EFFECTS OF CRYOSTABILIZERS AND CRYOPROTECTANT ON FROZEN CHINESE STEAMED BREAD DOUGH

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2011

EFFECTS OF CRYOSTABILIZERS AND CRYOPROTECTANT ON FROZEN CHINESE STEAMED BREAD DOUGH

by

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Thesis submitted in fulfillment of the requirements for the degree of Master of Science

July 2011

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere appreciation to my main supervisor, Dr. Cheng Lai Hoong for her invaluable guidance, support, and encouragement throughout the research works and writing of this thesis. Besides, I would like to thank my co-supervisors, Assoc. Prof. Dr. Noor Aziah Abdul Aziz and Prof. Teng Tjoon Tow. Their continuous support, suggestions and insightful comments are much indeed appreciated.

Deep appreciation is extended to all the laboratory staffs for their advices and helps dealing with the apparatus, equipments, and laboratory work. Besides, I wish to express sincere gratitude to my labmates and friends for their encouragement, advices and valuable helps.

Special thanks go to Kuok Foundation and Malayan Sugar Manufacturing Co. Bhd for providing funding for the research through a grant (304/PTEKIND/650441/K132). I would also like to acknowledge Institute of Postgraduate Studies, Universiti Sains Malaysia for fellowship support.

Last but not least, I'm deeply indebted to my beloved family for their understanding, patience, endless support and encouragement throughout my life.

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LIST OF SYMBOLS AND ABBREVIATIONS

%SR	Percentage of stress relaxation
ΔH	Heat enthalpy
$\Delta H_{1,2}$	Heat enthalpy difference between freeze-thaw cycles
A	Work applied to stretch dough
ALG	Sodium alginates
CMC	Carboxymethylcellulose
CSB	Chinese steamed bread
DMA	Dynamic Mechanical Analysis
DSC	Differential Scanning Calorimetry
EU	Extensograph Unit
Ext	Extensibility
F_o	Initial force
F_t	Decaying force after t time
FU	Farinograph Unit
G’	Storage modulus
G”	Loss modulus
HMW subunits	High molecular weight glutenin subunits
Hz	Hertz
k_1	Intercept of normalized graph (Constant)
k_2	Gradient of normalized graph (Constant)
KGM	Konjac glucomanan
LBG	Locust bean gum
LMW subunits	Low molecular weight glutenin subunits

Mal	Maltodextrin
MTI	Mixing tolerance index
M_W	Molecular weight
NSP	Non-starch polysaccharide
PSY	Psyllium husk powder
R_{50}	Resistance at constant deformation
R_{50}/Ext	Ratio of resistance at constant deformation to extensibility
R_{max}	Maximum resistance
R_{max}/Ext	Ratio of maximum resistance to extensibility
tan δ	Loss tangent
T_{bw}	Boiling point
T_g	Glass transition temperature
$T_{g,midpoint}$*	Midpoint glass transition temperature
T_g'	Sub-zero glass transition temperature
T_{gs}	Glass Transition temperature of dry solids
T_m	Melting temperature
T_m'	Onset ice-melting temperature in a maximally freeze-concentrated solution
T_{ms}	Melting point of dry solids
T_o	Onset temperature
T_p	Peak temperature
Tre	Trehalose
T_S	Storage temperature
T_u	Eutectic point

T_β	β-transition temperature
W_g'	Unfrozen water at sub-zero glass transition temperature
WLF	Williams-Landel-Ferry
X_s	Solids content
ΔT	Temperature difference between sub-zero glass temperature and storage temperature

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KESAN KRIO-PENSTABIL DAN KRIO-PEMELIHARA TERHADAP DOH PAU SEJUKBEKU

ABSTRAK

Dalam kajian ini, pelbagai gam makanan (natrium alginat, karboksimetil selulosa, gam kacang lokus, serbuk sekam psyllium dan glukomanan konjak), maltodekstrin dan trehalosa telah ditambah untuk mengawet doh pau sejukbeku. Sifat-sifat pengadunan dan tensil doh telah dianalisa masing-masing dengan menggunakan "farinograph" dan "extensograph". Differensial pemindaian kalorimetri (DSC) digunakan untuk mengetahui sifat-sifat termal doh terhadap penyimpanan sejukbeku dan kitaran sejukbeku-cair. Analisis dinamik mekanikal (DMA) digunakan untuk menentukan sifat dinamik mekanikal doh. Sifat-sifat pau yang disediakan juga diukur dari segi (i) nisbah penyebaran, (ii) isipadu spesifik, (iii) kekerasan pau dan (iv) sifat-sifat pengurangan tekanan remah pau. Sifat reologi doh adalah didapati bergantung pada jenis dan tahap penggunaan gam makanan yang ditambah. Keseimbangan antara ketegangan dan kekenyalan doh amat penting untuk memastikan supaya pau yang diperoleh mempunyai nisbah penyebaran dan isipadu spesifik yang memuaskan. Tahap penambahan gam makanan yang tinggi didapati mengurangkan indeks penghapakan, nisbah penyebaran dan isipadu spesifik pau. Gam makanan memendekkan jurang antara suhu transisi kaca "sub-zero" (T_g') dan suhu penyimpanan. Ini mungkin menunjukkan implikasi yang praktikal di mana fasa yang tidak sejukbeku dapat dilindungi daripada degradasi fizikal rangkaian gluten dengan lebih baik. Kestabilan pengadunan doh berkurangan dengan peningkatan tahap penambahan gam makanan. Ketegangan doh meningkat sedikit pada permulaan tempoh penyimpanan sejukbeku. Namun, suatu penurunan yang ketara

berlaku dengan peningkatan tempoh penyimpanan sejukbeku. Tambahan pula, pau yang disediakan daripada doh sejukbeku menunjukkan pengurangan secara progresif dari segi nisbah penyebaran, isipadu spesifik dan sifat kekenyalan rembah pau manakala kekerasan pau meningkat. Antara gam makanan yang dikaji, penambahan sekam psyllium pada tahap 0.2% menunjukkan sifat-sifat doh dan pau yang lebih baik. Selain itu, doh yang ditambah dengan 0.1% trehalosa dan 2% maltodekstrin memberikan kualiti doh sejukbeku dan pau yang lebih baik.

