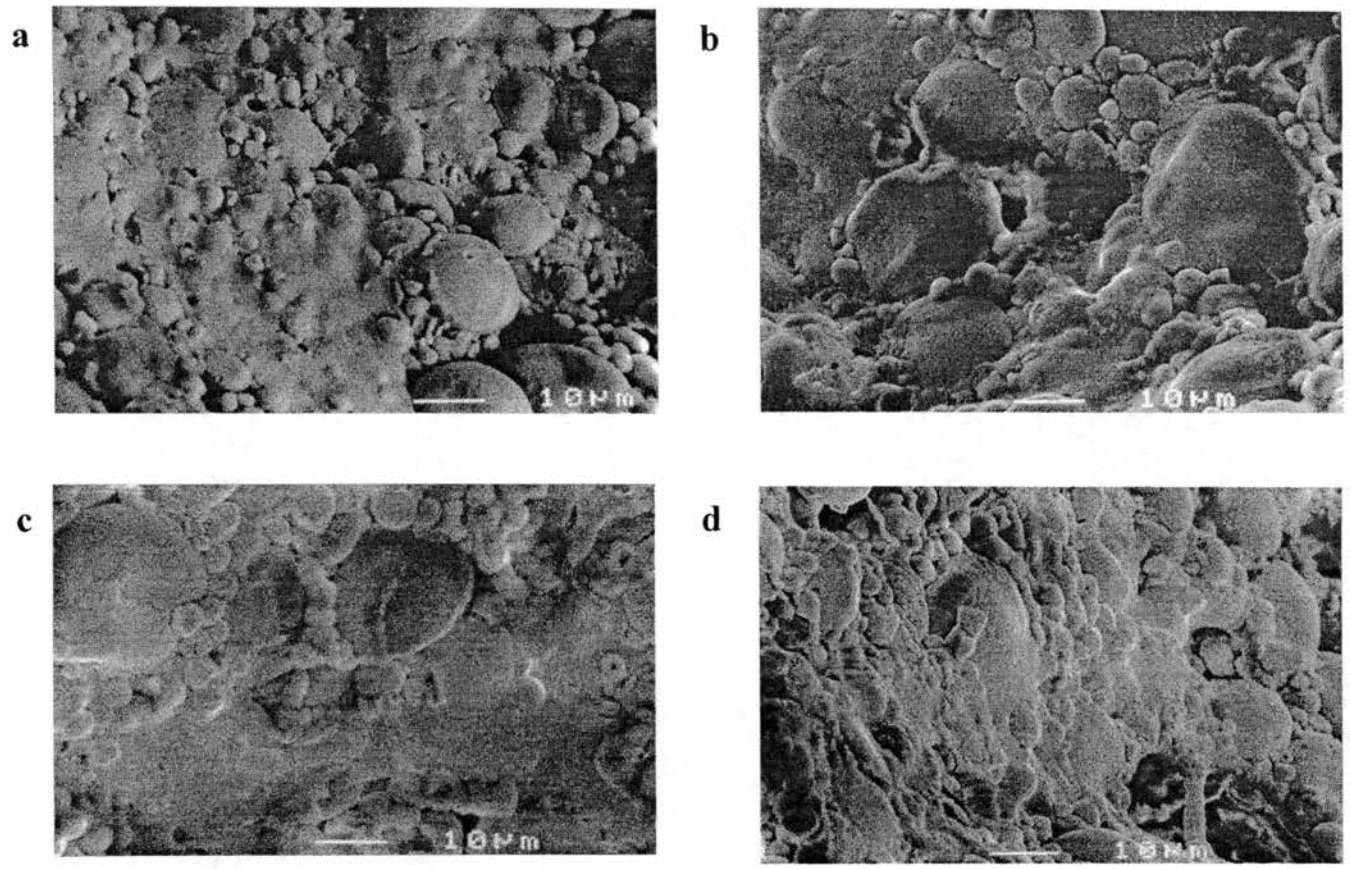


Fig. 11. Scanning electron micrographs of freeze dried dough, accelerating voltage 10 kV, magnification 1000x. **a)** control, 0 day, **b)** control, 6 weeks of frozen storage, **c)** 80 ppm GSH, 0 day, **d)** 80 ppm GSH, 1 day frozen storage. Scale bar = 10 μm.

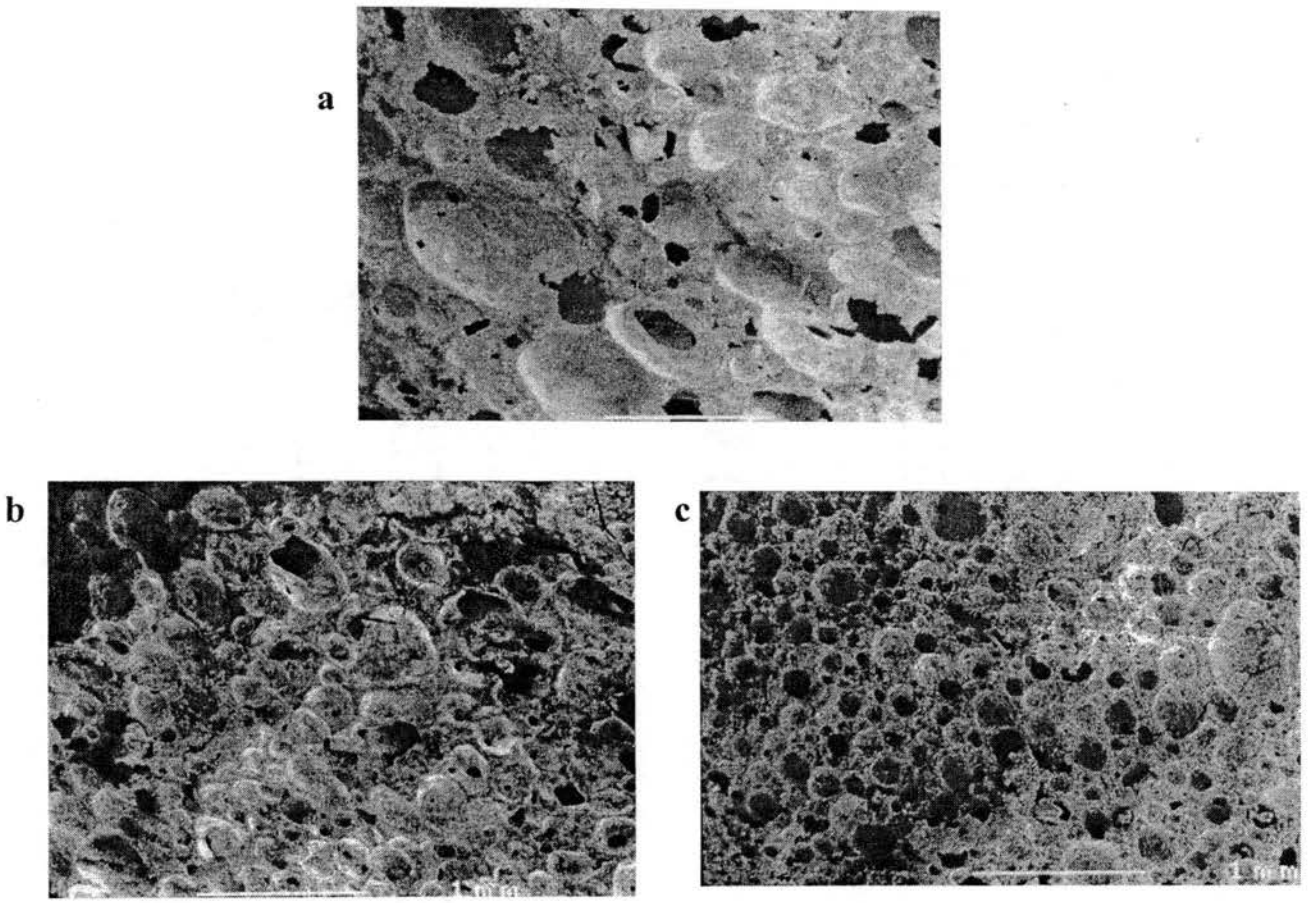


Fig. 12. Scanning electron micrographs of freeze dried dough, accelerating voltage 10 kV, magnification 25x. **a)** control, 0 day, **b)** control, 1 day frozen storage, **c)** control, 6 weeks frozen storage. Scale bar = 1 mm.

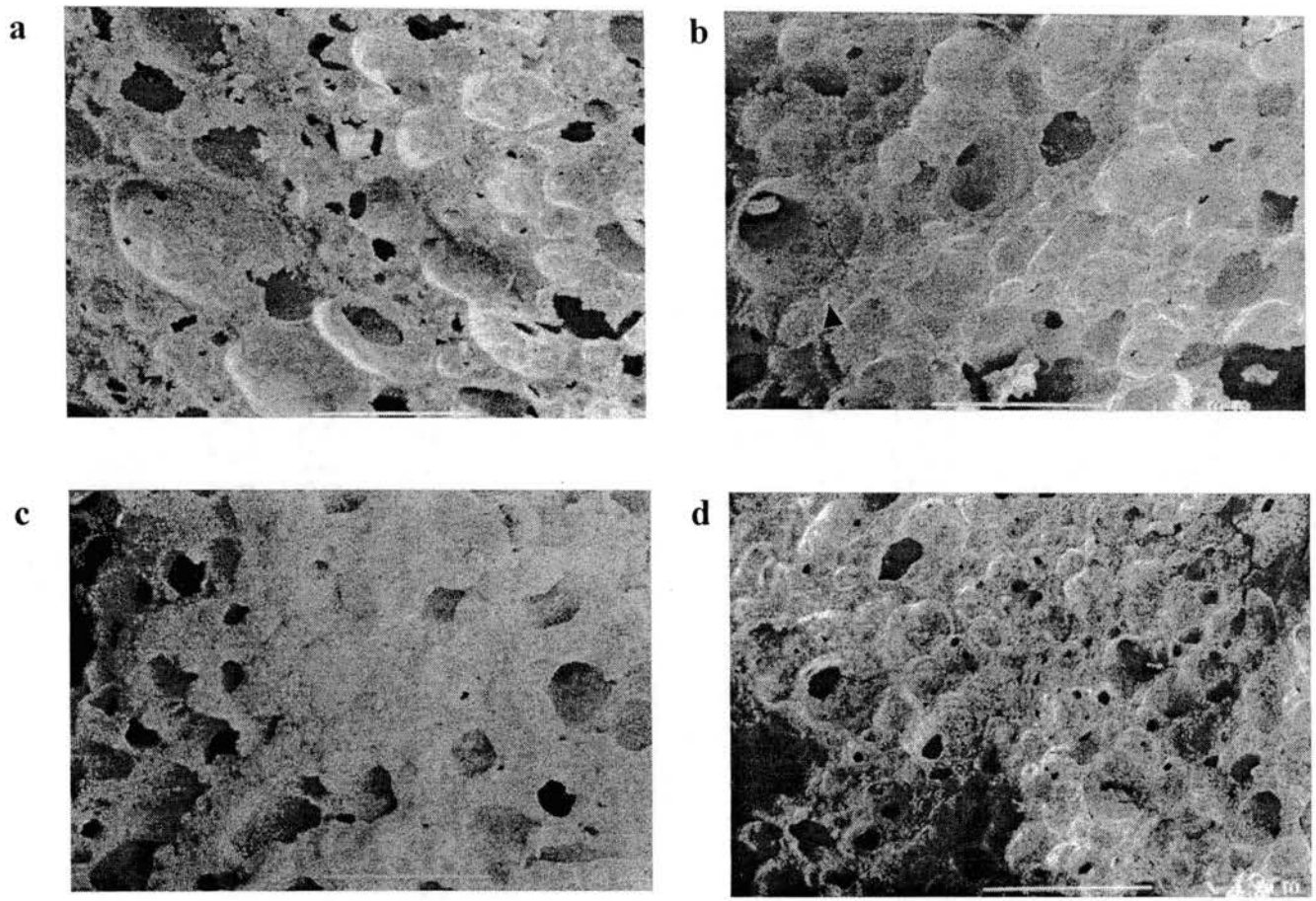


Fig. 13. Scanning electron micrographs of freeze dried dough, accelerating voltage 10 kV, magnification 25x. **a)** 0 ppm GSH, 0 day; **b)** 80 ppm GSH, 0 day; **c)** 160 ppm GSH, 0 day; **d)** 80 ppm GSH, 1 day frozen storage. Scale bar = 1 mm.

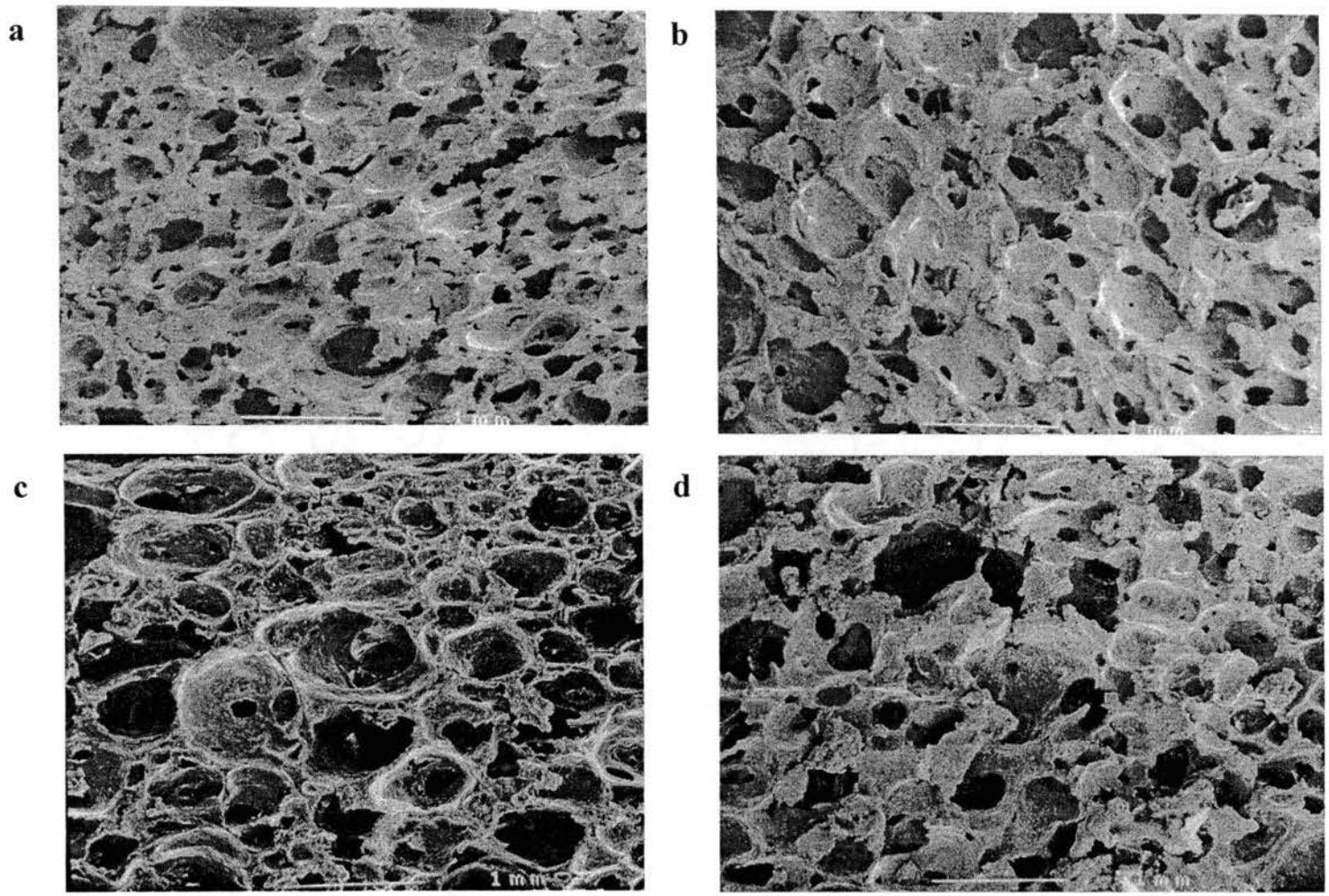


Fig. 14. Scanning electron micrographs of control bread crumb, accelerating voltage 10 kV, magnification 25x. **a**) fresh dough, **b**) frozen dough stored at 1 day, **c**) frozen dough stored at 2 weeks, **d**) frozen dough stored at 6 weeks. Scale bar = 10 mm.

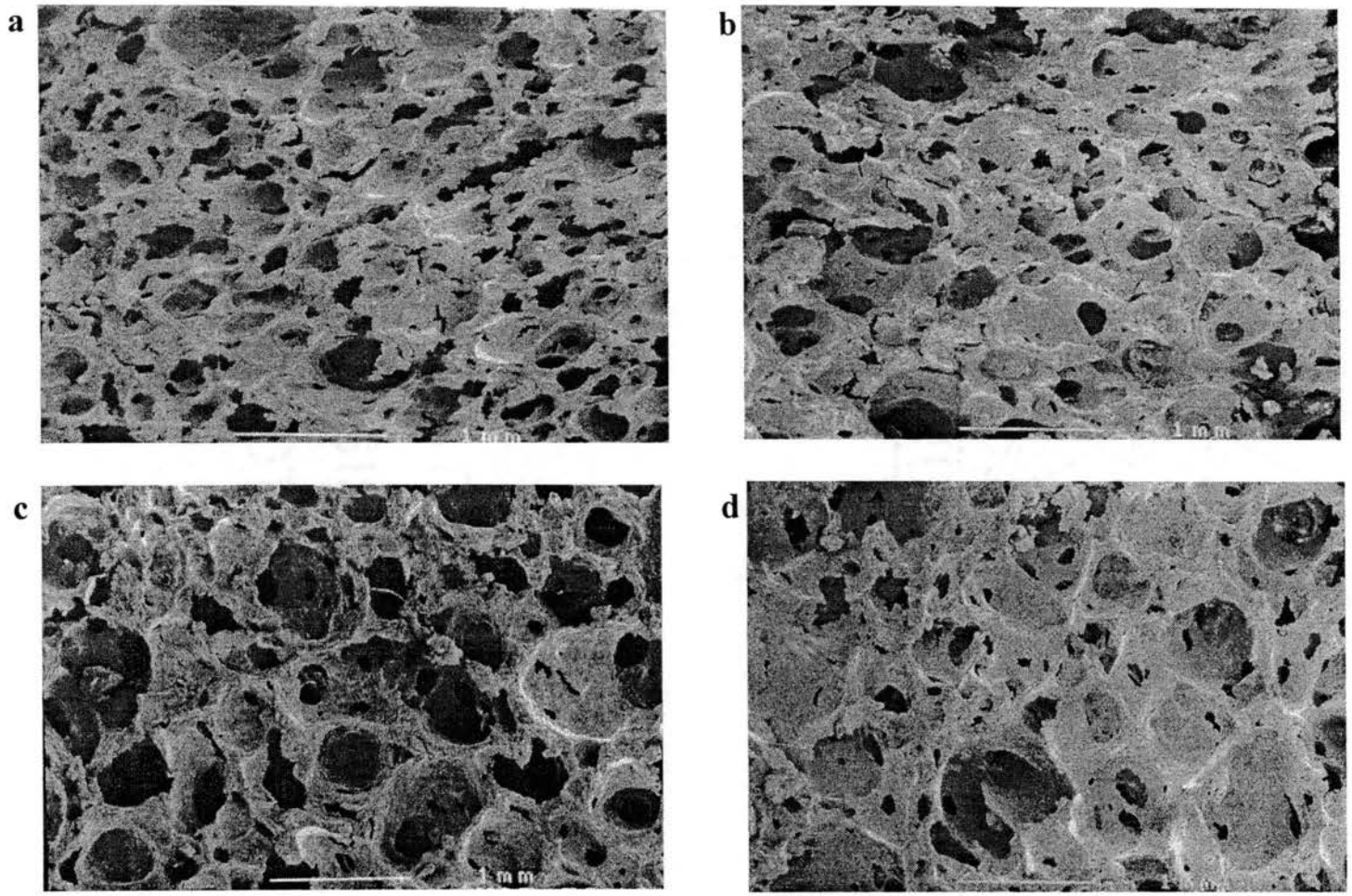


Fig. 15. Scanning electron micrographs of bread crumb with hard red spring flour at fresh (0 day storage) dough, accelerating voltage 10 kV, magnification 25x. Glutathione (GSH): **a**) 0 ppm, **b**) 80 ppm, **c**) 160 ppm, **d**) 240 ppm. Scale bar = 10 mm.

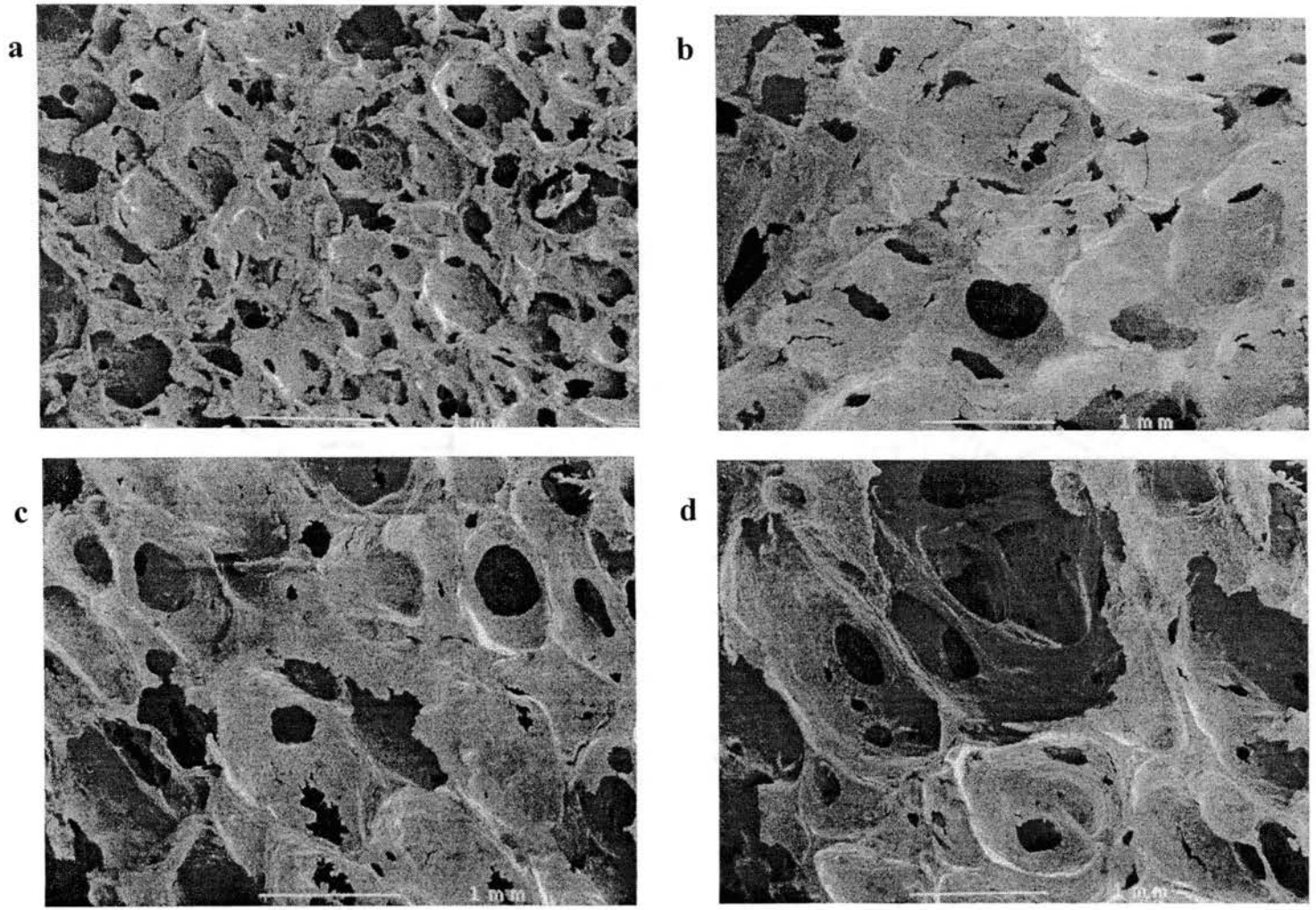


Fig. 16. Scanning electron micrographs of bread crumb from 1 day frozen storage dough, accelerating voltage 10 kV, magnification 25x. Glutathione (GSH): **a**) 0 ppm, **b**) 80 ppm, **c**) 160 ppm, **d**) 240 ppm. Scale bar = 10 mm.

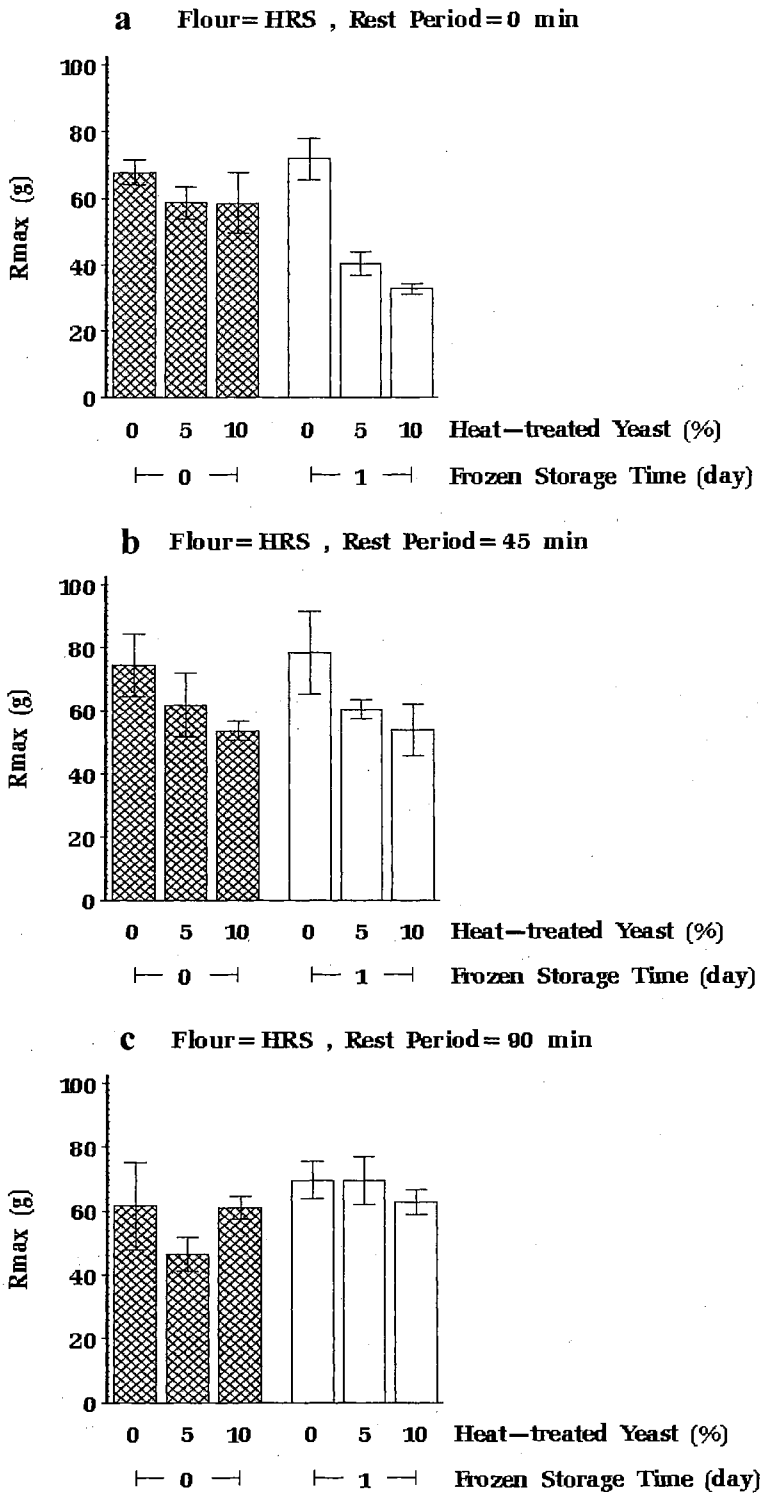


Fig. 17. Resistance to extension (Rmax) of dough made with hard red spring (HRS) flour as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Rest period: **a**) 0 min, **b**) 45 min, and **c**) 90 min. Bar = standard deviation, n = 14.

