Effect of Barley Flour Rich in Beta-Glucan on Rheological Properties of Frozen Dough and Quality of Bread and Cookies

By

Abdelmagid Hamed

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements for the degree of
Master of Science
in
Food Science

Guelph, Ontario, Canada

© Abdelmagid Hamed, December, 2013
ABSTRACT

EFFECT OF BARLEY FLOUR RICH IN β-GLUCAN ON RHEOLOGICAL PROPERTIES OF FROZEN DOUGH AND QUALITY OF BREAD AND COOKIES

Abdelmagid Hamed
University of Guelph, 2013

Co-Advisor:
Dr. Elsayed Abdel-Aal
Co-Advisor:
Dr. Massimo Marcone

Storage of dough/batter at freezing temperature unfavorably affects the final quality of baked products. The objective of this study was to investigate the effect of air-classified barley flour rich in β-glucan (~25%) on the rheological properties of frozen dough and batter and quality of their end product. Doughs and batters were made from control wheat flour and barley/wheat flour blend without or with vital gluten and stored at -18°C for up to 8 weeks. Dough rheology was affected by frozen storage to different extent with composite doughs exhibiting the least changes compared to wheat flour dough. Composite doughs contained higher amount of bound water, less freezable water content and less gluten network changes as indicated by Nuclear Magnetic Resonance, Differential Scanning Calorimetry and Scanning Electron Microscopy. Up to 4 weeks of storage, bread made from both control and composite frozen doughs had exhibited similar loaf specific volume and crumb texture profile compared to their corresponding once from fresh dough. Frozen storage resulted in no changes in composite batter hardness and stickiness and cookie spread factor compared to the control. While cookie breaking force decreased as frozen storage period increased. Both composite bread and cookie contained high amount of soluble β-glucan with slight changes molecular weight or solubility during frozen storage. This study has shown a good potential for developing fiber-rich bread and cookie from frozen dough and batter by incorporating air-classified barley flour as a rich source of β-glucan without compromising the quality of the end products when dough/batter was stored up to 4 weeks at -18°C.
DEDICATION

I would like to dedicate this thesis to my wonderful family; my beloved parents—my mother Mrs. Amina, my father Mr. Nageeb, my younger brother Ibrahem and sisters; Aisha and Ahlam for their unconditional, selfless love, support and understanding.

Thank you very much for all your help.
ACKNOWLEDGEMENTS

First and foremost I would like to thank Allah the Gracious, the Merciful.

I would like to take this opportunity to express my gratitude to my co-advisors, Dr. Elsayed Abdel-Aal and Dr. Sanaa Ragaee for their guidance, patience, and encouragement throughout my MSc study. Thank you for providing your knowledge and experience to assist me during my graduate studies. I am also thankful to my advisor Dr. Massimo Marcone for his valuable input and support to complete my MSc Thesis. Drs. Elsayed Abdel-Aal and Sanaa Ragaee deserve special mention and thanks for their support and efforts during the period of my studies to help me understand every concept of my research and in writing my thesis. I would also like to express my gratitude to Dr. Koushik Seetharaman for giving me the chance to be one of the students at the Department of Food Science, University of Guelph under the supervision of Dr. Sanaa Ragaee. Last but not least I would like to express my gratitude to Dr. Loong-Tak Lim for being my examiner.

I wish to extend my sincerest thanks and appreciation to all those who have helped and supported me all throughout my endeavor. I also want to convey my appreciation to the members of the cereal and bake laboratories at the Food Science Department, University of Guelph, and Ms. Iwona Rabalski at Guelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph.

Lastly and most importantly, I wish to thank my family who always pray for my success. They taught, loved and supported me to achieve higher goals in life. Their concern in me can never be fully returned but will always be remembered.
# TABLE OF CONTENTS

Dedication .......................................................................................................................... iii
Acknowledgements ............................................................................................................... iv
Table of Contents ................................................................................................................ v
List of Tables ........................................................................................................................ x
List of Figures ....................................................................................................................... xi

1. CHAPTER 1: Introduction ............................................................................................... 1
2. CHAPTER 2: Literature Review ....................................................................................... 5
   2.1. Importance of Frozen Dough ................................................................................... 5
   2.2. Frozen Dough ......................................................................................................... 6
   2.3. Quality of Frozen Dough ....................................................................................... 7
   2.4. Rheological Properties of Frozen Dough ............................................................... 9
      2.4.1. Empirical Rheological Methods (Large Deformation Rheology) Dough Extensibility ................................................................. 9
      2.4.2. Empirical Rheological Methods (Large Deformation Rheology) Dough Stickiness ............................................................... 10
      2.4.3. Fundamental Rheological Analysis (Small Deformation Rheology) .......... 11
      2.4.4. Differential Scanning Calorimetry (DSC) analysis, Nuclear Magnetic Resonance ($^1$H-NMR) spectrometer analysis and Scanning Electron Microscopy Analysis ............................................................................................................... 12
   2.5. Basic Ingredients of Baked Products and their Roles in the Dough/Batter System ............................................................................................................................... 17
      2.5.1. Wheat Flour ..................................................................................................... 17
3.3. Materials and Methods...........................................................................................................48
  3.3.1. Materials..........................................................................................................................48
  3.3.2. Sample Preparation...........................................................................................................48
  3.3.3. Dough Frozen Storage and Thawing Conditions..............................................................49
3.4. Methods...................................................................................................................................49
  3.4.1. Chemical Composition of Wheat, Barley, and Composite Flours.................................49
  3.4.2. Quality of Wheat and Composite Flours...........................................................................49
  3.4.3. Rheological Properties of Dough......................................................................................50
  3.4.4. Freezable Water Content using Differential Scanning Calorimetry..............................52
  3.4.5. Water Mobility in Dough using Nuclear Magnetic Resonance......................................53
  3.4.6. Weight Loss, Moisture Content and Water Activity of Doughs.......................................54
  3.4.7. Micro-Structure of Dough using Scanning Electron Microscopy...................................54
  3.4.8. Physicochemical Characteristics of β-Glucan.................................................................55
  3.4.9. Statistical Analysis............................................................................................................56
3.5. Results and Discussion............................................................................................................56
  3.5.1. Quality of Wheat and Composite Flours.........................................................................56
  3.5.2. Moisture Content, Water Activity and Weight Loss of Dough.........................................59
  3.5.3. Rheological Properties of Dough.....................................................................................60
  3.5.4. Differential Scanning Calorimetry....................................................................................65
  3.5.5. Water Mobility in Dough using Nuclear Magnetic Resonance.......................................67
  3.5.6. SEM Micro-Structure of Dough.......................................................................................68
  3.5.7. Physicochemical Properties of β-Glucan..........................................................................70
3.6. Conclusion.................................................................................................................................72
4. CHAPTER 4: Effect of β-Glucan-Rich Barley Flour on Quality of Bread Baked from Yeasted Frozen Dough

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1. Abstract</td>
<td>90</td>
</tr>
<tr>
<td>4.2. Introduction</td>
<td>91</td>
</tr>
<tr>
<td>4.3. Materials</td>
<td>93</td>
</tr>
<tr>
<td>4.4. Preparation of Composite Flours and Doughs</td>
<td>93</td>
</tr>
<tr>
<td>4.5. Storage, Thawing and Baking Conditions of Frozen Dough</td>
<td>93</td>
</tr>
<tr>
<td>4.6. Methods</td>
<td>94</td>
</tr>
<tr>
<td>4.6.1. Chemical Composition of Wheat, Barley, and Composite Flours</td>
<td>94</td>
</tr>
<tr>
<td>4.6.2. Bread Loaf Specific Volume</td>
<td>94</td>
</tr>
<tr>
<td>4.6.3. Bread Crumb Texture Profile Analysis (TPA)</td>
<td>95</td>
</tr>
<tr>
<td>4.6.4. Physicochemical Characteristics of β-Glucan</td>
<td>95</td>
</tr>
<tr>
<td>4.6.5. Statistical Analysis</td>
<td>96</td>
</tr>
<tr>
<td>4.7. Results and Discussion</td>
<td>97</td>
</tr>
<tr>
<td>4.7.1. Bread Loaf Specific Volume</td>
<td>97</td>
</tr>
<tr>
<td>4.7.2. Bread Crumb Moisture Content and Water Activity</td>
<td>99</td>
</tr>
<tr>
<td>4.7.3. Crumb Texture Profile of Breads</td>
<td>99</td>
</tr>
<tr>
<td>4.7.4. Physicochemical Properties of β-Glucan</td>
<td>101</td>
</tr>
<tr>
<td>4.8. Conclusion</td>
<td>102</td>
</tr>
</tbody>
</table>

5. CHAPTER 5: Effect of β-Glucan-Rich Barley Flour on Quality of Cookies Baked from Frozen Batter

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1. Abstract</td>
<td>109</td>
</tr>
<tr>
<td>5.2. Introduction</td>
<td>110</td>
</tr>
</tbody>
</table>
5.3. Materials....................................................................................................................... 111
5.4. Preparation of Composite Flours and Batter......................................................... 112
5.5. Frozen Storage and Thawing of Batter and Cookie-Baking................................. 112
5.6. Methods..................................................................................................................... 113
   5.6.1. Chemical Composition...................................................................................... 113
   5.6.2. Pasting Characteristics of Wheat and Composite Flours............................ 113
   5.6.3. Hardness and Stickiness of Batter................................................................. 114
   5.6.4. Moisture Content and Water Activity of Batter and Cookies..................... 115
   5.6.5. Spread Factor and Breaking Force of Cookies.............................................. 115
   5.6.6. Physicochemical Analysis of β-Glucan......................................................... 116
   5.6.7. Statistical Analysis......................................................................................... 117
5.7. Results and Discussion............................................................................................ 117
   5.7.1. Pasting Characteristics of Wheat and Composite Flours............................. 117
   5.7.2. Hardness and Stickiness of Batter................................................................. 118
   5.7.3. Moisture Content and Water Activity of Batter and Cookies..................... 120
   5.7.4. Spread Factor and Break Force of Cookies.................................................. 121
   5.7.5. Physicochemical Properties of β-Glucan....................................................... 124
5.8. Conclusion............................................................................................................... 125
6. CHAPTER 6: Conclusions......................................................................................... 138
7. References Cited......................................................................................................... 140
LIST OF TABLES

Table 3.1: Bread Formulation Based on 100g Flour..................................................73
Table 3.2: Approximate Chemical Composition of the Flour Samples (Dry Base)........74
Table 3.3: Quality of Wheat and Composite Flours.....................................................75
Table 5.1: Cookie Formulations Based on 225g Flour.............................................127
Table 5.2: Approximate Chemical Composition of the Flour Samples..................128
Table 5.3: Average RVA Starch Pasting Properties of Soft Wheat, Barley and Composite
Flours.........................................................................................................................129
LIST OF FIGURES

Figure 2.1: Process Flow Diagram of Conventional and Frozen Dough/Batter Baking...42

Figure 2.2: Flowchart of the Air-Classification Process for Barley Flour..........................43

Figure 2.3: Schematic Representation of β-Glucan..........................................................44

Figure 3.1: Example of DSC Curve Showing Ice Fusion Enthalpy (ΔH_{fw}), Onset (T_{onset})
and Peak (T_{peak}) Temperatures......................................................................................76

Figure 3.2: Water Absorption of Control and Composite Flour Samples at 500 Brabender
Units..................................................................................................................................77

Figure 3.3: The Summarized Results of Farinograph Profiles for Different Flours.........78

Figure 3.4: Gluten Peak Tester Analysis of Control (C) and Composite Flours without
(ACB) and with 1.4% Vital Gluten (ACB-G)....................................................................79

Figure 3.5: Weight Loss, Moisture Content and Water Activity of Fresh and Frozen-
Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks....80

Figure 3.6: Empirical Rheology Analysis (Large Deformation Rheology) of Fresh and
Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8
Weeks................................................................................................................................81

Figure 3.7: Fundamental Rheology Analysis (Small Deformation Rheology) of Fresh and
Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8
Weeks................................................................................................................................82

Figure 3.8: Differential Scanning Calorimetry Analysis of Fresh and Frozen-Thawed
Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks..................83
Figure 3.9: A: NMR Curves for Water Distribution. B: Relaxation Time of Water Molecules from Nuclear Magnetic Resonance Analysis of Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks

Figure 3.10: Approximate Percentage of Bound Water Calculated from NMR Data from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks

Figure 3.11: Scanning Electron Microscope Images of Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks

Figure 3.12: Increase (%) in Solubility of β-Glucan in Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks

Figure 3.13: Reduction (%) in β-Glucan molecular weight (Mw) in Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks

Figure 4.1: Loaf Specific Volume of Bread Made from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks

Figure 4.2: Appearance of Bread Made from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks

Figure 4.3: Moisture Content and Water Activity of Bread Crumb Made from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks

Figure 4.4: Texture Profile Analysis (TPA) of Bread Crumb Made from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 4.5: A: Increase (%) in Soluble β-Glucan Amount. B: Reduction (%) in Molecular Weight of β-Glucan in Bread Crumb Made from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks ........................................108

Figure 5.1: Rapid Visco Analyser (RVA) Viscograms of Soft Wheat and Barley Composite Flours..........................................................................................................................130

Figure 5.2: Hardness and Stickiness of Fresh Cookie Batter and Spread Factor Their Cookies with Different Levels of Air-Classified Barley Flour and Water Added to the Formula........................................................................................................................................131

Figure 5.3: Hardness and Stickiness of Fresh and Frozen-Thawed Control and Composite Batters Stored at -18°C for 4 Weeks..................................................................................................132

Figure 5.4: Moisture Content and Water Activity of Fresh and Frozen-Thawed Control and Composite Batters Stored at -18°C for 4 Weeks.................................................................133

Figure 5.5: Moisture Content and Water Activity of Cookies Made from Fresh and Frozen-Thawed Control and Composite Batters Stored at -18°C for 4 Weeks.....................134

Figure 5.6: Spread Factor and Breaking Force of Cookies Made from Fresh and Frozen-Thawed Control and Composite Batters Stored at -18°C for 4 Weeks..............................135

Figure 5.7: A: Increase (%) in Soluble β-glucan Amount. B: Reduction (%) In Molecular Weight of β-Glucan in Fresh and Frozen-Thawed Control and Composite Batters Stored at -18°C for 4 Weeks ..................................................................................................................136

Figure 5.8: A: Increase (%) in Soluble β-Glucan Amount. B: Reduction (%) in Molecular Weight of β-Glucan in Cookies Made from Fresh and Frozen-thawed Control and Composite Batters Stored at -18°C for 4 Weeks.........................................................137
CHAPTER 1:
INTRODUCTION

In recent years, frozen dough market has grown rapidly due to a big demand for convenient and fresh-taste bakery products by consumers and food services. Therefore, the bakery industry has been exploiting the advantages and applications of freezing technology in several frozen dough bakery products such as; par-baked or fully baked breads and rolls, pizza dough balls, etc. A major shortcoming of frozen dough is substantial deterioration of baking quality with increasing freezing storage period (Huang et al. 2008; Asghar et al. 2011). This requires more research to understand changes in dough during freezing storage.

Research in frozen dough, which is considered one of the fastest growing food processing industries, has been carried out to improve the quality characteristics to be comparable to the freshly prepared bakery products. The use of additives has become a common practice in the business of baking to improve the quality of frozen dough. Some additives have been used to overcome certain problems that face frozen dough production and utilization. The main role of most of the additives used is to interact with water in dough, which could lessen the damaging effect caused by ice crystals formed during freezing. The additives that have been used in frozen dough are safe and natural, and exhibit thickening functions as well as water binding and gelling properties such as hydrocolloids.

Several studies have investigated the effect of different additives such as; egg yolk and sugar ester (Hosomi et al. 1992), locust bean gum and xanthan gum (Lo and Ramsden 2000) and whey protein, polyols (sugar alcohol), carboxy methyl cellulose and gum Arabic (Asghar et al. 2005a, b, 2009) on the quality of frozen dough. Sharadanant and Khan (2006) observed better
retention of the starch-gluten network when incorporate selected gums (locust bean gum and gum Arabic) into basic bread dough formula (100%wheat flour) after different periods of frozen storage at −23°C from 4 to 16 weeks. Leray et al. (2010) reported that dough resistance to freezing and frozen storage was increased with the addition of a blend of dietary fiber (inulin and oat fibers). Most of the studies concluded that stability of frozen dough can be enhanced with the addition of food gums, but no study so far has investigated barley flour rich in β-glucan on the quality and functionality of frozen dough.

It is well known that incorporation of dietary fiber or sources of dietary fiber have negative effects on the quality of the final baked product. Incorporation of large amounts of barley or oat as rich sources of β-glucan could lead to quality defects of the end products. This brought the need to develop new processing technologies to obtain more concentrated sources of β-glucan and consequently minimize or even eliminate any negative effects on the food product quality (Vasanthan and Temelli 2008). Air-classification technology was introduced as an effective way to produce barley flour with high β-glucan content (Ferrari et al. 2009).

Foods rich in β-glucan have shown several health benefits and a lowering effect of the risks of numerous diseases including cardiovascular disease, diabetes, obesity and cancer (Brownlee 2011; Ahmad et al. 2012; Aldughpassi et al. 2012). The viscous property of β-glucan may result in reducing the absorption of lipids and bile acid (Wood 2010). This encourages highly competitive companies to exploit β-glucan as a functional food ingredient in many products, particularly cereals and baked products such as; bread, muffins, cookies, etc. The high water holding capacity of β-glucan allows it to have a significant effect on one of the most important processing parameter in baking industry which is the water behaviour of flour-based products.
This effect extends to affect rheological and functional properties like viscosity and consistency (Wood 2010).

The functional and nutritional effects of β-glucan are controlled not only by concentration, but also by solubility and molecular weight (Mw) (Wood 2010). Thondre and Henry (2009) showed that the higher the β-glucan Mw, the better benefits since low Mw β-glucan did not exhibit the expected health benefits. Processing, cooking and storage are critical factors that are capable of altering β-glucan solubility and Mw in the food matrix. As a result, affecting the viscosity generated by β-glucan that is responsible for β-glucan health benefits (Regand et al. 2009; Tiwari and Cummins 2009). Therefore, it is recommended to wisely select the right processing and cooking technique in order to preserve the desired nutritional functionality of β-glucan (Aldaghpassi et al. 2012).

At present there is no enough data available in the literature about the effect of frozen storage on β-glucan properties. However, several previous studies (Cleary et al. 2007; Flander et al. 2007; Andersson et al. 2008) have tried to introduce the beneficial effects of β-glucan to the consumers in bread. It was concluded that the beneficial effects of β-glucan are influenced when incorporated into the bread system particularly due to depolymerization of the molecule during dough mixing and fermentation. On the contrary, Moriartey (2010) reported that incorporating barley β-glucan concentrate of low solubility in bread at levels of 3.75, 5 and 7.5g/100g wheat flour resulted in no significant effect on β-glucan solubility. Andersson et al. (2004) also reported that there is no significant difference in the molecular weight of β-glucan between the final dough and the baked bread. However, the extent at which β-glucan can be degraded and still maintain its physiological effects still under investigation (Cleary et al. 2007; Flander et al. 2007; Andersson et al. 2008) and all of these previous studies was done on fresh doughs.
As a result, only the β-glucan degradation effect of the dough mixing and proofing processes were reported and no data available about the effect of low temperature storage of dough on β-glucan characteristics.

**Hypothesis:**

Incorporation of air-classified barley flour rich in β-glucan in bread dough and cookie batter stored at low temperature (-18°C) will increase their shelf life through the restriction of water mobility in the dough or batter system during frozen storage.

The main goal of the study is to improve bread dough and cookie batter stability under low temperature storage (freezing at -18°C) by incorporating air-classified barley flour rich in β-glucan. In specific the following objectives were investigated:

1. Determining the effect of incorporating barley flour rich in β-glucan on rheological properties of frozen bread dough and cookie batter.
2. Investigating the effect of β-glucan on water redistribution in frozen-thawed dough.
3. Investigating the changes in β-glucan molecular weight characteristics and solubility during frozen storage.
4. Evaluating baking quality of bread and cookie made from frozen dough and batter with the presence of air-classified barley flour rich in β-glucan.
CHAPTER 2:

LITERATURE REVIEW

2.1. Importance of Frozen Dough

In recent decades, centralization of retail distribution of food products has become necessary as a response to the demographic evolving of societies and economic constrains. Frozen dough allow dough manufacturing facilities to prepare huge amounts of dough, freeze it, and ship it to the retailers, food services and in store bakeries for final baked product manufacturing. The major shortcoming of the frozen dough is the short shelf life of six to eight weeks.

In many countries around the world, freezing technology is commonly used for the storage of different food products as it has drastically improved and became relatively inexpensive. Along with that, the production of frozen dough has increased tremendously because the fresh baked products have a very short shelf life, the increasing demand for quick and convenient food, and the rapid growth in number of in-store bakeries (Schroeder 1999; Laaksonen 2001).

In addition, the production of baked products from frozen dough allows easier and more profitable baking. In other words, it reduces the production costs by facilitating transportation and avoiding the need to skilled labour since frozen dough is ready to bake and easy to handle. It also allows fresh baked products, with desirable quality and sensory characteristics comparable to the conventional fresh baked products, to be available around the clock (Gelinas et al. 1995). Nowadays, frozen dough become the superior product in the market of frozen bakery products and developed significantly in the industry.
2.2. Frozen Dough

Freezing is one of the oldest methods of food preservation. The physical principle of freezing involves the separation of water from dough by decreasing the temperature to a point somewhere below the temperature where ice crystals are formed inside the food. The smaller the ice crystals formed during the freezing process, the better the quality and texture of frozen foods. Generally, the size of ice crystals depends on the freezing rate at which the ice crystals are formed. Large ice crystals are formed in slow freezing processes, while in rapid freezing forms a relatively large number of small ice crystals. Rapid freezing not only produces large number of small ice crystals in the frozen food material, but also promotes more uniform crystallization of water throughout the material leading to a higher quality frozen product. Commercially Blaster freezers and contact freezers are most widely used at -35 to -40°C. After the dough ingredients are mixed, the formed dough is molded and then rapidly frozen to avoid yeast activation, which is detrimental to storage stability (Gelinas et al. 1995). Once the dough is placed in the freezer it starts to freeze from the outside and slowly towards the center since dough, similar to other foods, has poor thermal conductivity compared to metal (Nesvadba 2009).

Compared to freshly baked products, quality of baked goods prepared from frozen dough are often poorer. Frozen storage may result in physical state changes of frozen dough, which contribute to the quality of the final baked product. There are many factors affect the quality of frozen dough such as, dough ingredients, processing steps, frozen storage temperature and period, and thawing conditions (Reid 1990).

The freezing process is basically a formation of ice. This process can be categorized in three major steps (Luyet 1968): Nucleation or initiation step, which is the formation of the ice
crystal seed, followed by propagation (crystal growth) or growth step and the formation of the main body of the ice crystal. The rate of propagation of solutions is lower than that of pure water. It decreases as the concentration of impurities increases. As the temperature decreases, the amount of ice crystals increases. Along with that, the solute concentration of the unfrozen phase increases. Therefore, in a certain concentration of solutes the unfrozen phase reaches its maximum. At that point the crystallization will stop as the unfrozen matrix solidifies (Roos 1995) and the maturation or recrystallization step could occur. This step includes any changes in the number, size, shape or orientation of ice crystals after the initial ice crystals formation. As frozen storage time increases, ice crystals increase in size and decrease in number through redistribution of water from small to large crystals (Fennema 1973).