EFFECTS OF CRYOSTABILIZERS AND CRYOPROTECTANT ON FROZEN CHINESE STEAMED BREAD DOUGH

ABSTRACT

In this study, different food gums (sodium alginates, carboxymethylcellulose, psyllium husk powder, locust bean gum, and konjac glucomannan), maltodextrin, and trehalose were added to preserve frozen Chinese steamed bread (CSB) dough. Dough mixing and stretching properties were analyzed using farinograph and extensograph, respectively. Differential scanning calorimetry (DSC) was used to study the thermal properties of dough upon frozen storage and freeze-thaw cycles. Dynamic mechanical analysis (DMA) was employed to determine the dynamic mechanical properties of dough. In addition, CSB prepared were characterized in terms of (i) spread ratio, (ii) specific volume, (iii) firmness of CSB and (iv) stress relaxation properties of CSB crumb. Dough rheological behaviors were found to be dependent on the types and usage level of the food gums added. A balance between dough resistance and extensibility is important to ensure acceptable spread ratio and specific volume of CSB. High addition level of food gums reduced staling index, spread ratio and specific volume of CSB. On the other hand, food gums brought the difference between sub-zero glass transition temperature (T_g') and storage temperature closer. This may have a practical implication whereby the unfrozen phase could be better protected against physical degradation of the gluten network. The mixing stability of dough was decreased with increasing food gums addition level. Dough resistance was increased slightly at the initial frozen storage period. However, it exhibited a significant decrease with extended frozen storage period. In addition, CSB prepared from frozen dough showed progressively decreased in spread ratio, specific volume and elastic properties of bread crumb while the firmness of

CSB was increased. Among the food gums studied, addition of psyllium husk at 0.2% level showed better dough and CSB characteristics. Apart from this, dough with 0.1% trehalose and 2% maltodextrin produced better quality frozen dough and CSB.

CHAPTER 1

INTRODUCTION

1.1 Background and rationale

Asian foods are gaining popularity worldwide. Chinese steamed bread (CSB) is one of the Asian delicacies that serves as a staple food for the people resides in China, especially in the Northern region. However, CSB is now available in several Southeast Asian countries as well as Western countries due to food culture intercommunication (Su *et al.*, 2005). Evidently, demand for Asian cuisine is growing high and shows potential for a global market (Yue & Rayas-Duarte, 1997; Te Yeh *et al.*, 2009). This has created an urgent need for better preservation method that would provide a larger distribution network in order to cater for the increasing demand for Asian foods, such as CSB. In this regard, freezing technology could be the solution as it helps to preserve food texture, nutrition, color, and flavor (Zaritzky, 2000; Hsu *et al.*, 2003).

Several works have been carried out to study the properties of CSB prepared from different variety of wheat flours (Addo *et al.*, 1991; Huang *et al.*, 1996; Koike *et al.*, 1998; Lijuan *et al.*, 2007). He *et al.* (2003) determined the suitability of different Chinese wheat cultivars to make Northern style CSB and compared the quality of CSB which prepared under manual and mechanized processing methods. In addition, Rubenthaler *et al.* (1990), Huang *et al.* (1998), and Kim *et al.* (2009) studied the effects of processing parameters such as dough fermentation time, proofing time, and sheeting on the CSB properties. A number of works on the effects of ingredients on dough and CSB properties have been reported. The ingredients studied are waxy flour (Qin *et al.*, 2007), enzyme (Su *et al.*, 2005; Jiang *et al.*, 2010),

salt-stressed baker's yeast (Te Yeh *et al.*, 2009), and diacetyl tartaric acid ester of monoglycerides (Xiujin *et al.*, 2007; Bi *et al.*, 2009).

Demand in ready-to-eat CSB products is increasing due to the fact of convenience (Wen *et al.*, 1996; Huang, 1999). However, CSB is normally served hot whereas re-steaming can affect the eating quality of CSB (Wen *et al.*, 1996; Fu *et al.*, 2005; Hou & Popper, 2007). Furthermore, frozen CSB requires more spaces for packaging if compared to those of frozen CSB dough as its size becomes bigger after proofing and steaming. Small size of packaging is preferable for easier transportation. In view of these facts, frozen CSB dough could be an alternative to ensure better eating quality and facilitates transportation. In addition, frozen dough can be manufactured in a centralized factory and distributed to retail outlets. This makes fresh product available throughout the day and quality of the product can be controlled easily. This directly helps to reduce the production costs (Asghar *et al.*, 2005; Yi & Kerr, 2009).

Despite the convenience and economic advantages of frozen dough, there are several quality issues that need to be addressed in order to ensure good quality attributes of finished product (Giannou *et al.*, 2003). These include long proofing time, low specific volume, unacceptable crumb texture, and loss of dough performances (Kenny *et al.*, 1999). Freezing and frozen storage caused decreased number of viable yeast cells and hence a decline in gassing power. In addition, the variable capability of the gluten network to retain carbon dioxide arised as a result of the gradual loss of dough strength (Räsänen *et al.*, 1997b; Ribotta *et al.*, 2004; Selomulyo & Zhou, 2007). These are attributable to the fact that water in foods is not entirely frozen at subfreezing temperature (Kobs, 1997). The frozen wheat dough consists of an ice phase and an unfrozen phase with a lower freezing point owing to

the freeze concentration of the solutes in which water is removed as ice crystals. Within this unfrozen phase, deteriorations are able to take place even at very low temperatures (Ribotta & Le Bail, 2007).

During frozen storage, ice crystal growth and recrystallization will inflict deterioration in food matrix and temperature gradients within a frozen stored product may cause moisture migration and leads to freezer burn, weight loss, as well as frost formation on product surface (Kobs, 1997; Hui *et al.*, 2004; Reid & Perez-Albela Saettone, 2006). In addition, subsequent to freezing, many solutes may be supersaturated within the unfrozen phase. Eventually, these materials may recrystallize or precipitate out causing an alteration in the ionic strength within the unfrozen phase and affecting the stability of other molecules. For instance, "salting out" of protein leads to protein denaturation as a result of increase in ionic strength (Kobs, 1997; Hui *et al.*, 2004). The aforementioned situation becomes worse when the food matrix is subjected to temperature fluctuations during storage and transportation, which is believed to be a commonplace (Berglund *et al.*, 1991; Kobs, 1997; Bail *et al.*, 1999; Reid & Perez-Albela Saettone, 2006).

Stability of a frozen food product is mainly relied on its storage temperature. It is of critical importance to store a food product below the glass transition temperature (T_g') of its maximally freeze-concentrated phase or manipulate the food formulation in order to elevate its T_g' so that it is above the practical freezer storage temperature but below the ice melting temperature (Slade & Levine, 1991; Goff, 1992; Torreggiani *et al.*, 1999). When a frozen food is maintained at a temperature below its T_g', it is believed to be comprised of ice phase and a freeze-concentrated phase present in a glassy state. With that, long shelf-life is anticipated as the frozen food is subjected to less detrimental changes such as ice recrystallization and

moisture distribution (Fennema, 1996; Zaritzky, 2008). Nevertheless, from an economical point of view, it is perhaps not a practical way to store frozen food in the glassy state because low frozen storage temperature is required. On the other hand, modification on food formulation in order to increase the T_g' has its limitation due to the fact that undesirable sensory characteristics might be imparted by some of the high molecular weight stabilizers. In view of this, the temperature range between T_g' and the onset of ice melting in turn becomes important and hence the Williams-Landel-Ferry (WLF) kinetics has been well accepted in studying the frozen food stability (Goff, 1992).