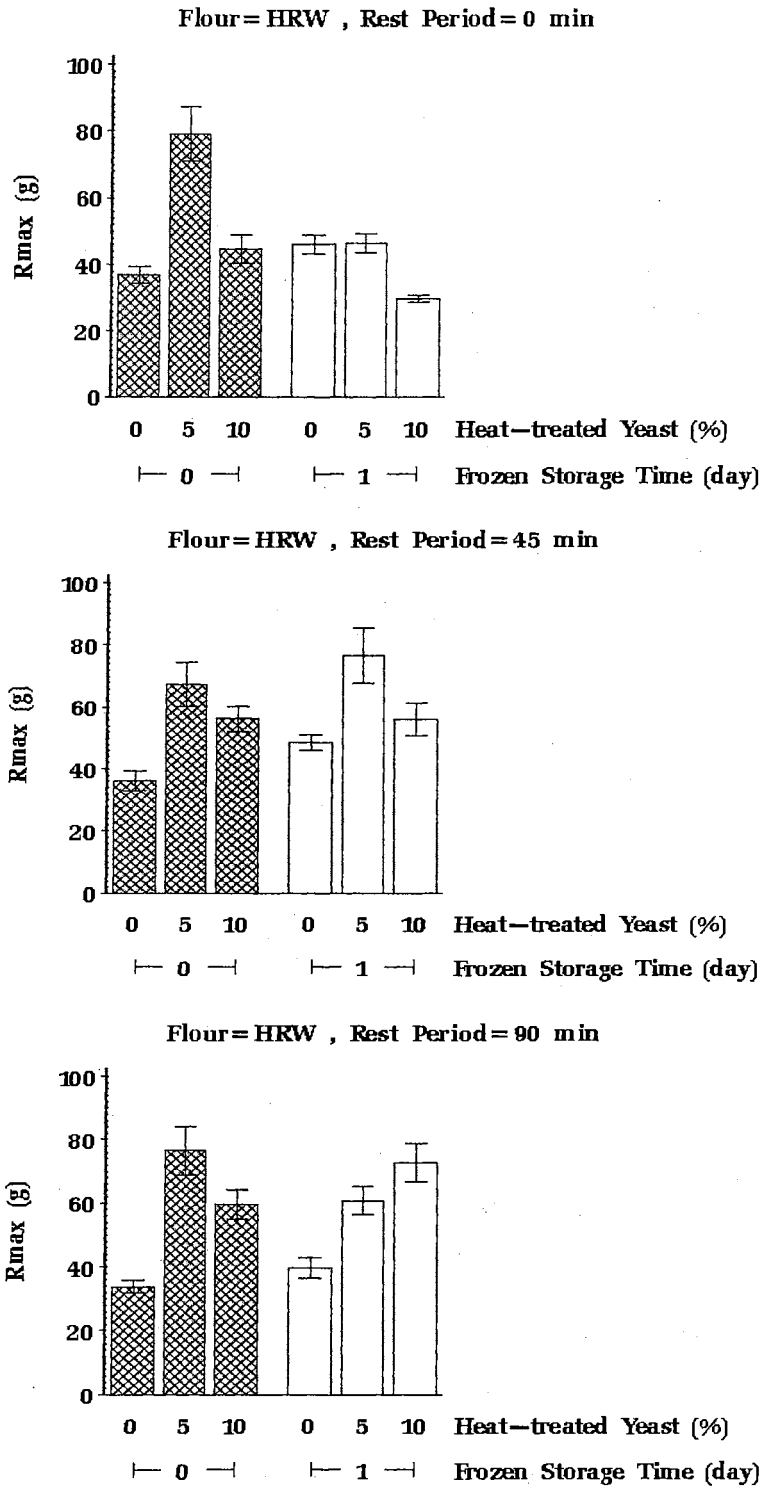


Fig. 18. Resistance to extension (Rmax) of dough made with hard red winter (HRW) flour as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Rest period: **a**) 0 min, **b**) 45 min, and **c**) 90 min. Bar = standard deviation, n = 14.

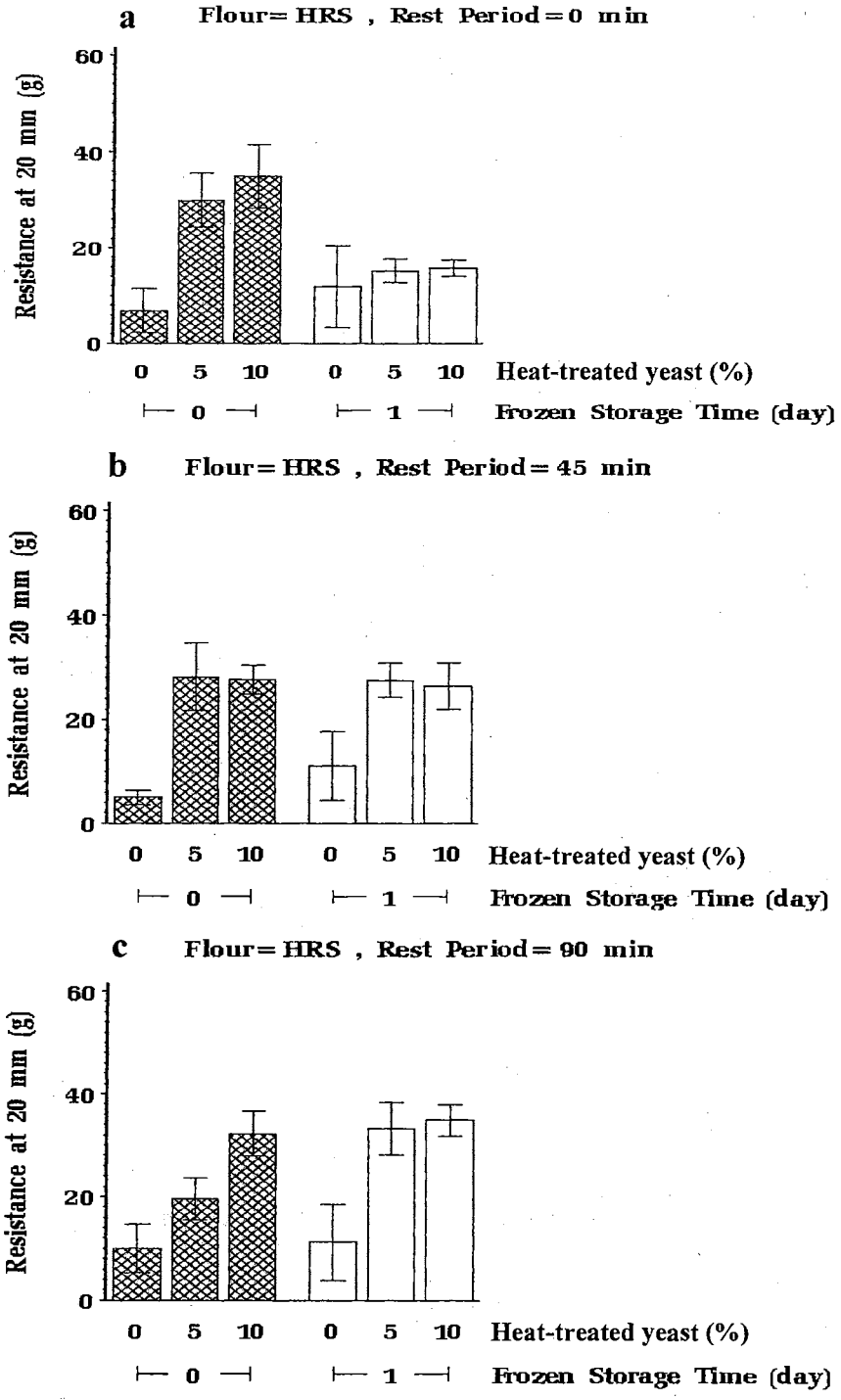


Fig. 19. Resistance to extension at 20 mm (R20mm) of dough made with hard red spring (HRS) flour as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Rest period: **a**) 0 min, **b**) 45 min, and **c**) 90 min.
Bar = standard deviation, n = 14.

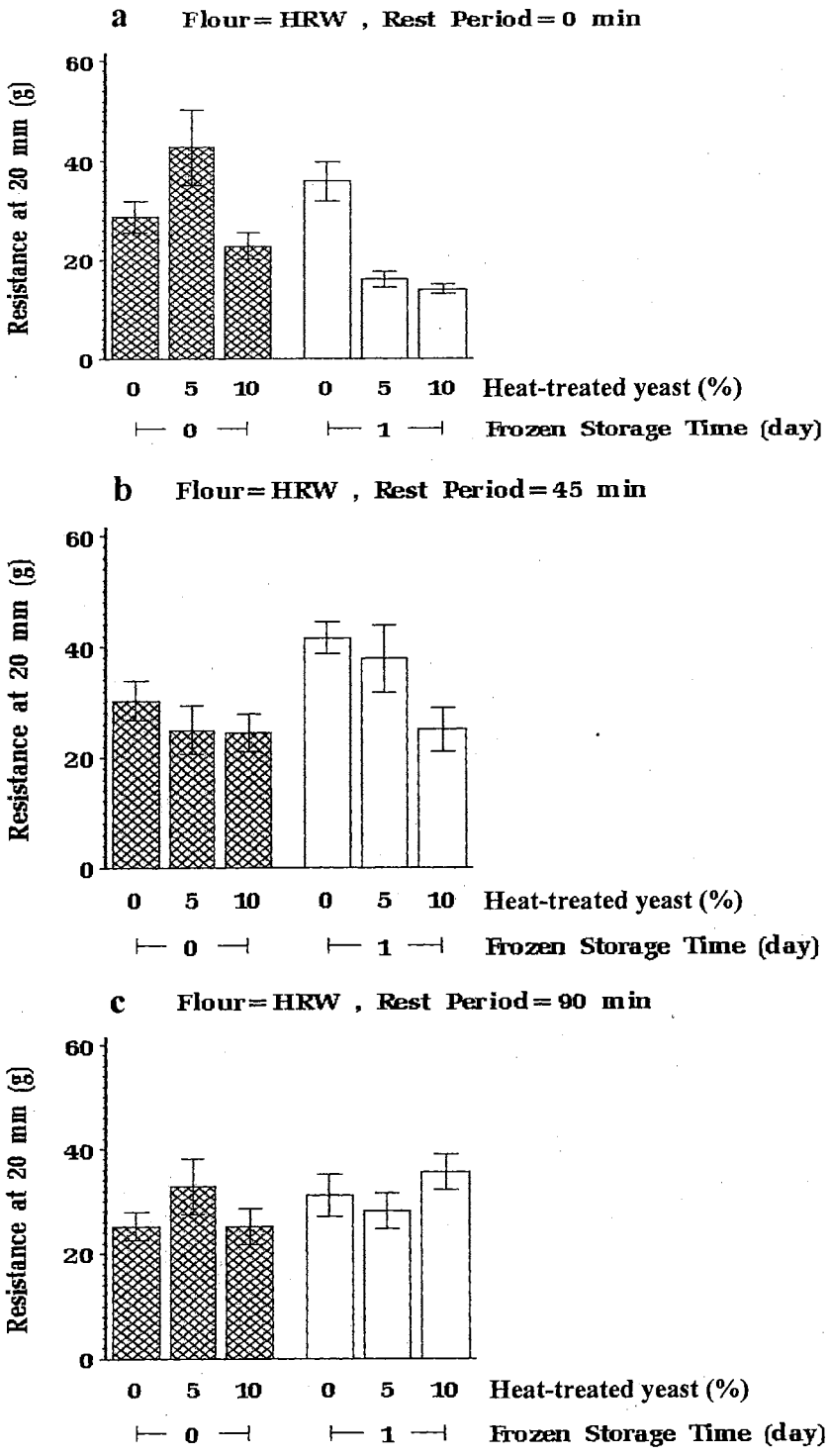


Fig. 20. Resistance to extension at 20 mm (R20mm) of dough made with hard red winter (HRW) flour as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Rest period: **a**) 0 min, **b**) 45 min, and **c**) 90 min.
Bar = standard deviation, n = 14.

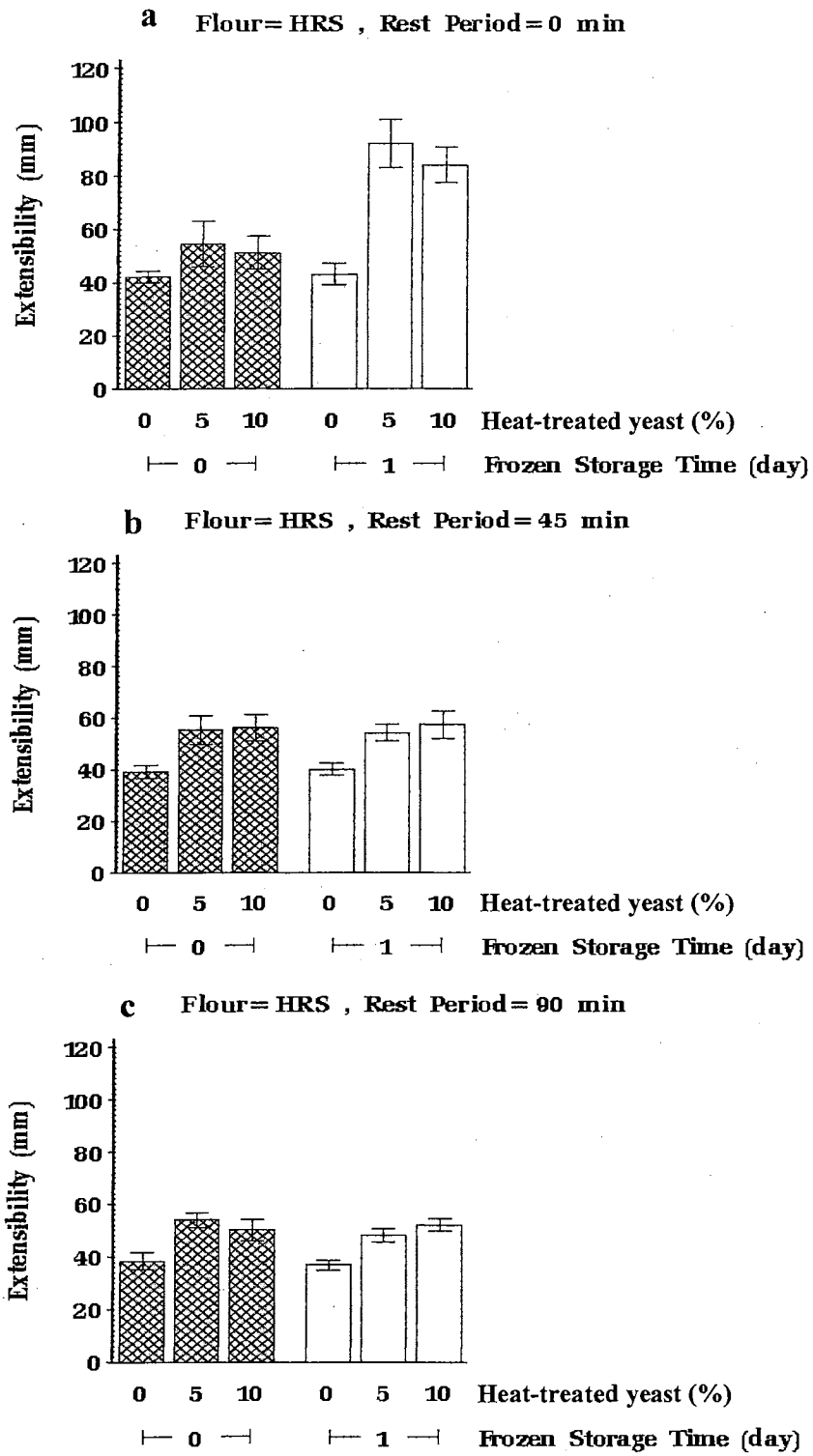


Fig. 21. Extensibility (E) of dough made with hard red spring (HRS) flour as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Rest period: **a)** 0 min, **b)** 45 min, and **c)** 90 min. Bar = standard deviation, n = 14.

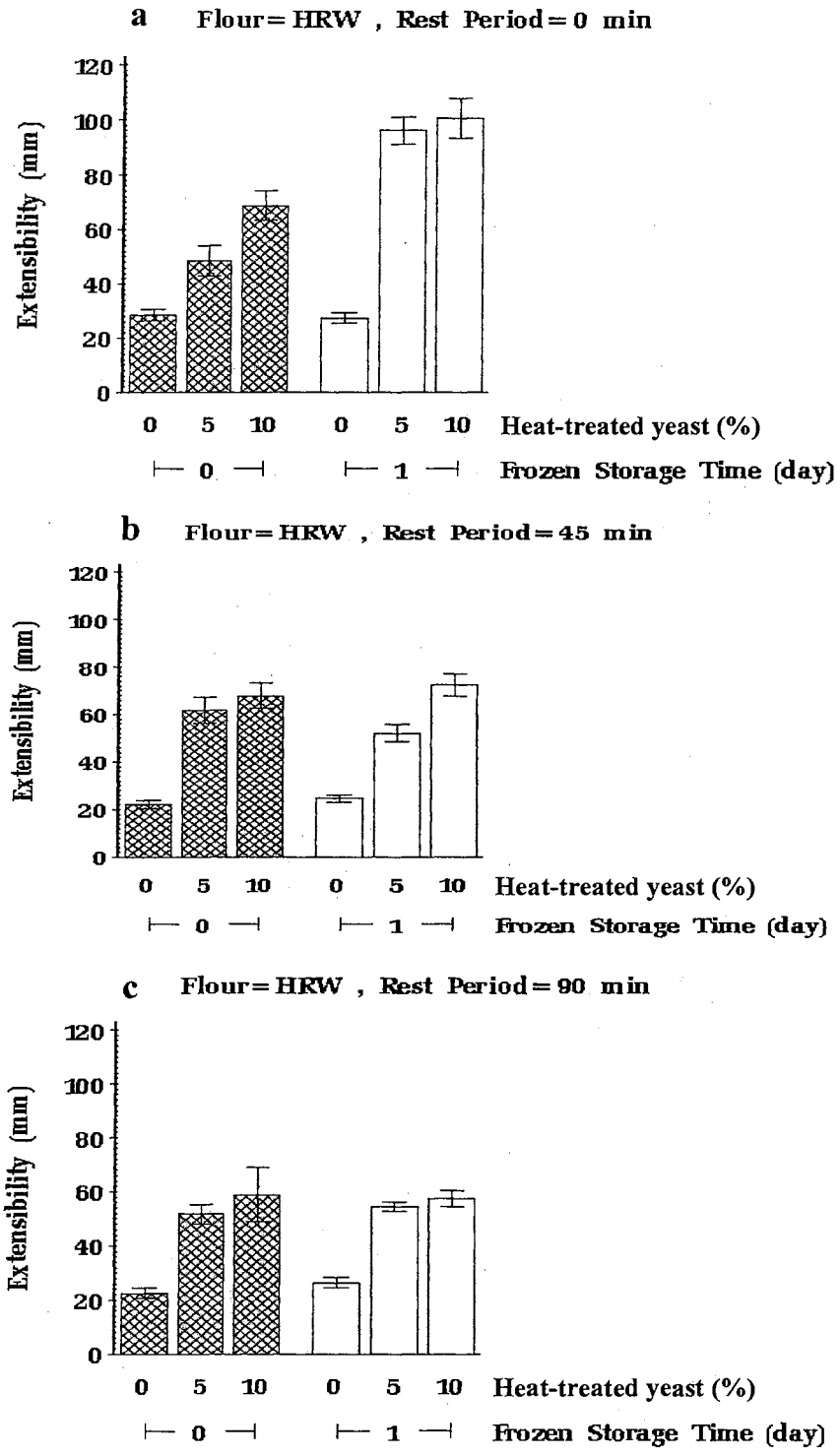


Fig. 22. Extensibility (E) of dough made with hard red winter (HRW) flour as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Rest period: a) 0 min, **b)** 45 min, and **c)** 90 min. Bar = standard deviation, n = 14.

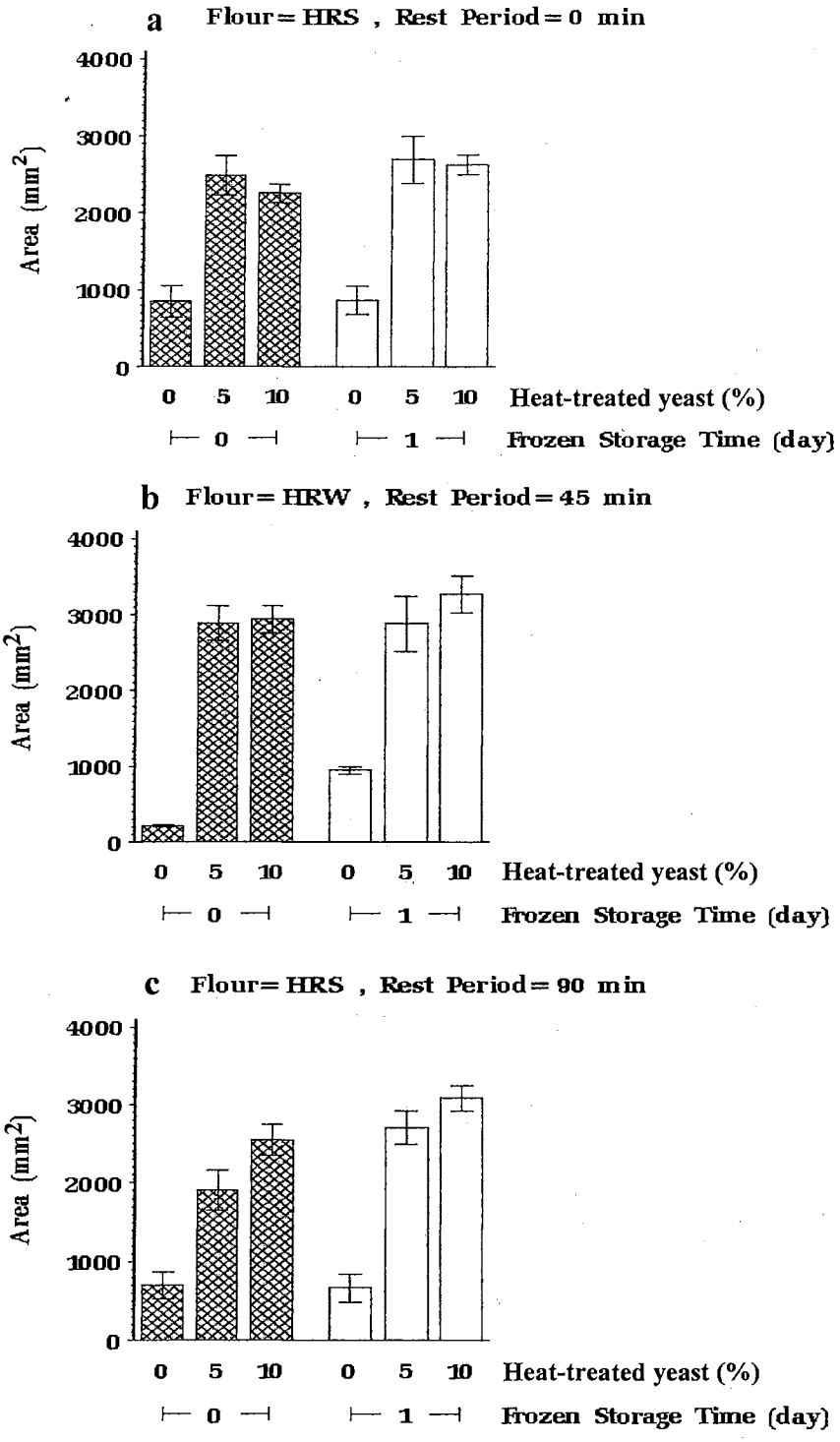


Fig. 23. Area (A) of dough made with hard red spring (HRS) flour as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Rest period: **a**) 0 min, **b**) 45 min, and **c**) 90 min. Bar = standard deviation, n = 14.

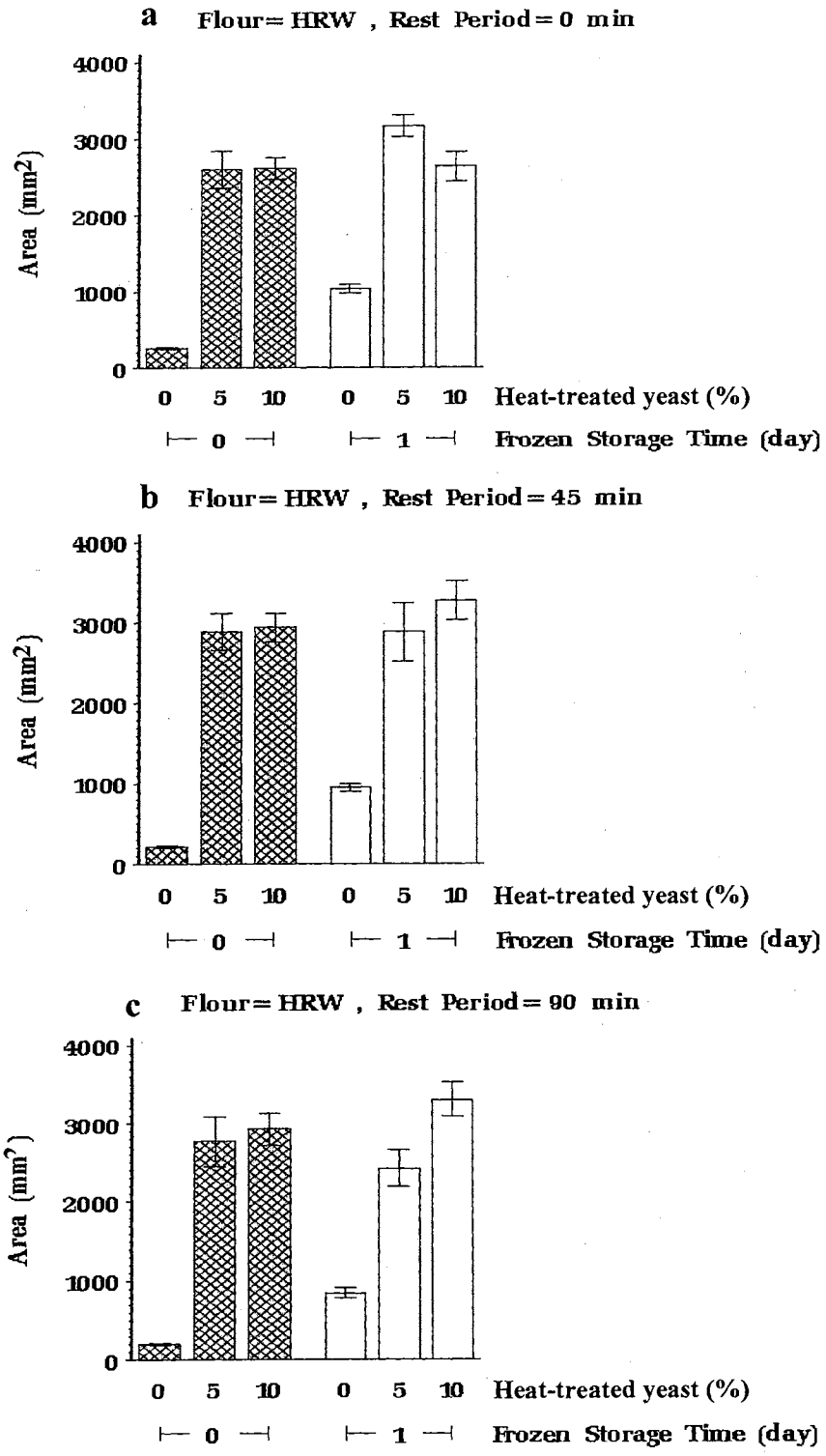


Fig. 24. Area (A) of dough made with hard red winter (HRW) flour as a function of heat-treatedyeast addition of fresh and 1 day stored frozen dough. Rest period: **a**) 0 min, **b**) 45 min, and **c**) 90 min. Bar = standard deviation, n = 14.

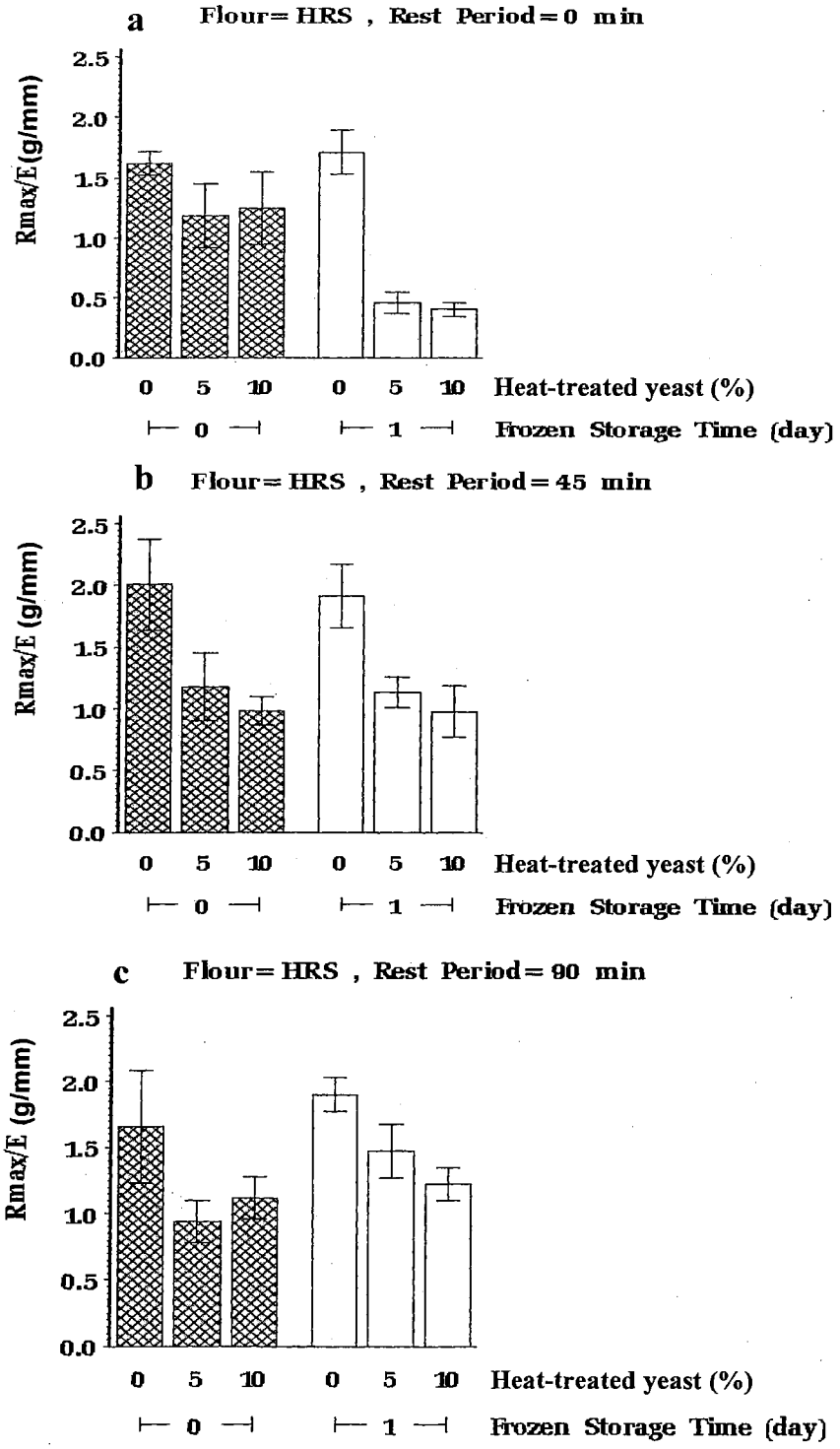


Fig. 25. Ratio of resistance to extension and extensibility (Rmax/E) of dough made with hard red spring (HRS) flour as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Rest period: **a**) 0 min, **b**) 45 min, and **c**) 90 min. Bar = standard deviation, n = 14.