Recrystallization occurs by different mechanisms (Roos 1995; Pham and Mawson 1997) including; Accretion, in which two close ice crystals contact directly by the surface and a bridge formed; isomass, the ice crystal enters a lower energy state without mass transfer with surroundings, which causes a change in the crystal shape or volume, migratory recrystallization, where small crystals melt and large crystals grow and pressure-induced recrystallization, a recrystallization caused by applying an external force to crystal. According to Fennema (1973) migratory and accretion are the main recrystallization mechanisms, which result in loss of frozen food quality.

2.3. Quality of Frozen Dough

Frozen storage of dough for a long time at low temperature (below -20°C) results in weakening the three dimensional gluten network that is responsible for gas retention in dough and eventually bread quality. The destruction of yeast during freezing attributes to increasing proof time by decreasing gas production and releasing reducing substances from dead yeast,
which weaken the gluten network. Other factors that increase the proof time of frozen dough are water redistribution, which mainly alters the properties of gluten and starch, especially the water binding capacity of gluten and starch as well as other dough constituents. Such damage occurs as ice grows and causes migration of water out of cells or from one region to another and reduction of gluten cross-linking.

Moreover, Casey and Foy (1995) observed that starch granules, which are firmly implanted in the gluten network, become more separated from the surrounding gluten strands in frozen dough. Along with that, the longer freezing period, the thinner the gluten strands become. Loss of frozen dough quality could also result from mishandling during transportation and storage (Laaksonen and Roos 2001a, b; Ribotta et al. 2001, 2003b).

Several studies have reported that the quality of baked products made from frozen dough is not only influenced by freezing rate, frozen storage temperature and time and thawing rate, but also wheat flour quality, dough additives, dough formulation and fermentation, as well as processing parameters such as dough mixing time (Le Bail et al. 1999).

The above mentioned factors result in lower loaf specific volumes and undesired changes in textural properties such as coarser internal structure and flattened top of bread baked from frozen dough such as. Storage of dough at low temperature (-20°C) or lower has a negative effect on the quality of bread. The longer the frozen storage time, the lower the quality of baked product baked from frozen dough (Bot 2003). Using appropriate processing conditions of frozen dough such as; proper mixing time, low dough temperature during and after mixing, and minimum resting time after mixing are very important parameters that can inhibit yeast damage and increase the ability of gluten network to retain gas by avoiding fermentation before freezing.
(Kulp et al. 1995). On the other hand, sheeting and molding conditions before freezing have no significant effect on the stability of frozen dough (Gélinas et al. 1995).

2.4. Rheological Properties of Frozen Dough

Dough rheology characteristic in the cereal industry is considered of great importance. This is due to the strong relationship between rheological properties of dough and quality of the final baked product (Ktenioudaki et al. 2010). Empirical and fundamental rheological analyses of dough give an estimation of dough viscoelastic balance. Good bread dough is the dough that exhibits balanced extensible and elastic rheological properties (e.g. form viscoelastic dough) in order to produce bread with desirable volume and crumb characteristics (Hoseney 1994).

2.4.1. Empirical Rheological Methods (Large Deformation Rheology) Dough Extensibility

Dough extensibility determines the expansion ability of dough caused by CO₂ during proofing and the resistance to extension is a measure of dough ability to retain CO₂ (Yi and Kerr 2009). Dough extensibility is mainly attributed to the elastic cross-linked network formed by gluten proteins in the wheat flour. Some authors suggested that the reduction of gluten cross-linking during dough freezing could be caused by the release of disulphide reducing substances from dead yeast (Ribotta et al. 2001; Giannou et al. 2003). Also the formation of ice crystals during freezing storage of the dough would result in progressive dehydration of gluten caused by water migration to form the ice crystals results in physical disruption of the dough matrix (Bhattacharya et al. 2003). Sharadanant and Khan (2003a, b) reported that an excessive extensibility would result in soft-weak dough that would collapse during proofing or baking.

There are contradictory data on the effect of freezing storage on dough extensibility and maximum resistance. For instance, some studies (Sharadanant and Khan 2003a,b; Yi and Kerr 2009) reported increase in dough maximum extensibility and reduction in maximum resistance to
extension with frozen storage time. In contrast, others (Inoue and Bushuk 1991; Inoue et al. 1994; Bhattacharya et al. 2003) reported increase in maximum resistance relative to extensibility with frozen storage or freeze-thaw cycles. Simmons et al. (2012) were able to minimize the changes in dough extensibility and resistance to extension by incorporating 48.5% soy ingredients leading to minimal changes in bread hardness.

2.4.2. Empirical Rheological Methods (Large Deformation Rheology) Dough Stickiness

Dough stickiness (or adhesiveness) is the measurement of the force required to separate the dough after dough adhering or sticking to a surface. It depends on both the adhesive and cohesive properties of the dough. Too sticky dough is very difficult to handle and may adhere to machine surfaces. In addition, it may result in bread that is too chewy, adheres to the mouth or seems to be under-baked (Hoseney and Smewing 1999; Fiszman and Damasio 2000). Previous studies (Angioloni et al. 2008; Yi and Kerr 2008, 2009) on frozen dough reported that dough adhesiveness increased as the frozen storage period increased. These results were the same for both 100% wheat dough as well as wheat dough with different additives such as food gums. Yi and Kerr (2009) reported that stickiness of frozen dough stored for 30, 90 and 180 days increased at different levels of waxy wheat flour, water added and frozen storage times. Angioloni et al. (2008) and Yi and Kerr (2009) suggested that the increase of frozen dough stickiness could be because of ice crystals redistribution through re-crystallization, deterioration of the gluten network and water redistribution during freezing and after thawing could lead to separation of water from the gluten strands. When the dough is thawed, the ice crystals melt but the water is not in the original state. Water becomes less closely associated with the gluten and starch network and more free water amount is accessible at dough surface, thus increasing adhesiveness.
2.4.3. Fundamental Rheological Analysis (Small Deformation Rheology)

Fundamental Rheological are parameters that can be measured using dynamic oscillatory rheometry to determine the response of a material to an applied force (stress) or deformation (strain). Bread dough is a viscoelastic mass that exhibit both viscous and elastic properties, which are the result of the presence of the three dimensional network of gluten proteins, which is formed by thiol-disulfide exchange reactions among gluten proteins (Belton 2005; Song and Zheng 2007). Dough viscoelastic measurements involve compression of a dough piece between parallel circular plates by which a sinusoidal stress is applied in the form of small amplitude rotational oscillations. As a result, the ability of a material to store and/or dissipate the applied energy can be identified using the amplitude of the resulting strain and its phase difference from stress (Loveday et al. 2012). The moduli $G'$ (elastic or storage moduli) and $G''$ (viscous or loss moduli) describe the character solid/elastic and the viscous/liquid state of the sample, respectively. These terms are measured within the linear viscoelastic range of dough (Loveday et al. 2012).

Ribotta et al. (2004) investigated the effect of guar gum on dynamic rheological behaviour of frozen dough stored at -18°C for 60 days and reported major decreases on the moduli on week 1 of frozen storage period indicating a reduction in dough firmness and elasticity. Meziani et al. (2011) also observed similar results after freezing 100% wheat flour doughs at different freezing rates (-20°C, -30°C, -40°C and immersion in liquid nitrogen). The same authors also reported that freezing process especially slow freezing may denature gluten proteins by increased acidity of the concentrated liquid phase. This indicates that these changes on rheological properties upon frozen storage could be attributed to the changes in dough protein composition of the dough.
During dough frozen storage of dough at -18°C, the loss in the polymer cross-linking and depolymerization of glutenin aggregates caused by ice recrystallization and/or by release of reducing substances from yeast leads to a reduction in dough firmness and elasticity (Ribotta et al. 2003a). Along with that is the dehydration of gluten network induced by freezing (Angioloni et al. 2008). Meziani et al. (2011) observed that freezing and thawing treatment process attributes to reduction in the dough resistance to deformation. Leray et al. (2010) reported that frozen dough composite with dietary fiber stored at two different temperatures -18°C and -30°C for 1, 7 and 28 days exhibited a significant decrease in G’ moduli during the first week of storage, and then it remained constant. It was concluded that addition of dietary fiber (blend of inulin and oat fiber) at the level of 10% increased wheat dough resistance to freezing and frozen storage. Leray et al. (2010) suggested that the positive effect of dietary fiber on frozen dough rheological properties could be attributed to their ability to bind most of the available water in the dough system. As a result, it inhibits the formation of ice crystals and consequently their damaging effect. β-Glucan has a great number of hydroxyl groups, which are capable of forming a viscoelastic gel network, increasing dough viscosity, and inhibiting diffusion of solutes and/or water between pores (Goff et al. 1999; Regand and Goff 2003).

2.4.4. Differential Scanning Calorimetry (DSC) Analysis, Nuclear Magnetic Resonance (1H NMR) Spectrometer Analysis and Scanning Electron Microscopy Analysis

Water is believed to play an important role during dough preparation and bread baking as well as in the structural, physical, chemical and sensory properties of the final product. It is the most abundant component in most foods that significantly affect structural transitions and phase transitions of foods. The effect of water on the rheological properties of frozen dough can be investigated using different methods such as Differential Scanning Calorimetry (DSC), Nuclear
Magnetic Resonance (¹H-NMR) and Scanning Electron Microscopy with a Cryogenic Preparation System.

Differential Scanning Calorimetry (DSC) analysis can be performed to investigate the thermal properties of fresh and frozen-thawed doughs based on calculating the ice melting enthalpy ΔH of dough during a freeze/thaw cycle (Sablani et al. 2002). The enthalpy recorded is directly correlated to the amount and state of water in the dough system and used as an indicator of the amount of freezable water present in dough (Simmons et al. 2012). Less changes in ΔH between freeze-thaw cycles of fresh and frozen-thawed dough indicates high freeze-thaw stability (Sim et al. 2012). When water sorption isotherm and state diagram of various doughs, dough components, and ingredients are known, processing and environmental conditions during storage can be controlled. That reduces the production costs and produces better quality of final product (Laaksonen 2001).

Upon frozen storage of wheat dough with and without additives, water molecules are transformed to ice crystals and become less associated with protein and starch compared to fresh dough system. This results in changes in the water distribution during frozen storage and an increase in the freezable water content, ice crystals amount, and consequently gluten network deterioration and injury of yeast cells (Sim et al. 2012). Additives such as dietary fiber are able to bind water molecules that are released from the gluten-starch network restricting their mobility and inhibiting ice crystals growth. As a result of this ice crystals growth and melting properties modifications, less freezable water (FW) content in the frozen dough system is obtained, while frozen dough stability is improved (Leray et al. 2010).

DSC results reported by Sharadanant and Khan (2003a,b) and Sim et al. (2012) showed that addition of food gums to frozen dough decreased the amount of FW in fresh as well as
frozen-thawed dough. Food gums also minimized the difference in melting enthalpy between fresh and frozen-thawed dough. However, it was reported that the amount of FW increased in control and food gums composite doughs as frozen storage time increased with significantly lower amount of FW with the presence of food gums. This indicates that additives such as food gums have the ability to increase freeze-thaw stability in frozen-stored dough samples.

Nuclear Magnetic Resonance (\(^1\)H-NMR) analyses can also be carried out as a complementary method to DSC. The proton spin-spin relaxation studies can determine the water mobility in bread dough at fresh and frozen-thawed statuses (Lu and Seetharaman 2013). The transverse relaxation time (T2) can be determined following the Carr–Purcell–Meiboom–Gill technique with the 90–180° pulse sequence. The NMR is used to measure the proton relaxation phenomena when the sample is placed in the magnet. The goal is to confirm DSC data through investigating the redistribution of water in dough during frozen storage and its effect on the quality parameters of the baked products (Jasrotia 2011). NMR spectroscopy measurement determines the water status or binding characteristics in fresh and frozen-thawed dough by obtaining spin-spin relaxation times (T2). NMR applies radio-frequency pulses on a sample surrounded with a magnetic field. While that sample nucleus, at a characteristic rate depending on its chemical and physical environment, is recovering its equilibrium status, NMR measures the electromagnetic energy emissions produced during its relaxation process. Results obtained from such a relaxation measurements can provide information on the location and surroundings of molecules containing these nuclei (Ruan and Chen 1998). NMR measurement does not require heating or cooling of samples, thus no physical or chemical changes occurs in the sample system.

Jasrotia (2011) and Lu and Seetharaman (2013) reported that the spin-spin relaxation times (T2) measurements are better representative and sensitive indicator of the overall mobility of
water molecules in a dough system. Free water is damaging in a dough system and yeast is sensitive to water during freezing and thawing cycles (El-Hady et al. 1996; Fennema 1996). NMR determines the content of bound water in dough based on water molecules mobility, which is highly affected by dough ingredients, freezing rate and frozen storage time. The lower the water molecules mobility, the higher the bound water content in the system (Ruan et al. 1999; Jasrotia 2011; Lu and Seetharaman 2013).

Esselink et al. (2003) and Yi (2008) reported that frozen storage reduces the relaxation time, however redistribution of water was observed due to formation and recrystallization of ice crystals. Yi (2008) reported that frozen-thawed doughs contain 15 and 30% waxy wheat flour had lower (T2), higher gas production and higher loaf volume compared to the 100% wheat flour dough. A study by Jasrotia (2011) examined the effect of emulsifiers such as calcium stearoyl-2-lactylate (CSL) and Diacetyl-tartaric acid ester of monoglycerides (DATEM) as well as cooling rate and frozen storage time on relaxation times (T22) of fresh and frozen-thawed dough. It was concluded that CSL had no significant effect, while DATEM significantly reduced the relaxation time value in frozen–thawed dough. Another study by Lu and Seetharaman (2013) assessed water behavior in freshly prepared (25°C), refrigerator-stored (4°C, one day), or freezer-stored (-35°C, one day) doughs that contain 5, 10, or 30% whole grain, air-classified β-glucan-diminished, and air-classified β-glucan-enriched barley flours. Addition of barley flour showed a significant effect on reducing the relaxation time value and consequently water mobility. This indicates that the damaging effect of water redistribution from gluten, starch and other dough components during frozen effect can be minimized by some additives. Air-classified β-glucan-enriched contain large number of available hydroxyl groups offered by β-glucan increasing the amount of water associated with starch and gluten matrix.
Scanning Electron Microscope with a Cryogenic Preparation System is commonly used to study the structure features of frozen dough including orientation of gluten strands and distribution of starch granules (Jasrotia 2011). Esselink et al. (2003) highlighted that during extended frozen storage, gluten dehydration occurs. SEM images from previous studies on frozen dough (Zounis et al. 2002a,b; Baier-Schenk et al. 2005) reported that water in frozen bread dough (with and without yeast) under different frozen storage conditions and times (up to 24 weeks), separated from the gluten-starch network and forms large ice crystals on the inside of the dough system. The ice-crystals formation and expansion leads to the disruption of gluten matrix and rendering the network separated from starch granules.

After thawing the dough, the redistribution of water in the dough system does not allow it to return to its initial state. As a result, water forms pools in the dough system and it becomes difficult to have equal water distribution throughout the dough, even with punching, leading to an increase in proofing time and decrease in loaf volume (Lu and Grant 1999). The effect of different freezing rates (19-69°C/h) and frozen storage time up to 180 days at four different temperatures (-10, -20, -30 and -35°C) was also investigated by Yi and Kerr (2009). The authors concluded that the least changes in water distribution and gluten network were obtained from dough’s stored at very low temperature (-30 and -35°C). Despite that, strength of gluten network decreased as frozen storage time increased for all different freezing rates and temperatures. The authors suggested that since different doughs have their unique system, adjustment of freezing process should be done for each product to minimize frozen storage deterioration effect on dough. Results obtained by Zounis et al. (2002b) also confirmed that dough structure changes are not only resulted from frozen storage, but also from a number of different factors. These
factors include dough mixing conditions, formulation and specific ingredients, which may inhibit or enhance structural damage in frozen doughs.

2.5. Basic Ingredients of Baked Products and their Roles in the Dough /Batter System

2.5.1. Wheat Flour

Wheat flour is the basic ingredient used in baking due to its ability to form dough or viscoelastic gluten system when it is mixed with water. It mainly consists of protein, starch and dietary fiber. If highly elastic dough is needed, it is preferred to use flour with high protein content. For example, in bread dough both the amount and the quality of flour protein are important. Usually hard wheat flour with high protein content (12-14%) and high protein strength is recommended. Strong gluten network is required in frozen dough to minimize the destructive effects of freezing, storing, and thawing processes that the dough will undergo (Sahlstrom et al. 2004).

Bhattacharya et al. (2003) reported that good quality frozen dough with good baking properties is obtained from moderately high gluten strength. Lu and Grant (1999) investigated the effect of frozen storage on baking quality of doughs made from 4 wheat cultivars and stored at -18°C for 16 weeks. They reported that the wheat cultivar with the overly strong gluten characteristics exhibited the highest proofing time and lowest loaf volume compared with the other three cultivars. This could be related to the negative effect of the too strong gluten network, which inhabits the expansion of dough especially with of the limited CO₂ produced by limited amounts of yeast that survived the frozen storage. On the other hand, pastry products are generally made from soft wheat flour with lower protein level about 8% (Bennion and Bamford, 1997).
There are four types of wheat proteins. They are classified according to their solubility; water-soluble albumin, salt soluble globulins, prolamins (gliadins) soluble in 70% ethyl alcohol and glutelin (glutenins) soluble in dilute acids and bases. Glutenins, which make up 40-50% of flour protein, and gliadins, which constitute 30-35% of total proteins, are the main protein fractions of the wheat grain. When water is added to wheat flour, the endosperm storage proteins (gliadin and glutenin) are hydrated and gluten is formed (Stauffer 1993). They play significant roles in forming viscoelastic dough from hydrated wheat flour. They complement each other in order to form strong, extensible and elastic dough. Yamauchi et al. (1999) showed that the glutenin fraction plays a dominant role in baking quality of frozen doughs, while gliadin and starch fractions contribute at a lower degree, followed by the water-soluble fractions, which usually have the minimum effect on frozen dough quality compared to the other fractions.

Wetted glutenins can form large polymers in dough by forming both intra- and inter-molecular disulphide bonding via cysteine residues. These polymers provide the dough with strength and elasticity, which are responsible for the mixing characteristics of dough. While wetted gliadins have the ability of filling the dough structure (filling the spaces between glutenins large polymers) and can only form the intra-molecular disulphide bonds, which makes the gliadins responsible for plasticity and extensibility of the dough (Lindsay and Skerritt 1999, 2000). As a result, a cohesive and viscoelastic water-insoluble complex (Gluten) is formed. Gluten is capable of retaining gas, which is responsible for doughs expansion during proofing as well as light, palatable loaf after baking.

Flour type and protein quality are important variables for the stability of frozen dough. In general, high protein content in flour provides the dough with better ability to trap and retain carbon dioxide gas and produce high bread volume (Cauvain 2003). Vital gluten can be used to
boost the gluten level and to strengthen flour with low protein content in order to produce good quality baked goods from frozen doughs.

2.5.2. Water

Water is the most abundant component in most foods. It is also one of the most important ingredients in dough. Water in a dough system acts as bound water, which is the amount of water necessary to fully hydrate and plasticize the wheat components, or as free water phase, which is partly responsible for the flow and mobility properties of dough and in which soluble compounds such as salts, sugars, soluble proteins, etc, are dissolved (Stear 1990). The amount of water exerts a strong influence on mixing characteristics and final product quality (Kulp and Ponte 2000). The bound water content is estimated to be between 27-32% (dry base), but it varies based on moisture content and characteristics of the wheat flour components. Water either in bound or free status interacts with flour components (starch, protein, pentosan). The understanding of these interactions is considered essential in the realization of the physicochemical events occurring during dough formation and cereal processing.

As bound water is immobile or has low mobility compared to free water, free water become problematic in dough during cold and frozen storage. This is due to its damaging effect in dough system during freezing and thawing cycle. Moreover, yeast is very sensitive to water during low temperature storage of dough. As a result, lower water absorption of flour is desirable for frozen doughs (El-Hady et al. 1996). Inoue and Bushuk (1996) recommended that water absorption of high quality flour (55-70%) should be decreased by about 3-5% for frozen doughs compared to fresh baking. Kulp and Ponte (2000) suggested that water absorption should be decreased by 3-5% for frozen doughs compared to fresh baking. High quality flour usually has high water absorption and retention of moisture content without producing excessively sticky
dough (Lai et al. 1989a, b; Sultan 1990). In addition of providing good processing conditions, flour exhibiting high water absorption produces the best loaf volume. However, loaf volume does not only depend on the amount of water added to dough, but also on the mixing time applied. Therefore, for the best loaf volume both water absorption and mixing time should be optimized (Lai et al. 1989a, b).

At the freezing point, water molecules converted to ice crystals, the solid form of water, which damage the yeast cell walls (Stauffer 1993). This is because of the increase in specific volume of water after formation of ice crystals by about 9% (Fennema 1973). To minimize the damage effect of freezing in frozen dough, yeast content of frozen dough should be higher than normal to neutralize the inevitable loss of activity during freezing and storage, while the amount of salt should not be more than 2% (based on flour weight) due to its osmotic pressure effect on yeast (Wolt and D’appolonia 1984).

2.5.3. Yeast

The most common yeast used in bread making and most of the fermented baked products is *Saccharomyces Cerevisiae*. Yeast cells are responsible for producing carbon dioxide and flavour compounds via the metabolism of fermentable sugars under anaerobic conditions. Carbon dioxide expands dough volume to the required level. This function introduces the yeast as a leavening agent and makes it a vital component in a yeast fermented dough system (Stauffer 1993). Yeast also develops the dough through the action of fermentation on the gluten structure and contributes to the rheological properties of dough as well as flavour and aroma (Giannou et al. 2003).

There are three types of *Saccharomyces* yeast available for baking (Stear 1990; Sultan 1990): compressed yeast (also called “fresh yeast”), contains approximately 70% moisture,
which makes it highly perishable, so it should be refrigerated, active dry yeast (ADY), it is substantially less active compared to compressed yeast and instant dry yeast (IDY). The performance of IDY under optimal conditions is very close to that of compressed yeast. It is usually added directly to the dough without rehydration for maximal action, while ADY is usually rehydrated in hot water (43-46°C) for 5-15 min and then added to flour/water mixture.

Usually yeast is added to bread dough formula at 3% on flour dry basis. When long fermentation time is desired, less yeast amount is recommended and vice versa. The activation of yeast starts once the yeast is hydrated. At that point, yeast starts consuming fermentable sugars and producing carbon dioxide, ethanol and flavour compounds. As dough temperature increases, the rate of gas production as well as flavour compounds increases until the optimum temperature at about 40ºC is reached. Above that temperature a progressive thermal killing of the yeast can be observed (Rogers 2004).

Yeast cells in dough are affected by many factors. The level of water available to the yeast is one of the very important factors that affect gassing power. As water content increases gassing power increases indicating an increase in yeast activity (Lee 1970). On the other hand, Salt has an opposite effect to water as it suppresses yeast activity. Similarly, high levels of sugar in dough inhibit gas production (Saalfeld and Freund 1998).

In freshly baked products, high final product quality can be obtained with long fermentation time. However, this relationship does not apply to frozen dough because dough fermentation before frozen storage increases the deterioration of dough and decreases yeast viability and activity after thawing (Sluimer 2005). Therefore, no-time and short-time dough methods are often applied in frozen dough industries (Marston 1978).
Yeast is sensitive to freezing, which causes a major problem in frozen yeasted doughs. In the dough system the yeast is under osmotic pressure and in a state of active fermentation. Under these circumstances the yeast cells have a thinner plasma membrane than dormant cells. Therefore, they become more susceptible to cell damage. Moreover, during freezing a concentrated aqueous phase is formed and contributed to the yeast cell damage. Yeast cells are also liable to mechanical damage during frozen storage caused by; first, the fast freezing, which results in the growth of intracellular ice crystals, second, by the recrystallization of ice crystals, which results in cell membrane damage (Stauffer 1993; Casey and Foy 1995; Lorenz and Kulp 1995). Consequently, broken cells might release wastes such as glutathione, which accumulate prior to freezing and known for its negative effects on the gluten network contributing to the weakening of the gluten network by reducing the gluten cross-linking (Miller 2006). Bhattacharya et al. (2003) reported that slow freezing rate is preferable to preserve yeast activity.