Lo & Ramsden (2000) stated that food gums commonly serves as additives to control the shelf-life stability and texture of frozen food products. Food gums when incorporated into frozen foods are able to minimize the negative effects of freeze-thaw cycles as it has high water holding capacity and helps to control water migration and also dough rheology (Kobs, 1997; Mandala *et al.*, 2007). Immobilization of water would lessen the formation of ice crystals and consequently prevent gluten network deterioration, decrease in frozen dough strength as well as injure of yeast cells (Sharadanant & Khan, 2003a).

In addition, food gums have been used to retard the baked goods from staling and improve the quality of the fresh produce (Bárcenas & Rosell, 2005), and to enhance frozen dough shelf-life (Asghar *et al.*, 2007). Incorporation of food gums into frozen dough helps to improve moisture retention of bread. In addition, the growth of ice crystals and water mobility within wheat dough system during frozen storage can be controlled (Shon *et al.*, 2009). Several studies have been carried out on the effects of food gums on frozen dough stability as well as quality attributes of finished products made therefrom. Dodić *et al.* (2007) studied the effects of type and

concentration of hydrocolloids (such as xanthan gum, κ-carrageenan and carboxymethylcellulose) on rising time of dough, fermentation activity of yeast and characteristics of final product as a function of frozen storage time of dough. Sharadanant & Khan (2003b) evaluated the effectiveness of hydrophilic gums, namely Arabic gum, carboxymethylcellulose, κ-carrageenan, and locust bean gum in reducing ice crystallization and recrystallization in frozen dough. Matuda *et al.* (2008) reported a work on the addition of guar gum and xanthan gum on proofing rate and thermal properties of frozen dough. In addition, Asghar *et al.* (2007) investigated the influence of Arabic gum and carboxymethylcellulose on dough rheology and baking quality of pizza made from frozen dough. Studies on the effects of food gums on frozen dough with the combination of other ingredients such as emulsifier and milk protein were reported by Ribotta *et al.* (2004) and Shon *et al.* (2009), respectively.

Most of the works on this issue have been reported on high protein wheat dough system. However, effects of food gums on medium protein wheat dough system which is more suitable for making of CSB is relatively scarce (Bi *et al.*, 2009). Furthermore, type and usage level of food gums affect the dough and bread characteristics to different extent (Sharadanant & Khan, 2003b; Guarda *et al.*, 2004; Mandala, 2005; Dodić *et al.*, 2007). There are also limited studies on frozen CSB dough stability and characteristics of finished product prepared therefrom.

In this work, wheat dough formulation was manipulated with the addition of cryostabilizers (food gums and 10 DE maltodextrin) and cryoprotectant (trehalose). Food gums and maltodextrin serve as cryostabilizers which could help to increase T_g' of a food system due to its high molecular weight. Addition of food gums into wheat flour mixture has been reported to show potential to improve texture and freeze-thaw stability besides serving as an anti-staling agent (Guarda *et al.*, 2004). It was reported

that food gums could minimize the deteriorative effects on starch and gluten during frozen storage due to their hygroscopicity (Asghar *et al.*, 2007). Maltodextrin also has been reported to show anti-staling properties (Martin & Hoseney, 1991; Defloor & Delcour, 1999). On the other hand, cryoprotectant possesses lower T_g' and help to induce high amount of unfrozen water (W_g') which is useful in conferring a desirable soft-frozen texture. The soft-frozen texture was ascribed to the high W_g' that indicates a lower amount of ice and a larger temperature difference between T_g' and the frozen storage temperature that reflects the relatively high degree of softening of the non-ice fraction in the food matrix (Slade & Levine, 1991). It was hypothesized that addition of cryostabilizers and cryoprotectant into frozen CSB dough would help in reducing the temperature difference between T_g' and storage temperature as well as provide a soft-frozen texture. Thus, storage stability of frozen CSB dough can be improved.

1.2 Objectives

The present study dealt with the development and storage study of frozen Chinese steamed bread (CSB) dough and the characterization of CSB made therefrom in terms of physical properties, thermal properties and storability. The general objective was to produce a good quality and stable CSB dough system that can withstand physical and chemical changes that occurred during frozen storage.

The specific objectives were as follows:

a. To determine the effects of selected food gums at low addition level on wheat dough rheological properties and the possibility of retarding staling and enhancing shelf-life of CSB stored at room temperature.

b. To evaluate the thermal and dynamic mechanical properties of frozen wheat dough with the addition of selected food gums.
c. To assess the storage stability of frozen CSB dough added with cryostabilizers and cryoprotectant at practical freezer storage temperature (-18 ℃).

1.3 Research protocol

The experimental work for this study has been divided into four main phases as listed below:

a. The effects of selected food gums on physical properties of medium protein dough and Chinese steamed bread (CSB) made therefrom.

b. The effects of selected food gums on thermal and dynamic mechanical properties of frozen dough.

c. Characterization of physical properties of dough added with locust bean gum or psyllium husk powder as a function of addition levels.

d. The effects of trehalose and maltodextrin addition on physical properties of CSB made from fresh dough and frozen dough.

Although several studies have been carried out on the effects of food gums in bakery products, the outcomes are unpredictable due to different addition levels of food gums on different variety of wheat flours (Armero & Collar, 1996; Mandala, 2005; Tavakolipour & Kalbasi-Ashtari, 2007). In addition, food gums exist in different chemical structures and can be extracted from different sources. Therefore, at the preliminary stage, five food gums were selected to study their effects on physical properties of medium protein dough and CSB made therefrom. The selected

food gums were sodium alginates, carboxymethylcellulose, konjac glucomannan, locust bean gum, and psyllium husk powder. The selection was in order to cover a broad range of food gums of different chemical structures and functionalities. The addition levels were fixed at 0.2 and 0.8% w/w for screening purposes to cover both the highest and lowest limits use of the food gums.

Characterization of rheological behaviors of wheat flour dough is the key step in determining the dough mechanical handling properties, dough stability during processes such as proofing and baking or steaming as well as quality of the end products (Stojceska *et al.*, 2007). In the first phase of the work, empirical rheological tests on wheat dough were carried out using farinograph and extensograph. Farinograph test determines the mixing properties of flour while extensograph test provides information about dough extensibility and resistance towards stretching (Stojceska *et al.*, 2007). In addition, the effects of food gums on CSB characteristics were assessed in terms of specific volume, spread ratio and staling index upon storage at room temperature. This is in order to investigate the effects of food gums on medium protein wheat dough rheological properties as well as their anti-staling properties on CSB.