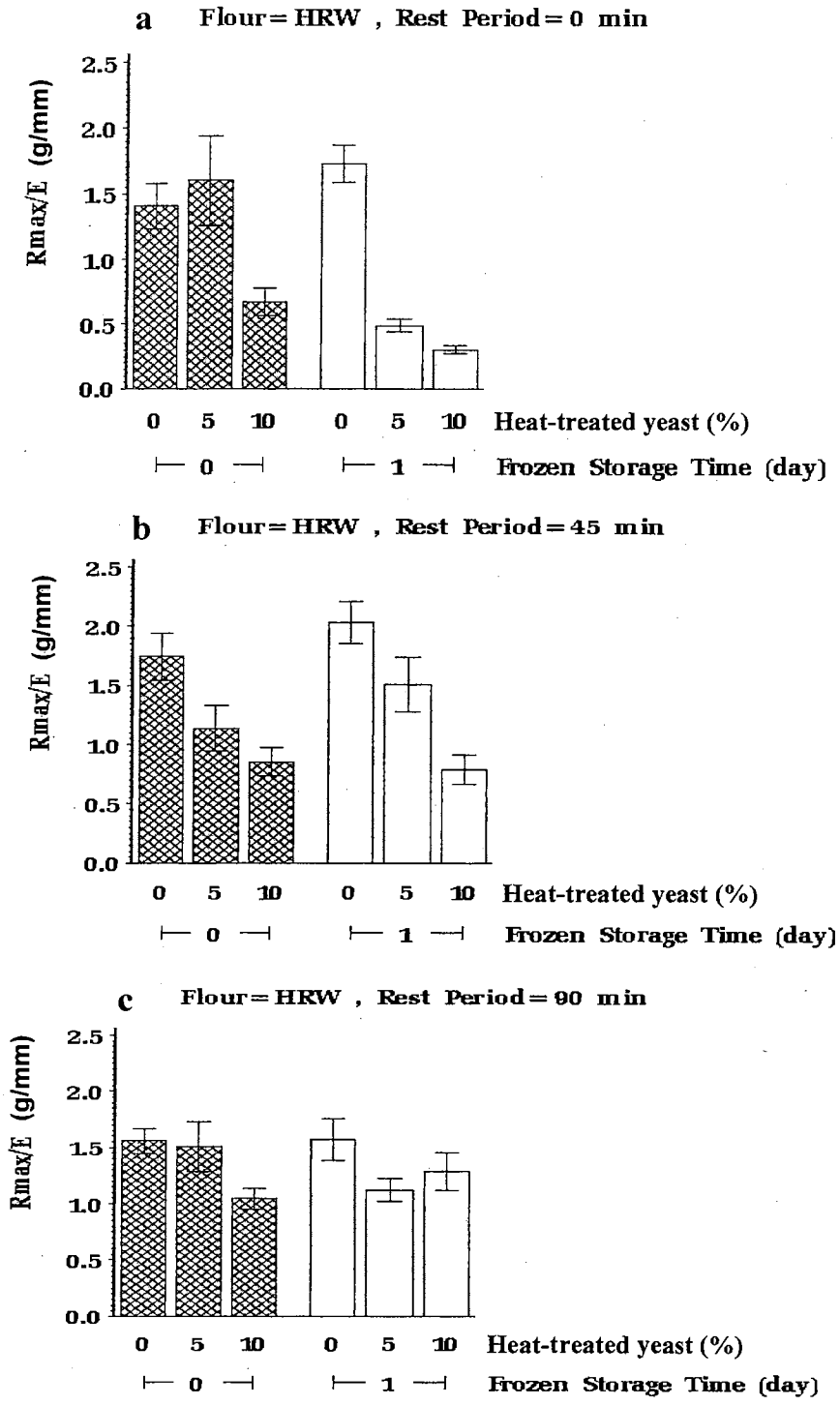


Fig. 26. Ratio of resistance to extension and extensibility (Rmax/E) of dough made with hard red winter (HRW) flour as a function of heat-treated yeast addition of fresh and 1 day stored frozen dough. Rest period: **a**) 0 min, **b**) 45 min, and **c**) 90 min. Bar = standard deviation, n = 14.

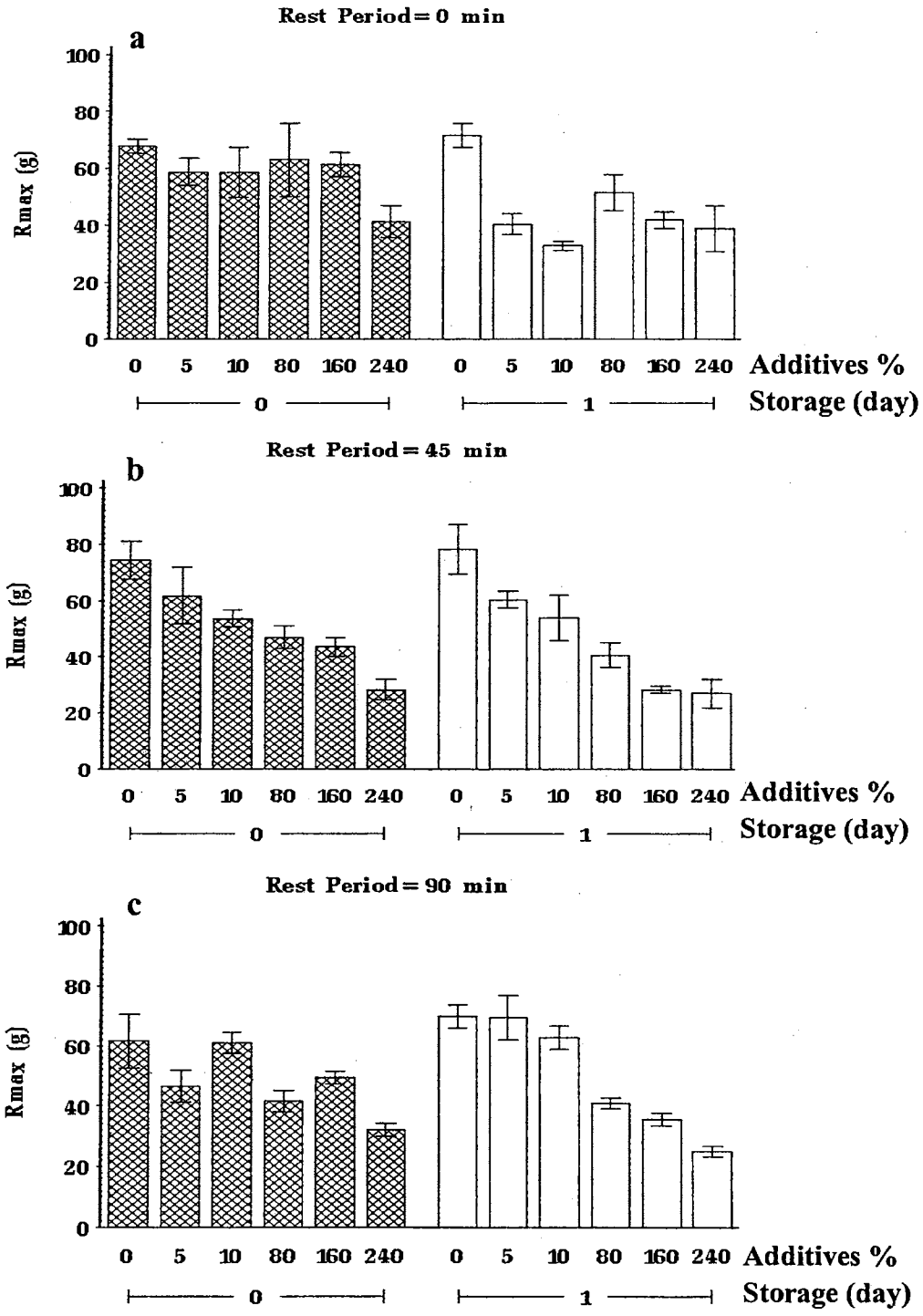


Fig. 27. Resistance to extension (Rmax) of dough made with hard red spring (HRS) flour as a function of heat-treated yeast (5 and 10%), GSH (80, 160, and 240 ppm) and frozen storage time (0 and 1 day). Rest period: **a**) 0 min, **b**) 45 min, and c) 90 min. Bar = standard deviation, n = 14.

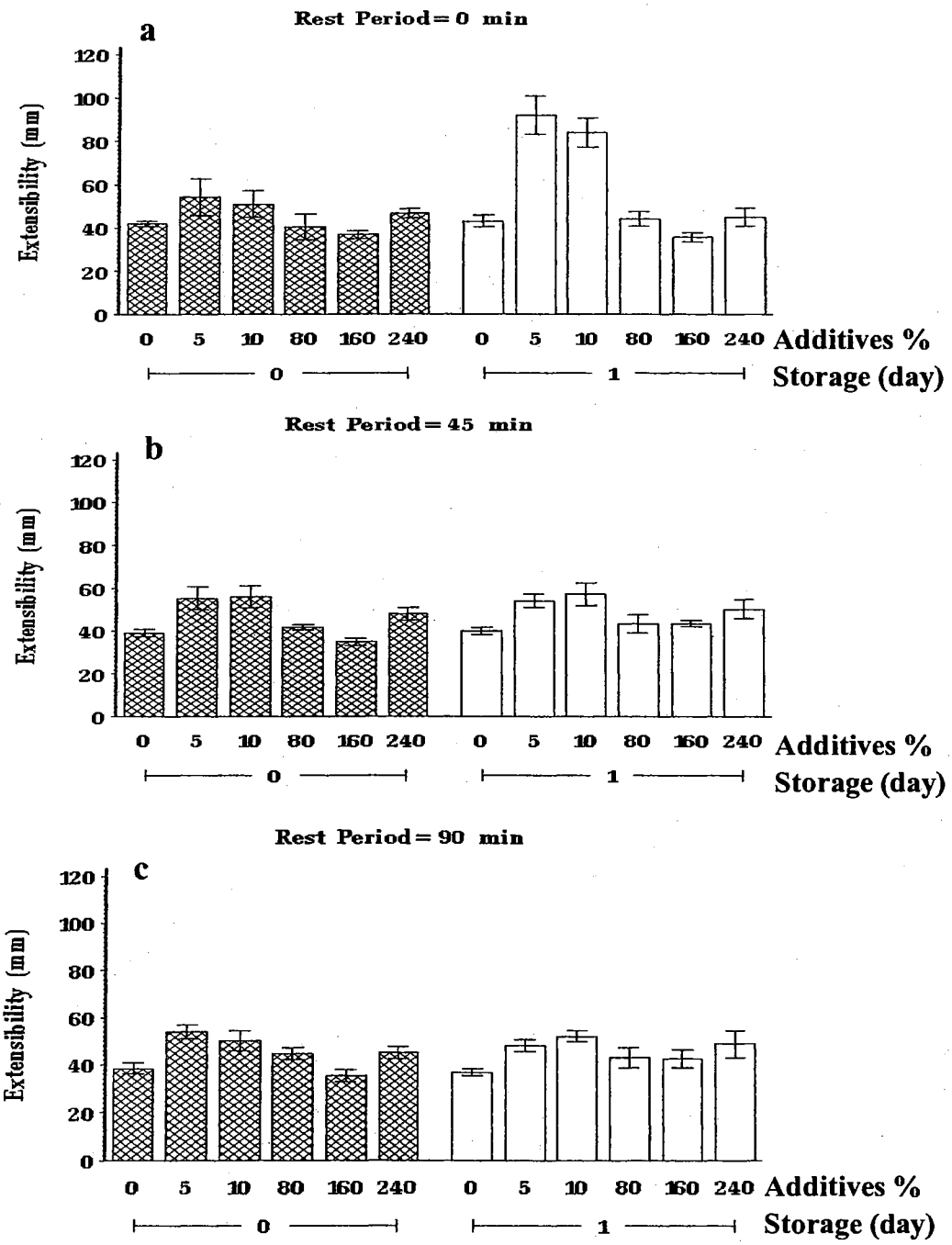


Fig. 28. Extensibility (E) of dough made with hard red spring (HRS) flour as a function of heat-treated yeast (5 and 10%), GSH (80, 160, and 240 ppm) and frozen storage time (0 and 1 day). Rest period: **a**) 0 min, **b**) 45 min, and **c**) 90 min. Bar = standard deviation, n = 14.

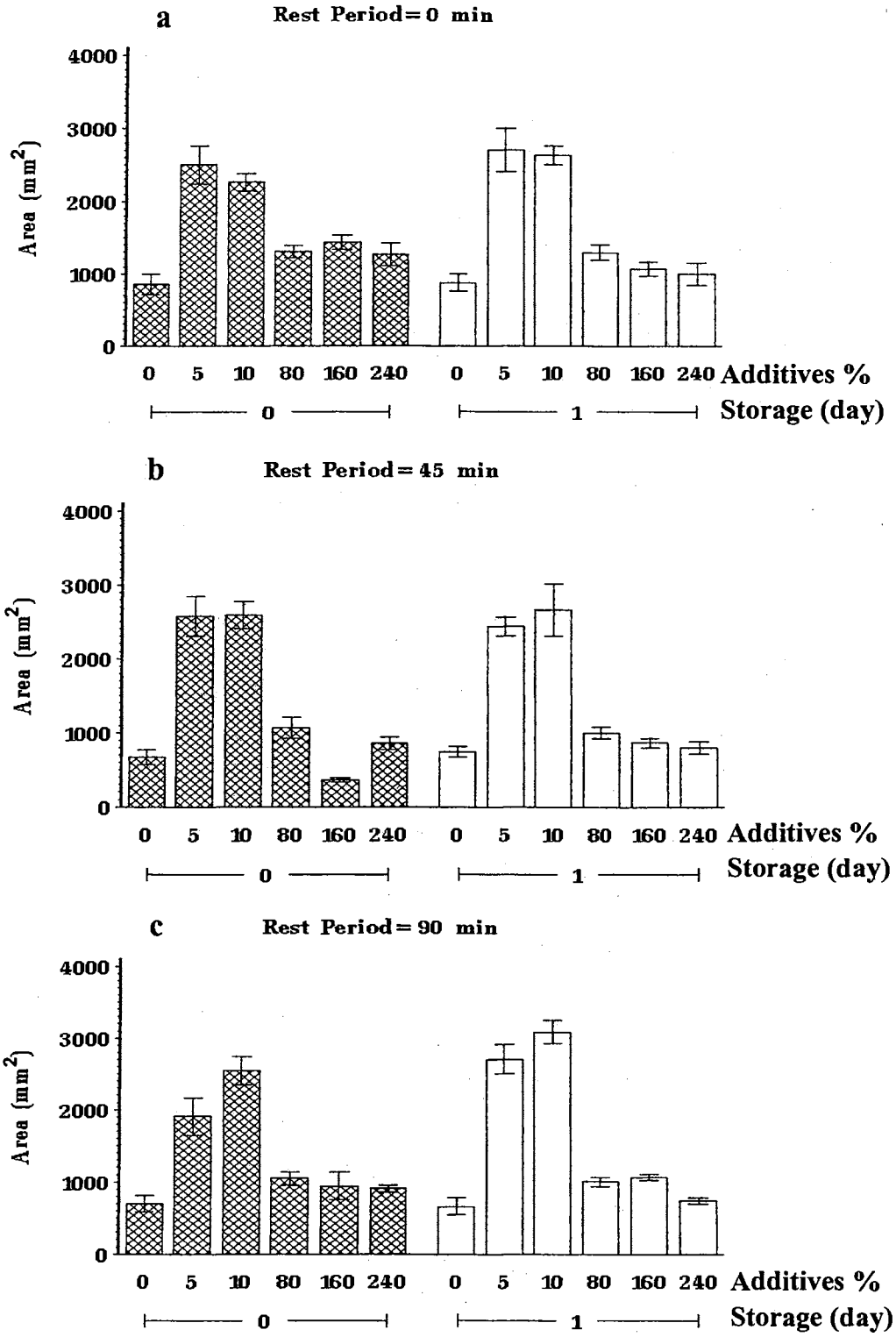


Fig. 29. Area (A) of dough as a function of frozen storage(0 and 1 day) and additives. Additives: heat-treated yeast (5 and 10%), GSH (80, 160, and 240 ppm) . The dough made from hard red spring (HRS) flour and had three rest periods: **a)** 0 min, **b)** 45 min, and **c)** 90 min. Bar = standard deviation, n = 14.

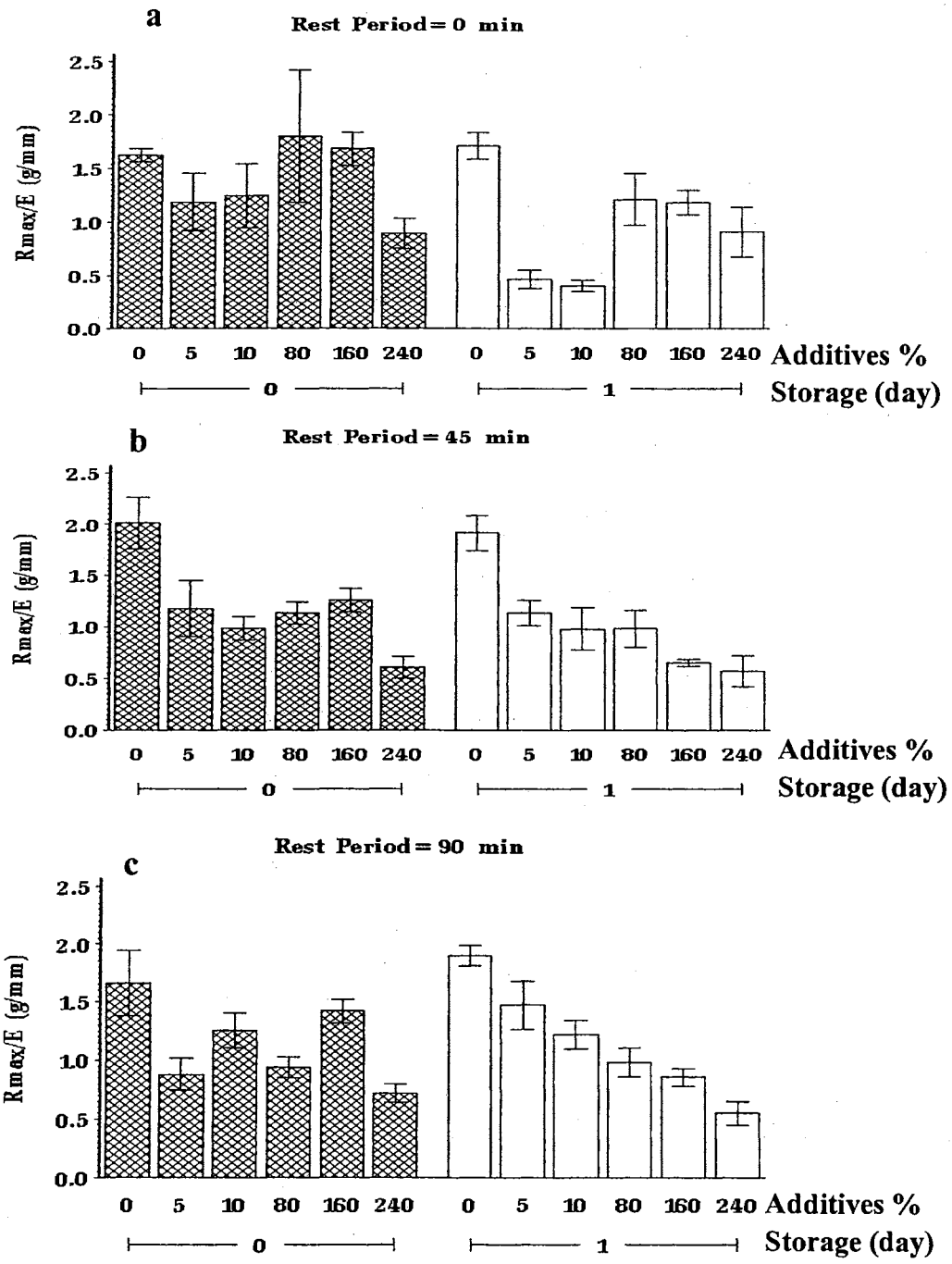


Fig. 30. Ratio of resistance to extension and extensibility (Rmax/E) of dough made with hard red spring (HRS) flour as a function of heat-treated yeast (5 and 10%), GSH (80, 160, and 240 ppm) and frozen storage time (0 and 1 day). Rest period: **a**) 0 min, **b**) 45 min, and **c**) 90 min. Bar = standard deviation, n = 14.

LITERATURE CITED

American Association of Cereal Chemists. 1995. Approved Methods of the AACC, 9 th ed. Method 08-01, final approval April 1961, revised October 1976 and October 1981, reviewed October 1994; Method 44-15A, approved October 1975, revised October 1981 and October 1994; Method 46-11A, approval October 1976, revised October 1982 and September 1985, reviewed October 1994; and Method 54-21, final approval November 1995. Method 74-09, final approval November 1995, reviewed October 1996. The Association: St. Paul, MN.

Anonymous. 1998. Precise color communication. Color control from perception to instrumentation. Page 20. Minolta Co., Ltd. Osaka, Japan.

Autio, K., and Sinda, E. 1992. Frozen doughs: Rheological changes and yeast viability. Cereal Chem. 69:409-413.

Bechtel, D. B., Pomeranz, Y., Francisco, A. 1978. Bread making studied by light and transmission electron microscopy. Cereal Chem. 55:392-401.

Berglund, P. T.; Shelton, D. R., and Freeman, T. P. 1991. Frozen bread dough ultrastructure as affected by duration of frozen storage and freeze-thaw cycles. Cereal Chem. 68:105-107.

Black, S., Harte, E. M., Hudson, B., and Wartofssky, L. 1960. A specific enzyme reduction of L (-) methione sulfoxide and related nonspecific reduction of disulfides. J. Biol. Chem. 235:2910-2916.

Cauvain, S. P. 1998. Dough retarding and freezing, in Technology of Breadmaking. Pages 149-179. S. P. Cauvain and L. S. Young ed.. Blackie Academic & Professional, London, UK.

Cauvain, S. P., and Young, L. S. 2000. The contribution of water during processing, baking, cooling and freezing. Pages 72-94 in: Bakery Food Manufacture and

Quality, S. P. Cauvain and L. S. Young ed. Blackie Academic & Professional, London, UK.

Chiang, B. Y., and Johnson, A. J. 1977. Measurement of total and gelatinized starch by glucoamulase and o-Toluidine reagent. Cereal Chem. 54:429-435.

Dubois, D. K., and Blockcolsky, D. 1986. Frozen bread dough. Effect of dough mixing and thawing methods. AIB Tech. Bull. Vol. VIII, No. 6. American Institute of Baking, Manhattan, KS.

El-Hady, E. A., El-Samahy, S. K., Seibel, W., and Bruemmer, J. M. 1996. Changes in gas production and retention in non-prefermented frozen wheat doughs. Cereal Chem. 73:472-477.

Ellis, G. P. 1959. The Millard reaction. Pages 63-134 in: Advances in Carbohydrate Chemistry, M. L. Wolfrom, ed. Academic Press: New York.

Fretzdorff, B., Bechtel, D. B., Pomeranz, Y. 1982. Freeze-fracture ultrastructure of wheat flour ingredients, dough and bread. Cereal Chem.59:113-120.

Gan, Z., Angold, R. E., Williams, M. R., Ellis, P. R., Vaughan, J. G., and Galliard, T. 1990. The microstructure and gas retention of bread dough. J. of Cereal Sci. 12:15-24.

Gélinas, P., Lagimoniere, M., and Dubord, C. 1993. Baker's yeast sampling and frozen dough stability. Cereal Chem. 70:219-225.

Gélinas, P., Lagimoniere, M., and Rodrigue, N. 1994. Performance of cream or compressed yeast in frozen and nonfrozen doughs. Cereal Chem. 71:183-186.

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

Havel, K., Pritss, K., and Wielgos, T. 1999. Quantitation of oxidized and reduced

glutathione in plasma by micellar electrokinetic capillary electrophoresis. J, Chromat.A. 853:215-223.

Havet, M., Mankai, M., and Le Bail, A. 2000. Influence of the freezing condition on the baking performances of French frozen dough. J. Food Eng. 45(3):139-145.

Hayman, D., Hoseney, R. C., and Faubion, J. M. 1998b. Effect of pressure (crust formation) on bread crumb grain development. Cereal Chem. 75:581-584.

Hsu, K. H., Hoseney, R. C., and Seib, P. A. 1979a. Frozen dough. I. Factors affecting stability of yeasted doughs. Cereal Chem. 56:419-424.

Hsu, K. H., Hoseney, R. C., and Seib, P. A. 1979b. Frozen dough. II. Effects of freezing and storing conditions on the stability of yeasted doughs. Cereal Chem. 56:424-426.

Inoue, Y., and Bushuk, W. 1991. Studies on frozen doughs. I. Effects of frozen storage and freeze-thaw cycles on baking and rheological properties. Cereal Chem. 68:627-631.

Inoue, Y., Sapirstein, H. D., Takayanagi, S., and Bushuk, W. 1994. Studies on frozen doughs. III. Some factors involved in dough weakening during frozen storage and thaw-freeze cycles. Cereal Chem. 71:118-121.

Inoue, Y., Sapirstein, H. D, and Bushuk, W. 1995. Studies on frozen doughs. IV. Effect of shortening systems on baking and rheological properties. Cereal Chem. 72:221-225, 228.

Ishida, N., Takano, H., Naito, S., Isobe, S., Uemura, K., Haishi, T., Kose, K., Koizumi, M., and Kano, H. 2001. Architecture of baked breads depicted by a magnetic resonance imaging. Magnetic Resonance Imaging. 19:867-874.

Javes, R. 1971. The ingredients and the processes. Effect on shelf life of frozen, unbaked yeast-leavened dough. Baker's Dig. 45(2):56-59.

Junge, R. C., Hoseney, R.C., and Varriano-Marston, E. 1981. Effect of surfactants on air incorporation in dough and the crumb grain of bread. Cereal Chem.58:338-342.

Kline, L., and Sugihara, T. F. 1968. Frozen bread doughs. I. Prepared by the straight dough method. Baker's Dig. 42(5):44-50.

Kenny, S., Wehrle, K., Dennehy, T, and Arendt E. K. 1999. Correlations between empirical and fundamental rheology measurements and baking performance of frozen bread dough. Cereal Chem. 76:421-425.

Kohama, Y., Teramoto, T., Kayamori, Y., Itoh, M., Mimura, T., Inada, A., and Nakanishi, T. 1990. Isolation and identification of hydroquinone in Baker's yeast. Agric. Biol. Chem. 54(11):3051-3052.

Kuninori, T., Yagi, M., and Matsumoto, H. 1968. Glutathione leached from yeast cells. Hakko Kogaku Zasshi. 46:196-201.

Laaksonen, T. J., and Roos, Y. H. 2000. Thermal, dynamic-mechanical and dielectric analysis of phase and state transitions of frozen wheat doughs. J. Cereal Sci. 32(3):281-292.

Le Bail, A., Pasco, M., Meric, L., and Cahagnier, B. 1996b. Influence of freezing rate on yeast activity in frozen bread dough. In: The 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., and Quilin, E. 1999. Influence of storage conditions on frozen French bread dough. J. Food Eng. 39:289-291.

Levine, H., and Slade, L. 1990. Influences of the glassy and rubbery states. Pages 157-331 in: Dough Rheology and Baked Product Texture: Theory and practice. H. Faridi, and J. M. Faubion, eds. Van Nostrand Reinhold/AVI: New York.

Lorenz, K. 1974. Frozen dough. Bakers' Dig. 48(2):14-15, 18-19, 22, 30.

Maillard, L. C. 1912. Action of amino acids on sugars. Formation of melanoidins in a methodical way. Compt. Rend. 154:66-68.

Marston, P. E. 1978. Frozen dough for breadmaking. Bakers' Dig. 52(5):18-20, 37.

Mazur, P., and Schmidt, J. J. 1968. Interactions of cooling velocity, temperature, and warming velocity on the survival of frozen and thawed yeast. Cryobiology 5:1-17.

Meric, L., Lambert-Guilois, S., Neyreneuf, O., and Richard-Molard, D. 1997. Cryoresistance of Baker's yeast Saccharomyces cerevisiae in frozen dough: contribution of cellular trehalose. Cereal Chem. 72:609-615.

Merritt, P. P. 1960. The effect of preparation on the stability of frozen unbaked, yeast leavened doughs. Baker's Dig. 34(4):57-61.

Moss, R. 1974. Dough microstructure as affected by the addition of cysteine, potassium bromate, and ascorbic acid. Cereal Science Today. 19(12):557-561.

Neyreneuf, O., and van der Plaat, J. B. 1991. Preparation of frozen French bread dough with improved stability. Cereal Chem.68:60-66.

Noll, B. 2000. Functional application of wheat fibre in frozen dough products. European Food & Drink Review, Spring, 53-56.

Nonami, Y., Saito, M., Shikano, K., Modeki, F., and Suzuki, A. 1984. Effects of chicken egg yolk on the ultrastructure and loaf volume of white bread. Nippon Shokuhin Hogyo Gakkaishi. 31:570-576.

Ponte, J. G., Glass, R. L, and Geddes, W. F. 1960. Studies on the behavior of active dry yeast in bread making. Cereal Chem. 37:263-279.

Räsänen, J., Harkonen, H., and Autio, K. 1995. Freeze-thaw stability of prefermented frozen lean wheat doughs: effect of flour quality and fermentation time. Cereal Chem. 72:637-42.

Räsänen, J., Laurikainen, T., and Autio, K. 1997a. Fermentation stability and pore size distribution of frozen prefermented lean wheat doughs. Cereal Chem. 74:56-62.

Räsänen, J., Blanshard, J. M. V., Siitari, K., M., and Autio, K. 1997b. Water distribution in frozen lean wheat doughs. Cereal Chem. 74:806-13.

Reed, G., and Nagodawithana, T. W. 1991. Use of yeast in baking. Pages315-368 in: Yeast Technology, 2nd eds. Van Nostrand Reinhold/AVI: New York.

Reed, G., and Peppler, H. J. 1973. Use of yeast in baking. Pages 103-164 in: Yeast Technology. AVI Publishing Co. Westport, Conn.

