Effects of Microwave Heating on Baking Quality of Wheat

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ABSTRACT

In 2011-2012, wheat production in Canada was 20.1 Mt. As a major staple food globally, wheat is one of the grain widely used in baking industry as well. Both the physical and chemical characteristics of flours affect their quality and the subsequent products from them.

The chemical compounds, namely starch and protein contribute to the functional and strength property of dough. Freshly harvested wheat may need a few years of storage to enhance the quality of these compounds. However, heat treatment with microwave is an efficient method to accelerate the process of aging.

The physical properties such as ash content and gelatinization temperature of starch play an important role in the dough stability during baking and other characteristics of final products as well.

In this study, two factors which include temperature and moisture content was applied. There were three levels of each: temperatures were 60 °C, 75 °C and 90 °C while moisture contents were set at 12%, 15% and 18% on wet basis. Combinations with different factors studied were obtained from the JMP 10 software (SAS Institute Inc., Cary, NC, USA) using a Central Composite Design (CCD).

Total protein content was determined by bicinchoninic acid method, while starch content was estimated by enzymatic method. A muffler furnace was used
to burn the wheat at 585 °C in order to measure the mineral content of wheat flour. The gelatinization temperature of starch was determined by using the differential scanning calorimetry (DSC).

Data were analyzed by analysis of variance (ANOVA) with JMP 10. It was found that, following the increase in temperature, the gelatinization temperature increased compared to the untreated samples. Starch and protein were increased initially followed by the decreasing trend when the heating temperature reached 75 °C. Similarly, ash content increased first and decreased when the temperature reached 90 °C. However, moisture content was found to be insignificant in the range used in this study.

In conclusion, the effects of microwave heating treatment on starch, protein and gelatinization temperature were determined which are important for baking. The model explained the effects of the factors on the parameters, which helps its use in baking industry.
RÉSUMÉ

En 2011-2012, le Canada a produit 20.1 Tg de blé. Une denrée alimentaire de base à l’échelle mondiale, le blé est une des céréales les plus utilisées dans l'industrie de la boulangerie. Les caractéristiques physiques et chimiques des farines de blé en affectent la qualité ainsi que les produits qui peuvent en découler. Les composés chimiques, notamment l’amidon et les protéines contribuent aux propriétés fonctionnelles et à la résistance de la pâte. Le blé nouvellement récolté peut gagner à être entreposé pour quelques années afin d’affiner la qualité de ces composés. Cependant, un traitement thermique par microondes présente une méthode efficace pour accélérer le processus de vieillissement. Les propriétés physiques tel la teneur en cendres et la température de gélatinisation de l’amidon jouent un rôle important dans la stabilité de la pâte durant la cuisson, ainsi que d’autres propriétés des produits finis. Deux facteurs expérimentaux, dont la température et la teneur en humidité, furent appliqués à trois niveaux chacun: températures de 60 °C, 75 °C et 90 °C, teneurs en humidité de 12%, 15% et 18% par rapport au poids frais. Les combinaisons des différents facteurs à l’étude dans un design expérimental composite central furent analysées avec le logiciel JMP 10 du SAS Institute. La teneur globale en protéine fut dosée par la méthode de l’acide bicinchoninique, tandis que la teneur en amidon fut dosée par une méthode enzymatique. Un four à moufle opérant à 585 °C servit à brûler la farine de blé afin d’en mesurer la
terre minéraux. La température de gélatinisation de l'amidon fut déterminée par analyse calorimétrique différentielle (DSC). Une analyse de variance (ANOVA) des données utilisant JMP 10 indiqua qu'une augmentation de la température par rapport au traitement témoin donna lieu à une augmentation de la température de gélatinisation. Une hausse de la température jusqu'à 75°C donna lieu à une augmentation, par rapport au témoin, des teneurs en protéines, amidon et cendres, mais une tendance décroissante au-delà de cette température. Cependant, la teneur en humidité n'eut aucun effet significatif. Les effets d'un traitement de la farine de blé par microondes eut donc un effet sur la teneur en protéines, en amidon et sur la température de gélatinisation de l'amidon, tous des facteurs importants dans la cuisson du pain. Le modèle statistique expliqua l'effet des différents facteurs du système de traitement aux microondes sur les paramètres étudiés, permettant à l'industrie de la boulangerie d'en entrevoir les avantages.
Chapter I

INTRODUCTION

Wheat is a cereal grain which originated from the Levant region of the Near East and Ethiopian Highlands (Belderok et al., 2000). On a world scale, wheat is the second most produced crop in the world, following only world corn production. However, unlike corn, it is primarily used for human consumption. The four major types of wheat (hard red, soft red, white and durum wheat) are harvested every month somewhere in the world. Wheat is the leading supplier of plant protein in human diets, which has more protein compared to maize or rice. In terms of total production of food, it is the second biggest source as human food crop just behind rice. In 2010-2011, the global production of wheat was 647.6 million tons, which was decreased by 35 Mt from 2009-2010, while the durum production was 34.5 Mt, which was reduced by 6.5. In Canada, the production in 2011 was 20.1 Mt, which was decreased by 1.3 Mt compared to the year before (Canada, 2011).

Wheat is consumed in various forms in a daily meal, which makes it as an absolutely essential part of the diet globally; it is also an important commodity in the third world countries. Grounded kernels of wheat converted to flour is used extensively in baking industry for breads, cookies, cakes, crackers and other processed staple foods like spaghetti, macaroni and other kinds of pasta in various forms.
Bread is a kind of product fermented mainly from wheat flour, yeast and water by a series of steps of processing manipulations such as mixing, kneading, proofing, shaping and baking (Miličević et al., 2012, Stojceska and Butler, 2012). Most types of bread are made with wheat flour; however, there exists other forms containing rye or oat.

1.1 Economics of Wheat

Wheat is the primary staple food all around the world. Wheat is grown throughout Canada, while the main area is in the western Prairie region. As a whole, there are two types of wheat, spring wheat and winter wheat. Spring wheat is grown from April to May in Canada and harvested in the later summer months (August to early September). Winter wheat is planted in fall from September to November in eastern Canada and along the US border and is harvested in early summer (June and July).

Globally, Canada is a major wheat producer. However, the Canadian production is not stable due to the weather changes, which affects significantly its production leading to problems in worldwide wheat supply.

1.2 Baking Quality

The series of steps involved in baking process of bread starts with the mixing of flour, water, yeast and other quality enhancing ingredients. Gluten forms a viscoelastic continuous phase when the dough is mixed well. The network has a dominant rheological impact on the dough (Georgopoulos et al.,
The microstructure and rheological behavior of dough are affected by variables such as pH, water and sodium chloride addition (Jekle and Becker, 2012).

There are many important elements for high quality baked products:

a. Protein content: Wheat flour contains gliadin and glutenin which form as gluten when mixed with liquid. The gluten has the ability to trap carbon dioxide formed by fermentation of sugar and starch by yeast, causing dough to “rise”. The quality of gluten determines the texture of end production.

b. Mixing: Significant amount of mixing time is necessary to achieve proper mixing. The wheat dough, which withstands the beating action by external forces, is preferred while mixing.

c. Mineral or ash content should be low.

d. Fermentation time: It is required for the dough to rise.

e. Water absorbance: Capacity of the flour to absorb water is an important attribute in the baking process.

1.3 Microwave heating

Thermal technology is by far the most sensitive aspect of food processing, which dictates the quality, environmental impact and economics of most processing of plants (Venkatesh and Raghavan, 2004). There has been an increasing trend of microwave use not only in food industry but also in household kitchen as well. As a unique thermal processing technique,
researchers have done studies on microwave treatment of food for disinfesting of insects in agricultural storages (Wang et al., 2003), drying of vegetables (Schiffmann, 1992), inactivation of enzymes, pasteurization of breads, sterilization of food products (Venkatesh and Raghavan, 2004) and acid pasteurization of eggs (Dev et al., 2012).

Characteristics such as efficiency and energy saving can be obtained by using microwaves compared to the conventional heating. According to the studies which has been done before, microwave treatment has the following advantages: shorter drying time (Campbell et al., 1957), energy saving and maintain the quality during production (Watanabe et al., 1998, Vallejo et al., 2003, Nair et al., 2011).

1.4 Problem Statement

When one substance converts into another, chemical change is taking place as a result of chemical reaction. The alcohol and carbon dioxide fermented from sugar by yeast is one of such chemical reaction. This is the fundamental step in preparing the bread dough. There is a phase where bread dough is allowed to rise before they are baked. It is the moment when fermentation occurs. During fermentation initialed by yeast, carbon dioxide, alcohol and other chemical compounds are produced, which make the dough to rise and modify its physical properties. After the correct fermentation procedure, the baker will obtain flour with proper external and internal characteristics that can be used to make
various end products. When combing with alcohol, a few other unstable compounds are known to be released which contribute to the taste and flavors of the baked products. Most of these compounds, and all of the alcohol, evaporate while baking.

Studies have shown that differential quality in bread-making depends on gluten characteristics which consists of 80% protein, 8% fats and 12% carbohydrates as well as minerals (Hoseney, 1990).

Starch, which accounts for most of the weight of wheat (70 to 75 percentage dry weight), has been known to influence the functional properties of wheat flours used in baking bread, cookie, and cake (Zhu and Wang, 2013). The starch, which is damaged during milling, interacts with other constituents of the baking process by exposing its components (amylose and amylopectin), affects the water absorption and fermentation time required by the bread-making dough, and the staling and crumb texture of bread as well.

Protein content in wheat varies from 8 to 17 percent which is based on genetic make-up and external factors associated with crops (Yousaf et al., 2013). Gluten takes up most of the protein content in wheat. Therefore, gluten impact importantly most of the viscoelastic properties of wheat flour dough. Gluten viscoelasticity, is commonly known as flour or dough strength for end-use purposes (Battacharya and Corke, 1996).

Heat treatment developed high ratio recipes, which generate products with
finer texture, longer shelf life, moist crumb and sweeter taste. The mechanism of heat treatment is not fully understood; however, protein denaturation and partial gelatinization of starch occurs. There is an increase in batter viscosity as well. Thus, it is very important to optimize the flour heat treatment process in order to enhance baking quality of wheat (Neill et al., 2012).

