Accelerated Aging of Wheat Grains- A Prelude

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ABSTRACT

Wheat (*Triticum spp.*) is one of the cereal grains highly used in the baking industries to make bread. The flour obtained from the wheat has a unique property of elasticity and extensibility. Both the properties contribute to the high specific volume of the loaf of bread. These two parameters have a great impact on the baking quality of the flour. The presence of gluten protein in significant amount in the wheat flour contributes extensively to the bread making characteristics.

Besides proteins, numerous other flour ingredients namely starch, lipids also contribute to the functional and rheological properties of the dough, which are the sources of baking potential. Aging of freshly harvested wheat grains for several years helps in enhancing the characteristics of flour components, thereby improving their functionality in bread making. This usual process of aging is highly time consuming, hence there is a need for an alternative process for improvement of the baking quality of flour in a shorter period of time of storage.

In this study, effect of accelerated aging of the freshly harvested grains on physico-chemical and thermal properties of the flour constituents has been studied. The aging is accelerated by storing the wheat grains at a temperature between 30˚C and 50˚C for a period of 2-8 days with moisture contents of 12-18% w.b. (wet basis).

Laboratory work on the chosen cultivar A.C. Walton was carried out. It was found that the total starch content and protein content of the flour decreased at the elevated temperatures and with longer storage periods, irrespective of the moisture content of the grains. On the other hand, the thermal properties of the aged wheat flour enhanced with the increase in the gelatinization temperature of starch, as compared to the freshly harvested grains.

Hence the effect of accelerated aging of wheat grains on the physico-chemical and thermal properties of the flour was studied. The regression model developed clearly explained the effects of the three parameters including storage temperature, moisture content of grains and duration of storage, on the baking qualities of the flour.

RÉSUMÉ

Le blé (*Triticum spp*.) est une céréale nutritive utilisée couramment pour faire du pain. Les pâtes faites à partir de la farine de blé possèdent des propriétés uniques caractérisées par son élasticité et son extensibilité. Ces deux caractéristiques ont un grand impact sur la qualité boulangère des farines car elles permettent de faire gonfler les pâtes afin d'obtenir des pains tendres et moelleux. C'est la présence de protéines de gluten en quantité suffisante dans la farine de blé qui contribue aux caractéristiques recherchées pour la fabrication de pain.

Outre les protéines et leurs propriétés élastiques, de nombreux ingrédients de la farine, dont l'amidon et les lipides, contribuent aux propriétés fonctionnelles et rhéologiques des pâtes. Le vieillissement des grains de blé contribue à améliorer les caractéristiques des composants de la farine de blé. Ce processus de vieillissement naturel est long et l'industrie est présentement à la recherche de procédés non chimiques qui permettraient d'accélérer le vieillissement des grains de blé afin d'améliorer des farines de qualité supérieure rapidement après la récolte.

Dans cette étude, l'effet de vieillissement accéléré des grains fraîchement récoltés sur les propriétés physico-chimiques et thermiques des constituants de la farine a été étudié. Le vieillissement a été accéléré en exposant les grains de blé dont la teneur en eau était entre 12 et 18% (base humide), à des températures allant de 30 à 50°C pendant des périodes de temps allant de 2 à 8 jours.

La variété de blé AC Walton a été utilisée pour les essais sur le vieillissement accéléré. Les résultats ont indiqué que l'augmentation de la température et du temps de stockage ont eu pour effet de réduire les teneurs en amidon et en protéines, et que ces dernières n'avaient pas été affectées par la teneur en eau des grains de blé. De plus, les farines obtenues à partir des

grains ayant subits les traitements de vieillissement accéléré ont exhibé des températures de gélatinisation supérieures à celles des farines faites à partir de grains frais non-traités. Cette étude a permis d'établir les relations mathématiques entre les paramètres (température, durée, et teneur en eau) utilisés pour accélérer le vieillissement du blé et les qualités boulangères des farines obtenues.

Chapter I

GENERAL INTRODUCTION

Wheat is one of the most important food crops in the world. It belongs to the Poaceae family (genus *Triticum)* and looks a lot like yard-grass, when it is small. Wheat is grown all over the world. About 20 billion bushels are grown during a year. Canada, China, France, India, Russia, and the United States grow most of the wheat grains. In case of developed nations (except Canada), the proportion of the population and GDP devoted to agriculture fell dramatically over the 20th century, but wheat production and export remains an important element of the Canadian economy. A wide range of agricultural practices are applied in Canada for wheat production.

Bread is used as staple food in most American countries, including Canada, and hence wheat grains are used in large quantities in the baking industries. Wheat is grown throughout Canada, but the major producers are located in the western Prairie region. The vast majority of bread is traditionally produced from wheat flour. Apart from starch, which is its major constituent, wheat flour also contains many other types of substances. Gluten, non-starch polysaccharides as well as lipids are the most important constituents, in terms of their impact on the processing properties of the raw material and the quality of final products.

1.1. Canadian wheat sector:

Wheat is Canada's most important crop. It is mainly grown in spring, although small amount of wheat is also produced in winter. The centers of wheat production are the Provinces of Alberta with plain areas, Saskatchewan, Manitoba, and the peninsula of Ontario. The spring-wheat belt of Canada adjoins with the spring-wheat section of the United States. Over 75 per cent of the spring-wheat is grown in the Provinces of Manitoba and Saskatchewan. The spring-

wheat belt is limited on the north by a short growing season and low summer temperature; and on the southwest by insufficient rainfall (Coleman, 1930).

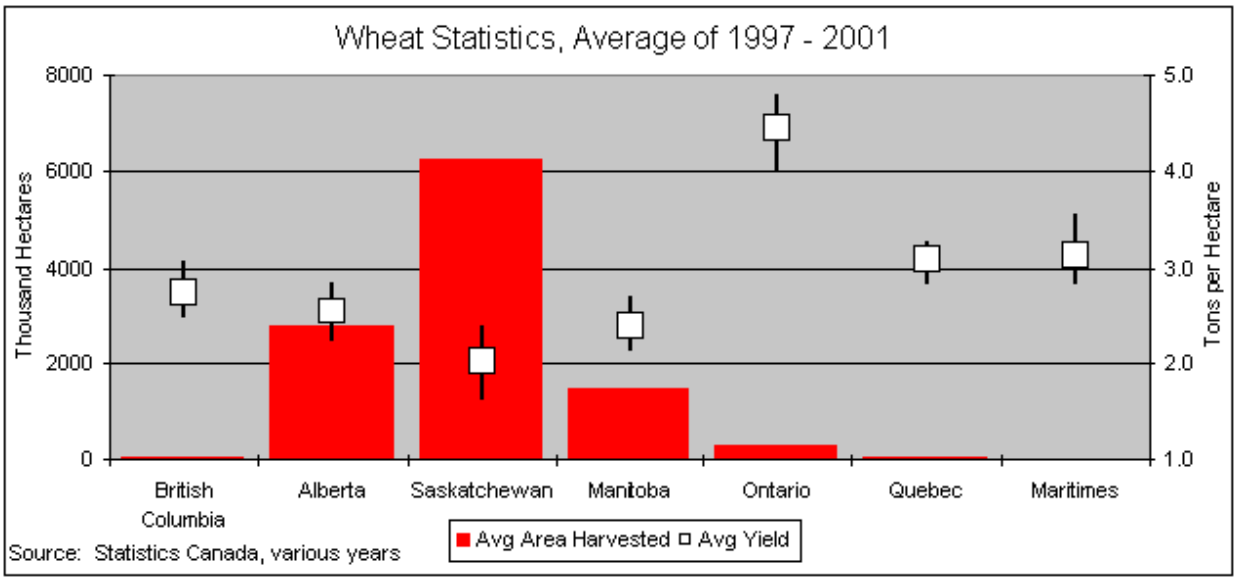

Fig. 1.1: Average area harvested and yields of wheat in different provinces

(Source: Statistics Canada)

The population of Canada does not require the entire supply of wheat produced. According to Bureau of Agricultural Economics, Canada ranks first among the wheat-exporting nations of the world (FAO). Canada, on an average, produces 25,717 MT of wheat, which makes Canada, the sixth largest producer of wheat in the world. Based on the average production, Canada consumes 7,922 MT of wheat. Canada exports about 18,385 MT, making them second among world wheat exporters (USDA Economics and statistics system).

1.2. Baking properties of flour:

To decide the best type of flour, it is helpful to understand the nature of each flour component. The main constituents of flour include carbohydrates (or starch), proteins, and in the case of whole-wheat flour, a bit of fat. Among these three nutrients, protein content of the

flour matters most to the baker. The proteins present in wheat include gluten-forming proteins, and the quantity and quality of these proteins determine the quality of the end product.

A high percentage of protein in flour is best suited for preparation of chewy, crusty breads and other yeast-risen products. Less protein content is best for tender and chemically leavened baked goods, like pie crusts, cakes, cookies, and biscuits. Since the protein content of wheat can range from 5% to 15%, the flour industry has established labeling standards that help us in finding the right flour according to our needs.

Differences in the expansion of dough during the baking phase are generally attributed to differences in protein content and quality. However, on a purely quantitative basis, the role of wheat starch in the unique bread making ability of wheat flour should not be overlooked. Starch has several distinct roles in the bread making process. It is the substrate for the amylases that produce fermentable sugars for yeast fermentation and it serves as a reservoir for water absorption and as a diluent for gluten, thereby contributing to the optimal viscoelastic properties of the dough (Burhans and Clapp 1942, Sandstedt et al 1954, Sandstedt 1955).

1.3. Problem statement:

The price of fresh bread is decided according to its taste, aroma, quality, appearance and texture. Retaining its freshness is important to keep it appetizing. Flour is a product made from wheat grain, where grain has been ground to a powdery consistency. Flour provides the primary structure to produce the final baked bread. Commonly available flours are made from rye, barley, maize, and other grains, but wheat flour is most commonly used for breads. Each of these grains provides the starch and protein needed to form bread.

The quantity of the protein contained in the flour serves as the best indicator of the quality of the bread dough and the finished bread. While bread can be made from all-purpose wheat flour, for quality bread, specialty bread flour containing more protein is recommended. If one uses flour with a lower (9–11%) protein content to produce bread, a longer mixing time will be required to develop gluten strength properly. This extended mixing time leads to oxidization of the dough, which gives the finished product a whiter crumb, instead of the cream color preferred by most artisan bakers.

Wheat flour, in addition to its starch content, contains two water-soluble protein groups (albumin and globulin) and two water-insoluble protein groups (glutenin and gliadin). The amount of protein content in the flour accounts for the functionality of the flour in terms of their baking quality. A generally accepted measure of objective bread quality is the volume of bread baked from an invariable amount of wheat flour. The bread volume is related to quantity and quality of gluten (gliadins and glutenins). When flour is mixed with water, the water-soluble proteins dissolve, leaving the glutenin and gliadin to form the structure of the resulting bread. When relatively dry dough is kneaded, or wet dough is allowed to rise for a long time, glutenin forms strands of long, thin, chainlike molecules, while gliadin forms bridges between the strands of glutenin. The resulting networks of strands produced by these two proteins are known as gluten. Gluten development improves if the dough is allowed to autolyse.

Wheat grain, as a living organised unit, contains a natural multitude of enzymes and enzyme systems. For many of these enzymes, the physiological functions have not been yet clearly established. However, enzymes can be active also in non-physiological conditions.

During wheat processing, the activities of different endogenous enzymes may have considerable effects on the quality of the end-products (Chloupek et al., 2008).

The quantity of free radicals in gluten, formed in the wheat dough after prolonged storage was studied. The concentration of radicals appeared to be age dependent, because highest content of radicals was detected between 13 and 15 years of aging, over 36 years of storage (Calogero Pinzino et al., 1999). Specific labelling of the sulfhydryl groups of gluten proteins enabled comparative EPR studies of the rigidity of the protein chains. A progressive stiffening of polymeric gluten with seed storage was found.

The fine quality and the large volume of the bread loaves are based on the protein and starch content of the wheat flour that ultimately depends on the aging of the wheat grains. The natural aging of wheat grains is attainable only through storage of freshly harvested grains for many years. An alternative method is needed to get rid of this prolonged storage of grains for aging. Over the years, experts found that the quality of bread from wheat grains improves over time. Currently, it takes several months after harvest, for the wheat grains to reach the quality required to optimize the milling for production of the flour, which meets the expectations of the customers.

1.4. Proposed solution (Hypothesis):

Seeds, like other organisms, age and die, although their life period varies greatly among species and within a species because of differences in genotype and origin. Influences of origin on their life period result from the cumulative effect of environment during seed maturation, harvesting, drying, and pre-storage conditions and the time of seed harvest (Hong and Ellis, 1996). Life period of the seed will then depend on the subsequent conditions of storage, which would maintain the seed's viability in the medium or long term.

Aged seeds show a variety of symptoms ranging from reduced viability to more or less full viability, but with abnormal development of the seedling. The reduction in the rate of germination may be an expression of aging of the embryo, but changes in the remainder of the seed could also contribute to the viability of the seed. Important processes during seed germination, such as the establishment of respiration, ATP production, and protein synthesis, are often perturbed by seed aging (Bewley and Black, 1994). However, it is generally accepted that loss of viability with seed aging is mainly connected with the loss of plasma membrane integrity (Senaratna et al., 1988).

In an effort to attain high-quality bread, it is recommended to accelerate and control the aging process of wheat without the addition of chemicals. Studies have shown that it is possible to accelerate the aging of wheat seeds within a few days, in an environment of high temperature and humidity. This method is used to condition the grain seeds before measuring the rate of germination. This environment can not only initiate the process of aging of wheat, but also can control it in order to extract high-quality flour.

1.5. Objectives:

Accelerated aging of wheat grains is the process of altering the physico-chemical properties of the grains that influences the baking characteristics of the flour. It has a greater effect on the physico-chemical properties of the flour than the natural aging of grains. The grains undergo specific conditioning under different sets of parameters like temperature, duration of storage and moisture content of the grains. After this treatment, the grains are milled to flour and the following are to be determined:

a. Total starch content of flour, i.e. resistant starch (RS) and non-resistant starch (NRS).
b. Total protein content, i.e. gluten content of flour.

c. Thermal properties of flour using differential scanning calorimeter (DSC).

Once all these properties of flour are determined, the best result can be correlated to the baking properties of the grains.

Chapter II

GENERAL REVIEW OF LITERATURE

2.1. Wheat flour:

The most frequently used and therefore, the most important ingredient in the bakery shop is wheat flour. Flour provides bulk and structure to baked goods. Flour is produced by milling wheat kernel. A wheat kernel has an outer covering called bran. It is composed of several layers that protect the endosperm, which contains starch and protein. The innermost part is the germ, which contains fat and serves as the wheat seed.