Reid, D. S. 1990. Optimizing the quality of frozen foods. Food Technology. 4478-82.

Rouille, J., Le-Bail, A., and Corcoux, P. 2000. Influence of formulation and mixing conditions on breadmaking qualities of French frozen dough. J. of Food Engineering 43(4):197-203.

Sapirstein, H. D., Roller, R., and Bushuk, W. 1994. Instrumental measurement of bread crumb grain by digital image analysis. Cereal Chem. 71:383-391.

Suchy, J., Lukow, O. M., and Ingelin, M. E. 2000. Dough microextensibility method using a 2-g mixograph and a texture analyzer. Cereal Chem. 77:39-43.

Van Horn, D. 1989. Cream yeast. Pages 144-153 in: Proc.Ann. Mtg. Am. Soc. Bakery Eng., 65th. The Society: Chicago.

Van Uden, N. 1971. Kinetics and energetics of yeast growth. In The Yeasts, Vol. 2, A. H. Rose and J. S. Harrison, ed. Academic Press, NY.

Varriano-Marston, E., and Hsu, K. H, and Mahdi, J. 1980. Rheological and structural changes in frozen dough. Bakers' Dig. 54(1):32-34, 41.

Verbruggen, I. M., Veraverbeke, W. S., and Delcour, J. A. 2001. Significance of LMW-GS and HMW-GS for dough extensibility: 'Addition' versus 'Incorporation'

protocols. J. of Cereal Science.33:253-260.

Whistler, R. L. and Daniel, J. R. 1985. Carbohydrates. Pages 69-138 in: Food Chemistry, O. R. Fennema, ed. Marcel Dekker, Inc.: New York.

Wolt, M. J., and D' Appolonia, B. L. 1984a. Factors involved in the stability of frozen dough. I. The influence of yeast reducing compounds on frozen-dough stability. Cereal Chem. 61:209-212.

Wolt, M. J., and D' Appolonia, B. L. 1984b. Factors involved in the stability of frozen dough. II. The effects of yeast type, flour type, and dough additives on frozen-dough stability. Cereal Chem. 61:213-221.

CHAPTER VI

SUMMARY AND FUTURE RESEARCH

Summary

Hard red spring (HRS) and hard red winter (HRW) flours used for making bread sticks from frozen dough showed different dough rheological properties and baking quality. Bread sticks made from fresh dough with HRS flour had better baking quality in terms of volume, appearance and rheological properties than HRW flour. Overall, baking properties of frozen dough made with HRS flour subjected to a short frozen storage (1 day frozen) were better than those from HRW. However, for periods of frozen storage from 1 up to 12 weeks, there was no significant difference of baking quality of HRS and HRW flours. The baking quality and rheological properties showed differences in numerical values due to genetic differences in the molecular structure of gluten proteins, but overall, similar pattern of deterioration was observed in both flours. In summary, baking quality and rheological properties showed significant changes during the initial frozen storage (1 day) and remained similar up to 4 weeks of frozen storage. As the frozen storage continues up to 12 weeks, more changes are evident with a different rate of modification.

This study confirmed that the traditional flour analysis (protein, ash, and Farinograph analysis) do not predict the baking quality of frozen dough. Initial freezing and frozen storage time caused deterioration effects of frozen dough shown as a reduction of bread volume, crust and crumb appearance, and firmness of the bread. The changes in rheological properties of the dough as a function of freezing and

frozen were observed by the reduction of total gas volume, gas retention, gas produced and an increase of dough permeability using a rheofermentometer. An increase in extensibility and a reduction of the ratio of resistance to extension and extensibility ratio of frozen dough using micro-extensibility indicates a deterioration of the dough during the frozen storage. The frozen dough also showed an increase of the elastic (storage modulus G') and viscous (loss modulus G") behavior as evaluated in an oscillation test as the frozen storage time increased.

The rheofermentometer parameters provided information related to viable yeast, yeast activity and gas retention of the frozen dough. Freezing and frozen storage reduced yeast activity with less CO_2 produced resulting in a reduction of total gas volume. The reduction of gas retention of dough was due to the changes in molecular structure caused by reduced glutathione (GSH) from yeast and formation of ice crystals. Freezing and frozen storage caused damage to yeast cells and leached out GSH into the frozen dough. Reaction of GSH as a reductant in gluten network resulted in disulfide-sulphydryl interchange and depolymerization of gluten protein, thereby changing elasticity and extensibility of the frozen dough. The formation of ice crystals in the gluten sheets during freezing and prolonged frozen storage ruptured gluten network and separated starch granules from the gluten sheet.

The performance of baking quality and rheological properties with the addition of methylcellulose (MC), commercial dough conditioner (CDC) and the combination of CDC+MC was investigated. The results showed that MC and CDC+MC improved bread volume and maintained crumb softness over 12 weeks of frozen storage for the HRS flour dough. However, MC and CDC+MC could improve HRW flour baking

quality of frozen dough only for short periods of frozen storage (up to 1 week). Compared to the control, when CDC+MC was added to HRW dough, higher values of total gas volume, gas retained and maximum dough height of frozen dough were observed up to 12 weeks of storage, except at 8 weeks. While in HRS flour, CDC+MC produced higher values of these parameters up to 3 weeks. Addition of MC in frozen dough made from HRS flour reduced the gas permeability (Tx) of the dough. Thus, MC could protect yeast and gluten network from the damaging effects of freezing and frozen storage. The gas permeability (Tx) of frozen dough containing MC was shorter compared to the control. Baking quality and rheofermentometer parameters had significant correlations ($P < 0.001$), r range |0.62 to 0.89|, which indicated that some rheofermentometer parameters could be used to predict frozen dough stability.

Three levels of reduced GSH (80, 160 and 240 ppm) were used to study the effects of baking quality and rheological properties of frozen dough. A modified oscillation tests with 3 different relaxation times (1, 13 and 26 min) of the dough after loading the sample on the rheometer were investigated. Long relaxation times (13 and 26 min) showed significant changes in the elastic and viscous behavior (G', G", G*, and η^*) due to the addition of GSH and the effects of freezing and storage time. The oscillation tests of the dough with relaxation time 26 min showed significant correlation with the micro-extensibility area ($P < 0.001$, r range 0.62 to 0.69). The analysis showed interaction between frozen storage time, GSH and relaxation time (using rheometer) or rest time (using micro-extensibility) ($P < 0.01$). GSH levels of 160 and 240 ppm in frozen dough lowered G', G", G*, and η^* compared to 80 ppm

GSH and control. In contrast, freezing and frozen storage showed an increase of G', G", G*, and η^* as the frozen storage time increased. GSH caused the frozen dough to have more liquid-like behavior, while freezing and frozen storage made the dough more rigid with more solid-like behavior.

Phase separation analysis using ultracentrifugation supported the rheological properties measured with the oscillation test. There were no interactions between the amount of solid phase and water in solid phase of the dough with the addition of GSH and frozen storage time. The addition of GSH significantly reduced the amount of solid phase ($P < 0.05$) in fresh and frozen dough. GSH had a depolymerizing effect on the gluten fibrils, causing a reduction of the solid phase and an increase in the liquid phase of the dough as corroborated by a more liquid-like material of the dough as GSH increased.

Frozen storage time was the main factor that showed a significant reduction of the amount of water in the solid phase of the dough. Freezing and increasing frozen storage time appeared to form a structure with more solid and liquid-like behavior frozen dough (increased of both G' and G"). Only one study is found in the literature in which the determination of the elastic (G') and viscous (G") behavior of a yeasted preproofed dough was reported (Räsänen et al 1997). The results from the study reported here do not agree with the trends of the elastic and viscous behavior of deceased G" and G' by Räsänen et al (1997). Previous reports using non-yeasted dough or unproofed yeasted dough showed a reduction of G' and G". The reduction of percentage of gelatinized starch from bread sticks made from frozen dough at 8 week frozen storage supported the evidence of a reduction of water in the solid phase

as the frozen storage time increases. Scanning electron micrographs were able to show surface structural differences of the gluten matrix and starch caused by the addition of GSH, initial freezing, and frozen storage time.

The parameters obtained with the micro-extensibility test of frozen dough showed interactions with frozen storage time and dough rest periods ($P<0.001$). Maximum resistant (Rmax), and ratio of Rmax and extensibility (E) of frozen dough reduced while extensibility increased as the concentration of GSH and frozen storage time increased. The viscoelastic behavior parameters of dough obtained in the oscillation and micro-extensibility tests and phase separation had significant correlations. Significant linear correlations were also observed with specific volume of the bread sticks.

Prolonged frozen storage time (> 4 months) caused large brown areas and blisters on the crust of bread sticks. This study demonstrated that the GSH and dead yeast had different effects in the crust. The frozen dough with addition of GSH produced crust with small brown spots and their number increased with higher level of GSH. The frozen dough with the addition of dead yeast produced crust with pale background and brown blister covering about 30% of crust area of fresh and 1 day frozen dough. This suggests a more complex phenomenon occurring in the crust when dead yeast is added compared to the reducing effect of GSH alone. A combination of GSH with residual enzymatic activity could be contributing to the observations with the addition of dead yeast. The rheological properties of the dough with the additional of GSH and dead yeast showed significant correlation with baking quality.

Future Study

More studies of the fundamental rheological properties of yeast-prefermented dough are needed to fully describe the kinetics of freezing, yeast damage and the improvement of frozen dough products with additives. The challenges of yeasted prefermented dough are the complex and transient properties of dough with time due to the effects of yeast activity on the relaxation of the dough (Surrnacka-Szczesniak 1988, Spies 1989, Bloksma 1990a,b, Räsänen et al 1997. Only one report using yeast-fermented dough is found in the literature (Räsänen et al 1997) and its results are different from the findings of this study. Thus, future studies should clarify the differences in reports. Among the recommendations to continue this work include:

1. Investigate the baking performance and changes in rheological properties of different frozen dough with non-yeasted, yeasted-unproofed and yeasted-preproofed using dynamic oscillation test. Having results of the same laboratory will enable to compare side by side the rheological properties with more detail as well as the description of possible correlations with specific baking parameters.
2. Expand the study of the composition of liquid and solid fractions separated by ultrafiltration. Molecular differences in the gluten structure should identify any shift in the molecular ratio of polymeric to nonpolymeric proteins. Glutenins and gliadins can be extracted, quantitated and follow any possible changes in structure.

3. Devise a methodology to quantitate fine modifications of starch and gluten structures and perhaps their interactions.
4. Study the influence of additional starch and gluten in the rheological properties of frozen dough.
5. Investigate the effects of sugars (glucose, fructose, maltose, and sucrose), wheat flour and yeast enzyme, moisture and heat including extracted GSH and dead yeast cells on browning reaction related to the blisters observed on the crust of bread sticks. By including electron micrographs or other visual methods, perhaps confocal microscopy to describe changes in the microstructure of frozen dough, changes can be followed chemically and structurally.
6. Explore a quantitative methodology to evaluate the breadsticks beyond the baking scores; perhaps a digital imaging technique to describe the crumb and crust.
7. Explore more the use of the Rheofermentometer parameters by selecting those that showed higher correlation coefficients.
8. Investigate the residual enzyme activity – including proteases, invertases, and thionic acid reducing enzyme in dead yeast extracts.

LITERATURE CITED

Bloksma, A. H. 1990a. Rheology of bread-making process. Cereal Foods World 35:228-236.

Bloksma, A. H. 1990b. Dough structure, dough rheology and baking quality. Cereal Foods World 35:237-244.

Räsänen, J., Blanshard, J. M. V., Siitari-Kauppi, M., and Autio, K. 1997. Water distribution in frozen lean wheat doughs. Cereal Chem. 74:806-813.

Spies, R. 1989. Application of rheology in the bread industry. In: Dough Rheology and Baked Product Texture. H. Faridi and J. M. Faubion, eds. Van Nostrand Rein hold. New York.

Surrnacka-Szczesniak, A. 1988. Applying rheology to dough and baked goods. Cereal Foods World 33:841.

Wolt, M. J., and D' Appolonia, B. L. 1984. Factors involved in the stability of frozen dough. I. The influence of yeast reducing compounds on frozen-dough stability. Cereal Chem. 61:209-212.

APPENDIX A

FARINOGRAPH RESULTS OF HRS FLOUR

Brabender® Farinograph

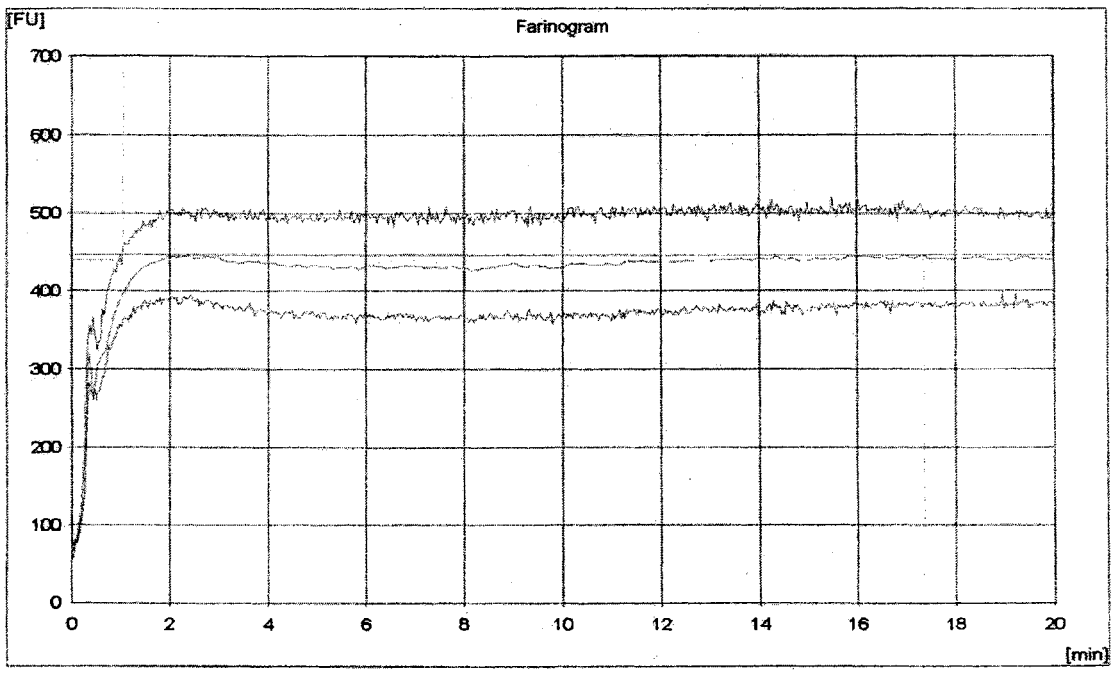
Sample: Heinz
Date: 9/30/98 9:15:46 AM

Method: AACC
Operator: Jan/Renee'

Mixer; 300 g
Consistency 447 FU with waterabsorption 59.8 %

Moisture content: 14.0 %

Waterabsorption (corrected for 500 FU):	58.5 %
Waterabsorption (Corrected to 14.0 %):	58.5 %
Development time:	17.3 min
Stability:	18.9 min
Toleranceindex (MTI):	447 FU
Time to breakdown:	1200.0 min
Farinograph quality number:	200
Remarks:	HWW sample with water circulator set at 30 C, not colder - secc



APPENDIX B

FARINOGRAPH RESULTS OF HRW FLOUR

Brabender® Farinograph

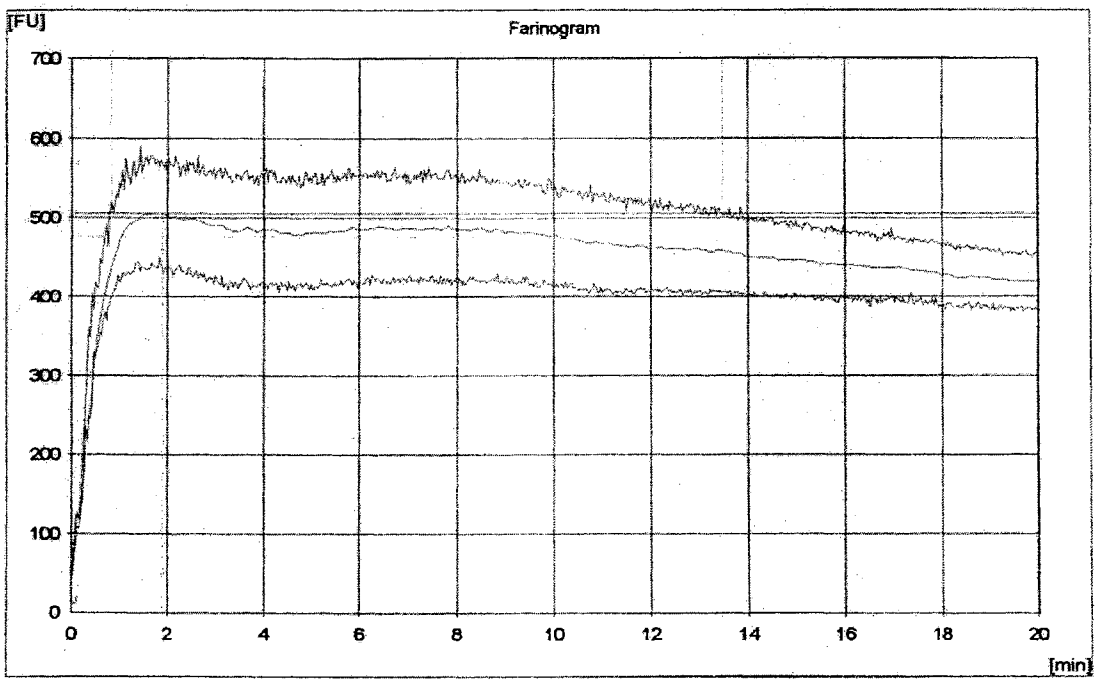
Sample: Shawnee High Gluten
Date: 11/6/98 3:24:17 PM

Method: AACC
Operator: Jan/Pong

Mixer: 300 g
Consistency 506 FU with waterabsorption 59.4 %

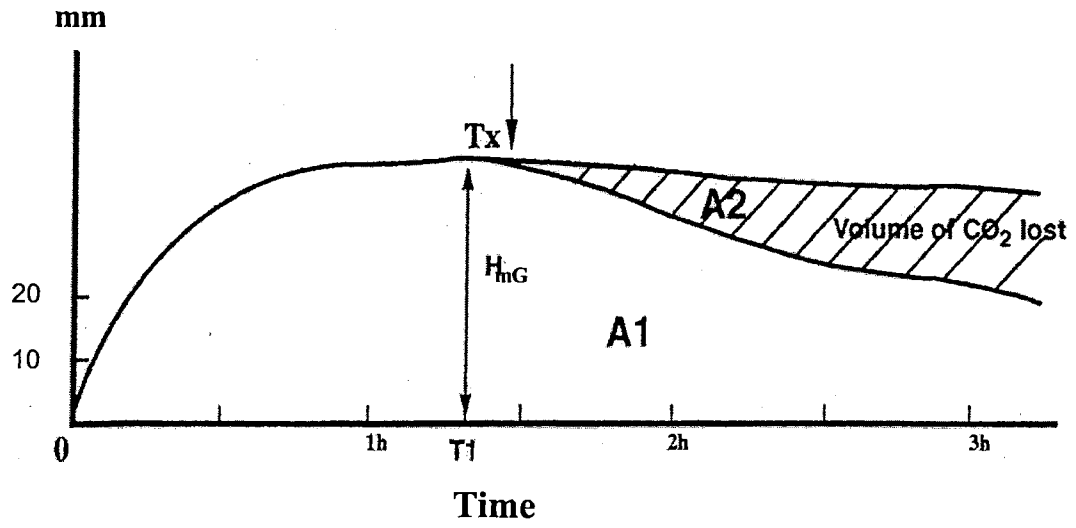
Moisture content: 13.0 %

Waterabsorption (corrected for 500 FU):	59.6 %
Waterabsorption (Corrected to 14.0 %):	58.4 %
Development time:	1.9 min
Stability:	12.7 min
Toleranceindex (MTI):	18 FU
Time to breakdown:	606.0 min
Farinograph quality number:	101
Remarks:	Shawnee High Gluten flour, received 11/04/98



APPENDIX C

THE CALCULATION OF PARAMETERS IN GAS PRODUCTION PROFILE



A1 = Retention volume (V_R).

A2 = Volume of CO2 lost (mL).

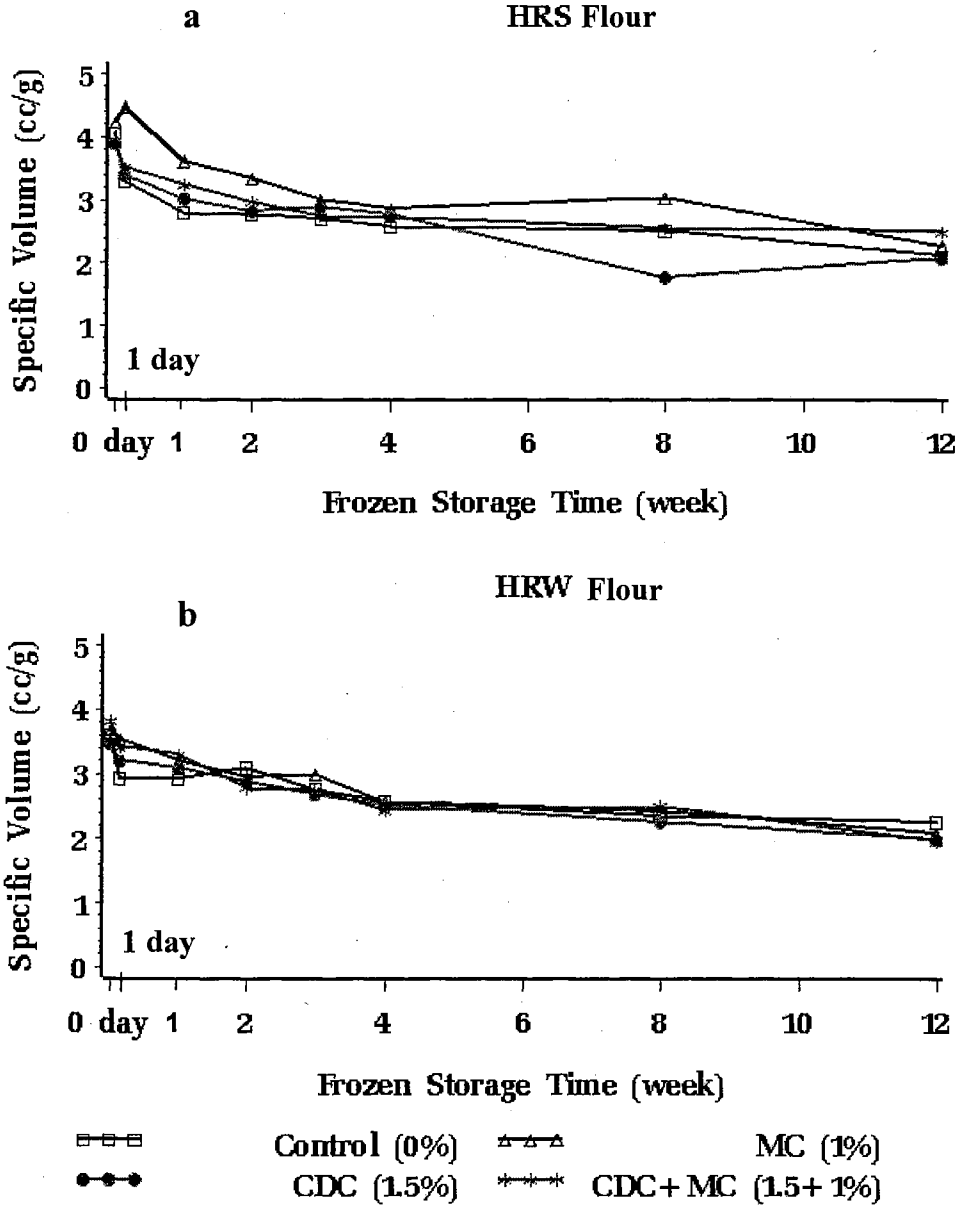
A1 + A2 = Total volume (mL).

Tx = Time at CO2 start to release (hr).

T1 = Time at maximum dough height (hr).

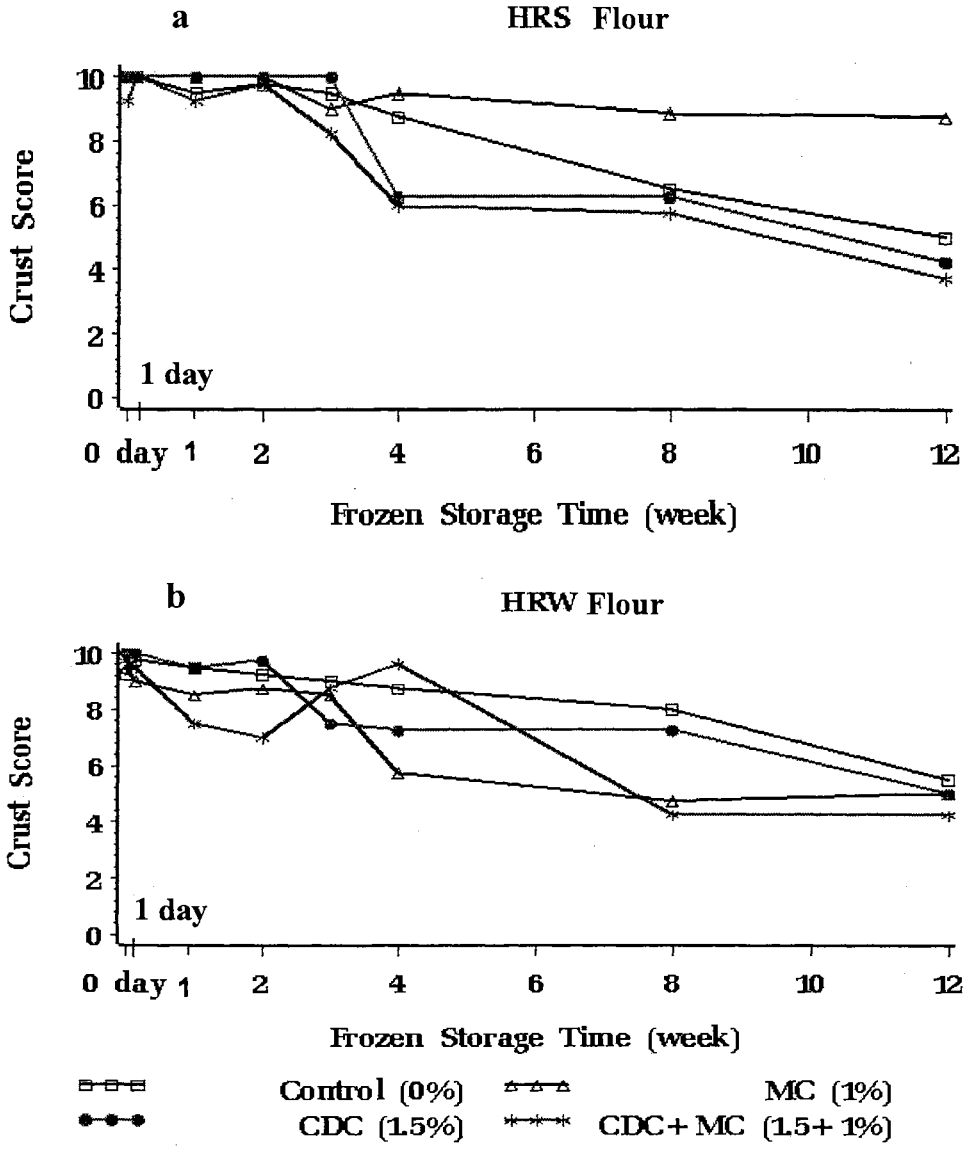
H_{mG} = Maximum height of dough.

APPENDIX D
SPECIFIC VOLUME AS A FUNCTION OF FROZEN STORAGE TIME OF BREAD STICKS MADE FROM HARD RED SPRING (HRS) FLOUR (a) AND HARD RED WINTER (HRW) FLOUR (b).



APPENDIX E

CRUST SCORE AS A FUNCTION OF FROZEN STORAGE TIME OF BREAD STICKS MADE FROM HARD RED SPRING (HRS) FLOUR (a) AND HARD RED WINTER (HRW) FLOUR (b).



APPENDIX F
CRUMB FIRMNESS OF BREAD STICKS MADE FROM HARD RED SPRING (HRS) AND HARD RED WINTER (HRW) FLOUR[a]

	Frozen Storage Time	Crumb Firmness (g)	
		HRS	HRW
Control	**0 day**	124 ± 28	115 ± 19
	1 day	143 ± 21	109 ± 17
	1 week	144 ± 22	121 ± 30
	2 weeks	152 ± 14	148 ± 28
	3 weeks	138 ± 23	163 ± 24
	4 weeks	217 ± 43	176 ± 54
	8 weeks	242 ± 65	357 ± 107
	12 weeks	476 ± 99	391 ± 126
CDC[b], 1.5%	**0 day**	106 ± 22	98 ± 12
	1 day	107 ± 18	106 ± 13
	1 week	111 ± 9	111 ± 14
	2 weeks	128 ± 20	133 ± 18
	3 weeks	137 ± 21	186 ± 33
	4 weeks	161 ± 32	202 ± 34
	8 weeks	318 ± 74	302 ± 45
	12 weeks	466 ± 97	435 ± 54
CDC+MC[b], 1.5+1%	**0 day**	120 ± 21	120 ± 20
	1 day	121 ± 22	124 ± 22
	1 week	119 ± 16	134 ± 27
	2 weeks	134 ± 25	168 ± 25
	3 weeks	167 ± 45	172 ± 31
	4 weeks	235 ± 66	210 ± 29
	8 weeks	314 ± 74	222 ± 59
	12 weeks	457 ± 110	351 ± 77
MC[b], 1%	**0 day**	105 ± 21	106 ± 22
	1 day	95 ± 13	111 ± 19
	1 week	101 ± 18	113 ± 12
	2 weeks	114 ± 15	179 ± 24
	3 weeks	133 ± 18	153 ± 37
	4 weeks	120 ± 18	196 ± 43
	8 weeks	226 ± 111	229 ± 69
	12 weeks	319 ± 52	225 ± 37

[a] Mean ± standard deviation, each value is a mean from 24 measurements. Analysis was done in two independent batches with two subsamples per batch.

