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# WHEAT BREAD QUALITY AS INFLUENCED BY THE SUBSTITUTION OF WAXY AND REGULAR BARLEY FLOURS IN THEIR NATIVE AND HEAT-MOISTURE TREATED FORMS

by

Sandip Kaur Gill

A thesis submitted to the Faculty of Graduate Studies and Research in the partial fulfillment of the requirements for the degree of Master of Science

in

# FOOD SCIENCE AND TECHNOLOGY

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DEDICATED TO MY PARENTS

#### ABSTRACT

Barley flour from two barley cultivars, Candle (waxy starch) and Phoenix (regular starch) in their native and two heat-moisture treated (conventional pan cooking and extrusion cooking) forms was used as substitutes for wheat flour in bread at 5, 10 and 15% levels and the influence of barley flour substitution on bread quality was examined. The substitution of wheat flour with barley flours altered the bread loaf volume, color and bread crumb firmness. These changes were found to be dependent on the barley cultivar, substitution level and flour pretreatment.

In native form, Phoenix barley flour at 15% substitution produced breads with bigger loaf volume and softer crumb than Candle barley flour. However, when the barley flours were heat-treated before substitution Candle barley flour produced better quality breads in terms of loaf volume, crumb firmness and crust color than the Phoenix counterparts. The baking functionality of Candle barley flour was markedly improved when added after heat-moisture treatment. Use of Candle barley flour extruded at high temperature (130°C) and high (50%) moisture (HTHM) at 15% substitution produced breads with acceptable physicochemical properties and higher soluble (SDF) and total (TDF) dietary fiber contents than the control (100% wheat) breads. These breads can substantially contribute to the elevation the TDF content in human diet.

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# LIST OF ABBREVIATIONS

a	Redness
b	Yellowness
DE	Dextrose equivalent
DF	Dietary fiber
DP	Degrees of polymerisation
HB	Hull-less barley
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
HTHM	High temperature high moisture
HTLM	High temperature low moisture
IDF	Insoluble dietary fiber
L	Lightness
L/D	Length/diameter
LDL	Low density lipoprotein
LTHM	Low temperature high moisture
LTLM	Low temperature high moisture
RTE	Ready to eat
SCFA	Short chain fatty acids
SD	Standard deviation
SDF	Soluble dietary fiber
SEM	Scanning electron microscopy
SSE	Single screw extruder

- TDF Total dietary fiber
- TSE Twin screw extruder
- WBC Water binding capacity
- WHC Water holding capacity

## 1. INTRODUCTION AND THESIS OBJECTIVES

Hull-less barley (HB) is receiving a considerable research attention in food, feed and industrial applications. Currently, barley is mostly being used for livestock feeds and the remainder for food (raw material for malt) and other industrial applications. It is a good source of a dietary fiber,  $\beta$ -glucan, that has a number of human health benefits such as lower blood glucose responses and glycemic index, higher satiety ratings, cholesterollowering and anti carcinogenic effects. Barley is rich in tocols (42 to 80 mgKg<sup>-1</sup>) that have vitamin-E and antioxidant activity and are potent inhibitors of cholesterogenesis. The nutritional values of whole or pearled barley grains have increased its desirability among humans. There is a growing research interest in enhancing the food uses of barley and thus making fiber-enriched ( $\beta$ -glucan) products that could possibly be used as functional foods.

Baked goods are the common source of grain products. HB has been successfully used in chemically leavened products such as muffins, pancakes, biscuits and cookies. However, it is less suitable for making yeast leavened products such as bread, which is the most widely consumed source of grain product. Efforts have been made in the past to produce barley breads. However, bread is a sensitive product in which the sensory quality differences are easily noticeable. Hence, the level of substitution, usually < 5% was small to be considered for the preparation of barley bread with appreciable amount of  $\beta$ -glucan as a health benefiting functional component. Therefore, research is warranted in this area to find ways to increase barley flour substitution levels to white wheat flour without seriously impairing the bread qualities. There have been efforts to find out the acceptable substitution levels for barley flour, however, little research has been done to comparatively evaluate the effect of replacing a portion of wheat flour (white) with barley flours (from different varieties), which substantially differ in their functionality.

Extrusion is highly adaptable, cost-effective, and energy efficient technology that is most commonly used for the production of breakfast cereals, which are a good source of dietary fiber. It can lead to physical and macromolecular changes in flour components and can also improve the nutritional quality of dietary fibers. Chemical, enzymatic and physical treatments can alter the functional properties of fiber. Studies have shown that incorporation of modified dietary fibers into baked products such as cakes and cookies have improved their texture, enhanced their sensory properties and increased their shelf life. Changes in the behavior of soluble fiber in waxy and regular barley starch as a result of physical (extrusion) modification have been studied recently. However, the effects of the extrusion induced changes to barley grain components on the baking functionality of barley flour have not been reported. Also the addition of cooked barley flours [prepared by conventional pan cooking at 100°C with excess water or extrusion cooking (at different temperatures) with limited amount of water (at different levels)] and its effect on bread quality has received scant attention.

Therefore, the objectives of this thesis were:

 to replace a portion of wheat flour with a waxy and a regular barley flour, in their native and cooked states (conventional pan cooking), and to examine their effects on the bread quality characteristics such as loaf volume, crumb firmness, and crumb and crust color. 2. to replace a portion of wheat flour with a waxy and a regular barley flour, in their native and extruded (extrusion cooked under different temperature and moisture combinations) states, and to examine their effect on the bread quality characteristics such as loaf volume, crumb firmness, and crumb and crust color.

#### 2. LITERATURE REVIEW

#### 2.1. BARLEY

Barley (*Hordeum Vulgare L.*) is a grass belonging to the family Poaceae, the tribe Triticeae, and the genus Hordeum. Barley is a highly versatile crop that can be grown over a wide range of environmental conditions. It has three spikelets at each rachis node. Barley, which has all the three spikelets fertile is called six-rowed, while in two-rowed barley, only the central spikelet is fertile. Two-rowed barley contains (plump and large kernels) lower husk content than six-rowed barley (lean and long kernels). Barley grain can be hulled or hull-less. Hull-less barley (HB) is another cultivar of barley, which unlike hulled barley has naked or hull-less caryopsis (Duffus and Cocharane, 1993). It is nutritionally superior to hulled barley and has many potential applications in food (ground, pearled, steamed, boiled, baked, extruded, roasted or flaked) and malting industry (Bhatty, 1986b, i999).

#### 2.1.1. History and Utilization

Barley is one of the oldest cereals recorded in history. Archaeological studies indicate the existence of two-rowed barley cultivation by about 8000 BC in Iran and sixrowed barley cultivation around 6000 BC. However, other evidences indicate that barley (wild or domestic) was cultivated at least 17,000 years ago in the Nile River Valley of Egypt (Nilan and Ullrich, 1993). Barley was considered as an important food grain in prehistoric times. Earlier, it was first cultivated as a food crop for human consumption (Mclelland, 1983). It was used as one of the several food sources including porridge, fermented beverage and flat breads (Matz, 1991) for thousands of years until animals were domesticated and a grain supplement to forages was required (Foster and Prentice, 1987). Over the centuries, wheat and other cereals surpassed barley in the food applications (Matz, 1991). Currently, barley is being used mostly for livestock feeds and the remainder for food (raw material for malt) and other industrial applications.

#### 2.1.2. Current Production

Barley is the fourth major cereal grown worldwide after wheat, corn and rice (Nilan and Ullrich, 1993). Canada is the largest producer of barley in the world, with an average production of 13.2 Mt of barley grain estimated for the 1999/2000-crop year. This is 10% of world production, compared with 5% produced by USSR. Canada is also the world's largest barley exporter. In Canada, most of the barley is produced on the prairies (Alberta, Saskatchewan, Manitoba) with about half coming from Alberta alone (Alberta Agriculture Food and Rural Development 2000). However, in Canada, the food market for barley for human use is very small (up to 5%), while up to 75% of the total barley production is used as feed for animals (chicken, cattle, pigs) and the remaining 20% is used in malting and brewing industry (Alberta Barley Commission, Calgary, AB, personal communication, 2000).

#### 2.1.3. Barley Grain Structure

A longitudinal section of barley is given in Fig. 2.1. The structure of barley is relatively similar to that of other cereals. It consists of a one-seeded fruit called caryopsis. The hull or husk is an outer tissue of the caryopsis (kernel) and constitutes up to 10-13%



Fig. 2.1. Diagram representing a longitudinal section cut of a barley grain.

of the dry weight of barley grain. Hull-less barley has loosely attached hull that falls of during harvesting and threshing. The lemma and palea of the hull covers the caryopsis, which include pericarp, seed coat, germ and endosperm. The weight of barley kernels ranges from 32 to 40 mg. The aleurone, the outermost layer of endosperm, consists of 3 to 4 cells of thickness and white to blue in color depending on cultivars. Endosperm is the largest tissue in kernel and mainly composed of granular starch embedded in a protein matrix. As compare to wheat, barley has a softer endosperm due to which the size reduction in milling is difficult.

#### 2.1.4. Composition

A typical barley grain is composed of starch, proteins, lipids, fibers and minerals. The approximate composition of barley grain is outlined in Table 2.1. The quality of barley grain and its chemical composition (especially protein, starch and  $\beta$ -glucan concentration) are influenced by growing, environmental (temperature, day, length, water supply, availability of soil minerals) and genetic conditions (MacGregor and Fincher, 1993). However, the grain component distribution patterns remain unaffected by the aforementioned conditions (Zheng et al., 2000).

#### 2.1.4.1. Carbohydrates

Carbohydrates are the main constituents comprising 80% by weight of barley grains (MacGregor and Fincher, 1993). Starch is the major constituent of carbohydrate, accounting for up to 65% (Table 2.1). Other carbohydrate components include cell wall polysaccharides,  $(1\rightarrow3),(1\rightarrow4)-\beta$ -D-glucans ( $\beta$ -glucans),  $(1\rightarrow3)-\beta$ -glucans, arabinoxyla-

Component	% (w/w)
	(dry wt. basis)
Carbohydrates	78-83
Starch	63-65
Sucrose	1-2
Other sugars	1
Water-soluble polysaccharides	1-1.5
Alkali-soluble polysaccharides	8-10
Cellulose	4-5
Lipids	2-3
Proteins	10-12
Albumins and globulins	3.5
Hordeins	3-4
Glutelins	3-4
Nucleic acids	0.2-0.3
Minerals	2
Others	5

Table 2.1. Chemical composition of barley grain<sup>1</sup>.

<sup>1</sup>Reprinted from MacGregor and Fincher (1993), with permission.

-ns (pentosans), cellulose and a number of simple sugars and oligosaccharides (Bhatty, 1999). Sugars and cell wall polysaccharides represent more than 10% of grain weight (MacGregor and Fincher, 1993).

#### 2.1.4.1.1. Starch

Starch, major reserve polysaccharide, is a polymer of  $\alpha$ -D-glucose. It has two main components: amylose and amylopectin. Amylose consists of long chain of  $\alpha$ - $(1\rightarrow 4)$ -linked-D-glucose residues. It is a minor element of starch and has low level of branching. Amylopectin is a highly branched molecule comprising a principal fraction of starch. It is composed of  $\alpha$ - $(1\rightarrow 4)$ -linked-glucose residues interconnected through  $\alpha$ - $(1\rightarrow 6)$  bonds (MacGregor and Fincher, 1993). Depending upon the ratio of amylose to amylopectin, starch is classified as waxy (0-5% amylose), normal (25-30% amylose) and high amylose types (40-50% amylose).

Starch occurs as granules in the cytoplasm of cells that essentially remain intact during most types of processing such as milling, separation and purification of starch. Barley starch is a mixture of small- and large- granules that are oval to round in shape with diameter ranging from 2-10 and 12-26  $\mu$ m respectively (Vasanthan and Bhatty, 1996). The large and small granules of barley starches differ in their physiochemical properties such as crystallinity (X-ray diffraction relative intensities), swelling factor, amylose leaching, Brabender pasting, resistance to acids and  $\alpha$ -amylase hydrolysis. However, these differences are greater among the barley starches of different genotypes (waxy, regular and high amylose) than between the small and large starch granules of same genotype (Vasanthan and Bhatty, 1996).

The small starch granules constitute 80-90% of the total number of starch granules, but only 10-15% of the total starch weight. On the other hand, large granules smaller in number (10-20%) contribute 85-90% of the total weight of starch (MacGregor and Fincher, 1993). Large granule starches have higher degree of crystallinity than the small granule starches (Vasanthan and Bhatty, 1996). Compared to large granules, small starch granules are hydrolyzed at a faster and greater extent (Morrison et al., 1986). The swelling factor of small granules is higher than the large granules for regular and waxy barley and lower for high amylose barley (Vasanthan and Bhatty, 1996). They also have higher amylose content especially true amylose, but lower apparent amylose, than the large granules (Szczodark and Pomeranz, 1991; Vasanthan and Bhatty, 1996).

#### Heating Starch in Water

Starch when heated in water undergoes several changes that are responsible for its unique character in the food products. When placed in cold water, starch granule hydrates and holds about 30% of its dry weight as moisture. The granule swells slightly and the change is reversible (Hoseney, 1994). However, starch granules when heated in presence of water, absorbs water, swells irreversibly and increases the viscosity. Further heating results in distortion of starch granule, loss of birefringence, loss of granule crystallinity and increase in starch solubility. This phenomenon is known as gelatinization. The temperature at which the granule begins to swell rapidly and loses its birefringes is called gelatinization temperature. Starch gelatinizes over a range of temperatures (i.e. 56-62°C) and is normally affected by the type of starch (Hoseney, 1994). Gelatinization temperature, swelling power and viscosity producing potential of barley starches influences the food uses of barley flour. Amylose and the associated lipid content suppress the granule swelling (Tester and Morrision, 1990). Waxy barley starches, due to their lower levels of amylose and lipids, swell to a greater extent than the normal starches (Lorenz, 1984; Morrision et al., 1986).

#### Pasting

Continuous heating of starch after gelatinization results in more starch solublization that increases the viscosity and forms paste. Further cooking reduces the paste viscosity because of granular disruption. This phenomenon is known as shear thinning. It is an important property of starch paste and is higher for more soluble starches. On cooling there is a rapid increase in viscosity referred as setback (Hoseney, 1994).

The peak viscosity of barley starches differs considerably among different genotypes. In general hull-less varieties have higher peak viscosity than the covered types (Georing et al., 1970). Waxy barley starch has very high paste viscosity followed by regular and high amylose barley starch. Moreover, the pasting temperature is considerably lower (Fig. 2.2) than other barley starches (Vasanthan and Bhatty, 1996). High amylose starches have maximum shear stability, as indicated by progressive increase in viscosity, during heating and holding periods. However, in waxy and regular barley starches the viscosity increases to maximum viscosity and then breaks down. The loss of viscosity is higher in waxy barley starch (Gudmundsson and Eliasson, 1992; Vasanthan and Bhatty, 1996).



Fig. 2.2. Viscoamylographs of small- (top) and large- (bottom) granule starches from SB 89528 (waxy), Condor (regular), and Glacier (high-amylose) barleys. Reprinted from Vasanthan and Bhatty (1996), with permission.

#### **Retrogradation**

During starch gelatinization/pasting, amylose leaches out of the starch granules. Upon cooling amylose and linear fraction of amylopectin reassociate by hydrogen bonding to form a three-dimensional network, which traps water. Continuous reassociation between the starch chains result in syneresis i.e. separation of starch and water. Overall the process of reassociation is known as retrogradation (MacGregor and Fincher, 1993; Hoseney, 1994). Starch retrogradation is considered as the main reason for staling of breads and other baked products. Amylose reassociates rapidly, where as amylopectin reassociate slowly with time. Waxy barley starch has higher enthalpies and gelatinization temperatures than the normal barley starch (Gudmundsson and Eliasson, 1992; Lorenz, 1984). Despite of the slower retrogradation rate during the initial days of storage, waxy barley starches have been found to retrograde to a greater extent than the normal barley starches (Gudmundsson and Eliasson, 1992). Retrogradation in starch gels is controlled by the amount of water present in the gel. (Zeleznak and Hoseney, 1986).

#### 2.1.4.1.2. Non-starch Polysaccharides

Apart from starch, the main polysaccharides found in barley grain are:  $(1\rightarrow 4)$ - $\beta$ -D-glucans (cellulose),  $(1\rightarrow 3)$ , $(1\rightarrow 4)$ - $\beta$ -D-glucans ( $\beta$ -glucans),  $(1\rightarrow 3)$ - $\beta$ -glucans, arabinoxylans and glucomannans (Table 2.1) (Duffus and Cochrane, 1993).  $\beta$ -Glucans and arabinoxylans are the major constituents. Barley contains 4-5% of cellulose (Table 2.1) concentrated in the cell walls of aleurone testa tissues. It is a linear structural polysaccharide in plants and is associated with lignin as well as other polysaccharides. Cellulose along with lignin and resistant starch is characterized as insoluble dietary fiber. Arabinoxylan content in barley grain ranges from 4-7% by weight. These are noncellulosic polysaccharides concentrated mostly in the cell walls of aleurone and endosperm. They have  $(1\rightarrow 4)$ - $\beta$ -xylopyransol as a backbone, with some xylosyl residues substituted with arabinose (mono, di, or oligo) residues (MacGregor and Fincher, 1993). The high viscous solution forming ability of arabinoxylan markedly affects the end-use potential of barley in food, feed and industrial applications.

#### β-Glucan

β-Glucan is the major soluble dietary fiber component of barley grain. Compared with other cereals, barley has higher levels of β-glucan. Barley varies in its β-glucan content ranging from 4-6% in normal barley and 6-8% in waxy hull-less barley (Fastnaught et al., 1996; Bhatty, 1999). Few studies reported β-glucan content of waxy HB to be >6% (Ullrich et al., 1986; Oscarsson et al., 1996; Lee et al., 1997). However, some varieties of waxy HB such as Arizona and waxy hulled barley such as Prowashonupana may contain upto 11% and 17.4% of β-glucan respectively (Miller and Fulcher, 1994; Bhatty, 1999). β-Glucan content ranges from 4.3% to 11.3% in barley, from 3.9% to 9.0% in flour, and from 4.9% to 15.4% in bran (Bhatty, 1992). Genetic and environmental factors affect the level of β-glucan content in barley (Fastnaught et al., 1996; Perez-Vendrel et al., 1996; Lee et al., 1997). Waxy hull-less cultivars generally have higher level of β-glucan than the normal cultivars (Ullrich et al., 1986; Fastnaught et al., 1996).

#### Structure

 $\beta$ -Glucans [(1 $\rightarrow$ 3),(1 $\rightarrow$ 4)- $\beta$ -D-glucan] are linear, unbranched, mixed linkage non-starchy polysaccharide comprising of mainly cellotriosyl and cellotetraosyl units of (1 $\rightarrow$ 4)-linked  $\beta$ -glucosyl residues (~70%) separated by single (1 $\rightarrow$ 3)-linked glucose residues (~30%) (Fig. 2.3). Its degree of polymerization goes up to 1,400 glucose residues. The presence of mixed linkages give rise to less compact and more watersoluble structure of  $\beta$ -glucan (Bamforth, 1982). A simple structure of  $\beta$ -glucan is shown below where G represents  $\beta$ -glucosyl residues and (1 $\rightarrow$ 3),(1 $\rightarrow$ 4)-linkages are shown by 3 and 4, respectively (MacGregor and Fincher, 1993).

# G3G4G4G4G3

The structure of barley  $\beta$ -glucan contains approximately one-fourth of  $(1\rightarrow 3)$ -linked cellotetrasyl units, whereas oat  $\beta$ -glucan comprises of one-third of this structural unit (Wood et al., 1991).