1.5 Proposed Solution

A decrease in starch and protein content in flour has been found under bad wheat storage conditions (McDonald, 1998). Moreover, a decline of wheat storage protein constituting gluten during storage can be detected (Galleschi et al., 2002, Calucci et al., 2004).

In order to achieve high-quality bread, it is recommended to accelerate and control the aging process of wheat without the addition of chemicals. Flour might enhance its yield of bread loafs due to the effect of artificially aged seeds due to the properties associated with gluten upon hydration. As a result, the quality of dough may be affected. It is possible to accelerate the aging of wheat seeds (Modarresi et al., 2001) in few days, with higher temperature and humidity environment. This environment, however, could have the ability to initiate the process of aging wheat, but it also controls it in an effort to extract high-quality flour. This effect also studied by Deepika (2012). In continuation to this work, different heating method will be studied.
1.6 Hypothesis and objectives

Heat treatment of wheat grain is the positive process of changing the physico-chemical properties that impacts the baking quality of flour. The key objective of this study is to determine the microwave heating effect as a thermal treatment, which improves the quality of the flour when processed. To determine the quality due to treatment, it is important to determine total protein, starch, ash content; also to establish the gelatinization temperature of starch. The study includes three temperatures and three moisture content levels of the seeds achieved through microwave heating.

Once all these properties of flour are determined, the best combination can be chosen to enhance the baking quality of grains in an efficient way.
2.1 Wheat Flour

2.1.1 Wheat Seed to Flour

Stone grit mill is the original method to make flour from wheat seeds. However, roller mills soon replaced it, which has historically driven technological development. Obviously, roller mills such as using water and wind power make the grit mill more efficient and labor-intensive. According to these terms, the water and wind power has been widely used rather than milling. The unified mill was developed in the mid-20th century.

Substantial interest in the milling industry was awakened by reports that generated byproducts with unique functional and nutritional properties (Dexter and Wood, 1996), and debranning (also referred to as pre-processing or pearling) of wheat prior to roller milling which improved durum wheat semolina milling performance (DEXTER et al., 1994). Likewise, evidence for improvement of common wheat milling performance is less definite (DEXTER et al., 1994, Doblado-Maldonado et al., 2012, Waters et al., 2012).

Normally, the whole processing contain following steps:

i) Preparing the wheat: Weighing inspecting and grading the wheat.

ii) Cleaning: Removing impurities like stones, dirt, metals and other seeds.
iii) Tempering: Soaking wheat in water to make it easier to remove the outer bran layer.

iv) Gristing: Mixing different wheat to create a specific kind of flour.

v) Milling: Involves a number of repeated steps. The wheat is ground by a machine equipped with rollers that break it into pieces.

vi) Blending: Different components are blended back together to form different flours. For instance, whole-wheat flour is a blend of white flour and wheat bran.

vii) Enriching & fortifying: Adding vitamins and minerals were followed as required by government regulations.

Cleaning is accomplished by means of separators, scourers, magnets, aspirators and washer-stoners. Wheat is conditioned under specific temperature in water to toughen the bran in order to reduce the fragmentation when it is removed. At the same time, absorbing water results in particles of the size desired. During the drying process with steam, minerals such as potassium and phosphorus migrate to the endosperm; however the minerals with strong binding characteristics like calcium and magnesium would not migrate. These may have positive effects on increasing the content of certain minerals in refined flour. In the milling process, steel rollers crush the grain; the flour is released and separated by sifters into various grades or streams, based on the fineness. As a result, each of these may contain different minerals or proteins. They will be
recombined to form a variety of flours to meet the demand for diverse baking purposes of various products. After the process, the bran and germ, which make up about 28% of the wheat, are totally removed. However, they can be used in pharmaceutical laboratories for making diet supplements or in the production of animal feeds.

2.1.2 Wheat Flour Usage

Wheat flour is a main staple food with high nutrient content. It is the first choice of health conscious people, because of its fiber properties. Wheat flour is obtained from milling wheat. Flours are of various types that come from different varieties of wheat, processed and mixed at various proportions of its constituents. There are diverse uses such as in bread and other bakery products as well as in many other recipes in which wheat flour is used as the main ingredient.

Wheat flour is the most common commodity used in baking industry. They can be distinguished by the amount of gluten they contain. There are two types of flour according to the protein content: strong flour and weak flour. Strong flour is the flour made from hard, high protein varieties of wheat which containing high gluten content. The weak flour is made from soft, low protein wheat, which is low in gluten (Sliwinski et al., 2004). Gluten, wheat’s natural protein, contributes to the good structure of baked products.

Wheat flour is an excellent source of series of carbohydrates. Wheat flour contains B-vitamins, calcium, iron, magnesium, phosphorus, potassium, zinc,
sodium, minerals and other trace elements (Bekes, 2012, Pasha et al., 2013). At the same time, it is a good supplier of protein as well. Because of its distinguished nutritional value, wheat flour has become the most consumed flour around the world.

The whole wheat flour is dark brown in color and is used in breakfast breads, muesli bars and animal feeds. Whereas the white wheat flour which is also called as enriched wheat flour is used in baking bread, cookies, cake and to thicken gravies and soups or in the other products such as cream cones, wafers and pet foods as well. People are opting to use whole wheat flour in their diets nowadays because it had been found that whole wheat flour has higher nutritional value than the enriched wheat flour (Steinfurth et al., 2012, Yamsaengsung et al., 2012, Yildiz and Bilgicli, 2012). Simultaneously, wheat extracts are used in the production of glue, licorice and animal feeds. Gluten is added to some flours and starch is used as a thickener in soups, spreads and puddings.

2.1.3 Bread making process and reactions

The raw material includes flour, yeast, water and substances that alter the texture (such as milk) and agents that catalyze the chemical reactions occurring (e.g. malt flour), flavoring agents (such as salt and sugar), preservatives (often vinegar). Even a single ingredient performs several functions.

The most substantial chemical reaction in bread baking is the CO$_2$-producing reaction caused by yeast feeding on sugar (Equations 1 and 2):
\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy} \quad \text{(Equation 1)} \]

Glucose + oxygen $\rightarrow$ carbon dioxide + water \quad \text{(Equation 2)}

Released energy during the reaction helps the continuation of growth of yeast, and the bread expansion leading to volume increase which is caused by the gas (CO$_2$). The kneading of the dough helps to produce bubbles of same size that is distributed throughout the dough. Both the starch and the gluten stiffen to form a solid network base for the bread (Edwards, 2007).

### 2.2 Wheat Flour Nutrients

Wheat can be considered as a good source of protein, minerals, B-group vitamins and dietary fiber (Shewry, 2007). It is easy to store, transport and ability to be processed into several kinds of food. The nutrient content of grains can be significantly different because of cultivar, crop year, area, and fertilization condition as well as soil type. The nutrient composition of different wheat products is shown in Table 2.1.

<table>
<thead>
<tr>
<th>Wheat Product</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Starch (g)</th>
<th>Sugar (g)</th>
<th>Carbohydrate (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>14.1</td>
<td>5.5</td>
<td>66.8</td>
<td>1.7</td>
<td>68.5</td>
</tr>
<tr>
<td>Whole meal flour</td>
<td>12.6</td>
<td>2.0</td>
<td>61.8</td>
<td>2.1</td>
<td>63.9</td>
</tr>
<tr>
<td>White flour (self-raising)</td>
<td>8.9</td>
<td>1.2</td>
<td>74.3</td>
<td>1.3</td>
<td>75.6</td>
</tr>
<tr>
<td>Wheat flour (bread-making)</td>
<td>11.5</td>
<td>1.4</td>
<td>73.9</td>
<td>1.4</td>
<td>75.3</td>
</tr>
</tbody>
</table>

Data taken from Food Standard Agency (2002).
The outer bran layer, the endosperm and germ constitute the kernel wheat. It contains several of nutrients. Many of them are concentrated in the bran and germ. It is important that, the kernel contains the entire B group vitamins except the B12. The B vitamins function as cofactors in many metabolic reactions, which were involved in the release of energy.

Also, other vitamins and plentiful of other minerals are found in the wheat kernels; however, they are in small amounts. The vitamins include vitamin B6 or pyridoxine, carotene, pantothenic acid, and folic acid, biotin, vitamin C, and vitamin K. At the same time, minerals are sodium, calcium, manganese, zinc, copper, chlorine, cobalt, nickel chromium, molybdenum, fluoride, iodine, boron, selenium, lead, aluminum, and silicon oxide (Lv, Sipos et al., 2006).

The germ, which includes the scutellum, is especially rich in vitamins B and E, high quality protein, unsaturated fats, carbohydrates and minerals. Most of the insoluble carbohydrate cellulose exists in the bran that contains minerals especially iron and B vitamins as well. The largest part of the grain is endosperm that consists mostly of the incomplete protein, carbohydrate starch and amounts of vitamins and minerals.

Wheat germ is promoted as a health food, and even as a cure for disease because of its high content of vitamin E. Recently, studies have shown that vitamin E can increase the necessary high density lipoprotein (HDL) cholesterol in women; however, in men only if they had lower levels initially. Vitamin E may be
helpful for fibrocystic breast disease as well (Guthrie and Bagby, 1989). From medical point of view, vitamin E is used to treat intermittent claudication, which includes pains in the calf muscles during night and while walking.

The quality of protein is determined by the category and composition of its constituent amino acids. When all essential amino acids are appeared in the proportions capable of promoting growth, the protein is complete with good quality, high biological value and may affect the body with high net protein utilization. Body tissue repair mechanism will occur while the protein has a relatively small amount of one essential amino acid (Guthrie and Bagby, 1989).

Wheat has been shown to maintain the nitrogen equilibrium or a slightly positive impact for the nitrogen balance and maintaining it from bread diet, so that it is adequate for adults (Bolourch.S et al., 1968, Betschart et al., 1985, Young and Pellett, 1985). Lysine, as a limiting essential amino acid in cereal, is especially important for children. The necessary intake amount of Lysine is three times more for children than adults. Protein from rye has higher biological value than wheat because of its greater amino acid composition, though wheat contains about 20% more protein than rye. Nevertheless, rye has 30% more amino acid Lysine than wheat. More calcium and fluoride are found in rye as well.