In wheat the changes in plasma membrane permeability appear to involve not the whole seed, but only the embryo axis and aleurone layer, because the scutellum cells do not show an increased permeability of the plasma membrane as the above-reported seed tissues (Golovina et al., 1997a). Lipid peroxidation caused by free radicals appears to be the main cause of plasma membrane deterioration during seed aging (Wilson and MacDonald, 1986). Among the other causes involved in the loss of seed viability, protein modifications might play an important role. These modifications can occur by nonenzymatic glycation with reducing sugars (Sun and Leopold, 1995) and/or by reaction with aldehydes produced by the oxidation of fatty acids mediated by free radicals (Priestley and Leopold, 1983; Priestley et al., 1985). These aldehydes seem to react with sulfhydryl groups of proteins (Stadtman, 1992; Mudgett and Clark, 1993). Nevertheless, an infrared microspectroscopy study failed to demonstrate protein aggregation and denaturation, in naturally aged seeds of different species (Golovina et al., 1997b).

During milling, the kernels first pass through metal rollers to crack them, and then the bran and germ are removed through repeated stages of sifting and separation. The remaining endosperm is then ground into flour. Flour derived from the portion of the endosperm closer to the germ is finer; flour derived from the portion of the endosperm nearer the bran is coarser and darker. The character of the wheat determines the character of the flour.

Wheat flour is an important raw material in the manufacture of several wheat-based foodstuffs such as bread, breakfast cereals, infant foods, snacks, and pasta. The protein quality of the wheat flour plays a major role in the preparations of these products (Schofield and Booth, 1996). Traditionally, dough is prepared by a combination of flour and water. The distribution and swelling of flour particles after mixing favor the formation of a protein matrix that holds starch (Campos et al., 1997).

During the mixing process, dough increases the resistance to extension. It is only after the dough has been developed to its optimum point, that the full bread making potential of that dough is achieved, displaying specific rheological properties (Spies 1990). These properties in wheat dough are important in the evaluation of flour quality (Lindborg et al., 1997), and in the study of the different process steps and the texture properties of the resulting products.

The characterization of these dough properties has been traditionally conducted in the bread making industry with the aid of farinographs and mixographs. However, several studies on basics and fundamental rheology have been performed through dynamic techniques (Janssen et al., 1996; Miller and Hoseney, 1999; Schluentz et al., 2000), providing information regarding the chemical structure of the dough. The knowledge of dough structure is valuable in explaining previously observed rheological phenomena and allows the modeling of responses to variations during processing and formulation (Faubion and Hoseney, 1990).

2.2. Flour constituents:

The vast majority of bread is traditionally produced from wheat flour. Apart from its major constituent starch, wheat flour also contains many other components, of which gluten, non-starch polysaccharides as well as lipids are most important in terms of their impact on the processing ability of the raw material and in terms of the quality of final product.

For several thousand years, bread has been one of the major constituents of the human diet, making the baking of yeast-leavened and sourdough breads one of the oldest biotechnological processes. Wheat is by far the most important cereal in bread making, although in some parts of the world the use of rye is quite substantial. Other cereals are used to a lesser extent.

In wheat bread making, flour, water, salt, yeast and/or other micro-organisms (often with the addition of non-essential ingredients, such as fat and sugar) are mixed into visco-elastic dough, which is fermented and baked. Wheat flour is the major ingredient and consists mainly of starch (70–75%), water (14%) and proteins (10–12%). In addition, non-starch polysaccharides (2–3%), in particular arabinoxylans (AX) and lipids (2%) are also important for bread production and quality.

During all the steps of bread-making, complex chemical, biochemical and physical transformations occur, which are affected by the various flour constituents. In addition, many substances are nowadays used to influence the structural and physico-chemical characteristics of the flour constituents in order to optimise their functionality in bread making.

2.3. Starch:

Yellow berry condition, commonly referred as starchiness, is a condition frequently encountered in hard common wheat and durum wheat. Starchy kernels are opaque, and cross-

sections of their endosperms are completely white and starchy. Piebald kernels are partly starchy and vitreous in sharply defined areas. Soil fertility, weather and the inherent susceptibility of varieties of wheat influence the incidence of starchiness. Starchy kernels are lower in protein content than vitreous kernels. The estimation of vitreous kernel content in hard common wheat and durum wheat is an important international grading factor because of the primary importance of protein content in determining bread loaf volume. Gluten protein quality is not influenced by vitreosity, leading some to suggest that protein content may be a more satisfactory index than vitreosity of the commercial value of wheat (Dexter et al., 1989).

2.3.1. Structural and physico-chemical aspects:

Starch, the most important reserve polysaccharide and the most abundant constituent of many plants, including cereals, occurs as semi-crystalline granules (Eliasson & Gudmundsson, 1996; Hizukuri, 1996; Parker & Ring, 2001). It has some unique properties, which determine its functionality in many food applications, in particular bread making. Its structure and physico-chemical properties have been the subject of many extensive reviews (Bule´on, Colonna, Planchot, & Ball, 1998).

2.3.2. Starch granule structure:

The major components of starch are the glucose polymers amylose and amylopectin. Amylose is an essentially linear molecule, consisting of α-(1, 4)-linked D-glucopyranosyl units with a degree of polymerisation (DP) in the range of 500–6000 glucose residues. It is now well recognised that a fraction of the amylose molecules is slightly branched by α-(1, 6)-linkages (Hizukuri, et al., 1981; Shibanuma, et al, 1994) (Fig. 2.1(e)). In contrast, amylopectin is a very large, highly branched polysaccharide with a DP ranging from $5 \times 10^5 \; to \; 3 \times 10^6$ glucose

units. It is composed of chains of α-(1, 4)-linked D-glucopyranosyl residues which are interlinked by α-(1, 6)-bonds (Zobel, 1988).

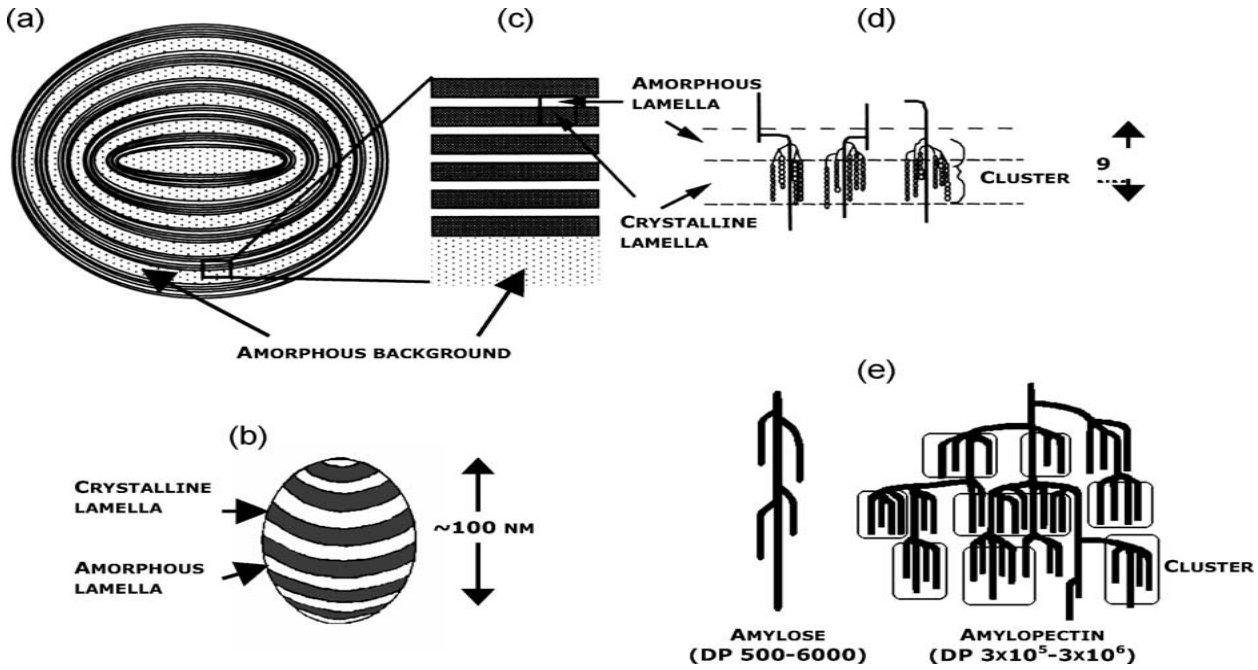

Fig: 2.1 Schematic representation of different structural levels of a starch granule: (a) granule with alternating amorphous and semi-crystalline shells; (b) expanded view of a blocklet structure, the building blocks of the shells; (c) expanded view of the semi-crystalline layer, consisting of alternating crystalline and amorphous lamellae; (d) the cluster structure of amylopectin within the semi-crystalline shell; (e) schematic representation of amylose and amylopectin. (Source: Donald et al., 1997)

These chains can be classified as the unbranched outer chains (A) or the branched inner chains (B). The later can be further divided into B1, B2, B3 and B4 chains. In addition, there is a single C chain per molecule which contains the sole reducing residue (Peat et al., 1956). The cluster model of the amylopectin structure is nowadays widely accepted (French, 1984; Robin, et al., 1974) (Fig. 2.1e). In this model, the short (A and B1) chains form double helices, which are organised in discrete clusters, while the longer B2, B3 and B4 chains extend into 2, 3 or 4 clusters, respectively.

The amylose/amylopectin ratio differs between starches, but ideal levels of amylose and amylopectin are 25–28% and 72–75%, respectively (Colonna & Bule´on, 1992). However, the

starches of some mutant genotypes of maize, barley and rice contain either increased amylose content (i.e. high amylose or amylostarch with up to 70% amylose) or increased amylopectin content (i.e. waxy starch with 99–100% amylopectin). In the past 10 years, several waxy wheat cultivars have been developed as well (Graybosch, 1998).

Starch is present as intracellular water-insoluble granules of different sizes and shapes, depending on the cultivar. In contrast to most plant starches, wheat, rye and barley starches show a bimodal size distribution. The small (B) granules are spherical with a diameter up to 10 mm, while the large (A) granules are lenticular with a mean diameter of 20 mm (Karlsson, et al., 1983; Moon and Giddings, 1993). At the lowest structural level, the starch granule can be defined in terms of alternating amorphous and semi-crystalline growth rings or shells with a radial thickness of 120–400 mm (Fig.2.1a).

The amorphous shells are less dense and contain amylose and probably less ordered amylopectin, while the semi-crystalline shells are composed of alternating amorphous and crystalline lamellae of about 9–10 nm (Jenkins, et al., 1993). The later are made up of amylopectin double helices packed in a parallel fashion, while the former consist of the amylopectin branching regions (Fig.2.1c and d). There are indications that these lamellae are organised into larger, somewhat spherical structures, named ‘blocklets’, which range in diameter from 20 to 500 nm (Gallant, et al., 1997) (Fig.2.1b).

Different packing of the amylopectin side-chain double helices gives rise to different crystal types. The A type is found in most cereal starches, while the B type is found in some tuber starches, high amylose cereal starches and retrograded starch. The B crystal type is a more highly hydrated and open structure. A significant fraction of the starch granules (8%) is damaged during milling. This mechanical damage to the granule structure greatly affects starch

properties. Damaged starch loses its birefringence, has higher water absorption capacity and is more susceptible to (fungal) enzymatic hydrolysis (Hoseney, 1994).

2.3.3. Properties of starch:

Upon cooling, the solubilised amylose forms a continuous network, in which swollen and deformed starch granules are embedded and interlinked. Because of its rapid retrogradation, amylose is an essential structural element of bread and is a determining factor for initial loaf firmness (Eliasson & Larsson, 1993). Indeed, flours containing no amylose were not suitable for bread making as they yielded breads with very poor crumb characteristics.

During storage, bread gradually loses its freshness and stales. The staling process comprises several steps: the crust toughens, the crumb becomes more firm and less elastic and moisture and flavour is lost (Hoseney, 1994). Bread staling is often evaluated by measuring crumb firmness. However, this property is also influenced by loaf volume and crumb structure. Bread staling is a complex phenomenon in which multiple constituents and mechanisms take part (Gray and BeMiller, 2003).

Water migration and transformations in the starch fraction are the most important steps in this process. Some researchers proposed that crumb firming can be attributed to some extent to gluten–starch interactions. However, in most staling models, the firming of the crumb during aging is mainly attributed to amylopectin retro gradation, in particular the formation of double helical structures and crystalline regions. Since amylose is already almost completely retrograded in the bread after cooling, it is considered to have little contribution to crumb firming. The amylopectin side-chain reorganisation leads to an increased rigidity of the swollen granules. However, the formation of ordered amylose structures in the centre of the granules may also contribute to granular rigidity (Hug-Iten et al., 2003; Hug-Iten et al., 1999).

In addition, the starch is slowly transformed from an amorphous structure to a partially crystalline state and a B type X-ray diffraction pattern can be observed. However, starch crystallinity is often poorly correlated with crumb firmness. This may indicate that the formation of a structured network, as is the case when large starch molecules (amylopectin and/or amylose) pass through multiple crystalline and amorphous regions, can be a more important factor in gel or bread rigidity than the extent or quality of crystallinity (Zobel & Kulp, 1996).

In this respect, both molecular reorganisation of the amylopectin rich and amylose rich regions in the starch granules, result in an increased granular rigidity, and the formation of a structured network consisting of interlinked crystallites, which contributes to crumb firming. Bread firming is often delayed due to a combination of acidification by the lactic acid bacteria, which affects retrogradation, and microbial starch and protein hydrolysis (Corsetti et al., 1998).

2.3.4. Starch functionality in bread making:

Based on structural and amino acid sequence similarities, a variety of amylolytic enzymes capable of hydrolysing α-(1, 4) - and/or α-(1, 6)-linkages in starch are grouped into glycoside hydrolase family 13 (α-amylase family). α-Amylases (EC 3.2.1.1), which are typical endo-amylases, more or less randomly hydrolyse the α-(1, 4)-linkages of starch, yielding low molecular weight α-dextrins. Other glycoside hydrolase family 13 amylolytic enzymes include maltogenic (EC 3.2.1.133) and other maltooligosaccharide- producing (e.g. EC 3.2.1.60, EC 3.2.1.98) amylases and debranching enzymes. The former are mainly exo-acting amylases which mainly release maltose or other maltooligosaccharides, like maltotetraose or maltohexaose, from starch.

Debranching enzymes, like pullulanase (EC 3.2.1.41) and isoamylase (EC 3.2.1.68), hydrolyse α-(1, 6)-bonds, thus removing the side-chains. Typical inverting exo-amylases, such as β -amylase (EC 3.2.1.2; glycoside hydrolase family 14) and glucoamylase (EC 3.2.1.3; glycoside hydrolase family 15), hydrolyse α-(1, 4)-linkages at the non-reducing ends of the starch molecules. β-Amylase cannot hydrolyse α-(1, 6)-linkages and its action stops at the branch points. It potentially degrades starch to β-maltose and β-limit dextrins. Glucoamylase has a limited activity on α-(1, 6) linkages. Theoretically, this enzyme can completely convert starch to β -glucose (Bowles, 1996; Hoseney, 1994).