In general, freezing, frozen storage, and thawing of dough resulted in longer proof time compared with fresh doughs (Autio and Mattilla-Sandholm 1992). The proof time increases with the frozen storage time as well as the loss of yeast viability and activity. Previous studies (Sharadanant and Khan 2003a,b, 2006) have used fresh compressed yeast within a week of its receipt, whereas, other studies (primo-Martin et al. 2003; Kim et al. 2008) have used active dry yeast in frozen dough. All of them agreed that as long as the yeast used in frozen dough is fresh there should be no significant variability in yeast leavening activity.

Stauffer (1993) and Inoue and Bushuk (1996) recommended that a higher level of yeast can be used (4-6% on flour basis) in order to minimize the damaging effect of freezing and maintain good performance and product quality in frozen dough products. The addition of high
amounts of yeast to dough (higher than 8% flour based) has a negative effect on flavor and aroma of baked goods prepared from frozen doughs (Stauffer 1993).

2.5.4. Salt

Salt in baked products refers to Sodium Chloride (NaCl) and is commonly used at low levels (1.5 – 2.0% flour basis). Salt has several functional effects on dough such as strengthening the gluten network by inhibiting the action of flour proteases and shielding the charges on the dough proteins (Hoseney 1994). It also improves flavor and controls the action of yeast via enhancing the action of amylases, which help to maintain a supply of maltose as food for the yeast. However, the higher the concentration of salt, the lower the rate of fermentation with the same yeast level, and vice versa due to the suppressing effect that salt has on yeast (Kulp and Ponte 2000).

In the absence of salt, yeasted dough becomes sticky and difficult to manipulate and the bread produced is quite tasteless. Salt also increases dough stability, firmness, and capability to retain fermentation gases. Moreover, salt plays an important role in dough prepared for frozen storage, which is delaying the yeast fermentation process during mixing and therefore slowing the production of carbon dioxide. As a result, minimum yeast activity is obtained during and after mixing (Kulp and Ponte 2000)

2.5.5. Sugar

The sugars that present in dough include sugars originally present in the flour, sugars enzymatically cleaved from oligosaccharides or polysaccharides present in flour after hydration and during mixing and sugar added as a dough ingredient. Sucrose either from cane or beet is the most common sweetener in the bakery industry. However, other sweeteners are used such as brown sugar, dextrose, maltose, molasses, corn syrup and invert sugar (Calval et al. 2001). The
amount of sugar used in dough depends on the type of the product and desired crust characteristics, but the average amount of sugar used is 3-6% based on the weight of flour used (Stear 1990; Sultan 1990).

Sugar plays an important role in dough. It works as the basic source of energy for yeast during the initial fermentation stages of dough. Sugar also binds water and function as an inhibitor for starch recrystallization or retarding, which has the undesired staling effect on baked products, a process that depend on water availability (Levine and Slade 1990). It is known for its hygroscopic characteristics, which increases the amount of water absorbed. Therefore, it is recommended to add higher level of sugar (8-10% on flour basis) in frozen dough in order to reduce the amount of free water in dough and decrease the yeast damage (Stauffer 1993).

Sugar does not affect only the taste of the baked product, but also its texture and appearance. In addition, it controls the viscosity and degree of gelatinization of starch and retards gluten development during mixing resulting in more tender dough and proper expansion of air bubbles (Kim and Walker 1992). In pastry product such as cookies, high fructose corn syrup (42%), which is a mixture of glucose and fructose, is utilized due to its lowering effect on water activity and freezing point as well as its higher sweetening effect compared with sucrose. Moreover, it promotes less undesirable browning reactions on the surface and in the interior of the baked goods and form less amounts of acrylamide and hydroxymethylfurfural (HMF) during baking compared to pure sugars such as glucose, fructose, lactose, dextrose, etc (Boldon et al. 2004; Gokmen 2007). However, in cookies high sugar (sucrose) to flour ratio is used about 33.3–42% sugar based on flour weight to attain the desired cookie sweetness and texture. Sugar also inhibits the development of gluten cross-linking because it competes with the flour for the small amount of water available in the batter formula (Perego et al. 2007; Ktenioudaki et al. 2010).
Besides, high Sucrose level and low water level minimize starch gelatinization during baking by increasing the temperature to be higher than the optimum temperature for gelatinizing all the starch available. As a result, a little amount of starch is gelatinized (Delcour and Hoseney 2009; Pareyt et al. 2009a,b, 2010b).

2.5.6. Lipids

Lipids (shortening or oil) are another important ingredient in baked products. They are considered as an optional ingredient in some types of bread, while they are essential ingredients in cookies, in which they significantly affect the rheology of the cookie dough and subsequently their final product quality (Jacob and Leelavathi 2007).

Lipids improve the quality of dough as well as the final product. They amend dough handling and structure and also ensure better final product texture giving it softer and shorter bite, better appearance and imparts desirable flavor (Sultan 1990; Stauffer 1993). During dough mixing stage, lipids interact with proteins to maintain proper structural of gas cell membrane and contribute to the viscoelastic properties of the gluten network, which is necessary for a better expansion and gas retention during proofing, resulting in a larger final loaf volume, a less crisp crust and better final product quality (Autio and Laurikainen 1997).

As a standard practice, lipids usually added to some baked products dough such as bread at a level of 0.7-3% of the flour weight to maintain good final product quality (Stauffer 1993). Inoue et al. (1995) reported that higher shortening level up to 40% oil in water (O/W) emulsion system is recommended for good stability of frozen dough during frozen storage and quality of final product made from frozen doughs.

In pastry batters such as cookies batter, high fat to flour ratio is used (e.g. 9.4–18%) based on flour weight (Wade 1988). Fat functions start very early in the mixing stage as it prevents the
excessive formation of the gluten network by covering the surface of the flour (Manohar and Rao 1999). It was found that the higher the fat level, the lower the amount of protein cross linking present in the batter structure (Pareyt et al. 2010a). Along with that, fat incorporates air bubbles into cookie batter and stabilizes the walls of the air bubbles formed during mixing (Pareyt et al. 2009a, b).

2.6. Conventional Baking

2.6.1. Bread

Bread has been well known as the oldest, most popular and widely consumed food product in the world. This fact is due to its valuable nutritional, sensory and textural characteristics. Bread making technology is basically processing wheat flour and other ingredients into a light, aerated, and palatable food. It starts once the primary ingredients (flour, water, yeast, shortening and salt) are mixed to form a cohesive mass of dough. Both dough composition and processing steps affect the quality of the baked product. Bread dough processing steps involve mixing and kneading, molding, fermentation, proofing, followed by a final baking step.

During the mixing, a mechanical energy is applied on the hydrated ingredients and solubilisation and swelling of the water soluble flour components occurs. The moistened flour proteins form gluten strands, which bind through two types of chemical bonds: the covalent bonds, e.g., disulfide bonds, which form inter- and intra- molecular cross bonds in the proteins during dough formation by the sulfide-disulfide interchange, and the secondary bonds involving hydrogen, hydrophillic, and ionic bonds and polar interactions. The ability of forming such a network depends on the gluten, which is limited to wheat and few other cereal seeds. Gluten particles form a large and cohesive system that exhibits unique rheological properties. In fully developed dough, the other components (lipid, starch, non-starch carbohydrates, salts, sugar) also
participate in the dough formation by being dispersed in the formed gluten network. Research has shown the continuous protein phase that surrounds the starch granules is responsible for the viscoelastic properties of dough (Rojas et al. 2000; Cauvain 2001; Bot and de Bruijne 2003).

Then, mixing, the homogenisation of the ingredients, and kneading, the development of the dough (gluten) structure by applying more mechanical energy after the initial mixing. These two steps (mixing and kneading) are very critical and must be properly controlled to prepare dough for frozen storage. This is because only during these steps gluten formation and modification takes place. Moreover, mixing the ingredients for an adequate time is also important to get the desired strong and elastic gluten network. For example, very short or long mixing time has a negative effect on dough structure, in which very short mixing time generates under-developed gluten, while long mixing time generates heat and enhances fermentation, which deteriorates frozen dough. In addition, applying an excessive mechanical energy, in the case of over mixing, will result in weakening of the gluten strands and then breaking them down (Potter and Hotchkiss 1998).

After kneading, the dough is left to rest and the fermentation or proofing step starts. In this step, bakes’ yeast (Saccharomyces cerevisiae) consumes sugars that are available in the dough and produce carbon dioxide (CO$_2$), ethanol and water. The formed dough traps gases and expands during fermentation. A series of punching steps are performed during fermentation to ensure that the expanded cohesive mass will produce the desired soft, light and palatable food after baking (Cauvain 2007). After the dough is fermented for the required time, it is then prepared to be baked by molding it to the desired shape. Ultimately, the molded dough undergoes the last step, which is baking in an oven.
Baking step is mainly heating the dough (gluten) until a fairly rigid structure with air cells is formed e.g. bread crumb. As the temperature of the dough increases from room temperature to around 40°C, solubilization and swelling of dough components, enzymatic activity, and yeast growth all accelerate. Once dough temperature reaches 50°C, dough and yeast enzymes become thermally deactivated and as the temperature increases from 50 to 70°C, partial dehydration of gluten as well as a coagulation of gluten elastic films occurs. Between 55°C and 65°C the moistened starch starts to gelatinize and form a stiff paste. Starch swelling and gelatinization is facilitated by the migration of water from other dough components to the starch granule. At this stage, the formation of the semi-rigid structure starts as gluten strands become more rigid, viscous and elastic. While dough temperature continue to increase, moisture evaporation (through pores in the crust), starch gelatinization and coagulation of dough proteins increases to reach its maximum level at 98-99°C resulting in a baked crumb structure (Sablani et al. 2002). The temperature of outer surface of dough increases to 130°C after 20 minutes, while the dough centre never exceeds 100°C because of the slow heat transfer from the dough surface to the crumb centre due to the temperature gradient between them (Cauvain 2001).

2.6.2. Cookies

Cookies, as it is called in North America or biscuit as it is known internationally is wheat flour based baked pastry product (AACC 2011). Compared to doughs cookies mix often has lower moisture content, so it is rather called batters. In contrast to bread dough, cookie as well as other pastry products’ batters are inelastic and have high flow properties. This is due to the minimum gluten network development desired in such products. Moreover, batters have high sugar and fat to flour ratio and they are chemically leavened with baking soda, e.g., sodium, potassium, or ammonium bicarbonate, etc., as a source of carbon dioxide with smaller amount of
water added about 1–5% (Pareyt and Delcour 2008). Batters usually have water activity less than 0.85 to about 0.55, which extend microbial shelf stability and improve frozen batter handling characteristics (Boldon et al. 2004). Typically, all batter ingredients (flour 47.5–54%, sugar 33.3–42%, salt, water, skimmed dry milk and fat 9.4–18% (e.g., vegetable oil or shortening and/or butter), chemical leavening, and various optional other ingredients) are blended, creamed, stirred or whipped to disperse all ingredients in the continuous batter medium and produce a thin structure. The result is a soft, moist, crumbly and stable product with long shelf life and good quality such as cookies, muffins, etc. (Boldon et al. 2004).

Generally, soft wheat flour is recommended for cookies making. Soft wheat flour is preferred due to its low hydration properties, low protein content 8-10%, low damaged starch and arabinoxylans and weak gluten network. As a result, the greatest cookie spread factor, which is width (W) to thickness (T) ratio (W/T), and cookies with a tender bite are produced (Barak 2013). The larger the spread factor, the higher the quality of cookies. Kweon et al. (2011) reported that there is a strong negative relationship between cookie spread factor and flour content and characteristics of damaged starch, protein, pentosans and non-starch polysaccharides in flour, which are responsible for water-holding properties. Abboud et al. (1985a,b) compared soft wheat with hard wheat and found that the best cookies with a greater expansion and longer time of expansion are produced from the soft wheat flour.

Dogan (2006) reported that cookie batter can be stored for 6 months at -18°C with no deterioration signs and no changes in the final product spread ratio, surface characteristics and density. Kulp et al. (1995) reported that even though cookies batters are chemically leavened and have low moisture content, improper handling and fluctuation in freezing temperature negatively affect frozen cookies batter quality.
During baking, as cookies temperature increases, gluten polymers start to form a continuous matrix through entanglement and cross-linking of polymers. As a result, the batter viscosity is increased and cookie with small spread ratio is produced. At the same time, the remained undisclosed sugar starts to dissolve and fat starts to melt and both contribute to the increase in cookie spread ratio (Hoseney 1994; Pareyt et al. 2009b). Pareyt et al. (2009b) showed that high sucrose and fat levels inhibit the formation of gluten network, decrease cookies height, and increase cookies diameter. Pareyt et al. (2010b) found a significant increase in batter’s elasticity and hardness when the fat level is reduced in the cookie batter formula. They also found that the changes in the batter’s rheological properties are due to the increment in the level of protein cross-linking during baking.

2.7. Baking of Frozen Dough

An important requirement for frozen dough is to keep its functional properties at the level of freshly mixed dough (Kulp et al. 1995). In frozen dough technology, the dough should be strong and durable and the changes that may occur during the storage period should be at the minimum level. Processing of frozen dough is usually different from that of unfrozen dough (Figure 2.1). However, the basic formulation and characteristics are essentially the same.

2.7.1. Mixing and Molding

Mixing and kneading used for the preparation of frozen dough are slimier to that used in fresh dough. Dough prepared for frozen storage must be transferred immediately to the freezer after mixing, kneading and molding, in which the gluten network is fully developed and shows optimal rheological properties such as dough extensibility and strength, to avoid any fermentation caused by the yeast activation.
After mixing dough/batter is divided into pieces of specific weight and is molded to the desirable shape according to the product specifications. Molding contributes as well to the final development of the gluten network as it results in a coalescence of small gas cells into larger ones in yeasted dough (Autio and Laurikainen 1997). It is believed that slabs and cylinders shapes are better than round-shaped dough pieces to produce more satisfactory bread (Gélinas et al. 1995). Stauffer (1993) reported that even with cookie batters the shape of cylinders is the most proper molding shape for frozen storage.

2.7.2. Packaging and Freezing

A variety of Packaging materials and shapes are available for frozen bakery products. The selection of a packaging material and shape for frozen dough depends mainly on the product specifications. The most common packaging materials used in frozen bakery product industry are plastic (films, membranes, etc.) and aluminium. Packaging material selected must be capable of preventing any contamination, providing good oxygen and moisture barrier characteristics and withstanding the stresses it is likely to meet during storage and transportation (Gélinas et al. 1995).

2.7.3. Thawing

When frozen dough is taken out of freezer to be prepared for baking, it needs to be defrosted and fermented. Thawing or defrosting process can be done at various time, temperature and humidity conditions and it primarily involves the rehydration of the system, mainly the gluten matrix and yeast cells. It is recommended that the dough undergoes a stepwise increase in temperature due to the large difference in temperature between the dough surface and the surrounding air and the gradual change in temperature from outer regions to the frozen center of the dough (Kenny et al. 2001a,b).
In the case of frozen batter, it can be baked immediately after being placed into the baking tray without need for defrosting. If desired, however, the frozen batter can be allowed to rest or thaw for short times, e.g., up to four hours without any significant changes (Boldon et al. 2004). Stauffer (1993) recommended a minimum amount of thawing time before baking and reported that frozen batters are stable after thawing for several days at 5°C but longer baking time is required in the later situation.

2.7.4. Proofing

Before baking yeasted dough, the thawed dough pieces should be proofed in order to obtain desirable volume. Once the dough is thawed, the yeast, which was inactive during frozen storage, is rehydrated and it starts its fermentation activity. The major products of yeast fermentation are carbon dioxide and ethanol. As carbon dioxide is produced, the gas cells in the dough become larger and larger. Punching or remixing are applied to first, bring the yeast cells and fermentable sugars together since yeast cells do not have mobility in dough, and Second, to subdivide the gas cells to produce many smaller cells. Optimum rheological properties (optimum balance of extensibility and elasticity) are used as indictors for properly proofing process, which produces a baked product with desirable volume and crumb characteristics (Cauvain 2001).

2.8. Improvement of Frozen Dough

Research in frozen dough, which is considered one of the fastest growing food processing industries, has been carried out to improve the quality characteristics comparable to the freshly prepared bakery products. As a matter of fact, quality of baked goods prepared from frozen doughs is often poorer than that of fresh dough. Frozen dough deterioration caused by the changes in water distribution that takes place during extended frozen dough storage and freeze-thaw cycles, which contribute to; a longer proofing time and reduced loaf volume of breads from
frozen dough. The ice crystals formation also contributes to the weakening of the protein network in the dough system, which is responsible for gas retention (Rasanen et al. 1997).

Mechanisms that damage food during freezing can be classified to three main mechanisms (Nesvadba 2009): Mechanical damage in food structure, which caused by the ice crystal growth, Cross-linking damage of proteins where the decrease in water availability leads to aggregation and denaturation of proteins and re-absorption of water during thawing process. Mechanisms number 1 and 2 are considered to be the main cause of the deterioration of quality in frozen foods. In order to minimize the damaging effect of frozen storage, using fast cooling rate is recommended. This is because it results in the formation of uniform and small ice crystals throughout the tissues, while slow cooling rates results in large ice crystals growth. It is also important to avoid any temperature fluctuations during frozen storage and maintain low and constant subfreezing temperatures for high quality frozen food (Petzold and Aguilera 2009). On the other hand, study by Neyreneuf and Vanderplaat (1991) indicated that the maximum yeast activity in frozen-thawed dough is usually obtained with slow freezing rates.

In this regard, different ways to minimize the effect of freezing on doughs/batters are suggested in the literature. These include finding new yeast strain more resistant to freezing, improving the dough/batter preparation process, or using suitable additives and ingredients for frozen dough/batter. The use of additives has become a common practice in the business of baking.

Some additives have been used to overcome certain problems that face frozen dough production and utilization. The main role of most of the additives used is interacting with water in dough, which can affect the quality of the end bakery product. These compounds are safe and natural food additives that exhibit thickening functions as well as water binding and gelling
properties, for example, hydrocolloids, starches, and other thickeners in food systems (Yi 2008). Several studies have investigated the effect of different additives such as egg yolk and sugar ester (Hosomi et al. 1992); guar, locust bean and xanthan gums (Lo and Ramsden 2000; Matuda et al. 2008); whey protein (Asghar et al. 2009); polyols, carboxy methyl cellulose and gum Arabic (Asghar et al. 2005); dietary fiber (blend of inulin and oat fibers) (Leray et al. 2010); Inulin from the root of Jerusalem artichoke (Filipovic and Filipovic 2010); starches (maize, rice and wheat) and two non-starch polysaccharides (xanthan and locust bean gum galactomannan) (Lo and Ramsden 2000); mono- and diacetyltartaric acid (DATEM) (Ribotta et al. 2004); soy ingredients (Simmons 2012) on the quality of frozen dough.

Sharadanant and Khan (2006) observed damaging in the gluten network and separation of starch granules from the gluten when control dough (100% wheat flour) was stored in the freezer at -23°C for 4 to 16 weeks, while better retention of the gluten network was observed after the addition of selected gums (locust bean gum and gum Arabic). Most of the studies concluded that stability of frozen dough can be enhanced with addition of food gums, up to date no study investigated the effect of barley flour rich in β-glucan on the quality and functionality of frozen dough. Therefore, this aspect will be the focus of the present study to understand the effect of barley β-glucan on the quality of frozen dough and final baked product.

2.8.1. Barley Flour as a Valuable Source of β-Glucan

β-Glucan content in barley genotypes varies from 2 to 10% (McCleary et al. 2006; Baik and Ullrich 2008). The major barleys that are commonly used as human food are the Hull-less barley due to its ease in processing and high β-glucan content (Taketa et al. 2004). Incorporation of large amounts of barley or oat as rich sources of β-glucan could lead to quality defects of the end products. This brought the need to develop new technologies to obtain more concentrated
sources of β-glucan and consequently minimize or even eliminate any negative effects on the food product quality (Vasanthan and Temelli 2008).

Several attempts of extracting β-glucan from barley were reported using aqueous solvents (Burkus and Temelli 1998) or water with subsequent centrifugation and freeze-drying of the supernatant (Cavallero et al. 2002). Another way of increasing the β-glucan content is the separating β-glucan rich fractions through milling and sieving of flours (Kiryluk et al. 2000; Andersson et al. 2003; Izydorczyk et al. 2003). Air-classification technology was introduced as an effective way to produce barley fractions with high β-glucan content (Ferrari et al. 2009)

2.8.2. Air-Classification of Barley Flour

Air-classification technology is utilization of gravity force to separate the fine particles from the course materials of dry mixtures. In other words, it is a separation process based on the particle size to produce different size fractions that falls in groups or grades at cut points ranging from 10 mesh to very small particle sizes approximately 1 µm. In air-classification process there is no need for using chemicals or applying a drying process, in this way the production of harmful by-products is avoided and the production cost is minimized. Moreover, the protein denaturation caused by the chemicals is avoided. However, neither the fine nor the coarse fractions are pure since they may contain undesirable components. This is considered a limitation for this technology (Ferrari et al. 2009; Verardo et al. 2011; Beull 2013). Air-classification process of barley flour involves three steps (PCAC 2013) (Figure 2.2): the dissipation of the material using air force and directing it to the separation site, the separation of fractions based on their particle size, at least into two fractions; the lighter fraction, which is carried by air and have high fiber content, and the heavier fraction, which have high β-glucan
and starch content (Nghiem et al. 2010; Srinivasan et al. 2012) and the separation between coarse and fine fractions using air force.

There are continuous efforts devoted for developing the air-classification technology. For instance, Fedec (2003) reported that dehulling of barley makes a more successful separation by air-classification technique. In addition, Ferrari et al. (2009) optimized an air-classification system and reported that the optimized system improves both β-glucan concentration and flour yield. Double the amount of β-glucan and a yield of about one-third of the whole barley flour were obtained from two commercial varieties. Their goal was to use the β-glucan enriched fraction as an ingredient in bakery products to improve textural properties and to prevent coronary heart disease.

2.8.3. β–Glucan as a Food Functional Ingredient

Cereal β-glucan is non-starch polysaccharides and major components of the water-soluble dietary fiber present in the endosperm cell walls of cereal crops, especially barley and oat. The structure of cereal β-glucan is similar to cellulose (Figure 2.3). It is a linear, non-branched and semi-flexible polymer consists of β-D-glucose units joined by (1→4)-glycosidic bonds. Every two or three (1→4)-glycosidic bonds are separated by (1→3)-glycosidic bond and the ratio of glycosidic bonds present in β-glucan is 30% β - (1→3) and 70% β - (1→4) linkages. The presence of β-(1→3)-linkages gives stability to β-glucan and reduces its tendency to form aggregates (Regand et al. 2011; Ahmad et al. 2012).

There has been a remarkable increase in consumers’ concern and demand for healthier foods. Since carbohydrates represent a major portion of the human diet. Several studies have focused on the importance of non-starch polysaccharides like β-glucan as the substrates for colonic fermentation and healthy ingredients.
β-Glucan as soluble dietary and one of the most important members of the dietary fiber family is well known through media and advertising news as well as the numerous studies investigating and documenting its cholesterol-lowering benefits as well as regulating blood glucose levels (Bourdon et al. 1999). The viscous property of β-glucan may result in reducing the absorption of lipids and bile acid (Wood 2010). This encourages highly competitive companies to exploit β-glucan as a functional food ingredient in many products, particularly cereals and baked products such as; bread, muffins, cookies, etc.