#### Distribution of $\beta$ -Glucan

As reported by Miller and Fulcher (1994), barley grains had a more uniform distribution of  $\beta$ -glucan throughout the endosperm than the oats that have high subaleurone concentrations of  $\beta$ -glucan. However, Zheng et al. (2000) showed that  $\beta$ -glucan distribution varies according to variety. Low  $\beta$ -glucan HB had higher concentrations in subaleurone region, whereas high  $\beta$ -glucan HB had greatest and more uniform distribution of  $\beta$ -glucan in the endosperm.





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#### 2.1.4.2. Proteins

Protein content of barley varies from 10-12% (Table 2.1) depending upon the environmental conditions and genotypes. Protein, being non-uniform in distribution throughout the endosperm, is more concentrated at the endosperm periphery than its center. Aleurone cells are also rich in protein (MacGregor, 1998). Depending on the function, barley proteins are separated into two groups: non-storage and storage. Albumin (water-soluble) and globulin (salt soluble) are non-storage proteins that function as structural and metabolic (i.e. enzymes) proteins. Storage proteins include the alcohol soluble prolamin (Hordein) and acid/base soluble glutelins. Barley proteins are rich in prolamins. However, the contents of essential amino acid lysine and threonine are very limiting (Foster and Prentice, 1987; Shewry, 1993).

#### 2.1.4.3. Lipids

Barley contains about 2-3% of lipids (Table 2.1). Lipids are the important components of cereals. They are present in free or bound state. They can also be classified as polar or non-polar lipids. Whole barley lipids contain 67-78% non-polar lipids, 8-13% glycolipids and 14-21% phospholipids. Most of the lipids are present in germ, followed by aleurone layer and bran. Starch granules contain traces of lipids part of which remain as complexed (bound) with amylose (Morrison, 1993).

#### 2.1.4.4. Vitamins and Minerals

Barley is a good source of B vitamins. However, it lacks vitamin A, C and  $B_{12}$ . It is rich in vitamin E (Foster and Prentice, 1987). Among cereals, barely is one of the best

sources of tocols (tocotrienols and tocopherol). It contains 42 to 80 mg/kg of tocols concentration that have vitamin E and antioxidant activity (Morrison, 1993; Peterson and Qureshi, 1993). Barley contains all eight isomers of tocols ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  tocotrienols and  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  tocopherols) (Qureshi et al., 1986; Wang et al., 1993a; Bhatty, 1999), amongst which  $\alpha$ -tocotrienol and  $\alpha$ -tocopherol are the most predominant isomers (Peterson and Qureshi, 1993). Mineral level in barley grain varies depending upon the cultivar (Foster and Prentice, 1987). Barley contains 2-3% ash content (Table 2.1), which is mainly composed of phosphorous (P), potassium (K), magnesium (Mg) and calcium (Ca). It contains small amount of sodium (Na), iron (Fe), zinc (Zn) and other trace elements. Up to 50-80% of P in barley is present in the form of phytic acid (inositol hexaphosphate) and remains unavailable to monogastric animals (Foster and Prentice, 1987).

# 2.1.5. Nutritional Importance of Barley

Barley is a nutritious grain. It is a rich source of soluble dietary fiber,  $\beta$ -glucan, that has various health promoting properties (Klopfenstein, 1988). The cholesterol lowering effects of barley have been demonstrated in both animal (Klopfenstein and Hoseney, 1987; Newman et al., 1992; Wang et al., 1992; Kahlon et al., 1993; Wang, 1997) as well as human studies (Newman et al., 1989b; McIntosh, 1991; Lupton, 1994; Lia et al., 1995).

Klopfenstein and Hoseney (1987) reported that rats fed with  $\beta$ -glucan enriched breads had lower serum cholesterol and liver cholesterol levels. Hence, they suggested that  $\beta$ -glucan enriched baked products could be useful in the dietary control of blood cholesterol levels. Wang et al. (1992) reported that chicks fed with barley diet had the lowest total and LDL (low density lipoprotein) cholesterol (3.15 mmol/L and 1.06 mmol/L, respectively) compared to corn diets (control) that had the highest total and LDL cholesterol (4.34 mmol/L and 2.04 mmol/L, respectively). In the chicks and rats fed study, significant cholesterol lowering (total and LDL) effects of barley meals were reported and the soluble  $\beta$ -glucan was suggested to be the strongest predictor of the serum cholesterol response in both species (Newman et al., 1992). Kahlon et al. (1993) reported that  $\beta$ -glucan enriched barley fraction due to its viscous properties resulted in a significant reduction in liver cholesterol in Hamsters. Wang et al. (1997) reported the hypocholesterolemic effect in hamsters fed with barley  $\beta$ -glucan.

In human studies (Newman et al., 1989ab; McIntosh et al., 1991), consumption of barley relative to wheat foods resulted in a significant decrease in plasma cholesterol (6%), and in LDL cholesterol (7%). Lupton et al. (1994) reported that the addition of barley bran flour (30 g/day –70% DF) decreased the total serum cholesterol by 7.7%, and the LDL cholesterol by 6.5% comparative to the diets containing 20 g of cellulose (100% DF). Barley is rich in tocols especially  $\alpha$ -tocotrienol that act as an inhibitor of cholesterogenesis (Qureshi et al., 1986).  $\beta$ -Glucan interferes with the sterol metabolism by increasing the cholesterol excretion and thus lowers the blood lipid concentrations. The excretion of cholesterol in  $\beta$ -glucan rich (13 g/day) barley diet was found to be higher than in oat bran diets (3.8 g  $\beta$ -glucan/day treated with  $\beta$ -glucanase) and wheat diets (1.2 g  $\beta$ -glucan/day) (Lia et al., 1995).

The hypocholesterolemic effect of barley is mainly due to its water-soluble fraction of  $\beta$ -glucan that results in increased viscosity and higher absorption of nutrients in the small intestine (McIntosh et al., 1991). Other studies (Anderson and Bridges, 1982;
Chen et al., 1984; Cummings and Branch, 1986) suggest mechanisms that involve colonic bacterial fermentation of the soluble fibers to short chain fatty acids (SCFA), such as butyric, propionic and acetic acids, which inhibit cholesterogenesis. Further, it was evidenced that higher concentrations of SCFA (especially butyric acid) in colon have a protective effect with regard to colon cancer in human (Kruh, 1982; Klopfenstein, 1988). The insoluble dietary fiber (IDF) from barley (spent barley grain) was found to be more effective at preventing dimethylhydrazine induced gastrointestinal tumors than soluble fiber rich barley bran (McIntosh et al., 1993). The hypoglycemic effects of barley have been studied as well. All the barley products resulted in reduced glycemic index values, higher satiety ratings and lower blood glucose responses than the relative wheat counterparts (Granfeldt et al., 1994; Yokoyama et al., 1997).

## 2.1.6. Pearling

Pearling is a primary process in barley flour milling. It produces dehulled, pot and pearled barley and barley flour. Pearling involves the removal of outer layers of barley grain (pericarp, seed coat, aleurone, subaleurone and parts of endosperm) and germ by abrasive action of stone mill. A pearler generally composed of 6-8 abrasive carborundum or emery coated disks, which revolves at a high revolution (about 450 rpm) within a perforated cylinder or closed chamber (Leonard and Martin, 1963). The outer layers of grain are removed uniformly. The pearling process involves cleaning, conditioning, tempering (~15% moisture), bleaching, dehulling, aspiration, sifting, groat cutting, pearling, sifting and polishing (Bhatty, 1993b).

Pearling is carried out to various degrees and depending upon the range (12-55%, dry grain wt. basis), barley products can be classified as dehulled, pot or pearled. Pearling of barley to a degree of 30-40% is most desirable to ensure its bright color and stability in barley based foods. The outer layers of the barley grain contain a lot of phenolic compounds that may add undesirable flavor the barley product. Also, the germ is rich in unsaturated fatty acids that are highly prone to auto-oxidation and subsequent rancid odor development. Therefore, the storage stability and overall quality of pearled barley due to the removal of outer grain layers and germ is superior to that of unpearled barley (Vasanthan, 1999). Pearling decreases insoluble fiber content, whereas the soluble fiber and  $\beta$ -glucan content gradually increases (Bhatty, 1993b). The degree of pearling has different meaning in different places. For example, "30% pearled" in North America signifies 30% pearling flour and 70% pearled grain. However, in places like Japan pearled barley is used at higher levels, "70% pearled" means 70% pearled grain and 30% pearling flour.

## **2.2. DIETARY FIBER**

The importance of high-fiber diet, with wheat bran as a laxative was recognized by Hippocrates in as early as 400 BC (Colomey, 1978). It was considered as "roughage", an abrasive component of the diet, that should be replaced by food of low fiber content whenever possible (Vratanina and Zabik, 1980). However, an extensive research on dietary fiber proved its nutritional benefits indicating fiber as an important component of human diet. Nutritionist recommends 20-30 g of fiber per person per day. However, dietary fiber intake in western countries is estimated to be 11 g of fiber per day, which is far below the recommended levels (U. S. Food and Drug Administration, 1998).

## 2.2.1. Definition and Composition of Dietary Fiber

Dietary fiber (DF) may be defined as "oligosaccharides, polysaccharides and the hydrophilic derivatives (including lignin) which cannot be digested by human digestive enzymes to absorbable components in the upper alimentary tract" (Thebaudin et al., 1997). They are a combination of chemically heterogeneous substances (Fig. 2.4) that includes structural polysaccharides (celluloses, hemicelluloses,  $\beta$ -glucan and pectins) associated with plant cell walls; structural non-polysaccharides (lignin); and other non-structural polysaccharides such as secreted gums (gum arabic), reserve gums (bean gums, guar gums) and polysaccharides from sea weeds or bacteria (Gillis and LeBlanc, 1991; Thebaudin et al., 1997).

Based on the solubility in water and the proportion of soluble and insoluble components, DF can be classified as soluble (pectins, gums and mucilages) and insoluble (cellulose, lignins and some hemicelluloses) fiber (Gillis and LeBlanc, 1991; Thebaudin et al., 1997). Vegetables, wheat and most other grains are rich in insoluble fiber, whereas fruits, oat, barley and legumes contain higher proportions of soluble fiber (Gillis and LeBlanc, 1991).

#### 2.2.2. Properties of Dietary Fiber

Dietary fiber possesses a range of properties depending upon their nature and source of origin. DF owing to its unique physiochemical and functional properties such as





water binding capacity (WBC), cation-exchange capacity, particle size, density (bulk volume), adsorption of organic molecules, microbial degradation, viscosity and gelation, are associated with a number of food applications and physiological benefits (Dreher, 1987a). Insoluble fibers have strong WHC (water holding capacity i.e. the quantity of water bound to fibers without the application of external suction force), and can swell and absorb up to 20 times their weight in water. Soluble fibers (referred to as hydrocolloids) form gel network and bind water. They are known for their high WBC (i.e. the quantity of water that remains bound to the hydrated fibers following the application of external suction force) (Thebaudin et al., 1997). WBC of dietary fiber is influenced by its chemical composition (higher for gums and hemicelluloses, and lower for cellulose and lignin), particle size, pH, ionic strength and preparation conditions. Reduction in particle size increases the density, and reduces the WBC and bile salt binding capacity (Dreher, 1987a). Hydrocolloids such as gums are used primarily for viscosity, thickening (in soups, gravies, bakery filling) and gelling (jams, jellies, preserves, puddings) applications. They are also used as stabilizers (salad dressings), adhesives (bakery glaze) and binding agents (sausages) (Dreher, 1987a).

Physiological properties of DF are associated with prevention of certain diseases. DF is associated with weight control and act as natural barrier to excess energy intake (Dreher, 1987b). Fiber can prevent constipation by restoring normal bowel function. It helps to increase stool weight, produce softer and more bulky stool, and reduce gastrointestinal time (Story and Kritchevsky, 1976; Schrijver et al., 1992). Gastrointestinal disorders (gallstone and appendicitis), diverticular diseases, diarrhoea, irritable bowel syndrome and duodenal ulcer can be prevented or cured with high fiber diet (Dreher, 1987b; Kritchevsky, 1999).

Fibers have protective effect against colonic cancer. Colonic bacteria can produce carcinogens either by metabolizing dietary substrates or from secretions produced in response to diet. Fecal bile acids were suggested as possible carcinogens. Prolonged transit time increases the degradation of bile acids to carcinogens (Eastwood, 1987). The bulking effect of fibers dilutes oncogenic potential of carcinogens by reducing their interactions with the intestinal mucosa and hence prevents colonic cancer (Mitchell and Eastwood, 1976; Eastwood, 1987). Dietary fibers in colon are metabolized or fermented to produce short chain fatty acids (SCFA) (acetic, propionic and butyric acid) and gases (hydrogen, carbon dioxide and methane). Butyrates have anti-tumor properties (Kruh, 1982) and propionates are implicated in lipid and glucose metabolism (Anderson and Bridges, 1982; Chen et al., 1984). Fermentation of dietary fibers from different sources produces different proportions of SCFA. Compared with fruit dietary fiber (pear, apple and fig), the dietary fiber from cereal sources (oat and soy) produce higher amounts of propionate and butyrate (Casterline et al., 1997).

Dietary fibers have been studied extensively for their cholesterol lowering effects (Klopfenstein and Hoseney, 1987; Newman et al., 1992; Wang et al., 1992; Kahlon et al., 1993; Wang et al., 1996; Wang, 1997; Newman, 1989; McIntosh, 1991; Lupton, 1994; Lia et al., 1995). They tend to lower total serum and LDL cholesterol in blood, whereas high density lipoprotein (HDL) cholesterol remains unaffected (Newman, 1989; Newman et al., 1992; Lupton, 1994). It was suggested that the binding of bile salts by fiber prevented the micellar formation, essential for cholesterol absorption which in turn lead

to cholesterol excretion from the body (Story and Kritchevsky, 1976). Soluble dietary fibers such as oat gum (80%  $\beta$ -glucan) delays glucose absorption, lowers post prandial plasma glucose and insulin concentrations in humans (Braaten et al., 1991).

Along with the potential health benefits of consuming dietary fiber, there have been concerns about the nutrient (mineral and vitamin) availability. Chelation of nutrients with dietary fibers has a possibility for reducing mineral and electrolyte absorption in the body. The effect of fiber on mineral bioavailability depends mainly on the kind of fiber, level of fiber intake, level of mineral intake and presence of mineral binding agents such as phytic acid and oxalic acid (Kelsay, 1982). Interactions between the above given components influences the calcium, magnesium, phosphorous, zinc and iron balances (decreases absorption) have been studied. With increase in fiber (cellulose) in diet, calcium and magnesium balances decreased in few studies, while they remained unaffected in others. Hemicellulose decreased the magnesium balance whereas pectin had no effect on calcium and magnesium balance (Kelsay, 1982). The detrimental effects of wheat and corn fibers on iron and zinc absorption have been shown by many studies while others have proved their non-inhibitory function. Hence, the effect of dietary fiber on mineral bioavailability continues to remain a controversial issue (Thebaudin et al., 1997). Compared to minerals, limited research has been carried out on the effect of DF on vitamin bioavailability. The effect of fiber is highly variable for vitamins. It may encourage riboflavin absorption, but on the other side it may cause deficiency of pyridoxine and vitamin  $B_{12}$ . It also interferes with the availability of vitamin A, D and E, whereas, niacin and pantothenic acid remains unaffected (Munoz, 1982; Dreher, 1987c).

# 2.2.3. Applications of Fibers in Baked Products

Numerous high fiber ingredients are available to the food industry (Table 2.2) and their functionality have been tested in various baked products as listed below.

Product	Fiber Source	Reference	
Biscuits	Wheat bran	Leelavathi and Rao, 1993	
Breads	Cellulose, wheat	Pomeranz et al., 1977	
	bran and oat hulls	<b>,</b>	
	Alpha-cellulose	Volpe and Lehmann, 1977	
	Brewers' spent grain	Prentice and D'Appolonia, 1977	
	Hull-less barley fractions	Bhatty, 1986a	
	Rye flour	Kuhn and Grosch, 1989	
	Barley bran flour	Chaudhary and Weber, 1990	
	Waxy hull-less barley	Berglund et al., 1992	
	Potato pulp	Nebensy, 1995	
	Barley shorts	Newman et al., 1998	
Cakes & doughnuts	Powdered cellulose	Ang and Miller, 1991	
Cookies	Wheat bran	Vratanina and Zabik, 1980	
	Oat fibers	Dougherty et al., 1988	
	High fiber wheat flour	Ranhotra et al., 1991	
	Powdered cellulose & soy fiber	Bullock et al., 1992	
Muffins	Oat bran, rice bran	Hudson et al., 1992	
	Potato peels	Arora and Camire, 1992	
	Barley fiber factions	Newman et al., 1998	
RTE cereals	Barley	Berglund et al., 1994	

Fibers act as bulking agents and it serves to reduce the calorie content in food (Burge and Duensing, 1989). The bulking nature and water retention properties of dietary fiber results in the formation of low calorie soft-type cookies that retains more moisture after baking and require lesser force to break than the control (Bullock et al., 1992;

Fiber course	
Fiber source	Amount of fiber (g)/100g
Whole wheat flour <sup>1</sup>	12.6
Rye flour <sup>1</sup>	
Dark	12.8
Medium	4.5
Light	3.0
Wheat bran <sup>1</sup>	42.4
Corn bran <sup>1</sup>	84.6
Oat bran <sup>1</sup>	22.0
Soy fiber <sup>1</sup>	75.0
Pea fiber <sup>1</sup>	45.0
Rice bran <sup>1</sup>	21.7
Purified cellulose <sup>1</sup>	96-98
Barley (high $\beta$ -glucan) fraction <sup>2</sup>	42.1
Hull-less barley bran <sup>3</sup>	16-24

Table 2.2. Various high-fiber ingredients available to the food industry.

Modified from <sup>1</sup>Gillis and LeBlanc (1991), <sup>2</sup>Hudson et al (1992), and <sup>3</sup>Bhatty 1995.

Dougherty et al., 1988). Compared to commercial muffins, the caloric content in oat bran and barley  $\beta$ -glucan muffin was reduced to 20-30% and about 6% in rice bran muffin. Total  $\beta$ -glucan content in oat bran and barley muffin was four to five times higher than commercial muffin (Fig. 2.5). High fiber muffins contained more moisture, protein and ash content (Hudson et al., 1992). Higher volumes and improved textures were observed in cakes made with addition of powdered cellulose. The sensory evaluations of cakes made with powdered cellulose were rated better than control. Doughnuts made with dietary fiber (cellulose) had increased moisture content, greater volume and pliability, and reduced fat content (Ang and Miller, 1991). The sensory scores of the barley fiberenriched products (except for muffin) and the physical measurements (except for bread) were rated similar to the standard version (Table 2.3) (Newman et al., 1998). The addition of fibers to baked products alters the physical and sensory properties of food such as the texture, volume, spreadability, color and flavor. These effects depend greatly on the type of fiber chosen and its addition levels (Arora and Camire, 1994). For instance, muffins prepared with potato peels imparted gritty mouthfeel, off flavor and off color and had lower sensory scores (Arora and Camire, 1994). However, muffins prepared with oat bran and barley  $\beta$ -glucan had positive sensory effects (Hudson et al., 1992). Breads made with 15% of various fiber ingredients had more moisture and lesser calories than the control (Chaudhary and Weber, 1990).

## 2.2.4. High Fiber Breads

Bread continues to be the most widely consumed source of grain products (Connard, 1999). It supplies a significant portion of most nutrients required in human



Fig. 2.5. Total and soluble dietary fiber (TDF, SDF) and  $\beta$ -glucans in most highly rated muffins. Reprinted from Hudson et al.(1992), with permission.