Amylases are routinely used in bread making. Their functionality depends mainly on their specificity, degradation products and thermal stability. In general, fungal enzymes have a low thermal stability and most of their activity is lost during starch gelatinisation. In contrast, some bacterial α-amylases are very thermostable and may survive the baking process. They are hence difficult to control during baking and storage and can result in reduced crumb structure properties upon overdosing. Addition of amylases mainly aims at optimising the amylase activity of the flour (i.e. flour standardisation) and at retarding bread staling.

2.3.5. Amylases for flour standardisation:

Amylases are endogenously present in flour, but amylase activity can vary considerably. In general, unmalted flour has high levels of β-amylase, while its α-amylase activity is low, resulting in low bread volume and quality (Drapron & Godon, 1987). Therefore, flour is routinely supplemented to optimise the amylase activity. It is generally acknowledged that the primary goal of amylase addition is to increase the levels of fermentable and reducing sugars in flour. By degrading the damaged starch particles during the dough preparation and generating

low molecular weight dextrins, supplemented α-amylase facilitates maltose production by the endogenous β-amylase (Linko et al., 1997).

The maltose can then be used as fermentable sugar by the yeast or the sourdough microbial population. In addition, increased levels of reducing sugars promote the generation of Maillard reaction products, which intensify bread flavour and crust colour. In contrast, amylase supplementation may primarily affect dough viscosity during the initial stages of starch gelatinisation (Kragh, 2003). In this view, by delaying the viscosity increase due to amylose leaching during gelatinisation, amylases allow a prolonged oven spring (rapid enhancement in the volume of dough) and an increased loaf volume.

2.4. Proteins:

The end-use characteristics of flours produced from bread wheat are strongly determined by the gluten proteins. Gluten proteins are particularly important for bread making quality and consist of two major fractions: the monomeric gliadins and the polymeric glutenins. As mentioned before, flour proteins interact in the presence of water by forming gluten, which provides the unique viscoelastic properties needed for bread making (Shewry et al., 2002). The glutenin fraction comprises a mixture of HMW and LMW polymers, with a wide range of size distribution, ranging from dimers to polymers with molecular weights up to millions (Wrigley, 1996).

The polymers are formed by intermolecular disulphide bond linking HMW and LMW. These polymeric proteins are known to be the most important determinants of bread making quality. Due to the large size and structural complexity of the polymeric proteins, they have been studied to a lesser extent than specific HMW and LMW proteins (Field et al., 1983).

Most of the studies regarding polymeric proteins are related to the impact of individual protein classes, HMW and LMW proteins, or the genes encoding them. However, glutenin subunits explain only a small proportion of the variation in quality (Weegels et al., 1996). The relative amount of polymeric protein increases with increasing gluten strength. The effects of the glutenin polymer function on dough and on flour end-use quality have also been investigated.

Changes in the gluten protein network during dough formation have been investigated using rheological methods, electrophoretical methods, microscopical methods and gel-filtration techniques (Lee et al., 2002). Many protein structural studies, as well as mixing and baking studies, have postulated that disulphide bonds are present in gluten polymer structure and contribute to the process of dough formation through the disulphide-sulphydryl exchange. However, a full understanding of the structure of the gluten polymer during dough processing, as well as of the changes in molecular associations, are still far from being reached. (Tilley et al., 2001)

2.4.1. Classification based on solubility:

Wheat proteins are classified based on sequential extraction in the following series of solvents: (1) water, (2) dilute salt solution, (3) aqueous alcohol and (4) dilute acid or alkali. Using Osborne classification scheme, wheat proteins were classified as albumins, globulins, gliadins and glutenins, respectively (Goesaert et al., 2005) (Table 2.1). However, a significant fraction of wheat proteins is excluded from the Osborne fractions because they are unextractable in all of the above-mentioned solvents.

2.4.2. Classification based on functionality:

From a functional point of view, wheat proteins are distinguished into two groups: the nongluten proteins, with either no role or just a minor role in bread making, and the gluten proteins, with a major role in bread making.

The non-gluten proteins accounting for 15% to 20% of total wheat proteins are highly concentrated on the outer layers of wheat kernels than in the endosperm region. Most of these proteins are extractable in dilute salt solutions, and therefore are likely to be found in the Osborne albumin and globulin fractions. They are mostly monomeric physiologically active or structural proteins. Triticins are non-gluten protein which belongs to the minor group of polymeric wheat storage proteins. They are found as residue in the extraction and their role in bread making is not clear (Veraverbeke & Delcour, 2002).

On the other hand, the gluten proteins, the major storage proteins of wheat account for 80 to 85% of total proteins. These proteins belong to the prolamin class of seed storage proteins (Shewry & Halford, 2002; Shewry et al., 1995). Gluten proteins are found in the endosperm of the mature wheat grain where they form a continuous matrix around the starch granules. These proteins are largely insoluble in water or dilute salt solutions.

Two functionally distinct groups of gluten proteins can be distinguished into monomeric gliadins and polymeric (extractable and unextractable) glutenins. Gliadins and glutenins are usually found in more or less similar amounts in wheat. Gliadins represent a highly polymorphic group of monomeric gluten proteins with molecular weights ranging between 30,000 and 80,000. Three types (α, γ and ω) have been identified biochemically. They are all readily soluble in aqueous alcohols and are therefore the main components in the Osborne gliadin fraction (Table 2.1).

Table 2.1: Overview of different groups of wheat protein (Source: H. Goesaert et al. 2005)

Osborne fraction	Solubility behaviour	Composition	Biological role	Functional role
Albumin	Extractable in water	Non-gluten proteins (mainly monomeric)	Metabolic and structural proteins	Variable
Globulin	Extractable in dilute salt	Non-gluten proteins (mainly monomeric)	Metabolic and structural proteins	Variable
Gliadin	Extractable in aqueous alcohols	Gluten proteins (mainly monomeric gliadins and low molecular weight glutenin polymers)	Prolamin-type seed storage proteins	Dough viscosity/ plasticity
Glutenin	Extractable in dilute acetic acid	Gluten proteins (mainly high molecular weight glutenin polymers)	Prolamin-type seed storage proteins	Dough elas-ticity/strength
Residue	Unextractable	Gluten proteins (high molecular weight poly-mers) and polymeric non-gluten proteins (triticins)	Prolamin-type (gluten) and globulin-type (triticin) seed storage proteins	Variable

2.4.3. Gluten functionality in bread making:

Bread making quality of wheat flour is largely determined by its proteins. Indeed both the quantity and composition (quality) of proteins are important for bread making. It is distinct from this observation that bread making performance of wheat flour is linearly proportional with its protein content and this linear relationship differs among different wheat varieties (Finney & Barmore, 1948). Although different non-gluten proteins play minor roles (e.g. certain enzymes, enzyme inhibitors, lipid binding proteins and possibly also triticins) in bread making, the main quality determinants of bread making are the gluten proteins.

Eventually, the unusual properties of the gluten proteins in the wheat flour tend to be transformed into dough with suitable properties for bread making. These properties are completely unique and are hardly found in cereals closely related to wheat such as barley and rye. Gluten proteins undergo various changes during the different steps of the bread making process. Even though the nature of these changes is clear, the native gluten protein structure itself is poorly understood.

During dough preparation, wheat flour is hydrated and as a result of the mechanical energy input, discrete masses of gluten protein are disrupted. As visualised by microstructural studies, the gluten proteins are transformed into a continuous cohesive visco-elastic gluten protein network (Singh and MacRitchie, 2001). This process is accompanied by a dramatic increase in the extractability of the gluten proteins. The development of a gluten protein network during dough mixing can be observed and monitored with recording dough mixers such as the Farinograph and the Mixograph (Walker and Hazelton, 1996).

During the process, the resistance of dough mixing first increases, then reaches an optimum and finally decreases during 'over-mixing'. During dough fermentation, further changes in the gluten protein network are evident from the observation that gluten proteins again become less extractable. Once the protein network is formed, the fermenting dough plays a major role in retaining the carbon dioxide gas produced during fermentation and during the initial stages of baking (Gan, Ellis, & Schofield, 1995).

The quantity and quality of gluten proteins largely determine dough mixing requirement and are highly sensitive to over-mixing. Furthermore, they determine the rheological properties of the optimally mixed dough and as such contribute to the gas retention properties of the fermenting dough (Gan, Ellis, & Schofield, 1995), which in turn determine loaf volume and crumb structure of the resulting bread. These two important factors are believed to mainly determine gluten protein quality in bread making.

The first important factor is the gliadin/glutenin ratio of the gluten proteins. This is a direct consequence of the fact that, gliadins and glutenins perform a different role in the visco-elastic gluten protein network of wheat flour dough. Due to their large size, glutenin polymers form a continuous network that provides strength (resistance to deformation) and elasticity to the

dough (Belton, 1999; Ewart, 1972). On the other hand, the monomeric gliadins are believed to act as plasticisers of the glutenin polymeric system. The quality bread making depends on the degree of balance between the dough viscosity and elasticity. Up to a certain limit, higher dough strength increases loaf volume. However, too strong dough hinders their rise (Shewry et al., 1992).

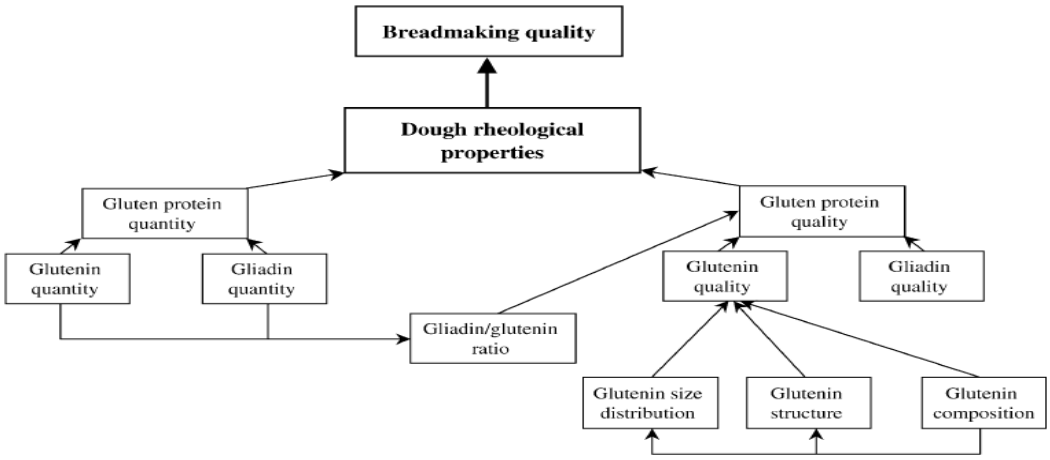

Fig. 2.2: Factors governing bread making quality and wheat dough rheological properties. (Source: Veraverbeke & Delcour, 2002)

The second factor in gluten protein quality is the quality of its (extractable as well as unextractable) glutenin fraction. Though the differences in gliadin properties might certainly also have a decreased effect, it is now generally considered that differences in glutenin properties are more important in explaining gluten protein quality differences in bread making. Although there is lack of detailed knowledge of the molecular structure of glutenin and its contribution to elasticity, it can be assumed that differences in glutenin functionality in bread making result from differences in (i) composition, (ii) structure and/or (iii) size distribution of the glutenin polymers (Fig.2.2).

2.5. Factors determining the milling and baking qualities of wheat:

Quality in wheat is an expression which conveys different ideas to the minds of producers, millers and bakers. To the wheat producer, quality in wheat means high yields per acre of land, plump wheat of high test weight (Coleman, 1930). Supplementary to this definition, the protein content of the wheat is gaining importance in some quarters.

From a miller's point of view, quality wheat in addition to being plump and of high test weight per bushel, should likewise be of good color, reasonably free from foreign material, practically free from damaged kernels of every description, and should have characteristics of easy milling and should be free from admixtures of wheat of other commercial classes.

For the baker, there is no fixed standard of quality, since there is no universally standardized method for making bread. Baking characteristics differ in degree of importance, depending upon the kind and quality of product desired. Length of time for fermentation and proofing, water absorption rate of the flour, volume, weight and shred of the loaf, color, grain and texture of crump are the most important baking characteristics to the baker.

The length of time for which the dough can be allowed to ferment and proof before deterioration of gluten begins, is highly indicative of the strength of the gluten. When the dough is allowed to ferment to the point at which the loaf of the greatest possible size can be produced, the loaf volume may be considered an expression of the relative strength of flour both in commercial and household baking.

The water absorbing capacity of the flour is of great importance as it is used to measure the quality and quantity of the flour. Other things being equal, the degree of the amount of gluten content in flour is directly proportional to the amount of water absorbed. There are frequent exceptions found in terms of the quality factor of gluten. In other words, flour

containing a high percentage of gluten of low quality will absorb less water than the flour containing a lower percentage of high quality gluten. Water absorption is likely related to the weight of the loaf, as a flour containing gluten of good quality will absorb and hold the added water against the heat of the baking oven.

2.6. Types of wheat:

There are two broad categories of wheat varieties: durum and non-durum. Depending on quality, durum wheat is used by the pasta industry, and non-durum is used by either the milling industry or the livestock feed industry. Spring wheat (durum and non-durum) is planted throughout Canada between May and June, and harvested between August and October. All durum wheat in Canada is spring wheat.

Winter wheat is planted from September to November in eastern Canada and along the U.S.A. border in the Prairie Provinces. Winter wheat is harvested in July and August. Spring non-durum wheat is the largest category of wheat grown in Canada, on 75 % of the harvested area and consists 75 % of production. Winter wheat is grown on 3 % of the harvested area and consists 6 % of production. Durum wheat is grown on 22 % of harvested area and consists 19 % of production (USDA 2004).

2.7. Natural aging of wheat:

Aging process causes changes that improve its characteristics and hence enhances their utilization in breads. Age- related changes have great influence on viscosity of the batter and the water-binding ability of the wheat flour. Also, the starch granule surface protein is found to be increased up to three to four times after various aging treatments compared to the control wheat flour (Shelke et al., 1992).

The need to produce cereal-based products all year round means that grains such as wheat may be stored for some time before processing. Even though some aging of flours and grains before use enhances their functionality, especially for cake and batters (Seguchi, 1993), grain quality may be compromised by prolonged storage (Pomeranz et al 1968). There have been reports of increased mixing times, lower loaf volumes, and poorer crumb structure when using aged flours for baking (Pomeranz et al., 1968; Shearer et al., 1975; Pomeranz., 1992). Some of the biochemical changes that have been reported to occur in grains or flour stored for 6 to 18 months include a reduction in starch and non-starch polysaccharides (Kim et al., 2003), vitamins (Rehman, 2004), available lysine (Rehman and Shah, 1999; Rehman, 2004), and free sulfhydryls (Rahman, 2005).

