FUNDAMENTAL BASES FOR THE IMPROVING ACTION OF NOVEL ENZYME-OXIDANT COMBINATIONS IN FROZEN DOUGH

by

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Abstract

The market for frozen goods is expanding and the frozen dough goods sector still has potential to expand its market. It is well known that deterioration in bread quality occurs during frozen dough/bread production. In addition, it is known that dough rheology influences bread quality. To prevent deterioration of bread quality, many additives have been used and researched. Combinations of oxidants (potassium bromate and ascorbic acid) are widely used worldwide. However, potassium bromate may be carcinogenic to humans, and it has been detected in bread after baking. Since it has been prohibited or strictly limited in many countries, many researchers have tried to find a replacement. Ascorbic acid is safe for human intake, and does not persist in bread. However, it is not as effective as potassium bromate. Possible replacements in frozen doughs include oxidant (ascorbic acid)-enzyme combinations. This study evaluated the effects of ascorbic acid-specific enzyme combinations as a replacement for the potassium bromate in frozen dough and related the effects to dough behavior (gluten network strength) as evaluated by dynamic oscillation rheometry. Bread quality was evaluated by test baking.

Based on the results from fresh baking studies, potassium bromate can be replaced by an optimum level combination of ascorbic acid and hemicellulase/endoxylanase. This combination clearly improved loaf volume, and crumb grain over both control and potassium bromate containing doughs.

For frozen dough/bread production, the addition of all additives improved bread quality, but ascorbic acid and endoxylanase containing dough resulted in higher volume, and better crumb structure than did dough containing potassium bromate.

Dough rheology experiments show that rheology was affected by both the process and additives. Strain sweeps gave the information about dough stability. Both the additives and proofing improved dough stability. Dough behavior (gluten network strength) was assessed by frequency sweeps. Dough containing ascorbic acid and endoxylanase was most stable during frozen dough processing.

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Dedication

This document is in dedication to my grandfather, and my parents.

CHAPTER 1 - Introduction

1.1 Bread making

Bread originated in the Mesopotamian civilization (B.C. 6000). At that time, dough was produced from stone crushed wheat and water. The first bread was simple and flat in shape because of a lack of fermentation. In succeeding years, un-fermented bread spread to the Egyptian civilization (B.C. 4000). Bread making developed greatly in this era when people discovered that flour and water dough swelled when it was leavened. This improved baked product quality (improved volume and palatability). This was the discovery of the fermented bread process which was applied to beer preparation as well. Consequently, bread, beer, and onions became peoples' staple foods in this period (Tannahill, 1973, Varilek and Walker, 1983). Bread ingredients, bread making processes, and equipment have been researched and developed ever since. Now, people all over the world eat bread, and it is a staple food for millions. However, bread has the disadvantage that its shelf life is short because of the phenomenon known as staling. Frozen dough technology was developed as a partial solution to this. Using this technique, the baker or retailer can provide bread similar quality to fresh to customers at any time. In terms of production the frozen dough process has a number of advantages; reduced labor requirements, ease of operation, and expansion of distribution area. The technology has been actually researched since early in the 1950s (Jackel, 1991). These studies have addressed areas as diverse as the effect of various ingredients and their interactions on dough/baked product quality, optimum condition of mixing, freezing, storing, thawing, proofing, and the stability or shelf life of the frozen dough product (Lorenz and Kulp, 1995). As a result of that research, frozen dough technology is popular in the baking industry. The field is continuing to be researched and developed with the goal of producing ever higher quality frozen dough.

Yeast is the most studied ingredient in frozen dough for several reasons. Yeast is an essential ingredient for bread making and the viability of yeast after freezing has a big influence on frozen dough product quality. In the freezing process, ice crystals can damage yeast cell walls. The damaged yeast releases glutathione, a protein reducing agent. As a result, dough weakens and final product quality becomes low (Hites, 1947; Lorenz and Bechtel, 1964; Anonymous, 1967; Hsu et al., 1979ab; Dubois and Blockcolsky, 1986ab; Spooner, 1998; Ribotta et al., 2003).

More recently, Berglund et al. (1991) observed that changes in water distribution occurred during extended frozen storage and freeze-thaw cycles, and the observed changes in the ultrastructure of the starch granules and gluten may contribute to reduced dough/bread quality. Naito et al. (2004) reported that the gluten fibrils forming the skeletal framework of crumb cell walls were cut and became coarse and non-uniform strings and that many knots were generated on gluten fibrils because of freeze damage. Therefore, some researchers assume that the existence of water (ice) in dough is a cause of deterioration in bread quality (Lu and Grant 1999; Zounis et al., 2002; Bot, 2003; Esselink et al., 2003; Naito et al., 2004; Seguchi and Morimoto, 2003, 2011).

To partially counter this problem, oxidants such as ascorbic acid (AA) and potassium bromate ($KBrO_3$) can be added to the dough (Hites, 1947; Lorenz and Bechtel, 1964, 1965; Jackel, 1978; Hsu et al., 1979ab; Inoue and Bushunk, 1991). These oxidants function to strengthen the gluten network at specific points during bread making, (hence, the term dough strengthener.) However, research indicates that residual unreacted potassium bromate in food is not safe for humans (Silverglade and Sperling, 2005). Even though residual levels are quite low, some countries ban the use of potassium bromate. The United States (FDA) established limitations on the amount of $KBrO_3$ use in food. By FDA regulation, the legal limit of use of potassium bromate is 75 ppm based on the flour weight (Code of Federal Regulations Title 21). However, some states in the United States prohibit its use. Thus, the trend in use of potassium bromate is toward worldwide prohibition. Consequently, potassium bromate replacement is necessary in the food and baking industry, and many researchers and product developers are interested in the use of one or more enzymes as replacements (Mathewson, 1998).

The food industry has tried to use a variety of food additives such as amino acids and enzymes for bread making as substitutes for potassium bromate (Morita et al., 1997). Much research has concluded that enzymes play the key role in bread. It improves final product quality such as softness (extend shelf life), volume and so on. Furthermore, one study (Haarasilta et al., 1991) reported that enzyme (hemicellulase) containing dough resulted in improved dough processing and final product quality. A subsequent study was done based on these results (Lin, 2008). In that research, "the researcher studied hemicellulase, endoxylanase, lipase, and ascorbic acid (AA) as possible replacements for potassium bromate in frozen dough (Lin, 2008)". Results showed that enzymes when combined with oxidants other than potassium bromate were able to

effectively replace the combination of potassium bromate with AA in frozen dough. However, the combination of potassium bromate and AA still provided the highest specific volume for frozen dough bread. The results also showed that the combination of AA and hemicellulose/endoxylanase might be a viable replacement for the combination of potassium bromate and AA, because the specific volumes were close to control. However, using only the enzyme (hemicellulose/endoxylanase) weakened the dough and provided no benefit to loaf volume (specific volume). The research also demonstrated that frozen storage time influenced bread staling rate, crumb texture, wall thickness and brightness. Longer frozen storage resulted in breads with coarser texture, thicker cell walls, and darker crumb colors.

1.2 Rheology

In 1928, the word "Rheology" was coined by Eugen Cook Bingham (Reiner, 1964). It means "everything flow" (Reiner, 1964). As research on the topic advanced, rheology is now defined as "the science of the deformation and flow of matter" (Dogan and Kokini, 2007). Generally, a rheological property is measured by controlled stress or strain applied to a material over a given time. The resulting force response is measured and it gives an indication of material properties such as stiffness, modulus, viscosity, hardness, strength or toughness of the material (Dobraszczyk and Morgenstern, 2003). Dobraszczyk and Morgenstern (2003) described 3 main purposes of rheological property measurement: 1) To obtain a quantitative description of the materials' mechanical properties; 2) To obtain information related to the molecular structure and composition of the material; 3) To characterize and simulate the material's performance during processing and for quality control. All materials have rheological properties, so rheology is studied in many scientific fields. There are many test methods used to measure rheological properties.

Rheological principles and theory can be used in process control, design, and as a tool in the simulation and prediction of the material's response to complex flows and deformation conditions (Ferry, 1980; Barnes et al., 1989; Whorlow, 1992; Dobraszczyk and Morgenstern, 2003). The food industry is one example of many applicable science fields. In the food industry, many areas need and use rheological data. Rheological data is applied to engineering calculations for designing equipment, determining ingredient functionality and product development, etc. (Steffe, 1996). Many researchers have employed rheological testing of foods (Sherman, 1970; Carter, 1990; Rao and Steffe, 1992; Dobraszczyk and Vincent, 1999; van-Vliet, 1999) and cereal products (Muller, 1975; Faubion and Faridi, 1986; Abdelrahman and Spies, 1986; Bloksma and Bushuk, 1988; and Faubion and Hoseney, 1990). It is common to categorize rheological techniques according to the type of strain imposed, e.g. compression, extension, shear, torsion, etc. Bloksma and Bushuk (1988) explained that the main measurement techniques of cereal product rheological testing can be categorized as descriptive empirical techniques and fundamental rheological techniques.

In the cereal industry, especially baked products, dough rheology or batter flow directly influences final product quality. Originally, dough/batter properties were judged by the

experience of each baker and/or empirical physical testing (by windowpane test or butter flow & viscosity). Dough rheology/batter flow changes by adding the ingredients; consequently, rheological research was expected to the able to clarify each ingredient's functionality and interaction with other ingredients. However, dough and batter are complex, not homogenous, and it is difficult to access dough/batter during processing without interrupting the baking process or disturbing the structure of the material. Therefore, this is a difficult area to research. The grain industry has measured the dough rheology /batter flow by an empirical described measurement for a long time. Many descriptive empirical rheological measurement devices are used in the cereal industry; the Penetrometer, Texturometer, Consistometer, Amylograph, Farinograph, Mixograph, Extensigraph, Alveograph, various flow viscometers and fermentation recording devices (Muller, 1975, Shuey, 1975). Rheological methods used for cereal products testing are shown in Table 1.1.

Table 1.1 Rheological methods used for cereal products

Method	Products	Measured property
Descriptive empirical method		
Mixer: farinograph, mixograph, reomixer	Dough	Mixing time/torque apparent viscosity
Extensigraph	Dough	Extensibility
Taxt2/Kieffer Rig	Dough, gluten	Extensibility
Alveograph	Dough, gluten	Biaxial extensibility
Amylograph, RVA	Pastes, suspensions	Apparent viscosity, gelatinization temperature
Consistometer	Sauces, fillings	Apparent viscosity
Flow cup	Fluids, sauces, batters	Apparent viscosity
Falling ball	Fluids	Apparent viscosity
Flow viscometers	Fluids, pastes	Apparent viscosity
Fermentometers	Dough	Height, volume
Penetorometers	Semi-solid foods, gels	Firmness, hardness
Texturometer, TPA	Solid foods	Texture, firmness
Fundamental methods		
Dynamic oscillation, concentric cylinders, parallel plate	Fluids, pasts, dough, gel dough, batters	Dynamic shear moduli, Dynamic viscosity
Tube viscometers: capillary, pressure, extrusion, pipe flow	Fluids, sauces, Pastes, dough	Viscosity, In-line viscosity
Transient flow: Concentric cylinders, parallel plate	Semi-solid (visco-elastic) materials	Creep, relaxation, Moduli and time
Extension: uniaxial, biaxial, dough inflation system , lubricated compression	Solid foods, doughs	Extensional viscosity, strain hardening

(Source: Muller, 1975, Shuey, 1975)

Dobraszczyk and Morgenstern (2003) concluded that descriptive empirical tests are easy to use and provide data which is useful in evaluating performance during processing and for quality control. The instruments are often robust and capable of withstanding demanding factory environment, and do not need highly trained operators. These instruments have provided a great deal of information on the quality and performance of cereal products such as consistency, hardness, texture, viscosity etc. However, these instruments don't meet the requirement for a fundamental rheological test. Dobraszczyk and Morgenstern (2003) explained the reasons that descriptive empirical tests are not fundamental rheological measurement. The reasons are as follows; 1) The sample geometry is variable and not well defined; 2) The stress and strain states are uncontrolled, complex and non-uniform; 3) It is; therefore, impossible to define any rheological parameters such as stress, strain, strain rate, modulus or viscosity. Therefore, these tests are purely descriptive and dependent on the type of instrument, size and geometry of the test sample and the specific conditions under which the test was performed (Dobraszczyk and Morgenstern, 2003).

On the other hand, fundamental rheology measurements can control the stress and strain states. Therefore, it is possible to define all rheological parameters. However, fundamental tests have disadvantages such as expense and complex instrumentation, time consuming tests, difficult to maintain in an industrial environment. They require a well-trained operator, results can be difficult to interpret, and slip and edge effects can occur during testing (Dobraszczyk and Morgenstern, 2003). Typical types of fundamental rheological tests in cereal product research are: 1) Small deformation dynamic shear oscillation; 2) Small and large deformation shear creep and stress relaxation; 3) Large deformation extensional measurements; and 4) Flow viscometry (Muller, 1975, Shuey, 1975). Fundamental rheological methods are shown in Table 1.1.

Dynamic oscillatory measurement is one of the most popular and widely used fundamental rheological techniques for measuring doughs and batters. Steffe (1996) explained that dynamic oscillation measurement results are related to chemical composition and physical structure, so they can be used for gel strength evaluation, monitoring starch gelatinization, studying the glass transition phenomenon, observing protein coagulation or denaturation, evaluating curd formation in dairy products, cheese melting, texture development in bakery and meat products, shelf-life testing, and correlation of rheological properties to human sensory perception. Consequently, this testing is a valuable tool for product research and development.

Typical commercial instruments operate in the shear deformation mode, the predominant testing method used for food. Dynamic oscillation test has begun to be used in the area of frozen dough rheology (Autio and Sinda, 1992; Kenny et al., 1999; Newberry et al., 2002; Meziani et al., 2012ab).

In summary, measurement of dough/ batter rheological properties is difficult to research. Still knowledge of a dough rheology / batter flow is helpful for designing new products, as well understanding functionality and interactions. Therefore, rheological property research continues to be carried out by descriptive empirical rheological test and fundamental rheological testing.

1.3 Objectives

The purpose of this study was to evaluate the effects of specific enzyme-oxidant combinations as a replacement for the potassium bromate-ascorbic acid combination in frozen dough. Specifically, dough behavior (gluten network strength) was evaluated by dynamic oscillation testing. Final product quality was evaluated by test baking.

The main objectives were:

1. To optimize the oxidants and oxidants-enzyme combination in the fresh baking (non-frozen system).

2. To evaluate frozen dough/ bread quality obtained using oxidants and oxidants-enzyme combinations at levels optimized for fresh baking.

3. To evaluate dough rheological properties (gluten network characteristics) by dynamic oscillation testing of each frozen dough making process step and various frozen storage condition.

CHAPTER 2 - Literature Review

The grain based frozen product market is a relatively new (~50 years) area and it is still growing. Following market expansion, many researchers have been research actively. The final frozen product quality is affected by many factors, such as dough formulation, essential ingredients quality and quantity, types or amounts of dough additives, and condition of each processes. The biggest challenge in producing high quality frozen dough is yeast survival during dough freezing and storing and the effects of yeast death on dough behavior.

2.1 Frozen product marketing

Retail markets for frozen doughs were not active during the 1950's to 1960s. Most likely, this was due to the limited shelf life, inconvenient preparation, complex process, and unexpected poor quality of the consumer prepared bread (Vetter, 1979). During the 1960s, researchers who believed in the potential of frozen dough tried to improve dough/bread quality (Lorenz and Bechtel, 1964, Anonymous, 1967). One company (Weston) began to sell retail packaged frozen dough in the 1970s. Additionally, the company began to sell to in-store bakeries almost as a sideline. That is the business which has greatly grown since 1985 and it led to the expansion of the market for the frozen product. Actually, frozen dough sales and in-store bakeries grew a lot during late 1980s to early 1990s (Palmer, 1994). In fact, frozen dough products sales are still increasing (The freedonia Group, Inc., 2011).

Grain- based food shipments increased by 3.5 % annually during the 2005-2010 period. Total shipments reached $85.4 billion at 2010. Grain based food shipments by category in 2010 are shown in Fig. 2.1. As Fig. 2.1 shows that commercial and retail bakery products accounted for the largest share. Additional, contributors were cookies and crackers; frozen goods; breakfast cereal; and other products such as corn, chip, pasta, and prepared flour mixes. (The Freedonia Group, Inc., 2011)

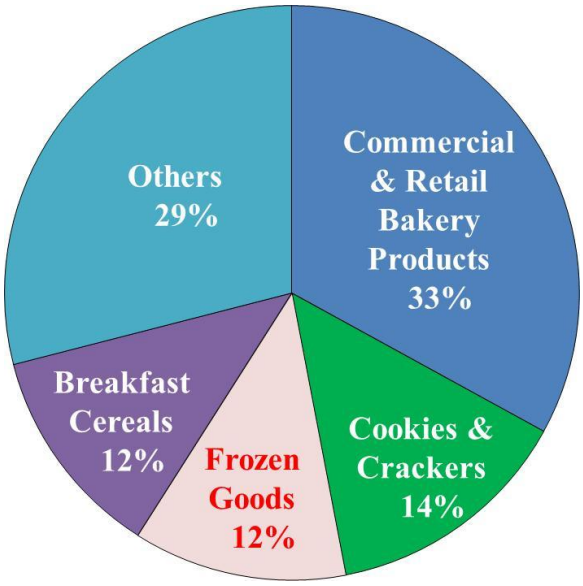

Figure 2.1 Types of grain-based food shipments at 2010

(Source: The Freedonia Group, Inc., 2011)

Shipment value and annual growth are shown in Table 2.1. Shipment value of frozen goods reached $10.6 billion in 2010. Frozen goods annual growth was 2.4 % during 2005-2010. This category includes frozen dough, cakes, pies, crullers, doughnuts and other pastries; and frozen specialty foods such as pizzas, bagels, pot pies, pasta and breakfast items (e.g., French toast, waffles and pancakes). Higher price, innovative new products, and the advantages of cost and convenience affected the gain in this segment. This is particularly true for frozen pizza product quality which improved greatly, making these items more attractive to consumers. Shipment of frozen grain-based products is predicted to be $12.1 billion in 2015, based on 2.7 % annual gains from 2010. This growth ratio is the fastest paced among other major product segments. Clearly, frozen dough goods still have potential to expand its market, so retail producers need to improve the quality, and invent new products as well (The Freedonia Group, Inc, 2011).

Table 2.1 Estimate of grain-based food shipment by types

	Shipments (Billion Dollars)			Annual Growth %	
Item \ Year	2005	2010	2015	2005-2010	2010-2015
Grain-Based Food (total)	72.1	85.4	93.9	3.5	1.9
Commercial & Retail Bakery Product	26.0	28.6	30.5	1.9	1.3
Cookie & Crackers	10.2	11.6	11.9	2.6	0.5
Frozen Goods	9.4	10.6	12.1	2.4	2.7
Breakfast Cereals	9.1	10.2	10.9	2.3	1.3
Other	17.4	24.4	28.5	7.0	3.2

(Source: The Freedonia Group, Inc, 2011)

2.2 Roles of essential ingredients in frozen dough

The essential ingredients in frozen dough are the same as those in normal bread making; flour, yeast, water, and salt. However, the flour specification, yeast type, water absorption, and salt levels needs to considered and modified for frozen dough making.

2.2.1 Flour

Wheat flour is a necessary structural ingredient in bread making. To make different bread products the flour specification must match the product and process requirements. Properties such as protein quality and quantity, water absorption, α-amylase activity, and starch damage have to be adjusted to meet product and process requirements while taking into account the influence of other ingredients in the formula. Flour for frozen dough production must have greater strength than that for an unfrozen product because of the stress imposed by the freezing, storing, and thawing process (Marston, 1978; Sideleau, 1987; Lorenz and Kulp, 1995). During frozen storage the gluten slowly deteriorates in quality. This is shown by scanning electron microscopy (SEM) and extensigraph measurements on frozen, thawed doughs. SEM microphotographs show progressive breakdown of the gluten membranes, with formation of fibrils (Varriano-Marston et al., 1980). SEM examination of dough weakened by other means (i.e. over-mixing, treatment with reductants) showed similar changes in gluten structure. Extensigraph measurements on yeasted doughs that had been frozen and thawed, showed a decrease in resistance to extension relative to regular frozen dough, i.e. the dough was somewhat slacker (Inoue and Bushuk, 1991). Also, Inoue and Bushuk (1992) showed that strong flour can maintain higher oven-spring during baking of frozen dough, and that protein quality is more important than protein quantity in this regard. Other researchers found that a low level of damaged starch is desirable (Marston, 1978). Consequently, in the United States hard spring wheat flour (patent flour) with 12.5-13.5 % protein level and a low level of damaged starch is used for frozen dough (Jackel, 1978, Spooner, 1998). Wang and Ponte (1994) reported that added 2 % (flour weight base) vital wheat gluten in low protein flour produced good frozen stability and improved bread quality. If sufficiently strong flour is not available, supplementing

native protein such as vital wheat gluten (up to 2 %) is a popular adaptation. Recently, Sandhu et al. (2011) reported that ozone treated flour improved flour and dough/ bread characteristics. Bread made from flour treated with ozone gas had specific loaf volumes similar to those containing potassium bromate and larger volumes than bread made without potassium bromate. As a result, potassium bromate might be replaced by ozone gas treatment of flour or blending of fully ozone treated flour in bread making. This might be able to be applied to frozen dough production.

2.2.2 Yeast

As described in the introduction, the single most studied ingredient in frozen dough has been yeast. Many of yeast's properties, such as stability, strain type, rates of freezing, and rates of degradation, etc. are continuing to be researched. Such interest is due to the fact that yeast is the essential ingredient necessary to provide proper gas production for dough leavening during fermentation, and thereby affecting the quality of the finished product (Brninsma and Giesenschlag, 1984).

Many frozen dough researchers reported that yeast performance after freezing is problem (Merritt, 1960; Kline and Sugihara, 1968; Hsu et al., 1979ab; Wolt and D'appolonia 1984ab; Bruinsma and Giesenschlag, 1984; Hino et al., 1987; Gélinas et al., 1993, 1994; Ribotta et al., 2003). It is now well known that yeast is damaged during freezing, dough is weakening in frozen storage, and gas production (yeast activity) is decreased during proofing. Therefore, final baked product quality is poor. Some researchers (Kline and Sugihara, 1968, Hsu et al., 1979ab) contend that dough weakening and reduced gas production is related to yeast. The gassing power of yeast depends on the strain, the numbers of yeast cells, the cell activity, and the amount of fermentable sugar. Fast freezing process reduces both gassing power (Autio and Sunda, 1992; Gélians et al., 1993; Inoue et al., 1994; El-Hady et al., 1996) and the number of viable yeast cells (Lorenz, 1974). There are two hypotheses to explain the decreased number of viable yeast cells. The first is a physical effect. Ice crystals form is in the aqueous phase surrounding yeast cells, and subsequently in the cytoplasm (internal aqueous phase) of the cells during freezing. The ice crystals (particularly those formed internally) may physically disrupt the outer membrane of the

cell, causing it to lose the cytoplasmic contents, and die. Dormant cells have a somewhat thicker membrane than do activated cells, and so are more resistant to this kind of damage (Stauffer, 1993). The second explanation turns attention to the metabolic products formed by yeast and bacteria during fermentation (in a sponge or preferment broth), namely ethanol, acetic acid, lactic acid, and smaller quantities of esters such as ethyl acetate and ethyl lactate. During freezing of the dough aqueous phase these materials are concentrated in the unfrozen phase. (This phenomenon is used to make a strong hard cider; the fermented cider is partially frozen, and the liquid portion, with elevated alcohol content, is decanted from the ice crystals.) The concentrated solution of organic compounds can cause autolysis of yeast cells, i.e. rupture of the cell membrane and cell death. Again, activated yeast cells are more susceptible to this autolytic action (Hsu et al., 1979ab).

Dough weakening is also observed as poor gas retention during proofing. The cause was concluded to be damage to the three-dimensional gluten protein network. There are several hypotheses to explain this damage. One is dead or damaged yeast during freezing resulting in release of reducing substances such as glutathione during frozen storage (freeze-thaw cycle) (Kline and Sugihara, 1968; Hsu et al., 1979ab). Oszlanyi (1983) reported on thiol production by yeast in regular bread-making. Table 2.2 shows the amounts of thiol released by various types of yeasts in mixed dough and in fully proofed dough. The amount of thiol is lower with the instant yeast than with compressed yeast. In addition, two types of conventional active dry yeasts released high amounts of thiol during the dough-making process. This accounts presumably for their gluten weakening effect.

Table 2.2 Thiol Production by yeast

Type of yeast	Mixed dough	Proofed dough
Compressed	88.07	127.41
Instant	74.52	111.65
Active dry yeast 1	176.84	169.90
Active dry yeast 2	110.68	152.11

(Source: Oszlanyi, 1983)

On the other hand, other workers (Varriano-Marston at el., 1980; Wolt and D' Appolonia 1984ab; Autio and Sinda 1992) have suggested that the structural changes in freeze-thawed dough are not associated with the release of reducing substances from yeast cells but with a lack of gluten cross-linking. Berglund et al. (1991) showed that the formation of ice-crystals in non-fermented dough stored for 24 weeks led to a disruption of the gluten matrix rendering a network separated from starch granules. They also explained that less free water was associated with either the gluten or starch fractions, concentrating instead into large patches of ice crystals. They also reported that gluten strands were observed to become thinner with time. Consequently, they conclude that these ultrastructure changes would help to explain the extended proof times and reduced loaf volumes of frozen bread dough. Based on that study, many researchers think it is possible that the ice crystals produced during freezing and the frozen storage process greatly influence the dough (gluten matrix) character. Recently, frozen dough study has been guided by this hypothesis (Lu and Grant, 1999; Zounis et al., 2002; Bot, 2003; Naito et al., 2004). While leached glutathione is certainly involved, the precise mechanism by which yeast contributes to increased slackening remains the subject to debate (Casey and Foy, 1995). Thus, Selomulyo and Zhou (2007) concluded that the reason dough weakens during freezing and thawing is still unclear.

Commercial baker's yeast (*Saccharomyces cerevisiae*) has been used for these baking studies. It can classified into two different forms. The first type is fresh yeast. This type comprises cream yeasts, compressed yeast (also called "wet", or "fresh" yeast), and bulk yeast (also termed "crumbled"). The second type is dry yeasts. It includes active dry yeast (ADY) or instant dry yeast (IDY) (Pyler, 2008). Both types of yeast are used for frozen dough research. Fresh compressed yeast performed better than did active dry yeast and instant dry yeast when used in frozen dough at comparable actively levels (Wolt and D' Appolonia, 1984ab, Sideleau, 1987). On the other hand, some workers concluded that dry yeast may be superior to compressed yeast in maintaining the frozen shelf life of frozen dough (Zahringer et al., 1951; Merritt, 1960; El-Hady et al., 1996). Bruinsma and Giesenschlag (1984) compared Red Star™ instant dry yeast to Red Star compressed yeast. They reported that either instant dry yeast or compressed yeast will function well in frozen dough. Both of types of yeast lose a significant amount of yeast activity after the initial freeze thaw cycle (Table 2.3).

Table 2.3 Freeze-thaw effect on gas production & proof time on Red Star yeast

Freeze-Thaw Cycle Number	Gas production [cc/hr]		Proof Times [minutes]	
	Compressed yeast	Instant dry yeast	Compressed yeast	Instant dry yeast
1	789	775	87	79
2	808	880	78	70
3	835	828	75	70
4	753	705	80	78
5	728	755	-	-
6	758	738	86	71
7	673	758	85	81

(Source: Bruinsma and Giesenschlag, 1984)

According to Table 2.3, gas production was relatively similar by both types of yeasts, but instant dry yeast exhibited a relatively shorter proof time than did compressed. In practice, the amount of yeast needed will depend on the average time in frozen storage, the formulation of the dough and the desired proof time after thawing. All of these factors must be considered when determining yeast levels in frozen dough. Generally frozen bread dough requires 4-6 % compressed yeast, a level higher than that used in fresh baking.

Currently, many studies try to use new freeze-tolerant (cold- tolerant) yeasts for frozen dough making (Hino et al., 1987; Oszlanyi, 1989; Takano et al., 2002). Oszlanyi (1989) described preparation of frozen dough yeast. The procedure was based on the use of IADY. The new yeast was dried to 25 % moisture content using a fluid bed dryer that removed only unbound water. Because of the relatively high water content, the yeast was not stable at room temperature even when packaged under vacuum, so the company froze the yeast to preserve it. All water was tightly bound within the cell; no free water was available. Therefore, when frozen, no ice crystals formed, and there was no cell damage (Pyler, 2008). Beside this method, freeze tolerant yeast strains are being researched and developed (Alves-Araújo et al., 2004, Ando et al., 2007). Oszlanyi (1989) compared yeast activity and baking performance of compressed yeast and cold tolerant yeast. Dough containing cold tolerance yeast dough produced more yeast activity (gas production) and better baking performance. In lean hearth bread, dough containing cold tolerant yeast kept its baking performance even after long term frozen storage (160-180 days). This

baking performance was shown not only for lean hearth bread but also for Danish pastry and hamburger buns. It resulted in good quality that approached that of fresh baked for all frozen dough types. Substituent research also reported the same result (Hino et al., 1987, Takano et al., 2002).

2.2.3 Water

The main functions of water in dough are hydration and plasticization. In general, optimum water absorption for frozen dough is slightly (2-3 %) lower than that of a regular bread formulation (Jackel, 1991; Lorenz and Kulp, 1995; Spooner, 1998). Commercially, water absorption is in the range of 55-60 % (flour weigh basis). This is to reduce the time necessary for optimum mixing, and to limit the amount of free water in the dough. A high level of free water results in more ice crystals, and thus is damaging to the dough and yeast during freezing and frozen storage (Javes, 1971, Sideleau, 1987). Complete hydration of the flour particles with a minimum amount of free water is important for frozen dough. Lower absorption produces stiff and dense dough which helps to maintain its shape during freezing and thawing cycles. Chilled dough water is used to reduce the dough temperature to less than 20 ºC (70 ºF). This slows the yeast activity and accelerates freezing of the dough piece (Javes, 1971). However, Fuhrmann (1985) warned about the use of ice in the mixer. When added in large amounts, ice will be still in the process of melting by the time the dough mass has already hydrated and, so, cannot properly absorb the remaining water from the melted ice.

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2.2.4 Salt

Salt (Sodium Chloride) has three functions in baking. The first function is flavor enhancement. Bread without salt has an insipid and flat taste and flavor and is normally unsalable except to consumers who must adhere to a low-sodium diet. When used at the proper level, salt does not impart a salty taste to the product. Rather salt imparts greater fullness to mouth feel, masking possible off-taste and, most important improving flavor balance (Gillette, 1985).

The second function of salt is to inhibit yeast activity. This is reflected as reduced gassing rates in the presence of salt (Gross et al., 1966). Salt alters the osmotic pressure in foods, causing microorganisms, including yeast, to lose moisture to the briny surroundings, reducing their vitality. Therefore, it controls fermentation, and influences proof time & gas production (Matz, 1992).

The third function of salt is its strengthening and tightening effect on the gluten in dough, due in part to its ability to inhibit proteolytic enzymes (suggested by Miller and Johnson, 1947). Other evidence indicates a more direct interaction of the salt with flour protein. Hoseney and Danno (1982) showed mixograms of doughs containing different amounts of sodium chloride. Increasing sodium chloride content delayed peak time. In addition, excess amounts of sodium chloride inhibited make gluten formation. Thus, it is well known in the baking industry that salt lengthens the mixing time. This phenomenon was also shown by the farinograms & mixograms by Miller and Hoseney (2008) who also explained the effect of salt on mixing time and dough strengthening. "Dough pH is usually about 6.0, and the gluten protein has a net positive charge at this pH. These positive charges repel each other, and it allow the gluten to hydrate faster (shorter mixing time) and keeps the protein chains from interacting with each other, resulting in weak dough. On the other hand, smaller amounts of added salt shield the charges allowing the protein chains to approach each other. This causes the flour to hydrate more slowly (longer mixing time) and allows the protein chains to react more tenaciously to form a stronger dough" (Miller and Hoseney, 2008). Due to its osmotic effect on yeast, the suitable amount of salt in frozen formulation is not more than 2 percent (based on flour). Thus, the suggested range is 1.5-2.0 %. In addition, salt is usually added late in the mixing process to minimize the effect of salt on mixing time.

2.3 Dough additives

Simple fresh and frozen dough/bread can be made using only 4 essential ingredients. However, the final quality is poor. Therefore, consumer products contain dough additives. These result in positive effects on the dough or final product.

2.3.1 Sweetener

Sugar is a very popular dough additive for bread making. Some include it as an essential ingredient. Sugar has three main functions in bread making; 1) As a substrate for the yeast during fermentation, 2) To confer sweetness to particular products, 3) For reducing sugars to be part of the Maillard reaction responsible for crust browning (Pyler, 2008). The sugars found in wheat flour (and their respective amounts) include the monosaccharides glucose (0.03 to 0.09 %), fructose (0.06 to 0.08 %), and galctose (0.02 %); the disaccharides sucrose (0.54 to 1.55 %) and maltose (0.04 to 0.18 %); the trisaccharides glucodifructose (0.26 to 0.41 %) and raffinose (0.19 to 0.68 %); and other oligosaccharides, or glucofructans (0.94 to 1.14 %), summarized by Lineback and Rasper (1988). Monosaccharaides and sucrose can be metabolized directly by the yeast. Yeast will metabolize maltose in the absence of the flour's natural sugar. Maltose accumulates in the dough due to the combined action of the flour's diastatic enzymes (α and β-amylase) on the damaged starch fraction of the flour. Yeast will only metabolize maltose if there is no other source of sucrose or its derivatives left in the dough. If sucrose or glucose is added to the dough as ingredients, the yeast will metabolize these before maltose (Brown, 1993). Depending on the amount of yeast metabolism and types of sugar, dough sweetener containing results in an increased sweetness, loaf volume, crust color, flavor, and improved shelf life (Brown, 1993; Lorenz and Kulp, 1995; Pyler, 2008). Sweetness and crust color preference are depend on the producer, so a wide range (2-10 %) of sugar is found in practice. Sugar has a 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 additional sugar to ensure adequate gas production (Brown, 1993). For frozen dough, sugar levels depend on the type of product and crust characteristics required. However, Lorenz and Kulp (1995) explained that levels of

sweeteners are slightly higher than in freshly baked products (usually 8 to 10 %). Products that are higher in sugar generally show greater freezer stability due to the sugar's hygroscopic properties. Sugar binds with water and this reduces the level of free water low, reducing damage to the yeast (Heid, 1968, Dubois and Dreese, 1984).

In a study of different sweeteners in frozen dough Dubois and Dreese (1984) found that increased sweetener solids level (6 to 10 %) did not increase the dough proof time of fresh dough. In the frozen dough, bread with three levels of 62 D. E. corn syrup (6, 8, and 10 %) had about the same proof times. However, increasing the sucrose or HFCS levels resulted in a longer proof time. Higher levels (8 and 10 %) of 62 D. E. corn syrup had shorter proof times than did the sucrose and high fructose corn syrup (HFCS) containing doughs. Based on final product characteristics, corn syrup containing doughs generally resulted in lower volume bread than did HFCS or sucrose. In addition, corn syrup produced bread having poorer crumb grain than was produced by using sucrose or HFCS. The bread made with sucrose and HFCS were about equal in quality, and sweetener level and (frozen) dough age did not appear to have any effect on loaf volume. Sucrose and HFCS produced bread having a darker crust color than that of bread produced with corn syrup. Increased sweetener levels produced darker crusts. These differences narrowed with frozen dough age, and after 20 weeks of storage, the crumb grain in breads from all sweeteners was about equal and poor. As a result, Dubois and Dreese (1984) concluded that HFCS and sucrose were better than corn syrup in frozen dough. Fuhrmann (1985) reported that glucose and HFCS is used to some frozen dough. HFCS could generate some savings. However, Fuhrmann (1985) warned that glucose and HFCS produced relatively higher yeast activation than sucrose and so caused rapid acceleration of fermentation during make-up. Thus, sucrose is the most commonly used sweetener in frozen dough. Fuhrmann (1985) concluded that HFCS and sucrose blends are a desirable approach from the stand point of product stability and ingredient cost savings.

2.3.2 Shortening

Fats and oils are also popular dough additives in bread making (Pyler, 2008). Bread baked with added fat possesses larger volume, exhibits greater oven spring, and has a softer

crumb and longer shelf life those equivalent formulas without other fat. Fat contributes tenderness, gives a moist mouth feel, confers structure, lubricates during chewing, and contributes flavor to baked products. Up to 5 % fat (flour weight basis) may be used in bread, although the usual levels is 3-4 % of a plastic fat such as all-purpose shortening, bread shortening, lard (rarely), or 2-3 % vegetable oil (Stauffer, 1993). These amounts produce the optimum effects in both fresh and frozen dough breads. Generally, all-purpose shortening or bread shortening is used for bread making. All-purpose shortening is designed to function optimally in a wide variety of applications. Bread shortening is more suitable for bread making as it contains dough conditioners such as ethoxylated monoglycerides or sodium stearoy-lactylate, in addition to the mono-, diglycerides. Emulsifier containing shortening (bread shortening, cake shortening) was created in the 1930s. Initially emulsified shortening contained only mono- and diglycdrides of fatty acids. These emulsifiers imparted to shortenings greatly improved aerating and creaming properties. They also improved the dispersibility of the shortenings in dough, which resulted in a perceptible softening effect in the bread crumb (Werner, 1981). Shortenings are traditionally produced from hydrogenated base oil, and their plastic range is extended by the addition of 4 to 12 % of hard fats (Pyler, 2008). The tenderizing effect on the crumb comes from the liquid phase of the shortening (Stauffer, 1993). Because all-purpose shortening contains about 25 % solid fat at room temperature, 3 kg of vegetable oil is equivalent to 4 kg of plastic fat, in terms of its softening effect on bread crumb. The tenderizing effect also slows down the staling process, so bread containing shortening is more palatable after storage for several days than is the same formula without fat in the dough. On the other hand, the solid phase of shortening contributes to loaf volume (Stauffer, 1993). When bread is made with oil only instead of plastic fat, dough strengtheners such as sodium stearoyl lactylate (SSL), or diacetyltartrate esters of monoglyceride (DATAEM) must be used to get good volume. Loaf volume increases as the amount of plastic shortening increases up to 5 % (flour basis), then remains roughly constant (Stauffer, 1993). This effect is because the dough expands in the oven for a longer time when shortening is present, as compared to dough made without added fat. In other words, in bakery terminology, the addition of shortening increases the oven spring of the bread (Stauffer, 1993). Thus, plastic shortening is better to use for bread making than is oil.

2.3.3 Surfactants

As described briefly in the previous section, a variety of emulsifiers (surfactants) are used in baked products. Basically, emulsifiers provide dough/batter strengthening and/or softening, and improved final product quality. The main emulsifiers used for baked products are as follows: lecithin, sodium stearoyl-2-lactylate (SSL), calcium stearoyl-2-lactylate (CSL), diacetyl tartaric acid esters of mono- and diglycerides (DATEM), ethoxylated mono- and diglycerides (EMG), polysorbate 60, succinylated mono- and diglycerides, mono- and diglycerides, and distilled mono- and diglycerides. These surfactants' functionalities and product use levels are shown in Table 2.4 (Lallemand Inc., 1996). In these cases the emulsifiers are added with the shortening or batter before mixing.

Table 2.4 Surfactants functionality & regular usage level

Surfactants	Use level	Functionality	Use products
Lecithin	0.25-1 %	Natural softener	Margarine, chocolate manufacture, wetting agent in cocoa powder, fillings, and beverage goods, and shortening mix with other dough ingredients
SSL	0.25-0.5 %	Strengthens and softens	Dough conditioner, coffee whiteners, puddings, and low-fat margarine
CSL	0.25-0.5 %	Strengthens and softens	Yeast leavened product, whipping agent in frozen& liquid egg white, whipped vegetable oil topping
DATEM	0.25-0.5 %	Strengthens	Dough conditioner, coffee whitener, chocolate coverture
EMG	0.25-0.5 %	Strengthens	Whipped topping, dough conditioner/emulsifier in baked goods, emulsifier in coffee whiteners, icing, and frozen desert
Polysorbate 60	0.25-0.5 %	Softens	Gloss enhancer in chocolate coating, coffee whiteners, cake, and icings
SMG	0.25-0.5 %	Strengthens and softens	Baked goods, and shortening
Mono- and diglyceride	0.25-1 %	Softens	Baked goods, frozen desserts, whipped topping, margarines
Distilled monoglycerides	0.25-1 %	Softens	Margarine, peanut butter, shortening, bakery goods, and whipped desserts

(Source: Lallemand Inc., 1996, Igoe and Hui, 2001)

Both SSL and DATEM have been shown to be effective in maintaining both volume and crumb softness in bread produced from dough subjected to extended frozen storage (Marston, 1978; Varriano-Marston et al., 1980; Davis, 1981; Dubois and Blockcolsky, 1986a). Davis (1981) reported data showing that SSL provides a longer period of dough stability in terms of loaf volume. However the study did not include information on proof-time stability, which is a critical parameter in judging the overall shelf-life of the dough. Wolt and D'appolonia (1984b) studied the effect of SSL and DATEM on proof time and loaf-volume stability during frozen storage. They concluded that the roles of SSL and DATEM in counteracting rheological changes that occur in frozen storage could be studied with the extensigraph. Neither additive was

effective in altering proof time. SSL produced in a greater loaf volume than control dough (without SSL), due to its greater oven spring. The use of DATEM was less effective than SSL in counteracting dough rheological changes and in maintaining loaf volume (Lorenz and Kulp, 1995). SSL was believed to increase the interactions between gluten proteins more than DATEM. This results in an increasing ability of the dough to retain gas (CO_2) formed during proofing, and also increasing the amount of oven spring. Finally, SSL containing bread possessed somewhat finer crumb grain.

The use of lactylates in baked products has been the subject of many publications and patents. Thompson and Buddemeyer (1954) reported that calcium stearoyl-2-lactylate (CSL) increased the mixing tolerance of dough. Although CSL has been widely used in the baking industry since 1961, it has very limited emulsifying ability in water-oil systems as the calcium ion imparts very little hydrophilic character to the lactylated fatty acid. In bread making, these characteristics have little to do with the product functionality. The calcium salt exhibits a dough conditioning effect in breads containing relatively low fat levels than is not observed with the other acyl lactylates. The need for a product to act both as a dough conditioner as well as emulsifier in high fat, yeast leavened baked products prompted the development of sodium stearoyl-2-lactylate (SSL), a reaction product of stearic and lactic acids neutralized with sodium salts. See Fig. 2.2 for its structure, insoluble in water, but soluble in oil (Tenney and Schmidt, 1968). SSL is a highly functional fat phase emulsifier for use in cakes (Thompson and Buddemeyer, 1961).

SSL's effects exist as optima which differ based on formula, and product and production methods. In practice levels up 1.0 % (flour weight base) generally produce the best results (Tenney and Schmidt, 1968). Therefore, manufactures recommend 0.25-0.5 % amount based on flour weight.

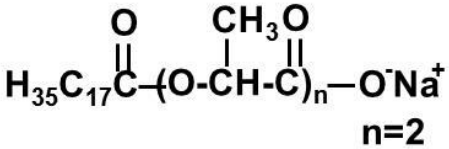


Figure 2.2 Average molecular structure of sodium searoyl-2-lactylate

(Source: Tenney and Schmidt, 1968)

2.3.4 Oxidants

Doughs made with proper levels of oxidation are more elastic, resistant to extension and less sticky than are un-oxidized doughs (Pyler, 2008). Properly oxidized dough exhibits good oven spring. Therefore, the final baked product has good volume, smooth crust break, soft and smooth texture, uniform small cell structure, and thin cell walls. On the other hand, doughs become excessively bucky, resist deformation during moulding and tear easily when oxidants are present in excess a phenomenon called over-oxidation. An over oxidized dough can break open during proofing. As a result, the final product has a lower loaf volume with a rough, uneven crust, and large unsightly breaks. In addition, its crumb has many ruptured cells and large holes. (Pyler, 2008)

There are two other reasons to add oxidant to frozen dough. First, frozen doughs are generally produced by a no-time dough method (Godkin and Cathcart, 1949; Merritt, 1960; Marston, 1978; Fuhrmann, 1985; Dubois and Blockcolsky, 1986b). This method lacks fermentation before make up, so final product quality is worth than that of regular, sponge-dough product. Second, there is a possibility that dead or damaged yeast cells release reducing materials (especially glutathione) during storage resulting in gluten weakness when the dough is thawed and proofed. More oxidant is, therefore, needed to offset the reducing action (Stauffer, 1993). Thus, frozen dough requires oxidants and the usage level is relatively higher than that found in fresh baking. Commercially popular oxidants are follows: potassium bromate, potassium iodate, calcium bromate, calcium iodate, calcium peroxide, azodicabonamide (ADA), and Ascorbic acid. These oxidant's functionalities and common usage levels are shown in Table 2.5 (Pyler, 2008). Most oxidants are thought to react in an essentially similar manner; by oxidizing the gluten's thiol groups, yet their overall effects differ considerably, mainly because they act at different stage of dough development as shown in Table 2.5. Both potassium bromate and ascorbic acid are widely used for fresh and frozen dough.

Table 2.5 Commercial oxidants

Oxidants	Maximum permitted level	Reaction rate	Stage of function
Potassium bromate	75ppm	Slow	Oven
Potassium iodate	75ppm	Fast	Mix/proof box
Calcium bromate	75ppm	Slow	Oven
Calcium iodate	75ppm	Fast	Mix/proof box
Calcium peroxide	75ppm	Fast	Mix/proof box
ADA	45ppm	Fast	Mix/proof box
Ascorbic acid	Unlimited	Intermediate oxidizer	Mix/proof box

(Source: Pyler, 2008)

2.3.4.1 Potassium bromate ($KBrO_3$)

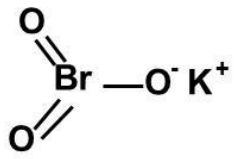


Figure 2.3 Structure of potassium bromate

(Source: Guide Chem)

Potassium bromate structure is shown in Fig. 2.3. According Table 2.5, potassium bromate is a slow acting oxidant. It is a called flour improver in that it acts only as a maturing agent without any perceptible bleaching action. This material exerts this action principally during baking. It is added after milling and acts in baking. In baking, it is effective in the late stages of proofing and the early stages of baking. The reason for this is that bromate needs high temperature to react (Pyler, 2008). Potassium bromate use in baking was patented in 1914 and has been used since (ABA and AIB, 2008). The effect of potassium bromate was reported Jørgensen (1945) along with the actions of other oxidizers and enzymes. That research concluded that it can help strengthen the baking performance of flours of widely varying quality. Slow acting bromate appears to work by oxidizing the thiol groups of flour during late proofing and early baking. This action suggests that sulfhydryl groups on the protein molecules are oxidaized

to disulfides, creating a cross-linked network of protein that is the primary determinant of dough structure and rheology. This oxidation is crucial in poor crop years when low-grade flours with an increased content of thiol groups are common. Others of researchers studied the addition of potassium bromate in doughs (Freilich and Frey, 1939ab; Hites, 1947; Tsen, 1964). Results indicated that it can improve final product volume at low levels. Bromate has an optimum level of addition to dough. If added in excess, it "over oxidizes" the dough and decreases final product quality. During the 1970s and 1980s, many researchers tested the use of oxidants for frozen dough (Marston, 1978; Varriano-Marston et al., 1980; Davis. 1981; Dubois and Blockcolsky, 1986ab; Inoue and Bushuk, 1991; El-Hady et al., 1999). They concluded that added oxidants or combination of oxidants were good for use with frozen dough. According to Inoue and Bushuk (1991) and El-Hady et al. (1999), AA & potassium bromate in combination in dough formulation is superior to using potassium bromate alone.

However, residual bromate can be detected in bread by ion chromatographic determination (Oikawa et al., 1982), and Kurokawa et al. (1982) reported mutations in rats resulted from intake of potassium bromate. Therefore, the Joint FAO/ World Health Organization (WHO) Expert Committee on Food Additives considered the use of potassium bromate as a flour improver or flour treatment agent and found it to be not acceptable (Joint FAO/ WHO Expert Committee on Food Additives (JECFA) Evaluation, 1995) due to its possible mutagenic and carcinogenic effects in humans (Kurokawa et al., 1990; Umemura et al., 1993, International Agency for Research on Cancer (IARC), 1986, 1999). In addition, Silverglade and Sperling (2005) reported that residual bromate may be left in the bread if it is not baked long enough or if baking temperature is not high enough.

It was thought that bromate was entirely decomposed (converted to bromide) during the baking process. However, a small amount (PPBs) of bromate has been detected (Oikawa, 1982). Consequently, use of the potassium bromate came under scruting during the late 80's to the 90's, and a highly accurate detection method for residual bromate in bread was requested. Oikawa (1982) had reported that residual bromate was detected in bread; however, this method was not accurate (detection limit was 500 ppb). Based on Oikawa's (1982) work, some researchers developed a method of measuring of residual bromate in bread (Mitsuhashi et al., 1988; Himata et al., 1994; Himata et al., 1997; Himata et al., 2000; Akiyama et al., 2002; Kawasaki et al., 2002). Nakamura et al. (2002) developed the sensitive determination method for bromate. This

method can detect as little as 0.5 ppb of residual bromate in bread. Along with the development of a highly accurate detection method, Nakamura et al. (2004) reported that no residual bromate was detected in Pullman-type breads with 13-15 ppm potassium bromate addition (flour weight base). On the other hand, residual bromate in open-top type (loaf type) bread with 9-30 ppm potassium bromate addition per kg of flour was found localized on the top crust. Risk assessment of potassium bromate by FDA established 20 parts per billion (ppb) as a 1 in 1 million upper-bound cancer risk at the 90^{th} percentile for intake of baked products (Pyler, 2008).

For several years, the American Bakers Association (ABA) and American Institute of Baking International (AIB) have been working with FDA and with the Japanese baking industry to improve testing and baking technology to permit continued use of bromate as a functional ingredient in baking in a manner that is safe and reliable, and Cogswell (1997), and Japan's Yamazaki Baking CO. (Himata et at., 2006) reported that different baking condition did not make a measurable difference in potential residual levels bromate. To this end, and in consultation with the FDA, the wholesale baking industry has progressively reduced potassium bromate usage (ABA & AIB, 2008). Therefore, FDA believes that 50 ppm or less of potassium bromate as an improvers in white flour and 75 ppm or less in whole wheat flour are safe (CFSAN, 2005). Consequently, the United States FDA has ruled that potassium bromate can be added into the dough at up to 75 ppm. However, in California, strict labeling is required if bromate used, and some countries are prohibiting potassium bromate use. Recently, FDA recommended voluntary reduction in bromate usage levels. Cauvain (1994) pointed out that the most important issue is consumer wanting "clean" labels, causing many bakeries to completely eliminate chemical oxidizers, relying instead on ascorbic acid. Thus, a lot of researchers began to investigate effective alternatives to potassium bromate.

2.3.4.2 Ascorbic acid (AA)

Ascorbic acid (popularly known vitamin C) is present in many green vegetable and fruits. It is an essential component in the diet (Cauvain and Young, 2001). It had long been recognized as an effective flour or dough improver (Jørgensen, 1945). In some Europe countries, such as Germany and France, it is the only improver permitted by law. Adding ascorbic acid to dough cannot enhance the nutritive value of the bread. This AA improves dough properties, but

contributes nothing to the nutritive value. Because AA is the least stable of the vitamins, it can be expected that all its vitamin activity will be destroyed during the baking process (Feaster and Cathcart, 1941). According to Tsen (1964), the effectiveness of ascorbic acid as an improving agent is only about two-thirds that of potassium bromate when their optimum levels of usage are compared. This comes from the mechanism of ascorbic acid action. In terms of its chemistry, AA is a reducing agent (and sometimes referred to as an anti-oxidant). However, during dough mixing AA is readily converted to dehydro-ascorbic acid (DHA) in the presence of oxygen and the enzyme ascorbic acid oxidase (Tsen, 1964). The sequence of oxidation and reduction reaction is shown in Fig. 2.4. The oxygen for the conversion comes from the gas bubbles incorporated during dough mixing and the conversion is enabled by the ascorbic acid oxidase enzyme, which occurs naturally in wheat flour. The oxidation product of ascorbic acid which is DHA functions in a manner similar bromate, iodate and azodicabonamide by oxidizing the thiol groups of flour during the dough mixing process. The chemistry of AA oxidation process in dough mixing is complex (Williams and Pullen, 1998) but probably involves the oxidation of the —S—H (sulphydryl) groups of gluten-forming proteins and the formation of —S—S— (disulphide) bonds. The net result of the AA effect is to improve the ability of the dough to retain gas (as seen by increased oven spring) and to yield bread with a finer (smaller average cell size) crumb cell structure. These changes also result in bread crumb that is softer to the touch yet has the resiliency to recover much of its original shape after compression (Yamada and Preston, 1992; Nakamura and Kurata, 1997; Cauvain and Young, 2001, Selomulyo and Zhou, 2007).