Product	Standard	Fiber-enriched	
Sensory evaluation <sup>a</sup>	·		
Bread	$6.8 \pm 1.2$	$6.6 \pm 1.8$	
Muffin	$6.7 \pm 1.6$	$7.5 \pm 1.2$	
Biscuits	$5.3 \pm 2.1$	$5.3 \pm 1.9$	
Cookies	$6.3 \pm 1.4$	$6.6 \pm 2.0$	
Physical measurement			
Bread, vol (cm <sup>3</sup> )	799	570	
Muffin, vol (cm <sup>3</sup> )	84	78	
Biscuits, height (cm <sup>3</sup> )	3.2	2.9	
Cookies, width/ thickness (mm)	59/8.9	59/8.5	

**Table 2.3.** Comparison of sensory evaluation and physical measurements of standard and fiber-enriched baked products<sup>1</sup>.

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<sup>a</sup> Overall quality, based on product hedonic values. <sup>1</sup>Modified from Newman et al., 1998. diet. Supplementation of bread with vitamins, minerals, proteins and amino acids (lysine) have played an important role in either preventing or recovering from diseases like atherosclerosis, carcinogenicity, obesity, etc (Wehrli and Pomeranz, 1968). The consumer awareness about the importance of fiber in diet increased the demand of high-fiber foods. Most of the research for use of fibrous material in bakery foods was focussed on bread because it is a sensitive product in which the differences are easily noticeable (Colmey, 1978).

#### 2.2.4.1. Effect of Fiber Addition

Baking functionality of high-fiber breads made from different cereal sources (wheat, oats, barley, rye, soybean and corn) have been studied in the past (Pomeranz et al., 1977; Prentice and D'Appolonia, 1977; Volpe and Lehmann, 1977; Shogern et al., 1981; Bhatty, 1986a; Swanson and Penfield, 1988; Burge and Duensing, 1989; Kuhn and Grosch, 1989; Chaudhary and Weber, 1990; Nebensy, 1995; Newman et al., 1998; Porter and Skarra, 1999). The addition of more than 10% of any fibrous material leads to deleterious changes in bread quality (loaf volume, crumb grain, color and flavor). Lower loaf volume, lower gas retention, increased firmness, impaired crumb grain texture, slightly darkened crumb color, altered flavor of bread are some of the changes brought about by fiber addition (Prentice and D'Appolonia, 1977; Pomeranz et al., 1977; Volpe and Lehmann, 1977; Dubois, 1978; Bhatty, 1986a; Chaudhary and Weber, 1990).

The reduction in loaf volume is attributed to dilution of functional gluten proteins, which causes weakening of cell structure. The fibrous materials cut the gluten strands that reduce the gas retention of dough (Pomeranz et al., 1977; Dubois, 1978). In general, as the level of fiber addition increases, the volume decreases whereas water absorption and mixing times of dough increases (Table 2.4). The fibrous materials from different sources vary in their effects on loaf volume, mixing times and water absorption. At 15% substitution level water absorption in oat hulls was reduced, whereas in bran and cellulose it was increased by 4% and 10% respectively. Mixing times for oat hulls were increased by little, cellulose increased them considerably and wheat bran had no consistent effect (Pomeranz et al., 1977). Compared to other fiber-substituted breads, alpha-cellulose required highest amount (78%) of water (Chaudhary and Weber, 1990). The deleterious effects of fibers on loaf volume and crumb grain were minimized by adding vital wheat gluten in combination with surfactants (diacetyl tartaric acid, ethoxylated monoglycerides, lecithin, sucrose monopalmitate and sodium stearoyl-2-lactylate) and shortenings (Shogern et al., 1981).

There have been efforts to increase the fiber content in breads by using various formulations and altering the processing techniques. Volpe and Lehmann (1977) replaced 10% of wheat flour (supplemented with vital wheat gluten) by alpha-cellulose and found that the overall quality of bread was lower than the control. Bhatty (1986a) reported that 10% of barley flour could be added to wheat flour without seriously affecting the bread quality. Swanson and Penfield (1988), by altering the salt level (2%), were able to incorporate 20% of barley flour into bread without significantly affecting the overall quality. However, in their study the experimental breads containing 20% barley, 30% whole wheat and 50% bread flour were compared to the control that contained 50:50 whole wheat: bread flour. When used at 15% substitution levels the baking performance of barley bran flour outperformed other fiber (corn bran, oat bran, soy bran, cellulose and

Source of fiber	Loaf volume	Mixing	Water	References
	(cm <sup>3</sup> )	times (min)	absorption (%)	
Control	973	3.52	64.2	Pomeranz et al., 1977b
Celluloses	751	6.00	74.6	
Wheat Bran	779	3.52	68.2	
Oat hulls	773	4.37	62.5	
Control	1077	4.22	67.0	Shorgen et al., 1981
White wheat bran	880	4.45	75.0	1901
Corn bran	858	5.07	74.3	
Soy bran	848	5.30	82.3	
Coconut residue	671	10.45	84.4	
Control	845	3.2	*	Bhatty, 1986
Barley flour	653	3.3	*	
Control	Specific volume (%) of control	4.5	69.0	Chan the second
Collubi	100	4.3	69.0	Chaudhary and Weber, 1990
Barley bran	92	5.0	72.5	-
Alpha-cellulose	61	6.5	78.0	
Corn bran	59	5.5	74.0	
Oat bran	75	4.5	72.0	
Soy bran	59	5.5	75.0	
Wheat bran	76	5.5	71.0	
Whole wheat flour	73	7.0	70.0	

Table 2.4. Characteristics of fiber enriched breads baked with 15% addition of various fiber ingredients.

whole-wheat flour) ingredients in terms of increased dietary fiber, highest loaf volume and maximum quality score (Chaudhary and Weber, 1990). Nebensy (1995) reported that only 5% fiber from potato pulp could be added to bread without affecting its organoleptic and physiochemical properties.

Due to potential health benefits a lot of research is focused on addition of soluble fiber in food products. Besides the nutritional importance, dietary fibers are also being explored for their textural, stabilizing and anti-staling properties (Wrathesen, 1995; Thebaudin et al., 1997). Fibers are modified by chemical (Gould et al., 1989), enzymatic (Thebaudin et al., 1997) and physical (Gaosong and Vasanthan, 2000) treatments and their behavior in baked products is under further investigations.

# 2.3. STALING OF BREAD

The term 'staling' refers to the changes that occur in bread after baking other than those associated with spoilage by microorganisms (Kim and D'Appolonia, 1977a). Baked products being perishable in nature undergo various physiochemical and sensory changes and stale upon storage. The various changes that take place during staling are general loss of flavor, firming of crumb, increase in crumbliness and opacity of crumb, increase in starch crystallinity, (Knightly, 1977) and decrease in water absorption capacity as well as starch solubility. Among these the prime change is firming of bread crumb (Kim and D'Appolonia, 1977a).

#### 2.3.1. Role of Starch

Starch plays an important role in bread firming. Bread staling is basically characterized by retrogradation of starch in crumb. Earlier, the retrogradation of the linear fraction of starch (amylose) was assumed to be the prime cause of staling (Knightly, 1977; Kim and D'Appolonia, 1977a). However, based on the water-soluble starch extracted from bread crumb, Schoch and French (1947) concluded that bread staling was primarily due to amylopectin recrystallisation and amylose being already in inert retrograded form had no influence on staling. During baking wheat starch granules swell, amylose partially dissolves and leach out into the surrounding aqueous medium, and amylopectin dilates (Fig. 2.6). However, the amount of swelling is restricted due to limited amount of water in bread. By the time the loaf is baked, the linear molecules associate in the interstitial water present between the starch granules and results in the formation of firm and permanent gel network. Hence, the firming of crumb during storage is attributed to physical changes in amylopectin fraction (such as folding up and association of dilated branches) within the swollen granules (Osman, 1975). However, the results of Kim and D'Appolonia (1977) and Ghaisi et al. (1984) indicated that amylose does contribute to staling during the first day of storage (but not for breads stored for longer periods), even though majority of it is retrograded during baking and subsequent cooling. Nevertheless, amylopectin retrogradation is considered as a prime cause of staling. Amylose, because of its rapid retrogradation, is believed to be responsible for setting bread crumb structure. Breads made with 100% waxy barley starch collapsed upon cooling (Hoseney et al., 1978) or shrinks excessively (Ghaisi et al., 1984). Breads and cakes made with waxy barley starches were not of acceptable quality



**Fig. 2.6.** Role of starch (amylose and amylopectin) during baking and aging of bread. Modified from Osman 1975.

(Lorenz, 1995), indicating the importance of amylose. During baking starch granules absorb water and swell. They are separated from each other by a continuous matrix of protein. Starch and protein remain separated in dough with starch being embedded in a protein matrix (Bechtel et al., 1978). However, during baking interactions (cross-links) occur between starch and gluten (Fig. 2.7) (Bechtel et al., 1978; Martin et al., 1991). Bread firming results from cross-links between gluten (continuous phase) and remnants of starch granules (discontinuous phase). The crumb loses kinetic energy during staling and interactions (hydrogen bonds) increase in number and strength. The degree of starch swelling plays an important role in bread firming. Higher degree of starch swelling results in increased starch solublization and more surface area exposed to gluten. This results in greater and stronger cross-links with protein and increase bread firming (Martin et al., 1991; Inagaki and Seib, 1992). Dextrins of intermediate size interfere with these cross-links between starch and protein (Fig. 2.8), and reduce the bread firminess (Martin and Hoseney, 1991).

Barley starch was found nearly equaled to wheat starch for bread making (Hoseney et al., 1978). Recrystallization of amylopectin is associated with crumb firming (Inagaki and Seib., 1992). It was found that amylopectin recrystallized faster in breads containing higher levels of amylopectin, which in turn have higher firming rates (Inagaki and Seib, 1992).

## 2.3.2. Role of Moisture

Standard bread dough constitutes about 40% water. Crumb of baked bread contains over 35% of moisture (Czuchajouiska et al., 1989). Bread staling does not occur



Fig. 2.7. Model of a mechanism of bread firming and the role of starch swelling. Reprinted from Martin et al. (1991), with permission.



Fig. 2.8. Mechanism of bread firming and the antifirming role of dextrins. Reprinted from Martin and Hoseney (1991), with permission.

due to moisture loss. The moisture content in stale, firm bread is often similar to that present in fresh bread. Presently, research identifies the states of water i.e. characteristics molecular motion (water mobility) to play an important role in bread firming (Ruan et al., 1996). But, loss of moisture from the loaf might accelerate the staling of bread (Kim and D'Appolonia, 1977a).

Crust of freshly baked bread had lower moisture content (Fig. 2.9) than the crumb With storage the crumb lost moisture to crust thereby setting up a moisture gradient for water mobility between crust and crumb (Czuchajowska and Pomeranz, 1989; Piazza and Massi, 1995; Ruan et al., 1996). Breads with crust removed (Fig. 2.10) had constant moisture. Also, breads with higher crumb moisture (baked for 8 min) had lower firming rates than breads with lower moisture (baked for 24 min) (He and Hoseney, 1995). During staling, the mobility of less mobile water fraction decreases, where as the mobility of more mobile water fraction increases. This change might be attributed to the physiochemical changes taking place during staling such as expelling of water molecules from starch matrix due to retrogradation or interaction with other components (Ruan et al., 1996).

Apart from moisture content, bread staling is also influenced by additional factors such as degree of starch gelatinization, processes, formulations and additives which delays the onset of staling by slowing down the dehydration phenomenon (Piazza and Massi, 1995). The water present during aging also controls retrogradation. Anti-staling agents in bread decrease the rate of crystallization by altering the moisture relationship during aging. Bread contains 35-40% of moisture content, which is recognized as optimum condition for starch recrystallization. Anti-staling agents function by altering



Fig. 2.9. Changes in moisture content in crust ( $\bullet$ ), center crumb (+), and near crust ( $\blacktriangle$ ) zones of 100-g flour loaves for up to 168 hr. Reprinted from Czuchajowska and Pomeranz (1989), with permission.



Fig. 2.10. Effect of baking times and packaging methods on changes in bread moisture content during storage. Reprinted from He and Hoseney (1990), with permission.

(raising or lowering) water availability to the level outside the optimum range (Zeleznak and Hoseney, 1986). Soluble fiber can play a role by binding water that is available for staling.

## 2.3.3. Role of Proteins

The protein of bread crumb undergoes certain changes during baking and subsequent storage. These changes are mild, first order transformation that may be associated with an increase in the extent of denaturation of the protein and may involve the configurational modification in the protein. These changes, unlike those in starch retrogradation, are not reversible by heat (Willhoft, 1973). Since starch is embedded in a continuous matrix of protein, the irreversible changes in protein structure leads to the firming of bread crumb (Kim and D'Appolonia, 1977a). The recovery of soluble starch extracted from fresh bread crumb was inversely related to the protein content of flour. The amylose content was almost negligible in the soluble starch extracts from the breads made with protein enriched flours. These results implies that the role of amylose diminishes as the flour protein content increases (Kim and D'Appolonia, 1977b).

## 2.4. EXTRUSION

Extrusion is a high temperature short time cooking process, which involves continuous mixing, kneading and shaping of food (Faubion et al., 1982; Akdogan, 1999). Originally developed for applications in plastic industry, extrusion is now the most widely practiced technology in food industry (Faubion et al., 1982). It is a highly adaptable, cost-effective, and energy efficient technology that has the ability to control the thermal changes of food constituents as well as improve their textural and flavor characteristics. Applications of extrusion in food industry include manufacturing of ready-to-eat (RTE) breakfast cereals, snacks, dry and semi-moist pet foods, confectionery products, and texturized vegetable proteins (Harper, 1989).

#### 2.4.1. Equipment Design and Process

Food extruders of various configurations and models are available to the food industry. Extruder consists of a *screw(s)* rotating in a large tightly fitting barrel (heat exchanger) and propelling the feed material forward while generating the pressure and shear, and a *nozzle or die* through which the product is forced (Miller, 1993). The degree of mixing and shear as well as the pressure and temperature profiles experienced by the processing material are influenced by the process variables such as screw design, nozzle size and shape, feed moisture and barrel temperature (Janssen, 1989).

Extrusion process can be divided into three different sections that include: 1) Feed section; where raw material is fed, mixed, compressed to fill the screw flights and conveyed uniformly into the extruder. 2) Transition or cooking or compression section; is having a rapid rise in pressure, temperature and shear. 3) Metering section; which involves further increase in pressure, temperature and compression. The pressure rises steadily along the screw and reaches its peak in the final metering section before the die, after which it goes down. The pressurized (homogeneous, thermoplast) fluid is forced through the die, which can be of various shapes (circular, annular, rectangular, irregular) and dimensions. Upon product exit from die, the rapid loss in temperature and pressure

results in moisture loss (flash evaporation) that causes product expansion (Faubion et al., 1982; Miller, 1993).

Food extruders can be classified as single screw (with or without grooved barrel) and twin screw (closely intermeshing, counter rotating, corotating, self-wiping, and nonintermeshing) extruders (Janssen, 1989). Commonly used extruders are the ones with single screw. However, due to the higher capacity and precise control over the process corotating twin screw extruders are getting popular (Huber, 1991).

Single screw extruders (SSE) consist of a screw rotating in a closely fitting barrel (Harper, 1989). Prior to extrusion, the raw material is preconditioned with steam that provides extra mechanical energy (heat) to the extruded product (Demetrakakes, 1998). Preconditioning is advantageous due to its lowest cost energy (steam), bigger capacity and improved product quality (less shear) (Demetrakakes, 1998). Twin screw extruders (TSE) have two parallel screws placed in a figure-eight sectioned barrel. Preconditioning is not a pre-requirement in TSE because everything is done in the same extruder. The heat necessary for cooking is provided mostly by mechanical energy inputs, while the rest comes via heat exchange through the barrel walls.

Compared to SSE, the TSE have greater conveying angle (i.e. screw angle  $\sim 30^{\circ}$  in TSE and 10° in SSE), self-wiping features, less residence time distribution, improved mixing and superior heat transfer. While SSE accepts only flowing granular materials having 12-35% moisture content, TSE offers a larger operating range capable of extruding wide variety of ingredients having low ( $\sim 6\%$ ) to very high moisture content (Harper, 1989). SSE, being economical, is commonly used for cooking and making pet foods. TSE costs 1.5-2.5 times more than SSE. Nevertheless, the processes where precise

control and greater flexibility is required employ TSE. Corotating TSE due to their greater pumping action are the most popular in food industry (Harper, 1989).

#### 2.4.2. Effect of Extrusion on Flour Components

Extruded products (RTE breakfast cereals, snacks and pet foods) are manufactured mainly from cereal flours and starches. The extrusion processing variables such as barrel temperature, screw configuration, die/nozzle size and shape, screw speed, shear rate and moisture content of feed have significant effect on extruded product quality (Mercier and Feillet, 1975; Chiang, 1977; Gomez, 1983; Chinnaswamy and Hanna, 1988; Colonna et al., 1984).

#### Starch degradation

Extrusion processing resulted in gelatinization and later macromolecular degradation of starch molecules that lead to increased starch solubility. Gelatinization of starch increased at higher extrusion temperature (Chiang and Johnson, 1977; Lee et al., 1982; Bhattacharya and Hanna, 1987b). Water solubility index, an indicator of starch solubility, was more at higher extrusion temperature (Mercier and Feillet, 1975; Gomes and Aguilera, 1983; Colonna et al., 1984; McPherson and Jane, 2000b) whereas the reverse was true for moisture content (Chinnaswamy et al., 1990; Jackson et al., 1990; McPherson and Jane, 2000a,b). Starch content remained unaffected by extrusion (Mercier and Feillet, 1975). Degradation of starch did not result in the formation of any maltodextrins (Mercier and Feillet, 1975; Jackson et al., 1990; Govindsamy et al., 1996). However, Chiang and Johnson (1977) showed significant increase in fructose, glucose,

melibiose, maltotriose and maltotetrarose due to breakdown of the  $(1\rightarrow 4)$  glucosidic bonds of malto-oligosaccharides and starch during extrusion cooking.

Colonna et al. (1984) suggested that the molecular degradation of starch was due to random chain fragmentation of both amylose and amylopectin molecules. However, Davidson et al. (1984) suggested that the reduction in molecular size was due to amylopectin fragmentation caused by mechanical rupture of covalent bonds that act similar to the action of pullulanase. Fragmentation of amylopectin molecules was reported to be higher than the amylose molecules (Chinnaswamy and Hanna, 1990). The molecular weight of amylopectin decreased from 7.7 x  $10^8$  in native state to  $1.0 \times 10^8$  after extrusion (30% moisture, 100°C temperature) (McPherson and Jane, 2000b). Starch crystallinity was maintained at high moisture and low temperature extrusion (Govindsamy et al., 1996) and decreased under severe extrusion conditions (McPherson et al., 2000a).

## Moisture

The effect of moisture content on starch gelatinization was pronounced only at higher (95° and 100°C) temperatures (Chiang and Johnson, 1977). The most suitable moisture-temperature combinations for making noodle-like and snack or flake like product reported were 25-35% moisture/120°C and 25% moisture/150°C, respectively (Lee et al., 1982). Fragmentation of starch at low moisture and high shear results in dextrinization of corn extrudates (Gomes and Aguilera, 1983). The reduction in moisture content (below 20%) lead to changes from gelatinized-like to dextrinized-like properties (Gomez and Aguilera, 1984). For high moisture (34-47%) low temperature (81-101°C)

extrusion, gelatinization rather than the dextrinization was the prime mode of degradation (Govindsamy et al., 1996). Also, the increase in moisture content (from 30 to 40%) and the level of cross linking (from 0.0 to 0.028% POCl<sub>3</sub>) results in higher viscosity of corn starch extrudates (McPhereson and Jane, 2000a).

#### Shear

Degree of starch gelatinization decreases with increasing shear rate (screw speed) (Chiang and Johnson, 1977). Higher screw speeds increase shear, but on the other hand reduce the residence time that suppresses starch swelling making granules less vulnerable to disintegration by shear (Bhattacharya and Hanna, 1987b). At screw speed greater than 410 rpm, the shearing action predominates over residence time and enhances gelatinization (Govindsamy et al., 1996). The effect of screw speed becomes less significant at high moisture content of feed (Chinnaswamy and Hanna, 1988, 1990; Govindsamy et al., 1996).