The high water holding capacity of β-glucan allows it to have a significant effect on one of the most important processing parameter in baking industry which is the water behaviour of flour-based products. This effect extends to affect rheological and functional properties like viscosity and consistency of dough and batter (Wood 2010).

The Food and Drug Administration (1997-2005); European Food Safety Authority (2006) and Health Canada (2010) recommended that foods should contain at least 0.75g/serving of oat or barley derived β-glucan for each serving portion to meet the health claim of β-glucan. However, the expected health benefits from β-glucan are controlled not only by concentration, but also by its solubility and molecular weight (Mw) (Wood 2010). Thondre and Henry (2009) showed that the higher β-glucan Mw, the better since low β-glucan Mw did not exhibit the expected health benefits. Moreover, processing, cooking technique and storage are critical factors that are capable of altering β-glucan solubility and Mw in the food matrix. As a result, affecting the viscosity generated by β-glucan that is responsible for β-glucan health benefits (Regand et al. 2009; Tiwari and Cummins 2009). Therefore, it is recommended to wisely select the right processing and cooking technique in order to preserve the desired nutritional functionality of β-glucan (Aldaghpassi et al. 2012).
2.8.4. Physicochemical Properties of β-Glucan

Previous studies (Brennan and Cleary 2005 and Vasanthan and Temelli 2008) reported that the physiological effectiveness of β-glucan depends not only on the β-glucan dose, but also its molecular weight and solubility. In addition, it was reported that the higher β-glucan Mw (Thondre and Henry 2009) and the higher β-glucan solubility (Lan-Pidhaity et al. 2007), the better since low β-glucan Mw and low β-glucan solubility did not exhibit its expected health benefits. Along with that, they reported that various conditions, such as; processing, baking technique and storage directly affect the β-glucan health benefits when incorporated into different baked food matrices (Regand et al. 2009; Tiwari and Cummins 2009). Therefore, it has become essential, when conducting studies on incorporation of β-glucan in a food product, to pay attention to β-glucan characteristics, especially molecular weight and solubility, in order to provide a food product with the desired physiologically effective β-glucan. It is believed that the health benefits of β-glucan depend mainly on its viscosity (Guillon and Champ 2000; Wood 2004). Therefore, the molar mass of β-glucan is very important since it directly affects its viscosity. Based on that, it must be taken into account when considering the positive health effects of β-glucan incorporated into food product.

The health benefits of β-glucan depend mainly on its viscosity (Guillon and Champ 2000) and the molar mass of β-glucan directly affects its viscosity (Cleary et al. 2007; Moriartey 2009). Previous studies (Cleary et al. 2007; Flander et al. 2007; Andersson et al. 2008) have tried to introduce the beneficial effects of β-glucan to the consumers. Thus, they incorporated it in bread, which is a staple food for great number of people. It was concluded that the beneficial effects of β-glucan are decreased when incorporated into the bread system particularly due to depolymerization of the molecule during dough mixing and fermentation. The decrease in β-glucan
\( M_p \) after mixing could be because of \( \beta \)-glucan enzymatic degradation by the endogenous \( \beta \)-glucanase present in the food preparation and/or during mixing and processing steps, e.g. hydration and enzymes present in the added yeast during mixing of bread dough (Cleary et al. 2007). On the contrary, Moriartey (2009) reported that incorporating barley \( \beta \)-glucan concentrate of low solubility in bread (at 3.75, 5 and 7.5g/100g wheat flour levels) resulted in no significant effect on \( \beta \)-glucan solubility. In agreement with that, Andersson et al. (2004) reported that there was no significant difference in the molecular weight of \( \beta \)-glucan between the final dough and the baked bread. However, the extent at which \( \beta \)-glucan can be degraded and still maintain its physiological effects still under investigation (Cleary et al. 2007; Flander et al. 2007; Andersson et al. 2008).

There is not enough data available in the literature about the effect of frozen storage on \( \beta \)-glucan properties. In contrast to the current study, bread in all of these previous studies was made from fresh doughs. As a result, only the \( \beta \)-glucan degradation effect of the dough mixing and proofing processes were reported and no data available about low temperature storage of dough. Other studies investigated whether or not the cold storage of food products that contain \( \beta \)-glucan affects the physicochemical properties of \( \beta \)-glucan. For example, when bread, composite with barley \( \beta \)-glucan concentrate, was stored at ambient, refrigeration and frozen conditions, a reduction in \( \beta \)-glucan molecular weight and viscosity was observed. It was concluded that it is better to consume bread with \( \beta \)-glucan fresh to maintain highest bread quality and \( \beta \)-glucan solubility and viscosity (Moriartey 2009). In agreement with that, reduction in \( \beta \)-glucan solubility increased as frozen storage period of muffins composite with \( \beta \)-glucan increased (Beer et al. 1997a,b). Moreover, Lan-Pidhainy et al. (2007) reported similar results with oat bran muffins upon increasing in the number of freeze-thaw cycles.
The contradicting results obtained in different studies could be because β-glucan used was from different sources, such as; oats, barley and rye. Besides, different extractions as well as physiological effectiveness analysis techniques have been used, which may yield different and less realistic results (Lazaridou and Biliaderis 2007; Moriartey 2009). The molecular weight of barley β-glucan has been reported to be in the range of 31-2700 x 10^3, this large Mp range is due to different environmental conditions, extraction procedures and analytical techniques (Lazaridou and Biliaderis 2007).

Many different techniques have been used to study the structural differences between β-glucan from different sources, such as; oats, barley and rye. For instance, some researchers Jiang and Vasanthan (2000); Panagiotopoulos et al. (2001) and Talaga et al. (2002) used liquid chromatography equipped with different columns and detectors, while others (Olson et al. 1988; Pettersen and Schandt 1991) used Gas chromatography (GC).

Another effective method is high–performance size-exclusion chromatography (HPSEC) (Wood et al. 1991a,b; Vatandoust 2012). In general, most of the previous methods analyse β-glucan through the oligo- and mono-saccharides produced after β-glucan hydrolysis either by enzymes (e.g., lichenase) (Izydorczyk et al. 1998a,b; Jiang and Vasanthan 2000) or strong acids such as trifluoroacetic acid and sulphuric acid (Olson et al. 1988; Pettersen and Schandt 1991).

However, HPSEC is believed to be superior to the other methods, particularly for determining average molecular weight (Mw) of β-glucan. This is because it separates β-glucan according to its molecular size, then the Calcofluor detector binds selectively to β-glucan, which ensure good analysis results even from unpurified and crude extracts (Wood et al. 1991a,b; Vatandoust 2012).
2.9. Conclusion

The future of frozen dough industry is very promising. The use of frozen dough has increased in bakeries due to its advantages for bakeries as well as consumers. An important requirement for frozen dough products is to keep functional properties at the level of freshly prepared dough. However, quality of baked goods prepared from frozen dough is often poorer. The major shortcoming of the frozen dough is the short shelf life of six to eight weeks. In addition, freezing and thawing process play a vital role in determining the quality of frozen food products in terms of its texture, taste and appearance. Different ways to minimize the effect of freezing on doughs/batters are suggested in the literature, but no study so far has used β-glucan to enhance the quality and functionality of frozen dough. Therefore, this aspect will be a focus of this study to understand the effect of barley β-glucan on the quality of frozen dough/batter and final baked product.
Figure 2.1. Process Flow Diagram of Conventional and Frozen Dough/Batter Baking

Bread Dough ———, Cookie Batter———
Figure 2.2. Flowchart of the Air-Classification Process for Barley Flour

- Hulled Barley → De-hulling → De-hulled barley → Pearling (6%) → Pearled Barley
  - Pin-milling 2×7000 rpm → Barley Flour
  - Air Classification

- Coarse Fraction (CF) Yield 40% particle size 120-477μm (high β-Glucan and starch content)
- Fine Fraction (FF) Yield 60% particle size 45-120μm
Figure 2.3. Schematic Representation of β-Glucan
CHAPTER 3:

EFFECT OF β-GLUCAN-RICH BARLEY FLOUR ON RHEOLOGY AND QUALITY OF FROZEN YEASTED BREAD DOUGH

3.1. Abstract

Frozen storage of dough for a long time affects gluten structure and yeast survival which reduce quality of end product. The aim of this study was to investigate the effect of air-classified barley flour rich in β-glucan (~25%) on the quality of yeasted frozen bread dough. Wheat flour was replaced by barley flour at 10% replacement level without or with 1.4% vital gluten. Three dough formulations were tested: control dough (C), barley-composite dough without vital gluten (ACB) and with vital gluten (ACB-G). Doughs were stored at -18°C for 8 weeks. Dough rheology was affected by frozen storage to different extent with composite doughs exhibiting the least changes. By the end of the 60 days of frozen storage, dough extensibility increased for samples C, ACB and ACB-G by 50, 11 and 10%, respectively. Similar viscoelastic solid behavior among doughs was observed as indicated by the decrease in storage and loss modulus of frozen doughs as frozen storage time increased with the most significant effect observed on the first week of frozen storage. DSC results indicated that ACB and ACB-G composite doughs maintained lower enthalpy by 5% and less freezable water amount by 10% compared to C dough. NMR showed that ACB and ACB-G doughs exhibited 9% more bound water than C dough. 10%ACB and 10%ACB-G composite doughs also exhibited less gluten network changes as shown by SEM photographs. Dough mixing exhibited significant effect on β-glucan molecular weight and solubility in dough, while freezing affected only β-glucan solubility as shown by high performance size exclusion chromatography and flow injection analysis. The results showed that addition of air-classified barley flour at 10% level in frozen wheat dough minimizes the deterioration effects caused by frozen storage via minimizing water redistribution and maintaining the rheological properties of frozen dough.

Key words: Frozen dough, Bread, Molecular weight of β-glucan
3.2. Introduction

In recent years, frozen dough market has grown rapidly due to a big demand by the food industry and food services. Therefore, baking industry has been exploiting advantages and applications of freezing technology in several frozen dough bakery applications. The production of baked products from frozen dough allows easier and more profit, as it reduces the production costs by facilitating transportation and avoiding the need to skilled labour since it is ready to bake and easy to handle.

Bread has been well known as the oldest, most popular and widely consumed food in the world. This fact is due to assorted bread types, delectable taste and valuable nutritional properties. Bread has been traditionally homemade using recipes that differ from household to household and from culture to another (Cauvain and Young 1999). Bread making technology is basically processing wheat flour and other ingredients into a light, aerated, and palatable food. These products have a short shelf-life and lose their freshness quickly, which negatively influences the product quality and consumer acceptance. Frozen dough allows fresh baked products, with desirable quality and sensory characteristics, to be available around the clock (Gelines et al. 1995).

A major shortcoming of frozen dough is the substantial deterioration of baking quality with increasing frozen storage period (Huang et al. 2008). Frozen storage of dough for a long time affects gluten network structure and yeast survival. The destruction of yeast during freezing resulted in increasing proof time by decreasing gas production and releasing reducing substances from dead yeast, which weaken the gluten network. Frozen storage affects water distribution in the dough system, which mainly alters the properties of gluten and starch, especially the water binding capacity of gluten and starch as well as other dough constituents (Loveday et al. 2012).
Several studies have reported that the quality of baked products made from frozen dough is not only influenced by freezing rate, thawing rate and frozen storage temperature and period, but also wheat flour quality, dough additives, dough formulation and fermentation, as well as processing parameters such as dough mixing time (Inoue and Bushuk 1991, 1992; Neyreneuf and Vanderplaat 1991; Le Bail et al. 1999).

The use of suitable additives has become a common practice in baking to minimize the effect of freezing on dough. The main role of most of the additives used is to interact with water in dough, which can affect the quality of the end bakery product (Hudson et al. 2000; Yi 2008). For example, egg yolk and sugar ester (Hosomi et al. 1992), locust bean gum and xanthan gum (Lo and Ramsden 2000; Matuda et al. 2008), whey protein, polyols, carboxy methyl cellulose and gum Arabic (Asghar et al. 2005a,b, 2009), dietary fiber (blend of inulin and oat fibers) (Leray et al. 2010), starches (maize, rice and wheat), and two non-starch polysaccharides (xanthan and locust bean gum galactomannan) (Lo and Ramsden 2000), mono- and diacetyltartaric acid (DATEM) (Ribotta et al. 2004), soy ingredients (Simmons 2012) were used to study their effect on the quality of frozen dough. Sharadanant and Khan (2006) observed damaging in the gluten network and separation of starch granules from the gluten when control dough (100% wheat flour) was stored in a freezer at -23°C for 4 to 16 weeks, while better retention of the gluten network was observed after the addition of selected gums (locust bean gum and gum Arabic) after different periods of storage. Most of the studies concluded that stability of frozen dough can be enhanced with addition of food gums, but up to date no study investigated the effect of air-classified barley flour enriched in β-glucan on the functionality and quality of frozen dough.
The high water holding capacity of β-glucan is expected to have a significant effect on water behaviour of frozen dough system, which consequently will affect its rheological and functional properties (Wood 2010). The aim of this study is to investigate the effect of β-glucan-rich barley flour on dough rheology and structure and to maintain bread dough quality during frozen storage.

3.3. Materials and Methods

3.3.1. Materials

Vieenna strong bakers’ wheat flour (control) and air-classified barley flour rich in β-glucan (~25%) were kindly provided by Parrish and Heimbecker Millinng Group, Dover Flour, Cambridge. Vital wheat gluten was kindly provided by ADM (Archer Daniels Midland, Candiac, Ca). Bakery yeast (Fleischmann’s, Traditional, Active Dry Yeast, product of Canada) was purchased from a local grocery. All assay kits and enzymes were purchased from Megazyme (Megazyme International, Bray, Co. Wicklow, Ireland).

3.3.2. Sample Preparation

Composite flours (90% wheat flour and 10% air-classified barley flour and 88.6% wheat flour, 10% air-classified barley flour and 1.4% vital gluten) were thoroughly mixed to produce uniform flours. Vital gluten was added at close portion of gluten dilution in wheat flour, which is due to the addition of barley flour. Yeasted bread doughs were made on a 100g scale from control and composite flours (Table 3.1). Doughs were prepared according to the approved method AACC10-10-03 (2011) using the recommended 90min fermentation and 33min proof times.
3.3.3. Dough Frozen Storage and Thawing Conditions

Dough were weighed and put in a polyethylene Zip-lock bags and then the dough samples were put in a covered plastic box to control temperature. Then the plastic boxes were stored in a freezer (ThermoScientific, Revco Value Series, CA) at -35°C for 3 hours followed by frozen storage in a -18°C freezer (Traulsen, G-Series, USA) for 8 weeks.

Before analysis, frozen dough was allowed to defrost for 2h at 31°C and 70% RH in a Hobart proofer (Hobart, USA). Analysis were conducted on day 0 (immediately after mixing) for fresh dough and every week for frozen-thawed dough,

3.4. Methods

3.4.1. Chemical Composition of Wheat, Barley and Composite Flours

Ash and total fiber insoluble and soluble dietary fibre were determined according to the AACC (2011) methods 08-01.01 and 32-07-01, respectively. Moisture content was determined using a Moisture Analyzer (Ohaus Halogen Moisture Analyzer, Ohaus, Switzerland), Protein was determined by Dumas method based on the combustion method using a nitrogen analyzer (FP-528 Leco Instrument Ltd Mississauga, ON. Canada). β-Glucan content was determined using β-glucan enzymatic assay kit (AACC 32-23 2011). All assay kits were supplied by (Megazyme International Ireland Ltd, Bray, Wicklow, Ireland).

3.4.2. Quality of Wheat and Composite Flours

Brabender Farinograph-E (GmbH & Co. KG. Duisburg, Germany), equipped with a 50g bowl was used to determine water absorption (%), dough development time (min), stability
(min), and mixing tolerance index (BU) according to the AACC approved method 54-21-02 (AACC 2011).

Gluten Peak Test (GPT) (Brabender GmbH and Co. KG. Duisburg, Germany), was used to measure gluten strength and quality according to Kaur and Seetharaman (2012). Solvent (9.5g, 0.5M CaCl2) was weighed in the GPT stainless steel mixing cylinder. Flour (8.5g) was added to the solvent and the test was immediately commenced at 1,900 rpm for 10min. Test temperature was adjusted at 34°C using a Brabender water bath connected to the GPT. The torque (Brabender equivalents, BE) and peak maximum time (PMT) resulting due to formation of gluten network were calculated using GPT software (version 1, Brabender GmbH and Co KG, Duisburg, Germany).

3.4.3. Rheological Properties of Dough

Dough extensibility was investigated using Texture Analyzer (TA.XT2. plus, Texture Technologies, Corp. Scarsdale, NY, USA) with a Kieffer extensibility rig and 5kg load cell to measure the stretching properties of dough following the procedure described by Yi and Kerr (2009) with some modifications. Approximately 50g fresh or frozen-thawed dough sample was placed into a Teflon-coated block and cut into dough strips approximately 7mm in diameter and 60mm in length using a mould cutter. Samples were left to rest in the Teflon grooved plate for 15min. Then, the dough strips were pulled at a crosshead speed of 3.3mm/s for a distance of 75mm. The resistance to extension (the maximum height of the curve, mean max force g) and extensibility from start to rupture (mean distance at max force mm) of fresh and frozen-thawed doughs were automatically calculated from the force deformation curves using Texture Exponent 32 software (Texture Technologies, Corp. Scarsdale, NY, USA).
Texture Analyzer was also used with a Chen–Hoseney stickiness rig as described by Chen and Hoseney (1995) to investigate the stickiness of fresh and frozen-thawed doughs as described by Chen and Hoseney (1995). Dough samples were placed in a cylindrical cell, which was then sealed by a lid with a perforated hole and a small amount of dough was extruded through the hole. The cylindrical cell with the sample was placed on the TA base. The TA with a 25mm Perspex cylinder probe was used to provide a constant compression force and to measure the tension force. Within a probe travel distance of (4mm), the 25mm Perspex cylinder probe was brought in contact with the exposed dough to adhere to it at compression force (40g-force), trigger force (5g-force), probe travel speed (2mm/s) and probe returning speed (10mm/s). Both the maximum force and the area under the force–deformation curve resulted from separating the probe from the test sample was automatically calculated using Texture Exponent 32 software (Texture Technologies. Corp. Scarsdale, NY, USA) and used as measures of dough stickiness as the maximum force of the positive peak (g).

Fundamental rheology methods under small deformations were performed to complement the empirical rheological tests and to characterize macromolecular interactions between the main components of the dough samples. Dynamic rheological analysis of dough was performed using a controlled stress Rheometer TA AR 2000 (TA Instruments, New Castle DE, USA) and a 40mm diameter parallel geometry. The dough samples were placed between two parallel geometries (40mm diameter) with adjusting the gap at 1mm and the extra dough was trimmed using a spatula. To prevent moisture loss and drying during the measurement, the edge of the sample was coated with Silicone oil (S159-500, Fisher Scientific). Strain sweep tests at a constant frequency of 1Hz and a relative strain range of 0.01–100% after 2min equilibration were performed to determine the linear viscoelastic region. Dynamic moduli were collected and
plotted as a function of the applied strain. Oscillatory tests with a frequency sweep from 0.01 to 100Hz were conducted at 25°C for all the samples under strain of 0.5%, which was within the linear viscoelastic behavior region for wheat and composite flour fresh doughs as previously determined by the strain sweep test. Dough portions were removed from the Rheometer after measurement and the plate surfaces were thoroughly cleaned with distilled water and dried to remove excess residue. The dynamic rheological properties of samples measured were the storage modulus G’ (elastic modulus), which provides information about the elastic property (the property of dough to recover and return back to its initial shape after deformation), and the loss modulus G” (viscous modulus), which refers to the amount of energy dissipated per cycle. The tests were replicated three times and values were analyzed using TA Rheology Advantage Data Analysis software. Average measurements were calculated and reported in the graphs that show the viscoelastic properties of fresh compared to frozen doughs stored at -18°C f for 8 weeks (Zhang et al. 2011)

3.4.4. Freezable Water Content using Differential Scanning Calorimetry

The freezable water content of fresh and frozen-thawed dough was investigated using a TA Q1000 DSC (TA Instruments, New Castle, DE, USA) equipped with an external cooling system (RCS) and a purge system. Nitrogen gas flow of 20ml/min was used to avoid any water condensation in the calorimeter head at the surface of the pan during thawing of the dough. The DSC was calibrated with indium and sapphire. A sealed and empty aluminum pan was accurately weighed and used as a reference. Approximately 10 to15mg of frozen-thawed dough samples were placed in a hermetic alodined aluminum pan (TA Instrument, USA) and hermetically sealed. The sealed pan with sample were equilibrated at 25°C for 5min, cooled from 25 to -40°C at a rate of 10°C/min, held for 5min at -40°C, and then heated to 25°C. After the thermal analysis,
the ice-melting curve (thermogram) was obtained (Figure 3.1). The ice melting enthalpy was obtained with Universal Analysis software (TA Instruments, New Castle, DE) by integrating the ice melting peak located at about 0°C on the thermogram. For each sample, relative amounts of freezable water measurements were carried out in triplicate (Jia et al. 2012; Meziani et al. 2011). The quantity of freezable water in percentage was calculated by dividing the ice melting enthalpy (in J/g of product) by the latent heat of ice fusion (Lf= 333 J/g). This freezable water quantity was next calculated as a (%) of total water by dividing the result by the percentage of total water in the dough and multiplied by 100 (Leray et al. 2010).

3.4.5. Water Mobility in Dough using Nuclear Magnetic Resonance

The proton spin-spin relaxation studies were carried out at 20MHz with a Bruker Minispec PS/20 NMR spectrometer (Bruker Optics, Milton, ON, Canada) for the determination of water mobility in wheat and composite bread dough at fresh and frozen-thawed statuses as described by Lu and Seetharaman (2013) with modifications. The $^1$H-NMR device was connected to a water bath (Isotemp 3006D, Fisher Scientific, USA) adjusted at 10°C to achieve a temperature of 22°C inside the cell holder. The transverse relaxation time (T2) was determined following the Carr–Purcell–Meiboom– Gill technique with the 90–180° pulse sequence. Acquisition parameters were: pulse separation (τ) was 0.25, the number of data points for fitting was 2,000 and the number of echoes not fitted, where no sampling points are collected, was 1. Four scans were accumulated to increase the signal-to-noise ratio. The recycle delay, which is waiting time after the last train of pulses applied and the new scan starts, was 5sec. Dough samples (~0.4g) were weighted in 8mm diameter, 18cm length NMR tubes and an air tight protective cap was placed on the tubes. A tape was placed at 11cm from the bottom of the 18cm NMR tubes in order
to make sure that the position of the sample is in the centre of the coil inside the measurement cell. The NMR was calibrated on the previous sittings using the control dough sample.

The data obtained were analyzed with the continuous distribution model (CONTIN) application (Provencher 1982) along with Minispec software (Minispec Application pool version 5.2 relaxation/contin t₁t₂, Bruker, Milton, ON, CA). The CONTIN application was used to illustrate the distribution of the magnetized protons while they are relaxing at a given moment with their respective rate constants. Peak fitting was performed with Igor Pro 6 software (Oregon, USA), with the use of a Gaussian distribution and the ratio of bound to free water was calculated using the area under the curve in a plot of T2 peak relaxation time versus relative intensity obtained from Igor Pro 6 software (Oregon, USA).

