ผลของอัตราการแช่เยือกแข็งและการเปลี่ยนแปลงอุณหภูมิการเก็บรักษาต่อคุณภาพของขนมปังพร้อมอบที่แช่เยือกแข็งและการประยุกต์เครือข่ายประสาทเทียมในการทำนายคุณภาพ

นายยุทธนา พิมลศิริผล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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EFFECTS OF FREEZING RATE AND FLUCTUATING STORAGE TEMPERATURE ON QUALITY OF FROZEN BREAD DOUGH AND APPLICATIONS OF ARTIFICIAL NEURAL NETWORK FOR QUALITY PREDICTION

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy Program in Food Technology

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ยุทธนา พิมลศิริผล: ผลของอัตราการแช่เยือกแข็งและการเปลี่ยนแปลงอุณหภูมิการเก็บรักษาต่อคุณภาพขนมปังพร้อมอบที่แช่เยือกแข็งและการประยุกต์เครือข่ายประสาทเทียมในการทำนายคุณภาพ (EFFECTS OF FREEZING RATE AND FLUCTUATING STORAGE TEMPERATURE ON QUALITY OF FROZEN BREAD DOUGH AND APPLICATIONS OF ARTIFICIAL NEURAL NETWORK FOR QUALITY PREDICTION)
อ. ที่ปรึกษา: ผศ. ดร. อุบลรัตน์ สิริภัทราวรรณ, อ. ที่ปรึกษาร่วม: Prof. Donald J. Cleland, Ph.D., รศ. ดร. วรรณา ตุลยธัญ, 247 หน้า

งานวิจัยนี้ศึกษาผลของอัตราการแช่เยือกแข็ง อุณหภูมิการเก็บรักษา และการเปลี่ยนแปลงอุณหภูมิระหว่างการเก็บรักษาของโดและขนมปังพร้อมอบแช่เยือกแข็ง และการประยุกต์ใช้เครือข่ายประสาทเทียมในการทำนายคุณภาพ โดยมีคุณภาพที่ศึกษาได้แก่ การสูญเสียน้ำหนัก อัตราการสร้างแก๊สคาร์บอนไดออกไซด์ จำนวนยีสต์ที่เหลือรอด วิทยากระแสของโด โครงสร้างของโด การเปลี่ยนแปลงน้ำในโด ปริมาตรของขนมปัง ความแน่นเนื้อของขนมปัง และลักษณะโครงสร้างของขนมปัง โดยศึกษาการเก็บรักษาที่อุณหภูมิคงที่ต่างกัน 2 ชุดการทดลอง (-10, -15, -20 และ -25 องศาเซลเซียส และ -8, -13, -18 และ -23 องศาเซลเซียส) และอุณหภูมิการเก็บรักษาที่จำลองภาวะการเก็บรักษาที่อุณหภูมิคงที่ (±0.1 องศาเซลเซียส) ดี (±1 องศาเซลเซียส) ไม่ดี (±3 องศาเซลเซียส) และไม่ดีมากที่สุด (±5 องศาเซลเซียส) รวมถึงช่วงอุณหภูมิที่จำลองการเปลี่ยนแปลงที่เกิดขึ้นจริงในระหว่างการเก็บรักษาและจัดจำหน่าย (เพิ่มขึ้นถึง 10 องศาเซลเซียส) จากผลการทดลองพบว่า ทั้งอัตราการแช่เยือกแข็งและการเก็บรักษาในภาวะแช่แข็งมีผลทำให้คุณภาพของโดและขนมปังลดลง อัตราการแช่เยือกแข็งที่ช้ามีผลทำให้คุณภาพโดและขนมปังที่ได้ดีกว่าอัตราการแช่เยือกแข็งที่เร็ว คุณภาพของโดและขนมปังลดลงเมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้น การสูญเสียของน้ำหนักโด การสร้างแก๊สคาร์บอนไดออกไซด์ และปริมาตรของขนมปังสามารถใช้เป็นดัชนีบ่งชี้คุณภาพโดยรวมของโดและขนมปัง (มีความแปรปรวนน้อยและสัมพันธ์กับการวัดค่าทางประสาทสัมผัส) การสูญเสียของน้ำหนักโด และความสามารถในการสร้างแก๊สคาร์บอนไดออกไซด์ระหว่างการเก็บรักษาสามารถอธิบายด้วยสมการจลนพลศาสตร์ของปฏิกิริยาลำดับศูนย์ การสูญเสียน้ำหนักของโดในระหว่างการเก็บรักษาที่อุณหภูมิคงที่เป็นสัดส่วนกับความดันไอของน้ำ ซึ่งสอดคล้องกับทฤษฎีการระเหยของน้ำที่มีผลต่อการสูญเสียน้ำหนักในอาหารที่หีบห่อ อุณหภูมิการเก็บรักษาที่มีช่วงการเปลี่ยนแปลงมาก (±3 ถึง ±5 องศาเซลเซียส) และการเก็บในภาวะที่มีการเปลี่ยนแปลงอุณหภูมิขึ้นๆ ลงๆ มีผลทำให้คุณภาพของโดและขนมปังลดลงมากกว่าการเก็บที่อุณหภูมิคงที่ หรือที่อุณหภูมิต่ำกว่า ดังนั้นการเปลี่ยนแปลงอุณหภูมิการเก็บรักษาควรน้อยกว่า ±3 องศาเซลเซียส เครือข่ายประสาทเทียมสามารถทำนายการสูญเสียน้ำหนักได้ดี (R^2>0.98) โดยสามารถทำนายการลดลงของปริมาตรของขนมปังได้ค่อนข้างดี (R^2>0.7) แต่สามารถทำนายการลดลงของแก๊สคาร์บอนไดออกไซด์ได้ไม่ดี (R^2<0.5) และต้องการข้อมูลในการฝึกหัดให้ครอบคลุมทุกภาวะที่ศึกษา

ภาควิชาเทคโนโลยีทางอาหาร ลายมือชื่อนิสิต .....
สาขาวิชาเทคโนโลยีทางอาหาร ลายมือชื่ออาจารย์ที่ปรึกษา.....
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YUTHANA PHIMOLSIRIPOL: EFFECTS OF FREEZING RATE AND FLUCTUATING STORAGE TEMPERATURE ON QUALITY OF FROZEN BREAD DOUGH AND APPLICATIONS OF ARTIFICIAL NEURAL NETWORK FOR QUALITY PREDICTION.
THESIS ADVISOR: ASST. PROF. UBONRAT SIRIPATRAWAN, Ph.D., THESIS CO-ADVISOR: PROF. DONALD JOHN CLELAND, Ph.D. AND ASSOC. PROF. VANNA TULYATHAN, Ph.D., 247 pp.

This study investigated the effects of freezing rates, storage temperature and temperature fluctuations during frozen storage on frozen dough and bread quality. Quality changes were quantified by measuring dough weight loss, CO_2 production rate, yeast viability, dough rheological properties, dough microstructure, dough water mobility (NMR), bread specific volume, bread crumb firmness and bread crumb characteristic. Two sets of quality kinetic data were measured at constant storage temperatures (-10°C, -15°C, -20°C and -25°C and -8°C, -13°C, -18°C and -23°C). Fluctuating storage regimes represented constant conditions (±0.1°C) good (±1.0°C), poor (±3.0°C) and very poor (±5°C) storage temperature control. Another regime mimiced temperature changes likely to be experienced in the cold chain (increases by up to 10°C). Both freezing rate and subsequent frozen storage had a significant deteriorative effect on all quality parameters. Slow freezing gave significantly better dough and bread quality than fast freezing. All quality parameters deteriorated further with increasing storage duration. Weight loss, CO_2 production and bread specific volume were the most useful indicators of overall quality (low variability and correlated to sensory measures). Weight loss and decline in CO_2 production during storage were adequately described by zero-order reaction kinetics. Weight loss during constant temperature storage was proportional to water vapor pressure consistent with the standard theory for evaporative weight loss from packaged foods. Large temperature fluctuations during frozen storage of ±3 to ±5°C and intermittent storage at higher temperatures resulted in significantly more rapid loss of dough and bread quality than storage at constant and/or lower temperatures. It is recommended that temperature variations should be less than ±3°C. An artificial neural network achieved a good fit (R^2>0.98) between experimental and predicted data for weight loss prediction and seem to be promising for predicting bread specific volume loss (R^2>0.7) but gave a poor fit (R^2<0.5) for CO_2 production and requires training data across the full range of conditions of interest.

Department.....Food Technology.....	Student's signature.....
Field of study.....Food Technology.....	Advisor's signature.....
Academic year.....2007.....	Co-advisor's signature.....

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NOMENCLATURE

a	network output
a_w	the water activity of product
A	constant in the Arrhenius law (%/day)
A	the amplitude for half the range of maximum and minimum temperatures (^oC)
A_p	the product surface area (m^2)
A_p	the fraction of protons of a fast decay component attributed to water protons in the ice (%)
A_s	the fraction of protons of a slow decay component attributed to non-frozen water protons (%)
$b(T)$	the temperature-dependent coefficient (location factor)
B	one quarter of the period of fluctuation (min)
C_1	the universal constant for WLF model
C_2	the universal constant for WLF model
C'_1	the system-dependent coefficient obtained from WLF fitting
C'_2	the system-dependent coefficient obtained from WLF fitting
C	the quality retention at time t or measured quality value (%)
C_0	the initial quality retention (%)
C_{eq}	the equilibrium quality retention (%)
E_a	the Arrhenius activation energy (kJ/mol)
G'	shear storage modulus (Pa)
H_r	the air relative humidity (%)
k	rate constant depending on temperature (%/day)
k	the process's acceleration beyond T_c
k_{av}	the average reaction rate calculated from the isothermal approach or the integrated approach (%/day)
k_g	the reaction rate at the glass transition temperature (%/day)
k_{gp}	the mass transfer coefficient between air and product ($kg/m^2s.Pa$)
k_{ref}	the kinetic rate at the reference temperature T_{ref} (%/day)
n	a fixed averaged or representative power
n_r	a reaction order
n_s	the slope

$n(T)$	temperature-dependent coefficient (the distribution's shape factor and the reciprocal of, $b(T)$, its location factor
N	number of samples
N_w	the rate of weight loss (kg/s)
P_{wa}	vapour pressure of water/ice at air temperature T_a (Pa)
p_{wp}	vapour pressure of water/ice at product temperature T_p (Pa)
Q	the quality characteristic (%)
Q_{10}	the ratio of reaction rate at temperatures 10°C different
Q_A	the ratio of the reaction rate at mean temperature plus the amplitude ($T_0 + A$) to that at T_0
Q_{max}	maximum value determined from the experiment (%)
Q_{min}	minimum value (%)
$Q_{(t)}$	the quality characteristic value at any time t (%)
R	the universal gas constant (J/mol.K)
$\overline{R}$	the average rate of reaction during this period is a function of the rate (%/min)
R^2	regression coefficient of determination
R_0	the rate reaction at the mean temperature (%/min)
R_{2p}	the relaxation rate of a fast decay component attributed to water protons in the Ice (ms)
R_{2s}	the relaxation rate of a slow decay component attributed to non-frozen water Protons (ms)
$S(t)$	sum of two exponential curves of the relaxation at time t
t	targets
t	time (min or day)
T	temperature at time t (°C or K)
T_0	the mean temperature (°C)
T_a	air temperature (°C)
T_{av}	the average temperature (°C)
T_c	the temperature range in which the degradation intensifies (°C)
T_e	the effective temperature (°C)
T_g	glass transition temperature (°C)
T_p	product temperature (°C)

T_{ref} the absolute reference temperature (K)

T_1 spin-lattice relaxation time (ms)

T_2 spin-spin relaxation time (ms)

α a dimensionless quantity which expresses the extent to which the effective temperature exceeds the mean temperature in relation to the amplitude of the cycle

CHAPTER I

INTRODUCTION

Frozen food is one of the largest and most dynamic sections of the food industry (Mallet, 1993). Rapid increases in sales of frozen foods in recent years are closely associated with increased ownership of domestic freezers and microwave ovens. Frozen foods and chilled foods consistently outsell canned or dried products due to their perceived fresh quality (Fellows, 2000). The range of frozen foods is becoming more diverse and many items are now multi-component (Shaevel, 1996).

Baked goods are highly perishable and their attractiveness declines rapidly within a few hours of being taken from the oven. Freezing is the best-known preservation method that will significantly retard any changes in quality, and the market for frozen bakery goods has grown rapidly (Stauffer, 1993a). Demand and market opportunities for value-added wheat-based products have been growing rapidly. The frozen dough market has steadily grown in recent years due to consumer demand for convenience and high quality baked products (Bhattacharya, Langstaff, and Berzonsky, 2003). Use of frozen dough has increased in bakeries, supermarkets and restaurants all over the world (Laaksonen and Roos, 2000). The rise in popularity of frozen baked goods has been driven mainly by the economic attractions of centralized manufacturing and distribution (Best, 1995). Frozen bakery products are characterized by quick preparation time and affordable price (Giannou, Kessoglou, and Tzia, 2003). The frozen dough method considerably reduces the labor content and cost of breadmaking by separating a long process into dough preparing and bread baking processes (Naito et al., 2004).

Quality of frozen foods is generally highest before freezing. The freezing process, temperature level during storage, storage duration, and thawing conditions are important with regard to the final quality of a frozen food (Lebail et al., 1999). The quality losses in most frozen foods are much slower in lower temperature storage (Martino and Zarizky, 1988; Martins and Silva, 2003). Chemical and biochemical reactions occurring in the product strongly depend on the time-temperature history

(Fennema, 1973; Labuza, 1980; Martins and Silva, 2003). It has been postulated that temperature fluctuations during storage and distribution cause increased rates of frozen food quality deterioration, particularly due to changes in structure of ice crystals (Mazur and Schmidt, 1968; Berglund, Shelton, and Freeman, 1991; Gormley et al., 2002). More frozen dough quality is damaged during transportation (in trucks with inadequate refrigeration capacity) and in the bakery (by improper handling or storage) than at any other stage of the process. Temperature fluctuations have been shown to lead to accelerated quality loss of dough and bread (Stauffer, 1993b).

For dough and bread, quality parameters that are affected by freezing and frozen storage include moisture location, carbon dioxide production, yeast viability, rheological properties, dough microstructure, loaf volume and crumb texture. Formation and growth of ice crystals during freezing and frozen storage is one of the major causes of quality losses due to water migration and changes to rheological properties and microstructure, which results in a loss of gas-retaining capacity and gas production. After baking, loaf specific volume, bread textural properties and bread characteristics indicate the relationship between dough quality and bread quality. Bread qualities such as loaf specific volume, textural properties and bread characteristics are the final quality criteria for consumer acceptance (Hsu, Hoseney, and Seib, 1979a; Autio and Sinda, 1992; Havet, Mankai, and Le Bail, 2000; Zounis et al., 2002b; Sharadanant and Khan, 2003b). Because of the relationships between these quality parameters, several techniques have been used to assess dough and baked bread quality.

Many researchers have investigated the effect of freeze-thaw cycle on frozen bread dough quality. However, temperature fluctuations in the coldstore and cold chain distribution are not the same as full freeze-thaw cycles. Also, there are limited data for quality kinetics and the effect of temperature fluctuations on frozen bread dough. Therefore, the overall objective of this research was to measure basic quality kinetic data for frozen dough under constant temperature conditions and to evaluate the effect of fluctuating/oscillating conditions during storage on the quality of frozen bread dough.

CHAPTER II

LITERATURE REVIEW

2.1 FROZEN BREAD DOUGH

Bread is one of the most common staple foods. Bread products have evolved to take many forms, each based on quite different and very distinctive characteristics. However, bread products have a very short shelf life. Their quality is highly dependent on the period of time between baking and consumption (Barcenas et al., 2003). The use of frozen bread dough by retail bakers for production of fresh bread, rolls and pastries offers economic advantages and convenience. These include cost and labor reduction, high product turnover and preparation time reduction (Best, 1995).

Improvements in frozen dough quality have made its use increasingly attractive. Basing a successful bakery operation on frozen doughs requires, first of all, good quality in the dough itself. The prime factor is gas production and retention by the dough. High quality dough has adequate ovenspring and small pores in the internal crumb structure that are maintained during storage for several weeks in the frozen state (Stauffer, 1993b). Berglund et al. (1991) stated that frozen bread dough is used by more than 50% of in-store supermarket bakeries as well as by retail customers. Many types of bakery products are prepared from frozen dough. Good quality frozen dough should provide at least 3 months storage life, depending on the freezing and frozen storage conditions (Jackel, 1991). Dough strength and frozen storage play an important role in the quality of bread produced from frozen dough, since dough must withstand harsh freezing and thawing conditions (Bhattacharya et al., 2003). Another major problem is the loss of quality that results from mishandling in transportation and storage (Berglund et al., 1991). The development of frozen dough in terms of the production and formulation of products has led to an improvement in the quality (Ribotta, Leon and Anon., 2003).

2.2 BASIC INGREDIENTS OF BAKERY PRODUCTS

2.2.1 FLOUR

Flour is the most important ingredient in bakery products because it modulates the specific characteristics of bakery products. It consists of protein, starch and other carbohydrates, ash, fibers, lipids, water and small amounts of vitamins, minerals and enzymes (Charley and Weaver, 1998). Wheat flour is the most common flour used. Wheat is unique among cereal grains as flour milled from it provides a light, palatable, well-risen loaf of bread when processed into fermented dough (Bushuk and Rasper, 1994).

The two basic types of protein which wheat flour contains are gliadin and glutenin. When wetted separately these proteins present totally different behavior. Gliadin forms a viscous liquid and is sticky and inelastic. Glutenin forms a rubbery material and is more elastic and tenacious (Singh and MacRitchie, 2001). When their mixture, which is called gluten, is wetted during the preparation of dough, they form a cohesive and elastic network. Gluten gives dough the ability to form thin sheets that will stretch and hold gas (Bushuk and Rasper, 1994). The peculiar viscoelastic properties of wheat dough are a result of the presence of three dimensional network of gluten proteins, which is formed by thiol-disulfide exchange reactions among gluten proteins (DeMan, 1990). The factors that determine wheat type are hardness, gluten strength and protein content. Even relatively poor quality wheat can produce bread that is significantly more palatable than that made from flour from other cereal grains. Generally, hard wheat with strong gluten and high-protein content is preferred in breadmaking (Bushuk and Rasper, 1994). Typical white flour protein content would be 12% or greater (Cauvain, 1998a).

Although strong gluten wheats are recommended for frozen dough production, wheat cultivars exhibiting overly strong gluten characteristics may not provide desirable frozen dough baking performance. Lu and Grant (1999b) studied the effects of different flour fractions on frozen dough quality. The fractions from the strong gluten flour had a positive impact on frozen dough baking performance, whereas the

weak gluten flour fractions had a negative impact. Glutenin plays a predominant role in frozen dough quality. The effects of the gliadin and starch fractions on frozen dough quality are also significant, however not as strong as those observed for the glutenin fraction. The effect of the water-soluble fractions is small but positive. The contribution of each flour fraction to frozen dough quality depends on the interactions between the fractions and other flour components.

2.2.2 YEAST

Saccharomyces cerevisiae is the most common yeast used in breadmaking. Yeast cells metabolize fermentable sugars (glucose, fructose, sucrose and maltose) under anaerobic conditions producing carbon dioxide. They act as a leavening agent and enhance dough volume. Yeast also supports both gluten network development and aromatic compound production. Active cells of yeast are available as a compressed cake or in dried form. The compressed cake contains approximately 70% moisture so it is highly perishable unless it is refrigerated. Active dry yeast is produced by extruding cake yeast in fine strands, which are dried to low moisture content. Instant yeast is made from more active strains of yeast and dried faster and to a lower level of moisture. Although active dry yeast has a long shelf life at room temperature, it must be hydrated before it is incorporated with other ingredients. In contrast, instant yeast can be incorporated with flour and other ingredients without prior hydration (Charley and Weaver, 1998).

At the present time, a good grade of regular baker's compressed yeast (30% solid) is considered the best choice for making frozen yeast-raised dough. Instant dry yeast (95% solid) performs poorly satisfaction because fermentation after thawing of the dough is considerably slower. The cool dough temperature inhibits rehydration of the dry yeast cells. Dry yeast must be prehydrated, and this produces fermentation compounds before freezing (Casey and Foy, 1995). Yeast levels in frozen dough formulations are higher than for the same doughs intended for immediate baking. The higher initial concentration of yeast cells compensates for the inevitable partial loss of fermentation capability during freezing and storage (Stauffer, 1993b).

Autio and Mattila-Sandholm (1992) indicated that freezing and frozen storage cause loss of yeast activity and delay in the gas production. During freezing ice crystals first form in the aqueous phase surrounding yeast cells, and subsequently in the cytoplasm (internal aqueous phase) of the cells. The ice crystals may physically damage the outer membrane of the cell, causing it to lose the cytoplasm contents and die but water migration from the cell due to the extracellular freeze concentration may also be significant (Mazur and Schmidt, 1968; Mazur, 1970). Dormant cells have a thicker membrane than activated cells and also are more resistant to the damage (Autio and Sinda, 1992).

2.2.3 WATER

Water is necessary for the formation of dough and is responsible for its fluidity. It is used for the dissolution of salt and sugars and assists the dispersion of yeast cells. It is the medium for food transportation to the yeast through cell membranes. Water is needed for starch and sucrose hydrolysis. It is important for starch gelatinization during baking and contributes to oven spring through vaporization. The water added to the flour activates enzymes, brings about the formation of new bonds between the macromolecules in the flour, and alters the rheological properties of dough. The amount of added water is related to the moisture content and the physicochemical properties of the flour (Gil, Callejo and Rodríguez, 1997).

Water, an important structural and chemical component of frozen dough, plays a significant role in frozen dough quality. Freezable water is the water that can form ice in a dough system when subjected to freezing and frozen storage (Davies and Webb, 1969). Ice crystallization and recrystallization are closely related to water movement in the dough during freezing, frozen storage and transportation of the frozen dough to bakery outlets (Kulp, 1995). A major part of the water is bound to starch surface-gluten matrix and is affected by dough processing and storage. During frozen storage, the water separates from the gluten and crystallizes. At prolonged storage times, large ice crystals also are formed in the gas cells. During thawing of frozen dough, the water does not return to its original state in the gluten matrix. The

gluten can be damaged due to the changing of water distribution (Esselink et al., 2003).

2.2.4 SUGAR

Sugar is normally used by yeast during the early stages of fermentation. Later more sugars are released for gas production by the action of enzymes in the flour. In some cases, extra sugar may be added to increase gas production, to improve the crust color and to sweeten the bread. Sugars also act as antiplasticizers retarding pasting of native starch or function as anti-staling ingredients inhibiting starch recrystallization (Faridi and Faubion, 1990). The quantity of sugar affects bread baking in terms of flavor, texture and product shelf life (Williams and Pullen, 1998). Sugar has retarding effect on yeast activity because it increases the osmotic pressure of the dough liquid phase and extra yeast must be added in direct proportion to the additional sugar to ensure adequate gas production (Brown, 1993).

2.2.5 SALT

Salt is considered as an ingredient with a functional role in the production of many bakery products. Salt strengthens the gluten, controls the action of yeast and thus controls the loaf volume. A small amount of salt in dough improves flavor and favors the action of amylases to maintain a supply of maltose which is food for the yeast (Wood, 1989). Salt inhibits the action of flour proteases, which otherwise would depolymerize proteins from the gluten complex. Yeast dough without salt is sticky and difficult to manipulate. In frozen dough products, salt slows the production of carbon dioxide by the yeast delaying their fermentation (Charley and Weaver, 1998). The normal quantity of salt addition is about 2% of flour weight. However, this level can be reduced to 1% when sugar is included (Williams and Pullen, 1998).

2.2.6 LIPIDS

Lipids can be used in bakery products either in the form of fats or oils and are usually referred to as shortening. They are an optional ingredient in bread but can improve dough handling and crumb appearance and contribute to product flavor (Stauffer, 1999). Lipids also improve the keeping quality, softness and moistness and contribute to bread texture. Both endogenous lipids and added fats are known to play an important role during breadmaking and staling of bread (Collar, Armero and Martinez, 1998). Lipids embedded into the protein matrix are essential as they interact with proteins during dough mixing and contribute to the viscoelastic properties of the gluten network required for expansion and gas retention during proofing (Demiralp, Celik and Koksel, 2000). Incorporation of lipids into bread dough results in a larger final loaf volume, a less crisp crust and improved keeping quality of bread (Autio and Laurikainen, 1997).

2.3 PROCESSING OF FROZEN BAKERY PRODUCT

The basic steps in the production of frozen bakery products are described and analyzed below.

2.3.1 MIXING

Dough is produced when all ingredients of the formula are mixed together for a certain period of time. The major purposes of mixing are to blend the ingredients into a quasi-homogeneous mixture, to develop the gluten matrix in wheat dough, and to incorporate air (Autio and Laurikainen, 1997).

In the first step of mixing, proteins are hydrated, and then during subsequent mixing they interact with each other. In addition to protein interaction, other flour components (e.g. lipids, salts, non-starch polysaccharides, and starch) also participate in the formation of the gluten matrix. The viscoelastic properties of doughs are primarily the result of a continuous protein phase that, in fully developed dough, surrounds the starch granules. The chemical bonds that stabilize gluten proteins in

bread doughs are covalent and secondary bonds. The covalent bonds are disulfide bonds which form inter- and intramolecular crossbonds in the proteins during dough formation by the sulfide-disulfide interchange. The secondary bonds are hydrogen, hydrophilic, hydrophobic, and ionic bonds and polar interactions (Dobraszczyk and Morgenstern, 2003). If dough is undermixed, starch and proteins are unevenly distributed, and compact protein masses are stretched out into sheets during mixing (Autio and Laurikainen, 1997). When dough is overmixed, gluten proteins become stressed, some disulfide bonds are broken to form thiyl radicals and gluten proteins are partially depolymerized resulting in greater solubility and decreased extractability of lipids (Demiralp et al., 2000). Overmixing usually results in a sticky dough, partly because the mechanical forces applied to the dough decrease the molecular weight of the protein (Autio and Laurikainen, 1997). Prolonged mixing can enhance the effects of oxidants on disaggregation of large protein aggregates, probably because of oxidation of more SH groups (Demiralp et al., 2000).

In order for dough to obtain the desirable structure, flour should be mixed with the required amount of water. When too little water is used, the transformation of starch into gel cannot be achieved successfully. As a result, the crumb dries and stales quicker. On the contrary, when excess water is used, it is not entirely constrained during starch gelatinization. Too much water in the recipe results in the crumb being moist and sticky. The water holding capacity of flour depends on its type, origin and other properties. Flour that has been properly stored has better holding capacity than freshly milled flour (Faridi and Faubion, 1990).

Mixing conditions are important as well. Mixing should be quick, homogenous and temperature controlled (Faridi and Faubion, 1990). After mixing, dough contains occluded gas cells whose diameters are typically in the range of 10-100 mm. The number and size of the gas bubbles have a great effect on the final bread characteristics. High-speed mixers with blades that shear the dough produce large numbers of small bubbles and result in a fine-structured bread. On the other hand, low-speed mixers, such as spiral-type mixers, occlude more air but result in an uneven pore size distribution (Autio and Laurikainen, 1997). Charun et al. (2000) stated that the number or volume of air bubbles in the dough decreased when the mixing

temperature increased. It is widely accepted that a dough temperature in the range of 18-20^{o}C is needed after mixing to achieve yeast stability (Neyreneuf and Van Der Plaat, 1991).

Since ionic strength increases with salt addition, water absorption between flour and water decreases. In the absence of salt, the gluten in dough develops more rapidly than when the salt is present from the beginning of the mixing process. To shorten the time for full mixing of the dough, the salt may be added when the gluten has fully developed. Mixing time may be reduced by as much as 25% (Stauffer, 1993b).

Rouille, Le Bail and Courcoux (2000) studied the influence of ascorbic acid, alpha amylase with hemicellulase activity and mixing time on bread making qualities of frozen French bread dough. The bread stick length, dough volume, gas volume during proofing and the bread volume were measured. They concluded that mixing time was the most influential factor. Longer mixing time resulted in pieces with larger volume.

2.3.2 MOLDING

After mixing, dough is divided into pieces of certain weight and is molded to obtain the desired shape. Dividing and molding modify the structure of gas cells as they induce coalesce of small cells into larger ones and subsequently contribute to the final development of the gluten network (Autio and Laurikainen, 1997). In regard to frozen bakery products, dough shape is influential on their stability and final quality. Furthermore, excessive molding can cause heat generation and enhance fermentation prior to freezing (Singh and MacRitchie, 2001).

2.3.3 PACKAGING

Packaging materials and shapes vary according to products specifications. Materials usually applied to frozen bakery products are plastic (films, membranes, etc.) and aluminum (Matz, 1989). Films used for frozen dough should possess the

following characteristics: good moisture and oxygen barrier characteristics, physical strength against brittleness and breakage at low temperature, stiffness to work on automatic machinery, and good heat sealability (Brown, 1992). Many packaging materials used for frozen dough products are based on co-extrusions of ethylene vinyl acetate (EVA) and linear low density polyethylene (LLDPE) or polymer blends of LLDPE with high density polyethylene. LLDPE is less clear and more expensive than low density polyethylene (LDPE) (Varriano-Marston, 1995).

2.3.4 FREEZING

Dough pieces, immediately after mixing or after a short fermentation period, are frozen and then stored at the appropriate temperature. Freezing technology can be categorized as mechanical (air-blast, plate, spiral, impingent, immersion, belt or fluidized bed freezers) or cryogenic. The selection of the technology is most commonly based on product requirements, availability and cost. Freezing, as a method of preservation and extension of a food product shelf life, involves mainly two processes. First, temperature reduction and second, phase transition from liquid water to ice (Gaman and Sherrington, 1990).

For most foods, it is known that the faster the freezing rate, the lower the degree of structural damage in frozen foods due to ice crystallization and to growth of ice crystals (Martino and Zaritzky, 1989; Roy, Taylor and Kramer, 2001). However, for dough too fast freezing rate causes loss of yeast viability. Yeast viability is correlated with the gas production after freezing and frozen storage (Autio and Sinda, 1992). Freezing and prolonged frozen storage influence dough properties by reducing gas retention and yeast cell viability. Loss of viability of cells upon freezing has been attributed to intracellular freezing and increased internal solute concentrations. Freezing and prolonged frozen storage effects are low pH, dehydration of the surface, ionic toxicity, damage to essential membrane processes, impairment of cytoskeletal elements and lowering the activities of glycolytic enzymes (Myers and Attfield, 1999).

Freezing damage is due to water movement across the cell membrane (e.g. cell membrane rupture, enzymes denature). With conventional freezing, the viability of organisms is enhanced as the freezing rate increases probably due to the diminished contacting time of the susceptible organisms with harmful high solute concentrations in the unfrozen water. With more rapid freezing rate, viability decreases probably due to the formation of internal ice crystals, which lead to destruction of the cell membranes. With extremely fast freezing rate, ice crystal formation is reduced and replaced by vitrification (Kulp, 1995). Forsythe and Hayes (1998) suggested that freezing dough at low rates (<2^{o}C/min) is preferable in order to get high yeast survival and good quality bread such as high specific loaf volume and firmness. Freezing causes negligible changes to pigments, flavors or nutritionally important components, although these may be lost during subsequent preparation processes or may deteriorate during frozen storage.

2.3.5 STORAGE

After freezing, the frozen dough is packaged, palletized and moved to the storage freezer. The storage freezer is usually maintained at -15^{o}C to -25^{o}C. The internal temperature equilibrates throughout the product over a period of 6 to 12 hours. When the dough pieces have been frozen, it is important to prevent internal temperature fluctuations. Even at frozen temperatures a certain amount of moisture migration is possible. The warm air that enters the freezer each time personnel enter to insert or remove product, temporarily raises the air temperature of the freezer space. The temperature gradient between the surface layer and the center of the dough pieces favors moisture migration and osmotic pressure changes. This causes a certain amount of yeast cell death (Stauffer, 1993b). The effects of freezing and frozen storage of dough will be further discussed in Section 2.4.4.

2.3.6 THAWING

Frozen doughs must be thawed before proofing. This process can be conducted under various time–temperature conditions. During thawing, it is important that the product traverses the rapid staling zone (-5^{o}C to 10^{o}C) as fast as possible. The

thawing process often resembles the freezing process, but with warm air (e.g. 40^{o}C) replacing refrigerated air (Stauffer, 1993a). Thawing is necessary for best performance of the dough as it involves rehydration of the system, mainly of gluten matrix and yeast cells by melting of ice (Lorenz and Kulp, 1995).

The effect of thawing on sensory quality of frozen parotta dough (FPD) and ready-to-bake frozen parotta dough (R-FPD) was determined by Indrani et al. (2002). Thawing conditions were at room temperature (1, 2 and 3 hours), in a microwave (1, 1.5 and 2 minutes) and in a refrigerator at 4^{o}C (8, 16 and 24 hours). The results indicated that the optimum thawing conditions for FPD or R-FPD was 1 minute using microwave or 16 hours in a refrigerator as the appearance of FPD and baked parotta were normal and the overall quality scores were higher than the other treatments.

Thawing can be performed either with a constant temperature or with stepwise increasing temperature. The second is more favorable for two reasons. Firstly, during thawing, condensation may occur on the dough surface, as the dough is colder than the surrounding air. A large difference in temperature between the dough surface and the surrounding air results in spotting and blistering of the crust especially after baking. A stepwise increase in temperature minimizes this effect. Secondly, excessively fast thawing raises the temperature in the outer regions of the dough, which becomes ready for proofing, while the center of the dough remains frozen. Retarder–proofer units are used for stepwise thawing and proofing of frozen dough. They allow the temperature of frozen dough pieces to be raised gradually and minimize the temperature differential within them (Kenny, Grau and Arendt, 2001).

2.3.7 PROOFING

Thawed dough pieces should be left to proof before baking either for a certain period of time or until they obtain the desired volume. Proofing is mainly attributed to the action of yeast, which contributes to many changes that are collectively termed dough maturing or ripening. Properly matured dough exhibits optimum rheological properties (optimum balance of extensibility and elasticity) as well as good machinability and produces bread with optimum volume and crumb characteristics.

During dough maturing yeast produce alcohol and carbon dioxide. Since alcohol is water-miscible, if appreciable amounts are formed it influences the colloidal nature of the flour proteins and alters the interfacial tension within the dough (Lorenz and Kulp, 1995).

Additionally, carbon dioxide partly dissolves in the aqueous phase of the dough and forms weak carbonic acid that lowers the pH of the system. Carbon dioxide also contributes to dough distension (Beuchat, 1987). Growth of gas cells depends in part on cell size. Greater pressure is needed to expand a small gas cell than a larger one, and it is possible that the smallest bubbles will not expand at all. Gas cell stabilization and gas retention are of considerable interest as they determine crumb structure and volume of wheat bread. In the case of frozen fermented doughs, gas cell structure significantly affects frozen storage stability. A dough that contains a large number of small bubbles with a narrow size distribution and thick walls will be more stable than a dough containing bubbles with less uniform size distribution and thin walls surrounding the larger bubbles (Autio and Laurikainen, 1997).

Proofing time for thawed frozen dough needs to be longer than that for conventional dough because frozen dough has lower initial dough temperature, lower gas retention power and lower yeast activity caused by the freezing process (Lorenz and Kulp, 1995).

2.3.8 BAKING

Baking is the last part of the breadmaking procedure. It results in a series of physical, chemical and biochemical changes in a bakery product, which include volume expansion, evaporation of water, formation of a porous structure, denaturation of protein, gelatinization of starch, crust formation and browning reactions, protein crosslinking, melting of fat crystals and their incorporation into the surface of air cells, rupture of gas cells and sometimes fragmentation of cell walls (Sablani, Baik and Marcotte, 2002). The most dramatic change at macroscopic level is the expansion of gas cells into an open network of pores (Autio and Laurikainen, 1997). During baking, the combination of gas production and evaporation together with the change in

rheological properties result in the loss of gas retention, turning the foam structure of dough into bread's open sponge structure with interconnected cells. The microstructure of flour is continuously modified during these processes until the structure of the final product is stabilized (Rojas et al., 2000). These changes are dependent on the temperature, humidity and duration of baking (Autio and Laurikainen, 1997).

The role of baking is to alter the sensory properties, to improve the palatability and to extend the range of tastes, aromas and textures in bread. Baking also destroys enzymes and microorganisms (Fellows, 2000). The flavor, especially of white bread, is mainly formed during fermentation and baking. Freshly baked bread has a delightful aroma that is rapidly lost on cooling and storage. During fermentation, a number of alcohols are formed (e.g. ethanol, n-propanol, isoamyl alcohols and phenol alcohol). However, most of these alcohols are lost to the oven air during baking. A large number of organic acids are also formed and several carbonyl compounds have been identified in bread, which are believed to be important flavor components. The formation of the crust and browning during baking appear to be primary contributors to the formation of bread flavor. The browning is mainly the result of Maillard-type browning reactions rather than of caramelization (DeMan, 1990). For frozen dough products, the baking process usually does not differ much from the conventional, especially when the dough is properly thawed.

2.4 FREEZING AND FROZEN STORAGE OF FOOD

2.4.1 EFFECTS OF TEMPERATURE AND STORAGE ON QUALITY OF FOOD

2.4.1.1 Effect of Freezing

The freezing process affects the quality of frozen foods in many ways. The methods of freezing affect freezing rate and freezing time in combination with the physical properties of foods such as the thermal properties. The important physical and thermal properties include density, water content, initial freezing point, latent heat

specific heat and thermal conductivity. Because of the large variations in thermal properties of food products, the theoretical calculations on freezing time are difficult (Reid, 1993).

Reynolds, Park and Choi (2002) investigated the effects of various freezing methods on the biochemical and physical properties of surimi. The freezing methods were divided into 3 methods (conventional plate freezing, fast freezing and freeze-drying). All the frozen samples were stored in a freezer at -18^{o}C. After 1 month of storage, the salt-extractable protein (SEP) of both conventional and fast frozen samples decreased approximately 10%, whereas the freeze-dried samples dropped approximately 50%.

Ngapo *et al*. (1999) studied the effect of freezing rate on ice crystals growth in pork using a cryo-scanning electron microscope. Cavities created after sublimation of the ice crystals were quantitatively analyzed using an image analysis software package. During the nucleation phase, crystal growth and nucleation occur simultaneously. Meat samples frozen in liquid N_2 showed cavities with cross-sectional areas about 10-fold smaller than those using the slower freezing rates. With liquid N_2 freezing, more than 90% of cavity sizes were less than $6x10^{-4}$ mm^2 while slow freezing rate had a bigger cavity size. Due to ice recrystallization during freezing and storage, drip loss appeared to reach a maximum and then plateau with storage time. These results suggest that there is a maximum crystal size formed during freezing in the meat system studied (Bevilacqua and Zaritzky, 1982).

Roy et al. (2001) investigated the effect of freezing rate on firmness retention and microstructural changes in the cell wall and middle lamella of carrot tissues. Baby-cut carrots cut into 1 cm^3 cubes were subjected to 4 freezing conditions to create temperature profiles with freezing rates of -0.05, -0.19, -2.4 and -4.5^{o}C/min. Freezing at the fastest rate retained 26.8% of the firmness of the control ($194x10^4$ N/m^2) when compared to 12.9% at -2.4^{o}C/min, 6.9% at -0.19^{o}C/min and 4.4% at -0.05^{o}C/min. Softening was further enhanced in blanched-frozen carrots. Severe

structural damage due to growing ice crystals and substantial loss of pectin material were found at slower freezing rate.

2.4.1.2 Effect of Thawing

Thawing can be divided into two methods depending upon whether heat is supplied to the frozen foodstuff by conduction or by electro-magnetic radiation. Martins and Silva (2004) investigated the effects of thawing at ambient and refrigeration temperatures on the quality profile of packaged frozen green beans using a simulation system based on object-oriented programming. Results emphasized that the principle of high-temperature-short-times is not directly applicable for thawing of frozen green beans. Furthermore, simulations led to the conclusion that the overall quality profile is maximized by thawing under refrigeration temperatures.

2.4.1.3 Effect of Storage Temperature

The effects of storage temperature have been studied by several researchers. Boggs et al. (1960) observed the quality change of frozen peas at -18^{o}C, -15^{o}C, -12^{o}C, -9^{o}C, -6^{o}C and -3^{o}C. The average times when color first changed were 202, 98, 48, 23, 11 and 5 days and the times when flavour changed were 305, 166, 90, 49, 27 and 14 days respectively.

Martino and Zaritzky (1989) examined the ice recrystallization in beef tissues stored at different storage temperature. The rate of ice recrystallization increased with increasing storage temperature. Kinetic constants of recrystallization in beef tissue stored at -5^{o}C, -10^{o}C, -15^{o}C and -20^{o}C were 339, 200, 142 and 107 $\mu m^2.day^{-1}$ respectively.

Flores and Goff (1999) found that the rate of recrystallization was higher in ice creams stored at higher temperatures. At higher temperatures, the amount of unfrozen water in the ice cream is greater and results in increased recrystallization. The storage temperature influenced the rheological properties of the ice cream, which

in turn affected the ice crystal migration. Storing ice cream at -30°C did not affect the ice crystal size. This could be due to its proximity to the glass transition temperature. When ice creams were stored at -16°C, an increase in the crystal size was observed. They reported that at constant storage temperature of -16°C, stabilizers did not directly affect recrystallization, but samples without stabilizers were more susceptible to structural failure due to air channeling.

Martins and Silva (2002) confirmed that color losses of frozen green beans occurred at significantly faster rates when stored at -7^{o}C rather than -15^{o}C. The color changes relates to degradation of chlorophyll pigment in the green beans. However, the correlation of color and chlorophyll losses was not linear at -7^{o}C, -15^{o}C and -30^{o}C.

2.4.2 TIME-TEMPERATURE SURVEYS IN FROZEN FOOD CHAIN

Once frozen, it is imperative that effective control of temperature is maintained throughout the storage life of the product, including all primary storage, transportation, intermediate or secondary storage, retail display and storage in the domestic freezer. Two aspects of temperature control are important. The first one is actual storage temperature of the product at any one time and the second one is the degree of fluctuation of the storage temperature over periods of time. Fluctuations in the air temperature should be kept as low as possible (Albela and Reid, 1998). Otherwise, product quality will suffer and there may be a significant build-up of free ice in the product due to sublimation of water from the product and its deposition on the internal wall of the package. Temperature-related quality deterioration is more severe at warmer storage temperature. The quality losses which occur even during short periods of temperature abuse, are cumulative and irreversible (Kennedy, 2000).

The conditions which frozen food experiences in the frozen food chain have been subjected to research in many countries all over the world. Spiess and Folkers (1984) observed the frozen food chain in Germany and divided the frozen food chain into four steps of frozen food production.

In step one, products intended for low-temperature frozen storage are usually frozen directly after production. Freezing is generally carried out in such a way that the product temperature, after an equilibration time, is below -18°C. Equilibration takes place in freezers in case of small products, and in frozen storage facilities for large products. Step two comprises control of air temperature in the coldstores. Temperatures observed in products are usually below -24°C; the lowest being -28°C. The relevant trade association reported this to hold for 87% of commercial coldstores. Step three comprises the distribution chain and local coldstores. These are generally operated at the same temperatures as central coldstores. The average temperature may be slightly higher than in the latter, however, due to more frequent deliveries to and from the store. Fluctuations up to -18°C may occur near doors and in the transferring areas. Step four comprises retail display units where product must be well displayed and easily accessible for consumers. Maintaining low air temperatures in retail display units during business hours is difficult. It was found that the air temperature varied between -1°C and -32°C. About 76% of deep frozen food samples in open retail freezer cabinets, had center temperatures above -18°C (Spiess and Folkers, 1984). For the UK frozen food chain, the mean temperature of product samples on retail display was found to be -15.4°C (Wignall, Potter and Storey, 1984).

In manufacturing coldstores, temperature fluctuations could occur because of infiltration of warm moist air through doorways into cold storage rooms during loading and unloading or because of imprecision in the temperature control system. Other effects of air infiltration include increased costs for running and defrosting the refrigeration system (Chen et al., 2002). Chen *et al*. (1999) found that strip curtains in good condition reduce air infiltration by about 92%, however for a damaged strip curtain the infiltration was about three times higher.

During transportation, products need to be loaded in the truck or trailer in such a way that the air circulates over all external surfaces. When product is placed against the walls or ceiling, or placed directly on the floor, heat is conducted into the refrigerated product. Inadequate air distribution is probably the principle cause of product deterioration and loss of shelf life during transportation. Conventional forced air units usually discharge air over the stacked or suspended products either directly

from the cooling unit or through ducts on the ceiling. Aside from long haul distribution, the difficulty of maintaining the temperature in local delivery vehicles is also well-known. These vehicles can be subjected to 40-70 door openings during an 8 hour delivery period in often high ambient temperature (James, 1996). Tso et al. (2002) reported that two minutes after the door was opened the average air temperature had risen from -10^{o}C to 8^{o}C for a refrigerated truck with plastic strip door protection.

Ben-Yoseph and Hartel (1998) compiled the mean temperatures, temperature fluctuations and storage times of ice cream being storage from a variety of sources. The conditions of the storage and distribution system were divided according to storage site. At the manufacturing plant, the mean storage temperature ranged from -18°C to -30°C. Mean air temperature was -22°C and up to 2°C fluctuations occurred over a 2 weeks period. At the distribution depot or central warehouse, the typical storage temperature was between -23°C and -26°C. During transportation, the temperature of the ice cream can increase by 3°C to 8°C depending on the type of distribution vehicle. Mean air temperature and time during transport from plant to central warehouse, storage at central transport and transport from warehouse to supermarket and supermarket storage were -19±2.8°C for 6 hours, -24±6°C for 4 weeks, -19±2.8°C for 3 hours and -15.6±2.8°C for 1 week respectively. Ice cream is displayed in the supermarket at a wide range of temperatures that can reach as high as -9°C (Ruland, 1992).

Retail display is possibly the weakest link in the commercial cold chain (James and Evans, 1992b). Retail cabinets, which require ready access and good product visibility, are often subject to larger fluctuations in temperature (Cortella, 2000). A temperature survey in the retail store of frozen fish made in many towns in Britain showed that roughly 60% of the retail packs examined were being kept at temperatures above -15^{o}C, some of them as high as -10^{o}C to -6^{o}C (FAO, 2001). Likar and Jevsnik (2006) surveyed and measured temperature conditions of ice cream in retail stores. Temperature in retail display store varied from -23^{o}C to -10^{o}C, depending on the size of the store.

Finally the consumer must return home with their purchase. Although the use of insulated freezer bags is increasing, the producer can certainly not rely on their widespread use. Most products are therefore likely to experience at least one large step up and down in temperature between purchase and storage in the domestic freezer (James and Evans, 1992a). A survey has shown that consumers take between 2 and 510 minutes to transport chilled foods from retail shops to their homes and up to a further 90 minutes to empty their cars and/or shopping bags and place the products in refrigerators (Evans et al., 1991). Ben-Yoseph and Hartel (1998) reported that the temperature conditions in the home-freezer were -12±2.8°C for 1 week.

Surveys in the retail and the consumer storage sector confirm that temperature abuse is common for frozen foods. The distribution of temperature in domestic freezers were as follows: 7% from -30^{o}C to -26^{o}C, 12% from -26^{o}C to -22^{o}C, 32% from -22^{o}C to -18^{o}C, 25% from -18^{o}C to -14^{o}C, 14% from -14^{o}C to -10^{o}C, 8% from -10^{o}C to -6^{o}C and 2% from below -6^{o}C (Taoukis and Giannakourou, 2004).

2.4.3 EFFECTS OF TEMPERATURE CHANGES AND FLUCTUATIONS ON THE QUALITY OF FROZEN FOODS

The effects of temperature fluctuation on the quality changes of frozen foods have been investigated by many researchers. Temperature fluctuations during storage and transportation of frozen foods may result in a reduction in food quality and shortening of the shelf life of the foods (Martins, Almeida and Silva, 2004). The mechanism postulated for accelerated loss of quality includes growth of ice crystals and recrystallization (Fennema, 1973; Blanshard and Franks, 1987).

Product weight loss is one quality parameter for frozen product. The product tends to lose moisture during frozen storage. Pham and Willix (1985) indicated that relative humidity was the main factor affecting lamb carcass weight loss. Pham (1987) stated that temperature fluctuations caused by lighting or heat conduction (through inadequate insulation or excessive underfloor heating) will always increase the rate of desiccation, and even small temperature rises can have major effects.

In a domestic freezer, the compressor "on" and "off" cycles contribute to temperature fluctuations of air, packages and products. There is a difference between the amplitude of air temperature variation and that of products due to thermal inertia. Fluctuations lead to frost formation in packages, which is often accompanied by surface dehydration for certain products (Laguerre and Flick, 2007). Bak et al. (1999) showed that temperature fluctuations resulted in very pronounced formation of frost in packages of frozen shrimp. After 6 to 9 months of frozen storage, the amount of frost corresponded to the weight of the glazing layer applied before storage.

Martino and Zaritzky (1988) studied the influence of thermal oscillation during storage on the morphology of frozen beef tissue and showed that the temperature oscillations lead to an increase of the average crystal size during frozen storage. The average equivalent diameter of crystals in frozen beef samples, stored at the constant temperature (18 days at -20^{o}C), was 45 micron. The average equivalent diameter of crystals in frozen beef samples stored under fluctuating temperature conditions was 61-62 micron.

The effect of temperature fluctuations compared with constant frozen storage temperature regimes of selected food products was investigated by Gormley et al. (2002). Frozen raw salmon, smoked mackerel, stewed pork pieces, ice cream, pizza (with a mozzarella cheese topping), hollandaise sauce, strawberries, and blanched broccoli were used in their experiments. Samples of the product were subjected to different storage temperature regimes for 8 months. The first regime was mild temperature abuse (fluctuation regime), the second was storage at -60^{o}C (superfreezing regime) and the third was storage at -30^{o}C (control regime). The fluctuating regime involved three cycles of -30^{o}C to -10^{o}C to -30^{o}C on consecutive weeks followed by storage at a steady -30^{o}C for 8 months. Each fluctuation was achieved by transferring product from a chest freezer at -30^{o}C to one at -10^{o}C and back again after 48 hours. Patterns of free fatty acids (FFAs) and peroxide values (PVs) were similar for each product type. Superfreezing gave the lowest values and the fluctuating regime gave the highest values.

Alvarez and Canet (1998) investigated the effect of temperature fluctuations during frozen storage on the quality of potato tissue (cv. Monalisa). The temperature fluctuating regimes used represented the storage temperatures at different phases of the cold chain (production, -24°C; transport, -18°C; and distribution and sale, from -18°C to -12°C). Once all the treatment patterns were completed, the product was air-blast thawed at 20°C. Five ranges of temperature fluctuation were used -24°C to -18°C, -18°C to -12°C, -12°C to -6°C, -24°C to -12°C and -18°C to -6°C. The large fluctuations at higher temperature had significant detrimental effects on the texture of frozen potato when compared to the lower temperature range of -24°C and -18°C. Mechanical damage and dehydration increased where temperatures were higher and fluctuation ranges were wider. Of the rheological parameters, compression was the most sensitive to temperature fluctuation. The shear test parameters showed the smallest differences.

Growth of ice crystals by recrystallization was shown to be the major factor in determining structural damage in unpacked potato, whereas the higher structural deterioration in polyethylene-packed samples was associated with greater drying of the tissue by sublimation of ice on the sample surface (Canet, 1989; Alvarez and Canet, 1998). Alvarez and Canet (1998) stated that it is possible to estimate cumulative loss of texture quality in a product during storage and distribution based on the combined effect of time and temperature during storage. For the -12°C to -6°C and -18°C to -6°C conditions, the temperature fluctuations were close to ice melting temperature. Increases up to -6°C accelerated melting of small ice crystals, thus increasing the amount of available water, which re-froze immediately, causing an increase in the size but a decrease in the number of ice crystals. Even though the range was wider, the fluctuations from -24 to -12°C caused less mechanical damage than did fluctuations from -12°C to -6°C.

Attempts have been made to establish methods for the prediction of the physical state and rates of deteriorative changes of amorphous foods based on the glass transition temperature (T_g) concept. It is assumed that shelf life and quality stability is greatest in the glassy state and that faster changes may occur above T_g with rates determined by the temperature difference (T-T_g) (Levine and Slade, 1986; Roos,

1995). Rates of deteriorative changes in frozen foods increase by orders of magnitude with small increases in temperature above T_g of the maximally freeze-concentrated solute matrix (Levine and Slade, 1986). The increase in the rate of the deteriorative changes was considered to follow the Williams-Landel-Ferry (WLF) equation which gives a faster increase than would be predicted by the Arrhenius equation.

Comparisons of the simulated shelf life tests using the Arrhenius and WLF predictions of rates of deteriorative changes in frozen foods under steady and fluctuating storage temperatures were made by Karel and Saguy (1991). It was shown using Arrhenius kinetics that shelf life was slightly affected by temperature fluctuations of 5°C. The WLF model predicted that temperature fluctuation of 3°C decreased shelf life from 12 months (at constant temperature) to less than 10 months. When the fluctuation was 5°C, shelf life was predicted to be less than 4 months.

The effects of storage temperature and controlled temperature fluctuations on the recrystallization rate in ice cream were studied by Donhowe and Hartel (1996b). Ice cream containers were allocated randomly among various storage treatments. Containers were subjected to fluctuating or constant temperature storage at four mean temperatures: -5°C, -7°C, -10°C and -15°C. The results showed that an increase in recrystallization rate occurred when both storage temperature and amplitude of temperature fluctuations increased. During distribution, the ice cream undergoes a series of temperature changes which cause the mean size of the ice crystals to increase and the overall number of crystals to decrease. Ice crystals increased in size between drawing and hardening before storage. However, the ice crystals at the surface were slightly smaller after hardening than the crystals towards the interior. This is due to the slower rate of cooling and hence, more rapid recrystallization. This difference between surface and interior was significant in virtually all containers tested after hardening. After 4 or 8 days storage under oscillating-temperature conditions, crystals at the surface of the container were larger than crystals in the interior. This effect was due to the larger temperature fluctuations at the surface (±1°C) than in the interior (±0.2°C). For the constant-temperature experiments at -5°C, the initial difference in size distributions between interior and surface ice crystals became negligible during

storage. Crystals did not become quite as large and size distributions were somewhat narrower as those with 5°C oscillating conditions.

Aparicio-Cuesta, Mateos-Notario and Rivas-Gonzalo (1992) studied the effect of fluctuating temperature during storage on the sensory quality of frozen green beans. The frozen green beans were placed for 10 hours in a freezer at -22°C, and then transferred to the unfrozen compartment of a refrigerator at 4°C for 14 hours and then returned to freezer section, and compared to those held constant at -22°C. The fluctuating operation was repeated daily for 30 days. Five sensory characteristics (color, flavor, texture, fibrousness and skin loss) of the green beans after boiling were evaluated. There were significant differences among the samples with respect to skin loss, texture and flavor. The soft texture of samples was increased with storage under these conditions, although the changes did not become significant until after 30 days. Significant differences in skin loss and flavor occurred after 15 days, which is reflected in a distinct loss of quality. Skin loss during boiling was very low in the recently frozen samples. It increased with time and after 15 days of storage under these conditions it became very important. Flavor became weaker as storage progressed. Additionally, the panelists referred to the development of an unpleasant taste after 15 days.

Home storage is the final step of the distribution chain of frozen foods and usually causes temperature fluctuations. Martins et al. (2004) studied the effect of thermal cycles inside the refrigerator by comparing the simulated quality losses with cycling against isothermal storage. They concluded that the effect of the refrigeration dynamics is strongly dependent on the storage temperature. Thermal fluctuations are generally more detrimental at higher storage temperatures.

2.4.4 EFFECTS OF FREEZING AND STORAGE ON THE QUALITY OF FROZEN DOUGH

Frozen doughs are increasingly being produced in the baking industry. However, the quality of the baked products made from frozen dough is not always satisfactory. The quality of frozen dough as studied by several researchers as discussed below.

2.4.4.1 Freezable Water, Glass Transition Temperature (T_g) and Ice Crystal Distribution

The quality of a bread product baked from a frozen dough decreased with increasing frozen storage time (Slade, Levine and Finley, 1989; Inoue and Bushuk, 1996). A possible explanation for the quality loss involves the changes in dough rheology as a result of water transport during storage from the hydrated gluten to the ice phase (Bot and de Bruijne, 2003), especially at temperatures near the glass transition temperature (T_g) of the dough. If frozen dough is stored well below T_g, it is expected to be relatively stable throughout the storage time (Slade et al., 1989). The T_g of the dough ranges from -34^{o}C to -28^{o}C, depending on the techniques of determination and the dough composition (Laaksonen et al., 2002). Rasanen et al. (1998) reported that the T_g of non-annealed frozen doughs were between -30^{o}C to -43.5^{o}C, depending on the flour used. Unfortunately, T_g is significantly lower than the commonly used freezer temperature of -18^{o}C (Bot, 2003).

The freezable water content of frozen dough measured by differential scanning calorimetry (DSC) increased with increasing frozen storage time, indicating water migration and ice recrystallization. Lu and Grant (1999a) indicated that water migration in frozen doughs would affect gluten structure. Gluten proteins contain a large proportion of amino acids with nonpolar chains. According to Fennema (1973), these nonpolar amino acid groups have a structure-forming action on the adjacent water. The interaction between water and non-polar groups of protein, mainly hydrophobic interaction, has an important bearing on the native conformation of gluten proteins. In addition to the non-polar amino acid groups, gluten also contains

ionic and other groups capable of participating in hydrogen bonding. Water may also be involved in the gluten structure by forming cross-links among the polar groups. Relocation of water, which occurs during the freezing process and continues during frozen storage, would offer an opportunity for additional intermolecular and intramolecular protein bonding, including hydrophobic and hydrogen bonding. This interaction may change the gluten structure irreversibly.

The water-binding properties of dough decreased as the amount of free water and the number of ice crystals increased (Rasanen, Laurikainen and Autio, 1997b). Bhattacharya et al. (2003) found that the enthalpy of frozen dough was initially 76 J/g and increased to 84 J/g after 4 weeks and to 86 J/g after 12 weeks of frozen storage due to increased freezable water fraction. The moisture migration and increase in freezable water immediately after freezing was attributed to the deterioration of gluten network due to ice crystal growth. Moreover, there was a further increase in the enthalpy of dough after two partial-thaw cycles within 12 weeks. This could be attributed to the melting and recrystallization of ice during freeze-thaw cycles, with subsequent damage to the gluten network and separation of water molecules (Varriano-Marston, Hsu and Mahdi, 1980).

Water in the dough can be determined by the centrifuged liquid from the dough. Seguchi, Nikaidoo and Morimoto (2003) studied the centrifuged liquid separated from the dough using 38,900 g centrifugation for 120 minutes at 4^{o}C. The amount of centrifuged liquid and breadmaking properties (bread height and specific volume) were strongly correlated. Moreover, the liquid oozed from dough was increased by a freezing and thawing cycle, resulting in the deterioration of bread properties. The deterioration of breadmaking properties of thawed frozen-dough may be due to a decrease in the water binding ability of dough.

However, the amount of freezable water did not show significant changes during frozen storage as reported by Baier-Schenk, Handchin and Conde-Petit (2005). They indicated that growth of ice crystals leads to a redistribution of water in the dough, which affects the polymeric compound properties in dough and reduces the baking performance.

Another technique for studying water distribution in dough is nuclear magnetic resonance (NMR) or magnetic resonance imaging (MRI). NMR is a powerful tool in that it can access the distribution and mobility of water in a non-invasive manner. The mobility of water in dough is an important parameter because it relates directly to the quality of gluten network (Leung, Magnuson and Bruinsma, 1979). In starch, a significant part of water is strongly bound to crystalline polysaccharide chains (Kulik, da Costa and Haverkamp, 1994; Le Bothlan et al., 1998). Time-domain NMR techniques have demonstrated that water is no longer bound in the physical sense at high water contents. Esselink et al. (2003) reported that time-domain NMR showed increased water mobility after frozen storage, which could be attributed to a release of water from the gluten matrix. The MRI results confirmed structural changes of dough due to the ice crystal growth, leading to increased relaxation parameters. Lucas et al. (2005) confirmed that ice fraction could be monitored from the MRI signal in transient thermal conditions.

2.4.4.2 Carbon Dioxide (CO_2) Production and Gas Retention

Frozen doughs are increasingly being produced in the baking industry even though they generally provide bread with lower loaf volume than does fresh (unfrozen) dough. Consumer acceptability depends on the quality of the frozen dough. Frozen dough stability has been related to dough formulation, yeast quality, sulfhydryl (SH) compounds released from yeast fermentation before freezing and freeze-thaw rates (El-Hady et al., 1996). Yeast activity of the frozen dough was measured in term of gassing power or CO_2 production. The gas production by yeast was affected by freezing rate. Gas production with a freezing rate of 9.2^{o}C/min was lower than at 1^{o}C/min (Gelinas, Lagimoniere and Dubord, 1993). Havet et al. (2000) confirmed that CO_2 volume decreased with increasing freezing rate. Baker's yeast samples retained about 70% of their fermentative activity in doughs stored for 12 weeks at -20 or -30^{o}C. Gas production of frozen dough stored for three months was between 69% and 72% of that for non-frozen dough (Gelinas et al., 1993).

El-Hady et al. (1996) studied the yeast activity of frozen dough using a risograph. The yeast activity of frozen dough decreased after one day storage at -20±2°C. After seven days storage, the depression in total gas volume was about 10% when compared with one day storage. The total gas production of frozen dough was decreased by 33.4% after 4 weeks storage. Moreover, the reduction was 49.7% when the dough was subjected to three freeze-thaw cycles.

However, the dough storage temperature also affects the rate of gas production. Meric et al. (1995) indicated that CO_2 production of non-frozen dough at 22°C was higher than the freeze-thawed dough stored at 4°C by about 21-28%, depending on yeast strain. This decrease in fermentative activity was not caused only by yeast damage; the temperature condition before measurement is also important. If yeast cells had time to adapt their metabolism to the medium, the CO_2 production reduced by only 5-10%. In fact, when dough was rapidly cooled down to 4°C and immediately transferred into the risograph, the CO_2 production was further reduced.

2.4.4.3 Yeast Viability

Baker's yeast is an important ingredient in frozen dough production. Several investigators have reported that the major changes in doughs that have been frozen are related to yeast. Yeast viability is a relative concept. It has been shown that yeast cells can be viable yet not active (Autio and Mattila-Sandholm, 1992). In the case of frozen dough, dead yeast cells release reducing agents such as glutathione, which weaken the gluten network, resulting in poor gas retention and longer proofing time (Kline and Sughiara, 1968; Hsu, Hoseney and Seib, 1979b). The quality of yeast directly influences the stability of the frozen dough (Hsu et al., 1979b). Good yeast performance after freezing was associated with yeasts having protein contents higher than 57% (Hsu et al., 1979a). During dough fermentation, yeast produces carbon dioxide and flavor compounds. The gas-forming ability of yeast depends on the strain, the number of yeast cells, cell activity and the amount of fermentable sugars. However, Gelinas et al. (1993) showed that the strain of a regular baker's yeast was not a major factor for frozen dough stability when the yeasts were grown under similar

conditions. The freezing rate is the more critical factor on the yeast cell cryoresistance.

Hsu et al. (1979a) reported that freezing at different rates caused different levels of damage to yeast. Under rapid freezing conditions (9.2°C/min), survival of yeast dropped to 24-32% compared to that of non-frozen dough; under slow freezing conditions (about 1°C/min) followed by storage at -23°C, survival of yeast was higher (48-61%). This indicated that rapid freezing in dough was much more deleterious to yeast than slow freezing (Gelinas et al., 1993). Cauvain (1998b) indicated that very low freezing rate (less than -0.21°C/min) allows too much gas production, resulting in loss of product quality.

Some of the detrimental changes in frozen dough are related to the freezing and thawing conditions. Baguena et al. (1991) found that pre-fermentation before freezing reduced the yeast viability in frozen dough after 30 days storage compared with doughs without pre-fermentation. Autio and Sinda (1992) found that the yeast viability decreased slightly during two weeks of storage in a home-type freezer at -18±0.5°C. Stecchini et al. (2002) stated that yeast viability was mostly affected by the freezing and storage temperature. With temperatures below the glass transition temperature (T_g) of dough (lower than -30°C), the yeast cell survival was highest.

However, Hsu et al. (1979a) stated that dough temperature after freezing had a greater effect than the freezing rate on frozen dough quality. Storage temperatures lower than freezing temperatures were more harmful than freezing and storing at the same temperature because of yeast damage. Moreover, freeze-thaw damage was more severe after longer frozen storage than after short frozen storage. The proofing time of the frozen dough increased proportionally with the number of freeze-thaw cycles. Teunissen et al. (2002) found that losses in yeast viability in frozen dough increased with number of freeze-thaw cycles and the frozen storage duration that the dough experienced.

There are two main approaches to improve yeast viability and dough quality after freezing and frozen storage. The first is yeast selection based on studies of gene expression. Oda, Uno and Ohta (1986) selected eleven yeast strains suitable for frozen dough from over 300 *Saccharomyces* strains. Tanghe et al. (2000) and Teunissen et al. (2002) used repetitive freezing and thawing for up to 200 cycles to isolate freeze resistant yeast strains from frozen dough. Another approach is to use a physical treatment to induce freeze tolerance in normal baker's yeast. Berry and Foegeding (1997) indicated that most microorganisms must accommodate a variety of changes and stresses in their environment in order to survive and multiply. Because of the impact of temperature on all cellular reactions, adaptations to fluctuations in temperature are possibly the most common. Beales (2004) stated that the application of physical stress to microorganism is the most widely used method to induce cell inactivation and promote food stability. Nakagawa and Ouchi (1994) showed the improvement of freeze-tolerance of commercial baker's yeast in dough by heat treatment of the dough before freezing. Diniz-Mendes et al. (1999) found that a mild cold shock before freezing (10^{o}C for 3 hours) increased yeast survival in a cell suspension at -20^{o}C for 28 days.