Ice crystallization happens during freezing of food matrix, giving rise to alterations in water distribution, structure and function of biochemical constituents. Upon freezing and thawing cycles, the dough is left exposed to various detrimental stresses as a result of ice crystallization and recrystallization that can cause gradual decrease in the strength of dough network as well as yeast viability and gassing power. This leads to deterioration of end product quality (Sharadanant & Khan, 2003a; Dodić *et al.*, 2007). Furthermore, knowledge of the phase and state transitions taking place in doughs during freezing and frozen storage as well as the effects of

food ingredients on dough properties is essential to ensure premium quality wheat dough is produced (Laaksonen & Roos, 2001). Therefore, in the second phase, thermal and dynamic mechanical properties of frozen CSB dough were determined by means of differential scanning calorimetry and dynamic mechanical analysis, respectively. These analyses were done to evaluate the effectiveness of different food gums addition on controlling ice crystallization and recrystallization in frozen wheat dough upon freeze-thaw cycles, and also the possibility to reduce the temperature difference between sub-zero glass transition temperature, T_g' of the dough and the practical frozen storage temperature (-18 ℃).

Changes in water distribution, moisture loss and ice recrystallization might take place in food matrix during extended frozen storage and freeze-thaw cycles (Hui *et al.*, 2004; Xu *et al.*, 2009). As a result, deterioration of dough structures might occur. Food gums is believed to be able to help in solving this problem due to its high hygroscopicity as well as ability to develop a better gluten network. Based on the first phase results, two food gums that have been selected were locust bean gum and psyllium husk powder. Therefore, in the third phase, farinograph and extensograph tests were performed to determine the effects of psyllium husk powder and locust bean gum addition on mixing properties of wheat flour and extensographic properties of wheat dough upon frozen storage. The tests were carried out as a function of addition levels of food gums (0.2, 0.4, 0.6 and 0.8% w/w). The purpose is to select the most suitable food gum and its addition level to be applied in frozen CSB dough for the later stage of the study.

Storage study is important in food product development as it provides a reliable estimation of the quality deterioration of a food product that might take place at a real storage condition. In the last phase, storage study was done to assess the

effects of selected cryostabilizers and cryoprotectant on frozen CSB dough prepared. Based on the previous results, psyllium husk powder at 0.2% w/w addition level was used. Storage study at freezing temperature up to 5 months was carried out on dough added with 0.2% psyllium husk powder in the presence of trehalose and maltodextrin at different addition levels.

As aforementioned in section 1.1, trehalose serves as a cryoprotectant and is able to confer soft-frozen texture to a food matrix while maltodextrin has a potential to serve as an anti-staling agent as well as a cryostabilizer. Binary mixtures of these two ingredients at two different levels each were varied in this study to get a better insight of the possible synergistic effect between the two ingredients in stabilizing frozen CSB dough. Where necessary, univariate general linear model procedure was then used to compare significant difference between results. Furthermore, excessive formulations was purposely avoided in this phase so that sufficient samples for storage study of all formulations can be prepared from the same batch of yeast and the yeast was used within a week of receipt. Physical properties of CSB prepared from fresh dough and frozen dough were characterized in terms of spread ratio, specific volume, staling index and stress relaxation properties.

CHAPTER 2

LITERATURE REVIEWS

2.1 Wheat dough

Wheat flour dough is a product developed after mixing of wheat flour, water, salt and other ingredients (Peressini *et al.*, 2008). The development of gluten matrix and the dough quality is governed by both the covalent and non-covalent bonds interactions (Peressini *et al.*, 2008). Gluten strands form the continuous matrix and starch granules are embedded within the gluten network to serve as filler (Angioloni & Collar, 2009b). The dough performances such as viscoelasticity or gas holding capability are largely relied on the composition of wheat flour and its characteristics, the amount of water used, the type of additives added as well as the processing conditions employed (Peressini *et al.*, 2008; Asghar *et al.*, 2009).

Processing steps involved in breadmaking are found to alter the rheological behaviors of wheat flour dough. In the mixing stage, wheat flour in the presence of water is transformed into a viscoelastic dough mass upon shear and extensional deformations to develop gluten. During fermentation and proofing stage, with the expansion of air bubbles, dough matrix is aerated and this will help to produce a foamed crumb structure. Baking converts the dough into an elastic bread to give the final loaf volume and bread texture (He & Hoseney, 1991; Autio & Laurikainen, 1997; Shah *et al.*, 1998; Collar & Bollaín, 2005; Angioloni & Collar, 2009b). Since wheat dough is a viscoelastic material, the rheological properties of the dough are associated with dough proofing time, loaf volume, and quality attributes of the end product (Kenny *et al.*, 1999; Angioloni & Collar, 2009b).

2.1.1 Composition of wheat flour

Protein and starch are the two main components of wheat flour, which are present in the range of 8 - 15% and 62 - 75%, respectively on a dry basis depends on the wheat variety. In general, wheat flour contains 72 - 80% starch, 10 - 15% protein, some dietary fiber, lipid, and ash (Khan & Nygard, 2006).

(a) Starch

Starch, presents as semi-crystalline granules, is the most important storage carbohydrates in plants (Goesaert *et al.*, 2005). Starch is the chief constituent of wheat flour in which it is a polymer composed of α-D-glucose, a ring-shaped molecule that contains six carbon atoms (Murphy, 2000; Yoo & Jane, 2002). Starch primarily make up by two types of glucose polymers, long linear amylose and highly branched amylopectin (Figure 2.1) (Hoseney, 1986; Belderok, 2000b; Murphy, 2000; Hu *et al.*, 2010). Amylose is a polymer of glucopyranose units linked by α-D-(1→4)-glucosidic bonds, while branching of this linear amylose chains through α-D-(1→6)-glycosidic bonds produce the highly branched amylopectin (Murphy, 2000; Hu *et al.*, 2010). The amorphous parts of starch granules are formed by amylose and the branching points of amylopectin, whereas the major crystalline part is consisted of the short branching chains in amylopectin (Liu *et al.*, 2010). The ratio of these two polysaccharides is different depends on the botanical source of starch. Normal starches, waxy starches, and high amylose starches consist of 20 – 35%, less than 15%, and more than 40% of amylose, respectively (Tester *et al.*, 2004). The chain length of amylose varies with botanical source of starch, usually comprised of 500 to 6000 glucose units (Murphy, 2000). Whereas each unit chain in amylopectin just have 20 - 25 glucose units. However, numerous branching of amylopectin

contributes to its high molecular weight of about 10^8 (Hoseney, 1986; Belderok, 2000b; Murphy, 2000).