#### Die nozzle size

Starch gelatinization is inversely dependent on the nozzle size; the greater the nozzle size the lesser is the degree of gelatinization and vice versa (Chiang and Johnson, 1977). The expansion ratio of starch, which is the ratio between the cross-sectional area of the rod-shaped product and the area of the die, is dependent on the degree of starch gelatinization (Chinnaswamy and Hanna, 1987; Gomez and Aguilera, 1984). On the contrary there are results, which state that with the increase in nozzle length/diameter (L/D) ratio from 2.5 to 3.4, the expansion ratio increased sharply from 4.5 to 13 and then

declined (Chinnaswamy and Hanna, 1987). Higher moisture and protein content of flour reduce the expansion ratio, while sugar, salt and oil content increase the expansion ratio (Mohamed, 1990).

## Amylose content

Water solubility of extrudates decreases with increasing amylose content (Mercier and Feillet, 1975; Jackson et al., 1990). Bhattacharya and Hanna (1987b) reported that under similar conditions waxy corn starch extrusion gelatinized to a greater extent than the ordinary corn starch. The textural properties of extrusion cooked waxy (1% amylose) and non-waxy (30% amylose) corn grits were affected by the degree (17.8-42.2% moisture content, 116-166°C temperature and 94-169 rpm screw speeds) of extrusion cooking. Increase in temperature and moisture increased the shear strength and water holding capacity, respectively, for waxy corn starch, but the reverse was true for nonwaxy starch (Bhattacharya and Hanna, 1987a). Waxy corn had greater expansion than the non-waxy corn, but it was highly prone to shear damage (Bhattacharya and Hanna, 1987a). However, Chinnaswamy and Hanna (1988) reported that as the amylose content of blended (10, 25, 50 and 65% amylose content on dry basis) corn starches increased from 0 to 50% the expansion ratio increased from 8 to 16.4, but decreased with higher amounts afterwards, suggesting that the structural differences between amylose and amylopectin plays an important role in expansion properties of starch.

Extrusion processing, in addition to starch, also modifies the properties of other flour components. An increase in water solubility (from 20 to 40%), water absorption capacity (270 to 375%) and soluble fiber content (from 8 to 16%) was observed for

extruded wheat bran. Solubility of glucuranoarabinoxylans and  $\beta$ -glucans was increased (Ralet et al., 1990). Extrusion of barley flour causes fragmentation of  $\beta$ -glucan that increases the  $\beta$ -glucan solubility, higher for waxy barley flour than regular barley flour (Gaosong and Vasanthan, 2000).

## 2.4.3. Nutritional Properties

Extrusion is most commonly used for the production of breakfast cereals, which are a good source of dietary fiber. Increased consumer awareness about high-fiber diets had shifted the trend towards the manufacturing of fiber-enriched extruded products. Severe extrusion conditions can result in slight increase in DF content i.e. non-starch polysaccharides and lignin due to formation of enzymatically indigestible starch fractions (Bjorck et al., 1984; Asp, 1986; Ostergard et al., 1989). A redistribution of insoluble to soluble dietary fiber occurred in extrusion cooking (Bjorck et al., 1984; Siljestrom et al., 1986; Shinnick et al., 1988; Camire and Flint, 1991). Moreover, a reduction in protein solubility was noticed after extrusion (Wen et al., 1990; Wang et al., 1993b). Feed exposed to greater shear resulted in higher starch solublization and had higher soluble fiber (Bjorck et al., 1984; Wang et al., 1993b). Similar results were reported by Siljestrom et al. (1986), however the TDF content in their samples remained unchanged.

A higher fermentability of extruded DF was observed in rat intestine than its native counterpart (Bjorck et al., 1984). Proportions of soluble  $\beta$ -glucans and neutral sugars were increased (Shinnick et al., 1988). The hypocholesterolemic effect of fibers (oat, rice, corn, barley and wheat) was improved by extrusion cooking (Shinnick et al., 1988; Wang and Klopfenstein, 1993; Kahlon et al., 1998). Interestingly, rats fed with extruded barley diets showed the highest hypocholesterolemic effect compared to extruded oat and wheat diets (Wang and Klopfenstein, 1993). Also, the soluble  $\beta$ -glucan content of several cereals (containing blends of barley and rice or barley and wheat) was increased after extrusion (Berglund et al., 1994).

Vitamins are sensitive in nature and therefore are susceptible to heat damage during extrusion. Riboflavin, niacin, pyridoxine and folic acid remain stable during extrusion cooking. The activity of vitamin A and E is affected by extrusion. However, vitamin C activity can be prevented from damage by low moisture cooking (Asp and Bjorck, 1989). Thiamin destruction is dependent on extrusion processing variables (Ilo and Berghofer, 1998). Extrusion cooking is effective in reducing Fumonisin B1 (mycotoxin) concentrations in corn and corn based foods (Castelo et al., 1999). Decreased extractability of fat had been reported after extrusion (Asp and Bjorock, 1989).

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# 3. WHEAT BREAD QUALITY AS INFLUENCED BY THE SUBSTITUTION OF WAXY AND REGULAR BARLEY FLOURS IN THEIR NATIVE AND COOKED FORMS<sup>1</sup>

# **3.1. INTRODUCTION**

Hull-less barley (HB) is receiving considerable research attention in food, feed and industrial applications (Bhatty, 1986b, 1999; Vasanthan and Bhatty, 1997). It is a good source of dietary fiber,  $\beta$ -glucan, which confer a number of human health benefits (Klopfenstein, 1988). Barley products are known to lower cholesterol (Newman et al., 1989; McIntosh et al., 1991; Lupton et al., 1994; Lia et al., 1995), blood glucose concentrations and glycemic index (Yokoyama et al., 1991; Granfeldt et al., 1994; Conard et al., 1999). Anti carcinogenic properties of barley have also been reported (McIntosh et al., 1993). Barley is rich in tocols (42 to 80 mgKg<sup>-1</sup>) that have vitamin E and antioxidant activity (Morrison, 1993, Peterson and Qureshi, 1993) and are potent inhibitors of cholesterogenesis (Qureshi et al., 1986). The nutritional value of whole or pearled barley grains makes it highly desirable as a food for health conscious consumers (Newman et al., 1988).

HB has been successfully used in chemically leavened products such as muffins, pancakes, biscuits and cookies (Berglund et al., 1992). Muffins prepared with barley fiber fraction contained more than 7 g of total dietary fiber per100 g muffin, compared to about 3 g in commercial oat bran muffin (Hudson et al., 1992). Wheaten bread and other baked products enriched with  $\beta$ -glucan could be used as functional foods (Newman et al.,

<sup>&</sup>lt;sup>1</sup>A version of this chapter is to be submitted to Journal of Cereal Science for consideration for publication.

1998). However, the addition of fibrous material to wheat flour leads to dilution of wheat gluten protein, which causes weakening of cell structure (crumb grain) (Pomeranz et al., 1977). Also, the fibrous materials, especially the insoluble fraction, tends to cleave the gluten strands during mixing that not only impairs the gas retention of the dough (gluten network), but also changes the texture and appearance of the baked product (Dubois, 1978). In some cases, the fiber material imparts undesirable color and flavor (Prentice and D'Appolonia, 1977; Kuhn and Grosch, 1989; Gillis, 1991; Nebensy, 1995). Hence, it is less suitable for making yeast leavened products such as bread (Bhatty, 1999).

Fibers may be modified using enzymatic or physical treatments (Gould et al., 1989; Wrathesen, 1995; Thebaudin et al., 1997). Studies have shown that incorporation of such modified dietary fibers into baked products, such as cakes and cookies, has improved the texture, sensory properties and shelf life of properties (Mark et al., 1988; Jasberg et al., 1989; Bullock, 1992; Ang and Miller, 1991). Therefore, developing an ideal fiber can counteract the deleterious effects brought about by addition of fiber into baked products.

The type of starch (i.e. waxy, regular or high amylose) also plays an important role in bread quality. When wheat starch was replaced with waxy barley (very low amylose) starch in reconstituted dough, the loaves collapsed upon cooling, suggesting that the amylose fraction of starch, perhaps by rapid retrogradation is responsible for setting the crumb structure of bread (Hoseney et al., 1978). Studies have shown that 10 and 26% replacement of wheat flour with barley flour gave breads with acceptable quality. It must be noted that, these supplemented breads compared well with the control, which was prepared by replacing a portion of all purpose flour with whole wheat flour at 26% and 30% levels, respectively (Prentice and D'Appolonia, 1977; Berglund et al., 1992). Bhatty (1986a) reported that barley flour could be added to wheat flour up to 5% (w/w) level without seriously affecting the loaf volume and bread appearance. This level of substitution is small to be considered for the preparation of barley bread with appreciable amount of  $\beta$ -glucan as a health benefiting functional component. Therefore, research is warranted in this area to explore ways to increase barley flour substitution levels to white wheat flour without seriously impairing the bread qualities.

There have been research efforts to find out the acceptable substitution levels for barley flour, however, little research has been done to comparatively evaluate the effect of replacing a portion of wheat flour (white) with barley flours (from different cultivars), which substantially differ in their functionality. Furthermore, the effects of substitution of cooked barley flours [prepared by conventional pan cooking at 100°C with excess water or extrusion cooking (at different temperatures) with limited amount of water (at different levels)] and their effect on bread quality have received scant attention. The objectives of this study were to replace a portion of wheat flour with a waxy and a regular barley flour, in their native and cooked states (conventional pan cooking), and to examine their effects on the bread quality characteristics of bread such as loaf volume, crumb firmness, and crumb and crust color.

## **3.2. MATERIALS AND METHODS**

## 3.2.1. Materials

Waxy barley grains (CDC Candle) were obtained from Mr. Jim Gray, Agricore, Calgary, AB. Regular barley grains (Phoenix) were obtained from Dr. Jim Helm, Alberta Agricultural, Food and Rural Development, Lacombe, AB. All-purpose wheat flour (Western Family Foods, Vancouver, BC), crisco shortening (Proctor & Gamble, Toronto, ON), non-fat dry milk (Carnation, Nestle, 1185 Eglintone, Don mills, ON), sugar (Rogers sugar Ltd, Vancouver, BC), salt (Windsor, The Canadian Salt Company, Canada) and yeast (Fleischmann's active dry yeast, Division of Burns Philip Food Ltd, LaSalle, QC) were purchased from a local store. Calcium propionate was obtained from UFL Foods Group Co., Edmonton, AB. The analytical kits for total starch,  $\beta$ -glucan and total dietary fibers were purchased from Megazyme International Ireland Ltd, Wicklow, Ireland. All other chemicals were of analytical grade and were purchased from Sigma Chemical Co., St Louis, MO, Anachemica, Champlain, NY, and Fisher Scientific, Fair Lawn, NJ.

#### 3.2.2. Pearling and Milling of Barley Grains

Pearling and milling of barley grains was carried out at the POS pilot pant, Saskatoon, SK. Grains were pearled to 32% in a Satake mill (Model RMB 10G; Satake, Houston, TX). The pearled grains were pin-milled (Alpine Contraplex wide chamber mill Type A250, Hosokawa Micron Systems, Summit, NJ) into flour at 6,000 rpm and a feed rate of 150 kg/h.

## 3.2.3. Preparation of Cooked Barley Flour

Barley flour (1 part) and distilled water (10 parts) were mixed for 1 hour at room temperature in order to obtain uniform slurry. The slurry was then heated for 25 min with occasional stirring in a steam jacketed water bath maintained at 100°C. The cooked/gelatinized slurry was then dried in a draft air-oven at 80°C. The dried material was coarse ground in a crushing mill (Model 4-E; Quacker City Mill, Philadelphia, USA) and later fine ground in Quadro mill (Model 197-S; Quadro Engineering Inc, Waterloo, ON) and screened through a 60-mesh sieve.

#### 3.2.4. Analysis of Flour

Moisture, crude fat and ash contents were determined according to AACC methods 44-19, 08-01 and 30-25, respectively. Total (TDF), insoluble (IDF) and soluble (SDF) dietary fiber contents, and the total starch and  $\beta$ -glucan contents, were determined according to Megazyme methods. Protein content was analyzed with Leco nitrogen (N X 5.7) determinator (Model FP-428; Leco, Leco corporation, St. Joseph MI). Apparent amylose content was determined according to the method explained by Chrastil (1987).

# 3.2.5. Scanning Electron Microscopy (SEM)

Flour samples were sprinkled on a double sided adhesive tapes mounted on aluminum stubs, coated with gold and examined under a JEOL (JSM 6301  $F \times V$ ) scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 5 KV.

# 3.2.6. Determination of Mono-, Di- and Oligo- Saccharides

Flour and bread (bread crumbs rapidly frozen in liquid nitrogen, freeze dried at –  $50^{\circ}$ C, ground and sieved through 60 mesh size) samples (0.1 g) were mixed with 80% ethanol (1 mL), incubated at 40°C for 15 hours in a shaking water bath and centrifuged at 10,000 rpm for 10 min. An aliquot of (10 µl) supernatant was used for mono-, di- and

oligo- saccharide determinations. An HPLC system, equipped with a Shimadzu Ezchrom chromatography data system, a solvent delivery system (Varian 9010, Sunnyvale, CA), an HP Series 1050 autosampler, a polyamine column (250 mm length  $\times$  4.6 mm, Jordi Gel DVB, Bellingham, MA), and an evaporative light scattering detector (Alltech 500 ELSD, Mandel Scientific, Guelph, ON) was used. Different combinations of mobile phases. distilled water (A) and acetonitrile (B), were used according to the method of Gaosong and Vasanthan (2000). The solvent gradient used was 10% A and 90% B (v/v) at the beginning; 40% A and 60% B (v/v) after 25 min; 0% A and 100% B (v/v) after 26 min; 10% A and 90% B (v/v) after 30 min. The detector temperature was set at 125°C, and a flow rate of 1.0 mL/min was maintained. A series of authentic oligosaccharide mixtures (glucose, DP1; maltose, DP2; maltotriose, DP3; maltotertose, DP4; maltopentose, DP5; maltohexose, DP6) was used for quantification purpose.

# 3.2.7. Water Binding Capacity (WBC) of Barley Flour

Barley flour (5 g) was mixed with 25 g of distilled water, rested for 15 minutes and heated in a boiling water bath (100°C) for 15 min using a Rotavapor (RE 121, Brinkmann, Switzerland). The condensed water was collected and, the moisture loss and WBC were calculated as mentioned below.

Moisture loss (%) =  $\frac{W1-W2}{W3} \times 100$ Where, W1 = Initial weight of flask and sample (g)

> W2 = Final weight of flask and sample (g), after evaporation of water W3 = Amount of water (25 g) taken

WBC = 100 - Moisture loss (%).

### 3.2.8. Dextrose Equivalent (DE)

DE of flour samples was determined using the method of Novo Nodisk Biochem North American Inc. (Franklinton, NC, USA). Flour was screened through a 60-mesh sieve. Sample (0.1 g) was added to 24.9 g of distilled water in a 250 mL flask. To this, Fehling A and Fehling B solution (10 mL each) were added and the mixture was boiled. Boiling beads were added to suppress foaming. After 2 min of boiling, the flask was quickly cooled to  $<30^{\circ}$ C and 30% KI solution and 26% H<sub>2</sub>SO<sub>4</sub> (10 mL each) were added. The solution was then titrated using sodium thiosulphate with pale mauve or pink as an end point. For water blank, 25 mL of distilled water was used. For standard, 20 mL of distilled water and 5 mL of a 1% dextrose solution were used.

 $DE = [(T_{wb} - T_a) \times 500] / [(T_{wb} - T_{dex}) \times W \times \% DS]$ 

 $T_{wb}$  Titre of water blank, mL

T<sub>a</sub> Titre of sample, mL

T<sub>dex</sub> Titre of dextrose, mL

W Weight of mash sample, g

%DS %dry solids of mash added to flask

#### 3.2.9. Bread-making

Breads were baked using 100 g of flour. Control bread contained 100% allpurpose wheat flour. Test Breads were prepared by replacing all-purpose wheat flour with barley flour at 5, 10 and 15% levels. Barley flour from two different varieties (Candle and Phoenix) was used. Barley flour was added at native as well as cooked states. Bread formula included flour (100 g), water (60 g), sugar (6 g), non-fat dry milk (4 g), shortening (3 g), salt (1.5 g), active dry yeast (0.76 g) and calcium propionate (1500 ppm). Besides flour, all the ingredients and their amounts listed in this formula were the same for control as well as test breads.

The dough was made in a Hobart Kitchen-aid mixer (Model K45SS; Hobart Corporation, Troy, OH). Breads were prepared according to straight dough method (AACC 10-10). For dough proofing, a Cres-Cor Proofer (Crescent Metal Products, Cleveland, OH) set at 30°C and 95% relative humidity was used. A moulder [Model B&B 860; Bloemhop Industries Ltd. (1986), Edmonton, AB] was used for sheeting as well as moulding purpose. Baking was done in conventional household type kitchen ovens (General Electric Brand). Baking pans were always placed in the middle rack positioned in the center of the oven. Five loaves of control and five loaves of each treatment were baked and stored for seven days at room temperature. Volume of all loaves was recorded while one loaf from each treatment was used for color measurements. On each day of evaluation, a new loaf from each treatment was used for firmness measurements.

# 3.2.10. Bread Quality Characteristics

Loaf Volume: After one hour of cooling, loaf volume was measured using a loaf volumeter, which used rapeseed displacement method (National Mfg. Co., Lincoln, NE). The loaves were then repacked in polyethylene pouches and stored for seven days at ambient temperatures.

Color: A Hunter lab colorimeter (Model 1836; Labscan XE, Hunter Associates Laboratory, Reston, VA) was employed to obtain L (lightness), a (redness) and b

(yellowness) values of flour as well as bread (crust and crumb). The cooked, dried, ground flours, were screened through 60-mesh sieve before taking the measurements. Firmness: Bread firmness was measured using an Instron Universal Testing Instrument (Model 4201; Instron Corporation, 100 Royal Street, Canton, MA) attached to an electronic data acquisition system. The firmness measurements of breads were taken on day 1, 3, 5 and 7 after baking. A separate loaf was used for each evaluation. Loaves were sliced into 12.5 mm thick slices with a Berkel meat slicer (Model 1836; Berkel Products Co. Ltd., Toronto, ON). To avoid the effect of air on crumb firmness, loaves were repacked in polyethylene bags immediately after slicing. The firmness was measured 30 min after slicing. Two slices were stacked on each other to obtain 25 mm thick sample and a flat plunger (36 mm diameter) was used for compression. Crust was removed using an electric knife (Hamilton Beach/ Proctor Silex, Inc., Washington, NC). The end slices of loaves were discarded and the remaining slices were picked up randomly. Samples were tested against 25% compression using 50 N load cell and 100 mm/min of cross head speed.

# 3.2.11. Statistical Analysis

All experiments were carried out in triplicates and the analyses were done in duplicates. Data was analyzed using SAS Statistical Software, Version 6 (SAS Institute, 1989). Analysis of variance was performed using General Linear Model procedure. The significance (P<0.05) of differences, observed among treatment means, was established using Tukey's Studentized range test.

#### **3.3. RESULTS AND DISCUSSION**

#### 3.3.1. Flour Analysis

The proximate composition and the contents of  $\beta$ -glucan and amylose of native flours used in bread making are given in Table 3.1. Starch content of wheat flour (74%, w/w) was slightly higher than that of barley flours [72% (w/w) for Phoenix, and 71% (w/w) for Candle]. Candle had the highest  $\beta$ -glucan content (6%, w/w) followed by Phoenix (4%, w/w) and wheat (0.3%, w/w). Candle, being waxy in nature, had starch with low amylose content (2%), while wheat and Phoenix had regular amylose contents (24 and 26%, w/w, respectively). The dextrose equivalent (DE; not shown in any tables) of native wheat and barley flours was <1, whereas cooked Phoenix had lower DE (8.4) than in cooked Candle (20.5). The DE is a measure of reducing capacity and expected to have an influence on the browning of bread crust. The mono-, di- and oligo- saccharide (DP3 – DP6) compositions of wheat and barley flours are given in Table 3.2. Their content was found to be significantly (P < 0.05) higher in cooked than in native flours. The saccharide content of cooked Candle was found to be significantly (P < 0.05) higher than the cooked Phoenix. The increase in DE might be attributed to heat-induced fragmentation of polysaccharides, such as starch and β-glucan, into di- and oligosaccharides.