2.8. Accelerated aging of wheat:

As the name suggested, natural aging of wheat grains is a time consuming process for the bakers to produce good quality flour. Natural aging is the practice of storage of freshly harvested grains for several years under room temperature to obtain suitable flour quality after milling. On the other hand, accelerated aging of wheat is accomplished by the application of higher temperature for a particular period of time with ambient moisture content for the storage of grains. The important factors which determine the rate of aging are the surrounding temperature and seed moisture content at which they are stored and the seed quality, which is an ill-defined parameter.

The moisture content of the grains and the storage temperature and relative humidity have been shown to exert dramatic changes in the acidity, pH, free amino nitrogen, crude protein, and protein quality. Significant changes in soluble sugars and amylase contents of the grains have also been reported during storage at elevated temperature.

In light of the above, this study is aimed at improving the baking characteristics of the wheat flour by performing the accelerated aging of wheat grains as well as by controlling them.

Chapter III

EFFECT OF ACCELERATED AGING OF WHEAT ON PHYSICO-CHEMICAL PROPERTIES OF FLOUR

3.1. Abstract:

Accelerated aging of wheat grains has a great impact on the rheological properties of the flour. Starch is a quantitatively very important component present in wheat grains accounting for 80% of its composition. The distinct role of starch in bread making is unique which serves as a substrate for the enzyme amylases. The enzymes present in dough produce fermentable sugars for yeast fermentation and acts as a reservoir for water absorption and also acts as diluents for gluten, thereby contributing to optimal visco-elastic properties of dough. Starch has the main property of coagulating the gas cells, thus it controls the expansion of dough during baking.

The total amount of gluten in the flour is 11- 14%. Since the quality and quantity of the bread loaf depends on their composition, the role of gluten in bread making is very important despite their small amount in the flour. The end-product characteristics of the flour are highly determined by the gluten proteins. The visco-elastic and the structural properties of the dough are strongly dependent on the proteins that form gluten matrix. The effect of aging and their implications determined by various treatments of gluten proteins are studied in this paper.

In the first phase of this study, the process of accelerated aging was performed by controlling three different factors, which include temperature, moisture content and the duration of storage of grains in a well equipped chamber. The temperature range of storage of grains included in this study was 30 -50˚C. The freshly harvested grains were stored for a period of 2-8 days with moisture content levels of 12- 18%. The experimental set up was

designed by using response surface methodology and the three distinct factors were structurally combined by central composite face-centered (CCF) design.

Once the grains were conditioned and their aging was accelerated and controlled, the second part of the study was conducted. Age-related changes and their effect on physico-chemical properties of starch and protein molecules were analysed. Total starch is the combination of both resistant starch (RS) and non-resistant starch (NRS). Starch was estimated by the enzymatic method of digestion by the enzymes namely, α-amylase and amyloglucosidase (AMG). The whole protein content was estimated using Lowry's method.

The total starch content and protein content was found to be decreasing with storage of wheat grains at elevated temperatures and increase in duration of storage. Moisture content of the grains had a less significant effect on the aging process.

Keywords: Accelerated aging, Total starch, Gluten protein, Physico-chemical properties.

3.2. Introduction:

Wheat is one of the major food commodities in Canada; hence there is a need of production all year round. Even though aging of flours and grains before use enhances their functionality and rheological properties to a certain extent, particularly for cake and batters (Seguchi, 1993), grain quality may be compromised by prolonged storage (Pomeranz et al., 1968).

Flour is a complex mechanical mixture of various organic and inorganic materials with some fixed chemical or physical components; however they vary widely in content, according to different wheat cultivars. Starch and the allied carbohydrates make up approximately 70 % of the composition of flour, and the proteins range from 6 to 18%. There is a very small amount of fat which has no significant effect on its baking properties.

Determining moisture content is an essential step in analyzing wheat or flour quality, as it is used in other studies as well. Flour millers adjust the moisture in wheat to a standard level before milling. Moisture content of 14 % (w.b.) is commonly used as a conversion factor in other studies, in which the results are affected by moisture content.

Starch plays a major role in the physical properties and the quality of baked products. Wheat flour contains appreciable quantities of 'resistant starch' (RS) i.e. starch that are resistant to the action of amylolytic enzymes, both in case of *in vivo* and *in vitro* conditions. Total starch, which is the combination of both RS and NRS, is to be determined by an assay procedure (AOAC Method 2002.02, AACC Method 32-40).

Protein content is a key characteristic for wheat and flour purchasers. It is related to many processing properties, such as water absorption and gluten strength. Protein content also can be related to finished-product attributes, such as texture and appearance. Low protein content is

desired for crisp or tender products, such as snacks or cakes. High protein content is desired for products with chewy texture, such as pan bread and hearth bread. Bakers use protein content results to anticipate water absorption and dough development time, because higher protein content usually leads to more water consumption and a longer mixing time, to achieve optimum dough consistency.

Cereal grains can be stored for long periods without microbial spoilage; however, biochemical changes also occur during aging. Eventually, the grain respires, dry matter is lost and functional and nutritional aspects of the grain are altered (Reed, 1992). Grain is stored at ambient temperature and moisture levels for extended periods without significant mold deterioration, but the functionality of the grain still suffers (Kirleis and Stroshine, 1990).

Some researchers have used moderate or high temperature conditions at ambient (12%) moisture levels to assess seed vigor in wheat and barley seeds and cooked rice quality (Gujral and Kumar, 2003). A model for accelerated aging at 30–60ºC at moderate relative humidity levels was developed which indicated that the condition of the seeds was crucial in determining seed life period and the accelerated aging model can be used to accurately predict seed life period in case of barley (Bason et al., 1993).

It is to be noted from various studies that the protein content of wheat grains and flour do not change significantly over time (Kim et al 2003). While protein quantity may not change, protein quality does. The relative amounts of soluble and insoluble glutenins were closely related to dough strength and that cultivars that gave the weakest dough strength had a higher proportion of soluble glutenins (of the total glutenin fraction) as compared to the cultivars which gave stronger dough. This explains the inverse relationship between dough strength and glutenin solubility (Sapirstein and Fu, 1998). It is also found that the amount of soluble

glutenin is negatively correlated to dough strength and loaf volume, and that the amount of insoluble glutenins is the most accurate predictor of protein quality for baking performance. Therefore this study was undertaken with the following objectives:

a. To accelerate the aging process of freshly harvested wheat grains by conditioning them with water to attain moisture content ranges of 12-18% (w.b.) and storing them for 2-8 days period at temperature range of 30-50º C.
b. To determine the total starch content (RS and NRS) of all the treated flour by using enzyme assay done by AOAC method.
c. To estimate the total protein content using Lowry's method.

3.3. Materials and Methods:

In this study, the accelerated aging test is performed for the cultivar AC Walton, which is hard red spring wheat. 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 were obtained from Les Moulins de Solanges, St Polycarp, Quebec. All wheat samples were free from insect infestation and no chemicals were used for preservation. About 10.00 kg of each sample with initial moisture content of 12% were placed in the air tight buckets of uniform size (Fig 3.1).

Fig 3.1: Storage of wheat grains in air tight buckets

These buckets were placed in well-built wooden chamber with a provision of heating to meet the required temperature, and a fan to circulate the air and maintain uniform humidity for the entire storage period (Fig. 3.2). The grains were stored at different temperatures for different durations. The accelerated aging of these freshly harvested grains was initiated by the control of three major factors, including temperature, moisture content and duration of storage of grains.

Fig 3.2: Storage of air tight buckets in the wooden chamber

Response surface methodology with central composite design (CCD) was applied to minimize total number of experimental runs and to determine the significant factors and obtain best combination of factors for accelerated aging. The experimental design was constructed using central composite face centered (CCF) design, with three factors with three levels each.

Measurement of moisture content:

The initial moisture content of the grains was determined by drying the grains in hot air oven at 65ºC for 24 hours. The initial and final weights of the samples were recorded and the w.b. moisture content of the grains was calculated using the formula (AOAC method) 1:

$$Moisture\ content\ (MC)\% = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100 \qquad (1)$$

The initial moisture content of the freshly harvested wheat grains was found to be 10% (w.b.). Three separate samples of freshly harvested wheat grains were prepared which weighed

10.00 kg each. After that about 235 mL, 605 mL and 1000 mL of water was added to each sample of freshly harvested wheat grain to bring the moisture content to 12%, 15% and 18% respectively. Once the grains were mixed with water and brought to the three different moisture levels, they were emptied into huge air tight buckets and stored in specially well designed wooden chamber, that had the temperature control via the heater and the hot air circulated through the entire chamber through the operation of a fan. The experimental levels of all the three factors are as follows:

Table 3.1. Experimental levels of the three parameters

Factor levels	Factors		
	Temperature (ºC)	Moisture content (%)	Duration (days)
1	30	12	2
2	40	15	5
3	50	18	8

After the grains were stored for assigned number of days, they were emptied into wooden boxes with the walls made of nets to bring back the grains to room temperature before milling. The conditioned wheat grains were milled and the effect of accelerated aging on various flour components were analysed.

3.3.1. Estimation of total starch content:

The total starch in the flour constitutes approximately for about 70% and is the combination of both resistant starch (RS) and non-resistant starch (NRS). The total starch was measured using the Megazyme Assay procedure. The assay follows the principle of digestion

of the starch in the flour to D- glucose by the combined enzymatic activity of pancreatic α-amylase and amyloglucosidase (AMG).

Resistant starch (RS) is that portion of starch that is resistant to the action of amylolytic enzymes, either *in vivo* or *in vitro.* Factors affecting the RS content of autoclaved starch pastes include autoclaving temperature, water content, the number of heating/cooling cycles and the botanical origin of the starch4 not broken down by human enzymes in the small intestine and hence not soluble.

On the other hand, non-resistant starch (NRS) is easily broken down and hence is the solubilised starch content of the flour. The D-glucose obtained after digestion of starch is measured with glucose oxidase/peroxidase reagent (GOPOD) which is the measure of the RS content of the sample.

Principle:

Thermostable α-amylase hydrolyses starch into soluble branched and unbranched maltodextrins (2)

$$\text{Starch} + H_2O \xrightarrow{\text{α-amylase, pH 7.0 or 5.0, 100°C}} \text{maltodextrins} \qquad (2)$$

Where necessary, resistant starch in the sample is pre-dissolved by stirring the sample with 2 M KOH at approx. 4°C, followed by neutralisation with sodium acetate buffer and hydrolysis with α-amylase (3).

$$\text{Resistant starch} + H_2O \xrightarrow{\text{KOH then neutralization+ α-amylase}} \text{maltodextrins} \quad (3)$$

Amyloglucosidase (AMG) quantitatively hydrolyses maltodextrins to D-glucose (4).

$$\text{Maltodextrins} \xrightarrow{\text{AMG}} \text{D-glucose} \qquad (4)$$

D-Glucose is oxidised to D-gluconate with the release of one mole of hydrogen peroxide (H_2O_2) which is quantitatively measured in a colourimetric reaction employing peroxidase and the production of a quinoneimine dye.

$$\text{D-glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{D-gluconate} + H_2O_2 \quad (5)$$

$$2H_2O_2 + \text{p-hydroxybenzoic acid} + \text{4-aminoantipyrine} \xrightarrow{\text{(Peroxidase)}} \text{Quinoneimine dye} + 4\,H_2O \quad (6)$$

Assay procedure:
Materials:

Commercial Canadian wheat grains from the cultivar AC Walton was purchased from Les Moulins de Soulanges inc., St- Polycarpe, Quebec and was milled to flour. The untreated wheat flour was kept as the reference for all the treated flour samples. The moisture content of the flour was determined by placing the flour in hot air oven for 1 hour at 130˚C.

Hydrolysis and solubilisation of non-resistant starch:

100 mg of different flour samples were weighed accurately and about 4.0 mL of pancreatic α-amylase (10mg/ml) was added to each sample. The contents were vortex mixed and placed in a shaking water bath. The samples were incubated for exactly 16 hours at 37ºC. The sample tubes were then removed from the shaker and the contents were treated with 4.0 mL of 99% ethanol and mixed well. The tubes were then centrifuged at 1,500 g (approx. 3000rpm) for 10 min.

The supernatants were decanted and the pellets were re-suspended in 8 mL of 50% ethanol, mixed well and the contents were centrifuged again at 1500 g for 10 min. The supernatants were separated from the pellets and the pellets contain the RS.

Measurement of Resistant Starch:

The pellets were re-suspended in 2 mL of 2M potassium hydroxide (KOH) and were mixed well for 20 min in an ice/water bath. Further 8 mL of 1.2M sodium acetate buffer (pH 3.8) containing glacial acetic acid (1.05 g/mL) was added and immediately 0.1 mL of AMG solution was added and mixed well. The contents were placed in the water bath at 50ºC for 30 min incubation. Since the wheat flour contains less than 10% RS content, the contents were directly centrifuged at 1,500 g for 10 min without any dilution.

0.1 m L aliquots of the supernatants were transferred in duplicate into a glass test tube and 3.0 mL of GOPOD reagent was added and incubated for 20 min at 50ºC. After incubation period, the absorbance of the solution containing RS was measured using Ultraspec 2100 pro UV/Visible Spectrophotometer at 510 nm against the reagent blank. The reagent blank solution was prepared by mixing 0.1 mL of 100 mM 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.

Measurement of Non-Resistant (solubilised) Starch:

The supernatant solutions obtained from the subsequent two 50% ethanol washings were combined and the volume was adjusted to 100 mL with 100 mM sodium acetate buffer (pH 4.5) and mixed well. Then 0.1 mL aliquots of this solution were incubated in duplicate with 10 μL of dilute AMG solution (300 U/mL) in 100 mM sodium maleate buffer (p H 6.0) for 20 min at 50ºC. Further, the contents were incubated for 20 min at 50ºC with addition of 3.0 mL of GOPOD reagent. The absorbance of the contents was measured at 510 nm against a reagent blank.

Calculation of total starch:

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

$$= \Delta E \times F \times \frac{10.3}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

$$= \Delta E \times \frac{F}{W} \times 9.27 \quad (7)$$

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

$$= \Delta E \times F \times \frac{100}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

$$= \Delta E \times \frac{F}{W} \times 90 \quad (8)$$

Total starch = Resistant starch + Non- resistant starch

Where,

$\Delta \boldsymbol{E}$ = absorbance (reaction) read against the reagent blank.

F = 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 this100 µg of glucose.

100/0.1 = volume correction (0.1 mL taken from 100 mL).

1/1000 = conversion from micrograms to milligrams.

W = dry weight of sample analyzed

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

100/W = factor to present RS as a percentage of sample weight.

162/180 = factor to convert from free glucose, as determined, to anhydro-glucose as it occurs in starch.

10.3/0.1 = 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.

3.3.2. Estimation of protein:

Gluten, the major protein in wheat flour constitutes to about 90 % of total protein. Wheat flour when hydrated yields gluten, which is the composite of two components namely, gliadin

and glutenin. Biochemical changes in the wheat grains occurred to various extent during storage at different temperatures. Gluten is one of the components of the flour, which has to be estimated over the change.