3.4.6. Weight Loss, Moisture Content and Water Activity of Doughs

Weight loss of frozen dough was determined according to (Phimolsiripol et al. 2008). Frozen dough samples were taken out of the polyethylene Zip-lock bag and weighed in grams using a laboratory balance immediately after their withdrawn from freezer and before defrosting. The weight loss was the difference in percentage between the initial value (weight of dough at day 0) and the final weight. Moisture content was determined using (Ohaus Halogen Moisture Analyzer MB45, Ohaus, Switzerland). Dough sample (~0.6g) was placed in the MA tray and moisture content tested at 130ºC for 5min. Dough water activity was recorded using Water Activity Analyzer (Aqua Lab 4TE, Decagon Devices, USA).

3.4.7. Micro-Structure of Dough using Scanning Electron Microscopy

A Hitachi S-570 Scanning Electron Microscope (Hitachi High Technologies, Tokyo, Japan) with a K1250X Cryogenic Preparation System (Quorum Technologies Ltd, Ashford, UK)
was used to investigate the changes in micro-structure of frozen dough. The dough samples were taken from the center of the dough piece to avoid areas near the surface, fractured under liquid nitrogen (~ -196°C) and 2 small pieces were placed in a cryo-specimen holder. The holder was transferred into the cryo-unit in the frozen state and under vacuum. The stage was maintained at -170°C in the cryo-chamber and -150°C in the SEM with liquid nitrogen. The sample surface was partially freeze-dried in the cryo-chamber or in the SEM at -80°C for 45min to remove some of the ice. The surface was sputter coated with platinum (<30nm) in an argon atmosphere, then transferred to the cold stage of the microscope where it was observed at 10kV and <-140°C. Dough samples were examined on day 0 (after mixing) and after 1, 4 and 8 weeks in frozen storage. Images were digitally captured from different locations and at a range of magnifications per sample and those selected for discussion are considered to be representative.

3.4.8. Physicochemical Characteristics of β-Glucan

β-Glucan was extracted from dough samples with hot-water and thermostable α-amylase extraction method with some modifications according to Rimsten et al. (2003). Dough samples (about 4g containing about 0.1mg β-glucan/1ml final extract) was broken into small pieces and placed in a 45ml centrifuge tube with a magnet stirrer and 25ml deionized water with CaCl₂ (0.28 mg/ml of H₂O) and 0.2% of Sodium Azide) and 200µl thermostable α-amylase were added. The sealed 45ml centrifuge tube was placed in a boiling water bath on a laboratory heater stirrer plate for 90min. After cooling, the tube was centrifuged (1,500×g for 15min) and the β-glucan extract supernatant was analysed on the same day for molecular weight and solubility of β-glucan.

The β-glucan extract was diluted (1:1 for control and 1:20 for composite dough samples) with deionized water to reach desirable concentration (~0.1mg/ml) and filtered (0.45µm). Peak
molecular weight ($M_p$) of β-glucan in the extracts was determined using high-performance size exclusion chromatography (HPSEC) equipped with calcofluor detector as described by Ragaee et al. (2008). The chromatographic system consists of a Perkin Elmer ISS-100 autosampler and injector, a Shimadzu model AD-vt HPLC pump, a Shimadzu RF-10Axl fluorescence detector, a Waters (Milford, CT) model 510 HPLC pump for post column addition of calcofluor, and the Viscotek DM 400 data manager. Data integration was performed using TriSEC 3.0 software (Viscotek, Houston, TX). Six β-glucan molecular weight standards (ranging from 20,000 to 1,300,000 g/mol) obtained commercially from (Megazyme) were used to construct a calibration curve for β-glucan by plotting retention time versus log peak molecular weight ($M_p$) (Wang et al. 2003). Soluble β-glucan concentration in the extract was determined using the Flow Injection Analysis (FIA) system according to Beer et al. (1997a).

3.4.9. Statistical Analysis

All analyses were performed at least in duplicate and the mean values are reported. Analysis of variance was performed using IBM SPSS Statistics 20 software. Significant difference ($p<0.05$) among means were detected using the Tukey’s multiple range test at a fixed level of $\alpha = 0.05$.

3.5. Results and Discussion

3.5.1. Quality of Wheat and Composite Flours

Composition of wheat flour, air-classified barley flour and their composite flour is presented in Table 3.2. Protein content was quite similar in wheat flour and composite flour, while barley had higher protein content. Barley flour also contained more dietary fiber fractions and β-glucan contents compared with wheat flour. Thus the composite flour had higher levels of
dietary fiber and β-glucan at 10.5% and 2.7% compared with 6.1% and 0.4% for wheat flour, respectively.

Quality of wheat flour is a key in making appropriate dough for a given end product. In the current study, flour quality was assessed using Farinograph and GPT. Farinograph profiles and data of control and composite flours are presented in Figure 3.3 and Table 3.3. As expected, Farinograph data showed significant increase in water absorption for composite flour by 16% to reach 500BU without and with gluten compared to control (Figure 3.2), while no significant effect on the rest of Farinograph parameters was observed. The presence of barley flour rich β-glucan would disturb the formation of the intermolecular disulfide bridges that allow longer stability (Autio et al. 2001). Izydorczyk et al. (2001) reported an increase in dough strength when added whole barley meal, small amounts of starch or non-starch polysaccharides (arabinoxylans and β-glucans) separately to wheat flour. In the current study a slight increase in time to breakdown and Farinograph quality number was observed for the composite flour with gluten. This indicates that β-glucan rich barley flour may have a potential to replace a portion of wheat flour at a replacement level of 10% without causing detrimental consequences on dough quality. The addition of 1.4% vital gluten seems to minimize the negative effect caused by barley flour.

The significant increase in water absorption is probably due to the high β-glucan content in the barley flour. β-Glucan with its high water-binding capacity would compete with other dough constituents especially the formed gluten for available moisture and affect water distribution in the dough system. At the same time, β-glucan would minimize the amount of free water in dough (Rieder et al. 2012). Therefore, higher amount of water is required to reach a fully developed gluten network in flour containing β-glucan.
Gluten Peak Teter (GPT) was used as a rapid shear-based method for discriminating gluten quality and functionality of flour in an aqueous solution (Kaur and Seetharaman 2012). The GPT results were in agreement with Farinograph data. Maximum torque (MT) and peak maximum time (PMT), also known as gluten aggregation time, are obtained as a result of gluten network formation. Basically, high torque response and short PMT are indications of high quality gluten. The addition of 10% barley flour resulted in a significant increase in MT and PMT compared to the control, while addition of both 10% barley flour and 1.4% vital gluten resulted in a MT similar to that of control with significantly higher PMT. The long PMT is due to the addition of barley flour with or without vital gluten indicates a delaying in developing gluten network. The dilution of gluten by the addition of barley flour was found to disturb the development of gluten network (Kaur and Lok 2013). Kaur and Lok (2013) blended Millet and Kamut flours with soft wheat flour at 0, 25, 50, 75 and 100 % replacement levels and reported that as the level of Kamut flour increased, higher PT and shorter MPT were obtained, while increased millet flour caused the opposite effect due to the high protein content in Kamut flour which increased the gluten network strength.

β-Glucan is capable of forming rigid, rod-like conformation solutions when mixed with water, which may contribute to the overall elasticity and strength of the dough (Izydorczyk et al. 2001). Therefore, the high PMT obtained with the addition of barley flour alone cannot be considered as an indication of high quality gluten as Figure 3.4 illustrates that composite flour curve increased sharply once water is added showing higher PMT and faster dough development compared to control. But this could be related to the resistance of the elastic networks formed by β-glucan and not to the gluten network formation. The addition of 1.4% vital gluten to composite flour may counteracts the effect of β-glucan elastic network as indicated by its PMT, which was
similar to that of control. However, the dilution effect of the barley flour on the wheat gluten network still exist and can be identified by the significantly extended long PMT that gluten needs to aggregate and form a gluten matrix compared to the control.

Wheat dough is expected to entrap gas produced by yeast and expand to the maximum level without breaking down. Peighambardoust et al. (2010) and Sivam et al. (2010) reported that these properties are mainly attributed to the appropriate cross-links among wheat proteins (gluten proteins) that generate a continuous viscoelastic network during dough development. Having the above mentioned effects, the fibrous nature of air-classified barley flour (52.3% total dietary fiber) could be the most critical factor for bread dough during processing, storage and baking (Peighambardoust et al. 2010; Sivam et al. 2010).

3.5.2. Moisture Content, Water Activity and Weight Loss of Dough

In general, no significant changes were observed in moisture content and water activity of fresh and frozen-thawed dough for all samples. A significant change in the weight of control frozen dough was observed after the first week of frozen storage. However, no changes in dough weight were observed with the presence of 10% barley flour with or without gluten (Figure 3.5).

Lo and Ramsden (2000) reported that freeze/thaw cycle showed no significant effect on wheat starch gels water holding capacity even with the addition of non-starch polysaccharides such as, xanthan and locust bean gum galactomannan. In agreement with that, Leray et al. (2010) concluded that fiber-enriched wheat dough becomes more resistant to freezing than the reference wheat dough with a slight change in moisture content during the first week of storage, and then returned to its initial value at the long storage time. Phimolsiripol et al. (2008) reported a constant dough weight loss for different storage regimes -18±0.1°C (control), -18±1°C (good practice), -18±3°C (poor practice), and -18±5°C (very poor practice, VP), which increased with
increasing storage time. The authors suggested that the frozen dough may become a frost inside the polyethylene bag during the transfer of water/ice. They suggested that two mechanisms could involve in the dough weight loss during the first week of frozen storage. First, the difference in the saturated vapour pressure of water on the frozen dough surface and the polyethylene bag partial pressure of water vapour. Once there is a difference in temperature between the bag and dough, the water vapour will diffuse from the dough to the bag or vice versa due to the changes in the saturated vapour pressure of water on the frozen dough surface. Second, the dough weight loss could be due to the loss of carbon dioxide gas produced by yeast during fermentation. At the beginning of frozen storage period in this study, dough weight loss is more possibly attributed to the second mechanism taking into account the slow fermentation occurred inside the dough before the dough is fully frozen.

3.5.3. Rheological Properties of Dough

Empirical and fundamental rheological measurements of dough give an estimation of dough’s viscoelastic balance. Good bread dough is the dough that exhibits optimum balance of extensibility and elasticity in order to produce bread with desirable volume and crumb characteristics (Hoseney 1994).

Dough extensibility determines the expansion ability of dough caused by CO₂ during proofing and the resistance to extension is a measure of dough ability to retain CO₂ (Yi and Kerr 2009). Results obtained from texture analyzer (Figure 3.6), by performing extensional deformation on a fully developed dough, showed that fresh control dough exhibited significantly lower dough resistance to extension (the measured force that the dough resisted during stretching), while it showed significantly higher extensibility (the distance to which dough can be stretched before it ruptured). It can also be seen that in the first two weeks of frozen storage
composite sample with the presence of vital gluten exhibited higher resistance to extension compared to the control and the composite sample without vital gluten.

During the first 4 weeks of frozen storage slight changes were observed in dough resistance to extension and extensibility for composite doughs as compared to control, which showed a significant increase in both parameters. No significant changes were observed during the rest of the 8 weeks frozen storage time for all the samples.

Addition of 10% barley flour with and without vital gluten showed a marked physical improvement during frozen storage by reducing alteration in frozen dough extensibility and resistance to extension. Results obtained are in agreement with that of Simmons et al. (2012) who concluded that supplementation of frozen wheat dough with 48.5% soy protein resulted in minimizing the changes in dough extensibility and resistance to extension leading to minimal changes in bread hardness. This could be because of the ability of β-glucan in barley flour to inhibit ice crystal growth through binding water, which was confirmed by the DSC and NMR analyses.

Sharadanant and Khan (2003a,b) and Yi and Kerr (2009) reported that dough maximum extensibility increased with frozen storage time, whereas the maximum resistance to extension decreased. Some studies reported an increase in maximum resistance relative to extensibility with frozen storage or freeze-thaw cycles (Bhattacharya et al. 2003), whereas others reported the opposite tendency (Inoue and Bushuk 1991; Inoue et al. 1994).

Dough extensibility is mainly attributed to the elastic cross-linked network formed by gluten proteins in the wheat flour. The progressive dehydration of gluten caused by water migration to form ice crystals and the formation of ice crystals during freezing could attribute to physical disruption of the dough matrix (Rasanen et al. 1997; Lu and Grant 1999; Bhattacharya...
et al. 2003). Some authors also suggested that the reduction of gluten cross-linking could be caused by release of disulphide reducing substances from dead yeast (Ribotta et al. 2001; Giannou et al. 2003). Sharadanant and Khan (2003a,b) reported that an excessive extensibility would result in soft and weak dough that would collapse during proofing or baking. It is important that the dough undergoes minimal changes during frozen storage to maintain high quality (Simmons et al. 2012).

Texture analyzer was also used to measure dough stickiness properties. Dough stickiness (or adhesiveness) is the measurement of the force required to separate the dough after dough adhering or sticking to a surface. It depends on both the adhesive and cohesive properties of the dough. Too sticky dough is very difficult to handle and may adhere to machine surfaces. In addition, it may result in bread that is too chewy, adheres to the mouth or seems to be underbaked (Armero and Collar 1997; Hoseney and Smewing 1999; Fiszman and Damasio 2000). No significant differences were observed in stickiness values between freshly mixed control and composite samples (Figure 3.6). During the 8 weeks of frozen storage period, all samples exhibited a significant increase in stickiness compared to its stickiness measured immediately after mixing. This indicates that adhesive and cohesive properties of composite frozen doughs were comparable to that of control. Similar results were reported by Angioloni et al. (2008) and Yi and Kerr (2008, 2009) on frozen dough. Yi and Kerr (2009) reported increase in stickiness of frozen dough stored for 30, 90 and 180 days in the presence of waxy wheat flours (WWF). Angioloni et al. (2008) and Yi and Kerr (2009) suggested that the increase of frozen dough stickiness could be because of the effect of different factors together such as ice crystals redistribution through recrystallization, deterioration of the gluten network and water redistribution during freezing and after thawing, which would lead to separation of water from
the gluten strands. When the dough is thawed, the ice crystals melt but the water is not in the original state. Water becomes less closely associated with the gluten and starch network and more free water amount is accessible at dough surface, thus increasing adhesiveness.

Dough rheological properties, which highly affect final products’ textural and sensory properties, were also determined using dynamic oscillatory rheometry to measure the response of a material to an applied force (stress) or deformation (strain) during 8 weeks of frozen storage.

The presence of the three dimensional network of gluten proteins, which is formed by thiol-disulfide exchange reactions among gluten proteins, is responsible for the viscous and elastic properties of the dough (Dobraszczyk and Morgenstern 2003; Belton 2005; Song and Zheng 2007). Dough viscoelastic measurements involve compression of a dough piece between parallel circular plates by which a sinusoidal stress is applied in the form of small amplitude rotational oscillations. As a result, the ability of a material to store and/or dissipate the applied energy can be identified using the amplitude of the resulting strain and its phase difference from stress (Loveday et al. 2012).

The moduli $G'$ (elastic or storage moduli) and $G''$ (viscous or loss moduli) describe the character solid/ elastic and the viscous/liquid state of the sample, respectively. These terms are measured within the linear viscoelastic range of dough. For Fresh and frozen composite doughs, 10% barley flour without or with vital gluten, $G'$ was higher than $G''$ in the entire range of frequency following a typical viscoelastic solid behaviour (Song and Zheng 2007; Loveday et al. 2012).

The presence of 10% barley flour without or with vital gluten were more elastic and firmer than the control as indicated by significantly higher $G'$ and $G''$ moduli compared to the fresh control dough. After 1 week of frozen storage, significant reduction in $G'$ and $G''$ moduli was
observed for all the samples indicating reduction in dough firmness and elasticity (Figure 3.7). However, composite doughs showed less significant decrease in $G'$ and $G''$ moduli compared to control. All the samples did not show any extra drop in $G'$ and $G''$ moduli after the first week up to 8 weeks of freezing. Interestingly, $G'$ and $G''$ moduli increased slightly during the rest of the storage period with the 10% barley flour composite dough showing the most significant increase in $G'$ and $G''$ moduli followed by 10% barley flour with vital gluten then the control dough. During dough storage at -18°C, the loss in the polymer cross-linking and depolymerisation of glutenin aggregates caused by ice recrystallization and/or by release of reducing substances from yeast leads to a reduction in dough firmness and elasticity (Ribotta et al. 2003). Along with that is the dehydration of gluten network induced by freezing (Angioloni et al. 2008). Meziani et al. (2011) suggested that freezing and thawing treatments attribute to reduction in the dough resistance to deformation. Air-classified barley flour added in the present study contains high concentration of β-glucan, a soluble dietary fiber that has a great number of hydroxyl groups. It is capable of forming a viscoelastic gel network, increasing dough system viscosity, and inhibiting diffusion of solutes and/or water between pores (Goff et al. 1999; Regand and Goff 2003).

Our dynamic rheological data is in agreement with several studies. Ribotta et al. (2004) investigated the effect of mono- and diacylglycerols esterified to mono- and diacetyltartaric acid (DATEM) and guar gum on dynamic rheological behaviour of frozen dough stored at -18°C for 60 days and reported similar results. Angioloni et al. (2008) obtained similar results for 100% wheat flour dough at the same storage temperature and time. Meziani et al. (2011) also observed similar results after freezing 100% wheat flour doughs at different freezing rates (-20°C, -30°C, -40°C and immersion in liquid nitrogen). A study by Leray et al. (2010) illustrated that addition of
dietary fiber (blend of inulin and oat fibers) at the level of 10% increased wheat dough resistance to freezing and frozen storage. They also reported that addition of dietary fiber to frozen dough stored at two different temperatures, -18°C and -30°C for 1, 7 and 28 days showed a significant decrease in G’ moduli during the first week of storage and then remained constant through the rest of the storage time. These changes in rheological properties upon frozen storage could also be attributed to changes in dough protein physical properties. Meziani et al. (2011) reported that freezing process may denature gluten proteins by increasing the acidity of the concentrated liquid phase. The positive effect of dietary fiber on frozen dough rheological properties could be attributed to their ability to bind most of the available water in the dough system. As a result, it inhibits the formation of ice crystals and consequently their damaging effect.

3.5.4. Differential Scanning Calorimetry

Structural and phase transitions of foods are significantly affected by water in the food system. Differential Scanning Calorimetry (DSC) analysis was performed in order to study the thermal properties of fresh and frozen-thawed yeasted bread doughs. It is a common method used to study the thermal properties of bakery products based on calculating the ice melting enthalpy (ΔH) of dough during a freeze/thaw cycle (Sablani et al. 2002). The enthalpy recorded is directly correlated to the amount and state of water in the food system and used as an indicator of amount of freezable water present in dough (Bot 2003; Sharadanant and Khan 2003a,b; Simmons et al. 2012). Less changes in ΔH between freeze-thaw cycles of fresh and frozen-thawed dough indicates high freeze-thaw stability (Sim et al. 2012). The frozen water data obtained by DSC should be considered only as relative, because the latent heats determined and the calculations differ from absolute thermodynamic values (Laaksonen 2001).
During frozen storage of dough, ice melting enthalpy as well as freezable water content increased as frozen storage time increased for control dough. Frozen-thawed control dough also exhibited the highest ice melting enthalpy and freezable water content at different extents compared to the composite samples during the whole period of frozen storage (8 weeks). Incorporation of 10% barley flour without and with vital gluten in the wheat dough system resulted in a significant reduction in ice melting enthalpy (105-109 J/g) compared to the control (117 J/g) (Figure 3.8). Upon frozen storage of wheat dough with and without additives, water molecules are transformed to ice crystals and become less associated with protein and starch compared to fresh dough system. This results in changes in water distribution in the dough system during frozen storage and an increase in the freezable water content, ice crystals amount and consequently gluten network deterioration and injure of yeast cells (Sim et al. 2012).

In comparison to the control sample, composite samples also showed significantly lower freezable water content during the first 4 weeks of frozen storage followed by fluctuation changes during the rest of the freezing period. Our results were in agreement with that obtained by Sharadanant and Khan (2003a,b) and Sim et al. (2012) who reported that addition of food gums (sodium alginate, carboxymethylcellulose, psyllium husk powder, locust bean gum, gum Arabic and konjac glucomannan) to frozen dough decreased the amount of freezable water (FW) content in fresh as well as frozen-thawed dough. Along with that, food gums minimized the difference in melting enthalpy between fresh and frozen-thawed dough. This indicates that additives such as food gums have the ability to increase freeze-thaw stability in frozen-stored dough samples. Air-classified barley flour used in this study contains high concentration of β-glucan (~25%), which is known for its high water holding capacity. This could restrict the
mobility of water molecules and inhibit ice crystals growth leading to less FW content in the frozen dough system.

3.5.5. Water Mobility in Dough using Nuclear Magnetic Resonance

$^1$H Nuclear Magnetic Resonance (NMR) analyses were conducted as a complementary method to DSC to confirm DSC data. NMR spectroscopy determines the water status or binding characteristics in fresh and frozen-thawed dough by obtaining spin-spin relaxation times (T2). It does not require heating or cooling of samples, thus no physical or chemical changes occur in the sample system (Ruan and Chen 1998).

NMR measurements of control and composite dough samples (Figure 3.9) obtained in the present study showed that despite the significant difference in water absorption between control and composite samples, three populations of water were detected tightly ($T_{21}$, 0.5~2 ms), less tightly ($T_{22}$, 10~15 ms), and weakly ($T_{23}$, 80~120 ms) bound water (Figure 3.9 and Figure 3.10). Jasrotia (2011) and Lu and Seetharaman (2013) reported that the spin-spin relaxation times (T2), especially (T22), measurements are better representative and sensitive indicator of the overall mobility of water molecules in a dough system.

The presence of barley flour resulted in reduction in relaxation time, e.g. T22 decreased from 13.8ms to 12.8ms (Figure 3.9). In general, composite doughs maintained shorter relaxation time during the entire period of frozen storage compared to control sample. The composite doughs contained significantly higher amount of bound water (58%) than control (51%). This indicates that barley flour decreased the water mobility or at least water molecules had less rotational freedom in frozen dough.

NMR studies: Jasrotia (2011); Lu and Seetharaman (2013) reported that NMR determines the content of bound water in dough based on water molecules mobility, which is highly
affected by dough ingredients, freezing rate and frozen storage time. The lower that water molecules mobility, the higher the bound water content in the system. Rasanen et al. (1998); Esselink et al. (2003) and Yi (2008) reported that frozen storage reduces the relaxation time, however redistribution of water was observed due to formation and recrystallization of ice crystals. Yi (2008) reported that frozen-thawed doughs contain 15 and 30% waxy wheat flour had lower (T2), higher gas production and higher loaf volume compared to the control.

A study by Lu and Seetharaman (2013) was carried out to assess water behavior in freshly prepared (25°C), refrigerator-stored (4°C, one day), or freezer-stored (-35°C, one day) doughs that contain 5, 10, or 30% whole grain, air-classified β-glucan-diminished, and air-classified β-glucan-enriched barley flours. Addition of air-classified β-glucan-enriched barley flours showed a significant effect on reducing the relaxation time value and consequently water mobility. This indicates that the damaging effect of water redistribution from gluten, starch and other dough components during frozen effect can be minimized by some additives. Air-classified β-glucan-rich barley flour contain large number of available hydroxyl groups offered by β-glucan, which binds relatively large amounts of water in the dough matrix and reduces water mobility compared to the control dough.