The dependence on oxygen for the AA to DHA conversion means that the quantities of air incorporated during dough mixing play a significant role in promoting oxidation.

The oxidizing effect of AA is limited mainly to the dough mixing period because bakers' yeast will remove any oxygen remaining in the air bubbles by the end of mixing or soon after its completion (Chamberlain, 1979). Thus, in the dough that leaves the mixer the gaseous mixture of nitrogen (from the air) and carbon dioxide (from yeast fermentation) that remains provides an environment in which AA can act as a reducing agent. If AA is used in dough making processes with extended periods of fermentation the opportunity exists for the reducing effect of AA to weaken the gluten structure with subsequent loss of gas retention in the dough. Therefore, ascorbic acid cannot over-oxidize the dough and so, is best suited to no-time dough making systems.

The action of AA during mixing also brings about changes in the rheology of the dough, making it more resistant to deformation (Cauvain et al., 1992). On the other hand, potassium bromate does not exert its full effect until the dough reaches the late stages of proofing and the early stages of baking. Therefore, Pyler (2008) explained that L-ascorbic acid "exhibits an intermediate reaction rate and is, therefore, capable of sustained action through most of the dough phase." Thus, the combination of AA and potassium bromate is popular in fresh & frozen dough production.

In many studies of frozen dough (Marston, 1978; Varriano-Marston et al., 1980; Davis. 1981; Dubois and Blockcolsky, 1986ab; Inoue and Bushuk, 1991; Kenny et al., 1999), researchers used AA or the combination of potassium bromate and AA. Inoue and Bushuk (1991) concluded that this combination in dough formulations is superior to using potassium bromate alone. The reason for this was explained by a fresh dough study by Tsen (1964) in which he used AA & potassium bromate combination. He concluded that the enzymatic oxidation of AA largely takes place during mixing, so the use of AA would be inefficient in a mixing process where the oxygen supply is quite limited. Under such conditions, bromate can oxidize AA to DHA chemically to speed up the oxidation. Some bromate, left over from the oxidation, can also supplement the oxidation of sulfhydryl groups by DHA. As a result, the improving effect of AA and bromate together is greater than that of bromate or AA alone. Consequently, the combination of AA & bromate improved definitely the dough and bread quality in fresh and frozen systems. Economically, most researchers believe it is also profitable to replace part of the AA with bromate, for bromate was less expensive than AA at that time. However, as mentioned above, the use of potassium bromate has been severely limited worldwide recently. Therefore, a material (alternative potassium bromate) that can be used together with AA is necessary and indispensable.

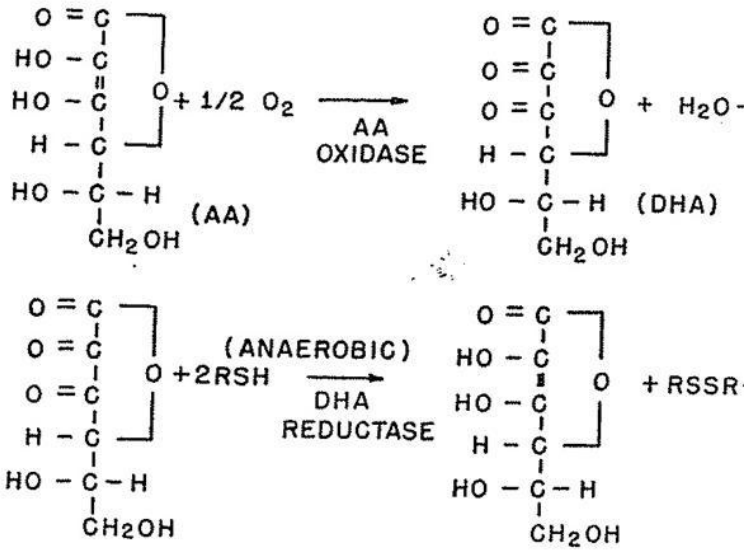


Figure 2.4 Sequence of oxidation and reductions involving L-ascorbic acid.

(Source: Tsen, 1964)

2.3.5 Enzymes

Enzymes are organic catalysts, facilitating and assisting chemical reactions. Enzymes are also proteins; polymers of amino acids connected by peptide linkage and folded in to specific 3-dimensional arrangements. The most important behavior or properly of enzymes is their ability to speed up rates of reaction. In baking, amylase enzymes affect starch and protease, protein, although many other enzymes exert improving effects on dough. Specific enzymes can be used producing high quality baked products. Although enzymes catalyze a wide variety of reactions, perhaps the most essential role they play in baking is to facilitate hydrolysis, the chemical process that splits a compound by oxidation, which in turn takes up a molecule of water (Pyler, 2008). Beginning in the 1970s, many researchers investigated enzyme use in baking. They concluded enzymes in baking can be used in the optimization of dough properties and quality improvement of bakery products (Barrett, 1975; Dubois, 1980abc; Chamberlain et al., 1981; Krueger and Lineback, 1987; Haarasilta et al., 1991; Hamer, 1992). Most research reported the improvements affected by enzymes are evident in loaf volume and external characteristics (loaf symmetry, smoothness of break and shred) and internal characteristics (texture, grain quality) of breads. One important effect of certain enzymes is the reduction of crumb firmness. This extends the shelf life and the period of marketability of the products.

Kulp (1993) explained the effects on doughs that can result from the application of enzymes: A) Generation of fermentable sugar to increase the fermentation rate by the action of amylase; B) Reduction of dough mixing time by proteases; C) Increases or decreases in dough stability by oxidases and proteases, or sulfhydryl reductases, respectively; D) Adjustment of dough extensibility, an important property in proper handling and machining of dough- addition of proteases enhances the extensibility while oxidases reduce this property, producing less extensible and drier doughs; and E) Alteration of the dough consistency during processing by the action of amylases on starch, proteases on gluten, and pentosanases on pentosans. In summary, adding enzymes to dough aims to improve fresh / frozen dough and product quality.

Enzymes might be expected to be potential replacements for potassium bromate. However, several authors (Kulp, 1993; Mathewson, 1998; and Boll, 1999) reported that researchers have tried to find a single enzyme to act as a bromate replacement but no single enzyme has been found to replace its oxidative effect. The problem is that bromate is a slow

acting oxidant. On the other hand, they pointed that an effective bromate replacer might be made by blending non-bromate oxidants with enzymes, emulsifiers and xylanase enzyme, or combinations of enzymes. The benefit would be an overall strengthening of the dough, which is essential when reducing or eliminating use of potassium bromate in favor of other oxidants such as ascorbic acid. Enzymes also help dough stand up to the physical abuse of high-volume production equipment. Based on previous study (Lin, 2008), enzymes such as xylanese (bacterial hemicellulase, and fungal endoxylanase) and lipase were used with ascorbic acid in the research reported here.

2.3.5.1 Xylanase

Xylanase is also called arabinoxylanase, hemicellulase and pentosanase (Pyler, 2008). Xylanase acts on specific non-starch polysaccharides which are called pentosans, hemicellulose, or most correctly, arabinoxylan (AX). AX is present at 2-3 % in wheat flour, 4 to 7 % in whole wheat flour (Hille and Schooneveld-Bergmans, 2004). AX makes up 60-70 % of the endosperm cell walls polysaccharide of wheat (Fincher and Stone, 1986). It is present at higher level in whole-wheat, whole-grain and high fiber formulations (Pyler, 2008). AX is able to absorb up to 20-23 % of its weight of water (Pyler, 2008). Arabinoxylans in wheat can be divided into a water-extractable fraction (WE-AX) and a water-unextractable fraction (WU-AX) (Hille and Schooneveld-Bergmans, 2004). Wheat flour consists 25-30 % of WE-AX and 65-70 % of WU-AX (Hille and Schooneveld-Bergmans, 2004). The WU-AX have a strong tendency to absorb water and swell, being reported to be able to hold 6.7 (Jelaca and Hlynca, 1971), 7.0 (Meuser and Suckow, 1986), 9.9 (Kim and D'Appolonia, 1977), 10 (Izydorezyk and Biliaderis, 1995) times their weight in water. WE-AX are said to have high water holding capacity with retention of 6.3 (Jelaca and Hlynca, 1971), 4.4 (Meuser and Suckow, 1986), and 3.5 (Kim and D'Appolonia, 1977) times their weight in water being reported. The result describes the effect in terms of the impact that AX have on the farinogram. Water is a very critical factor in dough. When WU-AX is treated with alkali, bridges between AX molecules are broken (Cole, 1967 and Gruppen et al., 1992). A large part of the WU-AX molecules is set free from the cell wall matrix and is rendered water-soluble (Gruppen et al., 1992). These can therefore be referred to as alkali-solubilized AX (AS-AX). Treatment of WU-AX with endoxylanases also results in solubilization, with the

generation of enzyme-solubilized AX (ES-AX) (Petit-Benvegnen et al., 1998, Courtin and Delcour, 2001). Xylanase breaks down (endo-hydrolysis) the water-unextractable arabinoxylans converting them to the enzyme-solubilized AX (ES-AX). ES-AX has reduced molecular weight because of hydrolysis of the xylan backbone. The enzyme with this functionality has been identified as endoxylanase (EC 3.2.1.8, endo-β-1, 4-endoxylanase). It attacks the AX backbone in a random manner, causing a decrease in the degree of polymerization of the substrate and liberating oligomers, xylobiose and xylose with retention of their configuration (Dekker and Richards, 1976, Reilly, 1981). This action is shown in Fig. 2.5.

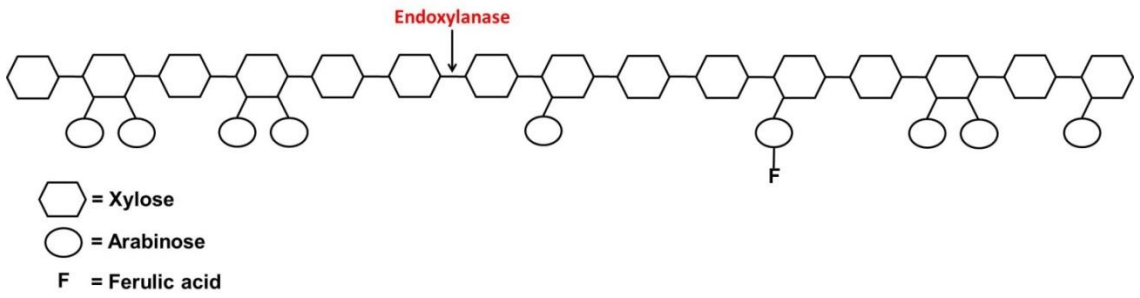


Figure 2.5 Sites of hydrolysis for endoxylanase on arabinoxylan

(Source: DSM Food specialties B.V., 2006)

In the 1960s, Cawley (1964) and Tracy (1964) used endoxylanases in a crude form (i.e. snail digestive juice) to demonstrate the importance of AX in bread making. The effect of endoxylanases activity in a general bread-making is a more recent discovery. Since then, endoxylanse has been known as dough improvers. Flour contains low levels of xylanase but not enough to be effective in baking. Furthermore, flour also contains natural xylanase inhibitors (Pyler, 2008). In the late 1990s, research was published indicating that cereals contained inhibitors of endo-(1.4)-β-D-xylanase activity (Debyser et al., 1997; Debyser and Delcour, 1997; Rouau and Surget, 1998). Subsequently, two distinct types of xylanase inhibitors with different structure and specificities were isolated from wheat flour. McLauchlan et al. (1999), and Hessing and Happe (2000) isolated xylanase inhibitor from wheat (*Triticum aestivum L.*). The inhibitor is called xylanase inhibitor proteins (XIP's). McLauchlan et al. (2000) reported that this inhibitor was also isolated from rye (*Secale cereal L.*) Debyser et al. (1999) found the other type of

inhibitor in wheat (*Triticum aestivum L.*) It is called *Triticum aestivum* Xylanase inhibitor (TAXI's). The characterization of these endogenous xylanase inhibitors has been helpful in identifying xylanase uses in baking.

Now, more of the mechanism behind xylanase functionality is known, but the interactions in which the xylanases participate are still not fully understood. However, it is well known that some xylanases perform better than others in stabilizing dough systems. Courtin and Delcour (2002) summarized AX and endoxylanase action in each step of wheat flour baking. They concluded that flour arabinoxylan degradation by xylanse is done during mixing and the effect is apparent in proofing (fermentation) and baking. At mixing, WE-AX effected dough consistency (Jelaca and HIynca, 1972). They reported that dough consistency was increased and the dough became stiff when native WE-AX was added to dough. Comparing the consistency value of control and WE-AX fortified dough, WE-AX dough has increased baking absorption. This increase is related to the WE-AX addition in the linear relationship over the range of 0 to 2 % (w/w). At optimum water absorption, WE-AX containing dough mixing time was the same as control or greater (Michniewicz et al., 1991, Biliaderis et al., 1995). Jelaca and HIynca (1972) also reported WE-AX containing dough development time was extended, but the required energy input to reach optimum mixing has decreased. Furthermore, WE-AX containing dough extensibility was decreased. However, resistance to extension was clearly enhanced by the addition of WE-AX (Jelaca and Hlynca, 1972, Courtin et al., 1999). Some researchers tried to find a relationship between dough handling and WE-AX addition (Rouau et al., 1994, Roels et al., 1993). However, results conflicted. Therefore, the relationship between dough handling and WE-AX content is still not completely known. A similar result was reported for WU-AX addition (Kulp, 1968, Jelaca and Hlynca, 1971). Kulp (1968) and Jelaca and Hlynca (1971) reported that addition of native WU-AX to dough results in higher dough consistency with short mixing time. Comparing at the same consistency value for control and WU-AX dough, WU-AX dough had increased baking absorption (Kulp, 1968, Jelaca and Hlynca, 1971). They reported this increase as being related to the WU-AX addition in the linear relationship within the range of 0 to 2% (w/w) addition, and mixing time increases. Kulp and Bechtel (1963) and Jelaca and Hlynca (1971) reported that dough extensibility was not changed by WU-AX addition. A relationship between flour WU-AX content and baking absorption was similarly shown for endogenous WU-AX through fractionation reconstitution bread-making experiments by Courtin

et al. (1999). Using this method, increasing the WU-AX content of the flour resulted in dough reduced extensibility decreased and increased resistance to extension (Casier et al., 1969).

Positive correlation between general dough characteristics and the percentage of endogenous WE-AX in total AX in dough was shown by Rouau et al. (1994). Rouau et al. (1994) also reported that negative correlation exists between general dough characteristics and the percentage of endogenous WU-AX in total AX in dough. The endogenous WU-AX is partially solubilized (around 10 to 15 % of the total WU-AX content) during the dough mixing stage (Rouau et al., 1994; Cleemput et al., 1997; Courtin et al., 2001).

Hillhorst et al. (1999) researched added endoxylanase effects on dough properties, and found that endoxylanase containing doughs had slightly decreased dryness and stiffness, but increased elasticity, extensibility, coherence and stickiness. At the optimum level of endoxylanase addition, there was a significant improvement in general dough characteristics, with poor quality flours being much more improved than good ones (Rouau et al., 1994). The first improvement comes from an increased solubilization of WU-AX (converted ES-AX). Dough viscosity was increased as a consequence (Popper, 1997, Sprossler, 1997). Moreover, Popper (1997) and Sprossler (1997) defined the parameter of an increase of dough viscosity as an important function of endoxylanase. The solubilization of WU-AX (ES-AX) reduced its water holding capacity. Therefore, previously bound water is redistributed among the other components of the gluten, increasing its extensibility (Maat et al., 1992). This water release is also partially counteracted by the increased viscosity of the dough aqueous phase, so dough slackness is increased (Rouau et al., 1994, Courtin et al., 2001). This results in an improved development and extensibility of the gluten (DSM Food specialties B.V., 2006). This proposed mechanism was shown in Fig. 2.6.

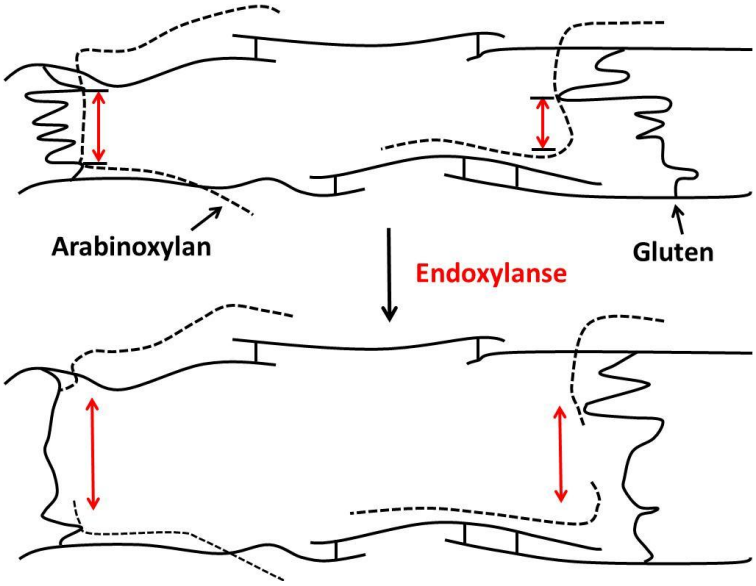


Figure 2.6 Interactions of arabinoxylan and gluten in dough

(Source: DSM Food specialties B.V., 2006)

A similar dough effect in was also reported Mullins (1990). As Mullins (1990) explained, when the dough is treated with xylanase, water becomes more available to plasticize protein, thus aiding development of the gluten network. He also pointed out unlike reducing agents. The "mellowing" effect does not decrease tolerance to mechanical abuse experienced during oven loading. Endoxylanase for bread-making degrades WE-AX and ES-AX to low molecular weight fragments, rather than primarily solubilizing WU-AX (Courtin and Delcour, 2002). Less suitable enzymes do not negatively affect dough quality in mixing and short fermentation; however, the dough becomes unacceptable slack, soften, and impairing machinability during longer fermentation (Courtin and Delcour, 2002)

Gan et al. (1995) and Sarker et al. (1998) suggested that during fermentation, stabilization of dough foams was improved by WE-AX. They explained that increasing dough viscosity effects on the stability of the liquid films surrounding gas cells. In addition, Hoseney et al. (1969) and Patil et al. (1976) concluded that the non-dialyzable water-soluble fraction, (containing the water-soluble pentosans,) contributes to gas retention. Kulp and Bechtel (1963) reported that gas retention of dough with the addition of WU-AX were similar to those of the control doughs. Sprossler (1997) reported that when active endoxylanase was added to the formula, the stability of the fermentation of the dough (measured as time that dough keeps the

optimal volume) and stability to mechanical stress increased. The explanation was that addition of endoxylanases mainly hydrolyzes WE-AX and ES-AX to small AX fragments and reduces water holding capacity of the WU-AX. As a result, dough viscosity is increased, thereby, limiting gas diffusion and maximizing gas retention (Courtin and Delcour, 2002). Sanders (1990) concluded that enzyme treated dough exhibited superior floor time and processing tolerance. During floor time and processing, fibers and gluten continue to absorb water, but the enzyme works to release water from AX. This action results in a more uniform, extensible dough during the entire run. Bread doughs can be sheeted more thinly without tearing, and sealing of the curl and seam during moulding are improved. Pan flow during proofing is increased due to continued enzyme action. Reduction in bake time or temperature can be achieved.

The effect of AX or endoxylanases at the baking stage is not clearly identified, but it can be assumed as an extension of the fermentation process (Courtin and Delcour, 2002). At the beginning of baking, dough expansion progresses at a much fast rate because of the increased yeast activity at high temperature. During this stage, the ability to retain fermentation gases which is one function of visco-elastic behavior of gluten which is critical. Gas cell perforation by WU-AX can enhance coalescence and decrease gas retention, but the stabilization of gas cells by WE-AX/ES-AX will delay the oven spring and improve crumb homogeneity (Courtin and Delcour, 2002). Though this theory is a hypothesis, it is supported by observations during bread-making and assessment of the final product.

Besides the above-mentioned research, many researchers reported that xylanase improves properties of dough, including internal (symmetry, crumb texture and softness) and external (final volume) quality of baked bread (Haarasilta et al., 1991; Hammond, 1994; Guy 2001; Hille and Schooneveld-Bergmans, 2004). Hille and Schooneveld-Bergmans (2004) reported that both fungal and bacterial hemicellulases are able to improve the internal and external quality of the final product. A blend of “strengthening” enzymes (xylanases, oxidation enzymes and lipases) can replace a portion of added gluten in dough formula. An extra benefit is that the blend will also save mixing time (Rees, 2008). Xylanases are often blended with fungal α-amylase. Hammond (1994) and Guy (2001) showed that added hemicellulase and fungal α-amylase a resulted in significantly greater volume than when using fungal alpha-amylase alone. Together, these enzymes make the flour’s native β-amylase more efficient at generating substrate for yeast

fermentation. Combinations of hemicellulase and fungal alpha-amylase have a synergistic effect on volume (Forman, 2004).

There is an optimum amount for xylanse addition as explained before. Over-dosing with xylanase reduces the overall water-holding capacity of the flour, so water release is excessive. As a result, dough becomes slack, sticky and difficult to handle (Rouau et al., 1994 and Courtin et al., 2001). Courtin et al. (2001) reported that this seems related to solubilization of the WU-AX and degradation of the ES-AX/ WE-AX and it might be counteracted by reducing the water content of the doughs. McCleary (1986) convincingly showed the impact of endoxylanase over-dosage on dough properties: consistency dropped dramatically and sticky doughs resulted. The deleterious effects of strong over dosage of endoxylanases on loaf crumb structure, crumb color, gas cell volume and crus color are not representative of the effects obtained when optimal use is made of active endoxylanses. Only the increase in loaf volume is common to both circumstances.

2.3.5.2 Lipase

Increased mechanization designed to increase production and demands for better quality and longer shelf-life have led to application of various additives by the baking industry in the last few decades. Lipase is one such additive. Lipases exist in all living organism, and take part in metabolizing lipid in the cell. Underkofler (1972) pointed out that "lipase activity in flour for baking is undesirable because free fatty acids have a detrimental effect in doughs." Therefore, Games (1976) concluded that use of lipase in the baking industry has been thought to be undesirable. In addition, one paper (JP patent, 1987) concluded that when lipase is used alone, other properties of the bread such as bread volume, elasticity of the crumb and mouth-feel deteriorate. Thus, it was recognized that lipase was not a dough improver at that time. At the bakery, additives as mentioned before (emulsifiers, oxidants and enzymes), SSL and DATEM have been used as dough conditioners and/or crumb softeners in many years (Stampfli and Nersten, 1995). The mechanisms of emulsifiers to improve dough handling properties and to provide longer crumb freshness have long been ascribed to their ability to bind to gluten proteins (Carr et al., 1992; Chung et al., 1981; Inoue et al., 1996; Riisom et al., 1984). In addition, emulsifiers are thought to form complexes with starch, particularly with linear amylose and to a lesser extent with branched amylopectin (Biliaderis et al., 1986; Ghiasi et al., 1982;

Gudmundsson and Eliasson, 1990; Krog et al., 1989; Raphaelides and Karkalas, 1988; Riisom et al., 1984). As emulsifier research progressed, lipase use gained interest again as a substitute for baking additives in recent (Abdullah and Hazim, 2012).

Lipase is an enzyme belonging to the class of glycerol ester hydrolases which catalyzes hydrolysis of ester bonds in triglycerides. In other word, lipase hydrolyzes a triglyceride into mono- and diglycerides. Trigrycerides in conventional bread dough containing no added fat come from flour, and constitutes about 1-3 % by weight of the dough. It has been reported by Weegels and Hamer (1992), Bekes et al. (1992), and Bushuk et al. (1984) that lipids present in dough interact with specific gluten complex proteins to form lipid-gluten aggregates during dough preparation. Mono- and diglycerides function as an emulsifier in dough that improves bread quality (Sluimer, 2005).

The use of lipase enzymes in the baking industry is quite recent as compared to other enzymes such as protease and α-amylase. However, lipase application is becoming important in an industry now. Lipases (1.3.-specific, phosphor-, and glycolipases) from fungal and bacterial sources having broad substrate specificity have been applied baking (Olesen et al., 2000; Qi Si and Hansen, 1994; Siswoyo et al., 1999). This research concluded that lipase addition can improve bread-making characteristics, in particular having strong, positive effects on dough stability and gas holding capacity. As a result, the final product had more uniform crumb structure, whiter crumb color, improved crumb softness, and increased loaf volume (Olesen et al., 2000; Qi Si and Hansen, 1994; Siswoyo et al., 1999). Hense, it is presently well known that lipase use modifies the interaction between lipid and gluten protein and thereby improves properties of dough and baked products. Moreover, Olesen et al. (2000), Qi Si and Hansen (1994) and Siswoyo et al. (1999) reported that lipase has anti-staling properties. In addition, Olsen et al (2000) found that dough treated with lipase has been found to have improved consistency, which results in a more machinable dough. However, excessive lipase addition has been reported to induce the dough to become dry and stiff with reduced volume (Qi Si and Hansen, 1994). Currently our knowledge is mostly based on knowledge of the effect of lipids in bread-making. The mechanisms underlying the technological effect of lipases are closely linked to the hydrolysis of one or more fatty acids from nonpolar triglycerides and/or polar lipids (phosphor-, and glycolipids) to form the corresponding more polar mono- and diacyl-forms (Castello et al., 1998; Poulson et al., 2006; Primo-Martín et al., 2006). Lipases, therefore, offer

the opportunity to generate surface active compounds in situ, and possibly to substitute or reduce the use of traditional emulsifiers. According to Abdullah and Hazim (2012), lipases improved dough handling properties similar to or to a greater extent than does DATEM. They reported that the formation of amylose-lipid complexes by lipases was much greater in extent than by DATEM. They also suggested that lipases probably play roles in delaying starch retrogradation. Thus, lipase is widely known to benefit bread quality (Sahi and Guy, 2004, Sluimer, 2005).

2.4 Frozen dough processing

Frozen dough making processes are basically the same as normal bread making. The biggest differences are that freezing, frozen storage, and thawing processes are added. In addition, the each process conditions needs to considered and modified for frozen dough making.

2.4.1 Mixing

Mixing is the first and critical stage of bread making. Mixed dough condition has a big influence on subsequent stages and on bread quality. The objectives of mixing are: 1) Hydration of the ingredients, 2) Homogenous distribution of the ingredients 3) Developing the gluten. 4) The initiation of fermentation (Doerry, 1995).

In North America, bread dough is produced by seven different methods: 1) Straight dough, 2) Sponge dough, 3) Liquid sponge, 4) Continuous mixing, 5) No-time dough, 6) Chorleywood, and 7) Authentic Sourdough method (O'Donnell, 1996). All have advantages and disadvantages (Table 2.6).

Table 2.6 Advantages and disadvantages of dough systems

Doguh System	Advantages	Disadvantages
Straight dough	Good flavor Medium process time Good mixing tolerance	Difficult dough handling Long mixing times Poor fermentation tolerance
Sponge and dough	Good fermentation tolerance Superior product score Good dough handling Longer product shelf life	Poor mixing tolerance Long process time High cost of equipment Large space requirement
Liquid sponge	Uniformity of product Medium process time Good dough handling Longer product shelf life	High cost of equipment Limited to 50-60 % of flour in sponge Lack of flavor and shelf life with low flour in sponge
Continuous Mix	Same advantages as liquid sponge if fermented Less equipment, labor, and space used	Limited to 50-60 % of flour in sponge Lack of crumb strength Lack of flavor and shelf life with less fermentation
No-time Dough	Short production time Greater flexibility Less equipment and space Superior yeast survival in freezing	Lack of flavor Lack of shelf life Higher ingredient cost Problem with floor time
Chorleywood Process	Tolerant to low protein flours Short production time Greater flexibility No floor time problems	High equipment costs High energy cost Lack of flavor Lack of product shelf life
Authentic Sourdough Process	Sourdough flavor Increased shelf life "Bristerd" appearance Chewy, resilient texture	Very long process time Nurturing of sponge Less consistency Increased space requirement

(Source: O'Donnell, 1996)

From the mid-1950s to roughly 1970, the production trend was toward continuous mixing. However, since in 1970, there has been a return to sponge dough and straight dough mixing. This is because the crumb characteristics and aroma of the final product made by continuous mixing are different from the quality made by other methods, and the demand for a natural type of product has increased (Jackel, 1978). At the same time of this trend change, "No-time straight dough method" began to spread. This method has been used widely in Australia since 1965 (Stenvert et al., 1978). At the same time, it became popular in the United Kingdom and for soft bread and roll baking in Canada. Somewhat later, this process was gradually accepted in the United States, especially by retail bakers and wholesale bakers (Kamman, 1979).

Research on mixing processes for frozen dough followed much the same trend as for fresh baking. Lorenz and Bechtel (1964) reported that the best storage life for frozen dough was obtained by the continuous-mix process. However, Javes (1971) concluded that the process was not satisfactory for frozen dough. Anonymous (1967) researched mixing conditions for frozen dough making. Godkin and Cathcart (1949) and Merritt (1960) researched the relationship between fermentation and frozen storage stability. As a result, Anonymous (1967) concluded that a high speed mixer with a refrigerating jacket to keep the low dough temperature (18-21 °C, 65-70 °F) was best. This retards fermentation and produces dense, plastic dough. This resulting dough is easy to handle and suitable for fast freezing. Fully developed dough is desired. If not, it results in poor final product quality. Furthermore, delayed salt and fat addition is preferred, so as to reduce mixing times and improve dough development and extensibility. Actually, dense dough has the best heat conductivity and facilitates rapid chilling and freezing (Marston, 1978). Godkin and Cathcart (1949) and Merritt (1960) concluded that frozen dough stability is inversely related to the amount of fermentation before freezing. For these reasons, a rapid, or "No-time (straight) dough" process with cold temperature and delayed salt incorporation is most suitable for frozen dough. On the other hand, Fuhrmann (1985) pointed out that direct expansion jackets on mixers can be a problem for straight no-time doughs because the liquid ingredients may freeze to the jacket before the dough has developed. Flour chilling systems can be advantageous in holding down dough temperatures, and liquid carbon dioxide injected directly into the mixing chamber to displace oxygen not only cools the dough but improves the reducing action of ascorbic acid to cut mixing time.

Using on no-time dough with the cold temperature method, Dubois and Blockcolsky (1986) studied the effect of several mixing methods on the quality of bread produced from frozen dough. They found that the delayed salt method produced dough with good gas production. Consequently, frozen dough must be fully developed in the mixer as rapidly as possible to minimize yeast activity and the gas generation before freezing.

After mixing by the no-time dough method, all doughs are transferred to the makeup process (dividing, rounding, and moulding) immediately. Sideleau (1987) researched the relationship between intermediate proof times and frozen storage period and concluded that longer intermediate proof times shorten the stable freezer storage period of the product. Therefore, make up processes should be finished as quickly as possible.

2.4.2 Freezing

The dough, once mixed, is transferred to the freezing process immediately. Freezing equipment can be divided into four types: quiescent (typical storage areas for frozen foods); blast (cold air is circulated through the chamber at velocity of 100-400 m per minute); impingement (jets of cold air are blown over the product surface); and cryogenic (liquid nitrogen or liquid carbon dioxide is sprayed on and around the product). These have been researched in detail in numerous papers on freezing of food (Tressler et al., 1968, Mallett, 1992). The most commonly used dough freezing method is blast freezing. Dough pieces are placed on trays on racks, or else conveyed on a spiral conveyor, and introduced into the freezer. Refrigerated air is blown over the product at a high linear velocity, cooling the product. A temperature gradient from the core to the surface is established. Because of the salt and sugar and other solubilized material, the freezing point of the internal water is depressed to about -3 °C to -5 °C (Stauffer, 1993). The core temperature of dough pieces is rapidly reduced from 20 °C to -5 °C, but freezing liberates heat of fusion that must be removed, and a plateau in the plot of the temperature versus time is observed. Freezing time is shorter when freezing temperature is lower. When all the water has frozen the temperature again decreases rapidly. The relationship was shown Fig. 2.7 (Lehman and Dreese, 1981).

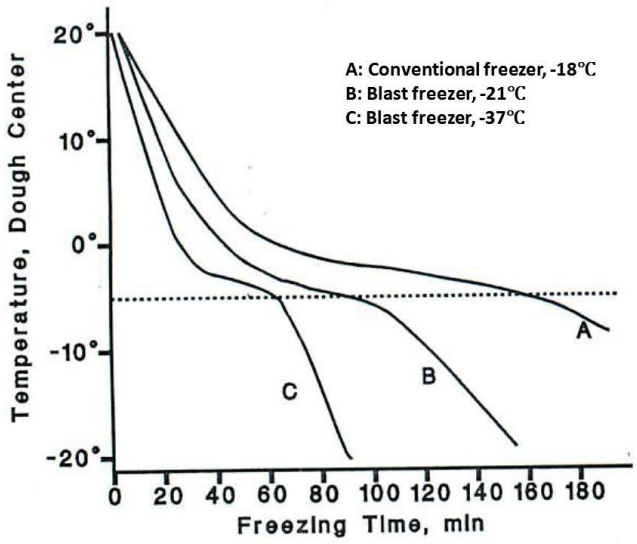


Figure 2.7 Relationship freezing time and core temperature at 500 g dough with three different conditions

(Source: Lehman and Dreese, 1981)

Studies on the preservation of microorganisms by freezing have shown that freezing and thawing rates affect yeast viability. Slow freezing is generally believed to allow cells to adjust to the freezing environment by transferring intercellular water to the external ice. Fast freezing, on the other hand, causes intracellular freezing 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 thawing and hence become lethal to the cells (Hsu et al., 1979b). Lehman and Dreese (1981) researched the influence of freezing conditions on frozen dough shelf-life. Their results are summarized in Fig. 2.8.

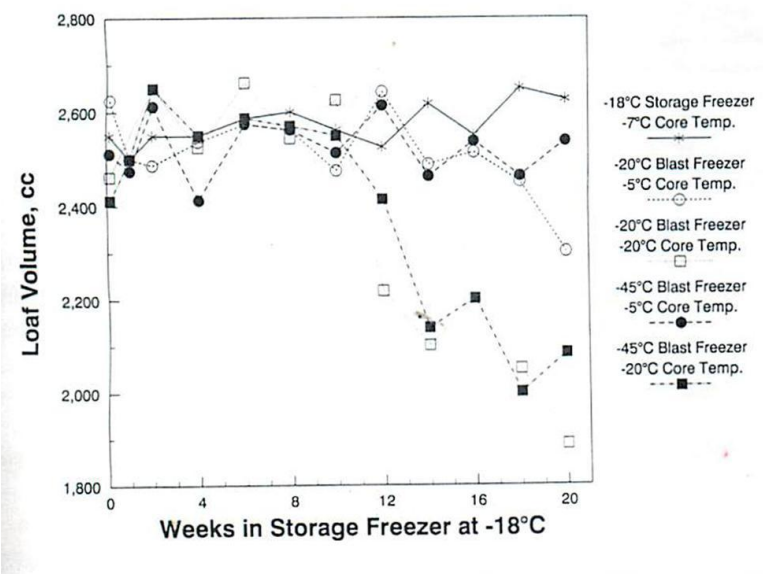


Figure 2.8 Relationship frozen storage and loaf volume at 500 g dough with different conditions

(Source: Lehman and Dreese, 1981)

When the dough was proofed to a constant height, proof time increased with longer storage, (about 50 % greater after 20 weeks than at 1 week). The lower loaf volume indicates decreased oven spring, i.e. weaker gluten strength. According to Fig. 2.8, rapid freezing to a lower core temperature harms dough keeping quality. For storage up to 11 weeks all the dough gave the same volumes, but after that time the samples frozen to an initial core temperature of -20 °C degraded rapidly. On the other hand, freezing to an internal core temperature of -7 °C (20 °F) gave high loaf of volume and kept dough quality until 20 weeks of storage. Therefore, it is generally recommended not to freeze solidly in freezing, because this will shorten the freezer storage life of the product and eventually lengthen proof times when the product is used by the retail bakers (Lorenz, and Kulp, 1995).

The remaining freezing of the dough occurs during what is called the equilibration period. Thus, the dough is thoroughly frozen at the point where yeast activity is least. This process occurs during the packaging and frozen storage of product (Sideleau, 1987). Internal core temperatures of products must be monitored and adjustments made to the dwell time in the freezer to maintain the proper freezing of dough. It is also extremely important that the products be indexed properly before entering the freezer to ensure that there is separation between items

for proper freezing. Products that touch each other will not freeze properly. By utilizing the two stage freezing operation, the dwell time in the main freezing camber is reduced and both the productivity of the plant and the product quality are improved (Lorenz, and Kulp, 1995).

2.4.3 Packaging

After freezing, doughs are packaging and stored in freezers. Today, good quality shelf life of yeast-leavened products is at least 3 months and, depending on the freezing and storage conditions, up to 6 to 9 months (Jackel, 1991).

Packaging designed for frozen doughs must perform a number of functions. It must contain, protect, identify, and merchandise the food. One of the factors responsible for the decline in product quality during frozen storage is loss of moisture. Cold air has low moisture content and therefore dehydrates any un-protected product with higher moisture content. A good packaging material must keep this this loss of moisture to a minimum (Klein, 1971ab). Klein (1971b) suggested that films to be used for frozen dough should possess the following characteristics: 1) Good moisture protection, 2) Good oxygen-barrier characteristics, 3) Physical strength against brittleness and breakage at low temperature, 4) Stiffness to work on automatic machinery, 5) Good heat seal ability. Therefore, Kline (1971b) concluded that 1.5 to 3.0 mil polyethylene or PVDC-coated polyethylene is suitable for frozen dough with a low shortening content, such as bread dough. Therefore, polyethylene bags are very popular packaging system.

2.4.4 Frozen storage

Packaged products are stored in a warehouse freezer at temperatures of -18 °C to -23 °C (0 °F to -10 °F). The air temperature in storage should be kept as consistent as possible, with a minimal amount of fluctuation. Fluctuation of the temperature of the product during cold storage or shipment reduces dough performance and shortens the freezer life of the product due to ice crystal formation and growth. Yeast, even at these temperatures, is not totally dormant, and extreme temperature fluctuations are detrimental to product quality and performance. The

temperature gradient between surface and the core of the dough piece favors moisture migration, and osmotic pressure (concentration of salt and sugar) changes (Sideleau, 1987).

Changes in the temperature between freezing and storage clearly affect yeast viability (Hsu et al., 1979b). Lower storage temperature than freezing temperatures were more harmful than freezing and storing at the same temperature. For instance, yeast activity, as judged by proofing time, was significantly lower in samples of dough frozen at -18 °C (0 °F) and stored at -34 °C (-30 °F) than in samples frozen and stored at-18 °C. The damage from freezing at -18 °C and storing at -34 °C was even more pronounced than from freezing and storing at -34 °C. Damage seemed to result from transferring a frozen sample to a lower temperature. Yeast damaged caused by slow freezing to -34 °C was similar to that caused by freezing at -18 °C and storing at -34 °C. Change of storage temperature to a higher level did not cause much additional yeast damage (Hsu et al., 1979b).

Lu and Grant (1999) showed that water separates from the protein and starch components in frozen dough and accumulates into pools that subsequently crystallize. During prolonged frozen storage the amount of freezable water in the frozen doughs increases significantly. Zounis et al. (2002) observed that the cause of this disruption apparently was a change in the ice crystal structure as indicated by the appearance and increase in the size of angular voids in doughs during frozen storage. Bot (2003) reported that in gluten stored at -15 °C, the water content in the gluten phase decreased by around 1 % over the first three weeks, and the same changes occurred in dough stored at either -15 °C or -25 °C as in gluten at -15 °C. Naito et al. (2004) reported that scanning electron microscopic images of dough's pore walls that were washed with distilled water (20 °C) clearly showed that gluten fibrils forming the skeletal framework of pore walls were cut and became coarse and non-uniform strings and that many knots were generated on gluten fibrils from freeze damage. Esselink et al. (2003) reported that at the macroscopic level, ice crystals are not evenly distributed over the moluded dough nor are the gas cells homogenously distributed throughout the dough.

2.4.5 Thawing

After a period of frozen storage, dough is thawed. As with the freezing process, numerous methods for thawing have been proposed. Dubois and Blockcolsky (1986b) published a systematic investigation into this process. Dough is thawed at three temperatures: 5 °C (retarder or refrigerator); or 20 °C to 25 °C (room temperature); or 30 °C to 40 °C (proofing cabinet). Three factors are considered by the baker: the time required to thaw the dough piece; the time required to proof to a certain height; and the volume, external appearance, and internal grain of the baked bread. Dubois and Blockcolsky (1986b) investigated four thawing procedures: 16 hours at 5 °C; 24 hours at 5 °C; 1 hour at 22 °C; and directly from the freezer to the proofing chamber at 32 °C. Twenty four hours at 5 °C condition was shortest proof time, and 16 hours at 5 °C condition resulted in the highest specific volume. However, all four specific volumes were quite acceptable for white pan bread. The thawing procedure showed only minor and inconsistent effects on the other quality factors (external appearance, internal grain.) Dubois and Blockcolsky, (1986b) concluded that dough should be thawed for 16 hours in the retarder. The total time required to obtain fully proofed dough is less than if dough is thawed at room temperature first, and the quality factors are equivalent. Finally, it is important to cover dough pieces as they are thawing in the retarder. If they are left uncovered overnight, a dry skin develops on the surface, which produces unsightly patchiness on the crust of the baked bread. Nicolas et al. (2003) indicated that after thawing, gluten exhibited a microstructure similar to that of fresh gluten, with the structure looking like a sponge (a fine gluten structure with tiny water pockets.) Seguchi et al. (2003) examined the relationship between loss of bread baking properties and increase in the amount of the centrifuged liquid from frozen and thawed dough and reported that the amount of centrifuged liquid from bread dough was increased by freezing and thawing.

Antifreeze or ice structuring proteins (ISPs) can lower the freezing point of solutions and inhibit ice crystal growth and recrystallization during freezing (Barrett, 2001; Kristiansen and Zachariassen, 2005; Yeh and Feeney, 1996). Research has been conducted on the effects of ISPs on the physicochemical, rheological and textural characteristics of frozen dough (Kontogiorgos and Goff, 2007, Zhang et al., 2007ab). Changes in the distribution and size of ice crystals formed and delaying recrystallization by ISPs had direct effects on product quality. Based on this research, Xu et al. (2009) evaluated water holding capacity (WHC) and bread making properties

of frozen dough containing ice structuring proteins from winter wheat. They reported prolonged frozen storage time and freeze-thaw cycles resulted in a decrease in WHC of dough and in bread making properties reflected by an increase in proof time and a decrease of bread specific volume. The ice structuring proteins (ISPs) had a protective effect on dough against the damage promoted by frozen storage and freeze thaw cycles, which was reflected by better WHC and bread making properties. With increases in WHC, the bread specific volume increased and the proofing time decreased. The reason is unclear why depression of bread making properties such as bread height, and specific volume were caused by freezing and thawing of bread dough (Selomulyo and Zhou, 2007).

2.4.6 Proofing

Proofing of the dough allows air cells to expand biaxially and carbon dioxide gas to be produced. This is the final step where the air bubble size is increased. The number of air cells and the quality creates a smoother texture and finer crumb grain in the baked bread loaf; therefore, it is an important process step (Dobraszczyk and Morgenstern, 2003). At the end of the retarding period (thawing), the dough temperature is the same as that of the retarder and it is ready to proof. For frozen dough, proof box temperatures are in the range of 32 °C to 43 °C (90 °F-110 °F), with relative humidity about 70-75 % (Stauffer, 1993, Lorenz and Kulp, 1995). The range is necessary for accommodating different dough sizes and weights. Higher proofing temperatures may be used with smaller dough pieces, while large pieces require lower proof temperatures. If bread is proofed at higher temperatures, the large temperature gradient (the center of the dough pieces may be barely above 0 °C) will cause the outer part of the dough to over proof relative to the center and the baked bread will have a close, under-proofed center and coarse grain near the crust. The relative humidity recommended is slightly lower than that for fresh dough. At a higher relative humidity (85-90 %) some condensation on the surface of the cold dough pieces is likely to occur, which cause blisters and/or light blotches to appear on the crust during baking.

Occasionally reference is made to ‘reworking’ thawed doughs. This is most often tried with dough that has been stored longer than its useful shelf-life. When thawed, proofed and

baked the product has low volume and coarse grain. The response of the experienced baker is to try to improve volume and grain by sheeting and moulding the dough pieces. Unfortunately, experience has shown that this is not effective. Once the dough has lost its gluten strength during extended frozen storage, further mechanical work does not reverse the process. Recently, Seguchi and Morimoto (2011) reported that the bread making properties of frozen dough was restored by addition of sugar, yeast, and subsequent processing.

2.4.7 Baking

During baking, starch gelatinization, protein denaturation and evaporation of water occur and the bread loaf structure is set (Bloksma, 1990). In baking, gas cells created by fermentation are expanding biaxially as they are during fermentation (Bloksma, 1990, Dobraszczyk and Morgenstern, 2003). This phenomena is called "oven spring". Baking conditions and theory for fully proofed doughs from frozen doughs are essentially the same as in conventional bread production. The fully proofed doughs from frozen dough have a significantly lower internal temperature than do those from a fresh dough system, which may result in slightly different oven-spring. However, this difference is due more to the different action of various oxidizing agents than directly to the cooler dough in the retarded-dough process. A moderate oven temperature of 200-220 °C (400-425 °F) is usually most suitable for the baking of frozen dough. This allows for some extension of proofing and further expansion of the dough before the structure sets, and it avoids too dark crust color (Marston, 1978).

2.5 Final products quality evaluation

Volume and/or specific volume is an important characteristic in the evaluation of bread quality, relating to the underlying gluten (structural) development of the bread. Volumes of bread are determined using two methods, rapeseed displacement and 3D laser scanning. Rapeseed displacement has been used for more than 60 years, and it is well known (Approved Method 10-05.01, AACC 2000). The 3D laser scanning method (Caley et al., 2005, Sato, 2003, 2007) was developed recently and it's begun to be used as an alternate method to rapeseed displacement.

The crumb texture (structure) is critical as well as the volume. Originally, crumb texture was subjectively judged (scored) by bakers. Recently, digital image analysis technology has developed and applied to bread crumb analysis (Whitworth et al., 2004). Therefore, bread crumb can be efficiently and objectively analyzed.

2.5.1 Loaf of volume

The measurement of loaf volume has been studied for a long time. The reason is loaf volume (LV) is the principal indication of bread quality evaluation (Caley et al., 2005). The American Association of Cereal Chemists has approved one method of volume determination for bread; rapeseed displacement. The idea of the displacement originated when human sat first in a full tub of water. Similarly, baked products can be measured using rapeseed instead of water. Rapeseed displacement determines the LV of oddly shaped baked products from the volume of rapeseeds they displace (Approved Method 10-05, AACC 2000). This method may crush a cake by weight of the falling rapeseed, skewing the volume measurement, so it's not perfect volume measurement method for tender baked products. However, rapeseed has a relatively uniform particle size, and is cheap (Takeya, 2005). Therefore, rapeseed displacement has been used for more than 60 years, and it is well known now. In the beginning, volume measurement was carried out using on hour-glass type of device (Cathcart and Cole, 1938). Actual loaf volume was measured by rapeseed or mustard seed, but the calibration of the loaf volume box and the accompanying burette was measured (calculated) using water. Therefore, the estimated bread volume introduced the error of the measurement almost invariably (Bailey, 1930). To prevent

measurement error, various methods were researched (Harrel, 1928; Heald, 1929; Bailey, 1930). Harrel (1928) explained that three procedures are possible for finding the loaf volume. First, LV can be determined as the box volume minus volume of seed retained in box. Second, by use of a constant volume of seed equal to the box volume, the overflow represents loaf volume. Third, placing the known volume of a standard object as the ordinate and the volume of the retained seed as the corresponding abscissa, it is assumed that the slope of curve equal 1. Increments in the abscissa represent corresponding decrease in volume and vice versa. Based on this theory, Harrel (1928) proposed a procedure that overcame the difficultly in the method of LV measurement. It involved the use of a rubber balloon filled with varying quantities of water. The volume of the filled balloon could be determined by weighing and correcting for thermal expansion of the water as a function of temperature. Two points on a graph could thus be determined by using two different quantities or volumes of water in the balloon and plotting the volume of seed as measured in the burette as abscissas and the true volume of the filled balloon as ordinates. Heald (1929) made four observations with the balloons, establishing as many points on his graph, and proceeded to plot "apparent" volume against "true" volume. He found that these four values fell on a straight line. The data included in his tabulation indicate that the error or difference in cubic centimeters between the true volume and the apparent volume tended to diminish as the volume of the balloon increased. The use of the rubber balloon filled with varying quantities of water, while satisfactory, is open to certain objections in the matter of convenience. Moreover, it is possible that the errors in filling the loaf volume box may vary somewhat with the shape of the object to be measured. If this were true, then the nearer one could approach to the shape of a loaf of bread, the more satisfactory would become the calibration of the devices, particularly in view of the fact that this calibration is essentially empirical at best. There are other obvious advantages associated with the calibration of a number of loaf devices in as many laboratories when the "true" volume points are in the same position on the graph in each instance. This cannot be attained satisfactorily through the use of balloons. However, Bailey (1930) pointed it is possible if solid models of loaves was employed.

The volume measurement device was modified and adapted to small loaves by Geddes and Binnington (1928). Malloch and Cock (1930) have modified the design still further in order to increase the ease of construction and the accuracy of operation. Based on such research results, the present day rapeseed displacement apparatus is designed with a metal box connected with the

hopper containing rapeseed through a rectangular chute. There are various sizes and types of volumeters (Bourne, 2002). A dummy loaf of standard size is provided with each volumeter to calibrate the rapeseed level in the hopper. After calibration, one sample (loaf of bread) is placed in the box, which is closed, a slide in the chute is pulled out, and the rapeseed is allowed to fill the box. A calibrated scale on the face of the volumeter column gives the direct reading of the volume of the bread in cubic centimeters. This device and method are approved by AACC (Approved Method 10-05, AACC 2000) and widely used in the baking industry to measure loaf volume (Cathcart and Cole, 1938, Funk et al., 1969).

Optical technology has developed a 3D laser scanning method and it is starting to be used as an alternate method to rapeseed displacement. There are problems in rapeseed displacement; time required for one sample volume measurement; error of measurement by operator; sample shape destruction by contact with rapeseed (Sato, 2003 and 2007). The laser scanning method was developed and used to deal with these problems. With this device a sample is put on a rotating table, laser light projected on the sample is captured with a CCD camera and processed with a PC to yield shape data, and from this value the volume is calculated (Sato, 2003 and 2007). Caley et al. (2005) compared rapeseed displacement and 3D laser scanning methods and reported the differences in LV values obtained between the two methods depend on the LV values of samples. However, LV values were highly correlated between the two methods and not related to wheat class, baking method, or sample size. The correlations between the two methods were highest for pup and pound loaves produced from winter wheat, and lowest for spring wheat. The R-square value for all data was 0.996. Thus, Caley et al. (2005) concluded that rapeseed LV values could be predicted accurately using the laser scanning instrument values such as LV, width, max depth, and area.

2.5.2 Image analysis

All bakers and baking researchers hope to make a perfect loaf. Because there are many different types of bread and many different opinions, it is difficult to define the perfect loaf. All bakers and baking researchers evaluate crumb grain as one of several bread quality parameters (as well as volume measurement) (Zayas, 1993). Different systems of scoring bread and different

grading scales have been used (Zayas, 1993). Early on, terms or descriptors of crumb parameters were different for each operator, so The Bakers Committee of the Hard Winter Wheat Quality Group (HWWQG) of the Wheat Council defined bread grain as visually perceived traits dealing with the size, shape, uniformity, and wall thickness of crumb cell (Rogers, 1995). Pyler (2008) explained that bread crumb grain appearance is affected by many technological factors such as baking ingredients, storage time, and temperature. In addition, Pyler (2008) pointed out that crumb color and crumb grain greatly influence baked product evaluation.