Mechanism: Lowry's method:

The method combines the reactions of copper ions with the peptide bonds under alkaline condition (Biuret test) with the oxidation of aromatic protein residues. The Lowry method is best used with protein concentrations of 0.01-1.0 mg/mL and is based on the reaction of Cu^{+}, produced by the oxidation of peptide bonds, with Folin-Ciocalteu reagent (a mixture of phosphotungstic acid andphosphomolybdic acid in the Folin-Ciocalteu reaction).

The reaction mechanism involves the reduction of the Folin reagent and oxidation of aromatic residues (mainly tryptophan, also tyrosine). The concentration of the reduced Folin reagent is measured by absorbance at 540 nm. As a result, the total concentration of protein in the sample can be deduced from the concentration of Trp and Tyr residues that reduce the Folin reagent.

Assay procedure:

Estimation of protein by Lowry's method was performed for 20 different samples of flour. About 0.05±0.002 g of flour was mixed well with 50 mL of distilled water in a magnetic stirrer. They were mixed well to make a uniform suspension of samples. About 1 mL of this suspension was taken in a clean test tube to have an initial concentration of 1 mg/ml.

Similarly protein standard solution was prepared using bovine serum albumin (BSA) of sigma grade. About 0.01 g of BSA was dissolved well in 10 mL of distilled water to make a protein concentration of 1mg/ml. From this stock solution, about 0.1 to 0.45 ml of solution was taken in different test tubes and distilled water was added to each test tube to make up the

volume to 1 ml of solution. The corresponding concentration of BSA was from 0.1 mg/ml to 0.45 mg/ml.

All reagents used were of analytical grade. Alkaline reagent was prepared by combining three different chemical solutions of 2 % sodium carbonate in 0.1 M NaOH,

1 % copper sulphate and 2 % K-Na tartrate in the ratio of 2:1:1. This alkaline reagent was prepared just before the use. 1N solution of Folin- ciocalteu phenol reagent (FCR) was prepared by mixing 2N solution with water in the ratio of 1:1.

Development of color:

About 10 mL of the alkaline solution was added and mixed well with both 1mL of sample suspensions and the standard BSA solutions of concentrations 0.1 to 0.45 mg/ml and left for 30 min at room temperature. Then about 1 mL of 1N Folin- ciocalteu phenol reagent was added further to all the samples and vortex mixed vigorously and immediately. This solution was again left for about 45 min at room temperature for the proteins to react with the reagents. Once the full blue color was developed, the absorbance of the samples were read in a suitable UV/ Visible spectrophotometer at 510 nm against the blank in duplicate and the mean absorbance was taken for calculation.

Calculation:

The content of protein is calculated on the basis of absorbance (corrected with respect to absorbance of blank sample) according to the expression 9:

$$\text{Protein } \% = \frac{a}{b} \times 100 \qquad (9)$$

Where:
a = mg BSA for measured absorbance from calibration graph

b = weight of sample in aliquot [mg].

Statistical analysis:

Statistical analysis was performed using the JMP 8 software (SAS Institute Inc., Cary, NC, USA). The data were analyzed by analysis of variance (ANOVA) and the adequacy of the response surface model was determined by evaluating the lack of fit and coefficient of determination (R^2). The statistical significance of the model and its variables was determined at 5% probability level ($p<0.05$). The surface response of all the three factors for total starch content and protein content were obtained based on modeling and desirability function that could be visually explained in terms of three-dimensional response surface plots and prediction profiler.

3.4. Results and discussion:

Analysis of total starch content of flour:

The total starch content of the wheat flour is the combination of resistant starch (RS) and non-resistant starch (NRS). Starch is a major constituent of wheat flour. Therefore, it appears likely that its characteristics and condition may contribute to the baking quality of flour. The analysis of quantity of total wheat starch in the flour does matter to determine the baking quality of flour.

Experimental model:

A three-level central composite design (CCD) of the three parameters was constructed using the response surface methodology (RSM). The studied factors and their three levels were: storage temperature of 30◦C, 40◦C and 50◦C, moisture content of grains at 12%, 15% and 18%, and duration of storage for 2, 5 and 8 days. The total starch content of the flour of each combination of factors was determined using the enzymatic assay procedure. The content of starch was measured in percentages in triplicates and the mean value was obtained. The

different combinations of independent variables and their corresponding response in terms of total starch content are presented in table 3.2.

Table 3.2: Observed responses for the total starch content in the experimental design.

Design points	Temperature, ◦C	MC, %	Duration, days	Total starch content, %		
				Actual	Predicted	Stderror
R1	30	18	8	55.00	54.27	± 2.59
R2	30	12	2	63.00	62.45	± 2.59
R3	30	12	8	58.00	58.08	± 2.59
R4	30	15	5	60.00	60.37	±2.08
R5	30	18	2	57.00	57.79	±2.59
R6	40	15	5	51.00	49.45	±1.24
R7	40	18	5	47.00	48.16	±2.08
R8	40	15	5	52.00	49.45	±1.24
R9	40	12	5	49.00	51.50	±2.08
R10	40	15	8	41.00	44.20	±2.08
R11	40	15	2	47.50	48.43	±2.08
R12	40	15	5	52.50	49.45	±1.24
R13	50	15	5	33.00	37.50	±2.08
R14	50	18	2	36.00	34.56	±2.59
R15	50	18	8	31.00	30.63	±2.59
R16	50	12	2	38.00	37.84	±2.59
R17	50	12	8	35.00	32.29	±2.59
Control flour	25	10	0	75.00		

The model obtained for total starch content was found to be highly significant with $p<0.0003$, with a R^2 value of 0.96. The lack of fit was not significant as expected ($p>0.05$), suggesting that the model was well fitted and could be used to predict the total starch content from enzymatic assay procedure.

The model indicated that the linear effect of the temperature had the greatest significance on the total starch contents of the flour. The value of (p) less than 0.05 indicate the significant terms in the model. The linear effect of moisture content of grains was found to be significant

since their (p) value = 0.0368. Also the linear effect of duration of storage of grains was found to be significant with (p) value= 0.0117. The quadratic effect of $duration\ of\ storage^2$ was found to be significant with (p) value= 0.0187. The bilinear effects of temp. × Duration of storage, temp. × MC and Duration of storage × MC and also the quadratic effect of $temp.^2\ and\ MC^2$ were found to be insignificant sine their p values were above 0.05 (Table 3.3).

Table 3.3: ANOVA for the total starch content of the aged wheat flour

Total starch content of aged flours (%)				
Source	SS	DF	MS	*P* value
Model	1553.78	4.00	388.44	<0.0001*
Lack of fit	61.66	10.00	6.16	0.0895
Error	62.82	12.00	5.23	
Term	Estimate	Std error	*t* ratio	*P* value
Intercept	49.21	0.86	56.91	<0.0001*
Temp.(30,50)	-12.00	0.72	-16.58	<0.0001*
MC (12,18)	-1.70	0.72	2.35	0.0368*
Duration of storage (2,8)	-2.15	0.72	2.97	0.0117*
Duration of storage × duration of storage	-3.06	1.12	-2.72	0.0187*

The predictive model for the total starch content of wheat flour by enzymatic assay in terms of uncoded factors' levels is shown in equation 10. It indicated the quadratic second-order relationship between total starch content and duration of storage.

$$Total\ starch\ \% = 49.21 - 12\left[\frac{temp.-40}{10}\right] - 1.7\left[\frac{MC-15}{3}\right] - 2.15\left[\frac{duration\ of\ storage-5}{3}\right] - 3.06\left[\frac{duration\ of\ storage-5}{3}\right]^2 \quad (10)$$

Pan breads were prepared using starch-gluten blends while keeping gluten content constant and varying the starch component to show the baking potential of different starches (Kulp K.,

1972). The data in Table 3.2 demonstrates the variation of starch content from 32 % to 62% getting decreased from the untreated (control) wheat flour with a starch content of 70 %. The wheat starches produce higher volume of bread compared to other cereal starches.

From the Fig 3.3, it is well understood that the model is significant and hence the total starch level gets decreased as there was a rise in the temperature. It is relatively simple to determine the exact optimum conditions for single response using RSM (Bezerra et al., 2008, Lundstedt et al., 1998).

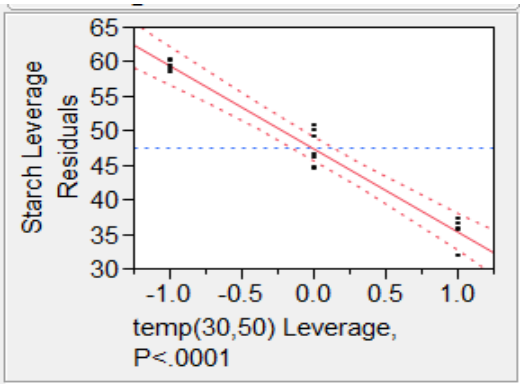

Fig. 3.3: Leverage plot of total starch content Vs temperature

The response surface plot explains the relationship of temperature and duration of storage with the starch content (Fig.3.4). At 30˚C, the starch content is found to be at its highest level and found decreasing gradually as the temperature increases. Hence there is an inverse correlation between the temperature and the starch content.

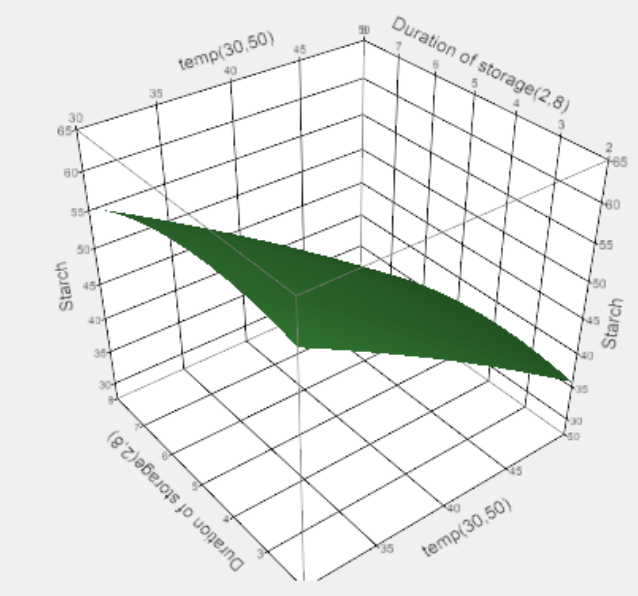

Fig.3.4: Response surface plot for total starch content

Analysis of protein content of flour:

Gluten proteins play a key role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesiveness, viscosity and elasticity on dough. Grains of two wheat (*Triticum aestivum* L.) cultivars, Sunco and Sunsoft, were stored at 4°C and 30°C for 270 days to examine changes in proteins during storage. When whole meal flour extracted from the grains was analyzed using an unfractionated protein extraction procedure, significant changes were found in protein content for either cultivar in samples stored at 30°C compared with those stored at 4°C (Meredith Wilkes and Les Copeland, 2007).

Experimental model:

A three-level central composite design (CCD) of the three parameters was constructed using the response surface methodology (RSM). The studied factors and their three levels were: storage temperature of 30˚C, 40˚C and 50˚C, moisture content of grains at 12%, 15% and 18%, and duration of storage for 2, 5 and 8 days. The protein content of the flour of each combination of factors was determined using the Lowry's assay procedure. The content of

protein was measured in percentages of triplicates and the mean value was obtained. The different combinations of independent variables and their corresponding response in terms of protein content are presented in Table 3.4.

Response surface model was fitted to experimental data. The leverage effect was emphasised to specify the model. The ANOVA of the quadratic regression of the model for total protein is given in Table 3.5.

The model obtained for total protein content of the flour was found to be significant with a p value < 0.0001 and R^2 value of 0.99. The lack of fit was not significant as expected (p>0.05), suggesting that the model was well fitted and could be used to predict the total protein content from Lowry's assay procedure.

Table 3.4: Observed responses for total protein content in experimental design

Design points	Temperature, ˚C	MC, %	Duration,days	Total protein content, %		
				Actual	Predicted	Stderror
R1	30	18	8	11.00	10.97	±0.19
R2	30	12	2	12.00	11.87	±0.19
R3	30	12	8	11.50	11.52	±0.19
R4	30	15	5	11.78	11.71	±0.15
R5	30	18	2	11.96	12.14	±0.19
R6	40	15	5	13.50	13.56	±0.09
R7	40	18	5	13.75	13.52	±0.15
R8	40	15	5	13.65	13.56	±0.09
R9	40	12	5	13.32	13.62	±0.15
R10	40	15	8	12.90	12.99	±0.15
R11	40	15	2	14.00	13.98	±0.15
R12	40	15	5	13.70	13.56	±0.09
R13	50	15	5	10.00	10.14	±0.15
R14	50	18	2	10.90	10.85	±0.19
R15	50	18	8	9.10	9.20	±0.19
R16	50	12	2	10.50	10.50	±0.19
R17	50	12	8	9.90	9.69	±0.19
Controlflour	25	10	0	13.80		

The model indicated that the linear effect of the temperature had the greatest significance also on the total protein contents of the flour. The value of (*p)* value = 0.0001, less than 0.05 indicate the significant terms in the model. The linear effect of duration of storage was also found to be very significant since their (*p)* value was 0.0001. The bilinear effects of MC × Duration of storage with (p) = 0.0145, and the quadratic effects of $temp.^2$ with (p) = 0.0001 are found to be significant sine their p values were below 0.05.

Table 3.5: ANOVA for total protein content of wheat flour

Total protein content of aged flours (%)				
Source	SS	DF	MS	*P* value
Model	38.59	5	7.71	<0.0001*
Lack of fit	0.43	9	0.48	0.1989
Error	0.45	11	0.01	
Term	Estimate	Stderror	*t* ratio	*P* value
Intercept	13.55	0.08	177.07	<0.0001*
Temp.(30,50)	-0.78	0.06	-12.25	<0.0001*
MC (12.18)	-0.05	0.06	-0.80	0.4740
Duration of storage (2,8)	-0.50	0.06	-7.75	0.0001*
MC×Duration of storage	-0.21	0.07	-2.90	0.0145*
Temp.×temp.	-2.68	0.10	-26.89	<0.0001*

The linear effect of the factors, temperature and duration of storage is highly significant for the total protein contents of the flour. As the temperature and the storage period increases, the protein level was found to be decreasing.

The response surface plot clearly depicts the trend of total protein content with respect to the storage temperature and duration of storage (Fig.3.5). The Pearson correlation co-efficient for total protein content was 0.99. It was found that the bilinear effect of MC and duration of storage had a significant effect increasing the protein content gradually.