To guarantee a satisfied intake of Lysine, bread should be consumed with combination of milk, nuts or meat. That is the reason one would need animal
products since they are the only source of vitamin B12. Large deficiencies of this vitamin lead to anemia (Guthrie and Bagby, 1989). The missing vitamin A and C can be found in fresh fruits and vegetables. Fats are needed to provide essential fatty acids, since they are found in small quantities in wheat (about 2%).

Despite all the nutritional values, wheat cannot meet all nutritional needs since it lacks necessary amounts of certain essential nutrients: vitamins A, B12 and C, amino acid Lysine and fats; these must come from other sources.

2.2.1 Protein in wheat

2.2.1.1 Protein structure

There are many efforts to reveal the structure of the gluten, however, they have obstacles associated with its low solubility attributes and lack of crystallinity of protein. The primary structures of individual proteins and also their interactions with non-covalent forces (Belton et al., 1998) and covalent disulphide bonds (Shewry and Halford, 2002) are the conditions which establishes the solubility properties of gluten protein.

The protein structure is still not fully understood (Veraverbeke and Delcour, 2002). The mature wheat seed contains 8-20% proteins (Dupont and Altenbach, 2003). The gluten protein which is made up with gliadins and glutenins, constitute 80-85% of total flour protein, it affects the elasticity and extensibility property that are significant for baking quality of wheat flour. Among the gluten protein, gliadins and glutenins each constitutes around 50%.
The first theory associated with classification of wheat grain proteins is attributable with their solubility which was established by Osborne. He categorized glutenin protein into four types: albumins (soluble in water), globulins (salt), gliadins (aqueous water) and glutenins (dilute acid or alkali) (Osborne and Clapp, 1906). Methods of protein fractionation have now been improved, due to findings of the Osborne fractions being heterogeneous and containing protein types overlapping each other. Nowadays, the protein classifying system is based on biological characteristics of proteins combined with their chemical and genetic relationship, which leads to different states of accumulation in dissociating solutions (Shewry and Tatham, 1990, Shewry et al., 1992). Therefore, gliadins are mixtures of glutenins consisting of polypeptides combined by disulphide bonds and monomeric polypeptides (Shewry and Tatham, 1990, Singh and MacRitchie, 2001). Similarly, glutenin is formed by a mixture of polymers, high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). The large glutenin polymers are stable through inter-chain disulphide bonds (Field et al., 1983).

The wheat proteins possess unique features which are often attributed to their structure (Weegels et al., 1996c) while they are complex under natural condition. The type of wheat protein determines flour baking quality directly. Most of the technological attributes related to dough-making and baking were based on the wheat proteins (Shewry and Halford, 2002). Gluten is composed of
gliadins and glutenins; also, it has lipids, minerals, and carbohydrates (Wieser, 2007). The wheat quality can be indicated by the rheological parameter of gluts. Poor quality wheat shows the feature of less elasticity and more viscous than those of high quality wheat because the rheological parameters of gluts is poor in quality (Khatkar et al., 2002).

2.2.1.2 Protein classification

The wheat contains four types of proteins: glutenins (soluble in dilute acid or alkali), albumins (soluble in water and dilute buffers), globulins (not soluble in water but soluble in saline solutions) and prolamins (soluble in 70–90% ethanol). Most of the albumin proteins which are identified from proteomic analyses of mature grain endosperm of wheat (Singh et al., 2001) and barley belong to the α-amylase/trypsin inhibitors family (Finnie et al., 2002). The gliadins and glutenins protein fractions are defined as proteins which are accumulated in the grain-filling period (Shewry and Halford, 2002).

Gliadins and glutenins, which occupy 80-90% of the total wheat flour protein, are the two primary classes of storage proteins. They are necessary for producing an appropriate balance of viscous and elastic properties in gluten and flour dough. It is widely accepted that gliadins contributes to viscous properties whilst glutenins affect strength and elasticity (Shewry et al., 1986). Gliadins are monomeric proteins, which can be further classified into four groups of α, β, γ and ω. Glutenins are polymeric proteins which are composed of low molecular
weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS).

Glutenins, gliadin/glutenin ratio and HMW-GS/LMW-GS ratio determines the viscoelasticity of glutsens (Popineau et al., 1994). Subunit composition and HMW-GS can modify the size and distribution. Also they affect protein aggregation leading to viscoelastic condition through crosslinking (Cornec et al., 1994, Popineau et al., 1994).

Increasing the gliadin/glutenin ratio may cause a decrease in elasticity since gliadins act as plasticizers in gluten (Popineau et al., 1994). Also, intermolecular interactions determine the viscoelasticity of gluten network. Under mechanical actions, slow destruction of intermolecular interactions causes the nonlinear rheological performances beyond a critical value. Normally, the critical strain of gluten dough can reach 5-10%, which is much larger than those in flour dough at around 0.2% (Wang and Kokini, 1995).

Gluteins in wheat can behave differently through different bread making quality. The good quality glutenins induces a greater elastic character than those which come from the wheat with poor quality glutenins (Khatkar, 2005). It has been found that the interactions within large complex formed by HMW-GS and LMW-GS determine Glutenin viscoelasticity (Cornec et al., 1994). At the same time, HMW-GS plays a major role in influencing the bread making quality.

The quality of wheat can be assessed by the molecular structure of storage proteins.
proteins from wheat which can control the interactions of the proteins during the bread making process (Bolourch.S et al., 1968). The variation in composition as well as the protein content of wheat flour can significantly change the baking quality of flour (Weegels et al., 1996b, Lafiandra et al., 1999, Branlard et al., 2001).

Wieser and Kieffer (2001) reported that the primary responsibility for the viscoelastic properties of flour dough and even the high molecular glutenin is associated with the molecular weight distribution of wheat proteins. The molecular weight of glutenin polymers reaching over 20 million Daltons which makes glutenin proteins becoming the largest protein molecules among those available in nature (Wrigley, 1996). These proteins are diverse mixtures of polymers formed by disulphide-bonded linkages of polypeptides (Payne et al., 1979, Payne et al., 1985).

Lawrence and Payne (1983) reported that 40% of the total seed protein are made up with glutenin fraction of hexaploid (*Triticum aestivum* L.) which is significant in establishing the strength and elasticity of wheat flour dough since it contains protein aggregates of high molecular weight (up to several million) formed by the association of numerous essential polypeptide chains.

Ohm and Chung (1999) determined that gluten hydration amounts and contents to have significant connections with not just water absorption, but also play a dominant role in bread making process. Under acidic condition, gliadin
shows higher surface activity than glutenins among the major gluten proteins. (Takeda et al., 2001).

2.2.1.3 Measurement of wheat protein

Both protein content and protein quality have major influence on the baking potential of wheat flours (Macritchie, 1987, Weegels et al., 1996a, Xu et al., 2006). Protein quality refers to the protein's ability to lend a good balance of characteristics such as extensibility, elasticity and fermentation tolerance to the dough. The composition of glutenin subunits are indirectly related to baking quality via the quantity or the size distribution of the glutenin polymers (Weegels et al., 1996a, MacRitchie, 1999), which are essential for the mixing requirement and the resistance of the dough. In other words, increasing protein content, generally increases dough extensibility.

Protein in biological tissue can be quantified by colorimetric assays such as the Bradford assay (Bradford, 1976) and the bicinchoninic acid (BCA) protein assay (Smith et al., 1985) which are sensitive or the elder Lowry assay (Lowry et al., 1951). These methods play a significant role in the determination of protein content rapidly (Davis, 1988).

The methods described above are accurate when they are used among soluble proteins; however, cereal proteins are largely insoluble in nature, which pose problems with using colorimetric assays. More recently, Stich (1990) directly assays protein bound to agarose beads which is a variation of the BCA
This procedure is based on the reduction of Cu$^{2+}$ to Cu$^{+}$ by the protein, which can derive the insoluble proteins. A purple chromophore, complexes by Cu$^{+}$ and BCA, is then released in aqueous solution. According to Chan and Wasserman (1993), a clarification step is added to remove suspended material. At the end, absorbance readings are obtained at 562 nm and protein content can be calculated by comparing it to a standard curve.

2.2.2 Starch in wheat

2.2.2.1 Wheat starch usage

As a main composition of cereal grain and plant root, starch can be widely used in food industry, paper and textile industries after chemical, enzymatic or physical modification (Thomas and Atwell, 1999). Enzymatic modification of starch through hydrolysis can be accomplished in order to convert some parts of starch into a lower molecular weight of starch, which is called maltodextrin, or dextrin. Starches after enzymatic modification are widely used in food and pharmaceutical industries. Physical modification involves heat treatment or pregelatinization of starch, etc. Pregelatinized starches are called “convenience products”, such as precooked and dried by producers and reconstituted in water to give viscous paste (Stute, 1992).

2.2.2.2 Influence of starch on baking quality

It is obvious that starch is the primary component since it occupies around 80% of the wheat total weight. Starch plays a notable role as a determinant of
the quality of food product. Most of the functional attributes of starch are related to the temperature dependent interactions of starch with water in the processes known as pasting, gelatinization, and retrogradation.

Starch is the most abundant component in wheat flour; however its significance in flour quality is not clear yet. The relevance of the starch will show up during the several steps including milling, mixing, fermentation and baking and storage of intended products manufacturing. It is interesting to notice that the differences in starch quality might affect baking quality, but it is given little attention. Endosperm hardness is one of the important elements of milling wheat grain quality. This property has been suggested to relate to the interactions between starch and protein (Barlow et al., 1973, Simmonds et al., 1973, Greenwell and Schofield, 1986). The specific interaction between starch and protein could be related to the amount of protein or to the protein matrix, which should be examined for continuity. Wheat proteins absorbance ability is less than the other plants such as potato starch (Eliasson and Tjerneld, 1990).

In bread making process, wheat protein, especially gluten, plays an important role in affecting the texture of dough. Nevertheless, starch is also a major component that contributes to the formation of texture and quality of dough and end products such as bread. It has been reported that starch has the following functions in bread making; diluting the gluten to a suitable level, interweaving with gluten, and absorbing water from the gluten through
gelatinization, therefore providing a bread structure permeable to gas so that the bread does not collapse while cooling. Starch is temperature-triggered water-sink in bread making procedure. Starch competes with other components for water availability in the system when temperature increases and starch gelatinizes. The final state of starch affects texture of the baking products.