#### 3.3.2. Dough Characteristics – a Subjective Evaluation

The native barley flour substitution increased the dryness and hardness of dough as compared to control. At all levels of substitution, the dough made with native-Phoenix

Component	Wheat	Phoenix	Candle
Starch	74.1 ± 1.2	$72.4 \pm 1.5$	71.1 ± 1.6
Lipids	$0.6 \pm 0.1$	$1.1 \pm 0.0$	$1.1 \pm 0.1$
Protein	$11.9 \pm 0.1$	$8.4 \pm 0.0$	$9.2 \pm 0.1$
Moisture <sup>2</sup>	$9.5 \pm 0.2$	$9.0 \pm 0.3$	$7.8 \pm 0.2$
Ash	$0.5 \pm 0.0$	$0.8 \pm 0.0$	$0.7 \pm 0.0$
Amylose	$23.6 \pm 2.2$	$25.6 \pm 0.6$	$1.9 \pm 0.1$
β-Glucan	$0.3 \pm 0.11$	$3.9 \pm 0.2$	$6.4 \pm 0.2$

**Table 3.1.** Chemical composition (% dry wt.) of wheat and barley flours used in bread making<sup>1</sup>.

<sup>1</sup> Expressed as mean  $\pm$  SD. <sup>2</sup> Wet basis.

Sample		DP1	DP2	DP3	DP4	DP5	DP6
Native	Wheat	$0.00 \pm 0.00$	$0.02 \pm 0.01$	0.05 ± 0.01	0.11 ± 0.01	$0.03 \pm 0.02$	0.02 ± 0.01
	Phoenix	$0.00 \pm 0.00$	$0.02 \pm 0.00$	$0.15 \pm 0.00$	$0.08 \pm 0.00$	$0.02 \pm 0.00$	0.01±0.00
J	Candle	$0.02 \pm 0.00$	$0.07 \pm 0.00$	$0.39 \pm 0.02$	$0.28 \pm 0.02$	$0.05 \pm 0.00$	$0.06 \pm 0.00$
Cooked	Phoenix	0.09 ± 0.01	4.97 ± 0.08	0.15 ± 0.05	0.08 ± 0.01	0.04 ± 0.00	0.01 ± 0.00
0	Candle	$0.21 \pm 0.01$	<b>24.69 ± 0.17</b>	0,54 ± 0,05	$0.40 \pm 0.03$	$0.06 \pm 0.01$	$0.08 \pm 0.04$

flour was softer than that made with native-Candle. The consistency of dough made with 5% cooked-barley flour was comparable to that of control dough. However, at higher substitution levels, dough with cooked-Candle flour was softer, more moist and stickier. On the contrary, dough with cooked-Phoenix flour was found to be dryer and harder.

Barley flour is rich in  $\beta$ -glucan, which is an excellent hydrocolloid. Also, barley flour had a relatively large amount of small particles as compared to wheat flour (data not shown). These small particles due to their large surface area influence water absorption and other functional properties. This is in agreement with the results of (Bhatty, 1986a). The dry and firm consistency of dough may be due to the high water-binding capacity of  $\beta$ -glucan as well as smaller particle size of barley flour that tends to absorb water more quickly than wheat flour. Candle flour, being higher in β-glucan content, absorbs more water and results in drier and firmer dough than that made with Phoenix flour. The high amount of  $\beta$ -glucan in barley flour, owing to its water soluble and viscous nature, had been a problem when used in baked products (Bhatty, 1986a). Despite its high  $\beta$ -glucan content, the cooked-Candle flour resulted in dough with soft consistency, where the softness increased at high substitution levels. On the other hand, the addition of cooked-Phoenix flour increased the dough firmness. The reasons for this divergence in functionality are unclear. However, the heat-induced changes in  $\beta$ -glucan molecules and the presence of sugars, formed due to thermal cleavage of polysaccharides, might be involved (Table 3.2).

#### 3.3.3. Quality of Wheat and Barley Breads

Breads were baked from 100% wheat flour as well as barley flour (5, 10 & 15%) supplemented wheat flours and their quality characteristics were measured. The photographs of their cross-sectional views are presented in Fig. 3.1 (a & b). Subjectively (personal observation), it was observed that the substitution of wheat flour with barley flours altered the loaf volume, color and firmness/texture of the bread loaves. These changes were found to be dependent on the barley variety and flour pretreatment (i.e. native or cooked). The aforementioned physical characteristics were then evaluated objectively and an attempt was made to explain the observed trends.

#### 3.3.3.1. Loaf Volume

#### Native flour substitution

Loaf volumes of control (100% wheat) and barley breads are given in Fig. 3.2. Control bread (100% wheat) had the highest loaf volume. When the substitution of wheat flour with native barley flour increased from 5% to 15% levels, the loaf volume decreased progressively. The 15% native barley flour substitution resulted in small and compact loaves with a blistered crust surface. Between the two varieties, i.e. Candle and Phoenix, an insignificant (P>0.05) difference in volume was noticed at 5% and 10% substitution levels. However at 15%, breads made with native Candle flour had a significantly (P<0.05) lower loaf volume than those made with native Phoenix flour.

# Cooked flour substitution

For Phoenix, cooked flour substituted breads (at all three levels) had significantly (P<0.05) lower loaf volume than those made with its native counterparts. For native and

Fig. 3.1. Cross-sectional views of breads, showing the effect of native (a) and cooked (b) barley flour substitution.







Fig. 3.2. Loaf volume of wheat and barley breads. N = Native; C = Cooked; WF = Wheat flour; BF = Barley flour.

cooked Candle, the difference in loaf volume observed at 5% and 10% substitution levels was insignificant (P>0.05). However, at 15% substitution level, a significantly (P<0.05) higher loaf volume was observed in cooked-Candle breads. The loaf volume of cooked-Candle breads was found to be significantly (P<0.05) higher than that of cooked-Phoenix breads at all substitution levels. At 15% substitution level, native-Phoenix breads had significantly (P<0.05) higher volume than the native-Candle breads. However, in the cooked barley flour breads an opposite trend was observed where Candle showed significantly (P<0.05) higher volume than the Phoenix. Also, the loaf volume of 15% cooked-Candle bread was significantly (P<0.05) higher than that of 5% cooked-Phoenix bread. This suggested that the use of cooked-Candle flour as opposed to native-Candle flour in barley bread products could increase the substitution level of barley flour. In addition, the crust of cooked-Candle bread, at 15% substitution level bread at the same substitution level.

#### 3.3.3.2. Bread Crumb Firmness

The effect of barley flour substitution on crumb firmness over a 7-day storage period is given in Fig. 3.3. As compared to control, barley breads (both Candle and Phoenix) had significantly (P<0.05) higher firmness, which increased progressively with substitution levels from 5% to 15%. At day one of storage after baking, the firmness of 5% and 10% substituted native Candle and Phoenix breads was statistically similar (P>0.05). However at 15% substitution level, Candle had significantly (P<0.05) higher firmness than Phoenix. Amongst the breads prepared with cooked barley flour, the



Fig. 3.3. Crumb firmness of native and cooked barley substituted bread over a 7-day storage period. • = Control,  $\Box = 5\%$  Phoenix,  $\Delta = 10\%$  Phoenix,  $\delta = 15\%$  Phoenix,  $\equiv 5\%$  Candle,  $\Delta = 10\%$  Candle and  $\phi = 15\%$  Candle.

Candle breads had significantly (P<0.05) lower firmness than Phoenix at all substitution levels. Interestingly, the breads made with 15% cooked-Candle flour showed lower firmness than those made at 5% and 10% substitution, and closely resembled that of control. The firmness of control as well as barley breads increased rapidly up to day-3 of storage and slowed down thereafter. However, in all the cases firmness increased throughout the entire storage period.

# 3.3.3.3.Bread Color

The Hunter (crumb and crust) color values (L, a and b) of wheat and barley breads are given in Table 3.3. Control breads had golden brown crust color. Native barley flour substitution, as increased from 5 to 1 5%, caused a gradual decrease in brownness of the crust while increasing its lightness/paleness (higher L-crust color values). The effect of cooked-barley flour substitution orn bread crust color was variety-dependent. The brownness of cooked-Phoenix breads progressively decreased with the increasing levels (from 5 to 15%) of substitution whereas the golden brown crust color of cooked-Candle breads remained comparable to that of control at all substitution levels. This may be attributed to the higher sugar content of cooked barley flours (Candle > Phoenix) as compared to the native flours (Table 3.2). There were no significant (P>0.05) differences among the L-crumb color values of control and native barley substituted breads at all three substitution levels. However, the L-crumb color values of cooked-barley flour substituted breads were significantly (P<0.05) lower than that of control. Both native and cooked barley flour substitutions had little effect on Hunter a-crumb and b-crumb color values.

	L		æ		-	q
	Native	Cooked	Native	Cooked	Native	Cooked
Crust color						
Control						
100%WF + 0% BF	$43.0 \pm 2.4$		$16.5 \pm 0.3$		$30.7 \pm 1.7$	
Phoenix					•	
95%WF + 5% BF	$41.6 \pm 1.2$	$47.7 \pm 2.7$	$17.1 \pm 0.4$	155±11	30 K + 0 R	30 4 9 62
90%WF + 10% BF	$47.1 \pm 3.3$	52.9 + 5.0	156408	10 9 4 3 0	33.7 + 1.0	00 0 T 0 0 C
85%WF + 15% BF	$50.2 \pm 2.1$	$54.4 \pm 5.0$	$15.2 \pm 0.6$	95±25	34 1 + 0.6	28.7 + 1 K
Candle						
95%WF + 5%BF	$43.1 \pm 3.7$	$43.6 \pm 2.9$	$16.5 \pm 0.9$	$17.1 \pm 0.5$	314±18	317+73
90%WF + 10% BF	$45.9 \pm 3.3$	$43.9 \pm 2.3$	$16.2 \pm 0.6$	$16.7 \pm 0.8$	32.5 ± 1.7	318+00
85%WF + 15% BF	57.1 ± 3.3	$43.5 \pm 3.8$	$11.9 \pm 2.0$	$16.3 \pm 1.2$	$33.4 \pm 0.9$	$31.2 \pm 2.4$
Crimb color						
Control 100%WF <sup>a</sup> + 0% BF <sup>a</sup>	74.9 ± 0.7		$0.1 \pm 0.3$		19.4 ± 0.6	
Phoenix						
95%WF + 5% BF	$75.6 \pm 0.6$	$71.3 \pm 1.9$	$0.7 \pm 0.2$	$1.3 \pm 0.1$	$19.0 \pm 0.4$	$17.4 \pm 0.7$
90%WF + 10% BF	<b>75.6 ± 0.7</b>	$70.3 \pm 3.3$	$1.0 \pm 0.1$	$2.1 \pm 0.2$	$19.3 \pm 0.4$	$17.5 \pm 0.9$
85%WF + 15% BF	75.1 ± 0.5	$70.0 \pm 1.3$	$1.4 \pm 0.1$	$2.7 \pm 0.1$	$19.8 \pm 0.3$	$18.2 \pm 0.6$
Candle						•
95%WF + 5%BF	$75.9 \pm 1.1$	$72.8 \pm 1.5$	$0.7 \pm 0.1$	$1.2 \pm 0.2$	$19.3 \pm 0.4$	$18.3 \pm 0.4$
90%WF + 10% BF	$75.9 \pm 0.5$	$71.3 \pm 1.5$	$1.0 \pm 0.1$	$2.1 \pm 0.2$	$19.7 \pm 0.3$	$18.9 \pm 0.5$
85%WF + 15% BF	$74.9 \pm 0.7$	$68.5 \pm 1.1$	$1.3 \pm 0.1$	$2.8 \pm 0.2$	$20.4 \pm 0.5$	$19.9 \pm 0.2$

The low loaf volume and firm crumb texture of barley breads as compared to control wheat breads may be attributed to a number of factors. The direct factor could have been the gluten dilution (Pomeranz et al., 1977; Dubois, 1978). The physicochemical properties of barley  $\beta$ -glucan and starch can also affect bread volume and texture indirectly.  $\beta$ -Glucan in barley flour, when added to wheat flour during bread making, could tightly bind to appreciable amounts of water in the dough, and suppressing the availability of water for the development of gluten network. Underdeveloped gluten network can lead to reduced loaf volume and increased firmness in breads.

Furthermore, in yeast leavened bread systems, in addition to  $CO_2$ , the steam is an important leavening agent. The extent of steam generation within the dough during baking would also have influenced the loaf volume and texture (firmness/softness). The WBC of Phoenix and Candle flour in native state (36% and 52%, respectively) was higher than in the cooked state (28% and 4%, respectively) (values not shown in any table). Due to its high affinity to water,  $\beta$ -glucan can suppress the extent of steam generation, resulting in a reduction in loaf volume and an increase in firmness. Gaosong and Vasanthan (2000) reported that the  $\beta$ -glucan content and its water solubility are substantially higher in native Candle flour (6.4% and 41.5%, w/w, respectively) than in native Phoenix flour (3.9% and 26.8%, w/w, respectively), which implied the " $\beta$ -glucan effect" would be more prominent with Candle flour substitution.

Hoseney et al. (1978) reported that wheat starch when replaced with barley starch (regular starch) resulted in satisfactory performance in bread making. However, the barley starch composition (i.e. the ratio between amylose and amylopectin) and physicochemical properties (i.e. swelling and water holding/binding during gelatinization) differ substantially with variety. The Candle starch belongs to the waxy type (<5% amylose content) and the Phoenix starch belongs to the regular type (approximately 20-25% amylose). During gelatinization, waxy starches swell and bind/hold substantially higher amount of water than regular starches (Vasanthan and Bhatty, 1996). Therefore, during baking, the starch in Candle flour would gelatinize and bind a substantial amount of available water. This could suppress the steam generation within the loaf, resulting in a lower volume and a higher firmness in Candle than in Phoenix breads. Lorenz (1995) demonstrated the importance of amylose in breadmaking, stating its importance for holding the starch granules intact and giving structure to the bread crumb. The author further concluded that waxy starch is not suitable as an ingredient in bakery products due to its low amylose content. Thus, in the present study, the lack of amylose (<5%) in Candle barley starch would have negatively contributed towards crumb setting (lower crumb strength), resulting in a collapse in crumb structure after baking (Hoseney et al., 1978). This, in turn, would have resulted in a reduced loaf volume and an increased firmness.

The slurry of Candle barley flour observed to be much thinner than of Phoenix barley flour towards the end of cooking. Lorenz (1995) reported similar observation and explained that waxy barley starch, owing to its high swelling power, gives a high peak viscosity than regular starch. The intermolecular forces in the granules of waxy starch are weak. This leads to rapid breakdown of granules on further cooking under stirring. In regular barley starch, prolonged stirring at high temperatures have resulted in minimal breakdown of starch granules. In the present study, the scanning electron microscopy of the native and cooked barley flours (Fig. 3.4) indicated that the cooked-Phoenix flour had Fig. 3.4. Scanning electron micrographs of native and cooked barley flours (bar width =  $10 \mu m$ ). Native Phoenix flour, a; cooked Phoenix flour, b; native Candle flour, c; cooked Candle flour, d.



relatively higher amount of large particles than in cooked-Candle flour. This may be due to the minimal disruption of Phoenix starch during cooking and the presence of amylose (a good binder) in Phoenix starch, which can leach out during cooking (i.e. gelatinization) and subsequently bind flour particles together (agglomeration) during its retrogradation. However, the retrogradation capacity of Candle starch is low due to its high amylopectin content and the disrupted starch granules and other flour particles remain as small particles due to minimal agglomeration. These small particles, especially the disrupted starch particles could increase overall surface area, which can effectively contribute towards crumb setting through improved starch-protein interactions. This may be responsible for the observed improvement in loaf volume and texture in breads made with cooked-Candle flour. However, in the native-Candle breads the disruption of Candle starch granules during baking would be minimal due to limited availability of water in the dough (limited water limits the swelling and subsequent disruption of starch granules).

The differences in water binding capacity of  $\beta$ -glucan and starch in native and cooked flours can influence the steam generation within the dough during baking and thereby influence loaf volume and firmness as explained earlier. For instance, Gaosong and Vasanthan (2000) reported that Candle  $\beta$ -glucan undergoes molecular fragmentation when subjected to heat and shear. Fragmented  $\beta$ -glucan is less viscous and holds less water than its native counterpart. However, the Phoenix  $\beta$ -glucan did not show any evidence of fragmentation.

The observations made in the bread storage study, where the crumb firmness increased rapidly during the first three days and slowed thereafter, were in agreement with those reported in previous studies (Volpe and Lehmann, 1977; Ghiasi, 1984).
Amylopectin plays an important role in crumb firming during storage. It was reported that amylopectin recrystallization was faster in the breads containing high levels of amylopectin. Breads made with waxy starch firmed faster than those made with regular starch (Inagaki et al., 1992). In the present study, the firmness data obtained from breads made with native barley flour substitution are in agreement with those reported by Inagaki et al. (1992) and Lorenz (1995). It was observed that the native-Candle breads with high levels of amylopectin firmed at a faster rate than the native-Phoenix breads containing normal level of amylopectin. However, the low firmness of breads made with cooked-Candle flour adds a discrepancy to the above finding. This implies that native and cooked barley flours deliver different functionalities in the production of baked products.

Martin and Hoseney (1991) have demonstrated the antifirming properties of low molecular weight dextrins (broken starch). As shown in the Fig. 3.3, the firmness rate decreased with high substitution levels of cooked-Candle flour. Unlike the 15% native-Candle bread that had the highest firmness, the firmness of 15% cooked-Candle bread was similar to that of control. This may be attributed to high DE and high saccharide contents (Table 3.2) of cooked-Candle flour, perhaps resulting from the breakage of starch as well as  $\beta$ -glucan during cooking. The higher DE, di- and oligo- saccharide contents of cooked-Candle than those of cooked-Phoenix indicated a greater extent of molecular breakage in Candle flour than in Phoenix.

He et al. (1990) reported a decrease in crumb moisture (acting as plasticizer) during storage (as moisture migrates from crumb towards crust), which accelerated starch-gluten interactions and bread firming. Due to their high water binding capacity, broken starch and  $\beta$ -glucan in cooked-Candle bread are capable of retaining more moisture in the crumb, resulting in a reduced crumb firming rate.

Martin et al. (1991) reported that the degree of starch swelling during baking has an important role in bread firming. Anti-firming agents such as monoglycerides and shortenings are known to restrict the swelling of wheat starch during baking, leading to a lessened disruption and solublization of starch molecules and thus less surface area exposed to gluten. This minimizes the starch-gluten interactions and reduces the firming rate. In the present study, the broken/fragmented starch and  $\beta$ -glucan in the cooked barley flour added to wheat flour might have competed for water with native wheat starch in the dough for water. This, in turn might have restricted the swelling and solublization of starch during baking, and thus reduced the firmness.

# 3.3.4. Total (TDF), Insoluble (IDF) and Soluble (SDF) Dietary Fiber Contents of Flours and Breads

The TDF, IDF and SDF contents of flours and breads are given in Table 3.4. The Candle flour had the highest TDF, IDF and SDF contents (12, 4 and 8%, respectively) whereas Phoenix (9, 4 and 5%, respectively) and wheat (4, 2 and 2%, respectively) had comparatively lower contents. The dietary fiber content of barley breads increased as the barley flour substitution level increased from 5% to 15%. As expected Candle barley breads had the highest TDF, SDF and IDF contents. As compared to control, the percentage increases in TDF, IDF and SDF contents in barley breads at 15% substitution level were 19, 18 and 21%, respectively, for Phoenix and 32, 23 and 39%, respectively, for Candle.