[b] CDC = Comercial Dough Conditioner, MC = Methylcellulose.

APPENDIX G
MAXIMUM HEIGHT OF GAS PRODUCTION AND DOUGH DEVELOPMENT USING RHEOFERMENTOMETER FOR DOUGH SAMPLES MADE FROM HARD RED SPRING (HRS) AND HARD RED WINTER (HRW) FLOUR[a]

		HRS		HRW	
	Storage Time	H_{mG}[b] (mm)	H_{mD}[c] (mm)	H_{mG} (mm)	H_{mD} (mm)
Control	0 day	35.0 ± 2.1	56.1 ± 12.2	26.0 ± 0.5	52.1 ± 0.8
	1 day	29.4 ± 1.8	44.5 ± 6.1	22.4 ± 0.8	42.3 ± 2.7
	1 week	29.8 ± 0.3	45.9 ± 1.4	23.3 ± 0.2	41.4 ± 4.4
	2 weeks	31.0 ± 1.1	46.4 ± 1.9	23.8 ± 0.3	36.8 ± 1.1
	3 weeks	30.4 ± 0.5	42.1 ± 2.5	22.7 ± 0.5	36.1 ± 3.2
	4 weeks	28.1 ± 2.7	38.3 ± 1.6	22.1 ± 1.1	34.5 ± 0.6
	8 weeks	17.7 ± 5.9	21.2 ± 0.0	18.4 ± 0.9	22.2 ± 1.5
	12 weeks	8.3 ± 3.8	4.2 ± 5.9	5.5 ± 0.3	0.0 ± 0.3
CDC[d], 1.5%	0 day	34.4 ± 1.1	57.9 ± 1.3	28.2 ± 1.6	43.5 ± 0.3
	1 day	32.4 ± 1.3	53.1 ± 3.1	24.0 ± 0.1	34.7 ± 0.7
	1 week	28.7 ± 0.4	47.5 ± 6.8	24.9 ± 2.0	36.1 ± 4.2
	2 weeks	28.9 ± 1.9	50.0 ± 2.8	25.1 ± 1.8	34.5 ± 6.0
	3 weeks	25.6 ± 0.1	40.5 ± 5.7	22.1 ± 0.7	33.0 ± 3.0
	4 weeks	24.1 ± 1.2	37.8 ± 4.3	19.8 ± 1.4	30.4 ± 1.4
	8 weeks	12.4 ± 3.8	15.0 ± 5.8	13.9 ± 0.6	14.3 ± 1.8
	12 weeks	6.9 ± 0.1	0.0 ± 0.0	8.9 ± 2.8	4.6 ± 6.5
CDC+MC[d], 1.5+1%	0 day	31.9 ± 0.4	69.9 ± 2.1	27.0 ± 1.1	55.4 ± 10.3
	1 day	31.0 ± 1.1	59.4 ± 4.9	24.4 ± 1.7	52.9 ± 3.9
	1 week	31.4 ± 0.8	57.0 ± 0.3	24.3 ± 0.3	47.6 ± 9.5
	2 weeks	32.6 ± 0.8	58.0 ± 1.9	23.5 ± 0.6	49.7 ± 1.3
	3 weeks	28.6 ± 4.2	49.1 ± 2.2	25.0 ± 0.9	56.0 ± 0.3
	4 weeks	20.1 ± 3.7	35.4 ± 5.6	23.5 ± 1.0	47.7 ± 0.3
	8 weeks	15.0 ± 7.5	22.7 ± 12.6	15.3 ± 0.5	17.2 ± 0.4
	12 weeks	10.1 ± 3.9	0.0 ± 0.0	13.9 ± 2.3	15.9 ± 4.9
MC[d], 1%	0 day	30.2 ± 4.2	62.3 ± 4.9	23.8 ± 3.8	60.8 ± 12.4
	1 day	28.5 ± 2.4	32.4 ± 2.5	21.8 ± 1.1	41.9 ± 4.7
	1 week	29.2 ± 0.5	43.9 ± 0.0	23.3 ± 1.4	42.6 ± 3.6
	2 weeks	28.7 ± 2.5	36.8 ± 9.2	20.7 ± 1.9	41.2 ± 3.3
	3 weeks	27.0 ± 1.5	34.8 ± 4.1	22.2 ± 1.4	40.1 ± 6.5
	4 weeks	27.3 ± 0.5	29.6 ± 2.5	19.6 ± 2.2	35.9 ± 6.5
	8 weeks	16.3 ± 4.7	16.8 ± 7.4	10.8 ± 0.8	9.1 ± 0.4
	12 weeks	9.2 ± 2.5	4.3 ± 6.0	6.7 ± 2.5	0.0 ± 0.5

[a] Mean ± standard deviation, each value is a mean from 4 measurements. Analysis was done in two independent batches with two subsamples per batch.

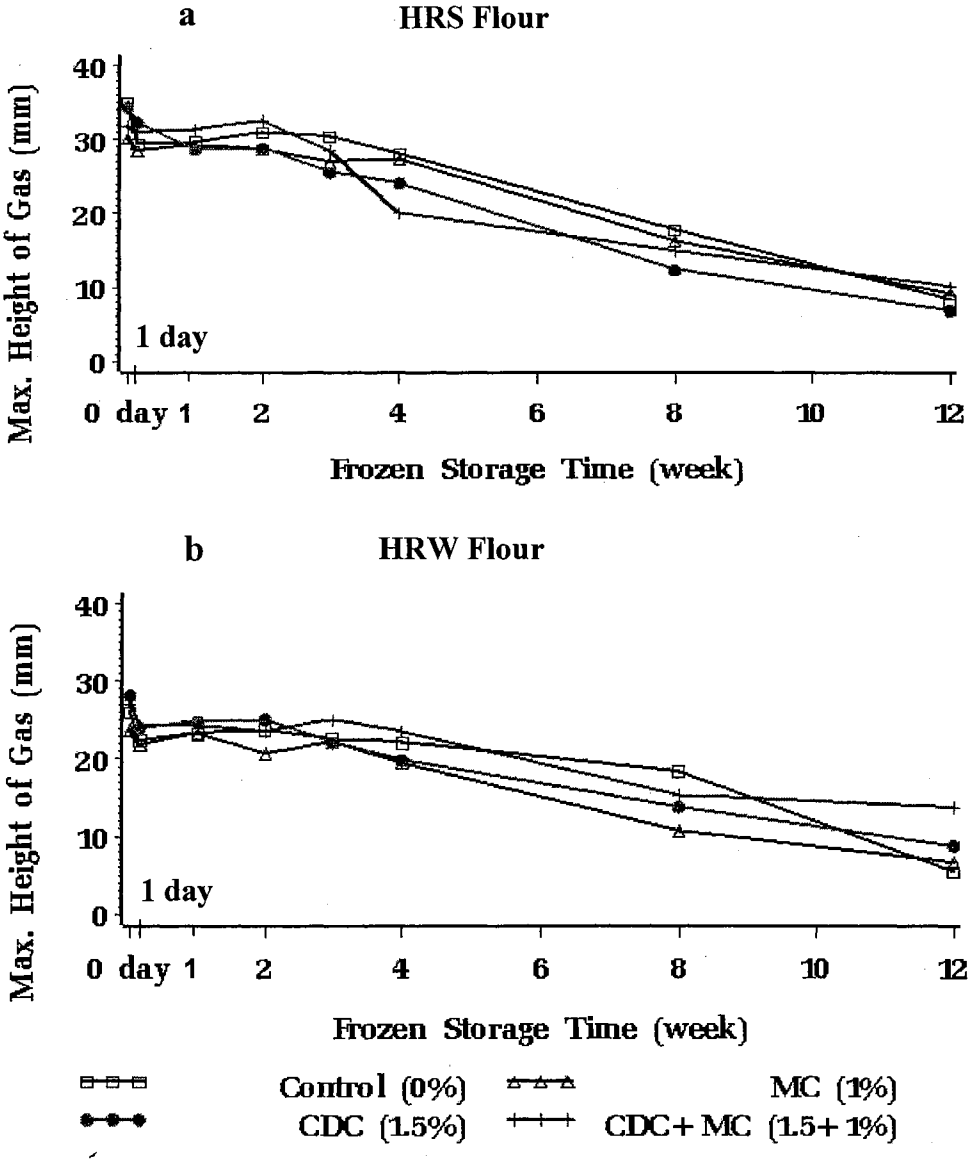
[b] H_{mG} = Maximum height of gas production.

[c] H_{mD} = Maximum height of dough development.

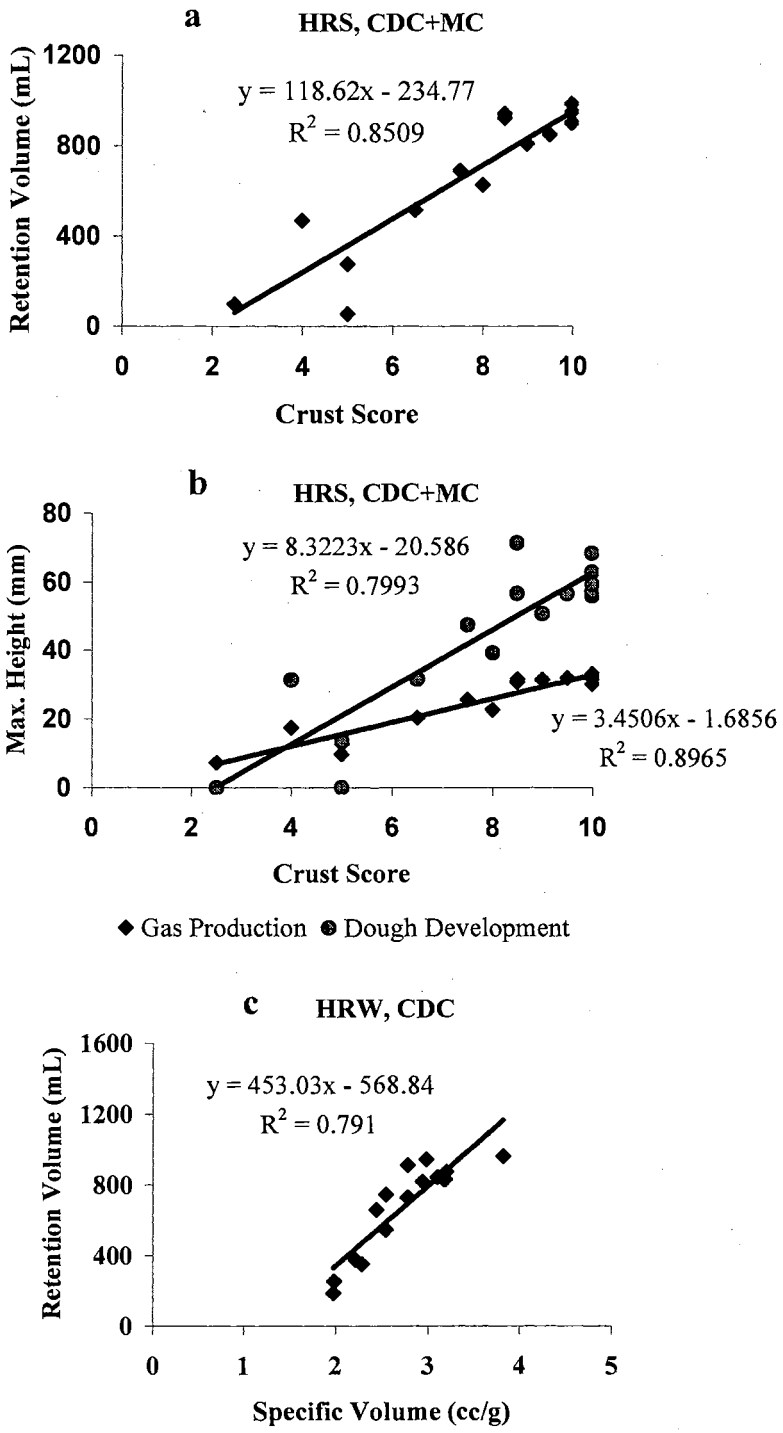
[d] CDC = Comercial Dough Conditioner, MC = Methylcellulose.

APPENDIX H

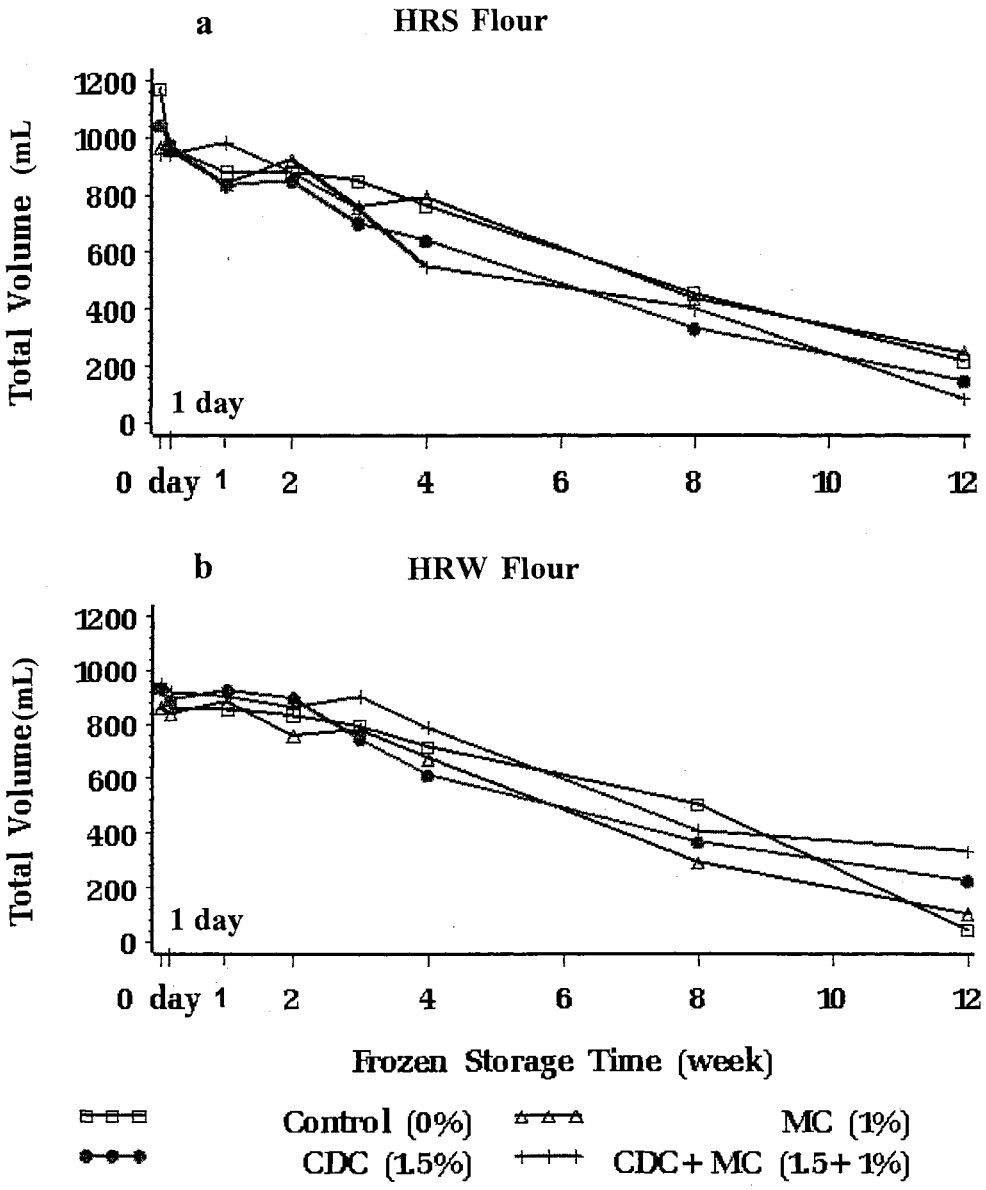
MAXIMUM HEIGHT OF GAS PRODUCTION (HmG) AS A FUNCTION OF FROZEN STORAGE STORAGE TIME OF BREAD STICKS MADE FROM HARD SPRING (HRS) FLOUR (a) AND HARD RED WINTER (HRW) FLOUR (b)



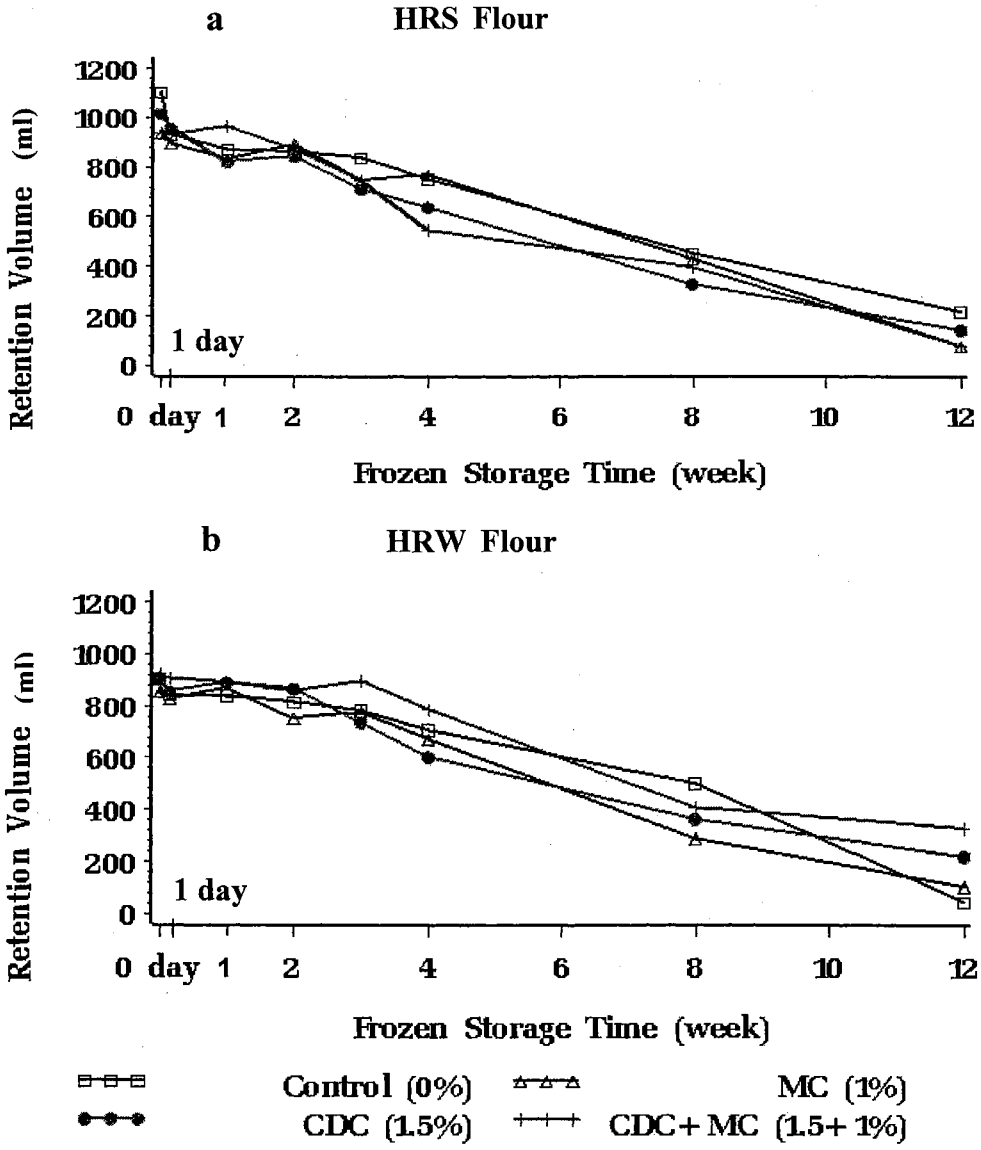
APPENDIX I
RELATIONSHIP BETWEEN RHEOFERMENTOMETER AND BAKING PARAMETERs, RETENTION VOLUME VS CRUST SCORE (a), MAXIMUM HEIGHT VS CRUST SCORE (b), RETENTION VOLUME VS SPECIFIC VOLUME (C), N = 64



APPENDIX J
TOTAL GAS VOLUME (VT) AS A FUNCTION OF FROZEN STORAGE TIME OF BREAD STICKS MADE FROM HARD RED SPRING (HRS) FLOUR (a) AND HARD RED WINTER (HRW) FLOUR (b)

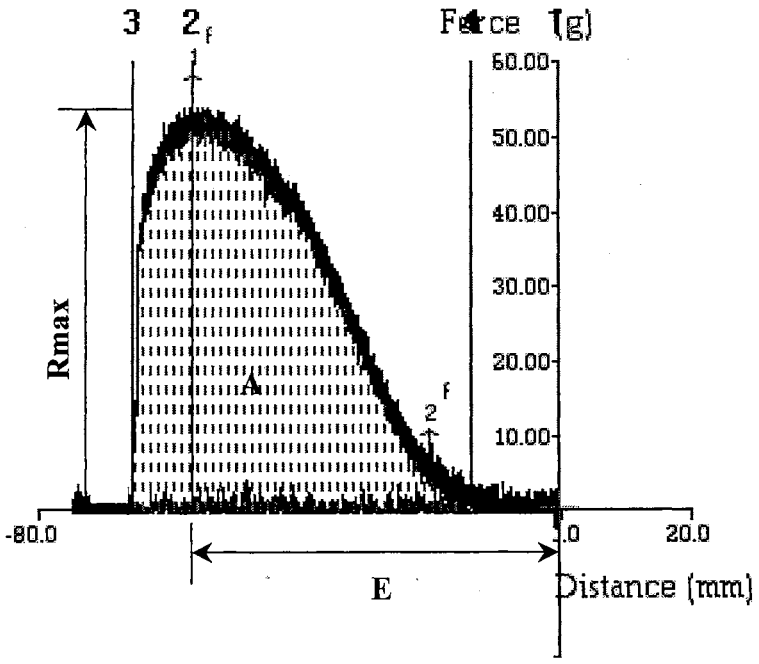


APPENDIX K
GAS RETENTION VOLUME AS A FUNCTION OF FROZEN STORAGE TIME OF BREAD STICKS MADE FROM HARD RED SPRING (HRS) FLOUR (a) AND HARD RED WINTER (HRW) FLOUR (b)



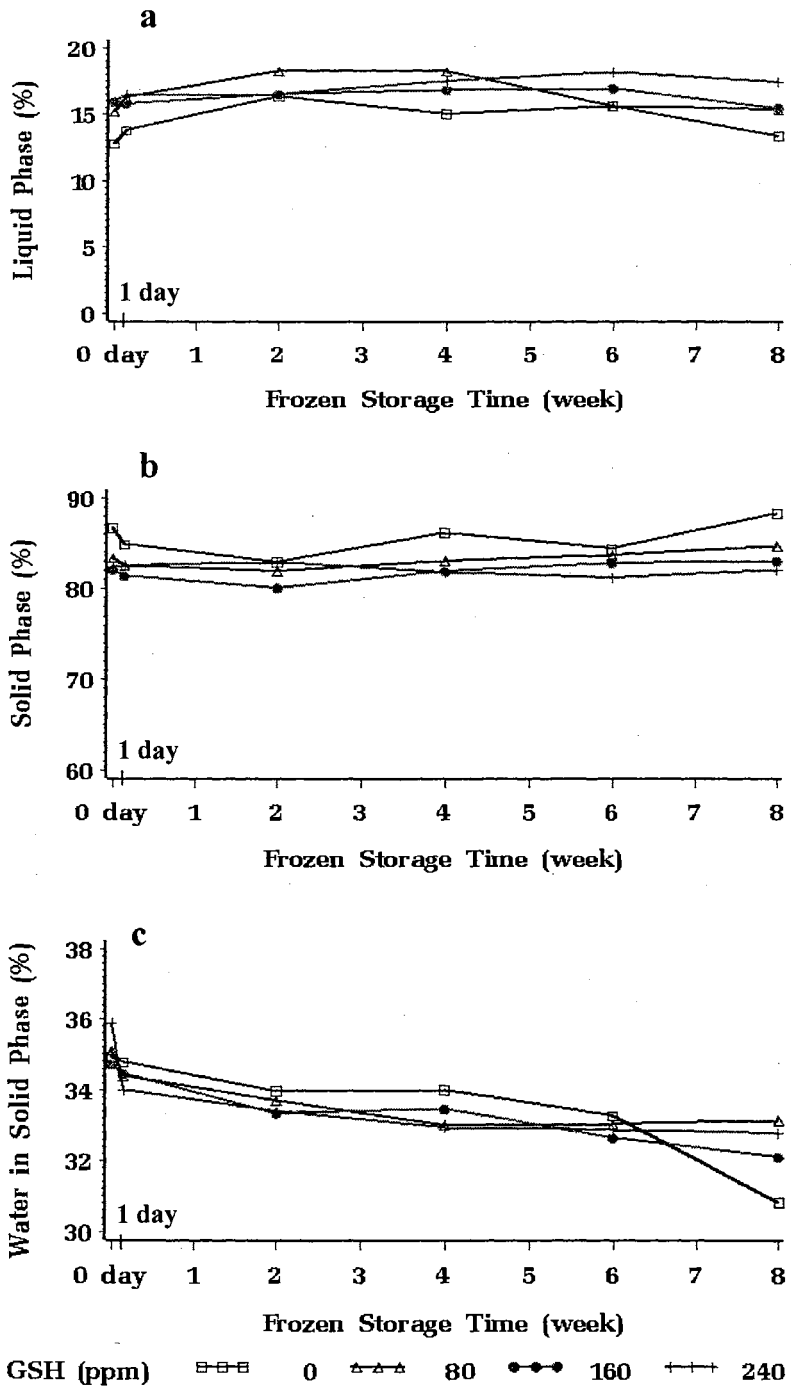
APPENDIX L

MICRO-EXTENSIBILITY CURVES USING TA.XT2 TEXTURE ANALYZER

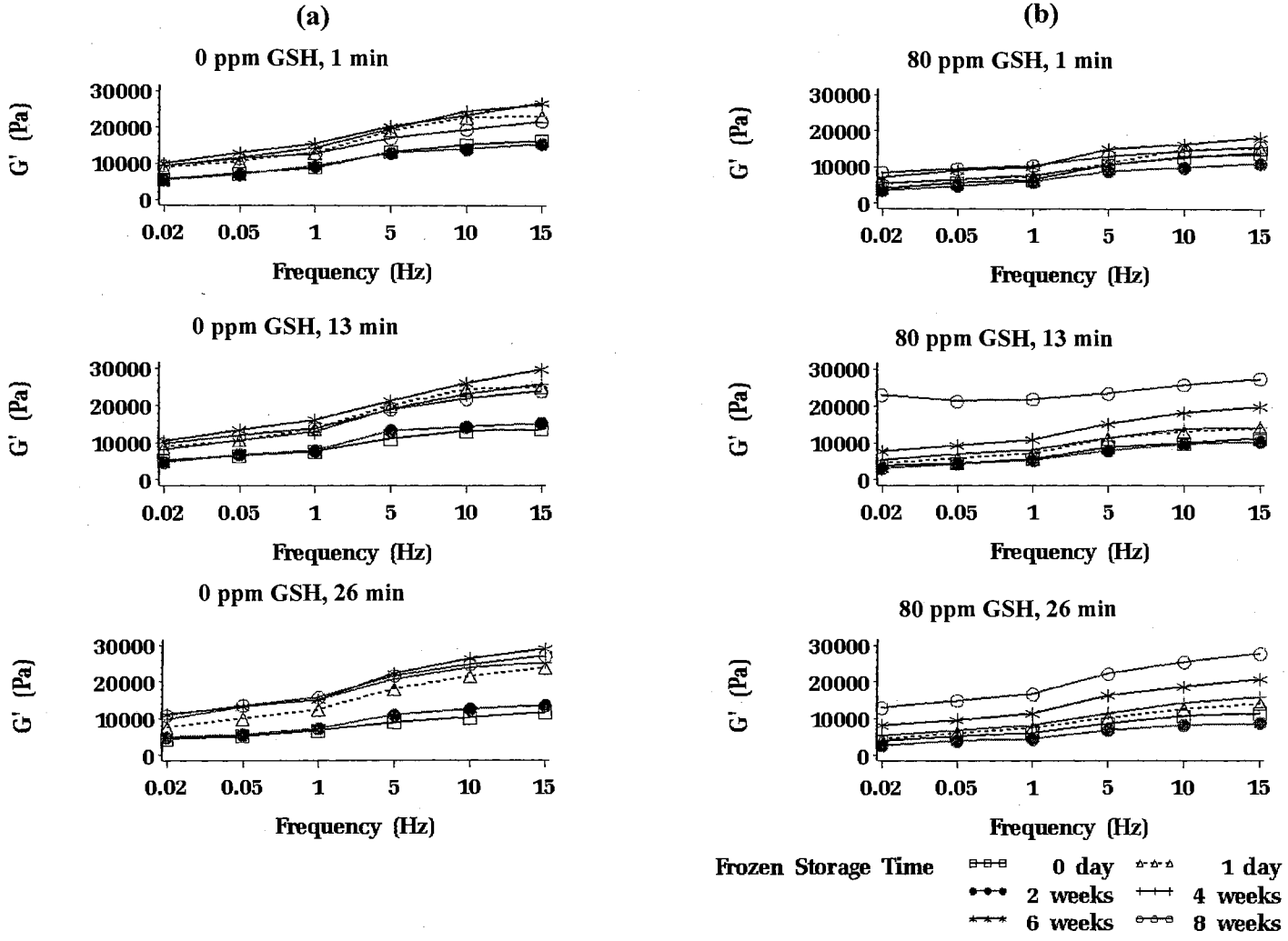


A = Area (mm^2)
E = Extensibility (mm)
Rmax = Resistance to extension (g)