Researchers have paid much attention to the relationship between insoluble wheat protein, mainly gliadin and glutenin, and texture and quality of bread (Cuq et al., 2000, Uthayakumaran et al., 2000b, Uthayakumaran et al., 2000a, Wesley et al., 2001). More recently, studies discovered that modified starches which were developed to overwhelm undesirable properties of native starches, also affected the properties of dough and quality of bread (Van Hung and Morita, 2004, Miyazaki et al., 2005a, Miyazaki et al., 2005b, Van Hung and Morita, 2005).

2.2.2.3 Effect of heat treatment on wheat starch

Heat treatment processes include high temperature and humidity strengthening treatments, both of which causes a physical modification of starch without any gelatinization, damage to granular integrity, or loss of birefringence (Stute, 1992). The high temperature and humidity treatment can improve starch consistency and lead to stable viscosity in food with a pH of less than 4.5, while the strengthening treatment has the ability to enhance viscosity profile which was considered in native starch and consequently labeled as “food starch” (Thomas and Atwell, 1999). Starch with chemical modification is considered as a
mainstream throughout the last century. Various chemically modified starches have been developed and improved to apply for food, paper and textile industry (Wang, 1997, Han et al., 2005).

Hoover and Vasanthan (1994) reported that the heat treatment did not change granule size and shape after the high temperature treatment of wheat, oat, potatoes, lentil and yam starches. The X-ray diffraction intensities increased in wheat starches, while decreased in potato and yam. The X-ray patterns of wheat and oat starches remained unchanged, while those of lentil, potato, and yam starches became more cereal-like. In all starches, the swelling factor and amylase leaching decreased after heat treatment. Heat treatment induces complex formation between amylase and native lipids. Differential scanning calorimeter (DSC) of the heat-treated samples showed a broadening of the gelatinization-temperature range and a shifting of the endothermic transition towards higher temperatures. Also, heat treatment increased the viscosity of wheat starch when the temperature reaches 95°C. They found that, in all starches, thermal and shear stability increased after heat treatment. The gelatinization enthalpy of wheat starch remained the same due to thermal treatment of wheat starch, while acid hydrolysis decreased.

2.2.2.4 Measurement of wheat starch

Englyst et al. (1982) first recognized the resistant to enzymatic hydrolysis of starch. The RS content was then measured by Berry (1986) who developed the
procedure by using the α-amylase employed by Englyst et al. (1982) and also modified the procedure by omitting the initial heating temperature at 100 °C.

The role of starch in baking has been widely recognized as an important component (Banks et al., 1973, Stevens, 1976, Cauvain et al., 1977). Recent research shows that there is appreciable variation in the amylose and lipid contents, granule size distributions, and gelatinization temperatures of wheat starches (Soulaka and Morrison, 1985a). Since these are properties which could affect flour baking quality, it was decided to evaluate the baking performance of a representative selection of these starches.

More recently, some modifications were done to include changes in concentrations of different enzymes used, pre-treatment methods and addition (or not) of ethanol after α-amylase incubation as suggested by Faisant et al. (1995), Goni et al. (1996) and Akerberg et al. (1998).

The enzyme method developed by Megazyme (McCleary et al., 2002) is a robust and reliable method as reflected in vivo condition. The procedure has been subjected to inter-laboratory evaluation under the auspices of AOAC International and AACC and accepted by both.

2.3 Thermal property of wheat

2.3.1 Gelatinization temperature of starch

It is evident that starch plays a specific role on its own during the dough mixing. The difference between dough and dough without starch are substantial
The baking experiments with glass beads replacing starch shows that starch is not just filler since the result is totally unacceptable ‘bread’. Furthermore source of starch can also relate to the final result. Wheat, rye and barley starches perform the best, whereas potato and maize starches cannot be used for baking (Kulp, 1972). Amylose is essential for obtaining a good loaf of bread which has been found in the baking experiments with waxy barley starch (Ghiasi et al., 1984).

It has been proved that wheat starch is the most suitable starch to be baked; however, there is still some unclear question such as the differences among wheat starches in baking performance. It has been reported that the particle size distribution of the starch granules will influence the baking quality, with the normal distribution found in the wheat as the best (Soulaka and Morrison, 1985b).

Starch gelatinization is a process of breaking down the intermolecular bonds of starch molecules under the varying condition of heat application at different moisture levels, which allows the hydrogen bonding sites to engage more water. Starch granule can be dissolved irreversibly during this procedure. Penetration of water increases randomness in the general starch granule structure and decreases the number and size of crystalline regions. Crystalline regions do not allow water to come in. Heat treatment makes such regions to become more diffusive. As a result, the chains begin to separate into an amorphous form.
Gelatinization temperature of starch depends upon plant type and water quantity, pH, sugar, fat and protein and the derivatisation technology used.

Gelatinization of starch is widely used in food processing area. Products such as baking bread, gelling of pie filling, pasta products, thickening of sauces and fabrication of starch based foods all use the gelatinization property of starch to produce products with desired texture and consistency of final product. There are several types of starch, which can be used in food industry. Tuber starch, such as potato and tapioca, and grain starches, such as corn and wheat, are most commonly used. A lot of researches have been aimed at differentiating various starches and their gelatinization behavior since the availability and cost of starches are varied. Miller et al. (1973) explained the rise in consistency during gelatinization.

In general, wheat starch is considered as insoluble in water (Knight, 1965). This may be correct in practical use; however, starch and water do have interaction before initial temperature of gelatinization is reached. Gough and Pybus (1971) treated undamaged wheat starch granules in water bath at 50 °C for 72 h. As a result, the gelatinization temperature increased. At the same time, gelatinization occurred more suddenly than the control samples, which indicates modification of the granules’ internal structure. Various phases are distinguished in breakdown of the starch molecule structures. During the first phase, which refers to the stage before the onset of gelatinization, water is slowly and
reversibly taken up. Jaska (1971) confirmed this phenomenon by using Proton Magnetic Resonance (PMR). Mobility of water decreased affecting the increase of temperature from 20 to 60 °C. Water is being reversibly complexed with the starch molecules. Initially, this change was reversible before reaching the onset temperature. However, with the exposure of starch to water can cause changes in granule itself (Gough and Pybus, 1971).

Differential scanning calorimetry (DSC) is a simple technique used by industries to check the gelatinization properties of starch. By detecting the difference in heat flow between sample and reference, DSC can measure the amount of heat absorbed and released as well during the heating procedure. It is widely used in industrial settings as a quality control instrument due to its applicability in evaluating sample purity and for studying polymer curing. With the data investigated by DSC, it has been shown that a relation between starch gelatinization temperature and loaf volume exists (Soulaka and Morrison, 1985b, a). Differences between ordinary bread wheat and durum wheat can be achieved through the gelatinization parameters, which are determined by DSC.

2.3.2 Thermal property of wheat protein

Effect of temperature on proteins has been studied (Bull and Breese, 1973a, b, Stellwagen and Wilgus, 1978), while there are few reports concerning dough quality at the temperature beyond 70°C (Guerrieri et al., 1996, Lavelli et al., 1996). As a result of heating, an irreversible change in molecular structure
occurred at thermal denaturation condition. Stellwagen and Wilgus (1978) reported the relationship between protein thermal stability and its solvent accessible surface area by using 30 different well-characterized water-soluble proteins. The pH of unbuffered water-soluble proteins under continuous heating is monitored to detect the changes in the protein structure, and observed a single inflection point in the pH versus the temperature curve. The result indicates the level of thermal denaturation of the proteins. During the procedure, the temperature midpoint is recorded as a measure of thermal stability (Bull and Breese, 1973a).

Recently, Elmehdy et al. (2003) found that thermal conversion occurs when heating temperature is above the gluten protein denaturation temperature by using the low frequency longitudinal ultrasonic velocity measurements to detect the structural and functional changes of wheat under high temperature condition. When glutenin was heated to above 55°C or gliadins (monomeric proteins) to above 70 °C (Payne et al., 1987), exchange reactions occurred (Schofield et al., 1983). Guerrieri states that after prolonged heating at 110°C for 18 hours, the aggregates could not be dissolved (Guerrieri et al., 1996, Lavelli et al., 1996). Once the temperature rose above 90 °C, all the proteins, except omega-gliadins, formed disulfide bonded aggregates.

2.4 Factors determining the baking quality of wheat

Baking quality is a main target for the wheat improvement, which is
essential for both producer and farmers. After the value of cross selection and hybridization was recognized, diversities have been chosen to perform better in mills and processing facilities. Full-scale mill and bakery testing is not performed regularly due to the restrictions of population size and sample size. Small-scale testing equipment is applied to predict variety performance. Many characteristics are measured to predict baking quality. These may include parameters such as grain hardness, flour protein content, water absorption, dough resistance and dough extensibility. Therefore, these small-scale assessments are costly, laborious and require quantities of grain in excess of that available at the early stages of a breeding program.

Environmental variation may reduce the heritability of phenotypic quality selection associated with both field and laboratory tests. With the beginning of molecular marker-assisted selection (MAS), new chances exist to replace or improve the current phenotype based selection activities with genotype based selection.

Many researchers have stated success in testing differences among the complex genetic basis of wheat quality. Payne et al. (1987) assessed some alleles at the high molecular weight (HMW) glutenin loci and noted their importance to wheat quality. The investigation of combined effect of alleles on dough strength has also been done by others (Gupta et al., 1989, Nieto-Taladriz et al., 1994, Eagles et al., 2002). In the report of Eagle et al., almost half the phenotypic
variation in dough resistance and extensibility was explained by the main and two-way interaction effects of the six glutenin loci. Other genes have contributed in the control of grain hardness as identified by Giroux and Morris (Giroux and Morris, 1997). Their effects on flour and dough performance have been detected as well (Cane et al., 2004).

Furthermore, alleles at the starch synthase loci has an associated effect with the amylose fraction of the grain starch and noodle texture (Nakamura et al., 1993). Elsewhere, few studies reported additional genetic loci, which influence dough rheology and baking quality (Perretant et al., 2000, Groos et al., 2004).

2.5 Microwave heating

Microwave heating applications have been researched since the late 1940s. For the past decades, there have been investigations on the development of microwave heating applications in grain drying (Shivhare et al., 1994) and insect control. Since then, numerous food applications have been studied with successful developments. In recent years, industrial microwave processing in the food industry has been firmly established.