Cell shape is a typical characteristic of crumb grain. Fineness and uniformity of the cell structure are preferable. Rogers (1995) pointed out that there are two problems with this grain scoring system. The first is that the bread scoring varies widely in all scale levels, and second, that personal, regional, and cultural preferences affect crumb grain score. Therefore, an objective bread crumb evaluation method is needed.

To solve this problem, several research groups have studied the feasibility of adapting digital image analysis (DIA) for crumb grain analysis. Smolarz and coworkers (1989) were among the first to apply classical image analysis to baked products. Based on these results, numerous researchers have used this quantitative tool for the assessment of crumb features such as cell size, cell size distribution, number of cells per unit area, cell wall thickness, void fraction an shape factor (Bertrand et al., 1992; Zayas 1993; Sapirstein et al., 1994; Rogers et al., 1995; Zghal et al., 1999; Takano et al., 2002; Lagrain et al., 2006; Gonzales-Barron and Butler, 2006; Calderón-Domínguez et al., 2008). Other researchers utilized digital scanners to capture bread crumb in two dimensional (2D) high resolution images (Esteller et al., 2006; Datta et al., 2007; Lassoued et al., 2007; Esteller and Lannes 2008) and product volume (Chevallier et al., 2012). Based on crumb characteristics, Bertrand et al. (1992) concluded that consumers preferred bakery products with fine structure. Zayas (1993) used the Kontron image processing system for image analysis. Sapirstein and coworkers (1994) concluded that electronic image analysis is objective, rapid, and precise. Zghal et al. (1999) showed a relationship between bread crumb density and bread crumb grain assessed by image analysis. According to Whitworth et al. (2004), CCFRA (Campden and Chorleywood Food Research Association, Station Road, Chipping Campden, Gloucestershire, GL55 6LD, UK) developed a high efficiency image analyzer that is called "C-Cell®". In this instrument, sliced bread crumb images are captured using a monochrome framing camera at a resolution of 1296 × 1026 pixels and field of view of 182 ×

143 mm. The software provided (C-Cell Software) analyzes 48 different sliced crumb data properties and 6 imaging (raw, brightness, cell, elongation, shape, and volume) parameters automatically. Samples can be measured in a few seconds by this instrument. Bread crumb evaluation instruments such as “C-Cell” have been developed, but there is not yet a standardized technique for this evaluation. Differences among the reported DIA methodologies for acquiring images by scanning or when pre-processing or processing these images to obtain crumb features are found (Farrera-Rebollo et al., 2012). Some of these differences among the reported methodologies are due to the scanning resolution, where researchers report using 200 dpi (Esteller et al., 2006, Esteller and Lannes 2008), 300 dpi (Lagrain et al., 2006), 350 dpi (Gonzales-Barron and Butler, 2006) or even without reporting the scanning resolution applied to the analysis. Farrera-Rebollo et al. (2012) warned that these differences in methodologies could result in different data for similar breads, making it difficult to compare information among published reports. Thus, the clarification and the standardization of the technique are required along with producing a high performance instrument.

2.6 Dough rheology

Wheat flour dough is made by mixing wheat flour and water, but it generally contains various additives such as sugar, salt, yeast, shortening, and oxidants for bread baking. These are commonly added to improve bread quality (improve palatability, loaf of volume and texture). In other words, bread dough is composite materials made of multiple components that have complex rheology. The dough's rheology is greatly influenced by the types and amount of ingredients added and the processing (mixing, proofing, and baking) conditions (Lee et al., 2001; Dobraszczyk and Morgenstern, 2003; Davidou et al., 2008). Defining and evaluating dough rheology is critical and essential, because it provides an understanding of each ingredient's interaction in dough and quality controlling each bread-making process. Originally, dough rheology was judged by empirical physical methods and each baker's experience (gluten film test). Later, descriptive empirical methods were developed and applied to dough rheology research (Muller, 1975, Shuey, 1975). More recently, fundamental rheology measurement methods were developed and applied to dough rheology research. In the study of dough rheology, fundamental measurement methods have contributed to understanding of dough quality and response to processing. At the same time, it was recognized that wheat dough rheology research was difficult to interpret because dough was a non-uniform and complex material. Therefore, dough rheology research is a big challenge.

2.6.1 Rheological properties in dough

All foods have their own intrinsic rheological properties and this information is very useful in a large number of industrial applications. Rheological property measurement and evaluation is the most valuable way to characterize the rheological behavior of fluid and semi-solid foods. Steady shear viscosity is a property of all fluids and semi-solid products. However, many phenomena or conditions cannot be described by only the viscosity function, and the elastic behavior must be considered (Steffe, 1996). Dough elasticity and viscosity are two important rheological properties of wheat flour dough and their combination is called viscoelasticity. Dough is viscoelastic because both behaviors exist together. Dough

viscoelasticity is affected by added ingredients and processing condition, so the measurement of dough viscoelasticity is critical in dough rheology (Uthayakumaran et al., 2000; Lee et al., 2001; Dobraszczyk and Morgenstern, 2003; Davidou et al., 2008). van-Vliet (1992, 2008) described the behavior of strain hardening as an indicator of bread-making quality, especially, in fermentation (proofing) and baking. After van-Vliet (1992), many researchers applied this theory to bread-making. Each of these dough rheological properties is described below.

2.6.1.1 Viscoelasticity

Dough viscoelasticity is an especially important behavior because it has a great influence on the dough machinability (processing), and the texture characteristics of the final product (Uthayakumaran et al., 2000). Dough viscoelastic behavior has been attributed mainly to the gluten fraction of dough. It is greatly changed by the types and amount of additives, mixing condition, and the glutenin to gliadin ratio in the flour (Uthayakumaran et al., 2000; Lee et al., 2001; Dobraszczyk and Morgenstern, 2003; Davidou et al., 2008). Dough viscoelasticity (dough condition) is greatly altered during the bread making process. These viscoelastic properties are determined by conducting empirical physical measurement, empirical descriptive measurement, and fundamental measurement (Dobraszczyk and Morgenstern, 2003). Many researchers measure dough viscoelasticity by descriptive empirical methods such as Farinograph or Mixograph. The results are used identify and understand the effect of additives and their interaction on dough (Muller, 1975; Shuey, 1975; Weak et al., 1977; Miller and Hoseney, 2008). More recently, fundamental tests have been applied to dough (Ferry, 1980; Faubion, and Faridi, 1986; Barnes et al., 1989; Faubion and Hoseney, 1990; Weipert, 1990; Bloksma, 1990; Amemiya and Menjivar, 1992; Steffe, 1996; Walker and Hazelton, 1996). Bloksma (1990) and Walker and Hazelton (1996) concluded that proper fundamental measurement can specifically relate to the rheological viscoelastic properties during processing.

2.6.1.2 Strain hardening

Strain hardening is a complex property. Strain hardening was first observed with amorphous glassy materials and polymer melts due to unstable necking when they are stretched uniaxially. Its importance was first recognized for metals (Considere, 1885). This phenomenon was observed in polymeric systems later (Vincent, 1960). Now, "Strain hardening" is defined as the phenomenon whereby the stress required deforming a material increases more than proportional to the strain (at constant strain rate and increasing strain) (van-Vliet et al., 1992). van-Vliet et al.(1992) proposed that strain hardening is an important property of dough, especially in the biaxial extension of dough, the dominant deformation with respect to gas cell coalescence during proofing and baking. The dough must remain extensible enough to allow further expansion of gas cells and elastic enough to prevent failure of the loaf structure during proofing and baking. Sroan and MacRitchie (2008) showed the ability of a cell to undergo biaxial extension and not rupture is affected by strain hardening and that it has a large influence on the stability of gas cells. In bread (dough) making theory, it is well known that gliadin provides dough extensibility (viscosity) and glutenin provides dough elasticity. These proteins create gluten when mixed with water and sufficient mechanical energy input. Bloksma (1990) reported that extension of long molecules, such as glutenin into elongated conformations is achieved through input of sufficient mechanical energy into the dough; thereby creating dough which has good machinability and gas retention properties. Based on baking theory and research, it was recognized that gluten in dough provides the stain hardening. Numerous publications have shown strain hardening of dough and gluten relates to baking performance (Dobraszczyk and Roberts, 1994; Janssen et al., 1996; Kokelaar et al., 1996; Fan et al., 1999; Zghal et al., 2002; Dobraszczyk et al., 2003; Tronsmo et al., 2003; Sliwinski et al., 2004; Dobraszczyk and Salmonowicz, 2008). Furthermore, various methods have been described to measure strain hardening characteristics of dough in uniaxial extension and bi-axial extension (van-Vliet et al., 1992; Dobraszczyk and Roberts, 1994; Sliwinski et al., 2004). Some researchers reported that good strain hardening dough characteristics resulting in a finer crumb texture (e.g. smaller gas cells, thinner cell walls and an even distribution of bubble sizes) and larger baked volume than did doughs with poor strain hardening properties (Dobraszczyk and Roberts, 1994; Dobraszczyk, 1997; Dobraszczyk and Morgenstern, 2003).

In bread quality evaluation, loaf volume (specific volume) and crumb structure of bread are considered to be very important quality indicators. As a result of Dobraszczyk and Roberts (1994), Dobraszczyk (1997) and Dobraszczyk and Morgenstern (2003) research, gluten strain hardening is known to influence gas retaining capacity of dough and the distribution of the gas cells within the gluten phase of the dough. Consequently, it affects final bread quality.

Thus, strain hardening is especially important because it provides the dough a mechanism that allows for expansion without damage during fermentation and oven spring. In addition, strain hardening provides good bread making performance. This parameter is important in dough rheology. van-Vliet et al. (1992) pointed out the most relevant deformation to be considered is biaxial extension as that is the prevalent deformation of dough around growing gas cells. A parameter characterizing strain hardening in relation to bread making performance is $\Delta \ln \sigma / \Delta \varepsilon$, where σ is the stress and ε the strain. In their studies, good baking performance resulted from biaxial strain hardening rates varying from 1 to 2 (van-Vilet et al., 1992; Dobrazyck and Roberts, 1994; Dobrazayck and Morgensten, 2003). In addition, Sliwinske et al. (2004) concluded that during proofing and baking, dough deformation involves both uniaxial and biaxial extension and that strain hardening in uniaxial and biaxial extension are not directly correlated.

2.6.2 Measuring rheological properties in dough

Many food and bread making processes operate under large deformation extensional flow (e.g. proofing, baking), but most rheological tests on foods are performed by small deformation shear in oscillation (Dobraszczyk and Morgenstern, 2003). Therefore, both small and large deformation measurement of dough is critical and essential to determining and understanding dough rheology. As described earlier, dough rheology measurement can be categorized by two parameters; empirical descriptive methods, and fundamental methods (Table 1.1). Empirical descriptive methods are easy to use, have high tolerance in industrial environments. They have used for a long time in the cereal industry (Muller, 1975, Sheuy, 1975). They are better accepted than as fundamental methods. Weipert (1990) observed that fundamental methods often do not provide good correlations with final bread quality. However, Dobraszczyk, and Morgenstern (2003) explained that the sample geometry of empirical descriptive methods is variable and not

well defined. Also, stress and strain states are uncontrolled, complex and non-uniform. It is; therefore, impossible to measure any rheological parameters such as stress, strain, strain rate, modulus or viscosity.

Because understanding dough properties and interactions, and defining all rheological parameters are useful, fundamental methods are applied to the measurement of dough rheology. Of the fundamental methods, dynamic oscillation measurement is one of the most popular and widely used techniques for measuring dough and batter rheology (Newberry et al., 2002). Because yeast leavened dough is difficult to study with fundamental rheological testing, non-yeasted dough is often employed to improve the reproducibility.

2.6.2.1 Dynamic oscillatory measurements

Dynamic oscillatory measurement is adapted from techniques developed for measuring viscoelastic properties of polymer melts and concentrated solutions (Ferry, 1980, Barnes et al., 1989). This method has been extensively used to determine fundamental mechanical characteristics of wheat flour dough (Faubion and Faridi, 1986; Faubion and Hoseney, 1990; Amemiya and Menjivar, 1992). This test applies sinusoidally oscillating strain or stress to samples, and measures the response (Weipert, 1990 and Steffe, 1996). As a result, rheological properties such as elastic and viscous moduli are determined. Dynamic oscillatory rheometer can be a one of the controlled stress or strain instruments. Oscillatory testing has the following advantages: 1) Well-developed theoretical background; 2) Readily available instrumentation; 3) Simultaneous measurement of elastic and viscous moduli, while the non-destructive nature of the test enables multiple measurements to be performed as temperature, strain or frequency is varied (Debraszyk and Morgenstern, 2003).

On the other hand, Debraszyk and Morgenstern (2003) pointed out this method has disadvantages. Oscillatory tests must be performed in the material's linear viscoelastic range of frequencies in order to be accurate and reproducible. Consequently, that range must be determined first. The magnitude of strain used in the test is very small, usually on the order of 0.1-2%, where the material is in the linear viscoelastic range. This linear viscoelastic range is not particularly sensitive to the molecular structures responsible for baking quality (Dobraszczyk and Morgenstern, 2003). In addition, Dobraszczyk and Morgenstern (2003) contended that the strain

rates and frequencies by amplitude oscillatory measurement are not relevant to practical bread making process conditions such as dough mixing, proofing, and baking. Bloksma (1990) reported that commercial proofing and oven spring extensional rates are several orders of magnitude different as the rates utilized in oscillatory testing. Amemiya and Menjivar (1992) reported the magnitude of strain applied to the measurement is in the range of 0.1-2 % because it is within the linear viscoelastic range. However, they showed the strains in gas cell expansion during proof is known to be in the region of several hundred percent.

Though the dynamic oscillation test appears to have disadvantages, Dobraszczyk and Morgenstern (2003) mentioned that it might be a very useful technique if applied under proper conditions. Identification and characterization of dough as well as wheat flour component at the molecular level is possible by applying dynamic oscillation methods (Tronsmo et al., 2003, Connelly and McIntier, 2008). A lot of researchers conclude that dynamic oscillation testing will be a useful method in the future as well as present (Weipert, 1990; Steffe, 1996; Salvador et al., 2006).

2.6.2.1.1 Oscillatory stress and strain

In oscillatory tests, materials are subjected deformation or stress which varies harmonically with time. Steffe (1996) explained that application in oscillatory testing. The parameter and equations are shown below. For this shear or stress, a simple sinusoidal (sine wave) with frequency (ω) is typically used. Stress of the sinusoidal (sine wave) of frequency (ω) is applied to the material and the oscillating strain response is measured along with the phase difference between the oscillating strain and stress. The input strain varies with time as shown Eqn.1

$$\gamma = \gamma_0 \sin(\omega t) \qquad \text{[Eqn.1]}$$

Where (γ_0) is the amplitude of the strain. For sinusoidally varying strain, a periodic shear rate is shown in Eqn.2 and Eqn.2'.

$$\frac{d\gamma}{dt} = \frac{d(\gamma_0 \sin(\omega t))}{dt}$$

[Eqn.2]

$$\dot{\gamma} = \gamma_0 \omega \cos(\omega t)$$

[Eqn.2']

When the material behaves in a linear viscoelasitic manner with small strain amplitude, the shear stress is produced by the strain input. The corresponding stress (σ) was represented as Eqn.3.

$$\sigma = \sigma_0 \sin(\omega t + \delta)$$

[Eqn.3]

Where (σ_0) is the amplitude of the shear stress and (δ) is the phase lag or phase shift relative to the strain. The shift angle (δ) is depends on material properties (Steffe, 1996). Darby (1976) showed input and response functions differing phase by the angle (δ) (Fig. 2.9).

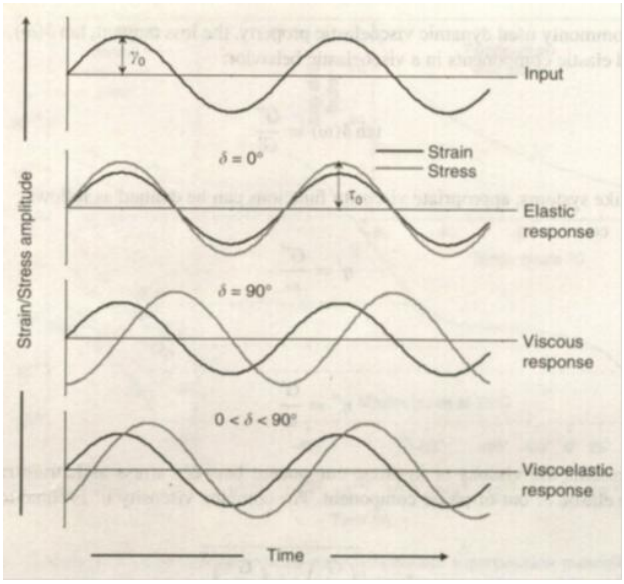


Figure 2.9 Input and response function differing in phase by the angle δ

(Source: Darby, 1976)

δ = 0 is a Hooken solid, δ = 90° is a Nwetonian fluid, and 0 < δ <90° is a viscoelastic material.

Dividing both side of [Eqn.3] by γ_0 yields.

$$\frac{\sigma}{\gamma_0} = \left(\frac{\sigma_0}{\gamma_0}\right)\sin(\omega t + \delta) \qquad \text{[Eqn.4]}$$

The complete result of small amplitude oscillatory tests can be described by plots of the ratio (σ_0/γ_0) and the phase shift (δ) as frequency dependent functions. The shear stress output produced by a sinusoidal strain input is generally written as [Eqn.5].

$$\sigma = G'\gamma + (G''/\omega)\dot{\gamma} \qquad \text{[Eqn.5]}$$

G' (called the dynamic shear storage modulus) and G" (called the dynamic shear loss modulus) are both functions of frequency and can be expressed in terms of the amplitude and the phase shift.

$$G' = \left(\frac{\sigma_0}{\gamma_0}\right)\cos(\delta) \qquad \text{[Eqn.6]}$$

and

$$G' = \left(\frac{\sigma_0}{\gamma_0}\right)\sin(\delta) \qquad \text{[Eqn.7]}$$

Additional frequency dependent material functions include the complex modulus (G*), complex viscosity (η*), dynamic viscosity (η'), and the out of phase component of the complex viscosity (η").

$$G^* = \frac{\sigma_0}{\gamma_0} = \sqrt{(G')^2 + (G'')^2} \qquad \text{[Eqn.8]}$$

$$\eta^* = \frac{G^*}{\omega} = \sqrt{(\eta')^2 + (\eta'')^2} \qquad \text{[Eqn.9]}$$

$$\eta' = \frac{G''}{\omega} \qquad \text{[Eqn.10]}$$

$$\eta'' = \frac{G'}{\omega} \qquad \text{[Eqn.11]}$$

Another parameter used to describe viscoelastic behavior is the tangent of the phase shift or phase angle (called tan δ) which is also function of frequency. The equation is Eqn.12.

$$\tan(\delta) = \frac{G''}{G'} \qquad \text{[Eqn.12]}$$

2.6.2.1.2 Application of dynamic oscillation measurements to dough

Dynamic oscillatory data on dough should be obtained within the dough's linear viscoelastic region. Numerous researchers have tried to relate these rheological data to baking performance or final quality, but interpretation has been difficult and often showed contradictory results. One reason may be that proofing and oven spring extensional rates are different the rates utilized in oscillatory testing (Bloksma, 1990). In addition, strain range is applied between 0.1-2 % in oscillatory testing, but strains in gas cell expansion during proof is known to be in the region of several hundred percent (Amemiya and Menjivar, 1992). Furthermore, the linear viscoelastic range in small amplitude oscillatory testing show low sensitivity to polymer molecular weight differences (protein interaction), so it may not be suitable for predicting bread making performance (Dobraszczyk and Morgenstern, 2003). Although the bread-making performance and dough rheology cannot be related directly, measurement data and interpretation of dynamic oscillatory tests are very useful. Most research has focused on how dough rheological properties were affected by major components, such as gluten, starch, and water (Faubion et al., 1985, Dreese et al., 1988) and flour cultivar (Faubion and Hoseney, 1990).

More recently, fundamental rheology measurement such as dynamic oscillation testing began to be used in the area of frozen dough rheology (Autio and Sinda, 1992). Numerous

studies have been conducted on ingredients and process (e.g. freezing and frozen storage), and how they affect frozen dough. Kenny et al. (1999) applied dynamic oscillation testing on un-yeasted, frozen dough with various additives (e.g. ascorbic acid, DATEM, and SSL). They reported that all additives addition improved frozen dough properties and baking quality (Kenny et al., 1999). In their research, over extended frozen storage time, frozen dough that contained the additive maintained a higher complex modulus than did control which containing no additives (Kenny et al., 1999). The phase angle vs. frequency measurement over eight weeks frozen storage found the ascorbic acid containing dough value was lower than that of control. This research concluded frozen doughs that performed best in baking had a high resistance to extension, a high complex modulus, and a low phase angle (Kenny et al., 1999). Newberry et al. (2002) compared dynamic elastic modulus (G') of fresh dough and freeze-thaw treated dough. The dynamic elastic modulus (G') of freeze-thaw treated dough was lower than that of the fresh. Similar changes were reported by other oscillatory shear studies (Autio and Sinda, 1992, Kenny et al., 1999). In addition, Newberry et al. (2002) reported freeze-thawed doughs had lower elongational viscosities than did fresh doughs, and had similar decreases in the extensigraph properties (Inoue and Bushuk 1992, Kenny et al., 1999). Berglund et al. (1990, 1991) assumed these freeze-thaw induced changes to be a result of physical interruption of the dough gluten matrix by ice crystals. Newberry et al. (2002) concluded that relative dough rheological properties are changed while freezing and thawing and that this is clearly shown during fermentation (proofing). Meziani et al. (2012a) confirmed that increased yeast amount compensates for the loss of yeast activity during freezing. Furthermore, rapid freezing overall gave better results in terms of fermentative activity, rheology and sensory properties in frozen sweet dough. In addition, Meziani et al. (2012b) reported that dough rheological parameters were not influenced by yeast level. However, dough hardness was increased and dough springiness and tan (δ) (G''/G') was decreased over four weeks of frozen storage. Meziani et al. (2012b) explained these modifications in the rheological properties to be due to ice crystal growth which induced a water redistribution causing mechanical damage to the gluten network of frozen sweet doughs (Berglund et al., 1990, 1991). Thus, fundamental rheology measurement such as dynamic oscillation test can be a useful and powerful tool for understanding and characterizing dough properties.

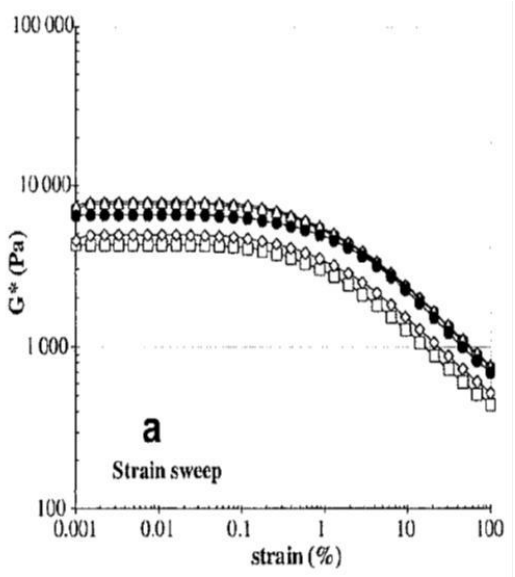
2.6.2.1.3 Dough preparation

The preparation of dough for dynamic oscillatory testing is important for the reproducibility of the results. Generally, dough is prepared using the mixograph (Rasanen, et al., 1997; Miller and Hoseney, 1999; Uthayakumaran, et al., 2000; Wang and Sun, 2002; Newberry, et al., 2002; Tronsmo, et al., 2003; Puppo, et al, 2005) or farinograph (Campos, 1997; Rasanen, et al., 1997; Miller and Hoseney, 1999; Lee, et al., 2001; Wang and Sun, 2002; Puppo, et al., 2005) until fully developed. Dough development affects dough rheology as well as final product quality (Campos et al., 1997). Campos et al. (1997) reported undeveloped and developed wheat doughs exhibit different and unique rheological behaviors. Undeveloped wheat dough is a viscoelastic material which exhibits linear behavior at low levels of stress (up to 50 Pa), corresponding to low strain levels (up to 0.2 %). The complex moduli (G*) of undeveloped wheat doughs are strong functions of frequency in dynamic oscillatory tests. In addition, developed dough has a higher complex modulus (G*) than does that of undeveloped dough. Therefore, fully developed dough was suitable for the dynamic oscillatory testing.

Immediately after mixing fully developed dough has strong mixing-generated stresses, water redistribution, enzymatic modification of gluten and starch, and sulfhydryl-disulfide interchange decreased average gluten molecular weight (Dong and Hoseney, 1995). Numerous researchers reported that storage modulus (G') and loss modulus (G'') decrease as the water content of doughs increase showing that oscillatory measurements are very sensitive to water content (Hibberd 1970; Hibberd and Parker, 1975ab; Navickis et al., 1982; Dreese et al., 1988). This mixing-generated stresses clearly affected dough rheology, and test result reproducibility. Consequently, fully developed dough is allowed rest for specific times before testing. Dong and Hoseney (1995) reported that rested dough used to test shows lower G' and larger loss tangent than dough immediately after mixing. Researchers concluded doughs had to rest at least 15 or 20 minutes rest before testing, and that less time might not be sufficient to obtain reproducible data (Dong and Hoseney 1995; Phan-Thien and Safari- Ardi 1998; Newberry et al., 2002).

2.6.2.1.4 Linear viscoelastic region

It is necessary to determine the linear viscoelastic region of the sample before measurement of rheological properties of the sample. When working within the linear viscoelastic range, data analysis can be conducted with the mathematical theory of linear viscoelasticity. To determine the linear viscoelastic region of a specific sample, the strain sweep technique is used. A specific range of strains are applied with a constant frequency (usually 1 Hz), and the resulting stresses are measured. G' and G" moduli are constant within the linear viscoelastic region. G’ and G” moduli decrease significantly over the linear viscoelastic region. Typical flour-water dough strain sweep test result is shown in Fig. 2.10.



Flour and water dough was shown circle of solid colored.

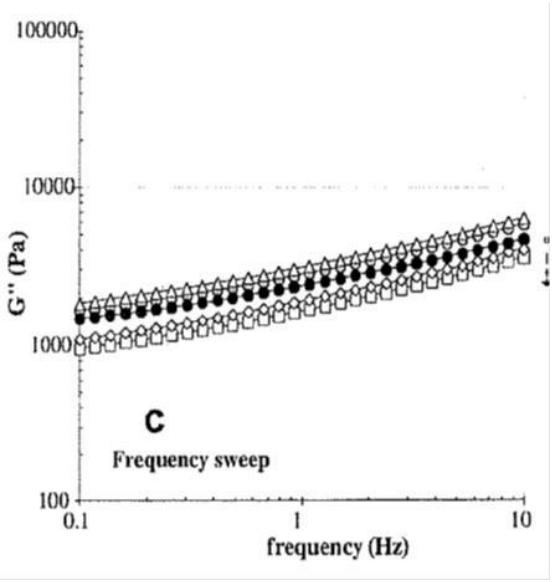
Figure 2.10 Typical strain sweep test

(Source: Mariotti and Alamprese, 2012)

According to Mariotti and Alamprese (2012), the linear viscoelastic region, determined from the strain sweep test, was 0.05 % strain for all their samples. The linear viscoelastic region within less of 0.1 % strain is generally used for oscillatory measurements of wheat flour dough rheology study (Weipert, 1990, Tanner et al., 2000).

2.6.2.1.5 Frequency sweep

Frequency sweep tests measure how the viscous and elastic behavior (viscoelastisity) of the material changes with the rate of application of strain or stress. It is a well-known mode of oscillatory testing (Steffe, 1996). Generally, materials have more solid like characteristics at higher frequencies. Steffe (1996) explained that frequency sweeps are very useful in comparing different food products, especially effects of various ingredients and processing treatments on viscoelasticity. In a frequency sweep test, dough is tested at frequencies of 0.1 to 10 Hz with a specific strain. The strain is determined by strain sweep test within the linear viscoelastic range. Researchers applied 0.05 % (Mariotti and Alamprese, 2012), 0.1 % (Clarke, et al., 2002), 0.2 % (Dus and Kokini, 1990; Angioloni and Dalla-Rosa, 2007; Connelly and McIntier, 2008), 0.22 % (Hibberd and Wallace, 1966), 0.25 % (Weipert, 1990), 0.5 % (Amemiya and Menjivar, 1992), 0.2 % to 0.8 % (Campos et al., 1997), and 0.8 % (Lindahl and Eliasson, 1992). Song and Zheng (2007) concluded frequency sweep tests under small deformations are very useful to clarify the structure of wheat flour component interaction in wheat flour dough.



Flour and water dough was shown circle of solid colored

Figure 2.11 Typical frequency sweep test

(Source: Mariotti and Alamprese, 2012)

CHAPTER 3 - Material & Methods

3.1 Materials

The following ingredients were used in this study. Hard wheat bread flour was supplied by General Mills, Carlisle, IA, USA. It had no treatment other than enrichment, and malt. According to the company specification sheet, the flour contained 12.8 % protein and 0.576 % ash (14 % m.b.). Flour moisture was measured by air oven (AACC approved method 44-15A) before each experiment. Instant dry yeast was used in all studies. It was supplied by Lesaffre Yeast Corporation, Milwaukee, WI, and AB Mauri Food Inc, Chesterfield, MO. The emulsifier sodium stearoyl-2-lactylate (SSL) was provided by Caravan Ingredients, Lenexa, KS. Potassium bromate and ascorbic acid, oxidants were supplied by Research Products Co., Salina, KS. Enzymes (fungal endoxylanase, bacterial hemicellulase, and lipase) were obtained from DSM Food Specialties USA, Inc., Parsippany, NJ. All-purpose shortening, sugar, salt, and pan spray were purchased from a local commercial market.

Dough was mixed in A-200 Hobart mixer (The Hobart MFG, Co., Troy, OH) equipped with a McDuffee Bowl (water jacketed) and two-pin fork (Total Manufacturing Co., Lincoln, NE). The mixed dough was sheeted and moulded by a sheeter/moulder (Oshikiri Machinery Ltd, Fujisawa, Kanagawa, Japan). For the frozen dough studies, the water jacketed mixing bowl was connected to a circulating refrigerated water bath (Fisher Scientific, Inc. Pittsburgh, PA). The water bath temperature was 5 ºC. Moulded dough was frozen by an air blast freezing system -21 ºC (-5 ºF) (Enersyst Development Co., Dallas TX) in freezer -18 °C to -23 °C (0 °F to -10 °F). Moulded or thawed dough was proofed in a proofer (Adamatic Inc., Eatontown, NJ) maintained at 40.6 ºC (104 ºF) and relative humidity 85-90 % for fresh dough, and maintained 40.6 ºC (104 ºF), and 40.6 ºC (104 ºF) and relative humidity 70-75 % for thawed dough. Proofed dough was baked in a gas reel oven (Reed Oven, Co., Kansas-city, MO). Baking temperature was 215 ºC (420 ºF), and bake time was 22 minutes.

The following instruments were used for product and data analysis. Volume measurement used rapeseed displacement (Total Manufacturing Co., Lincoln, NE). Loaf crumb structure was evaluated by C-Cell (Calibre Control International Ltd. Warrington WA4 4ST, UK). Dough

rheological tests, all of bake test data were performed in duplicate or triplicate. The data was analyzed by SAS computer software.

A temperature/stress controlled rheometer (Stress Tech HR, ATS Rheosystems, Bordentown, NJ), equipped with a 25mm serrated parallel plate system was used to assess rheological properties.

3.2 Flour moisture determination

Flour was stored in bulk in a retarder at 3-4 ºC (37-39 ºF) during this study. Before a baking test, one experiment worth of flour was scaled, and tempered to room temperature. Tempered flour was used for moisture determination and baking tests. The moisture content was measured by air oven (Approved method 44-15A, AACC 2000).

3.3 Dough formulation

All baking tests used the base formula shown below: 100 % flour (12.8 % protein), 59 % water, 4 % sugar, 3 % all-purpose shortening, 2 % salt, 2 % instant dry yeast, and 0.5 % sodiumstearoyl-2-lactylate (SSL). This formula was based on previous study (Lin, 2008). However, modifications had to be made because 3 % non-fat dry milk, 0.5 % vital wheat gluten were removed. Water absorption was modified as well. Oxidant types and quantity experiments, various ppm levels of potassium bromate (based on flour weight), and ascorbic acid (based on flour weight) were added to the dough. In enzyme type and amount studies, various levels of hemicellulase, endoxylanase, and lipase (based on flour weight) were added to the dough formulas individually. The amounts of oxidants and enzyme were determined based on dough handling properties and baked product quality and produced using fresh no-time dough method. Table 3.1 shows the base dough formula.

Table 3.1 Base dough formula

Ingredients	Bakers %
Flour (12.8% protein)	100
Water	59
Instant dry yeast	2
All-purpose shortening	3
Sugar	4
Salt	2
SSL	0.5
Total	170.5

3.4 Fresh no-time dough production

All of the baking tests in this study used a no-time dough method with delayed sugar and salt addition. This was done before proceeding to frozen dough studies, determines optimum levels of oxidants and enzymes, dough handling properties, and baked product quality. This defines the fresh no-time dough method.

In the method, all dry ingredients, except yeast, salt, and sugar were scaled together in a metal bowl. The excluded ingredients were scaled separately. Flour etc. was placed in the mixing bowl and mixed 15 seconds at speed 1. Then, yeast and water were added and mixed for another 15 seconds. Next, the dough was mixed for 3.5 minutes at speed 2. Afterwards, salt and sugar were added to the dough and mixed for 30 seconds at speed 1. That dough was mixed to optimum at speed 2. Optimum final mixing time was based on dough size and baker's observation. In this study, optimum final mixing time was 9 minutes and 30 second. One batch of dough was 2301.75 grams.

Dough temperature was measured immediately after mixing. The desired final dough temperature was 27 ºC ± 1 ºC (81 ºF ± 2 ºF). Immediately after temperature measurement, dough was divided into 540 ± 1 gram pieces and manually rounded. One batch produced 4 dough pieces. After manually rounding, the dough balls were allowed 5 minutes of rest at room temperature covered with a plastic bag, after which each dough ball was individually sheeted / moulded by sheeter/ moluder operated at the following settings; top roller 11.5, bottom roller 3.5/16, length 23.2 cm, spring pressure 2, and pressure board height 4.0. The dough pieces were placed in one side greased pans (25.4 cm L×10 cm W), and immediately moved into a proofer maintained at 40.6 ºC (104 ºF) and 85-90 % relative humidity. They were proofed until 2 cm above the top of the pan. Proofing time required 50 to 60 minutes. The core temperature of one dough piece from each batch was checked after proofing. The target range for proofed dough center temperature was 35-36 ºC (95-97 ºF). Fully proofed doughs were baked for 22 minutes at 215 ºC (420 ºF) in a gas fired reel oven, and cooled at room temperature for 24 hours. After cooling, the loaf weight and volume (AACC method-10.05, 2000) were measured. Afterwards, the loaves were sliced with an electric knife at 20 mm gaps (according to the template). Sliced loaves were placed in plastic bags prior to image analysis (C-Cell). Fig. 3.1 shows the fresh no-time dough flow chart.

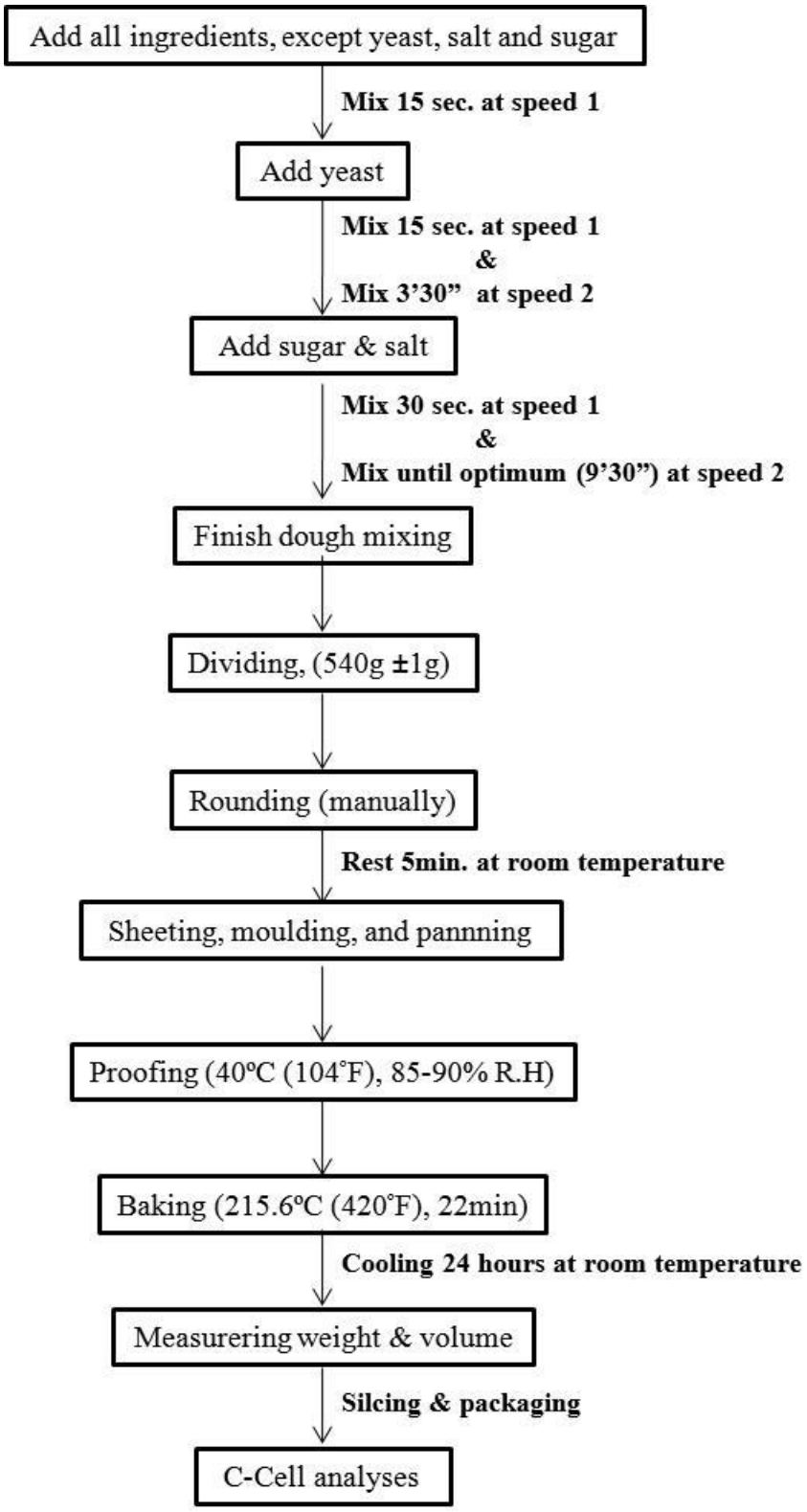


Figure 3.1 Process flow chart for fresh no-time dough

3.5 Frozen dough procedure

The frozen dough process was the same as the above (fresh no-time dough method) up to moulding. Subsequent changes are as follows. The mixing bowl was connected to a circulating water bath maintained at 5 ºC (41 ºF), and the mixing bowl was maintained at 6 ºC (43 ºF). Added water temperature was 0-1 ºC (32-34 ºF). The desired final dough temperature was 19 ± 1 ºC (66 ± 2ºF). After moulding, dough pieces were placed on a perforated sheet pan, and moved into a blast freezing system in freezer at -21 ºC (-5 ºF). Dough pieces were placed in the air blast until the dough's core reached -5 to -8 ºC (18 to 23 ºF), (35 minutes exposure). After freezing, the dough pieces were packed into plastic bags, and were stored at -18 to -20 ºC (-4 to 0 ºF) for specific periods of time before thawing. Before baking, the dough pieces were thawed for 16 hours in a retarder, maintained at 3 to 4 ºC (37 to 39 ºF) with 90% relative humidity. The thawed dough pieces were moved to room temperature conditions until core temperature reached 19 ºC (66 ºF), approximately 150-180 minutes, depending on ambient conditions. One dough was used to check core temperature. It was not used for baking data analysis. After the desired temperature was reached, the doughs were moved into a proofer. Proofing was 40.6 ºC (105 ºF) with 70-75 % relative humidity. Proofing time was approximately 30 minutes. Later processes (baking, cooling, etc.) and conditions were same as above (fresh no-time dough method). Fig. 4.2 shows the frozen dough procedure.

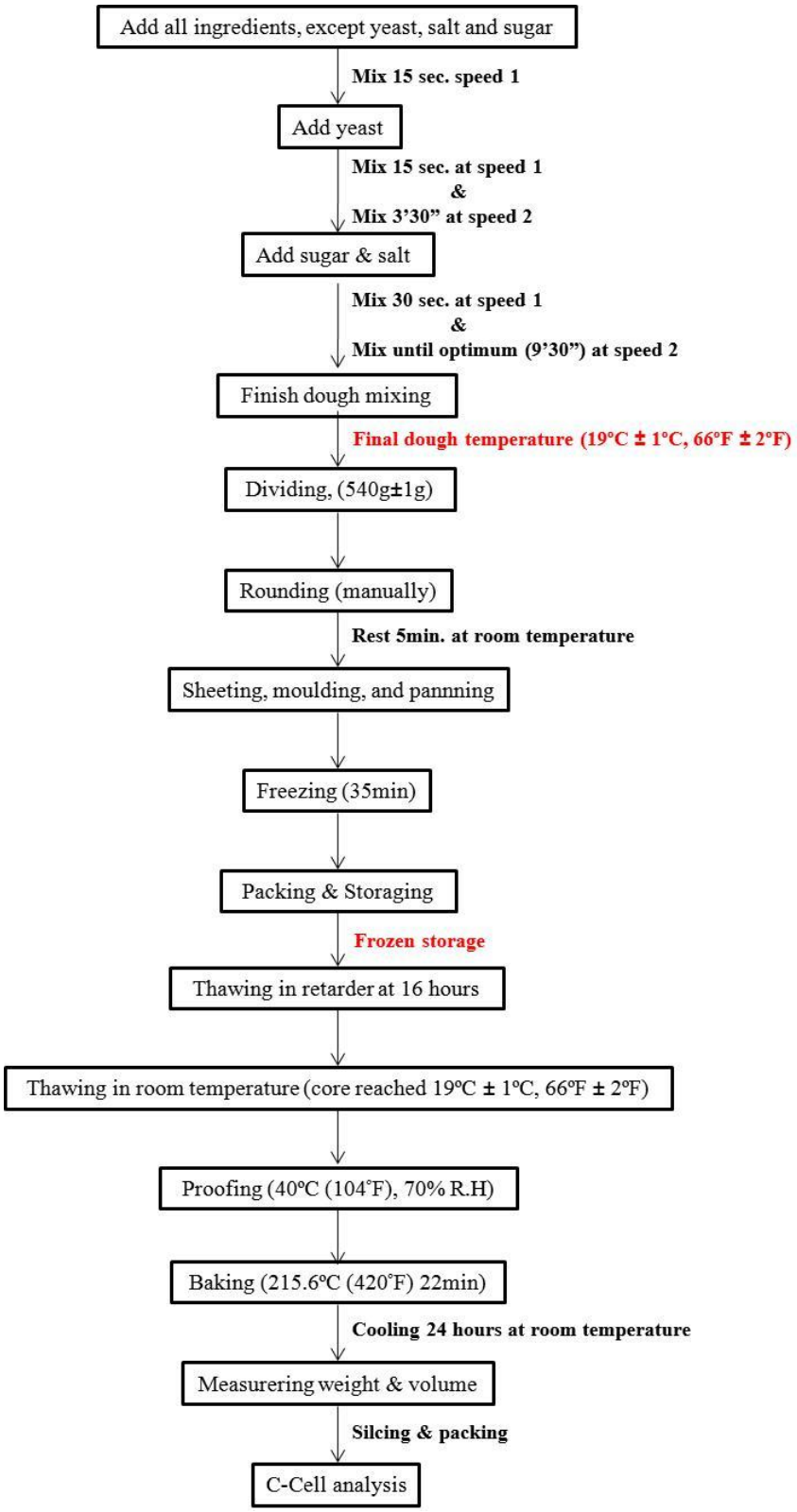


Figure 3.2 Process flow chart for frozen dough

3.6 Specific loaf volume measurement

Loaf weight and loaf volume were measured 24 hours after baking. Loaf weight was measured by electronic scales ± 0.1g, and loaf volume was determined with a rapeseed displacement volume meter (Approved Method 10-05, AACC 2000). Specific volume was calculated as follows.

$$\text{Specific Volume (SV)} = \frac{Loaf\ volume\ [cc]}{Loaf\ weight\ [grams]}$$

3.7 Image analysis

Loaf crumb structure was evaluated by image analysis using C-Cell. The instrument was connected to a PC, and used C-Cell software version 2.0 program. For sample preparation with weight and the volume measurement finished, the bread was sliced by an electric knife using the template. Middle top of the sliced part was used as the sample.

3.8 Statistical analysis

Data at the critical points in dough rheological tests, and the frozen dough bake procedure were collected in triplicate. The data was analyzed by SAS (Cary, NC).

Error bars for fresh and frozen dough/bread data were calculated using the standard deviations (STDEV) of triplicate loaf volume determination. All loaf volume data for frozen bread was analyzed by Tukey (-Kramer)'s statistic to detect significant difference. Error bars for dough rheology data at 1.1Hz were calculated as the grand coefficient variance (C.V. %). Grand coefficient variance was calculated as total average of coefficient variance (C.V. %) for an entire experimental condition. The calculated average of replicates for every frequency and corresponds to C.V. %. Calculate the average of C.V. % over the frequency range between 0.1 Hz and 9.1 Hz was calculated. This calculation used all processed and treatment dough rheology

data (totally 18 times, 6 process points with 3 treatments). All processed and treatment frozen dough rheology data analyzed by Tukey (-Kramer)'s adjustment to detect significant differences.

3.9 Measurement of dough rheological properties

A temperature/stress controlled rheometer (Stress Tech HR, ATS Rheosystems, Bordentown, NJ), equipped with a parallel plate measuring system (serrated 25 mm diameter corn and stage, gap 2.0 mm) and plate temperature held constant at 30 ºC, was used to measure the small deformation rheology of dough.

3.9.1 Sample preparation at after mixing

Post mixing dough was produced following the fresh/frozen dough making procedure. Immediately after temperature measurement of the fully developed dough, it was divided into approximately 10 gram pieces and manually rounded. After manually rounding, the dough balls were allowed 5 minutes rest at room temperature covered by plastic. After 5 minutes resting, each dough ball was individually sheeted (4.0 mm thickness, 4th level from right of gap adjustment dial) by a pasta sheeter (imperia®, TIPO LUSSO, SP150). The sheeting was two directions at the same setting for biaxial extension of dough. The sheeted dough was placed on a baking sheet, cut to 2.5 mm diameter by a handmade plastic dough cutter, and covered by aluminum pan. The dough piece was placed on the bottom serrated plate of rheometer using a spatula to avoid excess deformation. The rheometer was lowered to a gap of setting of 2.5 mm, and the excess dough was trimmed. Trimming was done with a small sharp spatula in a downward motion to avoid excess deformation of the dough while cutting it even with the edge of the top plate. Mineral oil was used to keep the edges of the dough from drying. After trimming, samples were allowed to rest for 20 minutes. After 20 minutes resting, the rheometer was lowered to a gap of 2.1 mm (0.1 mm above from target gap), and the excess dough was trimmed. After the 2nd trimming, the rheometer was lowered to the target gap of 2.0 mm, and sample was allowed to rest for 10 minutes. After the 10 minutes rest, dough rheology was measured by strain sweep and frequency sweep testing.

3.9.2 Sample preparation after thawing

"Post thaw" dough was produced by following frozen dough protocol. When the dough core temperature reached 19 ºC (66 ºF), the dough was taken out of the pan and placed on a baking sheet. The center of thawed dough was sliced approximately 10 cm wide by a bread knife. The edge of this sample was cut, and the core (center) of dough was obtained. The dough core was divided into approximately 10 g. This dough was sheeted by the pasta sheeter at the same setting as the post mix dough. After sheeting, the process followed the same procedure as the sample preparation for the post mix dough procedure.

3.9.3 Sample preparation after proofing

Post proof dough was produced by the fresh/frozen dough protocol. Dough was proofed until it reached 2 cm above top of the pan. Fully proofed dough was removed from the pan and placed on a baking sheet. The center of proofed dough was sliced approximately 10 cm in width with bread knife. After slicing, the process followed the same procedure as the sample preparation for the post thawed doughs.

3.9.4 Strain sweep (Linear viscoelastic region)

Strain sweeps were performed to determine the linear viscoelastic region of dough's response. Strain sweeps were run on post mixed, post fresh proof, post thawed 27 weeks frozen storage, and post proofed 28 weeks proofed control (no additives) dough. In addition, strain sweeps were run on post mix, and post fresh proof containing 50 ppm $KBrO_3$, and containing a combination of 200 ppm ascorbic acid and 100 ppm endoxylanase. This instrument operated with a 2.0 mm gap at 30 ºC with the sample loading method "To gap". The maximum loading force was 8.149E+4 Pa. The testing proceeded when residual force was below 4.074E+4 Pa or after waiting more than 1.000E+3 s. The final equilibrium time was 10 minutes, and all settings for number of measurement 1, measurement interval at 2.000E+1 s, constant frequency at 1.0 Hz,

Delay time 10 second, integration period 1.00, FFT size at 512, and strain range between 0.01 % and 10 %.

3.9.5 Frequency sweep

Frequency sweeps testing was performed at frequencies between 0.1 to 100 Hz with a constant stress of 15 Pa at 30 ºC, final equilibrium time 10 minutes, number of measurement 1, measurement interval 2.000E+1 s, Delay time 10 seconds, integration periods 1.00, FFT size at 512, and least three replicates (separate dough batches) were performed for each process and each ingredient variety. Data for elastic modulus (G'), viscous modulus (G"), complex modulus (G*), shear stress, phase angle, and complex viscosity were collected and used to compare the process and ingredients effects.

CHAPTER 4 - Result and Discussion

4.1 Determination of optimum amount of basic ingredients, oxidant and oxidants-enzyme replace and process condition in fresh no-time dough

Much research has concluded that the no-time dough process with cold temperature mixing and delayed salt incorporation is most suitable for frozen dough (Cathcart, 1949; Merritt, 1960; Fuhrmann, 1985; Dubois and Blockcolsky, 1986b). This protocol is the most popular method for frozen dough production. In this work, the basic ingredients, combination of oxidants and oxidants-enzyme, and the process condition were optimized for subsequent fresh no-time dough starting from the method of Lin (2008). The optimized control formula was presented in Table 3.1 and optimized process was shown in Fig. 3.1. Based on Table 3.1 and Fig. 3.1, the optimum combination of oxidants and oxidants-enzyme was determined.

4.1.1 Water absorption

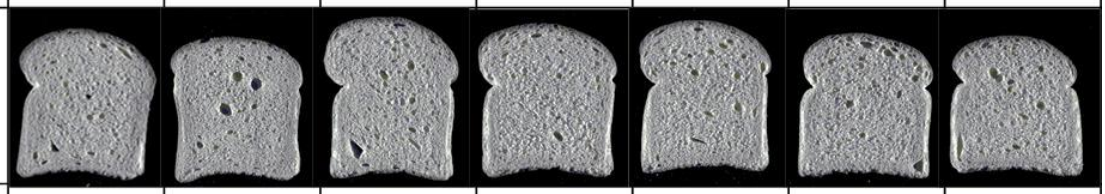
Using the modified Lin (2008) formula, determination of optimum water absorption was carried out using the following formulation in Table 4.1.

Table 4.1 Dough formula used to optimize water absorption

Ingredients	Bakers %
Flour (12.8% protein)	100
Water	Variable
Instant dry yeast	1.5
All-purpose shortening	3
Sugar	4
Salt	2

The final speed 2 mixing time was 4’30”. Water absorptions were tested from 55 % to 67 %. Absorptions of 55 %, 57 %, 63 %, 65 % were tested in 2 batches (6 loaves) each, and 59 %, 61 % were tested in 4 batches (12 loaves) each , and 67 % was tested in1 batch (3 loaves). Those results are presented in Table 4.2., and Fig. 4.1.