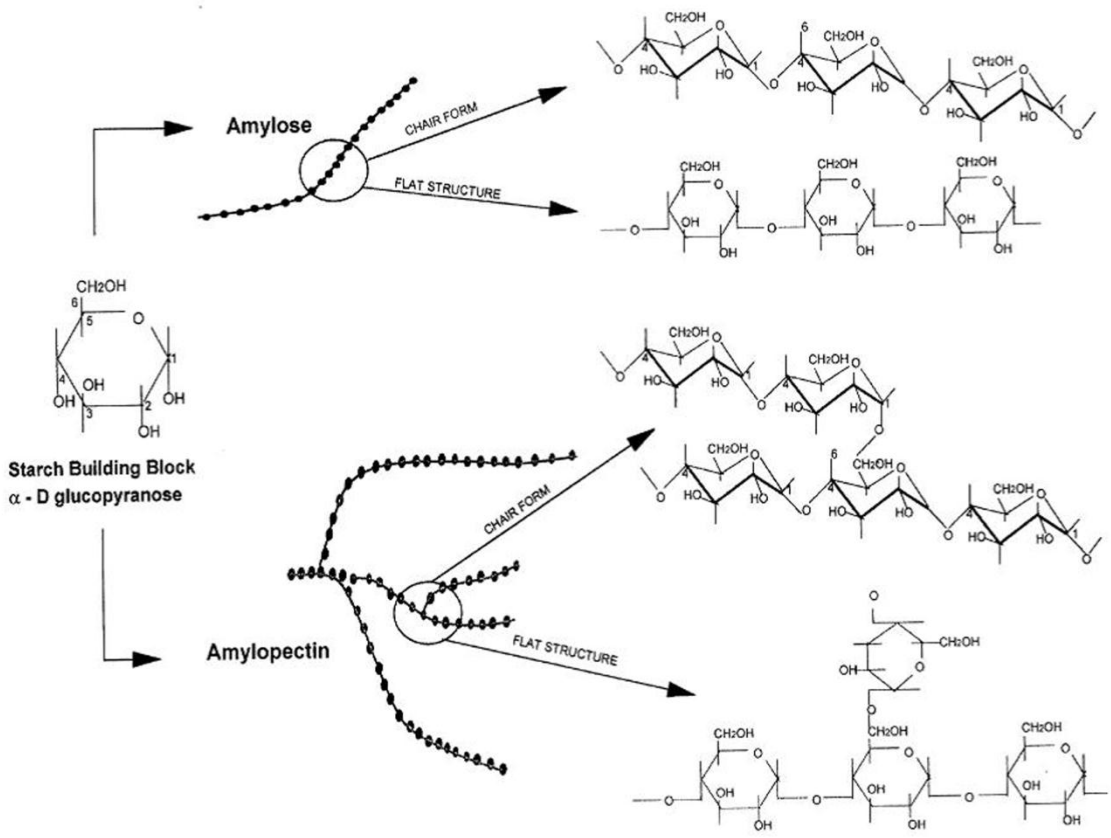

Figure 2.1 Structure of amylose and amylopectin of starch. (adapted from: Murphy (2000))

Some of the starch granules are crushed and damaged by the shearing action and pressure of the rollers during milling process. This produces damage starch (Hoseney, 1986; Belderok, 2000b). A certain quantity of damaged starch is required in wheat flour in order to have better breadmaking properties because the rate of water absorption of damaged starch in dough making is higher than that of undamaged starch. In addition, it can be degraded by amylolytic enzymes to form fermentable sugars such as dextrins and maltose sugars (Hoseney, 1986; Belderok, 2000b; Van Der Borght *et al.*, 2005).

(b) Protein

Wheat proteins can be categorized based on their solubility in solvents. Albumins are dissolve in water, globulins are soluble in dilute sodium chloride solutions but insoluble in pure water, gliadins are soluble in 70% ethyl alcohol, and glutenins are able to dissolve in dilute acid or alkali (Osborne, 1924; Belderok, 2000b). In general, proteins of wheat flour can be divided into two main groups, namely gluten proteins that consist of gliadins and glutenins, and non-gluten proteins that consist of albumins and globulins (Goesaert *et al.*, 2005; Van Der Borght *et al.*, 2005).

Gluten proteins are the major reserve proteins of wheat that cover about 80–85% of total wheat protein. They are present in the endosperm of a mature wheat grain and not found in germ or in seed coats (Belderok, 2000b; Goesaert *et al.*, 2005; Van Der Borght *et al.*, 2005). They are comprised of gliadins and glutenins (Goesaert *et al.*, 2005). Classification of gliadin and glutenin subunits in wheat flour is outlined in Figure 2.2. Glutenins made up of high molecular weight glutenin subunits (HMW subunits) and low molecular weight glutenin subunits (LMW subunits) which are bonded by disulphide bonds. Glutenins give elastic properties to dough (Verbruggen *et al.*, 2000). Gliadins can be separated into ω-gliadins, γ-gliadins and α/β-gliadins (Lindsay & Skerritt, 1999). The viscous characteristics of gluten network are attributed to gliadins (Khan & Nygard, 2006).

Gluten is the structure-building component in wheat flour dough that helps to produce desirable bread quality (Lindsay & Skerritt, 1999; Mariotti *et al.*, 2009). Gluten network is formed as a consequence of the interactions between monomeric gliadins and polymeric glutenins through both covalent and non-covalent linkages during mixing of wheat flour in the presence water (Lindsay & Skerritt, 1999; Khan

& Nygard, 2006; Bárcenas *et al.*, 2009; Mariotti *et al.*, 2009). Gluten possesses numerous disulphide bonds. It is these disulphide bonds that conferring rigidity and strength to the gluten network (Haegens, 2006). Glutenin subunits can be linked via disulphide bonds through both intra- and inter-chain interactions, whereas gliadins are only able to form inter-chain disulphide bonds (Lindsay & Skerritt, 1999).