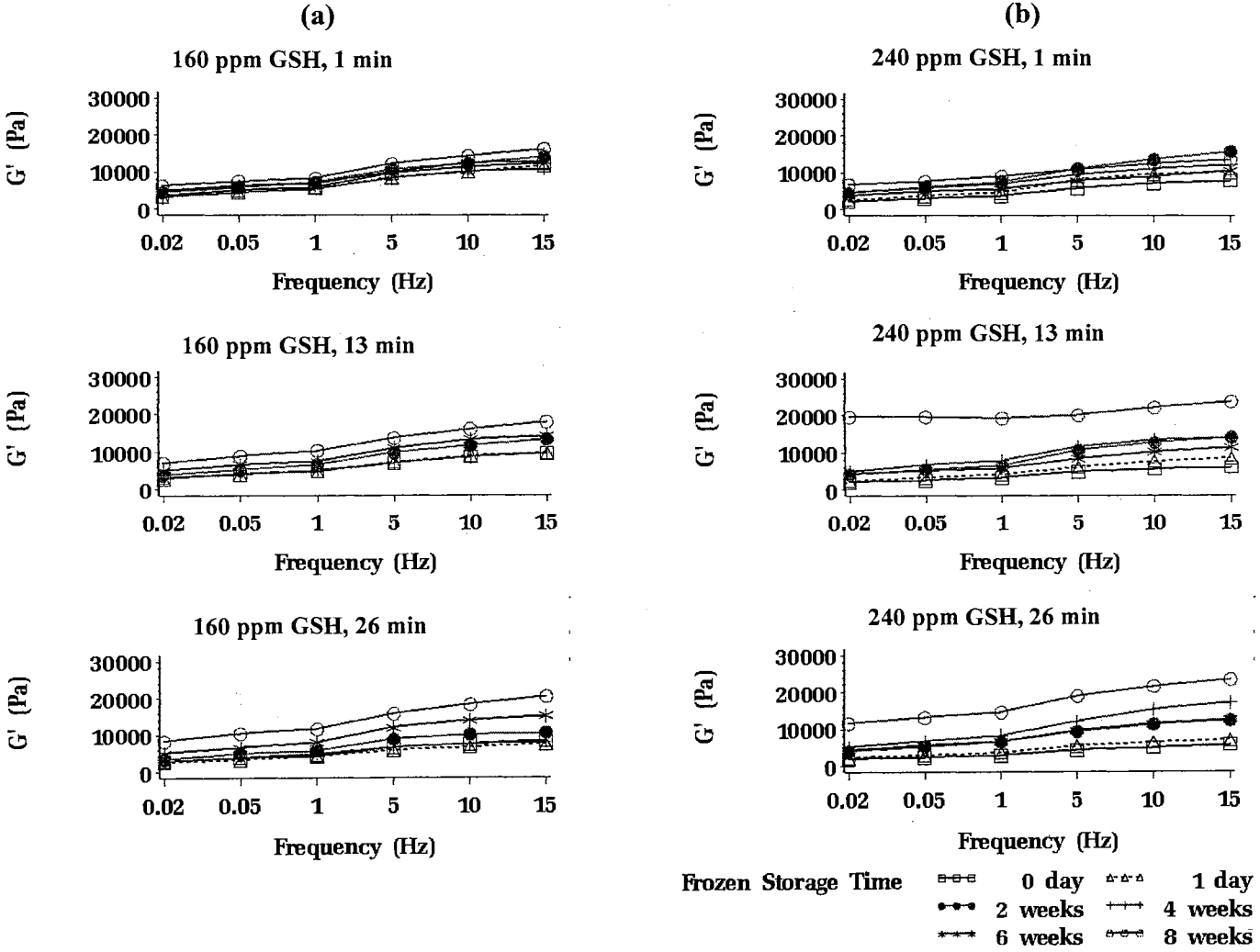
APPENDIX M
PHASE SEPERATION OF FROZEN DOUGH MADE FROM HARD RED SPRING FLOUR WITH ADDITION OF GLUTATHIONE, AS A FUNCTION OF FROZEN STORAGE TIME



APPENDIX N
STORAGE MODULUS (G') AS A FUNCTION FREQUENCY OF FROZEN DOUGH CONTAINING GLUTATHIONE (GSH) CONCENTRATIONS: a) 0 ppm, AND b) 80 ppm

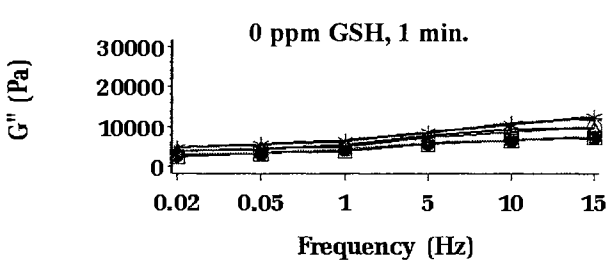


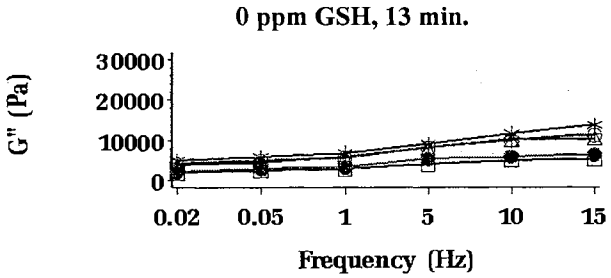
APPENDIX O
STORAGE MODULUS (G') AS A FUNCTION FREQUENCY OF FROZEN DOUGH CONTAINING GLUTATHIONE (GSH). CONCENTRATIONS: a) 160 ppm, AND b) 240 ppm

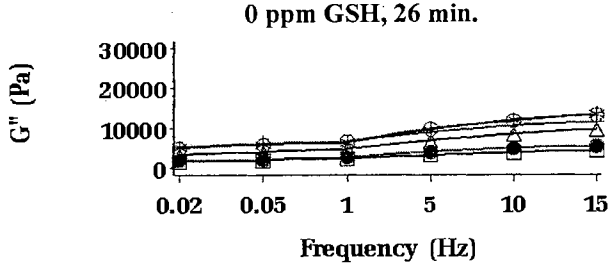


APPENDIX P
LOSS MODULUS (G'') AS A FUNCTION FREQUENCY OF FROZEN DOUGH CONTAINING GLUTATHIONE (GSH). CONCENTRATIONS : a) 0 ppm, AND b) 80ppm

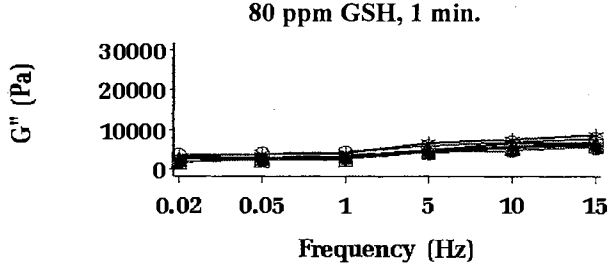
a

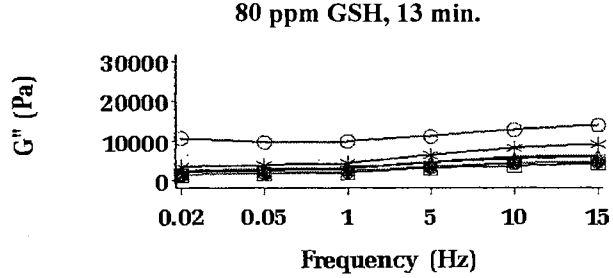


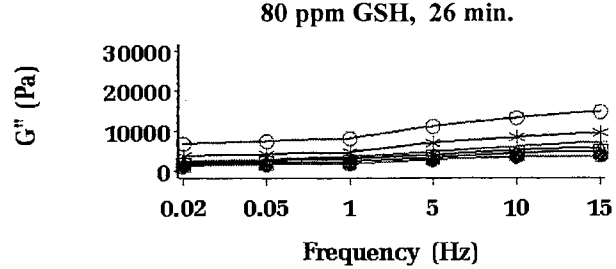




b



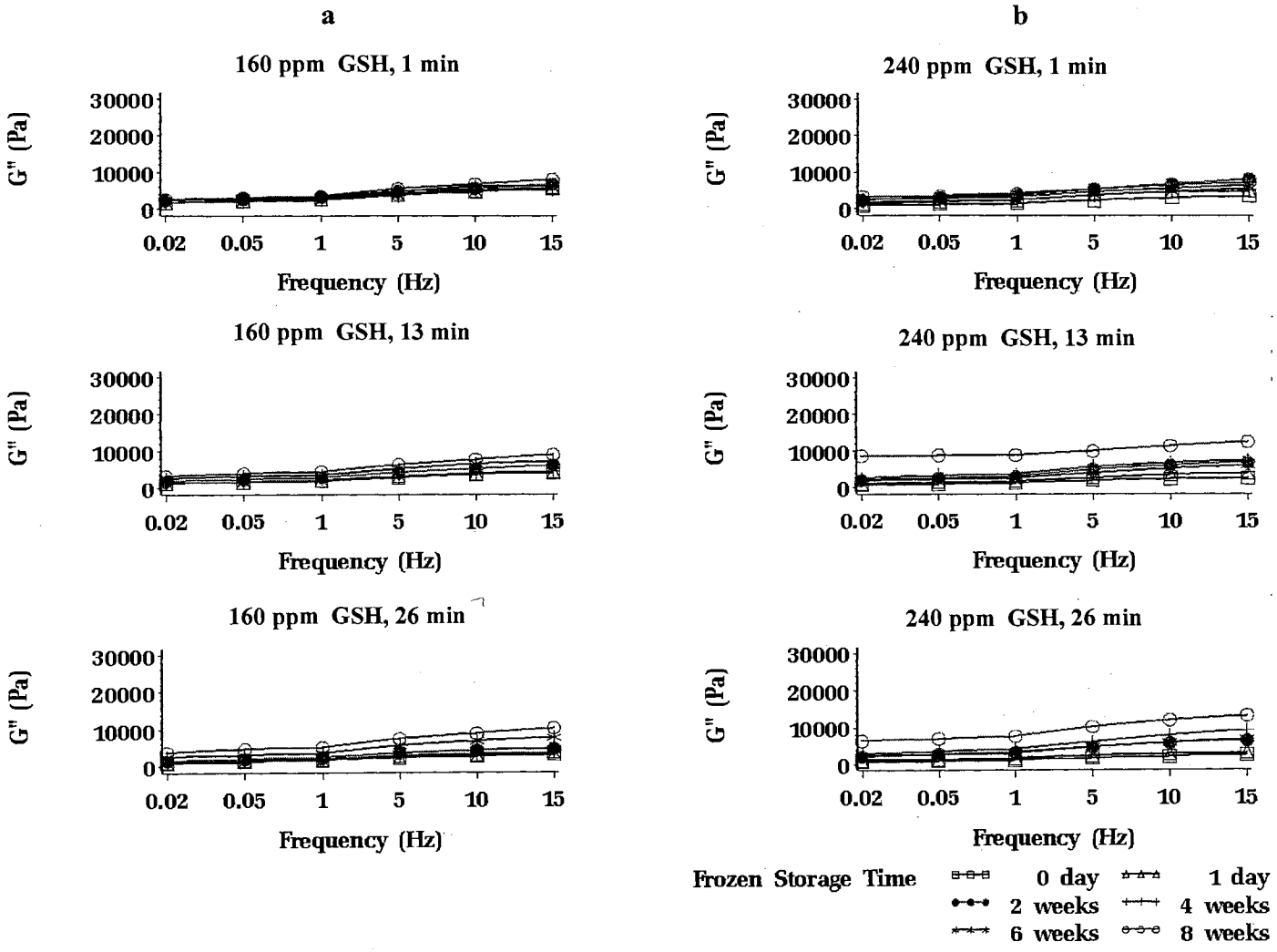




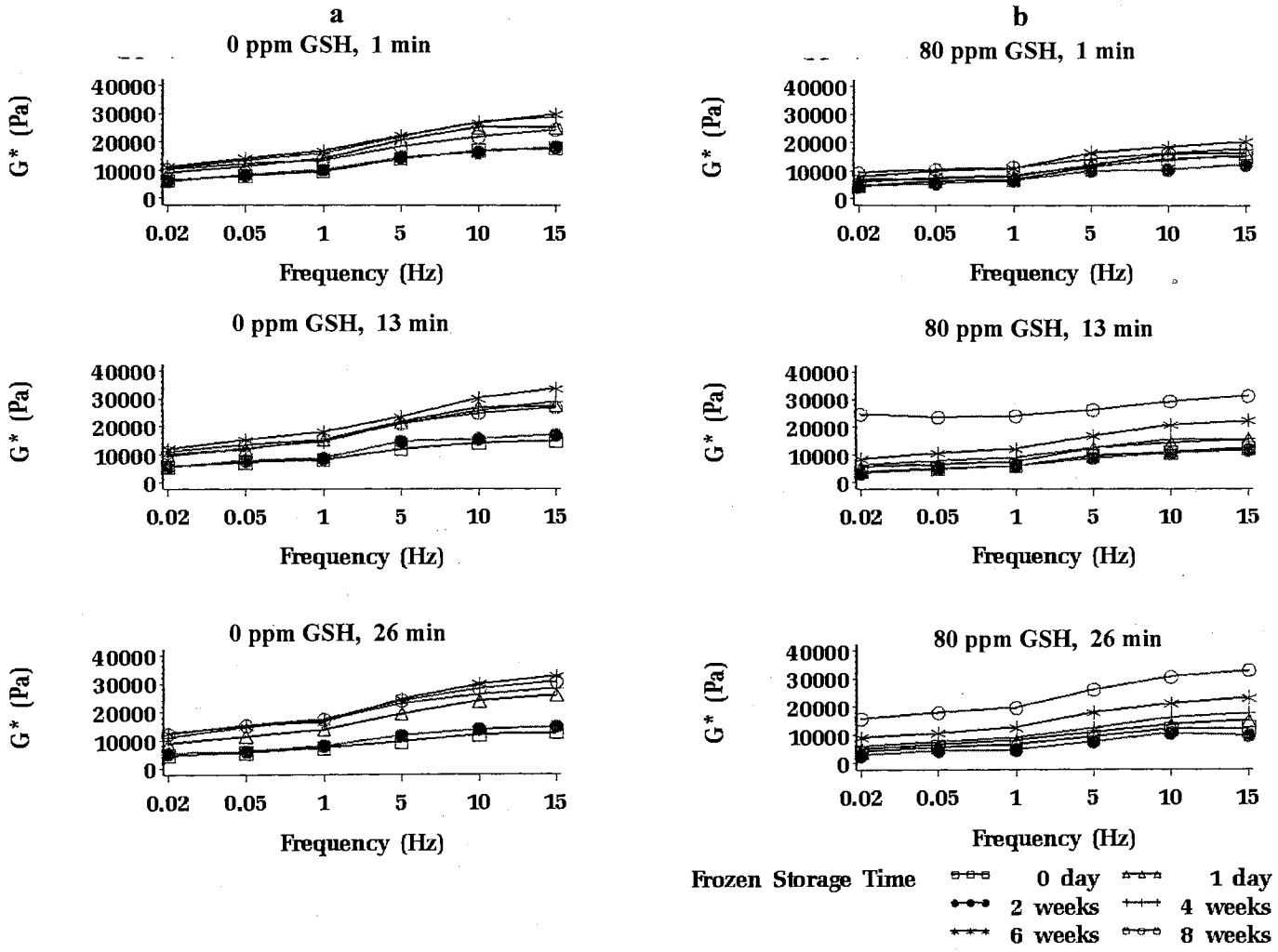
Frozen Storage Time — 0 day, 1 day, 2 weeks, 4 weeks, 6 weeks, 8 weeks

APPENDIX Q

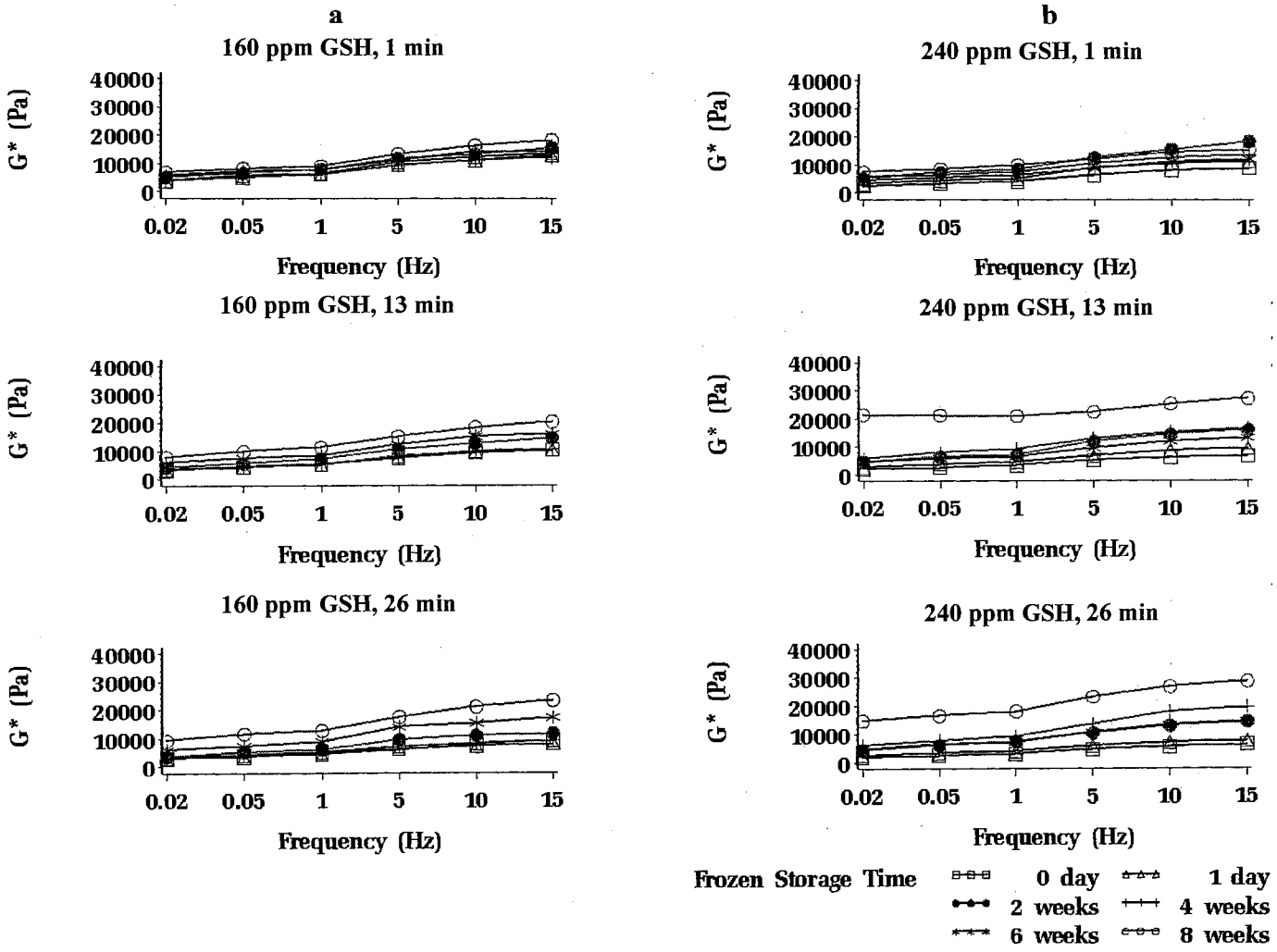
LOSS MODULUS (G") AS A FUNCTION FREQUENCY OF FROZEN DOUGH CONTAINING GLUTATHIONE (GSH). CONCENTRATIONS : a) 160 ppm, AND b) 240ppm



APPENDIX R
COMPLEX MODULUS (G'') AS A FUNCTION FREQUENCY OF FROZEN DOUGH CONTAINING GLUTATHIONE (GSH). CONCENTRATIONS : a) 0 ppm, AND b) 80 ppm

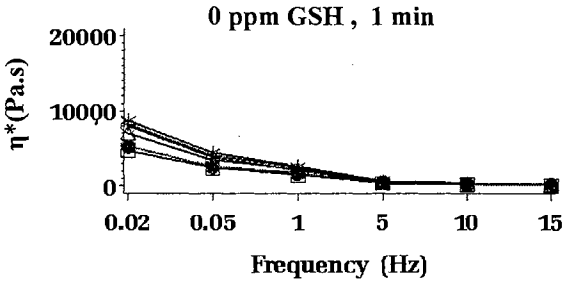


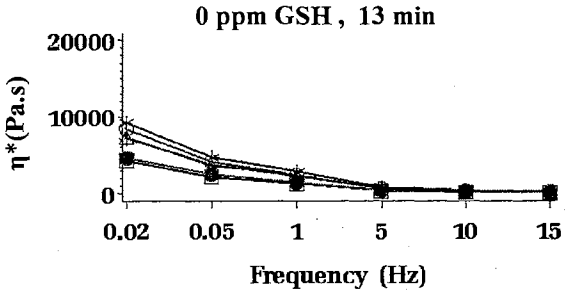
APPENDIX S
COMPLEX MODULUS (G*) AS A FUNCTION FREQUENCY OF FROZEN DOUGH CONTAINING GLUTATHIONE (GSH) . CONCENTRATIONS : a) 16 0 ppm, AND b) 240 ppm

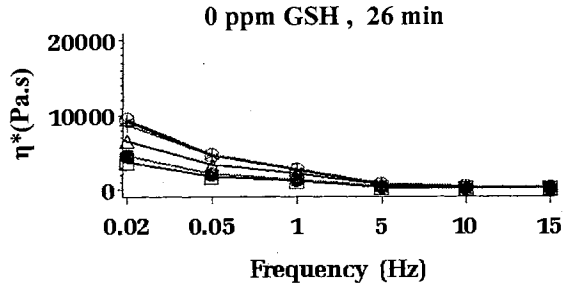


APPENDIX T
COMPLEX VISCOSITY (η*) AS A FUNCTION FREQUENCY OF FROZEN DOUGH CONTAINING GLUTATHIONE (GSH). CONCENTRATIONS : a) 0 ppm, AND b) 80 ppm

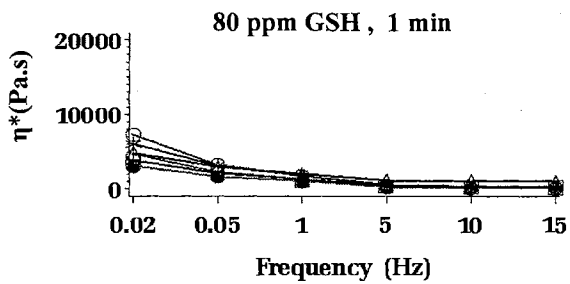
a

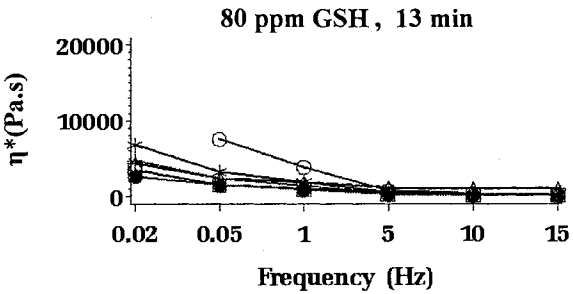


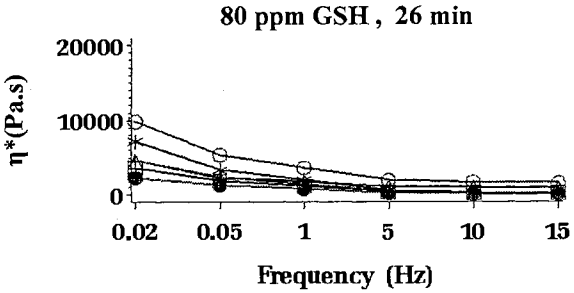




b



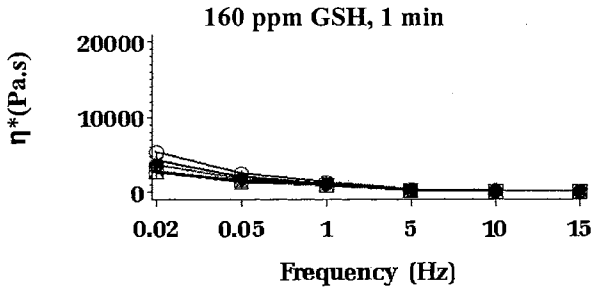


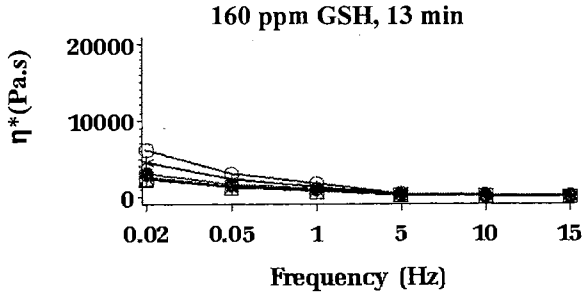


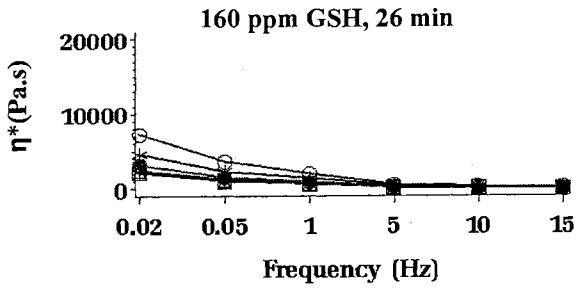
Frozen Storage — 0 day, 1 day, 2 weeks, 4 weeks, 6 weeks, 8 weeks

APPENDIX U
COMPLEX VISCOSITY (η*) AS A FUNCTION FREQUENCY OF FROZEN DOUGH CONTAINING GLUTATHIONE (GSH). CONCENTRATIONS : a) 160 ppm, AND b) 240 ppm