Samples	IDF <sup>2</sup>	SDF <sup>2</sup>	TDF <sup>2</sup>
Flour		······································	
Wheat	$1.91 \pm 0.1$	$2.28 \pm 0.1$	$4.19 \pm 0.1$
Phoenix	$4.06 \pm 0.3$	$5.25 \pm 0.2$	$9.31 \pm 0.4$
Candle	$4.18 \pm 0.1$	$7.80 \pm 0.7$	$11.98 \pm 0.8$
Breads			
$100\% WF^{a} + 0\% BF^{3}$	$3.57 \pm 0.4$	$2.48 \pm 0.1$	$5.95 \pm 0.4$
95%WF + 5% PBF <sup>3</sup>	$3.46 \pm 0.3$	$2.70 \pm 0.1$	$6.16 \pm 0.3$
90%WF + 10% PBF	$3.83 \pm 0.2$	$2.95 \pm 0.1$	$6.78 \pm 0.3$
85%WF + 15% PBF	$4.08\pm0.4$	$2.99 \pm 0.1$	$7.06 \pm 0.5$
95%WF + 5% CBF <sup>3</sup>	$3.89 \pm 0.7$	$2.79 \pm 0.1$	$6.68 \pm 0.6$
90%WF + 10% CBF	$4.22 \pm 0.2$	$3.10 \pm 0.1$	$7.32 \pm 0.1$
85%WF + 15% CBF	$4.39 \pm 0.3$	$3.45 \pm 0.1$	$7.84 \pm 0.3$

Table 3.4. Total, insoluble and soluble dietary fiber<sup>2</sup> contents (%) of flours (wheat and barley) and breads<sup>1</sup>.

<sup>1</sup>Expressed as mean ± SD. <sup>2</sup>IDF, Insoluble dietary fiber; SDF, Soluble dietary fiber; TDF, Total dietary fiber. <sup>3</sup>WF, All purpose wheat flour; BF, Barley flour; PBF, Phoenix barley flour; CBF, Candle barley flour.

One slice of 15% substituted Candle bread (approximately 28 g) contained 2.2 g (31.7% more) of TDF as compared to 1.67 g in control. The SDF was 40.5% higher (0.97 g) than that of control bread (0.69 g). Hudson et al. (1992) reported that muffins prepared with barley fiber fraction contained more than 7 g of total dietary fiber/100 g, compared to about 3 g/100 g in the commercial oat bran muffins. Newman et al. (1998) were able to incorporate 0.68 g of SDF per serving in barley enriched baked products. In the present study, the SDF in Candle bread was 0.97 g per serving, which is higher than the FDA requirement for oat fiber health claim of 0.75 g per serving. The recommended daily dietary fiber intake is 20-30 g (U. S. Food and Drug Administration, 1998). To meet these requirements 5-12 servings of grain products/day are recommended. Currently, average dietary fiber consumption in North America is 11 g/day, which is far below the recommended level.

#### **3.4. CONCLUSIONS**

The substitution of wheat flour with barley flour reduced the loaf volume and altered the color and firmness/texture of the bread loaves. However, these changes were found to be dependent upon the barley variety and flour pretreatment (i.e. native or cooked). Cooking of barley flour improved the baking functionality of Candle, while an opposite trend was noticed for Phoenix. Unlike the 15% native-Candle bread that had the highest firmness, the firmness of 15% cooked-Candle bread was close to control. The present study has indicated that breads made with 15% cooked-Candle flour had acceptable physicochemical properties. Therefore, the addition of cooked-Candle flour could be an effective way to increase the TDF and SDF of barley breads.

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## 4. WHEAT BREAD QUALITY AS INFLUENCED BY THE SUBSTITUTION OF WAXY AND REGULAR BARLEY FLOURS IN THEIR NATIVE AND EXTRUDED FORMS<sup>1</sup>

## **4.1. INTRODUCTION**

Barley, due to its high soluble fiber ( $\beta$ -glucan) content and nutritional significance (Klopfenstein and Hoseney, 1987; Klopfenstein, 1988; Newman et al., 1989; McIntosh et al., 1991; Yokoyama et al., 1991; McIntosh et al., 1993; Granfeldt et al., 1994; Lupton et al., 1994; Lia et al., 1995; Wang, 1997), has become a desirable food grain for human consumption. Consumer awareness about high-fiber diets has shifted the trend towards the manufacturing of fiber-enriched food products and thus, efforts have been made to produce high fiber baked goods (Prentice and D'Appolonia, 1977; Volpe and Lehmann, 1977; Bhatty, 1986; Dougherty et al., 1988; Chaudhary and Weber, 1990; Berglund et al., 1992; Hudson et al., 1992; Newman et al., 1998). HB has been successfully incorporated in chemically leavened baked goods such as muffins, pancakes, biscuits, cookies and noodles, however, little success has been achieved in yeast leavened baked products.

Waxy barley flour (very low amylose), contains higher  $\beta$ -glucan content than that of regular barley flour (Fastnaught et al., 1996; Oscarsson et al., 1996; Lee et al., 1997; Bhatty 1999). Therefore, it has gained importance as a useful means of incorporating substantial amounts of soluble fiber in baked goods. However, breads made from dough containing waxy barley starch collapsed upon cooling (Hoseney et al., 1978) and firmed faster than the breads with regular starch (Inagaki et al., 1992). Berglund et al. (1992)

<sup>&</sup>lt;sup>1</sup>A version of this chapter is to be submitted to Journal of Cereal Science for consideration for publication.

reported that the sensory characteristics of 26% waxy barley bread were comparable to those of 26% whole wheat bread, except for their significantly low color scores. However, Lorenz (1995) reported that waxy barley starch is unsuitable for bakery products. Newman et al. (1998) observed a decrease in bread volume in waxy barley breads while the overall quality (firmness not evaluated) was acceptable.

Extrusion is highly adaptable, cost-effective, and energy efficient technology that is most commonly used in the production of breakfast cereals (Harper, 1989). It can lead to physical and macromolecular changes in flour components (Mercier and Feillet, 1975; Chiang and Johnson, 1977; Lee et al., 1982; Gomez and Aguilera, 1983; Bhattacharya and Hanna, 1987; Jackson et al., 1990; Govindasamy et al., 1996; McPherson and Jane, 2000a). Extrusion cooking can improve the nutritional quality of dietary fibers. A higher fermentability rate of extruded dietary fiber from wheat flour was observed in rat intestine than its native counter part (Bjorck et al., 1984). Extrusion under severe conditions can result in slight increase in dietary fiber content (such as non-starch polysaccharides and lignin) because of the formation of enzymatically indigestible starch fractions, (Bjorck et al., 1984; Asp, 1986; Ostergard et al., 1989). A conversion of insoluble dietary fiber into soluble dietary counterparts has also occurred in extrusion cooking (Bjorck et al., 1984; Siljestrom et al., 1986; Shinnick et al., 1988; Camire and Flint, 1991). High degree of starch solublization in feed exposed to very high shear forces resulted in high soluble fiber contents (Bjorck et al., 1984; Wang et al., 1993b). Siljestrom et al. (1986) and Wang et al. (1993b) reported similar observations. However, a small increase in dietary fiber was reported by Wang et al. (1993b). The ß-glucan

contents of several breakfast cereals containing blends of barley and rice or barley and wheat were increased after extrusion (Berglund et al., 1994).

Extrusion cooking has improved the hypocholesterolemic effects of fibers in oat, rice, corn, barley and wheat, and the barley fiber had the greatest improvement (Wang and Klopfenstein, 1993). Chemical (Gould et al., 1989), enzymatic (Thebaudin et al., 1997) and physical (Gaosong and Vasanthan, 2000) treatments can alter the functional properties of fiber. Studies have shown that incorporation of such modified dietary fibers into baked goods, such as cakes and cookies, has improved the texture, sensory properties and shelf life of products (Mark et al., 1988; Jasberg et al., 1989; Bullock, 1992; Ang and Miller, 1991). Changes in the behavior of soluble fiber in waxy and regular barley starch as a result of physical (extrusion) modification have been studied recently (Gaosong and Vasanthan, 2000). However, the impact of extrusion induced changes in barley grain components on baking functionality of barley flour has not been evaluated.

The objectives of this study were to replace a portion of wheat flour with a waxy and a regular barley flour, in their native and extruded (extrusion cooked under different temperature and moisture combinations) states, and to examine their effect on the bread quality characteristics such as loaf volume, crumb firmness, and crumb and crust color.

## **4.2. MATERIALS AND METHODS**

## 4.2.1. Materials

Waxy barley grains (CDC Candle) were obtained from Mr. Jim Gray, Agricore, Calgary, AB. Regular barley grains (Phoenix) were obtained from Dr. Jim Helm, Alberta Agricultural, Food and Rural Development, Lacombe, AB. All-purpose wheat flour (Western Family Foods, Vancouver, BC), crisco shortening (Proctor & Gamble, Toronto, ON), non-fat dry milk (Carnation, Nestle, 1185 Eglintone, Don mills, ON), sugar (Rogers sugar Ltd., Vancouver, BC), salt (Windsor, The Canadian Salt Company, Canada) and yeast (Fleischmann's active dry yeast, Division of Burns Philip Food Ltd., LaSalle, QC) were purchased from a local store. Calcium propionate was obtained from UFL Foods Group Co., Edmonton, AB. The analytical kits for total starch, β-glucan and total dietary fibers were purchased from Megazyme International Ireland Ltd., Wicklow, Ireland. All other chemicals were of analytical grade and were purchased from Sigma Chemical Co., St Louis, MO, Anachemica, Champlain, NY, and Fisher Scientific, Fair Lawn, NJ.

## 4.2.2. Pearling and Milling of Barley Grains

Pearling and milling of barley grains was carried out at the POS pilot pant, Saskatoon, SK. Grains were pearled to 32% in a Satake mill (Model RMB 10G; Satake, Houston, TX). The pearled grains were pin-milled (Alpine Contraplex wide chamber mill Type A250, Hosokawa Micron Systems, Summit, NJ) into flour at 6,000 rpm and a feed rate of 150 kg/h.

## 4.2.3. Extrusion

Extrusion of barley flour was carried out in a corotating, conical C. W. Brabernder's twin screw extruder (Model 2003, C. W. Brabender Instruments, Inc., South Hackensack, NJ). The screws plated with hard chrome were fitted in a figure-eight grooved barrel having 20:1 L/D (length to diameter) ratio. The screws were of single flight and uniform pitch with a compression ratio of 2:1. The die opening was 2 mm in diameter. Total barrel volume with screws was 46 cm<sup>3</sup>. The 15-inch long barrel had two heat zones, heated externally by electrically controlled heating/cooling collars. Thermal

regulation of barrel and die was monitored by temperature control consol equipped with two thermocouples. A temperature/pressure transducer continuously monitored pressure and temperature near die.

Barley flour (waxy and regular) was extruded at different temperature-moisture combinations. These combinations were: low temperature (100°C) low moisture (30%), LTLM; low temperature (100°C) high moisture (50%), LTHM: high temperature (130°C) low moisture (30%), HTLM; and high temperature (130°C) high moisture (50%). HTHM. The screw speed (50 rpm) was kept constant. At each moisture level, the temperature was held constant throughout the barrel. The feed rates were 209 g/min and 110 g/min for feeds containing 50% and 30% moisture contents, respectively. Flours of varying moisture content were prepared by adding distilled water (dry wt. basis) and mixed for 2 hours in a ball mill (Model BP-71147; Notron, Chemical Products Division, U.S. Stoneware Inc., Akron, OH) to equilibrate the moisture. The flours were stored in sealed plastic bags until used. Collection of extruded samples was commenced after the temperature, pressure and torque had reached a steady state. Samples were dried in a draft oven at 75°C, ground in a comminuting mill (Fitz mill, Model D6, The Fitzpatrick Company Elmhurst, IL), sieved through 60-mesh screen, and stored in sealed in plastic bags.

## 4.2.4. Analysis of Flour

Moisture, crude fat and ash contents were determined according to AACC methods 44-19, 08-01 and 30-25, respectively. Total (TDF), insoluble (IDF) and soluble (SDF) dietary fiber contents, and the total starch and  $\beta$ -glucan contents were determined

according to Megazyme methods. Protein content was analyzed with Leco nitrogen (N X 5.7) determinator (Model FP-428; Leco, Leco corporation, St. Joseph MI). Apparent amylose content was determined according to the method explained by Chrastil (1987).

## 4.2.5. Scanning Electron Microscopy (SEM)

Flour samples were sprinkled on a double sided adhesive tapes mounted on aluminum stubs, coated with gold and examined under a JEOL (JSM 6301  $F \times V$ ) scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 5 KV.

## 4.2.6. Determination of Mono-, Di- and Oligo- Saccharides

Flour and bread (bread crumbs rapidly frozen in liquid nitrogen, freeze dried at –  $50^{\circ}$ C, ground and sieved through 60 mesh size) samples (0.1 g) were mixed with 80% ethanol (1 mL), incubated at 40°C for 15 hours in a shaking water bath and centrifuged at 10,000 rpm for 10 min. An aliquot of (10 µl) supernatant was used for mono-, di- and oligo- saccharide determinations. An HPLC system, equipped with a Shimadzu Ezchrom chromatography data system, a solvent delivery system (Varian 9010, Sunnyvale, CA), an HP Series 1050 autosampler, a polyamine column (250 mm length × 4.6 mm; Jordi Gel DVB, Bellingham, MA), and an evaporative light scattering detector (Alltech 500 ELSD, Mandel Scientific, Guelph, ON) was used. Different combinations of mobile phases, distilled water (A) and acetonitrile (B), were used according to the method of Gaosong and Vasanthan (2000). The solvent gradient used was 10% A and 90% B (v/v) at the beginning; 40% A and 60% B (v/v) after 25 min; 0% A and 100% B (v/v) after 26

min; 10% A and 90% B (v/v) after 30 min. The detector temperature was set at 125°C, and a flow rate of 1.0 mL/min was maintained. A series of authentic oligosaccharide mixtures (glucose, DP1; maltose, DP2; maltotriose, DP3; maltotertose, DP4; maltopentose, DP5; maltohexose, DP6) was used for quantification purposes.

## 4.2.7. Water Binding Capacity (WBC) of Barley Flour

Barley flour (5 g) was mixed with 25 g of distilled water, rested for 15 minutes and heated in a boiling water bath (100°C) for 15 min using a Rotavapor (RE 121, Brinkmann, Switzerland). The condensed water was collected and, the moisture loss and WBC were calculated as mentioned below.

Moisture loss (%) =  $\frac{W1-W2}{W3} \times 100$ Where, W1 = Initial weight of flask and sample (g)

W2 = Final weight of flask and sample (g), after evaporation of water

W3 = Amount of water (25 g) taken

WBC = 100 - Moisture loss (%).

#### 4.2.8. Bread-making

Breads were baked using 100 g of flour. Control bread contained 100% allpurpose wheat flour. Test Breads were prepared by replacing all-purpose wheat flour with barley flour at 5, 10 and 15% levels. Barley flour from two different varieties (Candle and Phoenix) was used. Barley flour was added at native as well as extrusion cooked forms. Bread formula included flour (100 g), water (60 g), sugar (6 g), non-fat dry milk (4 g), shortening (3 g), salt (1.5 g), active dry yeast (0.76 g) and calcium propionate (1500 ppm). Besides flour, all the ingredients and their amounts listed in this formula were the same for control as well as test breads.

The dough was made in a Hobart Kitchen-aid mixer (Model K45SS; Hobart Corporation, Troy, OH). Breads were prepared according to straight dough method (AACC 10-10). For dough proofing, a Cres-Cor Proofer (Crescent Metal Products, Cleveland, OH) set at 30°C and 95% relative humidity was used. A moulder [Model B&B 860; Bloemhop Industries Ltd. (1986), Edmonton, AB] was used for sheeting as well as moulding purpose. Baking was done in conventional household type kitchen ovens (General Electric Brand). Baking pans were always placed in the middle rack positioned in the center of the oven. Five loaves of control and five loaves of each treatment were baked and stored for seven days at room temperature. Volume of all loaves was recorded while one loaf from each treatment was used for color measurements. On each day of evaluation, a new loaf from each treatment was used for firmness measurements.

## 4.2.9. Bread Quality Characteristics

Loaf Volume: After one hour of cooling, loaf volume was measured using a loaf volumeter, which used rapeseed displacement method (National Mfg. Co., Lincoln, NE). The loaves were then repacked in polyethylene pouches and stored for seven days at ambient temperatures.

Color: A Hunter lab colorimeter (Model 1836; Labscan XE, Hunter Associates Laboratory, Reston, VA) was employed to obtain L (lightness), a (redness) and b (yellowness) values of flour as well as bread (crust and crumb). The extruded, dried, ground flours, were screened through 60-mesh sieve before taking the measurements. *Firmness:* Bread firmness was measured using an Instron Universal Testing Instrument (Model 4201; Instron Corporation, 100 Royal Street, Canton, MA) attached to an electronic data acquisition system. The firmness measurements of breads were taken on day 1, 3, 5 and 7 after baking. A separate loaf was used for each evaluation. Loaves were sliced into 12.5 mm thick slices with a Berkel meat slicer (Model 1836; Berkel Products Co. Ltd.. Toronto, ON). To avoid the effect of air on crumb firmness, loaves were repacked in polyethylene bags immediately after slicing. The firmness was measured 30 min after slicing. Two slices were stacked on each other to obtain 25mm thick sample and a flat plunger (36 mm diameter) was used for compression. Crust was removed using an electric knife (Hamilton Beach/ Proctor Silex, Inc., Washington, NC). The end slices of loaves were discarded and the remaining slices were picked up randomly. Samples were tested against 25% compression using 50 N load cell and 100 mm/min of cross head

## speed.

#### 4.2.10. Statistical Analysis

All experiments were carried out in triplicates and the analyses were done in duplicates. Data was analyzed using SAS Statistical Software, Version 6 (SAS Institute, 1989). Analysis of variance was performed using General Linear Model procedure. The significance (P<0.05) of differences, observed among treatment means, was established using Tukey's Studentized range test.