The predictive, second order, polynomial model for the total protein content of the aged wheat flour of the cultivar A.C.Walton is given in the equation 11.

$$Protein\ content\ \% = 13.55 - 0.78\left[\frac{temp-40}{10}\right] - 0.05\left[\frac{MC-15}{3}\right] - 0.50\left[\frac{duration\ of\ storage-5}{3}\right]$$
$$-0.21\left[\frac{MC-15}{3}\right]\left[\frac{duration\ of\ storage-5}{3}\right] - 2.68\left[\frac{temp-40}{10}\right]^2 \qquad (11)$$

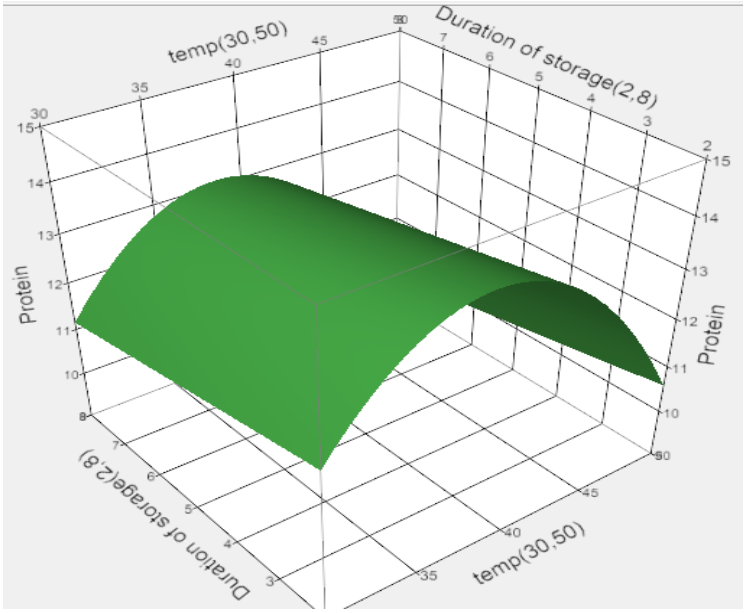

Fig.3.5: Response surface plot for total protein content

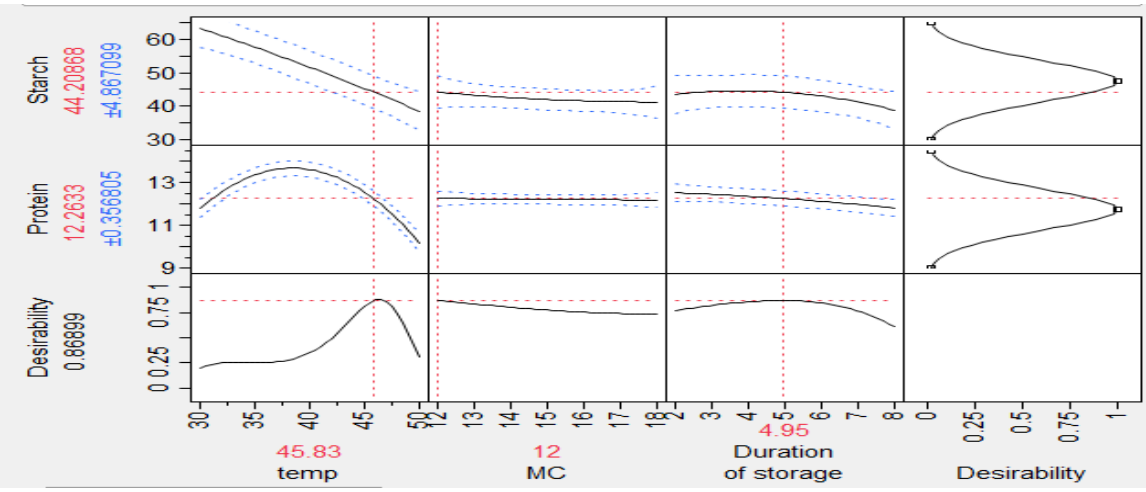

Fig. 3.6: Prediction profiler for total starch and protein content of flour

A higher desirability of 0.87 was obtained with the total starch content of 44.21±4.875% and protein content of 12.26±0.36% by the storage of grains at 45.83˚C with the moisture content of 12.00% for 4.95 days (Fig.3.6). From the prediction profiler, it is clear that the moisture content plays a minor role in the process of accelerated aging of wheat grains. Hence storage of grains at 45˚C for 5 days with the initial moisture content will enhance the aging of grains.

Most storage studies have shown that the protein content of wheat grains and flour do not change significantly over time (Pomeranz et al., 1968; Shearer et al., 1975; Lukow et al., 1995; Caddick and Shelton, 1998; Kim et al., 2003). While protein quantity may not change, protein quality does. Fractionation of the flour samples from stored grain into soluble and insoluble proteins revealed increases in soluble protein content for both cultivars stored at 30°C compared with 4°C. The soluble protein content, expressed as a percentage of the total protein, increased by 1.5% (P = 0.032) for Sunco and by 8.0 % (P = 0.158) for Sunsoft during storage at 30°C compared with those samples stored at 4°C (Meredith Wilkes and Les Copeland, 2008).

In correlation with Meredith wilkes's work, the protein content increased to about 15.0 % (P= 0.99) for the A.C.Walton cultivar during storage at 40˚C compared to those flour samples which were stored at 30˚C. This explains the significance of the temperature effect on the gluten, the total protein content of the flour for baking process.

3.5. Conclusions:

The physico-chemical property of the wheat flour was found to be affected as a result of storage of grains at elevated temperatures. The total starch content of the flour got decreased by 50 % when stored at 50˚C in comparison with the freshly harvested wheat grains. On the other

hand, the total protein content got decreased only by 10 % as a result of storage at 50˚C. Other two factors, namely moisture content and duration of storage had only a minimal effect on the enhancement of the physico-chemical properties.

The study of physico-chemical properties of the accelerated aged wheat flour of cultivar A.C.Walton had provided a good insight on the quantitative analysis of the components of the flour. Quantitative analysis of the total content of starch, both resistant starch and non-resistant starch helped in determining the variation of starch level in each treatment, thereby leading to find the maximum amount of starch content in flour. A regression model was developed to assist in further investigation and development of the quantitative analysis for starch and protein content.

Since the starch content by itself plays a major role in rheological properties of the wheat flour, it was investigated to determine their trend over the aging process. Wheat starch is unique among all other cereal starches that they contribute a lot to the loaf volume of the dough during the baking process. Also the amount of gluten, the major protein in wheat flour is important, since they contribute to the elasticity and extensibility of the dough. Thus optimization of the model for the three factors, namely temperature, moisture content and duration of storage of the grains would help determine the significant amount of starch and protein in the aged wheat flour.

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CONNECTING TEXT

The physico-chemical property of accelerated aged grain was studied in chapter 3. The properties gave an insight on the quantitative analysis of total starch content (resistant and non-resistant) and total protein content of the flour. On the other hand, the qualitative analysis of the flour is the most important criterion for determining the baking quality. Thus the determination of gelatinization temperature of starch would help analyse the thermal property of the flour, which can be correlated to the quality of the flour best suited for the baking process.

Chapter IV

EFFECT OF ACCELERATED AGING OF WHEAT GRAINS ON THERMAL PROPERTIES OF FLOUR

4.1. Abstract:

Accelerated aging of wheat grains is the storage of grains at a higher temperature range of 30- 50ºC for a maximum period of 8 days. This conditioning of the grains would have profound effect on the components of the flour namely, starch and protein. Differential scanning calorimetry (DSC) was used to analyse the changes in the properties of the components such as gelatinization temperature of starch and denaturation temperature of proteins. It measures the heat flow into the flour as a function of temperature as the components of the flour are heat-denatured.

In this study, the thermal property of accelerated aged wheat grains of cultivar AC Walton was studied. The aged flour was hydrated with water and heated in a temperature range from 20˚C to 160˚C at a constant heating rate of 5˚C/min. The gelatinization temperature of starch of each flour was found from the endothermic peak obtained. The accelerated aging had a great effect on the flour where the gelatinization temperature of starch increased from 55˚C in untreated freshly harvested flour to 67˚C in treated (aged) flour. Higher the gelatinization temperature of starch, larger is the loaf volume of the bread, thus ultimately improving the baking properties of the flour.

Key words: Accelerated aging, gelatinization temperature, differential scanning calorimeter, endothermic peak.

4.2. Introduction:

Gelatinisation of starch is a basis for many types of food production. Processes such as the baking of bread, the gelling of pie fillings, the production of pasta products, the fabrication of starch-based snack foods and the thickening of sauces are all dependent on proper gelatinization of starch to produce a desirable texture or consistency of the end product. Higher the gelatinization temperature of starch, largest the loaf volume of baked breads thus improving the baking qualities of the flour.

Wheat flour dough consists essentially of gluten gel with starch granules dispersed in the continuous gel matrix. The dough exhibits viscoelastic behavior that is predominantly controlled by the gluten protein fraction (Faubion and Hoseney, 1990) even though both starch and gluten contribute to the viscoelastic response (Larsson, 2000). Rheological properties of wheat flour doughs are of primary importance because the flow and deformation behavior of the dough is central to the successful manufacturing of bakery products (Menjivar, 1990).

Wheat starch contains about 30 % amylose and 70 % amylopectin. Commercially available wheat starches usually have about 12% moisture and 0.2% protein (Knight, 1965). The role of starch granules in the expansion of doughs during the process of baking is important. Starch granules should not gelatinize early in the baking cycle as potato starch does but should gelatinize later in the baking cycle as wheat starch does. This prevents early setting of the dough which inhibits expansion. Starch granules should not disrupt and fuse together during gelatinization as tapioca starch does, forming an impermeable gas membrane. Granules should gelatinize individually as wheat starch does, causing a disruption of cell membranes which prevents shrinkage of the loaf during cooling after baking.

4.2.1. Gelatinization of starch:

Starch granules in the wheat flour get hydrated and swell to several times their original size. By the increase in their size, the granules tend to lose their bi-refringence. Due to this process, the clarity in the mixture increases. A rapid increase in the consistency of the molecules occurs and reaches a peak. All the linear molecules dissolve and diffuse from ruptured molecules. Finally the mixture retrogrades to a paste-like mass or gel.

Starch has several distinct roles in the bread making process. It is the substrate for the amylases that produce fermentable sugars for yeast fermentation and it serves as a reservoir for water absorption and as a diluent for gluten, thereby contributing to the optimal viscoelastic properties of the dough (Burhans and Clapp, 1942, Sandstedt et al., 1954, Sandstedt, 1955). Rheological characterization of the flour could provide better understanding on functionality during heating process.

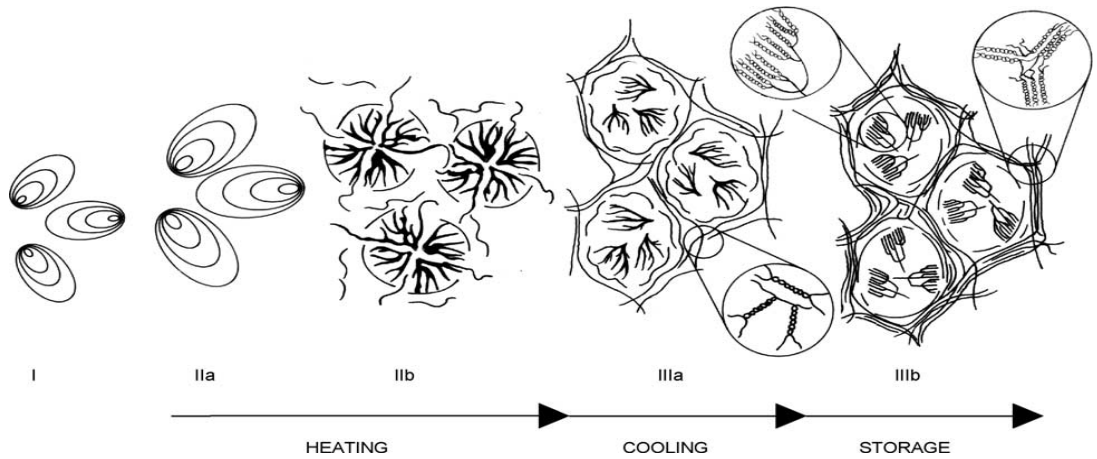


Fig.4.1. Schematic representation of changes that occur in a starch–water mixture during heating, cooling and storage. (I) Native starch granules; (II) gelatinisation, associated with swelling [a] and amylose leaching and partial granule disruption [b], resulting in the formation of a starch paste; (III) retrogradation: formation of an amylose network (gelation/amylose retrogradation) during cooling of the starch paste [a] and formation of ordered or crystalline amylopectin molecules (amylopectin retrogradation) during storage [b] (Source: Donald et al, 1997)

It is important to be able to compare experiments which determine the temperature ranges for denaturation of starch and protein with one another. There are two major considerations here. The first is the response time of instruments and temperature sensors to the changes which occur; the second is the behaviour of the polymers themselves, specifically, whether their behaviour is kinetically or thermodynamically limited.

Wheat is a cereal of unique properties because the resulting flour after milling has the ability to form gluten, a protein network which captures the gas produced during proofing and which leads to a desirable bread crumb structure after baking. The insoluble storage proteins are responsible for these functional properties.

Thermal analysis is a valuable tool for studying the effect of thermal processing on vegetable proteins. With DSC, the output between sample and reference is the differential power required to maintain sample and reference pans at the same temperature and this replaces temperature difference as the measured quantity. When proteins are heated they usually undergo thermal denaturation; the proteins unfold and become less ordered forming a random coil. The denaturation of proteins has been extensively studied by differential scanning calorimetry (DSC), where denaturation is observed as an endothermic peak on the thermogram.

Protein functionality is closely related to its conformational state, which in turn is completely influenced by processing conditions. Temperature is one of the most important factors in a process performance, since high temperature causes protein unfolding and loss of functionality. Indeed, there are a lot of common situations in which the functional properties of the wheat proteins are modified by heat. The drying process applied to wheat kernels for reducing the moisture content, promotes a decrease in the protein solubility and as a consequence the resulting bread has reduced volume (Alberto Leon et al., 2003).

Heat also plays an important role in the production of vital wheat gluten, which is obtained by wet processes and must be dried to prevent deterioration and to improve handling properties. Gluten drying is considered as one of the most critical factors determining the quality of vital gluten. In addition, denaturation takes place during the baking process and the 'setting' of the gluten network, which contributes significantly to the characteristics of the baked products.

Denaturation is the most important transition occurring in proteins during the baking process. The protein denaturation has been extensively studied by differential scanning calorimeter (DSC) where denaturation is indicated as an endothermic peak in thermogram (Privalov and Khechinashvili, 1974). Protein fractions of wheat consist of several proteins and, therefore, several denaturation endotherms are expected (Jasim Ahmed, Hosahalli S. Ramaswamy, Vijaya G.S. Raghavan, 2008).

In spite of the economic importance of wheat, and the great quantity of research effort devoted to wheat products, little is known about the thermal characteristics of wheat proteins. This could be because the most important wheat proteins (gliadins and glutenins) show little or no calorimetric response. Isolated globulins from wheat, reported two little peaks at 84˚C and 96˚C. When the flour was heated up in DSC pans, four endothermic peaks were observed, the most prominent of which were at 65˚C and 112˚C which were attributed to starch gelatinization and to complex starch-lipid melting, respectively (Fig 4.2.).