Table 4.2 Crumb structure (C-Cell) and specific volumes at varying levels of formula water

Water abs.[%]	55	57	59	61	63	65	67
C-Cell image							
Average SV [cc/g]	5.68	5.73	6.01	5.85	5.82	5.60	5.41
STDEV	0.21	0.09	0.30	0.14	0.12	0.12	0.05
C.V. [%]	3.64	1.57	3.31	2.39	2.04	2.07	0.99

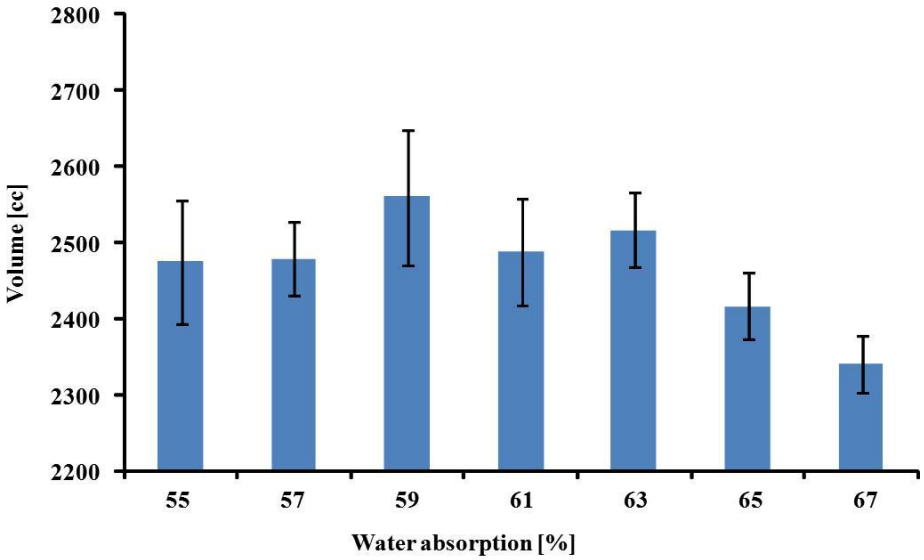


Figure 4.1 Average volumes at varying levels of formula water

Low absorption dough was relatively stiff, but dough texture increased with increasing water absorption. High water absorption doughs (65 and 67 % absorption) were sticky and difficult to handle. Based on the results (Table 4.2 and Fig. 4.1.), 59 % water absorption

produced highest volume and specific volume. C-Cell showed that 59 % water absorption bread had the best crumb structure. Volumes and specific volumes were acceptable, but slightly low. Some causes for this may be that the yeast level was relatively low, and the proofing time was long (approximately 85-95 minutes). It could also be that the dough was not fully developed during mixing or that the baking temperature may not have been proper. Crust color was slightly light and the loaf relatively soft in texture. Therefore, there formula and process were modified to test these hypotheses and improve the resulting bread. Most frozen dough papers recommend water absorptions slightly (2-3 %) lower than that of a regular bread formulation (Jackel, 1991; Lorenz, and Kulp, 1995; Spooner, 1998). Based on that and the result of the current work, 59 % water absorption was used in the following study.

4.1.2 Baking conditions

A baking temperature of 200-220 °C (400-425 °F) is most suitable for baking fresh/frozen dough (Marston, 1978). That said, baking temperature and time is variable depends on loaf size and the baker's judgment. Baking condition effect the crust color of final products and the effects are also related to ingredients (sugar, nonfat dry milk) and their amounts.

In this study, the final crust color was assessed by the baker's subjective judgment. In the previous experiment (Table 4.2, and Fig. 4.1), crust color was light and bread texture was soft. Those condition were 22 minutes at 210 ºC (410 ºF) as employed by Lin (2008). Tests to find the optimum baking condition were conducted using the formulation in Table 4.3.

Table 4.3 Dough formula for optimizing baking condition

Ingredients	Bakers %
Flour (12.8% protein)	100
Water	59
Instant dry yeast	1.5
All-purpose shortening	3
Sugar	4
Salt	2

The final speed 2 mixing time was 4’30”. Baking conditions were 22, 24, and 26 minutes at 210 ºC (410ºF), 20, 22, 24 minutes at 218 ºC (425 ºF), and 20, 22, 24 minutes at 215 ºC (420 ºF). A single batch (3 loaves) of each bake time and temperature condition was compared. All bake times at 210 ºC (410 ºF) produced slightly light crust color. All of the 218 ºC (425 ºF) baking were relatively darker. The crust color at 215 ºC (420 ºF) was subjectively judged best. Table 4.4 and Fig. 4.2 present the result of each baking time at 215 ºC (420 ºF). These comparisons at 215 ºC were based on 2 batches (6 loaves) each time.

Table 4.4 Crumb structure (C-Cell) and specific volumes at each baking time at 215 ºC (420ºF)

Baking time[min.]	20	22	24
C-Cell image			
Average SV [cc/g]	5.88	5.97	5.83
STDEV	0.25	0.08	0.16
C.V. [%]	4.32	1.39	2.77

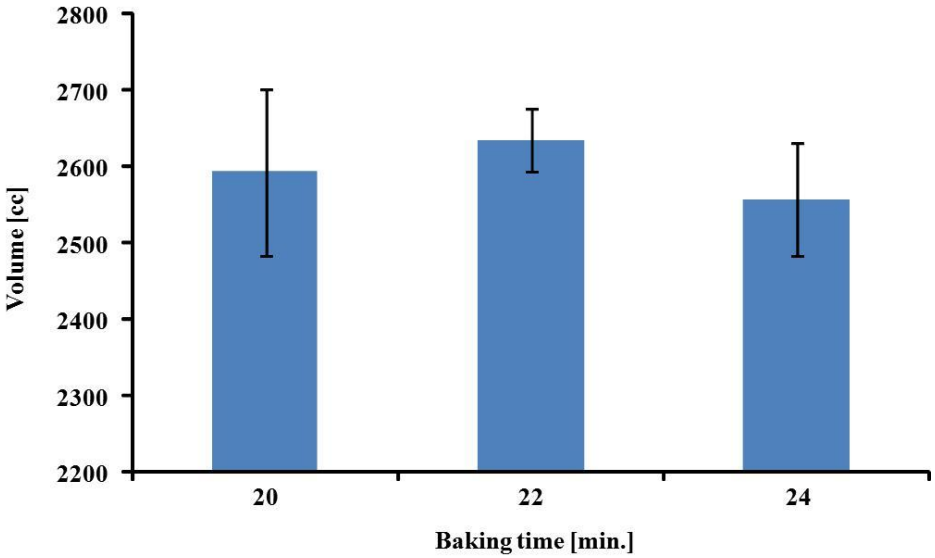


Figure 4.2 Average volumes baked at 215 ºC (420 ºF) with varying bake times

C-Cell images of product at 22 minutes baking time showed the best crumb structure. Consequently, at 22 minutes bake at 215 ºC (420 ºF) produced the highest volume, specific volume, the lowest standard deviation (STDEV) and percent of coefficient variation (C.V. %) although standard deviation overlapped. Therefore, baking conditions of 22 minutes at 215 ºC (420 ºF) were used in the following studies.

4.1.3 Yeast condition

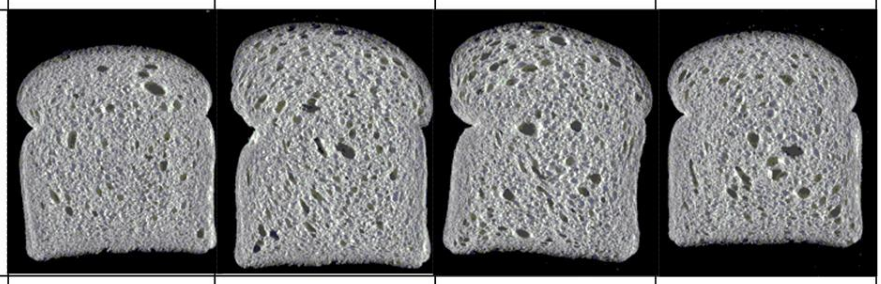
Lin (2008) used yeast (IDY) at 1.5 % (flour weigh base). Generally, proofing time requires 55 to 65 minutes, but here it took 85-95 minutes for fresh baking. Therefore, the optimum yeast level was investigated. Tests of the optimum yeast condition were conducted using the formulation in Table 4.5.

Table 4.5 Dough formula to optimize yeast condition

Ingredients	Bakers %
Flour (12.8% protein)	100
Water	59
Instant dry yeast	Variable
All-purpose shortening	3
Sugar	4
Salt	2

Yeast levels tested were 1.5 % to 3.0 %. The final speed 2 mixing time was 4’30”. The baking condition was 215 ºC (420 ºF) for 22 minutes. The dough containing 1.5% yeast was produced as 3 batches (9 loaves), and 2.0 %, 2.5 %, and 3.0 % dough was produced in 2 batches (6 loaves) each. The results at each yeast level are shown in Table 4.6 and Fig. 4.3.

Table 4.6 Crumb structure (C-Cell), proofing time, and specific volumes with varying levels of yeast

Yeast level [%]	1.5	2.0	2.5	3.0
Average proofing time [min.]	84	62	52.5	47.5
C-Cell image				
Average SV [cc/g]	5.85	6.11	6.05	5.74
STDEV	0.28	0.12	0.10	0.15
C.V. [%]	4.72	1.88	1.69	2.69

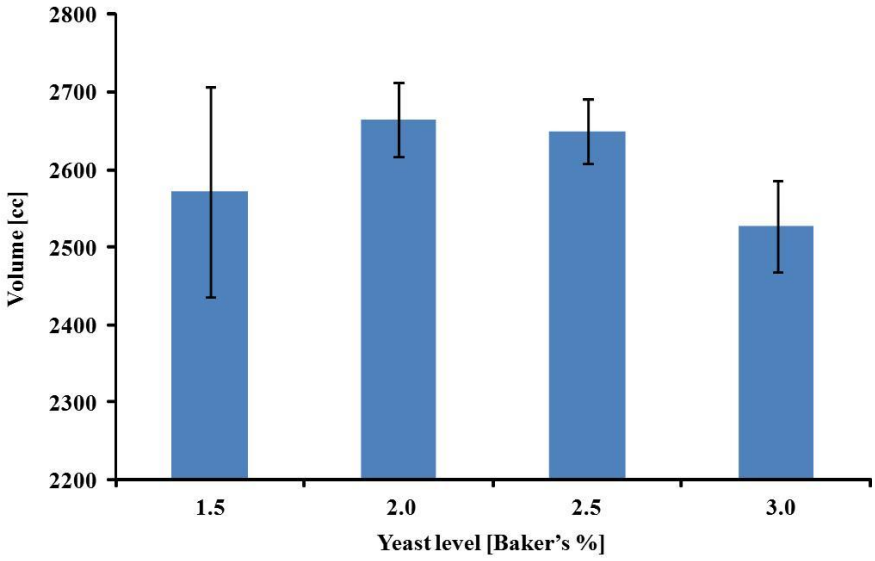


Figure 4.3 Average volumes with varying levels of yeast

The dough containing 2.0 % and 2.5 % had higher volume and specific volume, low standard deviation (STDEV), and percent of coefficient variation (C.V. %). C-Cell imaging indicated that bread containing 2.0 % yeast had the best crumb grain. Above 2.0 %, cell structure was rough and open. Two percent yeast also resulted in optimum proofing time. The proofing time decreased as yeast level increased above 2.0 %, but the crumb structure became rough and

coarse. On the other hand, doughs with 1.5 % yeast produced dense loaves and tight crumb structure. The dough containing 3.0 % yeast was low in both volume and specific volume. Consequently, the 2.0 % yeast level performed best and it is used in the following studies.

4.1.4 Mixing condition

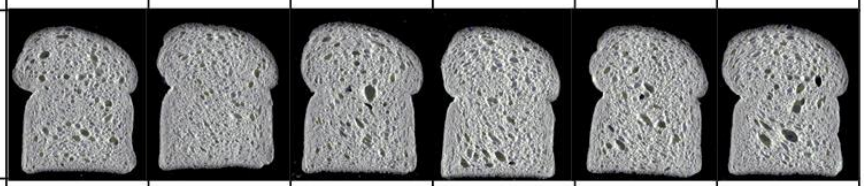
Mixing time is critical for bread making. The objectives of mixing are: 1) Hydration of the ingredients, 2) Homogenous distribution of the ingredients 3) Developing the gluten. 4) The initiation of fermentation (Doerry, 1995). Optimum mixing time is often determined by bakers' experience. In the precious (Lin, 2008), final mixing time was not given. To determine the optimum mixing time for this flour and dough studies were conducted using the formula in Table 4.7.

Table 4.7 Dough formula to optimize mixing time

Ingredients	Bakers %
Flour (12.8% protein)	100
Water	59
Instant dry yeast	2
All-purpose shortening	3
Sugar	4
Salt	2

The final speed 2 mixing time varied from 3'30" to 14'30". Baking was 22 minutes at 215 ºC (420ºF). A single batch for mixing times of 3'30", 4'30", 5'30", 7'30", 8'00", 10'30", 12'30", and 14'30" was produced. Mixing times of 6'30", and 10'00" were as 2 batches, and for mixing times 8'30", 9'00" and 9'30", 3 batches each were produced. The result of each mixing time is presented in Table 4.8 and Fig. 4.4.

Table 4.8 Crumb structure (C-Cell), and specific volumes with varying mixing time

Mixing time [min.]	3.5	4.5	5.5	6.5	7.5	8	8.5
C-Cell image							
Average SV [cc/g]	5.09	5.26	5.57	5.83	5.96	5.91	6.35
STDEV	0.06	0.07	0.09	0.10	0.09	0.11	0.14
C.V. [%]	1.10	1.38	1.63	1.63	1.53	1.86	2.22
Mixing time [min.]	9	9.5	10	10.5	12.5	14.5	
C-Cell image							
Average SV [cc/g]	6.27	6.47	6.36	6.32	6.36	6.35	
STDEV	0.14	0.17	0.11	0.14	0.04	0.06	
C.V. [%]	2.25	2.68	1.73	2.20	0.55	0.91	

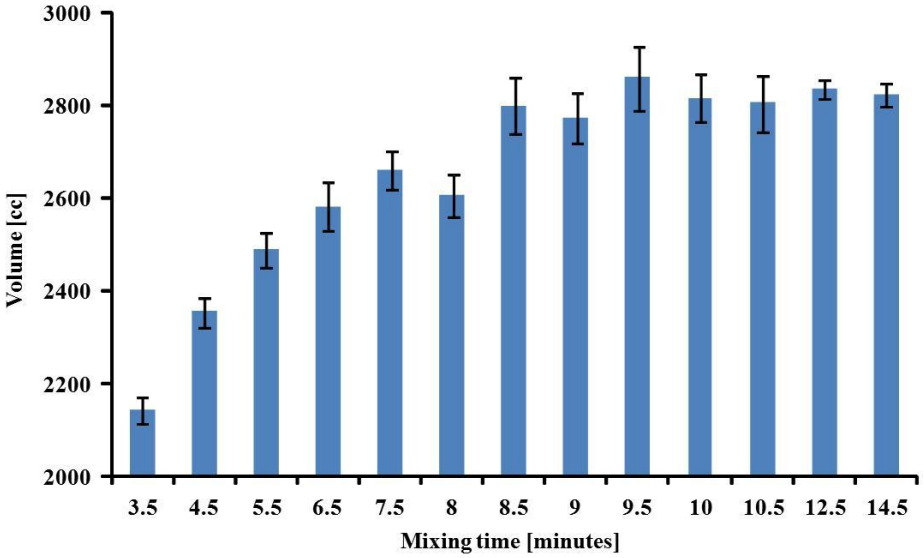


Figure 4.4 Average volumes with varying mixing time

The final mixing time has substantial effects on bread volume, specific volume and crumb structure. Short mixing time (under mixed) loaves had low specific volume and dense crumb structure. Increasing final mixing time, developed the dough gluten network and increased both volume and specific volume. Volume and specific volume gradually decreased and open crumb structure was apparent above 9’30” final mixing, suggesting over mixing. At mixing times of 12’30”, and 14’30” final dough temperature was too warm. The dough had less elasticity, excessive extensibility, and difficult handling. Comparing under mixing and over mixing, under mixed dough affected bread quality more dramatically than did over mixing. High protein flour was used for this study, so its dough stability was better, allowing the final product to remain high quality. From Table 4.8 and Fig. 4.4, final mixing time was determined to be 9’30”, and it used in the following study.

4.1.5 Potassium bromate without SSL

The optimization of basic ingredient amounts and the process condition with a simple formula was completed. The optimized control dough formula is as presented in Table 4.7, and the optimized process as shown in Fig. 3.1. Potassium bromate was added at levels ranging 10 from 75 ppm (flour weight base). Potassium bromate 0 ppm (control), and 50 ppm was produced as 3 batches each, 30 ppm, 40 ppm, and 60 ppm added was produced as 2 batches each, 25 ppm and 75 ppm added was produced as single batch each. Results are shown in Table 4.9 and Fig 4.5.

Table 4.9 Crumb structure (C-Cell), and specific volumes as a function of potassium bromate concentration

$KBrO_3$[ppm]	0(control)	25	30	40	50	60	75
C-Cell image							
Average SV [cc/g]	6.33	6.50	6.49	6.76	6.68	6.56	6.54
STDEV	0.17	0.06	0.21	0.11	0.12	0.08	0.05
C.V. [%]	2.65	0.87	3.21	1.58	1.78	1.20	0.77

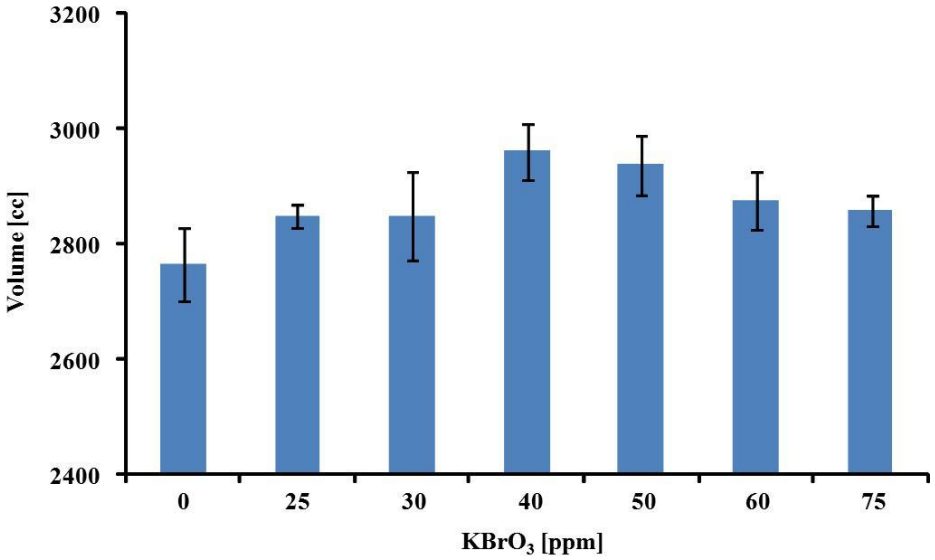


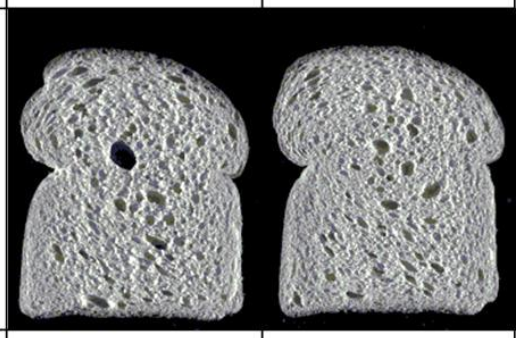
Figure 4.5 Average volumes as a function of potassium bromate concentration

According Table 4.9 and Fig 4.5 shows that potassium bromate affected bread volume, specific volume and crumb structure. Excessive amounts of potassium bromate cause over oxidization with lower final product loaf volume, a rough, uneven crust, and large unsightly breaks. The crumb has many ruptured cells and large holes. Table 4.9 and Fig. 4.5 also indicate that above 40 ppm addition the dough was over oxidized. At 50 ppm potassium bromate crumb structure was open, so 40 ppm potassium bromate addition determined to be optimum. At 60 or 75 ppm addition dough was definitely over oxidized.

4.1.6 SSL addition

Lin (2008) added 0.5% of sodium stearoyl-2-lactylate (SSL) to the control formula to improve dough/bread uniformity, crumb structure, standard deviation (STDEV), and coefficient of variation (C.V. %). In the work reported that above some conditions (control) also had high STDEV and C.V. %. Many researchers have concluded that SSL presence improved fresh and frozen dough/bread quality (Marston, 1978; Varriano-Marston et al., 1980; Davis. 1981; Dubois and Blockcolsky, 1986a). Therefore, 0.5 % (flour weight base) of SSL was added to Table 4.7, and the effect of 0.5 % SSL was tested. The doughs were produced by the optimized process (Fig. 3.1.). The dough without SSL (control) and with 0.5% SSL was produced as 3 batches each. The results are shown in Table 4.10 and Fig 4.6.

Table 4.10 Crumb structure (C-Cell), and specific volumes with SSL and without

Dough Treatment	Control	Control w/ SSL
C-Cell image		
Average SV [cc/g]	6.42	6.47
STDEV	0.20	0.07
C.V. [%]	3.09	1.14

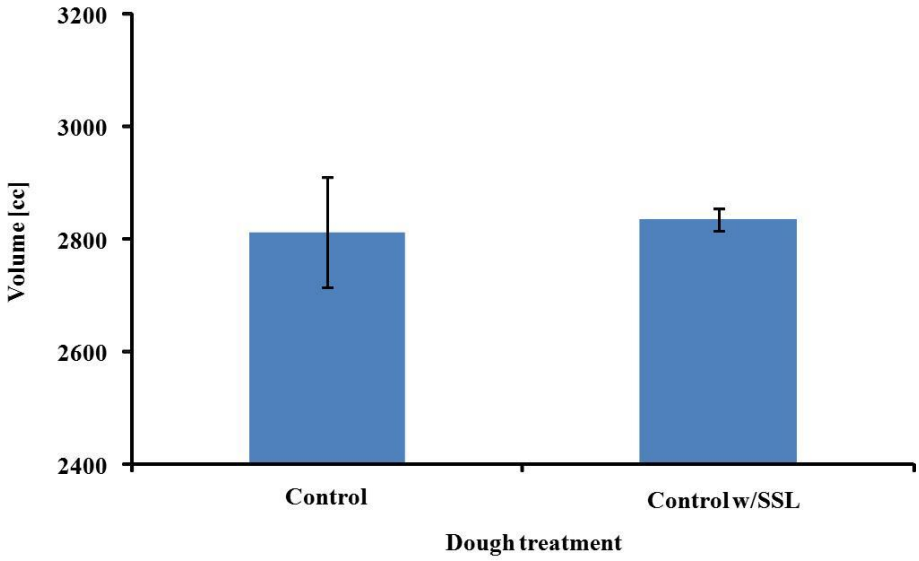


Figure 4.6 Average volumes with SSL and without

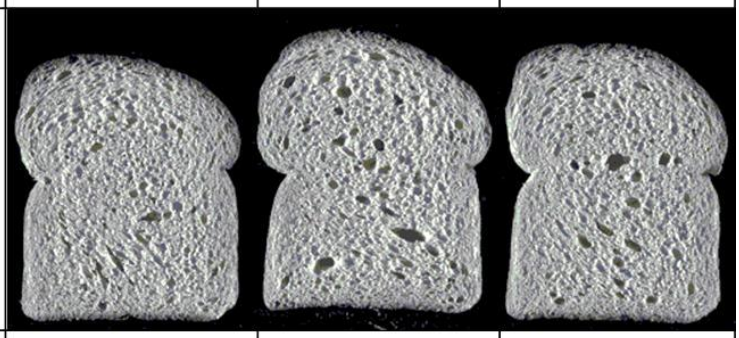
Table 4.10, and Fig. 4.6 shows that SSL addition did not cause a large volume and specific volume change. However, SSL addition affected crumb structure. SSL has two functions, as a dough conditioner and emulsifier. Quality was more uniform in dough containing SSL. The crumb structure was better and the volume (specific volume) was more uniform. The advantage of the addition of SSL was confirmed by this experiment. Therefore, the formula that contained

SSL was defined the basic dough formula (Table 3.1) and it was called control in subsequently studies.

4.1.7 Potassium bromate with SSL

Based on the result presented in Table 4.9 and Fig. 4.5, 40 to 50 ppm potassium bromate levels were best. For this experiment the control formula (Table 3.1) was used as control, plus 40 ppm, and 50 ppm potassium bromate dough was produced as 4 batches of each treatment. All doughs were produced by the optimized process (Fig. 3.1). Results of potassium bromate addition are shown in Table 4.11 and Fig 4.7.

Table 4.11 Crumb structure (C-Cell), and specific volumes for dough with SSL and varying levels of potassium bromate

Dough Treatment	Control	$KBrO_3$ 40ppm	$KBrO_3$ 50ppm
C-Cell image			
Average SV [cc/g]	6.48	6.52	6.65
STDEV	0.08	0.15	0.12
C.V. [%]	1.26	2.29	1.79

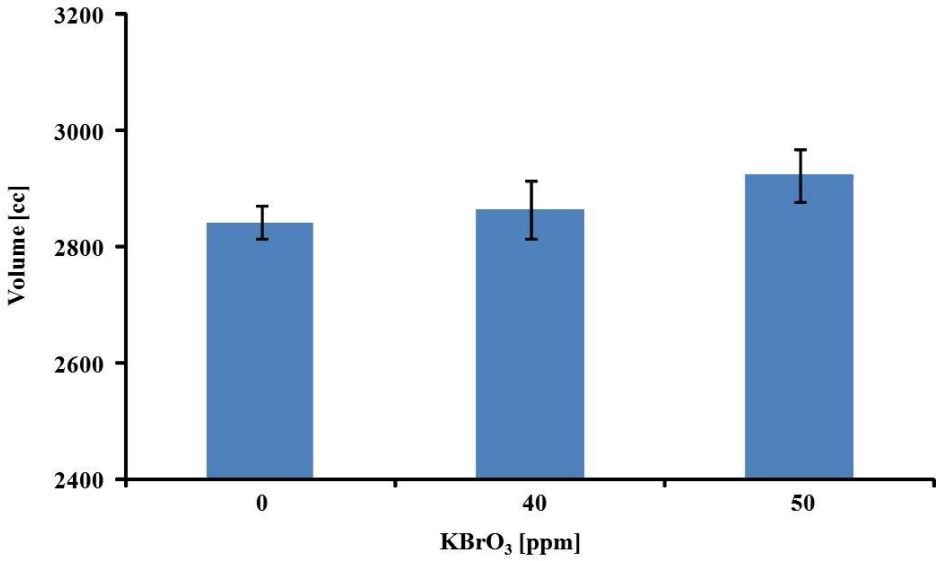


Figure 4.7 Average volumes for dough with SSL and varying levels of potassium bromate

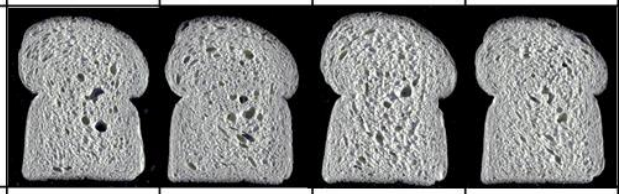
Table 4.11 and Fig. 4.7 show that 40 ppm and 50 ppm addition of potassium bromate affected bread volume, specific volume and crumb structure. Fig. 4.7 shows that 50 ppm addition produced the highest volume and specific volume. However, the crumb structure of 50 ppm addition was open (Table 4.11). This indicated that it was over-oxidized. Therefore, 40 ppm

potassium bromate was seemed best for fresh no-time dough making. Potassium bromate at 50 ppm addition was use for the frozen dough study.

4.1.8 Ascorbic acid

Next, the optimum amount of the ascorbic acid was determined. The control dough formula was used (Table 3.1). Ascorbic acid (AA) was added to across the range of 0 to 280 ppm (flour weight base). The AA 0 ppm (control), and 200 ppm additions were produced as 3 dough batches each, 250 ppm additions were produced as 2 batches, and 100, 150, 180, 230 and 280 ppm additions were produced in a single batch each. All doughs were produced by the optimized process (Fig. 3.1). The results are presented in Table 4.12, and Fig 4.8.

Table 4.12 Crumb structure (C-Cell), and specific volumes across ascorbic acid addition levels

Ascorbic acid [ppm]	0(control)	100	150	180
C-Cell image				
Average SV [cc/g]	6.45	6.46	6.53	6.47
STDEV	0.06	0.15	0.09	0.09
C.V. [%]	0.99	2.25	1.41	1.33
Ascorbic acid [ppm]	200	230	250	280
C-Cell image				
Average SV [cc/g]	6.54	6.44	6.52	6.51
STDEV	0.13	0.06	0.17	0.09
C.V. [%]	1.94	0.90	2.65	1.40

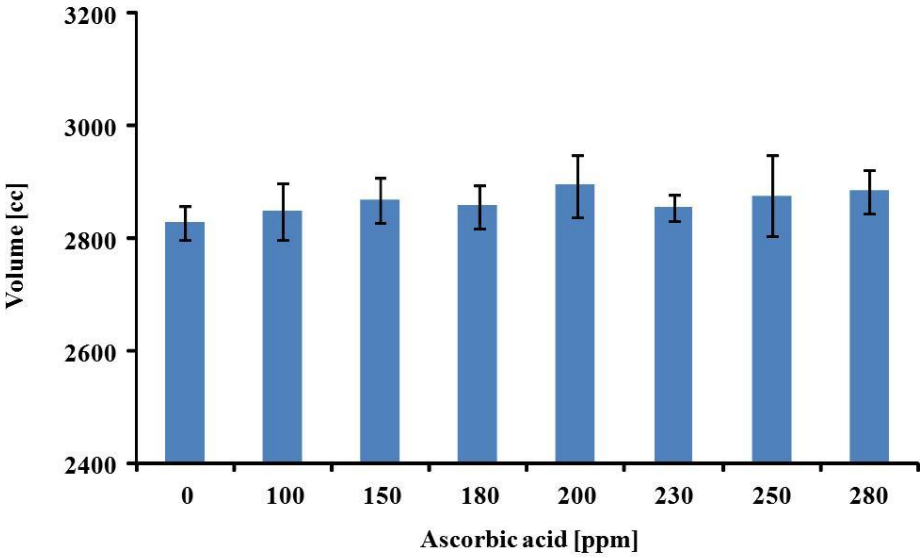
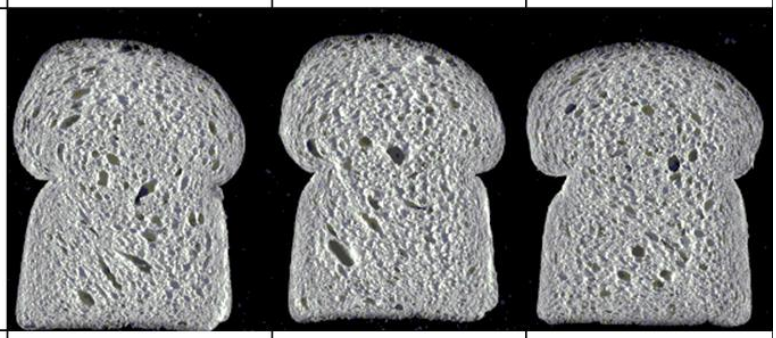


Figure 4.8 Average volumes at increasing ascorbic acid addition levels

Table 4.12, Fig.4.8 shows that ascorbic acid affected bread volume, specific volume and crumb structure somewhat. Generally, ascorbic acid is reducing agent, but ascorbic acid is converted to de-hydro ascorbic acid during mixing process (Tsen, 1964). De-hydro ascorbic acid is an oxidizing agent. If excess ascorbic acid remains dough, it then works on reducing agent. Comparing the dough handling properties of dough after mixing, above 200 ppm AA containing dough was difficult to handle as it became softer and sticker. C-Cell imaging indicated that bread containing 200 ppm AA had the best crumb grain. Fig. 4.8 shows that bread containing 200 ppm AA was highest volume. Therefore, the optimum AA level was established 200 ppm.

The possible effect of AA on mixing time was investigated. The control dough formula was used (Table 3.1). Final speed 2 mixing time varied 9’30”, 10’00”, and 10’30”. Baking condition was 22 minutes at 420 ºF. Each mixing time was tested as 3 batches. The results are shown in Table 4.13, and Fig 4.9.

Table 4.13 Crumb structure (C-Cell), and specific volumes at different mixing time (200 ppm ascorbic acid)

Mixing time [min]	9.5	10	10.5
C-Cell image			
Average SV [cc/g]	6.68	6.62	6.54
STDEV	0.11	0.06	0.09
C.V. [%]	1.59	0.98	1.33

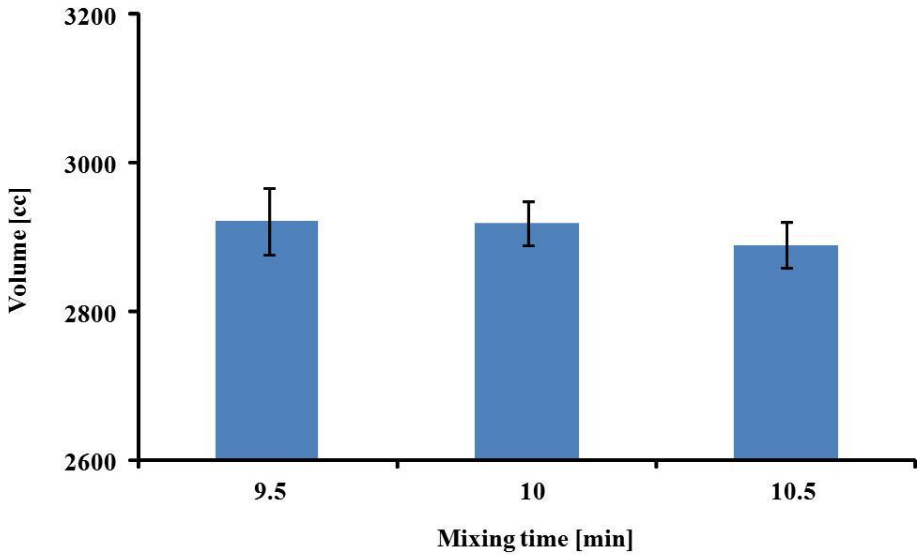


Figure 4.9 Average volumes at different mixing time (200 ppm ascorbic acid)

Table 4.13 and Fig.4.9 show that these mixing time differences did not affect bread volume, specific volume and crumb structure. Therefore, mixing time was not changed by AA addition and the same mixing time (9.5 minutes) was used for all studies.

4.1.9 Combination of potassium bromate and ascorbic acid

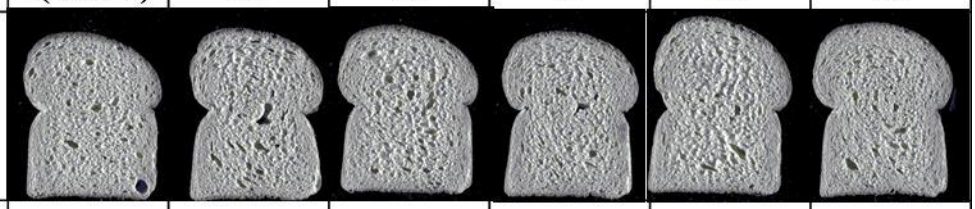
Previous experiment determined optimum levels of potassium bromate above, ascorbic acid above and mixing time. Based on those result, the combination of potassium bromate and ascorbic acid was evaluated. In the previous studies (Tables 4.12, 4.13 and Figs. 4.8, 4.9), the optimum levels and final mixing time of ascorbic acid (AA) for this flour and dough were determined. Based off these results, the combination of 200 ppm AA and potassium bromate was evaluated. The study was conducted using the formula in Table 4.14.

Table 4.14 Dough formula for combination of potassium bromate and 200 ppm ascorbic acid

Ingredients	Bakers %
Flour (12.8% protein)	100
Water	59
Instant dry yeast	2
All-purpose shortening	3
Sugar	4
Salt	2
SSL	0.5
Potassium bromate	Variable
Ascorbic acid	200 ppm

Potassium bromate content varied from 0 to 50 ppm. The potassium bromate 0 ppm (control), 10 ppm, 20 ppm, and 30 ppm containing doughs were produced as 3 batches each, 40 ppm, and 50 ppm potassium bromate containing doughs were produced as 2 batches each. All doughs were produced by the optimized process (Fig. 3.1). During this experiment, a mixer fork broke, so the potassium bromate 40 ppm and 50 ppm studies could only be produced as 2 batches each. Results are shown in Table 4.15 and Fig. 4.10.

Table 4.15 Crumb structure (C-Cell), and specific volumes varying $KBrO_3$ with 200 ppm ascorbic acid

$KBrO_3$[ppm]	0(control)	10	20	30	40	50
C-Cell image						
Average SV [cc/g]	6.42	6.52	6.48	6.44	6.70	6.71
STDEV	0.10	0.12	0.18	0.14	0.18	0.11
C.V. [%]	1.58	1.87	2.76	2.12	2.62	1.58

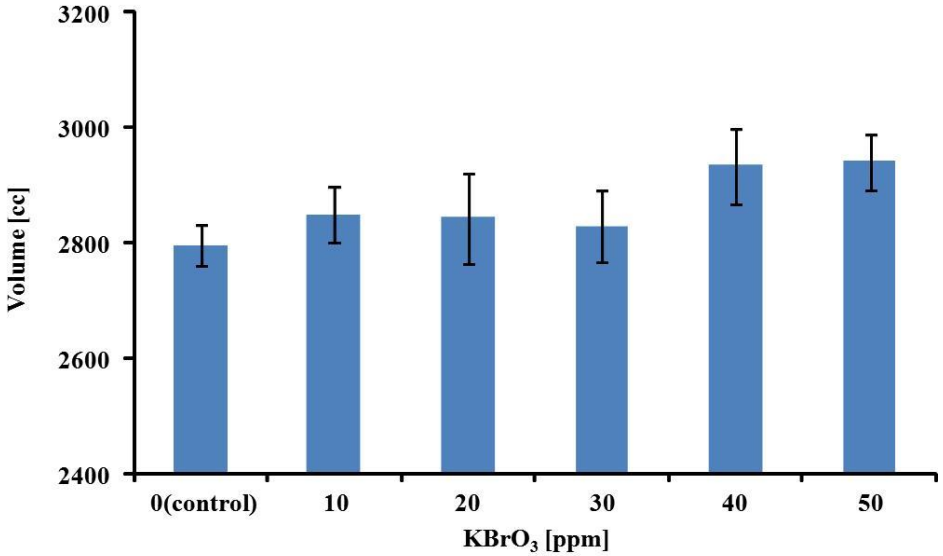


Figure 4.10 Average volumes varying $KBrO_3$ with 200 ppm ascorbic acid

Results showed that addition of 40 ppm and 50 ppm potassium bromate definitely improved bread volume, specific volume and crumb structure. The combination of AA and potassium bromate caused the dough to be stiffer (less slack). The 50 ppm addition produced highest volume. However, at 50 ppm cell structure was slightly open, suggesting that the bread was over-oxidized. Therefore, 40 ppm potassium bromate was deemed optimum for this study (fresh no-time dough).

4.1.10 Combination of ascorbic acid and hemicellulase

While enzymes might be expected to be potential replacements for potassium bromate, many researchers concluded that no single enzyme has been found to replace potassium bromate's oxidative effect (Kulp, 1993; Mathewson 1998; Boll, 1999). They pointed out that an effective bromate replacer might be made by blending non-bromate oxidants with enzymes, emulsifiers and xylanase enzyme, or combinations of enzymes. The benefit would be reducing or eliminating use of potassium bromate in favor of other oxidants such as ascorbic acid. Based on previous work (Lin, 2008), enzymes including xylaneses (bacterial hemicellulase, and fungal endoxylanase) and lipase were combined with ascorbic acid and tested frozen dough.

Tables 4.12, 4.13 and Figs. 4.8, 4.9 showed that optimum dose of AA for this flour and dough. Based off these results, the combination of AA and hemicellase was evaluated next. To determine the optimum combination of hemicellulase and 200 ppm ascorbic acid tests were conducted using the formula in Table 4.16.

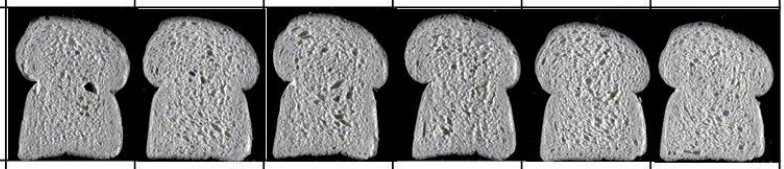
Table 4.16 Dough formula for combination of hemicellulase and 200 ppm ascorbic acid

Ingredients	Bakers %
Flour (12.8% protein)	100
Water	59
Instant dry yeast	2
All-purpose shortening	3
Sugar	4
Salt	2
SSL	0.5
Ascorbic acid	200 ppm
Hemicellulase	Variable

Hemicellase was present at levels varying from 0 to 250 ppm. The hemicellulase 0 ppm (control) was produced as 5 batches. The 120 ppm, 125 ppm, and 150 ppm additions were produced as 3 batches each while the 50 ppm, 100 ppm, and 200 ppm addition were produced as 2 batches each. The 110 ppm, 130 ppm, 140 ppm, 160 ppm, 175 ppm, and 250 ppm addition

were produced in a single batch each. All doughs were produced by the optimized process (Fig. 3.1). Results are presented in Table 4.17 and Fig. 4.11.

Table 4.17 Crumb structure (C-Cell), and specific volumes as a function of 200 ppm ascorbic acid and varying levels of hemicellulase

Hemicellulase [ppm]	0 (control)	50	100	110	120	125	130
C-Cell image							
Average SV [cc/g]	6.57	7.01	6.89	7.07	7.12	7.16	6.96
STDEV	0.09	0.17	0.14	0.19	0.10	0.14	0.15
C.V. [%]	1.31	2.41	2.01	2.69	1.42	1.90	2.21
Hemicellulase [ppm]	140	150	160	175	200	250	
C-Cell image							
Average SV [cc/g]	7.01	7.05	7.01	7.01	7.08	6.86	
STDEV	0.15	0.16	0.11	0.10	0.20	0.09	
C.V. [%]	2.11	2.29	1.63	1.39	2.82	1.29	

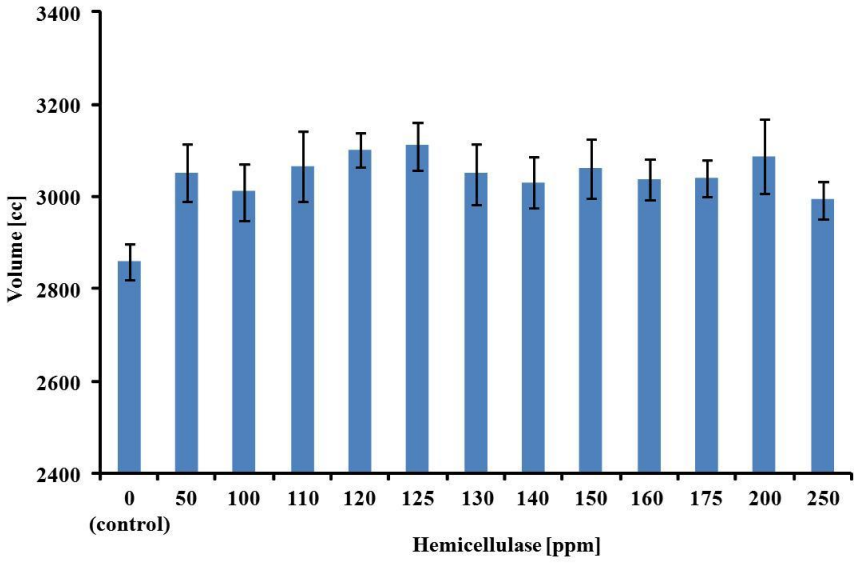


Figure 4.11 Average volumes as a function of hemicellulase levels at 200 ppm ascorbic acid

The results show that hemicellulas addition improved bread volume, specific volume and crumb structure. In addition, it had an optimum level of addition. Results indicate that 125 ppm addition produced the highest volume/specific volume. Hemicellulase (xylanase) breaks down (endo-hydrolysis) the water-unextractable arabinoxylans converting them to enzyme-solubilized AX (ES-AX). ES-AX has reduced molecular weight due to the hydrolysis of the xylan backbone (Petit-Benvegnen et al., 1998, Courtin and Delcour, 2001). The solubilization of WU-AX (ES-AX) reduced its water holding capacity. Therefore, previously bound water is redistributed amongst the other components of the gluten, increasing its extensibility (Maat et al., 1992). This water release is partially counteracted by the increased viscosity of the dough aqueous phase, so dough slackness is increased (Rouau et al., 1994, Courtin et al., 2001). This combination results in an improved dough development and extensibility of the gluten. Therefore, final bread quality is improved.

In this study, the dough became softer, sticker, and difficult to handle at enzyme levels above 125ppm. This phenomenon indicates over-dosing. Rouau et al. (1994) and Courtin et al. (2001) explained that over-dosing with xylanase reduces the overall water-holding capacity of the flour, so water release is excessive. As a result, dough becomes slack, sticky and difficult-to-handle. Consequently, optimum hemicellulase levels were set at 125 ppm.

4.1.11 Combination of ascorbic acid and endo-xylanase

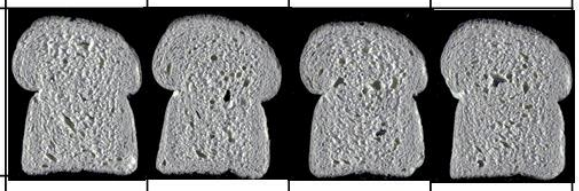
The effect of the combination of AA and endoxylanase was evaluated in a manner equivalent to that used to assess hemicellulase and AA combination. AA was held constant and the levels of endoxylanase varied. To determine the optimum combination of endo-xylanase and 200 ppm ascorbic acid was conducted using the formula in Table 4.18.

Table 4.18 Dough formula for combination of endo-xylanase and 200 ppm ascorbic acid

Ingredients	Bakers %
Flour (12.8% protein)	100
Water	59
Instant dry yeast	2
All-purpose shortening	3
Sugar	4
Salt	2
SSL	0.5
Ascorbic acid	200 ppm
Endo-xylanase	Variable

Endo-xylanase doses varied from 0 to 300 ppm. The endoxylanasee 0 ppm (control) was produced as 4 batches. The 100ppm was produced as 5 batches. The 75 ppm, 90 ppm, 110 ppm, 125 ppm addition were produced as 2 batches each. The 50 ppm, 150 ppm, 200 ppm, 250 ppm, and 300 ppm addition were produced in a single batch each. All doughs were produced by the optimized process (Fig. 3.1). Results are presented in Table 4.19 and Fig. 4.12.

Table 4.19 Crumb structure (C-Cell), and specific volumes as a function of 200 ppm ascorbic acid and varying levels of endo-xylanase

Endoxylanase [ppm]	0 (control)	50	75	90	100	110	125
C-Cell image							
Average SV [cc/g]	6.80	7.03	7.15	7.01	7.21	7.20	7.05
STDEV	0.13	0.07	0.16	0.18	0.19	0.22	0.18
C.V. [%]	1.85	0.99	2.28	2.60	2.59	3.02	2.60
Endoxylanase [ppm]	150	200	250	300			
C-Cell image							
Average SV [cc/g]	7.12	7.16	7.17	7.10			
STDEV	0.24	0.16	0.21	0.17			
C.V. [%]	3.31	2.28	2.89	2.35			

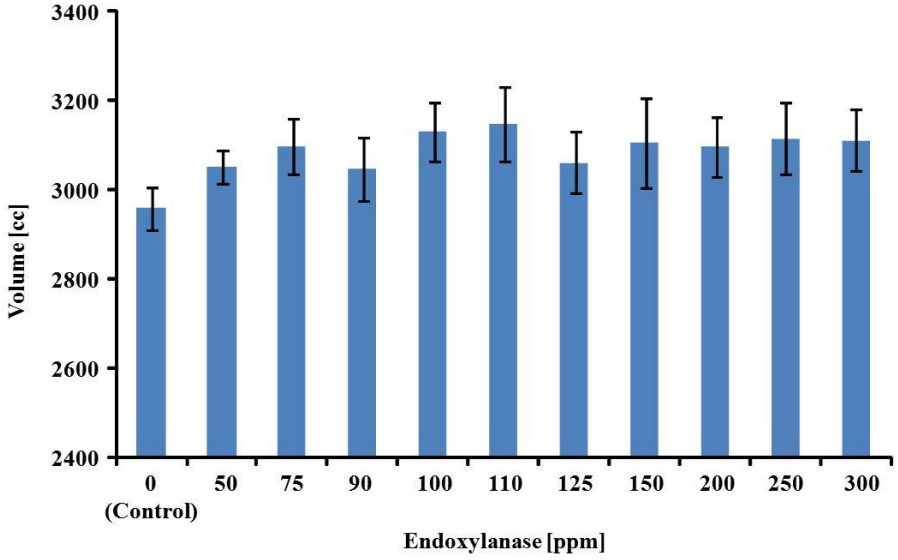


Figure 4.12 Average volumes as a function of endoxylanse levels at 200 ppm ascorbic acid

As Table 4.19 and Fig. 4.12 show endoxylamase addition improved bread volume, specific volume and crumb structure. In addition, it showed an apparent optimum amount. Adding 100 ppm gave the highest specific volume, and 110 ppm addition resulted in the highest volume. Therefore, a range of 100-110 ppm addition was the optimum level.

The dough became softer, sticker, and difficult to handle at addition above 110 ppm and the volume/specific volume decreased (Table. 4.19 and Fig. 4.12). As before, this phenomenon indicated over-dosing. Consequently, the optimum endoxylamase amount was set at 100 ppm by this study.

4.1.12 Combination of ascorbic acid and lipase

Finally, the effects of the combination of AA and lipase were evaluated, again by constant levels of AA, and varying the amount of lipase. Tests to determine the optimum combination of lipase and 200 ppm ascorbic acid were conducted using the formula in Table 4.20.

Table 4.20 Dough formula for combination of lipase and 200 ppm ascorbic acid

Ingredients	Bakers %
Flour (12.8% protein)	100
Water	59
Instant dry yeast	2
All-purpose shortening	3
Sugar	4
Salt	2
SSL	0.5
Ascorbic acid	200 ppm
Lipase	Variable

Lipase was added from 0 to 250 ppm. Endoxylanasee at 0 ppm (control) and at 50 ppm addition were produced as 2 batches each. The 10 ppm, 20 ppm, 30 ppm, 40 ppm, 100 ppm 150

ppm, 200 ppm and 250 ppm additions were produced in a single batch each. All doughs were produced by the optimized process (Fig. 3.1). Results are presented in Table 4.21 and Fig. 4.13.

Table 4.21 Crumb structure (C-Cell), and specific volumes as a function of 200 ppm ascorbic acid and varying levels of lipase

Lipase [ppm]	0 (control)	10	20	30	40
C-Cell image					
Average SV [cc/g]	6.81	6.80	6.73	6.74	6.61
STDEV	0.10	0.11	0.10	0.07	0.10
C.V. [%]	1.51	1.67	1.45	1.00	1.51
Lipase [ppm]	50	100	150	200	250
C-Cell image					
Average SV [cc/g]	6.57	6.55	6.29	6.09	6.12
STDEV	0.19	0.02	0.14	0.21	0.07
C.V. [%]	2.89	0.36	2.16	3.50	1.21

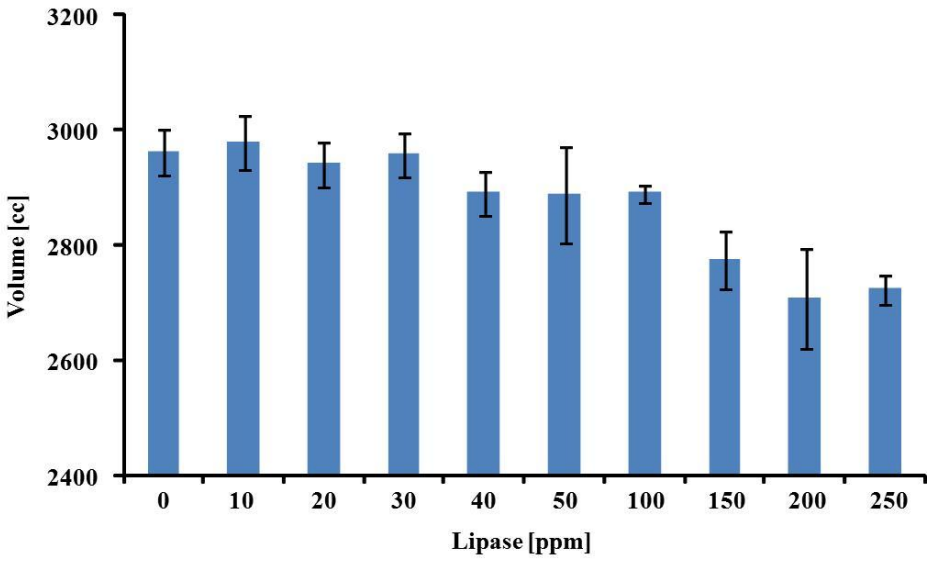


Figure 4.13 Average volumes as a function of lipase levels at 200 ppm ascorbic acid

Table 4.21 and Fig.4.13 show that lipase presence resulted in worse bread volume, specific volume, and crumb structure even low level additions. Therefore, no advantages of lipase addition were observed.