2.4.4.4 Dough Rheology

The quality of frozen dough is related to yeast activity, dough formulation and processing, storage conditions, and freezing and thawing condition. During frozen storage, the structure of gluten in the dough could be damaged by formation of ice crystals. The phenomenon of ice recrystallization could contribute to the weakening of the three-dimensional protein network responsible for gas retention in doughs. During recrystallization there is an increase in the size of ice crystals that results in a greater separation of the water molecules from the hydrophilic interactions (Inoune and Bushuk, 1991).

The rheological properties in bread doughs are considered to be important in relation to their stability and gas retention during proofing and baking. There are several methods for determining the rheological properties. Generally, rheological properties measurement of the dough can be divided into small

deformation and large deformation types. To obtain information about the structure of both flour and gluten doughs, mechanical tests involving small deformations are most useful. However, if information on the mechanical properties of dough under conditions similar to those in fermenting bread dough is required, biaxial extension tests involving large deformation should be performed (van Vliet et al., 1992).

Shear oscillation dynamic rheology is generally used under deformation conditions. It is inappropriate for breadmaking and shows little relationship with baking performance. The frequency range used in conventional shear oscillation tests is limited to the plateau region, which is insensitive to changes in the high molecular weight (HMW) glutenin polymers thought to be responsible for variations in baking quality. The optimal deformation conditions can be best assessed either by long-time creep or relaxation measurements, or by large deformation extensional measurements at low strain rates and elevated temperature (Uthayakumaran et al., 2000; Autio et al., 2001; Dobraszczyk et al., 2003)

Large deformation extensional rheological properties are more sensitive to changes in molecular weight distribution, polymer entanglements and branching than small deformation dynamic shear properties, based on polymer physics principles and experimental data. Insoluble HMW glutenins have been shown to be closely related to variations in baking quality, and to the presence of long relaxation times, indicating entanglements of the HMW polymers. Strain hardening has been shown to be a sensitive indicator of entanglements and long-chain branching in HMW polymers. Large extensional deformation of doughs and glutens is closely related to bubble wall stability (Dobraszczyk and Morgenstern, 2003). Kieffer et al. (1998) and Uthayakumaran et al. (2002) indicated that the elongation of the dough measured using uniaxial extension gave a good correlation with baking performance.

Rheological and structural characteristics of the dough exposed to freezing rate, fluctuating temperature and temperature changes during storage could be related to the quality of dough and bread after baking. Havet et al. (2000) studied the effect of freezing rate on the dough rheology using uniaxial compression. The results showed that the elasticity of the dough was reduced with increasing freezing rate. The

cause of direct change in gluten can be explained by disruption of certain gluten bonds by the mechanical action of ice crystal formation. A loss of bread loaf volume as a result of freezing and thawing might be attributable not only to decreased gas production but also to structural changes in the dough.

Lu and Grant (1999a) investigated the frozen dough rheology using a parallel plate dynamic rheometer. The storage modulus (*G′*) reflects the property of elasticity in a dough system (Abdelrahman and Spies, 1986). The results showed that G′ decreased with increasing frozen storage time. During frozen storage at -18±0.5^{o}C for 2 weeks, dead yeast cells did not affect the rheological properties of the doughs. A decreased *G′* in frozen and thawed dough suggested a loss of polymer crosslinking. Both the relaxation modulus and relaxation half-life decreased in frozen stored dough. The decrease of relaxation half-life indicates a weakening of the gluten network (Autio and Sinda, 1992). The rheological properties of the dough were susceptible to freeze-thaw cycles. The maximum gluten damage of frozen dough occurs during freeze-thaw cycles, rather than storage at constant storage temperature (Bhattacharya et al., 2003).

The extensigraph is regarded as a suitable instrument for frozen dough evaluation (Dobraszczyk and Morgenstern, 2003). Extensigraph results for yeast doughs showed that maximum resistance decreased significantly after one week of frozen storage and with increasing number of freeze-thaw cycles (Inoue and Bushuk, 1991; Inoue et al., 1994). Sharadanant and Khan (2003a) determined the dough rheology using a Kieffer dough extensibility rig for a texture analyzer (TA-XT2). The Kieffer dough extensibility rig was used to measure the extensibility and maximum resistance to extension of each dough sample. The advantage of the rig is that it uses a micro-extension method involving a very small sample size. It is highly correlates with the macro methods indicated by the baking performance. The peak force reached while stretching or extending the dough by means of a hook is considered to be the maximum resistance to extension of the dough. The distance travelled by the hook to reach the peak force value is the extensibility (Kieffer et al., 1998; Kieffer and Stein, 1999; Suchy, Lukow and Ingelin, 2000). The results of the Kieffer dough extensibility rig measurement showed that the maximum resistance to extension values decreased with frozen storage time from 0 to 16 weeks of frozen storage. The extensibility of the

frozen dough also increased with frozen storage time while the area under the curve decreased (Sharadanant and Khan, 2003a). Decreases in maximum resistance, area under the curve and increase in extensibility clearly indicated deterioration in the quality of the gluten (Inoue and Bushuk, 1992). An increase in extensibility resulted in poor gas retention of the dough and consequently the proofing time increased with an increase in frozen storage time (Sharadanant and Khan, 2003a).

2.4.4.5 Dough Microstructure

Bread dough from wheat flour is a nonlinear viscoelastic fluid. The effectiveness of dough rheology in bringing about good baked loaf structure is affected by the action of surface active molecules. The viscoelastic character of dough is created during mixing through the alignment of the unique glutenin proteins found in wheat, giving sufficient elasticity to retain gas during proving but sufficient extensibility to avoid premature rupturing (Dobraszczyk, Cambell and Gan, 2001). Changes of temperature during frozen storage resulting in ice recrystallization may cause changes to dough structure (Varriano-Marston et al., 1980). Disruption to the structure was related to stability of cell walls around the expanding gas bubbles, which is considered to be an important factor in determining baking quality. The limit of expansion is related directly to the expansion, stability and rupture of the bubble walls (Dobraszczyk et al., 2001). Structural characteristics of cells and tissues influence grain quality and performance in food processes. The microstructure determines the appearance, texture, taste perception and stability of the final product. It can give a visual explanation as to why cereal-based products of similar chemical composition have measurably different textures.

Berglund et al. (1991) examined the microstructure of frozen dough using a low temperature scanning electron microscope (LT-SEM). Twenty-four weeks after frozen storage at -23^{o}C, the gluten matrix appeared less continuous, more ruptured and separated from the starch granules. The gluten strands were also thinner. These structural characteristics may result in decreased loaf volume and increased proofing time of frozen dough stored for long periods. The gluten network damage from freeze-thaw cycles appeared to be much greater than that observed in freshly

mixed doughs and doughs frozen for 24 hours. Dough examined by SEM showed large ice masses that were formed during recrystallization. The effects of freeze damage on the crumb texture and on the underlying gluten fibrils of baked breads were studied using scanning electron microscopy (SEM). Sweet and white bread doughs were stored at -20^{o}C and subjected to freeze-thaw cycles. SEM images showed that gluten fibrils forming within the skeletal framework of pore walls were cut and became coarse and non-uniform strings with many knots were generated on the gluten fibrils. An increase in the number of freeze-thaw cycles increased both the coarseness of the gluten fibrils and size of the knots (Naito et al., 2004). Rasanen, Harkonen and Autio (1995) indicated that the thicker gluten structure is more resistant to the stress of freezing.

2.4.4.6 Protein Properties

The physiochemical and functional properties of wheat proteins are mainly attributed to the disulfide cross-links which play a significant role in the protein network formation in dough (Schofield and Chan, 1995; Weegels, Hamer and Schofield, 1996; Li and Lee, 1998). The noncovalent forces (namely hydrogen bonds, ionic bonds, and hydrophobic interactions) also contribute to the properties of the dough. The role of sulfhydryl groups of wheat proteins has attracted the attention of many cereal chemists and food technologists. Sulfhydryl groups are potentially able to undergo a sulfhydryldisulfide interchange which involves the cleavage or reformation of disulfide bonds mediated by endogenous sulfhydryl-containing components (such as proteins, reduced glutathione) or exogenous compounds (such as cysteine, reduced glutathione) (Dong and Hoseney, 1995; Schofield and Chan, 1995). Aminlari and Majzoobi (2002) stated that a slight increase in the protein solubility and disappearance of protein bands in the range of 55 to 70 KDa was observed when samples were frozen for 6 to 8 weeks. The results of this study suggest that high-molecular-weight proteins of wheat flour have a major influence on the properties of dough and that these proteins are affected by freezing.

2.4.4.7 Bread Volume

Bread volume is a key indicator of the quality of the whole breadmaking process (Gelinas et al., 1993). Specific loaf volume should not be too small or too large because it affects the crumb texture. Too small loaf volumes give a very compact and closed grain structure and too large volume gives a very open grain structure (Sharadanant and Khan, 2003b).

Changes in the specific volume of the bread as a function of the freezing conditions were reported by Havet et al. (2000). Bread volume reduced by at least 20% with different freezing rate, ranging from 1^{o}C/min to 3^{o}C/min at -20^{o}C. The effect of cryogenic temperatures on the baking performance was investigated by Neyreneuf and Delpuech (1993). Yeast doughs were frozen under different cooling velocities. Conventional mechanical refrigeration at -40^{o}C was compared to cryogenics at -40^{o}C, -60^{o}C, -80^{o}C, -100^{o}C and -120^{o}C. After three months of storage at -20^{o}C, the baking performance of the dough was not affected by the -40^{o}C and -60^{o}C cryogenic treatments, whereas decreasing the freezing temperature to -80^{o}C, -100^{o}C and -120^{o}C gave a drop in bread volumes by about 15%.

Sharadanant and Khan (2003b) confirmed that specific loaf volume decreased significantly with an increase in frozen storage from 0 to 16 weeks. Berglund et al. (1991) observed that with longer storage periods, dough proofing times increased, bread loaf volumes decreased, and bread firmness increased. The cause for extended proofing time of frozen dough could be the microstructure changes in the starch and gluten as well as decline in yeast viability. Freezing dough causes starch damage which may contribute to increased moisture retention resulting in increased loaf weight and lower loaf volume.

Inoue and Bushuk (1991) reported that bread dough was weakened during frozen storage and successive freeze-thaw cycles. As the number of freeze-thaw cycles increased, loaf volume decreased, and the tops of the loaves became flatter. Le Bail et al. (1999) investigated the influence of storage conditions on frozen French bread dough. The baking performance was estimated from the dough volume

after 140 minutes proofing at 28°C and from bread volume after baking. Temperature fluctuation had a large influence on the dough volume and on bread volume. Relative to fresh dough, a reduction of the dough volume by 6.7% was observed after 37 days at -22°C for minimal temperature fluctuation (±0.4°C), whereas it was reduced by 48% for large temperature fluctuations (up to -8°C for 40 minutes). Lu and Grant (1999a) stated that a prolonged proofing time was required to reach a predetermined proof height following frozen storage, which undoubtedly contributed to the lower loaf volume measured.

2.4.4.8 Bread Textural Properties

Textural properties of bread crumb have become a common criteria for evaluating bread quality and for assessing its tendency to lose freshness during storage (Kamel, 1987). The firming of bread crumb accompanying staling has been recognized as one of the most important factors in reducing acceptability to the consumer (Willhoft, 1971). Measurements of mechanical properties are frequently employed for assessing product quality changes resulting from ingredients, processing and duration of storage (Scanlon, Sapirstein, and Fahloul, 2000). Texture profile analysis (TPA) is one technique that attempts to use a common basis for both subjective and objective assessments of product eating qualities (Vulicevic et al., 2004). Liu and Scanlon (2002) used the Young's modulus and critical stress to evaluate the physical texture of bread crumb. The resulting regression showed that decreasing density led to a decrease in both Young's modulus and critical stress. Sharadanant and Khan (2003b) reported that an increase in storage time up to 16 weeks of the frozen storage doughs significantly increased bread firmness.

The changes in the microstructure of gluten and starch granules may contribute to increased bread firmness. Another cause of the particular textural feel of breads made from frozen dough may be the segregating of the gluten matrix from the ice crystals which destroys gluten sheets and isolates starch granules. This may be a result of the destruction of pore walls consisting of gluten fibrils by freezing or freeze-thaw cycles (Berglund et al., 1991). Berglund and Shelton (1993) studied the effect of frozen storage duration on firming properties of breads baked from frozen doughs.

The frozen doughs were baked and the firmness of baked bread was determined immediately after freezing (week 0) and after 4, 8, 12, 16 and 20 weeks of frozen storage. Their results showed that bread crumb firmness increased significantly with increasing storage time. After baking, a difference in bread texture of frozen dough compared with that of fresh dough remained (Naito et al., 2004).

2.4.4.9 Bread Sensory Characteristics

Bread characteristics relate to the consumer acceptability of bread products. A significant variation in the quality of products made from frozen doughs occurs normally without different treatments. There are many causes of quality losses of frozen dough. Some arise from factors which have their origins in the dough formulation and processing conditions. Appearance of the bread product provides the first clue for the consumer of the product's quality (Bushuk, 1985; Setser, 1993). Consumer acceptance is important to the food industry and, therefore, a good quality of bread product is essential. Critical factors are the appearance, flavour, texture and loaf volume (Bennion, 1990; Cambell, Penfield and Griswold, 1979).

Cauvain (1998b) stated that possible causes of quality losses after breadmaking can be summarized as follows:

2.4.4.9.1 Skinning

Skinning occurs in the low humidity during retarding. The upper crust will be hard and dry. Skinning becomes more pronounced as the storage time increases. It is an irreversible phenomenon and will carry through to the baked products, which may be small in volume and have a pinched appearance as a result of uneven expansion of the dough pieces during baking.

2.4.4.9.2 Crust fissures

Baked products may sometimes exhibit small fissures or cracks. The cracks may be observed on the surface of the dough. High yeast levels are the main causes. The cracks are more prevalent with dough pieces of large radius.

2.4.4.9.3 Ragged crust breaks

These occur most often when there is a large temperature difference between the centre of the dough piece and its surface. It can be a particular problem with frozen doughs of larger size where the low thermal conductivity of dough exaggerates the temperature differentials during baking.

2.4.4.9.4 Small volume

Volume losses occur through the loss of yeast activity and the release of proteolytic enzymes and glutathione from disrupted yeast cells. Improvements in volume may be achieved by reducing the length of time in storage.

2.4.4.9.5 White spots or small blisters

Small, translucent blisters or white spots sometimes occur on the top and bottom crusts of the baked product. The occurrence of white spots is associated with significant gas production before freezing. Solving white spot formation in frozen doughs by adjustment of the yeast level is difficult because of the need to maintain CO_2 production activity in the dough after thawing. Minimizing fermentations of doughs before freezing is a better option.

2.4.4.9.6 Waxy patches

Patches of uneven color may form on the side and bottom crusts of breads baked in the pans. They have a shiny or waxy appearance and are frequently observed on the lower corners of the loaves. This phenomenon increases as storage time increases.

2.4.4.9.7 Large blisters

The most common causes of blisters are poor molding and damage to the dough piece during processing. This problem relates to large gas bubbles trapped within the dough piece that rapidly expand due to the carbon dioxide gas released during the proofing phase. Delays in transferring dough from the blast freezer to deep-freezer storage can lead to partial thawing. Upon subsequent baking large blisters may be present under the top crust.

2.4.4.9.8 Uneven or open cell structure

Sometimes doughs which normally produce a fine cell structure yield a more open structure after freezing. The poor thermal conductivity of dough, exacerbated by large dough pieces, could be a cause.

2.4.4.9.9 Areas of dense crumb

This problem is often associated with ragged crust breaks. Uneven expansion of the dough piece during proof and the early stages of baking is usually caused by transferring cold doughs to a hot proofer. This problem can occur more often as frozen storage time increases.

Barrett et al. (2000) stated that correlations of consumer preference for bread with several trained panel attributes were highly significant. The relationships with preference included perceived cell size (r = 0.77), firmness (r = -0.84), denseness (r = -0.88) and chewiness (r = -0.87). Ribotta, Leon and Anon (2001) defined bread crumb properties in terms of gas cells per unit area (%gas cell) and the aspect ratio (height/width) of the loaf. Sahlstrom et al. (1999) measured bread characteristics including weight, aspect ratio, crumb score, bread score and loaf volume. The crumb and bread scores were evaluated subjectively by a skilled baker. The loaf volume can be used for describing the overall quality of bread.

Schwarzlaff et al. (1996) used the cell size of bread measured by photocopying the loaf half for identifying bread quality. Good quality dough shows thin grain walls with small holes (He and Hoseney, 1991), and a thin and smooth crust after baking (Takano et al., 2002). Jackel (1986) indicated that thin cell walls with uniform, elongated cells are preferred over thick cell walls and round cells. Open grain means large cells, and closed grain means small cells.

Ishida et al. (2001) used magnetic resonance image (MRI) to measure the pores in frozen baker's yeast dough. MRI showed that pore generation was small, expansion of the dough was low, and gluten fibrils were thick and undeveloped after fermentation. The crumb structures of bread prepared using frozen dough were characterized by thick network walls without any connection between pores, and by a thick crust with a rough surface due to pores generated near the surface. Image analysis of baked breads from frozen dough revealed that large round pores dominated and that the pores were non-uniformly distributed.

Sharadanant and Khan (2003b) characterized the external and internal bread attributes from frozen dough. The external characteristics include appearance and size of the loaf. Usually much attention is paid to the uniformity of the loaves and the crust break. There was significant deterioration in the external appearance of the bread loaves as storage time at -23^{o}C increased. The internal crumb characteristics such as texture, grain, cell wall structure, color and softness were graded on a 10-point scale, ranging from least favourable to most favourable. The overall bread score of

bread loaves decreased with increasing storage time. External and internal appearance scores at zero day decreased from 9.59 to be 6.03 and from 9.07 to be 4.95, respectively after 16 weeks frozen storage.

Greene and Bovell-Benhamin (2004) defined the attributes used in the sensory descriptive analysis of bread into three groups including appearance, flavor and texture. Most common appearance attributes of bread are cell size and cell uniformity.

Hersleth et al. (2005) investigated consumer's perception of bread and used principle component analysis (PCA) to identify the bread attributes. The PCA results could be divided into two components (PC1 and PC2). PC1 was described by attributes related to the texture of the breads. PC2 was described by flavour attributes. Correlations showed that 52% and 34% of the variation in bread attributes were explained by PC1 and PC2, respectively. These results indicated that textural attributes of bread were a significant consumers concern. Gambaro et al. (2004) found the best instrumental variables in relation to texture are cohesiveness and elasticity. The instrumental cohesiveness was measured according to the texture profile analysis (TPA) method using a TA-XT2.

Some researchers investigated the characteristics of baked bread made from frozen dough. Bruinsma and Giesenschlag (1984) found that crumb structure for loaves made from doughs that had been frozen and thawed were considered satisfactory, but rapidly deteriorated to very unsatisfactory levels after seven freeze-thaw cycles. Each successive freeze-thaw cycle caused the dough to become weak, fragile, difficult to handle and to have a moist appearance due to damaged dough microstructure from ice recrystallization. The crumb grains also became much less acceptable as they became harsh and coarse. Inoue and Bushuk (1991) found that the bread with successive freeze-thaw cycles were flat on the top of loaves. In addition, prominent blisters appeared on the crust surface, and the crumb structure became more open.

Ribotta et al. (2001) described bread crumb properties using gas cells per unit area of crumb (% gas cell) and the aspect ratio (height/width) of the bread prepared from fresh and frozen dough. Bread crumbs from frozen doughs showed higher values of % gas cell than the corresponding crumbs from non-frozen doughs, suggesting the presence of structures with a higher proportion of pores. The aspect ratio provides useful information related to the dough elasticity. Results indicated that fresh doughs produced bread with higher ratios than frozen doughs which were stored at -18°C regardless of the storage time. A high aspect ratio indicated that the dough had elastic properties while a low aspect ratio indicated viscous flow properties (Sahlstrom et al., 1999).

2.5 MODELING AND ARTIFICIAL NEURAL NETWORK (ANN)

Changes in food quality are the result of complex interactions in formulation, processing and storage. A model that relates formulation, processing and storage of food products to estimate product quality would benefit the food industry. Such a model would enable more efficient resource utilization and better understanding of critical factors which impact product attributes. A model of a food process or part of a process is a mathematical description relating the levels of the process variables and the raw materials attributes to the changes in the product attributes. A model is a codified systematic scheme, developed from one situation and then hopefully available to be fitted to all sorts of new situations (Earle and Earle, 2003).

2.5.1 MECHANISTIC APPROACHES

2.5.1.1 ISOTHERMAL MODELING

Kinetic modeling is gaining increasing interest in predicting chemical, physical and microbiological changes during food processing and storage. Most, if not all, foods are chemically or biologically active. Consequently, they undergo changes, the rate of which is temperature-dependent. The changes themselves can be undesirable (Peleg et al., 2002). Time and temperature are critical variables in food processing. Reaction rates are sensitive to temperature. Generally, reaction rates

increase with increasing temperature. The most common mathematical model to describe the effect of temperature on the rate of chemical and biochemical reactions has been the Arrhenius equation. The Arrhenius equation and its implications are often used for food processing reaction technology because temperature is the primary factor in the initiating and controlling the actual processing (Earle and Earle, 2003).

Traditionally, the degradation of nutrients in foods during their thermal processing and storage has been described in terms of zero, first or higher order kinetics (Mizrahi, 2004). Numerous research studies have been applied with zero- (Eq. 2.1) or first-order (Eq. 2.2) models to describe the degradation of food product quality:

$$C = C_0 - kt \qquad \text{(Eq. 2.1)}$$

$$C = C_0 \exp(-kt) \qquad \text{(Eq. 2.2)}$$

where C is the measured quality value (%), C_0 is the initial C (%), t is the storage time (day) and k is the reaction rate constant (%/day).

The Arrhenius equation relating reaction rate constants with absolute temperature is:

$$k(T) = Ae^{-E/RT} = A\exp(-E/RT) \qquad \text{(Eq. 2.3)}$$

where A is a constant (units), E is the "activation energy" (J/mol), R is the gas constant and T is the absolute temperature (K). The "activation energy" is usually calculated from the slope of $\ln k(T)$ *vs* $1/T$ data.

One of the most obvious advantages of the Arrhenius model is that in systems where it applies, knowing the values of k at any two temperatures is sufficient to calculate E/R. The linear $\ln k$ *vs* $1/T$ plots have been found to apply quite frequently in a variety of quite complex systems; hence the widespread use of this model.

There are systems, however, for which the Arrhenius model is clearly inadequate. This can be revealed by a noticeable curvature in their ln*k* *vs* 1/*T* plots. The effect of temperature on the kinetics of such systems has been described by a variety of alternative models. Among them, one that has become very popular in food research is the Williams-Landel-Ferry (WLF) equation. It was originally proposed for quantifying the effect of temperature on the viscosity of polymers above their glass transition temperature (T_g). The WLF equation is a flexible mathematical model. It can be fitted with any reference temperature in the pertinent temperature range (Corradini and Peleg, 2004). Its general form is (Williams, Landel and Ferry, 1955):

$$\log_{10}\left(\frac{k_{ref}}{k}\right) = \frac{-C_1(T - T_{ref})}{C_2 + (T - T_{ref})} \qquad \text{(Eq. 2.4)}$$

where k_{ref} is the reaction rate at a reference temperature T_{ref}, and C_1 and C_2 are constants.

The most commonly used form in the food literature uses T_g as the reference temperature:

$$\log_{10}\left(\frac{k_g}{k}\right) = \frac{-C_1^{'}(T - T_g)}{C_2^{'} + (T - T_g)} \qquad \text{(Eq.2.5)}$$

where k_g is the reaction rate at the glass transition temperature T_g, and $C_1^{'}$ and $C_2^{'}$ are constants whose values are different from those of C_1 and C_2 in Eq. (2.4).

Vulicevic et al. (2004) applied the kinetic model as described by Ateba and Mittal (1994) to study the changes in quality characteristics of par-baked frozen breads. The model is:.

$$\frac{dQ}{dt} = \pm kQ^{n_r} \qquad \text{(Eq. 2.6)}$$

where k is a rate constant depending on temperature and n_r is the reaction order.

The normalized quality characteristic is:

$$Q = \frac{Q_{\max} - Q_{(t)}}{Q_{\max} - Q_{\min}} \qquad \text{(Eq. 2.7)}$$

where Q_{max} represents the maximum value (%) determined from the experiment, Q_{min} represents the minimum value (%), and $Q_{(t)}$ represents the Q value (%) at any time t.

In the deterioration kinetics, the quality of product stored in a constant storage temperature was expressed by:

$$\ln(\frac{dQ}{dt}) = \ln(k) + n_s \ln(Q) \qquad \text{(Eq. 2.8)}$$

where n_s represents the slope and ln (k) represents the intercept on a ln-ln plot.

The prediction of quality at any time can be determined by integrating Eq. 2.6, where $n \neq 1$ giving:

$$Q = \sqrt[(1-n)]{(1-n)kt} \qquad \text{(Eq. 2.9)}$$

Vulicevic et al., (2004) showed that a plot between quality characteristic *Q* (sensory characteristic) *vs* storage time had an acceptable linear correlation which fitted the zero-order reaction kinetics for par-bread frozen breads. The slope of the plotted data had a negative trend, indicating that bread quality deteriorated over time.

A problem in processing is that the temperature may not be constant, subsequently affecting the food product quality. Martins et al. (2004) applied a mathematical model, using Arrhenius behaviour with temperature for the quality loss kinetic of frozen green beans. Quality losses were modelled by fractional conversion kinetics:

$$\frac{C - C_{eq}}{C_0 - C_{eq}} = \exp\left(- k_{ref} \exp\left[- \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right].t \right) \qquad \text{(Eq. 2.10)}$$

where C is the quality retention (%) at time t, C_0 is the initial quality retention (%), C_{eq} is the equilibrium quality retention (%), k_{ref} is the kinetic rate (%/day) at the absolute reference temperature T_{ref} (K), E_a is the Arrhenius activation energy (J/mol) and R is the universal gas constant (J/mol.K).

Martins et al. (2004) found that green beans quality losses during frozen storage were mostly influenced by temperature and kinetic properties. Quality losses convergence occurs at most of the studied storage temperatures. Temperature cycles inside refrigerators are relevant to quality losses. Despite the short periods of storage at +5^{o}C and -6^{o}C, deterioration of quality was very susceptible to the thermal fluctuations at these temperatures. Temperature cycles also had a long term effect at lower temperatures, such as at -12^{o}C and -18^{o}C. As more time is spent at temperatures below the set-point at low temperature, quality retention is higher than expected at a constant set-point temperature.

Curves from kinetic experiment data can also be described by power law models. Corradini and Peleg (2004) proposed that published isothermal degradation curves for chlorophyll A and thiamine in the range 100–150°C and the inactivation curves of polyphenol oxidase (PPO) in the range 50–80°C could be described by the model:

$$\frac{C(t)}{C_0} = \exp\left[-b(T)t^{n(T)}\right] \qquad \text{(Eq. 2.11)}$$

where $b(T)$ and $n(T)$ are temperature-dependent coefficients. This equation is the cumulative form of the Weibull distribution function, which has been a successful model of many processes that involve survival (van Boekel, 2002; Peleg et al., 2002). According to Eq. 2.11, $n(T)$ is the distribution's shape factor and the reciprocal of, $b(T)$, its location factor. However, since the slope of the survival curve has rate units, Eq. 2.11 can also be considered as a kinetic model. The familiar first order kinetics model is thus a special case of Eq. 2.11, where $n(T) = 1$.

In many cases, the distribution's shape factor, $n(T)$ in Eq. 2.11, has only a weak temperature dependence and sometimes none at all (van Boekel, 2002). Thus, for many systems, which include the enzymatic and microbial inactivation and the thermal degradation of thiamine and chlorophyll, Corradini and Peleg (2004) proposed the simplified model:

$$\frac{C(t)}{C_0} = \exp\left[-b(T)t^{n}\right] \qquad \text{(Eq. 2.12)}$$

where n is a fixed averaged or representative power. It should be stressed that the success of the Weibull model is an empirical observation and there is no compelling theoretical reason to assume that it will be universally applicable, especially with a constant shape factor, i.e. where $n(T) = n$. Whenever it is found applicable, the Weibull distribution is an extremely flexible and convenient model.

The temperature dependence of $b(T)$, and of $n(T)$ whenever it is not a constant, can be described by any ad hoc empirical models. In many biological systems, degradation becomes measurable only at a certain elevated temperature range. In such cases, the $b(T)$ versus temperature relationship can be described by the log logistic model (Peleg et al., 2002; Corradini and Peleg, 2004):

$$b(T) = \log_e \{1 + \exp[k(T - T_c)]\} \qquad \text{(Eq. 2.13)}$$

where T_c marks the temperature range in which the degradation intensifies and k the process's acceleration beyond T_c.

2.5.1.2 NON-ISOTHERMAL MODELING

Under non-isothermal situations, application of the Arrhenius and WLF models to food systems is more difficult because the rate of quality deterioration is not a linear function of temperature (Peleg, Corradini and Normand, 2004). In general, the average reaction rate is given by:

$$\overline{R} = \int_0^t \frac{k(T)dt}{t} \qquad \text{(Eq. 2.14)}$$

where T is temperature at time t, $k(T)$ is the reaction rate at temperature T and $\overline{R}$ is the average rate of reaction during this period is a function of the rate.

Schwimmer et al. (1955) proposed the calculation of reaction rates and effective temperatures in some simple periodically fluctuating temperature systems. Systems in which the temperature undergoes three modes of fluctuation- saw-toothed, square, and sine waves have been described. The temperature patterns are illustrated in Fig. 2.1.

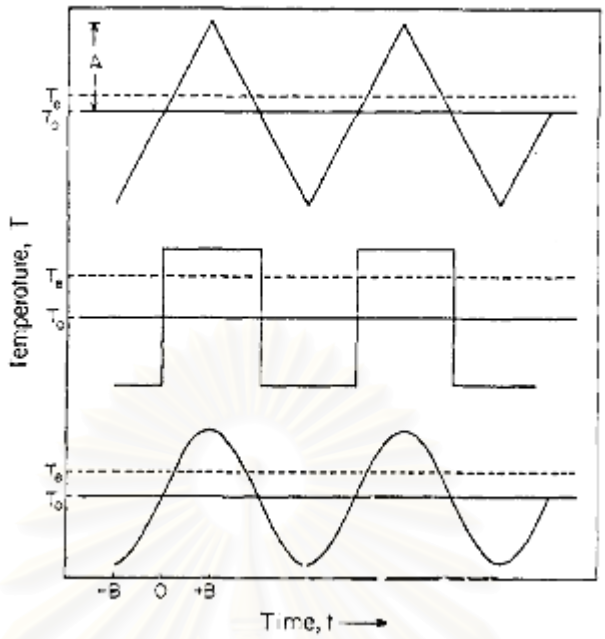


Fig. 2.1 Three modes of temperature fluctuation patterns. T_0 is the mean temperature, T_e is the effective temperature, *A* is the amplitude (half the range of maximum and minimum temperatures), and *B* is one quarter of the period of fluctuation.

Source: Schimmer et al. (1955)

The Saw-Toothed wave model for one half-cycle of amplitude *A* and duration time 2*B* is described by

$$T = T_0 + \frac{A}{B}t \qquad -B < t < B \qquad \text{(Eq. 2.15)}$$

where *T* is temperature at time, *t*. The average rate of reaction, $\overline{R}$, during this period is a function of the rate, R_0, at the mean temperature, T_0.

$$\overline{R} = \frac{R_0}{2B}\int_{-B}^{B} Q_A^{T-T_0/A} dt \qquad \text{(Eq. 2.16)}$$

where Q_A is the ratio of the reaction rate at $(T_0 + A)$ to that at T_0. This temperature coefficient is assumed to be constant throughout the temperature range of fluctuations. According to the classical Arrhenius theory, the ratio of reaction rate to temperature, Q_{10}, will not be strictly constant. The deviations become apparent over large temperature ranges and at temperature near absolute zero. Eq. 2.16 can be integrated to give:

$$\overline{R} = R_0\left[\frac{Q_A - Q_A^{-1}}{2\ln Q_A}\right] = R_0 Q_A^{\frac{T_e - T_0}{A}} = R_0 Q_A^{\alpha} \qquad \text{(Eq. 2.17)}$$

$$\alpha = \frac{T_e - T_0}{A} \qquad \text{(Eq. 2.18)}$$

where α is a dimensionless quantity which expresses the extent to which the effective temperature exceeds the mean temperature in relation to the amplitude of the cycle.

For the Square wave model, the effective rate when the temperature varies as a square wave can be treated as an average of the rates at $T_1 = T_0 - A$ and $T_2 = T_0 + A$:

$$\overline{R} = R_0\left[\frac{Q_A^{-1} - Q_A}{2}\right] = R_0 Q_A^{\frac{T_e - T_0}{A}} = R_0 Q_A^{\alpha} \qquad \text{(Eq. 2.19)}$$

For the Sine wave model, the effective rate when the temperature varies as a sine wave can be treated as an average of the rates at $T = T_0 + A\sin\frac{\pi t}{2B}$ and $T = T_0 - A\sin\frac{\pi t}{2B}$:

$$\overline{R} = \frac{R_0}{2B}\int_{-B}^{B} Q_A^{\sin\theta}\, dt \qquad \text{(Eq. 2.20)}$$

The advantages of expressing the relationship between the pertinent parameters (α and Q_A) is that the relationship holds independently of the order of the reaction or process and independently of the temperature scale. However, the reaction must have an essentially constant temperature coefficient. This relationship can be applied to any measurable process with a constant Q_{10} (e.g., evaporation, crystal growth, viscous flow, etc.).

Corradini and Peleg (2004) also proposed a model for non-isothermal vitamin degradation. Consider a non-isothermal process or thermal history, where the temperature variation with time is described by a mathematical expression, $T(t)$. According to Eq. 2.11 or Eq. 2.12, whenever $n(T) \neq 1$, or $n \neq 1$, the momentary isothermal logarithmic degradation rate is a function of time:

$$\left|\frac{dC(t)}{C_0 dt}\right|_{T=const} = -b(T)\exp\left[-b(T)t^{n(T)}\right]n(T)t^{n(T)-1} \qquad \text{(Eq. 2.21)}$$

Therefore, the degradation during any non-isothermal process or a temperature profile would be described by the differential equation:

$$\frac{d\log_{10}\left[C(t)/C_0\right]}{dt} = -b\left[T(t)\right]n\left\{\frac{-\log_{10}\left[C(t)/C_0\right]}{b\left[T(t)\right]}\right\}^{\frac{n-1}{n}} \qquad \text{(Eq. 2.22)}$$

2.5.1.3 WEIGHT LOSS PHYSICAL MODELING

Weight loss is also critical for frozen food products. Theoretical models have been proposed to predict simultaneous heat and mass transfer during freezing and storage. However, semi-empiric models have been used in most of the published works. Pham and Willix (1984) suggested the use of simple equations based on drying theory and on the use of the psychometric chart to calculate weight loss during frozen storage. They considered a dried layer of constant thickness and its resistance to heat and mass transfer; a similar approximation is made by Tocci and Mascheroni (1995).

Kuitche, Daudin and Letang (1996) proposed a mathematical model for the calculation of meat chilling. This model allows the prediction of both weight loss kinetics and internal temperature profile evolution. The model is based on analytical solutions of unsteady heat transfer in infinite cylinders. These solutions were adapted to account for product surface water evaporation to avoid fitting the effective heat transfer coefficient and the variable chilling conditions that exist in industrial chillers.

Campanone, Salvadori and Mascheroni (2001) also proposed a model for dehydration of unwrapped foods occurs during freezing and frozen storage. Coupled heat and mass balances were proposed incorporating solidification of water and sublimation of ice. The mathematical model was solved using an implicit finite-differences method, with a variable grid to follow the moving sublimation front. The model evaluates temperature and water concentration profiles and was used to predict the kinetics of weight loss for different products. Model predictions were favorably compared against experimental data for weight loss during storage of unwrapped meat, potato and tylose.

The basis physical model for weight loss relates to a difference in partial pressure around the product (Cleland, Cleland and White, 2002):

$$N_w = k_{gp} A_p (a_w p_{wp} - H_r p_{wa}) \quad \text{(Eq. 2.23)}$$

where N_w is the rate of weight loss (kg/s), k_{gp} is the mass transfer coefficient between air and product (kg/m^{2}s.Pa), A_p is the product surface area (m^2), p_{wp} is vapour pressure of water/ice at product temperature T_p (Pa), P_{wa} is vapour pressure of water/ice at air temperature T_a (Pa), a_w is water activity of product, H_r is the air relative humidity (%).

2.5.2 ARTIFICIAL NEURAL NETWORK (ANN) MODELING

A rational model could integrate, coordinate and evaluate the effects of individual input and output variables on the manufacture of a product and its quality and could be used to maximize economic benefits of the food product by improving decision-making. Although the first order reaction according to Arrhenius equation has been commonly used in kinetic studies, some food quality changes do not follow this relationship (Xie, Xiong and Church, 1998). The number of applicable factors affecting quality can be very large and they are often interact, and many of the processes and mechanisms are difficult to describe from first principles. Computer programs such as fuzzy logic and artificial neural networks have shown promise in analyzing complex interactive biological systems (Peters et al., 1996). Artificial neural networks (ANN) have been applied in food process modeling, food process controls and food process sensors. They can model a process without much a priori knowledge of the process because the network is taught from exemplar training data sets. It is especially good for modeling some food processes that are ill-defined, not well-known, nonlinear and multivariate or involve handling massive data sets. The modeling method is to identify the input and output variables of the process, select the proper neural network structure and learning rules, and train the network by a set of training data in supervised learning or its own output response in unsupervised learning (Singh and Ou-Yang, 1997).

ANN is a computational structure inspired by biological neural systems. The biological system can handle complicated tasks such as image and speech recognition, classification, generalization and adaptive learning. All ANN models attempt to achieve good performance through dense interconnection of simple computation elements (Singh and Ou-Yang, 1997). They can solve problems that are traditionally difficult or impossible using conventional computing techniques. These problems can be characterized as involving complex and nonlinear processes. Furthermore, the structure of neural networks provides not only structural parallelism, but also processing parallelism. This enables decisions to be made in real time (Bochereau, Bourgine and Palagos, 1992; Ruan, Almaer and Zhang, 1995). Therefore, ANN is well suited for food quality prediction, which is a complex task because of the nature of interrelationships among various quality parameters, compositions and processing conditions (Ni and Gunasekaran, 1998).

ANN consists of a great number of simple processing units (neurons) that are bound to each other. These units can be divided into functional layers, which are organized groups of processing units. A neural network usually has an input layer, one or more hidden layers and an output layer (Hu, 1999). The learning or training phase of a neural network typically requires paired input-output data. The input is fed into the network, transferred through the network layers and ultimately calculates a predicted output. This predicted output is subsequently compared with the actual output, and the connection weights between the processing elements are modified to minimize the deviation between the predicted and actual output. This process continues until a defined accuracy has been reached. This is the concept of back-propagation. During this training phase, many factors of a neural network structure, such as the number of hidden nodes, and the number of layers, are varied by a trial-and-error approach to obtain the optimum network. At this point, the network can be fed input data alone, and the model will accurately calculate the predicted output. Two of the key neural network variables were learning rate and momentum. Learning rate controls the degree at which connection weights are modified during the training phase. The larger the learning rate, the larger the weight changes, and the faster the learning will proceed. However, if learning rates are set too high, the neural network will not converge to its true optimum. Momentum weights the importance of the

previous iterations to the next connection weight modification (Bochereau et al., 1992; Ruan et al. 1995).

In recent years, ANN has attracted researchers in many disciplines of science and engineering, since it is capable of correlating large and complex datasets. Neural network modeling has generated increasing acceptance and is an interesting method in the estimation and prediction of food properties and process related parameters (Ni and Gunasekaran, 1998). Hussain and Rahman (1999) used the artificial neural network technique to predict thermal conductivity using a data set with 164 points.

Montague and Morris (1994) examined the contribution of various network methodologies to bioprocess modeling, control and pattern recognition. Mittal and Zhang (2000) developed ANN to predict the freezing time of food products of any shape. The Pham model was used to generate freezing time data and to train the ANN. The product thickness, width, length, convective heat transfer coefficient, thermal conductivity of frozen product, product density, specific heat of unfrozen product, moisture content of the product, initial product temperature, and ambient temperature were taken as input variables of the ANN to predict freezing time. The effects of the number of hidden layer nodes, learning rate, momentum on prediction accuracy were analyzed. Predicted freezing time using the ANN was proved to be simple, convenient and accurate. Selection of hidden nodes, learning rate and momentum were important to ANN predictions.

Xie (2001) used the mathematical solution to transient heat conduction into an isotropic and homogeneous finite cylinder, ANN and response surface (RS) models to predict roasting time and weight loss for beef joints. Predicted results from ANN and RS models were almost identical and better than the mathematical model. From the trained ANN models, it was found that higher air and initial beef temperatures decrease roasting time but increase weight loss. The ratio of beef radius to length was important for determining the weight loss. A critical ratio produces the largest weight loss. To improve the productivity and reduce the weight loss, small beef joints are recommended and the ratio of beef radius to length should be lower than the critical ratio.

Spectral stress strain analysis was used in combination with partial least squares (PLS) regression and artificial neural networks to predict nine sensory texture attributes of cooked rice (Sitakalin and Meullenet, 2001). The models calculated with ANN were significantly more accurate in predicting most of the sensory texture characteristics evaluated than those with the PLS models. Furthermore, ANN models were more robust and discriminating than PLS models.

A fuzzy neural network (FNN) is an ANN associated with fuzzy logic. FNN models make predictions or estimations as precisely as ANN models do; FNN models also elucidate the causal relationships between input and output as explicitly as multiple regression analysis (MRA) through an analysis of the fuzzy estimation rules of the acquired model (Patterson, 1996). Tominaga et al. (2002) investigated sensory modeling of coffee with a FNN. Models were constructed to predict sensory evaluation scores from the blending ratio of coffee beans. Twenty-two blended coffees were prepared from 3 representative beans and were evaluated with respect to 10 sensory attributes by 5 coffee cup-tasters and by models constructed using the response surface method (RSM), MRA and FNN.. The RSM and MRA models showed good correlations with some sensory attributes, but they lacked of sufficient overall accuracy. The FNN model exhibited high correlations with all attributes, clearly demonstrated the relationship between blending ratio and flavor characteristics, and was accurate enough for practical use.

Microbiological safety and quality testing in the food industry are important. Rapid method for microbiological testing is required. Siripatrawan and Harte (2007) developed a rapid method for prediction of the number of *S. typhimurium* from specific metabolic compounds using ANN. A multilayer perceptrons (MLPs) neural network based on back propagation was used. The MLP was trained to identify and quantify *S. typhimurium* in fresh produce under complex conditions. Good prediction was found as measured by the regression coefficient between actual and predicted data.

Sablani et al. (2002) developed an ANN to model the thermal conductivity of bakery products as a function of product moisture content, temperature and apparent density. The bakery products considered in this work were bread, bread dough, French bread, yellow cake, tortilla chip, whole wheat dough, baked chapati and cup cake. Data on thermal conductivity of bakery products were obtained from the literature for a wide range of product moisture contents, temperatures and apparent densities resulting from different baking conditions. The model was capable of predicting the thermal conductivity values of various bakery products for a wide range of conditions with a mean relative error of 10%, a mean absolute error of less than 0.02 W/mK and a standard error of about 0.003 W/mK.

Ruan et al. (1995) used a neural network to predict the rheological properties of dough from torque developed mixing. Dough rheological properties were determined using farinograph and extensigraph. The back-propagation neural network was designed and trained using the acquired mixer torque curve (input) and the measured rheological properties (output). The trained neural network accurately predicted the rheological properties (R^2>94%) based on the mixer torque curve. The ability to measure the rheology of every batch of dough enables online process control by modifying subsequent process conditions. This development has significant potential to improve product quality and reduce cost by minimizing process variability during dough mixing.

CHAPTER III

RESEARCH OBJECTIVES

3.1 SUMMARY OF LITERATURE

In Chapter II, the literature related to frozen dough production, effect of freezing and frozen storage on frozen food and frozen dough quality and modeling of food quality was reviewed. The literature can be summarized as follows:

- Freezing and cold storage are important preservation processes widely applied in the food industry. Sales of frozen foods are closely associated with increased ownership of domestic freezers and microwave ovens. The market for frozen bakery goods including frozen bread dough has grown rapidly (Section 2.1).

- Frozen storage research mainly considers isothermal storage on quality losses aimed at establishing shelf-life dates for different storage temperature (Section 2.4.1.3).

- Several researchers have related time-temperature profiles to food product quality (Section 2.4.2 and 2.4.3).

- Frozen dough quality is sensitive to freezing and storage conditions due to chemical and biochemical reactions occurring in the product that strongly depend on time-temperature history (Section 2.4.4).

- The mechanisms of quality losses of frozen dough includes losses in yeast viability leading to low baking performance, changes of the starch-gluten matrix leading to low gas retention, and ice recrystallization causing disruption to the yeast cell membranes and gluten matrix (Section 2.4.4).

- The research data on the effect of temperature fluctuations/oscillations on frozen dough and the interaction between freezing rate and temperature fluctuations/oscillations have been limited.

- Some researchers have reported the effect of freeze-thaw cycles on quality changes of dough (Section 2.4.4). Temperature fluctuations/oscillations in the cold store and cold chain distribution are not the same as full freeze-thaw cycles.

- Sensory evaluation is the ultimate test of quality but it is expensive, time consuming, lacks precision and is subjective.

- A wide range of analytical measures, both objective and subjective, to define quality changes have been proposed and used including (Section 2.4.4).

    - Dough weight loss.
    - DSC or centrifugation to measure freezable water.
    - Risograph or Rheofermentator to measure carbon dioxide production.
    - Yeast plate count to measure yeast viability.
    - Large and small deformation using Rheometer or TA-XTPlus to measure dough rheological properties.
    - SEM or CLSM or light microscope to measure dough microstructure including starch granule and protein matrix structure, ice crystals size distribution and air bubbles size distribution.
    - NMR to measure water distribution or ice fraction.
    - Seed displacement to measure bread volume.
    - Universal testing machine or TA-XTPlus to measure bread textural properties.
    - Scoring by panelists or experienced panels to measure bread sensory characteristics.

- The best analytical measurement and their relationship between instrumental and sensory quality is not clear. Therefore, several analytical measurements are generally used to assess the quality of dough and bread.

- A better understanding of quality kinetics in food systems facilitates improved formulation, storage and processing.

- Limited quality kinetics information is available for frozen bread dough.

- The basic quality kinetic data of frozen dough under constant storage temperature can be used to predict the effect of fluctuating temperature.

- Modeling provides a way to extrapolate and implement results. Mechanistic modeling may prove difficult for dough because dough properties are complex. Artificial neural network (ANN) modeling looks promising in such circumstances.

3.2 OBJECTIVES

The overall aim of this research is to study the effect of temperature fluctuations/oscillations and temperature changes through the cold chain distribution on the quality losses of frozen bread dough. The specific objectives of this study were to:

1) Identify a set of analytical measures to be used as de facto indicators of dough and bread sensory quality.
2) Measure basic quality kinetic data for frozen dough under constant temperature condition to provide a baseline to analyze the effect of fluctuating temperature.
3) Measure the effect of the following conditions on frozen dough and bread quality using both the methods and variables identified above:

- Freezing rate
- Average frozen storage temperature
- Oscillations in storage temperature likely to be experienced due to imperfect temperature control in storage facilities.
- Fluctuations and changes in storage temperature likely to be experienced as the dough moves through the cold chain from manufacturer to consumer.

4) Model the effect of freezing and storage condition on quality using both mechanistic and artificial neural network (ANN) approaches and select the best approach.

CHAPTER IV

METHODOLOGY

4.1 DOUGH PREPARATION

Dough samples were prepared using a straight dough formula described by Miller (2006). The dough recipe comprised 60% w/w commercial wheat flour (12% moisture content, 13% protein, 0.67% ash), 2% w/w compressed yeast, 1% w/w salt, 2% w/w sugar, 2% w/w canola oil and 33% w/w water (40% w/w of this water as ground ice). This corresponded to 3.3 g yeast, 1.7 g salt, 3.3 g sugar, 3.3 g oil and 55 g water for each 100 g of flour. Standard bakers' compressed yeast (Pinnacle brand, Auckland, New Zealand) was used in this work. This yeast is known as not being particularly freeze tolerant. Ground ice was used to delay yeast pre-fermentation (Le Bail et al., 1999).

All ingredients were mixed in a dough mixer (Model 7MX, Delta Food Equipment, New Zealand) for 4 minutes at low speed and for 10 minutes at high speed. The dough temperature was 18±1^{o}C at the end of mixing as recommended by Basaran and Gocmen (2003) as a compromise between excessive pre-fermentation and adequate development of the gluten network during mixing. After mixing, the dough was rested for 10 minutes and then divided into 100±2 g pieces, manually molded into round shapes (about 5 cm diameter), and placed each dough into 170 mm ×180 mm snaplock polyethylene bags before freezing. The resting, shaping and packaging processes took about 35 minutes at room temperature (about 20^{o}C). Dough preparation is shown schematically in Fig. 4.1.

All ingredients

↓

Mix in a dough mixer at low speed for 4 minutes
and high speed for 10 minutes

↓

Divide and molded the dough into 100±2 g pieces

↓

Place in snaplock polyethylene bag

↓

Freezing

Fig. 4.1 Dough preparation flowchart.

4.2 FREEZING

Cryogenic freezing using liquid nitrogen is the fastest practical way to commercially freeze a food item. However, preliminary trials of cryogenic freezing showed that the freezing rates were too fast, resulting in lower CO_2 production. The cryogenic freezing rate was estimated to be about -15.2°C/min between 18°C and -20°C. It is believed that the critical rate of dough freezing should be less than 1°C/min (Gelinas et al., 1993) and more than -0.21°C/min (Cauvain, 1998b). Therefore, the doughs were frozen using slow freezing (SF) or fast freezing (FF) conditions. The endpoint of freezing process was established as when the sample center temperature reached -20°C. The SF condition used an air blast freezer (Long Beck, Panel Systems Ltd., New Zealand) at about -25°C with air speed of 2.5 m/s for 120 minutes. The freezing rate was estimated to be about -0.28°C/min between 0°C and -20°C. The FF condition used air temperature at about -35°C with air speed of 5 m/s for 60 minutes. The freezing rate was estimated to be about -0.70°C/min between 0°C and -20°C.

4.3 THAWING, PROOFING AND BAKING

After frozen storage, frozen dough samples were thawed prior to quality assessment by immerging them in a water bath at 0°C for 90 minutes. After thawing, the dough pieces were put into 6 cm × 9 cm × 5 cm D2-Mini loaf pans (Wiltshire brand, item 9218, China) and proofed at 37±2°C (85% relative humidity) for 60 minutes in the proofer (Satchwellsun Vic, New Zealand). The dough pieces were baked in a 37 cm × 42 cm × 55 cm oven (AR85, Electrolux, Steelfort Engineering Company Ltd., Palmerston North, New Zealand) at 180°C for 15 minutes before cooling at ambient temperature (about 20°C) for 2 hours prior to quality assessment. The top of the dough was not cut before baking. Fresh dough was used as the overall quality level control.

4.4 FROZEN STORAGE SYSTEM

To achieve the various frozen storage regimes, dough samples were stored in cardboard boxes (Fig 4.2) in a walk-in coldstore at -28°C that was automatically defrosted every 8 hours. Each box was 84 cm × 62 cm × 25 cm and was constructed of 0.7 mm thick corrugated cardboard. A light bulb (between 40 W and 200 W) and 2 PC computer fans were located in one corner of the box to provide both heating and air circulation to ensure uniform temperature conditions throughout the box. A total of 84 dough samples were placed into each box including dummy samples immediately adjacent to the light bulb location. Dummy doughs were prepared in the same manner as the tested doughs. Dummy samples were used for preventing the heat from the light bulb overheating the test samples. The light bulb was controlled by an electronic thermostat with a defined set-point and dead band. The sizes of light bulb were selected to control the temperature fluctuations about the set-point in the range of 0.15 to 10 cycles per hour, depending on each storage temperature regime. For example, a 60 W light bulb gave a heating rate of about 0.13°C/min when controlled at -20°C. The cooling rate was about -0.13°C/min for all boxes at -18°C. Temperatures in the storage system were monitored using type T thermocouples connected to an Agilent datalogger (Model 34970A). The thermocouples were calibrated against an ice-point and a calibrated thermometer to within +/- 0.1°C accuracy.

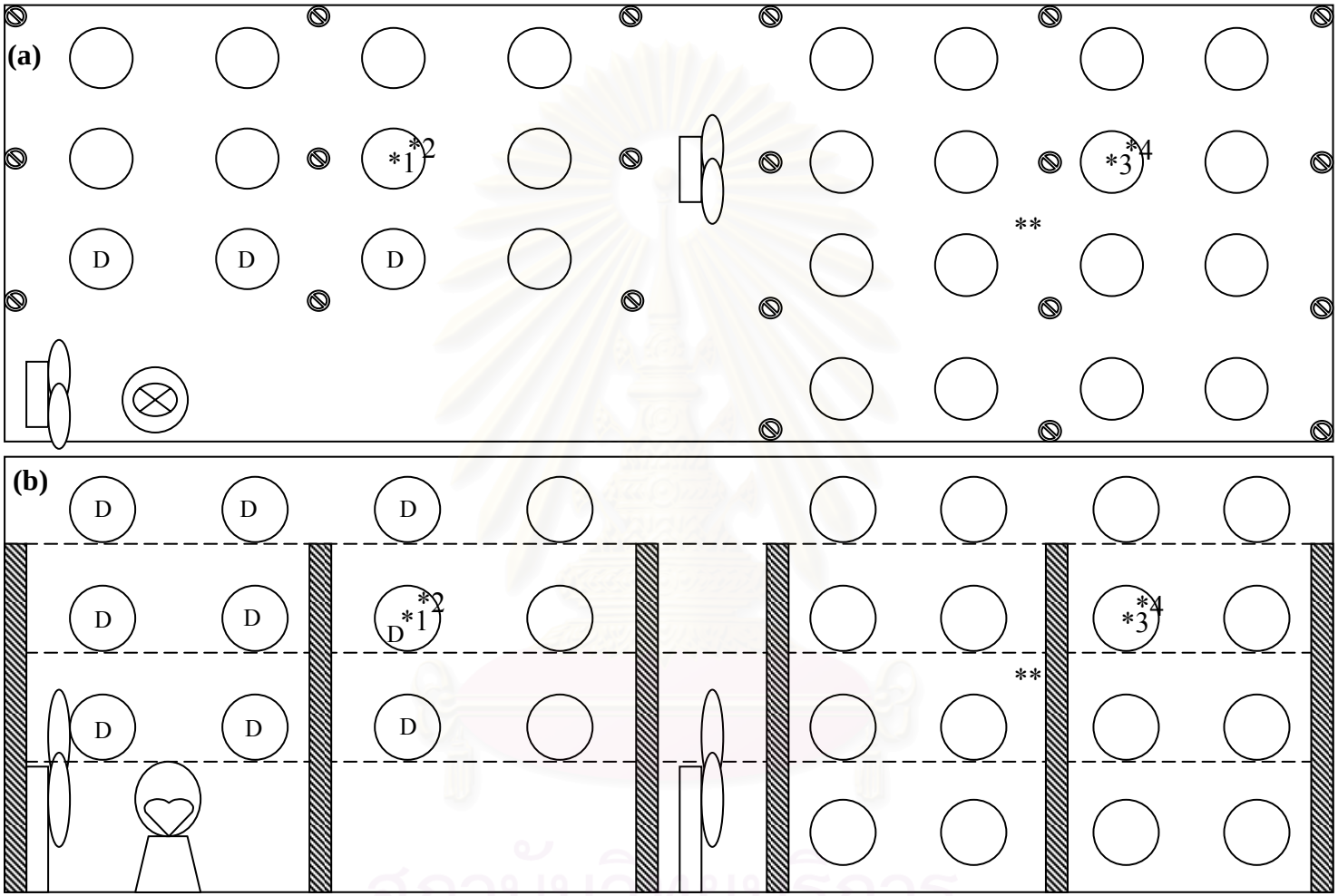


Fig. 4.2 Schematic diagram of the cardboard box storage system. (a) Top view (b) Side view. *1-4 indicates thermocouple positions. ** indicates temperature control sensor. D indicates dummy dough pieces. ---- indicates wire mesh trays. ⦸ indicates PVC support pipes in the top view. ▨ indicates PVC support pipes in the side view.

4.5 DOUGH AND BREAD QUALITY ASSESSMENT

There are many techniques to measure quality of dough and baked bread. The techniques to assess dough and baked bread quality are summarized below.

4.5.1 WEIGHT LOSS MEASUREMENT

As stated in Section 2.4.3, weight loss leads to reduction of both quality and saleable weight. Weight loss during frozen storage was measured.

To weigh the dough pieces, three dough samples were withdrawn from each controlled temperature box in the freezer and the dough pieces were removed from the polyethylene bag. The dough was weighed with ±0.01 g precision before being returned to the bag and the box. This whole process took less than 3 minutes. The weight loss was the difference between the initial weight and the measured weight at different storage periods.

4.5.2 CARBON DIOXIDE PRODUCTION MEASUREMENT

As reviewed in Section 2.4.4.2, CO_2 production is a key quality parameter for dough and bread. Preliminary trials of risograph measurements showed that the results for frozen storage dough were reproducible so it was used to determine the dough quality (Phimolsiripol et al., 2006a). The details of this study are provided in the Appendix A.

CO_2 production was measured using a risograph (R-Design, Pullman, WA) according to the method of El-Hady et al. (1996). For each replicate and treatment, 50 g sample of dough after thawing was placed into fermentation jars, and then placed in a water bath at 30^{o}C. The gas volume was measured every minute for 180 minutes after a 10 minute delay. Both cumulative CO_2 production (ml CO_2) and CO_2 production rate (ml CO_2/min) were measured. The percentage reduction in cumulative CO_2 production (gassing power) was calculated relative to fresh dough.

4.5.3 YEAST VIABILITY DETERMINATION

Loss of yeast viability results in low gassing power of dough and small bread specific volume (Section 2.4.4.3). Preliminary yeast plate count trials showed that yeast viability of frozen dough decreased with increasing storage time.

Yeast viability was measured using the AACC Approved Method 42-50 (AACC, 2000). Logarithmic dilutions were carried out in peptone water, and the diluted suspensions was cultured on a potato dextrose agar (Merck KgaA, Germany), adjusted to pH 3.5 with tartaric acid. The counts of surviving yeast in the dough were determined after 3 days of incubation at 25^{o}C. Samples were selected from the center of the dough pieces. Duplicate plates were prepared for each of 3 dough samples per treatment.

4.5.4 DOUGH RHEOLOGICAL PROPERTY MEASUREMENT

As outlined in Section 2.4.4.4, rheological characteristics of the dough exposed to freezing rate, freeze/thaw cycle and temperature changes during storage could be related to the final quality of dough and bread after baking. There are two main techniques (small and large deformation) to determine the dough rheological properties. The large deformation measurement shows good relationship with baking performance (Kieffer et al., 1998 and Uthayakumaran et al., 2002). A TA-XTplus with the SMS/Kieffer dough and gluten extensibility rig was chosen for dough rheology assessment because it used a small sample, it is a fast technique and the instrument was available. Phimolsiripol et al. (2006b) reported a successful technique to minimize the effect of fermentation on rheological measurement by holding yeasted dough in an ice/water bath for up to 90 minutes. The details of this study are provided in the Appendix B.

Uniaxial extension measurements were made using the SMS/Kieffer dough and gluten extensibility rig for a TA-XTplus texture analyzer (TA-XTplus, Stable Microsystems, Surrey, UK) following the large deformation method of Bhattacharya et al. (2003). Twenty grams of thawed dough at 0^{o}C was placed into a Teflon-coated

block, lined with parafilm, and cut into dough strips using a mould. The dough strips were allowed to rest for 30 minutes in air at 20°C, before being stretched by a hook extension at the speed of 3.3 mm/s for a distance of 100 mm (Suchy et al., 2000). All tests were carried out at a constant room temperature of 20°C. Dough extensibility (mm) from start to rupture and maximum force before rupture (g) were automatically calculated by the data processing software supplied with the TA-XTplus.

4.5.5 DOUGH MICROSTRUCTURE MEASUREMENT

The dough microstructure changes during frozen storage at the microscopic level as stated in Section 2.4.4.5. Dough microstructure has been investigated with different techniques including scanning electron microscope (SEM) and confocal laser scanning microscopy (CLSM).

Preliminary CLSM trials showed that the sample preparation before measurement affected the results. This was due to cutting the dough in the frozen state. The surface was not smooth, resulting in unclear micrographs and unidentifable dough microstructural characteristics. Hence, CLSM was not considered further for dough microstructure measurement. Preliminary trials showed that the SEM technique was effective in showing the effect of processing conditions on structure.

Measurement of dough microstructure was carried out using a SEM according to the method of Indrani et al. (2003). Samples were taken from the centre of the frozen dough, cut into 4 cm long and 4 mm diameter shape using a stainless puncture tube while frozen, and then freeze-dried. A fracture surface of the freeze-dried samples was mounted on the specimen holder and sputter-coated with gold at 0.05 mbar. Finally, each sample was transferred to a SEM (Model 250 Mark 3, Cambridge StereoScan, UK). The micrographs were made at 500x and 2000x magnification.

4.5.6 WATER MOBILITY DETERMINATION

As discussed in Section 2.4.4.1, one of the most direct techniques to measure the amount of water that can be retained by the dough is centrifugation immediately after thawing (Rasanen et al., 1997a; Seguchi et al., 2003). However, centrifugation of dough needs very high speed centrifuges and takes a long time. A suitable centrifuge was not available so, centrifugation for measuring the freezable water changes in the dough was not used.

Preliminary trials of a differential scanning calorimetry (DSC) for measuring the freezable water did not show any significant change in measurement as reported by Bot (2003) and Baier-Schenk et al. (2005). This was probably due to the DSC available was not sensitive enough. Therefore, the DSC technique was not used to assess the frozen dough quality. Nuclear magnetic resonance (NMR) has also been shown to be a powerful technique to calculate the amount of unfrozen water in a food sample and was available so it was used.

Water mobility was measured using a nuclear magnetic resonance (NMR) Bruker Avance 400 MHz spectrometer according to the method of Esselink et al. (2003). Transverse proton relaxation times (T_2) were measured using the Carr Purcell Meiboom Gill (CPMG) pulse sequence [90º-τ-180º-τ-…*n*…echo]. The echo spacing (τ) 100 μs was used and the number of CPMG cycles *n* ranged from 2 to 130. Data were averaged over eight acquisitions with a recycle delay of 5 s. T_2 relaxation measurements were performed at -20ºC in a 10 mm RF coil. The CPMG curves were approximated well by a sum of two exponential curves of the form:

$$S(t) = A_p \exp(-tR_{2p}) + A_s \exp(-tR_{2s}) \qquad \text{(Eq. 4.1)}$$

where A_p and R_{2p} are, respectively, the fraction of protons (%) and the relaxation rate of a fast decay component attributed to water protons in the ice, and A_s and R_{2s} are the fractions of protons and the relaxation rate of a slow decay component attributed to non-frozen water protons.

4.5.7 BREAD QUALITY EVALUATION

Bread sensory characteristics relate to the consumer acceptability as discussed in Section 2.4.4.9. However, sensory testing requires an experienced or trained panel. Full sensory panel testing needs a lot of sample and takes time. A photocopier has been applied to investigate the bread crumb characteristics (Schwarzlaff et al., 1996). This method is simple and quick. Thus, it was used to quantify bread crumb characteristics.

High loaf volume is positively correlated with a number of consumer-preferred quality characteristics of bread. The loaf volume is an end-use indicator of bread quality that can be used to identify the effect of other quality changes in dough (Section 2.4.4.7). The simplest method to measure the volume of baked bread is seed displacement.

Textural properties are a major quality factor for baked bread. The changes of microstructure may cause changes in textural properties as outlined in Section 2.4.4.8. The most frequency used instruments in bread crumb firmness are a TA-XTplus texture analyzer.

The bread quality parameters measured were specific volume, bread crumb image and bread crumb firmness. Two hours after baking, the volumes of the baked bread were measured using the seed displacement method and the specific volumes were calculated following the AACC Approved Method 55-50 (AACC, 2000). Bread crumb images were evaluated using a photocopier (HP 3300C, Hewlett-Packard Development Company, Japan). The photocopy image was subjectively assessed in terms of size and uniformity of the crumb cell structure. Bread crumb firmness was measured using a TA-XTplus texture analyzer (TA-XTplus, Stable Microsystems, Surrey, UK) with the SMS 45 mm diameter compression probe (P/45C) and according to the texture profile analysis (TPA) method. Firmness is the peak force during the first compression cycle. Two hours after baking, the central slices of each loaf were cut into 20 mm by 20 mm by 20 mm pieces using an electric knife (Breville brand, Model BEK5, Breville Holding Pty Ltd., China) to prevent structural damage. The

TPA method was conducted under following conditions: pre-test speed, 2 mm/s; post-test speed, 1 mm/s; rupture test distance, 1%; measurement distance, 40% deformation; force, 0.10 kg; time, 1.0 s; and auto trigger force, 0.020 kg (Kadan et al., 2001). All measurements were performed in triplicate using 3 bread samples per treatment.

4.6 EXPERIMENTAL PLAN

Frozen storage regimes were selected and designed to both obtain basic kinetic rate data for quality deterioration as a function of temperature (QK), and to mimic good and poor storage practice with temperature fluctuations (TF) likely to be experienced in the cold chain. The regimes were a compromise between practical time constraint to complete the work and providing representative changes of the dough temperature during the cold chain. Also, the regimes used were constrained by the characteristics of the storage equipment available. The storage regimes were similar to those used by Alvarez and Canet (1998), Ben-Yoseph and Hartel (1998) and Taoukis and Giannakourou (2004).

Two main experiments were undertaken to study the effects of freezing rates and frozen storage regime. QK1 and TF1 were designed to study the interactions between the freezing rates and the frozen storage regime. For subsequent experiments (QK2 and TF2), only one freezing rate was selected and the main purpose was to investigate the effect of frozen storage regime.