During microwave heating, continuous electromagnetic waves are formed in the magnetron and transmitted through a hollow metallic tube into a resonant cavity where the food is processed. Foods are heated based on the molecular friction caused by alternating polarization of molecules. Foods absorb microwave
energy in the form of ionic and orientation polarization which results from
dipolar molecules, such as water according to the applied electric field (Knoerzer
et al., 2004). The electric field oscillates at 2450 or 915 million times per second
(MHz) (depending on the equipment used), making the dipolar molecules
alternate, thus promoting molecular friction, which in turn results in heat
dissipation. When dissolved salts are present, Ionic polarization occurs. Positive
and negative ions tend to migrate to opposite-charged regions, while colliding
with other ions and converting kinetic energy into heat. As a microwave heating
mechanism, dipole rotation is more significant than ionic polarization.

Some heat sensitive nutrients such as vitamins and flavor ingredients can be
protected better through rapid heating than conventional heating methods.
However, only little evidence shows any real differences of flavor between
conventional and microwave heating (Malinger et al., 2004).

Microwave is widely used as heat treatment operations in the food
processing industries. Microwave heating provide the shorter heating time
compared to the conventional heat treatments to achieve the required
food-processing temperature. Similarly, microwave pasteurization and
sterilization promise fast heat processing (Dev et al., 2012).

To improve the quality of microwave baking products, dielectric properties
and penetration depth are the electrical properties, which have significant effects.
Dielectric properties can be separated into two factors, which are the dielectric
constant, and the dielectric loss factor. These properties reflect the ability of a material to collect and dissipate electrical energy, respectively (Mudgett, 1982).

Microwave food processes provide a lot of advantages such as less start-up time, faster heating, energy efficiency, space savings, precise process control, selective heating and food with high nutritional quality (Marra et al., 2010). The most significant difference between convection ovens and microwave ovens is the incapability of the microwave ovens to induce browning. As a result, the cool ambient temperature in the microwave oven causes surface cooling of microwave-heated products. The low surface temperature prevents Maillard browning reactions, which contributes to the production of many flavored and colored compounds. The Maillard reaction and caramelization of sugars result in the brown surface of products. They are the result of high temperature accompanied by dehydration. The short microwave baking time may have effect on flavor development as well, since these flavor compounds may not have opportunity to develop when they are under conventional baking. Microwave energy causes the flavor components turning to be volatilized at different rates and in different proportions than those, which occurs during conventional heating.
The above review sets the stage to proceed with the objectives set forth earlier for evaluating microwave heat treatment on wheat seeds which in turn lead to superior quality flour for baking.
Chapter III
MATERIALS AND METHODS

3.1 Experimental materials

In this study, the microwave heating treatment is performed for the hard spring wheat, which is the cultivar AC Walton. AC Walton is a high-yielding spring wheat cultivar, developed by Agriculture and Agri-Food Canada, Charlottetown Research Centre, Prince Edward Island. The freshly harvested grains (year 2011) were obtained from Les Moulins de Solanges, St Polycarp, Quebec. No chemicals were used for preservation for all wheat samples, which were free from insect infestation as well.

3.2 Experimental design

The initial moisture content of wheat grains was measured by drying them in hot air oven at 105 °C for 24 hours. The initial and final weights were recorded and the dry basis moisture content of wheat sample was calculated by using the follow equation (AOAC, 1984):

\[
\text{Moisture content (MC)}\% = \frac{\text{initial mass} - \text{final mass}}{\text{initial mass}} * 100 \quad \text{(Equation 1)}
\]

As a result, the initial moisture content of red spring wheat was 9.55 % (wet basis). According to the initial moisture content, calculating the water they need and mixing samples with the specific quantity of water can determine the required moisture content of the samples. After the calculation, about 11 mL, 46 mL, 38 mL of water was added to 450 g, 850 g, 450 g sample to increase the
moisture content to 12%, 15% and 18% respectively; samples were kept in the sealed bottle placed in a fridge at 7°C for 24 hours with 3 times shaking at every 8 hours. Final moisture contents were checked after 24 hours. Once the grains are brought to the moisture levels required, 100 g of each were placed in a microwave oven. The microwave heating will bring the sample to the three temperature levels, which are 60°C, 75°C and 90°C. The experimental levels of all two factors are given in Table 3.1.

**Table 3.1 Experimental Levels of two factors**

<table>
<thead>
<tr>
<th>Factor Levels</th>
<th>Factors</th>
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<tr>
<td></td>
<td>Temperature (°C)</td>
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<tr>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
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</table>

About 100 grams of each sample were placed in the breaker with initial moisture content as set by the levels 1, 2 and 3. These beakers were placed in a microwave (multimode cavity operating at a set power density of 1.5 W/g). Thus heating the sample rapidly to achieve the required set temperatures. There is a sensor to detect the temperature of each sample. The control programs used to control the time of operation for reaching the desire temperature (60°C, 75°C and 90°C). Once the temperature reached the desired value, microwave would stop automatically since a program controlled it. During the heating procedure, beaker was taken out; samples were mixed well to maintain the temperature
evenly in the overall mass contained in the beaker. The beaker was placed in the microwave again to continue heating; this was repeated two more times. The microwave heating of freshly harvested wheat was initiated by controlling two major factors, temperature and moisture content. Moisture content after heating is shown in appendix A.

In order to minimize the total number of experimental runs and to determine the significant factors to get best combination of factors affecting the baking quality of wheat flour, a response surface methodology with a Central Composite Design (CCD) was applied. The experimental design was constructed using central composite face centered (CCF) design; with two factors, which have three levels each. The design will provide maximum information with reasonable experimental runs.

After heating, the grains were put on a plate to cool down to room temperature before milling. The prepared samples were milled into whole wheat flour and the effects of microwave heating on physical properties were determined.

3.3 Estimation of total starch content

During the 16 h incubation at 37 °C with pancreatic α-amylase and amyloglucosidase (AMG), non-resistant starch is solubilized and hydrolyzed to glucose by the combined action of the two enzymes (Equation 2).
\( \alpha \)-amylase, pH 7.0 or 5.0, 100°C

\[
\text{Starch} + H_2O \rightarrow \text{Maltodextrins} \quad \text{(Equation 2)}
\]

An equal volume of ethanol terminates this action, and the RS existed in the pellet after centrifugation. RS in pellet is dissolved in 2 M KOH by vigorously stirring in an ice-water bath using a magnetic stirrer, followed by addition of sodium acetate buffer and \( \alpha \)-amylase (Equation 3).

\[
\text{KOH then neutralization} + \alpha \text{-amylase} \Rightarrow \text{RS} + H_2O \rightarrow \text{Maltodextrins} \quad \text{(Equation 3)}
\]

Next, amylloglucosidase (AMG) quantitatively hydrolyses maltodextrins to D-glucose (Equation 4).

\[
\text{Maltodextrins} \xrightarrow{\text{AMG}} \text{D-glucose} \quad \text{(Equation 4)}
\]

Finally, the maltodextrins are quantitatively hydrolyzed to glucose with AMG (Equation 4). Then, D-glucose is oxidised to D-gluconate accompanied by the release of hydrogen peroxide \( (H_2O_2) \), which can be measured in a colorimetric reaction employing peroxidase and producing quinoneimine dye (Equation 5, 6).

\[
\text{D-gluconate} + \text{Glucose oxidase} \rightarrow H_2O_2 \quad \text{(Equation 5)}
\]

\[
2H_2O_2 + \text{p-hydroxybenzoic acid} + 4\text{-aminoantipyrine} \rightarrow \text{Quinoneimine dye} + 4H_2O \quad \text{(Equation 6)}
\]
3.3.1 Measurement of Resistant starch

A 100±5 mg sample was placed in a screw cup tube and gently tapped the tube ensuring the sample falling to the bottom; 4.0 mL of pancreatic α-amylase (10 mg/ml) containing AMG (3 U/ml) was added to each tube. After tightly capping it, mixing was achieved through a vortex action. In the next step, the tubes were placed in an Incu-Shaker (Benchmark Scientific, USA) and incubated at 37 °C with a continuous shaking (200 rpm) for exactly 16 hours. Then the tubes were taken out from the Incu-Shaker and the caps were removed. Then 4.0 mL of ethanol (99%) was added and stirred vigorously on a vortex mixer; centrifuging the tubes at 3300 rpm was carried out for 10 min. Then the supernatants were decanted carefully and the pellets were re-suspended in 8 mL 50% ethanol, mixing on vortex mixer and centrifuging again at 3300 rpm for 10 min. The supernatants were decanted and the steps of suspension and centrifugation described above were repeated once more. Finally the pellets containing the resistant starch (RS) were obtained.

The pellets were re-suspended by addition of 2 mL of 2 M KOH, followed by magnetic stirring for 20 min in an ice/water bath. Further 8 mL of 1.2 M sodium acetate buffer (pH 3.8) were added and the magnetic stirring was continued. Immediately after 0.1 mL of AMG (3300 U/ml) was added and mixed well; incubating in water bath at 50 °C for 30 min was accomplished with intermittent mixing on a vortex mixer every 10 min for 3 times totally.
According to the guidance of the kit, the method applied for RS content was different based on amount of RS content in the samples (greater or less than 10%). Since the wheat flour contains less than 10% RS content, the tubes were centrifuged at 3300 rpm for 10 min directly after incubation. Pipetting of 0.1 mL aliquots of each supernatant into a clean tube was done followed by the addition of 3.0 mL of glucose oxidase/peroxidase (GOPOD) reagent. Tubes were placed in a water bath at 50 °C for 20 min. The absorbance of each solution at 510 nm was recorded against the reagent blank by using a spectrophotometer (Ultraspec 2100 pro, Biochom, UK). The reagent blank was prepared by mixing 0.1 mL of 0.1 M sodium acetate buffer (pH 4.5) and 3.0 mL of GOPOD reagent. Also the D-glucose standard was prepared by mixing 0.1 mL of D-glucose (1 mg/ml) and 3.0 mL of GOPOD reagent. The calculations are shown in Section 3.3.3.

3.3.2 Measurement of Non-Resistant starch

All the supernatant solutions obtained from the centrifugation of initial incubation and two 50% ethanol washings were combined. The volume was adjusted to 100 with 100 mM sodium acetate buffer (pH 4.5) in a 100 mL volumetric flask and mixed well.