Abdullah and Hazim (2012) reported that lipases improved dough handling properties equal to or greater than does DATEM. This suggests that lipase functions indirectly as an emulsifier. In the present experiment, emulsifier (SSL) was already present in control formula; therefore, lipase activity in flour for baking was undesirable because free fatty acids have a detrimental effect in doughs/and bread (Underkofler, 1972).

4.2 Determination of optimum process condition, the effect of oxidants and oxidants-enzyme combination in frozen dough

Optimum amount of oxidants, and enzyme levels as well as process conditions were determined the previous section. The frozen dough process conditions were optimized based on Lin (2008) studies. In actual frozen dough production, the room temperature thawing condition was changed to when dough core temperature reached 18 °C to 19°C. Because, it was the same temperature as at post mixing, it was good for comparing dough rheology at this point. Based on fresh dough baking results, dough treatments were; control (Table 3.1), 50ppm potassium bromate addition, and a combination of 200 ppm ascorbic acid with 100 ppm endoxylanase addition. That combination gave the best baking performance in fresh baking. This combination was the lowest enzyme usage level and produced the highest specific volume. Therefore, it was used for frozen dough production. Frozen doughs were produced following the optimized process (Fig. 3.2).

4.2.1 Mixing condition

The final dough temperature and dough mixing method are critical in frozen dough production. In frozen dough making, most researchers recommend that to minimize yeast fermentation during processing, dough mixing by the "no-time dough method" and the desired final dough temperature is 18-21°C (65-70°F), (Anonymous ,1967; Fuhrmann,1985; Dubois and Blockcolsky,1986).

As per Lin (2008), the mixing bowl was connected to a refrigerated circulating water bath and maintained at 6 °C (43 °F). It required two hours to stabilize the water bath at the set temperature and the mixing bowl temperature and water bath temperature had 1-1.5°C offset. Therefore, the water bath was set at 5 °C and final dough temperature was measured. This experiment was conducted using the formula in Table 3.1. The final speed 2 mixing time was 9'30". Water bath was set at 5.0 °C, and the mixing bowl maintained at ≈ 6.0 °C. In all 8 batches were produced. Results are presented in Table 4.22.

Table 4.22 Final dough temperature at a mixing bowl temperature of 6 °C

Room temperature: 23.3-27.5 °C

	Water bath temp.[°C]	Mixing bowl temp.[°C]	Flour temp.[°C]	Water temp.[°C]	Final Dough temp. [°C]
Batch 1	**5.0**	**6.2**	**18.7**	**0.8**	**19.2**
Batch 2	**5.0**	**6.2**	**18.9**	**1.0**	**19.1**
Batch 3	**5.0**	**6.2**	**20.1**	**0.6**	**19.3**
Batch 4	**5.0**	**6.2**	**21.4**	**1.2**	**18.9**
Batch 5	**5.0**	**6.2**	**22.1**	**0.8**	**18.9**
Batch 6	**5.0**	**6.2**	**22.8**	**1.0**	**19.1**
Batch 7	**5.0**	**6.1**	**23.7**	**1.1**	**19.7**
Batch 8	**5.0**	**6.3**	**23.9**	**0.8**	**18.9**

Table 4.14 shows that all dough batches were within the desired final dough temperature range. Therefore, water bath was set at 5.0 °C, and a mixing bowl temperature of ≈6.0 ºC was used for all of frozen dough study.

4.2.2 Freezing conditions

According Lin (2008) and others, the desired freezing condition is for the dough core temperature to reach -5 to -8 °C (17.5-23 °F). Freezing conditions were investigated by using one dough each from eight dough batches. The dough core temperature during freezing is shown in Fig. 4.14.

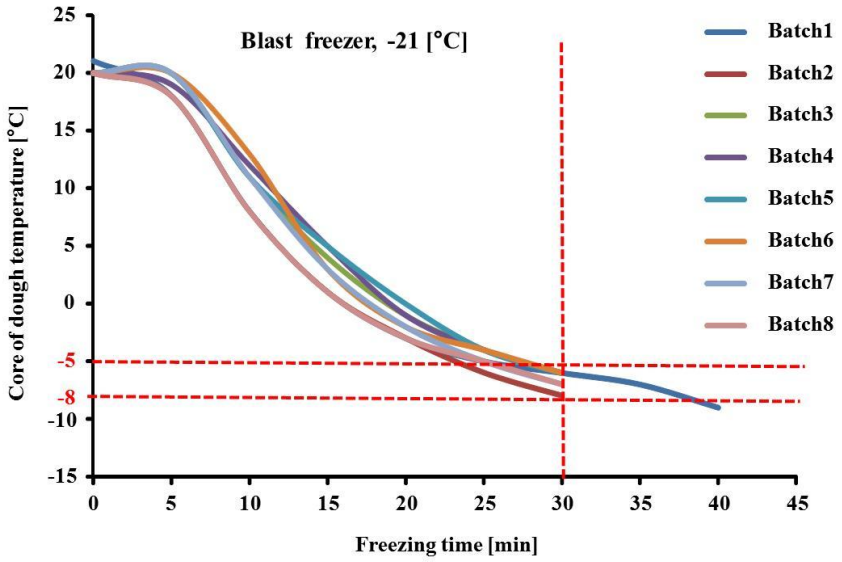


Figure 4.14 Relationship between freezing time and dough core temperature

Fig. 4.14 shows that at the core dough temperature generally reached the target temperature range between 30 minutes and 40 minutes. By ~40 minutes dough core temperature was lower than -10 °C. Freezing time was set at 35 minutes.

4.2.3 Thawing conditions

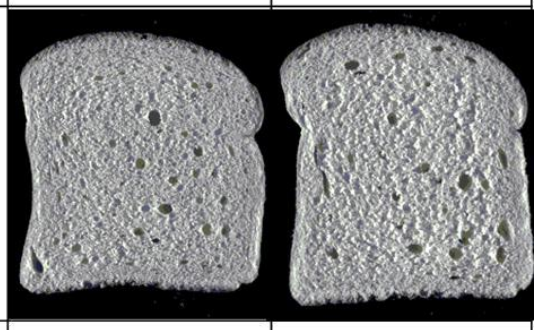
As per Lin (2008), frozen stored doughs were thawed in a retarder 16 to 18 hours and the thawed dough pieces were moved to room temperature until their core temperature reached 18 °C. Retarder thawing time was not given, so the optimum retarder thawing conditions were determined. This experiment used the doughs made during the research on freezing conditions (4.3.1). In that experiment (4.3.1), dough core temperature of batches 2 to 8 were within target range. All doughs were put in frozen storage for 24 hours. Batches 2 to 5 were thawed in the retarder for 16 hours and the remainder was thawed in the retarder for 18 hours. After retarder thawing, all doughs were placed in room temperature until their core temperature reached 18 °C. After dough core temperature reached 18 °C, the doughs were proofed at 40.6 °C (105 °F), 70 % relative humidity, and baked 22 minutes at 215 °C (420 °F). Results are shown in Tables 4.23, 4.24 and Fig. 4.15.

Table 4.23 Room temperature thawing required to reach a core temperature of 18 ºC at different retarder thawing conditions

Room temperature (R.T.): 23.3-26.1 °C

	Frozen storage [h]	Thawing in retarder [h]	Thawing required at R.T.	Core temp. of after R.T thawing [°C]
Batch 2	24	16	1h 48min	18.3
Batch 3	24	16	1h 44min	18.2
Batch 4	24	16	1h 47min	18.1
Batch 5	24	16	1h 45min	18.7
Batch 6	24	18	1h 46min	18.2
Batch 7	24	18	1h 46min	18.1
Batch 8	24	18	1h 41min	18.6

Table 4.24 Crumb structure (C-Cell), and specific volumes under different retarder thawing condition

Retarder thawing [h]	16	18
C-Cell image		
Average SV [cc/g]	6.18	6.13
STDEV	0.06	0.17
C.V. [%]	0.97	2.72

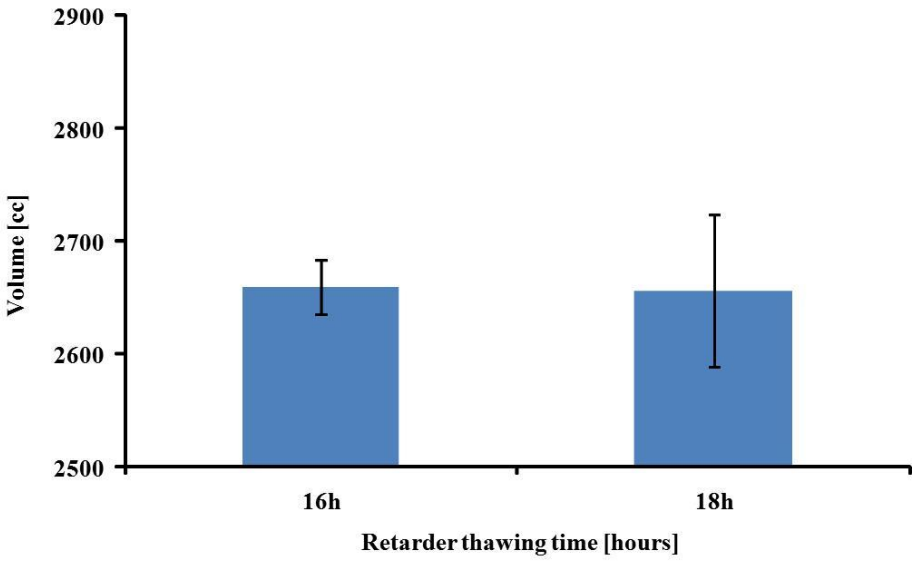


Figure 4.15 Average volumes at different retarder thawing times

Tables 4.23, 4.24 and Fig. 4.15 show that retarder thawing time did not affect the room temperature thawing time requirement or final bread quality (volume and specific volume). Therefore, time of retarder thawing did not have a large influence the dough/bread quality. Longer retarder thawing time had no advantages, so the time of retarder thawing was set at 16 hours.

To simplify the process and reduce the floor thawing, doughs were moved directly into the proofer after retarder thawing. The floor thawing effect was then investigated. The time of floor thawing and proofing are shown in Table 4.25, and final baking data shown in Table 4.26 and Fig. 4.16.

Table 4.25 Time of floor thawing and proofing

	Floor thawing time [min.]	Core temp.of floor thawed [°C]	Proofing time [min.]	Core temp. of post proof [°C]
Batch 1	**97**	**18.5**	**54**	**32.9**
Batch 2	**96**	**18.1**	**51**	**32.1**
Batch 3	**93**	**18.4**	**49**	**31.1**
Batch 4	-	-	**81**	**31.7**
Batch 5	-	-	**80**	**31.9**
Batch 6	-	-	**78**	**31.9**

Table 4.26 Crumb structure (C-Cell), and specific volumes effect of floor thawing

Thawing condition	With floor	Without floor
C-Cell image		
Average SV [cc/g]	6.24	6.20
STDEV	0.07	0.13
C.V. [%]	1.15	2.11

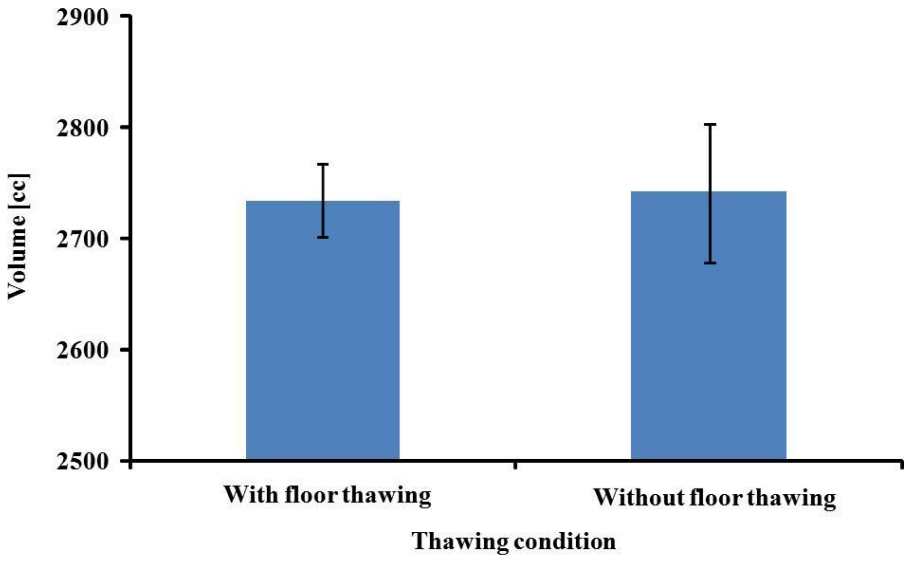


Figure 4.16 Average volumes; effect of floor thawing

Total processing time was decreased with no floor thawing (Table 4.25). The results in Table 4.26 and Fig. 4.16 suggest that thawing condition did not have an effect on final product volume (specific volume). However, bread without floor thawing was not uniform and showed big holes in middle of crumb. It may be that a rapid change in the dough temperature caused the yeast activating rapidly created a coarse crumb structure. Without floor thawing product values

had higher STDEV and C.V. % than with floor thawing. Floor thawing affected final bread quality and has advantages for frozen dough making, so it was retained.

4.2.4 Proofing condition

For frozen dough, proofing temperatures are generally in the range of 32 °C to 43 °C (90 °F-110 °F), with relative humidity about 70-75 % (Stauffer, 1993, Lorenz, and Kulp, 1995). At a higher relative humidity (85-90 %) some condensation on the surface of the cold dough pieces is likely to occur, which cause blisters and/or light blotches to appear on the crust during baking. Based on this study and that of Lin (2008), optimum proofing condition was investigated. The proofing time and core of dough tempterture post proof are presented in Table 4.27, and final baking data in Table 4.28 and Fig. 4.17.

Table 4.27 Proofing time and core of dough tempterture post proof

	Core temp.of thawed [°C]	Proofing condition	Proofing time [min.]	Core temp. of post proof [°C]
Batch 1	18.1	35 °C (95 °F), 70% r.h.	47	30.3
Batch 2	18.1	35 °C (95 °F), 70% r.h.	47	29.9
Batch 3	18.2	40.6 °C (105 °F), 70% r.h.	42	32.9
Batch 4	18.4	40.6 °C (105 °F), 70% r.h.	42	32.4
Batch 5	18.4	43.3 °C (110 °F), 70% r.h.	40	35.1
Batch 6	18.4	43.3 °C (110 °F), 70% r.h.	38	34.6

Table 4.28 Crumb structure (C-Cell), and specific volumes under different proofing condition

Proofing temp.	35 °C (95 °F)	40.6 °C (105 °F)	43.3 °C (110 °F)
C-Cell image			
Average SV [cc/g]	6.13	6.16	6.03
STDEV	0.12	0.14	0.14
C.V. [%]	1.97	2.26	2.34

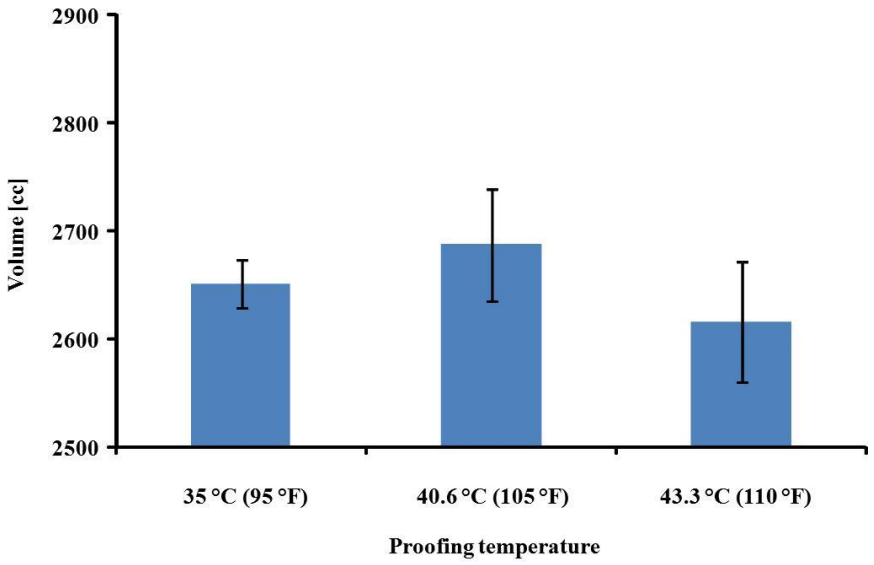


Figure 4.17 Average volumes under different proofing condition

Not surprisingly, (Table. 4.27) proofing time was reduced when proofing temperature increased. In addition, dough core temperature was increased. Proofing condition affected final product volume (specific volume) and 40.6 °C (105 °F) resulted in the highest volume and specific volume. C-Cell images showed that high temperature bread relatively rough crumb structure. Breads from high proof temperature had higher STDEV and C.V % than did lower

temperature products. Because, proofing conditions affected final bread quality, a proofing temperature of 40.6 °C (105 °F), and relative humidity 70 % was used for frozen dough production studies.

4.2.5 No frozen storage bread

Generally, frozen dough/bread was produced by no-time dough method with low mixing bowl temperature to avoid yeast activation during mixing. First, dough and bread was produced by the frozen dough (low temperature mixing) method with 0 frozen storage time. This experiment was conducted using the above mentioned treatments and optimized process (Fig. 3.2). All treatment doughs were produced as 3 batches each. Crumb structure (C-Cell), and specific volumes results are presented in Table 4.29. Each treatment's bread volume with statistical analysis is presented in Table 4.30, and Fig. 4.19.

Table 4.29 Crumb structure (C-Cell), and specific volumes different bowl temperature effect of additives

Dough Treatment	Control		$KBrO_3$		Ascorbic acid Endoxylanase	
Mixing bowl temp.	Ambient	Low (6 °C)	Ambient	Low (6 °C)	Ambient	Low (6 °C)
C-Cell image						
Average SV [cc/g]	6.51	6.29	6.65	6.53	7.21	7.07
STDEV	0.10	0.07	0.12	0.07	0.19	0.12
C.V. [%]	1.49	1.14	1.79	1.08	2.59	1.72

Table 4.30 Statistical analysis of volumes in 3 different formulas by mixing temperature

	Control [cc]	$KBrO_3$ [cc]	AA-EX [cc]
Ambient temp.	$2849^{A)}$	$2925^{B)}$	$3134^{C)}$
Low temp.	$2738^{A)}$	$2853^{B)}$	$3069^{C)}$

Superscripts A), B), and C) are significantly different from each other at $P < 0.05$.

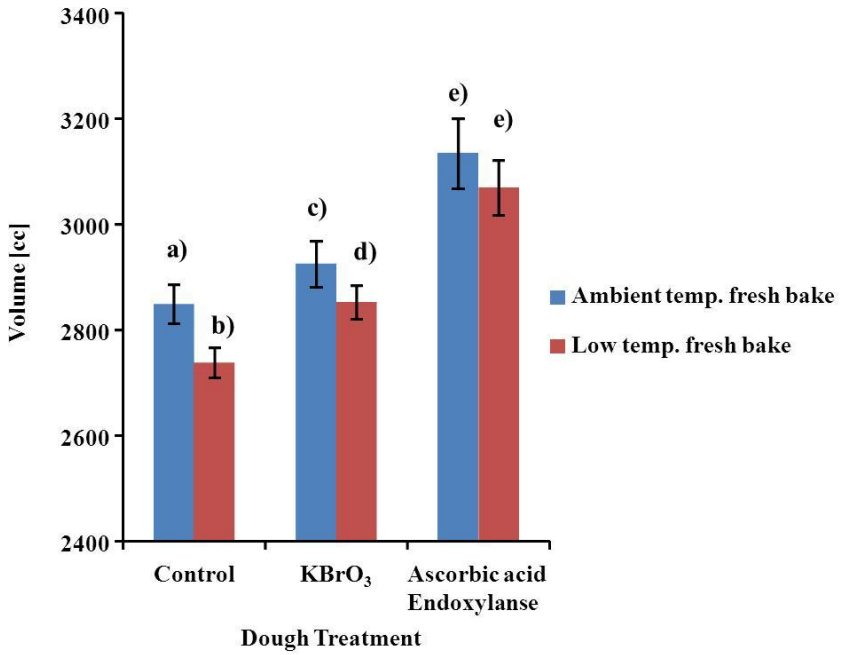


Figure 4.18 Average volumes different mixing bowl temperature effect of additives
a), b), c), d), and e) proved to be significantly different from each other at $P < 0.05$ in each formula.

Table 4.29, 4.30, and Fig. 4.18 showed that dough containing additives produced better quality (volume) bread than did controls regardless of mixing temperature. Furthermore, 200 ppm ascorbic acid and 100 ppm bread endoxylanse bread was better than that of 50 ppm potassium bromate levels. Statistical analysis (Table 4.30) shows that the 3 different treatment breads were significantly different from each other at both mixing temperature conditions.

Table 4.29, 4.30, and Fig. 4.18 showed that the low temperature produced dough/bread that was lower in volume than the ambient temperature system regardless formula. Statistical analysis (Fig. 4.18) shows that the volume of the ambient temperature mixed control, dough containing $KBrO_3$ and low temperature control, containing $KBrO_3$ were significantly different from each other. On the other hand, the volume of AA-EX dough (ambient temperature mixed and low temperature mixed) was not significantly different. In this study, final mixing time was same as ambient temperature condition (fresh baking). During the mixing, low temperature dough was stiffer than that produced at ambient temperature. As a result, mixing bowl temperature affected dough property and all low temperature produced doughs lower bread quality than ambient (high) temperature. The causes might be follows; 1) The gluten network

was damaged or inhibited by low temperature 2) Time required to create gluten network (full development time) was changed (increased), because of low temperature. In this case, re-optimizing the final mixing time at low temperature would solve the problem.

Based on statistical analysis, bread quality loss was reduced by the addition of additives though the mixing temperature was changed. The additives' effect was different depending on the additive type, but all affected dough texture positively during processing. In this study, 50 ppm potassium bromate and a combination of 200 ppm ascorbic acid and 100 ppm endoxylanse was used. During the frozen dough processing, additives were affected dough texture positively. Therefore, bread containing 50 ppm potassium bromate bread was higher in volume than controls. Furthermore, 200 ppm ascorbic acid and 100 ppm endoxylanse bread was higher volume than the 50 ppm potassium bromate.

4.2.6 1 week frozen storage bread

Following the formula determined for fresh no-time dough and frozen dough processes (Fig. 3.2), Bread was produced from dough frozen 1 week. This bread was produced as 3 batches each of 3 different treatments. Crumb structure (C-Cell), and specific volume results are presented in Table 4.31. Each treatment volumes with statistical analysis are shown in Table 4.32, and Fig. 4.19.

Table 4.31 Crumb structure (C-Cell), and specific volumes after 1 week frozen storage

Dough Treatment	Control			$KBrO_3$			Ascorbic acid Endoxylanase		
Mixing bowl temp.	Ambient	Low (6 °C)	Low (6 °C)	Ambient	Low (6 °C)	Low (6 °C)	Ambient	Low (6 °C)	Low (6 °C)
Frozen storage [week]	0	0	1	0	0	1	0	0	1
C-Cell image									
Average SV [cc/g]	6.51	6.29	5.99	6.65	6.53	6.44	7.21	7.07	7.04
STDEV	0.10	0.07	0.09	0.12	0.07	0.14	0.19	0.12	0.17
C.V. [%]	1.49	1.14	1.45	1.79	1.08	2.21	2.59	1.72	2.45

Table 4.32 Statistical analysis of volumes of 3 different formulas at 1 week frozen storage

	Control [cc]	$KBrO_3$ [cc]	AA-EX [cc]
1 week frozen storage	2658[A)]	2858[B)]	3114[C)]

Superscripts A), B), and C) proved are significantly different from each other at $P < 0.05$.

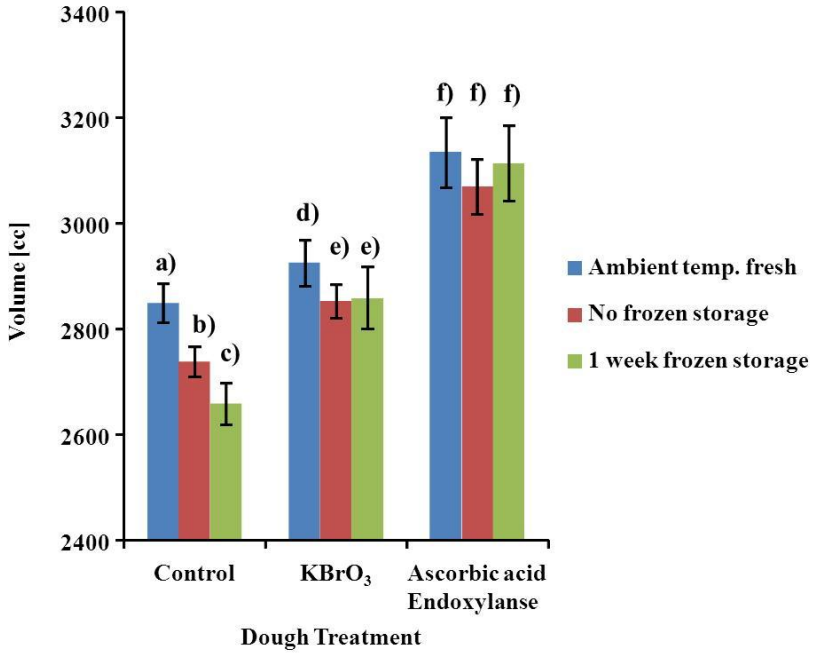


Figure 4.19 Average of volumes after 1 week frozen storage

Focus on each treatment, groups a), b), c), d) and e) proved to be significantly different from each other at $P < 0.05$.

Table 4.31 and Fig. 4.19 showed that 1 week of frozen storage resulted in lower volume for control dough, but no loss in volume for bromate containing bread and a slight increase in volume for AA-endoxylanase doughs. Statistical analysis (Table 4.32) shows that the volumes for each of the 3 treatments were significantly different of each other at 1 week frozen storage. Cleraly, additives were affecting the dough and final bread volume was improved. In addition, statistical analysis (Fig. 4.19) shows that control dough with no frozen storage and with 1 week frozen storage were significantly different of each other. The control dough was most influenced by freezing, frozen storage, and thawing. Among causes might be 1) Control dough texture

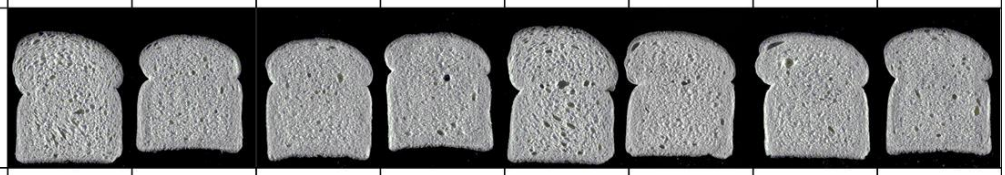
(gluten network) was changed (damaged) by freezing, frozen storage, and thawing 2) The water distribution of control dough was easy to change by freezing, frozen storage, and thawing.

As Table 4.31 and Fig. 4.19 shows, with no frozen storage both potassium bromate and AA-endoxylanase increased bread volume over control with the AA-endoxylanase having a larger positive effect. Statistical analysis (Fig. 4.19) shows that for each dough treatments with no frozen storage and 1 week frozen storage results were not significantly different from each other. In the case of dough containing potassium bromate it be; 1) The deterioration gluten structure caused by freezing, frozen storage, and thawing are reversed some extent by the action of potassium bromate at late proofing and early baking. 2) Water distribution of potassium bromate containing dough was not easy to influenced by freezing, frozen storage, and thawing. For containing AA-endoxylanase doughs, the causes might be that AA and endoxylanase affected dough during mixing, so post mixing gluten network was improved relative to the others. Therefore, dough containing this combination was less effected of freezing, frozen storage, and thawing. If the gluten network was improved after mixing, water distribution might be less affected by freezing, frozen storage, and thawing. Therefore, gluten conducting network was maintained during freezing, frozen storage, and thawing. Consequently, it produced slightly higher (almost equal) volumes though 1 week of frozen storage.

4.2.7 3 weeks frozen storage bread

Frozen storage was extended to 3 weeks for control, potassium bromate and AA-endoxylanase doughs. Crumb structure (C-Cell), and specific volumes results are shown in Table 4.33, and each treatment bread volume with statistical analysis showed in Table 4.34, and Fig. 4.20. Three weeks frozen dough/bread was produced as 3 bathes each in 3 different treatments by optimized process (Fig.3.2).

Table 4.33 Crumb structure (C-Cell), and specific volumes after 3 weeks frozen storage

Dough Treatment	Control				$KBrO_3$			
Mixing bowl temp.	Ambient	Low (6 °C)	Low (6 °C)	Low (6 °C)	Ambient	Low (6 °C)	Low (6 °C)	Low (6 °C)
Frozen storage [week]	0	0	1	3	0	0	1	3
C-Cell image								
Average SV [cc/g]	6.51	6.29	5.99	5.97	6.65	6.53	6.44	6.43
STDEV	0.10	0.07	0.09	0.06	0.12	0.07	0.14	0.12
C.V. [%]	1.49	1.14	1.45	0.96	1.79	1.08	2.21	1.83

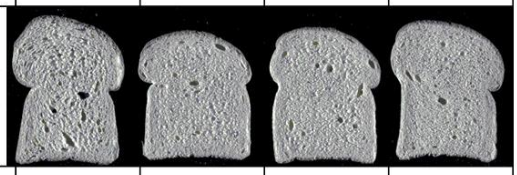
Dough Treatment	Ascorbic acid	Endoxylanase		
Mixing bowl temp.	Ambient	Low (6 °C)	Low (6 °C)	Low (6 °C)
Frozen storage [week]	0	0	1	3
C-Cell image				
Average SV [cc/g]	7.21	7.07	7.04	6.98
STDEV	0.19	0.12	0.17	0.11
C.V. [%]	2.59	1.72	2.45	1.63

Table 4.34 Statistical analysis for volumes in 3 different formulas at 3 weeks frozen storage

	Control [cc]	$KBrO_3$ [cc]	AA-EX [cc]
3 weeks frozen storage	$2638^{A)}$	$2842^{B)}$	$3083^{C)}$

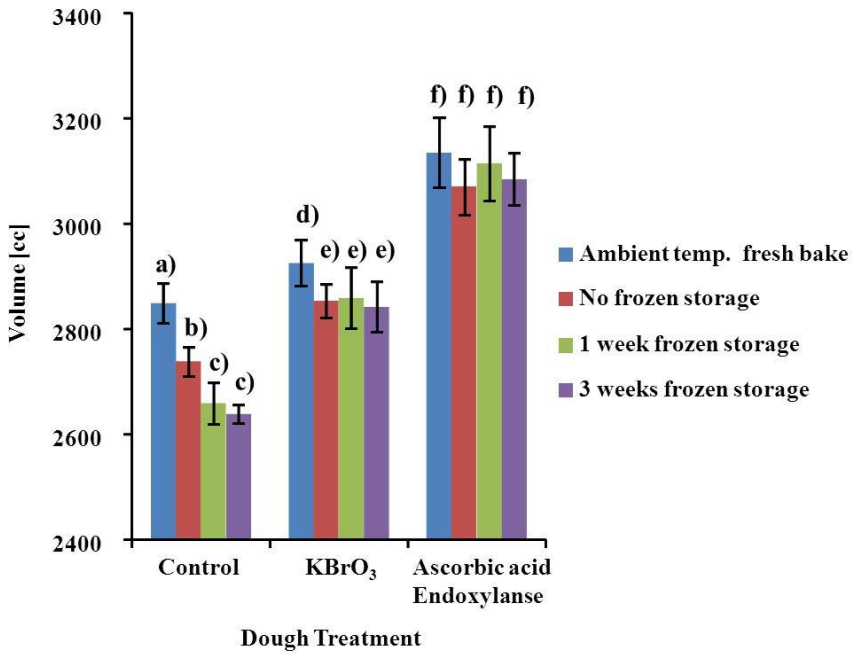


Figure 4.20 Average of volumes after 3 weeks frozen storage

Focus on each treatment, groups a), b), c), d) and e) proved to be significantly different from each other at $P < 0.05$.

Statistical analysis (Table 4.34) shows that the 3 treatment's volumes were significantly different from each other at 3 weeks frozen storage. The additives remained effective though 3 weeks of frozen storage. As Table 4.33, and Fig. 4.20 show additional storage further reduced control bread volumes. Comparing control dough with no frozen storage and 3 weeks frozen storage, the large volume loss is evident. On the other hand, dough containing additives was not observed large volume loss. The causes were freezing, 3 weeks frozen storage, and thawing was affected final bread quality (volume). Statistical analysis (Fig. 4.20) show that control dough of no frozen storage and 3 weeks frozen storage were significantly different. However, for doughs containing $KBrO_3$ or ascorbic acid-endoxylanase, the volumes of breads with no frozen storage and 3 weeks frozen storage were not significantly different. In addition, comparing the statistical analysis of all doughs a 1 week and 3 weeks frozen storage, these were not significant difference in each treatment. Volume loss due to longer frozen storage was minimal for potassium bromate, and AA- endoxylanase containing loaves. Even 3 after weeks frozen storage, bread containing potassium bromate showed similar volume to that with no frozen storage. As explained before, potassium bromate is slow acting oxidant, so it was affected during late prof and baking;

therefore, it showed similar volume as no frozen storage. Even though 3 weeks frozen storage, AA-endoxylanase containing dough show an ever greater volume, and maintained significantly better quality and volume relative to control. As described before, AA and endoxylanase active during mixing. Post mixing, dough containing this additives combination was more developed and improved in dough texture. Furthermore, dough containing this combination dough kept their performance texture though freezing frozen storage, and thawing.

4.3 Fundamental measurements of frozen and thawed dough rheology

As explained in Chapter 2.6.1, viscoelastic properties can be measured by experiments which examine the relationship between stress and strain. In small amplitude oscillatory flow experiments, a sinusoidal oscillating stress with a given frequency is applied to the material, and the oscillating strain response is measured along with the phase difference between the oscillating stress and strain. The storage modulus, G', corresponds to the elastic character of the fluid or the energy stared during deformation. The loss modulus, G", is related to the viscous character of the material or the energy dissipation that occurs during the deformation.

Small amplitude dynamic oscillatory measurements have been used extensively to determine wheat flour dough's fundamental mechanical characteristics (Faubion and Hoseney, 1990; Amemiya and Menjivar, 1992; Dobraszczyk and Morgenstern, 2003). The oscillatory method is the most useful technique for evaluating the viscoelastic properties of materials that cannot be investigated by steady-shear instruments due to their shear-sensitivity, such as dough (Gunasekaran and Ak, 2000; Dobraszczyk and Morgenstern, 2003; Connelly and McIntier, 2008).

Dough consistency, which can be adjusted and optimized by farinograph/mixograph water absorption, has significant influence on baking results. In bread making, flours producing doughs with balanced tensile and elastic properties are required to ensure optimal baking performance. This balance and its effects can be demonstrated using dynamic rheological tests. The tensile and elastic properties can be described by the viscosity and loss tangent (G"/G') of the dough. Stress/strain and frequency sweeps show differences in storage and loss moduli of doughs having differing sensory properties. Weipert (1990) reported that the wheat variety, which produces a resistant and poorly extensible dough (sensorially described as stiff and snappy), had a high G' and low G". Because the difference between the two was high, the loss tangent (a quotient of G" and G' values) was low. The wheat variety which mixed to a more extensible but still elastic dough (sensorially described as normal and silky), gave lower values for both G' and G" and a lower difference between the two. The combination of high G' and low G" reflects a more rigid and stiff material whose loss tangent is small. These results are consistent with baking tests results. Doughs characterized as moist and slack possessed lower

complex viscosity and a higher loss tangent than did doughs described as having a short texture and dry surface appearance (Weipert, 1987).

This section addresses the rheological behavior of 18 different dough samples obtained through 3 different formulations and combination of various processing and storage conditions as summarized in Table 4.35.

Table 4.35 Abbreviations of frequency sweep test

Formulation	Frozen storage/Process		Legends name	
	Frozen storage	Process	Long description	Abbreviation
Control	-	After mixing	Mixed low temperature (6 °C) control dough	D-C
	-	After proofing	Proofed low temperature (6 °C) control dough	D-P-C
	1 week	After thawing	1 week frozen thawed control dough	1wFD-C
	1 week	After proofing	1 week frozen proofed control dough	1wFD-P-C
	3 weeks	After thawing	3 weeks frozen thawed control dough	3wFD-C
	3 weeks	After proofing	3 weeks frozen proofed control dough	3wFD-P-C
$KBrO_3$	-	After mixing	Mixed low temperature (6 °C) dough containing $KBrO_3$	D-K
	-	After proofing	Proofed low temperature (6 °C) dough containing $KBrO_3$	D-P-K
	1 week	After thawing	1 week frozen thawed dough containing $KBrO_3$	1wFD-K
	1 week	After proofing	1 week frozen proofed dough containing $KBrO_3$	1wFD-P-K
	3 weeks	After thawing	3 weeks frozen thawed dough containing $KBrO_3$	3wFD-K
	3 weeks	After proofing	3 weeks frozen proofed dough containing $KBrO_3$	3wFD-P-K
AA-EX	-	After mixing	Mixed low temperature (6 °C) dough containing AA-EX	D-AE
	-	After proofing	Proofed low temperature (6 °C) dough containing AA-EX	D-P-AE
	1 week	After thawing	1 week frozen thawed dough containing AA-EX	1wFD-AE
	1 week	After proofing	1 week frozen proofed dough containing AA-EX	1wFD-P-AE
	3 weeks	After thawing	3 weeks frozen thawed dough containing AA-EX	3wFD-AE
	3 weeks	After proofing	3 weeks frozen proofed dough containing AA-EX	3wFD-P-AE

4.3.1 Linear viscoelastic region

The linear viscoelastic region (LVR) is important because it allows for simplified interpretation of resulting data. Assessment of physical properties and dough ingredient functionality becomes easier and more reliable in the linear region because the mathematical equations describing those properties are less complex, and continuous testing of time-dependent changes is made possible by the non-destructive nature of the testing (Weipert, 1990, Song and Zheng, 2007). Oscillatory measurements in the linear viscoelastic region allow measurements, but do not disturb or destroy inherent structure. Thus, they are of great value in studying the influence and action of additives, such as hydrocolloids in dough systems (Weipert, 1990). This is because dynamic mechanical parameters are highly sensitive to changes in polymer type and concentration (Ferry, 1980).

4.3.1.1 Strain sweep

In rheological testing, all materials have a linear viscoelastic response region. Only when working within LVR, rheological data analysis can be conducted with the mathematical theory of linear viscoelasticity. Therefore, determination of LVR is a necessary preliminary step for performing all dynamic rheology measurements. LVR is generally determined by either strain sweep tests or stress sweep tests. Strain sweep tests are conducted at a constant frequency (e.g. 1Hz) while the applied strain varies.

The effect on the LVR at different stages in the frozen dough baking process was tested using control formula dough. Results are shown in Fig.4.21 for the strain sweep test performed at a constant frequency of 1 Hz on eight different samples representing formulation (control dough, dough containing $KBrO_3$, dough containing AA-EX), storage condition (fresh or frozen storage) and process (before and after proof dough) effects. Frozen samples were stored 27 weeks (before proof, FD-C) and 28 weeks (after proof, FD-P-C) which provided long enough time (much longer than 3 weeks of the longest frozen storage time used in the experimental design of this study) to represent the effect of prolonged frozen storage.

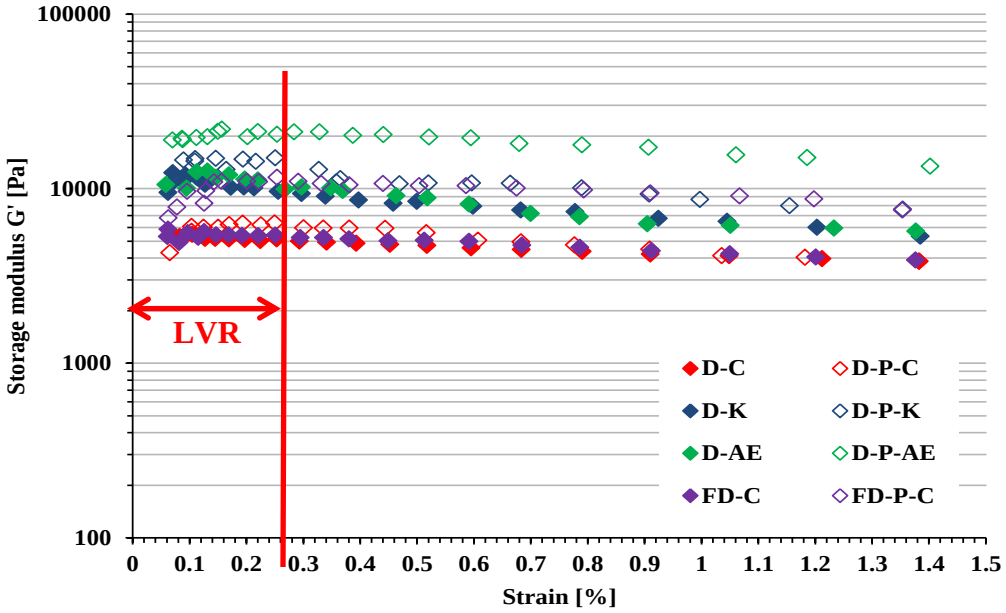


Figure 4.21 Determination of the linear viscoelastic region for control dough representing each processing and storage effects

Elastic modulus (G') of dough samples in LVR ranged between 5000 and 20000 Pa which is a typical for wheat dough (Salvador et al., 2006, Mariotti and Alamprese, 2012). The value of the storage modulus was relatively constant for strain values less than 0.25%; whereas moduli started to decrease at higher values, indicating the onset of nonlinear behavior. Drop in the elastic modulus, G′, started to occur above 0.25% strain and became large above 1% strain, indicating the breakdown of the dough structure beyond this deformation level. It has been previously found that wheat flour-water doughs exhibit linear viscoelasticity at strain levels lower than 0.25% (Phan-Thien and Safari-Ardi, 1998, Weipert, 1990). Fig.4.21 shows that all processed control dough had LVR at strain levels lower than 0.25%, so all processed control dough samples were not disrupted across that range.

The effects of additives (control, $KBrO_3$, and AA-EX) were tested at post mix and post proof to determine their influence on LVR (Fig. 4.21). Both treatment doughs had LVR at strain levels lower than 0.25%, so both treatment dough samples were also not disrupted across this range. Therefore, all processed and treatment doughs also had LVR at strain levels lower than 0.25%.

4.3.1.2 Stress sweep

As mentioned previously, the LVR can be determined by strain or stress sweep tests. There are two types of dynamic oscillatory instruments: Controlled stress instruments have fixed stress amplitude and measure deformation. Controlled strain instruments that have a fixed strain rate and measure stress (Steffe, 1996). Controlled stress rheometry (CSR) has been used successfully to illustrate the fundamental rheological properties of wheat flour dough systems (Dus and Kokini, 1990; Khatkar et al., 1995; Dobraszczyk and Morgenstern, 2003), but the more common method in oscillatory measurement of rheology is the controlled strain instrument. Each of these methods produces similar results, and often controlled stress instruments can be used as controlled strain instruments through software manipulation.

In this study, although the LVR was originally identified using strain sweep as explained in section (4.3.1.1), the data had to be converted to a stress sweep because the frequency sweep testing mode of the rheometer requires a constant stress value identified across the samples tested. To test the reliability of the conversion process, stress sweep curves for storage (G'), loss (G") and complex (G*) moduli of post mix control dough samples were compared. Two sets of curves are shown in Fig. 4.22 using data obtained directly from stress sweeps and data converted from strain sweeps.

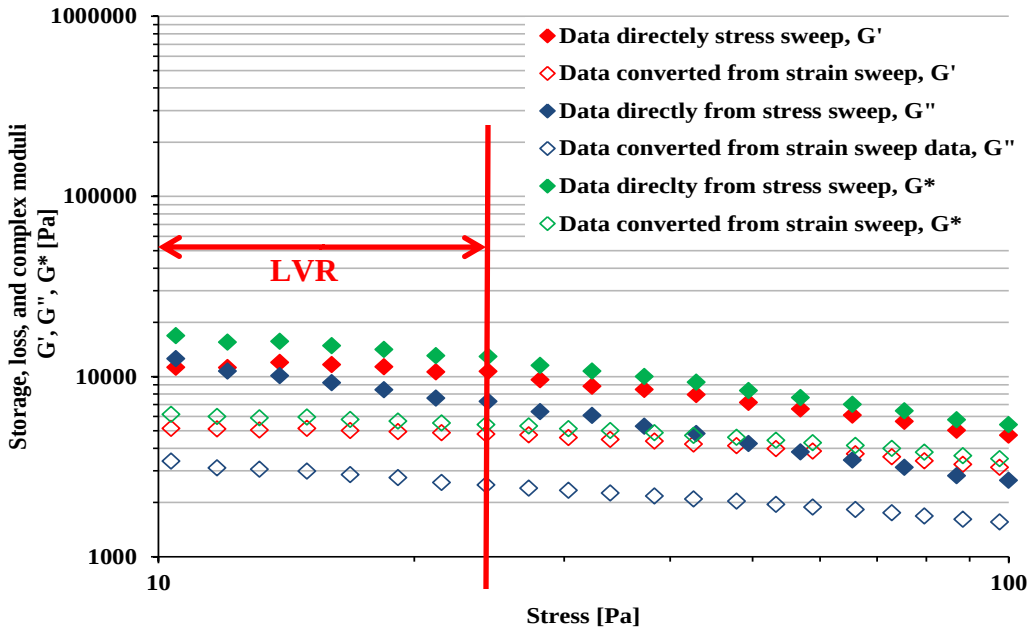


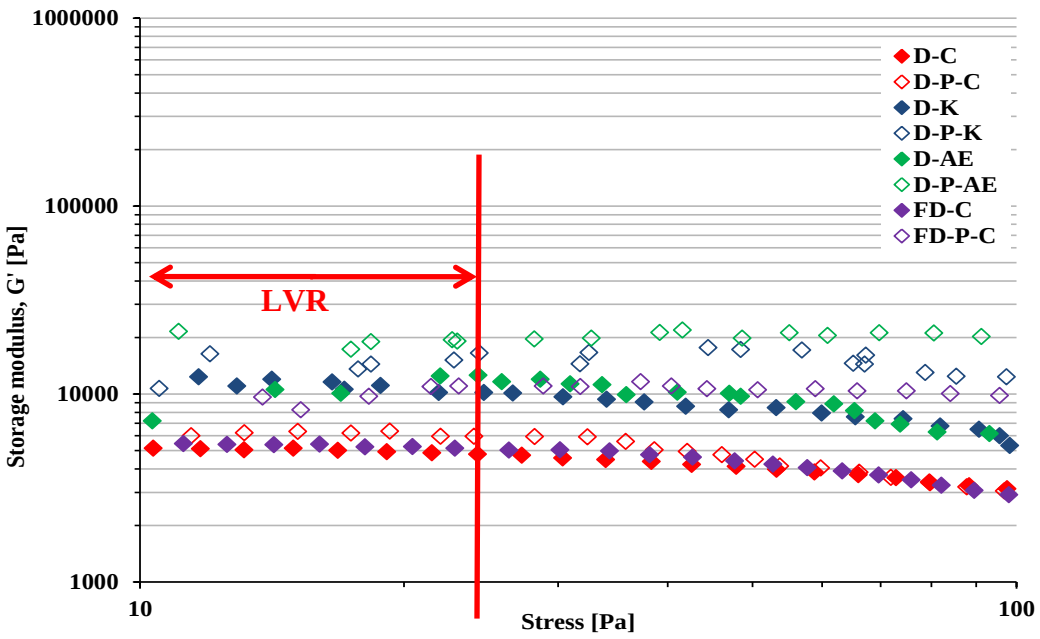
Figure 4.22 Stress sweep curves for storage (G'), loss (G") and complex moduli (G*) of post mix control dough. Two sets of curves are shown using data obtained directly from stress sweeps and data converted from strain sweeps

Fig. 4.22 illustrates that although G', G", and G* values (magnitudes) of converted stress sweeps and direct stress sweeps were different, the linear viscoelastic region (LVR) identified from each set of curves were comparable. Therefore, a constant stress value lowers than 23 Pa was identified and used in the subsequent frequency sweeps (section 4.3.3 & section 4.3.4) throughout the study. The differences in the magnitude of elastic modulus can be explained by batch effect. Moreover, the reported strain sweep data were average of samples tested from five batches while the stress sweeps were conducted for only one batch. Campos et al. (1997) reported that the strain responses of flour-water dough systems are reasonably proportional to the applied stress up to 50 Pa, which corresponds to the strain amplitude of 0.2 %. The reported strain values corresponding to 50 Pa differ as reported by different researchers: 0.2% (Dus and Kokini, 1990), 0.22% (Hibberd and Wallace, 1966), 0.25% (Weipert, 1990), 0.5% (Amemiya and Menjivar, 1992), or 0.8% (Lindahl and Eliasson, 1992).

Fig. 4.22 demonstrated that in order to confirm that less than 23 Pa is the common LVR for all samples across the board, strain sweep test results were converted to stress sweeps. Fig. 4.23 shows storage (a) and loss (b) moduli of eight different samples representing formulation

(control dough, dough containing $KBrO_3$, dough containing AA-EX), storage condition (fresh, frozen storage) and process (before and after proofed dough) effects.

(a)



(b)

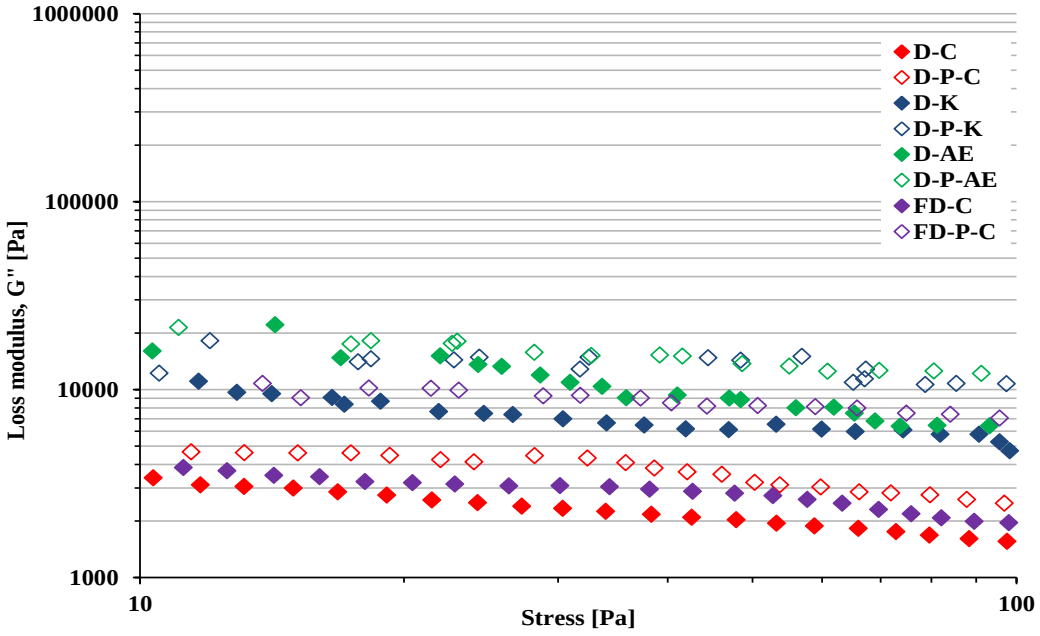


Figure 4.23 Stress sweep curves used to determine the linear viscoelastic region for dough samples representing combinations of ingredient, processing and storage effects. (a) Storage modulus, G', and (b) Loss modulus, G"

The Fig. 4.23 (a) and (b) demonstrated that the control dough at each process point can be characterized as a viscoelastic soft-solid material because G' (storage modulus, elastic moduli) is higher than G" (loss modulus, viscous modulus) within the linear region. At low stress levels (< 23 Pa), G' for all control doughs were constant and the structure was not disrupted. Fig. 4.23 (a) shows that dough at the post mix had the shortest LVR, an indication that the LVR was affected by the freezing process, and that the post mix dough breaks down more easily. The length of the LVR is a measure of dough stability, so a longer LVR reflects more stable dough and shorter LVR corresponds to less stable dough. This allows the conclusion that dough stability was changed during frozen dough processing.