3.5.6. SEM Micro-Structure of Dough

Scanning Electron Microscope (SEM) analysis was done to investigate structure of dough samples, including orientation of gluten strands and distribution of starch granules. Fresh control dough sample, analysed after mixing, showed a typical uniform dough structure where starch granules were embedded in the gluten network uniformly and entirely acting as inactive filler. On the other hand, the presence of barley flour without and with vital gluten in the dough system exhibited less uniform gluten network with more separated starch granules (Figure 3.11). This
could be because of the presence of dietary fiber components in particular β-glucans that interact with gluten proteins leading to strengthening and at the same time disruption of the gluten-starch network formation (Leray et al. 2010).

During frozen storage at -18°C for (1, 4 and 8 weeks), the gluten network of all samples appeared to be quite damaged and showed more porous structure while the thick gluten strands, white filmy materials, that surrounded the starch became thinner (Figure 3.11). However, frozen dough samples with 10% barley flour without and with vital gluten exhibited less structure changes compared to the control dough.

SEM images from previous studies (Berglund et al. 1991; Zounis et al. 2002a,b; Baier-Schenk et al. 2005) showed that water in frozen bread dough (with and without yeast) under different frozen storage conditions and times (up to 24 weeks), separated from the gluten-starch network and forms large ice crystals on the inside of the dough system. The ice crystals formation and expansion leads to the disruption of gluten matrix, rendering a network separated from starch granules. After thawing the dough, the redistribution of water in the dough system does not allow it to return to its initial state. As a result, water forms pools in the dough system and it becomes difficult to have equal water distribution throughout the dough, even with punching, leading to an increase in proofing time and decrease in loaf volume (Lu and Grant 1999). The effect of different freezing rates (19-69°C/h) and frozen storage time (up to 180 days) at four different temperatures -10, -20, -30 and -35°C was also investigated by Yi and Kerr (2009). It was concluded that the least changes in water distribution and gluten network were obtained from doughs stored at very low temperature -30 and -35°C. Despite that, strength of gluten network decreased as frozen storage time increased for all different freezing rates and temperatures.
Although addition of 10% barley flour with and without vital gluten restricted water mobility, reduced freezable water content, according to the DSC and NMR results, and retained the gluten network to a large extent, according to SEM images, the damaging effect of frozen storage on the dough structure was not totally avoided. Yi and Kerr (2009) suggested that since different doughs have their unique system, adjustment of freezing process should be done for each product to minimize frozen storage deterioration effect on dough. Results obtained by Zounis et al. (2002b) also confirmed that dough structure changes are not only resulted from frozen storage, but also from a number of different factors. These include dough mixing conditions, formulation and specific ingredients, which may inhibit or enhance structural damage in frozen doughs.

3.5.7. Physicochemical Properties of β-Glucan

Physicochemical properties of β-glucan such as solubility and molecular weight are important factors in determining quality and stability of dough during frozen storage. In the current study, Mw of β-glucan extracted from control and composite dough samples after mixing (fresh dough) and after frozen storage (every week for 8 weeks) were determined (Figure 3.12). In the present study, air-classified barely flour containing 25% β-glucan with molecular weight 250000g/mol and solubility 54% was used. The Mw results showed that the most significant changes in Mw observed after mixing and fermentation of composite doughs. Frozen storage of both composite dough samples showed no significant change in Mw during the entire period of frozen storage (8 weeks). However, the changes in Mw were inconsistency during the entire period of frozen storage in composite dough samples.

Solubility of β-glucan was also determined (Figure 3.13), the data showed that soluble β-glucan content in wheat flour increased significantly from up to ~50% after the addition of 10%
barley flour with and without vital gluten. Mixing of composite flours resulted in a significant change in soluble β-glucan amount in the freshly mixed composite doughs, while further changes were observed as frozen storage time increased with the minimum changes observed at the end of the frozen storage time.

The decrease in β-glucan Mw after mixing could be because of the degradation by endogenous β-glucanases present in the wheat flour during mixing (Wood et al. 1994, 2000; Cleary et al. 2007). There is not enough data available in the literature about the effect of frozen storage on β-glucan properties. Moriartey (2009) reported that incorporating barley β-glucan concentrate of low solubility at 3.75, 5.0 and 7.5g/100g wheat flour levels into bread dough resulted in no significant effect on β-glucan solubility. Andersson et al. (2004) reported that there was no significant difference in the molecular weight of β-glucan between the final dough and baked bread. Only the β-glucan degradation effect of the dough mixing and proofing processes were reported and no data available on low temperature storage of dough. Several studies investigated the effect of cold storage of baked food products containing β-glucan on its physicochemical properties. For example, molecular weight and viscosity of β-glucan in barley β-glucan concentrate composite bread reduced significantly when composite bread was stored at different temperatures (ambient, refrigeration and freezing) (Moriartey 2009). It was recommended that it is better to consume bread enriched with β-glucan fresh in order to maintain high bread quality and high solubility and viscosity of β-glucan. In agreement with that, reduction in solubility of β-glucan in muffins increased as frozen storage period increased (Beer et al. 1997b). Lan-Pidhainy et al. (2007) also reported reduction in solubility of β-glucan in oat bran muffins upon increasing the number of freeze-thaw cycles. The contradicting results obtained in different studies could be because of the different sources of β-glucan, different
extraction technique as well as physiological effectiveness analysis techniques, which may yield different and less realistic results (Lazaridou and Biliaderis 2007; Moriartey 2009).

3.6. Conclusion

The presence of 10% barley flour without and with vital gluten showed a marked physical improvement during frozen storage by reducing alteration in frozen dough empirical and fundamental rheological properties. The high fiber content in air-classified barley flour particularly β-glucan was able to bind water molecules that are released from the gluten-starch network restricting their mobility and inhibiting ice crystals growth. Therefore, higher amount of bound water and less freezable water content in the frozen dough system was obtained. Above that, there was a significant increase in soluble β-glucan amount in the composite doughs indicating that barley flour may have a potential to replace a portion of wheat flour and introduce the beneficial effects of β-glucan to a great number of consumers. Despite the improving effect of β-glucan during frozen storage of dough still some changes on the dough structure occurred as freezing storage period increased and additional research in this area is underway.
Table 3.1. Bread Formulation Based on 100g Flour

<table>
<thead>
<tr>
<th>ingredient</th>
<th>control dough (c)</th>
<th>composite dough with 10% barley flour</th>
<th>composite dough with 10% barley flour and 1.4% vital gluten</th>
</tr>
</thead>
<tbody>
<tr>
<td>wheat flour (g)</td>
<td>100</td>
<td>90</td>
<td>88.6</td>
</tr>
<tr>
<td>sucrose (g)</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>NaCl (g)</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>yeast (g)</td>
<td>5.3</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td>distilled water(^a) (ml)</td>
<td>64</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>shortening(^b) (g)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>ACB-E Flour(^c) (g)</td>
<td>-</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>vital Gluten (g)</td>
<td>-</td>
<td>-</td>
<td>1.4</td>
</tr>
</tbody>
</table>

\(^a\)Water absorption was determined using Brabender Farinograph. \(^b\)Crisco shortening. \(^c\)Air-Classified Barley flour rich in β-glucan.
Table 3.2. Approximate Chemical Composition of the flour Samples (Dry Base)

<table>
<thead>
<tr>
<th>component</th>
<th>control</th>
<th>ACB&lt;sup&gt;c&lt;/sup&gt;</th>
<th>composite flour&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ash %</td>
<td>0.71±0.1</td>
<td>2.5±0.0</td>
<td>0.9±0.0</td>
</tr>
<tr>
<td>protein %</td>
<td>14.96±0.1</td>
<td>17.1 ±0.0</td>
<td>15.04±0.3</td>
</tr>
<tr>
<td>moisture%</td>
<td>13.37±0.2</td>
<td>12.1±0.1</td>
<td>13.5±0.3</td>
</tr>
<tr>
<td>total dietary fiber %</td>
<td>6.1±0.0</td>
<td>52.3±1</td>
<td>10.52±0.0</td>
</tr>
<tr>
<td>SDF%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1±0.2</td>
<td>35.2±0.8</td>
<td>6.23±0.0</td>
</tr>
<tr>
<td>IDF%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1±0.2</td>
<td>17.10±0.2</td>
<td>4.3±0.1</td>
</tr>
<tr>
<td>β-glucan%</td>
<td>0.4 ± 0.0</td>
<td>24.9 ± 0.5</td>
<td>2.65±0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>SDF: Soluble dietary fiber. <sup>b</sup>IDF: Insoluble dietary fiber. <sup>c</sup>ACB: Air-classified barley flour rich in β-glucan. <sup>d</sup>Composite flour: 90% refined wheat flour+10% ACB.
Table 3.3. Quality of Wheat and Composite Flours

<table>
<thead>
<tr>
<th>farinograph parameters</th>
<th>control wheat flour</th>
<th>composite flour&lt;sup&gt;c&lt;/sup&gt; (ACB&lt;sup&gt;b&lt;/sup&gt;)</th>
<th>composite flour +1.4% vital gluten</th>
</tr>
</thead>
<tbody>
<tr>
<td>consistency [FU]&lt;sup&gt;a&lt;/sup&gt;</td>
<td>494.5±6.4a</td>
<td>503.5±2.12a</td>
<td>496.5±2.02a</td>
</tr>
<tr>
<td>waterabsorption [%]</td>
<td>64.1±0.14b</td>
<td>80.05±0.35a</td>
<td>80.6±0.3a</td>
</tr>
<tr>
<td>development time [min]</td>
<td>5.75±0.63a</td>
<td>5.7±0a</td>
<td>5.3±2.12a</td>
</tr>
<tr>
<td>stability [min]</td>
<td>8.1±0.3a</td>
<td>7.5±1.6a</td>
<td>8.6±0.42a</td>
</tr>
<tr>
<td>tolerance Index [FU]</td>
<td>33±2.82a</td>
<td>31±4.24a</td>
<td>30±2.82a</td>
</tr>
<tr>
<td>time to breakdown [min]</td>
<td>10.2±0.14a</td>
<td>9.15±1.9a</td>
<td>11.15±0.91a</td>
</tr>
<tr>
<td>farinograph quality number</td>
<td>102±1.41a</td>
<td>91.5±9.1a</td>
<td>111.5±9.19a</td>
</tr>
</tbody>
</table>

Statistical analysis within the same raw, same letter indicates no significant difference. <sup>a</sup>FU:Farinograph units. <sup>b</sup>ACB: Air-classified barley flour rich in β-glucan. <sup>c</sup>Composite flour: 90% refined wheat flour+10% ACB.
Figure 3.1. Example of DSC Curve Showing Ice Fusion Enthalpy ($\Delta H_{fw}$), Onset ($T_{onset}$) and Peak ($T_{peak}$) Temperatures
Figure 3.2. Water Absorption of Control and Composite Flour Samples at 500 Brabender Units
Figure 3.3. The Summarized Results of Farinograph Profiles for Different Flour
Figure 3.4. Gluten Peak Tester Analysis of Control (C) and Composite Flours without (ACB) and with 1.4% Vital Gluten (ACB+G)
Figure 3.5. Weight Loss, Moisture Content and Water Activity of Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks.
Figure 3.6. Empirical Rheology Analysis (Large Deformation Rheology) of Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 3.7. Fundamental Rheology Analysis (Small Deformation Rheology) of Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 3.8. Differential Scanning Calorimetry Analysis of Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 3.9. A: NMR Curves for Water Distribution of Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 3.9. B: Relaxation Time of Water Molecules from Nuclear Magnetic Resonance Analysis of Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 3.10. Approximate Percentage of Bound Water Calculated from NMR Data from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 3.11. Scanning Electron Microscope Images of Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 3.12. Increase (%) in Solubility of β-Glucan in Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 3.13. Reduction (%) in β-Glucan molecular weight (Mw) in Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
CHAPTER 4:

EFFECT OF β-GLUCAN-RICH BARLEY FLOUR ON QUALITY OF BREAD BAKED FROM YEASTED FROZEN DOUGH

4.1. Abstract

Storage of dough at freezing temperature unfavorably affects the final quality of baked products. The aim of this study was to investigate the effect of air-classified barley flour rich in β-glucan (~25%) on the quality of bread baked from yeasted frozen dough. Wheat flour was replaced by barley flour at 10% without and with 1.4% vital gluten. Three dough formulations were tested: control dough (C), barley-composite dough (ACB) and barley-composite dough with vital gluten (ACB-G). Doughs were stored at -18°C for 8 weeks and evaluated for bread baking weekly. The fresh C dough gave the highest loaf specific volume (LSV) compared to the fresh ACB and ACB-G doughs. Breads made from both C and ACB frozen doughs stored up to 4 weeks exhibited similar LSV compared to that of their corresponding fresh dough. Significant reduction in LSV was obtained with ACB-G dough after two weeks of storage. Bread crumb from frozen ACB dough maintained a similar texture profile to that of C up to 5 weeks of dough frozen storage, while ACB-G bread crumb showed significant adverse changes in hardness and chewiness after 1 week of storage. Molecular weight and solubility of β-glucan in breads baked from frozen doughs were slightly influenced by storage over the test period. The barley supplemented breads could be considered as a good source of β-glucan (0.53g β-glucan/serving of 33g slice) with good preserving ability up to 4 weeks of dough frozen storage.

Key words: Frozen dough, Barley flour, β-glucan, Bread quality
4.2. Introduction

Bread is evolving constantly as a response to the increasing consumer demand for freshly, healthy and safe products. Bread has been very well known as the oldest, most popular and widely consumed food product in the world due to its valuable nutritional and sensory characteristics. Bread making is basically processing of wheat flour and other ingredients into a light, aerated, and palatable food. These products have a short shelf-life, and the loss of freshness negatively influences the product quality as well as consumer acceptance.

Therefore, baking industry has been exploiting the advantages and applications of freezing technology in several frozen dough bakery applications. The production of baked products from frozen dough reduces the production costs by facilitating transportation and avoiding the need to skilled labour since frozen dough is ready to bake and easy to handle. It also allows fresh baked products with desirable quality and sensory characteristics comparable to the conventional fresh baked products, to be available around the clock (Gelinas et al. 1995).

On the other hand, frozen storage of food can involve damage to food texture through the disruption of food structure and a loss of integrity after thawing. The quality of baked goods prepared from frozen doughs is often poorer than that of fresh dough. Deterioration of frozen dough occurs when changes in water distribution takes place during extended storage and freeze-thaw cycles, which contribute to a longer proofing time and reduced loaf volume of breads baked from frozen dough. The ice crystals formation also contributes to the weakening of the protein network in the dough system, which is responsible for gas retention (Räsänen et al. 1997).

Storage of dough at freezing temperature has a considerable effect on the final quality of bread. It results in a lower specific loaf volume as well as flattened top and undesired changes in textural properties such as coarser internal structure of bread baked from frozen dough as
compared to that made from fresh dough. The longer the frozen storage time, the lower the quality of baked product baked from frozen dough (Bot 2003). Seguchi et al. (2003) highlighted that bread loaf properties such as bread height and specific volume are strongly influenced by the amount of liquid that is released from the frozen dough during thawing. Ribotta et al. (2001, 2003b) also reported that bread made from frozen dough showed faster increase in bread firmness which could be because of the faster starch retrogradation compared to bread made from non-frozen dough. The use of additives has become a common practice in the business of baking. The main role of most of additives is to interact with water in dough to absorb excessive moisture that is released following the breakdown of the gluten network. As a result, the use of additives can counteract the rheological changes that occur in frozen storage and retard the negative effect on the quality of the end bakery product (Yi 2008).

Examples of additives used in frozen dough industry include: soy ingredients (Simmons 2012), whey protein, polyols (sugar alcohol), carboxy methyl cellulose and gum Arabic (Asghar et al. 2005a,b, 2009). Better retention of the gluten network was observed after the addition of selected gums (locust bean gum and gum Arabic) to doughs stored at different periods from 4 to 16 weeks (Ribotta et al. 2004; Sharadanant and Khan 2006). Leray et al. (2010) and Filipovic and Filipovic (2010) reported that dough resistance to freezing and frozen storage was increased with the addition of a blend of inulin and oat fibre. Most studies concluded that stability of frozen dough can be enhanced with the addition of food gums, but up to date no study investigated the effect of barley flour rich in β-glucan on the quality of bread from frozen dough.

The high water holding capacity of β-glucan allows it to have a significant effect on one of the most important processing parameter in baking process, which is water behaviour in dough systems and consequently could modulate its functional and rheological properties. The aim of
this study was to investigate the effect of air-classified barley flour rich in β-glucan on the quality of bread made from frozen dough stored for 8 weeks.

4.3. Materials

Vieenna strong bakers wheat flour (control) and air-classified barley flour rich in β-glucan (~25% β-glucan) were kindly provided by Parrish and Heim Becker Milling Group, Dover Flour, Cambridge. Wheat vital gluten was kindly provided by ADM (Archer Daniels Midland, Candiac, Ca). Bakery yeast (Fleischmann’s, Traditional, Active Dry Yeast, product of Canada) was purchased from a local grocery store. All assay kits and enzymes were purchased from Megazyme (Megazyme International, Bray, Co. Wicklow, Ireland).

4.4. Preparation of Composite Flours and Doughs

Composite flours (90% wheat flour and 10% air-classified barley flour and 88.6% wheat flour, 10% air-classified barley flour and 1.4% vital gluten) were thoroughly mixed to produce uniform flours. Vital gluten was added at a close portion of gluten dilution in the wheat flour blend due to the addition of barley flour. Yeasted bread doughs were made on a 100g scale from control and composite flours (Table 3.1). Doughs were prepared according to the approved method AACC10-10-03 (2011) using the recommended 90-min fermentation and 33-min proof times.

4.5. Storage, Thawing and Baking Conditions of Frozen Dough

Doughs were divided based on weight. Cylindrical bread dough pieces were weighed and put in a polyethylene Zip-lock bag (9×12”, Uline, Ca) and all samples were placed in a covered plastic box. The plastic boxes stored in a freezer (Thermo Scientific, Revco Value Series, CA) at
-35°C for 3h. After that, samples were stored in a -18°C freezer (Traulsen, G-Series, USA) for 8 weeks.

Before baking, frozen dough was allowed to defrost for 2h at 31°C and 70% RH in a Hobart proofer (Hobart, USA). Baking was done at 215°C for 24min on day 0 for fresh dough and every week for frozen dough for 8 weeks. Bread baked from frozen dough was allowed to cool for 1h on a wired rack at room temperature, sealed in a polyethylene Zip-lock bag (9×12”, Uline, Ca) and kept at room temperature for further analysis. Bread crumb was dried at 40°C over night and then ground using a coffee grinder (SmartGrind, Black&Decker, China) prior to analysis.

4.6. Methods

4.6.1. Chemical Composition of Wheat, Barley and Composite Flours

Ash and dietary fiber fractions were determined according to the AACC (2011) methods 08-01.01 and 32-07-01, respectively. Moisture content was determined (Ohaus Halogen Moisture Analyzer, Ohaus, Switzerland), and water activity was recorded using Water Activity Analyzer (Aqua Lab 4TE, Decagon Devices, USA). Protein was determined by Dumas method based on dry combustion using a nitrogen analyzer (FP-528 Leco Instrument Ltd Mississauga, ON, Ca). β-Glucan content was determined using β-glucan enzymatic assay kit (AACC 32-23, 2011).

4.6.2. Bread Loaf Specific Volume

Each bread loaf was weighed in grams using analytical balance. Bread loaf volume was measured according to the rapeseed displacement method of measuring volume in cubic
centimeters (cm$^3$) as described by the AACC approved method 10-05-01 (2011). Specific volume was calculated as cm$^3$/g by dividing the volume of the bread loaf by its weight.

4.6.3. Bread Crumb Texture Profile Analysis (TPA)

Textural characteristics of bread crumb made from fresh and frozen-thawed doughs were analysed instrumentally using a Texture Analyzer (TA.XT2. plus, Texture Technologies. Corp. Scarsdale, NY, USA) according to the AACC approved method 74-09 (2011) and Ponzio et al. (2008) with some modifications. Texture profile analyses (TPA) also known as "Two Bites Test" were done on four separate loaves made from two dough batches and each two loaves were baked from the same dough batch for each sample. This allowed collecting four data points in order to minimize variation within each sample.

Bread loaves were sliced manually using a knife to obtain a 25mm bread slice on which the test was carried out. After placing the bread slice on the TA base, it was squeezed twice with a Perspex cylinder plunger with a diameter of 25mm to a depth of 17.5mm to reach a 70% compression. The test was conducted with trigger force 5g, plunger speed 5mm/sec and time between strokes was 5sec. The results obtained were calculated using Texture Exponent 32 software (Texture Technologies. Corp. Scarsdale, NY, USA), which recorded the plunger force as a function of time, to determine the following texture parameters: hardness, springiness, cohesiveness, chewiness, adhesiveness and resilience. Means and standard deviations for TPA parameters were reported.

4.6.4. Physicochemical Characteristics of β-Glucan

β-Glucan was extracted from dry grinded bread crumb using hot-water with thermostable α-amylase extraction method according to Rimsten et al. (2003) with some modifications.
Ground bread crumb samples (2.4g) was placed in a 45ml centrifuge tube with a magnet stirrer and 25ml of deionized water with CaCl$_2$ (0.28 mg/ml of H$_2$O), 0.2% of Sodium Azide and 200µl thermostable α-amylase added. The sealed tube was placed in a boiling water bath on a laboratory heater stirrer plate for 90min. After cooling, the tube was centrifuged (1,500×g for 15min) and the β-glucan extract supernatant was analysed.

The β-glucan extract was diluted 1:1 for control and 1:20 for composite dough samples with deionized water to reach desirable concentration (0.1 mg/ml), filtered (0.45µm) and then the peak molecular weight (M$_p$) of β-glucan in the extract was determined using high performance size exclusion chromatography (HPSEC) equipped with calcofluor detector as described by Ragaee et al. (2008). The chromatographic system consists of a Perkin Elmer ISS-100 autosampler and injector, a Shimadzu model AD-vt HPLC pump, a Shimadzu RF-10Axl fluorescence detector, a Waters (Milford, CT) model 510 HPLC pump for post column addition of calcofluor, and the Viscotek DM 400 data manager. Data integration was performed using TriSEC 3.0 software (Viscotek, Houston, TX). Six β-glucan molecular weight standards (ranging from 20,000 to 1, 300, 00 g/mol) obtained commercially from (Megazyme) were used to construct a calibration curve for β-glucan by plotting retention time versus log peak molecular weight (M$_p$) (Wang et al. 2003). Soluble β-glucan concentration in the extract was determined using the Flow Injection Analysis (FIA) system according to Beer et al. (1997a,b).

4.6.5. Statistical Analysis

All analyses were performed at least in duplicate for each loaf and the mean values are reported. Analysis of variance was performed using IBM SPSS Statistics 20 software. Significant difference (p<0.05) among means were detected using the Tukey’s multiple range test at a fixed level of α = 0.05.
4.7. Results and Discussion

4.7.1. Bread Loaf Specific Volume

Appearance of breads made from fresh and frozen-thawed (4 and 8 weeks) doughs using control and barley composite flours is shown in (Figure 4.2). As expected, fresh control bread exhibited significantly higher specific loaf volume (LSV) than the fresh composite breads without and with vital gluten (Figure 4.1). Vital gluten was added to compensate for the dilution in gluten and to restore the flour quality. However, the addition of gluten resulted in no improvement in LSV perhaps due to different gluten interactions in the presence of β-glucan. Bread volume measurement is an indirect evaluation of crumb structure since too small LSV indicates a very compact and closed grain structure, while too large LSV indicates a very open grain structure (Sharadanant and Khan 2003b). During frozen storage of doughs no significant drop in LSV was observed in the control and barley composite sample without gluten up to week 4. Control bread from frozen dough exhibited a significant drop in LSV in week 5 and then no extra drop in LSV was observed until week 8. Similarly there was continues drop in LSV of bread made from barley composite flour without gluten. Brad from barley composite flour with vital gluten exhibited significant reduction in LSV after 2 weeks of frozen storage and the LVS continued to drop until the end of experiment (8 weeks). Similar results were obtained by Mohamed et al. (2010) when banana peel flour was added to frozen bread dough formula. The results are also in agreement with Yi (2008) who investigated the effect of different freezing rates (19-69°C/h) and frozen storage times (up to 180 days) at four different storage temperatures (-10, -20, -30 and -35°C) on dough quality. The author concluded that loaf volumes for all breads decreased with increased storage time. Rosell et al. (2001) and Sharadanant and Khan (2003a) reported that the addition of hydrocolloids increased the stability of the interface dough system.
during proofing, conferring a capability of proofing to an optimum height and additional strength to the gas cells through baking. The reduction in both yeast activity and the ability of the dough gluten network to retain CO\textsubscript{2} during proofing could be a result of the ice recrystallization during frozen storage time (Berglund et al 1991).