#### **4.3. RESULTS AND DISCUSSION**

#### 4.3.1. Flour Analysis

The proximate composition and the contents of  $\beta$ -glucan and amylose of native flours used in bread making are given in Table 4.1. Starch content of wheat flour (74% w/w) was slightly higher than that of barley flours [72% (w/w) for Phoenix and 71% (w/w) for Candle]. Candle had the highest  $\beta$ -glucan content (6%, w/w) followed by Phoenix (4%, w/w) and wheat (0.3%, w/w). Candle, being waxy in nature, had starch with low amylose content (2%), while wheat and Phoenix had regular amylose contents (24 and 26%, w/w, respectively). The saccharide (DP1 – DP6) compositions of wheat and barley flours are given in Table 4.2. Extruded barley flours had higher contents of monoand di- saccharides than their native counterparts. Among the Phoenix flours, the DP3, DP4, DP5 and DP6 contents remained statistically similar, except in native and LTLM flour that had higher DP3 contents. Compared to the native, extrusion cooking of Phoenix flour significantly (P<0.05) decreased the DP3 content (except LTLM flour), whereas an increase in DP1 and DP2 contents was observed. However, DP4, DP5 and DP6 remained unchanged after extrusion. In Candle, the DP1 contents of LTHM and HTHM flours were statistically similar (P>0.05), but higher than those of native, LTLM and HTLM flours. The DP2 content was higher for LTLM and HTLM > HTLM and LTLM > native flour. However, extrusion cooking resulted in a decrease in DP3, DP4 and DP6 content, except that the DP3 and DP4 contents of HTLM flour and DP6 content of HTHM flour were statistically similar (P>0.05) to the native flour. Extrusion at high moisture content (LTHM and HTHM) resulted in high DP1 (Phoenix > Candle) and DP2 (Candle > Phoenix) contents as compared to those observed for low moisture (LTLM and HTLM)

Wheat	Phoenix	Candle
74.1 ± 1.2	72.4 ± 1.5	71.1 ± 1.6
$0.6 \pm 0.1$	$1.1 \pm 0.0$	$1.1 \pm 0.1$
$11.9 \pm 0.1$	$8.4 \pm 0.0$	$9.2 \pm 0.1$
$9.5 \pm 0.2$	$9.0 \pm 0.3$	$7.8 \pm 0.2$
$0.5 \pm 0.0$	$0.8 \pm 0.0$	$0.7 \pm 0.0$
$23.6 \pm 2.2$	$25.6 \pm 0.6$	$1.9 \pm 0.1$
$0.3 \pm 0.11$	$3.9 \pm 0.2$	$6.4 \pm 0.2$
	$0.6 \pm 0.1$ $11.9 \pm 0.1$ $9.5 \pm 0.2$ $0.5 \pm 0.0$ $23.6 \pm 2.2$	$0.6 \pm 0.1$ $1.1 \pm 0.0$ $11.9 \pm 0.1$ $8.4 \pm 0.0$ $9.5 \pm 0.2$ $9.0 \pm 0.3$ $0.5 \pm 0.0$ $0.8 \pm 0.0$ $23.6 \pm 2.2$ $25.6 \pm 0.6$

**Table 4.1.** Chemical composition (% dry wt.) of wheat and barley flours used in bread making<sup>1</sup>.

<sup>1</sup>Expressed as mean  $\pm$  SD. <sup>2</sup> Wet basis.

A I CALINCIIL	Flour	DP1	DP2	DP3	DP4	DP5	DP6
Native							
	Wheat	$0.00 \pm 0.00$	$0.02 \pm 0.01$	$0.05 \pm 0.01$	0.11 ± 0.01	$0.03 \pm 0.02$	$0.02 \pm 0.01$
	Phoenix	0.00 ±0.00	$0.02 \pm 0.00$	$0.15 \pm 0.00$	$0.08 \pm 0.00$	$0.02 \pm 0.00$	0.01 ± 0.00
	Candle	$0.02 \pm 0.00$	$0.07 \pm 0.00$	$0.39 \pm 0.02$	$0.28 \pm 0.02$	$0.05 \pm 0.00$	$0.06 \pm 0.00$
Extruded temp/moist	Phoenix						
100/30		$0.02 \pm 0.00$	$0.07 \pm 0.01$	$0.13 \pm 0.01$	$0.07 \pm 0.01$	$0.03 \pm 0.00$	$0.01 \pm 0.00$
100/50		$0.14 \pm 0.00$	$0.38 \pm 0.01$	$0.06 \pm 0.00$	$0.05 \pm 0.00$	$0.02 \pm 0.00$	$0.01 \pm 0.00$
130/30		$0.03 \pm 0.00$	$0.07 \pm 0.00$	$0.05 \pm 0.00$	$0.06 \pm 0.00$	$0.02 \pm 0.00$	$0.01 \pm 0.00$
130/50		$0.16 \pm 0.01$	$0.44 \pm 0.01$	$0.06 \pm 0.00$	$0.07 \pm 0.00$	$0.02 \pm 0.00$	$0.02 \pm 0.00$
	Candle						
100/30		$0.02 \pm 0.00$	$0.22 \pm 0.01$	$0.25 \pm 0.00$	$0.19 \pm 0.01$	$0.03 \pm 0.00$	$0.01 \pm 0.00$
100/50		$0.09 \pm 0.03$	$1.10 \pm 0.16$	$0.14 \pm 0.05$	$0.19 \pm 0.05$	$0.03 \pm 0.02$	$0.01 \pm 0.01$
130/30		$0.04 \pm 0.00$	$0.31 \pm 0.02$	$0.36 \pm 0.00$	$0.27 \pm 0.01$	$0.06 \pm 0.01$	$0.02 \pm 0.00$
130/50		$0.11 \pm 0.01$	$1.08 \pm 0.04$	$0.16 \pm 0.01$	$0.22 \pm 0.01$	$0.04 \pm 0.01$	$0.04 \pm 0.01$

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content. Increase in moisture content resulted in an increase in DP6 content, but DP4 and DP5 remained unaffected. The DP3 was found to be higher in low moisture than in high moisture extruded flour. Increasing the extrusion temperature from 100°C to 130°C had insignificant (P>0.05) effect on the DP1 and DP2 contents. Increase in temperature increased DP1, DP3, DP4 and DP6 contents, but high temperature had no effect on DP2 and DP5. In general, Candle had higher contents of DP2-6 than Phoenix, which had higher content of DP1. The changes in saccharide composition might be attributed to heat-induced fragmentation of polysaccharides, such as starch and  $\beta$ -glucan.

## 4.3.2. Dough Characteristics – a Subjective Evaluation

The native barley flour substitution increased the dryness and hardness of dough as compared to control. At all levels of substitution, the dough made with native-Phoenix flour was softer than that made with native-Candle. The consistency of dough made with 5% substitution of LTLM and LTHM Phoenix flours was comparable to that of control dough and showed a softer consistency than those substituted with HTLM and HTHM Phoenix flours. However, the dryness and hardness of Phoenix dough increased at higher substitution levels. Dough made with HTHM Phoenix flours resulted in dough with soft consistency as compared to extruded Phoenix flours, except the dough having 5% LTLM and LTHM Phoenix flours. On the contrary, the consistency of dough made with 5% substitution of LTLM, LTHM and HTLM Candle flours resembled that of the control. The stickiness of dough increased at higher substitution levels, nevertheless, the dough consistency remained comparable to control. The dough made with HTHM Candle flour had a soft consistency as compared to control. It had moist, soft but sticky consistency that increased at higher levels of substitution.

Barley flour is rich in  $\beta$ -glucan, which is an excellent hydrocolloid. Also, barley flour had a relatively large amount of small particles as compared to wheat flour (data not shown). This is in agreement with the report of (Bhatty, 1986). The dry and firm consistency of dough may be due to the high water binding capacity of  $\beta$ -glucan as well as smaller particle size of barley flour that tends to absorb water more quickly than wheat flour. Candle, flour being higher in  $\beta$ -glucan content, absorbs more water and results in drier and firmer dough than that made with Phoenix flour. The high amount of \beta-glucan in barley flour, owing to its water soluble and viscous nature, had been a problem when used in baked products (Bhatty, 1986). Despite its high  $\beta$ -glucan content, the extruded-Candle flours resulted in dough with soft consistency, where the softness increased at higher substitution levels. On the other hand, the addition of extruded-Phoenix flour increased the dough firmness. The reasons for this divergence in functionality are unclear. However, the heat-induced changes in  $\beta$ -glucan molecules and the presence of sugars, formed due to thermal change of polysaccharides, might have been involved (Table 4.2).

## 4.3.3. Quality of Wheat and Barley Breads

Breads were baked from 100% wheat flour as well as barley flour (5, 10 & 15%) supplemented wheat flours and their quality characteristics were measured. The photographs of their cross-sectional views are presented in Fig. 4.1 (a, b, c, d and e). Subjectively (personal observation), it was observed that the substitution of wheat flour

Fig. 4.1. Cross-sectional views of breads, showing the effect of native (a) and extruded (LTLM, b; LTHM, c; HTLM, d; HTHM, e) barley flour substitution.







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with barley flours (both native and extruded) altered the loaf- volume, color and firmness/texture of the bread loaves. These changes were found to be dependent on the barley variety and flour pretreatment (i.e. native or extruded). The aforementioned physical characteristics were then evaluated objectively and an attempt was made to explain the observed trends.

## 4.3.3.1. Loaf Volume

## Control vs barley breads

Loaf volumes of control (100% wheat) and barley (native and extruded) breads are given in Table 4.3. Control bread had the highest loaf volume. As compared to the control, the barley (native and extruded) breads had low loaf volumes.

## Native vs extruded barley breads

Among barley breads, the loaf volume of Phoenix native breads at all substitution levels remained significantly (P<0.05) higher than their extruded counterparts. However in Candle breads, the loaf volume decreased in the following order: HTLM and HTHM > native and LTLM > LTHM breads at 5% substitution level; HTHM > native, LTHM and HTLM > LTLM breads at 10% substitution level; and HTHM > HTLM > native and LTHM > LTLM breads at 10% substitution level; and HTHM > HTLM > native and LTHM > LTLM breads at 15% substitution level. It is noteworthy that the volume of 10 and 15% HTHM-Candle breads was statistically similar (P>0.05) to the volume of 5% native Candle and Phoenix breads.

## Extruded barley breads

The substitution of wheat flour with extruded barley flours resulted in decreased loaf volume, which progressively decreased with increased level of substitution. In

Treatment	Substitution	Wheat	Phoenix	Candle
	level (%)			
Native	0	558 ± 12		
	5		$488 \pm 31$	$475 \pm 24$
	10		$426 \pm 23$	$421 \pm 18$
	15		$394 \pm 22$	$340 \pm 13$
Extruded				
temp/moist				
100/30	5		$427 \pm 15$	$455 \pm 33$
100/30	10		$.323 \pm 06$	$383 \pm 18$
100/30	15		$263 \pm 13$	$307 \pm 11$
100/50	5		$393 \pm 11$	$446 \pm 19$
100/50	10		$318 \pm 11$	$415 \pm 06$
100/50	15		$258 \pm 12$	$342 \pm 12$
130/30	5		398 ± 24	$493 \pm 18$
130/30	10		$308 \pm 24$	$427 \pm 20$
130/30	15		$270 \pm 10$	$418\pm20$
130/50	5		$410 \pm 18$	$507 \pm 11$
130/50	10		$352 \pm 31$	457 ± 11
	15		$255 \pm 10$	$466 \pm 15$

Table 4.3. Loaf volume of wheat and barley breads<sup>1</sup>.

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addition to the substitution levels, the loaf volume was found to be significantly (P<0.05) affected by barley variety and extrusion conditions (temperature and moisture). Volume of extruded Candle bread remained significantly (P<0.05) higher than extruded Phoenix bread at their respective addition levels among which HTHM-Candle breads had the highest volume.

Among the extruded barley flours produced at low temperature (100°C), increase in moisture content from 30% to 50% resulted in lower loaf volume in Phoenix at 5% substitution level. However, the differences in loaf volumes observed at 10% and 15% substitution levels were insignificant (P>0.05). For Candle flours, except at 5% substitution level, increase in moisture significantly (P<0.05) increased the loaf volume. For the extruded barley flours produced at high temperature (130°C), increase in moisture (from 30% to 50%) did not significantly alter the loaf volume of Phoenix at 5 and 15% substitution levels. However, at 10% substitution level a small but significant (P<0.05) increase in volume was observed. In case of Candle at 5% substitution level, increase in moisture (from 30% to 50%) resulted in insignificant (P>0.05) differences among the loaf volumes of breads, but the volume remained highest among all barley breads substituted with extruded barley flour. However, at 10 and 15% substitution level, increase in the moisture (from 30% to 50%) significantly (P<0.05) increased the loaf volume of Candle breads.

For the extruded barley flours produced at 30% moisture, an increase in extrusion temperature from 100°C to 130°C decreased the loaf volume of 5% Phoenix breads, whereas statistically insignificant differences were observed at 10 and 15% substitution levels. However, for Candle, extrusion at high temperature (130°C) resulted in increased

loaf volumes. For barley flours extruded at 50% moisture, an increase in temperature increased the loaf volume of 5 and 10% Phoenix breads, but at 15% substitution level, the difference in loaf volume was insignificant (P>0.05). However, a significant increase in loaf volume was observed at all substitution levels for Candle. It is noteworthy that at a 15% substitution level, native and extruded forms of Candle and Phoenix flours showed an opposite effect on loaf volume. Also, the loaf volume of 15% HTHM-Candle bread was similar (P>0.05) to that of 5% substituted native and extruded (expect 5% HTLM-Candle bread) Phoenix and Candle breads. This suggests that use of HTHM Candle flour as opposed to native and other extruded barley flours in bread production could increase the substitution level.

## 4.3.3.2. Bread Crumb Firmness

## Control vs barley breads

The firmness of wheat and barley breads on day-1 of storage is given in Table 4.4. Control breads had the lowest firmness. As compared to control, the barley (native and extruded) breads had high firmness that progressively increased with substitution level, exception being the barley breads made with 15% extruded Candle flour (HTHM; extrusion temperature and moisture, 130°C and 50%, respectively). On day-1, the firmness of 5% native barley flour breads (Phoenix and Candle) and 5% HTHM-Candle breads was similar to that of control (Table 4.4). Furthermore, the firmness of breads made from HTHM-Candle flour was similar (P>0.05) to that of control at all three substitution levels.

Treatment	Substitution	Wheat	Phoenix	Candle
	level (%)			
Native	0	$0.9 \pm 0.2$		
	5		$1.2 \pm 0.3$	$1.1\pm0.1$
	10		$1.7 \pm 0.3$	$1.5 \pm 0.1$
	15		$1.8\pm0.2$	$2.3\pm0.2$
Extruded				
temp/moist				
100/30	5		$1.6 \pm 0.1$	$1.3 \pm 01$
100/30	10		$2.5 \pm 0.1$	$1.9 \pm 0.3$
100/30	15		$3.7 \pm 0.2$	$2.7\pm0.1$
100/50	5		$2.1 \pm 0.1$	$1.4 \pm 0.1$
100/50	10		$2.6\pm0.1$	$1.4 \pm 0.1$
100/50	15		$4.3 \pm 0.2$	$2.1\pm0.1$
130/30	5		$1.9 \pm 0.3$	$1.1 \pm 0.2$
130/30	10		$3.1 \pm 0.1$	$1.5 \pm 0.0$
130/30	15		$4.3 \pm 0.2$	$1.4 \pm 0.2$
130/50	5		$1.6 \pm 0.1$	$1.0 \pm 0.0$
130/50	10		$2.2\pm0.3$	$1.1 \pm 0.1$
130/50	15		$4.2 \pm 0.2$	$0.7 \pm 0.1$

**Table 4.4.** Firmness of wheat and barley (native and extruded) breads on day-1 of storage<sup>1</sup>.

<sup>1</sup>Expressed as mean  $\pm$  SD. Note: See Appendix 3a & b for statistical analysis.

## Native vs extruded barley breads

The day-1 firmness (Table 4.4) of all levels of native Phoenix substituted breads was significantly (P<0.05) lower than that of extruded Phoenix substituted breads. However, in Candle at 5% substitution level, native and extruded breads had equal firmness, but other substitution levels the crumb firmness decreased in the following order: LTLM > native, LTHM and HTLM> HTHM breads at 10% substitution; native, LTLM and LTHM >HTHM > HTHM breads at 15% substitution level.

## Extruded barley breads

Amongst the breads made from extruded barley flour, Phoenix had higher firmness than Candle. Generally, breads made with low moisture and low temperature extruded barley flours were found to be firmer than those made with high moisture and high temperature extruded barley flours. For Phoenix breads produced with low temperature extruded flour, increase in moisture content increased the firmness of breads at high (10 and 15%) substitution levels. However, among the breads produced with high temperature (130°C) extruded Phoenix flour, high content of extrusion moisture decreased the firmness at 10% substitution level. In Candle, at high levels of substitution, an increase in extrusion temperature (from 100°C to 130°C) and moisture (from 30% to 50%) decreased the firmness of barley breads.

The effects of barley flour substitution on bread crumb firmness over a 7-day storage period are given in Fig. 4.2 (a, b, c, d & e). For all treatments (native and extruded), bread firmness changed significantly (P<0.05) overtime and varied between varieties at different levels of substitution. The firmness of control and barley breads increased rapidly up to day-3 of storage and slowed down thereafter. However, in all the

Fig. 4.2. Crumb firmness of native (a) and extruded (LTLM, b; LTHM, c; HTLM, d; HTHM, e) barley substituted bread over a 7-day storage period. • = Control,  $\Box = 5\%$  Phoenix,  $\Delta = 10\%$  Phoenix,  $\Diamond = 15\%$  Phoenix,  $\blacksquare = 5\%$  Candle,  $\blacktriangle = 10\%$  Candle and  $\blacklozenge = 15\%$  Candle.






cases firmness increased throughout the entire storage period. The difference between the firmness of LTLM-Phoenix and LTLM-Candle flour substituted breads was insignificant (P>0.05). However, for the low temperature extruded barley flours, increase in the extrusion moisture content significantly (P<0.05) reduced the firmness of Candle breads as compared to Phoenix breads. It was observed that 5 and 10% substituted LTHM-Candle breads had similar firmness, whereas the firmness of 15% LTHM-Candle breads was similar to that of 5% LTHM-Phoenix breads.

For barley flours extruded at high temperature, an increase in moisture content (from 30% to 50%) resulted in a decrease in the firmness of Candle breads, while the firmness of Phoenix breads remained unaffected. Amongst the HTLM-barley flour substituted breads, the firmness of Candle breads at all substitution levels was significantly (P<0.05) lower than that of 5% Phoenix breads. Interestingly, for barley flour extruded at high temperature, increasing the moisture content (from 30% to 50%) not only decreased the firmness of Candle breads, but also made 15% substituted breads softer than control (100% wheat).

Also, the firmness of 5 and 10% Candle breads was somewhat similar to that of control. Similar results were reported in one of our previous study (chapter 3), where substitution with 15% kettle-cooked Candle flour resulted in firmness lower than that of 5 and 10% substitution, but higher and comparable to control. In the extrusion of Candle flour, increasing the extrusion temperature at both low and high moisture levels significantly reduced the firmness of Candle breads. Firmness of all extruded Phoenix breads at all substitution levels remained higher than that of control and Candle breads.

Breads made with 15% Phoenix flour extruded at different moisture and temperature combinations had maximum firmness.

## 4.3.3.3. Bread Color

### Bread Crust Color

Control breads had golden brown crust (subjective-personal evaluation). Generally, Candle breads (at all substitution levels) and Phoenix breads (only at lower substitution level) showed golden brown crust. The crust of breads made from high temperature and high moisture extruded barley flour was browner than that from low temperature and low moisture extruded barley flour. Hunter L-, a- and b-crust color values of breads are given in Table 4.5. The L-crust color value of native and extruded (5% LTLM, LTHM, HTLM and 5 & 10% HTHM) Phoenix breads was comparable to that of control. Similarly, the L-crust color value of native and extruded Candle breads, at all substitution levels were comparable to that of control, except that 10% LTLM and 15% native & LTHM breads had paler/lighter crust (higher L-crust color value) than the control. High temperature extruded (HTLM and HTHM) barley flour substituted breads (5, 10 and 15% Candle and 5% Phoenix) had lower L-crust color value, but in case of low temperature extruded barley flour, only 5% barley breads had L-crust color value comparable to that of control.