A 0.1 mL aliquots of this solution (in triplicate) received 10 µL of dilute AMG solution (300 U/mL in 100 mM sodium maleate buffer pH 6.0), followed by incubation at 50 °C for 20 min. Then, 3.0 mL of GOPOD reagent was added. Tubes
were placed in a water bath at 50 °C for further 20 min. Then the absorbance at 510 nm was measured and recorded against a reagent blank.

3.3.3 Calculation

Resistant starch (g/100g sample, flour containing < 10% RS):

\[
\Delta E \times F \times \frac{10.3}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} = \Delta E \times \frac{F}{W} \times 9.27
\]  
(Equation 7)

Non-resistant (solubilised) starch (g/100g sample)

\[
\Delta E \times F \times \frac{100}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} = \Delta E \times \frac{F}{W} \times 90
\]  
(Equation 8)

Total starch = Resistant starch + Non-resistant starch

Where,

\[\Delta E = \text{Absorbance (reaction) read against the reagent blank.}\]

\[F = \text{conversion from absorbance to micrograms (the absorbance obtained for 100 µg of glucose in the GOPOD reaction is determined and F = 100 (µg of glucose) divided by the GOPOD absorbance for this 100 µg of glucose.}\]

\[100/0.1 = \text{volume correction (0.1 mL taken from 100 mL).}\]

\[1/1000 = \text{conversion from micrograms to milligrams.}\]

\[W = \text{dry weight of sample analyzed}\]

\[= "as is" \text{ weight x (100-moisture content)/100].}\]

\[100/W = \text{factor to present RS as a percentage of sample weight.}\]

\[162/180 = \text{factor to convert from free glucose, as determined, to}\]
anhydro-glucose as it occurs in starch.

\[ 10.3/0.1 = \text{volume correction (0.1 mL taken from 10.3 mL) for samples} \]

containing 1-10% RS where the incubation solution is not diluted and the final volume is \(~ 10.3\) mL.

3.4 Estimation of protein content

The Pierce BCA Protein Assay is based on bicinchoninic acid (BCA) for the colorimetric reaction and quantification of total protein. \(\text{Cu}^{2+}\) is reduced to \(\text{Cu}^+\) by protein with the release of a soluble purple chromosphere. This water-soluble complex compound has strong absorbance at 562 nm, which can quantify the protein content by comparison with a standard curve.

The solid-phase assay suspends samples directly in BCA work reagent to maximize interaction of the reaction with the insoluble sample. After a clarification step by micro-centrifugation and dilution, absorbance can be read and protein content can be quantified.

3.4.1 Solid-phase protein assay procedure

A sample of 5 mg wheat flour was weighed and transferred to 1.5 mL micro-centrifuge tubes and suspended in 1.0 mL of BCA work reagent. The BCA work reagent was prepared by mixing 50 parts of BCA reagent A with 1 part of BCA reagent B (50:1, Reagent A: B). Samples were then incubated in water bath at 37 °C for 30 min with intermittent mixing on vortex mixer at every 10 min. After incubation, tubes were cooled in an ice bath for 5 min and centrifuged at
3300 rpm for 10 min in a microcentrifuge at room temperature.

Aliquot of supernatant sample of 0.2 mL was supplemented with 0.8 mL of BCA reagent A, followed by thorough mixing step and centrifuged at 3300 rpm for 30 sec. The absorbance at 562 nm was read by using a spectrophotometer.

A standard curve (200-600 μg) was constructed using bovine serum albumin provided by the kit. Samples were then brought to 1 mL with addition of BCA work reagent. Incubation at 37 °C for 30 min was done. After cooling to room temperature, taking 0.2 mL reagent and diluting with 0.8 mL BCA reagent A, following by thorough mixing step and centrifuging at 3300 rpm for 30 min. The absorbance value was recorded at 562 nm.

3.4.2 Calculation

The protein content is calculated on the basis of absorbance (Equation 9)

\[
\text{Protein Content (\%)} = \frac{c}{w \times 0.2} \times 100
\]

(Equation 9)

Where,

\(c\) = concentration read from standard curve

\(w\) = dry weight of sample analyzed

= "as is" weight x (100-moisture content)/100.

3.5 Estimation of ash content

3.5.1 Analysis procedure

About 2±0.1 gram of each sample was weighed and placed in a crucible, which had been previously weighed and its weight recorded. Flour was dried in
an oven at 105 °C for 24 hours to ensure the samples were completely dry. The weight of samples was noted and its moisture content calculated. Later the furnace was set to 585 °C as the set point temperature and the samples were in the unit for 930 min.

The samples were cooled down in a sealed cabinet to room temperature. Samples were then weighed and ash content was noted by deducting the dry weight.

3.5.2 Calculation

Ash content is calculated by the Equation 10 follows:

\[
\text{Ash content} \% = \frac{\text{final mass}}{\text{dry mass}} \times 100
\]  
(Equation 10)

3.6 Determination of thermal properties

3.6.1 Equipment condition

The equipment used to determine the gelatinization temperature of starch was TA Instruments Q 100 Differential Scanning Calorimeter (Newcastle, DE, USA). The instrument consists of four parts: namely furnace, auto sampler, cooling system and dedicated computer. The DSC is operated with a TA Instrument Q 100 DSC 7.0 equipped with a 244 software. Nitrogen was used as a purge gas flowing at a 50 ml/min rate.

3.6.2 Analysis procedure

Wheat flours were weighed and 5.00 mg sample was placed in an aluminum pan desired for DSC Q 100, followed by adding 5 μl of distilled water directly in to
the pan. The pans were then closed with an aluminum lid and sealed well carefully and placed at room temperature for 30 min to allow the flour and water to mix well. After that, samples were placed in the auto sampler of DSC.

The nitrogen gas flow was turned on and adjusted to 50 ml/min. At the same time, the refrigerated cooling system was turned on. The heating temperature from 20 °C to 160 °C was set up at a rate of 5 °C/min. The Universal Analysis Version 1.2 software can determine the onset temperature (T_o) and peak maximum temperature (T_m), which were associated with the changes of structure of major dough components. During the thermal treatment, an endothermic peak appeared at about 60 °C, which was the temperature of starch gelatinization (T_g). This temperature of treated samples was compared to the untreated flour.

3.7 Statistical analysis

JMP 10 software (SAS Institute Inc., Cary, NC, USA) was used to analyze the data obtained from the experiments. Data were analyzed by analysis of variance (ANOVA). The significance of factors was determined at 5% probability level (p value <0.05). Distribution was checked before running the model to ensure the goodness of fits. The lack of fit and coefficient of determination (R²) was evaluated by the adequacy of the response surface model. The surface of all the two factors and their three levels each for total starch which was obtained by adding the components of resistant starch and non-resistant starch and total
protein were obtained based on the modeling approach, the interrelation can be explained visually through the three-dimensional response surface plots.
Chapter IV
Results and Discussion

4.1 Analysis of total starch content

The total starch and total protein contents were constructed using a three-level Central Composite Design (CCD). There were two factors in the study to determine the effects on the content of these two chemical compounds.

The total starch content of the flour samples of each combination of factors was determined using the enzymatic assay procedure. All the samples were measured in triplicates and the mean value was obtained. The starch content of each sample is shown in Table 4.1.

Table 4.1 The total starch content obtained at various treatment levels in the experimental design

<table>
<thead>
<tr>
<th>$\mu$</th>
<th>Temperature $^\circ$C</th>
<th>MC $%$</th>
<th>Total starch content $%$</th>
<th>Actual</th>
<th>Predicted</th>
<th>Std error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>60</td>
<td>12</td>
<td></td>
<td>86.98</td>
<td>83.14</td>
<td>$\pm2.26$</td>
</tr>
<tr>
<td>B</td>
<td>75</td>
<td>12</td>
<td></td>
<td>78.75</td>
<td>80.95</td>
<td>$\pm2.52$</td>
</tr>
<tr>
<td>C</td>
<td>90</td>
<td>12</td>
<td></td>
<td>74.66</td>
<td>78.77</td>
<td>$\pm3.45$</td>
</tr>
<tr>
<td>D</td>
<td>60</td>
<td>18</td>
<td></td>
<td>83.45</td>
<td>84.50</td>
<td>$\pm3.88$</td>
</tr>
<tr>
<td>E</td>
<td>75</td>
<td>18</td>
<td></td>
<td>74.82</td>
<td>78.72</td>
<td>$\pm2.56$</td>
</tr>
<tr>
<td>F</td>
<td>90</td>
<td>18</td>
<td></td>
<td>73.02</td>
<td>72.93</td>
<td>$\pm4.11$</td>
</tr>
<tr>
<td>G</td>
<td>60</td>
<td>15</td>
<td></td>
<td>88.99</td>
<td>83.82</td>
<td>$\pm2.41$</td>
</tr>
<tr>
<td>H1</td>
<td>75</td>
<td>15</td>
<td></td>
<td>75.89</td>
<td>79.83</td>
<td>$\pm1.45$</td>
</tr>
<tr>
<td>H2</td>
<td>75</td>
<td>15</td>
<td></td>
<td>74.67</td>
<td>79.83</td>
<td>$\pm1.45$</td>
</tr>
<tr>
<td>H3</td>
<td>75</td>
<td>15</td>
<td></td>
<td>76.66</td>
<td>79.83</td>
<td>$\pm1.45$</td>
</tr>
<tr>
<td>H4</td>
<td>75</td>
<td>15</td>
<td></td>
<td>89.01</td>
<td>79.83</td>
<td>$\pm1.45$</td>
</tr>
<tr>
<td>H5</td>
<td>75</td>
<td>15</td>
<td></td>
<td>77.66</td>
<td>79.83</td>
<td>$\pm1.45$</td>
</tr>
<tr>
<td>I</td>
<td>90</td>
<td>15</td>
<td></td>
<td>83.14</td>
<td>75.85</td>
<td>$\pm2.48$</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>9.6</td>
<td></td>
<td>83.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Measured total starch contents obtained from the enzymatic assay were tested for normality using a Shapiro-Wilk test prior to the analysis of variance. The result indicated that temperature had an effect on the total starch content of this Canadian spring wheat although it was not significant at 0.05 level. However, the p value was 0.0699 (a bit more than 0.05), which indicated the effect of temperature processed by microwaves. The linear effect of moisture content of samples was found to be not significant with p value= 0.7361 (more than 0.05). The bilinear effects of temp.\(^2\), MC\(^2\) and temp. * MC were also insignificant since their p values were all above 0.05 (Table 4.2).