Quality and quantity of protein in wheat play an important role in frozen dough performance (Lu and Grant 1999). Inoue and Bushuk (1992) reported that the undesirable effect of frozen storage temperatures can be minimized using very strong flour or flour with added vital gluten for bread dough preparation. Gujral et al. (2003) also demonstrated that addition of vital gluten was used to overcome the deteriorating effect of barley flour on bread volume. In contrast, Lu and Grant (1999) studied the effect of frozen storage on the baking of frozen doughs made from 4 wheat cultivars. The frozen dough that had overly strong gluten characteristics but relatively low protein content (12.1\%) exhibited the greatest increase in proofing time and decrease in loaf volume. The gluten network was not able to expand with the limited CO\textsubscript{2} produced by the limited yeast available. Bhattacharya et al. (2003) reported that flours with moderately high gluten strength may be most typical for producing optimum quality frozen doughs, with good shelf life and baking properties. The reduction in LSV of bread made from barley and vital gluten composite flour could be due to changes in the ultra-structure of starch and gluten network and poor gas retention of gluten matrix. The presence of vital gluten along with β-glucan from barley in frozen dough could result in a too strong gluten network that is not able to expand.
4.7.2. Bread Crumb Moisture Content and Water Activity

Water plays an important role during dough development and bread baking. It also affects the quality of the end product. Bread produced from composite frozen doughs exhibited relatively higher moisture content and water activity compared to the control bread (Figure 4.3). All bread samples made from frozen dough maintained similar levels of moisture content and water activity compared to their fresh status during the entire frozen storage time (8 weeks).

Addition of dietary fiber to wheat flour modifies the rheological properties of the dough such as water absorption, moisture content and water activity of the bread crumb (Wang et al. 2002; Mandala et al. 2007). The increase of starch damage during frozen storage may be responsible for increased moisture retention resulting in a bread crumb with higher moisture content (Berglund et al. 1991). In the current study, the presence of vital gluten did not affect bread moisture content or water activity, which is similar to the results found by Wang et al. (2004a,b) who added five levels of vital gluten in bread formula and did not observe any significant changes in moisture content of bread crumb.

4.7.3. Crumb Texture Profile of Breads

Consumers expect bread made from frozen dough to have quality and sensory characteristics comparable to the fresh one (Mohamed et al. 2008). The texture profile analysis (TPA) is considered one of the good measures of wheat flour quality as well as the effect of added ingredients on the final bread quality characteristics. The textural characteristics of bread crumb made from fresh and frozen-thawed control and composite doughs were studied instrumentally using TPA. No significant differences in the crumb texture were observed among bread samples baked on day 0 from control and composite fresh doughs (Figure 4.4). This
indicates that the incorporation of barley flour rich in β-glucan without and with vital gluten had no effects on the textural characteristics of bread crumb made from freshly prepared doughs. All texture parameters of control and barley flour supplemented bread crumb did not exhibit any change up to week 5 of dough frozen storage. However, bread baked from barley flour with vital gluten frozen dough exhibited significant increase in crumb hardness and chewiness after 1 week of dough frozen storage. The same sample exhibited significant decrease in crumb adhesiveness after 3 weeks of dough frozen storage. In general, hardness, adhesiveness and chewiness of control crumb exhibited slight changes throughout the frozen storage period of the dough.

Changes in the gluten network during frozen storage of dough could affect crumb physical characteristics such as texture and crumb pores distribution (Lu and Grant 1999). Lu and Grant (1999), Asghar et al. (2005a,b) and Yi (2008) observed less oven spring, coarse internal structure, faster bread staling and loss of overall baking quality of bread made from frozen dough stored for 16 weeks. The incorporation of 20% barley flour into a baking formula resulted in a decrease in loaf volume and crumb cohesiveness and increase in crumb firmness of bread (Gujral et al. 2003). The significant changes in bread crumb observed with the addition of vital gluten and barley flour are attributed to the low loaf volume (Figure 4.2), which may result in a denser crumb with poor pores distribution (Sharadanant and Khan 2003b; Asghar et al. 2005b). The addition of 10% barley flour rich in β-glucan was found to lessen changes in rheological properties of frozen dough and improve water redistribution in the dough system (chapter 3). But prolonged frozen storage could alter dough structure. Gujral et al. (2003) reported that β-glucan in barley flour could be attributed to the lower staling rate of bread during storage. This is due to higher crumb moister content for bread made from β-glucan enriched composite flour compared to control.
4.7.4. Physicochemical Properties of β-Glucan

The physiological effectiveness of β-glucan depends on its molecular weight (Mw) and solubility (Brennan and Cleary 2005; Vasanthan and Temelli 2008). High Mw and solubility of β-glucan are keys to deliver the expected health benefits (Lan-Pidhainy et al. 2007; Thondre and Henry 2009). Processing, baking and storage conditions could directly affect the physicochemical properties of β-glucan and eventually its health benefits when incorporated into different baked food matrices (Regand et al. 2009; Tiwari and Cummins. 2009).

In the present study, air-classified barely flour containing 25% β-glucan with molecular weight 250000g/mol and solubility 54% was used. Major changes in β-glucan solubility were observed in both composite breads after the mixing and baking of fresh doughs as well as during the beginning of frozen storage. The composite bread that contains vital gluten showing significantly higher changes compared to composite bread without vital gluten. Changes in β-glucan solubility decreased in both composite frozen doughs as storage time increased. On the other hand, β-glucan molecular weight in composite breads exhibited insignificant variation during the entire period of dough frozen storage (8 weeks) (Figure 4.5). Several studies reported that the beneficial effects of β-glucan incorporated into bread decreased particularly due to depolymerization during dough mixing and fermentation (Cleary et al. 2007; Flander et al. 2007; Andersson et al. 2008; vatandoust et al. 2012). Moriartey (2009) reported that incorporating barley β-glucan concentrate of low solubility in bread at levels 3.75, 5 and 7.5g/100g wheat flour resulted in no significant effect on β-glucan solubility. There were also no significant differences in molecular weight of β-glucan between final dough and baked bread (Andersson et al. 2004).
There is no enough data available in the literature about the effect of frozen storage on β-glucan properties. Only few studies investigated whether or not the cold storage of food products that contain β-glucan affects its physicochemical properties. For example, when bread, contain barley β-glucan concentrate, was stored at ambient, refrigeration and frozen conditions, a reduction in β-glucan molecular weight and viscosity was observed. It was concluded that it is better to consume bread with β-glucan as fresh to maintain the highest bread quality and β-glucan solubility and viscosity (Moriartey 2009). In agreement with that, reduction in β-glucan solubility increased as frozen storage period of β-glucan composite muffins increased (Beer et al. 1997b). Lan-Pidhainy et al. (2007) also reported similar results with oat bran muffins underwent several freeze-thaw cycles. This demonstrates that challenges encounter the production of high-quality β-glucan enriched bread.

4.8. Conclusion

Incorporation of barley flour at 10% replacement level without and with vital gluten reduced loaf specific volume (LSV) compared to the control bread. Both control and barley flour supplemented bread made from frozen doughs stored up to 4 weeks of storage had similar LSV compared to their corresponding fresh breads. The barley supplemented bread also exhibited crumb texture profile similar to that of control bread up to 5 weeks of storage. Addition of vital gluten to the barley frozen dough resulted in significantly lower LSV compared to its corresponding fresh bread after 2 weeks of storage along with significant changes in bread crumb texture changes in Mw and solubility of β-glucan caused primarily by mixing, fermentation and baking, while frozen storage of doughs had slight effects on Mw up to week 8, and on solubility until week 6. The barley supplemented breads could be considered good sources of β-glucan.
(0.53g β-glucan/serving of 33g slice) with good preserving ability for dough stored at -18°C up to 4 weeks.
Figure 4.1. Loaf Specific Volume of Bread Made from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 4.2. Appearance of Bread Made from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 4.3. Moisture Content and Water Activity of Bread Crumb Made from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 4.4. Texture Profile Analysis (TPA) of Bread Crumb Made from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
Figure 4.5. A: Increase (%) in Soluble β-Glucan Amount. B: Reduction (%) in Molecular Weight (Mw) of β-Glucan in Bread Crumb Made from Fresh and Frozen-Thawed Control and Composite Yeasted Bread Doughs Stored at -18°C for 8 Weeks
CHAPTER 5:

EFFECT OF β-GLUCAN-RICH BARLEY FLOUR ON QUALITY OF COOKIES
BAKED FROM FROZEN BATTER

5.1. Abstract

Frozen batter offers an opportunity to produce fresh cookies around-the-clock at food services for consumer’s satisfaction. The objective of this study was to investigate the effect of air-classified barley flour rich in β-glucan (~25%) on the rheological properties of frozen batter and the quality of the end product. Batters were made from control wheat flour and barley flour blends at replacement levels 10, 15, 20 and 30% and various levels of water. The pasting characteristics of the blends exhibited a reduction in peak viscosity, trough paste viscosity, breakdown and final viscosity. Batter hardiness and stickiness and cookies breaking force increased, while cookies spread factor decreased compared to the control sample. Addition of 10% barley flour rich in β-glucan with added extra 8 ml water to the standard formula (224g composite flour) improved cookie-making process and quality of batter and cookies. Frozen storage of the optimal formula at -18°C for 4 weeks resulted in no changes in hardness and stickiness of batter and spread factor of cookie compared to the control, while cookie breaking force decreased as frozen storage period increased. Frozen storage had little effects on molecular weight and solubility of β-glucan. This study has shown a good potential for developing fiber-rich cookie from frozen batter by incorporating air-classified barley flour as a rich source of β-glucan without compromising the quality of the end products when batter was stored up to 4 weeks at -18°C.

Key words: Frozen batter, Barley flour, β-glucan, Cookies quality
5.2. Introduction

Cookies as it is called in North America or biscuit as it is known internationally is one of the most popular food products because of its convenience, general acceptability, ready to eat nature and long shelf life. In contrast to bread dough, cookie batter as well as other pastry products are inelastic and have high flow properties. This is due to the minimum gluten network development desired in such products. Therefore, soft wheat flour is preferred since it has low hydration properties and weak gluten network. Thus cookies that made from soft wheat flours possess the greatest cookie spread factor (the ratio of width (W) to thickness (T) or W/T) and tender bite (Barak 2013). The quality of the final product is largely influenced by the components of the batter (Pareyt and Delcour 2008). Frozen storage is aimed to produce cookies batter with minimum changes and high quality that is similar to freshly mixed one (Kulp et al. 1995). Dogan (2006) reported that cookie batter can be stored for 6 months at -18°C with no deterioration signs and no changes in the final product spread ratio, surface characteristics and density.

Research in frozen doughs and batters has been carried out as a response to consumers’ taste and lifestyle and more importantly their demand for food products that promote nutritional health, which is influencing their purchasing decisions. The use of additives has become a common practice in the business of baking and many studies have investigated the effect of different added ingredients on rheological behaviour of batter and on the quality of cookies. Piteira et al. (2006) reported that it is possible to use dietary fibre as a functional ingredient at levels up to 10g/224g flour with minimum effect on the processibility and the quality of cookies. In agreement with that, Šeremešic et al. (2012) found that addition of up to 15% resistant starch
(RS) has good potential for developing fiber-rich cookies and improve their nutritional quality. Seker et al. (2008) also reported that apricot kernel flour significantly increased the fibre content and provided acceptable cookies at 10 and 20% supplementation levels. Wheat bran, coarse wheat flour, and rice flour were also added to cookie batter at levels 0–10, 0–20 and 0–20%, respectively to produce high fibre cookie with overall acceptability that was in favour of rice flour and coarse wheat flour (Gujral et al. 2003).

Most of the studies concluded that there is a good potential for developing fiber-rich cookies with improved nutritional quality. However, addition of fibre to cookie batter may result in the reduction of cookie spread factor producing smaller cookies compared to wheat flour cookies. This could be because of the competition between fibre and sugar on the available water causing a decrease in batter viscosity and consequently spread ability of cookies during baking (Šeremešic et al. 2012). Up to date there is no study investigated the effect of air-classified barley flour rich in β-glucan on the quality of frozen batter and cookies made from it. The objective of this study was to investigate the effect of air-classified barley flour rich in β-glucan (~25%) on the rheological properties of frozen batter and the quality of their end product.

5.3. Materials

Sensation unbleached pastry wheat flour (control) and air-classified barley flour rich in β-glucan (~25%) were kindly provided by Parrish and Heimbecker Milling Group, Dover Flour, Cambridge, ON, Canada. Sucrose (Red Path sugar Ltd, Toronto, ON, Ca), Sodium bicarbonate (Selection, USA), Vegetable shortening (Crisco, USA), fat free instant skim milk powder (Carnation, Ca), Sodium chloride and ammonium bicarbonate were purchased from a local grocery store in Guelph, ON, Canada. High fructose corn syrup (42%) was purchased from
Archer Danials Midland Co (IL, USA). All assay kits and enzymes were purchased from Megazyme (Megazyme International, Bray, Co. Wicklow, Ireland).

5.4. Preparation of Composite Flours and Batter

Wheat flour was replaced with air-classified barley flour at 10, 15, 20 and 30% to composite flours, and thoroughly mixed to produce uniform flours. Cookie batters were prepared according to the AACC approved Method 10-53 (AACC 2011) with some modifications. Cookie formulations are listed in Table 5.1.

5.5. Frozen Storage and Thawing of Batter and Cookie-Baking

Cylindrical cookie batter were put in polyethylene Zip-lock bags (9×12”, Uline, Ca) and then batters were put in a covered plastic box, then the plastic boxes were placed in a freezer (Thermo Scientific, Revco Value Series, CA) at -35°C for 3h followed by storage in a -18°C freezer (Traulsen, G-Series, USA) for 4 weeks. Fresh cookie batter was analyzed immediately and frozen thawed batters were analyzed every week.

Before baking, frozen Cookie batter was allowed to defrost for 4h at room temperature according to Gupta et al. (2011). Frozen-thawed batter was divided into six relatively equal portions. All portions were placed on a lightly greased baking sheet and rolled with one forward stroke using a rolling pin, cut with a 60mm cookie cutter then immediately baked. After baking at 205°C (400°F) for 11min, cookies were cooled at room temperature for 5min then removed from baking sheet and placed on a wire cooling rack for 30min. Cookies were ground using a coffee grinder for analysis.
5.6. Methods

5.6.1. Chemical Composition

Ash and total fiber (soluble and insoluble dietary fiber) were determined according to the AACC (2011) methods 08-01.01 and 32-07-01, respectively. Moisture content was measured using (Ohaus Halogen Moisture Analyzer, Ohaus, Switzerland). Protein was determined by Dumas method based on the combustion method using a nitrogen analyzer (FP-528 Leco Instrument Ltd Mississauga, ON. Canada). β-Glucan content was determined using β-glucan enzymatic assay kit according to the approved Method 32-23 (AACC 2011).

5.6.2. Pasting Characteristics of Wheat and Composite Flours

Rapid Visco Analyzer (RVA-4) (Newport Scientific Pty, Ltd, Warriewood, Australia) was used to determine pasting properties of wheat and composite flours with containing barley flour following the method of Ragaee and Abdel-Aal (2006). A sample of 3.5g of flour and approximately 25±0.1ml distilled water (corrected to compensate for 14% moisture basis) was placed in a RVA canister and heated to 50°C and stirred at 960rpm for 10sec for thorough dispersion. At stirring speed of 160rpm, the test temperature was held at 50°C for the first minute, then heated to 95°C during the next 7.3min to be held again at 95°C for 5min and finally a gradual cooling from 95°C to 50°C was commenced during the following 7.7min. The pasting temperature (the temperature where an increase of viscosity by about 25cp is recorded over a 20sec period), peak time (the time at which peak viscosity occurred), holding strength or trough viscosity (the trough at the minimum hot paste viscosity), final viscosity (the paste viscosity recorded when the paste temperature is 50°C after the cooling stage at the end of test), breakdown viscosity (peak viscosity holding strength or trough viscosity) and setback viscosity
(final viscosity holding strength) were calculated from the pasting curve, using Thermocline software (TCW version 3, Newport Scientific Pty. Ltd. Warriewood, Australia).

When starch granules hydrate, swell while the slurry is heated, the swollen starch granules make the slurry thicker, which has more resistance to the paddle during stirring and has a higher peak viscosity (PV, The highest point during the heating cycle). The other primary starch pasting viscosity (centipoise (cP)) parameters derived from the RVA curve includes; onset of gelatinization or pasting temperature (indicates the dramatic increase in viscosity generally occurred at ~3–5min from the beginning of the test and associated with starch gelatinization), trough viscosity (the lowest viscosity value after the 5min hold at 95ºC), and final viscosity (maximum viscosity attained during cooling to 50ºC). From these primary parameters it is possible to calculate breakdown, peak viscosity minus minimum viscosity and total set back viscosities also known as re-association of starch molecules upon cooling, calculated as final viscosity minus trough viscosity, respectively (AACC 2011; Goode et al. 2005).

5.6.3. Hardness and Stickiness of Batter

Cookie batter hardness and stickiness were determined by compressing the cookie batter with a 25mm Perspex cylinder probe using a Texture Analyzer (TA.XT2. plus, Texture Technologies, Corp. Scarsdale, NY, USA). Compression tests were performed according to the method described by Pareyt et al. (2008). The TA settings were: pre- and post-test speeds 2.0mm/s, while test speed was 1.0mm/s. Dough pieces were precisely centered in a Perspex cup and compressed by 50%. The probe was held for 10sec at maximum compression. Values of cookie batter hardness (positive peak value) and batter stickiness (negative peak value) were
calculated using Texture Exponent 32 software (Texture Technologies, Corp. Scarsdale, NY, USA).

5.6.4. Moisture Content and Water Activity of Batter and Cookies

Moisture content and water activity of samples (fresh, frozen thawed batter and ground baked cookies) were determined. Moisture content was measured using (Ohaus Halogen Moisture Analyzer MB45, Ohaus, Switzerland). Sample (~0.6g) was placed in the moisture analyzer tray and moisture content tested at 130°C for 5min. Water activity was recorded using Water Activity Analyzer (Aqua Lab 4TE, Decagon Devices, USA).

5.6.5. Spread Factor and Breaking Force of Cookies

Cookies baked from fresh and frozen-thawed batters undergone several analyses. Cookies width (W) and thickness (T) were measured and spread factor was calculated as the ratio of W/T according to the AACC approved Method 10-53 (AACC 2011). Cookie break strength was determined using a Texture Analyzer (TA.XT2. plus, Texture Technologies. Corp. Scarsdale, NY, USA) adjusted on the compression mode with the 50kg load cell. Cookies breaking force was determined with the three point bending test as described in Pareyt et al. (2008) with some modifications. A cookie was placed on two support beams at a known distance (2.5cm) and a third beam, a metal blade (70mm wide and 3mm thick), was brought down through the product at a point equidistant from both support beams until the product breaks (snaps). The TA settings were: travel distance of the blade 40mm, pre-test, test and post-test speeds were respectively 2.5mm/s, 2.0mm/s and 10.0mm/s. The maximum peak force, an index of cookie break strength, was recorded during compression using Texture Exponent 32 software (Texture Technologies,
Two separate batches were prepared with six cookies being tested from each batch.

5.6.6. Physicochemical Analysis of β-Glucan

β-Glucan was extracted from fresh and frozen-thawed batters and ground cookies using the hot-water extraction with thermostable α-amylase method according to Rimsten et al. (2003) with some modifications. Samples (about 2.5g to obtain 0.1mg β-glucan /1ml final extract), were placed in a 45ml centrifuge tube with a magnet stirrer and 25ml deionized water with CaCl₂ (0.28mg/ml of H₂O), 0.2% of Sodium Azide and 200µl thermostable α-amylase were added. The sealed centrifuge tube was placed in a boiling water bath on a laboratory heater stirrer plate for 90min. After cooling for 10min at room temperature, the tube was centrifuged (1,500×g for 15min) and β-glucan extract was collected for further analysis.

The β-glucan extract was diluted 1:1 for control and 1:20 for composite batter samples with deionized water to reach desirable concentration to be used (0.1mg/ml or less), filtered (0.45µm) and peak molecular weight (Mₚ) of β-glucan was determined using high-performance size exclusion chromatography (HPSEC) equipped with calcofluor detector as described by Ragaee et al. (2008). The chromatographic system consists of a Perkin Elmer ISS-100 autosampler and injector, a Shimadzu model AD-vt HPLC pump, a Shimadzu RF-10Ax1 fluorescence detector, a Waters (Milford, CT) model 510 HPLC pump for post column addition of calcofluor, and the Viscotek DM 400 data manager. Data integration was performed using TriSEC 3.0 software (Viscotek, Houston, TX). Six β-glucan molecular weight standards (ranging from 20,000 to 1,300,00g/mol) obtained commercially from (Megazyme) were used to construct a calibration curve for β-glucan by plotting retention time versus log peak molecular weight.
Soluble β-glucan concentration in the extract was determined using the Flow Injection Analysis (FIA) system according to Beer et al. (1997a).

5.6.7. Statistical Analysis

All analyses were performed at least in duplicate and the mean values are reported. Analysis of variance was performed using IBM SPSS Statistics 20 software. Significant difference (p<0.05) among means were detected using the Tukey’s multiple range test at a fixed level of α = 0.05.

5.7. Results and Discussion

5.7.1. Pasting Characteristics of Wheat and Composite Flours

The Rapid Visco Analyser (RVA) has been widely used for determining the pasting properties of different flours and starches and hence their processing value for baking. It measures the resistance of flour slurry to the stirring action of a paddle at a specific stirring speed and heating and cooling rate.