4.6.1 EFFECTS OF FREEZING RATES AND FROZEN STORAGE REGIME (QK1 AND TF1)

The interaction between freezing rates and storage regimes during frozen storage were studied in QK1 and TF1 using a completely randomized factorial design. For QK1, two freezing rates, 4 constant temperature regimes and 5 storage durations were investigated. For TF1, two freezing rates, 4 fluctuating temperature regimes and 5 storage durations were investigated. The experimental plans are shown in Fig. 4.3.

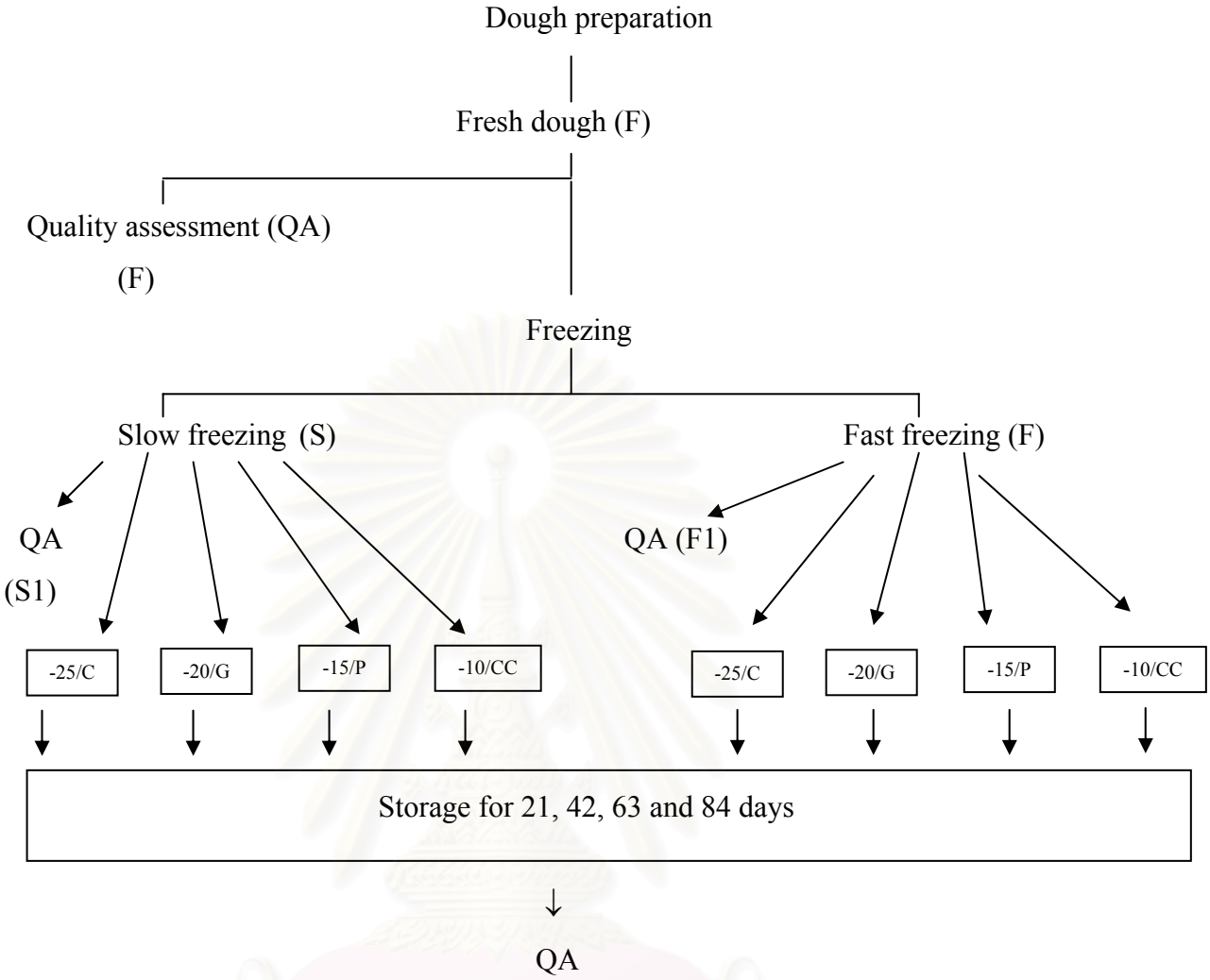


Fig. 4.3 Schematic of the overall experimental plan for the QK1 and TF1 experiments.

4.6.1.1 Dough Preparation and Freezing

Three replications were used for most quality assessment to quantify the uncertainty and variation yet to keep the time to complete all quality assessments practical. Each tripicate quality assessment required a total of 30,000 g of dough to do the 40 combinations of freezing rate, storage regime and storage duration. Therefore six 5.5 kg batches of dough were prepared for each of QK1 and TF1.

Dough samples were prepared according to method in Section 4.1. The doughs were frozen using slow freezing (SF) and fast freezing (FF) conditions as described in Section 4.2.

To reduce experimental uncertainty from sample to sample, the dough samples were prepared in six 5.5 kg batches (B1-B6) as follows. Batch 1 was prepared first. The batch was divided into 2 sets according to the freezing rate. Set 1 was frozen first with fast freezing at -35^{o}C for 1 hour. Set 2 was stored in an ice/water bath at 0^{o}C for 1 hour to prevent yeast fermentation prior to slow freezing at -25^{o}C. When batch 1 was finished, the frozen dough samples were stored at constant temperature (-20^{o}C). The other batches (Batch 2-6) were prepared in the same pattern. The dough preparation process for all 6 batches took 3 days. After finishing dough preparation, the dough pieces were allocated into the different storage regimes and the zero week dough samples (designated 1 day after freezing) were assessed for quality. The sample preparation process is summarized in Fig. 4.4.

4.6.1.2 Frozen Storage Regimes

The storage temperatures for determining the quality kinetics (QK1) of frozen bread dough comprised 4 constant storage temperatures (-25±0.1^{o}C, -20±0.1^{o}C, -15±0.1^{o}C and -10±0.1^{o}C). In parallel the four storage regimes for TF1 were -20±0.1^{o}C (Control, C), -20±1^{o}C (Good Practice, G), -20±3^{o}C (Poor Practice, P) and the cold chain (CC). For the cold chain regime, the temperature set-points were -20±1^{o}C for 4 days, -15±1^{o}C for 1 day, -10±1^{o}C for 1 day, and then -20±1^{o}C for 1 day

on a repeating weekly cycle. Samples were stored for 21, 42, 63 or 84 days prior to quality assessment.

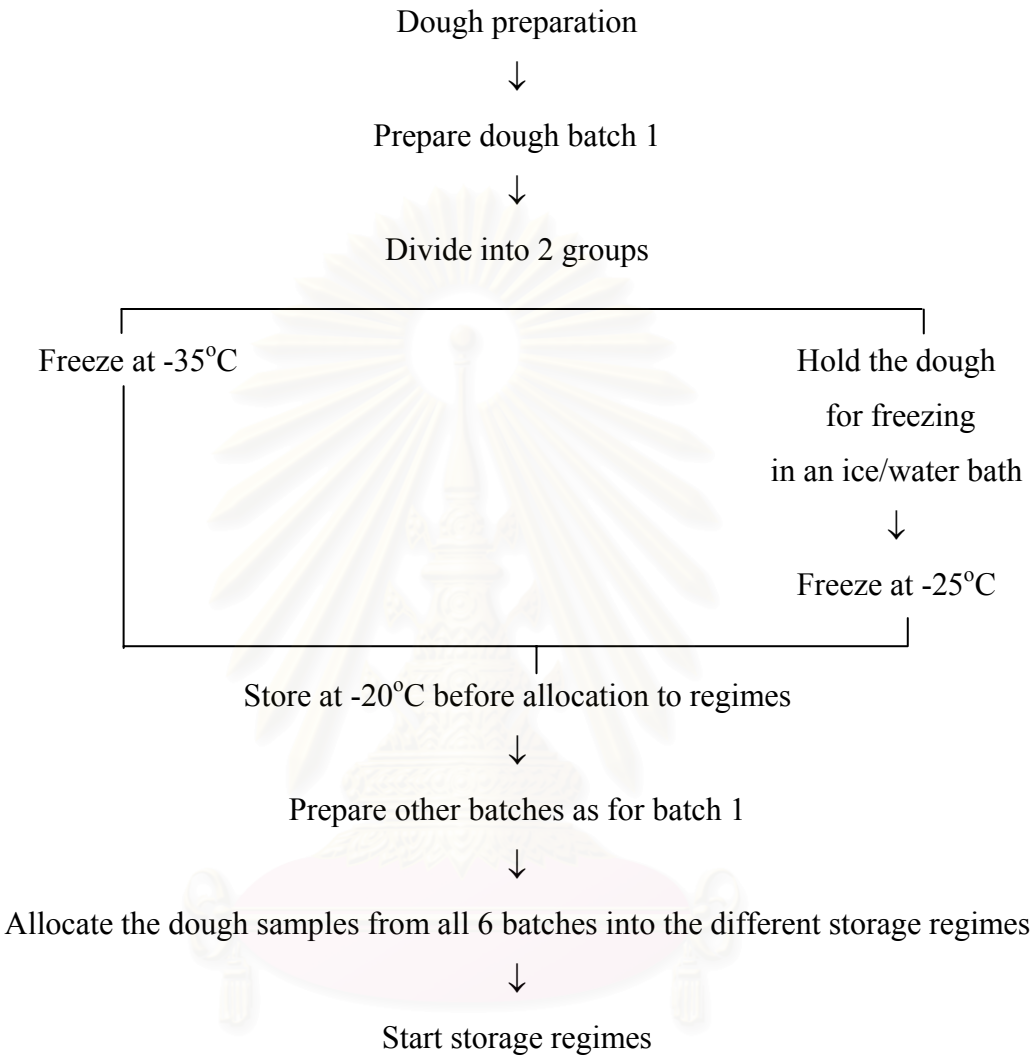


Fig. 4.4 Sample preparation process for the QK1 and TF1 experiments.

4.6.1.3 Quality Assessment (QA) for QK1 and TF1

After storage, the dough samples were thawed, proofed and baked according to the methods as described in Section 4.3. Quality parameters including dough weight loss, CO_2 production, yeast viability, dough rheological properties, dough microstructure, bread specific volume, bread crumb firmness and bread crumb image were measured according to the methods as described in Section 4.4.

Fig. 4.5 gives the allocation of dough samples from batches to quality assessment. The allocation of samples from batches was systematic to reduce the effect of variation from batch to batch. To determine weight loss, CO_2 production, yeast viability, rheological properties and microstructure, the dough samples were selected from batches 1, 3 and 5. To determine the loaf volume, bread firmness and bread image, the dough samples were selected from batches 2, 4 and 6.

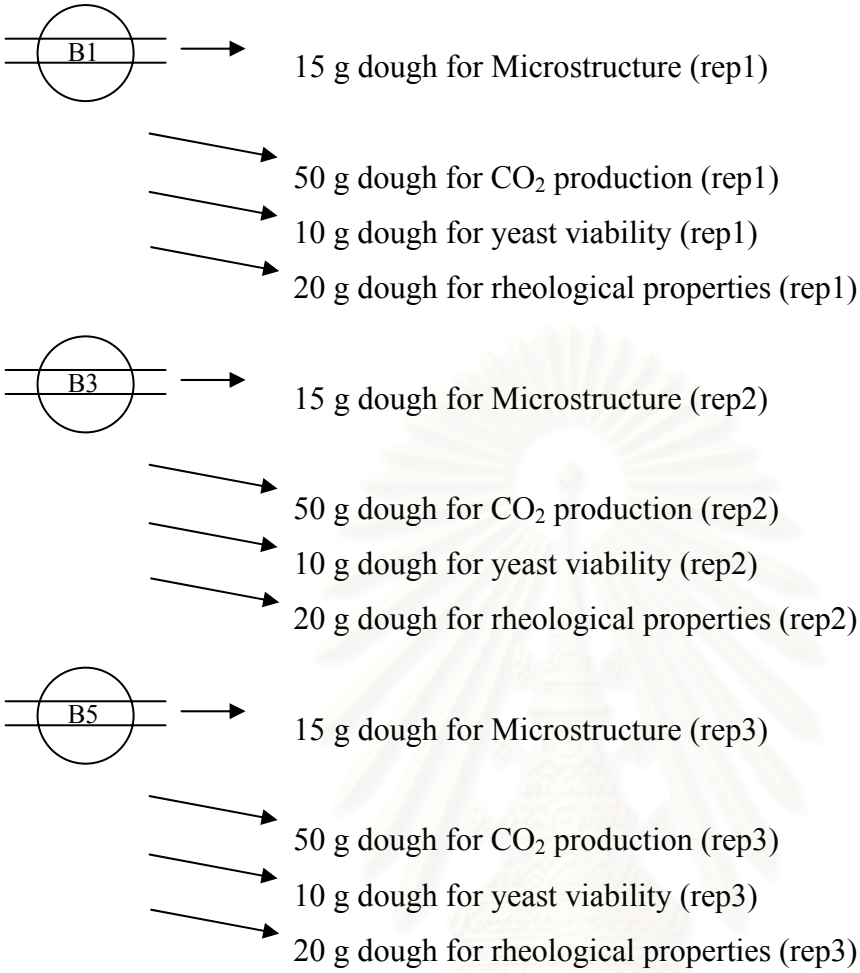


Fig. 4.5 Allocation of dough samples from preparation batches to quality assessment for the QK1 and TF1 experiments.

4.6.2 EFFECT OF FROZEN STORAGE REGIME (QK2 AND TF2)

A completely randomized factorial design was used for the QK2 and TF2 experiments. For QK2, four constant temperature temperatures and 7 storage durations were investigated. For TF2, four fluctuating temperature regimes and 7 storage durations were investigated. Fig. 4.6 shows overall experimental chart of both QK2 and TF2 experiments.

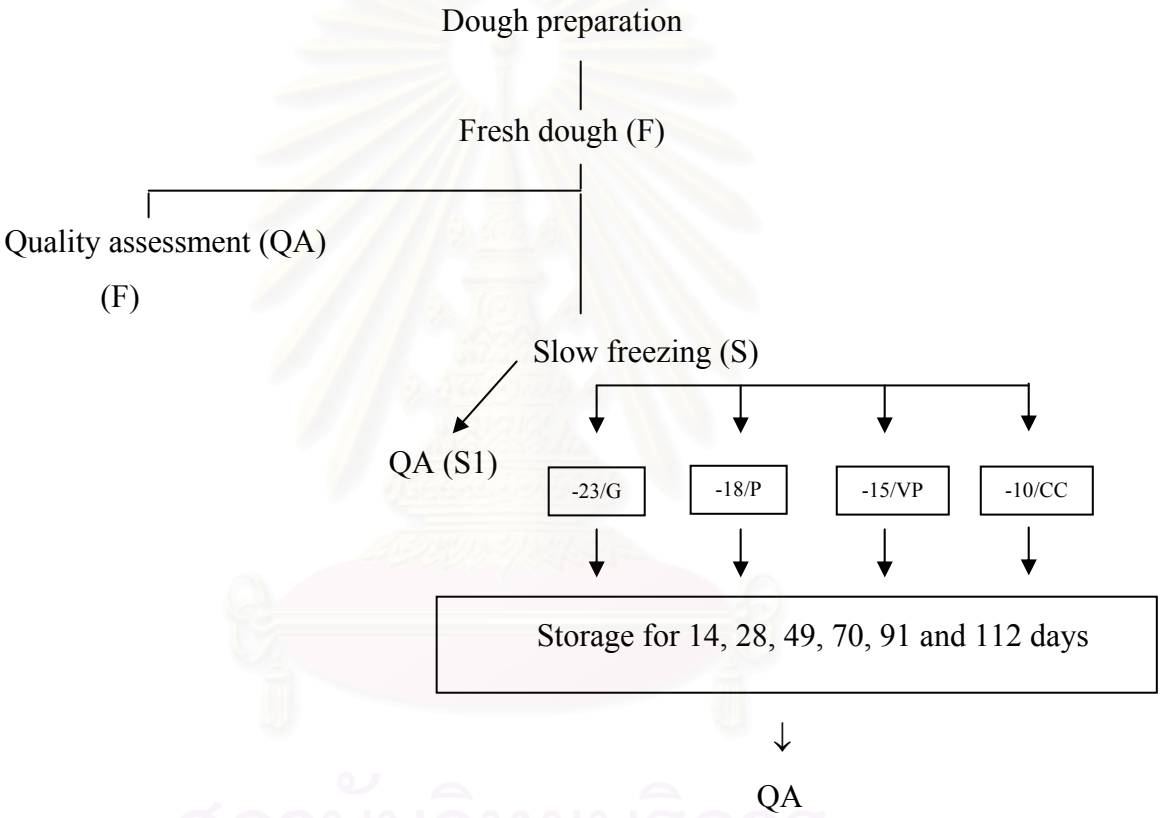


Fig. 4.6 Schematic of the overall experimental plan for the QK2 and TF2 experiments.

4.6.2.1 Dough Preparation and Freezing

As for QK1 and TF1, 3 replicates were used for most quality assessments. The 24 combinations of storage regime and storage duration meant that a total of 17,400 g of dough was required. Therefore, three 6 kg batches of dough were prepared as in Section 4.5.1.1. The sample preparation process is summarized in Fig. 4.7. The slow freezing rate was selected for the QK2 and TF2 experiments because it gave better quality of frozen dough and bread in experiments QK1 and TF1. Section 4.2 describes the freezing process.

4.6.2.2 Frozen Storage Regimes

The storage temperatures for the QK2 experiment comprised 4 constant temperature storage temperatures (-23±0.1^{o}C, -18±0.1^{o}C, -13±0.1^{o}C and -8±0.1^{o}C). The four temperature regimes for TF2 were -18±0.1^{o}C (Good Practice, G), -18±3^{o}C (Poor Practice, P), -18±5^{o}C (Very Poor Practice, VP) and the cold chain (CC). For the cold chain regime, the temperature set-points were -18±1^{o}C for 4 days, -13±1^{o}C for 1 day, -8±1^{o}C for 1 day, and then -18±1^{o}C for 1 day on a repeating weekly cycle. Samples were stored for 14, 28, 49, 70, 91 or 112 days prior to quality assessment.

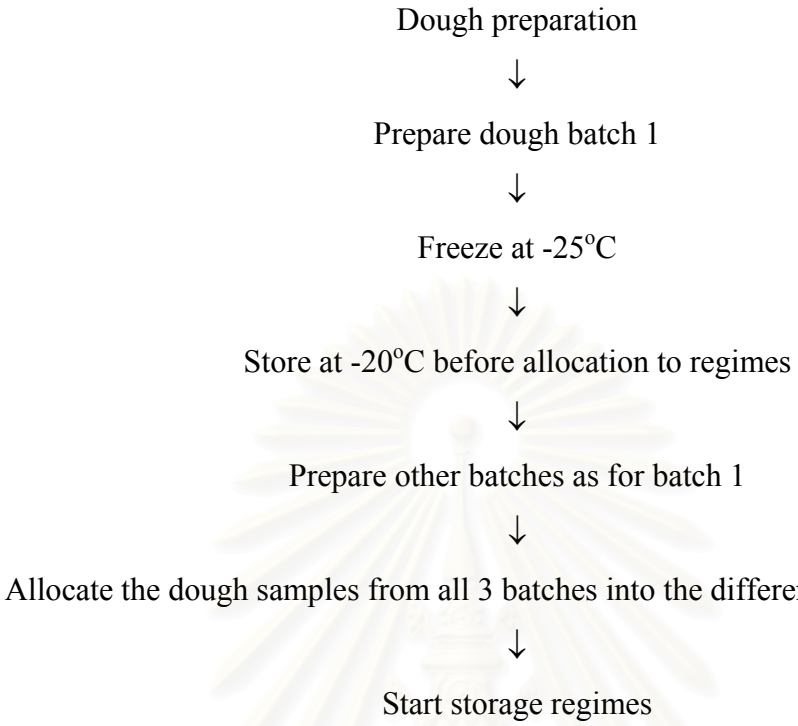
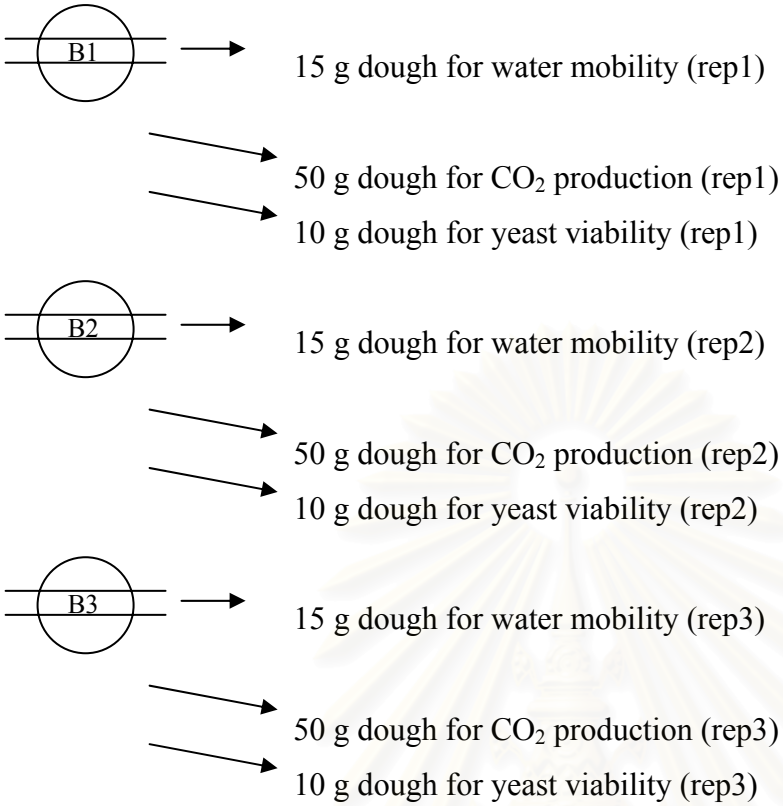


Fig. 4.7 Sample preparation process for the QK2 and TF2 experiments.

4.6.2.3 Quality Assessment (QA) for QK2 and TF2

After storage, the dough samples were thawed, proofed and baked according to the methods as described in Section 4.3. Quality parameters including dough weight loss, CO_2 production, yeast viability, water mobility, bread specific volume, bread crumb firmness and bread crumb image were measured according to the methods as described in Section 4.4. The allocations of samples from batches were systematic to minimize the effect of batch to batch variability as shown in Fig. 4.8.



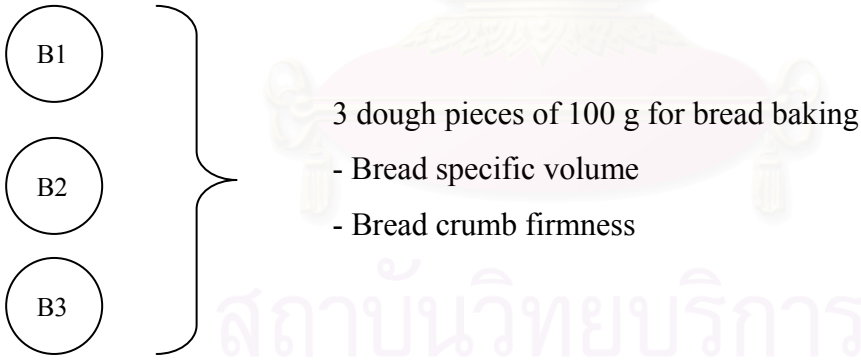


Fig. 4.8 Allocation of dough samples from preparation batches to quality assessment for the QK2 and TF2 experiments.

4.7 STATISTICAL ANALYSIS

The statistical analysis system (SAS Institute, Inc., version 8.0, 2000) was used to conduct an ANOVA using PROC GLM, to find out if the effects of different storage variables (temperature and time) and their interactions on the quality characteristics of frozen dough were significant. Duncan's multiple range test ($p<0.05$) was used to detect differences among treatment means.

4.8 MODELING

4.8.1 KINETIC MODELS

Quality kinetic data were fitted to the Arrhenius law and the Williams-Landel-Ferry (WLF) model.

4.8.1.1 Arrhenius Model

The Arrhenius law was used to fit the experimental data points using the two-step method described by Arabshahi and Lund (1985). Only weight loss, CO_2 production and bread specific volume data were used as key parameters representing frozen bread dough quality. In the first step, linear fits assuming zero-order reaction kinetics of the quality property were applied for each isothermal experiment to calculate the corresponding reaction rate constant (k):

$$C = C_0 - kt \qquad \text{(Eq. 4.2)}$$

In the second step, the rate constants were fitted using the Arrhenius equation:

$$k = A\exp\left(-\frac{E_a}{RT}\right) \qquad \text{(Eq. 4.3)}$$

where C is the quality retention (%) at time t, k is the rate constant (%/day) at temperature T (K), E_a is the Arrhenius activation energy (J/mol), A is a constant (%/day), R is the universal gas constant (J/mol.K), and t is time (day).

Each parameter's standard error was estimated from the variance-covariance matrix of the regression coefficients and the model variance was estimated by the mean standard error (Neter et al., 1996).

4.8.1.2 Williams-Landel-Ferry (WLF) Model

The WLF model, which is an alternative method to describe the temperature dependence of a reaction, was also used to fit the CO_2 production and the bread specific volume. The WLF equation is (Sapru and Labuza, 1993):

$$\log\frac{k_{ref}}{k} = \frac{-C_1(T - T_{ref})}{C_2 + (T - T_{ref})} \qquad \text{(Eq. 4.4)}$$

where C_1 and C_2 are system-dependent coefficients, and k_{ref} is the reaction rate at the reference temperature, T_{ref} (Ferry, 1980). Often T_{ref} is taken to be the glass transition temperature, T_g or higher. Rasanen et al. (1998) and Laaksonen et al. (2002) reported that the T_g of the dough varied from -25°C to -43°C, depending on dough composition and measuring technique. Therefore, T_{ref} of the frozen dough was assumed to be -26°C, -30°C or -43°C. If the model is applicable then a plot of $[\log k]^{-1}$ versus $\frac{1}{T - T_{ref}}$ will give a reasonable linear fit.

4.8.2 WEIGHT LOSS PHYSICAL MODEL

The basis physical model for weight loss relates to a difference in partial pressure between the product and the surrounding air (Cleland et al., 2002):

$$N_w = k_{gp} A_p (a_w p_{wp} - H_r p_{wa}) \qquad \text{(Eq. 4.5)}$$

where N_w is the rate of weight loss (kg/s), k_{gp} is the mass transfer coefficient between air and product (kg/m^{2}s Pa), A_p is the product surface area (m^2), p_{wp} is vapor pressure of water/ice at product temperature T_p (Pa), p_{wa} is vapor pressure of water/ice at the air temperature T_a (Pa), a_w is water activity of product, H_r is the air relative humidity (%).

Eq. (4.5) means that weight loss would be proportional to the difference in partial pressure of water vapor in the air and at the surface of the dough. A common empirical approach is to assume a constant surface water activity (usually near to 1.0) and a constant air relative humidity (H_r) inside the package (likely to be close to 100%). In this case, if the temperature is constant then the rate of weight loss should be proportional to the vapor pressure of water at the storage temperature as $p_{wp} = p_{wa}$. The vapor pressure data for water and ice as a function of temperature is shown in the Appendix C. If the rate of weight loss is a linear function of water/ice vapor pressure then this is consistent with this mechanistic explanation. Therefore, the measured rate of weight loss at constant temperature was fitted against the water/ice vapor pressure corresponding to the storage temperature.

4.8.3 NON-ISOTHERMAL QUALITY KINETICS MODEL

Two approaches were used to predict the dough weight loss, CO_2 production loss and bread specific volume loss rate kinetics for the non-isothermal experiments (TF1 and TF2).

In the Isothermal approach the Arrhenius model was used to estimate the average reaction rate (k_{av}) based on the average temperature (T_{av}) during the trials.

$$k_{av} = A\exp\left(-\frac{E_a}{RT_{av}}\right) \qquad \text{(Eq. 4.6)}$$

where k_{av} is the average reaction rate (%/day), T_{av} is the average temperature (^{o}C) during the trails.

In the Integrated rate approach the Arrhenius model was also used to estimate the average reaction rate by integrating over the measured temperature-time data for time period (t) of interest:

$$k_{av} = \frac{\int_0^t Ae^{-E_a/RT}dt}{t} \qquad \text{(Eq. 4.7)}$$

The rates were multiplied by the time interval to get the incremental change using Eq. (4.2) and all incremental changes were added up to get the cumulative quality loss at a particular point in time corresponding to each measured point.

For weight loss, the physical model for weight loss was also applied using both the isothermal and integrated approaches based on the linear fits to the rate of weight loss versus water/ice vapor pressure for the constant temperature trials.

4.8.4 ARTIFICIAL NEURAL NETWORK MODEL

The artificial neural network and all matrix calculations were performed using MATLAB Version 5.3 (The Mathworks, Natick, MA). A multi layer perceptron (MLP) neural network based on backpropagation was used to predict dough weight loss, CO_2 production loss and bread specific volume loss using time-temperature data. For the neural network, three steps were completed, including creating the network object, training the network and validating the network. Using this approach, the total data matrices (x*y) of 456 data were divided into 3 sets (training, validation, and test sets). The validation set is used to ensure that there is no overfitting in the final result. The test set provides an independent measure of how well the network can be expected to perform on data not used to train it. Twenty percent of the data was used for the validation set and 20% for the test set. Sixty percent of the data were used for the training set. All sets of the data were randomly selected from the original data.

The inputs of the neural network were storage period, amplitude of temperature, minimum temperature, maximum temperature, mean temperature and variations in temperature. Output of the system was the dough weight loss, CO_2 production loss or bread specific volume loss. The network architecture created for each output data matrix data matrix includes an input layer, one hidden layer of neurons and an output layer. The number of neurons in the hidden layer, and to a lesser extent, the number of hidden layers was varied to search for the optimal network architecture. Specifically, the number of neurons in a hidden layer was varied in order to examine the influence of the hidden layers on performance of neural network.

The ANN was trained using a Lavenberg–Marquardt (LM) algorithm. Transfer function between the input and the hidden layer was hyperbolic tangent sigmoid transfer function (tansig) and linear transfer function (purelin) was used for output layer. The training started with different initial random weights, and was optimized during training. The performance function performed during training feedforward neural networks was the mean sum of squares of the network errors (MSE).

$$MSE = \frac{1}{N}\sum_{i=1}^{N}(t_i - a_i)^2 \qquad \text{(Eq. 4.8)}$$

where a = network output, t = targets, and N = number of samples.

The difference between target value and actual neural output was propagated back through the network to the input. The learning process described herein is referred to as error-correction learning. For error-correction learning, the error was minimized by adjusting the weight. Minimization of the error leads to a learning rule generally referred to as a delta rule. One complete entire training process is called an epoch. The learning process continued epoch-by-epoch until the synaptic weights and bias level of the network stabilized and the mean square error over the entire training set converged to the minimum value. After learning, the target was achieved and the learning stage was completed. In order to test the trained network another data set was used and the input test set was presented to the network and the output was obtained. The output of the ANN was compared with the experimental data for the validation and testing data sets.

CHAPTER V

RESULTS AND DISCUSSION

5.1 ISOTHERMAL FROZEN STORAGE

Quality kinetics experiments (QK1 and QK2) were run to investigate the effects of freezing rate and frozen storage temperature on dough quality. QK1 considered both slow freezing (SF) and fast freezing (FF) rates and 4 storage temperatures (-10±0.1^{o}C, -15±0.1^{o}C, -20±0.1^{o}C and -25±0.1^{o}C) for up to 84 days while QK2 only considered SF and 4 storage temperature (-8±0.1^{o}C, -13±0.1^{o}C, -18±0.1^{o}C and -23±0.1^{o}C) for up to 112 days.

5.1.1 STORAGE TEMPERATURE PROFILES

Fig. 5.1 shows the measured air and dough temperatures in the temperature controlled boxes and the coldstore for QK1 trial. Table 5.1 gives the average and the standard deviation for the air temperature in the coldstore (ATC), the air temperature in the controlled box (ATB), the dough center temperature (DCT) and the dough surface temperature (DST). The average DCT and DST were very similar and close to the average ATB.

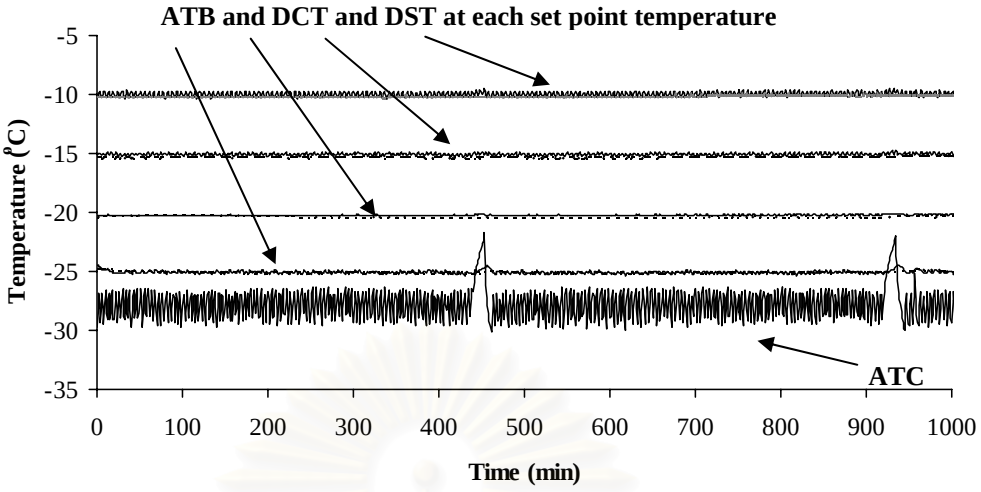


Fig. 5.1 Air and dough temperature variations at each storage temperature for the QK1 experiment. ATC indicates coldstore air temperature, ATB indicates box air temperature, DCT indicates dough center temperature and DST indicates dough surface temperature.

Table 5.1 Average air temperature and dough temperature under constant temperature conditions in the QK1 experiment

Storage temperature	Set-point	Air temperature	Dough temperature	
			Center	Surface
-25°C	-25±0.1°C	-25.0±0.13°C	-25.1±0.07°C	-25.1±0.07°C
-20°C	-20±0.1°C	-20.2±0.03°C	-20.5±0.06°C	-20.4±0.06°C
-15°C	-15±0.1°C	-15.1±0.11°C	-15.4±0.06°C	-15.4±0.07°C
-10°C	-10±0.1°C	-10.0±0.20°C	-10.1±0.10°C	-10.0±0.10°C

Values are the mean and standard deviation of the measurements.

For the QK2 trials, the average and the standard deviation for the ATC, the ATB, the DCT and the DST were shown in Table 5.2. Temperature profiles for the QK2 experiments are shown in Fig. 5.2. The average DCT and DST were similar and close to the average ATB. In this experiment, the defrosting system did not affect ATB at -23°C.

Table 5.2 Average air temperature and dough temperature under constant temperature conditions in the QK2 experiment

Storage temperature	Set-point	Air temperature	Dough temperature	
			Center	Surface
-23°C	-23±0.1°C	-23.1±0.06°C	-22.9±0.06°C	-22.9±0.06°C
-18°C	-18±0.1°C	-18.0±0.07°C	-18.0±0.06°C	-17.9±0.06°C
-13°C	-13±0.1°C	-13.1±0.06°C	-12.9±0.06°C	-12.9±0.06°C
-8°C	-8±0.1°C	-8.1±0.20°C	-8.0±0.05°C	-7.8±0.06°C

Values are the mean and standard deviation of the measurements.

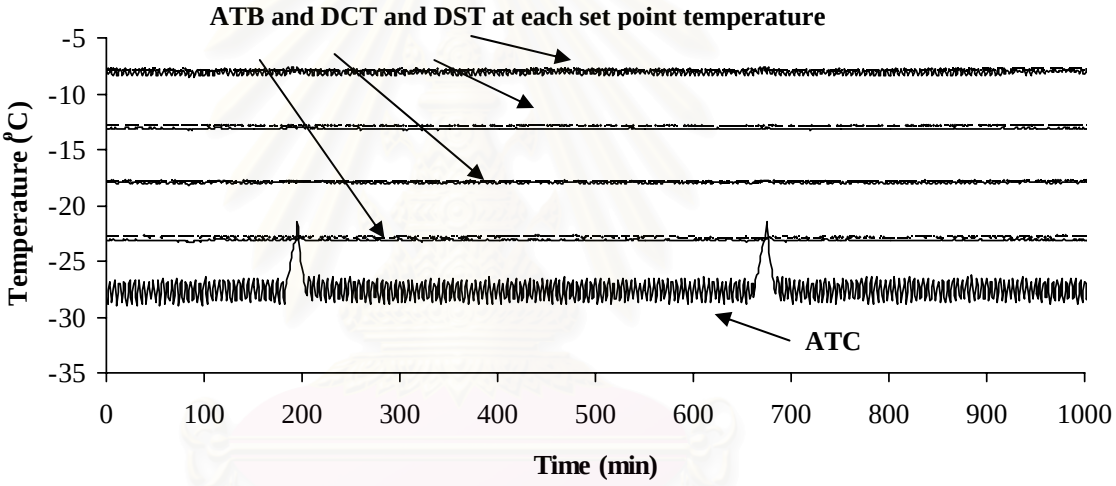


Fig. 5.2 Air and dough temperature variations at each storage temperature in the QK2 experiment. ATC indicates coldstore air temperature, ATB indicates box air temperature, DCT indicates dough center temperature and DST indicates dough surface temperature.

5.1.2 DOUGH WEIGHT LOSS

Fig. 5.3a and 5.3b show the cumulative weight loss of dough frozen with both SF and FF. For each storage temperature, the rate of weight loss was estimated by linear regression. It is clear that the dough weight loss results obtained from the QK1 and QK2 experiments were consistent. At all storage temperatures, the weight loss was increased significantly ($p<0.05$) with increasing storage time and increasing storage temperature. Dough weight loss is due to water/ice transfer from the frozen dough to become frost inside the polyethylene bag. Change in the water/ice distribution in the complex dough matrix could result in changes of the yeast's microenvironment, leading to reversible or irreversible cellular damage (Mazur, 1976).

The measured pattern of dough weight loss is consistent with the standard theory for evaporative weight loss from packaged foods (Laguerre and Flick, 2007). The mechanism is that the frozen dough exerts a partial pressure of water vapor in the air boundary layer associated with the surface depending on the water activity of the dough and the saturated vapor pressure (SVP) of water at the dough surface temperature. The air boundary layer associated with the polyethylene bag surface exerts a partial pressure of water vapor equal to the SVP at the bag temperature. If the bag and dough temperature become sufficiently different then the changes in SVP results in a difference in partial pressures so the water vapor will diffuse from the dough to the bag or vice versa. If the partial pressure of water vapor in the air boundary layers becomes larger than the equilibrium value then the water vapor will condense or freeze and if it is smaller then the water/ice will evaporate or sublime.

Campanone et al. (2005) indicated that freezing influences the subsequent weight loss during storage because different freezing rates lead to different thickness of the dehydrated layers. A lower weight loss during freezing produces a thinner dehydrated layer and thus, less resistance to water vapor diffusion during the storage than higher weight loss during freezing. However, our results showed that the freezing rates did not significantly affect dough weight loss during frozen storage. This is probably due to the small difference in freezing rates between SF (-0.28^{o}C/min) and FF (-0.70^{o}C/min).

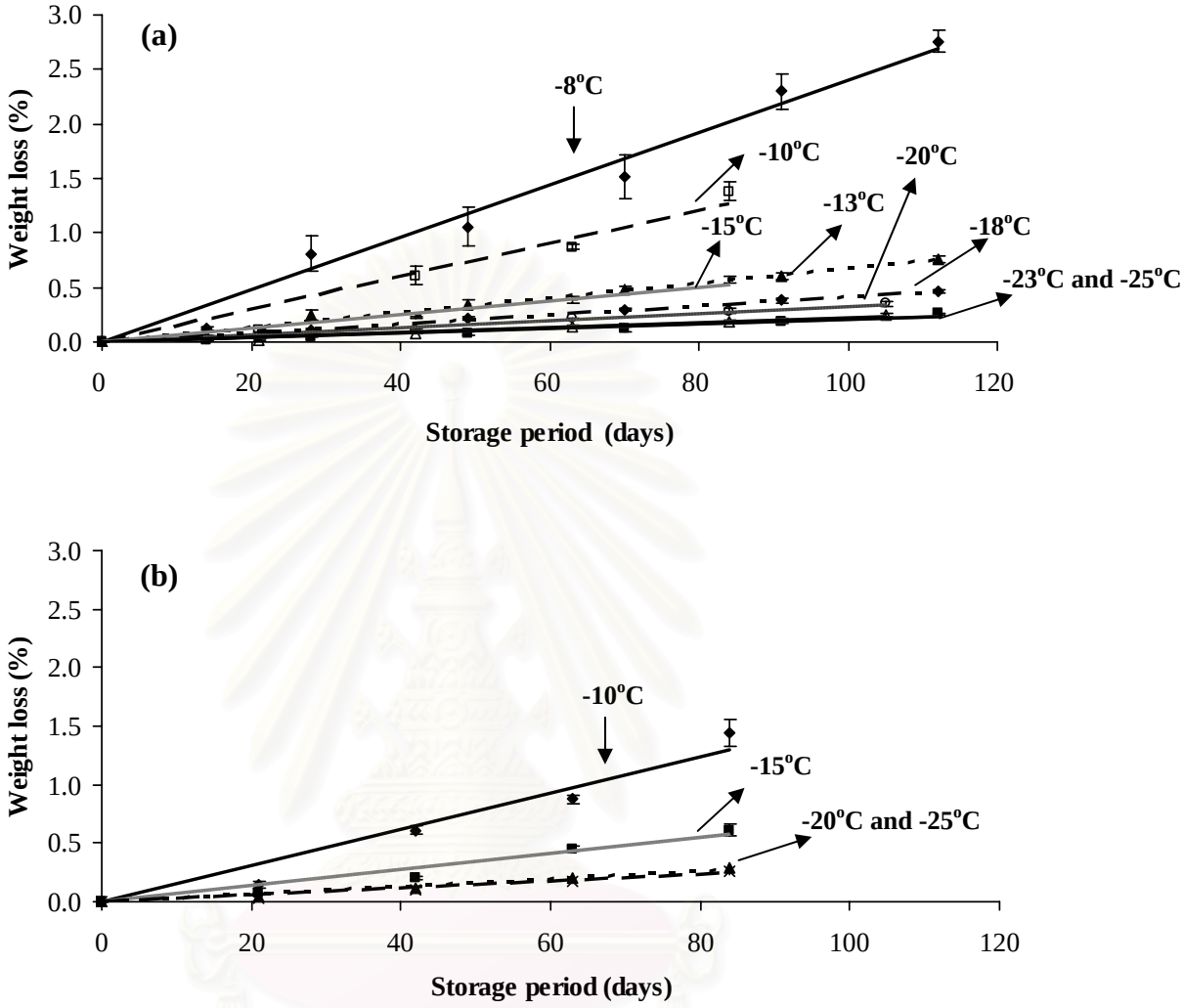


Fig. 5.3 Effect of frozen storage temperature on weight loss of dough frozen with (a) SF for QK1 and QK2 and (b) FF for QK1.

From a theoretical point of view, the factors influencing the rate of weight loss of stored frozen product are the air temperature, air velocity, relative humidity and proximity of warm radiating surfaces (Pham and Willix, 1984). Our results indicated that the rate of weight loss of frozen dough was strongly related to air temperature. Pham (1987) also found that weight loss of frozen product increased with higher air temperature and lower relative humidity for an unpackaged product.

Another possible contribution to weight loss is escaping carbon dioxide gas due to any slow but continuing yeast fermentation (Cauvain, 1998b). Mazur and Schmidt (1968) and Mazur (1970) indicated that the cell interior typically remains unfrozen until the temperature is -10^{o}C to -15^{o}C. Given that the storage temperatures were generally less than -15^{o}C most of the time, such fermentation weight loss was assumed to be insignificant.

5.1.3 CARBON DIOXIDE (CO_2) PRODUCTION

The cumulative CO_2 production and CO_2 production rate were both considered. Fast and effective proofing leads to high quality bread (Hino, Takano and Tanaka, 1987). Table 5.3 shows the effect of freezing rate and frozen storage temperature during frozen storage on CO_2 production for QK1 relative to fresh dough. Table 5.4 shows the similar results for QK2. The dough frozen with SF had higher cumulative CO_2 production (gassing power) than that frozen with FF. After freezing, the gassing power of SF and FF decreased by 3% and 20% respectively for QK1 and by 7% For QK2. CO_2 production of frozen dough showed that the dough stored at -23±0.1^{o}C after 112 days had higher CO_2 production than that stored at higher temperatures. CO_2 production of frozen dough also significantly decreased ($p<0.05$) with increasing storage duration.

These results are consistent with these reported by Neyreneuf and Delpuech (1993) and Havet et al. (2000) who indicated that a slow freezing rate is usually better for frozen dough production. For many foods, higher freezing rate is needed for a good quality frozen product but frozen dough is clearly different. Tanghe, Van Dijck and Thevelein (2003) stated that limited intracellular ice crystal formation occurs in slow freezing. If yeast cells had time to adapt their metabolism to the medium, in SF then yeast activity must be higher after freezing. Although Gelinas et al. (1993) recommended that the freezing rate of frozen dough production should be less than 1^{o}C/min, the fast freezing rate of -0.71^{o}C/min still had more damaging effect on the quality of frozen dough than slow freezing rate at -0.28^{o}C/min.

The gassing power of the doughs frozen with SF and FF decreased significantly ($p<0.05$) in all treatments with increasing storage period (Table 5.3). During the first 6 weeks storage, storage temperature also had a significant effect on CO_2 production with CO_2 production generally being higher at lower storage temperature.

Table 5.3 Effects of freezing rates and frozen storage temperatures on CO_2 production (gassing power) for QK1

Temperature (^oC)	**Storage period (day)**	**CO_2 production (%)**	
		SF	**FF**
-10	Fresh	100±0 a	100±0 a
	1	97±2 ab	81±9 c-i
	21	92±6 a-c	75±7 e-l
	42	86±8 c-f	64±11 l-o
	63	76±6 e-k	56±5 o
	84	57±11 no	34±6 p
-15	Fresh	100±0 a	100±0 a
	1	97±2 ab	81±9 c-i
	21	89±5 a-d	74±3 f-l
	42	76±6 e-k	69±9 i-m
	63	77±5 e-k	61±8 m-o
	84	73±5 f-l	55±8 o
-20	Fresh	100±0 a	100±0 a
	1	97±2 ab	81±9 c-i
	21	86±8 c-f	76±5 e-k
	42	84±5 c-g	74±6 f-l
	63	86±4 c-f	71±9 h-m
	84	81±3 c-h	67±7 j-n
-25	Fresh	100±0 a	100±0 a
	1	97±2 ab	81±9 c-i
	21	78±10 d-k	72±5 g-m
	42	75±5 e-l	68±8 j-n
	63	78±4 d-j	66±8 k-o
	84	70±5 h-m	67±3 j-n

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Table 5.4 Effects of frozen storage temperatures on CO_2 production (%) for QK2

Storage period (day)	Temperature (^oC)				
	-8	**-13**	**-18**	**-23**	**Average**
fresh	100±0 a	100±0 a	100±0 a	100±0 a	**100±0**
1	93±1 a	93±1 a	93±1 a	93±1 a	**93±1**
14	84±4 b	75±3 c-g	75±3 c-g	83±3 b	**79±5**
28	85±2 b	77±3 c-f	75±8 c-g	81±3 bc	**79±6**
49	72±8 e-g	71±4 fg	68±4 g	79±2 b-d	**73±6**
70	58±6 h	70±2 fg	68±7 g	73±4 d-g	**67±7**
91	49±0 i	70±2 fg	73±4 d-g	78±4 b-e	**68±12**
112	48±3 i	61±4 h	70±2 fg	74±6 c-g	**63±11**
Average	**74±19**	**77±13**	**78±12**	**83±9**	**78±14**

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

CO_2 production rate was also considered. Fig. 5.4 and 5.5 show the CO_2 production rate of dough before freezing and after frozen storage for 1 day, 42 days and 84 days for the SF and FF treatments respectively. After freezing, the CO_2 production rate decreased significantly ($p<0.05$) relative to fresh dough. The CO_2 production rate of the dough with both freezing rate reduced in all treatments as storage time increased. The CO_2 production rate of the dough stored at -10±0.1^{o}C dropped gradually after 84 days. With SF, the CO_2 production rate for the dough stored at -15±0.1^{o}C, -20±0.1^{o}C and -25±0.1^{o}C for 6 weeks and 12 weeks showed similar trends. In terms of both the cumulative CO_2 production and the CO_2 production rate, the dough with SF stored at -20±0.1^{o}C showed a better quality than those with FF and other storage temperatures. Fig. 5.6 shows the CO_2 production rate for QK2 decreased with longer storage at higher temperature (-8±0.1^{o}C and -13±0.1^{o}C). However, the CO_2 production rates of dough stored at -18±0.1^{o}C and -23±0.1^{o}C for 49 days and 112 days were similar. Based on this result, it is recommended that frozen storage of the doughs should be between -18^{o}C and -23^{o}C.

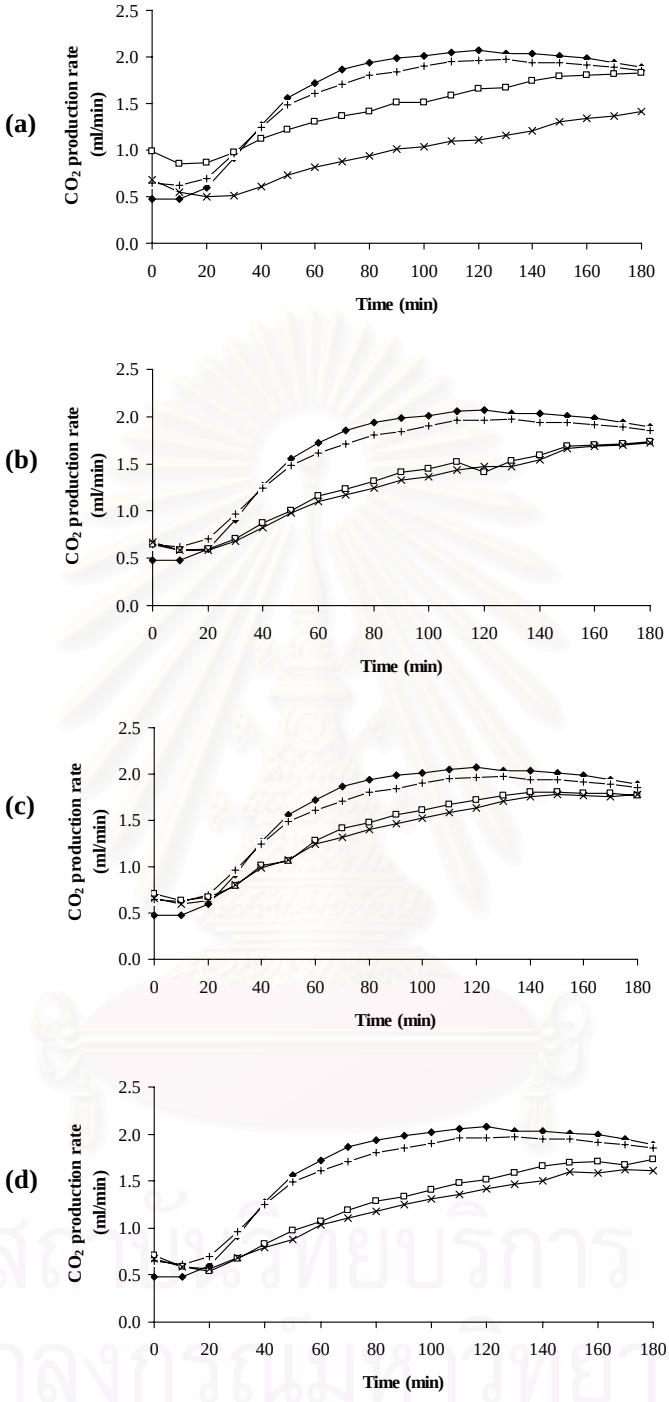


Fig. 5.4 Effects of freezing and isothermal frozen storage temperature on CO_2 production rate of the dough frozen with slow freezing (SF) for QK1.
(a) -10°C regime (b) -15°C regime (c) -20°C regime (d) -25°C regime.
--♦-- indicates fresh. --+-- indicates 1 day frozen storage.
--□-- indicates 42 days frozen storage. --×-- indicates 84 days frozen storage.

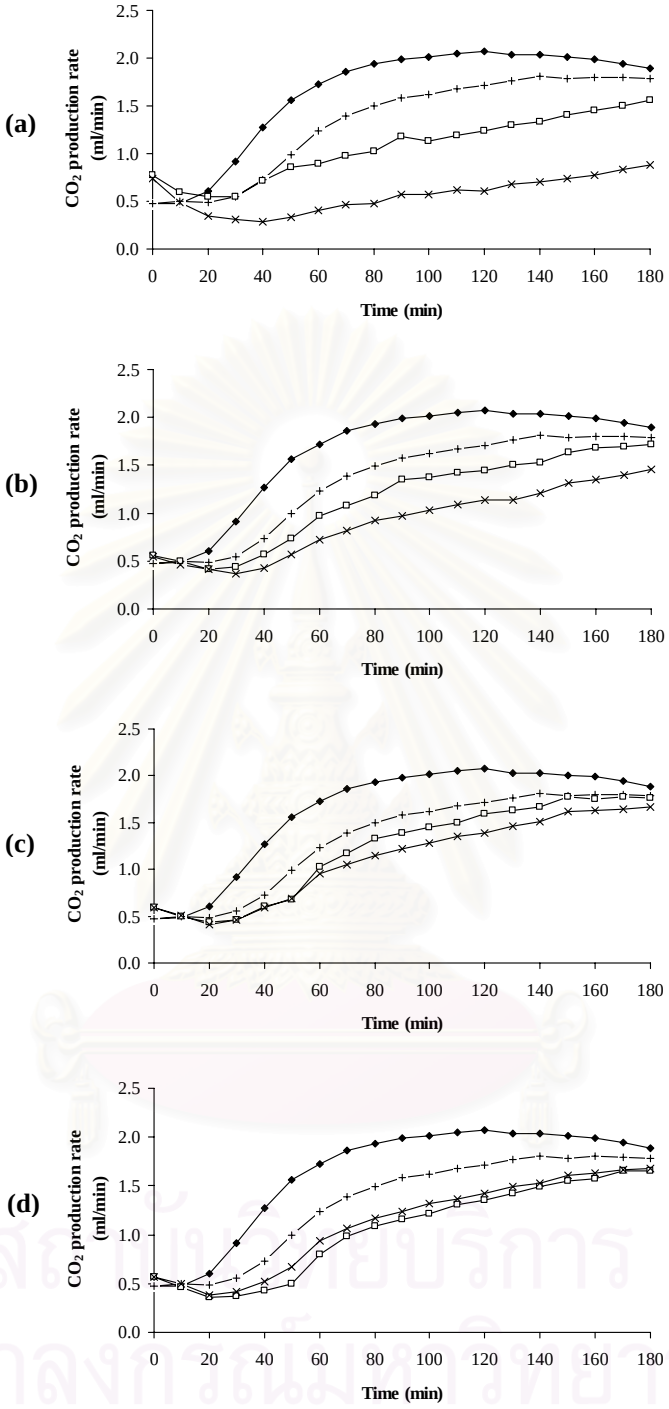


Fig 5.5 Effects of freezing and isothermal frozen storage temperature on CO_2 production rate of the dough frozen with fast freezing (FF) for QK1.
(a) -10°C regime (b) -15°C regime (c) -20°C regime (d) -25°C regime.
--♦-- indicates fresh. --+-- indicates 1-day frozen storage.
--□-- indicates 42 days frozen storage. --×-- indicates 84 days frozen storage.

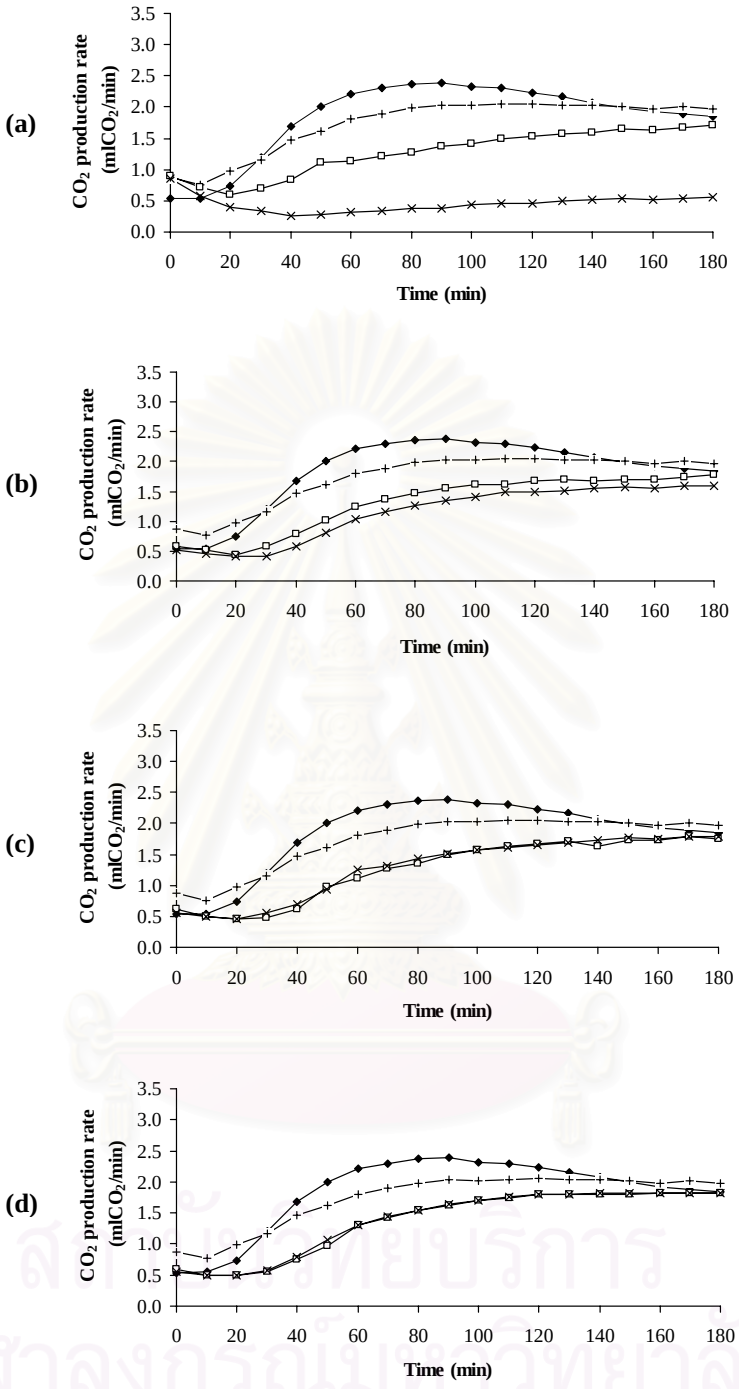


Fig. 5.6 Effects of freezing and storage temperature during frozen storage on CO_2 production rate for QK2. (a) -8°C regime (b) -13°C regime (c) -18°C regime (d) -23°C regime. --♦-- indicates fresh. --+-- indicates 1 day frozen storage. --□-- indicates 49 days frozen storage. --×-- indicates 112 days frozen storage.

5.1.4 YEAST VIABILITY

Table 5.5 shows the measured yeast viability as a function of frozen storage time for QK1 relative to fresh dough. Table 5.6 shows the similar results for QK2. After freezing, the yeast viability for the dough frozen with SF and FF decreased by about 20% and 30% respectively. Yeast cell viability decreased gradually with increasing storage time for both SF and FF samples. Yeast cell viability reduction was affected by freezing rate. Fast freezing had greater effect on viability reduction. After 84 days frozen storage, the dough frozen with SF and stored at -20±0.1^{o}C and -25±0.1^{o}C had viability of 65-66% relative to fresh dough, while the dough frozen with FF stored at the same storage temperature had dropped to less than 62%. The yeast cell viability gradually decreased with increasing storage time. There was no significant difference ($p>0.05$) in yeast viability with temperature when the dough was stored between -23^{o}C and -13^{o}C.

Yeast viability loss is caused by microstructure damage to frozen dough, resulting in loss of yeast performance (Autio and Sinda, 1992; Berglund et al., 1991). Neyreneuf and Delpuech (1993) indicated that faster freezing rate reduced the number of viable yeast cells. Rapid freezing also results in a much higher sensitivity to storage duration than slow freezing and the maximum yeast activity is obtained with a slow freezing rate of -0.19^{o}C/min (Le Bail et al., 1996). The loss of yeast viability in higher freezing rate is caused by injury to yeast cells. The injury sustained during freezing and thawing is caused by a combination of multiple types of stress imposed on the cells, including changes in temperature, water content, water state, pH, and free radical, ion and solute concentrations (Mazur and Schmidt, 1968) and water outflow from the cell (Dumont, Marechal and Gervais, 2003).

The freezing process constitutes a double stress for the cell in terms of thermal stress and hyperosmotic stress (Morris, Coulson and Clarke, 1988). Dumont et al. (2003) indicated that freezing rate strongly influences the viability of yeast cells during cold thermal stress. Two principal damage mechanisms associated with freezing rate have been suggested (Mazur, 1970; Muldrew and McGann, 1990). For slow freezing rates, the extracellular solutes concentrate in the remaining unfrozen

extracellular water and cause cell dehydration by osmosis as water diffuses from the cytoplasm into the more concentrated external solution. This means slow freezing allows cells to adjust to the freezing environment by transferring intracellular water to the external ice. On the other hand, fast freezing rates cause intracellular ice formation because temperatures change much faster than water permeates cell membranes. The small ice crystals formed during intracellular freezing are likely to recrystallize into larger crystals during warming and hence become lethal for the cells.

Table 5.5 Effects of freezing rates and frozen storage temperatures on yeast viability in frozen dough for QK1

Temperature (^oC)	**Storage period (day)**	**Yeast viability (%)**	
		SF	**FF**
-10	Fresh	100±0 a	100±0 a
	1	80±7 ab	68±2 c-h
	21	76±2 bc	70±6 c-h
	42	68±3 c-h	71±6 c-g
	63	63±7 e-j	52±11 j
	84	51±16 j	30±13 l
-15	fresh	100±0 a	100±0 a
	1	80±7 ab	68±2 c-i
	21	74±2 b-f	62±1 e-j
	42	79±5 bc	69±2 c-h
	63	59±6 g-j	55±11 ij
	84	59±7 g-j	40±6 kl
-20	fresh	100±0 a	100±0 a
	1	80±7 ab	68±2 c-h
	21	84±2 b	80±8 ab
	42	70±8 c-g	67±11 c-i
	63	69±4 c-h	61±19 f-g
	84	65±6 d-i	62±5 e-j
-25	fresh	100±0 a	100±0 a
	1	80±7 ab	68±2 c-i
	21	75±8 b-e	73±3 b-f
	42	71±4 b-g	67±1 j-n
	63	71±1 c-h	68±1 k-o
	84	66±3 d-i	58±3 h-j

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Table 5.6 Effect of frozen storage temperature on yeast viability (%) in frozen dough for QK2

Storage	**Temperature (^{o}C)**				
period (day)	**-8**	**-13**	**-18**	**-23**	**Average**
fresh	100±0	100±0	100±0	100±0	**100±0 a**
1	88±7	88±7	88±7	88±7	**88±6 b**
14	83±4	87±10	82±5	88±6	**85±6 b**
28	65±17	79±8	77±4	79±6	**75±10 c**
49	66±8	75±9	74±5	75±6	**73±7 cd**
70	64±2	70±5	72±7	74±6	**70±6 cd**
91	58±1	68±3	71±6	74±6	**68±8 de**
112	52±3	63±5	69±6	72±5	**64±9 e**
Average	**72±17 B**	**79±13 A**	**79±11 A**	**81±10 A**	**78±13**

Values are the mean and standard deviation of 3 samples. A–B means within the same row with different letters are significantly different ($p<0.05$). a-e means within the same column with different letters are significantly different ($p<0.05$).

Yeast viability loss during frozen storage was probably due to cell injury. Cells are injured by the formation of intracellular ice crystals, whose size increases with the time of frozen storage, leading to mechanical disruption of cell components (Morris et al., 1988; Kaul et al., 1992). Yeast viability losses during frozen storage are related to reduction of CO_2 production. Hsu et al. (1979b) indicated that metabolites, such as ethanol and other volatile compounds, formed during fermentation negatively affect fermentative activity of yeast cells. This has been demonstrated for yeast suspensions and frozen yeasted dough. In addition, glutathione reducing substances released from dead yeast cells could contribute to dough weakening (Kline and Sugihara, 1968) and loss of gas retention due to reduction of disulfide bonds (Wolt and D′Appolonia, 1984).

5.1.5 DOUGH RHEOLOGICAL PROPERTIES

Changes in maximum rupture force and extensibility of the dough with SF and FF as affected by frozen storage temperature and storage duration for QK1 are shown in Table 5.7 and 5.8. Table 5.9 and 5.10 show the similar results for QK2. Freezing rate had no significant effect ($p>0.05$) on maximum force after frozen storage but were significant for dough extensibility. This result is consistent with Kenny et al. (2001) who found that resistance to extension force did not change significantly during 15 weeks frozen storage.

The frozen storage temperature had a significant effect ($p<0.05$) on maximum rupture force with maximum rupture force increasing with increasing storage temperature. This result is probably due to higher moisture loss during frozen storage at higher temperature. However, storage temperature did not show a significant effect ($p>0.05$) on dough extensibility. Inoue and Bushuk (1991) and Lu and Grant (1999) found that maximum rupture force decreased and extensibility increased significantly for frozen dough stored for one week. Their results showed that all doughs weakened after freezing and thawing. Inoue and Bushuk (1992) reported a gradual decrease in resistance to extension for some doughs over a 10 weeks frozen storage, whereas we observed no change for any of the doughs tested. The inconsistency of rheological measurement in our results was probably due to moisture loss of the dough during frozen storage.