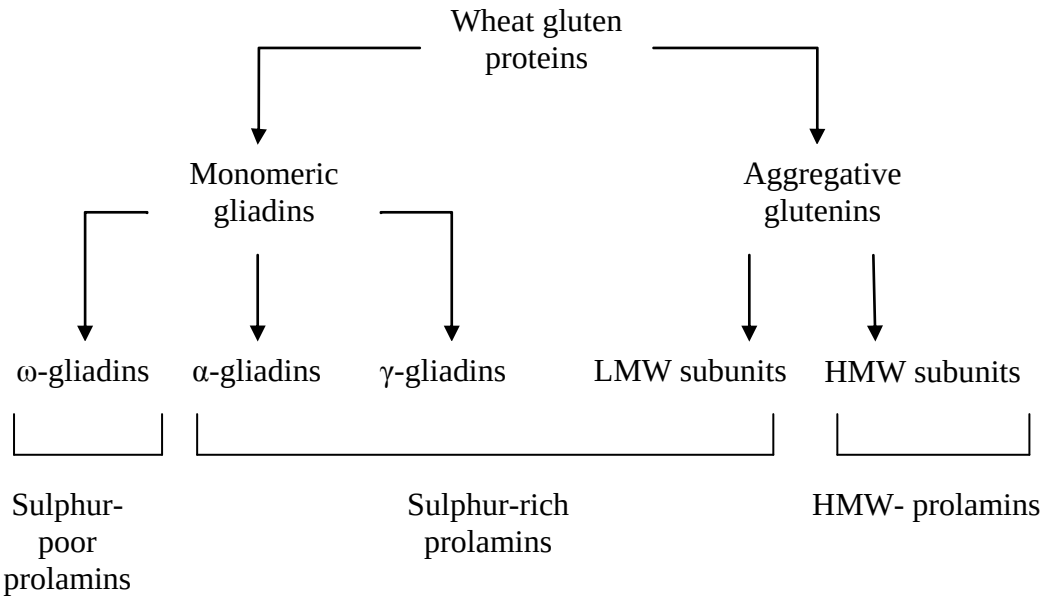

Figure 2.2 Classification of gliadin and glutenin subunits in wheat flour. (adapted from: Lindsay & Skerritt (1999))

A strong gluten network provides dough stability as its viscoelasticity is vital for dough structure as well as bread production (Hamer & Van Vliet, 2000; Kenny *et al.*, 2001). The viscoelastic properties of gluten confer an extensible dough with good gas retention properties and able to endure stresses during breadmaking (Lindsay & Skerritt, 1999; Gallagher *et al.*, 2004; Khan & Nygard, 2006; Mariotti *et al.*, 2009). The outcome is light and aerated bread products with good crumb grain. On the other hand, a gluten-free matrix without addition of other additive often gives rise to a

liquid batter rather than a viscoelastic dough. In addition, the finished products with poor crumb color and texture together with other post-baking quality defects are produced (Gallagher *et al.*, 2004; Khan & Nygard, 2006; Mariotti *et al.*, 2009).

Non-gluten proteins that cover approximately 15 - 20% of total wheat protein mostly located in the external layers of wheat kernel and with small amounts in endosperm. They are mainly monomeric proteins including albumins and globulins which are structural or physiologically active (Goesaert *et al.*, 2005; Van Der Borght *et al.*, 2005). Albumins are the smallest wheat proteins, followed by globulins (Belderok, 2000b). Besides that, non-gluten proteins also consist of some polymeric wheat storage proteins, known as triticins that belong to the globulin category of storage proteins in seed (Goesaert *et al.*, 2005).

(c) Other components

Non-starch polysaccharide (NSP) is a general term for arabinoxylans, arabinogalactan-peptides, β-glucan, and cellulose (Henry, 1985; Goesaert *et al.*). NSP is a minor constituent of wheat flour. It can be divided into water-soluble and water-insoluble component (Sasaki *et al.*, 2000).

Arabinoxylan is the major NSP present in wheat flour (Sasaki *et al.*, 2000). They are water-soluble and water-insoluble arabinoxylan (Goesaert *et al.*). Water-soluble arabinoxylan was shown to be able to improve the quality of dough and bread due to its viscosity and water-binding capability. However, addition of water-insoluble arabinoxylans reduces loaf volume (Sasaki *et al.*, 2000).

2.1.2 Breadmaking process

Conventional breadmaking process includes mixing and molding of wheat dough, proofing and steaming or baking of raising dough to obtain final bread products (Giannou *et al.*, 2003). The viscoelastic properties of dough depends on several aspects such as characteristics of wheat flour and other ingredients, temperature, energy input and duration of mixing, amount of water added and so on (Mirsaeedghazi *et al.*, 2008). Rheological behaviors of dough altered during every steps of the breadmaking process and hence will affect the characteristics of the finished products (Angioloni & Dalla Rosa, 2005).

(a) Dough mixing

The main idea of mixing is to hydrate and blend all the dough ingredients uniformly. Besides that, there are alignment and stretching motions to develop the three-dimensional dough matrix to obtain an optimum viscoelasticity for the retention and stabilization of gas cells in a dough system (Autio & Laurikainen, 1997; Martin *et al.*, 2004; Haegens, 2006; Lai & Lin, 2006; Rosell *et al.*, 2007).

A high shear rate during mixing assists the hydration process. This is due to the external layer of wheat flour particles was being removed once it becomes hydrated and consequently exposes another surface layer for hydration (Mirsaeedghazi *et al.*, 2008). In the mixing step, dough is subjected to both shearing and extensional actions in which the wheat proteins interact with each other. The development of wheat dough to a cohesive mass is happened along with an alteration in dough rheology and also a decrease in the size of the polymeric protein (Sutton *et al.*, 2000; Zheng *et al.*, 2000; Mirsaeedghazi *et al.*, 2008). This leads to the hydrated proteins forming gluten strands that aligned in the matrix and thus the resistance to

extension of the dough enhanced (Mirsaeedghazi *et al.*, 2008). Hence, aerated dough matrix with desired rheology and baking performance can be produced (Martin *et al.*, 2004).

Quality of wheat flour dough is depending on the mixing process. Dough handling or working behaviors will be altered when the dough was under-mixed or over-mixed (Haegens, 2006). According to Mirsaeedghazi *et al.* (2008), the protein molecules in a developed dough are mainly present in the unfolded form and are able to build cross-links at different positions, that including disulphide bonds through inter-chains interactions. As for the undeveloped dough, it is mostly made up of intra-chains disulphide bonds and non-covalent cross-links that may be broken down during subsequent mixing. Thus, a much bigger protein network may be built up with a fully developed dough that showed the greatest elasticity and rigidity, whereas the undeveloped dough shows less resistance to deformation (Mirsaeedghazi *et al.*, 2008).

Létang *et al.* (1999) proposed a molecular model to depict gluten development during dough mixing. Figure 2.3 illustrates the mechanism involved in the formation of wheat dough. At the initial phase of dough mixing process, glutenins are folded and the chains are in an unorganized orientation (Figure 2.3A). When the mixing continues, the glutenins align due to the shear and stretching forces imparted (Figure 2.3B). Gluten network becomes more developed due to the cross-linkages between protein strands with disulphide bonds and the dough strength increased (Létang *et al.*, 1999).

Over-mixing of dough will result in weakening of protein network (Figure 2.3C). Breaking of disulphide linkages that join the polypeptide subunits will occur. Dough strength will be decreased as a consequence of the mechanical shear stress

during mixing (Rosell *et al.*, 2007). The glutenin particles which are vital for quality of breadmaking are then partially depolymerized and separated into smaller fragments that act like free polymers. As a consequence, stickier dough is formed as compared to optimally developed wheat dough (Chu, 1989; L étang *et al.*, 1999; Don *et al.*, 2005; Rosell *et al.*, 2007).