### Table 4.2 ANOVA for total starch content of the wheat flour

| Term      | Estimate  | Std error | t ratio | Prob>|t| |
|-----------|-----------|-----------|---------|-----|
| Intercept | 102.0908  | 11.97294  | 8.53    | < .0001* |
| MC        | -0.23106  | 0.66665   | -0.35   | 0.7361 |
| Tem       | -0.25144  | 0.123916  | -2.03   | 0.0699 |
| Temp*MC   | -0.036936 | 0.030497  | -1.21   | 0.2537 |

The predictive starch content can be calculated based on the factors as shown in Equation 10. It indicated a relationship between total starch, temperature and moisture content.

\[
\text{Total Starch} \% = 102.09 + (-0.23 \times MC) + (-0.25 \times \text{Temp.}) + (-0.04) \times (\text{Temp.} -71.43) \times (MC - 14.61)
\]

(Equation 10)

The three-dimensional plot is shown in Figure 4.1 which clearly indicated
that total starch decreased when the temperature went above 60 °C. In general, the total starch increased slightly when the heating temperature went to 60 °C. With the continuous increasing of the temperature, total starch content decreased compared to the untreated sample.

![Response surface plot for total starch content.](image)

Research has been done by others using microwaves with similar conditions. In general, storability improved in all samples when they were treated using microwaves. The influence of microwave energy on certain chemical components was also investigated by MacArthur & D’Appolonia (1981).

Blaszcak et al. (2002) treated the wheat from 20 °C to 98 °C with microwaves. After his evaluation on the features with starch, he indicated visible
changes for temperatures above 64 °C. In the range of 79 °C to 98 °C, grain
denaturation of protein on the surface of starch granules was observed.

Compared to the current study, 60 °C was found to be a significant
temperature in the quantification of changes of starch content of wheat. It had
been reported that the main changes in starch occurred in the range of 49-60 °C
(Doliniska et al., 2004). Similar results were reported earlier by MacArthur and
D’Appolonia (1981) who analyzed flour and bread and found that important
qualities was adversely affected after 240 sec at 61 °C of microwave exposure
where the moisture content of flour was estimated at 14%. Zaied et al. (1996)
contradicted and noted that there were no changes in the content of starch
during the microwave heating.

4.2 Analysis of total protein content

The total protein content of the flour samples of each combination of
factors is determined using the solid-phase assay procedure. All the samples
were measured in triplicates and the mean value was obtained. The protein
content of each sample is shown in Table 4.3.
Table 4.3 The total protein content obtained at various treatment levels in the experimental design

<table>
<thead>
<tr>
<th>Design points</th>
<th>Temperature °C</th>
<th>MC %</th>
<th>Total protein content %</th>
<th>Actual</th>
<th>Predicted</th>
<th>Std error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>60</td>
<td>12</td>
<td>10.89</td>
<td>10.12</td>
<td>±0.84</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>75</td>
<td>12</td>
<td>10.17</td>
<td>9.54</td>
<td>±0.38</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>90</td>
<td>12</td>
<td>9.82</td>
<td>8.97</td>
<td>±0.42</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>60</td>
<td>18</td>
<td>11.12</td>
<td>10.63</td>
<td>±0.58</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>75</td>
<td>18</td>
<td>10.10</td>
<td>9.76</td>
<td>±0.65</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>90</td>
<td>18</td>
<td>10.12</td>
<td>8.89</td>
<td>±0.43</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>60</td>
<td>15</td>
<td>10.74</td>
<td>10.38</td>
<td>±0.69</td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>75</td>
<td>15</td>
<td>9.38</td>
<td>9.65</td>
<td>±0.40</td>
<td></td>
</tr>
<tr>
<td>H2</td>
<td>75</td>
<td>15</td>
<td>9.02</td>
<td>9.65</td>
<td>±0.24</td>
<td></td>
</tr>
<tr>
<td>H3</td>
<td>75</td>
<td>15</td>
<td>8.75</td>
<td>9.65</td>
<td>±0.24</td>
<td></td>
</tr>
<tr>
<td>H4</td>
<td>75</td>
<td>15</td>
<td>8.73</td>
<td>9.65</td>
<td>±0.24</td>
<td></td>
</tr>
<tr>
<td>H5</td>
<td>75</td>
<td>15</td>
<td>8.60</td>
<td>9.65</td>
<td>±0.24</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>90</td>
<td>15</td>
<td>8.12</td>
<td>8.93</td>
<td>±0.24</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>9.6</td>
<td>10.87</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Normality testing was done for all the data obtained from the solid-phase protein assay by using a Shapiro-Wilk test prior to the analysis of variance.

Results shown in Table 4.4 indicated that some of the factors affect the total protein content. Temperature was a significant factor which affect the protein content of the treated wheat with a p value of 0.0468 (p< 0.05). The other factors such as moisture content were found to be insignificant. The bilinear effect of
temp.\(^2\), MC\(^2\) and temp. * MC were also insignificant since their p values were all above 0.05.

Table 4.4 ANOVA for total protein content of the wheat flour

| Term       | Estimate | Std error | t ratio | Prob>|t| |
|------------|----------|-----------|---------|------|
| Intercept  | 12.461121| 2.0012    | 6.23    | <. 0001* |
| MC         | 0.0479737| 0.111426  | 0.43    | 0.6759 |
| Temp       | -0.046968| 0.020712  | -2.27   | 0.0468* |
| Temp*MC    | -0.003265| 0.005097  | -0.64   | 0.5362 |

The predictive protein content can be calculated based on the factors shown in Equation 11. It indicated the following relationship between total protein, temperature and moisture content.

Total protein (%) = 12.46 + 0.05 * MC + (- 0.05 * Temp.) + (- 0.003) * (Temp. - \(71.43\)) *(MC – 14.61) \hspace{1cm} (Equation 11)

From the three-dimensional plot in Figure 4.2, the effects of the key factors are clearly shown. As the temperature increased from 60 °C to 75 °C or even 90 °C, the total protein content decreased compared to the untreated sample. Instead, when the heating temperature reached 60 °C, the total protein content kept a high level or even increased a little compared to the room temperature which is an untreated blank (control).
Figure 4.2 Response surface plot for total protein content.

Rheological properties of wheat gluten are very sensitive to any chemical or physical modification of the gluten molecular and supramolecular structure (Shewry et al., 2002, Lefebvre et al., 2003, Belton, 2005). It could therefore be expected that even very satisfactory changes in arrangement and physicochemical properties of the grain storage proteins induced due to microwave energy input to wheat grain would affect the gluten structure and its viscoelastic behavior.

According to Blaszczaık et al. (2002), when the temperature reached 79 °C and 98 °C, the grain temperature denaturation of protein was observed. The enlarged view of cell fragments clearly indicated the denaturation of protein.

Technological tests performed on microwave-heated grain proved changes in starch and proteins fractions which could be seen in deterioration of baking
value (Blaszczak et al., 2002). Also analysis of grain microstructure by scanning electron microscopy (SEM) proved some changes in protein structure (Blaszczak et al., 2002). In addition Sodium Dodecyl Sulphate (SDS) and Zeleny sedimentation tests confirmed these results.

Although, there are some other contradictory reports, Grundas et al. (2008) investigated the physicochemical properties of winter wheat grain with the similar methods of Blaszczak (2002). According to his result, there were no direct effects on the total protein content. However, indirect effect was found as shown by the statistical analysis.

Wilkes and Copeland (2008) found the wheat protein to increase when they were stored at 30 °C compared to the ones stored at 4 °C. According to this, the slight increase of total protein at 60 °C followed by the decrease when the temperature went up was reasonable.

4.3 Analysis of total ash content

The total ash content of the flour samples of each combination of factors was determined by burning in the furnace for 16 h. All the samples were measured in triplicates and the mean value was obtained. The starch content of each sample is shown in Table 4.5.
Table 4.5 The total ash content obtained at various treatment levels in the experimental design

<table>
<thead>
<tr>
<th>Design points</th>
<th>Temperature °C</th>
<th>MC %</th>
<th>Total starch content %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual</td>
<td>Predicted</td>
<td>Stderror</td>
</tr>
<tr>
<td>A</td>
<td>60</td>
<td>12</td>
<td>1.97</td>
</tr>
<tr>
<td>B</td>
<td>75</td>
<td>12</td>
<td>2.01</td>
</tr>
<tr>
<td>C</td>
<td>90</td>
<td>12</td>
<td>1.93</td>
</tr>
<tr>
<td>D</td>
<td>60</td>
<td>18</td>
<td>2.04</td>
</tr>
<tr>
<td>E</td>
<td>75</td>
<td>18</td>
<td>2.05</td>
</tr>
<tr>
<td>F</td>
<td>90</td>
<td>18</td>
<td>1.87</td>
</tr>
<tr>
<td>G</td>
<td>60</td>
<td>15</td>
<td>2.09</td>
</tr>
<tr>
<td>H1</td>
<td>75</td>
<td>15</td>
<td>1.96</td>
</tr>
<tr>
<td>H2</td>
<td>75</td>
<td>15</td>
<td>1.97</td>
</tr>
<tr>
<td>H3</td>
<td>75</td>
<td>15</td>
<td>2.16</td>
</tr>
<tr>
<td>H4</td>
<td>75</td>
<td>15</td>
<td>2.01</td>
</tr>
<tr>
<td>H5</td>
<td>75</td>
<td>15</td>
<td>1.84</td>
</tr>
<tr>
<td>I</td>
<td>90</td>
<td>15</td>
<td>1.81</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>9.6</td>
<td>1.93</td>
</tr>
</tbody>
</table>

Measured ash contents obtained from the experiments were tested for normality using a Shapiro-Wilk test prior to the analysis of variance.

Results are shown in Table 4.5 indicating the factors that affect the total ash content. The analysis of the data is shown in Table 4.6. Temperature was a significant factor, which affects the ash content of the treated wheat with a p value of 0.0438 (p< 0.05). The other factors such as moisture content was found
to be insignificant (p value greater than 0.05). The bilinear effect of temp.$^2$, MC$^2$ and temp. * MC were also insignificant since their p values were all above 0.05.