Table 5.7 Effects of freezing rates and frozen storage temperatures on maximum rupture force of dough for QK1

Temperature (°C)	Storage period (day)	Maximum rupture force (g)	
		SF	FF
-10	Fresh	39.3±3.5 f-n	39.3±3.5 f-n
	1	40.9±8.7 d-l	45.8±8.5 b-h
	21	52.2±5.4 ab	55.2±4.0 a
	42	47.5±0.3 a-g	51.1±3.7 a-c
	63	48.2±3.1 a-f	48.3±4.0 a-f
	84	42.5±0.8 c-k	49.1±3.8 a-e
-15	fresh	39.3±3.5 f-n	39.3±3.5 f-n
	1	40.9±8.7 d-l	45.8±8.5 b-h
	21	43.8±1.9 b-j	40.1±1.3 f-m
	42	38.6±2.6 g-n	31.4±1.3 mn
	63	44.8±2.1 b-i	35.7±0.8 j-n
	84	36.5±2.4 i-n	42.3±2.9 d-k
-20	fresh	39.3±3.5 f-n	39.3±3.5 f-n
	1	40.9±8.7 d-l	45.8±8.5 b-h
	21	34.8±0.7 j-n	32.4±4.7 l-n
	42	49.8±7.5 a-d	35.9±0.9 i-n
	63	40.8±2.1 e-l	36.8±2.8 h-n
	84	43.5±4.3 b-j	36.2±2.1 i-n
-25	fresh	39.3±3.5 f-n	39.3±3.5 f-n
	1	40.9±8.7 d-l	45.8±8.5 b-h
	21	33.9±0.9 k-n	33.7±0.7 k-n
	42	34.4±0.4 k-n	30.4±1.4 n
	63	39.9±3.7 f-m	33.2±0.9 l-n
	84	32.7±0.8 l-n	36.2±0.9 i-n

Values are the mean and standard deviation of 3 samples with triplicate per treatment. Mean values with different letters are significantly different ($p<0.05$).

Table 5.8 Effects of freezing rates and frozen storage temperatures on dough extensibility for QK1

Temperature (°C)	Storage period (day)	Dough extensibility (mm)	
		SF	FF
-10	fresh	40.6±3.0 f-h	40.6±3.0 f-h
	1	42.4±3.0 d-h	44.6±2.5 c-g
	21	42.5±1.2 d-h	39.5±1.0 f-h
	42	39.5±1.9 f-h	41.0±4.6 e-h
	63	42.4±2.5 d-h	39.5±0.9 f-h
	84	57.5±4.4 ab	46.4±3.8 c-f
-15	fresh	40.6±3.0 f-h	40.6±3.0 f-h
	1	42.4±3.0 d-h	44.6±2.5 c-g
	21	42.3±4.5 d-h	44.1±3.3 d-g
	42	40.6±2.2 f-h	43.2±1.9 d-h
	63	42.9±1.4 d-h	41.7±1.9 d-h
	84	51.4±1.4 bc	42.3±1.7 d-h
-20	fresh	40.6±3.0 f-h	40.6±3.0 f-h
	1	42.4±3.0 d-h	44.6±2.5 c-g
	21	38.2±0.6 gh	60.5±15.7 a
	42	39.0±1.9 f-h	45.5±3.4 c-g
	63	40.7±2.3 f-h	43.5±1.1 d-g
	84	38.3±2.6 gh	41.3±1.6 d-h
-25	fresh	40.6±3.0 f-h	40.6±3.0 f-h
	1	42.4±3.0 d-h	44.6±2.5 c-g
	21	42.0±3.3 d-h	46.4±2.4 c-f
	42	38.1±1.5 gh	57.8±9.5 ab
	63	35.8±1.6 h	44.8±1.9 c-g
	84	48.4±3.8 c-e	48.8±4.2 cd

Values are the mean and standard deviation of 3 samples with triplicate per treatment. Mean values with different letters are significantly different ($p<0.05$).

Table 5.9 Effect of frozen storage temperatures on maximum rupture force of dough for QK2

Storage	**Temperature (^{o}C)**				
period (day)	**-8**	**-13**	**-18**	**-23**	**Average**
fresh	35.5±5.4 cd	35.5±5.4 cd	35.5±5.4 cd	35.5±5.4 cd	**35.5±4.6**
1	31.7±1.5 d-h	31.7±1.5 d-h	31.7±1.5 d-h	31.7±1.5 d-h	**31.7±1.2**
14	39.1±1.7 a-c	36.9±0.9 b-d	32.9±3.4 d-f	27.5±1.2 g-j	**34.1±4.9**
49	33.6±1.6 de	28.0±1.8 f-j	25.4±1.5 if	26.3±1.0 ij	**28.3±3.6**
70	24.4±0.8 jk	33.2±2.7 d-f	29.9±1.1 e-i	20.2±0.6 k	**27.0±5.4**
91	31.9±2.4 d-g	35.5±4.6 cd	32.3±0.4 d-g	40.9±0.9 ab	**35.1±4.4**
112	43.6±3.2 a	41.7±2.6 ab	26.5±2.5 h-j	34.3±2.5 c-e	**36.5±7.4**
Average	**34.3±6.2**	**34.6±4.9**	**30.6±4.1**	**30.9±6.8**	**32.6±5.8**

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Table 5.10 Effect of frozen storage temperatures on dough extensibility for QK2

Storage	**Temperature (^{o}C)**				
period (day)	**-8**	**-13**	**-18**	**-23**	**Average**
fresh	40.4±1.0 cd	40.4±1.0 cd	40.4±1.0 cd	40.4±1.0 cd	**40.4±0.9**
1	47.6±3.8 ab	47.6±3.8 ab	47.6±3.8 ab	47.6±3.8 ab	**47.6±3.2**
14	24.7±2.7 h-j	30.9±0.5 ef	29.9±1.5 e-h	24.3±3.0 h-j	**27.5±3.6**
49	9.5±3.6 l	14.5±3.7 kl	19.6±3.5 jk	35.6±1.3 de	**19.8±10.6**
70	22.0±0.5 j	22.6±1.8 ij	30.7±2.9 e-g	28.4±1.0 f-i	**25.9±4.2**
91	45.1±3.6 a-c	49.0±9.7 a	47.3±6.7 ab	41.7±0.9 b-d	**45.8±6.0**
112	41.1±3.6 cd	38.0±2.4 d	47.6±2.8 ab	48.9±2.2 a	**43.9±5.3**
Average	**32.9±13.8**	**34.7±12.7**	**37.6±10.9**	**38.1±9**	**35.8±11.7**

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

5.1.6 DOUGH MICROSTRUCTURE

Microstructure deterioration of frozen dough for QK1 was also investigated using a SEM. Fig. 5.7 shows the microstructure of dough frozen with SF and FF 1 day after freezing. SEM micrographs show that the dough frozen with FF had greater gluten network damage than the dough frozen with SF. The effect of the freezing on the microstructure of bread dough has been previously studied (Berglund et al., 1991; Ribotta et al., 2004). They concluded that freezing mainly damages the protein matrix. Freezing impairs the baking performance of dough, which is largely attributed to structural changes as induced by ice formation. Structural damage of frozen dough resulted in loss of textural properties in the bread from frozen dough (Naito et al., 2004).

SEM micrographs of frozen dough stored at -10^{o}C, -15^{o}C, -20^{o}C and -25^{o}C for 84 days are shown in Fig. 5.8, 5.9, 5.10 and 5.11 respectively. The fracture surface of frozen dough stored at -10^{o}C and -15^{o}C was less smooth and had more holes when compared with those of frozen dough stored at -20^{o}C and -25^{o}C. This was attributed to increased ice crystal growth and recrystallization due to higher storage temperature. Most starch granules in dough stored at -20^{o}C and -25^{o}C were embedded in gluten matrix whereas higher temperatures (-10^{o}C and -15^{o}C) caused more of the starch granules to be floating separately from the gluten matrix. More broken gluten strands were also found in dough stored under higher temperatures. These results are consistent with those of Zounis et al. (2002a) who found that the major structural changes were the growth of air voids and the separation of gluten from starch with increasing storage time. Such changes are likely to impact upon the shelf-life of frozen dough products. Disruption of dough structure at -10^{o}C was possibly due to minor amounts of yeast fermentation and water migration. This temperature is just below the freezing point of dough (-6^{o}C to -8^{o}C) and hence water movement and yeast activity are more likely at this temperature. The greater disruption to the dough structure under non-ideal storage conditions (-10^{o}C) is consistent with the water and ice crystal growth known in frozen food products (Goff, 1992). Storage at lower temperature (-20^{o}C and -25^{o}C) for several weeks led to slight structural damage caused by water migration and ice crystal growth.

Berglund et al. (1991) indicated that such structural changes may contribute to a decreased ability of the gluten to retain gas during proofing. The phenomenon of ice accumulation in the pores leads to a non-uniform distribution of water in dough and could be the reason for the impaired baking performance (Baier-Schenk et al., 2005). During frozen storage, the tendency of the ice crystals to minimize their surface to volume ratio promotes the formation of large ice crystals. A particular feature of bread dough is that large ice crystals are found in the pores as observed by cryomicroscopy (Baier-Schenk et al., 2005; Esselink et al., 2003).

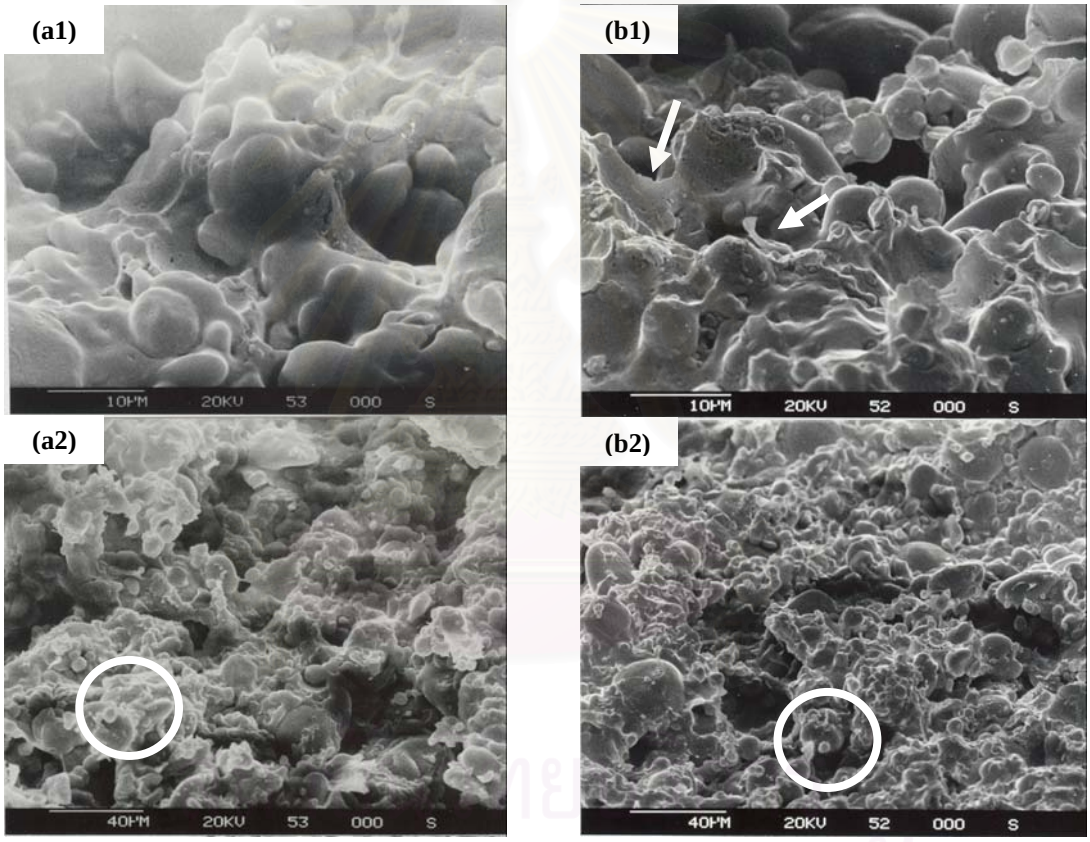


Fig. 5.7 SEM micrographs of frozen dough samples after 1 day storage for QK1. (a1-2) dough frozen with slow freezing (SF) under 2000x and 500x magnification. (b1-2) dough frozen with fast freezing (FF) under 2000x and 500x magnification. Arrow indicates examples of broken gluten matrix and strands. Circle indicates examples of floating starch granules.

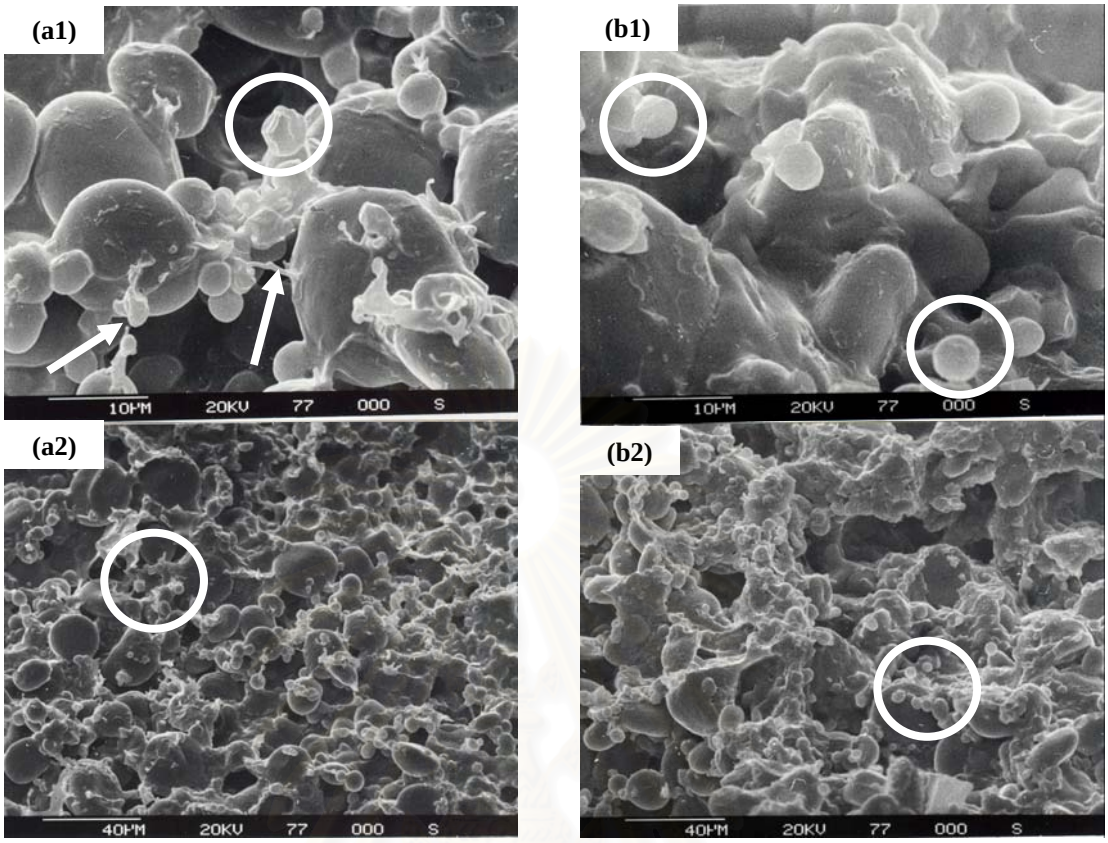


Fig. 5.8 SEM micrographs of frozen dough samples stored at -10^{o}C after 84 days storage for QK1. (a1-2) dough frozen with slow freezing (SF) under 2000x and 500x magnification. (b1-2) dough frozen with fast freezing (FF) under 2000x and 500x magnification. Arrow indicates examples of broken gluten matrix and strands. Circle indicates examples of floating starch granules.

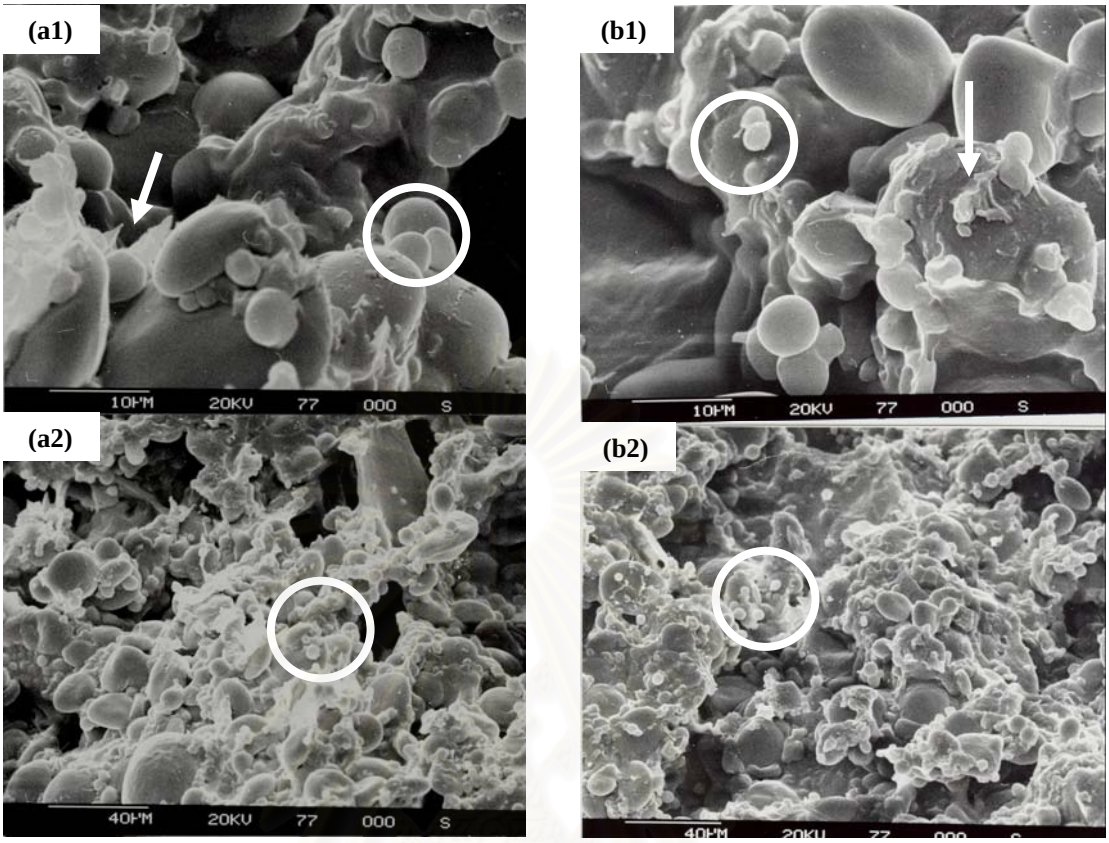


Fig. 5.9 SEM micrographs of frozen dough samples stored at -15^{o}C after 84 days storage for QK1. (a1-2) dough frozen with slow freezing (SF) under 2000x and 500x magnification. (b1-2) dough frozen with fast freezing (FF) under 2000x and 500x magnification. Arrow indicates examples of broken gluten matrix and strands. Circle indicates examples of floating starch granules.

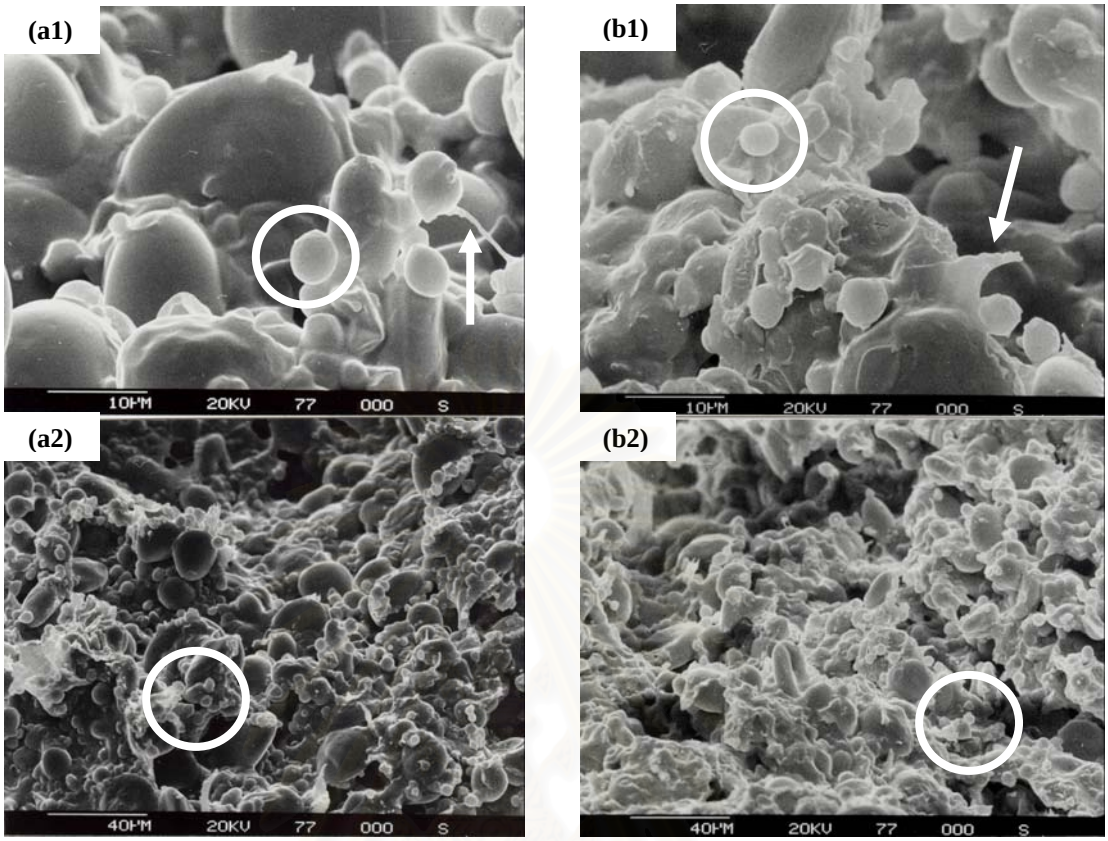


Fig. 5.10 SEM micrographs of frozen dough samples stored at -20^{o}C after 84 days storage for QK1. (a1-2) dough frozen with slow freezing (SF) under 2000x and 500x magnification. (b1-2) dough frozen with fast freezing (FF) under 2000x and 500x magnification. Arrow indicates examples of broken gluten matrix and strands. Circle indicates examples of floating starch granules.

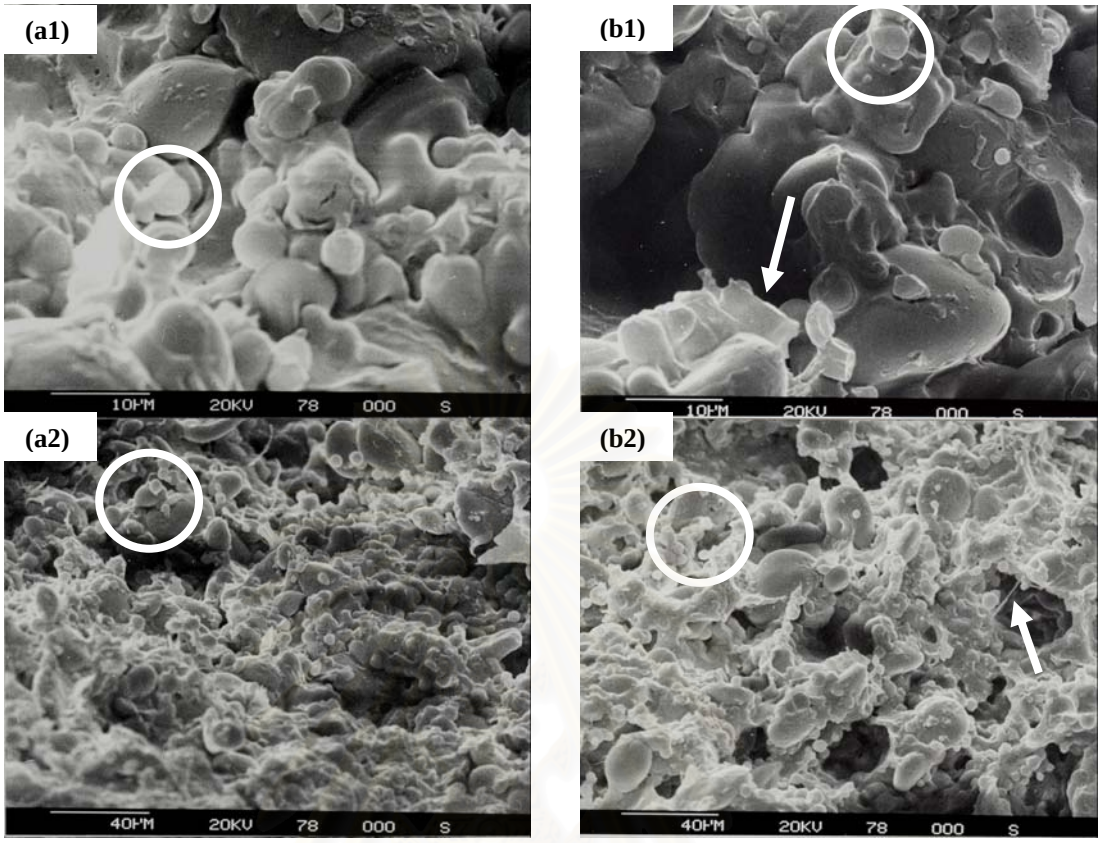


Fig. 5.11 SEM micrographs of frozen dough samples stored at -25^{o}C after 84 days storage for QK1. (a1-2) dough frozen with slow freezing (SF) under 2000x and 500x magnification. (b1-2) dough frozen with fast freezing (FF) under 2000x and 500x magnification. Arrow indicates examples of broken gluten matrix and strands. Circle indicates examples of floating starch granules.

5.1.7 DOUGH WATER MOBILITY

The T_2 relaxation time of frozen dough stored at different storage temperatures after 1 day and 112 days for QK2 is shown in Fig. 5.12. The T_2 values increased with increasing storage period from 6.7 ms after 1 day frozen storage to be about 8.3-9.6 ms after 112 days frozen storage. After 112 days, T_2 relaxation of frozen dough stored at higher temperatures (-8°C, -13°C and -18°C) was longer than that of frozen dough stored at lower temperature (-23°C).

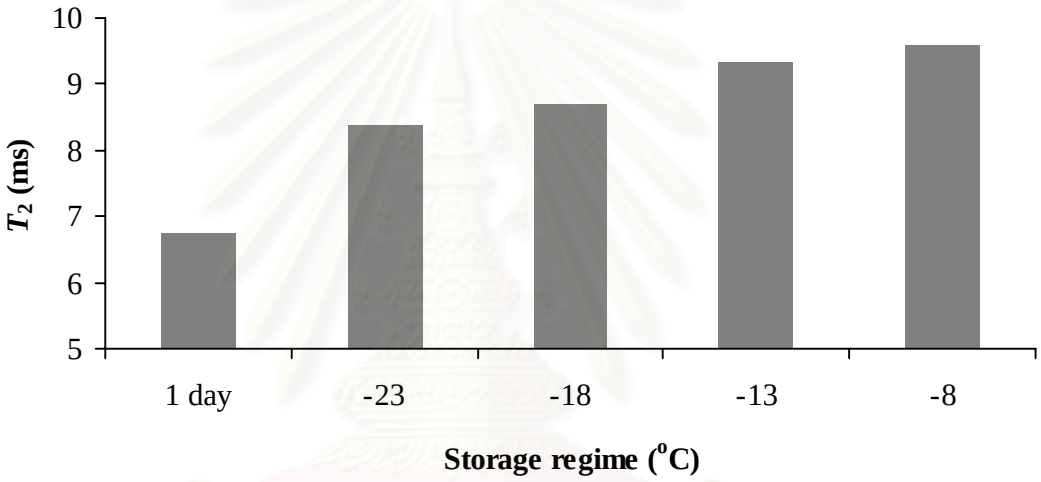


Fig. 5.12 Effects of storage temperature on T_2 values measured by NMR for frozen dough stored at different constant storage temperatures after 1 day and 112 days for QK2.

Longer T_2 relaxation time indicates more relaxation of water and it could be attributed to a release of water from the gluten matrix by recrystallizing of ice crystals. This water redistribution alters the properties of the gluten and starch phase. The water may not return to its original state in the gluten matrix, resulting in an increase in water mobility in the dough (Esselink et al., 2003). A simple description of water distribution in dough has been postulated. First, part of the water occurs in a rigid state primarily bound into the starch particles. A second part of the water is associated with the starch surface and the gluten matrix. The latter water is sensitive to the temperature variations which occurred during dough processing and storage. It is also

this type of water (freezable water) that can form ice in a dough system when subjected to freezing and frozen storage. Rasanen et al. (1998) indicated that the higher amount of freezable water results in a greater number of ice crystals. Lu and Grant (1999a) also found that freezable water increased with increasing frozen storage period due to damage to the gluten network and phase-separation of ice from the gluten-water system by the freezing and frozen storage. This can cause damage to the gluten-starch structure that can account for poor baking performance of frozen dough.

5.1.8 BREAD SPECIFIC VOLUME

Table 5.11 shows effects of freezing rates (SF and FF) and frozen storage temperature on bread specific volume for QK1. Table 5.12 shows similar results for QK2. Freezing had insignificant effect ($p>0.05$) on the bread specific volume. After 1 day frozen storage, bread specific volume decreased by 5% and 6% for SF and FF respectively relative to bread baked from fresh dough. Bread specific volume significantly decreased ($p<0.05$) with increasing storage time. After 84 days frozen storage at different temperatures, the reduction in bread specific volume of SF and FF was 24% and 28% respectively. Storage temperature had insignificant effect on bread specific volume. These results are consistent with those reported by Giannou and Tzia (2007) where a rapid loss of loaf volume was observed after freezing and during the first month of storage.

Table 5.11 Effects of freezing rates and frozen storage temperatures on bread specific volume for QK1

Temperature (^{o}C)	Storage period (day)	Bread specific volume (ml/g)	
		SF	FF
-10	fresh	2.57±0.18 a	2.57±0.18 a
	1	2.46±0.24 ab	2.40±0.30 a-c
	21	2.24±0.09 b-g	2.09±0.01 c-j
	42	2.08±0.13 c-j	1.85±0.05 i-k
	63	2.04±0.14 d-j	1.91±0.30 h-k
	84	1.83±0.06 i-k	1.67±0.13 k
-15	fresh	2.57±0.18 a	2.57±0.18 a
	1	2.46±0.24 ab	2.40±0.30 a-c
	21	2.32±0.19 a-f	2.19±0.08 b-h
	42	2.05±0.10 d-j	2.01±0.03 c-j
	63	2.03±0.03 ab	1.98±0.07 g-k
	84	1.99±0.08 g-j	1.87±0.18 i-k
-20	fresh	2.57±0.18 a	2.57±0.18 a
	1	2.46±0.24 ab	2.40±0.30 a-c
	21	2.33±0.19 a-e	2.09±0.10 c-j
	42	2.25±0.15 a-g	2.08±0.08 c-j
	63	2.22±0.11 b-h	1.91±0.05 h-k
	84	2.12±0.14 c-i	2.04±0.14 d-j
-25	fresh	2.57±0.18 a	2.57±0.18 a
	1	2.46±0.24 ab	2.40±0.30 a-c
	21	2.36±0.10 a-d	1.96±0.02 g-k
	42	2.15±0.07 b-i	1.92±0.04 h-k
	63	2.22±0.14 b-h	2.04±0.13 d-j
	84	1.87±0.12 i-k	1.79±0.07 jk

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Table 5.12 Effect of frozen storage temperature on bread specific volume for QK2

Storage	**Temperature (^{o}C)**				
period (day)	**-8**	**-13**	**-18**	**-23**	**Average**
fresh	2.78±0.17	2.78±0.17	2.78±0.17	2.78±0.17	2.78±0.14 a
1	2.51±0.04	2.51±0.04	2.51±0.04	2.51±0.04	2.51±0.04 b
14	2.42±0.11	2.15±0.14	2.27±0.06	2.17±0.05	2.25±0.14 c
28	2.52±0.04	2.38±0.18	2.39±0.17	2.43±0.07	2.43±0.13 b
49	2.28±0.05	2.30±0.12	2.30±0.09	2.33±0.10	2.30±0.08 c
70	1.98±0.19	1.97±0.10	1.98±0.07	2.00±0.18	1.98±0.12 d
91	1.96±0.05	1.94±0.12	1.97±0.04	2.03±0.04	1.98±0.07 d
112	1.87±0.02	1.90±0.06	1.93±0.03	2.01±0.01	1.93±0.06 d
Average	**2.29±0.32 A**	**2.24±0.31 A**	**2.27±0.30 A**	**2.28±0.28 A**	**2.27±0.30**

Values are the mean and standard deviation of 3 samples. a-d means within the same column with different letters are significantly different ($p<0.05$).

Bread specific volume is a key quality parameter as it indicates dough inflating ability and ovenspring. For bakery product, there is usually an ideal relation between dough weight and loaf volume that yields the most desirable texture and grain (Pyler, 1988). It is commonly accepted that an optimum bread volume is related to a properly developed gluten network, which is a cross-linked structure, via the SH–SS interchange reaction during dough mixing. This three-dimensional network enables retention of gas bubbles produced by yeast (Gan, Ellis and Schofield, 1995). The volume reduction of bread made from frozen dough could be due to the weakened gluten network either by depolymerization or ice crystals during the frozen storage period, leading to poor gas retention (Berglund et al., 1991). Ribotta et al. (2001) reported that the loss of bread volume could be attributed to the damaged gluten network and was increased by extended frozen storage at -18^{o}C. Varriano-Marston et al. (1980) and Berglund et al. (1991) pointed out that the formation of ice crystals led to the separation of starch granules from the gluten matrix. These disrupted the gluten matrix and weakened the gluten network responsible for gas retention in dough, causing a reduction in bread volume or an excessive proofing time. Lorenz and Kulp (1995) stated that the decrease in loaf volume during frozen storage may be caused by decreased yeast viability as well as gluten and starch damage. Although the precise

mechanism involved in the quality deterioration of frozen dough remains unknown, a reduction in bread volume is more likely due to the combined effects of damaged protein network and reduced gassing power of yeast in the frozen dough system, resulting in lower gas production and poorer gas retention, and consequently poor baking quality (Kennedy, 2000).

5.1.9 BREAD CRUMB FIRMNESS

The evaluation of the mechanical properties of bread crumb is important not only for routine quality assurance in the baking industry, but also for assessing the effects of changes in various dough ingredients and processing conditions, and also the effect of shelf life on acceptability of bread to the consumer (Cauvain, 2004). Effects of freezing rates and frozen storage temperature on bread crumb firmness for QK1 are shown in Table 5.13. Table 5.14 shows similar results for QK2. Freezing rate had a significant effect ($p<0.05$) on bread crumb firmness. One day after freezing, bread crumb firmness of the dough with SF and FF increased about 4% and 35% respectively relative to bread baked from fresh dough. The crumb firmness of bread made from the frozen dough increased with the frozen storage duration up to 12 weeks for all treatments. Bread crumb firmness gradually increased with increasing storage period, and was in the range of 3-9 N. The results are in agreement with the findings of other researchers (Kenny et al., 1999). Different frozen storage temperatures had no significant effect on bread crumb firmness. Giannou and Tzia (2007) also reported this behavior of crumb firmness of bread made from frozen dough. However, bread crumb firmness of the dough stored at -8^{o}C did decrease. This was probably due to loss in microstructure of the bread due to moisture loss.

Table 5.13 Effects of freezing rates and frozen storage temperatures on bread crumb firmness for QK1

Temperature (^{o}C)	Storage period (day)	Bread crumb firmness (N)	
		SF	FF
-10	fresh	4.5±0.4 g	4.5±0.4 g
	1	4.7±2.3 fg	6.1±2.0 e-g
	21	6.8±2.7 d-g	8.5±1.5 c-g
	42	11.1±2.7 b-d	13.2±1.4 ab
	63	11.7±5.4 a-c	11.9±2.5 a-c
	84	10.8±2.5 b-d	15.9±3.1 a
-15	fresh	4.5±0.4 g	4.5±0.4 g
	1	4.7±2.3 fg	6.1±2.0 e-g
	21	7.4±3.0 c-g	8.8±2.2 b-g
	42	9.8±1.8 b-e	10.1±3.3 b-e
	63	9.3±1.4 b-f	11.9±4.1 a-c
	84	11.1±3.3 b-d	11.8±1.2 a-c
-20	fresh	4.5±0.4 g	4.5±0.4 g
	1	4.7±2.3 fg	6.1±2.0 e-g
	21	6.9±3.4 d-g	10.2±2.4 b-e
	42	7.9±1.7 c-g	10.2±2.9 b-e
	63	8.0±1.8 c-g	11.4±2.0 b-d
	84	8.2±2.7 c-g	10.2±2.5 b-e
-25	fresh	4.5±0.4 g	4.5±0.4 g
	1	4.7±2.3 fg	6.1±2.0 e-g
	21	7.4±2.5 c-g	11.7±2.3 a-c
	42	8.4±2.6 c-g	11.9±1.0 a-c
	63	7.8±2.8 c-g	9.1±2.3 b-g
	84	8.9±2.9 b-g	10.6±1.7 b-e

Values are the mean and standard deviation of 3 samples with triplicate per treatment. Mean values with different letters are significantly different ($p<0.05$).

Table 5.14 Effect of frozen storage temperature on bread crumb firmness for QK2

Storage	**Temperature (^{o}C)**				
period (day)	**-8**	**-13**	**-18**	**-23**	**Average**
fresh	4.08±0.59 k	4.08±0.59 k	4.08±0.59 k	4.08±0.59 k	**4.08±0.50**
1	3.81±0.35 k	3.81±0.35 k	3.81±0.35 k	3.81±0.35 k	**3.81±0.30**
14	3.63±0.91 k	5.68±0.56 f-j	5.55±0.62 g-j	5.61±0.66 f-j	**5.12±1.08**
28	3.59±0.58 k	5.63±0.86 f-j	4.86±0.63 h-k	4.73±0.58 i-k	**4.70±0.95**
49	4.56±0.49 jk	6.20±0.33 d-g	7.30±1.19 b-d	6.81±0.67 c-g	**6.22±1.25**
70	6.72±0.94 c-g	7.70±0.72 bc	7.19±0.34 b-e	6.09±0.54 d-h	**6.92±0.85**
91	6.22±0.81 d-g	6.55±0.77 c-g	6.21±0.12 d-g	5.90±0.41 e-i	**6.22±0.56**
112	4.56±0.49 jk	9.08±0.69 a	8.12±0.39 ab	6.91±1.80 b-f	**7.17±1.96**
Average	**4.65±1.27**	**6.09±1.75**	**5.89±1.60**	**5.49±1.31**	**5.53±1.57**

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Firmness is linked with the force required to compress the food between the molars while elasticity represents the extent to which a compressed food returns to its original size when the load, which caused its compression, is removed. Crumb firmness is a common quality characteristic for bakery products since it is strongly correlated with consumers' perception of bread freshness (Faridi and Faubion, 1990). Berglund and Shelton (1993) and Sharadanant and Khan (2003b) also observed increased firmness with increasing frozen storage duration. The increase in firmness was probably related to the decrease in corresponding bread volume, due to the weakened gluten strength, reduced yeast activity (Berglund et al., 1991; Inoue and Bushuk, 1992), moisture loss during the extended frozen storage (He and Hoseney, 1990) and also starch recrystallization during shelf storage (Ribotta et al., 2003).

5.1.10 BREAD IMAGE CHARACTERISTICS

The cellular structure of bread crumb (crumb grain) is an important factor that contributes to the textural properties of bread and consumer acceptance (Scanlon and Zghal, 2001). Bread crumb made from fresh dough and 1 day after freezing with SF and FF showed a very similar grain structure (Fig. 5.13).

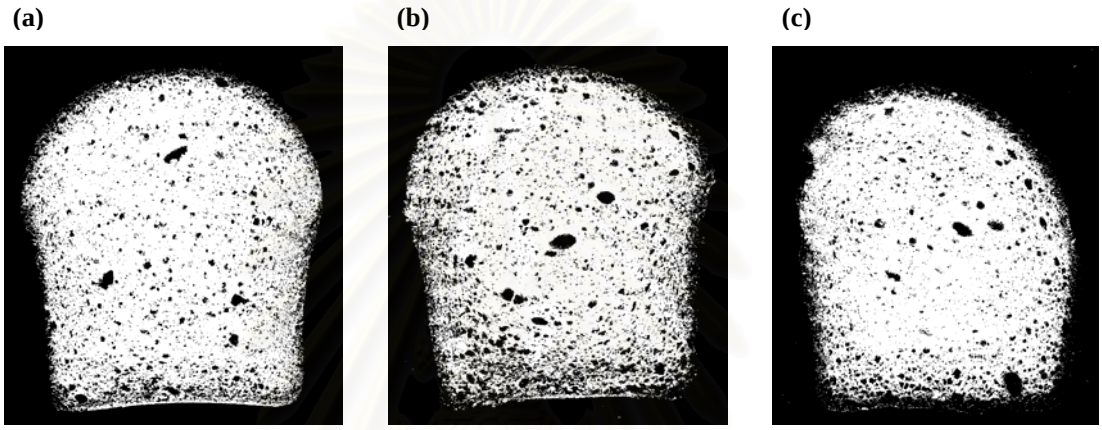


Fig. 5.13 Bread images baked after various conditions for QK1.
(a) bread made from fresh dough (b) bread made from dough frozen with SF
(c) bread made from dough frozen with FF.

Fig. 5.14 shows the effects of freezing rate and storage temperature on bread characteristics after 84 days frozen storage. Overall crumb structure of bread with SF had a better appearance than that of bread with FF. Crumb structure of bread made from dough stored at -20^{o}C was better than breads stored at -10^{o}C, -15^{o}C and -25^{o}C. The bread from dough stored at -10^{o}C had more holes and had a less smooth crumb grain than those from dough stored under lower temperature. Bigger holes and less smooth crumb structure indicated low quality bread (He and Hoseney, 1991). This is probably due to ice recrystallization during frozen storage (Inoue and Bushuk, 1991). The result is consistent with the studies of Sharadanant and Khan (2003b), who found more damage in appearance of bread made from frozen dough as storage duration increased.

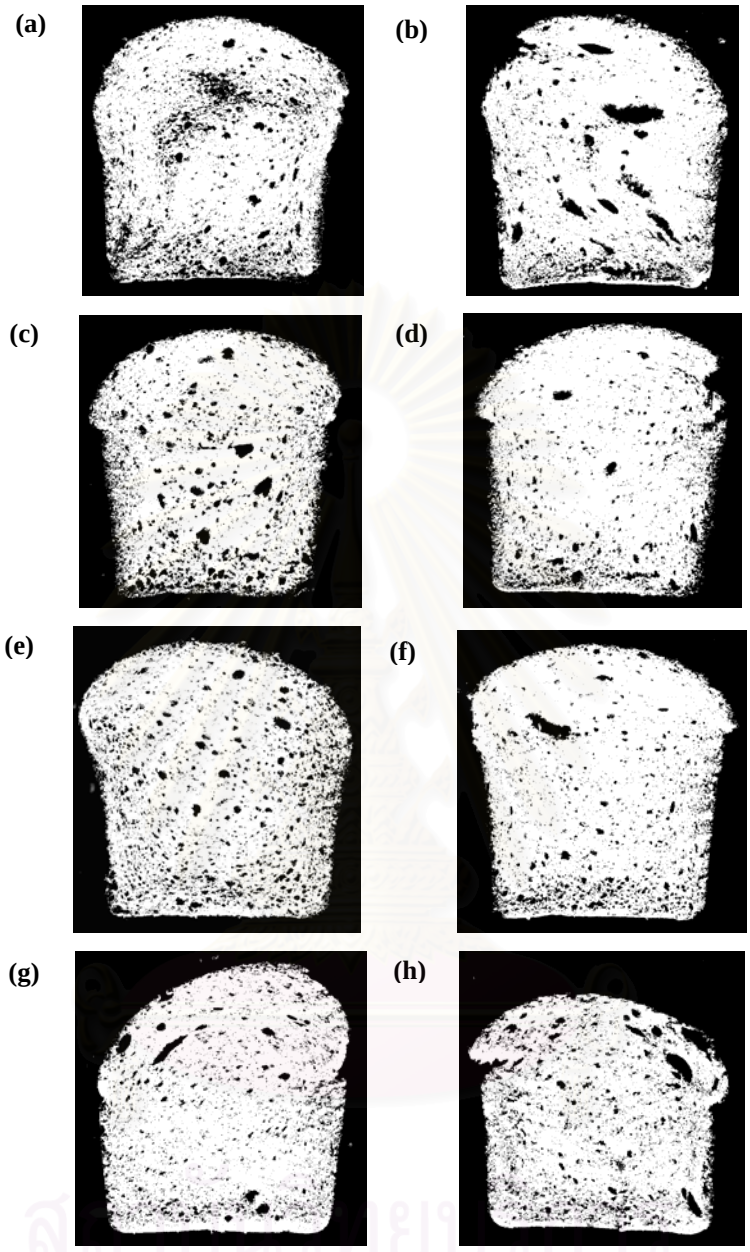


Fig. 5.14 Bread images baked after 84 days frozen storage under various constant storage temperature for QK1. Left column indicates bread made from dough with SF. Right column indicates bread made from dough with FF.
(a-b) -10^{o}C (c-d) -15^{o}C (e-f) -20^{o}C (g-h) -25^{o}C.

5.1.11 QUALITY PARAMETER RELATIONSHIPS

Dough and bread quality parameters after frozen storage were significantly correlated as shown in Table 5.15. Dough weight loss had a negative correlation with CO_2 production and yeast viability, resulting in a decreased bread specific volume and increased bread crumb firmness. The overall quality of dough and bread after freezing and frozen storage could be explained by 2 or 3 quality parameters. It is suggested that dough weight loss, CO_2 production and bread specific volume could be used as key parameters for frozen dough and bread quality.

Table 5.15 Pearson correlation coefficients between each pair of quality measurements for dough and bread for all trails

Dough		**Bread**		
CO_2 production	Yeast viability	Specific volume	Firmness	
-0.664^{**}	-0.500^{**}	-0.542^{**}	0.354^{**}	Weight loss
	0.717^{**}	0.740^{**}	-0.558^{**}	CO_2 production
		0.624^{**}	-0.547^{**}	Yeast viability
			-0.755^{**}	Specific volume

p-values below 0.01 (**) indicates statistically significant non-zero correlations at the 99% confidence level.

5.2 ISOTHERMAL KINETIC MODELS

The kinetics of dough quality during frozen dough storage have not be reported. This section attempts to quantify the reaction kinetics and to investigate the applicability of the Arrhenius relationship, Williams-Landel-Ferry (WLF) model and the standard theory for evaporative weight loss from packaged foods for specific frozen dough quality parameters.

Only weight loss, CO_2 production and bread specific volume were investigated because dough weight loss is an important for dough in terms of quality and sealable weight, and loss of CO_2 production is highly related to lower bread volume, resulting in reduced consumer acceptance.

5.2.1 DOUGH WEIGHT LOSS KINETICS

QK1 showed that the freezing rate did not affect on dough weight loss during frozen storage. The analysis of variance indicated that the dough weight loss data from QK1 and QK2 experiments was not significantly different ($p>0.05$). Therefore, dough weight loss data from QK1 and QK2 was combined. Table 5.16 gives the rate constant (k) for dough weight loss for each temperature assuming zero-order reaction kinetics. The Arrhenius plot of $\ln k$ *vs* $1/T$ for dough weight loss is given in Fig. 5.15. Weight loss was found to be adequately described by zero order reaction kinetics with rate constant being an Arrhenius function of temperature. Estimated kinetic parameters for weight loss are given in Table 5.17.

Table 5.16 Rate constants for dough weight loss assuming zero-order reaction kinetics

Temperature (^{o}C)	**$k_{0 \times 10}{}^{2}$ (% day^{-1})**
-8	2.41±0.25
-10	1.53±0.12
-13	0.68±0.07
-15	0.66±0.06
-18	0.42±0.03
-20	0.33±0.04
-23	0.21±0.02
-25	0.25±0.06

Values are the mean and standard deviation of the measurements.

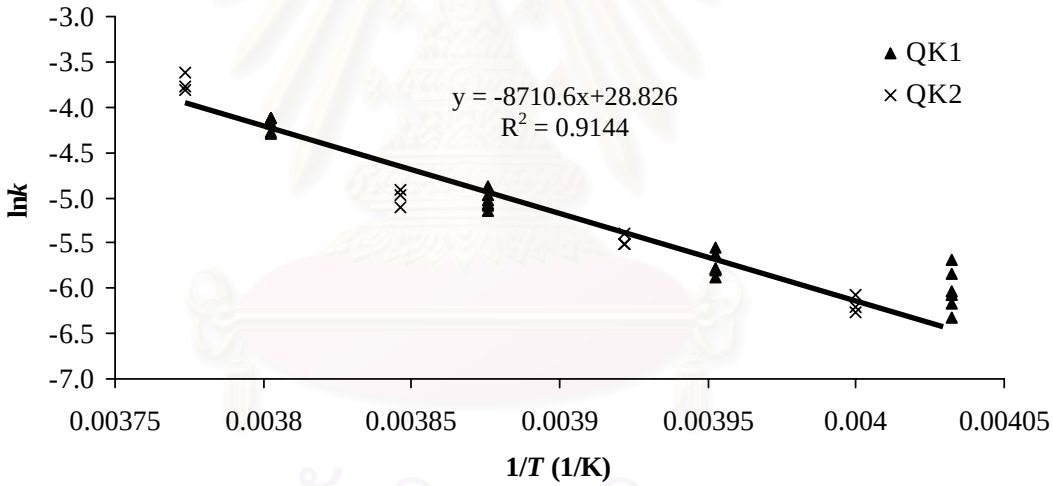


Fig. 5.15 Arrhenius plot (ln*k* *vs* 1/*T*) for dough weight loss rate constant during frozen storage.

Table 5.17 Estimated kinetic parameters for the degradation of quality parameters during frozen storage of frozen dough

Quality parameters	Data	Temperature range (^oC)	E_a (kJ/mol)	k_0 (% day^{-1})	Corr. coeff.[a]
Weight loss	Combined	-25 to -8	72.4±7.6	3.30×10^{12}	0.914
CO_2 production	Combined	-25 to -8	38.9±11.7	2.37×10^{7}	0.565
	Combined	-23 to -8	58.3±11.9	1.79×10^{11}	0.772
Bread specific volume	Combined	-25 to -8	9.3±0.2	0.17×10^{1}	0.139
	Combined	-23 to -8	20.1±10.1	2.40×10^{3}	0.456

[a] Correlation coefficient between model predicted and experimental data.

Fig. 5.16 shows measured rate of weight loss at each storage temperature against water vapor pressure. While the relationship may not be linear, if is obvious that there is a strong relationship suggesting that the physical model is a useful starting point. Linear regression of all the data gave an R^2 = 0.78. However, the weight loss data appear to have two parts with different trends - from -25^{o}C to -13^{o}C and above -13^{o}C. The rationale could be that above -13^{o}C a significant fraction of the water remains unfrozen leading to extra weight loss but other factors (e.g. non-constant water activity, slow fermentation etc.) may also be important especially at higher temperatures. Separate linear regressions for data from -25^{o}C to -13^{o}C and above -13^{o}C gave an R^2 = 0.88 and 0.95 respectively as shown in Fig.5.16. Delgado and Sun (2007) suggested that water activity is an important factor and should be incorporated in the modeling in order to improve the accuracy of the predictions.

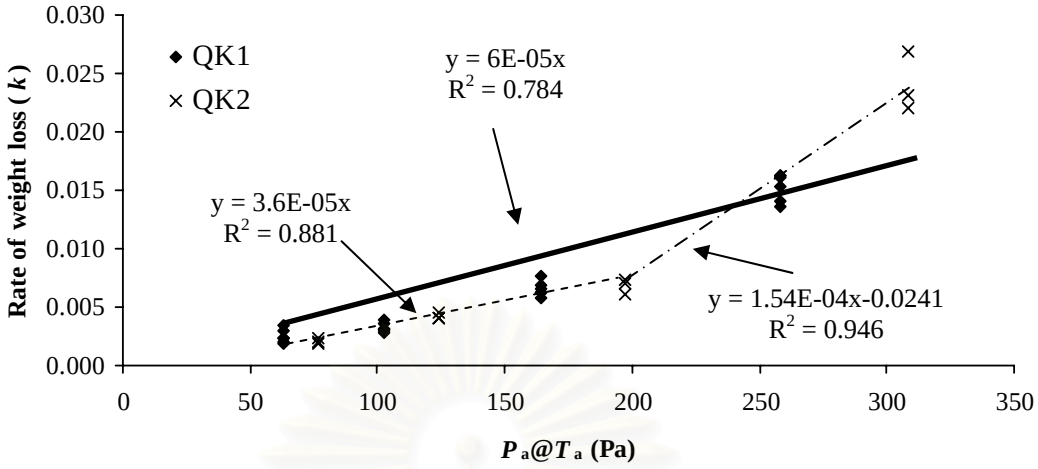


Fig. 5.16 Plot of dough weight loss rate constant (k) *vs* water vapor pressure (P_a) at the air temperature (T_a). — indicates all data fitting. -- indicates data fitting from -25°C to -13°C. – · indicates data fitting above -13°C.

5.2.2 CO_2 PRODUCTION LOSS KINETICS

Kinetic data for CO_2 production rate from both the QK1 and QK2 experiments were fitted to the Arrhenius law based on zero-order kinetic reactions. The analysis of variance indicated that the CO_2 production of the dough frozen with SF and FF for QK1 and with SF for QK2 was not significantly different ($p>0.05$) therefore the data for the QK1 and QK2 experiments were combined. Table 5.18 gives rates of CO_2 production loss at each storage temperature assuming zero-order reaction kinetics. The results indicated that CO_2 production of the dough stored at -25°C was lower than that of the dough stored at -23°C, -20°C and -18°C which was opposite to the general trend of reduce deterioration at lower temperature. Hsu et al. (1979b) reported that frozen dough were less stable if their storage temperature was lower than the temperature they were frozen using. Le Bail (2006) also found this effect and suggested out that this was due to yeast damage. However, further investigation for storage temperature is required to confirm the effect of low storage temperature on frozen dough and yeast cells. The effect of the coldstore defrosting on the dough temperature control -25°C trials may also have contributed to the poorer than expected storage stability at this temperature. Therefore, the rate data was analyzed both including and excluding the -25°C data.

Table 5.18 Rate constants for CO_2 production loss assuming zero-order reaction kinetics

Temperature (^oC)	**$k_{0 \times 10^2}$ (% day^{-1})**
-8	62.3±2.8
-10	56.6±13.7
-13	21.9±3.0
-15	33.6±9.5
-18	15.2±1.0
-20	17.4±2.1
-23	14.5±2.6
-25	22.6±6.5

Values are the mean and standard deviation of the measurements.

The Arrhenius plot of ln*k vs* 1/*T* for CO_2 production loss is given in Fig. 5.17. Estimated kinetic parameters for CO_2 production loss are shown in Table 5.17. The lower activation energy value than for weight loss indicated that CO_2 production was less sensitive to storage temperature. The activation energy of CO_2 production fitted from -25^{o}C to -8^{o}C was lower than that fitted from -23^{o}C to -8^{o}C but had low correlation coefficient (R^2 = 0.56). Giannakourou and Taokis (2002) also reported high variations in estimated activation energy for color and vitamin C loss of frozen peas. Martins and Silva (2002) stated that Arrhenius behavior with storage temperature has been assumed in most kinetic studies, but it may not be applicable during low storage temperatures. If solute concentration occurs in the unfrozen phase and the storage temperature is near the maximum freeze concentration and glass transition temperature, kinetics may not follow the Arrhenius law due to a sudden decrease in solutes molecular mobility.

While the QK2 data is consistently lower than the QK1 data overall the kinetics seem to be reasonable accurately described by the Arrhenius kinetics if the -25^{o}C data is omitted.

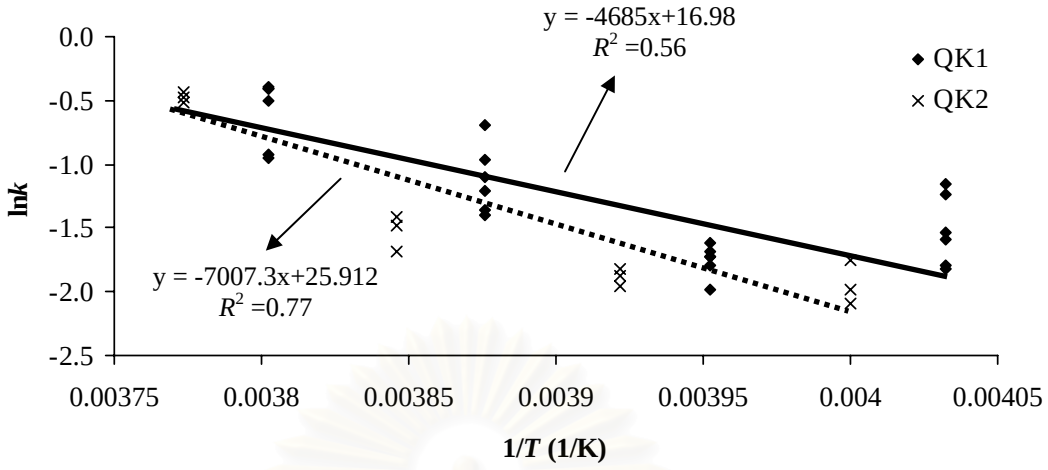


Fig. 5.17 Arrhenius plot of ln*k* *vs* 1/*T* for CO_2 production rate constant during frozen storage. — indicates fitted line for all data. --- indicates fitted line if -25°C data is omitted.

Calligaris et al. (2004) found that the application of the Arrhenius equation for the prediction of oxidative reaction rate in frozen foods is often precluded because of deviations from linearity. An abrupt change in the temperature dependence of hexanal formation rate was observed at temperature values close to -7°C, indicating that below this temperature, the advanced steps of the oxidation reaction proceed at a higher rate than that predicted by the Arrhenius equation. The reason for this non-Arrhenius behavior is that at subzero temperatures, food is a dynamic system in which physicochemical factors change as a function of temperature. Below freezing temperatures, a cascade of temperature-dependent events such as solute concentration, changes in physicochemical properties (reactant solubility, pH, ionic strength, water activity, residual volume of concentrated phase and viscosity), protein denaturation, and phase transitions of crystallizing components (water, sugar and lipids) could take place (Parker and Ring 1995; Fennema 1996; Champion, Blond and Simatos, 1997).

An alternative approach of fitting the Williams-Landel-Ferry (WLF) model was also applied. Fig. 5.18 shows a WLF plot for CO_2 production rate constant assuming the reference temperature to be -26°C, -30°C or -43°C.

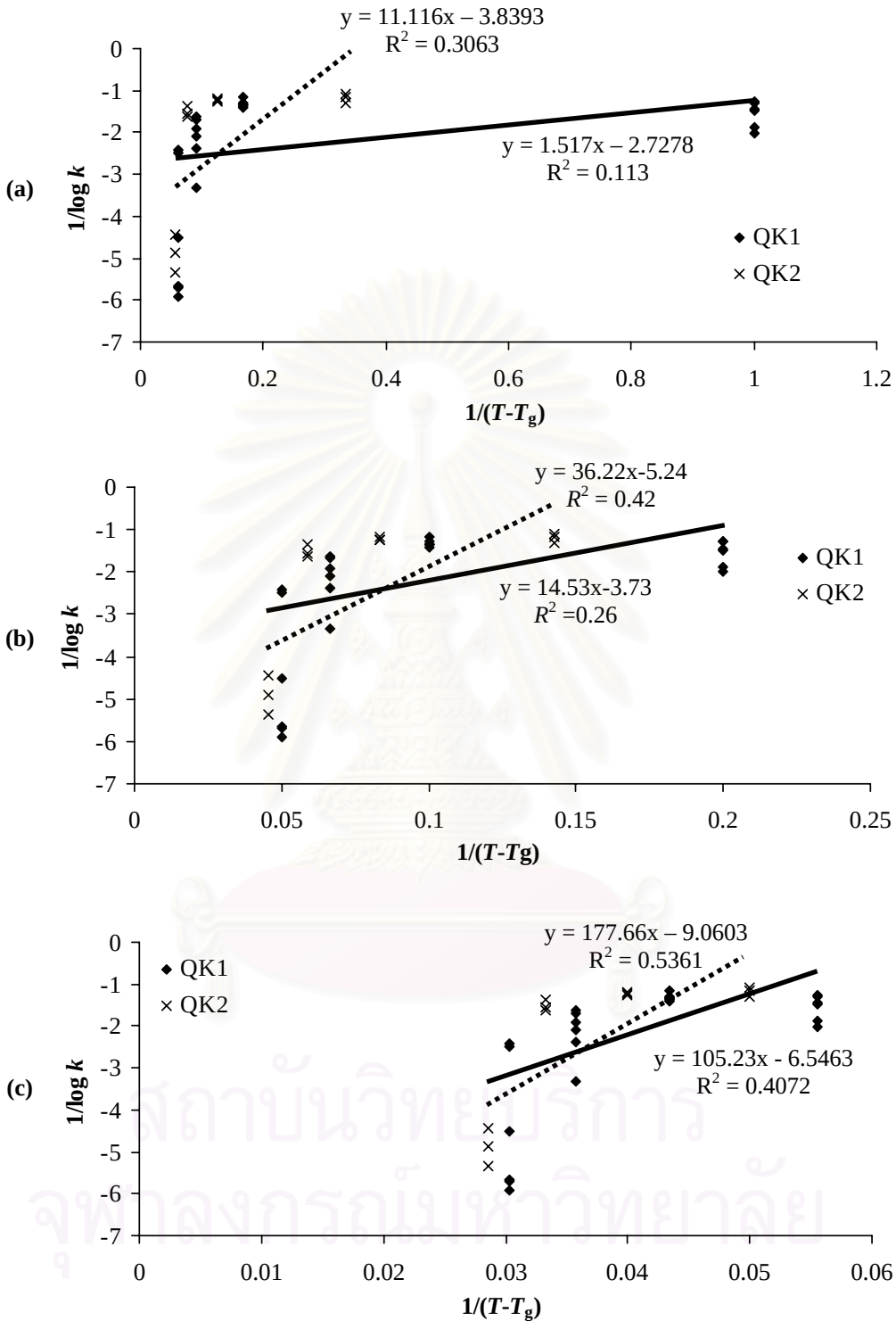


Fig. 5.18 WLF plot for CO_2 production rate constant during frozen storage with assuming the reference temperatures. (a) T_{ref} = -26°C, (b) T_{ref} = -30°C, (c) T_{ref} = -43°C. — indicates fitted line for all data.
--- indicates fitted line with -25°C data is omitted.

The regression coefficient of the WLF model from -25°C to -8°C was lower than 0.41 for all reference temperatures while a slightly better fit for all reference temperatures was achieved for the data range from -23°C to -8°C (R^2 = 0.31-0.54). Overall, it suggested that the CO_2 production loss did not follow the WLF behavior. Using different reference temperature values did not improve the WLF fits. Calligaris et al. (2004) also found that the WLF equation did not fit to predict the hexanol formation rate of sunflower oil at subzero storage temperatures.

5.2.3 BREAD SPECIFIC VOLUME KINETICS

Kinetic data for bread specific volume from both the QK1 and QK2 experiments were fitted to the Arrhenius law based on zero-order kinetic reactions. The analysis of variance indicated that the bread specific volume baked from the dough frozen with SF for QK1 and with SF for QK2 was not significantly different ($p>0.05$) therefore the data for the QK1 and QK2 experiments with SF were combined. Table 5.19 gives rates of bread specific volume loss at each storage temperature assuming zero-order reaction kinetics. The results showed that bread specific volume baked from the dough stored at -25°C was lower than that of the dough stored at higher storage temperature as found in CO_2 production results. This result seems to be relating to that of CO_2 production loss which was probably due to yeast damage. Therefore, the rate data was analyzed with and without utilizing the -25°C data.

The Arrhenius plot of ln$k$ *vs* 1/T for bread specific volume loss is given in Fig. 5.19. Estimated kinetic parameters for bread specific volume loss are also shown in Table 5.17. The lower activation energy value than for weight loss and CO_2 production loss indicated that bread specific volume was less sensitive to storage temperature. The activation energy of bread specific volume fitted from -25°C to -8°C was lower than that fitted from -23°C to -8°C but had low correlation coefficient (R^2 = 0.46).

As the same way as CO_2 production, the WLF model was also applied. Fig. 5.20 shows a WLF plot for bread specific volume rate constant assuming the reference

temperature to be -26°C, -30°C or -43°C. The regression coefficient of the WLF model from -25°C to -8°C was lower than 0.01 for all reference temperatures while a slightly better fit was achieved for the data range from -23°C to -8°C for all reference temperatures (R^2<0.37). Again, it suggested that the bread specific volume loss did not follow the WLF behavior.

Table 5.19 Rate constants for bread specific volume assuming zero-order reaction kinetics

Temperature (°C)	$k_{0 \times 10}{}^{2}$ **(% day^{-1})**
-8	25.6±1.1
-10	28.0±7.1
-13	19.9±1.3
-15	23.6±8.1
-18	20.8±3.7
-20	14.9±4.1
-23	16.6±2.6
-25	25.4±2.2

Values are the mean and standard deviation of the measurements.