The affects of additives (control, dough containing $KBrO_3$, and dough containing AA-EX) were tested at post mix and post proof to determine their influence on LVR. Fig. 4.23 (a) and (b) indicate that each treatment dough at each process points (post mix and post proof) can be characterized as a viscoelastic soft-solid material because G' was higher than G" within the linear region. Measured after mixing, the presence of either $KBrO_3$ or AA-EX caused the LVR to lengthen relative to the control (no additives) doughs. This reflects an increased degree of stability caused by the additive at this point in the process. As was the case for control doughs proofing (measurement post proof) extended the LVR for dough containing either additive.

4.3.2 Frequency sweeps by additives

Frequency sweep tests involve increasing the frequency of oscillation while keeping a constant strain or stress. Based on the results of the previous section (4.3.1), the linear viscoelastic region was found to be at stress levels lower than 23 Pa. Consequently, a stress level of 15 Pa was chosen and kept constant throughout the frequency sweep tests across 18 different dough samples obtained through 3 different formulations (control, dough containing 50 ppm potassium bromate, and dough containing combination of 200 ppm ascorbic acid and 100 ppm endoxylanase) and various processing (post mix, thaw and proof) and storage conditions (0, 1 and 3 weeks frozen storage) as summarized in Table 4.35.

4.3.2.1 Control

Control dough frequency sweeps are shown in Fig. 4.24.

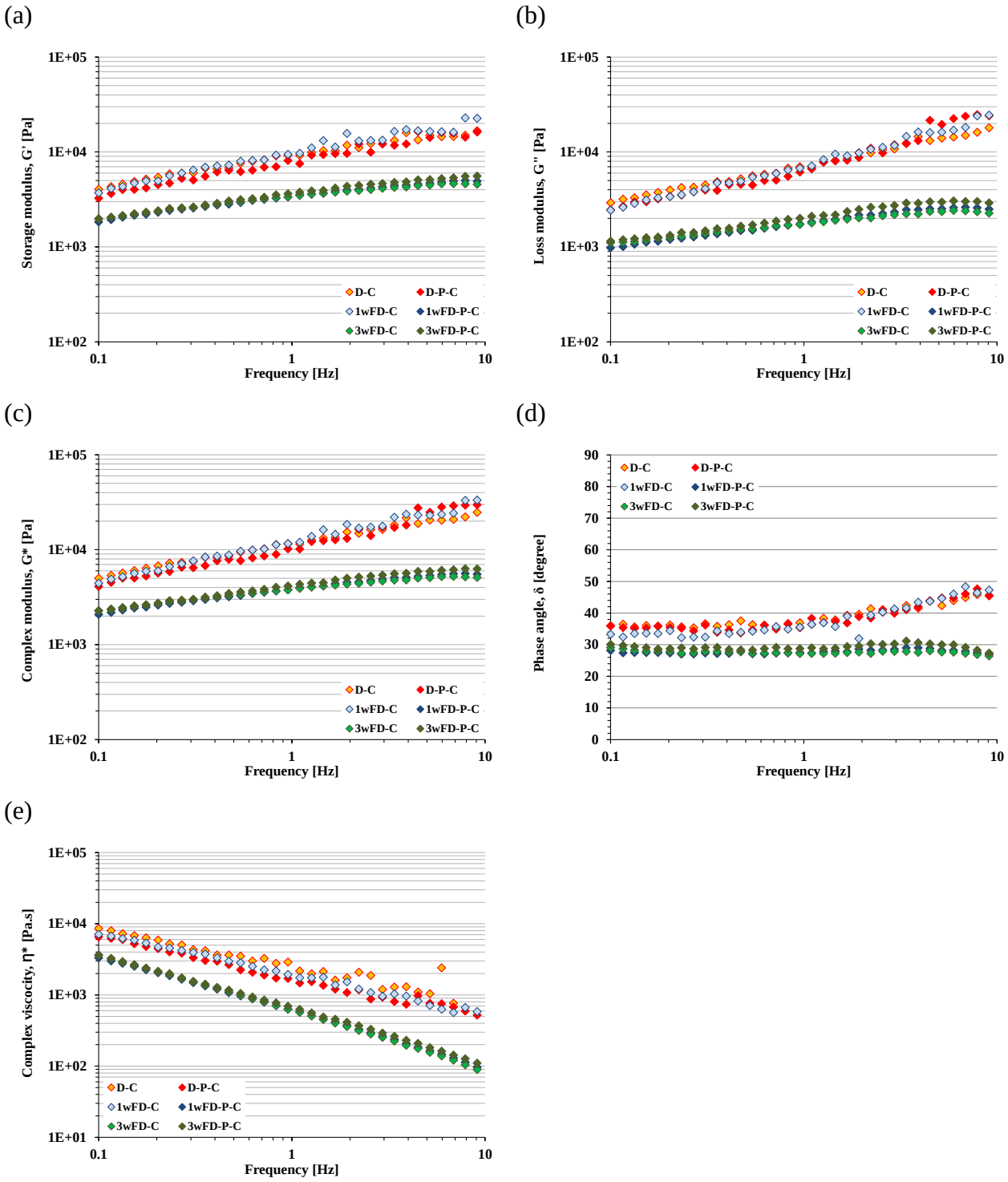


Figure 4.24 Frequency sweeps tests of control dough (a) Storage modulus G', (b) Loss modulus G", (c) Complex modulus, G*, (d) Phase angle, tan δ, (e) Complex viscosity, η*

Fig. 4.24 (a) and (b) demonstrate that because G′ is greater than G″ over all frequencies, the dough has a viscoelastic soft-solid nature phase angle values below or around 45 º reflect the slightly more solid-like material property of the dough. In a frequency range of 0.1 to 10 Hz (2 folds), elastic modulus (G') and complex modulus (G*) varied between 2000 and 30000 Pa, which is a typical range for wheat dough rheology frequency sweep studies (Miller and Hoseney, 1999, Tanner et al., 2008). The magnitude of G' and G" increased as the frequency increased as expected (Tanner et al., 2008) since at high frequencies the material has less time to recover (or relax).

The increase in G" was faster than the increase in G' for fresh doughs (before and after proof) and 1 week frozen before proof dough samples. This resulted in increasing tan δ values as the frequency increased. Tan δ of 3 week frozen before and after proof doughs was independent from frequency and remained constant around 30 ° (Fig. 4.24 (d)).

Post mix, fresh proofed and 1 week frozen thawed dough samples showed higher values of all moduli over the entire frequency range. There are studies reported that higher dough strengths correlate with lower moduli values at small deformations (Safari-Ardi and Phan-Thien, 1998, Uthayakumaran et al., 2002). This could explain the relatively small loaf volumes observed in breads made from 3 week frozen storage bread samples, (discussed in section 4.4).

The slope and magnitude of the storage modulus (G') are the most commonly used rheological measures for quantifying the network formation in biopolymers. When a polymer undergoes cross-linking, the molecular orientation results in a major increase in its solid-like properties. As the network density increases the molecular weight between entanglements/cross-links decreases, and G' increases and remains constant with frequency.

The slopes of G' and G" indicate that the dough elasticity (elastic modulus) and viscosity (viscous modulus) are dependent on the frequency change. Higher slope values signify that the dough elasticity or viscosity is more frequency dependent, and lower slope value suggests that the dough elasticity or viscosity is frequency independent. The slope values of G' and G" of each processed control dough are presented in Table 4.36.

Table 4.36 Slope of frequency sweeps for control dough

Control dough	G'	G"
Post mixing	0.31	0.40
Post proofing	0.35	0.52
1 week frozen thawed	0.38	0.50
1 week frozen thawed & proofed	0.22	0.22
3 weeks frozen thawed	0.19	0.18
3 weeks frozen thawed & proofed	0.23	0.23

The slopes of both moduli for post mix, proof and post 1 week frozen thawed doughs were higher than the other processed doughs. This demonstrated that these doughs were more sensitive to frequency change (more frequency dependent) than the other processed doughs. Phase angle is the ratio of G″ to G′. Therefore, an increase in the phase angle shows that G″ has a greater slope than G′ indicating the G" is increasing at a faster rate than G'. An ideal solid (elastic) material produces a shear stress in phase with the strain (i.e. 0 °), while an ideal fluid (viscous) material produces a stress 90 ° out of the phase with the applied strain/stress. If a material is an ideal elastic material, the stress and strain are in phase; therefore, G″ and η* are also equal to 0 ° because there is no viscous energy dissipation (Steffe, 1992; Miller and Hoseney, 1999; Belton, 2005). By definition, a viscoelastic material exhibits an intermediate phase angle between 0 ° and 90 º. A solid-like viscoelastic material exhibits a phase angle smaller than 45 º, while liquid like viscoelastic material exhibits a phase angle greater than 45 º.

Dough made from good quality (strong) flour has lower tan δ values than does dough from poor quality (weak) flour (Kokelaar et al, 1996; Miller and Hoseney, 1999). The tan δ values of glutens are ranked in the decreasing order as weak gluten > strong > extra strong glutens, while G′ and G″ values show the reverse trend (Song and Zheng, 2007). Soluble fractions in dough also play important role. Less tan δ and greater G′ may occur in the absence of the water-soluble fractions (Faubion and Hoseney, 1997; Rouillé et al., 2005). The high tan δ value of doughs that were made from poor quality flour could be a result of fewer entanglements that were easily dissociated (Miller and Hoseney, 1999).

Comparing phase angles (Fig.4.24 (d)), proofed dough after 1 week frozen storage, 3 weeks frozen before and after proof were below 45 º for the entire frequency range. This suggests that doughs were slightly more solid-like than at the other processing stage (post mix, post proof and 1 week frozen thaw). On the other hand, after proof and 1 week frozen after proof

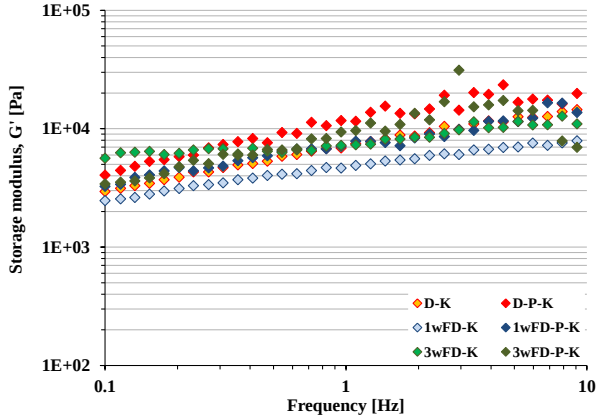
dough were slightly more liquid-like. Fig.4.24 (d) also illustrated that the phase angle of post mix, proof and post 1 week frozen thaw dough changed greatly as the frequency increased from 0.1 to 10 Hz indicating that those doughs are more sensitive to frequency changes (more frequency dependent). As described in Table 4.36, slope values of post mix, proof and post 1 week thaw doughs were higher, which is another indication of higher sensitivity to frequency changes compared to rest of the dough samples

Complex viscosity η* is the frequency-dependent viscosity function determined during dynamic measurements. It contains that the both in-phase viscosity η' (ratio of loss modulus to angular frequency) and out-of-phase viscosity η" (ratio of storage modulus to angular frequency). Fig. 4.24 (e) indicates that independent of storage and processing, all control dough samples showed shear-thinning behavior over frequencies between 0.1 and 10 Hz. Post mix, proofed, and 1 week frozen thawed samples had slightly higher dynamic viscosities compared to rest of the control doughs. A similar differentiation was observed in phase angle data (Fig. 4.24 (d)).

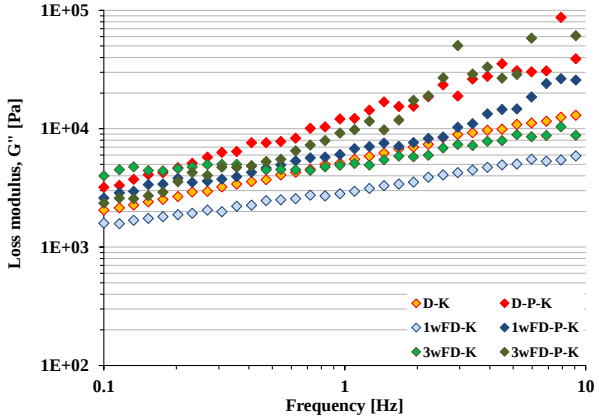
4.3.2.2 Potassium bromate

The rheology results of frozen dough containing 50ppm potassium bromate are shown in Fig. 4 25.

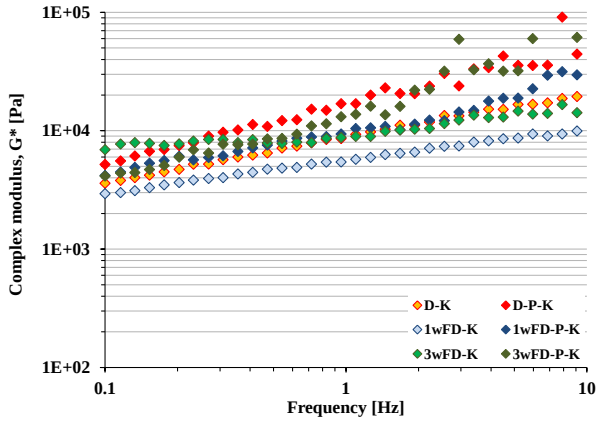
(a)



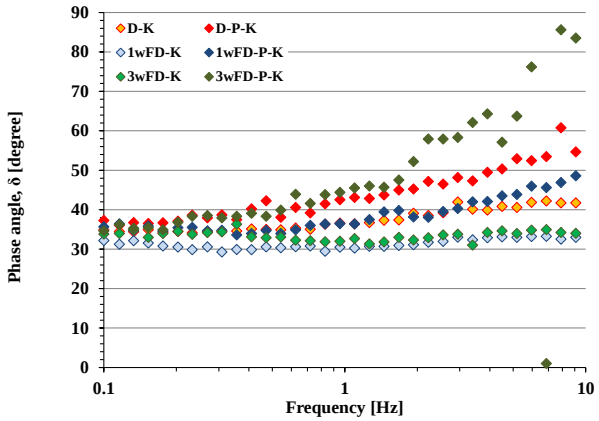
(b)



(c)



(d)



(e)

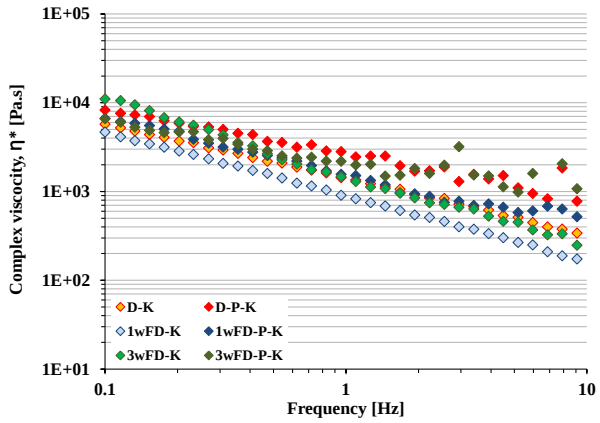


Figure 4.25 Frequency sweeps of the dough containing $KBrO_3$. (a) Storage modulus G', and loss modulus G", (b) Complex modulus, G*, (c) Phase angle, tan δ, (d) Complex viscosity, η*

Potassium bromate is a slow acting oxidant and it reacts in the late stage of proofing and early baking. Some researchers have shown that dough containing oxidants had increased elastic modulus (G'), and viscous modulus (G") relative to control (no additives) (Attenburrow, 1990, Miller and Hoseney, 1999).

Fig.4.25 (a) and (b) indicates that expect for proof doughs, G′ is greater than G″ over all frequencies, and the dough has a viscoelastic soft-solid nature. The slope of G" for proofed samples increased dramatically at frequencies higher than 1 Hz resulting in phase angle values above 45 º. In a frequency range of 0.1 to 10 Hz (2 folds) elastic modulus (G') and complex modulus (G*) varied between 2000 and 100000 Pa, which is slightly higher and a wider range compared to moduli of control dough as discussed in a previous section (4.3.2.1).

G′ and G″ slopes of all doughs containing potassium bromate were found to be highly frequency dependent (Fig. 4.25 (a), (b)). As explained earlier, the magnitude of G' and G" increased with increasing frequency as expected because at high frequencies the material has less time to recover (or relax) (Tanner et al., 2008). All of the proofed doughs containing potassium bromate had higher G' and G" values than did the other processed doughs. This means that these doughs exhibited higher consistency than the other doughs. This indicates that the gluten network was stronger with added potassium bromate, especially at proofing, resulting in the higher G' values of all proofed dough.

The increase in G" was faster than was the increase in G' for all proofed samples resulting in increasing phase angle as the frequency increased. The phase angles of proofed samples were distinctly higher with a sharper increase observed at frequencies higher than 0.5 Hz. Smaller phase angle values indicate a "stiffer" material while high phase angles are an indication of high "plasticity" or more viscous nature. Phase angle of before proof dough was independent from frequency and remained constant around 30-40 ° (Fig. 4.25 (d)).

The slope values of G' and G" of each processed dough containing $KBrO_3$ is presented in Table 4.37.

Table 4.37 Slope of frequency sweeps for dough containing $KBrO_3$

	G'	G"
Post mixing	0.35	0.42
Post proofing	0.34	0.59
1 week frozen thawed	0.25	0.30
1 week frozen thawed & proofed	0.32	0.48
3 weeks frozen thawed	0.16	0.18
3 weeks frozen thawed & proofed	0.43	0.74

As discussed earlier, the effects of frequency on dynamic moduli can reflect the stability of the system. The slope values of G′ and G″ for all doughs containing potassium bromate were frequency dependent. The slopes values for all proofed doughs were higher than those of dough before proof. This could be explained the slow action of $KBrO_3$ (at late stage of proofing and early baking). Comparing the G′, G″, and G* curves of all processed control dough (Fig. 4.24) and those doughs containing potassium bromate (Fig. 4.25), dough containing potassium bromate curves were closer to each other than the control. This suggests that the presence of potassium bromate helped to maintain the dough's rheological properties (elasticity and viscosity) though freezing, frozen storage, and thawing. This demonstrates that potassium bromate has the effect of maintaining dough stability in varying storage conditions.

All frozen dough containing $KBrO_3$, the phase angle of dough before proof was significantly lower than the dough after proof (Fig.4.25 (d)). This suggests that proofed doughs containing $KBrO_3$ became more frequency sensitive and displayed more liquid-like behavior particularly at higher frequency. As described in Table 4.37, slope values of the doughs after proof were higher, which is another indication of higher sensitivity to frequency changes compared to rest of the dough samples.

Complex viscosity η* is the frequency-dependent viscosity and provides additional mean for comparison of flow behavior of different samples. Fig. 4.25 (e) demonstrates that complex viscosity η* decreases with increasing frequency signifying that doughs containing potassium bromate also show shear-thinning behavior over frequencies between 0.1 and 10 Hz. All proofed doughs had slightly higher complex viscosity than did their before proofed counterparts. This reinforces that the proofed doughs observation were more plastic (more viscous in nature) than the other processed dough, a fact also reflected in the phase angle values (Fig. 4.25 (d)).

4.3.2.3 Ascorbic acid and endoxylanase combination

The frequency sweeps of doughs containing a combination of 200 ppm ascorbic acid and 100 ppm endoxylanase are shown in Fig. 4 26.

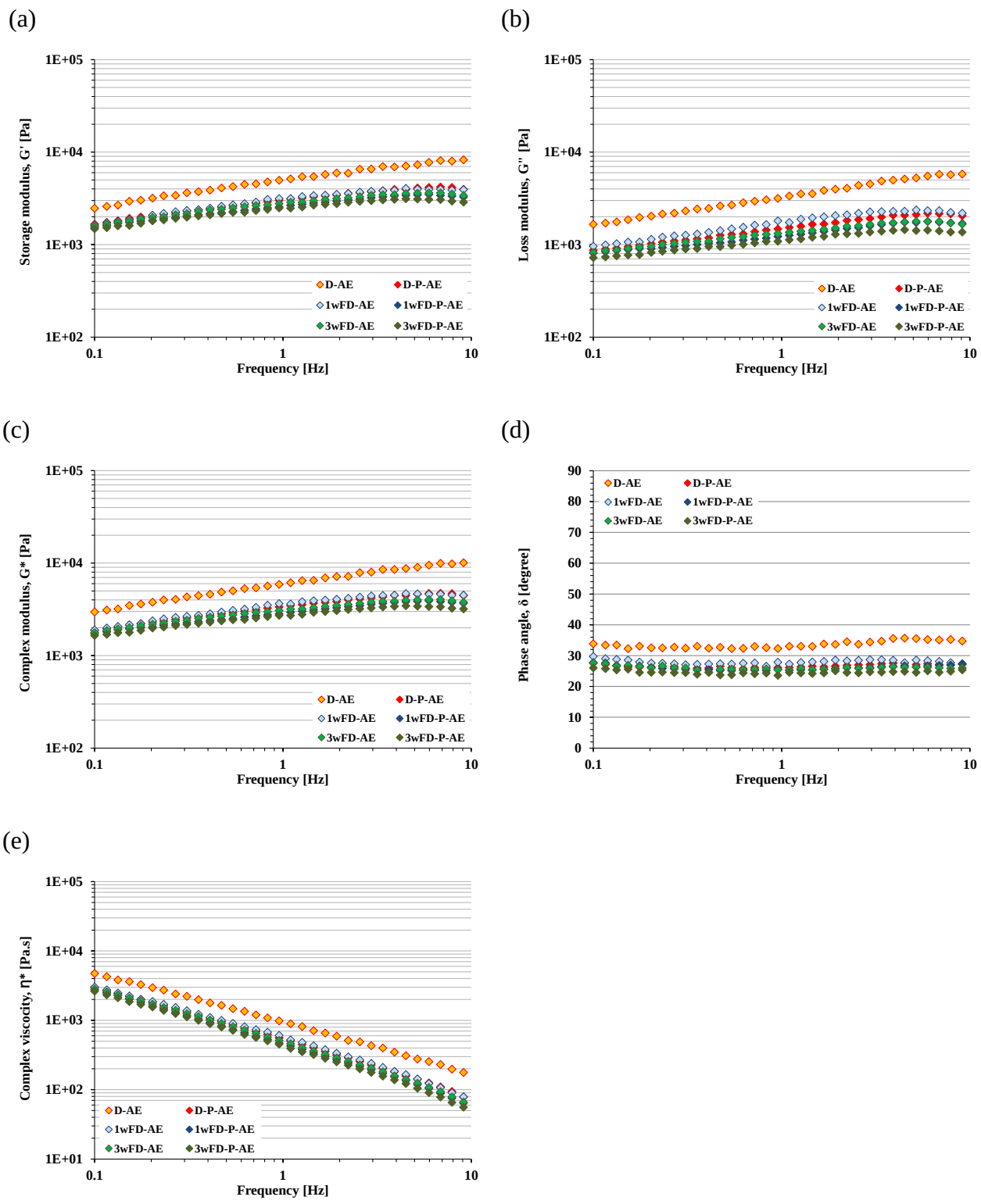


Figure 4.26 Frequency sweeps of doughs containing ascorbic acid and endoxylanase. (a) Storage modulus G', and loss modulus G", (b) Complex modulus, G*, (c) Phase angle, tan δ, (d) Complex viscosity, η*

Ascorbic acid is an intermediate acting oxidant and reacts during mixing. Endoxylanse also reacts during mixing and the effects appear in subsequent baking steps. Other researchers showed that dough containing endoxylanase had lower elastic modulus (G'), and viscous modulus (G") than control (no additives) (Hilhorst et al.,1999, Miller and Hoseney, 1999).

Fig.4.26 (a) and (b) illustrated that G′ is greater than G″ over all frequencies, so the dough has a viscoelastic soft-solid nature. Phase angles below 45º reinforce the characterization as slightly more solid-like material property of the dough. In a frequency range of 0.1 to 10 Hz (2 folds), elastic modulus (G') and complex modulus (G*) varied between 1500 and 10000 Pa. This is slightly lower and a narrower range compared to moduli of control and dough containing potassium bromate discussed in previous sections (4.3.2.1 & 4.3.2.2).

G′ and G″ of all doughs containing ascorbic acid and endoxylanse were also found to be frequency dependent (Fig. 4.26 (a), (b)). As explained earlier, the magnitude of G' and G" increased with increasing frequency expectedly since at high frequencies the material has less time to recover (or relax) (Tanner et al., 2008). Dough at post mix had higher G' and G" values than did the other processed dough. This means that dough affects mix exhibited higher consistency than the other doughs. Post mix dough did not have as much rest time (or relaxation) compared to the other processed doughs, resulting in the highest G', and G" values.

The increase in G" was slower than the increase in G' for all doughs containing ascorbic acid and endoxylanase samples resulting in a relatively constant phase angle as the frequency increased. Tan δ of all dough containing ascorbic acid and endoxylanase doughs were independent of frequency and remained constant around 30 ° (Fig. 4.26 (d)).

The slope values of G' and G" of each processed dough containing ascorbic acid and endoxylanase are presented in Table 4.38.

Table 4.38 Slope of frequency sweeps for dough containing AA-EX

	G'	G"
Post mixing	0.26	0.29
Post proofing	0.20	0.21
1 week frozen thawed	0.20	0.21
1 week frozen thawed & proofed	0.18	0.19
3 weeks frozen thawed	0.18	0.17
3 weeks frozen thawed & proofed	0.16	0.16

The effects of frequency on dynamic moduli can reflect the stability of the system. The slope value of G′ and G″ of all doughs containing ascorbic acid and endoxylanase were frequency dependent. The slopes values for all processed doughs were similar and low. This could be explained by fast/intermediate acting mechanism of ascorbic acid and endoxylanase. Its effects were complete mixing. Comparing the G′, G″, and G* curves of all processed control dough (Fig. 4.24) and those doughs containing ascorbic acid and endoxylanase (Fig. 4.26), dough containing ascorbic acid and endoxylanase G', G″, and G* curves were closer (more similar) to each other than were the controls. This suggests that the presence of ascorbic acid and endoxylanase also helped to maintain the dough rheological properties (elasticity and viscosity) through freezing, frozen storage, and thawing. Because ascorbic acid and endoxylanase reacts during mixing and the effects appears during baking, G′, G″, and G* values remained fairly constant during freezing, frozen storage, and thawing.

Complex viscosity η* is the frequency-dependent viscosity which provides additional mean for comparison of flow behavior of different samples. Fig. 4.26 (e) demonstrates that complex viscosity η* decreased with increasing frequency indicating that doughs containing ascorbic acid and endoxylanase show shear-thinning behavior over frequencies between 0.1 and 10 Hz. Post mix doughs had slightly higher complex viscosity than did the other doughs. It shows that at this point, the doughs were more plastic (more viscous in nature) than the other processed dough. This result was also reflected in the phase angle values (Fig. 4.26 (d)).

4.3.3 Frequency sweeps by process/storage

The previous section (4.3.2) investigated how dough rheological properties changed with respect to dough formulations as a result of different additive addition. This section compares the effects of process and storage history on rheological behavior of doughs of three formulations.

4.3.3.1 Fresh dough (post mix)

The rheology of fresh doughs at post mixing is presented in Fig. 4 27.

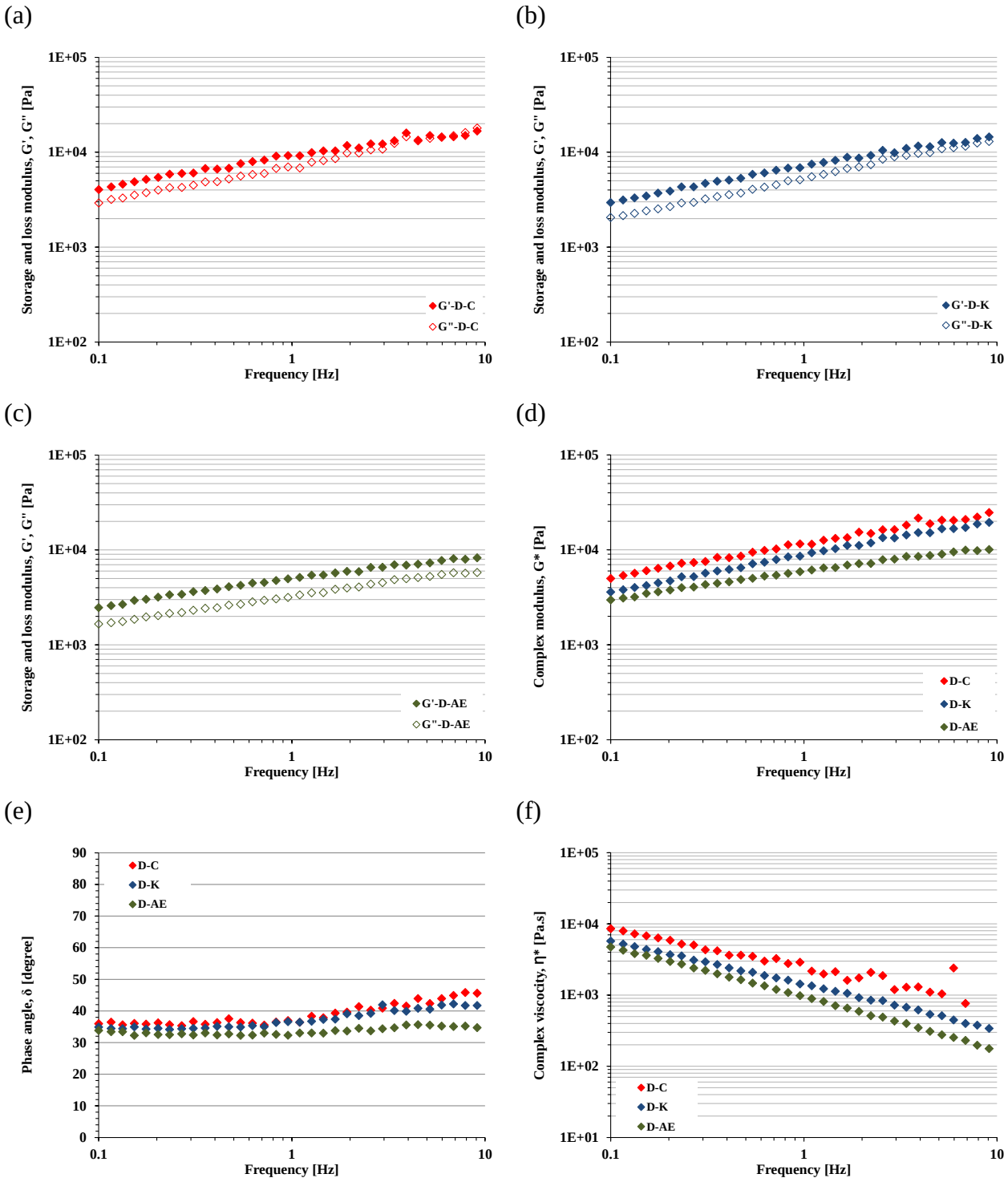


Figure 4.27 Frequency sweeps of doughs at post mixing. (a) Storage modulus G' and loss modulus G" of control dough, (b) Storage modulus G' and loss modulus G" of dough containing $KBrO_3$, (c) Storage modulus G' and loss modulus G" of dough containing AA+EX, (d) Complex modulus, G*, (e) Phase angle, tan δ, (f) Complex viscosity, η*

Moduli of all dough ranged from 1800 to 25000 Pa over a frequency range of 0.1 to 10 Hz. The control dough (Fig. 4.27 (a-d)) had the highest G', G", and G* after mixing demonstrating high dough consistency. This was followed by dough containing $KBrO_3$ and finally AA-EX. The consistencies of control and $KBrO_3$ doughs are expected to be very similar as $KBrO_3$ is known to react at later stages of proofing and early baking. Dough samples containing AA-EX had the lowest consistency. The chemistry of AA oxidation process in dough mixing is complex, but probably involves the oxidation of the —S—H (sulphydryl) groups of gluten-forming proteins and the formation of —S—S— (disulphide) bonds (Williams and Pullen, 1998). The action of AA during mixing also brings about changes in the rheology of the dough, making it more resistant to deformation (Cauvain et al., 1992). The addition of endoxylanase on dough rheological properties include slightly decreased dryness and stiffness, but increased elasticity, extensibility, coherence and stickiness (Mullins, 1990, Hillhorst et al., 1999). The net result of the AA and endoxylanse is to improve gas retention capacity/gas cell distribution of dough, and to yield bread with a finer crumb cell structure (Yamada and Preston, 1992; Nakamura and Kurata, 1997; Cauvain and Young, 2001; Courtin and Delcour, 2002; Selomulyo and Zhou, 2007; Haarasilta et al., 1991; Hammond, 1994; Guy 2001; Hille and Schooneveld-Bergmans, 2004). End product qualities of the resulting bread samples will be compared and discussed in section 4.4.

The values of G′ and G″ of all doughs increased with increasing frequency indicating frequency dependence of the moduli (Fig. 4.27 (d)). The slope values of G' and G" post mixing of each dough treatment are presented in Table 4.39.

Table 4.39 Frequency sweeps of doughs slope of G' and G" at post mixing

	G'			G"		
	Control	$KBrO_3$	AA-EX	Control	$KBrO_3$	AA-EX
Post mixing	0.31	0.35	0.26	0.40	0.42	0.29

The slope values for the control and potassium bromate containing doughs were slightly higher than the combination of ascorbic acid and endoxylanase, signifying that the control and potassium bromate containing doughs were more sensitive to frequency change than the ascorbic acid and endoxylanase containing dough. Similarities between control and dough containing $KBrO_3$ can be explained by slow acting mechanism of $KBrO_3$, as mentioned earlier. Oxidation of

the sulphydryl groups of gluten-forming proteins and the formation of disulphide bonds in AA-EX containing doughs might result in a stable dough network as indicated by smaller slope values.

It is of particular interest that the control dough (around 6-7 Hz) and dough containing $KBrO_3$ (around 9-10 Hz) displayed a "crossover" of G' and G" while dough containing AA-EX did not. This indicated that the dependence of G" on frequency was much higher than the dependence of G'. This faster increase in G" compared to increase in G' with increase in frequency resulted the crossover after which liquid like behavior become slightly more dominant than solid-like behavior. The same was observed for the phase angle (ratio of G" to G') values. While the phase angle values remained fairly constant around 30 ° over the frequency range at 0.1 to 10 Hz for doughs containing AA-EX, control dough and dough containing $KBrO_3$ showed an increase up to 48 ° within the same frequency range. Except for the high frequency range, all of the samples showed solid-like behavior (phase angle values below 45 °). Dough containing ascorbic acid and endoxylanase were the most elastic and stable (less frequency dependent). These results were also reflected in the slopes of doughs post mix (Table. 4.39).

Fig. 4.27 (f) demonstrated that, independent of formulation, all dough samples displayed shear-thinning behavior over frequencies between 0.1 and 10 Hz. The control and $KBrO_3$ containing doughs had slightly higher dynamic viscosities compared to dough containing AA-EX. A similar differentiation was observed in phase angle data (Fig. 4.27 (e)).

4.3.3.2 Fresh, proofed dough (post fresh proof)

The rheology of fresh doughs at post proofing is presented in Fig. 4 28.

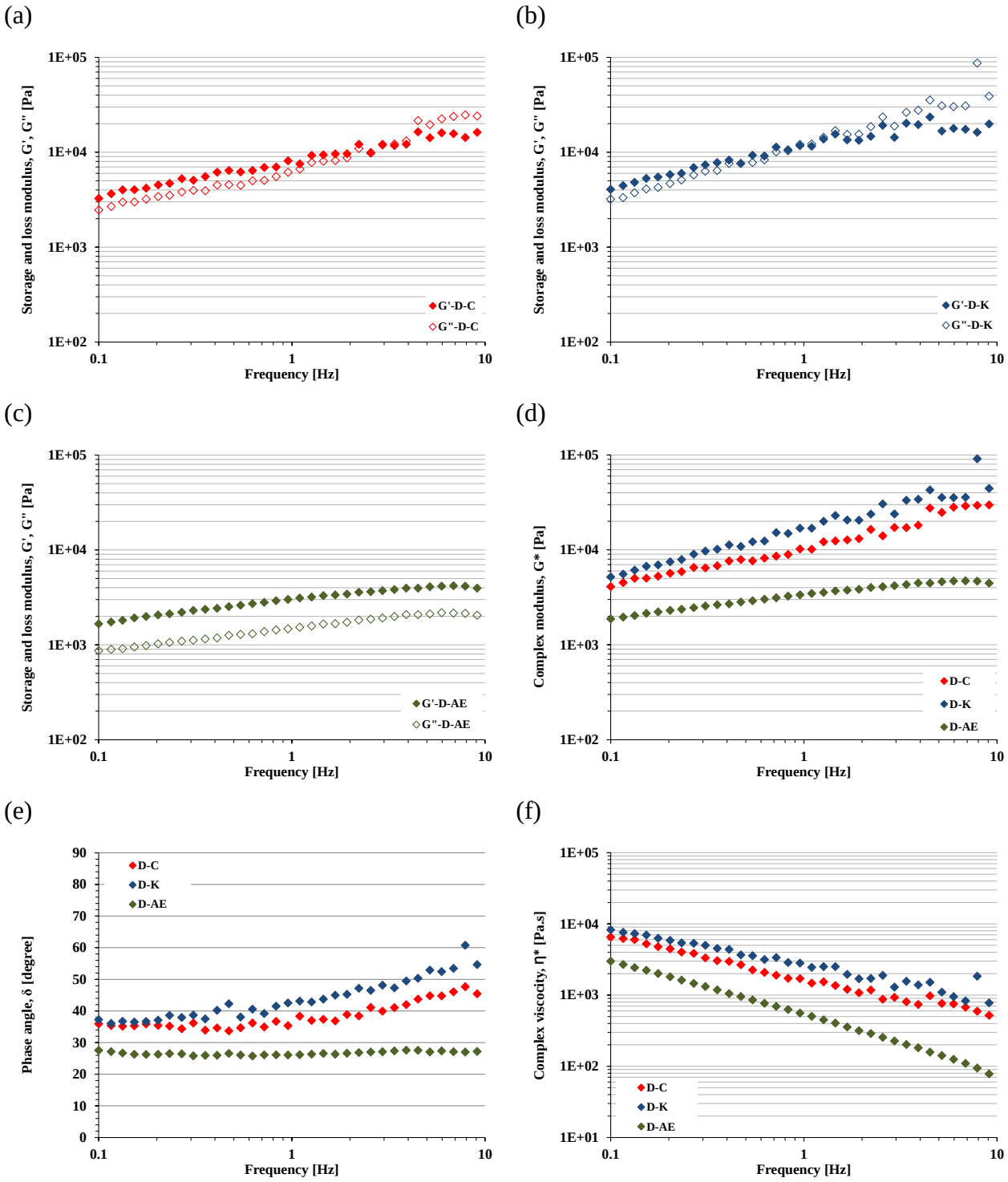


Figure 4.28 Frequency sweeps of fresh proofed dough. (a) Storage modulus G' and loss modulus G" of control dough, (b) Storage modulus G' and loss modulus G" of dough containing $KBrO_3$, (c) Storage modulus G' and loss modulus G" of dough containing AA+EX, (d) Complex modulus, G*, (e) Phase angle, tan δ, (f) Complex viscosity, η*

The elastic and viscous moduli of proofed dough containing potassium bromate were higher than the control (no additives) (Attenburrow, 1990, Miller and Hoseney, 1999). This result and the reason for this were explained in section 4.3.2.2.

Moduli of all dough ranged from 900 to 50000 Pa over the frequency range of 0.1 to 10 Hz. The dough containing $KBrO_3$ (Fig. 4.28 (a-d)) had the highest G', G", and G* after proofing demonstrating high dough consistency. This was followed by control and dough containing AA-EX. The consistencies of dough containing $KBrO_3$ and the other treatment doughs were expected to be different from each other because $KBrO_3$ is known to react at the later stages of proofing and early baking. Dough containing AA-EX had the lowest consistency. The reaction of $KBrO_3$ oxidation process in dough proofing was reported by Jørgensen (1945) along with the actions of other oxidizers and enzymes.

The slopes of G′ and G″ of all doughs increased with increasing frequency indicating frequency dependence of moduli (Fig. 4.28 (d)). The slope values of G' and G" post proofing of each dough treatment are presented in Table 4.40.

Table 4.40 Frequency sweeps of doughs slope of G' and G" at post proofing

	G'			G"		
	Control	$KBrO_3$	AA-EX	Control	$KBrO_3$	AA-EX
Post proofing	0.35	0.34	0.20	0.52	0.59	0.21

The slope values for the control and potassium bromate containing doughs were slightly higher than the combination of ascorbic acid and endoxylanase, signifying that the control and potassium bromate containing doughs were more sensitive to frequency change than the ascorbic acid and endoxylanase containing dough. The difference between dough containing $KBrO_3$ and other treatment dough can be explained by the slow acting mechanism of $KBrO_3$, as mentioned earlier. Oxidation of the sulphydryl groups of gluten-forming proteins and the formation of disulphide bonds in AA-EX containing doughs might result in a stable dough network, which was indicated by smaller slope values.

Again, the control dough and dough containing $KBrO_3$ displayed a "crossover" of G' and G" while dough containing AA-EX did not. The crossover points of proofed doughs were different at post mix. Proofed control dough had cross over point around 4-5 Hz and the dough containing $KBrO_3$ had cross over point around 1 Hz. The proofed dough crossover points were

shifted at lower frequency range than post mix. This demonstrated that the dependence of G" on frequency was much higher than the dependence of G'. Thus, faster increase in G" compared to increase in G' with increase in frequency resulted in a crossover point after which liquid like behavior become slightly more dominant than the solid-like behavior. Proofed dough crossover point was shifted lower frequency range is indicating that the proofed dough behavior was changed at lower frequency range than post mix. The same was observed at the phase angle (ratio of G" to G') values. While the phase angle values remained fairly constant around 30 ° in frequency range from 0.1 to 10 Hz for doughs contains AA-EX. However, proofed control and dough containing $KBrO_3$ had crossover point at lower frequency range, so the phase angle showed an increase up to 60 ° within the same frequency range. Following to crossover point shift, proofed dough was higher phase angle than post mix (up to 48 °). Except for high frequency range, all of the samples showed a solid-like behavior as indicated by phase angle values below 45 °. Dough containing ascorbic acid and endoxylanase was the most elastic and stable (less frequency dependent) dough. These results were also reflected in the slope of doughs at post proof (Table. 4.39).

Fig. 4.28 (f) demonstrated that independent from dough formulation, all dough samples displayed a shear-thinning behavior over frequencies between 0.1 and 10 Hz. The control and $KBrO_3$ containing doughs had slightly higher dynamic viscosities compared to dough containing AA-EX. A similar differentiation was observed in phase angle data (Fig.4.28 (e)).

4.3.3.3 Frozen (1w), thawed dough (post 1 week thaw)

One week frozen then thawed rheology is shown in Fig. 4 29.

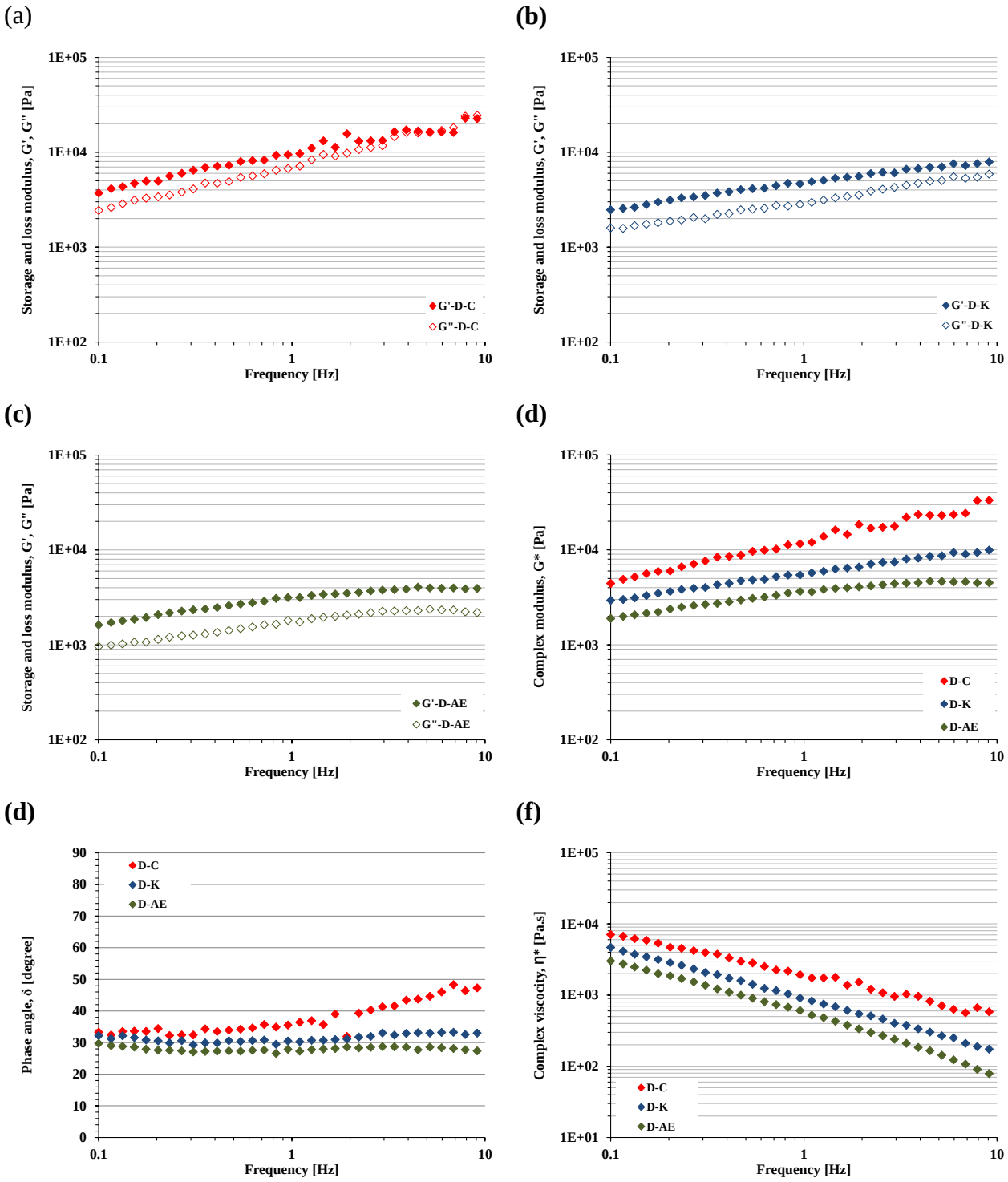


Figure 4.29 Frequency sweeps doughs after 1 week frozen. (a) Storage modulus G' and loss modulus G" of control dough, (b) Storage modulus G' and loss modulus G" of dough containing $KBrO_3$, (c) Storage modulus G' and loss modulus G" of dough containing AA+EX, (d) Complex modulus, G*, (e) Phase angle, tan δ, (f) Complex viscosity, η*

Moduli of all dough ranged from 1000 to 40000 Pa across the frequency range of 0.1 to 10 Hz. The control dough (Fig. 4.29 (a-d)) had the highest G', G", and G* after 1 week reflecting its high dough consistency. This was followed by dough containing $KBrO_3$ and AA-EX, and finally dough containing AA-EX had the lowest consistency. One factor to consider when interpreting post thaw dough rheological data is that these dough samples were prepared following the frozen dough production method in which thawing consisted of 2 steps. First, the frozen dough was thawed 16 hours in the retarder, then the dough was moved to room temperature until the dough core temperature reached 19 ºC. During this 2 step thawing, the dough was also fermenting (proofing). The dough fermenting (proofing) was clearly observed visually during room temperature thawing. Thus, post thawed dough was completely thawed and also partially proofed. Because the time required for the dough core temperature to reach 19 ºC was strongly dependent on ambient conditions, this data point (post thaw) might be more variable.

The slopes of G′ and G″ of all doughs increased with increasing frequency indicating their frequency dependence (Fig. 4.29 (d)). The slope values of G' and G" post 1 week thawing of each dough treatment are presented in Table 4.41.

Table 4.41 Frequency sweeps of doughs slope of G' and G" after 1 week frozen

	G'			G"		
	Control	$KBrO_3$	AA-EX	Control	$KBrO_3$	AA-EX
1 week frozen	0.38	0.25	0.20	0.50	0.30	0.21

The slope G' values for the control dough was slightly higher and those G" substantially higher than the other treatment doughs, signifying that the control dough was more sensitive to frequency change than the other treatment doughs. Again, AA-EX produced doughs with more stability than control and $KBrO_3$.

Again, AA-EX produced dough with more stability than control or $KBrO_3$. It is of particular importance that the control dough (around 6-7 Hz) displayed a "crossover" of G' and G" while dough containing $KBrO_3$ and AA-EX did not. Cross over indicated that the dependence of G" on frequency was much higher than the dependence of G'. The crossover is the point after which liquid like behavior becomes slightly more dominant than does solid-like behavior. The same was observed for the phase angle (ratio of G" to G') values. While the phase angle values

remained fairly constant around 30° in frequency range from 0.1 to 10 Hz for doughs containing $KBrO_3$ and AA-EX, control dough showed an increase up to 48 ° within the same frequency range. Except for high frequency range, all of the samples showed a solid-like behavior as indicated by phase angle values below 45 °. Dough containing ascorbic acid and endoxylanase was both the most elastic and stable (less frequency dependent). These results were also reflected in the modulus slopes of 1 week frozen doughs at post thaw (Table. 4.41).

Fig. 4.29 (f) demonstrated that regardless of dough formulation, all dough samples displayed a shear-thinning behavior over frequencies between 0.1 and 10 Hz. The control dough had slightly higher dynamic viscosities compared to the other treatment doughs. A similar differentiation was observed in phase angle data (Fig. 4.29 (e)).

4.3.3.4 Frozen (1w), thawed, proofed dough (post 1 week proof)

The rheological data of one week frozen then proofed is shown in Fig. 4 30.