Results obtained by RVA (Table 5.3 and Figure 5.1) show that addition of air-classified barley flour at different replacement levels (10, 15, 20 and 30%) to soft wheat flour resulted in a significantly lower peak, trough, break down (except 15 and 20%), final and setback viscosities compared to control (100% soft wheat flour). No trend in all viscosity parameters was observed with increasing the level of barley flour. However, there were significant differences within the composite flour samples. The highest replacement level of barley flour (30%) exhibited the highest trough viscosity followed by 10, 15 and 20% replacement levels, while it gave the lowest breakdown viscosity compared to the 15 and 20% replacement levels, which gave similar values to that of control. In agreement with the current study, Ragaee and Abdel-Aal (2006) investigated pasting properties of starch and protein of different grain whole meals including
barley and its blends with wheat flour. The authors reported different RVA curves for the different flours with the soft wheat flour giving the highest starch pasting characteristics. While barley flour exhibited much lower values of PV, TV and FV compared to soft wheat flour. Symons and Brennan (2004) also reported that replacement of 5% wheat flour with β-glucan-rich fiber fractions extracted from barley flour reduced the pasting characteristics of the composite flour as indicated by the reduction in PV and FV compared to the control. Sullivan et al. (2010) reported that the higher the level of pearled barley flour (from 30 up to 70%) incorporated in wheat flour, the higher the reduction in peak viscosity, breakdown, setback and final viscosity of the starch slurries of the formulations. Furthermore, Gray et al. (2010) reported that the viscosity and texture properties of barley flour vary based on its composition. High concentration of the soluble dietary fiber (β-glucan) in barley flour used in the present study could have competed with starch on the available water leading to a reduction in the degree of starch granule swelling (incompletely hydrated starch) and, consequently, the reduction in the pasting properties of wheat starch. Batey and Curtin (2000) and Gray et al. (2010) suggested that the starch quality and quantity as well as protein composition in wheat flour compared to other cereals such as barley may contribute, to some extent, to the superior pasting characteristics of wheat flour. Ragaee and Abdel-Aal (2006) reported that although replacing certain level of wheat flour with other cereal flour modifies the pasting properties of wheat flour, no significant detrimental effect was observed on physical properties or acceptability of the final product.

5.7.2. Hardness and Stickiness of Batter

Cookies tend to be highly influenced by the physico-chemical properties of their batters (Manohar and Rao 1997, 2002). From the food manufacturing perspective, understanding of the function of ingredients such as dietary fibre in batter is critical in order to enhance the quality of
the final product. Wire cut cookies batter must be sufficiently viscoelastic to separate cleanly when cut by the wire. At the same time, it must be cohesive enough to hold together during baking and spread with minimum rise (Tangkanakul et al. 1995).

Preliminary experiments using different replacement levels of air-classified barely flour 10, 15, 20 and 30% and different levels of water (8, 12, 16 and 24ml) indicated that the maximum replacement level of barley flour is 10% with the addition extra 8ml of water, which exhibited the lowest batter hardness and stickiness values and the highest cookies spread factor among the composite cookies (Figure 5.2).

The increase in water absorption caused by the inclusion of fiber in the cookie batter resulted in highly elastic batter, which was harder and produced smaller and thicker cookies compared to the control (Brennan and Samyne 2004). High water capacity of fibre could be the reason behind their ability to work as stabilizers to the batter matrix leading to higher hardness values compared to that of control. The increased hardness can be attributed to the higher levels of water-soluble carbohydrates in barley flour. Yamamoto et al. (1996) reported that cookie flour with high water absorption results in stiffness of the cookie batter and consequently a reduction in cookie diameter and spread factor. Brennan and Samyne (2004) investigated the effects of dietary fiber from different sources such as inulin, β-glucan enriched fraction, potato fiber, and resistant starch on cookie texture and properties. The authors reported that fibre can be added to cookie flour up to the replacement level of 10% without having any negative effect on the characteristics of the cookie. Cujral et al. (2003) investigated the effect of wheat bran (0–10%), coarse wheat flour (0–20%) or rice flour (0–20%) on the hardness and stickiness of batter. Compared to control, wheat bran was found to exhibit higher batter hardness and stickiness whereas coarse wheat flour reduced both parameters. Increasing levels of rice flour decreased
cohesiveness but increased adhesiveness. Dogan (2006) stored cookie batters (sugar snap, chocolate chip, and hazelnut cookies) at 4°C for 6 weeks and at -18°C for 6 months. The author concluded that batter refrigeration and frozen storage have no significant effect on the physical characteristics of the batter.

5.7.3. Moisture Content and Water Activity of Batter and Cookies

Although water is a minor component in cookie batter formula, the rheological behaviour and the machinability of cookie batter are largely influenced by the water content and its distribution within the batter (Agyare et al. 2005; Assifaoui et al. 2006; Lee and Inglett 2006). This was clearly seen in the increased moisture content with addition of barley flour, which caused an increase in the hardness and stickiness properties of batter at the optimum level of water in the formula, while addition of extra water decreased the hardness and stickiness properties of composite batter to be closer to that of control.

Water in the batter matrix provides lubrication when the bakery product is being eaten, affecting its texture. It is therefore useful to monitor the water content in batter as well as in cookies (Šeremešic et al. 2012). Also, attention should be paid to water activity because it is an important aspect of food stability and shelf-life (Sanchez et al. 1995; Swanson et al. 1999). Moisture content of all batters slightly increased with increasing frozen storage time, while there were no significant differences in water activity between all batters during the entire period of frozen storage. Similar results were obtained after baking the fresh and frozen-thawed batters (Figure 5.5). The most important player seems to be the fiber in barley flour that binds water. Cookies moisture content and water activity increased gradually as the frozen storage period of batter increased for all samples. An increase in the moisture content was observed in cookies
baked from the control and composite batters during frozen storage indicating that less water was removed during the baking process.

In agreement with the current study, Sanchez et al. (1995) and Swanson et al. (1999) observed increases in the moisture content and water activity when they used carbohydrate-based fat substitutes in cookie batter formula. The water activity of cookies produced from control (C), 10% barley flour (ACB) and 10% barley flour +8ml water (ACB+8ml) frozen batters was 0.40, 0.44, and, 0.45, respectively, which were lower than the limit of microbial growth, which is 0.5 (Beuchat 1981). Lai and Lin (2006) also highlighted the significance of the amount of water used for making cookies. This is because of its effect not only on the gluten development in the batter, but also cookie spread during baking, moisture retention, and eating quality of the end products. This was observed in the present study through the significant differences in hardness and stickiness values of batters that contain different levels of barley flour and water. During the baking process many changes take place in the cookie dough. One of the most important changes is the loss of moisture. Water has a marked influence not only during batter preparation but also in the end product during storage of cookies (Cornillon and Salim 2000). The authors hypothesized that redistribution of water molecules within the cookies occurs during cooling and storage leading to internal moisture gradients and/or water activity differences.

5.7.4. **Spread Factor and Break Force of Cookies**

Cookie spread factor represents a ratio of diameter to height. While cookies breaking force shows the force required to break/snap the cookies indicating its texture (Bourne 2002; Mamat et al. 2010; Pareyt et al. 2009a,b). Size (spread factor) and breaking force of cookies are two main factors that determine a good quality cookie. They are influenced by the flour, recipe, procedure
and conditions used in cookies production (Pareyt et al. 2009a, b; Mamat et al. 2010). Cookie batter expands during baking leading to the dramatic increase in cookie diameter after baking (Yamamoto et al. 1996). Cookies spread factor is influenced by batter viscosity. High batter viscosity will result in slower batter spread during the baking phase and consequently smaller cookie diameter (Pareyt et al. 2009a,b).

Spread factor of cookies made from fresh and frozen-thawed batters prepared from control and composite flours is presented in (Figure 5.7). It was observed that control cookies exhibited significantly higher spared factor compared to the composite cookies and maintained it during the entire period of frozen storage (4 weeks). While barley containing cookies showed a significant decrease in spread factor after the first week of frozen storage then they maintained a constant spread factor ratio to the end of frozen storage time.

Cookies made from frozen batters also exhibited changes in their breaking force after the first week of frozen storage of the batter (Figure 5.7). The breaking force of control and composite samples decreased as frozen storage period of the batter increased. Freshly baked cookies made from composite flours had the highest breaking force compared to the control and 10% barley flour +8ml water, which exhibited similar breaking force values to control.

In agreement with the current study, Cujral et al. (2003) studied fiber enriched cookies and reported that addition of wheat bran and rice flour lowered the spread factor whereas coarse wheat flour increased spread factor. Along with that, coarse wheat flour and rice flour lowered the breaking force whereas wheat bran increased it. Several studies (Kaldy et al. 1991; Tangkanakul et al. 1995 and Uysal et al. 2007) reported that reduction in spread factor of cookies with increasing the level of fiber in the formula. Increasing the replacement level of soft wheat
flour with barley flour from 10 to 20\% resulted in additional reduction in the spread factor and the force required break/snap the cookie and consequently an increase in the thickness of cookies (Gupta et al. 2011). Another study by Lee et al. (2005) reported reduced spreading characteristics and reduction in breaking force compared with the control when oat β-glucan fractions were incorporated in cookie flour at 10\% and 20\% replacement levels. During baking cookie batter flows (spreads) as temperature increases and continues to spread then the spreading stops due to the increase in viscosity (Hoseney 1994). Since no starch gelatinization occurs due to the low moisture content, it is believed that cookies spreading and increase in viscosity during baking is mainly due to the property of soft wheat flour proteins. Gaines (1990) and Lai and Lin (2006) stated that soft wheat proteins affect cookie texture, moisture retention, and the eating quality of the finished products. The most important players in cookie quality are those components that bind the water and thereby limit the spread of the cookie (Piazza and Masi 1997). Another theory could be that the relatively high amount of the water in the batter is held by fiber and consequently less water is available to dissolve the sugar. As a result, the viscosity is higher than it should be, which allows the cookie to spreads at a slower rate (Miller and Hoseney 1997).

Dogan (2006) studied the effect of frozen storage at -18°C for 6 months on sugar snap and chocolate chip, and hazelnut cookies. It was found that storage time in the freezer was not a critical factor affecting cookies spreading. Cookies produced from batter containing barley flour and underwent freeze-thaw cycles every 4h for 24h were crisper than the freshly baked ones (Gupta et al. 2011). This indicates that frozen storage of batter produces cookies with lower braking force, which was confirmed in the current study. It was also observed that the longer the frozen storage period of batter, the lower the breaking force of the produced cookie become. This must be attributed to the ice crystals formed during frozen storage, which weakens the
hydrophobic bonds and redistributes water in the dough resulting in a physical damage to the gluten protein structure (Rasanen et al. 1998).

5.7.5. Physicochemical Properties of β-Glucan

β-Glucan molecular weight (Mw) and solubility determines its expected physiological and functional benefits of food products (Lan-Pidhainy et al. 2007; Thondre and Henry 2009). Processing, baking and storage conditions directly affect physicochemical properties of β-glucan and eventually its physiological effects when it is incorporated into different baked food matrices (Regand et al. 2009; Tiwari and Cummins 2009). In the present study, air-classified barely flour containing 25% β-glucan with molecular weight 250000g/mol and solubility 54% was used. The major changes in β-glucan Mw and solubility were observed in batter after mixing. Several studies (Wood et al. 1994, 2000; Cleary et al. 2007; Vatandoust et al. 2012) reported that the decrease in β-glucan Mw after mixing could be because of β-glucan enzymatic degradation by the endogenous β-glucanases present in the flour. No significant changes in β-glucan Mw or solubility were observed after incorporation of 10% air-classified barley flour in the frozen batter as well as in the cookies made from it (Figure 5.8 and Figure 5.9). This indicates a good potential in using air-classified barley flour rich in β-glucan at the level of 10% as a functional ingredient in cookie formula without compromising the physicochemical properties of β-glucan.

It is believed that the health benefits of β-glucan depend mainly on its viscosity, which is correlated to β-glucan Mw. High β-glucan Mw exhibits higher viscous solutions compared to low Mw β-glucan (Guillon and Champ 2000). Previous studies (Cleary et al. 2007; Flander et al. 2007; Andersson et al. 2008) have tried to introduce the beneficial effects of β-glucan supplemented bread. The beneficial effects of β-glucan were reported to decrease when
incorporated into the bread system perhaps due to depolymerization of the molecule weight during dough mixing and fermentation. In batter no yeast is added and therefore there is no fermentation process. Cookies also characterised by their low moisture content (Gupta et al. 2011). Gupta et al. (2011) reported that it is possible to replace soft wheat flour used in cookie batter with whole barley flour up to 30% replacement level to produce acceptable cookies with increased fiber content. There is no enough data available in the literature about the effect of batter frozen storage on β-glucan properties. Beer et al. (1997b) stored oat bran baked muffins at -20ºC for up to five months. The authors concluded that β-glucan solubility significantly decreased as frozen storage time increased, while no changes occurred in β-glucan Mw. Lan-Pidhainy et al. (2007) also observed similar results with oat bran baked muffins when it was undergone under a number of freeze-thaw cycles.

5.8. Conclusion

This study has demonstrated that there is good potential for developing fiber-rich cookies from frozen batter with addition of air-classified barley flour rich in β-glucan (~25%) at 10% replacement level. The high fiber content of barley flour (~53% total dietary fiber) reduced the pasting characteristics of soft wheat flour leading to low peak viscosity, trough paste viscosity as well as break down and final viscosity. In consequence, it modified the characteristics of batter and cookies as it increased batter hardness and stickiness and cookies hardness, while it decreased cookies spread factor. However, it was possible to minimize these changes by adjusting water amount added to the formula. Frozen storage resulted in no changes in composite batter hardness and stickiness and cookie spread factor compared to the control, while cookie breaking force decreased as frozen storage period increased. Compared to control, cookie made
from composite flours contained higher amount of soluble β-glucan with slight changes in molecular weight or solubility during frozen storage. In order to introduce the beneficial effects of β-glucan to a great number of consumers, more research should be done on adjusting freezing process and water amounts in the formula of cookie batter that contain non-wheat flours rich in β-glucan and dietary fiber to minimize frozen storage deterioration effect on cookies.
Table 5.1. Cookie Formulations Based on 225g Flour

<table>
<thead>
<tr>
<th>ingredients</th>
<th>control batter (C)</th>
<th>batter fortified with 10% (ACB) flour</th>
<th>dough fortified with 10% (ACB) flour with extra 8ml water</th>
</tr>
</thead>
<tbody>
<tr>
<td>wheat flour (g)</td>
<td>224</td>
<td>201.6</td>
<td>201.6</td>
</tr>
<tr>
<td>ACB flour (g)</td>
<td>-</td>
<td>22.4</td>
<td>22.4</td>
</tr>
<tr>
<td>sucrose (g)</td>
<td>94.5</td>
<td>94.5</td>
<td>94.5</td>
</tr>
<tr>
<td>NaCl (g)</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>sodium bicarbonate (g)</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>distilled water (ml)</td>
<td>50.5</td>
<td>50.5</td>
<td>58.5</td>
</tr>
<tr>
<td>shortening (g)</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>high-fructose corn syrup (g)</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>free instant skim milk powder (g)</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>ammonium bicarbonate (g)</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*a Air-Classified Barley-Enriched Flour. *b Distilled water amount = (225g flour)+ 49.5. *c Crisco shortening.
Table 5.2. Approximate Chemical Composition of the Flour Samples

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>(ACB)*</th>
<th>Composite flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>ash %</td>
<td>0.6±0.0</td>
<td>2.5±0.0</td>
<td>0.8±0.0</td>
</tr>
<tr>
<td>protein %</td>
<td>9.1 ±0.0</td>
<td>17.1 ±0.1</td>
<td>9.88±0.3</td>
</tr>
<tr>
<td>moisture%</td>
<td>4.2±1.2</td>
<td>12.1±0.1</td>
<td>13.8±0.3</td>
</tr>
<tr>
<td>total dietary fibre %</td>
<td>5.4±0.3</td>
<td>52.3±1</td>
<td>9.6±0.2</td>
</tr>
<tr>
<td>SDF%</td>
<td>2.7±0.2</td>
<td>35.2±0.8</td>
<td>5.7±0.1</td>
</tr>
<tr>
<td>IDF%</td>
<td>2.6±0.1</td>
<td>17.10±0.2</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>β-glucan%</td>
<td>0.31±0.0</td>
<td>24.7±0.5</td>
<td>2.65±0.1</td>
</tr>
</tbody>
</table>

*Composite flour: 90% refined soft wheat flour+10% ACB.
Table 5.3. Average RVA Starch Pasting Properties of Soft Wheat, Barley and Composite Flours

<table>
<thead>
<tr>
<th>flour sample</th>
<th>peak viscosity (cp)</th>
<th>trough viscosity (cp)</th>
<th>breakdown viscosity (cp)</th>
<th>final viscosity (cp)</th>
<th>setback viscosity (cp)</th>
<th>peak time (min)</th>
<th>pasting temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft wheat*</td>
<td>1912±33 a</td>
<td>2227±24 a</td>
<td>685±17 a</td>
<td>2353±61 a</td>
<td>1126±38 a</td>
<td>9.2±0.0 a</td>
<td>86±0.6 a</td>
</tr>
<tr>
<td>10% ACB-E*</td>
<td>1576±20 b</td>
<td>949±6 c</td>
<td>627±15 b</td>
<td>1764±56 b</td>
<td>816±53 b</td>
<td>9.2±0.0 a</td>
<td>86±0.1 a</td>
</tr>
<tr>
<td>15% ACB-E</td>
<td>1637±54 b</td>
<td>952±26 c</td>
<td>685±29 a</td>
<td>1768±30 b</td>
<td>816±9 b</td>
<td>9.2±0.0 a</td>
<td>86±0.4 a</td>
</tr>
<tr>
<td>20% ACB-E</td>
<td>1606±4 b</td>
<td>913±9 c</td>
<td>694±12 a</td>
<td>1748±9 b</td>
<td>835±0 b</td>
<td>9.1±0.0 a</td>
<td>86±0.3 a</td>
</tr>
<tr>
<td>30% ACB-E</td>
<td>1640±4 b</td>
<td>1110±7 b</td>
<td>530±11 c</td>
<td>1758±7 b</td>
<td>828±11 b</td>
<td>9.1±0.1 a</td>
<td>85±0.3 a</td>
</tr>
</tbody>
</table>

*Soft wheat sample (control)
*Air-classified barley flour

Means in a column with the same lowercase letter are not significantly different between flours at P< 5%. Different letters within each column are significantly different between fractions at P< 5%.
Figure 5.1. Rapid Visco Analyser (RVA) Viscograms of Soft Wheat and Barley Composite Flours
Figure 5.2. Hardness and Stickiness of Fresh Cookie Batter and Spread Factor Their Cookies with Different Levels of Air-Classified Barley Flour and Water Added to the Formula
Figure 5.3. Hardness and Stickiness of Fresh and Frozen-Thawed Control and Composite Batters Stored at -18°C for 4 Weeks
Figure 5.4. Moisture Content and Water Activity of Fresh and Frozen-Thawed Control and Composite Batters Stored at -18°C for 4 Weeks
Figure 5.5. Moisture Content and Water Activity of Cookies Made from Fresh and Frozen-Thawed Control and Composite Batters Stored at -18°C for 4 Weeks
Figure 5.6. Spread Factor and Breaking Force of Cookies Made from Fresh and Frozen-Thawed Control and Composite Batters Stored at -18°C for 4 Weeks
Figure 5.7. A: Increase (%) in Soluble β-Glucan Amount. B: Reduction (%) in Molecular Weight (Mw) of β-Glucan in Fresh and Frozen-Thawed Control and Composite Batters Stored at -18°C for 4 Weeks
Figure 5.8. A: Increase (%) in Soluble β-Glucan Amount. B: Reduction (%) in Molecular Weight (Mw) of β-Glucan in Cookies Made from Fresh and Frozen-thawed Control and Composite Batters Stored at -18°C for 4 Weeks
CHAPTER 6:

Conclusions

The bakery industry has been exploiting the advantages and applications of freezing technology in several frozen dough/batter bakery products to meet the demand for convenient and fresh bakery products by consumers and food services. A major shortcoming of frozen dough is substantial deterioration of baking quality with increasing freezing storage period. As a result, research in frozen dough has been carried out to improve the quality characteristics to be comparable with the freshly prepared bakery products. The use of additives has become a common practice in the business of baking industry to improve the quality of frozen dough and consequently the quality of the end products. This research was designed to tackle this issue and investigate the effect of air-classified barley flour as a rich source of β-glucan (~25%) on the rheological properties of frozen dough and batter and the quality of their end products. The high water holding capacity of β-glucan was expected to affect water distribution in the dough system which consequently would affect the dough rheological and functional properties and behaviour of water during storage at low temperature.

In dough and batter systems, replacement of 10% wheat flour with air-classified barley flour along with water adjustment in the formulas was found to minimize detrimental effects of dough/batter characteristics occurred during frozen storage. For instance, frozen composite bread doughs without and with vital gluten exhibited marked physical improvement during frozen storage at -18°C for up to 4 weeks by alleviating alteration in frozen dough rheological properties. The composite doughs contained higher amount of bound water, less freezable water content and less gluten network damage compared to 100% wheat flour frozen dough used as a
control. Frozen storage of composite batters (contain 10% air-classified barley flour) at -18°C for 4 weeks resulted in no changes in neither control nor composite batters hardness and stickiness.

As expected, incorporation of barley flour at 10% replacement level without or with vital gluten reduced loaf specific volume (LSV) of the baked bread compared to the control. Up to 4 weeks of frozen storage, frozen control and composite without vital gluten breads exhibited similar LSV and texture profile compared to that of their corresponding bread made from fresh dough. The addition of vital gluten in frozen composite dough resulted in a significant reduction in LSV and significant changes in its crumb texture after the second week of storage. No changes were observed in cookie spread factor during the entire period of frozen storage (4 weeks), while cookies breaking force decreased as frozen storage period increased. Both bread and cookie made from composite flours contained high amount of soluble β-glucan with slight effects on its molecular weight or solubility due to frozen storage. This study has shown a good potential for developing fiber-rich bread and cookie from frozen dough and batter by incorporating air-classified barley flour as a rich source of β-glucan and dietary fiber without compromising the quality of the end products when dough/batter was stored up to 4 weeks at -18°C. In general, incorporation of air-classified barley flour rich in β-glucan along with water adjustment in the baking formula resulted in positive effects on frozen dough and batter and their end product quality but for a limited frozen storage period of 4 weeks. These findings approved the proposed hypothesis and showed the significance of β-glucan in frozen dough.
7. REFERENCES CITED


Beer MU, Wood PJ and Weisz J. 1997a. Molecular weight distribution and (1→3),(1→4)-
D-β-glucan content of consecutive extracts of various oat and barley cultivars. Cereal Chem 74:
476-480.

Beer MU, Wood PJ and Weisz J. 1997b. Effect of cooking and storage on the amount and
molecular weight of (1→3),(1→4)-D-β-glucan extracted from oat products by an in vitro

Belton PS. 2005. New approaches to study the molecular basis of the mechanical

Bennion EB and Bamford GST. 1997. The Technology of Cake Making, edited by Bent,

Berglund PT, Shelton DR and Freeman TP. 1991. Frozen bread dough ultra-
structure as affected by duration of frozen storage and freeze-thaw cycles. Cereal Chem.
68:105-107.

Beuchat LR. 1981. Microbial stability as affected by water activity. Cereal Foods World,
26, 345–349.

Bhattacharya M, Langstaff TM and Berzonsky WA. 2003. Effect of frozen storage and
freeze–thaw cycles on the rheological and baking properties of frozen doughs. Food Research


Buell Inc. Introduction to air classification.


144


EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific opinion on the substantiation of health claims related to oat beta-glucan and lowering blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of regulation (EC) no 1924/2006. EFSA journal 2010; 8(12):1885.


149


Jasrotia AKS. 2011. The effect of functional ingredients and frozen storage on wheat dough and bread quality studies using time-domain nuclear magnetic reasonance, dynamic


Miller SD. 2006. Yeast metabolism in fresh and frozen dough. A thesis presented in partial fulfilment of the requirements for the degree of doctor of philosophy in food technology at Massey University, Palmerston North, New Zelan. 15–90


Moriartey SE. 2009. Barley β-glucan in bread: The journey from production to consumption. A thesis submitted to the faculty of graduate studies and research in partial fulfillment of the requirements for the degree of doctor of philosophy in food science and


Yi J. 2008. Improving frozen bread dough quality through processing and ingredients. a dissertation submitted to the graduate faculty of the university of georgia in partial fulfillment of the requirements for the degree doctor of philosophy. University of Georgia, Athens, Georgia. 1-90.