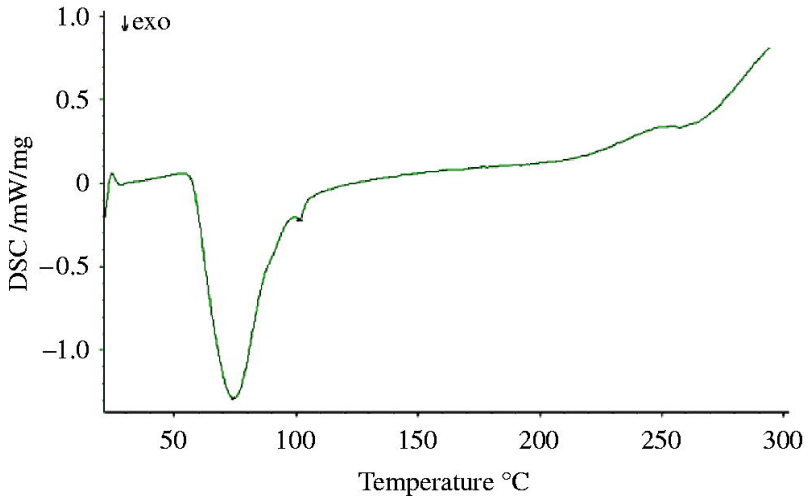


Fig. 4.2: A typical thermogram of wheat flour (Source: Cheng, 2009)

4.2.2. Enthalpy of heat:

The significance of the enthalpy (ΔH) value is that it represents the amount of thermal energy involved in the gelatinisation process of starch. At the molecular level, this may be expected to involve the cleavage of existing H-bonds between starch molecules, and the formation of new bonds involving water to give a less-ordered structure with increased entropy, so that the overall process is endothermic.

The enthalpy of denaturation is the net value of the combination of endothermic reactions, such as the disruption of hydrogen bonds, and of exothermic processes, such as the breakup of hydrophobic interactions and protein aggregations. The resulting residual enthalpy has been correlated to the remaining content of ordered secondary structure of a protein (Iesel Van der Planckenet al., 2006).

The present study intends to examine the thermal behaviour of the accelerated wheat flour samples to best correlate with their baking properties. Rheological behaviour was studied as the function of temperature keeping in view changes associated with heat of enthalpy and starch gelatinization at selected temperature range. This study would help baking and other

food processing industries to formulate, function and the possible use of accelerated aging method to produce best baking foods.

4.3. Materials and methods:

Materials:

Freshly harvested wheat grain of cultivar AC Walton was obtained from Les Moulins de Soulanges, St. Poly-Carp, Quebec. The accelerated aged wheat grains of the cultivar AC Walton were milled to fine particles of uniform sized flour and used for the analysis.

Preparation of sample:

About 10.00±0.10 mg of each flour sample was weighed accurately and placed in an aluminium pan designed for DSC Q100 and about 10.00 ± 0.10 µl of distilled water was added directly using a micropipette to the weighed flour and the total weight of each of the sample was noted. The pans were closed by aluminium lids and sealed well hermetically and the set up was left at room temperature for half an hour for the flour to mix well with water.

Equipment:

The instrument used to measure the gelatinization temperature of starch and denaturation temperature of protein was a TA Instruments Q100 Differential Scanning Calorimeter (Newcastle, DE, USA) operated with the TA Instruments Q100 DSC 7.0 Build 244 software. The instrument consists of four basic units, namely furnace; auto sampler; cooling system and computer. The instrument was calibrated with indium for heat flow and temperature. The instrument was coupled with refrigerated cooling system. Nitrogen was used as a purge gas at a flow rate of 50 ml/min (Fig. 4.3)

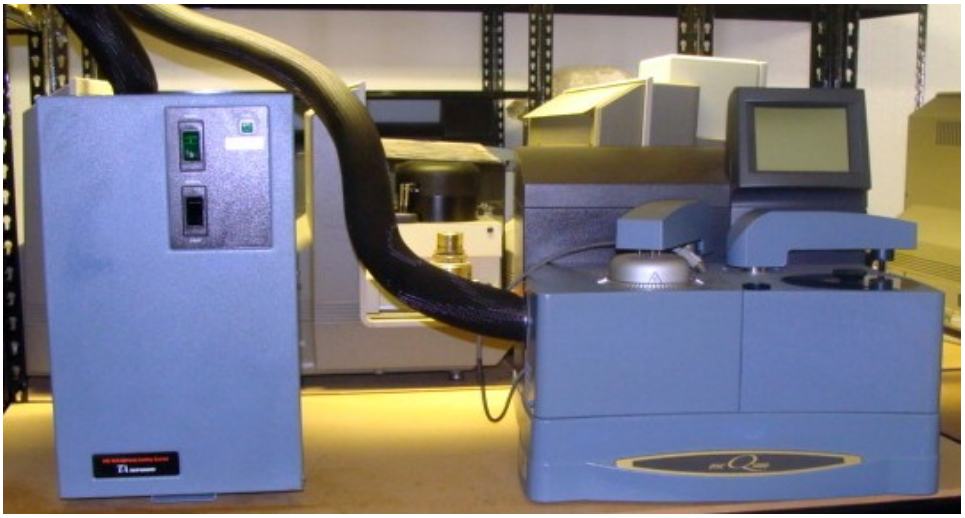


Fig. 4.3: Experimental setup of DSC Q100

Thermal analysis by DSC:

All the treated flour samples were placed in an aluminium pan weighed around 10±0.5 mg separately. Then distilled water of about 10 µl was added directly through pipette into the pan. The flour was treated with water, in order to assess the endothermic peaks. An empty polymer-coated aluminum sample pan was hermetically sealed and used as reference. The mixture of flour and water was allowed to set well for about 10 min. The pans were closed with aluminium lids and sealed well hermetically and left at room temperature for 30 min. Once the samples were equilibrated at room temperature, they were placed in the auto sampler.

The nitrogen gas flow was turned on and the flow was adjusted to 50 mL/ min. The refrigerated cooling system was turned on and the flour samples were heated from 20˚C to 160˚C at a heating rate of 5˚C/min. The onset temperature (T_O) and peak maximum temperature (T_m) associated with the structural changes of the major dough components were determined with the Universal Analysis Version 1.2 software supplied by the DSC Company. The samples underwent a thermal treatment and an endothermic peak was noticed to appear at about the

temperature of 60˚C, which was the gelatinization temperature of the starch (T_g). The gelatinization temperature of the samples was compared to that of the freshly harvested untreated flour.

Statistical analysis:

Statistical analysis was performed using the JMP 8 software (SAS Institute Inc., Cary, NC, USA). The data were analyzed by analysis of variance (ANOVA) and the adequacy of the response surface model was determined by evaluating the lack of fit and coefficient of determination (R^2). The statistical significance of the model and its variables was determined at 5% probability level ($p<0.05$). The surface response of all the three factors for the gelatinization temperature of starch (T_g) was obtained based on modeling and desirability function that could be visually explained in terms of three-dimensional response surface plots and prediction profiler.

4.4. Results and discussion:

4.4.1. Gelatinization temperature of starch (T_g):

The gelatinization temperature of wheat starch plays an important role in the dough strength during the baking process. The influence of water content on the gelatinization of wheat starch was examined by differential scanning calorimetry. Two endothermic transitions were observed when flour was heated to 160°C with 35 to 50% (w/w) water. The temperature of the first and second endotherms and the enthalpies of the corresponding endotherms varied with each flour.

Various aspects of gelatinisation of starch in water are considered, including swelling and interaction with water before the onset of gelatinisation, increase in consistency during gelatinisation, gelatinisation temperature, types of gelatinised system and gel and paste

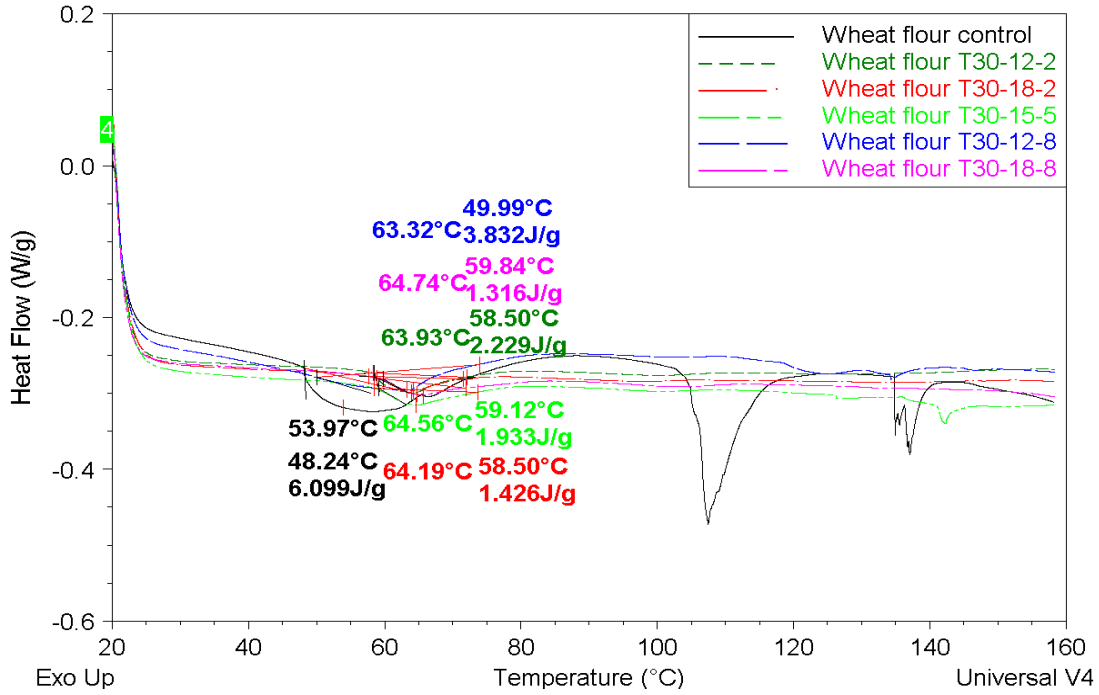
formation upon cooling. In addition, the traditional rationale of gelatinisation and gelatinisation from the viewpoint of water mobility are discussed.

Experimental model:

A three-level central composite design (CCD) of the three parameters was constructed using the response surface methodology (RSM). The studied factors and their three levels were: storage temperature of 30˚C, 40˚C and 50˚C, moisture content of grains at 12%, 15% and 18%, and duration of storage for 2, 5 and 8 days. The gelatinization temperature of starch of the flour of each combination of factors was determined using the DSC instrument. The temperature (T_g) of each flour was determined in duplicates and the mean value was obtained.

All the flour samples showed an endothermic transition in the range of 60˚C to 70˚C against the heat flow (W/g). The freshly harvested wheat flour sample ended up with a gelatinization temperature of about 57˚C comparatively lower than the treated flour samples, indicating their variation in the thermal property. The thermogram curves for each of the treated flour was obtained from the DSC and the results were analysed using the software Instruments Q100 DSC 7.0 Build 244 (Fig 4.4).

It was interesting to note that, each flour obtained from the grains stored at 30˚C and 40˚C had an onset temperature of gelatinization at 58˚C (T_o) and a peak temperature in the range of 63˚C- 64˚C (T_g), whereas the grains stored at 50˚C had an onset temperature T_O at 60˚C and T_g in the range of 65˚C to 70˚C. Also low moisture content of the grains had greater T_g compared to higher moisture content. Furthermore, the area under the curve was quite small indicating the heat of enthalpy to be minimum leading to reduction in energy being consumed by the system. Higher the gelatinization temperature of starch, largest is the expansion of the dough, thus producing huge volumes of bread.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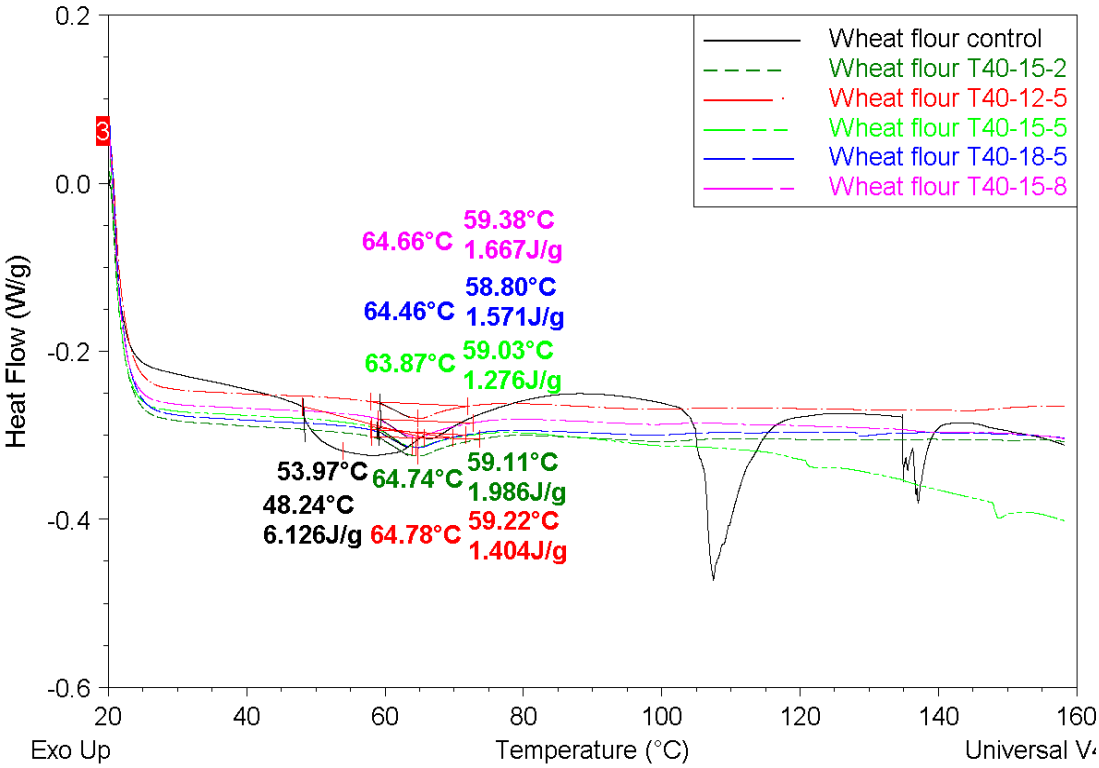
Wheat flour T30-12-2
Wheat flour T30-18-2
Wheat flour T30-15-5
Wheat flour T30-12-8
Wheat flour T30-18-8
49.99°C
63.32°C 3.832J/g
59.84°C
64.74°C 1.316J/g
58.50°C
63.93°C 2.229J/g
53.97°C 64.56°C 59.12°C
1.933J/g
48.24°C
6.099J/g
64.19°C 58.50°C
1.426J/g
Heat Flow (W/g)
Exo Up
Temperature (°C)
Universal V4