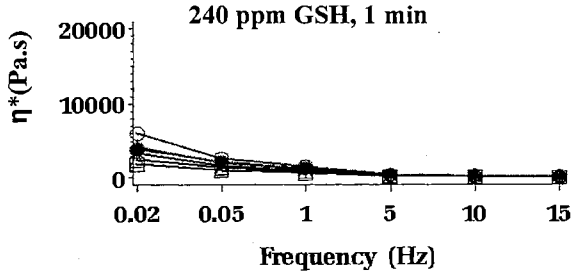
a

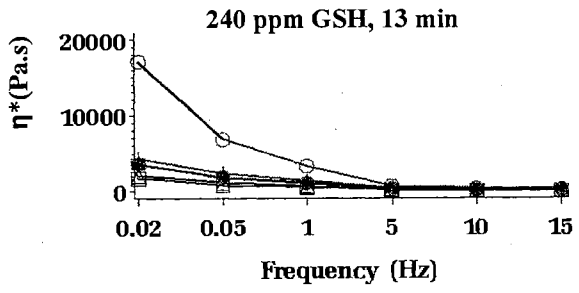


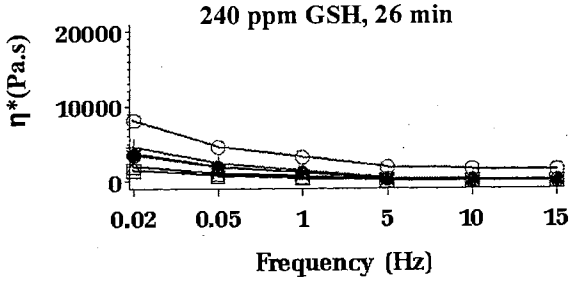




b



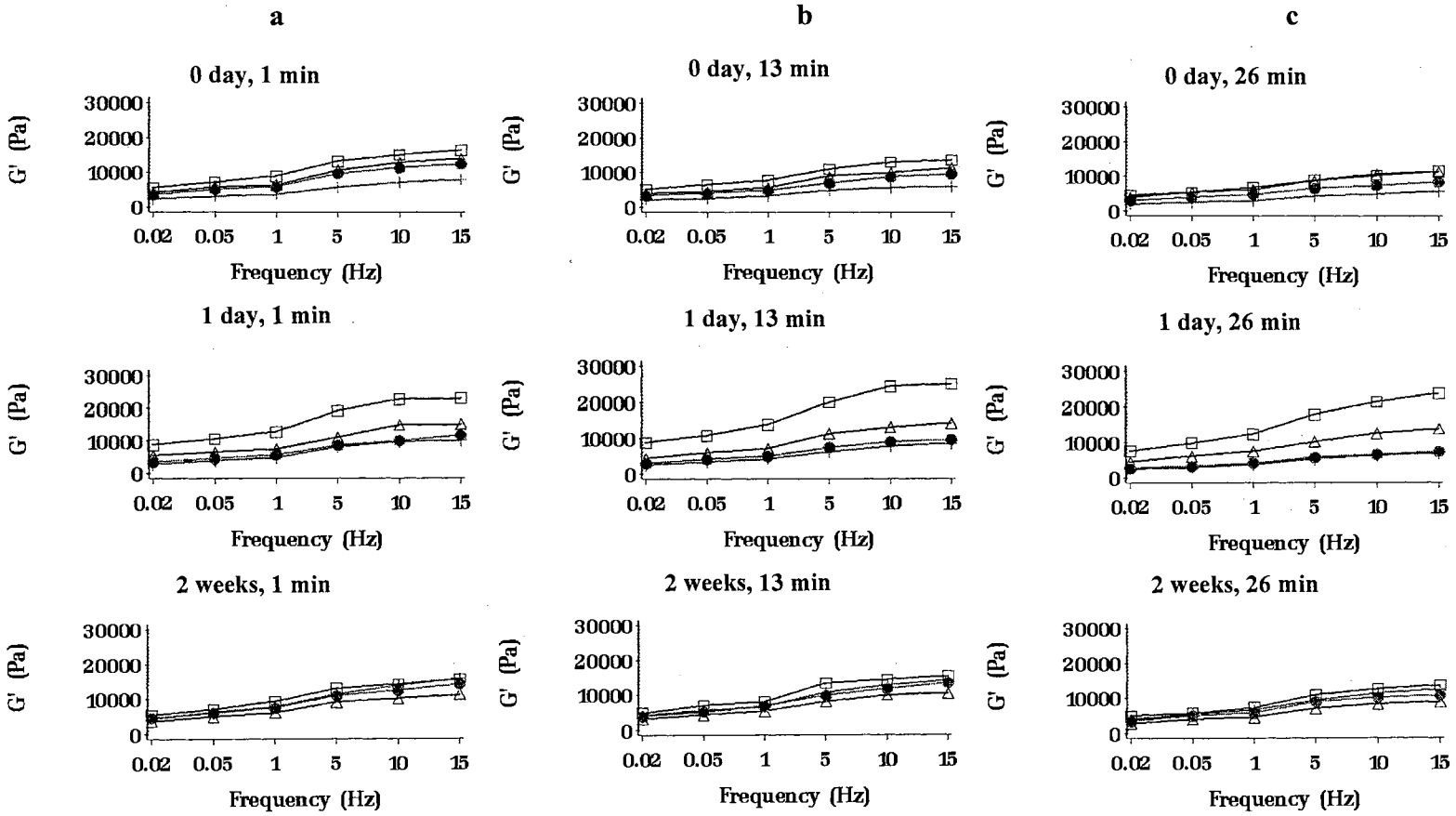




Frozen Storage — 0 day, 1 day, 2 weeks, 4 weeks, 6 weeks, 8 weeks

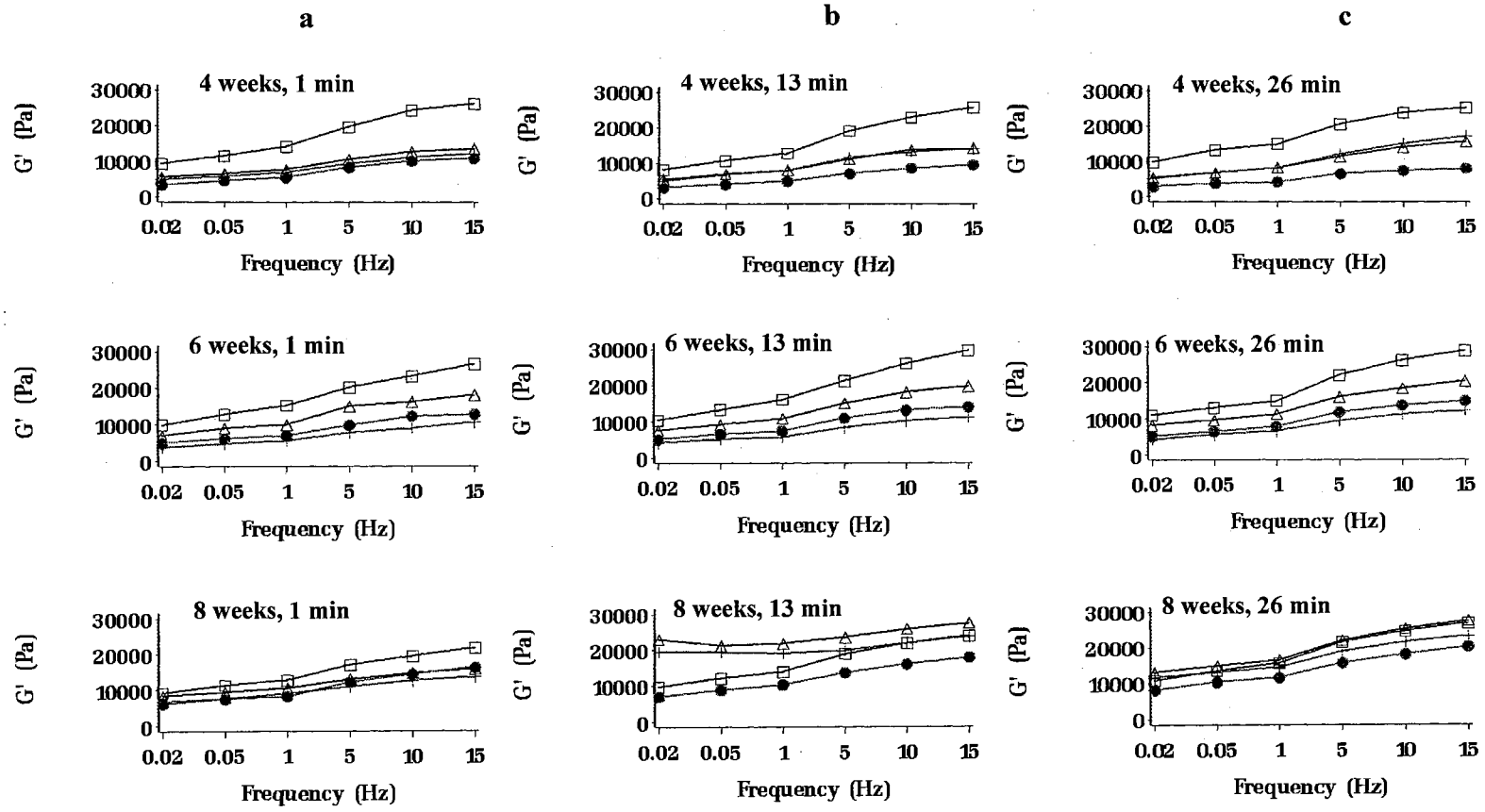
APPENDIX V

STORAGE MODULUS (G') AS A FUNCTION OF FREQUENCY OF DOUGH CONTAINING GLUTATHIONE (GSH). THE DOUGH WAS FROZEN FOR 0 DAY, 1 DAY AND 2 WEEKS



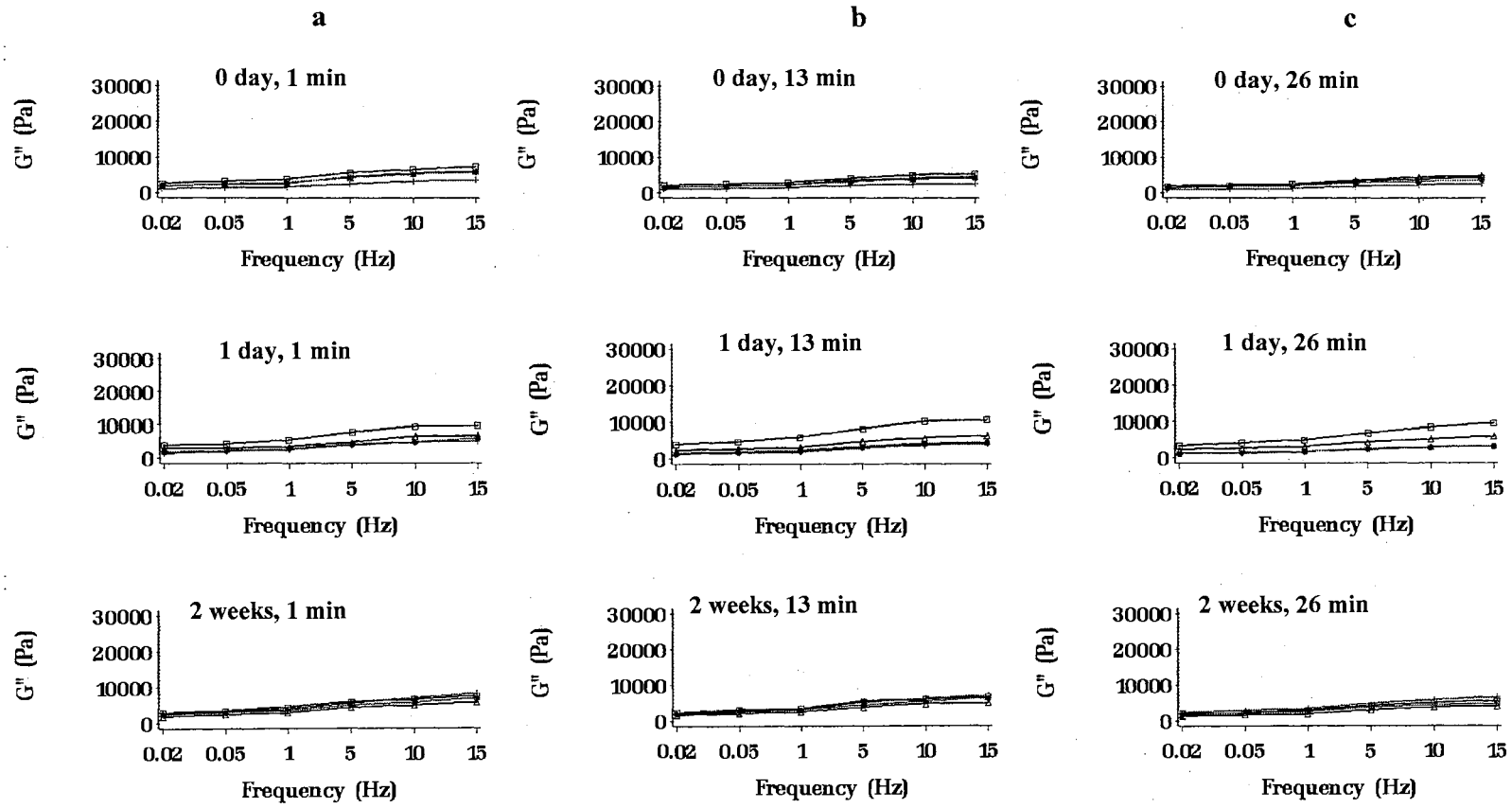
GSH: □ 0 ppm, Δ 80 ppm, ● 160 ppm, + 240 ppm

APPENDIX W
STORAGE MODULUS (G') AS A FUNCTION OF FREQUENCY OF DOUGH CONTAINING GLUTATHIONE (GSH). THE DOUGH WAS FROZEN FOR 4, 6 AND 8 WEEKS



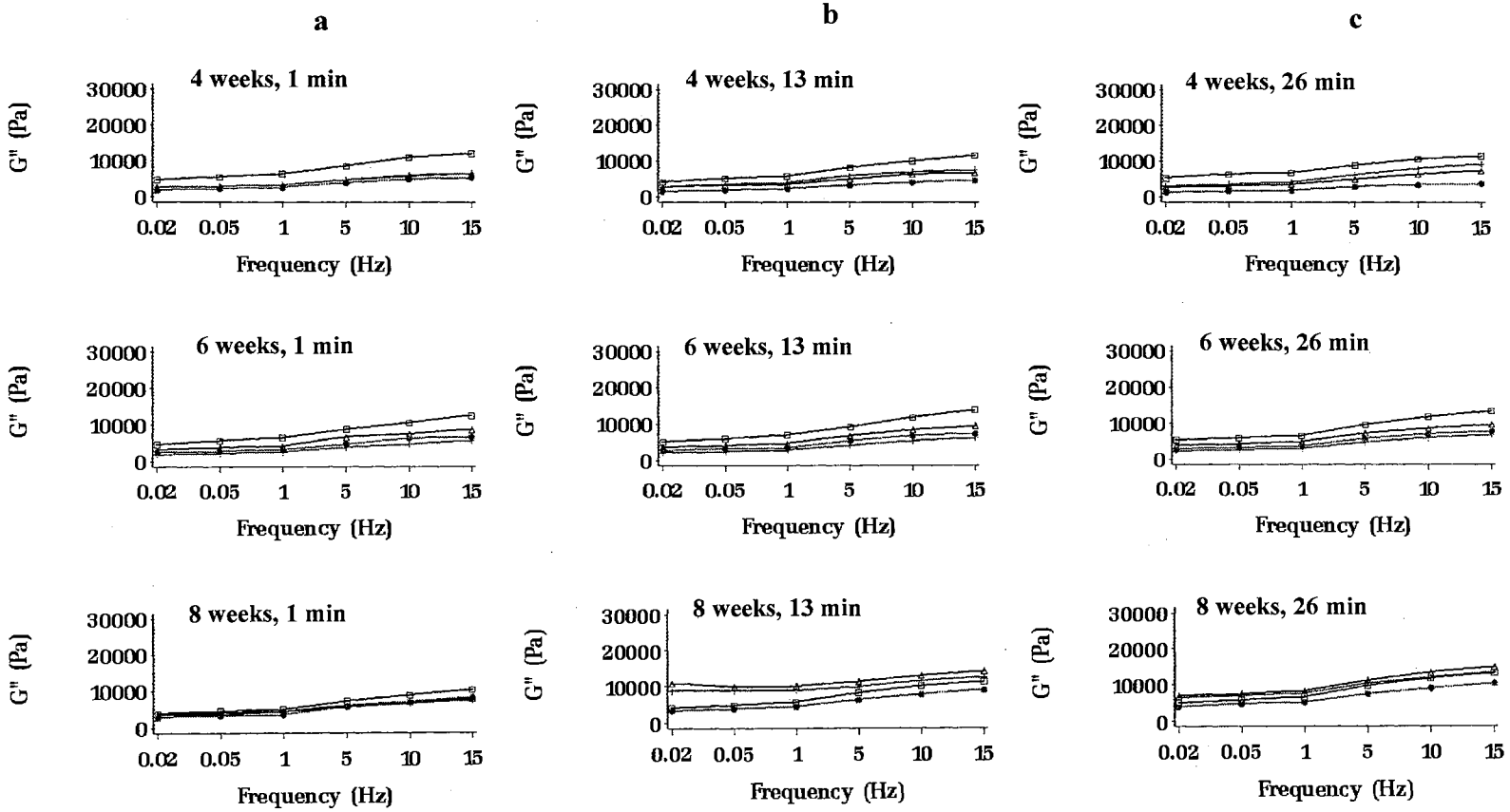
GSH: □ 0 ppm, Δ 80 ppm, ● 160 ppm, + 240 ppm

APPENDIX X
LOSS MODULUS (G") AS A FUNCTION OF FREQUENCY OF DOUGH CONTAINING GLUTATHIONE (GSH). THE DOUGH WAS FROZEN FOR 0 DAY, 1 DAY AND 2 WEEKS



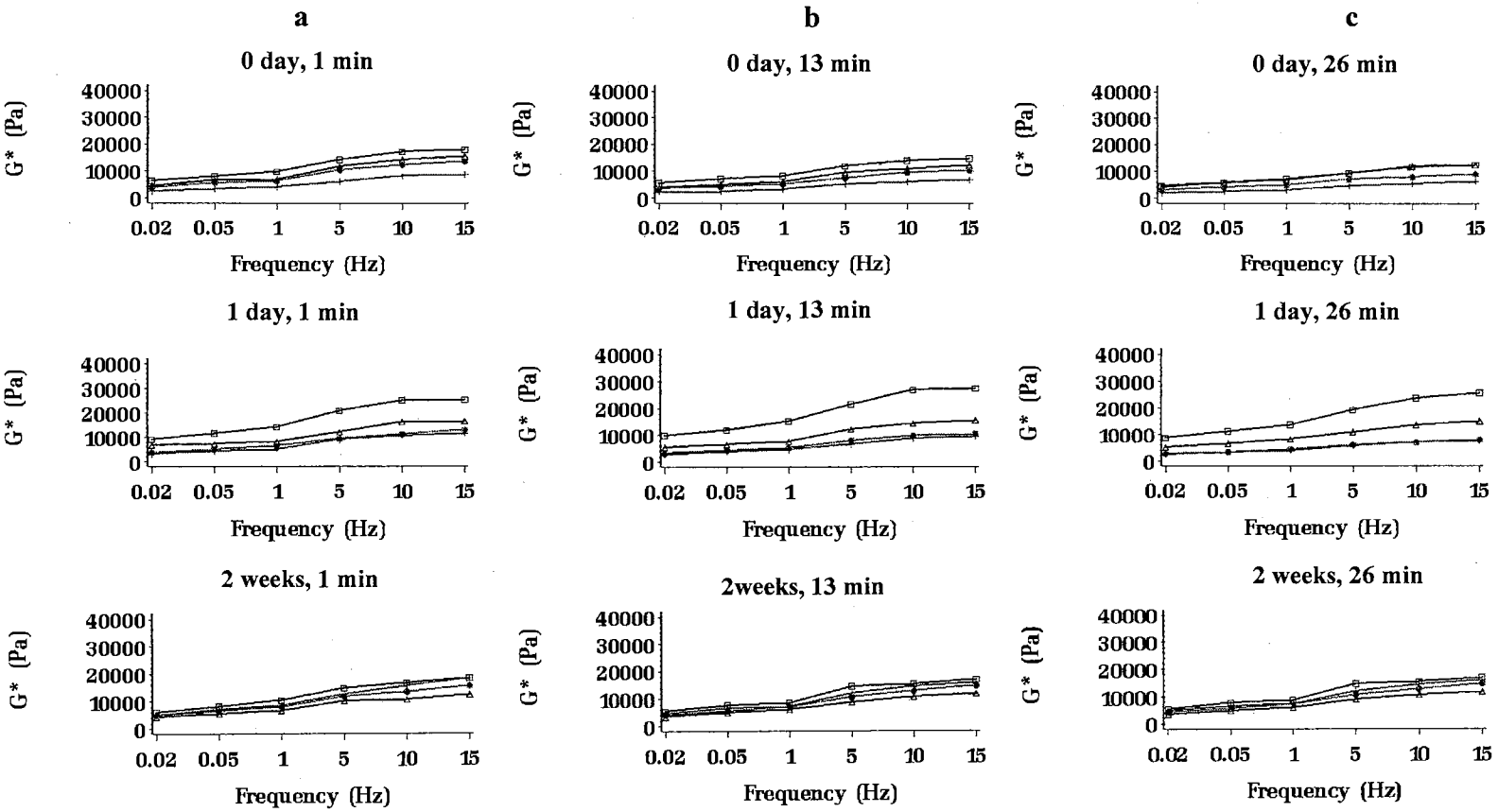
GSH: □ 0 ppm, Δ 80 ppm, ● 160 ppm, + 240 ppm

APPENDIX Y
LOSS MODULUS (G") AS A FUNCTION OF FREQUENCY OF DOUGH CONTAINING GLUTATHIONE (GSH). THE DOUGH WAS FROZEN FOR 4, 6 AND 8 WEEKS



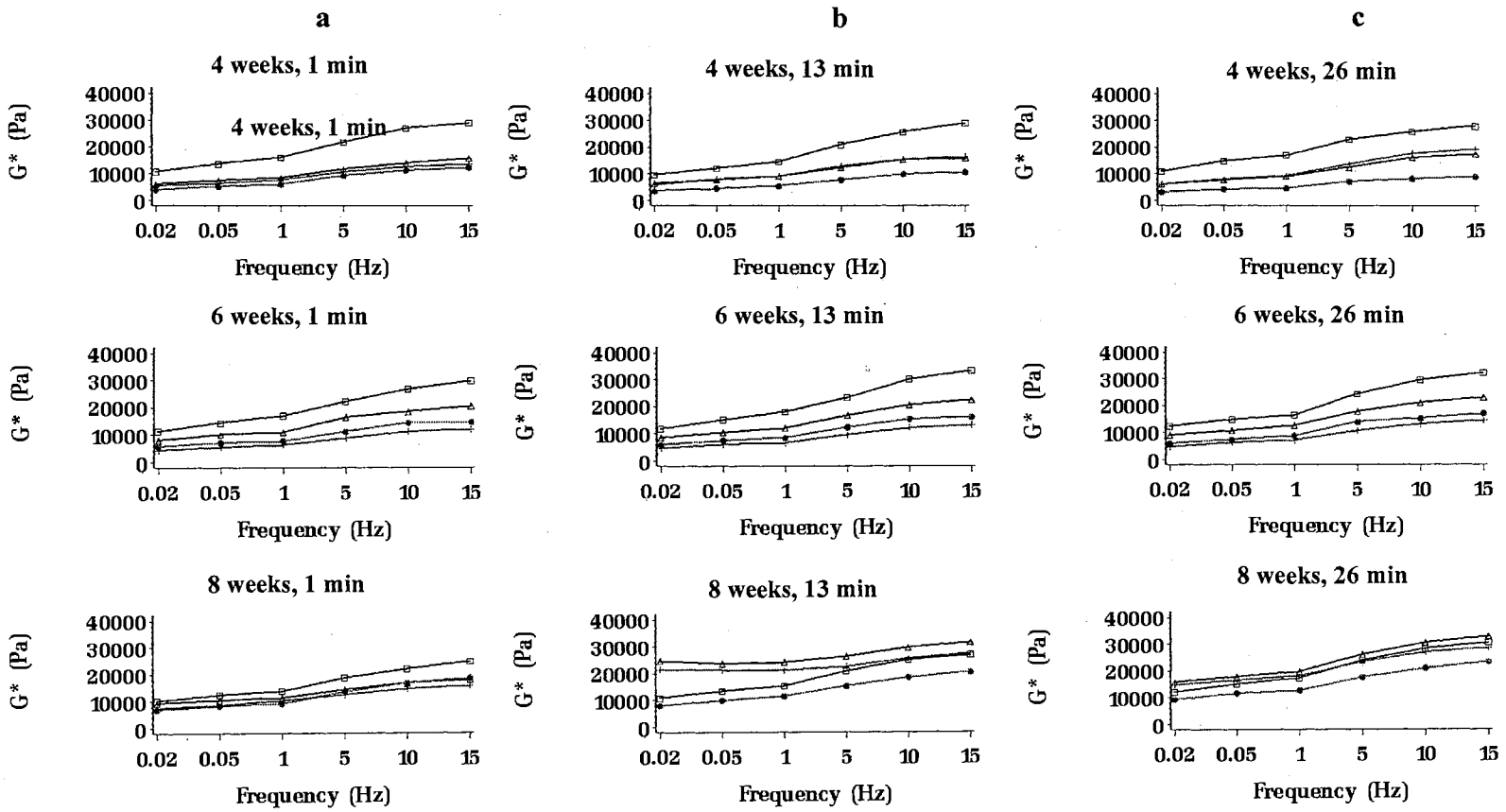
GSH: □ 0 ppm, Δ 80 ppm, ● 160 ppm, + 240 ppm

APPENDIX Z
COMPLEX MODULUS (G*) VS FREQUENCY AS A FUNCTION OF GLUTATHIONE (GSH). THE DOUGH WAS FROZEN FOR 0 DAY, 1 DAY AND 2 WEEKS



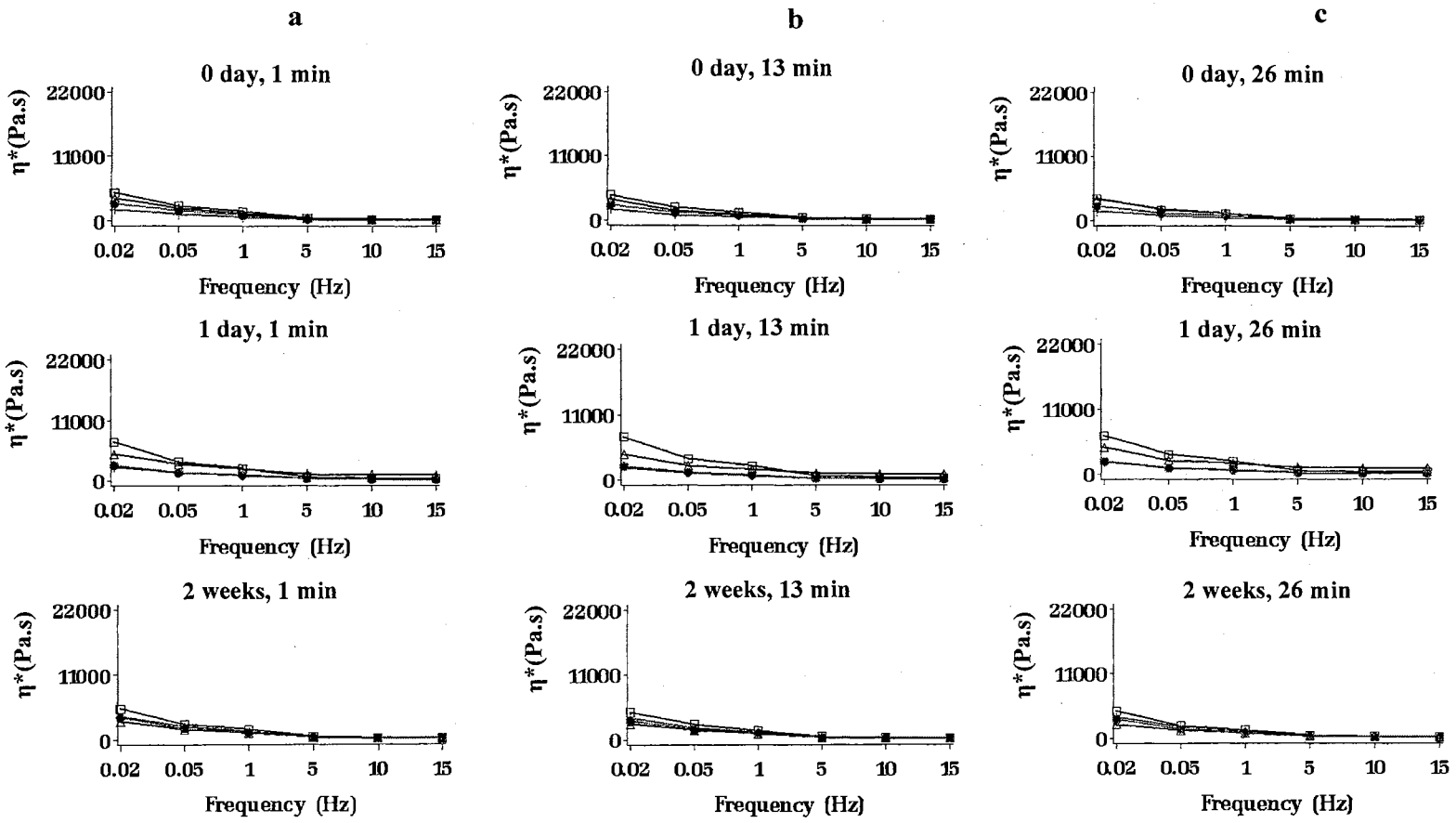
GSH: □ 0 ppm, Δ 80 ppm, ● 160 ppm, + 240 ppm

APPENDIX AA
COMPLEX MODULUS (G*) VS FREQUENCY AS A FUNCTION OF GLUTATHIONE (GSH). THE DOUGH WAS FROZEN FOR 4, 6, AND 8 WEEKS



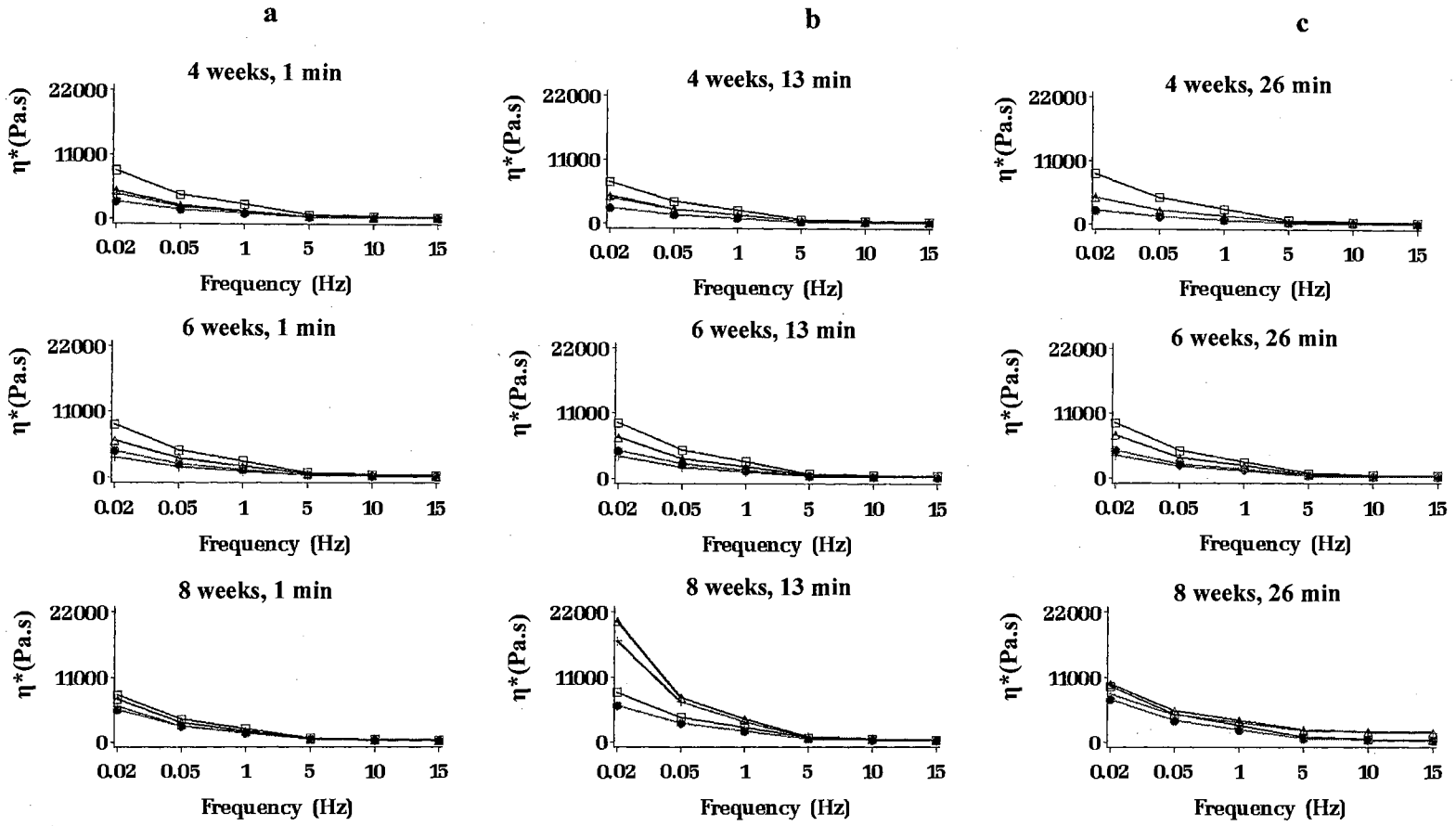
GSH: □ 0 ppm, Δ 80 ppm, ● 160 ppm, + 240 ppm

APPENDIX AB
COMPLEX VISCOSITY (η*) VS FREQUENCY AS A FUNCTION OF GLUTATHIONE (GSH). THE DOUGH WAS FROZEN FOR 0 DAY, 1 DAY AND 2 WEEKS