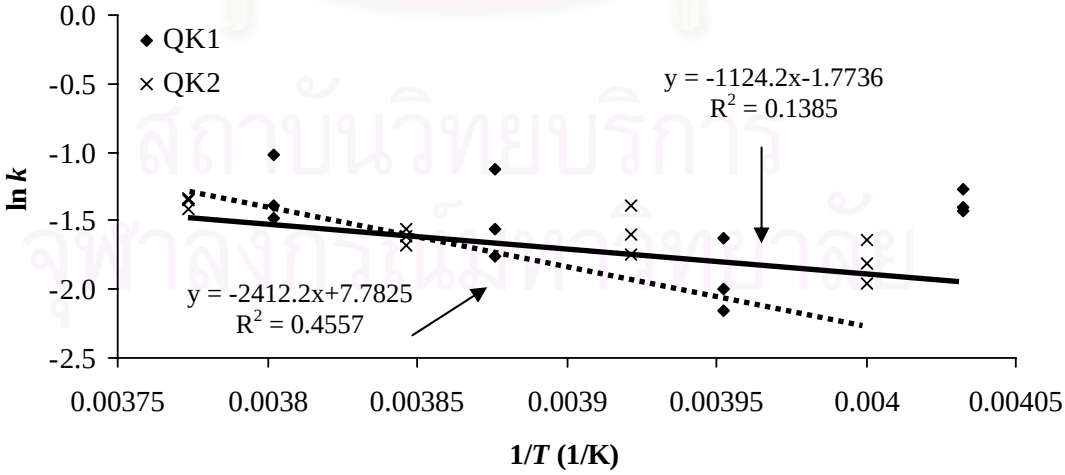


Fig. 5.19 Arrhenius plot of ln*k* *vs* 1/*T* for bread specific volume constant during frozen storage. ─ indicates fitted line for all data. --- indicates fitted line if -25°C data is omitted.

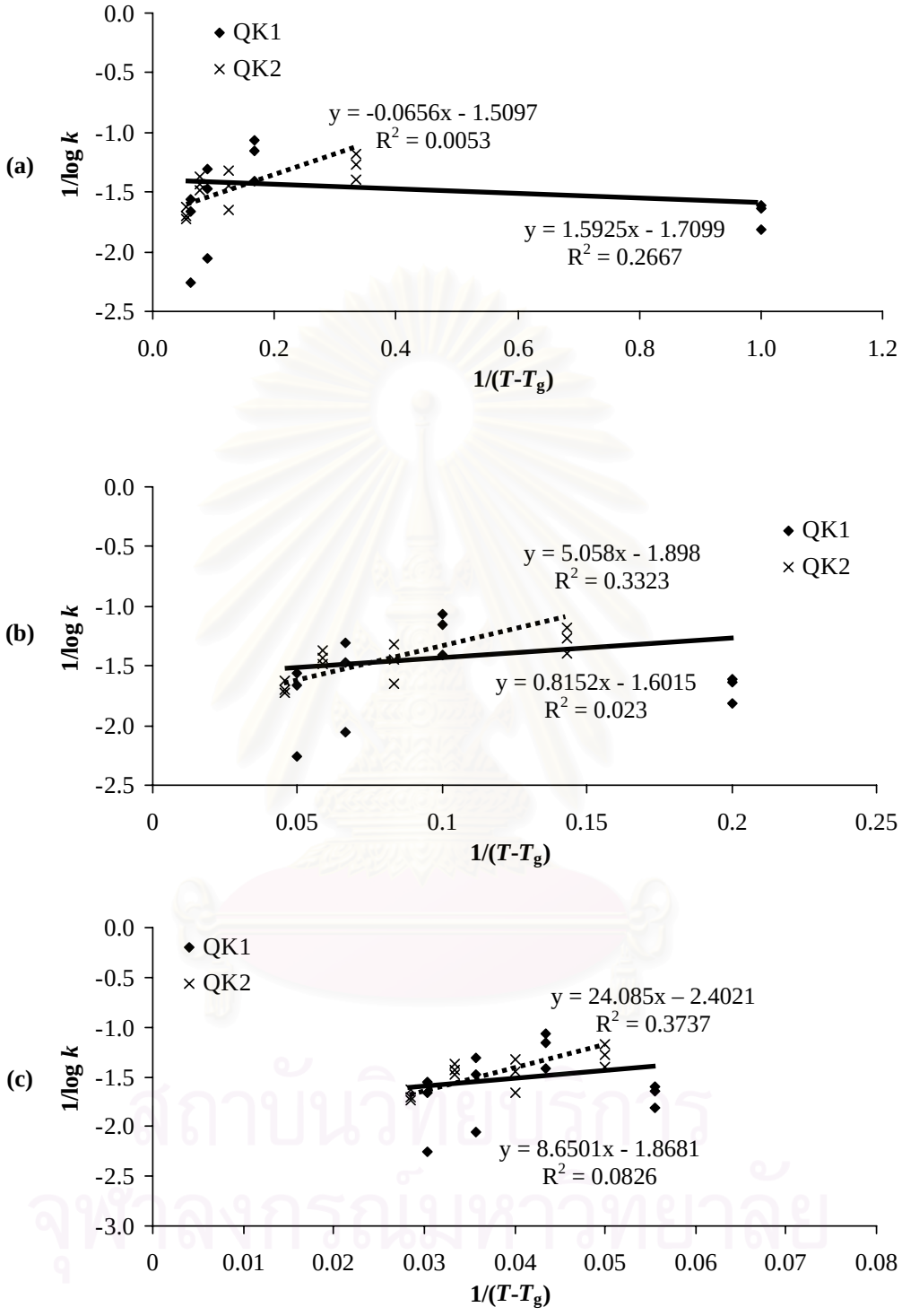


Fig. 5.20 WLF plot for bread specific volume rate constant during frozen storage with assuming the reference temperatures. (a) T_{ref} = -26°C, (b) T_{ref} = -30°C, (c) T_{ref} = -43°C. — indicates fitted line for all data.
--- indicates fitted line with -25°C data is omitted.

5.3 SUMMARY FOR QK

Freezing rate is an important factor for frozen dough and baked bread quality. Slow freezing rate gave a better quality of frozen dough and baked bread than fast freezing rate with higher CO_2 production, less yeast viability loss and smoother microstructure of dough and bread crumb structure. However, slow and fast freezing rate did not significantly affect dough weight loss and CO_2 production loss during frozen storage. The freezing process caused greater damage to dough quality than the subsequent frozen storage for up to 112 days.

Frozen storage duration had a significant effect on the frozen dough and bread quality. Increasing storage duration resulted in decreased overall dough and bread quality. Higher storage temperature led to increased freezable water, increased weight loss and reduced CO_2 production. Storage temperatures had no significant effects ($p>0.05$) on dough rheological properties, bread specific volume and bread crumb firmness. Nevertheless, there was possibility that the storage temperature did affect the rheological properties of the dough, but the measurement technique selected may not be sensitive enough to detect small changes. Dough microstructure and bread image characteristic seems to be good but subjective methods for quality assessment. Dough weight loss, CO_2 production and bread specific volume are quantitative and therefore could be used as a key parameter for frozen dough and bread. They are closely related to other quality parameters which indicate overall quality of frozen dough and bread after freezing and frozen storage yet are relative easy and low cost to measure.

Lower frozen storage temperature gave a better quality of dough and bread. A frozen storage temperature between -23^{o}C and -18^{o}C can retain adequate dough and bread quality during frozen storage for up to 12-16 weeks under constant storage temperature. However, storage temperatures lower than -23^{o}C gave poorer quality. Future research is required to investigate the impact of lower storage temperature on frozen dough quality.

Weight loss data were adequately fitted by the Arrhenius law assuming zero-order reaction kinetics. However, the Arrhenius model didn't give a good predictions for CO_2 production and bread specific volume prediction unless the -25^{o}C data was omitted. The WLF model was unsuitable to predict the CO_2 production and bread specific volume rate constants.

Weight loss of frozen dough was fitted to the standard physical model for evaporative drying. The rate of weight loss was proportional to vapor pressure of water at the storage temperature suggesting that model is appropriate.

5.4 FLUCTUATING FROZEN STORAGE REGIMES (TF)

The first temperature fluctuations experiment (TF1) was run to investigate the effects of both freezing rate and fluctuating frozen storage temperature during frozen storage on dough quality deterioration. The quality parameters of the dough were measured for both slow freezing (SF) and fast freezing (FF) rates and storage under 4 fluctuating temperatures regimes for up to 84 days. The four storage regimes for TF1 were -20±0.1^{o}C (Control, C), -20±1^{o}C (Good Practice, G), -20±3^{o}C (Poor Practice, P) and the cold chain (CC). For the cold chain regime, the temperature set-points were -20±1^{o}C for 4 days, -15±1^{o}C for 1 day, -10±1^{o}C for 1 day, and then -20±1^{o}C for 1 day on a repeating weekly cycle.

To investigate the effect of fluctuating frozen storage temperature on frozen dough and bread quality, only slow freezing was selected for TF2 experiment. The dough was stored under 4 fluctuating temperature regimes for up to 112 days. The four storage regimes were -18±1^{o}C (Good Practice, G), -18±3^{o}C (Poor Practice, P), -18±5^{o}C (Very Poor Practice, VP) and the cold chain (CC). For the cold chain regime, the temperature set-points were -18±1^{o}C for 4 days, -13±1^{o}C for 1 day, -8±1^{o}C for 1 day, and then -18±1^{o}C for 1 day on a repeating weekly cycle.

5.4.1 STORAGE TEMPERATURE VARIATION

The variations of the air temperature in the controlled temperature boxes (ATB), the air temperature in coldstore (ATC), the dough center temperature (DCT) and the dough surface temperature (DST) are shown in Fig. 5.21a-c for the C, G and P regimes and Fig. 5.22a-b for the CC regime for TF1. The average ATB, ATC, DCT and DST of the C, G, P and CC regimes are given in Table 5.20.

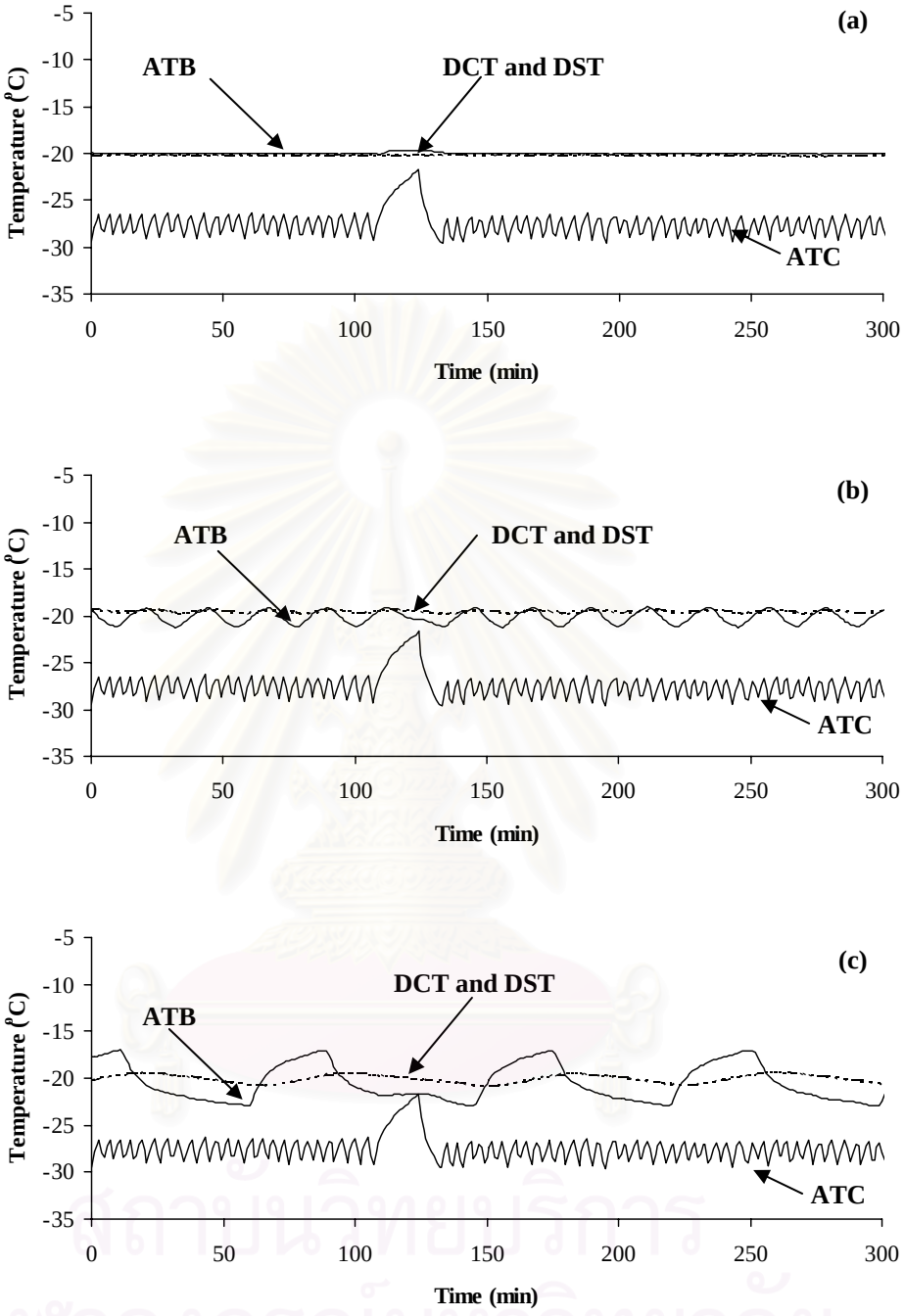


Fig. 5.21 Typical air and dough temperature variations in TF1. (a) C regime (b) G regime (c) P regime. ATC indicates coldstore air temperature, ATB indicates box air temperature, DCT indicates dough center temperature and DST indicates dough surface temperature.

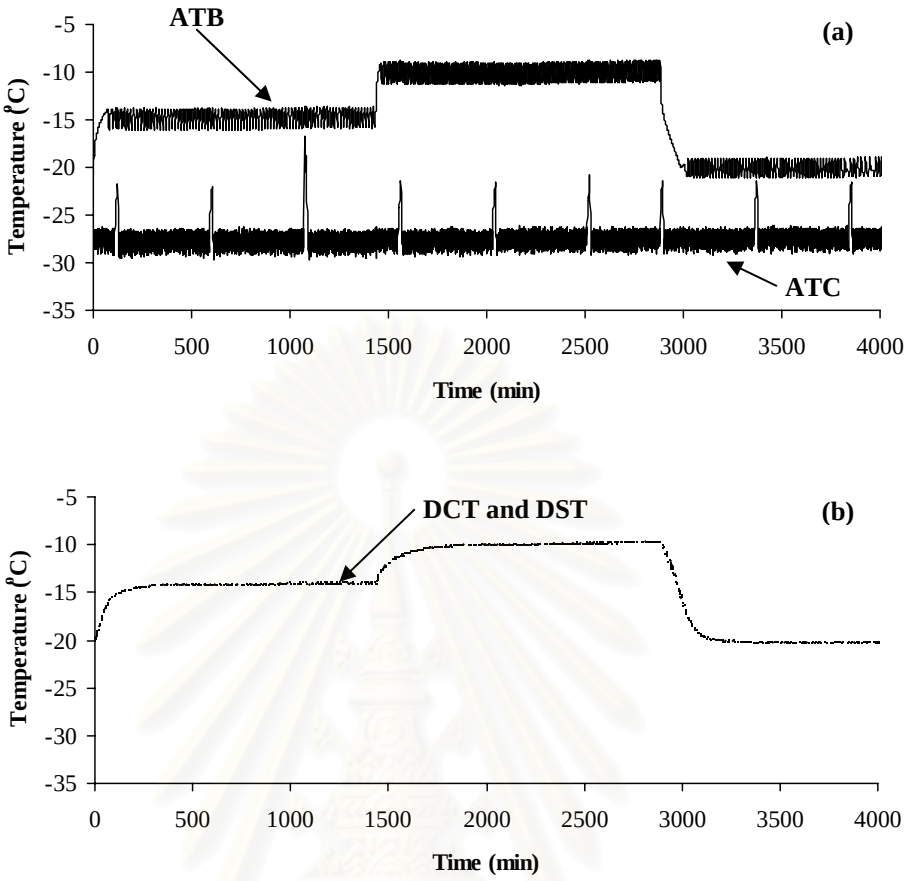


Fig. 5.22 Typical air and dough temperature variations for the CC regime in TF1.
(a) coldstore air temperature (ATC) and box air temperature (ATB),
(b) dough center temperature (DCT) and dough surface temperature (DST).

Table 5.20 Average air and dough temperatures for TF1

Regime	Set-point	Air temperature	Dough temperature	
			Center	Surface
Control (C)	-20±0.1°C	-20.0±0.1°C	-20.2±0.07°C	-20.2±0.07°C
Good Practice (G)	-20±1°C	-20.2±0.7°C	-19.6±0.14°C	-19.5±0.14°C
Poor Practice (P)	-20±3°C	-20.6±1.9°C	-20.5±0.45°C	-20.5±0.45°C
Cold Chain (CC)	-20±1°C	-20.1±0.7°C	-19.8±0.08°C	-19.8±0.09°C
	-15±1°C	-14.7±0.8°C	-14.7±0.08°C	-14.7±0.08°C
	-10±1°C	-10.1±0.8°C	-10.0±0.09°C	-10.0±0.09°C

Values are the mean and standard deviation of the measurements.

For TF2 experiment, the variations of the ATB, the ATC, the DCT and the DST of the C, G, P, VP and CC regimes are given in Table 5.21. The variations of the ATB, ATC, DCT and DST are shown in Fig. 5.23a-d for the C, G, P and VP regimes and Fig. 5.24a-b for the CC regime.

Table 5.21 Average air and dough temperatures for TF2

Regime	Set-point	Air temperature	Dough temperature	
			Center	Surface
Control (C)	-18±0.1°C	-18.1±0.1°C	-17.9±0.07°C	-17.9±0.07°C
Good Practice (G)	-18±1°C	-18.2±0.6°C	-18.3±0.07°C	-18.3±0.09°C
Poor Practice (P)	-18±3°C	-18.7±1.8°C	-18.5±0.39°C	-18.4±0.45°C
Very Poor Practice (VP)	-18±5°C	-18.3±3.3°C	-18.2±2.08°C	-18.1±2.11°C
Cold Chain (CC)	-18±1°C	-18.1±0.7°C	-18.1±0.09°C	-18.1±0.09°C
	-13±1°C	-13.2±0.8°C	-13.2±0.08°C	-13.3±0.09°C
	-8±1°C	-8.3±0.8°C	-8.4±0.09°C	-8.4±0.09°C

Values are the mean and standard deviation of the measurements.

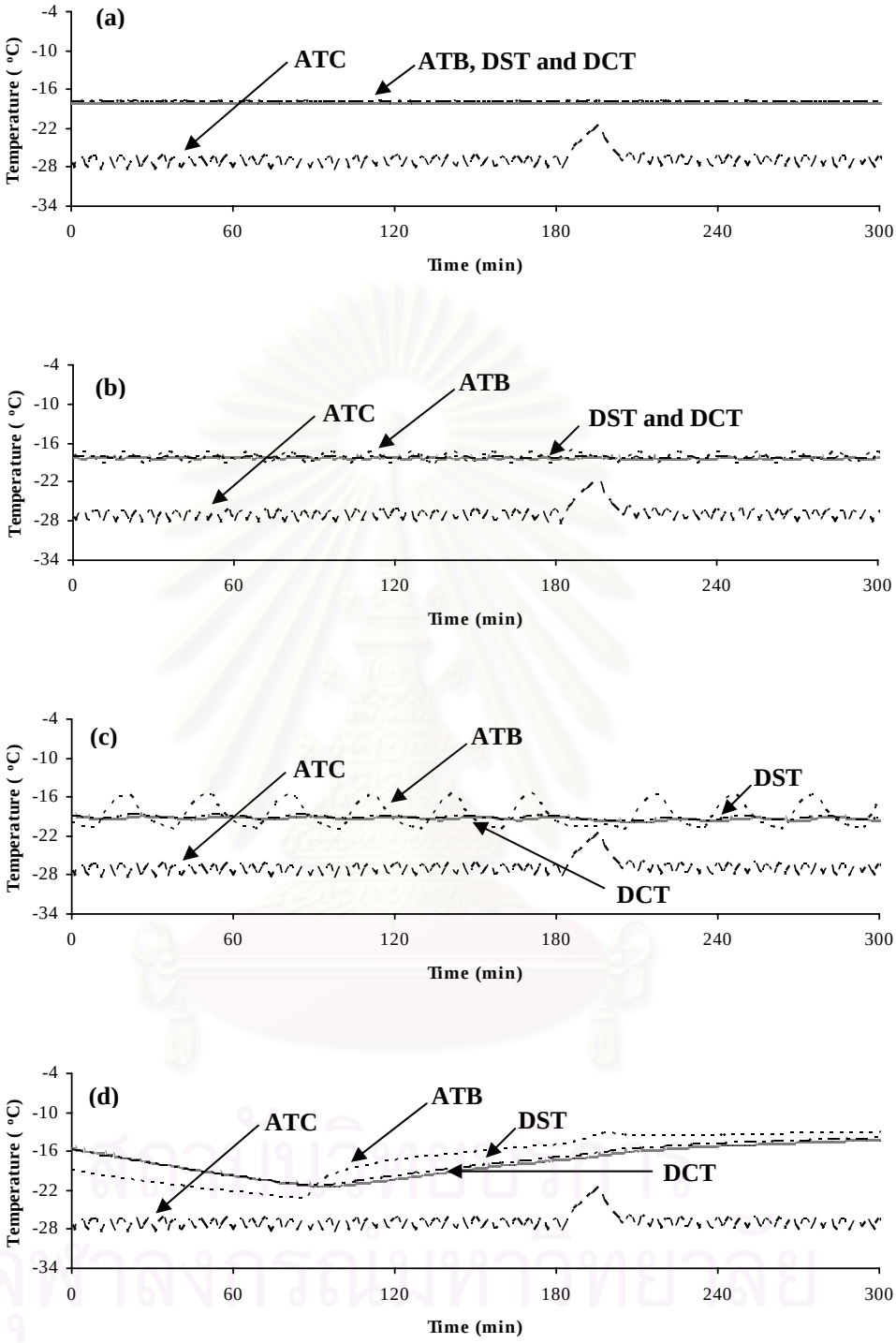


Fig. 5.23 Typical air and dough temperature variations in TF2. (a) C regime (b) G regime (c) P regime (d) VP regime. ATC indicates coldstore air temperature, ATB indicates box air temperature, DCT indicates dough center temperature and DST indicates dough surface temperature.

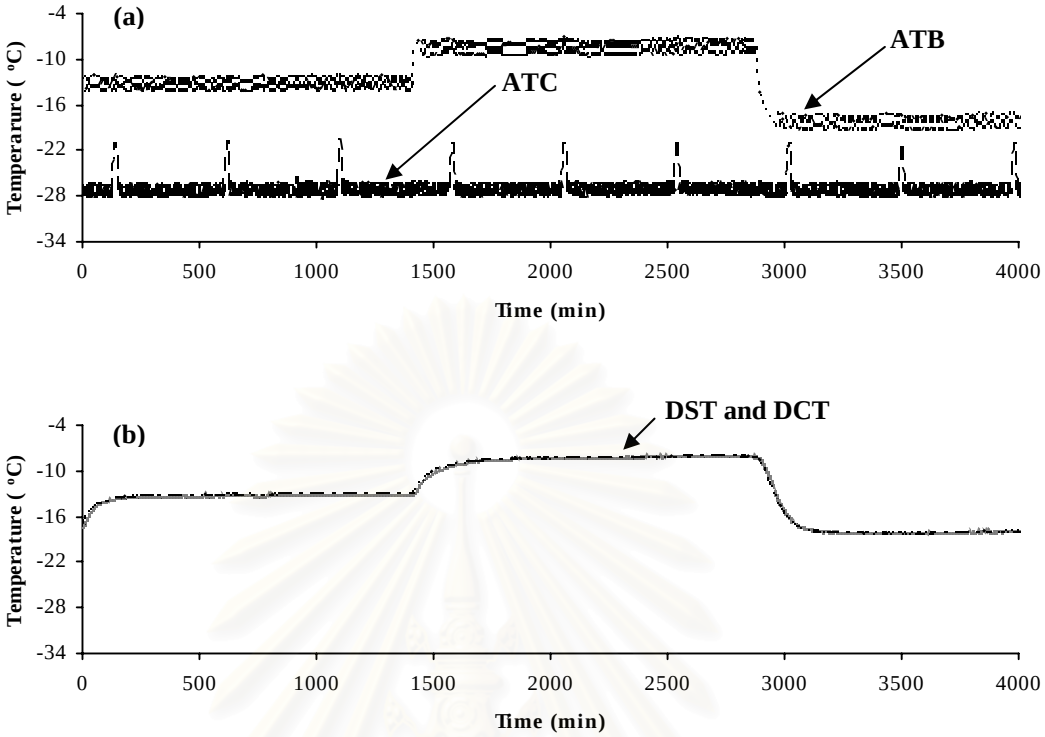


Fig. 5.24 Typical air and dough temperature variations for the CC regime in TF2.
(a) coldstore air temperature (ATC) and box air temperature (ATB),
(b) dough center temperature (DCT) and dough surface temperature (DST).

In TF2, the air temperature in the coldstore was about -27.0±0.9°C whereas for TF1 it was 26.8±1.2°C. Although the average air temperature of the C, G and VP regimes were nearly identical, the DCT and DST differed by 0.3 to 0.4°C (Table 5.21). This apparent difference probably reflects offset and uncertainty in the temperature measurement rather than any significant actual difference. For the VP regime, both the average air and the dough temperatures were slightly colder than other regimes. Despite significantly different levels ($p<0.05$) of variation in the air temperature for the C and G regimes (±0.1°C and ±0.6°C), the temperature variations of the DCT and DST were very similar (±0.07°C and ±0.09°C). However, the average DCT and DST for the C and G regimes were significantly different ($p<0.05$) being about -17.9°C and -18.3°C, respectively. Excluding the dummy samples adjacent to the lights that were not part of the experimental plan, differences in temperature between samples at different positions in the same box at any time were less than 0.25°C.

5.4.2 DOUGH WEIGHT LOSS

When unwrapped foods are frozen and/or stored in the frozen state or with a non-adhering packaging, weight loss takes place due to sublimation of the surface ice. Ice sublimation produces a dehydrated surface layer that changes the appearance, color, texture and taste (Camponone et al., 2001). During storage, temperature fluctuations are transferred, delayed in time, to the stored goods. Thus, there will be alternating periods in which dough surface temperature is higher than the room or packaging temperature, with subsequent ice sublimation. The cumulative weight loss throughout long storage periods may cause a significant quality loss. This effect is usually much more important than weight loss during freezing due to the longer duration in frozen storage. The effects of temperature fluctuations on weight loss during frozen storage were determined.

The weight loss of the dough during frozen storage for both SF and FF is shown in Fig. 5.25 and 5.26 for TF1. Freezing rate had no significant effect ($p<0.05$) on weight loss of frozen dough. Fig. 5.27 shows the dough weight loss as a function of storage regime and storage time for TF2. Increasing storage period resulted in an increase in weight loss for both TF1 and TF2. Pham (1987) also found that moisture loss during frozen storage was affected by temperature fluctuations.

The rate of weight loss was reasonably constant for all storage regimes. Doughs stored under the C and G regimes had no significant difference ($p>0.05$) in weight loss. For the P, VP and CC regimes the rate of weight loss was significantly higher than these for the C and G regimes, and was greater as the regime had larger temperature fluctuations (P and VP regimes) and/or higher average storage temperature (CC).

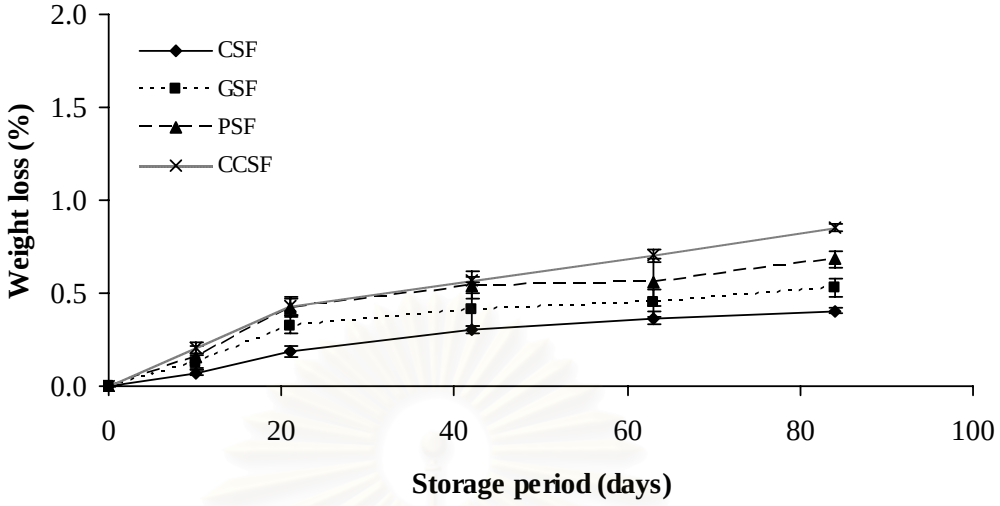


Fig. 5.25 Effect of fluctuating storage regime during frozen storage on weight loss of frozen dough with slow freezing (SF).

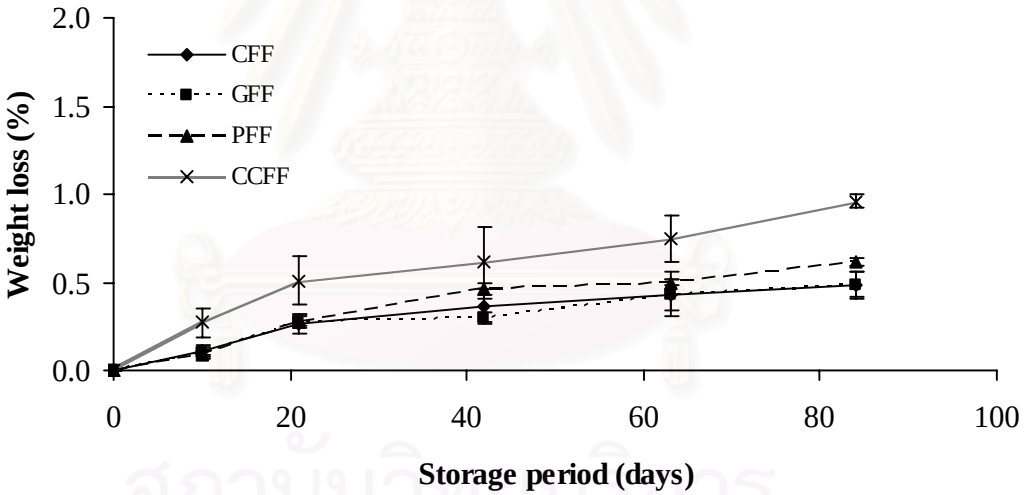


Fig. 5.26 Effect of fluctuating storage regime during frozen storage on weight loss of frozen dough with fast freezing (FF).

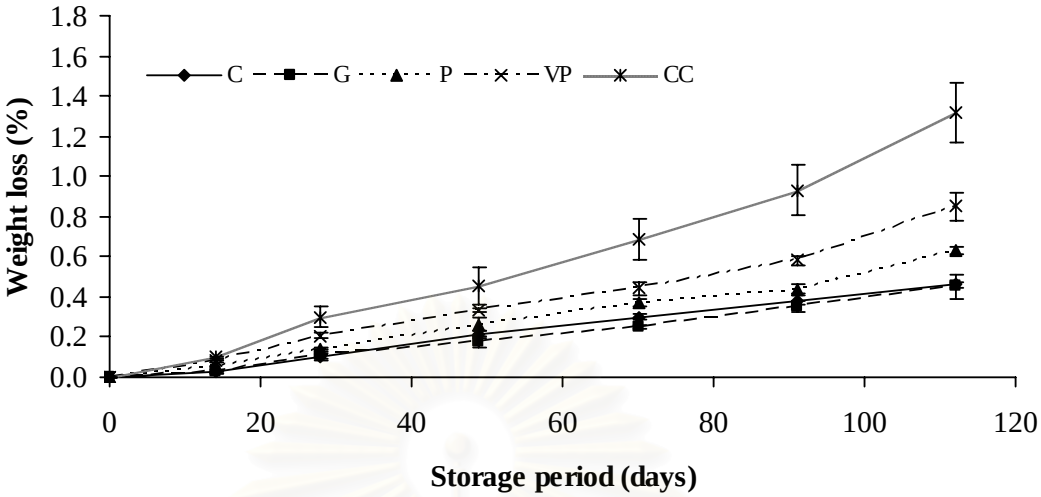


Fig. 5.27 Effect of fluctuating temperature during frozen storage on dough weight loss for TF2.

Fluctuations in storage temperature such that the dough and the plastic bag have differing temperatures can drive a net loss of weight from the dough because the relationship between water/ice saturated vapor pressure (SVP) and temperature is not linear and any frost forming on the inside of the bag tends to drop off the surface and accumulate which reduces the rates of reverse sublimation. Given this mechanism then as storage temperature increases the rate of weight loss will increase because the SVP of the water and hence the partial pressure of water driving force between the dough and the bag will tend to be higher. Also, as temperature fluctuations become larger then temperature differences and hence partial pressure of water vapor differences between the dough and the bag will increase, giving greater potential for weight loss. The results in Fig. 5.25, 5.26 and 5.27 confirm this behavior.

5.4.3 CO_2 PRODUCTION

Cumulative CO_2 production (gassing power) and CO_2 production rate were both investigated. Table 5.22 shows cumulative CO_2 production as function of frozen storage time for TF1. Table 5.23 shows the equivalent results fro TF2. The freezing rate had a significant effect ($p<0.05$) on CO_2 production (TF1). Slow freezing (SF) gave a better CO_2 production than fast freezing (FF), which was consistent with the

quality kinetics (QK1) experiments. In TF1 experiment after freezing, the cumulative CO_2 production of the dough with SF and FF declined by 2 % and 20% respectively and decreased by about 7% compared to fresh dough for TF2. Under more extreme temperature fluctuation and higher temperature storages (VP and CC regimes), the dough gassing power declined 43% and 55% respectively after 112 days frozen storage.

Table 5.22 Effects of freezing rates and fluctuating frozen storage regimes on cumulative CO_2 production (gassing power) for TF1

Regime	Storage period (day)	CO_2 production (%)	
		SF	FF
C	fresh	100±0 a	100±0 a
	1	99±2 ab	81±4 b-g
	21	90±5 ab	75±10 d-j
	42	86±4 b-d	75±11 e-k
	63	86±2 b-d	73±7 e-k
	84	79±3 b-h	73±1 f-k
G	fresh	100±0 a	100±0 a
	1	99±2 ab	81±4 b-g
	21	83±5 b-f	78±6 c-i
	42	81±5 b-g	73±2 e-k
	63	79±6 b-h	68±2 i-l
	84	81±10 b-g	64±5 k-l
P	fresh	100±0 a	100±0 a
	1	99±2 ab	81±4 b-g
	21	88±4 bc	79±10 b-h
	42	84±3 b-e	74±11 e-k
	63	76±6 d-j	71±11 g-l
	84	74±5 e-k	65±5 j-l
CC	fresh	100±0 a	100±0 a
	1	99±2 ab	81±4 b-g
	21	88±7 bc	73±8 e-k
	42	80±7 b-h	71±8 g-l
	63	74±10 e-k	67±5 i-l
	84	69±9 h-l	60±4 l

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Table 5.23 Cumulative CO_2 production (ml) for various frozen storage regimes and storage periods for TF2

Storage	**Regime**					
Period (days)	**C**	**G**	**P**	**VP**	**CC**	**Average**
Fresh	336±5 a	328±15 ab	328±15 ab	328±15 ab	328±15 ab	**330±12**
1	316±5 ab	295±31 bc	295±31 bc	295±31 bc	295±31 bc	**300±26**
14	252±8 d-f	256±18 d-f	256±14 d-f	233±29 d-g	264±25 cd	**252±20**
28	250±21 d-f	238±21 d-g	261±35 c-e	234±9 d-g	254±37 d-f	**248±25**
49	230±11 d-i	240±24 d-g	232±10 d-h	222±14 e-j	248±27 d-f	**234±18**
70	229±19 d-i	222±19 e-j	226±27 d-i	202±18 g-j	225±25 d-i	**221±21**
91	245±8 d-f	232±14 d-h	219±5 f-j	193±3 h-j	192±1 ij	**217±23**
112	234±2 d-g	238±2 d-g	218±6 f-j	185±14 i	146±29 k	**204±38**
Average	**262±40**	**256±39**	**255±42**	**236±51**	**244±59**	**257±44**

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

The main effect of storage regime was that the dough stored under the C, G and P regimes had a significantly higher ($p<0.05$) CO_2 production than that stored under the CC regime. Storage regime had significant effect ($p<0.05$) for the dough frozen with SF but insignificant ($p>0.05$) for the dough frozen with FF possibly because for FF the freezing process itself caused greater yeast damage. Cumulative CO_2 production significantly declined with increasing storage time. Decrease in CO_2 production for both SF and FF showed similar trends during frozen storage.

Fig. 5.28 and 5.29 show the CO_2 production rate of the dough frozen with SF and FF stored under fluctuating temperature regimes. Fig. 5.30 gives the similar results for TF2. After freezing, the CO_2 production rate of the dough frozen with FF had a greater decline than for dough frozen with SF. An increase in storage duration resulted in lower CO_2 production rate for all conditions. The dough stored under the P and CC regimes had lower CO_2 production rate than the dough stored under the C and G regimes. A lower plateau was observed for CO_2 production rate indicating possible rupture of the gluten network which was unable to retain CO_2. The corresponding volume appeared to be function of the frozen storage regime and storage period. This is consistent with the results of Le Bail et al. (1999) that showed that temperature fluctuations had a large influence on the dough volume. This result is probably due to ice recrystallization which is accelerated by temperature fluctuations, resulting in the reduction of yeast activity (Neyreneuf and Delpuech, 1993). However, changes to the dough gluten network and its ability to retain CO_2 due to ice recrystallization may also have contributed.

Laaksonen and Roos (2000) found that the glass transition temperature of dough was less than -30^{o}C. Normal frozen storage temperatures and the temperatures used in this study are significantly higher than this. Therefore relating the increased rate of deterioration to increased mobility of the water with more extreme storage temperature fluctuations or higher storage temperatures is a reasonable mechanistic explanation.

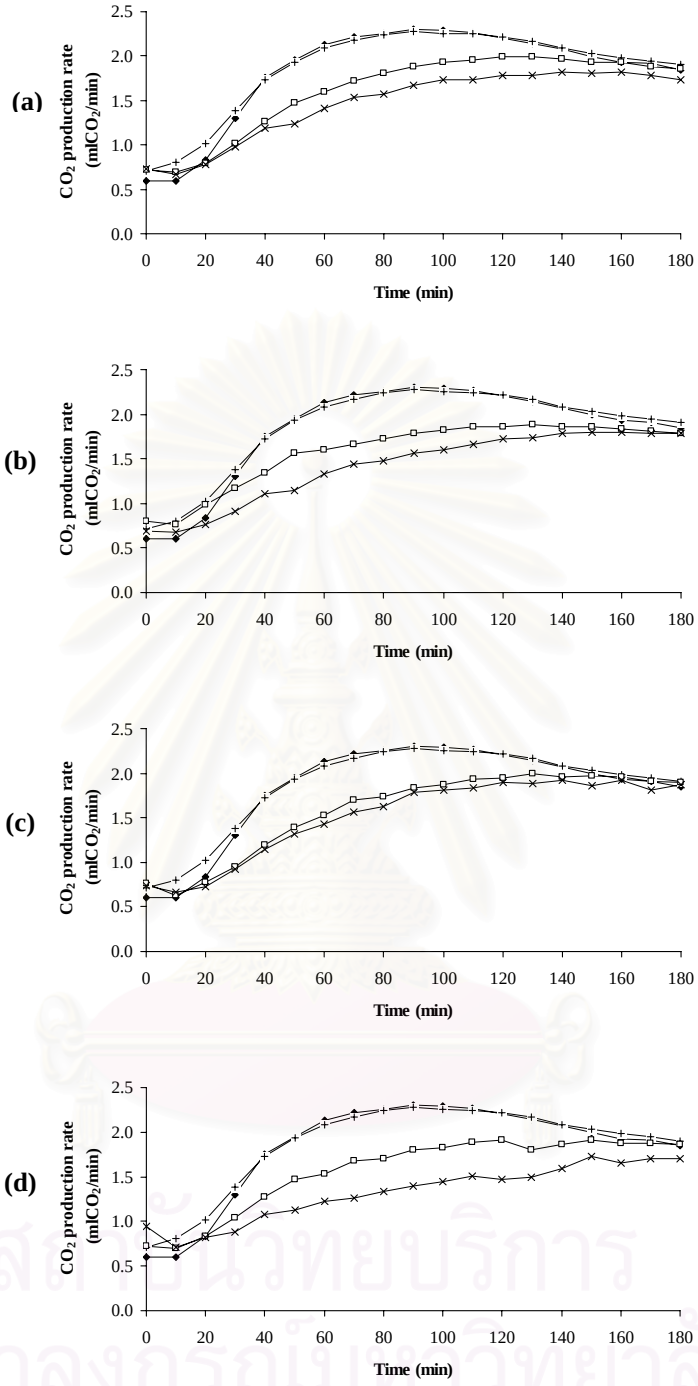


Fig. 5.28 Effects of freezing and fluctuating storage regime during frozen storage on CO_2 production rate of the dough with slow freezing (SF) for TF1.
(a) C regime (b) G regime (c) P regime (d) CC regime. --♦-- indicates fresh. --+-- indicates 1 day frozen storage. --□-- indicates 42 days frozen storage. --×-- indicates 84 days frozen storage.

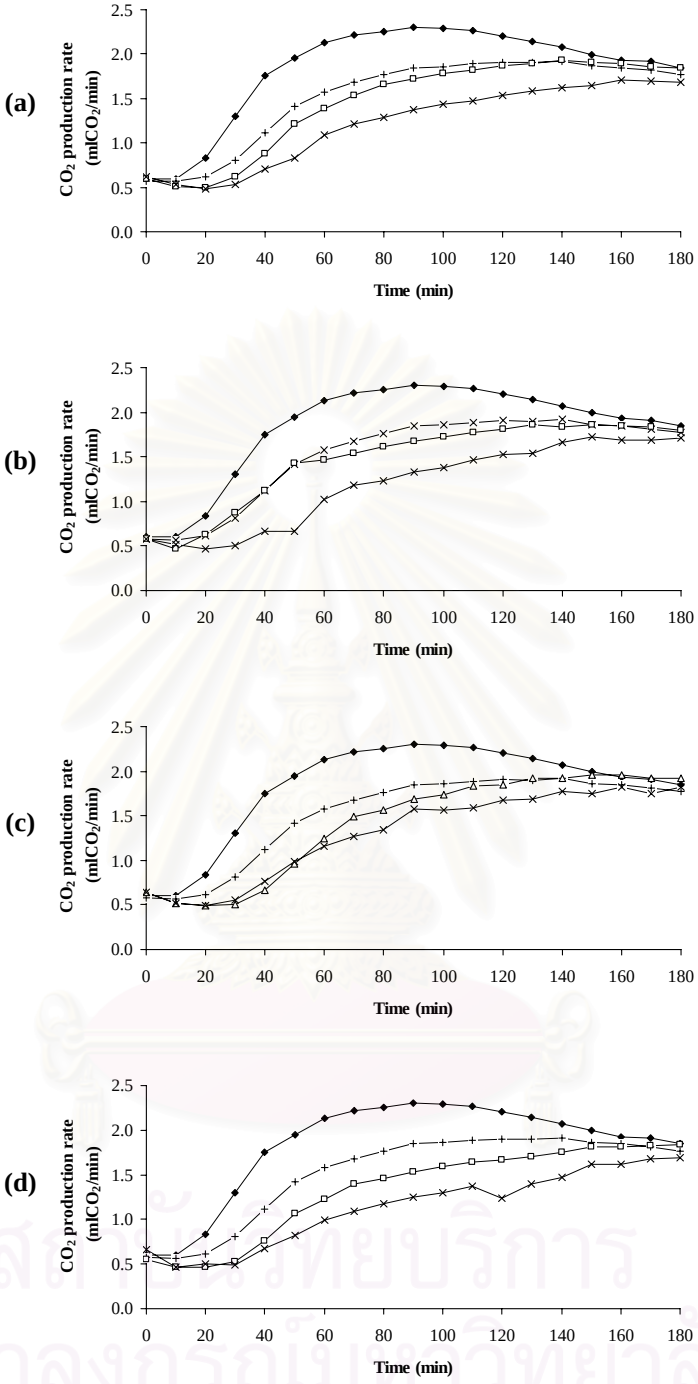


Fig. 5.29 Effects of freezing and fluctuating storage regime during frozen storage on CO_2 production rate of the dough with fast freezing (FF) for TF1.

(a) C regime (b) G regime (c) P regime (d) CC regime. --♦-- indicates fresh. --+-- indicates 1 day frozen storage. --□-- indicates 42 days frozen storage. --×-- indicates 84 days frozen storage.

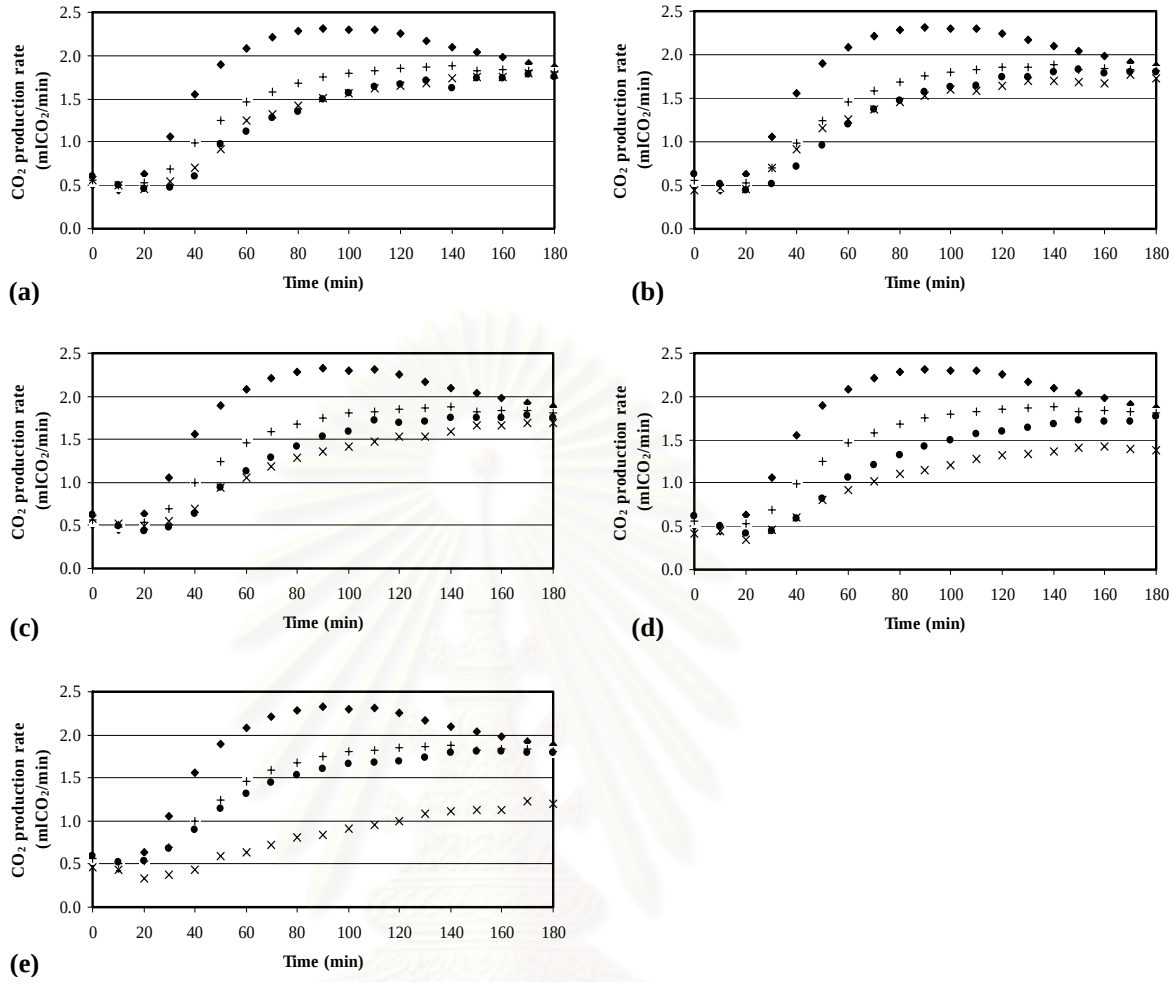


Fig. 5.30 CO_2 production rate for frozen dough stored under different storage regimes for TF2. (a) C regime (b) G regime (c) P regime (d) VP regime (e) CC regime. ♦: fresh; + : 1 day frozen storage; • : 49 days frozen storage; × : 112 days frozen storage.

5.4.4 YEAST VIABILITY

Table 5.24 shows yeast viability of frozen dough stored under temperature fluctuations with different freezing rates (TF1). Table 5.25 shows the equivalent results for TF2. One day after freezing, yeast viability of the dough frozen with SF and FF dropped by 6% and 15% respectively for TF1 and by 9% for TF2 consistent with the results of Havet et al. (1999). Yeast viability decreased with increasing storage period. During the first 42 days frozen storage, yeast viability loss did not show any significant difference for each regime. After that period, yeast viability loss for the dough stored under the CC regime declined significantly faster than that for the dough stored under other regimes. The doughs stored under the C, G, P and VP regimes had significantly higher yeast viability than those stored under the CC regime. Yeast viability gradually decreased with an increased storage period for all storage regimes. After 112 days frozen storage, the yeast viability for the CC regime had decreased by more than 50% relative to fresh dough. Salas-Mellado and Chang (2003) also found that dough yeast viability after 45 days frozen storage at -15^{o}C declined by 1% to 53%, depending on dough formulation and yeast type. Overall, the trends in CO_2 production and yeast viability were similar suggesting that yeast viability reduction was a significant contributor to reduction in CO_2 production.

Table 5.24 Effects of freezing rates and fluctuating frozen storage regimes on yeast viability for TF1

Regime	Storage period (day)	Yeast viability (%)	
		SF	FF
C	fresh	100±0 a	100±0 a
	1	95±4 ab	86±12 a-d
	21	90±20 a-c	85±2 a-d
	42	83±8 a-e	76±12 b-g
	63	78±15 b-g	78±18 b-g
	84	77±6 b-g	78±6 b-g
G	fresh	100±0 a	100±0 a
	1	95±4 ab	86±12 a-d
	21	80±12 b-f	85±21 a-d
	42	82±8 a-e	75±4 b-g
	63	77±12 b-g	71±1 c-g
	84	70±11 c-g	73±16 b-g
P	fresh	100±0 a	100±0 a
	1	95±4 ab	86±12 a-d
	21	87±1 a-d	88±1 a-c
	42	88±8 a-c	70±14 c-g
	63	83±15 a-e	78±22 b-g
	84	75±5 b-g	72±2 c-g
CC	fresh	100±0 a	100±0 a
	1	95±4 ab	86±12 a-d
	21	83±11 a-e	74±13 b-g
	42	74±9 b-g	71±5 c-g
	63	64±19 e-g	66±15 d-g
	84	60±13 fg	59±2 g

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Table 5.25 Effects of temperature fluctuations during frozen storage on yeast viability of frozen dough for TF2

Storage Period (days)	Regime					
	C	**G**	**P**	**VP**	**CC**	**Average**
Fresh	100±0	100±0	100±0	100±0	100±0	**100±0 a**
1	88±7	92±4	92±4	92±4	92±4	**91±5 b**
14	82±5	89±12	81±12	86±18	68±3	**81±12 c**
28	77±4	83±15	80±15	79±12	66±4	**77±10 cd**
49	74±5	73±5	75±5	70±12	66±13	**72±10 de**
70	72±7	67±10	69±10	64±2	62±2	**67±7 cf**
91	71±6	67±7	67±7	58±3	54±4	**64±8 fg**
112	69±6	66±6	65±6	57±4	46±2	**60±10 g**
Average	**79±11 A**	**80±14 A**	**79±14 A**	**76±17 A**	**69±18 B**	**76±15**

Values are the mean and standard deviation of 3 samples. A–B means within the same row with different letters are significantly different ($p<0.05$). a-g means within the same column with different letters are significantly different ($p<0.05$).

5.4.5 DOUGH RHEOLOGICAL PROPERTIES

Table 5.26 and 5.27 shows the effects of freezing rates and fluctuating frozen storage regimes on maximum rupture force and extensibility of frozen dough for TF1. Table 5.28 and 5.29 give equivalent results for TF2. The effect of freezing rate on the rheological properties was small for both maximum rupture force and extensibility. Maximum force before rupture reduced for all regimes during the first 42 days storage. However, an increase in maximum force was observed after 63 days storage.

Table 5.26 Effects of freezing rates and fluctuating frozen storage regimes on maximum rupture force of dough for TF1

Regime	Storage period (day)	Maximum rupture force (g)	
		SF	FF
C	fresh	39.2±3.6 b-f	39.2±3.6 b-f
	1	39.1±3.8 b-f	34.3±1.3 f-k
	21	31.1±1.0 i-m	36.7±1.9 c-h
	42	26.7±2.3 mn	33.0±2.8 h-l
	63	31.2±0.7 i-m	33.7±1.2 g-k
	84	33.1±2.4 h-l	33.0±1.2 h-l
G	fresh	39.2±3.6 b-f	39.2±3.6 b-f
	1	39.1±3.8 b-f	34.3±1.3 f-k
	21	30.0±2.7 k-n	29.7±1.5 k-n
	42	30.9±1.9 j-n	34.2±0.7 f-k
	63	28.4±3.8 l-n	26.1±0.0 n
	84	41.8±1.1 bc	37.5±1.2 c-h
P	fresh	39.2±3.6 b-f	39.2±3.6 b-f
	1	39.1±3.8 b-f	34.3±1.3 f-k
	21	36.1±1.7 d-i	38.7±1.8 c-g
	42	36.1±4.7 d-i	33.0±1.4 h-l
	63	35.4±1.2 e-j	29.8±0.3 k-n
	84	41.0±1.4 b-d	40.2±2.3 b-e
CC	fresh	39.2±3.6 b-f	39.2±3.6 b-f
	1	39.1±3.8 b-f	34.3±1.3 f-k
	21	36.6±3.0 d-h	37.3±1.3 c-h
	42	35.1±0.9 e-j	37.3±1.5 c-h
	63	40.7±2.8 b-d	37.0±2.5 c-h
	84	47.4±5.2 a	44.0±0.6 ab

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

For the dough extensibility, the dough stored under various fluctuating storage regime showed small changes during frozen storage for up to 84 days (Fig. 5.28). High variations in dough rheological measurement were observed. This is probably due to the moisture loss in the frozen dough affecting the rheological measurements. Other possible explanations for high variations in dough rheological properties measurement were that the rheological properties changes during frozen storage were small, resulting in lack of significant trends or the instrument used may not be sensitive enough.

Table 5.27 Effects of freezing rates and fluctuating frozen storage regimes on dough extensibility for TF1

Regime	Storage period (day)	Dough extensibility (mm)	
		SF	FF
C	fresh	41.0±0.6 b-d	41.0±0.6 b-d
	1	41.4±1.1 b-d	50.0±8.1 a
	21	41.0±1.5 b-d	43.4±2.1 bc
	42	38.1±2.4 cd	46.0±1.6 ab
	63	38.0±2.4 cd	41.0±3.7 b-d
	84	38.0±2.1 cd	39.4±1.8 cd
G	fresh	41.0±0.6 b-d	41.0±0.6 b-d
	1	41.4±1.0 b-d	50.0±8.1 a
	21	37.0±0.5 d	40.0±2.4 cd
	42	38.0±3.2 cd	42.8±1.0 b-d
	63	39.2±3.0 cd	42.5±1.1 b-d
	84	40.0±0.8 cd	51.2±1.5 a
P	fresh	41.0±0.6 b-d	41.0±0.6 b-d
	1	41.4±1.1 b-d	50.0±8.1 a
	21	41.0±2.8 b-d	41.6±4.2 b-d
	42	37.0±1.7 d	42.0±2.7 b-d
	63	36.8±3.3 d	41.2±1.2 b-d
	84	40.3±1.7 b-d	40.5±0.6 b-d
CC	fresh	41.0±0.6 b-d	41.0±0.6 b-d
	1	41.4±1.1 b-d	50.0±8.1 a
	21	37.2±0.2 d	49.0±3.2 a
	42	39.0±2.1 c-d	38.0±3.0 cd
	63	41.4±2.6 b-d	40.2±1.8 b-d
	84	38.1±0.3 cd	40.4±3.9 b-d

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Table 5.28 Effects of fluctuating frozen storage regimes on maximum rupture force of dough for TF2

Storage Period	**Regime**					
(days)	**C**	**G**	**P**	**VP**	**CC**	**Average**
Fresh	35.5±5.4 ab	36.3±1.1 a	36.3±1.1 a	36.3±1.1 a	36.3±1.1 a	**36.1±2.2**
1	31.7±1.5 c-e	28.9±1.1 d-h	28.9±1.1 d-h	28.9±1.1 d-h	28.9±1.1 d-h	**29.4±1.5**
28	32.9±3.4 bc	25.9±2.2 h-j	26.0±1.2 h-j	25.0±1.4 i-l	24.1±0.4 j-m	**26.8±3.7**
49	25.4±1.5 h-k	22.2±1.2 k-n	22.3±1.5 k-n	16.5±1.2 o	20.7±0.4 n	**21.4±3.2**
70	29.9±1.1 c-g	22.0±0.7 l-n	21.2±1.8 mn	20.0±1.7 n	24.9±0.1 i-l	**23.6±3.8**
91	32.3±0.4 cd	29.8±1.1 c-g	31.8±2.8 c-e	28.5±0.5 e-h	30.4±0.3 c-f	**30.6±1.8**
112	26.5±2.5 g-j	28.1±3.0 f-i	31.4±2.3 c-f	37.5±2.0 a	38.5±1.6 a	**32.4±5.4**
Average	**30.6±4.1**	**27.6±4.9**	**28.3±5.4**	**27.5±7.5**	**29.1±6.2**	**28.6±5.7**

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Table 5.29 Effects of fluctuating frozen storage regimes on dough extensibility for TF2

Storage Period (days)	Regime					
	C	**G**	**P**	**VP**	**CC**	**Average**
Fresh	40.4±1.0 d-f	40.8±2.0 d-f	40.8±2.0 d-f	40.8±2.0 d-f	40.8±2.0 d-f	**40.7±1.5**
1	47.6±3.8 bc	46.9±2.5 b-d	46.9±2.5 b-d	46.9±2.5 b-d	46.9±2.5 b-d	**47.0±2.4**
28	29.9±1.5 ij	35.3±2.5 f-i	30.6±4.0 ij	34.2±1.1 g-j	33.2±1.9 g-j	**32.6±3.0**
49	19.6±3.5 k	39.3±8.3 e-g	32.3±2.6 h-j	34.8±4.4 f-j	33.8±3.8 g-j	**31.9±8.0**
70	30.7±2.9 ij	37.8±2.9 f-h	30.2±1.4 ij	29.1±4.1 ij	28.5±0.3 j	**31.2±4.1**
91	47.3±6.7 bc	52.8±4.7 ab	55.2±4.3 a	44.5±1.3 c-e	56.5±2.6 a	**51.3±6.0**
112	47.6±2.8 bc	48.1±2.1 bc	55.0±5.2 a	47.4±2.9 bc	48.4±4.1 bc	**49.3±4.2**
Average	**37.6±10.9**	**43.0±7.0**	**41.6±10.9**	**39.6±7.2**	**41.1±9.7**	**40.6±9.3**

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

5.4.6 DOUGH MICROSTRUCTURE

SEM micrographs of frozen dough stored under the C, G, P and CC regimes after 84 days are shown in Fig. 5.31, 5.32, 5.33 and 5.34 respectively. The fracture surfaces of dough stored under the G, P and CC regimes had more damage than dough stored under the C regime. Ice crystal growth and recrystallization from greater fluctuations and higher storage temperature regimes probably led to greater structural damage in frozen dough. Most starch granules in dough stored under the C and G regimes were embedded in gluten matrix whereas higher fluctuating temperature regimes (P and CC regimes) caused more of the starch granules to be floating separately from the gluten matrix. More broken starch granules and gluten strands were also found in dough stored under more fluctuating conditions. Zounis et al. (2002a) found that when frozen dough was subjected to cycling temperature conditions, both yeast activity and ice recrystallization may have been factors causing damage to the dough structure. Temperature cycling between -20^{o}C and -10^{o}C exacerbated changes in ice-crystallinity and consequent physical damage to the dough structure, which were more extensive at longer storage times. Damage to dough structure under fluctuating storage temperatures during frozen storage is consistent with textural damage in other frozen products (Reid, 1990). Donhowe and Hartel (1996a) also found that ice recrystallization increased with increasing temperature. Increasing extent of temperature fluctuation also caused increasing recrystallization rate. Recrystallization still occurred at negligible temperature fluctuations (±0.01^{o}C) and increased gradually with increasing extent of temperature oscillations.

Aibara et al. (2005) also found that starch granules of the frozen dough became separate from the gluten matrix after two month frozen storage. Starch granules were dissembled and the close connection of the dough structures disappeared after repeating freeze-thaw cycle. Berglund, Shelton and Freeman (1990) stated that the loss of ability of gluten proteins to retained water resulted in the separation of starch granules and led to the deterioration of dough structures. However, microstructural changes are likely to arise from a number of different factors. These include dough mixing conditions and formulation, and specific ingredients, which may inhibit or accelerate structural damage in frozen doughs (Zounis et al., 2002a).

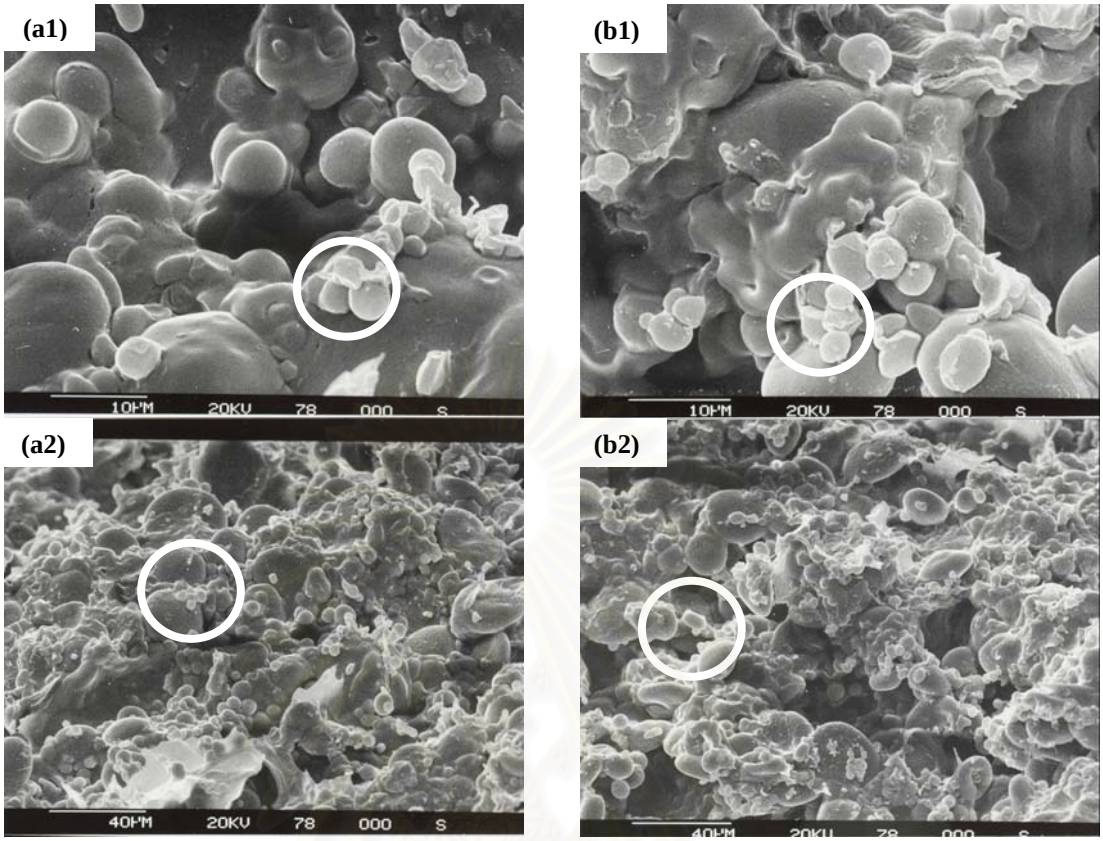


Fig. 5.31 SEM micrographs of frozen dough samples stored at -18±0.1°C (C regime) after 84 days storage for TF1. (a1-2) dough frozen with slow freezing (SF) under 2000x and 500x magnification. (b1-2) dough frozen with fast freezing (FF) under 2000x and 500x magnification. Circle indicates examples of floating starch granules.