Table 4.6 ANOVA for total ash content of the wheat flour

| Term     | Estimate   | Std error  | t ratio | Prob>|t| |
|----------|------------|------------|---------|-----|---|
| Intercept| 2.2305025  | 0.199843   | 11.16   | <. 0001* |
| MC       | 0.0070778  | 0.111426   | 0.64    | 0.5390 |
| Temp     | -0.004769  | 0.002068   | -2.31   | 0.0438* |
| Temp*MC  | -0.000998  | 0.000509   | -1.96   | 0.0785 |

The predictive ash content can be calculated based on the factors shown in Equation 12. It indicated a relationship between total ash, temperature and moisture content as shown below:

Total ash (%) = 2.23 + 0.007 * MC + (-0.005) * Temp. + (Temp - 71.43)*(MC - 14.61)*(-0.001)  
(Equation 12)

From the surface response plot shown in Figure 4.3, the effects of the factors are clearly shown. As the temperature increased from 60 °C to 75 °C, the total ash content increased compared to the untreated sample. When the heating temperature reached 75 °C, the total ash content kept a high level followed by a slight increase compared to the room temperature of the untreated blank (control).
The greater portion of minerals found in a kernel of wheat is contained in the germ, and husk, or bran, and the least amount in the endosperm. Ash content affects the color and other characteristics of the baking products. However, research has been done on ash content but it is not enough to show quality differences. Kathuria (1984) stated that ash content of semolina in comparison with whole grains decreased under the hot water treatment. A significant quantity of soluble compounds was leached out during the heat treatment. But he did not point out the specific experimental temperature of the wheat. According to the results of this experiment, wheat’s total ash content might decrease before 90 °C or lower.
4.4 Analysis of gelatinization temperature of starch

All the flour samples showed an endothermic transition between 60 to 70 °C. The thermogram curves for each treated samples, which indicate the various thermal property, were obtained from the equipment and results were analyzed by using the software of the Instrument Q100 DSC 7.0 Build 244. The curve for the untreated control sample is shown in Figure 4.4. It was easy to read the gelatinization temperature to be at 62.12 °C and 64.17 °C for one of the sample in duplicates.

Figure 4.4 DSC thermogram of untreated wheat flour

The different combinations of factors and their corresponding response are presented in Table 4.7. Overall, the gelatinization temperature of starch was within the range of 62-68 °C.
Table 4.7 Gelatinization temperature of starch obtained at various treatment levels in the experimental design

<table>
<thead>
<tr>
<th>Design points</th>
<th>Temperature °C</th>
<th>MC %</th>
<th>Gelatinization temperature of starch °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual</td>
<td>Predicted</td>
<td>Stderror</td>
</tr>
<tr>
<td>A</td>
<td>60</td>
<td>12</td>
<td>64.77</td>
</tr>
<tr>
<td>B</td>
<td>75</td>
<td>12</td>
<td>66.13</td>
</tr>
<tr>
<td>C</td>
<td>90</td>
<td>12</td>
<td>67.21</td>
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<td>18</td>
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</tr>
<tr>
<td>E</td>
<td>75</td>
<td>18</td>
<td>66.47</td>
</tr>
<tr>
<td>F</td>
<td>90</td>
<td>18</td>
<td>68.29</td>
</tr>
<tr>
<td>G</td>
<td>60</td>
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<td>64.39</td>
</tr>
<tr>
<td>H4</td>
<td>75</td>
<td>15</td>
<td>65.07</td>
</tr>
<tr>
<td>H5</td>
<td>75</td>
<td>15</td>
<td>65.11</td>
</tr>
<tr>
<td>I</td>
<td>90</td>
<td>15</td>
<td>67.98</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>9.6</td>
<td>63.15</td>
</tr>
</tbody>
</table>

Nomality of the statistics was checked before running the model. The value indicated that the statistics were well fitted using a Shapiro-Wilk test prior to the analysis of variance.

The ANOVA of the model for $T_g$ is shown in Table 4.8. It is clear to see from the Table 4.8 that temperature has significant effect on the gelatinization...
temperature of starch since the p value is less than 0.05. The other factors such as moisture content were found to be insignificant. The bilinear effect of temp.\(^2\), MC\(^2\) and temp. * MC where also insignificant since their p values were all above 0.05.

**Table 4.8 ANOVA for gelatinization temperature of starch of wheat flour**

| Term      | Estimate | Std error | t ratio | Prob>||t| |
|-----------|----------|-----------|---------|--------|
| Intercept | 58.096814| 2.010758  | 28.89   | <.0001*|
| Temp      | 0.0926185| 0.020811  | 4.45    | 0.0012*|
| MC        | 0.0456262| 0.111959  | 0.41    | 0.6922 |
| Temp*MC   | 0.0092211| 0.005122  | 1.80    | 0.1020 |

The predictive gelatinization temperature \(T_g\) can be calculated based on the factors shown in Equation 13. The equation indicated a relationship as shown between gelatinization temperature and moisture content.

\[
T_g \, (^\circ C) = 58.10 + 0.09 \times \text{Temp.} + 0.046 \times \text{MC} + (\text{Temp.-71.43}) \times (\text{MC - 14.61}) \times (0.009) \\
\text{(Equation 13)}
\]

From the three-dimensional plot in Figure 4.5, the trend of temperature, which is the significant factor, is shown. As the temperature increased from 60 \(^\circ C\) to 90 \(^\circ C\), the gelatinization of starch gets higher compared to the untreated sample.
Heat treatment with sufficient water present will cause gelatinization, which will increase susceptibility for starch degradation in the digestive tract, although a linear relationship between extent of gelatinization due to processing and digestibility has not been found. Svihus et al. (2005) reported that the gelatinization temperature and extent of gelatinization will be affected by the properties of the starch.

The best rheological property of the dough depends on the behavior of starch during thermal treatments. Thus monitoring of the gelatinization temperature of starch helped in controlling and assisting the thermal property of the wheat flour.
In some early studies, the heat treatment increases the gelatinization and its range were found by Biliaderis (2009). At the same time, heat treatment affects the water binding capacity and the stability of starch pastes to heat and shear as well. This confirms the results reported from the DSC measurements.
Chapter V

SUMMARY AND CONCLUSION

Wheat is used for many different purposes and baking is one of them.

Baking quality is both an important and complex characteristic of bread wheat.

Generally, the volume of the bread loaf has been considered as the most important criterion for produced bread. The desirable rheological characteristics of dough (stickiness, mixing requirement and water absorbance) together with the color and appearance of loaf are of importance as well. The balance and interaction between different flour components such as proteins, starch, lipids, water, ash etc., are of high importance for the baking quality.

The protein fraction is known to play the most essential role for baking process. Gluten itself, contribute to the elasticity and extensibility of the dough. At the same time, rheological property of the dough depends on the function of gluten and total starch content. The flour without heat treatment was found to have poor rheological attributes, since their gluten quality was unproductive resulting in sticky dough. Ash content contributes to the specific purpose of wheat flour use. Gelatinization is considered as a significant thermal parameter of wheat flour, which can determine the flour characteristic under high temperature process.

According to some early research, heat treatment can accelerate the aging of wheat. The aged grains had productive improved result in terms of the flour
components. Since the conventional heating is a time consuming and costly process, more efficient method with microwave heating is applied. The samples can reach the desired temperature within 10 min as shown in laboratory trials of this study.

The study was aimed to establish a model of heat treatment and determine the effects on physico-chemical properties of wheat grain. These objectives were achieved through a series of laboratory experiments.

As a result, following the increased heating temperature, the quantity of flour chemical compounds, namely starch and protein gets affected. For the physical properties, ash content and gelatinization temperature of starch, temperatures are also significant factors.

From the data obtained from the model, a better understanding of how heat treatment affects on the baking quality of wheat flour by quantitative change of the compounds. As the only significant factor to the properties, temperature at 75 °C was determined to be the best temperature to treat the wheat grain since the physico-chemical properties enhanced under such condition.

Effects of heat treatment on physical properties of wheat were assessed. The heat-treated flour has enabled recipes to be developed which generate products with finer texture and other characteristics. During the heat treatment process, partial gelatinization of starch granules occurs. Therefore, it is significant
to quantify these property variations as they have a marked effect on the quality characteristics of baked products.

Since microwave is a more efficient heating method, the time and cost saving could occur and can be used in the industry. It is easy to set up and manage the treatment procedure.
Chapter VI

RECOMMENDATIONS FOR FURTHER RESEARCH

The microwave heating affects physico-chemical properties under specific temperature (60°C, 75°C and 90°C) has been studied. Though, the quantitative changes are determined while the quality is still not clear after microwave heating. Repeated experiments are necessary to ensure the reliability of experimental conditions for scale-up. Different temperature range is to be employed for further studies.

For the baking industry, the elasticity and extensibility of dough are significant. In this study, all the samples were treated with laboratory scale. Large industrial scale experiment should be considered and performed. Accordingly the quality determination and more detailed condition (temperature, moisture content, pH), the best condition of microwave heating can be suggested and those conditions could be widely used in the industry.
Appendix A. Moisture content of samples after microwave heating
<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture Content (%)</th>
<th>Temperature (°C)</th>
<th>Moisture Content After Heating(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.55</td>
<td>RT</td>
<td>9.55</td>
</tr>
<tr>
<td>A</td>
<td>12</td>
<td>60</td>
<td>11.54</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>75</td>
<td>11.21</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>90</td>
<td>10.65</td>
</tr>
<tr>
<td>D</td>
<td>18</td>
<td>60</td>
<td>15.76</td>
</tr>
<tr>
<td>E</td>
<td>18</td>
<td>75</td>
<td>15.70</td>
</tr>
<tr>
<td>F</td>
<td>18</td>
<td>90</td>
<td>15.83</td>
</tr>
<tr>
<td>G</td>
<td>15</td>
<td>60</td>
<td>13.81</td>
</tr>
<tr>
<td>H1</td>
<td>15</td>
<td>75</td>
<td>13.38</td>
</tr>
<tr>
<td>H2</td>
<td>15</td>
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</tr>
<tr>
<td>I</td>
<td>15</td>
<td>90</td>
<td>12.45</td>
</tr>
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</table>
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