GSH: □ 0 ppm, Δ 80 ppm, ● 160 ppm, + 240 ppm

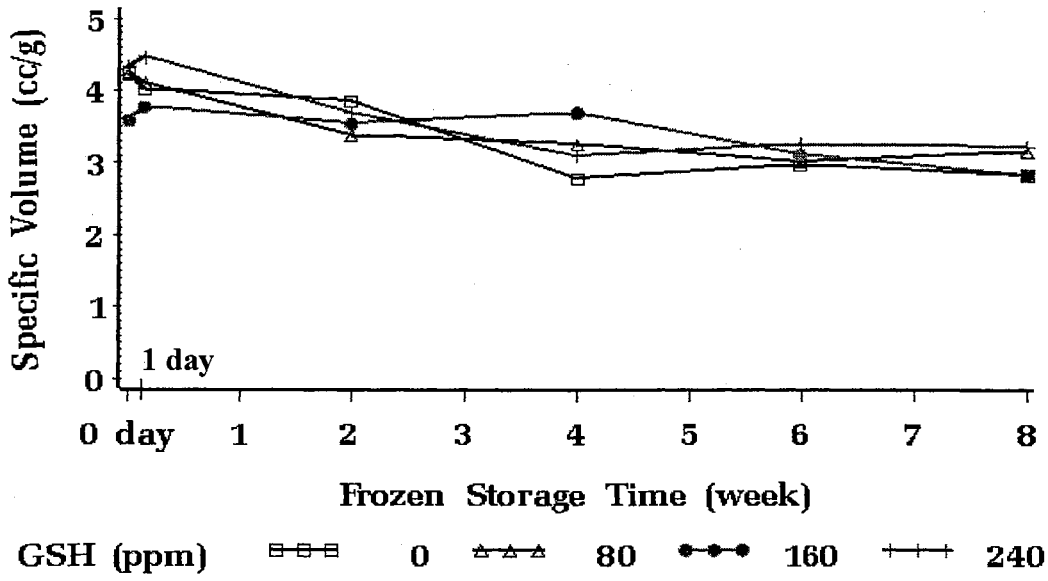
APPENDIX AC
COMPLEX VISCOSITY(η*) VS FREQUENCY AS A FUNCTION OF GLUTATHIONE (GSH). THE DOUGH WAS FROZEN FOR 4, 6 AND 8 WEEKS



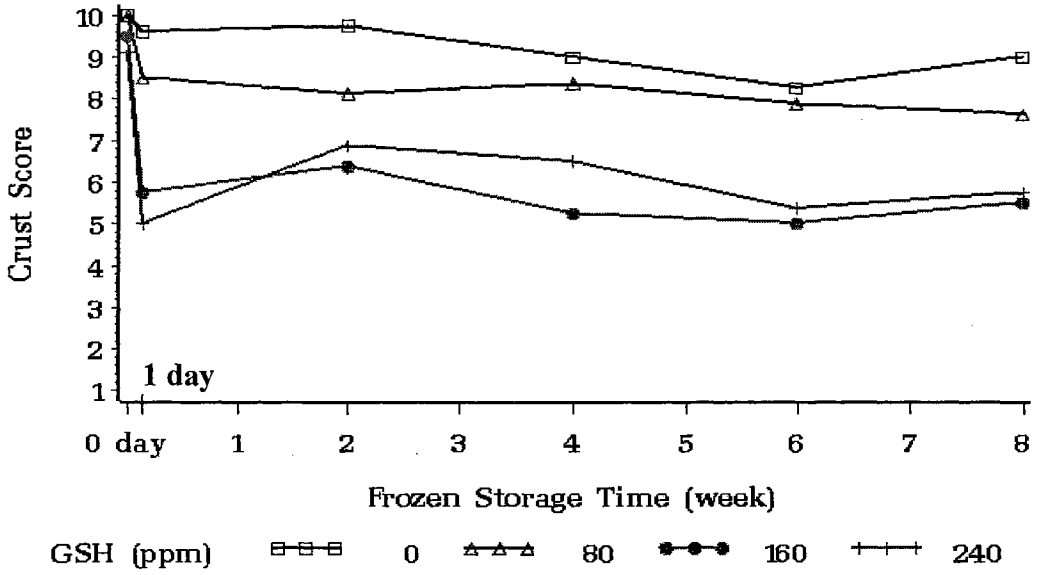
GSH: □ 0 ppm, Δ 80 ppm, ● 160 ppm, + 240 ppm

APPENDIX AD

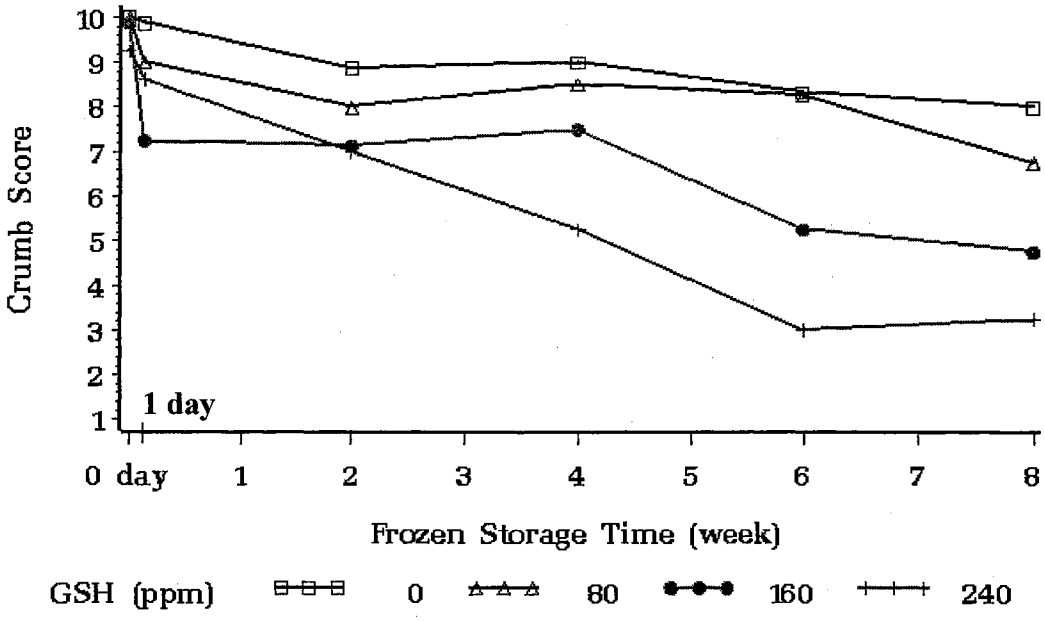
SPECIFIC VOLUME OF BREAD STICKS MADE WITH HARD RED SPRING (HRS) FLOUR AS A FUNCTION OF FROZEN STORAGE TIME AND GLUTATHIONE (GSH)



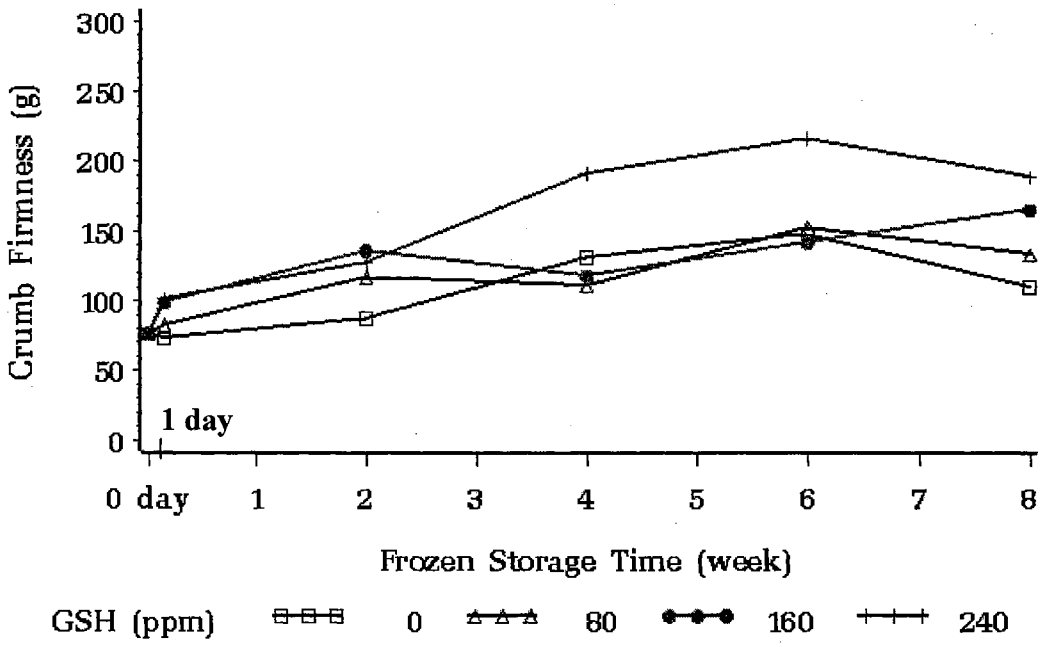
APPENDIX AE
CRUST SCORE OF BREAD STICKS MADE WITH HARD RED SPRING (HRS) FLOUR AS A FUNCTION OF FROZEN STORAGE TIME AND GLUTATHIONE (GSH).



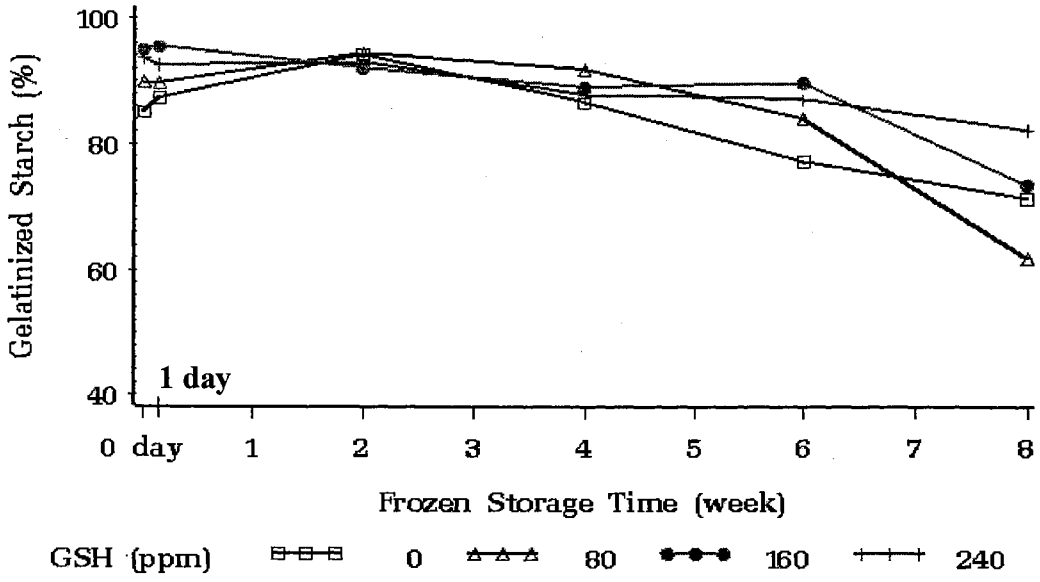
APPENDIX AF
CRUMB SCORE OF BREAD STICKS MADE WITH HARD RED SPRING (HRS) FLOUR AS A FUNCTION OF FROZEN STORAGE TIME AND GLUTATHIONE (GSH)



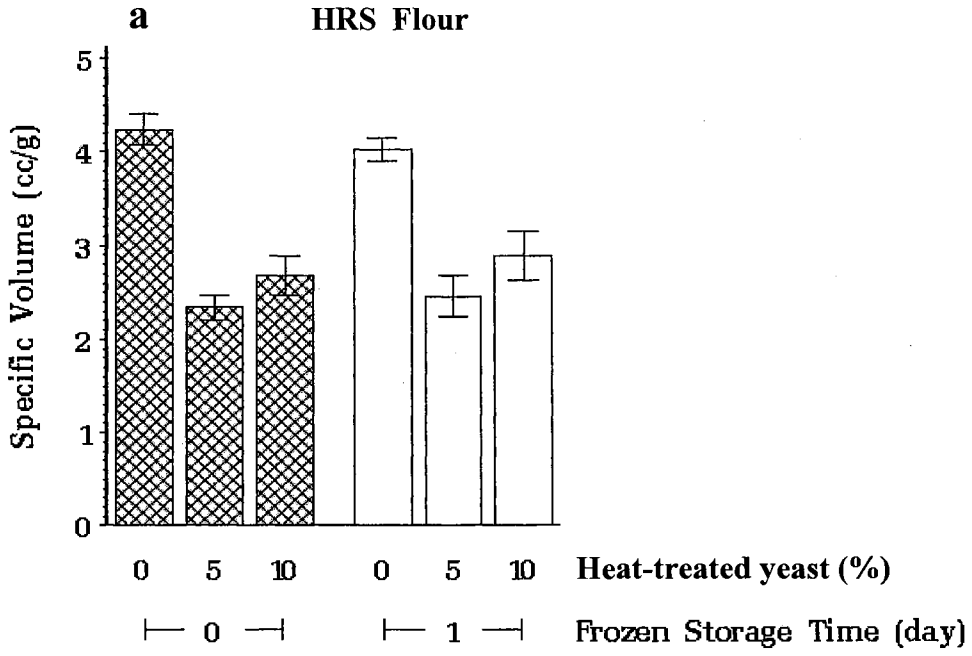
APPENDIX AG
CRUMB FIRMNESS OF BREAD STICKS MADE WITH HARD RED SPRING (HRS) FLOUR AS A FUNCTION OF FROZEN STORAGE TIME AND GLUTATHIONE (GSH)

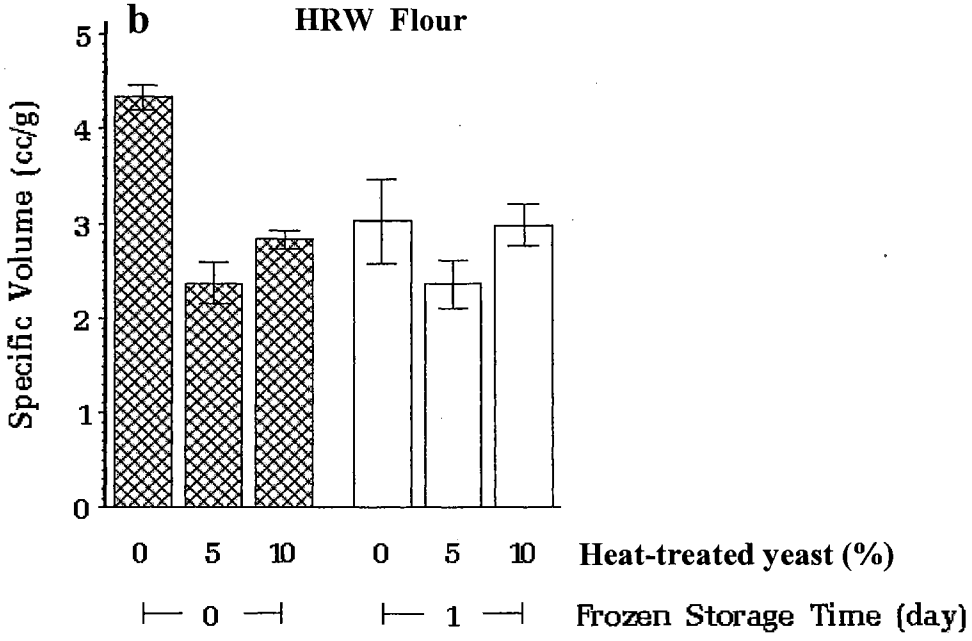


APPENDIX AH
PERCENTAGE OF GELATINIZED STARCH OF BAKED BREAD STICKS MADE FROM HARD RED SPRING (HRS) FLOUR AS A FUNCTION TIME AND GLUTATHIONE (GSH)



APPENDIX AI
SPECIFIC VOLUME OF BREAD STICKS AS A FUNCTION OF HEAT-TREATED YEAST ADDITION OF FRESH AND 1 DAY STORED FROZEN DOUGH. HARD RED SPRING (HRS) (a) AND HARD RED WINTER (HRW) FLOUR (b)





APPENDIX AJ
YEAST COLONY FORMING UNITS (CFU) FROM COMPRESSED BULK YEAST ANALYZED AFTER 7 DAYS INCUBATED AT ROOM TEMPERATURE USING POUR PLATE METHOD[ab]

Treatment	Yeast (CFU/mL)
5% Yeast (Control[c])	1.3×10^9
5% Yeast (Heat treated[d])	1.7×10^1
10% Yeast (Control)	2.3×10^9
10% Yeast (Heat treated)	6.3×10^3

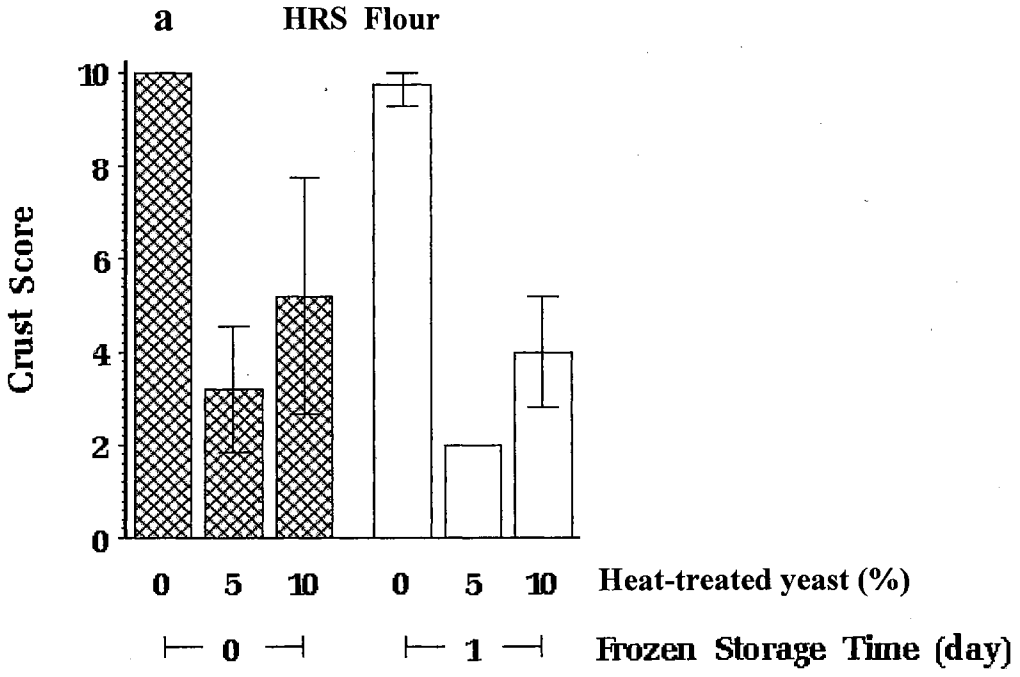
[a] Mean from two measurements of two independent batches.

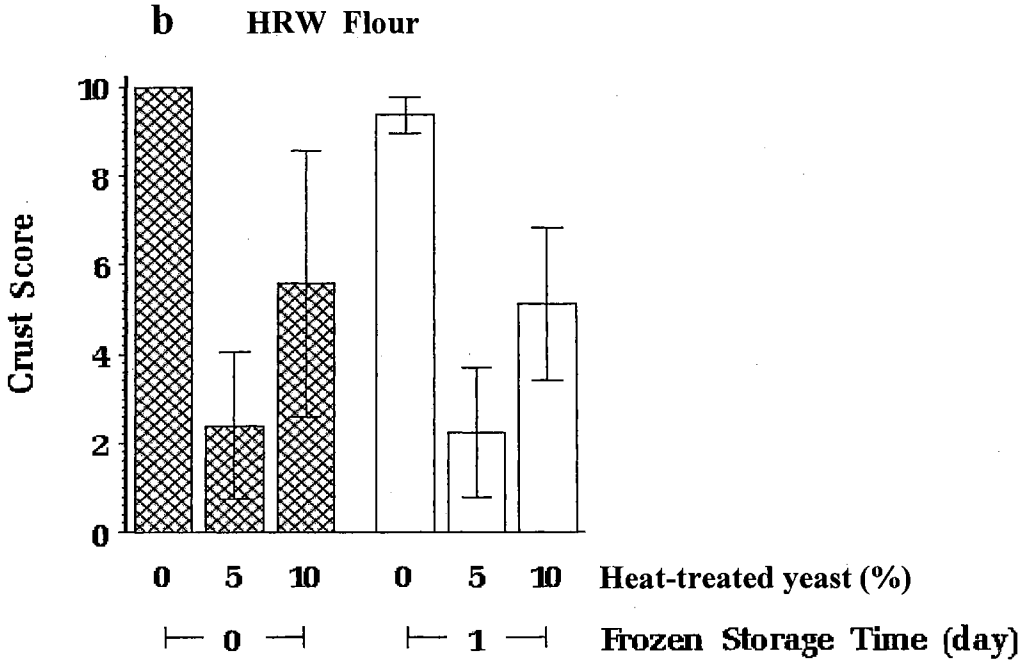
[b] Acidified Potato Dextrose agar.

[c] 25% Compressed yeast suspension without heat treated.

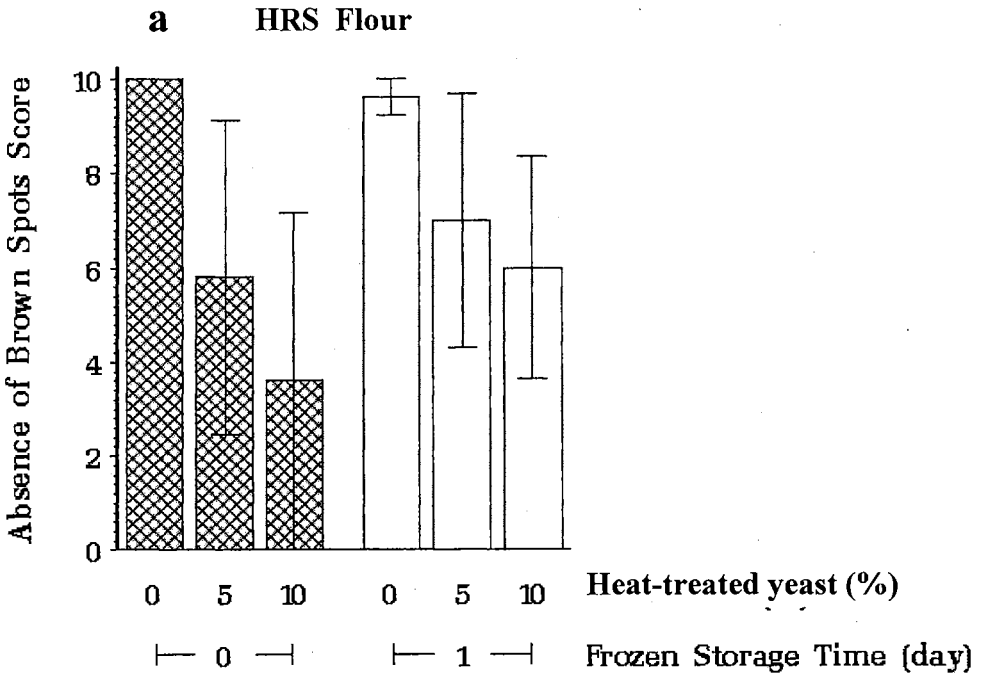
[d] 25% Compressed yeast suspension with heat treated at 50°C for 18 min.

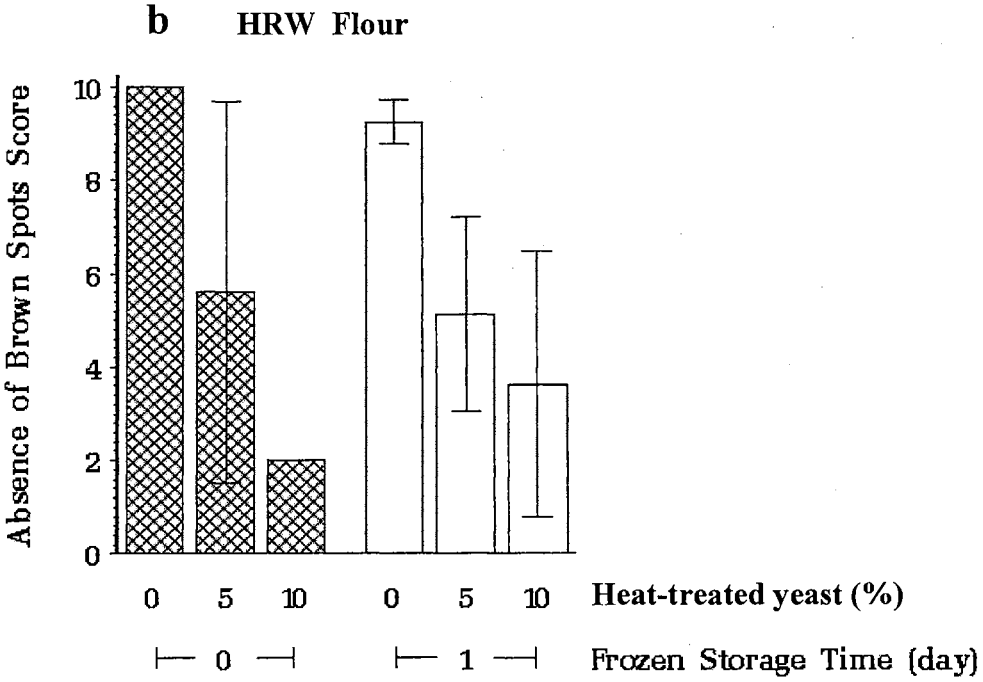
APPENDIX AK
CRUST SCORE OF BREAD STICKS AS A FUNCTION OF HEAT-TREATED YEAST ADDITION OF FRESH AND 1 DAY STORED FROZEN DOUGH. HARD RED SPRING (HRS) (a) AND HARD RED WINTER (HRW) FLOUR (b)





APPENDIX AL
ABSENCE OF BROWN SPOTS SCORE OF BREAD STICKS AS A FUNCTION OF HEAT-TREATEDYEAST ADDITION OF FRESH AND 1 DAY STORED FROZEN DOUGH. HARD RED SPRING (HRS) (a) AND HARD RED WINTER (HRW) FLOUR (b)





APPENDIX AM
MICRO-EXTENSIBILITY OF 0 AND 1 DAY FROZEN DOUGH OF CONTROL, WITH ADDITION OF HEAT-TREATED YEAST AND ADDITION OF GLUTATHIONE (GSH) IN THE DOUGH[a]

Treatments	Storage time	Rest period (min)	Rmax	E	A	R20mm	Rmax/E
Control	0 day	0	63.6	42.4	953.4	8.1	2.1
		45	69.7	39.6	785.6	6.3	2.5
		90	61.6	38.4	698.9	9.2	2.3
	1 day frozen	0	67.9	41.6	972.2	18.5	1.8
		45	74.4	38.6	839.0	13.9	2.0
		90	65.4	35.1	771.4	17.0	2.0
Heat-treated[b]	0 day	0	58.7	54.5	2494.7	29.9	1.2
yeast, 5%		45	61.9	55.4	2571.4	28.1	1.2
		90	46.6	53.8	1903.1	19.5	0.9
	1 day frozen	0	40.5	91.9	2697.8	15.1	0.5
		45	60.4	54.1	2431.3	27.5	1.1
		90	69.5	48.0	2703.3	33.2	1.5
Heat-treated	0 day	0	58.7	51.1	2256.3	34.9	1.2
yeast, 10%		45	53.7	56.0	2582.8	27.7	1.0
		90	61.1	50.0	2545.4	32.2	1.3
	1 day frozen	0	32.8	83.9	2628.4	15.7	0.4
		45	52.6	57.7	2615.3	25.6	0.9
		90	62.9	52.0	3084.0	34.8	1.2
GSH[c]	0 day	0	63.1	40.3	1306.4	20.7	1.0
80 ppm,		45	47.0	41.5	1068.7	9.4	0.3
		90	41.6	44.6	1044.1	7.7	0.1
	1 day frozen	0	51.8	44.4	1297.3	12.4	1.7
		45	40.5	43.2	1001.3	9.6	1.5
		90	40.8	42.9	1001.6	11.1	1.5
GSH,	0 day	0	61.5	36.8	1430.4	26.5	1.8
160 ppm		45	43.4	35.0	351.0	7.4	1.4
		90	49.7	35.3	960.7	19.7	1.6
	1 day frozen	0	42.0	35.8	1065.1	23.0	1.3
		45	28.2	43.5	864.6	11.7	0.7
		90	35.6	42.6	1065.6	17.3	0.9
GSH,	0 day	0	41.4	46.7	1258.9	8.3	0.9
240 ppm		45	28.3	48.1	858.0	4.9	0.7
		90	32.1	45.1	905.8	6.9	0.8
	1 day frozen	0	38.9	45.1	996.1	7.1	1.5
		45	27.0	50.2	793.2	5.7	1.2
		90	25.1	48.7	739.4	6.8	1.2

[a] Values are means of 14 measurements. Analysis was done in two independent batches of hard red spring wheat flour with two subsamples per batches.

[b] Yeast suspension was heated at 50°C for 18 min.

[c] GSH = Glutathione reduced form.

VITA

Juntanee Uriyapongson

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE EFFECTS OF ADDITIVES ON DOUGH RHEOLOGICAL PROPERTIES OF PRE-PROOFED FROZEN DOUGH AND BAKING QUALITY OF BREAD STICKS

Major Field: Food Science

Biographical:

Education: Received Bachelor of Science degree in Agronomy from Kasetsart University, Bangkok, Thailand in June 1983; received Master of Science degree with a major in Cereal Science, North Dakota State University in May 1994. Completed the requirements for the Doctor of Philosophy with major of Food Science, Oklahoma State University in May 2002.

Experience: Agricultural Researcher (1983-1989), Khon Kaen Field Crop Research Center, Khon Kaen, Thailand; Department Chair (1996-1997), Associate Chair (1995-1996), lecturer (1994-1997), Department of Food Technology, Khon Kaen University (KKU), Thailand.

Professional Memberships: Institute of Food Technologists (IFT), American Association of Cereal Chemists (AACC).

Honors and Awards: Honored with the Independent Reserved Peace Medal from Ministry of Interior of Thailand,1984; recipient, Research Assistant Scholarship of Cereal Science, North Dakota State University, 1991-1994; recipient, Scholarship of Food Science from the Royal Thai Government, 1997-2002.