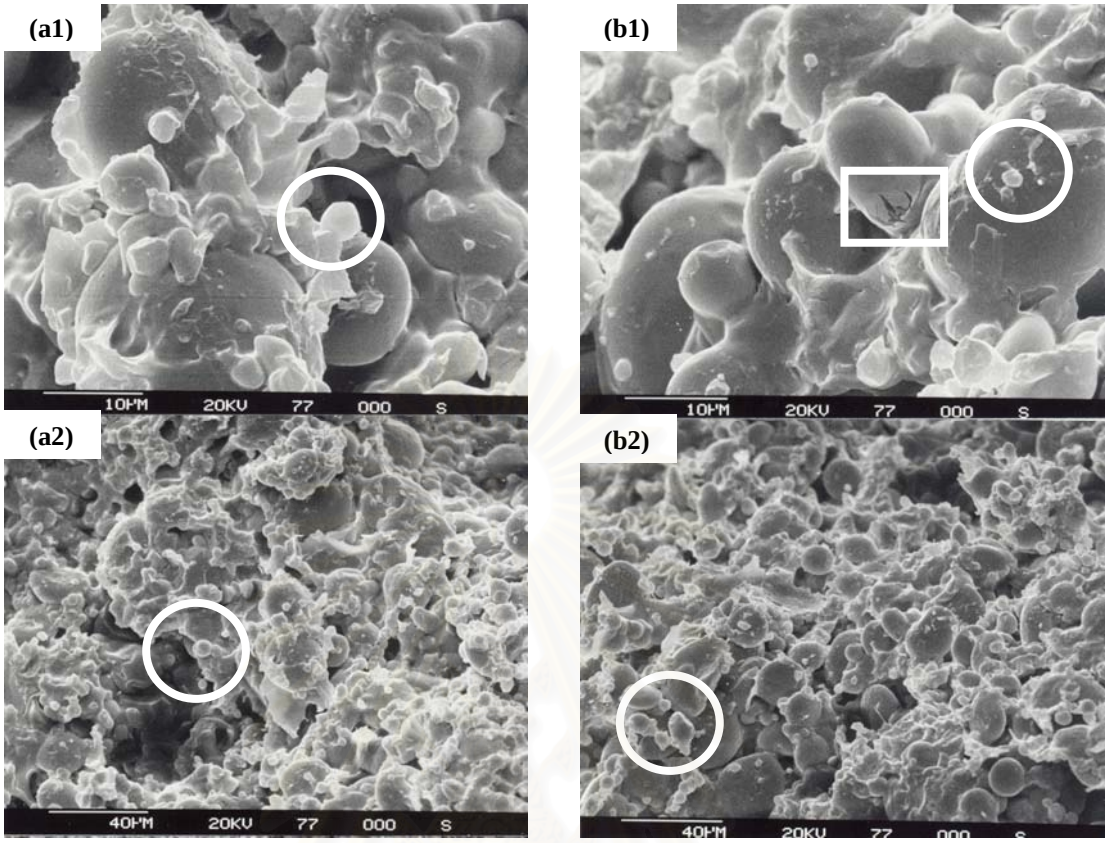


Fig. 5.32 SEM micrographs of frozen dough samples stored at -18±1°C (G regime) after 84 days storage for TF1. (a1-2) dough frozen with slow freezing (SF) under 2000x and 500x magnification. (b1-2) dough frozen with fast freezing (FF) under 2000x and 500x magnification. Circle indicates examples of floating starch granules. Square indicates examples of broken starch granules.

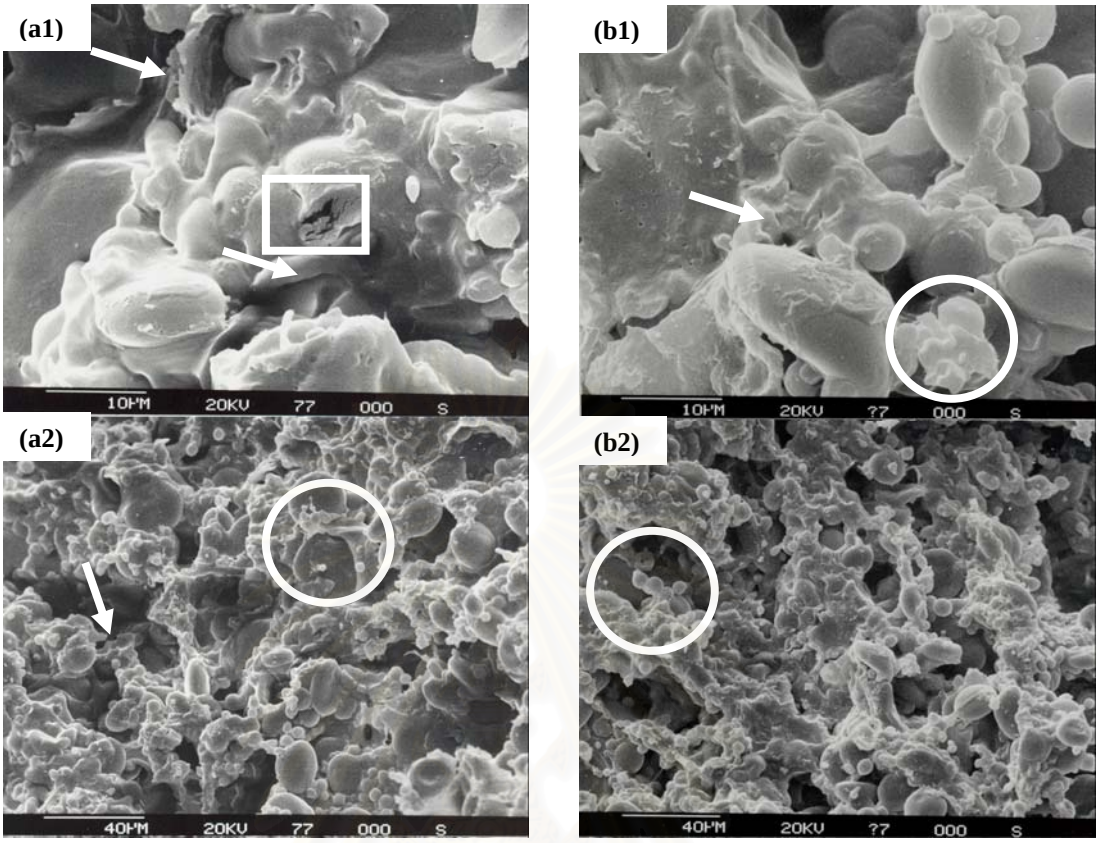


Fig. 5.33 SEM micrographs of frozen dough samples stored at -18±3°C (P regime) after 84 days storage for TF1. (a1-2) dough frozen with slow freezing (SF) under 2000x and 500x magnification. (b1-2) dough frozen with fast freezing (FF) under2000x and 500x magnification. Arrow indicates examples of broken gluten matrix and strands. Circle indicates examples of floating starch granules. Square indicates examples of broken starch granules.

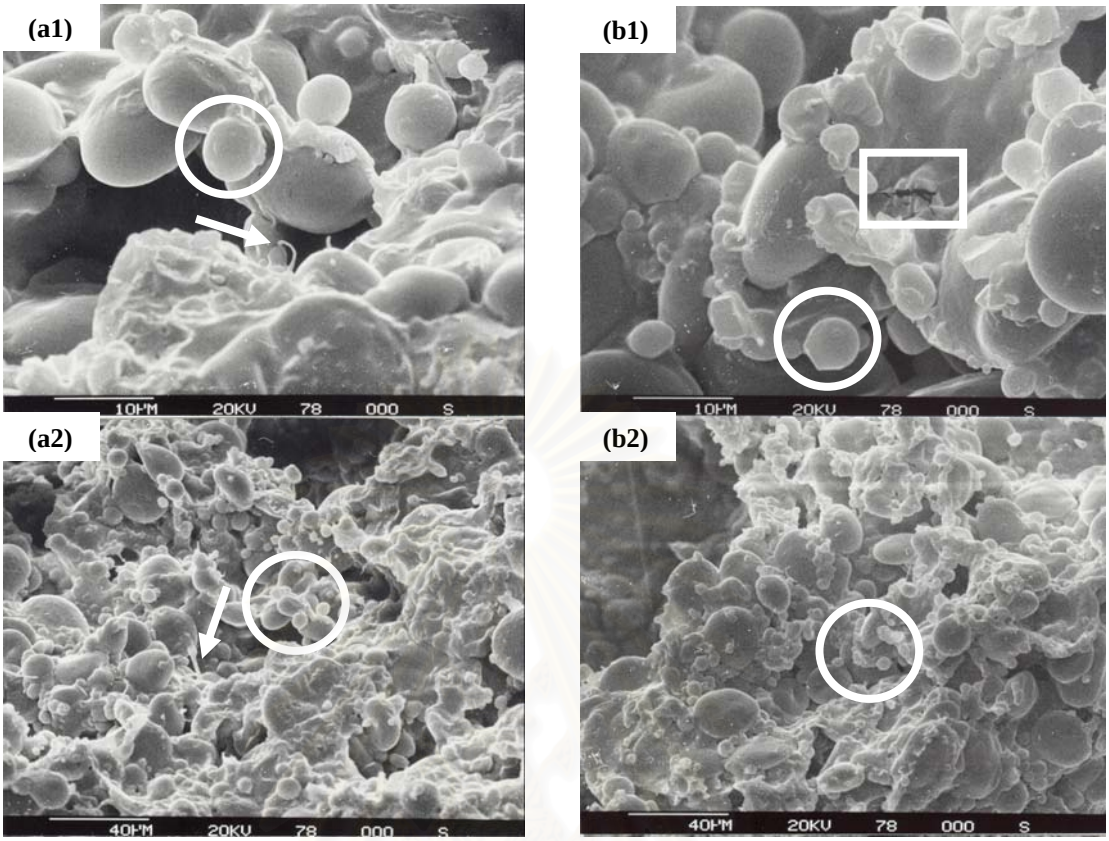


Fig. 5.34 SEM micrographs of frozen dough samples stored at higher temperature (a combination of -20°C, -15°C and -10°C; CC regime) after 84 days storage for TF1. (a1-2) dough frozen with slow freezing (SF) under 2000x and 500x magnification. (b1-2) dough frozen with fast freezing (FF) under 2000x and 500x magnification. Arrow indicates examples of broken gluten matrix and strands. Circle indicates examples of floating starch granules. Square indicates examples of broken starch granules.

5.4.7 DOUGH WATER MOBILITY

The T_2 relaxation time of frozen dough stored at different storage regimes after 1 day and 112 days is shown in Fig. 5.35. T_2 values increased with increasing storage period from 6.7 ms after 1-day frozen storage to be about 7.8-8.9 ms after 112 days frozen storage. After 112 days, T_2 relaxation values of frozen dough stored at more constant temperature regimes (G and P regime) were smaller than that of frozen dough stored under more fluctuating temperature (VP regime) and a higher temperature (CC regime). Longer T_2 of the dough stored under fluctuating temperature indicated greater separation of water bound to the starch-gluten matrix.

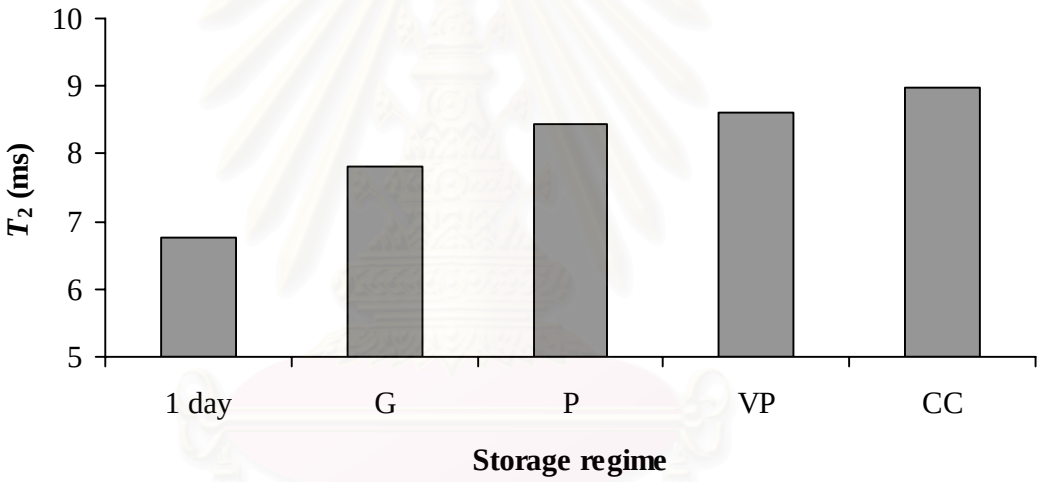


Fig. 5.35 T_2 values of dough after 1 day frozen storage and stored under different fluctuating storage regimes after 112 days frozen storage for TF2.

Fig. 5.36 shows examples of NMR spectrum of the dough stored under the G and CC regimes. A peak separation was observed in the dough stored under the CC regime as shown in Fig. 5.36b. This was attributed to the separation in components in dough system due to temperature changes and fluctuation during frozen storage. Aibara, Ogawa and Hirose (2005) indicated that water in the continuous protein phase of the frozen dough migrated to the damaged starch granules in the disperse phase from the gluten matrix during freeze-thaw treatment.

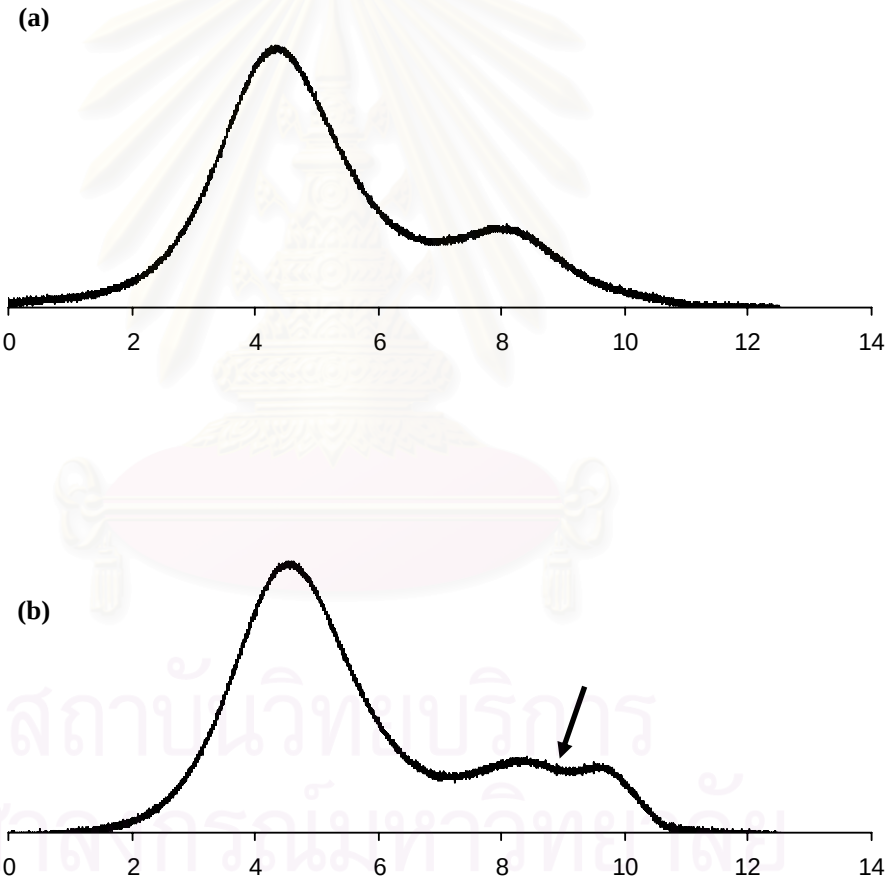


Fig. 5.36 NMR spectrum of frozen dough after 112 days storage. (a) G regime (b) CC regime. Arrow shows a peak indicating phase separation in dough components.

5.4.8 BREAD SPECIFIC VOLUME

Table 5.30 shows effects of freezing rates and fluctuating storage regimes on bread specific volume for TF1. Table 5.31 gives equivalent results for TF2. Freezing rate had a significant effect ($p<0.05$) on bread specific volume. After freezing, specific volume of the dough with SF and FF declined by 2% and 12% respectively for TF1 and for TF2 declined by 9% relative to bread baked from fresh dough. Specific loaf volume was greatest for the unfrozen bread dough and decreased as duration of frozen dough storage increased. The specific loaf volume decreased significantly ($p<0.05$) as the temperature fluctuations during storage increased in magnitude. The dough stored under the C, G and P regimes were not significantly different from each other ($p>0.05$) but were significantly better ($p<0.05$) than dough stored under the VP and CC regimes. These results agree with those of Inoue and Bushuk (1992), Ribotta et al. (2001) and Le Bail et al. (1999). The reduction in loaf volume was probably due to ice recrystallization causing both losses in yeast activity and reduced ability of the dough gluten network to retain CO_2 during proofing.

Table 5.30 Effects of freezing rates and fluctuating frozen storage regimes on bread specific volume for TF1

Regime	Storage period (day)	Bread specific volume (ml/g)	
		SF	FF
C	fresh	2.49±0.14 a	2.49±0.14 a
	1	2.44±0.11 ab	2.19±0.07 c-h
	21	2.41±0.15 a-c	2.08±0.11 f-j
	42	2.41±0.16 a-c	2.03±0.07 f-k
	63	2.37±0.11 a-d	2.09±0.05 e-j
	84	2.26±0.18 b-f	2.00±0.18 g-k
G	fresh	2.49±0.14 a	2.49±0.14 a
	1	2.44±0.11 a-b	2.19±0.07 c-h
	21	2.20±0.17 c-h	1.95±0.06 i-m
	42	2.14±0.15 d-i	1.90±0.14 j-m
	63	2.21±0.07 c-g	1.99±0.02 g-l
	84	2.19±0.16 c-h	2.01±0.08 g-k
P	fresh	2.49±0.14 a	2.49±0.14 a
	1	2.44±0.11 ab	2.19±0.07 c-h
	21	2.16±0.19 d-i	1.88±0.06 j-m
	42	2.17±0.09 d-i	1.90±0.07 j-m
	63	2.26±0.08 a-f	1.95±0.08 i-m
	84	2.20±0.14 c-h	1.83±0.11 k-n
CC	fresh	2.49±0.14 a	2.49±0.14 a
	1	2.44±0.11 ab	2.19±0.07 c-h
	21	2.31±0.09 a-e	1.97±0.08 h-l
	42	2.08±0.22 e-j	1.77±0.04 l-n
	63	1.95±0.07 i-m	1.73±0.04 mn
	84	2.00±0.13 g-k	1.64±0.04 n

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Table 5.31 Effects of temperature fluctuations during frozen storage on bread specific volume of frozen dough for TF2.

Storage Period	**Regime**					
(days)	**C**	**G**	**P**	**VP**	**CC**	**Average**
Fresh	2.78±0.17 a	2.74±0.17 ab	2.74±0.17 ab	2.74±0.17 ab	2.74±0.17 ab	**2.75±0.15**
1	2.51±0.04 c	2.54±0.10 bc	2.54±0.10 bc	2.54±0.10 bc	2.54±0.09 bc	**2.53±0.08**
14	2.27±0.06 d-g	2.51±0.10 c	2.38±0.16 cd	2.24±0.05 d-h	2.36±0.18 c-e	**2.35±0.14**
28	2.39±0.17 cd	2.21±0.01 d-i	2.20±0.12 d-i	1.94±0.05 j-m	2.09±0.18 g-l	**2.17±0.18**
49	2.30±0.09 d-f	2.17±0.06 e-j	2.15±0.07 f-k	1.94±0.03 lm	1.89±0.03 lm	**2.09±0.17**
70	1.98±0.07 j-l	2.03±0.02 i-l	2.03±0.07 i-l	1.94±0.05 k-m	1.75±0.11 mn	**1.95±0.12**
91	1.97±0.04 j-l	2.06±0.06 h-l	1.99±0.19 j-l	1.96±0.02 k-m	1.75±0.17 mn	**1.95±0.15**
112	1.93±0.03 lm	2.00±0.04 j-l	1.94±0.12 lm	1.89±0.03 lm	1.68±0.09 n	**1.89±0.13**
Average	**2.27±0.30**	**2.28±0.27**	**2.25±0.29**	**2.15±0.32**	**2.10±0.40**	**2.21±0.32**

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

5.4.9 BREAD CRUMB FIRMNESS

Freezing rate had a significant effect ($p<0.05$) on crumb firmness for TF1 as shown in Table 5.32. Table 5.33 shows equivalent results for TF2. Crumb firmness of bread with SF and FF increased by 16% and 34% respectively for TF1. Crumb firmness increased with an increase in storage duration. Crumb firmness of bread made from the dough stored under the C and G regimes was not significantly different ($p>0.05$) but had a significantly lower firmness than those stored under the VP and CC regimes. Increase in crumb firmness with storage was more pronounced when the temperature fluctuations during storage increased in magnitude. This effect may be due to the increased loss of moisture content during frozen storage. He and Hoseney (1990) found that moisture content significantly affected bread firming. Wang et al. (2006) reported similar results. The increase in firmness was also probably related to the decrease in bread volume due to the weakened gluten strength and reduced yeast activity as storage time increased (Berglund et al., 1991; Inoue and Bushuk, 1992).

Table 5.32 Effects of freezing rates and fluctuating frozen storage regimes on bread crumb firmness for TF1

Regime	Storage period (day)	Bread crumb firmness (N)	
		SF	FF
C	fresh	4.2±0.2 n	4.2±0.2 n
	1	5.0±2.2 l-n	6.4±0.4 h-n
	21	6.1±1.7 j-n	8.6±1.4 d-k
	42	4.2±0.8 n	7.9±1.7 f-m
	63	4.7±1.5 mn	7.7±1.0 f-n
	84	5.6±1.6 k-n	9.6±1.1 d-j
G	fresh	4.2±0.2 n	4.2±0.2 n
	1	5.0±2.2 l-n	6.4±0.4 h-n
	21	8.0±3.4 e-m	11.8±2.3 b-d
	42	7.4±2.0 g-n	9.8±2.3 c-h
	63	5.5±1.6 k-n	7.6±1.9 g-n
	84	6.3±2.3 i-n	9.8±1.8 c-i
P	fresh	4.2±0.2 n	4.2±0.2 n
	1	5.0±2.2 l-n	6.4±0.4 h-n
	21	8.8±2.8 d-k	11.4±2.4 c-e
	42	8.0±1.6 e-m	11.1±1.1 c-f
	63	4.8±1.7 mn	8.1±1.6 e-m
	84	8.5±3.1 d-l	10.6±0.9 c-g
CC	fresh	4.2±0.2 n	4.2±0.2 n
	1	5.0±2.2 l-n	6.4±0.4 h-n
	21	6.9±2.3 h-n	10.7±1.2 c-g
	42	9.6±4.5 d-j	14.6±1.1 b
	63	9.5±2.1 d-j	13.0±0.7 bc
	84	8.7±2.7 d-k	17.8±0.4 a

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

Table 5.33 Effects of temperature fluctuations during frozen storage on bread crumb firmness of frozen dough for TF2.

Storage Period	**Regime**					
(days)	**C**	**G**	**P**	**VP**	**CC**	**Average**
Fresh	4.0±0.6 op	4.8±0.7 l-p	4.8±0.7 l-p	4.8±0.7 l-p	4.8±0.7 l-p	**4.6±0.6**
1	3.8±0.3 p	4.6±0.3 m-p	4.6±0.3 m-p	4.6±0.3 m-p	4.6±0.3 m-p	**4.4±0.4**
14	5.5±0.6 j-o	4.3±0.5 n-p	4.2±0.7 n-p	6.1±0.1 i-m	4.3±0.6 n-p	**4.9±0.9**
28	4.9±0.6 l-p	5.1±0.5 k-p	6.3±0.4 h-l	8.7±0.3 b-e	7.4±0.7 d-i	**6.5±1.6**
49	7.3±1.2 e-i	5.8±1.2 i-n	5.8±0.8 i-n	8.4±1.5 b-f	7.3±0.5 e-i	**6.9±1.4**
70	7.2±0.3 e-i	5.3±0.5 j-p	6.2±0.1 i-m	6.8±0.6 f-j	8.7±0.8 b-e	**6.8±1.2**
91	6.2±0.1 i-m	6.7±1.2 g-k	9.0±0.2 b-d	10.0±1.6 ab	10.0±0.5 ab	**8.4±1.8**
112	8.1±0.4 c-g	7.9±0.8 d-h	9.6±2.1 bc	8.5±1.2 b-e	11.2±2.1 a	**9.1±1.8**
Average	**5.9±1.6**	**5.6±1.3**	**6.3±2.1**	**7.3±2.1**	**7.3±2.6**	**6.5±2.1**

Values are the mean and standard deviation of 3 samples. Mean values with different letters are significantly different ($p<0.05$).

5.4.10 BREAD IMAGE CHARACTERISTICS

Crumb structure appearance of fresh bread and bread from frozen dough 1 day after freezing with slow (SF) and fast freezing (FF) is shown in Fig. 5.37. Bread crumb appearances for fresh and 1 day after freezing with SF were similar. Bread crumb for 1 day after freezing with FF showed a flatter loaf. Fig. 5.38 shows bread crumb structure after 84 days frozen storage under various storage conditions. The bread crumb structure after 84 days frozen storage under different storage conditions did not show a different appearance. However, crumb structure for the P and CC regimes showed some non-continuous surface and big holes on the structure. The loaves for the P and CC regimes were flatter than those for the C and G regimes. This is probably due to temperature fluctuations during frozen storage resulting in loss of yeast activity and ice recrystallization. Bruinsma and Giesenschlag (1984) found that each successive freeze-thaw cycle caused the dough to become weak, fragile, difficult to handle and to have a moist appearance. The crumb grains became much less acceptable because of a harsh and coarser structure. Inoue and Bushuk (1991) also found that the bread with successive freeze-thaw cycles were flat on the top of loaves. In addition, prominent blisters appeared on the crust surface, and the crumb structure became more open.

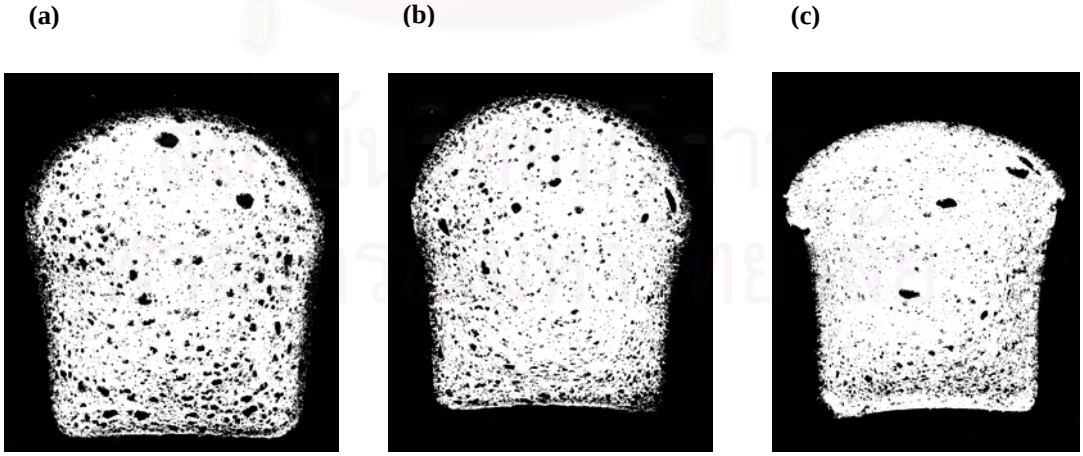


Fig. 5.37 Effect of freezing rates on bread crumb structure for TF1. (a) bread made from fresh dough (b) bread made from dough frozen with SF (c) bread made from dough frozen with FF.

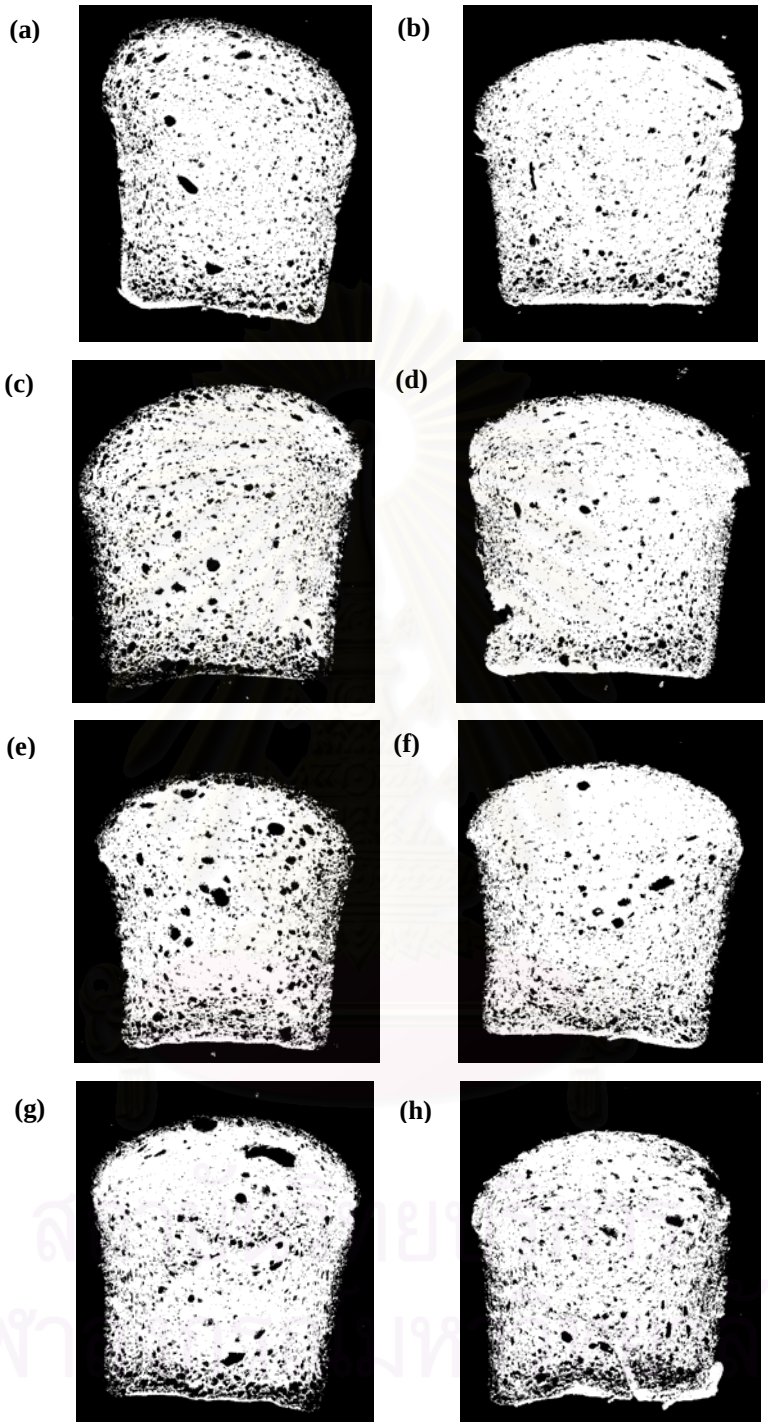


Fig. 5.38 Bread images baked after 84 days frozen storage under different fluctuating storage regimes for TF1. Left column indicates bread made from dough with SF. Right column indicates bread made from dough with FF. (a-b) C regime (c-d) G regime (e-f) P regime (g-h) CC regime.

5.5 NON-ISOTHERMAL KINETIC MODELS

Two approaches were used to predict quality loss for non-isothermal storage - predictions of rate based on the average temperature (isothermal) and an average rate based on the integrated rate over the measured temperature-time history (integrated). If the modeling approach is appropriate then the relationship between predicted and measured data will be linear with a high correlation coefficient and will have a slope close to 1.0. If the relationship is linear but the slope differs from 1.0 then it probably indicates that the physical mechanism inherent in the model is relevant but that other effects are also important. A slope greater than 1.0 indicates that the actual rate of the process is faster than that predicted by the modeling approach.

5.5.1 WEIGHT LOSS

Dough weight loss during frozen storage under various fluctuating temperature regimes was modeled using both the Arrhenius and physical models.

For the Arrhenius model, Fig. 5.39 shows the predicted values versus experimental values of dough weight loss under constant temperature (C regime) and various fluctuating storage regimes (G, P, VP and CC regimes). Table 5.34 gives the linear regression slope between the measured and predicted rates of weight loss. The relationships were linear with R^2 greater than 0.89 in all cases. The prediction with the isothermal rate and integrated rate were not significantly different ($p>0.05$). Slopes for the C and G regimes were not significantly different ($p>0.05$) from 1.0. However, slopes for the P, VP and CC regimes were significantly greater ($p<0.05$) than 1.0. This indicated that effect of fluctuations in temperature on weight loss is greater than that predicted by integrating the steady-state kinetic model alone. It suggests that fluctuations enhance the mechanism of weight loss in some manner not accounted for by temperature effect on vapor pressure alone (e.g. temperature gradients with product or between product and packaging). The difference between CC and P (both fluctuations of $\pm 1.0^{o}$C) showed that CC regime effect is greater than that due to fluctuations about each set-point alone (the transition between temperature set-points also contributes to enhanced weight loss).

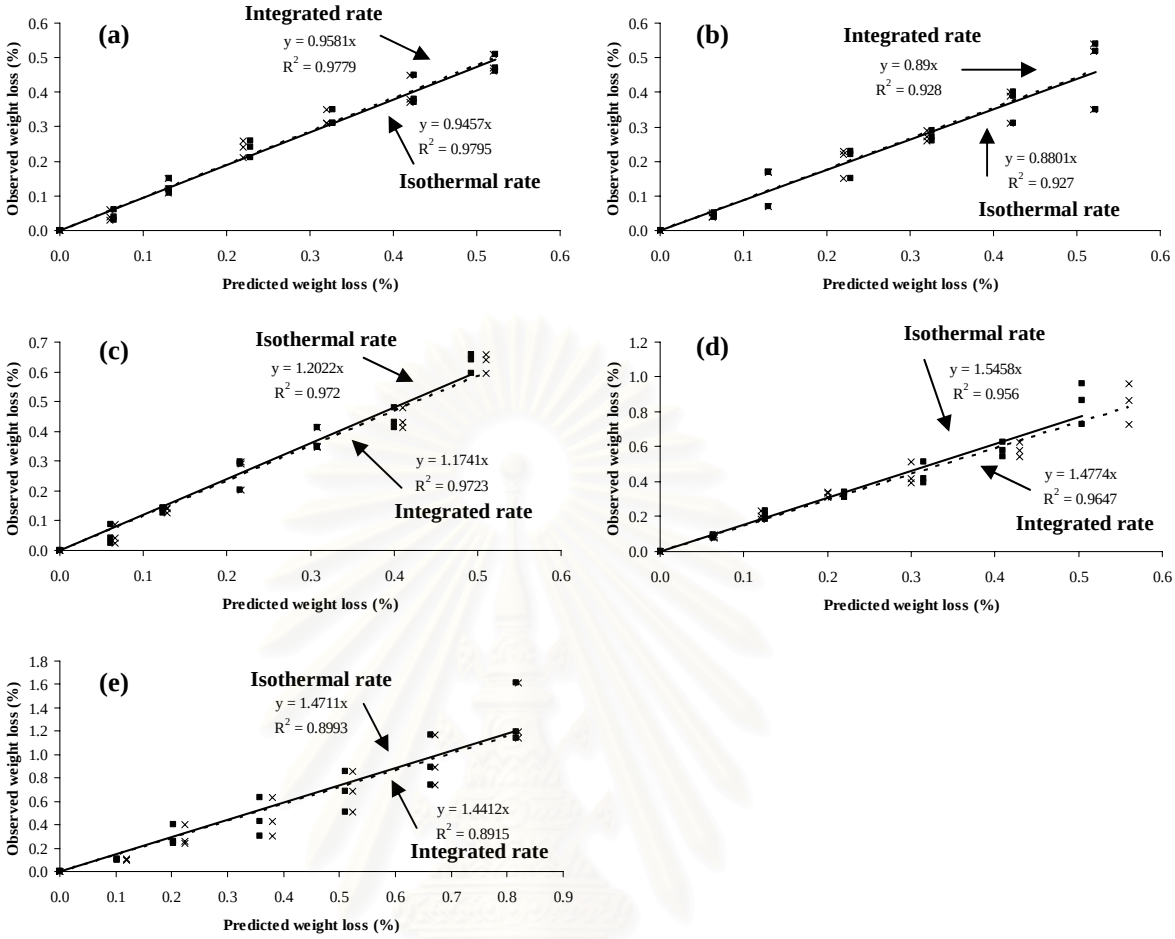


Fig. 5.39 The predictions using the Arrhenius dough weight loss model versus experimental values of dough weight loss under various fluctuating storage regimes. (a) C regime (b) G regime (c) P regime (d) VP regime (e) CC regime. — indicates fitted line for isothermal rate. --- indicates fitted line for integrated rate.

Table 5.34 Slopes of measured dough weight loss compared to predicted dough weight loss using the Arrhenius model under various fluctuating storage temperatures

Regime	Isothermal rate		Integrated rate	
	Slope ± S.E.	R^2	Slope ± S.E.	R^2
C ^{ns} (-18±0.1^{o}C)	0.946 ± 0.018	0.98	0.958 ± 0.019	0.98
G ^{ns} (-18±1^{o}C)	0.880 ± 0.033	0.93	0.890 ± 0.033	0.93
P ^{ns} (-18±3^{o}C)	1.203 ± 0.028	0.97	1.171 ± 0.028	0.97
VP ^{ns} (-18±5^{o}C)	1.543 ± 0.044	0.96	1.474 ± 0.038	0.96
CC ^{ns} (-18±1^{o}C, -13±1^{o}C, -8±1^{o}C)	1.470 ± 0.068	0.89	1.444 ± 0.069	0.89

ns indicates values in the same row are not significantly different ($p<0.05$).
R^2 indicates correlation coefficient

For the physical model, the predictions versus experimental values of dough weight loss under various fluctuating storage regimes are shown in Fig. 5.40. Table 5.35 gives the linear regression slopes between the measured and predicted rates of weight loss. The results were similar to those for the Arrhenius model. The linear relationship between measured and predicted rates of weight loss based on water vapor pressure showed that the proposed mechanism (physical model) has at least partial validity. Temperatures fluctuations could increase weight loss by other mechanisms (e.g. irreversible enhanced sublimation when packaging and dough differ in temperature).

Fig. 5.41 gives the slopes (relative rate of dough weight loss) as a function of temperature fluctuation magnitude. The relative rates increased with increasing magnitude of temperature fluctuations. This indicates that the effect of temperature fluctuations over and above the steady-state temperature effects is systematic and could probably be predicted by a more advanced physical model including dough and packaging temperature, dough water activity and air relative humidity changes with time.

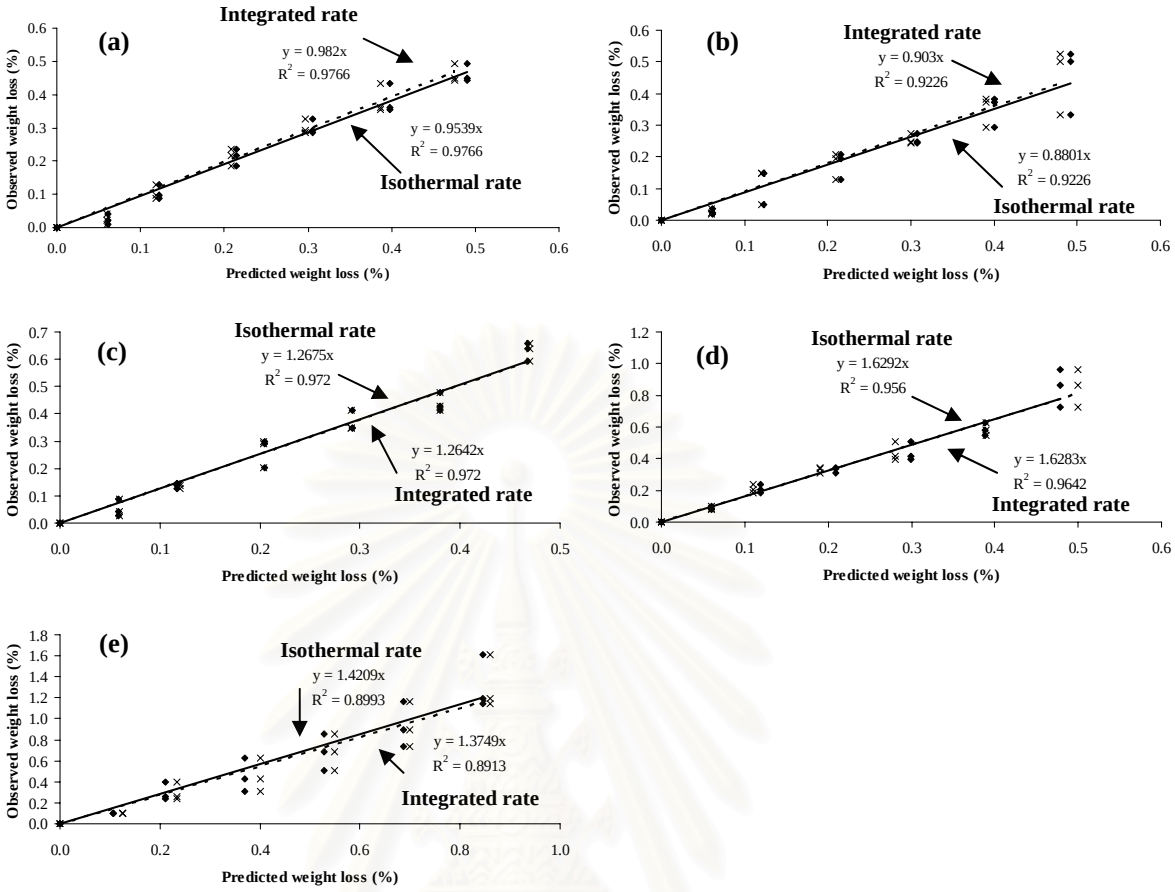


Fig. 5.40 The predictions using the physical dough weight loss model versus experimental values of dough weight loss under various fluctuating storage regimes. (a) C regime (b) G regime (c) P regime (d) VP regime (e) CC regime. — indicates fitted line for isothermal rate. --- indicates fitted line for integrated rate.

Table 5.35 Slopes of measured dough weight loss compared to predicted dough weight loss using the physical model under various fluctuating storage temperatures

Regime	Isothermal rate		Integrated rate	
	Slope ± S.E.	R^2	Slope ± S.E.	R^2
C ^{ns} (-18±0.1°C)	0.951 ± 0.020	0.98	0.972 ± 0.021	0.98
G ^{ns} (-18±1°C)	0.876 ± 0.036	0.92	0.899 ± 0.037	0.92
P ^{ns} (-18±3°C)	1.268 ± 0.030	0.97	1.268 ± 0.030	0.97
VP ^{ns} (-18±5°C)	1.620 ± 0.046	0.96	1.627 ± 0.042	0.96
CC ^{ns} (-18±1°C, -13±1°C, -8±1°C)	1.421 ± 0.066	0.89	1.375 ± 0.066	0.89

ns indicates values in the same row are not significantly different ($p<0.05$).
R^2 indicates correlation coefficient

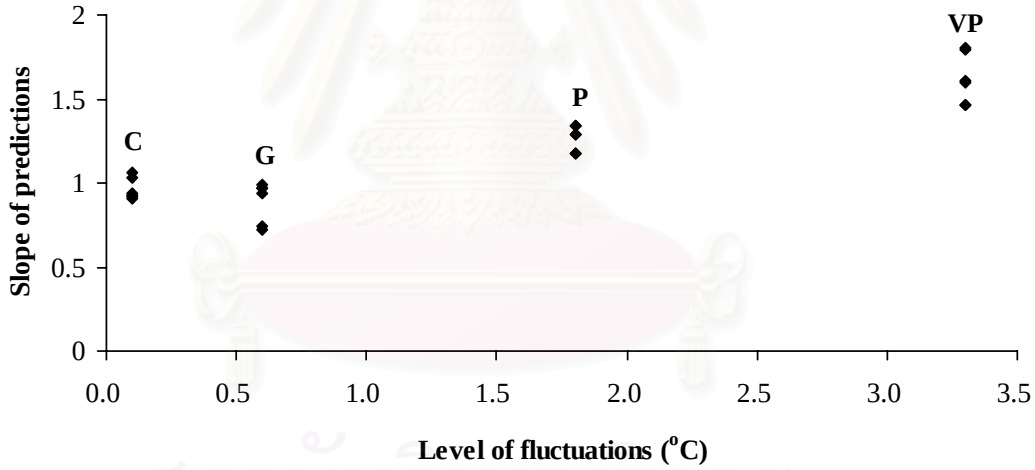


Fig. 5.41 Plot of slopes of the measured dough weight loss relative to isothermal predictions versus fluctuations magnitude under the C, G, P and VP regimes.

5.5.2 CO_2 PRODUCTION LOSS

The CO_2 production loss under fluctuating temperature during frozen storage was predicted based on the Arrhenius reaction rate equation at constant temperature over the temperature range from -25^{o}C to -8^{o}C (Section 5.2.2).

Fig. 5.42 shows predicted and measured CO_2 production loss constant and various fluctuating temperature storage regimes. Table 5.36 gives the slopes and standard error for CO_2 production loss predictions relative to the measured values. As for weight loss, the predictions using the isothermal approach were not significantly difference to those using the integrated approach. The correlations coefficients were low and the standard errors in the slope were high for all sets of predictions. This probably reflects the greater measurement uncertainty for CO_2 production than for weight loss, but may also indicate that fluctuations do not have a significant effect on CO_2 production.

Fig. 5.43 gives the slopes (relative rate of CO_2 production loss) as a function of temperature fluctuation magnitude. The relative rates increased with increasing magnitude of temperature fluctuations. For the C, G and P regimes, slopes were not significantly different from 1.0 but those for the VP and CC regimes were significantly different from 1.0. Overall, while the trend is less obvious that for weight loss, it appears that temperature fluctuations during storage do result in faster decline in CO_2 production than would be expected due to the temperature variation alone if the magnitude of the fluctuations is sufficiently large.

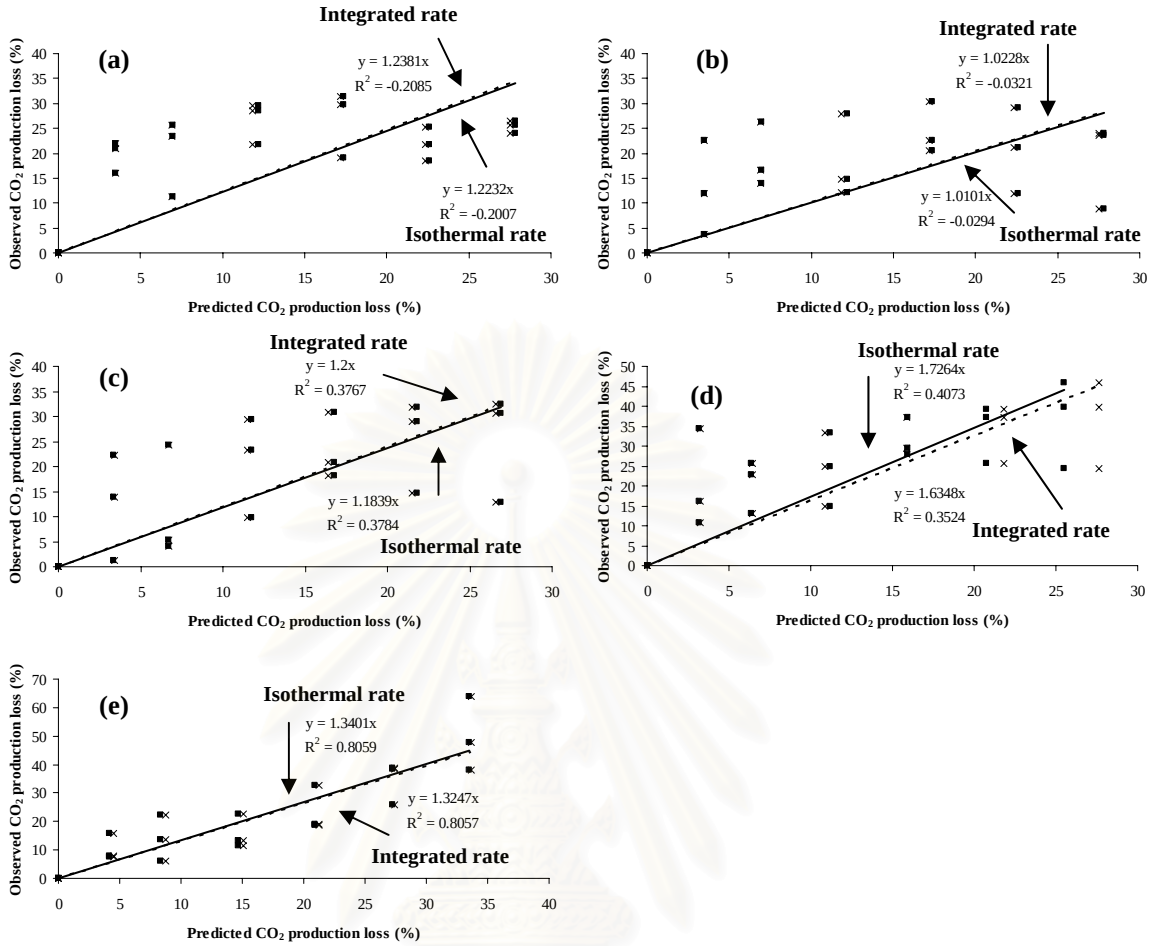


Fig. 5.42 The predictions using the Arrhenius model versus experimental values of CO_2 production loss in dough during frozen storage under various fluctuating temperature regimes. (a) C regime (b) G regime (c) P regime (d) VP regime (e) CC regime. — indicates fitted line for isothermal rate. --- indicates fitted line for integrated rate.

Table 5.36 Slopes of measured CO_2 production loss compared to predicted CO_2 production loss using the Arrhenius model under various fluctuating storage temperatures

Regime	Isothermal rate		Integrated rate	
	Slope ± S.E.	R^2	Slope ± S.E.	R^2
C ^{ns} (-18±0.1°C)	1.223 ± 0.144	-0.20	1.238 ± 0.146	-0.21
G ^{ns} (-18±1°C)	1.010 ± 0.135	-0.03	1.023 ± 0.137	-0.03
P ^{ns} (-18±3°C)	1.184 ± 0.131	0.38	1.200 ± 0.133	0.38
VP ^{ns} (-18±5°C)	1.727 ± 0.156	0.41	1.635 ± 0.155	0.35
CC ^{ns} (-18±1°C, -13±1°C, -8±1°C)	1.340 ± 0.085	0.81	1.375 ± 0.084	0.81

ns indicates values in the same row are not significantly different ($p<0.05$).
R^2 indicates correlation coefficient

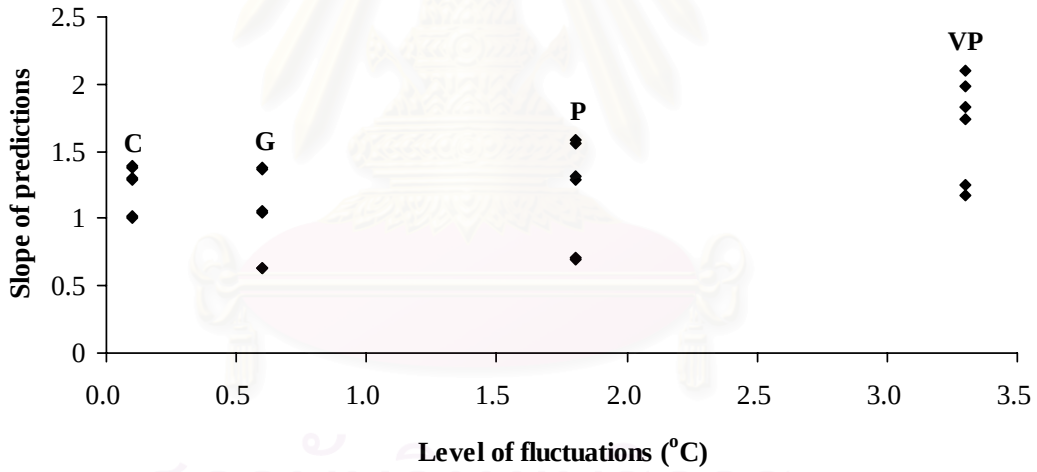


Fig. 5.43 Plot of slopes of the measured CO_2 production loss relative to isothermal predictions versus fluctuations magnitude under the C, G, P and VP regimes.

5.5.3 BREAD SPECIFIC VOLUME LOSS

The bread specific volume loss under fluctuating temperature during frozen storage was predicted based on the Arrhenius reaction rate equation at constant temperature over the temperature range from -25°C to -8°C (Section 5.2.3).

According to the non-isothermal model, bread specific volume loss showed similar trends as the CO_2 production loss. Slopes for the C and G regimes were not significantly different from 1.0 but those for the P, VP and CC regimes were significantly different from 1.0 as shown in Fig. 5.44 and Table 5.37. Fig. 5.45 gives the slopes (relative rate of bread specific volume loss) as a function of temperature fluctuation magnitude. The relative rates increased with increasing magnitude of temperature fluctuations. Overall, it appears that temperature fluctuations during storage do result in faster decline in bread specific volume than would be expected due to the temperature variation alone if the magnitude of the fluctuations is sufficiently large.

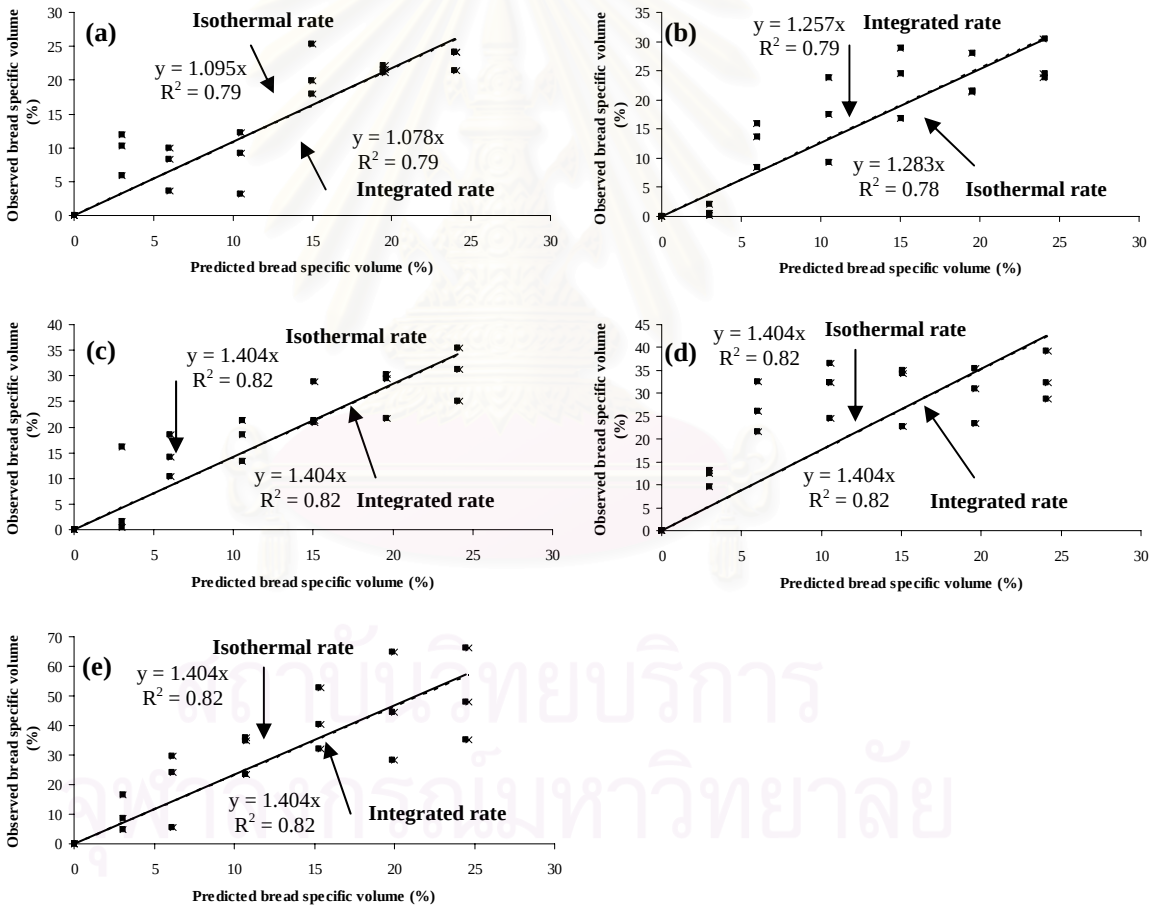


Fig. 5.44 The predictions using the Arrhenius model versus experimental values of bread specific volume made from frozen dough during frozen storage under various fluctuating temperature regimes. (a) C regime (b) G regime (c) P regime (d) VP regime (e) CC regime. — indicates fitted line for isothermal rate. --- indicates fitted line for integrated rate.

Table 5.37 Slopes of measured bread specific volume loss compared to predicted bread specific volume loss using the Arrhenius model under various fluctuating storage temperatures

Regime	Isothermal rate		Integrated rate	
	Slope ± S.E.	R^2	Slope ± S.E.	R^2
C ^{ns} (-18±0.1^{o}C)	1.095 ± 0.067	0.79	1.078 ± 0.066	0.79
G ^{ns} (-18±1^{o}C)	1.257 ± 0.079	0.79	1.283 ± 0.082	0.78
P ^{ns} (-18±3^{o}C)	1.404 ± 0.080	0.82	1.404 ± 0.080	0.82
VP ^{ns} (-18±5^{o}C)	1.749 ± 0.160	0.57	1.749 ± 0.160	0.57
CC ^{ns} (-18±1^{o}C, -13±1^{o}C, -8±1^{o}C)	2.364 ± 0.170	0.73	2.317 ± 0.170	0.72

ns indicates values in the same row are not significantly different ($p<0.05$).
R^2 indicates correlation coefficient

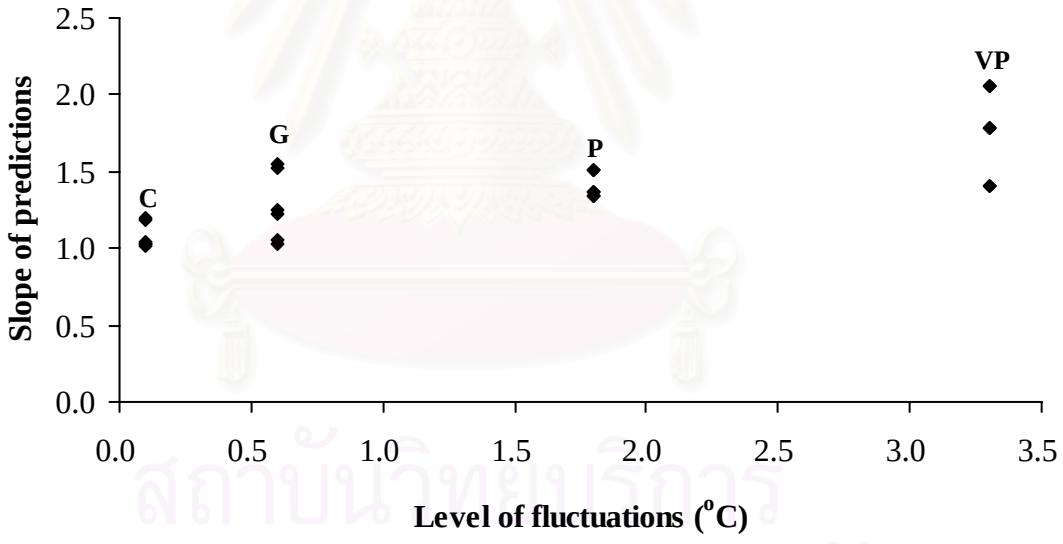


Fig. 5.45 Plot of slopes of the measured bread specific volume relative to isothermal predictions versus fluctuations magnitude under the C, G, P and VP regimes.

5.6 APPLICATIONS OF ARTIFICIAL NEURAL NETWORK (ANN) FOR NON-ISOTHERMAL PREDICTION

Artificial neural network (ANN) is an alternative method for predicting a non linear quality response to different freezing and storage regimes. The application of ANN was tested for predicting weight loss, CO_2 production loss and bread specific volume loss during frozen storage under fluctuating conditions.

Comparisons were made between the ANN output (predicted values) and the corresponding targets (experimental values). Fig. 5.46 presents the ANN predictions versus actual weight loss of frozen dough. The optimum ANN architecture for weight loss prediction comprised six neurons in an input layer, six neurons in a hidden layers and one neuron in an output layer. The mean sum of squares of the network errors (MSE) for weight loss prediction was about 0.0013. The high determination coefficient (R^2>0.98) and the slope of close to 1.0 indicates a good fit between experimental and predicted data for weight loss prediction.

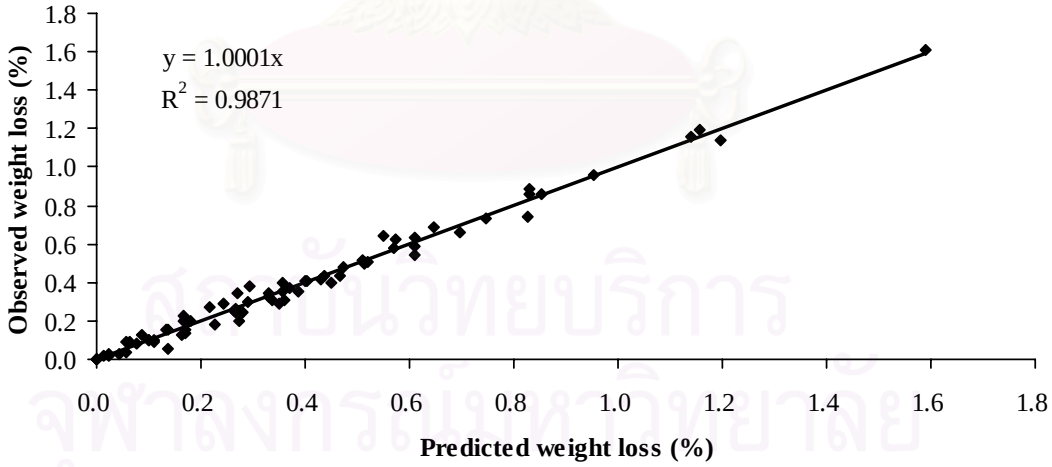


Fig. 5.46 The predictions using the ANN model versus experimental values of the dough weight loss during frozen storage under various fluctuating temperature regimes.

The ANN algorithms for predicting CO_2 production loss were also tested. Fig. 5.47 presents the ANN predictions versus actual CO_2 production loss of frozen dough. The optimum ANN architecture for CO_2 production loss prediction comprised nine neurons in an input layer, six neurons in a hidden layer and one neuron in an output layer. However, the prediction had low determination coefficient (R^2<0.5) with about 88.33 for MSE. The result is similar to the non-isothermal model for CO_2 production loss predictions. It is suggested that the CO_2 production loss of frozen dough during frozen storage is not dependent on temperature variations alone. The mechanism of CO_2 production loss is complex and the effect of temperature fluctuations is not simply a steady-state temperature effect.

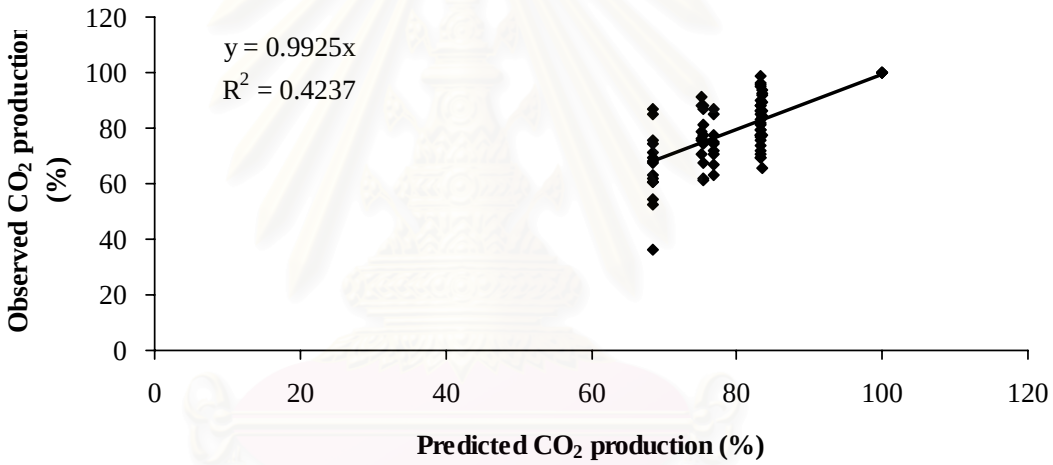


Fig. 5.47 The predictions using the ANN model versus experimental values of the CO_2 production loss during frozen storage under various fluctuating temperature regimes.

The ANN algorithms for predicting bread specific volume loss were also tested. Fig. 5.48 presents the ANN predictions versus actual bread specific volume loss of frozen dough. The optimum ANN architecture for bread specific volume loss prediction comprised nine neurons in an input layer, fifteen neurons in a hidden layer and one neuron in an output layer. The prediction had regression coefficient (R^2 = 0.76) with low MSE (0.0051) and the slopes of close to 1.0. The predictions gave slightly better than the non-isothermal model for bread specific volume loss. It is suggested that ANN predictions for bread specific volume provide fast speed and can

estimate the reaction rate without using the kinetic model. Bas, Dudak and Boyaci (2007) reported a good estimation of enzymatic reaction rate using ANN. The regression coefficient showed a good correlation between estimated and experimental data sets (R^2>0.96). However, the reaction kinetics and prediction of kinetic constants is required for understanding the mechanism of the quality loss.

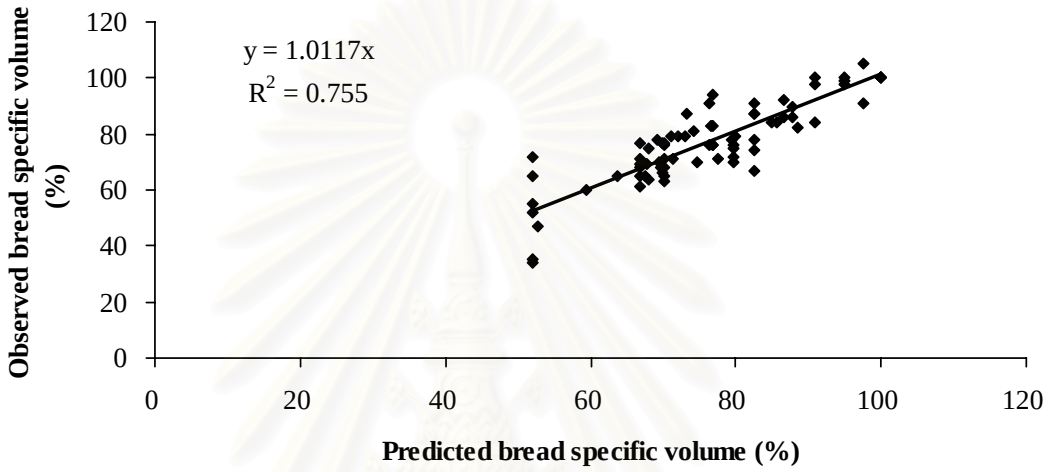


Fig. 5.48 The predictions using the ANN model versus experimental values of the bread specific volume loss during frozen storage under various fluctuating temperature regimes.