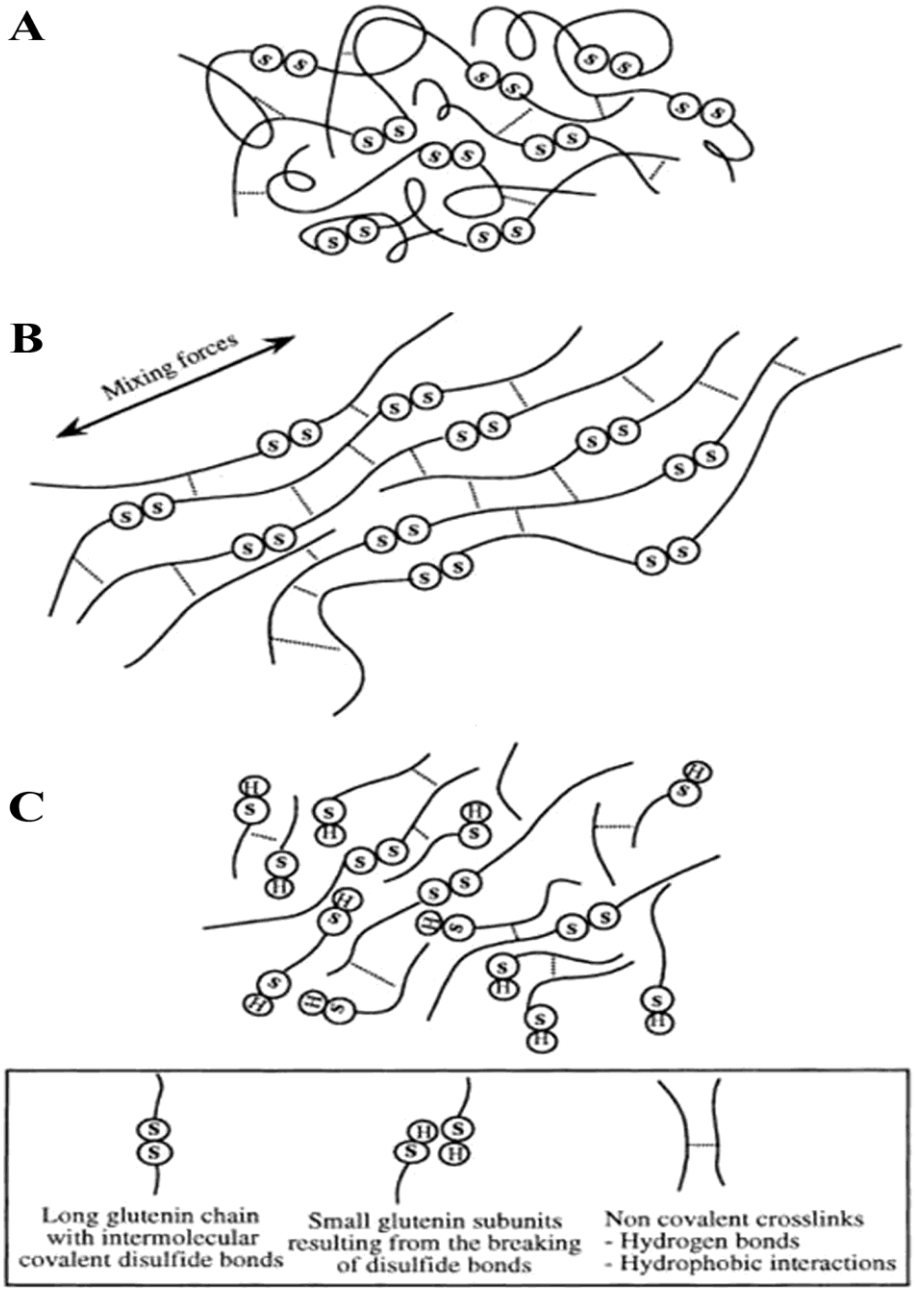

Figure 2.3 Gluten development (A) Initial stage of mixing, (B) Optimum gluten development, (C) Over-mixing. (adapted from: L étang *et al.* (1999))

(b) Dough proofing

During dough proofing, gas bubbles in a dough mass will be expanded due to carbon dioxide gas are produced by yeasts (Shah *et al.*, 1998; Matuda *et al.*, 2008). The growth of gas bubbles in dough during proofing are depending on the rate of carbon dioxide gas generated by yeasts, the rate of carbon dioxide gas diffusion into bubble cells, the extent of retention of carbon dioxide gas in the dough mass, and the rate of coalescence of bubble cells (Shah *et al.*, 1998). Dough that undergoes proper proofing stage shows optimum rheological behaviors and dough handling properties. Bread with desirable loaf volume and bread crumb will be obtained (Giannou *et al.*, 2003), depending on the stabilization of gas cells as well as gas retention properties of the dough matrix (Matuda *et al.*, 2008).

(c) Dough steaming/baking

During baking or steaming of dough, gas production and evaporation take place in conjunction with the alteration in rheological behaviors of the dough. The dough with a foamed structure that contains disconnected gas bubbles is changed into a sponge structure of bread which possesses interconnected gas cells (Shah *et al.*, 1998; Rojas *et al.*, 2000; Giannou *et al.*, 2003). Flour microstructure is continuously changing in this process until the bread structure is fixed (Rojas *et al.*, 2000; Giannou *et al.*, 2003). The protein matrix of the dough is fixed due to the denaturation of proteins by heat. This contributes to the texture, crumb structure, loaf volume and shape of the bread products (Belderok, 2000b; Khan & Nygard, 2006).

2.2 Shortcomings of fresh bread and advantages of frozen dough

Shelf-life of fresh bread is usually short even after being packaged and frozen dough could serve as an alternative to ensure fresh bread is supplied daily (Ribotta *et al.*, 2003; Asghar *et al.*, 2009). During the storage period, chemical and physical changes such as staling take place. These render the bread quality deteriorated. As a result, bread loses its freshness as reflected in the loss of the pleasing aroma and flavor, and stale taste becomes evident. At the same time, an increase in bread crumb firmness occurred (Ribotta *et al.*, 2006; Sahi & Little, 2006). Moisture loss of bread loaf and moisture migration from the crumb to the crust can cause bread quality changes as well. Firmness of bread increases with time and it is mainly attributed to starch retrogradation (Sahi & Little, 2006).

Retrogradation refers to a process in which starch chains start to reassociate in an ordered structure. Initially, at least two starch chains form a simple junction point, after which may develop into a more ordered regions. Eventually, starch chains will organize to form a crystalline order under favorable circumstances (Thomas & Atwell, 1999). Retrogradation starts to occur when amylose released from starch granules during gelatinization in the steaming or baking process. Storage of bread for a day or more can result in a crystalline structure of the crumb as the consequence of retrogradation that possibly involves alterations in the physical arrangement of amylopectin. When the bread is subjected to reheating, the retrogradation is reversible but happens again once the bread was left to cool again (Lai & Lin, 2006).