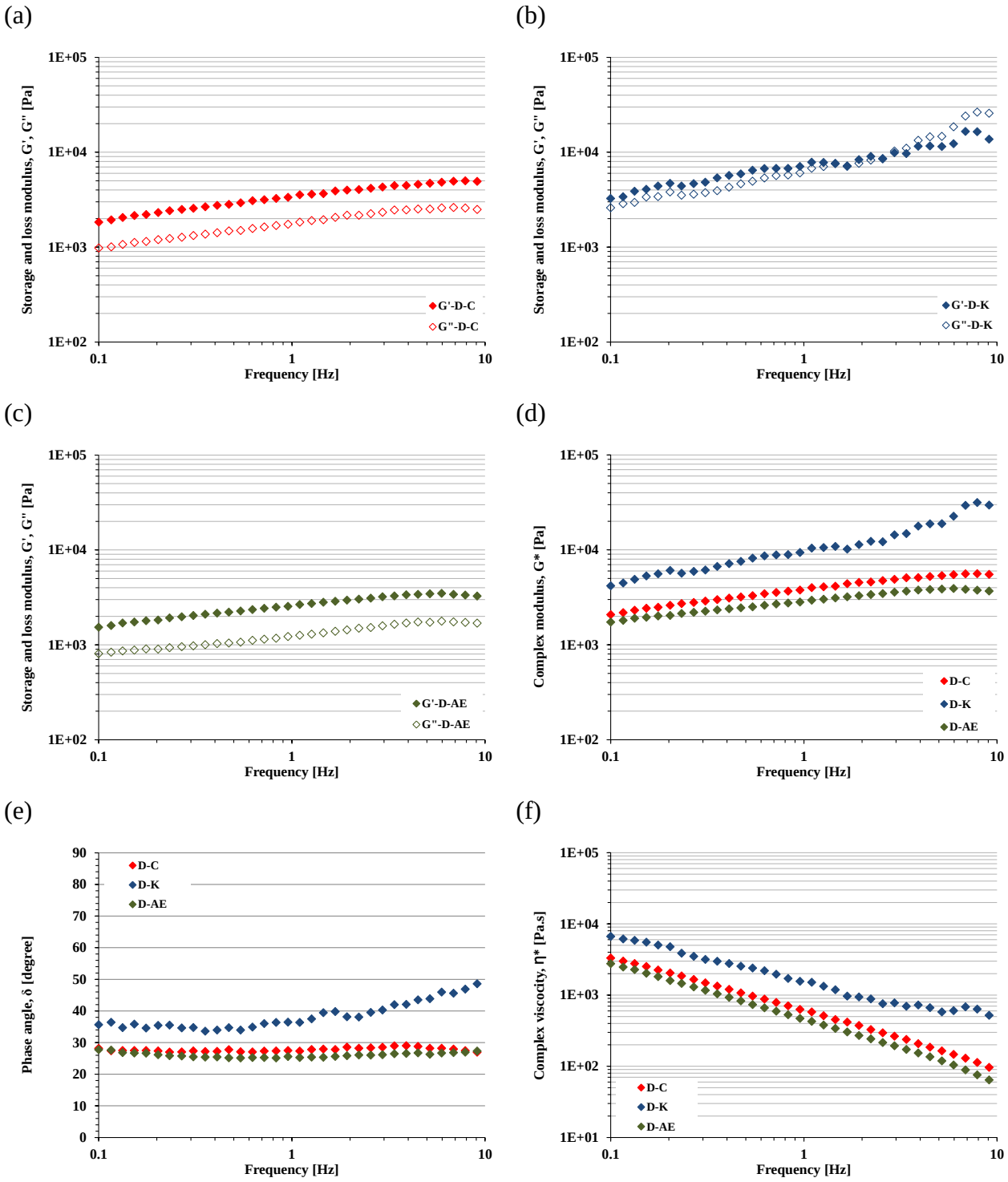


Figure 4.30 Frequency sweeps of proofed doughs after 1 week frozen. (a) Storage modulus G' and loss modulus G" of control dough, (b) Storage modulus G' and loss modulus G" of dough containing $KBrO_3$, (c) Storage modulus G' and loss modulus G" of dough containing AA+EX, (d) Complex modulus, G*, (e) Phase angle, tan δ, (f) Complex viscosity, η*

Again, the elastic moduli and viscous moduli of the proofed dough containing potassium bromate were higher than did control (no additives). This which results is similar to that of other researchers (Attenburrow, 1990, Miller and Hoseney, 1999). The result and reason were explained in section 4.3.2.2.

Moduli of all dough ranged from 900 to 40000 Pa in the frequency range of 0.1 to 10 Hz. Once proofed dough containing $KBrO_3$ (Fig. 4.30 (a-d)) had the highest G', G", and G*. This was followed by control and dough containing AA-EX. Consistencies of control and $KBrO_3$ doughs were expected to be different because $KBrO_3$ is known to react during later stages of proofing and early stages of baking. Dough samples containing AA-EX had the lowest consistency. The reaction of the $KBrO_3$ in dough proofing was explained in section 4.3.3.2. End product qualities of the resulting bread samples will be compared and discussed in section 4.4.

The slopes of G′ and G″ of all doughs increased with increasing frequency indicating the frequency dependence of moduli (Fig. 4.30 (d)). The slope values of G' and G" post proofing each dough treatment are presented in Table 4.42.

Table 4.42 Frequency sweeps of proofed doughs slope of G' and G" after 1 week frozen

	G'			G"		
	Control	$KBrO_3$	AA-EX	Control	$KBrO_3$	AA-EX
1 week frozen & proofed	0.22	0.32	0.18	0.22	0.48	0.19

The G' slope values for the potassium bromate containing dough and the G" substantially higher than control doughs, especially AA-EX doughs. The difference between dough containing $KBrO_3$ and other treatment doughs can be explained by slow acting mechanism of $KBrO_3$, as mentioned earlier.

The dough containing $KBrO_3$ (around 3-4 Hz) displayed a "crossover" of G' and G" while control and dough containing AA-EX did not. The dependence of G" on frequency was much higher than the dependence of G'. The after crossover point, liquid-like behavior became slightly more dominant than the solid-like behavior. The same was observed in the phase angle (ratio of G" to G') values. While the phase angle values remained fairly constant around 30 ° in the frequency range from 0.1 to 10 Hz for control and dough contains AA-EX, dough containing $KBrO_3$ showed an increase up to 48 ° within the same frequency range. Except for high frequency range, all of the samples showed a solid-like behavior as indicated by phase angle

values below 45 °. Dough containing ascorbic acid and endoxylanase was the most elastic and stable dough (less frequency dependent). These results were also reflected in the slope of 1 week frozen doughs at post proofing (Table. 4.42).

Fig. 4.30 (f) demonstrated that independent of dough formulation, all dough samples displayed a shear-thinning behavior over frequencies between 0.1 and 10 Hz. The dough containing $KBrO_3$ had slightly higher dynamic viscosities compared to control and dough containing AA-EX. A similar differentiation was observed in phase angle data (Fig. 4.30 (e)).

4.3.3.5 Frozen (3w), thawed dough (post 3 weeks thaw)

The rheological properties of doughs frozen for 3 weeks are shown in Fig. 4 31.

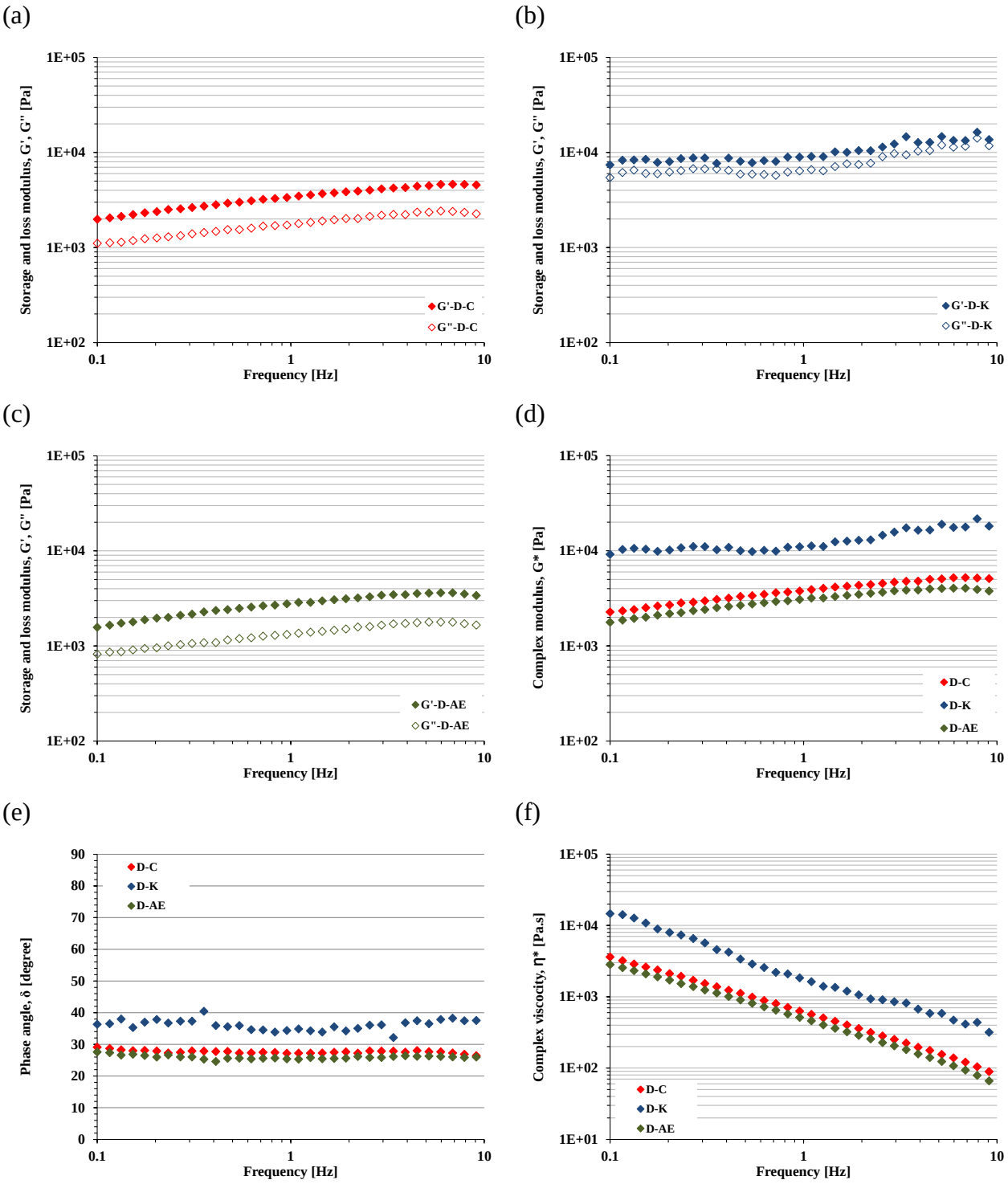


Figure 4.31 Frequency sweeps of doughs after 3 weeks frozen. (a) Storage modulus G' and loss modulus G" of control dough, (b) Storage modulus G' and loss modulus G" of dough containing $KBrO_3$, (c) Storage modulus G' and loss modulus G" of dough containing AA+EX, (d) Complex modulus, G*, (e) Phase angle, tan δ, (f) Complex viscosity, η*

Moduli of all doughs ranged from 900 to 20000 Pa in frequency range of 0.1 to 10 Hz. The dough containing $KBrO_3$ (Fig. 4.31 (a-d)) had the highest G', G", and G* after 3 weeks frozen thawed demonstrating high dough consistency. This was followed by control and dough containing AA-EX, and dough samples containing AA-EX had the lowest consistency. The post thawed data underwent the same treatment condition as 1 week frozen storage making the results more variable (4.3.3.3).

The slopes of G′ and G″ of all doughs increased with increasing frequency indicating frequency dependence of moduli (Fig. 4.31 (d)). The slope values of G' and G" post 3 weeks frozen thawing of each dough treatment are presented in Table 4.43.

Table 4.43 Frequency sweeps of doughs slope of G' and G" after 3 weeks frozen

	G'			G"		
	Control	$KBrO_3$	AA-EX	Control	$KBrO_3$	AA-EX
3 weeks frozen	0.19	0.16	0.18	0.18	0.18	0.17

The slope values for all treatment doughs were lower than the other points in the process. The reason for this is not clear. None of treatment doughs displayed a "crossover" of G' and G". This indicated that G' and G" were least dependent on frequency. Thus, the phase angle values remained fairly constant between 30 ° to 40 ° in frequency range from 0.1 to 10 Hz for all treatment doughs. All of doughs showed a solid-like behavior as indicated by phase angle values below 45 °. Dough containing ascorbic acid and endoxylanase was the most elastic and stable (less frequency dependent). These results were also reflected in the slope of 3 weeks frozen doughs at post thaw (Table. 4.41).

Fig. 4.31 (f) demonstrated that independent of dough formulation, all dough samples displayed a shear-thinning behavior over frequencies between 0.1 and 10 Hz. The dough containing $KBrO_3$ had slightly higher dynamic viscosities compared to the other treatment doughs. A similar differentiation was observed in phase angle data (Fig. 4.31 (e)).

4.3.3.6 Frozen (3w), thawed, proofed dough (post 3 weeks proof)

Three weeks frozen then proofed dough rheology is presented in Fig. 4 32.

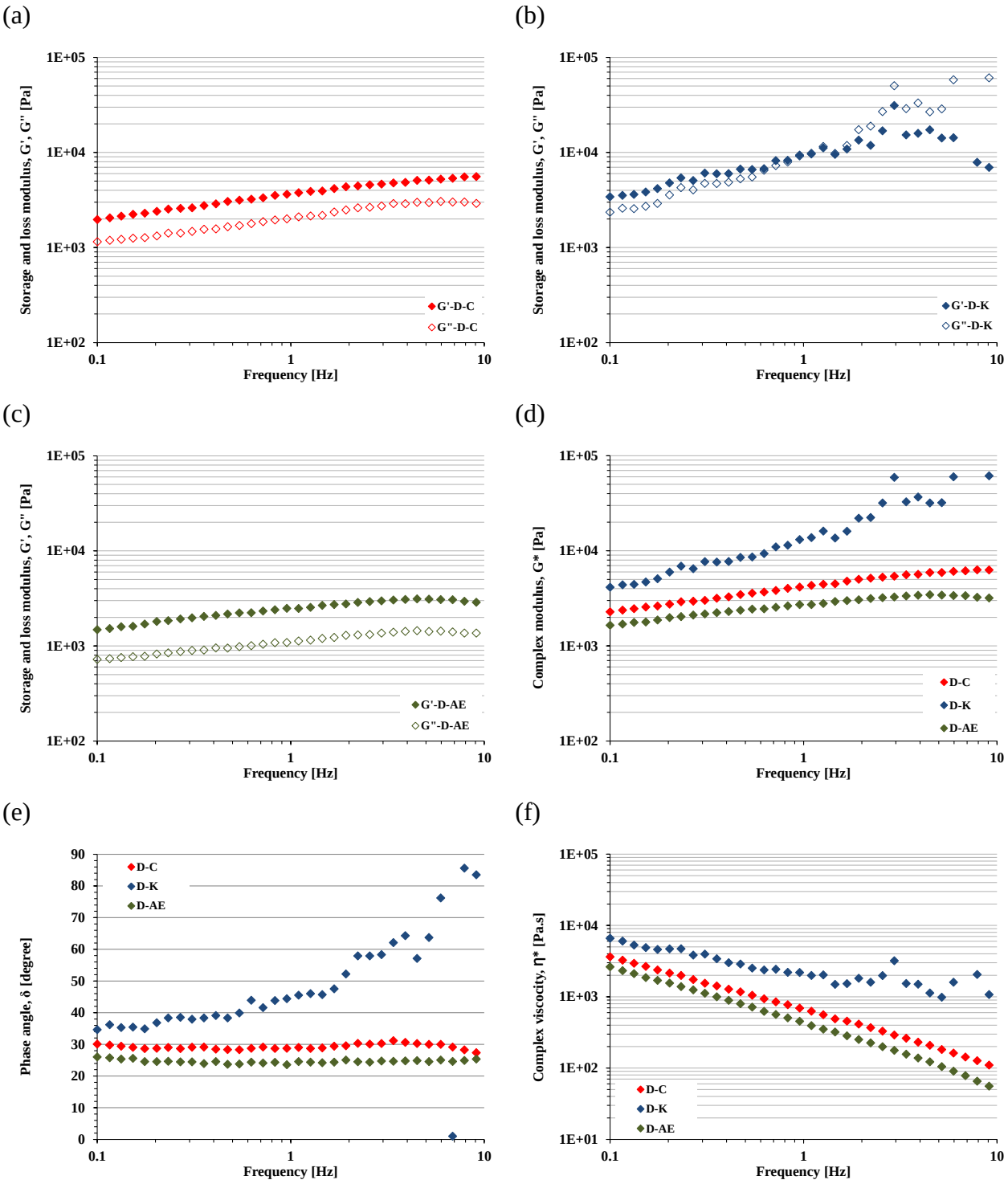


Figure 4.32 Frequency sweeps of proofed doughs after 3 weeks frozen. (a) Storage modulus G' and loss modulus G" of control dough, (b) Storage modulus G' and loss modulus G" of dough containing $KBrO_3$, (c) Storage modulus G' and loss modulus G" of dough containing AA+EX, (d) Complex modulus, G*, (e) Phase angle, tan δ, (f) Complex viscosity, η*

Again, the proofed dough containing potassium bromate elastic modulus and viscous modulus were higher than were those of control (no additives) a result similar to that reported by as other researchers (Attenburrow, 1990, Miller and Hoseney, 1999). This result and reason for it was explained in section 4.3.2.2.

The moduli of all doughs ranged from 700 to 60000 Pa over frequency range of 0.1 to 10 Hz. The dough containing $KBrO_3$ (Fig. 4.30 (a-d)) had the highest G', G", and G*. This was followed by control and dough containing AA-EX. Consistencies of control and $KBrO_3$ doughs were expected to be different because $KBrO_3$ is known to react during the later stages of proofing and early stages of baking. Dough samples containing AA-EX had the lowest consistency. The reaction of $KBrO_3$ oxidation process results for the bread samples will be compared and discussed in section 4.4.

The slopes of G′ and G″ of all doughs increased with increasing frequency demonstrating their frequency dependence (Fig. 4.32 (d)). The slope values of G' and G" post proofing of each dough treatment are presented in Table 4.44.

Table 4.44 Frequency sweeps of proofed doughs slope of G' and G" after 3 weeks frozen

	G'			G"		
	Control	$KBrO_3$	AA-EX	Control	$KBrO_3$	AA-EX
3 weeks frozen & proofed	0.23	0.43	0.16	0.23	0.74	0.16

The slope values for the potassium bromate containing dough was significantly higher than the other treatment doughs, indicating that the potassium bromate containing dough's structures were more sensitive to frequency than the other treatment doughs.

It is of particular interest that the dough containing $KBrO_3$ (around 1 Hz) displayed a "crossover" of G' and G" while control and dough containing AA-EX did not. Compared the crossover points of proofed, 1 and 3 week frozen proof, the crossover point was shifted to a lower range. This indicated that the dependence of G" on frequency was much higher than the dependence of G'. Thus, faster increase in G" compared to increase in G' with increase in frequency resulted in a crossover point after which liquid-like behavior become slightly more dominant than the solid-like behavior. The same was observed at the phase angle (ratio of G" to G') values. While the phase angle values remained fairly constant around 30 ° in frequency range from 0.1 to 10 Hz for control and dough contains AA-EX, dough containing $KBrO_3$ showed an

increase up to 85 ° within the same frequency range. Except for high frequency range, all of the samples showed a solid-like behavior as indicated by phase angle values below 45 °. Dough containing ascorbic acid and endoxylanase was the most elastic and stable (less frequency dependent). These results were also reflected in the slope of doughs at post proofing (Table. 4.44).

Fig. 4.32 (f) demonstrated that independent from dough formulation, all dough samples displayed a shear-thinning behavior over frequencies between 0.1 and 10 Hz. The dough containing $KBrO_3$ had slightly higher dynamic viscosities compared to control and dough containing AA-EX. A similar differentiation was observed in phase angle data (Fig. 4.32 (e)).

4.3.4 Comparison at 1.1 Hz

In the previous section (4.3.3) results were presented for the entire spectrum of frequency sweeps while keeping the stress constant at 15 Pa. Comparisons were organized and reported with respect to processing steps (post mix, proof, after frozen storage thaw, and after frozen storage proof) and treatments (control dough, dough containing 50 ppm potassium bromate, and dough containing combination of 200 ppm ascorbic acid and 100ppm endoxylanase). Since it is not practical to compare one set of data with other using the entire spectrum, often times a certain frequency level is chosen. In this section, G' and G" values are compared at one specific frequency (1.1 Hz) with statistical analysis. This frequency (1.1 Hz) is in the mid-point of the frequency range tested (0.1 to 10 Hz). Results are presented in Fig. 4.33. The error bars indicate the average of coefficient of variance for the entire experiment (described in Chapter 3.8). The grand average of G' for the coefficient of variance was found to be 26.3 %, 36.1 %, 29.8 %, 10.4%, 32.5 % for G', G", G*, tan δ, and η*, respectively.

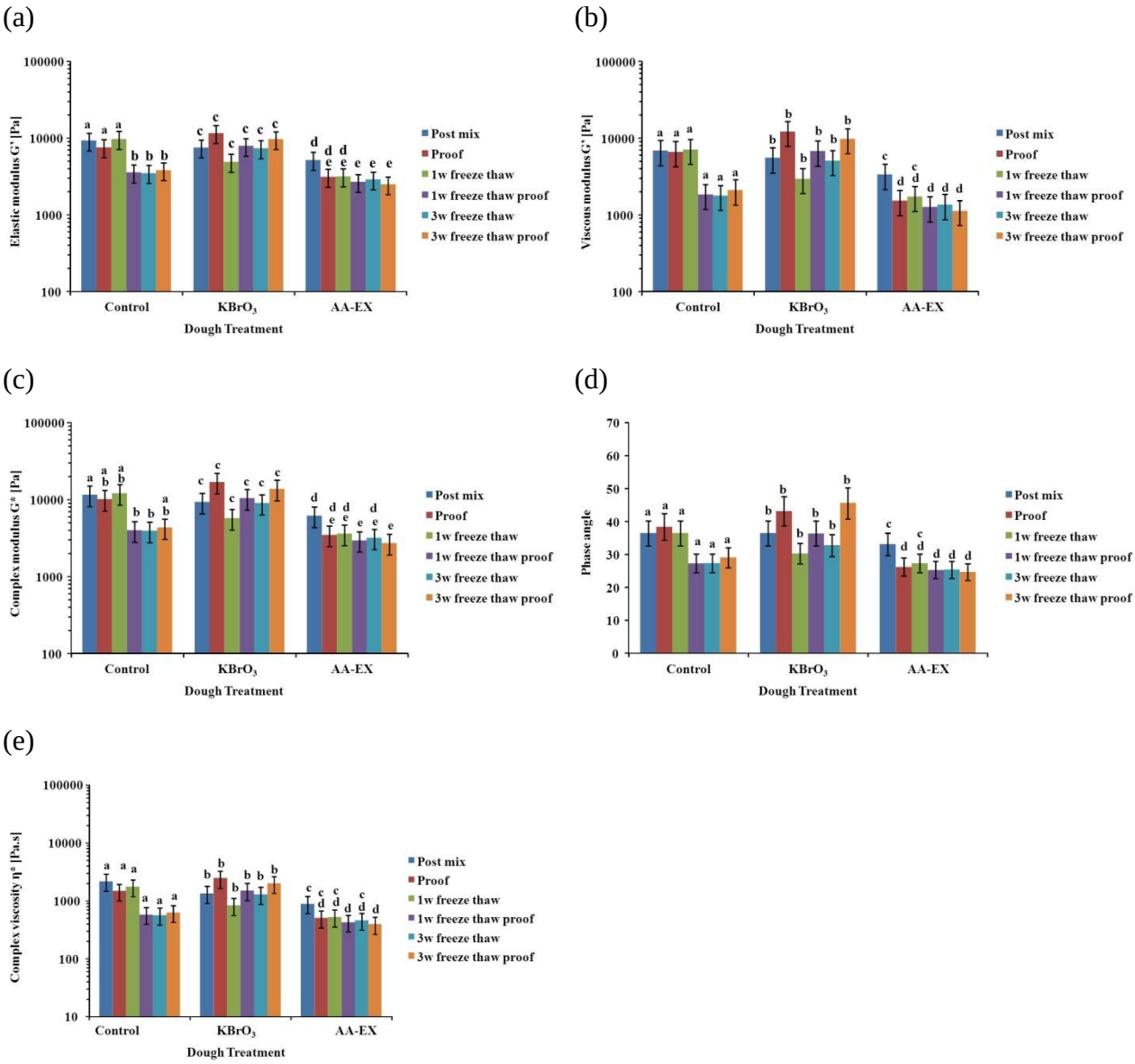


Figure 4.33 Comparison of each processed doughs at 1.1 Hz

Compared process effects by formula and letters (a, b, c, d, and e) indicates significantly different of between process stage ($P< 0.05$).

The magnitudes of moduli, phase angle and complex viscosity presented in Fig. 4.33 can be analyzed in two ways: Effect of process points (post mix, proof, 1week before and after proof, 3weeks before and after proof) within each treatment (control, $KBrO_3$, AA-EX), and the effect of treatment at each processing step. Statistical analyses of process effects by formula are presented by letters (a, b, c, d, and e) indicating significant differences between process stages ($P< 0.05$).

Post mixing, proofed and 1 week before proof for control dough G' values were significantly higher than the other process stages (Fig. 4.33 (a)). This means post mix and proof dough, and 1 week freezing control dough was stiffer (more elastic) than the other process points. Post mix, proof and 1 week freeze control dough G' values were not significantly different indicating that the dough elasticity was not significantly different at those 3 points. For the 1 week thaw control dough, data of only a single batch was available, so it was not very reliable. G" value of all points of the control dough was not significantly different by statistical analysis (Fig. 4.33 (b)). Therefore, all points of control doughs had comparable viscous characteristics. Proofed 1 and 3 weeks frozen and 3 weeks frozen before proof control dough G' values were not significantly different. Similar observations were done for G". This indicates that these three dough samples displayed similar elastic and viscous characteristics. The control post mix, proof, and proofed 1 week frozen dough samples were significantly different than the former three samples discussed above. This is indicates that the control dough was affected by the freezing process in a more dramatic way compared to the other two treatments, $KBrO_3$ and AA-EX.

G* value of post mix, 1 week frozen before and after proof dough was significantly different by statistically analysis. Since G* is derived from G' and G", the discussions provided on these two parameters also holds for the statistical differences observed between the treatments (at a given processing step) and between the processing steps within the same treatments.

All processed control dough phase angles were not significantly different (Fig. 4.33 (d)). The phase angle ranged between 25 and 40 º, indicating that all processed control doughs were solid-like viscoelastic at 1.1 Hz.

Complex viscosities η* of control doughs across the six-processing steps were not significantly different (Fig. 4.33 (e)). The complex viscosity η* ranged between 800 and 3000 Pa.s at 1.1 Hz.

The rheological properties (G', G", G*, phase angle, and complex viscosity) of doughs containing $KBrO_3$ displayed minimal changes during the entire process (Fig.4.33 (a-e)). None of the rheological parameters were across processing steps. Addition of potassium bromate improved frozen dough's tolerance to freezing, frozen storage, and thawing. In other words, addition of potassium bromate helped to maintain dough rheological properties during freezing, frozen storage, and thawing. Moreover, G' and G" values of post proofed frozen doughs containing $KBrO_3$ were higher than those of post proofed frozen control doughs. $KBrO_3$ affected

the dough at proof and increased its elasticity and viscosity. Since $KBrO_3$ is known to react during proofing, the gluten network apparently became stronger at this point. This was reflected by the frequency sweeps for the dough containing $KBrO_3$ in section 4.3.2.2.

The rheological properties (G', G", G*, phase angle, and complex viscosity) of the post mix dough containing ascorbic acid and endoxylanase were significantly different than the rest of the five-processing steps (proof, 1 week frozen before and after proof, 3 weeks frozen before and after proof). However, there was no significant difference between all proofed and 1 or 3 weeks frozen before proof doughs, indicating that all rheological properties of dough containing ascorbic acid and endoxylanase had only a small change during the entire process. This shows that these dough's rheological properties were less susceptible to changes during frozen storage and subsequent processes. Therefore, addition of ascorbic acid and endoxylanase improved dough's tolerance to freezing, frozen storage, and thawing. In addition, the fact that post mix AA-EX doughs G' and G" values were lower than those of the control and the $KBrO_3$ dough indicates that endoxylanase is most effective at dough at mixing, decreasing elasticity and viscosity (softer). Because, endoxylanase reacts during mixing, the gluten was more extensible and developed at this stage. This result was explained by the frequency sweeps for the ascorbic acid and endoxylanase (4.3.2.3).

4.4 Relationship with frozen dough rheology and bread quality

In this section, the relationships between the dough rheological properties and the final bread quality were investigated. As described earlier, dough rheology was affected by both the process stage and the treatment. Among the six-processing steps studied, the proofed doughs rheology (fresh proofed, proofed 1 week and 3 weeks frozen storage) was chosen and used for further comparisons. These three are the process point nearest to the baking process, and thus provide the best representation of the effects of the treatments on final loaf quality. Fig.4.34 (a-e) shows the frequency sweeps for these treatments.

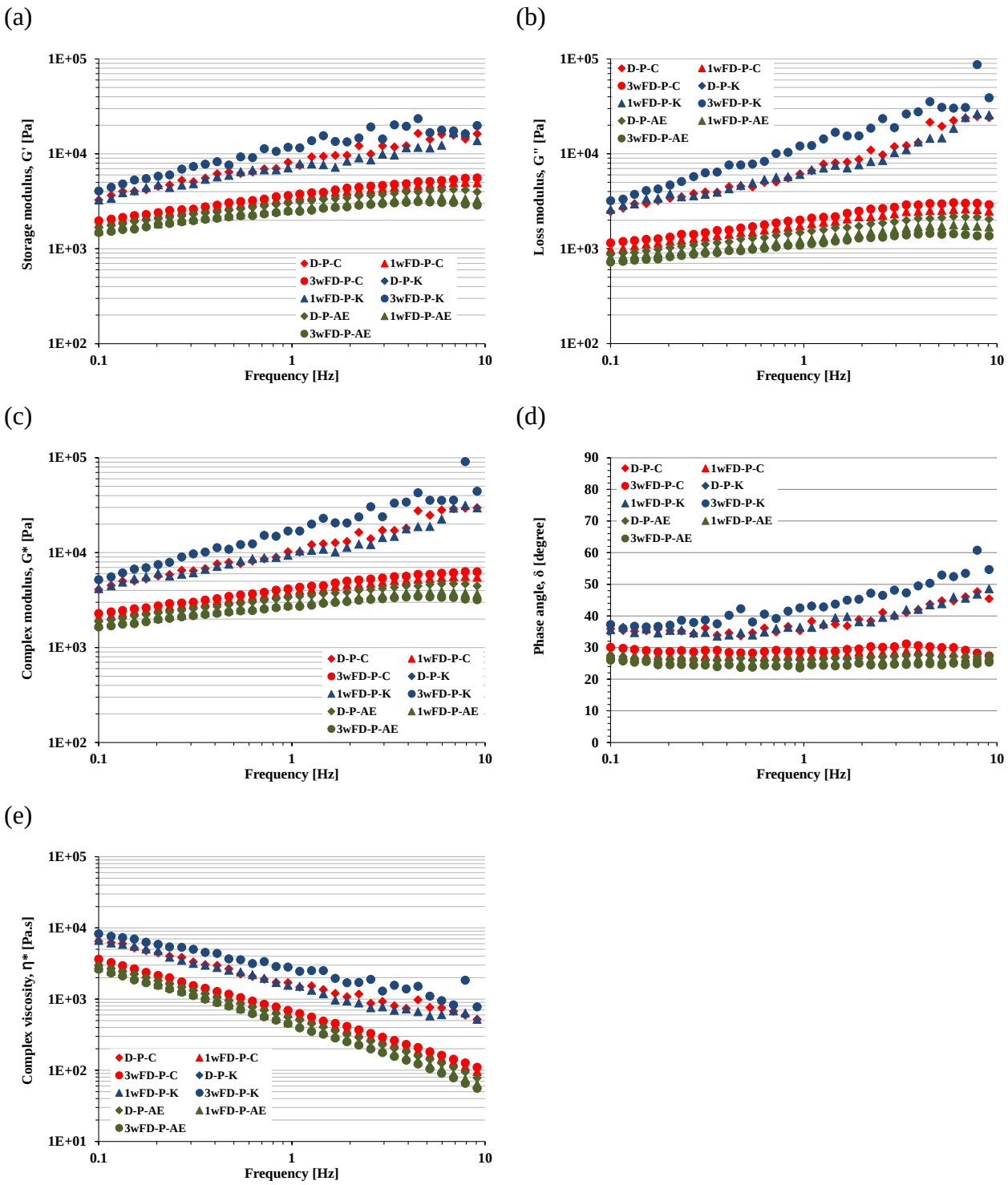


Figure 4.34 Frequency sweeps of all dough treatments at proof, 1 week frozen proof, and 3 weeks frozen proof. (a) Storage modulus G', (b) loss modulus G", (c) Complex modulus, G*, (d) Phase angle, tan δ, (e) Complex viscosity, η*

Fig. 4.34 (a), (b), and (c) show that fresh proofed control and all proofed doughs containing potassium bromate had higher G', G", and G* than did proofed 1 and 3 weeks of frozen control, and all proofed dough containing ascorbic acid and endoxylanase. This indicates that the fresh proofed control and all proofed potassium bromate doughs were stiffer (more elastic) than the other proofed treatment doughs. G', G", and G* of all proofed treatment doughs ($KBrO_3$ and AA-EX) were similar, so both treatments helped to maintain the dough's rheological property during frozen dough processing. On the other hand, fresh proofed control dough G', G", and G* values were higher than the proofed 1 or 3 weeks frozen control dough illustrated that control dough was affected by frozen dough process (freezing, frozen storage, and thawing) and its rheological properties (elasticity and viscosity) were decreased during frozen dough processing. This difference may be caused by the gluten network being damaged during frozen dough processing. The slope values for G' and G" of all proofed treatment doughs are presented in Table 4.45.

Table 4.45 Frequency sweeps of proofed all treatment doughs slope of G' and G"

	G'			G"		
	Control	$KBrO_3$	AA-EX	Control	$KBrO_3$	AA-EX
Post proofing	0.35	0.34	0.20	0.52	0.59	0.21
1 week frozen & proofed	0.22	0.32	0.18	0.22	0.48	0.19
3 weeks frozen & proofed	0.23	0.43	0.16	0.23	0.74	0.16

The slope values for G' and G" were positively frequency dependent for all proofed treatment dough. Table 4.45 shows that the slope of both moduli for fresh proof control and all proofed potassium bromate dough were higher than the other proofed treatment dough. This demonstrated that those doughs were more sensitive to frequency change (more frequency dependent) than the other proofed treatment doughs. Therefore, those properties of the doughs were more susceptible to change during frequency changes. The slope of G', and G" of proofed treatment doughs G', and G" were similar, so the treatments helped to maintain the dough rheological property (elastic and viscous) and were less sensitive to frequency change. On the other hand, the slope G' and G" of fresh proofed control dough were higher than that of proofed 1 or 3 weeks frozen dough. This suggests that the fresh proofed control doughs were more sensitive to frequency change while the proofed 1 or 3 weeks frozen dough were less sensitive. It also means that control dough was affected by frozen dough process (freezing, frozen storage,

and thawing) and its sensitivity to frequency change was altered during frozen dough processing. The gluten network was most likely damaged during frozen dough processing causing the difference in the data.

Phase angle (Fig.4.34 (d)) comparisons indicate that, proofed 1 or 3 weeks frozen control and all proofed doughs with ascorbic acid and endoxylanseendoxylanase had a lower phase angle than did the other doughs. This suggests that proofed 1 or 3 weeks frozen control and all proofed doughs containing ascorbic acid and endoxylanseendoxylanase were more solid-like than the other doughs.

On the other hand, fresh proofed control and all proofed potassium bromate dough were more fluid like (viscous) especially at high frequencies. Fig.4.34 (d) also illustrated that the phase angle for fresh proofed control and all proofed dough containing potassium bromate changed greatly during frequency change. This indicates that those doughs were more sensitive to the frequency change (more frequency dependent). This result was reflected in the slope of G' and G" (Table.4.45)

Phase angles of all proofed treatment were similar, so the treatments helped to maintain dough rheological properties (elasticity and viscosity) during frozen dough processing. However, fresh proofed control dough's phase angle was higher than proofed 1 or 3 weeks frozen dough. Thus, the control dough behavior had changed due to the frozen dough process (freezing, frozen storage, and thawing). Proofed frozen doughs displayed a more solid-like behavior. Phase angles of AA-EX containing dough were frequency independent in nature, while $KBrO_3$ containg doughs samples become progressively more liquid-like as indicated by increased phase angles exceeding 45 ° at frequencies above 1 Hz.

Fig. 4.34 (e) demonstrated that the η* value for fresh proofed control and all proofed dough containing potassium bromate were higher (η*) than the other doughs. All samples, independent of treatment and process history, displayed a shear-thinning behavior expectedly. AA-EX containing samples typically had lower complex viscosities. Storage history had little or no effect on these samples. However, the complex viscosity of dough samples containing $KBrO_3$ displayed a strong dependence on frozen storage history. Complex viscosity of these samples increased progressively with the storage time (fresh < 1 week < 3 weeks). An opposite relation was observed in the complex viscosity of the control samples: Fresh proof dough had the highest

viscosity, frozen storage resulted in significant decrease both in 1w frozen proof and 3 weeks frozen proof samples.

Fig. 4.35 compared G', G", G*, phase angle, complex viscosity of all proofed dough G', G", G*, phase angle, complex viscosity at 1.1 Hz and the final bread volume with statistical analysis.

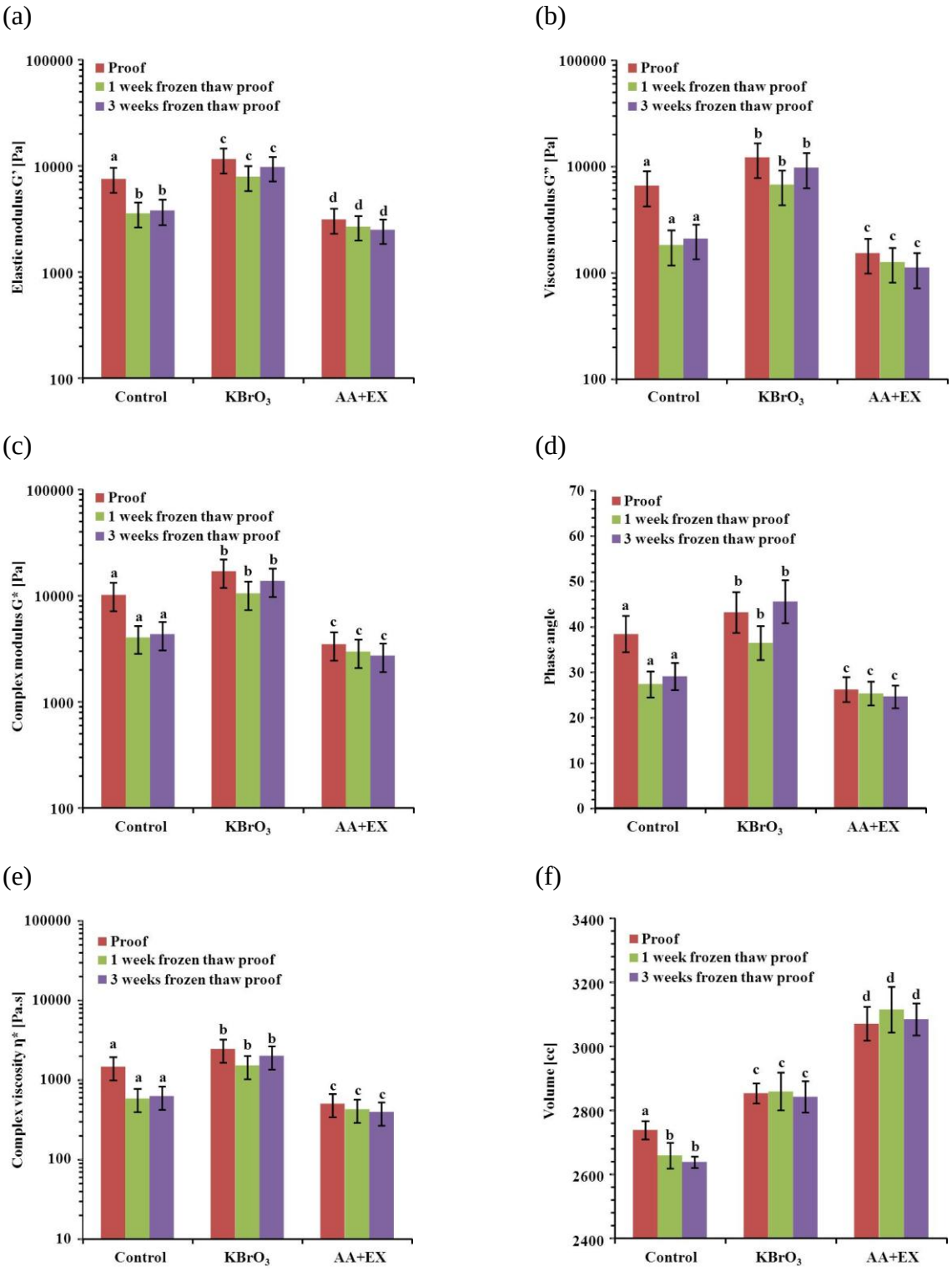


Figure 4.35 Comparing frequency sweeps of all proofed dough rheology at 1.1 Hz and bread volume. (a) Storage modulus G', (b) loss modulus G", (c) Complex modulus, G*, (d) Phase angle, tan δ, (e) Complex viscosity, η*, (f) Bread volume

The G' at 1.1 Hz (Fig. 4.35) for the control fresh proofed dough was significantly higher than that of 1 or 3 weeks frozen proofed control dough. On the other hand, the G", G*, phase angle, and complex viscosity η* at 1.1 Hz for all proofed doughs were not significantly different. This shows that the frozen proofed control dough had a greater loss only in elasticity, making the dough softer than the fresh proofed. For the bread volume, 1 and 3 weeks frozen bread showed significantly lower loaf volume than the never frozen bread. This difference may be because the gluten was damaged by frozen dough processing (freezing, frozen storage, and thawing) leading loss in elasticity. Comparing all rheological properties at 1.1 Hz for 1 week and 3 weeks proofed control frozen dough, no significant difference was observed between 1 week and 3 weeks frozen storage; therefore, the final bread volumes of these dough samples were not significantly different. It can be concluded that frozen control dough became softer than the never frozen because gluten structure was altered (damaged) by frozen dough process. Thus, the control bread volume decreased. The prolonged frozen storage did not affect dough rheological properties, so the bread volumes were comparable.

Fig. 4.35 also illustrated that all rheological properties of proofed doughs containing potassium bromate at 1.1 Hz were not significantly different. The inclusion of potassium bromate in proofed doughs helped the dough maintain its rheological properties and produced the same loaf volume. The potassium bromate prevented change in the dough during proofing and baking, and helped to reduce variation in the rheological properties after proof caused by storage. As a result, all loaf volumes of potassium bromate doughs were not significantly different. As discussed earlier, all proofed doughs containing potassium bromate had higher moduli (G', G', G*) compared to their un-proofed counterparts. This additive is known to be most effective at oxidizing the gluten network during the proofing and baking. Therefore, the proofed dough with potassium bromate had higher moduli (stiffer dough) resulting in loaf volumes significantly higher than the control.

Fig. 4.35 demonstrated that all rheological properties of proofed doughs containing ascorbic acid and endoxylanase at 1.1 Hz were not significantly different indicating minimal or no effect of frozen storage process. These additives helped to maintain the rheological properties of the doughs minimizing significant differences in loaf volume. The magnitude of moduli and complex viscosity of dough samples containing AA-EX were lower than those of control dough and dough containing $KBrO_3$. In general, addition of AA-EX created softer doughs than the

control and $KBrO_3$ treatment. The oxidant addition is known to improve gluten quality because the ascorbic acid and endoxylanase plasticize the protein. Soft dough is favorable for growth of air bubbles during baking promoting an increased in loaf volume in the baked bread. Therefore, final volume breads produced from AA-EX containing doughs was the highest, followed by $KBrO_3$ treatment.

Overall, data indicated that there is a relationship is between proofed frozen dough rheological properties, especially dough elasticity and bread volume. Because dough elasticity is changed by addition of additives, treatments cannot be compared to one another in order to estimate the bread volumes directly. Comparison of the elasticity of the same formulation within each storage condition provides a better prediction of loaf volume. Small elasticity changes of proofed not frozen and proofed frozen dough, thus are less susceptible to variations in loaf volumes.

CHAPTER 5 - Conclusion

In this study, the addition of oxidants individually and in combination with enzyme were optimized and evaluated in fresh baking. For frozen dough studies, frozen dough/ bread quality obtained using oxidants and oxidants-enzyme in combinations at the optimized levels for fresh baking were evaluated for dough rheological properties (gluten network characteristics) using dynamic oscillation testing at the each frozen dough making process steps and various frozen storage conditions.

This study determined that fresh baking results through the addition of additives, except lipase provided greater quality bread with good size and volume when the system was optimized. Test results also showed that the combination of ascorbic acid and hemicellulase/endoxylanase improved bread quality (crumb structure and volume) greatly. Loaf volume showed that these treatment combinations were much higher than the control and that containing potassium bromate. Therefore, these combinations could be used as a replacement for potassium bromate in fresh baking.

Based on fresh dough baking results, control (no treatment), 50 ppm potassium bromate addition, and a combination of 200 ppm ascorbic acid with 100 ppm endoxylanase addition was used for frozen dough/bread production. The optimized level of endoxylanase was lower than the hemicellulase, so endoxylanase was used for frozen dough/bread production. When comparing by storage condition only, loaf volumes for the control formula were significantly different between no frozen and 1, 3 week frozen storage. The control dough was affected by the frozen dough process (freezing, frozen storage, and thawing) and caused a loss in the bread quality, due to gluten being damaged. On the other hand, loaf volume for the treatment doughs was not significantly different between no frozen and 1, 3 week frozen. This test result showed that addition of additives improved and maintaining the dough quality during frozen dough process and provided similar quality bread. The addition of additives improved bread quality under frozen storage conditions. The combination of ascorbic acid with endoxylanase dough had significantly higher volume than those doughs with potassium bromate in frozen dough/bread production. The combination of ascorbic acid and endoxylanase can be a full replacement for potassium bromate in frozen dough making.

For rheological properties, the strain/stress sweep test results showed that the doughs' linear viscoelastic range (LVR) affected by process and additives and indicated the dough stability was changed by process and treatment. Process condition, especially proofing, and addition of additives extended the dough LVR. The extension of the LVR showed that proofing and the treatments improved dough stability.

Frequency sweep test results showed that all dough rheological properties (G', G", G*, phase angle, and complex viscosity) also were affected by process and additives. The elasticity or viscosity increase or decrease depended on the different treatments and storage conditions. Comparing the frozen control dough within each process stage at post mix, proof, 1 week frozen and 1 or 3 weeks frozen proofed, 3 weeks frozen, the elastic moduli (G') at 1.1 Hz were significantly different, but the other rheological properties (G", G*, phase angle, and complex viscosity) was not significantly different. This suggests that gluten structure damage was more related to elastic modulus (G') than viscous modulus (G"). On the other hand, all rheological properties of dough containing potassium bromate doughs at 1.1 Hz were not significantly different during entire frozen dough process. In addition, all rheological properties of dough containing ascorbic acid and endoxylanase dough at 1.1 Hz were also not significantly different at proof, 1week freeze thaw, 1 week freeze thaw proof, 3 week freeze thaw, and 3 week freeze thaw proof. Consequently, minimal changes were seen in the doughs elastic modulus (G') with either the added treatments during frozen dough processing. Therefore, addition of additives improved dough tolerance for frozen dough process.

There was a relationship between the added treatments and the influence of storage condition as there was less variation in dough rheology and loaf volume with the addition of the oxidants and oxidants-enzyme combinations. In addition, through the rheological testing, it was determined that the G' values were the most effective in determining or predicting bread quality based off of loaf volume. The effects of the treatment additions at optimized levels helped to maintain bread quality throughout the different stages and storage conditions and dynamic oscillation rheology proved to be an effective method for evaluating variations in the fundamental rheological properties of dough under these changing conditions.

CHAPTER 6 - Future study

1. Low temperature (6 ºC) produced doughs/breads of lower quality (volume) than did ambient temperature. It indicates that the dough rheological properties (elasticity and viscosity) decreased under those conditions. Therefore, study dough rheology following the addition of additives to the fresh dough and after each baking stage.
2. The required time for full gluten development may be different at ambient temperature and low temperature. Therefore, to optimize mixing time for frozen dough/bread production and test under those conditions.
3. In frozen dough/bread production, thawing process was influenced by ambient condition. The required time to completely thaw depended on ambient conditions and the doughs were also partially proofed. Therefore, dynamic oscillatory rheometer at post thawing data was variable, so require repeating the dough rheology study following a more controlled the thawing stage and so to collect more reliable data.
4. Frozen doughs require to at least 3 months frozen storage tolerance in baking industry. Therefore, extend the frozen storage (-18 ºC) for up to 3 months, then conduct bake test, and measurement dough rheological properties by dynamic oscillatory rheometer.
5. Proofed dough containing potassium bromate elastic modulus (G') (elasticity) was higher than control. It indicates that this treatment dough was stiffer than control. However, proofed dough containing ascorbic acid and endoxylanase elastic modulus (G') (elasticity) was lower than control, and on potassium bromate containing dough. It means that this treatment dough was the softest of the three. Consequently, the loaf volume containing potassium bromate was better than control, and the loaf volume containing ascorbic acid and endoxylanase was even better than the dough containing potassium bromate. This cause may be the difference of gas retaining capacity and the distribution of the gas cells within the gluten phase of the dough. The strain hardening test would be good test for this task.

References

AACC International Method 10-05, Guidelines for Measurement of Volume by Rapeseed Displacement; Method 44-15A, Moisture- Air-Oven Methods. Approved Methods of the American Association of Cereal Chemists, 10th ed. AACC International, St. Paul, MN, 2000

Abdelrahman, A., and Spies, R. 1986. Dynamic rheological studies of dough systems. In: Fundamentals of Dough Rheology. Faridi, H., Faubion, J.M. eds. Published American Association of Cereal Chemists, St Paul, Minnesota, USA.

Abdullah, S. C., and Hazim O. 2012. Potential use of endogenous lipases for DATEM replacement to modify the rheological and thermal properties of wheat flour dough. J. cereal Sci. vol.55: 397-404.

Akiyama, T., Yamanaka, M., Date, Y., Kubota, H., Hamano, Nagaoka, M., Kawasaki, Y., Yamazaki, T., Yomata, C. and Maitani, T. 2002. Specific determination of bromate in bread by ion chromatograpy with ICP-MS. J. Food Hyg. Soc. Japan., 43, 348-351.

Alves-Araújo, C., Almeida, M.J., Sousa, M. J., and Leão, C. 2004. Freeze tolerance of the yeast *Torulaspora delbrueckii:* cellular and biochemical basis. FEMS Microbiology Letters 240: 7-14.

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

American Bakers Association, and American Institute of Baking. 2008. Commercial Baking industry guide for the safe used of potassium bromate. https://www.aibonline.org/press/SafeUsePotassiumBromate%2009_08.pdf

Ando. A., Nakamura, T., Murata, Y., Takagi, H., and Shima, J. 2007. Identification and classification of genes required for tolerance to freeze-thaw stress revealed by genome-wide screening of Saccharomyces cerevisiae deletion strains. FEMS Yeast Res. 7: 244-253

Angioloni, A., and Dalla Rosa, M. 2005. Dough thermo-mechanical properties: influence of sodium chloride, mixing time and equipment. J. Cereal Sci. 41: 327-331.

Anonymous, 1967. Potential for frozen dough. Bull. 787. Econ. Res. Serv., U.S. Dep. Agric., Washington, DC.

Askeland, D. R and Phule, P.P. 2006. Chapter 6- Mechanical Properties: Part one; "The science and Engineering of Materials, 2nd edition". Published by Nelson, a division of Thomson Canada Limited.

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

Bailey, C. H. 1930. Calibration of loaf volume measuring devices with metal models. Cereal Chem. 5: 346-348

Barnes, H.A., Hutton, J.F., Walters, K., 1989. An Introduction to Rheology, Elsevier, Amsterdam.

Barrett, F. F. 1975. Enzyme uses in the milling and baking industries. In Enzymes in Food Processing, ed. Reed, G., Academic Press, New York, page 301-348.

Barrett, J., 2001. Thermal hysteresis proteins. International Journal of Biochemistry & Cell Biology 33, 105–117

Bekes, F., MacRitchie, F., Panozzo, J. F., Batey, I.L., and O'Brien, L. 1992. Lipid mediated aggregates in flour and in gluten. J. cereal Sci. vol.16. pp. 129-140.

Belton, P.S., 2005. New approaches to study the molecular basis of the mechanical properties of gluten. J.of Cereal Sci. 41, 203–212

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 dough. Cereal Chem. 67:139

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

Bernardin, J. E. 1978. Gluten protein interaction with small molecules and ions – the control of flour properties. Bakers Dig. 52 (4): 20.

Bertrand, D., Le Guerneve, C., Marion, D., Devaux, M. F., and Robert, P. 1992. Description of the Textural Appearance of Bread Crumb by Video Image Analysis. Cereal Chem. 69:257-261.

Biliaderis, C.G., Page, C.M., Maurice, T.J., 1986. On the multiple melting transitions of starch/monoglyceride systems. Food Chemistry 22, 279-295.

Biliaderis, C.G., Izydorczyk, M.S. and Rattan, O. 1995. Effect of arabinoxylans on bread-making quality of wheat flours. Food Chemistry 5: 165–171.