5.7 COMPARISON OF THE MODEL PREDICTION UNDER NON-ISOTHERMAL CONDITIONS

The predictions of weight loss showed that the Arrhenius model and weight loss physical model gave an adequate explanation for weight loss prediction under constant or good control temperature (±0.1°C or ±1°C). Slopes were not significantly different ($p<0.05$) from 1.0. However, both Arrhenius and weight loss physical models did not give a good fit if the frozen dough stored under large temperature fluctuations (±3°C or ±5°C) and also under higher temperature changes. Low R^2 and the significance from 1.0 for slopes were found for the P, VP and CC regimes. ANN model gave a good fit (R^2>0.98) and the slope of close to 1.0 for predicting weight loss of frozen dough under fluctuating temperature. This suggests that ANN model provided an alternative tool for fast and accuracy prediction for weight loss.

For CO_2 production prediction, the Arrhenius model can be applied for frozen dough stored under fluctuating conditions between $\pm 0.1^oC$ to $\pm 3^oC$. The low fitting results also found in large fluctuating storage temperature and higher temperature predictions (VP and CC regimes). All slopes for the VP and CC regimes were significantly different from 1.0. ANN model did not fit well (R^2<0.5) for predicting CO_2 production loss.

For bread specific volume prediction, the Arrhenius model can be applied for frozen dough stored under constant ($\pm 0.1^oC$) and good practice temperature control ($\pm 1^oC$). The precision of fits for large fluctuations in storage temperature and higher temperature predictions (P, VP and CC regimes) were poor. All slopes for the P, VP and CC regimes were significantly different from 1.0. However, ANN model looks promising for predicting bread specific volume loss with R^2>0.7.

The finding in this study suggests that multilayer perceptron provided a tool that can be used to determine the weight loss of frozen dough but did not success for CO_2 production loss prediction. For bread specific volume, the ANN model seems to be promising. Moreover, ANN could offer several advantages over actual quality determination, including faster speed of information processing, learning ability, fault tolerance, and multi-output ability for some systems. Bas et al. (2007) indicated the advantages of ANN were that it estimates reaction rate without requiring any kinetic model equation. Estimation of reaction rate without using a kinetic model eliminates the errors arising from the selection of kinetic model and the estimation of kinetic constants. ANN could be useful in a part of the complex calculation using an improved program. However, there are limited to apply ANN for some systems. Complex and large data may lead to lower speed data processing. The ANN model needs training data over the full range of conditions of interest.

5.8 SUMMARY FOR TF

The results from TF1 suggested that freezing rate has a major effect on quality of frozen dough and bread. Slow freezing rate is preferred for maintaining the frozen dough quality, resulting in a better bread quality. This is consistent with the results in the QK experiment.

The quality of frozen dough decreased with increasing frozen storage time. The degradation in quality of frozen dough and bread was accelerated by temperature fluctuations during frozen storage. Larger fluctuations in temperature resulted in increased dough weight loss and increased bread crumb firmness, lower CO_2 production, damaged microstructure and lower bread specific volume. As for the QK experiments, the rheological properties of frozen dough did not significantly change during frozen storage. It is recommended that temperature variations should be kept less than $\pm 3^{o}C$ (Phimolsiripol et al., 2008).

Weight loss predictions based on integrating the isothermal models over the actual temperature-time history gave a linear fit relative to the measured rates for fluctuating temperature regimes but under-predicted the measured rates. The enhancement of weight loss under temperature fluctuations was roughly proportional to the magnitude of the fluctuations. The weight loss is not explained by steady-state temperature models alone.

The Arrhenius model could be applied for predicting CO_2 production loss of frozen dough stored under constant and good practice temperature control. The under-predicted results were found in large fluctuating storage temperature and higher temperature predictions (P, VP and CC regimes). Unsuccessful predictions for CO_2 production loss indicated that large temperature fluctuations during storage gave a faster decline in CO_2 production than would be expected due to the temperature variation alone.

Artificial neural network (ANN) gave accurate predictions of weight loss under various fluctuating storage temperature regimes. The optimum ANN architecture for weight loss included six neurons in an input layer, six neurons in a hidden layer and one neuron in an output layer. For the prediction of CO_2 production loss, the ANN model performed poorly but seems to be good for bread specific volume prediction.

CHAPTER VI

CONCLUSIONS

Shelf-life of frozen dough products is limited by a decline in gassing power, leading to unacceptably long proofing time and resulting in decreased final product quality including lower bread volume and increased bread firmness. It has been postulated that storage temperature is a significant factor affecting the quality of frozen dough. Temperature fluctuations during frozen storage are generally considered to result in more rapid loss of quality than storage at constant and uniform temperature. This work investigated the quality change of bread dough for both constant and fluctuating storage conditions.

Freezing processes, particularly freezing rate, have significant effects on frozen dough and bread quality. Slow freezing gave a better quality of frozen dough and bread than fast freezing. A large decline in dough quality was found after freezing. However, the rate of freezing did not affect the rate of dough weight loss during frozen storage.

Quality of frozen dough and bread decreased with increase in storage time, at a rate depending on storage temperature. Storage temperatures had a significant effect on weight loss, CO_2 production, yeast viability, dough microstructure, water mobility, bread specific volume and bread crumb firmness. Higher storage temperature led to increased freezable water content.

Dough rheological properties were highly variable during the isothermal frozen storage study. Different storage temperatures had no significant effect on dough rheological properties. Dough microstructure and bread image characteristics gave only subjective measures of quality. Dough weight loss and CO_2 production gave quantitative measures of frozen dough and bread quality deterioration. They were closely related to other quality parameters and thus were effective to be used as overall quality indicators for frozen dough and bread after freezing and frozen storage.

Lower frozen storage temperature generally gave a better quality of dough and bread. Storage between -23^{o}C and -18^{o}C retained acceptable dough and bread quality for up to 12-16 weeks under constant storage temperature regimes. However, the lowest storage temperature of -25^{o}C gave significantly poorer quality than at -23^{o}C.

Dough weight loss and CO_2 production were fitted by zero-order reaction kinetics and the Arrhenius law or the WLF model for the effect of temperature on reaction rate. Dough weight loss was accurately described by the Arrhenius model. However, neither model gave particularly good predictions for CO_2 production.

The rate of weight loss at constant temperature was proportional to water vapor pressure consistent with the standard theory for evaporative weight loss from packaged foods.

Temperature fluctuations had a significant effect on frozen dough quality in terms of increased dough weight loss, lower CO_2 production, negative changes in dough microstructure, lower bread specific volume and higher bread firmness. The mechanism of quality loss is probably ice crystals growth and recrystallization, resulting in damage to gluten network and separation of starch granules.

Predictions of weight loss and CO_2 production for frozen dough under fluctuating temperature storage based on the constant temperature rate kinetics significantly under-predicted the measured changes. These results suggest that temperature fluctuations have an enhancing effect over and above that explained by the steady-state rate models.

An artificial neural network was also used to predict weight loss of frozen dough during storage under fluctuating temperature conditions. The optimum ANN algorithm for weight loss prediction included six neurons in input layer, six neurons in hidden layers and one neuron in output layer. The ANN achieved a good fit between experimental and predicted data for weight loss prediction but does not help to understand the physical mechanisms. However, the ANN prediction did not give good predictions for CO_2 production loss. The ANN model seems to be promising for

predicting bread specific volume loss with R^2>0.7. The ANN model gave slightly better performance than the non-isothermal prediction for bread specific volume loss. ANN may offer several advantages over actual quality determination, including faster speed of information processing, learning ability, fault tolerance, and multi-output ability but requires training data covering the full range of conditions of interest.

Frozen storage with constant and small fluctuating temperature gave significantly better frozen dough and bread quality. From our study, it is recommended that storage temperature for frozen dough should be kept at -20^{o}C and temperature variations should be less than ±3^{o}C.

CHAPTER VII

RECOMMENDATIONS FOR FUTURE WORK

Slow freezing rate is recommended for frozen dough production. However, the optimum freezing rate for frozen dough was not obvious because experiments were only performed at two rates. Future work is required to optimize the temperature and duration of the cold treatment, the rate of freezing, to determine if other yeast strains give similar effects, and to consider prolonged storage durations.

There is some evidence that frozen doughs were less stable if their storage temperature was lower than the temperature used during freezing (Hsu et al., 1979b). Future frozen dough research should examine the impact of lower storage temperature on dough quality.

The moisture loss of the dough during frozen storage should be minimized because it affects other quality parameters. Improved packaging systems may enable lower weight loss of frozen dough.

The yeast used in this work was a New Zealand compressed yeast, which probably had different frozen dough performance to strains commonly used for frozen dough manufacture. Gassing power was more sensitive to pre-fermentation, freezing and frozen storage than has been reported for other yeasts. The results should be confirmed for specialized frozen-dough yeasts used for manufacture in Europe or the US.

The dough ingredients used in this work were based on a typical dough formula. However, frozen dough is a complex system and then the results should be confirmed for different dough formula and processes.

Measurement of eating properties and bread characteristics is very subjective because it relies on human perception. Analytical techniques such as C-cell image analyzer to measure internal structure of the crumb and bread shape as reported by Cabrera (2006) may provide a quick analytical measurement that is highly correlated with the texture analyzer measurements and other consumer preferences. Also, there are no specific reports about exactly how bread specific volume affects consumer acceptance. Research is required to define this relationship.

Food systems are complex and the relationships between chemical and physical food properties may not be linear. Artificial neural networks could be useful and applicable for such systems but further investigation for quality prediction is required. Also, the performance of other algorithms (e.g. fuzzy logic, genetic algorithms etc.) should be explored.

REFERENCES

AACC. 2000. Approved Methods of the AACC. 10th ed. St. Paul, MN: The American Association of Cereal Chemists.

Abdelrahman, A., and Spies, R. 1986. Dynamic rheological studies of dough systems. In H. Faridi, and J. M. Faubion (eds.), Fundamental of dough rheology, pp. 87-103. St. Paul, MN: AACC.

Aibara, S., Nishimura, K., and Esaki, K. 2001. Effects of shortening on the loaf volume of frozen dough bread. Food Science and Biotechnology 10: 521-528.

Aibara, S., Ogawa, N., and Hirose, M. 2005. Microstructures of bread dough and the effects of shortening on frozen dough. Bioscience Biotechnology and Biochemistry 69: 397-402.

Albela, L. P., and Reid, D. S. 2004. Evaluating the quality implications of power load management: The sensitivity of product quality factors to a fluctuating temperature environment. Alexandria, Virginia: World Food Logistics Organization.

Alvarez, M. D., and Canet, W. 1998. Effect of temperature fluctuations during storage on the quality of frozen potato tissue (cv. Monalisa). zeitschrift für Lebensmitteluntersuchung und -Forschung A 206: 52-57.

Aminlari, M., and Majzoobi, M. 2002. Effect of chemical modification, pH change, and freezing on the rheological, solubility, and electrophoretic pattern of wheat flour proteins. Journal of Food Science 67: 2502-2506.

Aparicio-Cuesta, M. P., Mateos-Notario, M. P., and Rivas-Gonzalo, J. C. 1992. Sensory evaluations and changes in peroxidase activity during storage of frozen green beans. Journal of Food Science 57: 1129-1131, 1143.

Arabshahi, A., and Lund, D. B. 1985. Considerations in calculating kinetic parameters from experimental data. Journal of Food Process Engineering 7: 239-251.

Ateba, P., and Mittal, G. S. 1994. Dynamics of crust formation and kinetics of quality changes during frying of meatballs. Journal of Food Science 59: 1275-1290.

Autio, K., Flander, L., Kinnunen, A., and Heinonen, R. 2001. Bread quality relationship with rheological measurements of wheat flour dough. Cereal Chemistry 78: 654-657.

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

Autio, K., and Mattila-Sandholm, T. 1992. Detection of active yeast cells (*Saccharomyces cerevisiae*) in frozen dough sections. Applied and Environmental Microbiology 58: 2153-2157.

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

Baguena, R., Soriano, M. D., Martinez-Anaya, M. A., and De Barber, C. B. 1991. Viability and performance of pure yeast strains in frozen wheat dough. Journal of Food Science 56: 1690-1694, 1698.

Baier-Schenk, A., Handchin, S., and Conde-Petit, B. 2005. Ice in prefermented frozen bread dough-An investigation based on calorimetry and microscopy. Cereal Chemistry 82: 251-255.

Bak, L. S., Andersen, A. B., Andersen, E. M., and Bertelsen, G. 1999. Effect of modified atmosphere packaging on oxidative changes in frozen stored cold water shrimp (*Pandalus borealis*). Food Chemistry 64: 169-175.

Barcenas, M. E., Haros, M., Benedito, C., and Rosell, C. M. 2003. Effect of freezing and frozen storage on the staling of part-baked bread. Food Research International 36: 863-869.

Barret, A. H., Verdello, A. V., Mair, L., Maguire, P., Lrsher, L., Richardson, M., Priggs, J., and Taub, I. A. 2000. Texture optimization of shelf-stable bread: Effect of glycerol content and dough-forming technique. Cereal Chemistry 77: 169-176.

Bas, D., Dudak, F. C., and Boyaci, I. H. 2007. Modeling and optimization III: Reaction rate estimation using artificial neural network (ANN) without a kinetic model. Journal of Food Engineering 79: 622-628.

Basaran, A., and Göçmen, D. 2003. The effects of low mixing temperature on dough rheology and bread properties. European Food Research and Technology 217: 138-142.

Beales, N. 2004. Adaptation of microorganisms to cold temperature, weak acid preservatives, low pH, and osmotic stress: A review. Comprehensive Reviews in Food Science and Food Safety 3: 1-20.

Bennion, M. 1990. Batters and doughs and yeast breads. In J. Alexander, and H. McInnis (eds.), Introductory foods, pp. 491, 523-541. New York: Macmillan.

Ben-Yoseph, E., and Hartel, R. W. 1998. Computer simulation of ice recrystallization in ice cream during storage. Journal of Food Engineering 38: 309-329.

Berglund, P. T., and Shelton, D. R. 1993. Effect of frozen storage duration on firming properties of breads baked from frozen doughs. Cereal Foods World 38: 89-90, 92-93.

Berglund, P. T., Shelton, D. R., and Freeman, T. P. 1990. Comparison of two sample preparation procedures for low-temperature scanning electron microscopy of frozen bread dough. Cereal Chemistry 67: 139-140.

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 Chemistry 68: 105-107.

Berry, E. D., and Foegeding, P. M. 1997. Cold temperature adaptation and growth of microorganisms. Journal of Food Protection 60: 1583-1594.

Best, D. 1995. Economic potential of frozen and refrigerated doughs and batters. In K. Kulp, K. Lorenz, and J. Brummer (eds.), Frozen and refrigerated doughs and batters, pp. 1-18. Saint Paul, MN: AACC.

Beuchat, L. R. 1987. Food and beverage mycology. New York: AVI.

Bevilacqua, A. E., and Zaritzky, N. E. 1982. Ice recrystallization in frozen beef. Journal of Food Science 47: 1410-1414.

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

Blanshard, J. M. V., and Franks, F. 1987. Ice crystallization and its control in frozen-food systems. In J. M. V. Blanchard, and P. Lillford (eds.), Food structure and behaviour, pp. 51-65. Orlando: Academic Press.

Bochereau, L., Bourgine, P., and Palagos, B. 1992. A method for prediction by combining data analysis and neural networks: Application to prediction of apple quality using near infra-red spectra. Journal of Agricultural Engineering Research 51: 207-216.

Boggs, M. M., Dietrich, W. C., Nutting, M. -D., Olson, R. L., Lindquist, F. E., Bohart, G. S., Neumann, H. J., and Morris, H. J. 1960. Time-temperature tolerance of frozen foods. XXI. Frozen peas. Food Technology 14: 181-185.

Bot, A. 2003. Differential scanning calorimetric study on the effects of frozen storage on gluten and dough. Cereal Chemistry 80: 366-370.

Bot, A., and de Bruijne, D. W. 2003. Osmotic properties of gluten. Cereal Chemistry 80: 404-408.

Brown, J. 1993. Advances in breadmaking technology. In B. S. Kamel, and C. E. Stauffer (eds.), Advances in baking technology, pp. 38-87. New York: VCH.

Brown, W. E. 1992. Plastics in food packaging, properties, design and fabrication. New York: Marcel Dekker.

Bruinsma, B. L., and Giesenschlag, J. 1984. Frozen dough performance: Compressed yeast-instant dry yeast. The Bakers Digest 58: 6-7, 11.

Bushuk, W. 1985. Flour proteins: Structure and functionality in dough and bread. Cereal Foods World 30: 447-451.

Bushuk, W., and Rasper, V. F. 1994. Wheat–Production, properties and quality. New York: Chapman & Hall.

Cabrera, R. 2006. Exploring the link between the cell structure and eating properties in sanwich bread using C-cell and texture analysis. Proceeding of the World Grains Summit: Foods and Beverages, pp. 54. San Francisco, USA.

Calligaris, S., Manzocco, L., Conte, L. S., and Nicoli, M. C. 2004. Application of a modified Arrhenius equation for the evaluation of oxidation rate of sunflower oil at subzero temperatures. Journal of Food Science 69: 361-366.

Cambell, A. D., Penfield, M. P., and Griswold, R. M. 1979. Experimental study of food. Boston: Houghton Miffin.

Campanone, L. A., Salvadori, V. O., and Mascheroni, R. H. 2001. Weight loss during freezing and storage of unpackaged foods. Journal of Food Engineering 47: 69-79.

Campanone, L. A., Salvadori, V. O., and Mascheroni, R. H. 2005. Food freezing with simultaneous surface dehydration: Approximate prediction of weight loss during freezing and storage. International Journal of Heat and Mass Transfer 48: 1195-1204.

Canet, W. 1989. Quality and stability of frozen vegetables. In S. Thorne (ed.), Developments in food preservation, pp. 1-50. London: Elsevier.

Casey, G. P., and Foy, J. J. 1995. Yeast performance in frozen doughs and strategies for improvement. In K. Kulp, K. Lorenz, and J.-M. Brummer (eds.), Frozen & refrigerated doughs and batters, pp. 19-51. Minnesota: AACC.

Cauvain, S. P. 1998 a. Breadmaking processes. In S. P. Cauvain, and L. S. Young (eds.), Technology of breadmaking, pp. 18-44. London: Blackie Academic & Professional.

Cauvain, S. P. 1998 b. Dough retarding and freezing. In S. P. Cauvain, and L. S. Young (eds.), Technology of breadmaking, pp. 149-179. London: Blackie Academic & Professional.

Cauvain, S. P. 2004. Improving the texture of bread. In D. Kilcast (ed.), Texture in food volume 2: Solid foods, pp. 432-450. Cambridge: Woodhead.

Champion, D., Blond, G., and Simatos, D. 1997. Reaction rates at subzero temperatures in frozen sucrose solution: A diffusion controlled reaction. Cryo-Letters 18: 251-260.

Charley, H., and Weaver, C. 1998. Foods–A scientific approach. New York: Prentice Hall.

Charun, E., Abecassis, J., Contamine, A.-S., Roulland, T.-M., Vergnes, B., and Morel, M.-H. 2000. Effects of temperature and mechanical input on semisweet biscuit (Cookie) quality and dough characteristics. Cereal Chemistry 77: 265-271.

Chen, P., Cleland, D. J., Lovatt, S. J., and Bassett, M. R. 1999. Air infiltration into refrigerated stores through rapid-roll doors. Proceeding of the 20th International Congress of Refrigeration. IV: 925–932.

Chen, P., Cleland, D. J., Lovatt, S. J., and Bassett, M. R. 2002. An empirical model for predicting air infiltration into refrigerated stores through doors. International Journal of Refrigeration 25: 799-812.

Cleland, D. J., Cleland, A. C., and White, S. D. 2002. Cost-Effective Refrigeration. Palmerston North, New Zealand: Massey University.

Collar, C., Armero, E., and Martinez, J. 1998. Lipid binding of formula bread doughs: Relationships with dough and bread technological performance. Zeitschrift für Lebensmitteluntersuchung und -Forschung A 207: 110-121.

Corradini, M. G., and Peleg, M. 2004. A model of non-isothermal degradation of nutrients, pigments and enzymes. Journal of the Science of Food and Agriculture 84: 217-226.

Cortella, G. 2000. Retail display equipment. In C. J. Kennedy (eds.), Managing frozen foods, pp. 233-262. Boca Raton: CRC Press.

Davenel, A., Schuck, P., Mariette, F., and Brule, G. 2002. NMR relaxometry as a non-invasive tool to characterize milk powders. Lait 82: 465–473.

Davies, R. J., and Webb, T. 1969. Methods, apparatus: New product research, process development and design. Chemistry and Industry 16: 1138-1139.

Delgado, A. E., and Sun, D.-W. 2007. Influence of surface water activity on freezing/thawing times and weight loss prediction. Journal of Food Engineering 83: 23-30.

DeMan, J. M. 1990. Principles of food chemistry. London: Chapman and Hall.

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

Diniz-Mendes, L., Bernardes, E., de Araujo, P. S., Panek, A. D., and Pascoalin, V. M. F. 1999. Preservation of frozen yeast cells by trehalose. Biotechnology and Bioengineering 65: 572-578.

Dobraszczyk, B. J., Cambell, G. M., and Gan, Z. 2001. Bread: A unique food. In D. A. V. Dendy, and B. J. Dobraszczyk (eds.), Cereals and cereal products: Chemistry and technology, pp. 182-232. Maryland: Aspen Publishers.

Dobraszczyk, B. J., and Morgenstern, M. P. 2003. Rheology and the breadmaking process. Journal of Cereal Science 38: 229-245.

Dobraszczyk, B. J., Smewing, J., Albertini, M., Maesmans, G., and Schofield, J. D. 2003. Extensional rheology and stability of gas cell walls in bread doughs at elevated temperatures in relation to breadmaking performance. Cereal Chemistry 80: 218-224.

Dong, W., and Hoseney, R. C. 1995. Effects of certain bread making oxidants and reducing agents on rheological properties. Cereal Chemistry 72: 58-64.

Donhowe, D. P., and Hartel, R. W. 1996 a. Recrystallization of ice in ice cream during controlled accelerated storage. International Dairy Journal 6: 1191-1208.

Donhowe, D. P., and Hartel, R. W. 1996 b. Recrystallization of ice cream during bulk storage of ice cream. International Dairy Journal 6: 1209-1221.

Dumont, F., Marechal, P. -A., and Gervais, P. 2003. Influence of cooling rate on *Saccharomyces cerevisiae* destruction during freezing: Unexpected viability at ultra-rapid cooling rates. Cryobiology 46: 33-42.

Earle, M., and Earle, R. 2003. Fundamentals of food reaction technology. Surrey: Leatherhead Publishing.

El-Hady, E. A., El-Samahy, S. K., Seubel, W., and Brummer, J. -M. 1996. Change in gas production and retention in non-prefermented frozen wheat doughs. Cereal Chemistry 73: 472-477.

Esselink, E. F. J., van Aalst, H., Maliepaard, M., and van Duynhoven, J. P. M. 2003. Long-term storage effect in frozen dough by spectroscopy and microscopy. Cereal Chemistry 80: 396-403.

Evans, J. A., Stanton, J. I., Russell, S. L., and James, S. J. 1991. Consumer handling of chilled foods: A survey of time and temperature conditions. London: MAFF.

FAO. 2001. Fish display in retail shops. Ministry of Agriculture, Fisheries and Food.

Faridi, H., and Faubion, J. M. 1990. Dough rheology and baked product texture. New York: AVI.

Fellows, P. 2000. Food processing technology: Principles and practice. New York: Woodhead.

Fennema, O. R. 1973. Nature of freezing process. In O. R. Fennema, W. D. Powrie, and E. H. Marth (eds.), Low-temperature preservation of food and living matter, pp. 150-239. New York: Marcel Dekker.

Fennema, O. R. 1996. Freezing preservation. In O. R. Fennema (eds.), Physical principles of food preservation, pp. 173–215. New York: Marcel Dekker.

Ferry, J. D. 1980. Viscoelastic properties of polymers. New York: John Wiley.

Flores, A. A., and Goff, H. D. 1999. Recrystallization in ice cream after constant and cycling temperature storage conditions as affected by stabilizers. Journal of Dairy Science 82: 1408-1415.

Forsythe, S. J., and Hayes, P. R. 1998. Food hygiene, microbiology and HACCP. New York: Aspen Publications.

Fu, B., and Labuza, T. P. 1997. Shelf-life testing: Procedures and prediction methods. In M. C. Eriksson, and Y. C. Hug (eds.), Quality of frozen food, pp. 377-415. New York: Thomson.

Gaman, P. M., and Sherrington, K. B. 1990. The science of food. New York: Pergamon Press.

Gambaro, A., Fiszman, S., Gimenez, A., Varela, P., and Salvador, A. 2004. Consumer acceptability compared with sensory and instrumental measures of white pan bread: Sensory shelf-life estimation by survival analysis. Journal of Food Science 69: S401-S405.

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: 15-24.

Gan, Z., Ellis, P. R., and Schofield, J. D. 1995. Gas cell stabilisation and gas retention in wheat bread dough. Journal of Cereal Science 21: 215-230.

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

Giannakourou, M. C., and Taoukis, P. S. 2002. Systematic application of time temperature integrators as tools for control of frozen vegetable quality. Journal of Food Science 67: 2221-2228.

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

Giannou, V., and Tzia, C. 2007. Frozen dough bread: Quality and textural behavior during prolonged storage - Prediction of final product characteristics. Journal of Food Engineering 79: 929-934.

Gil, M. J., Callejo, M. J., and Rodríguez, G. 1997. Effect of water content and storage time on white pan bread quality: Instrumental evaluation. Zeitschrift für Lebensmitteluntersuchung und -Forschung A 205: 268-273.

Goff, H. D. 1992. Low-temperature stability and the glassy state in frozen foods. Food Research International 25: 317-325.

Gormley, R., Walshe, T., Hussey, K., and Butler, F. 2002. The effect of fluctuating *vs*. constant frozen storage temperature regimes on some quality parameters of selected food products. Lebensmittel-Wissenschaft Und-Technologie-Food and Science and Technology 35: 190-200.

Greene, J. L., and Bovell-Benjamin, A. C. 2004. Macroscopic and sensory evaluation of bread supplemented with sweet-potato flour. Journal of Food Science 69: SNQ167-SNQ173.

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

He, H., and Hoseney, R. C. 1990. Changes in bread firmness and moisture during long-term storage. Cereal Chemistry 67: 603-605.

He, H., and Hoseney, R. C. 1991. Gas retention in bread dough during baking. Cereal Chemistry 68: 521-525.

Hersleth, M., Berggren, R., Westad, F., and Martens, M. 2005. Perception of bread: A comparison of consumers and trained assessors. Journal of Food Science 70: S95-S101.

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

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

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

Hu, R. 1999. Food product design. Pennsylvania: Technomic.

Hussain, M. A., and Rahman, M. S. 1999. Thermal conductivity prediction of fruits and vegetables using neural networks. International Journal of Food Properties 2: 121-138.

Indrani, D., Prabhasankar, P., Rajiv, J., and Rao, G. V. 2002. Effect of storage on the rheological and parotta-making characteristics of frozen parotta dough. European Food Research and Technology 215: 484-488.

Indrani, D., Prabhasankar, P., Rajiv, J., and Venkateswara Rao, G. 2003. Scanning electron microscopy, rheological characteristics, and bread-baking performance of wheat-flour dough as affected by enzymes. Journal of Food Science 68: 2804-2809.

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

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

Inoue, Y., and Bushuk, W. 1996. Effects of freezing, frozen storage and thawing on dough and baked goods. In L. E. Jeremiah (ed.), Freezing effects on food quality, pp. 367-400. New York: Marcel Dekker.

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 Chemistry 71: 118-121.

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.

Jackel, S. S. 1991. Frozen dough opportunities keep heating up. Cereal Foods World 36: 529.

Jackel, S. S. 1986. Baking: Sensory evaluation. Cereal Foods World 31: 324-325.

James, S. 1996. The chill chain "from carcass to consumer". Meat Science 43: 203-216.

James, S. J., and Evans, J. 1992 a. Consumer handling of chilled foods: Temperature performance. International Journal of Refrigeration 15: 299-306.

James, S. J., and Evans, J. 1992 b. The temperature performance of domestic refrigerators. International Journal of Refrigeration 15: 313-319.

Kadan, R. S., Robinson, M. G., Thibodeaux, D. P., and Pepperman, A. B. Jr. 2001. Texture and other physicochemical properties of whole rice bread. Journal of Food Science 66: 940-944.

Kamel, B. S. 1987. Bread firmness measurement with emphasis on baker compressimeter. Cereal Foods World 32: 472.

Karel, M., and Saguy, I. 1991. Effects of water on diffusion in food systems. In H. Levine, and L. Slade (eds.), Water relationships in foods: Advances in the 1980s and trends for the 1990s, pp. 157-173. New York: Plenum Press.

Kaul, S. C., Obuchi, K., and Komatsu, Y. 1992. Cold shock response of yeast cells: Induction of 33 kDa protein and protection against freezing injury. Cellular and Molecular Biology 38: 553-559.

Kennedy, C. J. 2000. Freezing processed foods. In C. J. Kennedy (ed.), Managing frozen foods, pp. 137-158. Boca Raton: CRC Press.

Kenny, S., Grau, H., and Arendt, E. K. 2001. Use of response surface methodology to investigate the effects of processing conditions on frozen dough quality and stability. European Food Research and Technology 213: 323-328.

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 Chemistry 76: 421-425.

Kieffer, R., and Stein, N. 1999. Demixing in wheat doughs-First influence on dough and gluten rheology. Cereal Chemistry 76: 688-693.

Kieffer, R., Wieser, H., Henderson, M. H., and Graveland, A. 1998. Correlations of the breadmaking performance of wheat flour with rheological measurements on a micro-scale. Journal of Cereal Science 27: 53-60.

Kline, L., and Sugihara, T. F. 1968. Factors affecting the stability of frozen bread doughs. I - Prepared by the straight dough method. The Bakers Digest 42: 44-46 & 48-50.

Kuitche, A., Daudin, J. D., and Letang, G. 1996. Modelling of temperature and weight loss kinetics during meat chilling for time-variable conditions using an analytical-based method -- I. The model and its sensitivity to certain parameters. Journal of Food Engineering 28: 55-84.

Kulik, A. S., da Costa, J. R. C., and Haverkamp, J. 1994. Water organization and molecular mobility in maize starch investigated by two-dimensional solid-state NMR. Journal of Agricultural and Food Chemistry 42: 2803-2807.

Kulp, K. 1995. Biochemical and biophysical principles fo freezing. In K. Kulp, K. Lorenz, and J. Brummer (eds.), Frozen and refrigerated doughs and batters, pp. 63-89. St. Paul, MN: American Association of Cereal Chemists.

Laaksonen, T. J., Kuuva, T., Jouppila, K., and Roos, Y. H. 2002. Effects of arabinoxylans on thermal behavior of frozen wheat doughs as measured by DSC, DMA, and DEA. Journal of Food Science 67: 223-230.

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

Labuza, T. P. 1980. The effect of water activity on reaction kinetics of food deterioration. Food Technology 34: 36-41, 59.

Laguerre, O., and Flick, D. 2007. Frost formation on frozen products preserved in domestic freezers. Journal of Food Engineering 79: 124-136.

Le Bail, A. Scientific Director Food Process Engineering, ENITIAA-GEPEA, Nantes, France. Interview, 19 February 2006.

Le Bail, A., Grinand, C., Le Cleach, S., Martinez, S., and Quilin, E. 1999. Influence of storage conditions on frozen French bread dough. Journal of Food Engineering 39: 289-291.

Le Bail, A., Pasco, M., Meric, L., and Cahagnier, B. 1996. Influence of the freezing rate on yeast activity in frozen bread dough. International Congress of Refrigeration, pp. 16-18. Nantes, France.

Le Botlan, D., Rugraff, Y., Martin, C., and Colonna, P. 1998. Quantitative determination of bound water in wheat starch by time domain NMR spectroscopy. Carbohydrate Research 308: 29-36.

Le Meste, M., Champion, D., Roudaut, G., Blond, G., and Simatos, D. 2002. Glass transition and food technology: A critical appraisal. Journal of Food Science 67: 2444-2458.

Leung, H. K., Magnuson, J. A., and Bruinsma, B. L. 1979. Pulsed NMR study water mobility in flour dough. Journal of Food Science 44: 1408-1411.

Levine, H., and Slade, L. 1986. A polymer physico-chemical approach to the study of commercial starch hydrolysis products. Carbohydrate Polymers 6: 213-244.

Li, M., and Lee, T. C. 1998. Effect of cysteine on the molecular weight distribution and the disulfide cross-link of wheat flour proteins in extrudates. Journal of Agricultural and Food Chemistry 46: 846-853.

Likar, K., and Jevsnik, M. 2006. Cold chain maintaining in food trade. Food Control 17: 108-113.

Liu, Z., and Scanlon, M. G. 2002. Understanding and modeling the processing-mechanical property relationship of bread crumb assessed by indentation. Cereal Chemistry 79: 763-767.

Lorenz, K., and Kulp, K. 1995. Freezing of doughs for the production of breads and rolls in the United States. In K. Kulp, K. Lorenz, and J. Brummer (eds.), Frozen and refrigerated doughs and batters, pp. 135-165. St. Paul, MN: American Association of Cereal Chemists.

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

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

Lucas, T., Grenier, A., Quellec, S., Le Bail, A., and Davenel, A. 2005. MRI quantification of ice gradients in dough during freezing or thawing processes. Journal of Food Engineering 71: 98-108.

Mallet, C. P. 1993. Frozen food technology. London: Chapman & Hall.

Martino, M. N., and Zaritzky, N. E. 1988. Ice crystal size modification during frozen beef storage. Journal of Food Science 53: 1631-1637, 1649.

Martino, M. N., and Zaritzky, N. E. 1989. Ice recrystallization in a model system and in frozen muscle tissue. Cryobiology 26: 138-148.

Martins, R. C., Almeida, M. G., and Silva, C. L. M. 2004. The effect of home storage conditions and packaging materials on the quality of frozen green beans. International Journal of Refrigeration 27: 850-861.

Martins, R. C., and Silva, C. L. M. 2002. Modelling colour and chlorophyll losses of frozen green beans (*Phaseolus vulgaris*, L.). International Journal of Refrigeration 25: 966-974.

Martins, R. C., and Silva, C. L. M. 2003. Kinetics of frozen stored green bean (*Phaseolus vulgalis* L.) quality change: Texture, vitamin C, reducing sugars, and starch. Journal of Food Science 68: 2232-2237.

Martins, R. C., and Silva, C. L. M. 2004. Green beans (*Phaseolus vulgaris*, L.) quality loss upon thawing. Journal of Food Engineering 65: 37-48.

Matz, S. A. 1989. Bakery technology - Packaging, nutrition, product development, quality assurance. New York: Elsevier Science.

Mazur, P. 1970. Cryobiology: The freezing of biological systems. Science 168: 939-949.

Mazur, P. 1976. Mechanisms of injury in frozen-thawed cells cooled at suboptimal and supraoptimal rates. Cryobiology 13: 645-645.

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. 1995. Cryoresistance of baker's yeast *Saccharomyces cerevisiae* in frozen dough: Contribution of cellular trehalose. Cereal Chemistry 72: 609-615.

Miller, S. D. 2006. Yeast Metabolism in Fresh and Frozen Dough. Doctoral dissertation, Institute of Food, Nutrition, Human and Health, Massey University, New Zealand.

Mittal, G. S., and Zhang, J. 2000. Prediction of freezing time for food products using a neural network. Food Research International 33: 557-562.

Mizrahi, S. 2004. Accelerated shelf life tests. In R. Steele (ed.), Understanding and measuring the shelf-life of food, pp. 317-339. Cambridge, UK: Woodhead Publishing.

Montague, G., and Morris, J. 1994. Neural-network contributions in biotechnology. Trends in Biotechnology 12: 312-324.

Morris, G. J., Coulson, G. E., and Clarke, K. J. 1988. Freezing injury in Saccharomyces cerevisiae: The effect of growth conditions. Cryobiology 25: 471-482.

Muldrew, K., and McGann, L. E. 1990. Mechanisms of intracellular ice formation. Biophysical Journal 57: 525-532.

Myers, D. K., and Attfield, P. V. 1999. Intracellular concentration of exogenous glycerol in *Saccharomyces cerevisiae* provides for improved leavening of frozen sweet doughs. Food Microbiology 16: 45-51.

Naito, S., Fukami, S., Mizokami, Y., Ishida, N., Takano, H., Koizumi, M., and Kano, H. 2004. Effect of freeze-thaw cycles on the gluten fibrils and crumb grain structure of breads made from frozen doughs. Cereal Chemistry 81: 80-86.

Nakagawa, S., and Ouchi, K. 1994. Improvement of freeze tolerance of commercial baker's yeasts in dough by heat treatment before freezing. Bioscience Biotechnology and Biochemistry 58: 2077-2079.

Nelson, K. A., and Labuza, T. P. 1994. Water activity and food polymer science: Implications of state on Arrhenius and WLF models in predicting shelf life. Journal of Food Engineering 22: 271-289.

Neter, J., Kutner, M. H., Nachtsheine, C. J., and Wasserman, W. 1996. Applied Linear Statistical Models. Chicago: IRWIN.

Neyreneuf, O., and Delpuech, B. 1993. Freezing experiments on yeasted dough slabs. Effects of cryogenic temperatures on the baking performance. Cereal Chemistry 70: 109-111.

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

Ngapo, T. M., Babare, I. H., Reynolds, J., and Mawson, R. F. 1999. A preliminary investigation of the effects of frozen storage on samples of pork. Meat Science 53: 169-177.

Ni, H., and Gunasekaran, S. 1998. Food quality prediction with neural network. Food Technology 52(10): 60-65.

Oda, Y., Uno, K., and Ohta, S. 1986. Selection of yeasts for breadmaking by the frozen-dough method. Applied and Environmental Microbiology 52: 941-943.

Parker, R., and Ring, S. G. 1995. A theoretical analysis of diffusion-controlled reactions in frozen solution. Cryo-Letters 16: 197–208.

Patterson, D. W. 1996. Artificial neural networks: Theory and applications. Singapore: Prentice Hall.

Peleg, M. 1992. On the use of the WLF model in polymers and foods. Critical Reviews in Food Science and Nutrition 32: 59-66.

Peleg, M., Corradini, M. G., and Normand, M. D. 2004. Kinetic models of complex biochemical reactions and biological processes. Chemie Ingenieur Technik 76: 413-423.

Peleg, M., Engel, R., Gonzalez-Martinez, C., and Corradini, M. G. 2002. Non-Arrhenius and non-WLF kinetics in food systems. Journal of the Science of Food and Agriculture 82: 1346-1355.

Peters, G., Morrissey, M. T., Sylvia, G., and Bolte, J. 1996. Linear regression, neural network and induction analysis to determine harvesting and processing effects on surimi quality. Journal of Food Science 61: 876-880.

Pham, Q. T. 1987. Moisture transfer due to temperature changes or fluctuations. Journal of Food Engineering 6: 33-49.

Pham, Q. T., and Willix, J. 1984. A model for food desiccation in frozen storage. Journal of Food Science 49: 1275-1281, 1294.

Pham, Q. T., and Willix, J. 1985. Weight loss from lamb carcasses in frozen storage: Influence of environmental factors. International Journal of Refrigeration 8: 231-235.

Phimolsiripol, Y., Siripatrawan, U., Tulyathan, V., and Cleland, D. J. 2006 a. The effect of temperature fluctuations on the quality of frozen bread dough. Proceeding of the IIR-IRHACE Conference on Innovative Equipment and Systems for Comfort and Food Preservation, pp. 559-565. Auckland, New Zealand.

Phimolsiripol, Y., Siripatrawan, U., Tulyathan, V., and Cleland, D. J. 2006 b. Effect of holding time on CO_2 production and rheological properties in yeasted frozen dough. Proceeding of the 34th International Symposium on Agricultural Engineering, pp. 587-592. Opatija, Croatia.

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

Pyler, E. J. 1988. Baking science & technology. Kansas, US: Sosland.

Rasanen, J., Blanshard, J. M. V., Mitchell, J. R., Derbyshire, W., and Autio, K. 1998. Properties of frozen wheat doughs at subzero temperatures. Journal of Cereal Science 28: 1-14.

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

Rasanen, J., Harkonen, H., and Autio, K. 1995. Freeze-thaw stability of prefermented frozen lean wheat doughs: Effect of flour quality and fermentation time. Cereal Chemistry 72: 637-642.

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

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

Reid, D. S. 1993. Basic physical phenomena in the freezing and thawing of plant and animal tissues. In C. P. Mallett (ed.), Frozen food technology, pp. 1-19. London: Chapman & Hall.

Reynolds, J., Park, J. W., and Choi, Y. J. 2002. Physicochemical properties of Pacific whiting surimi as affected by various freezing and storage conditions. Journal of Food Science 67: 2072-2078.

Ribotta, P. D., Leon, A., and Anon, M. C. 2001. Effect of freezing and frozen storage of dough on bread quality. Journal of Agricultural and Food Chemistry 49: 913-918.

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

Ribotta, P. D., Perez, G. T., Leon, A. E., and Anon, M. C. 2004. Effect of emulsifier and guar gum on micro structural, rheological and baking performance of frozen bread dough. Food Hydrocolloids 18: 305-313.

Rojas, J. A., Rosell, C. M., de Barber, C. B., Perez-Munuera, I., and Lluch, M. A. 2000. The baking process of wheat rolls followed by cryo scanning electron microscopy. European Food Research and Technology 212: 57-63.

Roos, Y. H. 1995. Phase transitions in foods. New York: Academic Press.

Roos, Y., and Karel, M. 1991. Plasticizing effect of water on thermal behavior and crystallization of amorphous food models. Journal of Food Science 56: 38-43.

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

Roy, S. S., Taylor, T. A., and Kramer, H. L. 2001. Textural and ultrastructural changes in carrot tissue as affected by blanching and freezing. Journal of Food Science 66: 176-180.

Ruan, R., Almaer, S., and Zhang, J. 1995. Prediction of dough rheological properties using neural networks. Cereal Chemistry 72: 308-311.

Ruland, S. 1992. Battling heat shock. Dairy Field 175: 53.

Sablani, S. S., Baik, O. -D., and Marcotte, M. 2002. Neural networks for predicting thermal conductivity of bakery products. Journal of Food Engineering 52: 299-304.

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

Salas-Mellado, M. M., and Chang, Y. K. 2003. Effect of formulation of quality of frozen bread dough. Brazilian Archives of Biology and Technology 46: 461-468.

Sapru, V., and Labuza, T. P. 1993. Glassy state in bacterial spores predicted by polymer glass transition theory. Journal of Food Science 58: 445-448.

Scanlon, M. G., Sapirstein, H. D., and Fahloul, D. 2000. Mechanical properties of bread crumb prepared from flours of different dough strength. Journal of Cereal Science 32: 235–243.

Scanlon, M. G., and Zghal, M. C. 2001. Bread properties and crumb structure. Food Research International 34: 841-864.

Schofield, J. D., and Chan, X. 1995. Analysis of free reduced and free oxidized glutathione in wheat flour. Journal of Cereal Science 21: 127-136.

Schwarzlaff, S. S., Johnson, J. M., Barbeau, W. E., and Duncan, S. 1996. Guar and locust bean gums as partial replacers of all-purpose flour in bread: An objective and sensory evaluation. Journal of Food Quality 19: 217-229.

Schwimmer, S., Ingraham, L. L., and Hughes, H. M. 1955. Temperature tolerance in frozen food processing: Effective temperatures in thermally fluctuating systems. Industrial and Engineering Chemistry Research 47: 1149-1151.

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

Setser, C. S. 1993. Sensory evaluation. In B. S. Kamel, and C. E. Stauffer (eds.), Advances in baking technology, pp. 254-291. New York: VCH.

Shaevel, M. L. 1996. Manufacturing of frozen prepared meals. In C. P. Mellet (ed.), Frozen food technology, pp. 270-302. London: Chapman & Hall.

Sharadanant, R., and Khan, K. 2003 a. Effect of hydrophilic gums on frozen dough. I. Dough quality. Cereal Chemistry 80: 764-772.

Sharadanant, R., and Khan, K. 2003 b. Effect of hydrophilic gums on the quality of frozen dough: II. Bread characteristics. Cereal Chemistry 80: 773-780.

Singh, H., and MacRitchie, F. 2001. Application of polymer science to properties of gluten. Journal of Cereal Science 33: 231-243.

Singh, R. K., and Ou-Yang, F. 1997. Neuro-fuzzy technology for computerized automation. In G. S. Mittal (ed.), Computerized control systems in the food industry, pp. 119-178. New York: Marcel Dekker.

Siripatrawan, U., and Harte, B. R. 2007. Solid phase microextraction/gas chromatography/mass spectrometry integrated with chemometrics for detection of Salmonella typhimurium contamination in a packaged fresh vegetable. Analytica Chimica Acta 581: 63-70.

Sitakalin, C., and Meullenet, J. -F. C. 2001. Prediction of cooked rice texture using an extrusion test in combination with partial least squares regression and artificial neural networks. Cereal Chemistry 78: 391-394.

Slade, L., Levine, H., and Finley, J. W. 1989. Protein-water interactions: Water as a plasticizer of gluten and other proteins. In R. D. Phillips, and J. W. Finley (eds.), Protein quality and the effects of processing, pp. 9-124. New York: Marcel Dekker.

Spiess, W. E. L., and Folkers, D. 1984. Time-temperature surveys in the frozen food chain. In P. Zeuthen, J. C. Cheftel, C. Erikson, M. Jul, H. Lineger, P. Linko, G. Varela, and G. Vos (eds.), Thermal processing and quality of foods, pp. 585-604. Essex: Elsevier Applied Science.

Stauffer, C. E. 1993 a. Frozen bakery products. In C. P. Mallett (ed.), Frozen food technology, pp. 303-331. London: Chapman & Hall.

Stauffer, C. E. 1993 b. Frozen dough production. In B. S. Kamel, and C. E. Stauffer (eds.), Advances in baking technology, pp. 88-106. New York: VCH.

Stauffer, C. E. 1999. Fats and oils. London: Eagan Press.

Stecchini, M. L., Maltini, E., Venir, E., Del Torre, M., and Prospero, L. 2002. Properties of wheat dough at sub-zero temperatures and freeze tolerance of a baker's yeast (*Saccharomyces cerevisiae*). Journal of Food Science 67: 2196-2201.

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

Takano, H., Naito, S., Ishida, N., and Kano, H. 2002. Fermentation process and grain structure of baked breads from frozen dough using freeze-tolerant yeasts. Journal of Food Science 67: 2725-2733.

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

Tanghe, A., Van Dijck, P., and Thevelein, J. M. 2003. Determinants of freeze tolerance in microorganisms, physiological importance, and biotechnological applications. Advances in Applied Microbiology 53: 129-176.

Taoukis, P. S., and Giannakourou, M. C. 2004. Temperature and food stability: Analysis and control. In R. Steele (ed.), Understanding and measuring the shelf-life of food, pp. 42-68. Boca Raton: CRC Press.

Teunissen, A., Dumortier, F., Gorwa, M. -F., Bauer, J., Tanghe, A., Loïez, A., Smet, P., Van Dijck, P., and Thevelein, J. M. 2002. Isolation and characterization of a freeze-tolerant diploid derivative of an industrial baker's yeast strain and its use in frozen doughs. Applied and Environmental Microbiology 68: 4780-4787.

Tocci, A. M., and Mascheroni, R. H. 1995. Heat and mass transfer coefficients during the refrigeration, freezing and storage of meats, meat products and analogues. Journal of Food Engineering 26: 147-160.

Tominaga, O., Ito, F., Hanai, T., Honda, H., and Kobayashi, T. 2002. Sensory modeling of coffee with a fuzzy neural network. Journal of Food Science 67: 363-368.

Tso, C. P., Yu, S. C. M., Poh, H. J., and Jolly, P. G. 2002. Experimental study on the heat and mass transfer characteristics in a refrigerated truck. International Journal of Refrigeration 25: 340-350.

Uthayakumaran, S., Newberry, M., Keentock, M., Stoddard, F. L., and Bekes, F. 2000. Basic rheology of bread dough with modified protein content and glutenin to gliadin ratios. Cereal Chemistry 77: 744-749.

Uthayakumaran, S., Newberry, M., Phan-Thien, N., and Tanner, R. 2002. Small and large strain rheology of wheat gluten. Rheologica Acta 41: 162-172.

van Boekel, M. A. J. S. 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. International Journal of Food Microbiology 74: 139-159.

van Vliet, T., Janssen, A. M., Bloksma, A. H., and Walstra, P. 1992. Strain hardening of dough as a requirement for gas retention. Journal of. Texture Studies 23: 439-460.

Varriano-Marston, E. 1995. Packaging materials for frozen and refrigerated doughs. In K. Kulp, K. Lorenz, and J. Brummer (eds.), Frozen and refrigerated doughs and batters, pp. 245-254. St. Paul, MN: American Association of Cereal Chemists.

Varriano-Marston, E., Hsu, K. H., and Mahdi, J. 1980. Rheological and structural changes in frozen dough. The Bakers Digest 54: 32-34, 41.

Vulicevic, I. R., Abdel-Aal, E. -S. M., Mittal, G. S., and Lu, X. 2004. Quality and storage life of par-baked frozen breads. Lebensmittel-Wissenschaft Und-Technologie-Food and Science and Technology 37: 205-213.

Wang, R., Zhou, W., Yu, H.-H., and Chow, W. -F. 2006. Effects of green tea extract on the quality of bread made from unfrozen and frozen dough processes. Journal of the Science of Food and Agriculture 86: 857-864.

Weegels, P. L., Hamer, R. J., and Schofield, J. D. 1996. Functional properties of wheat glutenin. Journal of Cereal Science 23: 1-17.

Wignall, J., Potter, D., and Storey, R. M. 1984. Some observations on temperature and quality in the UK frozen food chain. In P. Zeuthen, J. C. Cheftel, C. Erikson, M. Jul, H. Lineger, P. Linko, G. Varela, and G. Vos (eds.), Thermal processing and quality of foods, pp. 605-613. Essex: Elsevier Applied Science.

Willhoft, E. M. A. 1971. Bread staling I. Experimental study. Journal of the Science of Food and Agriculture 22: 176.

Williams, M. L., Landel, R. F., and Ferry, J. D. 1955. The temperature dependence of relaxation mechanisms in amorphous polymers and other glass-forming liquids. Journal of the American Oil Chemical Society 77: 3701–3706.

Williams, T., and Pullen, G. 1998. Functional ingredients. In S. P. Cauvain, and L. S. Young (eds.), Technology of breadmaking, pp. 45-80. London: Chapman & Hall.

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 Chemistry 61: 209-212.

Wood, B. J. B. 1989. Microbiology of fermented foods. volume 1. New York: Chapman & Hall.

Xie, G. 2001. Use of neural networks to predict roasting time and weight loss for beef joints. Food Service Technology 1: 53-59.

Xie, G., Xiong, R., and Church, I. 1998. Comparison of kinetics, neural network and fuzzy logic in modelling texture changes of dry peas in long time cooking. Lebensmittel-Wissenschaft und-Technologie -Food and Science and Technology 31: 639-647.

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

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

APPENDICES

APPENDIX A

The Effect of Temperature Fluctuations on the Quality of Frozen Bread Dough

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Abstract

Temperature fluctuations during frozen storage are generally considered to result in more rapid loss of quality than storage at constant and uniform temperature. The quality change of bread dough was measured for both constant and fluctuating storage conditions. Quality was assessed as carbon dioxide production rate and dough microstructure by SEM. After 30 days frozen storage, the frozen dough stored at -20±1°C had significantly higher CO_2 production level than the frozen dough stored in conditions that fluctuated between -20°C, -15°C and -10°C. Compared with fresh dough, the cumulative CO_2 production after 30 days of storage at -20±1°C and under fluctuating conditions decreased by about 33% and 40%, respectively. The microstructure of frozen dough stored at -20±1°C was smoother than under fluctuating conditions. The SEM micrograph of dough stored under fluctuating conditions had more holes on the fracture surface consistent with accelerated ice crystal growth and recrystallization.

Keywords: frozen bread dough, temperature fluctuations, CO_2 production, microstructure

1. Introduction

Frozen bread dough is used by more than 50% of in-store supermarket bakeries as well as by retail customers. A major problem for frozen dough is the loss of quality during storage and transportation (Stauffer, 1993). A certain amount of temperature fluctuation during frozen storage is unavoidable (Berglund *et al*., 1991). It has been postulated that temperature fluctuations during storage and distribution cause enhanced rates of quality deterioration particularly due to changes in the structure of ice crystals and recrystallization (Mazur and Schmidt, 1968; Varriano-Marston *et al*., 1980; Gormley *et al*., 2002). Dobraszczyk *et al*. (2001) showed that disruption of dough structure affected the stability of cell walls around the expanding gas bubbles during proofing and was an important factor in determining baking quality. The textural quality changes of bread baked from frozen dough were measured by Berglund and Shelton (1993). Dough microstructure changes due to ice crystal growth and recrystallization led to poorer baking performance resulting in increased bread firmness.

Berglund *et al*. (1991) examined the microstructure of frozen dough using a low temperature scanning electron microscope (SEM). After twenty-four weeks of frozen storage at -23^{o}C and up to 3 freeze-thaw cycles, the gluten matrix appeared less continuous, more ruptured and more separated from the starch granules than 24 hours after freezing. These structural characteristics were postulated to explain decreased loaf volume and increased proofing time. Zounis *et al*. (2002) observed the dough microstructure during frozen storage at -20^{o}C, -10^{o}C or -20°C with cycling to -10°C for 66 hours per week. It was found that dough structure became increasingly disrupted with frozen storage time. The disruption was more extreme at -10°C than at -20°C, and was even greater with temperature cycling. Changes in ice crystal structure led to the appearance of and increase in the size of voids in the dough during storage. The effects of freeze damage on the crumb texture and on the underlying gluten fibrils of baked breads were studied by Naito *et al*. (2004). Sweet and white bread doughs were stored at -20^{o}C and subjected to multiple freeze-thaw cycles. SEM images of bread showed that gluten fibrils formed within the gluten matrix were shorter, coarser, and non-uniform with many knots. An increase in the number of

freeze-thaw cycles increased both the coarseness of the gluten fibrils and also the size and number of the knots.

However, temperature fluctuations in the coldstore and cold chain distribution are not the same as full freeze-thaw cycles. The objective of this study was to determine the effect of temperature fluctuations on the CO_2 production rate and the microstructure of frozen bread dough as indicators of dough quality deterioration.

2. Materials and Methods

2.1 Dough Recipe

Dough samples were prepared using the straight dough formula described by Miller *et al*. (2005). The dough recipe comprises 60% w/w flour, 2% w/w compressed yeast, 1% w/w salt, 2% w/w sugar, 2% w/w canola oil and 33% w/w water (40% w/w as ground ice).

2.2 Dough Preparation

All ingredients were mixed in a dough mixer (Model 7MX, Delta Food Equipment, New Zealand) for 4 minutes at low speed and for 10 minutes at high speed. The dough temperature was 15±1^{o}C at the end of mixing. After mixing, the dough was divided into 100±2 g pieces, manually molded into round shapes (ca 5 cm diameter), and placed into 170x180 mm snaplock polyethylene bags before freezing.

2.3 Freezing

The dough pieces were frozen in an air blast freezer operating at about -30^{o}C with an air speed of 4 m/s for 60 minutes. The freezing rate was estimated to be about -0.51^{o}C/min between 0^{o}C and -20^{o}C.

2.4 Frozen Storage Regimes

To achieve the various storage regimes, the samples were stored in cardboard boxes in a coldstore at -28^{o}C. Each box was 84 cm by 62 cm by 25 cm high and was constructed of 0.7 mm thick corrugated cardboard. A light bulb (between 60W and 150W) and 2 PC computer fans were located in one corner of the box to provide both

heating and air circulation to ensure uniform temperature condition throughout the box. The bulb was controlled by an electronic thermostat with a defined set-point and dead band. Temperatures in the storage system were monitored using T-Type thermocouples connected to an Agilent datalogger (Model 34970A). The sizes of light bulb were selected to control the temperature fluctuations about the set-point to between 2 to 10 cycles per hour. For example, a 60W light bulb gave a heating rate of about 0.13^{o}C/min and a cooling rate of about -0.13^{o}C/min when the box was controlled to -20^{o}C.

Storage regimes were selected and designed to mimic either good or poor practice likely to be experienced in the cold chain but they were constrained by the characteristics of the storage equipment described above. The storage regimes were similar to those used by Alvarez and Canet (1998) and Ben-Yoseph and Hartel (1998). The storage regimes were:

- Control (Fresh) - Samples were taken from the freshly mixed and molded dough without freezing. The dough samples were held in a cooling bath for 30 minutes to reduce the dough temperature to 0^{o}C before quality assessment.
- Good Practice (GP) - Samples were stored at -20±1^{o}C for 2 days (GP2) or 30 days (GP30).
- Poor Practice (PP) - Samples were stored at -20±1^{o}C, -20±3^{o}C, -15±1^{o}C or -10±1^{o}C for between 0.5 to 5 days at each temperature as shown in Figure 1. Samples were taken after 2 days (PP2) or 30 days (PP30).

2.5 Thawing

Frozen dough samples were thawed prior to quality assessment by transferring them to an ice/water bath at 0^{o}C for 90 minutes.

2.6 Carbon Dioxide Production Measurement

CO_2 production was measured using a risograph (R-Design, W. 700 Pullman, WA) according to method of El-Hady *et al*. (1996). Four samples of 50 g of dough each with core temperatures of 0^{o}C were placed into fermentation jars in a water bath at 30^{o}C. The gas level was measured every minute for 180 minutes after a 10 minute

delay. Average rate of gas production and total of gas production were expressed as ml CO_2/minute and ml CO_2 respectively.

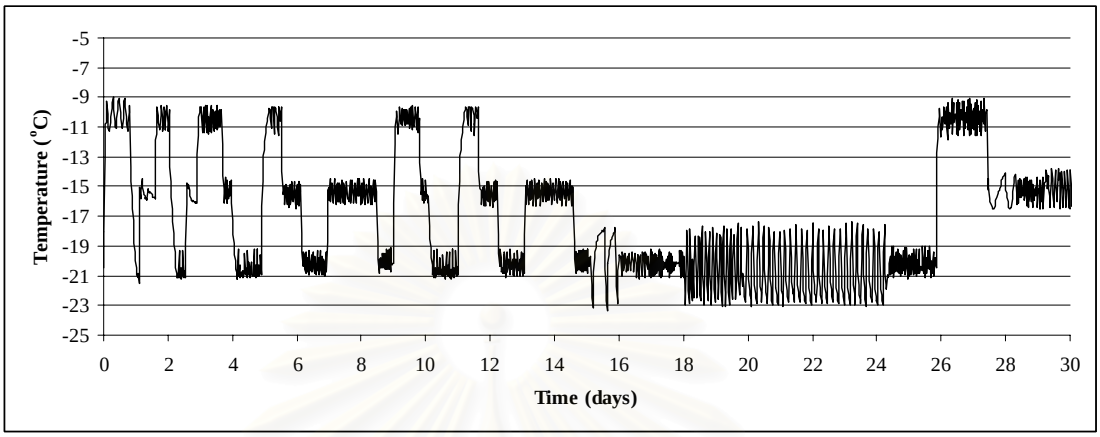

Figure 1 Storage temperature profile representing poor practice (PP).

2.7 Dough Microstructure

Measurement of dough microstructure was carried using a scanning electron microscope (SEM) according to the method of Indrani *et al*. (2003) on the 30 days stored samples only. Samples were taken from the centre of frozen dough, cut into 4 cm long and 4 mm diameter shapes while frozen, and then freeze-dried. A fracture surface of the freeze-dried samples was mounted on the specimen holder and sputter-coated with gold at 0.05 mbar. Finally, each sample was transferred to a SEM (Model 250 Mark 3, Cambridge StereoScan, UK). The micrographs were made at 100x, 500x and 2000x magnification.

2.8 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using PROC GLM (SAS Institute, Cary, NC, USA.). Duncan's multiple range test ($p \leq 0.05$) was used to detect differences among treatment means.

3. Results & Discussion

3.1 Carbon Dioxide Production

Decrease in the total CO_2 production indicates the loss of yeast activity and dough quality (Hsu *et al.*, 1979). Figure 2 and Figure 3 shows the rate of CO_2 production (ml/min) and cumulative CO_2 production (ml) measured after the different storage regimes. Table 1 shows the cumulative CO_2 production and the results of the statistical significant test. The rate of CO_2 production and cumulative CO_2 production of frozen dough stored under fluctuating condition was significantly lower compared with the dough stored at more constant temperature. The CO_2 production after 30 days was significantly lower than after 2 days for the dough stored under the fluctuating storage regime. Compared to fresh dough, the cumulative CO_2 production for the dough stored at -20±1^{o}C after 2 days and 30 days decreased by about 30% and 33%, respectively. For the dough stored under fluctuating conditions after 2 days and 30 days, the cumulative CO_2 production decreased by about 26% and 40%, respectively.

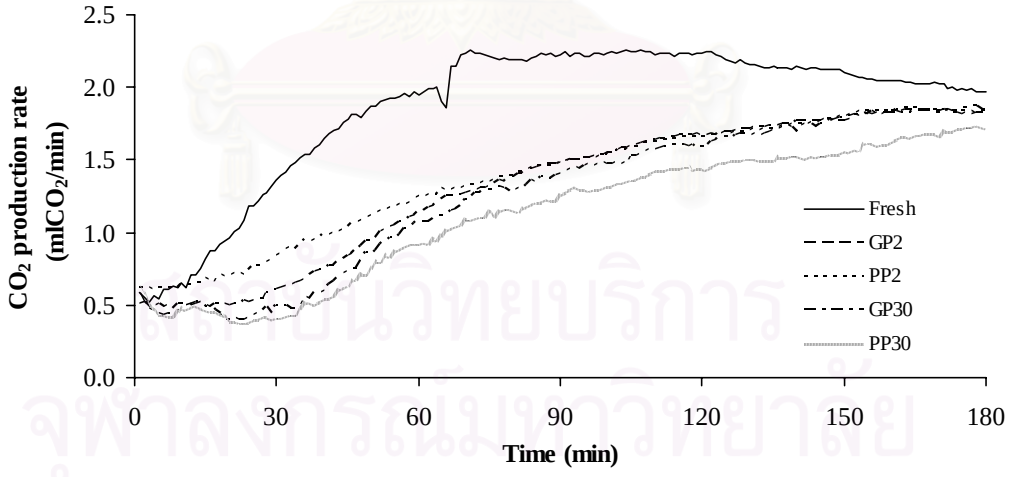

Figure 2 Rate of CO_2 production of frozen dough after different storage regimes.

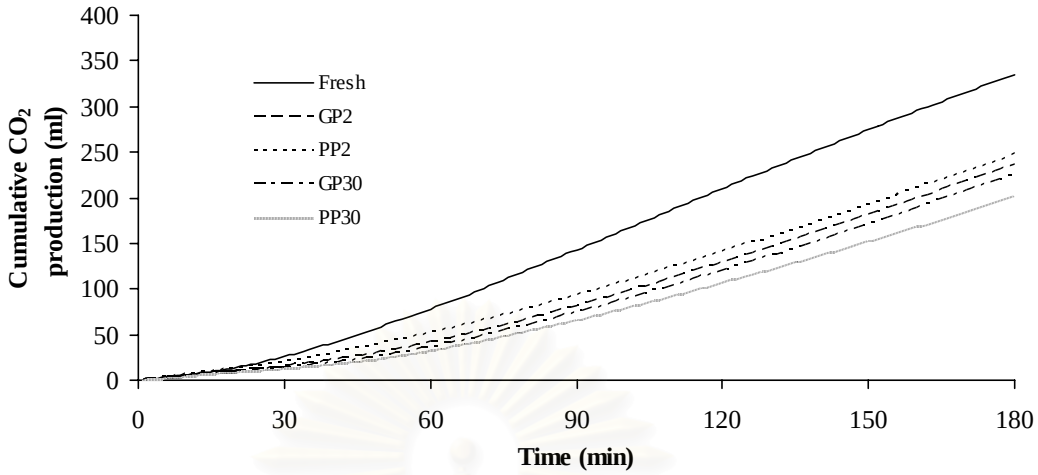

Figure 3 Cumulative CO_2 production of frozen dough after different storage regimes.

Table 1 Average cumulative CO_2 production for the 4 samples and results of the statistical significant test

Storage regime	**Cumulative CO_2 production (ml)**
Fresh	335.5±5.4 a
Good Practice after 2 days (GP2)	236.3±9.7 bc
Good Practice after 30 days (GP30)	226.2±9.0 c
Poor Practice after 2 days (PP2)	247.7±7.5 b
Poor Practice after 30 days (PP30)	202.2±6.7 d

Means within the same column with different letters are significantly different ($p \leq 0.05$).

3.2 Dough Microstructure

SEM micrographs show that frozen dough subjected to temperature fluctuations had greater damage to the gluten network than dough stored at more constant conditions (Figure 4). After 30 days frozen storage, the fracture surface of frozen dough subjected to fluctuating temperature was less smooth and had more holes compared with frozen dough stored at more constant temperature (Figures 4-A1 and 4-B1). This was attributed to increased ice crystal growth and recrystallization due to the temperature fluctuations. Most starch granules in dough stored at more constant

conditions were embedded in gluten matrix whereas fluctuating storage condition caused more of the starch granules to be floating separately from the gluten matrix (Figures 4-A2, 4-B2, 4-A3 and 4-B3). More broken gluten strands were found in dough stored under fluctuating conditions (Figures 4-A2 and 4-A3). Berglund *et al*. (1991) indicated that such structural changes might contribute to a decreased ability of the gluten to retain gas during proofing.

4. Conclusions

Extreme temperature fluctuations during 30 days frozen storage significantly affected the frozen dough quality compared to dough stored at more constant conditions. The frozen dough stored under fluctuating conditions had significantly lower CO_2 production. Greater changes in the dough microstructure were observed when stored under fluctuating condition. Weakening of the dough structure through damage to the gluten network during frozen storage can reduce gas-retaining ability and may lead to the extended proofing times and reduced loaf volumes of frozen bread dough.