Wheat flour control
Wheat flour T40-15-2
Wheat flour T40-12-5
Wheat flour T40-15-5
Wheat flour T40-18-5
Wheat flour T40-15-8
64.66°C 59.38°C
1.667J/g
58.80°C
64.46°C 1.571J/g
63.87°C 59.03°C
1.276J/g
53.97°C 64.74°C 59.11°C
1.986J/g
48.24°C
6.126J/g 64.78°C 59.22°C
1.404J/g
Heat Flow (W/g)
Exo Up
Temperature (°C)
Universal V4

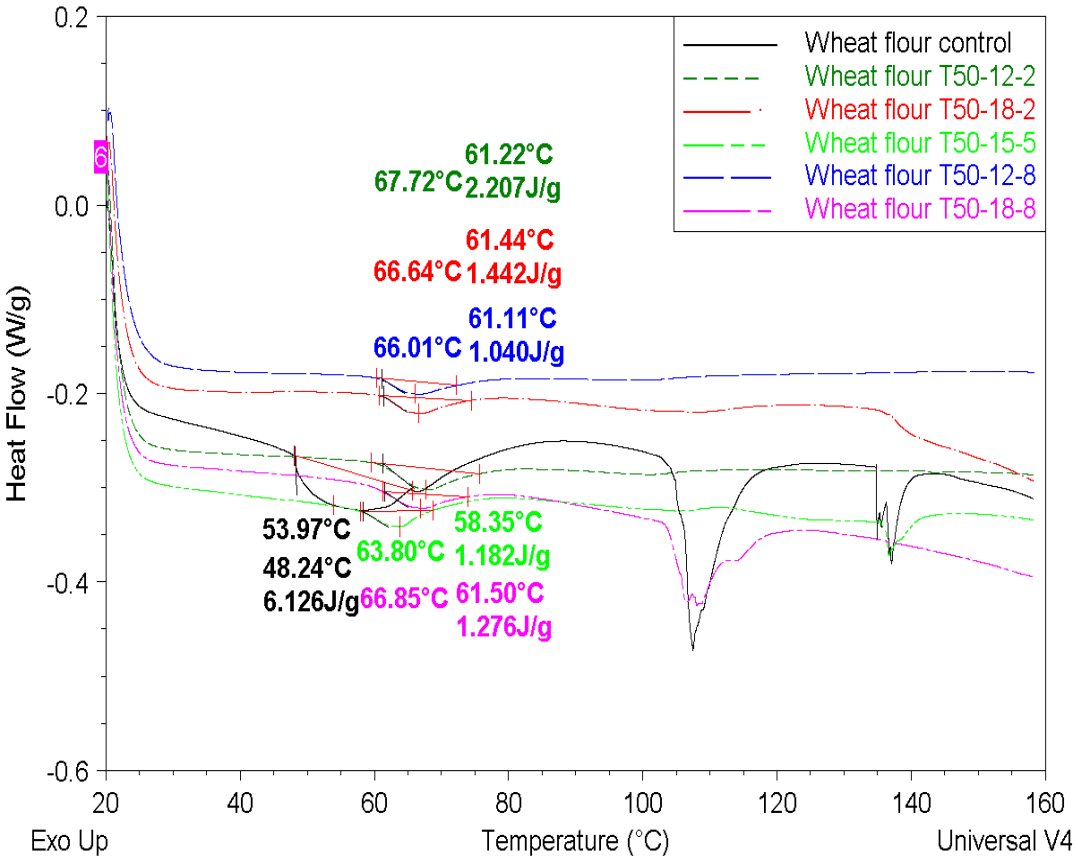


Fig. 4.4: DSC thermogram of wheat flour stored at various temperatures

The different combinations of independent variables and their corresponding response are presented in Table 4.1. The gelatinization temperature of starch of the treated flour samples varied from 59˚C to 70˚C. The endothermic transition of the flour occurred with the minimal absorption of energy from the system resulting in a lower enthalpy range compared to the flour from freshly harvested wheat grain.

Table 4.1: Observed response for the gelatinization temperature of starch in the experimental design

Design Points	Temperature, ◦C	MC, %	Duration, Days	Gelatinization temperature of starch,˚C		
				Actual	Predicted	Std error
R1	30	18	2	59.20	59.08	±0.86
R2	30	18	8	59.90	60.48	±0.86
R3	30	12	8	61.00	61.07	±0.86
R4	30	12	2	62.00	61.74	±0.86
R5	30	15	5	62.50	62.19	±0.69
R6	40	15	5	64.00	65.16	±0.41
R7	40	15	2	63.00	63.61	±0.69
R8	40	15	8	64.46	63.00	±0.69
R9	40	15	5	64.80	65.16	±0.41
R10	40	15	5	65.00	65.16	±0.41
R11	40	18	5	65.70	64.62	±0.69
R12	40	12	5	65.99	66.21	±0.69
R13	50	12	8	66.67	66.99	±0.86
R14	50	15	5	69.67	69.12	±0.69
R15	50	12	2	70.00	69.62	±0.86
R16	50	18	2	66.89	67.02	±0.86
R17	50	18	8	66.00	66.46	±0.86
Control Flour	25	10	0	53.75		

Response surface model was fitted to experimental data. The leverage effect was emphasised to specify the model. The ANOVA of the quadratic regression of the model for T_g is given in table 4.2.

The model obtained for gelatinization temperature was found to be significant with a p value <0.0001, with a R^2 value of 0.92. The lack of fit was not significant as expected (p>0.05), suggesting that the model was well fitted and could be used to predict the gelatinization temperature of starch using DSC.

The model indicated that the linear effect of the temperature had the greatest significance on T_g of the starch of the aged flour. The value of (p) value <0.0001, less than 0.05 indicating

the significant terms in the model. Also the linear effect of moisture content of the grains was found to be very significant since their (p) value = 0.0259. The quadratic effect of $duration\ of\ storage^2$ with (p) = 0.0110 was found to be significant since their p value was below 0.05. All the bilinear effects of the factors were found to be insignificant with the statistical model since their (p) was greater than 0.05.

Table 4.2: ANOVA for gelatinization temperature of starch of the wheat flour

Gelatinization temperature of starch, ˚C				
Source	SS	DF	MS	*P* value
Model	136.09	4	34.02	<0.0001*
Lack of fit	11.25	10	1.13	0.2156
Error	11.81	12	0.28	
Term	Estimate	Stderror	*t* ratio	*P* value
Intercept	65.38	0.37	174.36	<0.0001*
Temp.(30,50)	3.46	0.30	11.04	<0.0001*
MC (12,18)	-0.80	0.30	-2.54	0.0259*
Duration of storage (2,8)	-0.30	0.30	-1.00	0.3486
Duration of storage× duration of storage	-1.47	0.49	-3.00	0.0110*

The predictive, second order, polynomial model for the gelatinization temperature of starch of the aged wheat flour of the cultivar A.C.Walton is given in the equation 12.

$$Gelatinization\ temperature\ of\ starch\ T_g°C = 65.38 + 3.46\left[\frac{temp-40}{10}\right] - 0.80\left[\frac{MC-15}{3}\right] - 0.31\left[\frac{duration-5}{3}\right] - 1.47\left[\frac{duration-5}{3}\right]^2 \quad (12)$$

From the prediction profile, it is interesting to note that, a maximum desirability of 0.82 was obtained by the storage of wheat grains at the chosen temperature of 50˚C with a lowest

MC of 12% for a period of about 4.7 days. It was predicted from these combinations of storage, the gelatinization of starch to occur at a maximum temperature of 69.66˚C (Fig. 4.5).

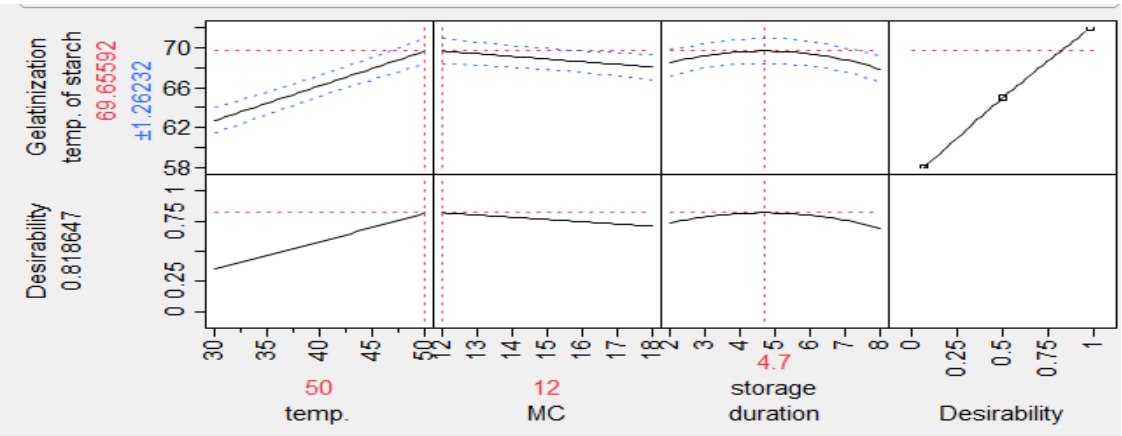


Fig.4.5: Prediction profiler for the gelatinization temperature of starch

The surface response plot of T_g of the starch explained the trend of storage temperature and MC (Fig.4.6). As the storage temperature of grains increased, the consistency of the starch improved, thus having a great effect on the gelatinization temperature. Eventually, MC of the grains also had a great impact on the starch molecules of the flour.

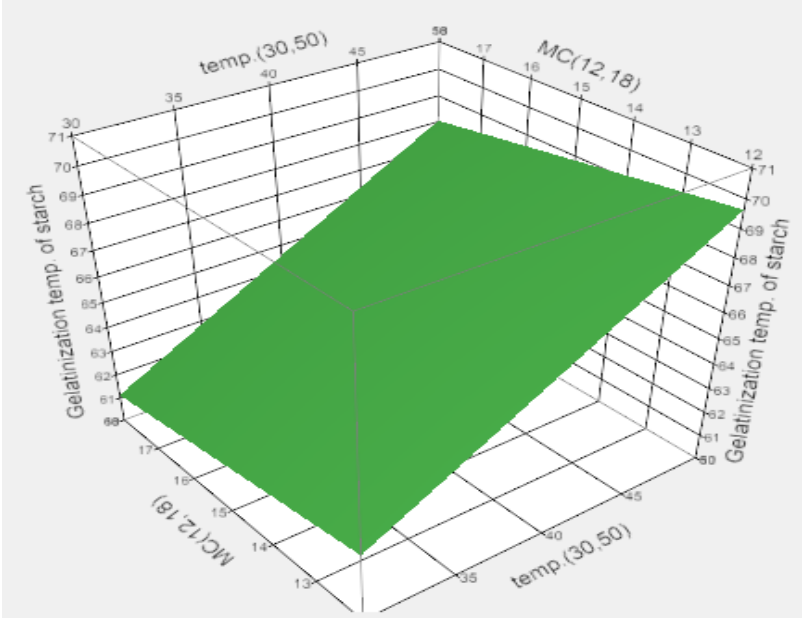


Fig.4.6: Surface response plot of T_g of starch

The best rheological property of the dough depends on the behaviour 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.

4.5. Conclusion:

The effect of accelerated aging of wheat grains on the thermal property of the flour was assessed and compared to that of freshly harvested grains. The study of thermal property of the stored wheat grains was highly beneficial to determine the rheological nature of the flour, which is highly recommended during baking. Baking properties of the dough depends on the behaviour of starch throughout the process and hence the thermal properties of the treated flour were studied.

The analysis of data obtained from statistical model, clearly explained the trend of the gelatinization temperature of starch with respect to the storage temperature of grains. The increase in the gelatinization temperature of starch is an indication of the improvement of the rheological property of the dough. Moisture content of the grains just before milling into flour also played a significant role in the baking behaviour of the starch in the dough. The studied model explained the trend of the storage condition of the grains towards the gelatinization temperature of starch and optimization of the factors studied.

Thus the accelerated aging of wheat grains by storage at higher temperatures with lower moisture contents helps enhance the baking properties of the flour.

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Chapter V

SUMMARY AND CONCLUSION

Bread remains as the staple food in almost all the Western countries. The wheat grains are in high use in the baking industries to produce various food commodities. The wheat flour milled from the grains has a unique property of components, which aids in the preparation of good quality and quantity of loaves of bread. Though there is no Universal definition for the quality of the wheat flour, it is the high concern of the bakers to produce best quality of loaves of bread.

The presence of gluten in the flour contributes to the elasticity and extensibility of the dough. The rheological property of the dough depends on the function of gluten and total starch content. The flour obtained from freshly harvested grains was found to have poor rheological attributes, since their gluten quality was unproductive resulting in sticky dough.

The possible solution to enhance the baking quality of the wheat was by storing the grains for years together by natural aging process. The aged grains had productive results in terms of the flour components. Since the process of natural aging is a time consuming work, accelerated aging of grains was chosen as an alternative solution in this study. The grains were subjected to higher temperatures in the range of 30˚C – 50˚C with a moisture content of 12-18% for a short duration of 2-8 days.

The study consisted of developing a regression model to analyse the effect of this accelerated aging on the physico-chemical as well as thermal properties of the flour. It was interesting to note that, as the temperature of storage kept increasing, the quantity of the flour components, namely starch and gluten content got affected. On the other hand, the thermal

property of the flour enhanced, which was clear from the increase in the gelatinization temperature of starch from 53˚C to 70˚C as a result of storage at 50˚C with 12% moisture content for a maximum period of 8 days.

The regression model developed from the data gave a better understanding of the effect of accelerated aging of grains on their baking qualities. Hence accelerated aging of freshly harvested wheat grains were achieved by storage of wheat grains at a maximum temperature of 50˚C for a period of 5 days with initial moisture content of 12%. This enhances both the physic-chemical and thermal properties of flour. The improvement in the rheological behaviour of the dough through this accelerated aging process by thermal treatment would be a promising boon for the bakers. Also the less time consumption of this aging process has proved to be a better alternative for the natural aging of wheat grains.

Successful commercial application of this accelerated aging process of wheat grains is possible, once optimization of the statistical model of all the parameters is performed.

Chapter VI

RECOMMENDATIONS FOR FURTHER RESEARCH

The effect of accelerated aging of wheat grains on their physico–chemical and thermal properties has been studied. It was found that the total starch content and protein content getting affected adversely as a result of storage at elevated temperatures. On the other hand, the thermal properties of the flour got improved as analysed from the gelatinization temperature of starch, which is one of the prime factors for good baking quality.

The regression model developed explained the trend of storage temperature, moisture content and duration over the flour properties. But optimization of these factors to obtain the best combination of factors is yet to be studied. Once the optimal parameters are determined using the statistical regression model, the commercial application of storage conditions for cultivar A.C.Walton can be suggested, thereby accelerating their aging process in a shorter period of duration.

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