Bloksma, A.H., Bushuk, W., 1988. Rheology and chemistry of dough. In: Pomeranz, Y., (Ed.), Wheat Chemistry and Technology II, AACC, St Paul, Minnesota, USA.

Bloksma, A.H. 1990. Rheology of the breadmaking process. Cereal Food World. 35: 228-236.

Boll, D. 1999. Improving dough strength with enzymes. Proc. Am. Soc. Bakery Engrs.75; 155.

Bot, A. 2003. Differential canning calorimetric study on the effect of frozen storage on gluten and dough. Cereal Chem. 80: 366-370.

Bourne, M. C. 2002. Volume Measuring Instrument. In: Food Texture and Viscosity: Concept and Measurment. Academic Press, San Diego, page 224-226.

Brinsma. B.L. and Giesenschlag, J. 1984. Frozen dough performance Compressed yeast – instant dry yeast. Bakers Dig.58 (6):6-11.

Brown, J. 1993. Chapter 3- Advances in bread making technology. Pages38-87. In: Advances in Baking Technology. Kamel, B. S., and Stauffer, C. eds. Published in the USA and Canada by VCH publishers, Inc., 220 East 23rd Street, Suite 909, New York, NY.

Bushyk, W. and Bekes, F. 1984. "Carbohydrate and Lipid Complexes with Gliadin and Glutennin" in; Gluten Proteins, Ed, Graveland, A and Moonen, J. H. E. Proc. of 2nd International workshop on Glten Proteins. Wageningen, the Netherlands.

Calderón-Domínguez, G., Chanona-Pérez, J., Ramos-Cruz, A. L., López-Lara, A. I., Tlapale-Valdivia, A. D., and Gutiérrez-López, G. F. (2008). Fractal and image analysis of Mexican sweet bread bubble distribution; influence of fermentation and mixing time. In G. M. Campbell, M. Scanlon, L. Pyle, & K. Niranjan (Eds.), Bubbles in food 2: Novelty, health and luxury (pp. 73–81). St. Paul: American Association of Cereal Chemists.

Caley, M.S., Park, S., Chung, O.K. 2005. Comparison of loaf-volume measuring methods: Rapeseed displacement vs. laser sensor. AACC International Meeting. Poster Paper No. 111.

Campos, D. T., Steffe, J .F., and NG. P. K.W. 1997. Rheological Behavior of Undeveloped and Developed Wheat Dough. Cereal Chem. 74(4):489-494

Carter, R.E., 1990. Rheology of Food, Pharmaceutical and Biological Materials with General Rheology, Elsevier Applied Science, London.

Casey, G., and Foy, J. 1995. Yeasted performance in frozen doughs and strategies for improvement. Pages 19-51. In: Frozen & Refrigerated Doughs and Batter. Kulp, K., Lorenz, K., and Brummer, J. eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

Casier, J.P.J., Stephan, H., De Paepe, G. and Nicora, L. 1969. Einfluss der Wasserunlo¨ slichen Endospermpentosane auf die Teig- und Geba¨ckeigenschaften bei der Ver- arbeitung von Weichweizen. Brot und Geba¨ck 12: 231–236.

Cathcart, W.H., and Cole, L.C. 1938. Wide-range volume measuring apparatus for bread. Cereal Chem. 15; 69-79.

Cauvain, S.P., Collins, T.H. and Pateras, I. 1992. Effects of ascorbic acid during processing. Chorleywood Digest No. 121, October/November, CCFRA, Chipping Campden, UK, pp. 111–114.

Cauvain, S. P. 1994. Improved dough oxidation. Proc. Am. Soc. Bakery Engrs. 70:69.

Cauvain, S., and Young, L. 2001. 4.2 What are the functions of ascorbic acid in Breadmaking? Baking problems solved. Woodhead Publishing Limited. Abington, Cambridge CB1 6AH, England. 2001.

Cawley, R.W. 1964. The role of wheat flour pentosans in baking. II Effect of added flour pentosans and other gums on gluten-starch loaves. J. of the Science of Food and Agriculture 15 (1964) 834–838.

Carr, N.O., Daniels, N.W.R., Frazier, P.J., 1992. Lipid interactions in breadmaking. Critical Reviews in Food Science and Nutrition 31, 237-258.

Castello, P., Jollet, S., Potus, J., Baret, J.-L., Nicolas, J., 1998. Effect of exogenous lipase on dough lipids during mixing of wheat flours. Cereal Chem. 75, 595-601

CFSAN/Office of Food of Safety, 2005. Food Additive Status List. United States Food and Drug Administration.

Chamberlain, N. 1979. Gases – the neglected ingredients, in Proceedings of the 49th Conference of the British Society of Baking, British Society of Baking, pp. 12–17.

Chamberlain, N. T., Collins, T. H. and McDermott, E. E. 1981. Alpha-amylase and bread properties. J. Food Technol., 16.127.

Chevallier S, Zúñiga R., and Le-Bail, A. 2012. Assessment of bread dough expansion during fermentation. Food Bioprocess Technology,5:609–617

Chung, O.K., Tsen, C.C., Robinson, R.J., 1981. Functional properties of surfactants in bread making: III. Effects of surfactants and soy flour on lipid binding in breads. Cereal Chem. 58, 220-226.

Clarke, C. I., Schober, T.J., and Arendt, E. K. 2002. Effect of Single Strain and Traditional Mixed Strain Starter Cultures on Rheological Properties of Wheat Dough and on Bread Quality. Cereal Chem. 79: 640–647

Cleemput, G., Booij, C., Hessing, M., Gruppen, H. and Delcour, J.A. 1997. Solubilisation and changes in molecular weight distribution of arabinoxylan and protein in wheat flours during bread-making, and the effects of endogenous arabinoxylan hydrolysing enzymes. J. of Cereal Sci. 26: 55–66.

Cogswell, T. S. 1997. Oxidants in the baking process. Proc. Am. Soc. Bakery Engrs 73:91.

Cole, E.W. 1967. Isolation and chromatographic fractionation of hemicelluloses from wheat flour. Cereal Chem. 44: 411–416.

Connelly, R., and McIntier, R. 2008. Rheological properties of yeasted and nonyeasted wheat doughs developed under different mixing conditions. J. Sci. Food Agric. 88: 2309-2323.

Considere, M., 1885. Memoire sur le´mploi du fer et de la´ cier dans les constructions. In: Annales des Ponts et Chause´ es, vol. 9. CH. Dunod, Paris, pp. 574–605.
Courtin, C.M., Van den Bulck, K. and Delcour, J.A. 1999. Understanding arabinoxylan functionality in bread-making: Use of a combination of reconstitution and in vitro endoxylanase breakdown of water-extractable and water-unextractable arabinoxylans in dough rheological measurements. Poster presentation at the AACC 84th Annual Meeting, Seattle, U.S.A.
Courtin, C.M., Roelants, A. and Delcour, J.A. 1999. Fractionation–reconstitution experiments provide insight into the role of endoxylanases in bread-making. Journal of Agricultural and Food Chemistry 47: 1870–1877.
Courtin, C.M., Gelders, G.G. and Delcour, J.A. 2001. The use of two endoxylanases with different substrate selectivity provides insight into the functionality of arabinoxylans in wheat flour breadmaking. Cereal Chemistry 78: 564–571.
Courtin, C.M. and Delcour, J.A. 2001. Relative activity of endoxylanases towards water-extractable and water- unextractable arabinoxylan. J. of Cereal Sci. 33: 301–312.
Courtin, C.M. and Delcour, J.A. 2002. Arabinoxylans and endoxylanses in wheat flour bread-making. J. of Cereal Sci. 35: 225–243.
Dannno, G., and Hoseney, R.C. Effect of sodium chloride and sodium dodecyl sulfate on mixograph properties. Cereal Chme. 59:202-204.
Darby, R. 1976. Viscoelastic Fluids. Marcel Dekker Inc., New York. Chapter 1- Rheological properties of Foods. Pages13 .In: Handbook of food engineering. Heldman, D. R., and Lund, D. B. eds. Published in Tayler and Francis group, LLC.
Datta, A. K., Sahin, S., Sumnu, G., and Keskin, S. O. 2007. Porous media characterization of breads baked using novel heating methods. Journal of Food Engineering, 79, 106–116.
Davidou, S., Michon, C., Thabet, I., and Launay, B. 2008. Influence of shaping and orientation of structures on rheological properties of wheat flour dough measured in dynamic shear and biaxial extension. Cereal Chem. 85: 403-408.
Davis, E. W. 1981. Shelf life studies on frozen doughs. Baker's Dig.58(6):6.
Debyser, W., Derdelinckx, G., and Delcour, J. A., 1997. J. Am. Soc. Chem.55: 153-156
Debyser, W., and Delcour, J. A. 1997. European patent application WO98/49278.
Debyser. W., Peumans, W. J., Van Damme, E.J.M., and Delcour, J.A. 1999. J. Cereal Sci. 30: 39-43.
Dekker, R.F. and Richards, G.N. 1976. Hemicellulases: their occurrence, purification, properties, and mode of action. Advances in Carbohydrate Chemistry and Biochemistry 32:277–352.
Dobraszczyk, B.J., Roberts, C.A., 1994. Strain hardening and dough gas cell-wall failure in biaxial extension. J. Cereal Sci. 20: 265–274.
Dobraszczyk, B.J., 1997. Development of a new dough inflation system to evaluate doughs. Cereal Food World 42, 516–519.
Dobraszczyk, B.J., Vincent, J.F.V., 1999. Measurement of mechanical properties of food materials in relation to texture: the materials approach. In: Rosenthal, A.J., (Ed.), Food Texture: Measurement and Perception, Aspen Publishers, Maryland.
Dobraszczyk, B. J., Morgenstern, M. P. 2003. Rheology and the baking. J. Cereal Sci. 38: 229-245.
Dobraszczyk, B.J., and Salmonowicz, B.P., 2008. Comparison of predictions of baking volume using large deformation rheological properties. J. Cereal Sci. 47: 292-301

Doerry. W. T. 1995. Chapter 7- Dough processing. In: Breadmaking technology vol.1. Published by American Institute of Baking, Manhattan, KS.

Dogan, H, and Kokini, J.L., 2007. Chapter 1- Rheological Properties of Foods; "Hand book of food engineering, 2nd edition", Heldman, D.R. and Lund D.B. eds. CRC Press Taylor and Francis Group. 6000 Broken Sound Parkway NW, suite 300 Boca Raton, FL 33487-2742

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

Dreese, P.C., Faubion, J.M., Hoseney, R.C., 1988. Dynamic rheological properties of flour, gluten and gluten–starch systems. I. Temperature-dependent changes during heating. Cereal Chem 65: 348–353.

DSM Food specialties B.V. 2006. BAKEZYME® BXP 5000 BG, BAKEZYME® HSP 5000 BG product specification sheet.

Dubois D. K. 1980a. Enzymes in Baking. Ⅰ Classification. Research department Technical Bulletin 2 (10). American Institute of Baking, Manhattan, KS.

Dubois D. K. 1980b. Enzymes in Baking. Ⅱ Applications. Research department Technical Bulletin 2 (11). American Institute of Baking, Manhattan, KS.

Dubois D. K. 1980c. Enzymes in Baking. Ⅲ Products. Research department Technical Bulletin 2 (12). American Institute of Baking, Manhattan, KS.

Dubois, D. K. and Dreese, P. 1984. Frozen white bread dough-Effects of sweetener level. AIB Tech. Bull. Vol.6, Issue 7. American Institute of Baking, Manhattan, KS.

Dubois, D. K., and Blockcolsky, D. 1986. Frozen bread dough, Effect of additives. AIB Tech. Bull. Vol.8, No.4. American Institute of Baking, Manhattan, KS.

Dubois, D. K., and Blockcolsky, D. 1986. Frozen bread dough. Effect of dough mixing and thanwing methods. AIB Tech. Bull. Vol.8, No.6. American Institute of Baking, Manhattan, KS.

Dus, S. J., and Kokini, J. L. 1990. Prediction of the nonlinear viscoelastic properties of a hard wheat flour dough using the Bird-Carreau constitutive model. J. Rheol. 34:1069-1084.

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

El-Hady, E. A., El-Samahy, S. K., and Brümmer, J.-M. 1999. Effects of oxidants, sodium-stearoyl-2-lactylate and their mixtures on rheological and baking properties on nonprefermented frozen dough. Lebensmittel Wissenschaft und Technologie 32, 446-454.

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 Chem. 80:396-403.

Esteller, M. S., Zancanaro, O., Jr., Santos-Palmeira, C. N., and Lannes, S. C. S. 2006. The effect of kefir addition on microstructure parameters and physical properties of porous white bread. European Food Research and Technology, 222, 26–31.

Esteller, M. S., and Lannes, S. 2008. Production and characterization of sponge-dough bread using scalded rye. Journal of Texture Studies, 39, 56–67.

Fan, J., Mitchell, J.R., Blanshard, J.M.V., 1999. A model for the oven rise of dough during baking. J. Food Engin. 41, 69–77.

Farrera-Rebollo, R. R., Paz Salgado-Cruz, Ma. de la, Chanona-Pérez, J., Gutiérrez-López, G. F., Alamilla-Beltrán, L. and Calderón-Domínguez, G. 2012. Evaluation of Image Analysis Tools for Characterization of Sweet Bread Crumb Structure. Food Bioprocess Technol. 5: 474-484.

Faubion, J. M. and Faridi, H. 1986. Dough Rheology: Its benefits to cereal chemist. In: Fundamentals of Dough Rheology. Faridi, H., Faubion, J.M. eds. Published American Association of Cereal Chemists, St Paul, Minnesota, USA.
Faubion, J. M., and Hoseney. R. C. 1990. The viscoelastic Properties of Wheat flour doughs. In: Dough Rheology and Baked Product Texture. Faridi, H., Faubion, J.M. eds. AVI Van Nostrand Reinhold, New York.
FDA, U.S. food and drug administration, "CFR - Code of Federal Regulations Title 21" http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.730
Feaster, J. F., and Cathcart, W. H. 1941. Value of ascorbic acid from various sources in baking. Cereal Chem. 18: 201-208
Ferry, J. D., 1980. Viscoelastic Properties of Polymers, Wiley, New York.
Fincher, G.B.,and Stone, B. A. 1986. Pages 207-295 in: Advances in cereal science and technology, Pomeranz, Y.,Eds., AACC., Vol.8.
Forman, T. 2003. ESL- where enzyme chemistry meets manufacturing. Proc. Am. Soc. Baking 79:166.
Forman. T. 2004. Enzymes used in bread making: and application update. AIB Tech. Bull.26 (10).
Freilich, J. and Frey, C. N. 1939a. Cereal Chem. 16: 495
Freilich, J. and Frey, C. N. 1939b. Cereal Chem. 16: 495
Fuhrmann, D. F. 1985. Frozen dough manufacturing – Ingredients and formulation. Proc. Am. Soc. Bakery Engrs. 61:149-154
Funk, K., Zabik, M. E. and Elgidaily, D. A. 1969. Objective measurements for baked products. J. Home Econ. 61; 119-123.
Gams, 1976. Getreide Mehl und Brot, Tchnologishce Zeitxchrift für Getreid, Mehl, Brot, Cackwaren, 30. Jahrgang, Heft 5, pp. 113-116.
Gan, Z., Ellis, P.R. and Schofield, J.D. 1995. Mini review: gas cell stabilisation and gas retention in wheat breaddough. J. of Cereal Sci. 21:215–230.
Geddes, W. F. and Binnigton, D.S. 1928. A volume measuring device for small loaves. Cereal Chem. 5: 215-220
Gélians, P., Lagimoniére, M., and Dubord, C. 1993. Baker's yeast sampling and frozen dough stability. Cereal Chem. 70: 219-225.
Gélians, P., Lagimoniére, M., and Rodrigue, N. 1994. Performance of Cream or Compressed Yeast in Frozen and Nonfrozen Doughs. Cereal Chem. 71:183-186.
Ghiasi, K., Varriano-Marston, E., Hoseney, R.C., 1982. Gelatinization of wheat starch: II. Starch-surfactant interaction. Cereal Chem. 59: 86-88.
Gillette, M. 1985. Food Techonol. 39:47
Godkin, W. J. and Cathcart, W. H. 1949. Fermentation activity and survival of yeast in frozen fermented and unfermented dough. Food Technol. 3 (April): 139.
Goldstein, S. 1957. Correlation between sulfhydryl and disulfide groups of gluten proteins and baking properties of bread flour. Mitt. Gebiete Lebensmitel. Hyg. 48 (2): 87.
Gonzales-Barron, U., and Butler, F. 2006. A comparison of seven thresholding techniques with the k-means clustering algorithm for measurement of bread-crumb features by digital image analysis. Journal of Food Engineering, 74(2), 268–278.
Gross, H., Bell, R.L., and Redfern, S. 1966. Cereal Science Today. 11

Gruppen, H., Hamer, R.J. and Voragen, A.G.J. 1992. Water- unextractable cell wall material from wheat flour. I. Extraction of polymers with alkali. Journal of Cereal Science 16: 41–51.

Gudmundsson, M., Eliasson, A.-C., 1990. Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifiers. Carbohydrate Polymers 13; 295-315.

Guide Chem. Structure of Potassium bromate. Chemical Trading Guide™ http://www.guidechem.com/products/7758-01-2.html

Gunasekaran S, MM Ak. 2000. Dynamic oscillatory shear testing of foods – selected applications. Trends in Food Science and Technology 11(3):115-127

Guy, R. 2001. Baking with Enzymes. The World of Food Ingredients. 26:28-29.

Haarasilta, S., Pullinen, T., Vaisanen, S., and Tammersalo-Larste. I. 1991. Enzyme product and method of improving the properties of dough and the quality of bread. United States Patent. Patent Number 4,990,343.

Hamer, R. J. 1992, Enzymes and the baking industry; friends or foes? In cereal chemistry and Technology: A long past and Bright Future, ed. P. Feillet, Institut National de la Recherche Agronomique, 9th International Cereal and bread Congress, Paris, 1992.

Hammond, J. 1994. Breadmaking with hemicellulase: overcoming the legal hurdles. Food technology international Europe. Pages: 19-20, 22-23.

Harrel, C. G. 1928. Calibration of loaf volume box. Cereal. Chem. 5: 220-222

Heald, W. L. 1929. Calibration of loaf measuring apparatus. Cereal Chem.6: 308-310.

Heid, M. 1968. Frozen batters and doughs. Pages 360-385 in: The freezing Preservation of Foods, Vol.4. D.K. Tressler, W.B. van Arsdel, and M. J. Copley, eds. AVI Publ. Co., Westport, CT.

Hessing, M. and Happe, R. P. 2000. European Patent EP 0979830 A1

Hibberd, G. E., and Wallace, W. J. 1966. Dynamic viscoelastic behavior of wheat flour doughs. I. Linear aspects. Rheol. Acta 5:193-198.

Hibberd, G. 1970. Dynamic viscoelastic behavior of wheat flour doughs. Part III: The influence of the starch granules. Rheol. Acta. 9: 501-505.

Hibberd, G.E., Parker, N.S., 1975a. Dynamic viscoelastic behaviour of wheat flour doughs, part IV. Non-linear behaviour. Rheologica Acta 14, 151–157.

Hibberd, G.E., Parker, N.S., 1975b. Measurement of the fundamental rheological properties if wheat flour doughs. Cereal Chem. 52, 1–23.

Hille, J. D. R. and Schooneveld-Bergmans M. E. F. 2004. Hemicellulases and Their Synergism in Breadmaking. Cereal Food World. 49:283-286.

Hillhorst, R., Dunnewind, B., Orsel, R., Stegeman, P., van Vliet, T., Gruppen, H. and Schols, H.A. 1999. Baking performance, rheology, and chemical composition of wheat dough and gluten affected by xylanase and oxidative enzymes. J. of Food Sci. 64: 808– 813.

Himata, K., Kuwahara, T., Ando, S., and Maruoka, H. 1994. Determination of bromate in bread by capillary gas chromatography with mass detector (GC/MS). *Food Addit. Contam.*,11 page; 559-569.

Himata, K., Noda, M., Ando, S., and Yamada, Y. 1997. Measurement of bromate in bread by high performance liquid chromatography with post-column flow reactor detection. *Food* Addit. Contam.,14 page; 809.

Himata, K., Noda, M., Ando, S., and Yamada, Y. 2000. Measurement of bromate in bread by high performance liquid chromatography with post-column flow reactor detection. *Y.J. AOAC Intl.*, 83. 347.

Himata, K., Nakamura, M., Murakami, T., Hosoya, S., and Yamada, Y. 2006. Procedures for the safe use of potassium bromate in bread making in the Japanese baking industry. *AACCI annual meeting poster*, San Francisco, US.

Hino, A., Takano, H., and Tanaka, Y. 1987. New Freeze-Tolerant for Frozen Dough Preparations. Cereal Chem. 64:269-275.

Hites, B. D. 1947. Review of Oxidation and reduction in flours and doughs. Baker's Dig. 21 (2) 34.

Hoseney, R.C., Finney, K.F., Pomeranz, Y. and Shogren, M.D. 1969. Functional and biochemical properties of wheat flour components. II. Role of water-solubles. Cereal Chem. 46 (1969) 117–125.

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

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

Igoe, R.S., and Hui, Y.H. 2001 Dictionary of Food Ingredients fourth edition. An aspen publication, Aspen publishers, Inc., Gaithersburg, Maryland

Inoue, S., Tugita, K., Koike, S., Suzuki, K., Kamoi, I., 1996. Effects of monoglyceride fatty acid species on the properties of dough protein. Food Science and Technology International-Tokyo 2, 97-102.

Inoue, Y., and Bushuk, W. 1991. Studies on Frozen Doughs. I. Effects of Frozen Storage and Freeze-Thaw Cycles on Baking and Rheological Properties. Cereal Chem. 68:627- 631.

Inoue, Y., and Bushuk, W. 1992. Studies on Frozen Doughs. 2. Flour Quality Requirement for Bread Production from Frozen Dough. Cereal Chem. 69:423- 428.

Inoue, Y., Saperstein, H. D., Takayanagi, S., and Bushunk, 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.

International Agency for Research on Cancer (IARC), 1986. Potassium Bromate. Summaries and Evaluations, vol.40, 207pp.

International Agency for Research on Cancer (IARC), 1999. Potassium Bromate (Group 2B). Summaries and Evaluations, vol.73, 481pp.

Izydorezyk, M. S., and Biliaderis, C. G. 1995. Cereal arabinoxylans: Advances in structure and physicochemical properties. Carbohydr. Polym. 28:33.

Jackel, S. S. 1978. Trends in bread production in Cereals '78: Better Nutrition for the World's Millions. Amer. Assoc. of Cereal Chemists, St. Paul, MN.

Jackel. S. 1979. Ascorbic acid as a basic dough conditioner. Bakery production & Marketing 14 (2): 88.

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

Janssen, A.M., van Vliet, T., Vereijken, J.M., 1996. Fundamental and empirical rheological behavior of wheat flour doughs and comparison with bread making performance. J. Cereal Sci. 23: 43–54.

Japan Institute of Baking. Introduction of laser scanning volume measurement. http://www.jibt.com/laser_volume_system.html

Javes, R. 1971. Frozen bakery foods- The ingredients and the process- Effect on shelf-life of frozen, unbaked yeast leavened doughs. Bakers Dig.45 (2):56.

Jelaca, S.L. and Hlynca, I. 1971. Water binding capacity of wheat flour crude pentosans and their relation to mixing characteristics of dough. Cereal Chem. 48: 211–222.

Jelaca, S.L. and Hlynca, I. 1972. Effect of wheat-flour pentosans in dough, gluten and bread. Cereal Chemistry 49; 489–495.
Joint FAO/WHO Expert Committee on Food Additives (JECFA) Evaluation, 1995. http://www.inchem.org/documents/jecfa/jeceval/jec_1969.htm.
Jørgensen, H. 1945. Studies on the Nature of the Bromate effect. Oxford University Press: London, UK.
JP patent, JP-A-62-285749 (published 1987-1-12)
Kamman, P. W. 1979. Recent Developments in No-time dough sysytems. Bakers Dig. 53(1): 26-29.
Kawaseki, Y., Kubota, H., Yomota, C., and Maitani, T. 2002. Improved sensitive determination method for bromate in bread. J. Food Hyg. 43. 221-224.
Kenny, S., Wehrle, K., Dennehy, T., Arendt, E. K., 1999. Correlations between empirical and fundamental rheology measurement and baking performance of frozen bread dough. Ceeral Chem. 76, 321-425.
Khatkar, B.S., Bell, A. E., Schofield, J.D. 1995. "The dynamic rheological properties of wheat glutens and gluten sub-fractions from wheats of good and poor breadmaking quality", J. Cereal Sci., 35(3), 307-313.
Kim, S.K. and D'Appolonia, B.L. 1977. Bread staling studies. III. Effect of pentosans on dough, bread and bread staling rate. Cereal Chem. 54: 225–229.
Kline, L., and Sugihara, T. F. 1968. Factors affecting the stability of frozen bread dough. Ⅰ. Prepared by the straight dough method. Bakers Dig.42 (5):44.
Kline, M, G. 1971a. Packaging – Special requirement for frozen bakery products. Bakers Dig.45 (2):58.
Kline, M, G. 1971b. Problems in baking. Bakers Dig.45 (6):64.
Kokelaar, J.J., van Vliet, T., Prins, A., 1996. Strain hardening properties and extensibility of flour and gluten doughs in relation to bread-making performance. J. Cereal Sci. 24: 199–214.
Kontogiorgos, V., Goff, H.D., 2007. Effect of aging and ice structuring proteins on the morphology of frozen hydrated gluten networks. Biomacromolecules 8, 1293–1299.
Kristiansen, E., Zachariassen, K.E., 2005. The mechanism by which fish antifreeze proteins cause thermal hysteresis. Cryobiology 51, 262–280.
Krog, N., Olesen, S.K., Toernaes, H., Joensson, T., 1989. Retrogradation of the starch fraction in wheat bread. Cereal Foods World 34, 281-285.
Krueger, J. E. and Lineback. D. R. 1987. Carbohydrate-degrading enzymes in cereals. In Enzymes their Role in Cereal Technology, eds. Krueger, J. E. and Lineback. D. R. and Stauffer, C. E., American Association of Cereal Chemists, St Paul, MN, p117.
Kulp, K. and Bechtel, W.G. 1963. Effect of water-insoluble pentosan fraction of wheat endosperm on the quality of white bread. Cereal Chem. 40: 493–504.
Kulp, K. 1968. Pentosans of wheat endosperm. Cereal Science Today 13: 414–426.
Kulp, K. 1981. Oxidation in baking processes. AIB Tech. Bull. 3 (6)
Kulp, K. 1993. Chapter 7- Enzymes as dough improvers. Pages152.In: Advances in Baking Technology. Kamel, B. S., and Stauffer, C. eds. Published in the USA and Canada by VCH publishers, Inc., 220 East 23rd Street, Suite 909, New York, NY.
Kurokawa, Y., Hayashi, Y., Maekawa, A., Takahashi, M., and Kokubo, T. 1982. Introduction of renal cell tumors in F-344 rats by oral administration of potassium bromate a food additives. *Gann*, 73, 335-338

Kurokawa, Y., Maekawa, A., Takahashi, T., Hayashi, Y. 1990. Toxicity and carcinogenicity of potassium bromate – A new renal carcinogen. Environ. Health Perspect., 87. 309-335.
Lagrain, B., Boeckx, L., Wilderjans, E., Delcour, J. A., and Lauriks, W. 2006. Non-contact ultrasound characterization of bread crumb: application of the Biot-Allard model. Food Research International, 39, 1067–1075.
Lallemand Inc., 1996. A Guide to Dough Conditioner Ingredients. Baking update 1:13
Lassoued, N., Babin, P., Della Valle, G., Devaux, M. F., & Réguerre, A. L. 2007. Granulometry of bread crumb grain: considerations of 2D and 3D image analysis at different scale. Food Research International, 40, 1087–1097.
Lee, L., Ng, P. K. W., Whallon, J. H., and Steffe, J. F. 2001. Relationship between rheological properties and microstructural characteristics of non-developed, partially developed, and developed doughs. Cereal Chem. 78 (4): 447-452
Lehman, T. A., and Dreese, P. 1981. Stability of frozen dough – effects of freezing temperatures. Tech. Bull. Am. Inst. Baking, 3(7), 1-5.
Lin, H.I. 2008, "Using enzymes to improve frozen dough bread quality", Kansas state university, Manhattan, KS
Lindahl, L. and Eliasson, A.-C. 1992. A comparison of some rheological properties of durum and wheat flour doughs. Cereal Chem. 69:30-34.
Lineback,D.R., and Rasper, V.F. 1988. Wheat carbohydrate. In: Wheat Chemistry and Techology, Vol.1. Y. Pomeranz, ed. American Association of Cereal Chemists. St. Paul, MN.
Lorenz, K., and Bechtel, W.G. 1964. Frozen bread dough. Bakers Dig. 38(6):59
Lorenz, K., and Bechtel, W.G. 1965. Frozen dough: variety bread; effect of bromate level on white bread. Bakers Dig. 39(4):53
Lorenz, K. 1974. Frozen dough present trend and future outlook. Baker's Dig. 48:14
Lorenz, K., and Kulp, K. 1995. Dough for Bread and Rolls in the United States. Pages 135-151. In: Frozen & Refrigerated Doughs and Batter. Kulp, K., Lorenz, K., and Brummer, J. eds. Am. Assoc. Cereal Chem.: St. Paul, MN.
Lu, W., and Grant, L. A. 1999. Effects of prolonged storage at freezing temperatures on starch and baking quality of frozen doughs. Cereal Chem. 76: 656-672.
Maat, J., Roza, M., Verbakel, J., Stam, H., Santos da Silva, M.J., Bosse, M., Egmond, M.R., Hagemans, M.L.D., van Gorcom, R.F.M., Hessing, J.G.M. and van den Hondel, C.A.M.J.J. 1992. Xylanases and their application in bakery. In 'Xylans and xylanases' (J. Visser et al., eds.), Elsevier Science Publishers, Amsterdam, The Netherlands. pp 349–360.
Mallett, C.P. ed, 1992. Frozen Food Technology, Blackie and Son Limited, Glasgow.
Mariotti, M. and Alamprese, C. 2012. About the use of different sweeteners in baked goods. Influence on the mechanical and rheological properties of the doughs. LWT - Food Science and Technology 48; page 9-15.
Marston, P. E. 1978. Frozen Dough for Breadmaking. Bakers' Digest. 52:18-20, 37.
Mathewson, P. R. 1998. Chapter5 – Application of Enzymes to Baked products. Enzymes. American Associate of Cereal Chemists, St. Paul, MN.
Malloch, J. G., and Cook, W.H. 1930. A volume-measuring apparatus for small loaves. Cereal Chem. 7: 307-310.
Matz, S. A. 1992. Water and Salt. Pages 123-150 in: Bakery Technology and Engineering, third edition. Van Nostrand Reinhold/AVI, New York.

McCleary, B.V. 1986. Enzymatic modification of plant poly sacharides. International J. of Biological Macromolecules 8: 349–354.
McLaunchlan, W. R., Garcia-Conesa, M.T., Williamson, G., Roza, M., Ravenstein, P. and Maat, J. 1999. Biochem. J. 338: 441-446.
McLaunchlan, W. R., Flatman, R.H., Sancho, A.I., Kakuta, J., Faulds, C.B., Elliot, G.O., Kroon, P. A., Furniss, C. S. M., Juge, N., Ravestein, P. and Williamson, G. 2000. The second European Symposium on Enzyme in Grain Processin, VTT Techinical Research Center, Finland.
Merrit, P.P. 1960. The effect of preparation on the stability and performance of frozen, unbaked, yeast-leavened doughs. Bakers Dig.34 (4):57.
Meuser, F. and Suckow, P. 1986. Non-starch polysaccharides. In 'Chemistry and Physics of Baking' (J.M.V. Blan-shard, P.J. Frazier and T. Galliard, eds.), The Royal Society of Chemistry, London, U.K. pp 42–61.
Meziani, S., Kaci, M., Jacquot, M., Jasniewski, J., Ribotta, P., Muller, J. M., Ghoul, M. Desobry, S. 2012a. Effect of freezing treatments and yeast amount on sensory and physical properties of sweet bakery products. J. Food Eng. 111: 336-342.
Meziani, S., Jasniewski, J., Ribotta, P., Arab-Tehrany, E., Muller, Jean-Marc., Ghoul, M. Desobry, S. 2012b. Influence of yeast and frozen storage on rheological, structural and microbial quality of frozen sweet dough. J. Food Eng. 109: 538-544.
Michniewicz, J., Biliaderis C.G. and Bushuk, W. 1991. Effect of added pentosans on some physical and technological characteristics of dough and gluten. Cereal Chemistry 68: 252–258.
Miller, B .S., and Johnoson, J. A. 1947. High levels of alpha amulase in baking (abstract). Bakers Digest 21 (5):38
Miller, K. A. and Hoseney, R.C. 1999. Dynamic Rheological Properties of Wheat Starch-Gluten Doughs. Cereal Chem. 76:105-109
Miller, R. A., and Hoseney, R. C. 2008. Role of salt in baking. Cereal Foods World 53 (1):4.
Mitsuhashi, T., Adachi, K., and Kaneda, Y. 1988. Determination of bromate in foods by gas chromatography. J. Food Hyg. Soc. Japan, 29, 301-305.
Morita, N., A, Arishima, Y., Tanaka,N., and Shiotubo, S., 1997. Utilization of Hemicellulase as Bread Improver in a Home Baker. J. Applied Glycoscience. 44:143-152
Muller, H.G., 1975. Rheology and the conventional bread and biscuit making process. Cereal Chem. 52, 89–105.
Mullins, M. M. 1990. Nonstarch carbohydrate enzymes – technical aspects. Proc. Am. Soc. Bakery Engrs. 66:199.
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 structures of breads made from frozen doughs. Cereal Chem. 81:80-86.
Nakamura, M., Kurata, T., 1997, Effects of L-ascorbic acid on the rheological properties of wheat flour dough. Cereal Chem. 74, 647-650.
Nakamura, M., Noda, M., Murakami, T., Himata., K. 2004. Effect of reducing agents and baking procedure on the residual bromate in bread. Nippon Shokuhin Kagaku Kogaku Kaishi. Vol.51. No5. 229-237.
Navickis, L. L., Anderson, R. A., Bagley, E. B., & Jasberg, B. K. (1982). Viscoelastic properties of wheat flour doughs: variation of dynamic moduli with water and protein content J. Texture Studies, 13, 249–264.

Newberry, M.P., Phan-Thien, N., Larroque, O.R., Tanner, R.I., and Larsen, N.G. 2002. Dynamic and elongation rheology of yeasted bread doughs. Cereal Chem. 79: 874-879.
Nicolas, Y., Smit, R. J. M., van Aalst, H., Esselink, F. J., Weegels, P. L., and Agterof, W. G. M. 2003. Effect of storage time and temperature on rheological and microstructural properties of gluten. Cereal Chem. 80:371-377.
O'Donnell, K. 1996. Methods of bread dough making. AIB Research Department Technical Bulletin Volume XVIII Issue 12.
Oikawa, K., Saito, H., Sakuzume, S., and Fujii, M. 1982. Ion chromatographic determination of bromate in bread. *Bunseki Kagaku*, 31, E251-255
Olesen, T., Qi-Si, J., and Donelyan. V. 2000. Use of lipase in baking. United States Patent. Patent Number 6,110,508.
Oszlanyi, A. G. 1983. Instant yeast. Proc. Am. Soc. Bakery Engrs. 61: 29-31
Oszlanyi, A. G. 1989. Stable yeast for frozen doughs. Proc. Am. Soc. Bakery Engrs. 65: 133
Palmer, E. 1994. Food companies are buying into frozen dough. Milling & Baking news, October 18: pp.28-30
Patil, S.K., Finney, K.F., Shogren, M.D. and Tsen, C.C. 1976. Water-soluble pentosans of wheat flour. III. Effect of water-soluble pentosans on loaf volume of re-constituted gluten and starch doughs. Cereal Chem. 53: 347–354.
Petit-Benvegnen, M.-D., Saulnier, L. and Rouau, X. 1998. Solubilization of arabinoxylans from isolated water-unextractable and wheat flour doughs by cell wall degrading enzymes. Cereal Chemistry 75: 551–556.
Phan-Thien, N, and Safari-Ardi, M. 1998. Linear viscoelastic properties of flour-water doughs at different water concentrations. J. Non-Newton. Fluid. Mech., 74: 137-150
Popper, L. 1997. Simple approaches for identification of baking active xylanases. In 'The first European symposium on enzymes and grain processing' (S.A.G.F. Angelino et al., eds.), TNO Nutrition and Food Research Institute, Zeist, The Netherlands. pp 110–120.
Poulson, C.H., Søe, J.B., Rasmussen, P., Madrid, S.M., Zargahi, M.R., 2006. Lipase and use of same for improving doughs and baked products. European Patent Application, EP 1721527.
Primo-Martín, C., Hamer, R.J., de Jongh, H.H.J., 2006. Surface layer properties of dough liquor components: are they key parameters in gas retention in bread dough? Food Biophysics 1, 83-93
Puppo, M. C., Calvelo, A. and Añón1, M. C. 2005. Physicochemical and Rheological Characterization of Wheat Flour Dough. Cereal Chem. 82:173–181
Pyler, E. J. 2008. Bakery Ingredients part A: Major Ingredients. Page 192-193, 222-225. Bakery Ingredients part B: Minor Ingredients. Page 287, 293, 395 398-399. Bakery Ingredients part C: 402-409 in; Baking Science & Technology. Fourth edition volume 1. Sosland Publishing Company, Kansas City, MO.
Pyler, E. J. 2009. Bakery Ingredients part B: Minor Ingredients. Page 287 in; Baking Science & Technology. Fourth edition volume 2. Sosland Publishing Company, Kansas City, MO.
Qi Si, J., Hansen, T.T., 1994. Effect of lipase on bread-making in correlation with their effects on dough rheology and wheat lipids. In: Proceedings of the International Symposium AACC/ICC/CCOA. The Association, St. Paul, MN
Rao, M.A., Steffe, J.F., 1992. Viscoelastic Properties of Foods, Elsevier Applied Science, New York.

Raphaelides, S., Karkalas, J., 1988. Thermal dissociation of amylose-fatty acid complexes. Carbohydrate Research 172: 65-82.
Rasanen, J., Blanshard, J. M. V., Siitari-kauppi, M, and Autio. K. 1997. Water Distribution in Frozen Lean Wheat DoughsCereal Chem. 74(6):806–813
Rees. N. 2008. Enzymes as processing aids. Cereal Foods World. 53 (3): 124.
Reilly, P.J. 1981.Xylanases: structure and function. In 'Trends in the Biology of Fermentation for Fuels and Chemicals' (A. Hollaender, ed.), Basic Life Sciences, Plenum Press, New York, pp 111–129.
Reiner, M. 1964, The Deborah Number. Phys. Today, 17: (1) 62
Ribotta, P. D., Leon, A. E., and Anon, M. C. 2003. Effects of Yeast Freezing in Frozen Dough. Cereal Chem. 80:454-458.
Riisom, T., Krog, N., Eriksen, J., 1984. Amylose complexing capacities of cis- and trans-unsaturated monoglycerides in relation to their functionality in bread. J. of Cereal Sci. 2: 105-118.
Rogers, D. E., Day, D. D., and Olewnik, M. C. 1995. Development of an Objective Crumb-Grain Measurement. Cereal Foods world. 40:498-501.
Rouau, X., El-Hayek, M-L. and Moreau, D. 1994. Effect of an enzyme preparation containing pentosanases on the bread making quality of flours in relation to changes in pentosan properties. Journal of Cereal Science 19: 259–272.
Rouau, X. and Surget, A. 1998. Evidence for the presence of a pentosanase inhibitor in wheat flours. J. of Cereal Sci. 28: 63-70
Rouillé, J., Valle, G.D., Lefebvre, J., Sliwinski, E., and Van Vliet, T. 2005. Shear and extensional properties of bread doughs affected by their minor components. J Cereal Sci.(42):45–57
Sahi, S. S., and Guy, R. C. E. 2004. New Lipase Functionality in Bakery Products. Proceedings of the 12th international ICC cereal and bread congress, Edts: Cauvain, S. P., Salmon, S. S., and Young, L. S., Woodhead publishing Ltd.
Salvador, A., Sanz, T., and Fiszman, S. 2006. Dynamic rheological characteristics of wheat flour-water doughs. Effect of adding NaCl, sucrose, and yeast. Food Hydrocolloid. 20: 780-786.
Sanders, S. W. 1990. Nonstarch carbohydrate enzymes- bakery applications. Proc. Am. Soc. Bakery Engrs. 66: 203.
Sandhu, H. P. S., Manthey, F.A., Simsek, S., Ohm, J. 2011. Comparison between potassium bromate and ozone as flour oxidants in bread making. Cereal Chem. 88 (1): 103-108.
Sapirstein, H. D., Roller, R., and Bushuk, W. 1994. Instrumental Measurement of Bread Crumb Grain by Digital Image Analysis. Cereal Chem. 71:383-391.
Sarker, D.K.,Wilde, P.J. and Clark, D.C. 1998. Enhancement of protein foam stability by formation of wheat arabinoxylan-protein crosslinks. Cereal Chem. 75:493–499.
Sato, K. 2003. Know the volume of food by three dimension volume measurement device. Techno Tokyo 21, vol. 127; 9.
Sato, K. 2007. Measured food volume by three dimension volume measurement device. Tokyo metropolitan food technology research center newsletters. No.3
Seguchi, M., Nikaidoo, S., Morimoto, N., 2003. Centrifuged liquid and bread making properties of frozen-and-thawed bread dough. Cereal Chem. 80, 264–268.
Seguchi, M., Morimoto, N., 2011. Restoration of Bread making Properties to Frozen Dough by Addition of Sugar and Yeast and Subsequent Processing. Cereal Chem. 88(4), 409–413.

Selomulyo, V. O. and Zhou, W. 2007. Frozen bread dough: Effects of freezing storage and dough improver. J. Cereal Sci. vol.45: 1-17

Sherman, P., 1970. Industrial Rheology, with Particular Reference to Foods, Pharmaceuticals, and Cosmetics, Academic Press, London.

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

Sideleau, P. 1987. Freezing and thawing of unbaked products. Proc. Am. Soc. Bakery Eng., p89

Silverglade, B. and Sperling, A. 2005. Published online at www.fda.gov/ohrms/dockets/dockets/98n0359/98n-0359-c000120-01-vol9.pdf. Re: Docket Number 1998N-0359, CFSAN Program Priorities for FY 2006, 70 Fed. Reg. 29328-29329 (May20, 2005). Center for Science in the Public Interest.

Siswoyo, T.A., Tanaka, N., Morita, N., 1999. Effects of lipase combined with α-amylase on retrogradation of bread. Food Science and Technology Research 5: 356-361.

Sliwinski, E.L., Kolster, P., van Vliet, T., 2004. On the relationship between large-deformation properties of wheat flour and baking quality. J. Cereal Sci. 39: 231–245.

Sluimer, P. 2005. Chapter 3- Optional ingredients and Chapter 8- Frozen Dough. Principles of Breadmaking: Functionality of Raw Materials and Process Steps. American Association of Cereal Chemists, St. Paul, MN.

Smolarz, A., Van-Hecke, E., and Bouvier, J. M. 1989. Computerized image analysis and texture of extruded biscuits. J. Texture Stud. 20: 223.

Song, Y., and Zheng, Q. 2007. Dynamic rheological properties of wheat flour dough and proteins. Trends Food Sci. Technol. 18: 132-138.

Spooner, T. F. 1998. Why frozen dough formulas are different. In: Milling & Baking News, December 1. Page:14-16

Sprossler, B.G. 1997. Xylanases in baking. In 'The first European symposium on enzymes and grain processing' (S.A.G.F. Angelino et al., eds.), TNO Nutition and Food Research Institute, Zeist, The Netherlands. pp 177–187.

Sroan, B. and MacRitchie, F. 2008. Mechanism of gas cell stability in breadmaking. Pages 299-306 in: Bubbles in Food 2: Novelty, Health and Luxury. G. Campbell, M. Scanlon, and D. Pyle, eds. AACC International: St. Paul, MN.

Stampfli, L., Nersten, B., 1995. Emulsifiers in bread making. Food Chemistry 52: 353-360.

Stauffer, C. 1993. Chapter 4- frozen dough production. Pages92.Chapter 13- Fats and fat replacers. Pages 356-357. In: Advances in Baking Technology. Kamel, B. S., and Stauffer, C. eds. Published in the USA and Canada by VCH publishers, Inc., 220 East 23rd Street, Suite 909, New York, NY.

Steffe, J.F. Rheological methods in food process engineering. 1st ed. Mich.: Freemans Press; 1992. Viscoelasticity; pp. 168–93

Steffe, J.F, 1996. Chapter 5- Viscoealstisity; "Rheological methods in food process engineering 2nd edition",

Stenvert, N. L., Moss, R., and Bond, E. E. 1978. Sixth Int. Cereal and Bread Congress, Winnipeg, Canada,Sept.21.

Sullivan, Betty, Howe, M., and Schmalz, F. D. 1936. Cereal Chem.: 665.

Takano, H., Naito, S., Ishida, N., Koizumi, M., and Kano, H. 2002. Fermentation Process and Grain Structure of Baked Breads from Frozen Dough Using Freeze-Tolerant Yeasts. J. Food Sci. 67: 2725-2733

Takeya, K. 2005. Chapter of Bake mathematics. Calculation of specific volume. In: New Baking Guide. Takeya, K. and Nishikawa, T. eds. Published in Pan news Co., Tokyo, Japan.
Tannahill, R, 1973. Part 2 – The near east, Egypt, and Europe, "Food in history", Printed in the United States of America Stein and day/ publisher/ 7 East 48 Street, New York, N.Y. 10017.
Tenney, R. J., and Schmidt, D. M. 1968. Sodium Stearoyl-2-Lactylate. Its functions in yeast-leavened bakery products. Bakery Dig.
The Freedonia Group, Inc. 2011. "FREEDONIA FOCUS ON Grain Based Foods", http://www.orbiscascade.org/index/cms-filesystem-action/electronic_resources/fredoniaus.pdf
Thompson, J. B. and Buddemeyer B.D. 1954. Improvement of flour mixing characteristics by a stearyl lactylic acid salt. Cereal Chem. 31: 296-302
Thompson, J. B. and Buddemeyer B.D. 1961. Cake mixes and improved shortenings therefore, U.S. Patent 2,789,992 (February 28)
Tracy,M.V.1964. The role of wheat flour pentosan in baking. I. Enzymic destruction of pentosans in situ. Journal of the Science of Food and Agriculture 15:607–611.
Tressler, D. K., Van-Arsdel, W. B. and Copley, M, U., eds. 1968. The freezing preservation of foods, 4th edn, vol.4. The AVI publishing Company, Westport, CT.
Tronsmo, M. K, Magnus,E. M., Baardseth, P., Schofield, J.D. Aamodt,A., andFærgestad, E. M. 2003. Comparison of small and large deformation rheological properties of wheat dough and gluten, Cereal Chem 80(5): 587-595
Tsen, C. C. 1964. Ascorbic acid as a flour improver, Baker's Dig. 38 (5): 44-47.
Umemura, T., Sai, K., Takage, A. Hasegawa, R., Kurokawa, Y. 1993. A possible role for cell proliferation in potassium bromate ($KBrO_3$) carcinogenesis. J. Cancer Res. Clin. Oncol., 119. 463-469.
Underkofler, L. A. 1972. Chapter 1 Enzymes in Handbook of Food Additives. 2nd Edition. Vol. I. CRC Press.
Uthayakumaran, S., Newberry, M., Keentook, M., Stoddard, F., and Bekes, F. 2000. Basic rheology of bread dough with modified protein content and glutenin-to-gliadin ratios. Cereal Chem. 77: 744-749.
van Vliet, T., Janssen, A.M., Bloksma, A.H., Walstra, P., 1992. Strain hardening of dough as a requirement for gas retention. Journal of Texture Studies 23, 439–460.
van Vliet, T., 1999. Rheological classification of foods and instrumental techniques for their study. In: Rosenthal, A.J., (Ed.), Food Texture: Measurement and Perception, Aspen Publishers, Maryland, USA
van Vliet, T., 2008. Strain hardening as an indicator of bread-making performance: A review with discussion. J. Cereal Sci. 48: 1–9
Varilek, P. and Walker, C.E, 1983, "Baking and ovens, history of heat technology", Bakers Dig. 57: p52-59
Varriano-Marston, E., Hsu, K. H., and Mahdi, J. 1980, Rheological and structural change in frozen dough, Bakers Dig, 54(1), 32-35
Vetter, L. J., 1979. "Frozen Unbaked Bread Dough: Past, Present, Future", Cereal Foods World, Vol.24: P42-43
Vincent, P.I., 1960. The necking and cold-drawing of rigid plastics. Polymer 1, 7–19.
Walker, C., and Hazelton, J. 1996. Dough rheological tests. Cereal Food World. 41: 23-28.

Wang, F. C. and Sun, X. S. 2002. Creep-Recovery of Wheat Flour Doughs and Relationship to Other Physical Dough Tests and Breadmaking Performance. Cereal Chem. 79(4):567–571

Wang, Z.J. and Ponte, J.G., 1994. Improving frozen dough qualities with the addition of vital wheat gluten. Cereal Foods World, Vol.39. No.7: P42-43

Weak, E. D., Hoseney, R. C., Seib, P. A., and M. Biag. 1977. Mixograph studies Ⅰ. Effect of certain compounds on mixing properties.

Weegels, P. L. and Hamer, R. J. 1992. Improving the bread-making quality of gluten. Cereal foods world. Vol.37. No.5 pp.379-385.

Weipert, D. 1987. Present stage and new feasibilites in bread dough rheology. Getreide Mehl Brot 41, (11): 325.

Weipert, D. 1990. The benefits of basic rheometry in studying dough rheology. Cereal Chem. 67: 311-317.

Werner, L. E. 1981. Shortening Systems. Proc. Am. Soc. Bakery Engrs. 57:61-70

Whitworth, M. B., Cauvain, S. P., and Cliffe, D. 2004. Measurement of bread cell structure by image analysis. In: Using cereal science and technology for the benefit of consumers, proceedings of the 12th International ICC Cereal and Bread Congress 23-26th May 2004, Harrogate, UK. Woodhead Publishing Ltd. 2008.

Whorlow, R.W., 1992. Rheological Techniques, 2nd ed., Rheological Techniques 2nd., Ellis Horwood, Chichester, UK.

Williams, A. and Pullen, G. (1998) Functional ingredients, in Technology of Breadmaking (eds S.P. Cauvain and L.S. Young), Blackie Academic & Professional, London, UK, pp. 45–80.

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

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

Xu, H., Huang, W., Jia, C., Kim, Y., and Liu, H. 2009. Evaluation of water holding capacity and bread making properties for frozen dough containing ice structuring proteins from winter wheat. J. Cereal Sci. 49, 250-253.

Yamada, Y., Perston, K. R. 1992. Effects of individual oxidants on oven rise and bread properties of Canadian short process bread. J. Cereal Sci. 15, 237-251.

Yeh, Y., Feeney, R.E., 1996. Antifreeze proteins: structures and mechanisms of function. Chemical Reviews 96, 601–618.

Zahringer, M. V., Mayfield, H. L., and Odland, L. M. 1951. The effect of certain variations in fat, yeast and liquid on the frozen storage of yeast doughs. Food Res. 16: 353-357.

Zayas, I. Y. 1993. Digital Image Texture Analysis for Bread Crumb Grain Evaluation. Cereal Food World. 38:760-762, 764-766.

Zghal, M. C., Scanlon, M. G., and Sapirstein, H. D. 1999. Prediction of Bread Crumb Density by Digital Image Analysis. Cereal Chem. 76:734-742.

Zghal, M.C., Scanlon, M.G., Sapirstein, H.D., 2002. Cellular structure of bread crumb and its influence on mechanical properties. J. Cereal Sci. 36: 167–176.

Zhang, C., Zhang, H., Wang, L., 2007a. Effect of carrot (Daucus carota) antifreeze proteins on the fermentation capacity of frozen dough. Food Research International 40, 763–769.

Zhang, C., Zhang, H., Wang, L., Gao, H., Guo, X., Yao, H., 2007b. Improvement of texture properties and flavor of frozen dough by carrot (Daucus carota) antifreeze protein supplementation. J. of Agri. and Food Chem. 55, 9620–9626.
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.