5. Acknowledgments

We gratefully acknowledge the financial support of the Royal Thai Government and also extend special thanks to Doug Hopcroft (HortResearch) for his assistance with the scanning electron microscopy.

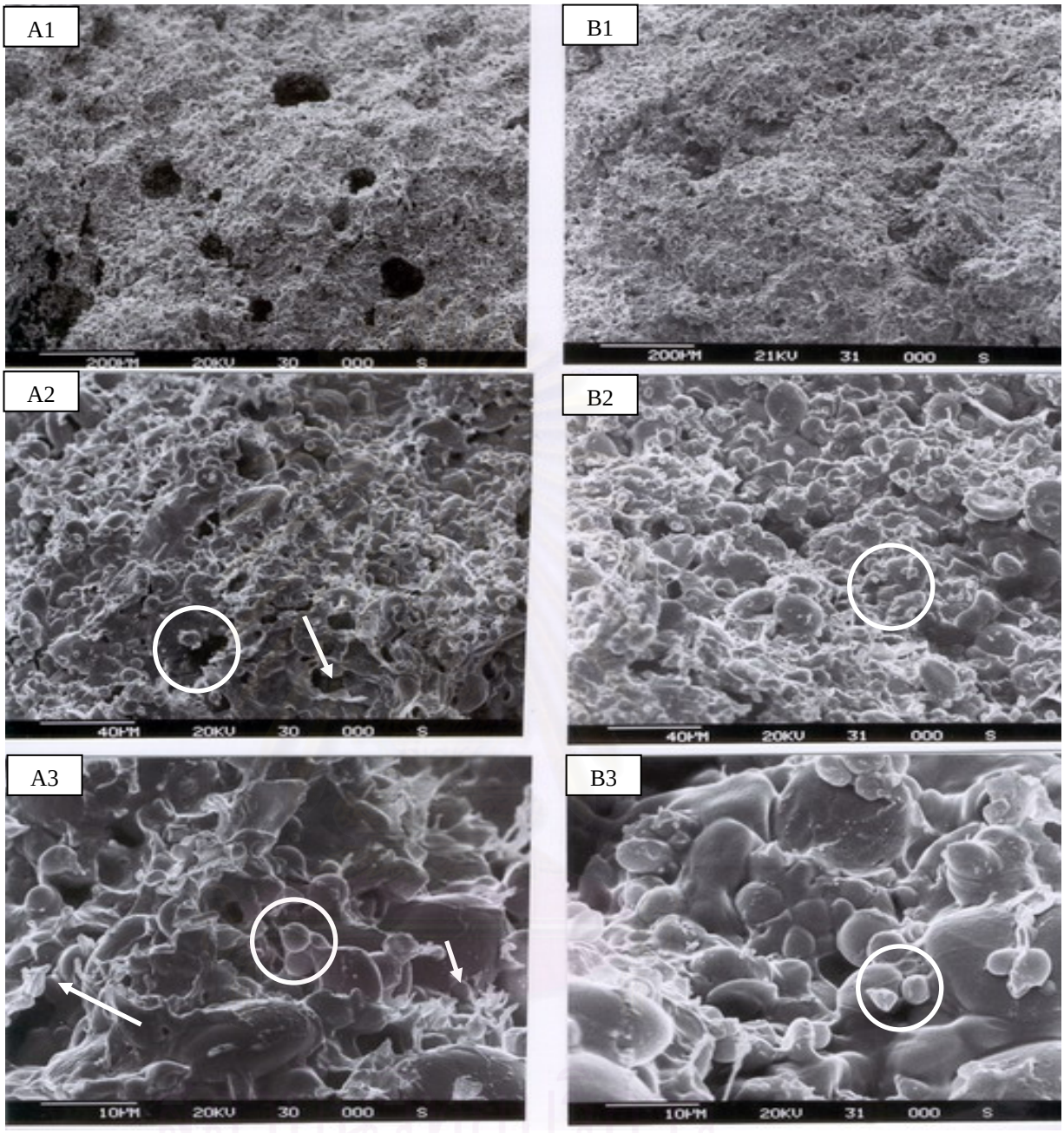

Figure 4 SEM micrographs of frozen dough sample after 30 days storage.

A1-3, frozen dough subjected to fluctuating temperature condition with 100x, 500x and 2000x magnification. **B1-3,** frozen dough stored at -20±1^{o}C with 100x, 500x and 2000x magnification. Circle indicates examples of floating starch granules. Arrow indicates examples of broken gluten matrix and strands.

6. References

Alvarez M.D., Canet W. 1998, Effect of temperature fluctuations during storage on the quality of frozen potato tissue (cv. Monalisa), *Z. Lebensm. Unters. Forsch. A.* 206: 52-57.

Ben-Yoseph E., Hartel R.W. 1998, Computer simulation of ice recrystallization in ice cream during storage, *J. Food Eng.* 38: 309-329.

Berglund P.T., Shelton D.R. 1993, Effect of frozen storage duration on firming properties of breads baked from frozen doughs, *Cereal Food World.* 38: 89-90, 92-92.

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

Dobraszczyk B.J., Cambell G.M., Gan Z. 2001, Bread: A unique food, *In*: Dendy D.A.V., Dobraszczyk B.J., *Cereals and Cereal Products: Chemistry and Technology*, Aspen Publishers, Maryland: 182-232.

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

Gormley R., Walshe T., Hussey K., Butler F. 2002, The effect of fluctuating *vs*. constant frozen storage temperature regimes on some quality parameters of selected food products, *Lebensm.-Wiss. u.-Technol*. 35: 190-200.

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

Indrani D., Prabhasankar P., Rajiv J., Rao G.V. 2003, Scanning electron microscopy, rheological characteristics, and bread-baking performance of wheat-flour dough as affected by enzymes, *J. Food Sci.* 68: 2804-2809.

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

Miller S.D., Winger R.J., Shannon E. 2005, To rise or not to rise-that is the question, *IFT Annual Meeting*, IFT: (in press).

Naito S., Fukami S., Mizokami Y., Ishida N., Takano H., Koizumi M., Kano H. 2004, Effect of freeze-thaw cycles on the gluten fibrils and crumb grain structure of breads made from frozen doughs, *Cereal Chem.* 81: 80-86.

Stauffer C.E. 1993, Frozen dough production, *In*: Kamel B.S., Stauffer C. E., *Advances in Baking Tecnology*, VCH Publishers, New York: 88-106.

Varriano-Marston E., Hsu K.H., Mahdi J. 1980, Rheological and structural changes in frozen dough, *Baker's Dig.* 54: 32-34, 41.

Zounis S., Quail K.J., Wootton M., Dickson M.R. 2002, Studying frozen dough structure using low-temperature scanning electron microscopy, *J. Cereal Sci.* 35: 135-147.

APPENDIX B

Effect of Holding Time on CO_2 Production and Rheological Properties in Yeasted Frozen Dough

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21st-24th February, 2006 in Opatija, Croatia

Summary

The CO_2 production and rheological properties of frozen dough are importance for breadmaking. Measuring the rheological properties of yeasted dough is particularly complicated and time consuming. A major difficulty is that the yeast continues to ferment and produce CO_2 during the measuring process. The simple technique of holding dough at low temperature (0^oC) in an ice/water bath was applied to control or delay yeast fermentation. After 24 hours frozen storage at -20^oC, dough samples were thawed at 0^oC in a water bath for 90 minutes. Thawed dough samples were held at 0^oC in an ice/water bath for 0, 30, 60, 120, 180 or 240 minutes before measurement of CO_2 production using a risograph and rheological properties using the large deformation technique with a SMS/Kieffer probe on a TA-XTplus texture analyzer. The CO_2 production level did not significantly change for holding times up to 240 minutes. The large deformation rheological properties were significantly different for holding times greater than 90 minutes. With increasing of holding time, the maximum force before rupture of the dough decreased and the dough extensibility increased. It was concluded that holding yeasted dough for up to 90 minutes at 0^oC did not significantly affect CO_2 production and rheological properties.

Keywords: holding time, CO_2 production, rheological properties, dough

1. Introduction

The frozen dough market has steadily grown in recent years due to consumer demand for convenience and high quality baked products (Bhattacharya *et al*., 2003). Quality problems associated with frozen dough include long proofing time, low bread volume, poor bread texture and variable baking performance. The CO_2 production and rheological properties are important indicators of the quality of frozen dough. Damage of yeast cells during frozen storage results in a decrease in gas production and loss of baking performance (Inoue *et al*., 1994). The rheological properties of gas cell walls in bread doughs affect the stability of gas cells and gas retention during proofing and baking. Dough rheological studies show how these properties changes with ingredients or other process conditions (Autio and Sinda, 1992; Lee *at al*., 2001). Weakening of the dough structure through damage to the gluten network during frozen storage can reduce gas-retaining ability (El-Hady *et al*., 1996).

There are several methods for determining the rheological properties. Generally, rheological measurement of the dough use either small deformation or large deformation techniques. In order to obtain information about the structure of both flour and gluten doughs, mechanical tests involving small deformations are most useful. However, if information on the mechanical properties of dough under conditions similar to those in fermenting bread dough is required, biaxial extension tests involving large deformation are preferred (van Vliet *et al*., 1992). Kieffer *et al*. (1998) and Uthayakumaran *et al*. (2002) indicated that the elongational properties of the dough measured using uniaxial extension gave a good correlation with baking performance.

Measuring the rheological properties of yeasted doughs is not easy and can be time consuming. The major problem is that the yeast continues to ferment during the measuring process producing CO_2 and thereby changing the rheological properties. Although fermentation is clearly important in breadmaking, research into dough rheological properties has often used non-yeasted dough to avoid yeast fermentation during the measurement (Amemiya and Menjivar, 1992; Morgenstern *et al*., 1996; Tronsmo *et al*., 2003). Newberry (2003) used an extreme freezing and thawing

procedure to inactivate the yeast. However, such a method does not make sense in a study of the quality of frozen yeasted-dough. Another option is to control yeast activity by keeping the dough cool using ice in the recipe, cooling the dough during mixing and by holding in an ice/water bath between thawing and measurement of the rheological properties and CO_2 production. The aim of this work is to evaluate the effect of holding time at 0^oC post thawing on CO_2 production and rheological properties of frozen yeasted dough.

2. Materials and Methods

2.1 Dough Recipe

Dough samples were prepared using the straight dough formula described by Miller *et al*. (2005). The dough recipe comprises 60% w/w flour, 2% w/w compressed yeast, 1% w/w salt, 2% w/w sugar, 2% w/w canola oil and 33% w/w water (40% w/w as ground ice).

2.2 Dough Preparation

All ingredients were mixed in a dough mixer (Model 7MX, Delta Food Equipment, New Zealand) for 4 minutes at low speed and for 10 minutes at high speed. Dough temperature was 15 ± 1^oC at the end of mixing. After mixing, the dough was divided into 100±2 g pieces, manually molded into round shapes (ca 5 cm diameter), and placed into 170x180 mm snaplock polyethylene bags before freezing.

2.3 Freezing

The freezing process used an air blast freezer (Long Beck Panel Systems Ltd., New Zealand) operating at about -30^oC with an air speed of 4 m/s for 60 minutes. The freezing rate was estimated to be about -0.51^oC/min between 0^oC and -20^oC.

2.4 Thawing

After 24 hours frozen storage at -20^{o}C, frozen dough samples were thawed for the quality assessment. The dough pieces were thawed by transferring them to a water bath (Neslab Istruments, Inc., Newlington, U.S.A) at 0^{o}C for 90 minutes. After thawing, the doughs were held in an ice/water bath at 0^{o}C for 0, 30, 60, 120, 180 or 240 minutes before measurement of CO_2 production and rheological properties.

2.5 Carbon Dioxide Production Measurement

CO_2 production was measured using a risograph (R-Design, W. 700 Pullman, WA) according to method of El-Hady *et al*. (1996). Three samples of 50 g of dough with core temperatures of 0^{o}C were placed into fermentation jars in a water bath at 30^{o}C. The gas level was measured every minute for 120 minutes after a 10 minute delay. Average rate of gas production and total of gas production were expressed as ml CO_2/minute and ml CO_2 respectively.

2.6 Rheological Measurement

Uniaxial extension measurements were made using the SMS/Kieffer dough and gluten extensibility rig for the TA-XTplus texture analyzer (TA-XTplus, Stable Microsystems, Surrey, UK) following the large deformation method of Bhattacharya *et al*. (2003). Twenty grams of thawed dough at 0^{o}C was placed into a Teflon-coated block, lined with parafilm, and cut into dough strips using a mould. The dough strips were allowed to rest for 30 minutes in air at 20^{o}C, before being stretched by a hook extension at the speed of 3.3 mm/s for a distance of 100 mm (Suchy *et al*., 2000). All tests were carried out at a constant room temperature of 20^{o}C. Dough extensibility (mm) from start to rupture and maximum force before rupture (g) were automatically calculated by the data processing software supplied with the TA-XTplus.

2.7 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using PROC GLM (SAS Institute, Cary, NC, USA.). Duncan's multiple range test ($p \leq 0.05$) was used to detect differences among treatment means.

3. Results and Discussion

3.1 Carbon Dioxide Production

Decrease in the total CO_2 production indicates the loss of yeast activity or prefermentation during the holding time (Hsu *et al.*, 1979). Figure 1 and 2 show the rate of CO_2 production (ml/min) and cumulative CO_2 production (ml) after each holding period. It was found that the rate of CO_2 production and cumulative CO_2 production did not significantly change with increasing holding time. These results showed that the yeast activity did not reduce during holding in an ice/water bath. The yeasted dough could be held in an ice/water bath for up to 240 minutes without significant loss of yeast activity.

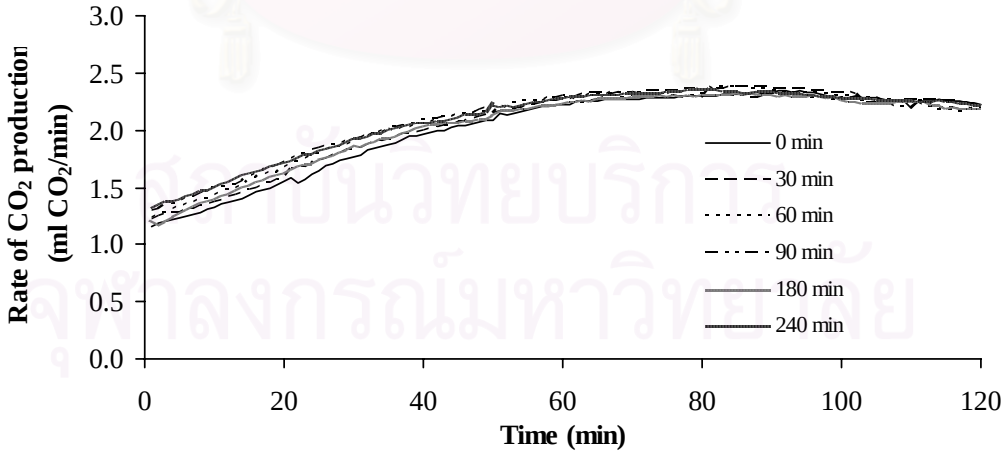

Figure 1 Rate of CO_2 production of yeasted dough after different holding time in an ice/water bath.

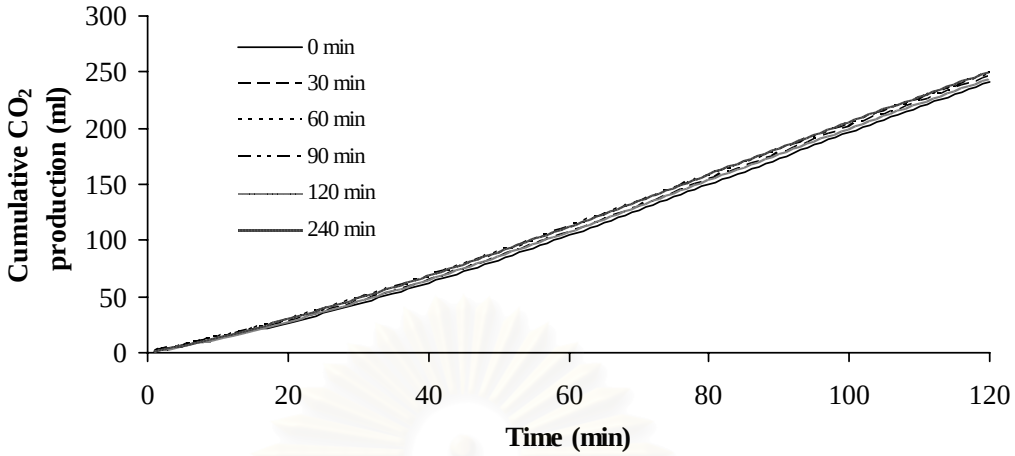

Figure 2 Cumulative CO_2 production of yeasted dough after different holding time in an ice/water bath.

3.2 Rheological Properties

The CO_2 gas bubbles from yeast fermentation play a highly significant role in the development of dough protein structure due to increased cross-linking between dough proteins. However, the rheological properties of the yeasted dough deteriorate with fermentation. Although the dough proteins become more cross-linked increasingly large gas bubbles interrupt the dough protein network and weaken it (Newberry, 2003).

Table 1 gives the maximum force before rupture and dough extensibility using the large deformation technique. The rheological properties of yeasted dough were significantly affected by different holding time in an ice/water bath. The maximum force before rupture decreased significantly after holding in an ice/water bath for more than 120 minutes. In contrast, the dough extensibility increased significantly after more than 90 minutes. Decreases in maximum force and increase in dough extensibility clearly indicated deterioration in the quality of the gluten (Inoue and Bushuk, 1992). An increase in dough extensibility would be expected to result in poorer gas retention of the dough and consequently, increased proofing time (Sharadanant and Khan, 2003).

Table 1 Rheological properties of yeasted dough after different holding time in an ice/water bath

Holding time (minutes)	Maximum force before rupture (g)	Dough extensibility (mm)
0	39.79±0.69 a	38.77±1.07 a
30	38.99±1.94 a	40.80±2.48 ab
60	38.08±1.35 ab	39.33±1.84 a
90	37.97±0.98 ab	41.41±1.05 ab
120	36.38±1.66 b	43.36±1.78 b
180	31.25±1.98 c	46.69±5.39 c
240	32.07±2.08 c	56.68±3.97 d

Means within the same column with different letters are significantly different ($p \leq 0.05$).

4. Conclusions

The yeast viability, CO_2 production and rheological properties of dough are all important for dough quality. However, yeast fermentation affects rheological measurement. Although the CO_2 production rate did not change after holding at 0^oC for up to 240 minutes, the rheological properties changed by the effect of yeast fermentation when held for greater than 90 minutes. Holding yeasted dough in an ice/water bath for up to 90 minutes was therefore recommended to minimize the effect of fermentation on rheological measurement.

5. Acknowledgment

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6. References

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.

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

Bhattacharya M., Langstaff T.M. and Berzonsky W.A., 2003, Effect of frozen storage and freeze-thaw cycles on the rheological and baking properties of frozen doughs, Food Res. Int, 36, 365-372.

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

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

Inoue Y. and Bushuk W., 1992, Studies on frozen doughs II. Flour quality requirements for bread production from frozen dough, 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.

Kieffer R., Wieser H., Henderson M.H. and Graveland A., 1998, Correlations of the breadmaking performance of wheat flour with rheological measurements on a micro-scale, J. Cereal Sci, 27, 53-60.

Lee L., Ng P.K.W., Whallon J.H. and Steffe J.F., 2001, Relationship between rheological properties and microstructural characteristics of nondeveloped, partially developed, and developed doughs, Cereal Chem, 78, 447-452.

Miller S.D., Winger R.J., Shannon E, 2005, To rise or not to rise-that is the question, *IFT Annual Meeting*, IFT, (in press).

Morgenstern M.P., Newberry M.P. and Holst S.E., 1996, Extensional properties of dough sheets, Cereal Chem, 73, 478-482.

Newberry M., 2003, The secret life of gas bubbles and their role in bread doughs, Flourmilling and baking research bulletin, 21, Autumn, 1-2.

Sharadanant, R. and Khan K., 2003, Effect of hydrophilic gums on frozen dough I. Dough quality, Cereal Chem, 80, 764-772.

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

Tronsmo K.M., Magnus E.M., Baardseth P., Schofield J.D., Aamodt A. and Faergestad E.M., 2003, Comparison of small and large deformation rheological properties of wheat dough and gluten, Cereal Chem, 80, 587-595.

Uthayakumaran S., Newberry M., Phan-Thien N. and Tanner R., 2002, Small and large strain rheology of wheat gluten, Rheologica Acta, 41, 162-172.

van Vliet T., Janssen A.M., Bloksma A.H. and Walstra P., 1992, Strain hardening of dough as a requirement for gas retention, J. Texture Stud, 23, 439-460.

APPENDIX C

Table 1 Vapour pressure data for water and ice (mm Hg) as a function of temperature (^{o}C)

Temp (^{o}C)	P_w (Pa)	Temp (^{o}C)	P_w (Pa)	Temp (^{o}C)	P_w (Pa)
water		**Ice**		**Ice**	
40	55.32	0	4.58	-20	0.78
38	49.69	-1	4.22	-22	0.64
36	44.56	-2	3.88	-24	0.53
34	39.90	-3	3.57	-26	0.43
32	35.66	-4	3.28	-28	0.35
30	31.82	-5	3.01	-30	0.29
28	28.35	-6	2.77	-32	0.23
26	25.21	-7	2.54	-34	0.19
24	22.38	-8	2.33	-36	0.15
22	19.83	-9	2.13	-38	0.12
20	17.54	-10	1.95	-40	0.10
18	15.48	-11	1.79	-42	0.08
16	13.63	-12	1.63	-44	0.06
14	11.99	-13	1.49	-46	0.05
12	10.52	-14	1.36	-48	0.04
10	9.21	-15	1.24	-50	0.03
9	8.61	-16	1.13	-52	0.02
8	8.05	-17	1.03	-54	0.02
7	7.51	-18	0.94	-56	0.01
6	7.01	-19	0.85	-58	0.01
5	6.54	-20	0.78	-60	0.01
4	6.10				
3	5.69				
2	5.29				
1	4.93				
0	4.58				
-1	4.26				
-2	3.96				
-3	3.67				
-4	3.41				
-5	3.16				
-6	2.93				
-7	2.72				
-8	2.51				
-9	2.33				
-10	2.15				

Source: Cleland et al. (2002)

These data can be represented by the equations,

$$P_w = 0.0075 \exp\left(28.7775 - \frac{6071.67}{T + 271.511}\right) \qquad \text{where } T < 0^oC$$

$$P_w = 0.0075 \exp\left(23.4795 - \frac{3990.56}{T + 233.833}\right) \qquad \text{where } T > 0^oC$$

where T = dry bulb temperature (^oC)
P_w = vapour pressure (mm Hg)

To convert P_w in Pascal (Pa), multiply by 133.

APPENDIX D

Table 1 ANOVA: overall effect of independent variables on weight loss, CO_2 Production and bread specific volume kinetic models

Independent variable	Sum of squares			
	df	Weight loss	CO_2 production	Bread specific volume
Rate	1	0.01	0.76	0.002
Time	10	17.69*	22369.90*	0.682*
Regime	6	21.23*	3934.50*	0.016
Rate * Time	4	0.01	244.461	0.051*
Rate * Regime	3	0.00	696.57*	0.019*
Time * Regime	30	13.81*	9320.18*	0.018
Rate * Time * Regime	12	0.01	460.536	0.027
Error	136	1.14	3586.13	0.193

* indicates significance at 5% level.

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Effects of freezing and temperature fluctuations during frozen storage on frozen dough and bread quality

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Abstract

The effects of freezing and temperature fluctuations during frozen storage on frozen dough and bread quality were investigated. Storage regimes were selected and designed to mimic either good or poor practice likely to be experienced in the cold chain (±0.1 °C, ±1 °C, ±3 °C or ±5 °C). Quality changes and dough weight loss were measured for both constant and fluctuating frozen storage conditions. Quality was assessed as CO_2 production rate, yeast viability, bread specific volume and bread crumb firmness relative to fresh dough. Both the freezing process and subsequent frozen storage had a significant effect on all quality parameters. Dough weight loss and bread crumb firmness increased with increasing storage time. CO_2 production rate reduced with increased storage period, however, constant storage conditions (−18 ± 0.1 °C) and good temperature control (−18 ± 1 °C) gave no significant difference in CO_2 production rate for up to 112 days after freezing. Large temperature fluctuations during frozen storage (−18 ± 5 °C) and storage at higher temperatures (a combination of −18 °C, −13 °C and −8 °C) resulted in significantly more rapid loss of dough and bread quality than storage at constant and/or colder temperatures.

Keywords: Frozen dough; Bread; Freezing; Temperature fluctuations; Frozen storage

1. Introduction

The bread bakery industry is increasingly using frozen dough. Use of frozen dough permits large scale centralized dough production, distribution and storage of dough in the frozen form and relatively small scale point-of-sale baking. A major issue for frozen dough is the loss of quality during storage and transportation (Stauffer, 1993). Frozen dough should have 16 weeks shelf-life if the dough has not been temperature abused during transportation and storage. A certain amount of temperature fluctuation during frozen storage is unavoidable (Berglund, Shelton, & Freeman, 1991). It has been postulated that temperature fluctuations during storage and distribution cause increased rates of quality deterioration particularly due to changes in the structure of ice crystals and recrystallization (Gormley, Walshe, Hussey, & Butler, 2002; Mazur & Schmidt, 1968; Varriano-Marston, Hsu, & Mahdi, 1980). In addition, there can be increased moisture loss leading to reduction of both quality and saleable weight.

Freezing and frozen storage can affect dough and bread quality in a number of ways. Maintenance of yeast viability and dough gas production properties during freezing and frozen storage are important if proofing is to be fast and effective leading to high quality bread (Hino, Takano, & Tanaka, 1987). Havet, Mankai, and Le Bail (2000) found that freezing rate is important and that the key indicator of dough quality is CO_2 production rate during proofing. The CO_2 production rate depends on yeast strain, numbers of yeast cells, cell activity and amount of fermentable sugars (Autio & Sinda, 1992; El-Hady, El-Samahy, Seubel, & Brummer, 1996; Teunissen et al., 2002). Kline and Sugihara (1968) indicated that poor gas retention during proofing can result from damage of the three-dimensional gluten

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protein network. Dough weakening during frozen storage is attributed to the release of reducing substances such as glutathione from yeast during freezing.

Berglund et al. (1991) suggested that structural changes during freezing and thawing of dough led to the damage of the gluten network. Naito et al. (2004) reported the effects of freeze damage on the crumb texture and the gluten fibrils of baked breads. SEM images of bread showed that gluten fibrils formed within the gluten matrix were shorter, coarser, and more non-uniform with many knots. An increase in the number of freeze–thaw cycles increased both the coarseness of gluten fibrils and the size and number of knots. Berglund and Shelton (1993) noticed firmness of bread baked from frozen dough increased with increasing storage time. The net effect was that the CO_2 production of yeast cells was reduced by freezing and frozen storage. This led to increased proofing time, lowered bread volume and increased bread crumb firmness (Aibara, Nishimura, & Esaki, 2001).

However, temperature fluctuations in the coldstore and cold chain distribution are not the same as full freeze–thaw cycles. To our knowledge, few researchers have investigated the effect of temperature fluctuations for frozen dough quality. Phimolsiripol, Siripatrawan, Tulyathan, and Cleland (2006) found that structural damage and reduced CO_2 production of dough occurred after 30 days of frozen storage under extreme fluctuating temperature conditions. There are limited data for the effects of various temperature fluctuations and prolonged storage of frozen dough and bread properties. Therefore, the objective of this study was to further investigate the effect of prolonged frozen storage and temperature fluctuations on frozen dough and bread quality properties.

2. Materials and methods

2.1. Dough preparation

Dough samples were prepared using a straight dough formula. The dough recipe comprised 60% w/w commercial wheat flour (12% moisture content, 13% protein, 0.67% ash), 2% w/w compressed yeast, 1% w/w salt, 2% w/w sugar, 2% w/w canola oil and 33% w/w water (40% w/w of this water as ground ice). This corresponded to 3.3 g yeast, 1.7 g salt, 3.3 g sugar, 3.3 g oil and 55 g water for each 100 g of flour. All ingredients were mixed in a dough mixer (Model 7MX, Delta Food Equipment, New Zealand) for 4 min at low speed and for 10 min at high speed. The dough temperature was 18 ± 1 °C at the end of mixing. After mixing, the dough was rested for 10 min and then divided into 100 ± 2 g pieces, manually moulded into round shapes (about 5 cm diameter), and placed into 170 mm × 180 mm snap lock polyethylene bags before freezing. The resting, shaping and packaging processes took about 35 min.

2.2. Freezing conditions

The dough pieces were frozen in an air blast freezer operated at about −25 °C with air speed of 2.5 m/s for 120 min. The freezing rate was estimated to be about −0.28 °C/min between 0 °C and −20 °C. After freezing, the dough pieces were allocated into different frozen storage regimes.

2.3. Frozen storage regimes

To achieve the various storage regimes, the samples were stored in cardboard boxes in a walk-in coldstore at −28 °C that was automatically defrosted every 8 h. Each box was 82 cm × 62 cm × 25 cm and was constructed of 0.7 mm thick corrugated cardboard. A light bulb (between 60 W and 150 W) and two PC computer fans were located in one corner of the box to provide both heating and air circulation to ensure uniform temperature conditions throughout the box. A total of 84 dough samples were placed into each box including dummy samples immediately adjacent to the light bulb location. The light bulb was controlled by an electronic thermostat with a defined set-point and dead band. The sizes of light bulb were selected to control the temperature fluctuations about the set-point in the range of 0.15–10 cycles per hour, depending on each storage temperature regime. For example, a 60 W light bulb gave a heating rate of about 0.13 °C/min when controlled at −20 °C. The cooling rate was about −0.13 °C/min for all boxes at −18 °C. Temperatures in the storage system were monitored using type T thermocouples connected to an Agilent datalogger (Model 34970A). The thermocouples were calibrated against an ice-point and a calibrated thermometer to within ±0.1 °C.

Storage regimes were selected and designed to mimic either good or poor practice likely to be experienced in the cold chain but they were constrained by the characteristics of the storage equipment described above. The storage regimes were similar to those used by Alvarez and Canet (1998) and Ben-Yoseph and Hartel (1998). The storage regimes used were -18 ± 0.1 °C (control, C), -18 ± 1 °C (good practice, G), -18 ± 3 °C (poor practice, P), -18 ± 5 °C (very poor practice, VP) and the cold chain (CC). For the cold chain regime, the temperature set-points were -18 ± 1 °C for four days, 13 ± 1 °C for one day, -8 ± 1 °C for one day, and then -18 ± 1 °C for one day on a repeating weekly cycle. Three replicate dough pieces were prepared and subjected to each storage regime.

2.4. Thawing and baking

After frozen storage, frozen dough samples were thawed prior to quality assessment by transferring them to a water bath at 0 °C for 90 min. After thawing, the dough pieces were put into 6 cm × 9 cm × 5 cm D2-Mini loaf pans (Wiltshire brand, item 9218, China) and proofed at 37 ± 2 °C (85% relative humidity) for 60 min in the proofer

(Satchwellsun Vic, New Zealand). The dough pieces were baked in a 37 cm × 42 cm × 55 cm oven (AR85, Electrolux, Steelfort Engineering Company Ltd., Palmerston North, New Zealand) at 180 °C for 15 min before cooling at ambient temperature for 2 h prior to quality assessment. The top of the dough was not cut before baking. Fresh dough was used as the overall quality level control.

2.5. Weight loss measurement

To weigh the dough pieces, three dough samples were withdrawn from each controlled temperature box in the freezer and the dough pieces were removed from the polyethylene bag. The dough was weighed with ±0.01 g precision before being returned to the bag and the box. This whole process took less than 3 min. The weight loss was the difference between the initial value and the final weight.

2.6. Carbon dioxide production measurement

CO_2 production was measured using a risograph (R-Design, W. 700 Pullman, WA) according to the method of El-Hady et al. (1996). For each replicate and treatment, 50 g sample of dough was placed into fermentation jars, and then placed in a water bath at 30 °C. The gas volume was measured every minute for 180 min after a 10 min delay. Both cumulative CO_2 production (ml CO_2) and CO_2 production rate (ml CO_2/min) were measured. The percentage reduction in cumulative CO_2 production (gassing power) was calculated relative to fresh dough.

2.7. Yeast viability measurement

Yeast viability was measured using the AACC Approved Method 42-50 (AACC, 2000). Logarithmic dilutions were carried out in peptone water, and the diluted suspensions was cultured on a potato dextrose agar (Merck KgaA, Germany), adjusted to pH 3.5 with tartaric acid. The counts of surviving yeast in the dough were determined after three days of incubation at 25 °C. Samples were selected from the center of the dough pieces. Duplicate plates were prepared for each of three dough samples per treatment.

2.8. Bread quality evaluation

The bread quality parameters measured were specific volume and bread crumb firmness. Two hours after baking, the volumes of the baked bread were measured using the seed displacement method and the specific volumes were calculated following the AACC Approved Method 55-50 (AACC, 2000). Bread crumb firmness was measured using a TA-XTplus texture analyzer (TA-XTplus, Stable Microsystems, Surrey, UK) with the SMS 45 mm diameter compression probe (P/45C) and according to the texture profile analysis (TPA) method. Firmness is the peak force during the first compression cycle. Two hours after baking, the central slices of each loaf were cut into 20 mm by 20 mm by 20 mm pieces using an electric knife (Breville brand, Model BEK5, Breville Holding Pty. Ltd., China) to prevent structural damage. The TPA method was conducted under these conditions: pre-test speed, 2 mm/s; post-test speed, 1 mm/s; rupture test distance, 1%; measurement distance, 40% deformation; force, 0.10 kg; time, 1.0 s; and auto trigger force, 0.020 kg (Kadan, Robinson, Thibodeaux, & Pepperman, 2001). All measurements were performed in triplicate using three bread samples per treatment.

2.9. Statistical analysis

The statistical analysis system (SAS Institute, Inc., version 8.0, 2000) was used to conduct an ANOVA using PROC GLM, to find out if the effects of different storage regimes and storage time on the quality characteristics of frozen dough and bread were significant. Duncan's multiple range test ($p < 0.05$) was used to detect differences among treatment means.

3. Results and discussion

3.1. Storage temperature variation

The variations of the air temperature in the controlled temperature boxes (ATB), the air temperature in the coldstore (ATC), the dough center temperature (DCT) and the dough surface temperature (DST) are shown in Fig. 1a–d for the C, G, P and VP regimes and Fig. 2a and b for the CC regime. The average ATB, ATC, DCT and DST of the C, G, P, VP and CC regimes are given in Table 1.

The air temperature in the coldstore was about −27.0 ± 0.9 °C. Although the average air temperature of the C, G and VP regimes were nearly identical, the DCT and DST differed by 0.3–0.4 °C (Table 1). This apparent difference probably reflects offset and uncertainty in the temperature measurement rather than any significant actual difference. For the VP regime, both the average air and the dough temperatures were slightly colder than for the other regimes. Despite significantly different levels ($p < 0.05$) of variation in the air temperature for the C and G regimes (±0.1 °C and ±0.6 °C), the temperature variations of DCT and DST were very similar (±0.07 °C and ±0.09 °C). However, the average DCT and DST for the C and G regimes were significantly different ($p < 0.05$) being about −17.9 °C and −18.3 °C, respectively. Excluding the dummy samples adjacent to the lights that were not part of the experimental plan, differences in temperature between samples in the same box at any time were less than 0.25 °C.

3.2. Weight loss

Dough weight loss is due to transfer of water/ice in the frozen dough to become frost inside the polyethylene bag.

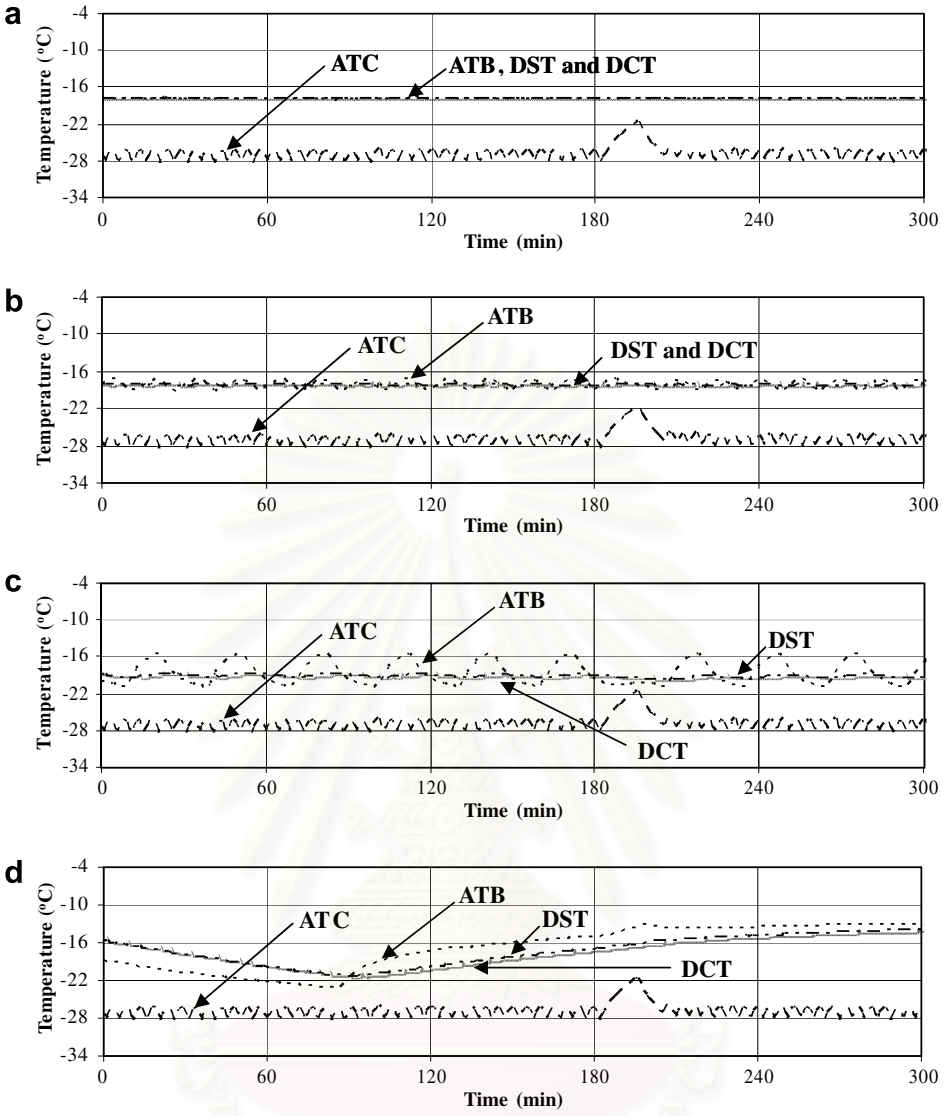

Fig. 1. Air and dough temperature variations: (a) C regime, (b) G regime, (c) P regime and (d) VP regime. ATC indicates coldstore air temperature, ATB indicates box air temperature, DCT indicates dough center temperature and DST indicates dough surface temperature.

Change in the water/ice distribution in the complex dough matrix could result in changes to the yeast's microenvironment, leading to reversible or irreversible cellular damage (Mazur, 1976). Fig. 3 shows the dough weight loss as a function of storage regime and storage time. The rate of weight loss was reasonably constant for all storage regimes. Doughs stored under the C and G regimes had no significant difference ($p > 0.05$) in weight loss throughout 112 days of frozen storage which is consistent with the temperature variations in these regimes being similar. For the P, VP and CC regimes the rate of weight loss was significantly higher than for the C and G regimes, and was greater as the regime had larger temperature fluctuations (P and VP regimes) and/or higher average storage temperature (CC).

This pattern of dough weight loss is consistent with the standard theory for evaporative weight loss from packaged foods (Laguerre & Flick, 2007). The mechanism is that the frozen dough exerts a partial pressure of water vapour in the air boundary layer associated with the surface depending on the water activity of the dough and the saturated vapour pressure (SVP) of water at the dough surface temperature. The air boundary layer associated with the polyethylene bag surface exerts a partial pressure of water vapour equal to the SVP at the bag temperature. If the bag and dough temperature become sufficiently different then the changes in SVP results in a difference in partial pressures so that the water vapour will diffuse from the dough to the bag or vice versa. If the partial pressure of water vapour in the air boundary layers becomes larger than the equilibrium value then the water vapour will condense or freeze and if it is smaller then the water/ice will evaporate or sublime. Fluctuation in storage temperature such that the dough and the bag have differing temperatures can drive a net loss of weight from the dough because the relationship between SVP and temperature is not linear and any frost forming on the inside of the bag tends to drop

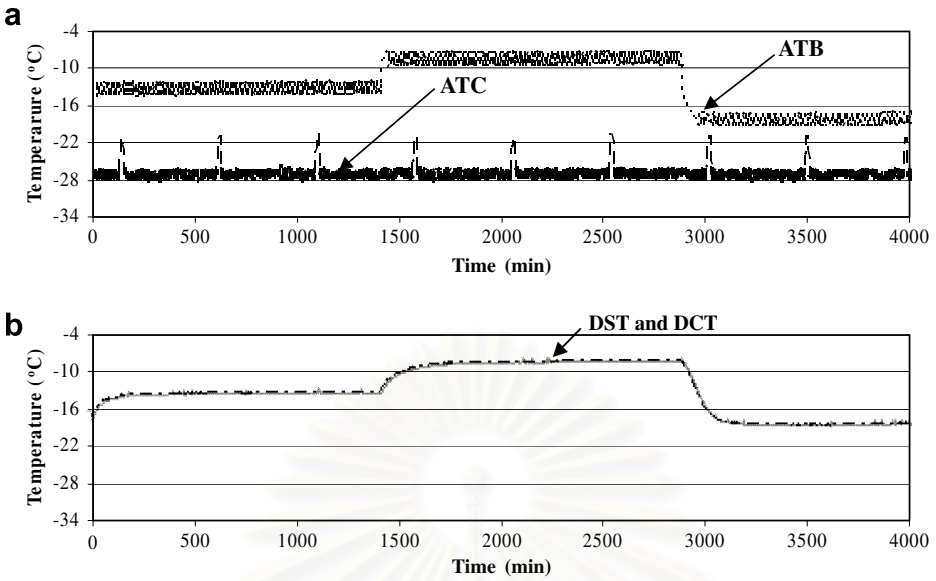

Fig. 2. Air and dough temperature variations for the CC regime: (a) coldstore air temperature (ATC) and box air temperature (ATB) and (b) dough center temperature (DCT) and dough surface temperature (DST).

Table 1
Average air temperature and dough temperature under fluctuating conditions

Regime	Set-point (°C)	Air temperature (°C)	Dough temperature (°C)	
			Center	Surface
Control (C)	−18 ± 0.1	−18.1 ± 0.1	−17.9 ± 0.07	−17.9 ± 0.07
Good practice (G)	−18 ± 1	−18.2 ± 0.6	−18.3 ± 0.07	−18.3 ± 0.09
Poor practice (P)	−18 ± 3	−18.7 ± 1.8	−18.5 ± 0.39	−18.4 ± 0.45
Very poor practice (VP)	−18 ± 5	−18.3 ± 3.3	−18.2 ± 2.08	−18.1 ± 2.11
Cold chain (CC)	−18 ± 1	−18.1 ± 0.7	−18.1 ± 0.09	−18.1 ± 0.09
	−13 ± 1	−13.2 ± 0.8	−13.2 ± 0.08	−13.3 ± 0.09
	−8 ± 1	−8.3 ± 0.8	−8.4 ± 0.09	−8.4 ± 0.09

Values are the mean and standard deviation of the measurements.

off the surface and accumulate which reduces the rates of reverse sublimation. Given this mechanism then as storage temperature increases the rate of weight loss will increase because the SVP of the water, and hence, the partial pressure of water driving force between the dough and the bag will tend to be higher. Also, as temperature fluctuations become larger then temperature differences, and hence, partial pressure of water vapour differences between the dough and the bag will increase, giving greater potential for weight loss. The results in Fig. 3 confirm this behaviour.

Another possible contribution to weight loss is escaping carbon dioxide gas due to any slow but continuing yeast fermentation (Cauvain, 1998). Mazur and Schmidt (1968) and Mazur (1970) indicated that the cell interior typically remains unfrozen until the temperature is from −10 °C to −15 °C. Given that the temperatures for the storage

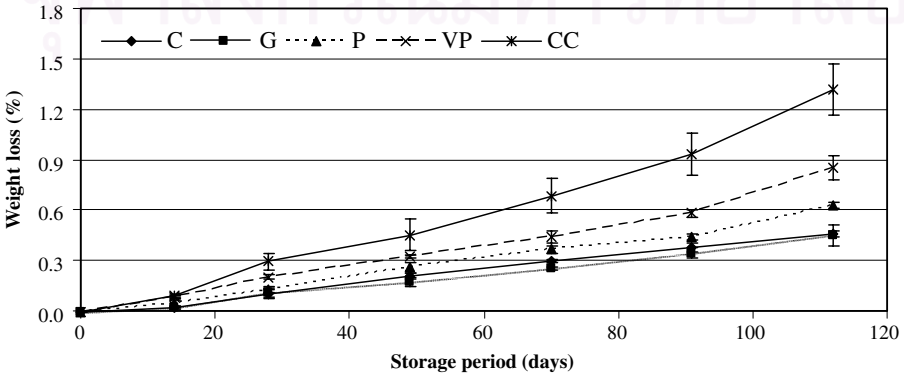

Fig. 3. Weight loss (%) of frozen dough stored under different storage regimes.

regimes were less than −15 °C most of the time, such fermentation weight loss was assumed to be insignificant.

3.3. Carbon dioxide production

CO_2 production is a key parameter for dough and bread quality. Loss of CO_2 production is highly related to consumer preference, resulting in lower bread volume (Hsu, Hoseney, & Seib, 1979). The effect of frozen storage times and storage regimes of frozen doughs on CO_2 production rate relative to fresh dough (storage time of 0 days) is shown in Table 2. One day after freezing, the cumulative CO_2 production (gassing power) of frozen dough decreased by about 7% compared to fresh dough.

There was a general decrease in gassing power with frozen storage duration. Surprisingly, the gassing power of the P and CC regimes after 49 days storage was slightly higher (although not statistically significant) than that of the C and G regimes. However, after 112 days the expected pattern of the more extreme storage regimes (greater fluctuations and/or higher temperatures) giving lower gassing power was clearly established. After 14 days frozen storage, the gassing power of frozen dough for the C, G, P and VP regimes was significantly lower than the dough after one-day storage. However, for the C and G regimes, the gassing power did not significantly decline ($p > 0.05$) further after 112 days frozen storage suggesting that these regimes would be likely to maintain acceptable dough quality. Under more extreme temperature fluctuation and higher temperature storages (VP and CC regimes), the dough gassing power declined 43% and 55%, respectively after 112 days frozen storage.

The cumulative CO_2 production and the CO_2 production rate were considered simultaneously. The CO_2 production rate showed how fast the proofing proceeded thereby indicating the effectiveness of the yeast. It was observed that the CO_2 production rate of frozen doughs declined for all regimes with increasing storage period (Fig. 4). The doughs stored under the P and CC regimes showed much slower CO_2 production rate after 112 days frozen storage. This is consistent with the results of Le Bail, Grinand, Le Cleach, Martinez, and Quilin (1999) that showed that temperature fluctuations had a large influence on the dough volume. This result is probably due to ice recrystallization which is accelerated by temperature fluctuations, resulting in the reduction of yeast activity (Neyreneuf & Delpuech, 1993). However, changes to the dough gluten network and its ability to retain CO_2 due to ice recrystallization may also have contributed.

Laaksonen and Roos (2000) found that the glass transition temperature of dough was less than −30 °C. Normal frozen storage temperatures and the temperatures used in this study are significantly higher than this. Therefore relating the increased rate of deterioration to increased mobility of the water with more extreme storage temperature fluctuations or higher storage temperatures is a reasonable mechanistic explanation.

3.4. Yeast viability

The freezing process alone resulted in about 9% yeast viability loss (Fig. 5) consistent with the results of Havet et al. (1999). Yeast viability significantly decreased ($p < 0.05$) during frozen storage. The doughs stored under the C, G, P and VP regime had a significantly higher yeast viability than those stored under the CC regime. Yeast viability gradually decreased with an increased storage period for all storage regimes. After 112 days frozen storage, the yeast viability for the CC regime had decreased by more than 50% relative to fresh dough. Salas-Mellado and Chang (2003) also found that dough yeast viability after 45 days frozen storage at −15 °C declined by 53–99%, depending on dough formulation and yeast type. Overall, the trends in CO_2 production and yeast viability were similar suggesting that yeast viability reduction was a significant contributor to reduction in CO_2 production.

3.5. Bread quality

3.5.1. Bread specific volume

High loaf volume is positively correlated with a number of consumer-preferred quality characteristics of bread, and is the end-use indicator of bread commonly used to identify the quality changes in dough (Aibara et al., 2001; Sharada-

Table 2
Cumulative CO_2 production (ml) for various frozen storage regimes and storage periods

Storage period (days)	Regime					
	C	G	P	VP	CC	Average
0	336 ± 5 a	328 ± 15 ab	328 ± 15 ab	328 ± 15 ab	328 ± 15 ab	330 ± 12
1	316 ± 5 ab	295 ± 31 bc	295 ± 31 bc	295 ± 31 bc	295 ± 31 bc	300 ± 26
14	252 ± 8 def	256 ± 18 def	256 ± 14 def	233 ± 29 defg	264 ± 25 cd	252 ± 20
28	250 ± 21 def	238 ± 21 defg	261 ± 35 cde	234 ± 9 defg	254 ± 37 def	248 ± 25
49	230 ± 11 defghi	240 ± 24 defg	232 ± 10 defgh	222 ± 14 efghij	248 ± 27 def	234 ± 18
70	229 ± 19 defghi	222 ± 19 efghij	226 ± 27 defghi	202 ± 18 ghij	225 ± 25 defghi	221 ± 21
91	245 ± 8 def	232 ± 14 defgh	219 ± 5 fghij	193 ± 3 hij	192 ± 1 ij	217 ± 23
112	234 ± 2 defg	238 ± 2 defg	218 ± 6 fghij	185 ± 14 i	146 ± 29 k	204 ± 38
Average	262 ± 40	256 ± 39	255 ± 42	236 ± 51	244 ± 59	257 ± 44

Values are the mean and standard deviation of three samples. Mean values with different letters are significantly different ($p < 0.05$).

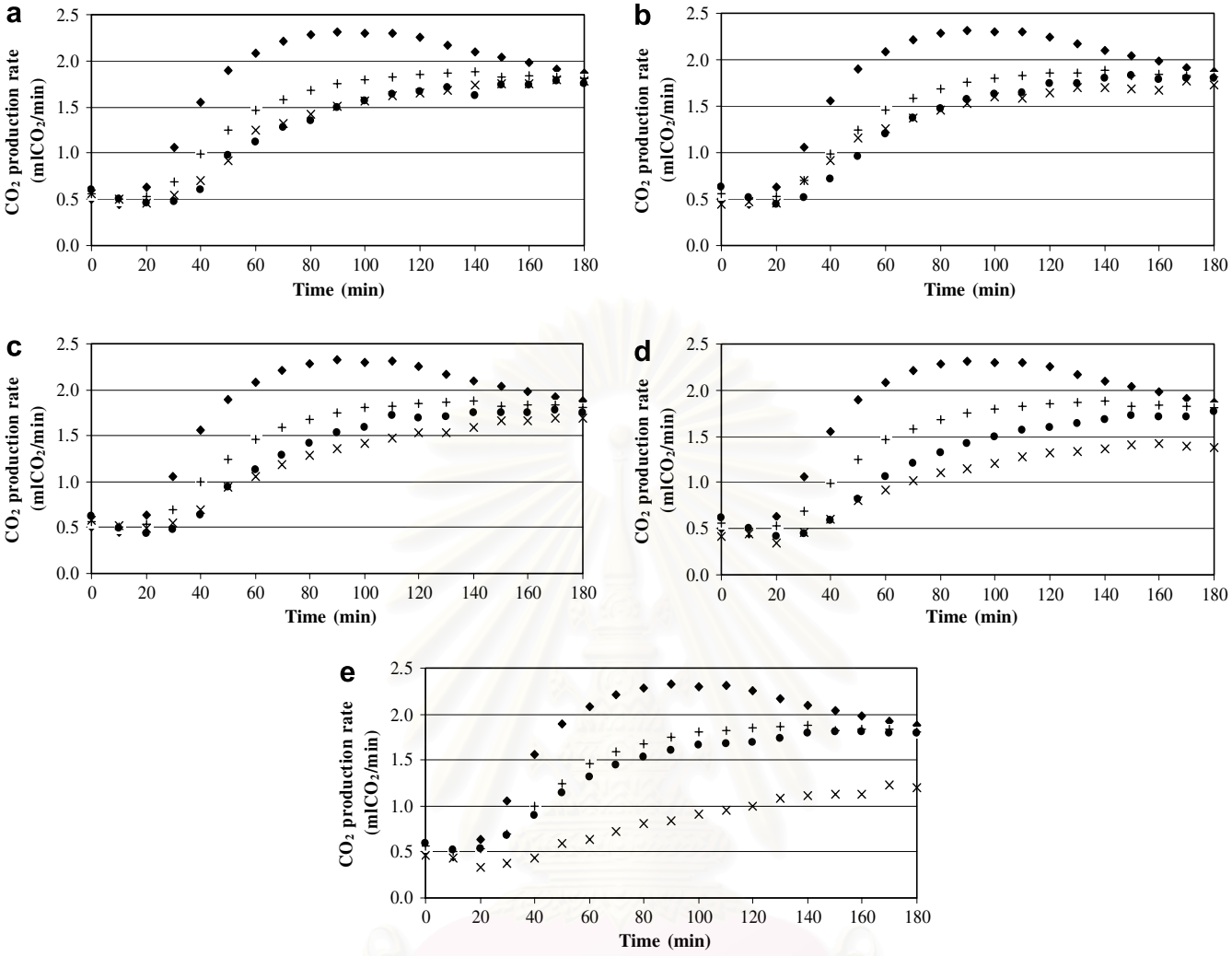

Fig. 4. CO_2 production rate for frozen dough stored under different storage regimes: (a) C regime, (b) G regime, (c) P regime, (d) VP regime and (e) CC regime. ♦: Fresh; +: one day frozen storage; ●: 49 days frozen storage; and ×: 112 days frozen storage.

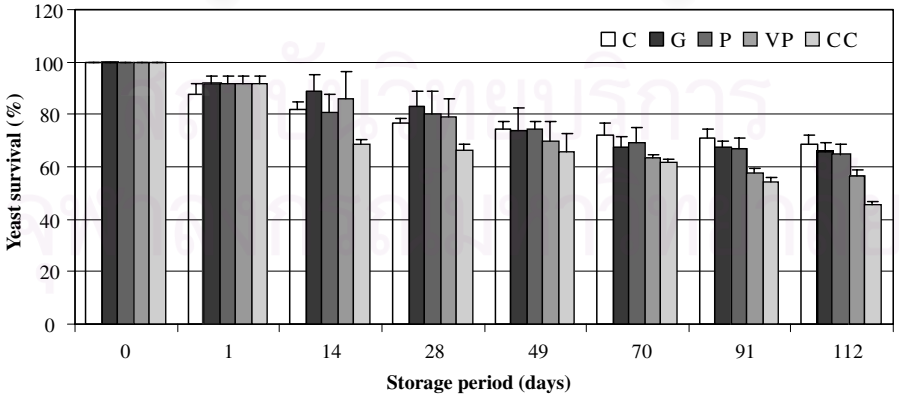

Fig. 5. Yeast survival (%) for frozen dough stored under different storage regimes.

nant & Khan, 2003). It is commonly known that a freezing process followed by storage in frozen condition affects the gassing power of yeast (El-Hady et al., 1996). A higher gas volume is necessary to increase the loaf volume. Bread specific volume declined after freezing of the dough by about 9% (Fig. 6). Specific loaf volume was greatest for the unfrozen bread dough and decreased as duration of frozen dough storage increased. As shown in Fig. 6, specific loaf volume decreased significantly ($p < 0.05$) with an increase in frozen storage for all treatments.

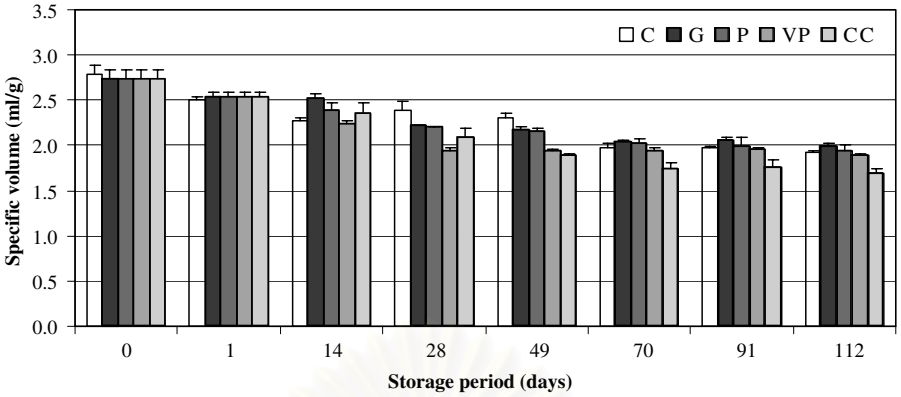

Fig. 6. Bread specific volume for frozen dough stored under different storage regimes.

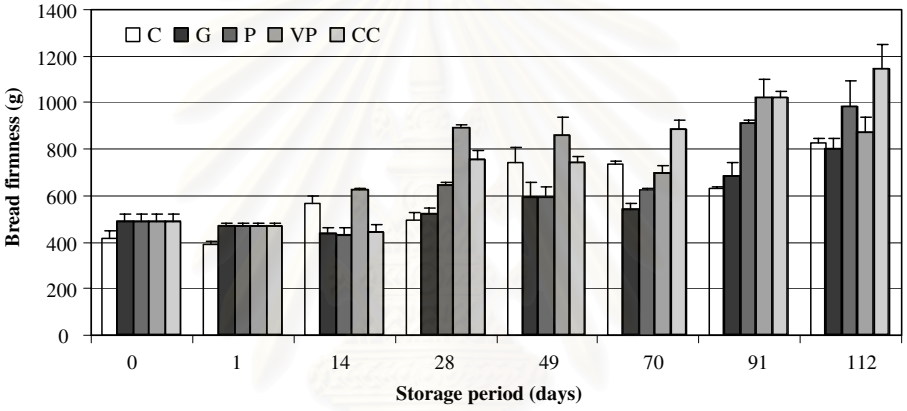

Fig. 7. Bread firmness for frozen dough stored under different storage regimes.

The specific loaf volume decreased significantly ($p < 0.05$) as the temperature fluctuations during storage increased in magnitude. The dough stored under the C, G and P regimes were not significantly different from each other ($p > 0.05$) but were significantly better ($p < 0.05$) than dough stored under the VP and CC regimes. These results agree with those of Inoue and Bushuk (1992), Le Bail et al. (1999) and Ribotta, Leon, and Anon (2001). The reduction in loaf volume was probably due to ice recrystallization causing both losses in yeast activity and reduced ability of the dough gluten network to retain CO_2 during proofing.

3.5.2. Bread crumb firmness

Low crumb firmness is a desirable quality characteristic. Firmness was significantly different for the storage regimes (Fig. 7). The freezing process had no significant effect on bread crumb firmness. Crumb firmness increased gradually with storage time for all regimes but did not change significantly until after 14 days storage. Crumb firmness of bread made from the dough stored under the C and G regimes was not significantly different ($p > 0.05$) but had a significant lower firmness than those stored under the VP and CC regimes. Increase in crumb firmness with storage was more pronounced when the temperature fluctuations during storage increased in magnitude. This effect may be due to the loss of moisture content during frozen storage. He and Hoseney (1990) found that moisture content significantly affected bread firming. Wang, Zhou, Yu, and Chow (2006) reported similar results. The increase in firmness was also probably related to the decrease in bread volume due to the weakened gluten strength and reduced yeast activity as storage time increased (Berglund et al., 1991; Inoue & Bushuk, 1992).

4. Conclusions

Freezing, frozen storage temperature and temperature fluctuations during storage generated loss of dough and bread quality as reflected by a lower CO_2 production, yeast viability and bread specific volume and increased bread crumb firmness and dough weight loss. The rates of quality and weight loss were significantly greater when temperature fluctuations were more extreme and/or storage temperatures were higher. Due to the fact that temperature fluctuations are unavoidable, it is suggested that temperature variations should be kept minimum or not more than ±3 °C.

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References

American Association of Cereal Chemists (2000). *Approved methods of the AACC* (10th ed.). St. Paul, MN: The Association.

Aibara, S., Nishimura, K., & Esaki, K. (2001). Effects of shortening on the loaf volume of frozen dough bread. *Food Science and Biotechnology, 10*, 521–528.

Alvarez, M. D., & Canet, W. (1998). Effect of temperature fluctuations during storage on the quality of frozen potato tissue (cv. Monalisa). *Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 206*, 52–57.

Autio, K., & Sinda, E. (1992). Frozen doughs: Rheological changes and yeast viability. *Cereal Chemistry, 69*, 409–413.

Ben-Yoseph, E., & Hartel, R. W. (1998). Computer simulation of ice recrystallization in ice cream during storage. *Journal of Food Engineering, 38*, 309–329.

Berglund, P. T., & Shelton, D. R. (1993). Effect of frozen storage duration on firming properties of breads baked from frozen doughs. *Cereal Foods World, 38*, 89–90, 92–93.

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

Cauvain, S. P. (1998). Dough retarding and freezing. In S. P. Cauvain & L. S. Young (Eds.), *Technology of breadmaking* (pp. 149–179). London: Blackie Academic & Professional.

El-Hady, E. A., El-Samahy, S. K., Seubel, W., & Brummer, J.-M. (1996). Change in gas production and retention in non-prefermented frozen wheat doughs. *Cereal Chemistry, 73*, 472–477.

Gormley, R., Walshe, T., Hussey, K., & Butler, F. (2002). The effect of fluctuating vs. constant frozen storage temperature regimes on some quality parameters of selected food products. *Lebensmittel-Wissenschaft und -Technologie – Food and Science and Technology, 35*, 190–200.

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

He, H., & Hoseney, R. C. (1990). Changes in bread firmness and moisture during long-term storage. *Cereal Chemistry, 67*, 603–605.

Hino, A., Takano, H., & Tanaka, Y. (1987). New freeze-tolerant yeast for frozen dough preparations. *Cereal Chemistry, 64*, 1987.

Hsu, K. H., Hoseney, R. C., & Seib, P. A. (1979). Frozen dough. II. Effects of freezing and storage conditions on the stability of yeasted doughs. *Cereal Chemistry, 56*, 424–426.

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

Kadan, R. S., Robinson, M. G., Thibodeaux, D. P., & Pepperman, A. B. Jr., (2001). Texture and other physicochemical properties of whole rice bread. *Journal of Food Science, 66*, 940–944.

Kline, L., & Sugihara, T. F. (1968). Factors affecting the stability of frozen bread doughs. I – Prepared by the straight dough method. *The Bakers Digest, 42*, 44–46, 48–50.

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

Laguerre, O., & Flick, D. (2007). Frost formation on frozen products preserved in domestic freezers. *Journal of Food Engineering, 79*, 124–136.

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

Mazur, P. (1970). Cryobiology: The freezing of biological systems. *Science, 168*, 939–949.

Mazur, P. (1976). Mechanisms of injury in frozen-thawed cells cooled at suboptimal and supraoptimal rates. *Cryobiology, 13*, 645.

Mazur, P., & Schmidt, J. J. (1968). Interactions of cooling velocity, temperature and warming velocity on the survival of frozen and thawed yeast. *Cryobiology, 5*, 1–17.

Naito, S., Fukami, S., Mizokami, Y., Ishida, N., Takano, H., Koizumi, M., et al. (2004). Effect of freeze–thaw cycles on the gluten fibrils and crumb grain structure of breads made from frozen doughs. *Cereal Chemistry, 81*, 80–86.

Neyreneuf, O., & Delpuech, B. (1993). Freezing experiments on yeasted dough slabs. Effects of cryogenic temperatures on the baking performance. *Cereal Chemistry, 70*, 109–111.

Phimolsiripol, Y., Siripatrawan, U., Tulyathan, V. & Cleland, D.J. (2006). The effect of temperature fluctuations on the quality of frozen bread dough. In *Proceeding of the IIR-IRHACE conference on Innovative Equipment and Systems for Comfort and Food Preservation* (pp. 559–565). Auckland, New Zealand.

Ribotta, P. D., Leon, A., & Anon, M. C. (2001). Effect of freezing and frozen storage of dough on bread quality. *Journal of Agricultural and Food Chemistry, 49*, 913–918.

Salas-Mellado, M. M., & Chang, Y. K. (2003). Effect of formulation of quality of frozen bread dough. *Brazilian Archives of Biology and Technology, 46*, 461–468.

Sharadanant, R., & Khan, K. (2003). Effect of hydrophilic gums on the quality of frozen dough: II. Bread characteristics. *Cereal Chemistry, 80*, 773–780.

Stauffer, C. E. (1993). Frozen dough production. In B. S. Kamel & C. E. Stauffer (Eds.), *Advances in baking technology* (pp. 88–106). New York: VCH Publishers.

Teunissen, A., Dumortier, F., Gorwa, M.-F., Bauer, J., Tanghe, A., Loïez, A., et al. (2002). Isolation and characterization of a freeze-tolerant diploid derivative of an industrial baker's yeast strain and its use in frozen doughs. *Applied and Environmental Microbiology, 68*, 4780–4787.

Varriano-Marston, E., Hsu, K. H., & Mahdi, J. (1980). Rheological and structural changes in frozen dough. *The Bakers Digest, 54*, 32–34, 41.

Wang, R., Zhou, W., Yu, H.-H., & Chow, W.-F. (2006). Effects of green tea extract on the quality of bread made from unfrozen and frozen dough processes. *Journal of the Science of Food and Agriculture, 86*, 857–864.

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