There is increasing popularity for frozen dough owing to consumer demand for convenience and good quality products besides variety types of breads. Frozen dough technology alleviates the labor conditions in bakery industry and enables fresh

breads to be easily available to consumers (Takano *et al.*, 2002; Bhattacharya *et al.*, 2003; Momose *et al.*, 2010). It facilitates dough production in a centralized manufacturing factory and steaming or baking of final bread products at the retail outlets (Rouillé *et al.*, 2000; Filipovic & Filipovic, 2010). With that, bread can be prepared whenever required with no major needs of space and equipments or utensils (Kenny *et al.*, 1999; Yi *et al.*, 2009).

2.3 Deterioration of wheat dough upon freezing and frozen storage

Frozen dough refers to dough that is kept in a frozen condition right after mixing and molding or after proofing stage. Freezing technology has become increasingly popular in the bakery industry due to its economic advantages and convenience (Baier-Schenk *et al.*, 2005). Usage of frozen dough may negatively affect physicochemical properties of dough although it may lengthen dough products' shelf-life (Giannou & Tzia, 2008). From the economical point of view, the main challenge of frozen bakery products is that one must keep the doughs frozen during storage, during transportation until the point of thawing and baking (Ribotta *et al.*, 2006).

Long-term frozen storage period together with freeze-thaw cycles of dough may deteriorate bread quality. This is revealed by the variable performance of dough, such as an excessively long proofing duration, decrease in bread loaf volume, and poor crumb texture. These may affect dough machinability and cause a loss in consumers' satisfaction for the finished products (Kenny *et al.*, 1999; Lu & Grant, 1999b; Pepe *et al.*, 2005; Xu *et al.*, 2009; Filipovic & Filipovic, 2010). Two possible reasons to account for the deterioration of dough performance upon freezing or freeze-thaw cycles are the decrease in yeast viability and its subsequent reduced

gassing power, and the gradual weakening of dough structure (Räsänen *et al.*, 1997b; Ribotta *et al.*, 2004; Ribotta *et al.*, 2006). Damage of yeast cells causes decline in gassing power whereas destruction of dough structure affects gas retention properties (Varriano-Marston *et al.*, 1980).

At freezing and frozen condition, ice crystals present in dough matrix physically disrupt the dough structure and hence deteriorate the dough network as well as decrease in yeast viability (Räsänen *et al.*, 1997a; Räsänen *et al.*, 1998; Sahlström *et al.*, 1999). Besides deterioration of bread quality, these can lead to reduce in consumers' acceptability as well as economic disadvantage to manufacturers. Therefore, it is essential to know ways to preserve quality of frozen products (Xu *et al.*, 2009).

2.3.1 Yeast viability

Yeast is crucial for dough leavening, characteristic and taste of bread (Casey & Foy, 1995). High survival rate of the yeast cells is required for dough leavening after freezing and frozen storage as yeast viability and activity in a frozen dough matrix may reduce due to ice crystallization (Pepe *et al.*, 2005; Huang *et al.*, 2008). Freezing damage is promoted with temperature fluctuations during frozen storage (Ribotta *et al.*, 2006).

At sub-zero temperature, cells are normally stays unfrozen, and therefore are supercooled (Mazur, 1965; Mazur, 1970). Nevertheless, the supercooled water that presents inside the cytoplasm possesses higher vapor pressure than the external ice. Water will move out from within the cells to outside the cells in response to the vapor pressure difference in order to achieve an equilibrium (Mazur, 1970). Cooling rate and water permeability of the cell will determine the way in which cells

equilibrate. If the cooling velocity is low or water permeability of cells is high, equilibrium will be done through dehydration. In contrast, the cells will regain equilibrium by intracellular freezing if their permeability to water is low or are subjected to rapid cooling (Mazur, 1970; Casey & Foy, 1995).

In any case, whether the cells achieve equilibrium through dehydration or through intracellular ice formation, they are subjected to a phenomenon, known as "solution effects". As the temperature decreases, cell water content decreases because water is removed as ice, concentration of extracellular and intracellular solutes increases, and there are changes in ionic strength because solutes precipitation occurred. In other words, cells under lower cooling rate than the optimum cooling velocity have lower survival rate due to longer exposure time to "solution effects" (Mazur, 1970; Casey & Foy, 1995). On the other hand, it is suggested that injury for cells that are subjected to cooling velocity which is greater than the optimum cooling velocity is a result of intracellular ice formation and ice recrystallization when subjected to warming (Bank & Mazur, 1973). The cells have inadequate time to equilibrate osmotically with the frozen external medium before intercellular freezing, hence intracellular ice formation increases as the water flow out from the cells during cooling becomes less (Mazur, 1966; Mazur, 1970; Bank & Mazur, 1973).

In addition, rapid cooling generates small ice crystals that have a tendency to recrystallize during warming, especially if during slow warming (Mazur, 1970). Hence, an optimum freezing rate should be applied in which it is low enough to avoid intracellular ice freezing but sufficiently high to reduce the exposure of cells to condition of high solute concentrations (Ribotta *et al.*, 2006).

2.3.2 Loss of gluten strength

Gluten protein plays an important role in wheat dough as it is capable to stabilize and retain gas bubbles during breadmaking. Nevertheless, the gas holding properties of dough might be seriously damaged after freezing and thawing (Inoue & Bushuk, 1996). This is apparent when there is a decline in bread volume because of the freezing process and longer frozen storage duration (Nicolas *et al.*, 2000). Hence, the strength of dough and frozen storage condition are the important factors that ensure the quality of bread made from frozen doughs. During harsh freezing and thawing condition, frozen storage and thawing process, gluten structure and dough integrity are subjected to physical stress (Bhattacharya *et al.*, 2003; Ribotta *et al.*, 2006). Results of Varriano-Marston *et al.* (1980) proposed that changes in rheological properties after freezing and thawing are not mainly associated to damage of yeast cells and simultaneous release of yeast metabolites. In addition, gluten network also deteriorated by ice recrystallization (Varriano-Marston *et al.*, 1980).

Ice recrystallization is a phenomenon in which ice crystals tend to minimize their free energy as apparent in changes of amount, size, and shape of ice crystals during frozen storage as compared to large ice crystals and thus be likely to decrease their surface energies through growth or through fusion with other smaller size ice crystals (Mazur, 1970). Ice crystallization and recrystallization are directly connected to water distribution in the dough throughout freezing, frozen storage, and transportation of the frozen dough from the manufacturing plant to the retail outlets (Lu & Grant, 1999a). Ice recrystallization weakens the protein network that plays an important role in gas retention of doughs. It is proposed that the growth of large ice crystals within the gas pores of dough causes them to expand. This can seriously