Candle flour extruded at high temperature (HTLM and HTHM) had the brownest crust color that observed to be similar at all substitution levels. Conversely, the crust of breads made with low temperature extruded Candle flours became less brown at higher (10 and 15%) substitution levels. High temperature and high moisture extruded Phoenix

Treatment	Treatment Substitution	Ľ	Γ			æ			a	
	level (%)	Wheat	Phoenix	Candle	Wheat	Phoenix	Candle	Wheat	Phoenix	Candle
Native	0	$44.3 \pm 0.5$			$18.1 \pm 0.3$			$32.8 \pm 0.2$		
	S		$41.6 \pm 1.2$	Ŧ		H	$16.5 \pm 0.9$		$30.6 \pm 0.8$	Η
	10		$47.1 \pm 3.3$	$45.9 \pm 3.3$		-++	$16.2 \pm 0.6$		-#1	-++
	15		$50.2 \pm 2.1$	57.1 ± 3.3		$15.2 \pm 0.6$	$11.9 \pm 2.0$		$34.1 \pm 0.6$	$33.4 \pm 0.9$
Extruded										
temp/moist										
100/30	5		47.8 ± 1.5	$48.0 \pm 1.7$		$18.0 \pm 0.1$	$17.8 \pm 0.1$		34.7 + 1.2	351+09
100/30	10		+	$56.2 \pm 2.3$		$12.6 \pm 3.8$	$15.5 \pm 1.6$		1+1	+
100/30	15		++	-+-		+	+		343+08	364+15
									ł	4
100/50	5		47.8 ± 2.3	47.5 ± 2.7		$16.9 \pm 0.8$	$17.7 \pm 0.5$		$34.5 \pm 1.0$	$34.8 \pm 1.3$
100/50	10		57.4 ± 4.2	$51.3 \pm 2.9$		$14.7 \pm 3.3$	$17.3 \pm 1.0$		+	$36.5 \pm 0.9$
100/50	15		H	$58.9 \pm 3.1$		+1	$13.4 \pm 2.4$		35.3±2.3	$36.4 \pm 1.3$
130/30	Ś		$49.6 \pm 3.3$	45.1 ± 1.1		17.6 + 0.7	186+04		350+14	2 1 + C PC
130/30	10		548+73	468+13		15 0 + 1 3	1 - 1		4 4	31 T T T T T T
130/30	15		58.9±2.8	7.6 ± 1			+ +		-1 -+1	н н
130/50	S		43.4 ± 2.3	43.5 + 1.6		185+02	186403		+ 2 + 2	+
130/50	10		5.1	1 ++		$17.5 \pm 0.5$	$18.9 \pm 0.3$			
130/50	15		1.1	$47.0 \pm 2.2$		$14.9 \pm 0.8$	$19.0 \pm 0.2$		1 +	35.7+1.5

had browner crust that became lighter (pale) with higher substitution levels.

No significant (P>0.05) differences were found between the a-crust color values of control and Phoenix (native and extruded) breads at all levels of substitution, except 15% native, LTLM and LTHM breads that had lower values. The a-crust color value of native and extruded Candle breads at all substitution levels (exception being, 15% native, LTLM and LTHM breads) was similar to that of control. Moisture level during extrusion of barley flour had no effect (P>0.05) on a-crust color value. However a-crust color value was significantly affected by barley variety (Candle>Phoenix), substitution level (decreased with increase in barley flour substitution) and extrusion temperature (greater at 130°C). The differences among the b-crust color values of control, native and extruded Phoenix breads were insignificant (P>0.05), except that the b-crust color values of 5% LTHM, 5 and 10% HTLM and 15% HTHM breads were higher (P<0.05) than that of control. However in Candle, 5% LTLM and LTHM breads had significantly (P<0.05) higher b-crust values than that of control. Barley variety as well as extrusion variables (temperature and moisture) had no effect (P>0.05) on b-crust color values. But, the level of barley flour substitution had a significant (P<0.05) affect on the b-crust color values. The b-crust color values for 10 and 15% barley breads were similar (P>0.05), but significantly higher than 5% barley breads.

#### Bread Crumb Color

The objective evaluation of Hunter L-, a- and b-crumb color values of breads are given in Table 4.6. The brightness of crumb decreased (lower L-crumb color value) with the increase in extrusion temperature, moisture as well as barley flour substitution.

Treatment	Treatment Substitution	-	Ŀ			R			٩	
	level (%)	Wheat	Phoenix	Candle	Wheat	Phoenix	Candle	Wheat	Phoenix	Candle
Native	0	$79.2 \pm 0.1$			$-0.4 \pm 0.0$			$17.4 \pm 0.1$		
	5		$75.6 \pm 0.6$	$75.9 \pm 1.1$		$0.7 \pm 0.2$	$0.7 \pm 0.1$		$19.0 \pm 0.4$	$19.3 \pm 0.4$
	10		$75.6 \pm 0.7$	$75.9 \pm 0.5$		$1.0 \pm 0.1$	$1.0 \pm 0.1$		$19.3 \pm 0.4$	$19.7 \pm 0.3$
	15		$75.1 \pm 0.5$	$74.9 \pm 0.7$		$1.4 \pm 0.1$	$1.3 \pm 0.1$		$19.8 \pm 0.3$	$20.4 \pm 0.5$
Extruded										1
temp/moist										
100/30	S		<b>79.1</b> ± 0.2	$78.9 \pm 0.3$		$0.5 \pm 0.1$	$0.5 \pm 0.2$		$18.1 \pm 0.1$	$18.1 \pm 0.1$
100/30	10		$78.0 \pm 0.6$	$77.8 \pm 0.4$		$0.9 \pm 0.1$	$0.7 \pm 0.1$		$17.5 \pm 0.5$	$17.9 \pm 0.3$
100/30	15		$77.3 \pm 0.9$	$77.2 \pm 0.4$		$1.2 \pm 0.1$	1.1 ± 0.1		$17.3 \pm 0.4$	$17.7 \pm 0.4$
100/50	ç		0 0 + 0 22	786407		0.7 + 0.1	03401			-
00/201	5 <u>-</u>					1.0 + 7.0			1014 101	10.1 ± 0.2
00/001	10		H	$8.0 \pm 0.2$		$1.2 \pm 0.1$	$0.7 \pm 0.1$		$17.7 \pm 0.4$	$18.1 \pm 0.1$
100/50	15		76.6 ± 0.6	<b>76.7 ± 0.5</b>		$1.6 \pm 0.0$	$1.2 \pm 0.1$		$17.5 \pm 0.1$	$18.2 \pm 0.4$
130/30	S		<b>79.0 ± 0.4</b>	79.1 ± 0.1		<b>0.8</b> ± 0.0	$0.3 \pm 0.1$		$18.1 \pm 0.3$	$17.8 \pm 0.1$
130/30	10		$77.9 \pm 0.3$	$78.2 \pm 0.2$		$1.2 \pm 0.0$	$0.7 \pm 0.1$		$18.1 \pm 0.4$	18.1 ± 0.3
130/30	15		77.5 ± 0.4	<i>77.5</i> ± 0.2		$1.5 \pm 0.0$	-++		$17.2 \pm 0.2$	18.1 ± 0.0
130/50	5			$78.0 \pm 0.1$		0.9 ± 0.1	0.3 ± 0.1			$18.0 \pm 0.1$
130/50	10		± 0.3	$77.6 \pm 0.1$		$1.5 \pm 0.0$	$0.7 \pm 0.1$		$17.6 \pm 0.1$	$18.1 \pm 0.2$
130/50	15		$76.3 \pm 0.0$	$76.2 \pm 0.1$		$1.7 \pm 0.0$	$0.8 \pm 0.1$			$17.8 \pm 0.0$

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However, the L-crumb color value was not influenced by barley variety. The Lcrumb color value was influenced to a greater extent by extrusion moisture than extrusion temperature. Hunter a-crumb color value of barley breads was significantly higher than that of control. Extrusion of barley flours either under high temperature or high moisture increased a-crumb color value. Also, the a-crumb color value increased with high substitution levels.

Among the breads made of extruded barley flours (at both moisture levels), Phoenix had significantly (P<0.05) high a-crumb color values as compared to Candle. The b-crumb color values of native Phoenix and Candle breads were higher than that of control and extruded breads. On the other hand, extruded Candle breads had higher bcrumb color values than their Phoenix counterparts. Candle breads had no significant differences in the b-crumb color values whereas b-crumb color value of Phoenix breads decreased with high substitution levels

The low loaf volume and firm crumb texture of barley breads as compared to control wheat breads may be attributed to a number of factors. The direct factor could be the gluten dilution (Pomeranz et al., 1977; Dubois, 1978). The physicochemical properties of barley  $\beta$ -glucan and starch can also affect bread volume and texture indirectly.  $\beta$ -Glucan in barley flour, when added to wheat flour during bread making, could tightly bind to appreciable amounts of water in the dough, suppressing the availability of water for the development of gluten-net work. Underdeveloped gluten network can lead to reduced loaf volume and increased firmness in breads. Furthermore, in yeast leavened bread systems, in addition to CO<sub>2</sub>, the steam is an important leavening agent. The extent of steam generation within the dough during baking could also have

influenced the loaf volume and texture (firmness/softness). Due to its high affinity to water,  $\beta$ -glucan can suppress the extent of steam generation, resulting in a reduction in loaf volume and an increase in firmness. Gaosong and Vasanthan (2000) reported that the  $\beta$ -glucan content and its water solubility are substantially higher in native Candle flour (6.4% and 41.5%, w/w, respectively) than in native Phoenix flour (3.9% and 26.8%, w/w, respectively).

Also, it was determined in this study that the WBC of native Candle flour (52%) was higher than the native Phoenix flour (36%). This implied the " $\beta$ -glucan effect", would be more prominent with Candle flour substitution. However, the WBC of native Phoenix flour and Candle flours was increased after extrusion cooking (Appendix 4). Therefore, the observed better baking performance of HTHM-Candle flour indicates the positive influence of other factors (as discussed below) overriding the negative influence by the increase in WBC.

Hoseney et al. (1978) reported that regular barley starch when replaced with wheat starch resulted in satisfactory performance in bread making. However, the barley starch composition (i.e. the ratio between amylose and amylopectin) and physicochemical properties (i.e. swelling and water holding/binding during gelatinization) differ substantially with variety. The Candle starch belongs to the waxy type (<5% amylose content) and the Phoenix starch belongs to the regular type (approximately 20-25% amylose). During gelatinization, waxy starches swell and bind/hold substantially higher amount of water than regular starches (Vasanthan and Bhatty, 1996). Therefore, during baking, the starch in Candle flour would gelatinize and bind a substantial amount of available water. This could suppress the steam generation within the loaf, resulting in a

lower volume and a higher firmness in Candle than in Phoenix breads. Lorenz (1995) demonstrated the importance of amylose in bread-making, stating its importance for holding starch granules as intact and giving structure to the bread crumb. The author further concluded that waxy starch is not suitable as an ingredient in bakery products due to its low amylose content. Thus in the present study, the lack of amylose (<5%) in Candle barley starch would have negatively contributed towards crumb setting (lower crumb strength), resulting in crumb collapse after baking (Hoseney et al., 1978). This, in turn, would have resulted in a reduced loaf volume and an increased firmness.

In the present study, the scanning electron microscopy of the native and extruded barley flours (Fig. 4.3) indicated that the extruded-Phoenix flour had relatively higher amount of large particles than in extruded-Candle flour. This may be due to the minimal disruption of Phoenix starch during extrusion and the presence of amylose (a good binder) in Phoenix starch, which can leach out during cooking (i.e. gelatinization) and subsequently bind flour particles together (agglomeration) during its retrogradation. However, the retrogradation capacity of Candle starch is low due to its high amylopectin content and the disrupted starch granules and other flour particles remain as small particles due to minimal agglomeration. HTHM-Candle flour performs better because crumb setting is efficient due to granule breakage as evident from the SEM micrographs (HTHM extruded flour showed broken starch granules). These small particles, especially the disrupted starch particles, could increase overall surface area, which can efficiently contribute towards crumb setting through improved starch-protein interactions. This may be responsible for the observed improvement in loaf volume and texture in breads made with extruded-Candle flours. However, in the native-Candle breads the disruption of

Fig. 4.3. Scanning electron micrographs of native and extruded barley flours (bar width =  $10\mu m$ ). Native Phoenix flour, **a**; HTHM Phoenix flour, **b**; native Candle flour, **c**; HTHM Candle flour, **d**.

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Candle starch granules during baking would be minimal due to limited availability of water in the dough (limited water limits the swelling and subsequent disruption of starch granules).

Native waxy barley breads did not show desirable sensory properties due to lack of amylose, which is important for the crumb setting process (Lorenz, 1995). Extrusion cooking results in molecular fragmentation of native starches (Colonna et al., 1984; Davidson et al., 1984). McPherson and Jane (2000b) reported that the molecular weight of amylopectin, in corn starch, decreased from  $7.7 \times 10^8$  to  $1.0 \times 10^8$  during extrusion. Chinnaswamy and Hanna (1990) showed an increase in amylose content in corn starch during extrusion cooking and reported that the fragmentation of amylopectin chains could be responsible. The authors also reported that the fragmentation in amylopectin chains was more pronounced than that in amylose chains, and the severity of amylopectin fragmentation increased with the decrease in amylose content. Such molecular fragmentation of amylopectin and the resulting increase in amylose content in Candle waxy barley flour are likely factors responsible for its better performance in baking. It is noteworthy that the loaves of 30% substituted HTHM-Candle breads did not collapse and were even comparable to its 5% substituted counterpart (Appendix 7).

The differences in WBC of  $\beta$ -glucan and starch in native and extruded/cooked barley flours can influence the steam generation within the dough during baking and thereby influence loaf volume and firmness as explained earlier. For instance, Gaosong and Vasanthan (2000) reported that Candle  $\beta$ -glucan undergoes molecular fragmentation when subjected to heat and shear. Fragmented  $\beta$ -glucan is less viscous and holds less water than its native counterpart. However, the Phoenix  $\beta$ -glucan did not show any evidence of fragmentation.

The observations made in the bread storage study, where the crumb firmness increased rapidly during the first three days and slowed thereafter, were in agreement with those reported in previous studies (Volpe and Lehmann, 1977; Ghiasi, 1984). Amylopectin plays an important role in crumb firming during storage. It was reported that amylopectin recrystallization was faster in the breads containing high levels of amylopectin. Breads made with waxy starch firmed faster than those made with regular starch (Inagaki et al., 1992). In the present study, the firmness data obtained from breads made with native barley flour substitution are in agreement with those reported by Inagaki et al. (1992) and Lorenz (1995). It was observed that the native-Candle breads with high levels of amylopectin firmed at a faster rate than the native-Phoenix breads containing normal level of amylopectin. However, the low firmness of breads made with extruded Candle flour adds a discrepancy to the above finding. These firmness data are in agreement with those observed in our previous study (chapter 3), where substitution with cooked Candle flour resulted in lower bread firmness. This implies that native and extruded barley flours deliver different functionalities in the production of baked products.

Martin and Hoseney (1991) have earlier demonstrated the antifirming properties of low molecular weight dextrins (broken starch). As shown in the results, the firmness rate decreased with high substitution levels of extruded-Candle flour. Unlike the 15% native-Candle bread that had the highest firmness, the firmness of 15% HTHM-Candle bread was lower than that of the control. This may be attributed to high saccharide contents (Table 4.2) of extruded-Candle flour, perhaps resulting from the breakage of starch as well as  $\beta$ -glucan during cooking. The higher di- and oligo- saccharide contents of extruded-Candle than those of extruded-Phoenix indicated a greater extent of molecular breakage in Candle flour than in Phoenix.

He et al. (1990) reported a decrease in crumb moisture (acting as plasticizer) during storage (as moisture migrates from crumb towards crust) which accelerated starchgluten interactions and bread firming. In the present study, increased water binding capacity of extruded-Candle might have improved the moisture retention in the extruded-Candle breads, resulting in reduced crumb firmness.

Martin et al. (1991) reported that the degree of starch swelling during baking has an important role in bread firming. Anti-firming agents such as monoglycerides and shortenings are known to restrict the swelling of wheat starch during baking, leading to a lessened disruption and solublization of starch molecules and thus less surface area exposed to gluten. This minimizes the starch-gluten interactions and reduces the firming rate. Similarly, in the present study, the broken/fragmented starch and  $\beta$ -glucan in the extruded barley flour added to wheat flour might have competed for water (as evidenced from the increased WBC of extruded barley flour being higher in Candle than in Phoenix – Appendix 4) with native wheat starch granules in the dough. This, in turn, might have restricted the swelling and solublization of starch during baking, and thereby reduced the firmness.

# 4.3.4. Total (TDF), Insoluble (IDF) and Soluble (SDF) Dietary Fiber Content of Flours and Breads

The TDF, IDF and SDF contents of flours and breads are given in Table 4.7. The Candle flour had the highest TDF, IDF and SDF contents (12, 4 and 8%, respectively) whereas Phoenix (9, 4 and 5%, respectively) and wheat (4, 2 and 2%, respectively) had comparatively lower contents. The dietary fiber content of barley breads increased as the barley flour substitution level increased from 5% to 15%. As expected Candle barley breads had the highest TDF, SDF and IDF contents. As compared to control, the percentage increases in TDF, IDF and SDF contents in barley breads at 15% substitution level were 19, 18 and 21%, respectively, for Phoenix and 32, 23 and 39%, respectively, for Candle.

One slice of 15% substituted Candle bread (approximately 28 g) contained 2.2 g (31.7% more) of TDF as compared to 1.67 g in control. The SDF was 40.5% higher (0.97 g) than that of control bread (0.69 g). Hudson et al. (1992) reported that muffins prepared with barley fiber fraction contained more than 7 g of total dietary fiber/100 g, compared to about 3 g/100 g in the commercial oat bran muffins. Newman et al. (1998) were able to incorporate 0.68 g of SDF per serving in barley enriched baked products. In the present study, the SDF in Candle bread was 0.97 g per serving, which is higher than the FDA requirement for oat fiber health claim of 0.75 g per serving. The recommended daily dietary fiber intake is 20-30 g (U. S. Food and Drug Administration, 1998). To meet these requirements 5-12 servings of grain products/day are recommended. Currently, average dietary fiber consumption in North America is 11 g/day, which is far below the recommended level.

Samples	IDF <sup>2</sup>	SDF <sup>2</sup>	TDF <sup>2</sup>
Flour			
Wheat	$1.91 \pm 0.1$	$2.28 \pm 0.1$	$4.19 \pm 0.1$
Phoenix	$4.06 \pm 0.3$	$5.25 \pm 0.2$	$9.31 \pm 0.4$
Candle	$4.18\pm0.1$	$7.80 \pm 0.7$	11.98 ± 0.8
Breads			
100%WF <sup>a</sup> + 0% BF <sup>3</sup>	$3.57 \pm 0.4$	$2.48 \pm 0.1$	$5.95 \pm 0.4$
95%WF + 5% PBF <sup>3</sup>	$3.46 \pm 0.3$	$2.70 \pm 0.1$	$6.16 \pm 0.3$
90%WF + 10% PBF	$3.83 \pm 0.2$	$2.95 \pm 0.1$	$6.78 \pm 0.3$
85%WF + 15% PBF	$4.08\pm0.4$	$2.99 \pm 0.1$	$7.06 \pm 0.5$
95%WF + 5% CBF <sup>3</sup>	$3.89 \pm 0.7$	$2.79 \pm 0.1$	$6.68 \pm 0.6$
90%WF + 10% CBF	$4.22 \pm 0.2$	$3.10 \pm 0.1$	$7.32 \pm 0.1$
85%WF + 15% CBF	$4.39 \pm 0.3$	$3.45 \pm 0.1$	$7.84 \pm 0.3$

Table 4.7. Total, insoluble and soluble dietary fiber<sup>2</sup> contents (%) of flours (wheat and barley) and breads<sup>1</sup>.

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<sup>1</sup>Expressed as mean ± SD. <sup>2</sup>IDF, Insoluble dietary fiber; SDF, Soluble dietary fiber; TDF, Total dietary fiber. <sup>3</sup>WF, All purpose wheat flour; BF, Barley flour; PBF, Phoenix barley flour; CBF, Candle barley flour.

### **4.4. CONCLUSIONS**

The substitution of wheat flour with barley flour reduced the loaf volume and altered the color and firmness/texture of the bread loaves. However, these changes were found to be dependent upon the barley variety and flour pretreatment (i.e. native or extruded). Extrusion of barley flour improved the baking functionality of Candle, while an opposite trend was noticed for Phoenix. Unlike the 15% native-Candle bread that had the highest firmness, the firmness of 15% HTHM-Candle bread was similar to control. The present study has indicated that breads made with 15% HTHM-Candle flour had highly acceptable physicochemical properties. Therefore, the addition of extruded-Candle flours, especially the HTHM Candle flour, would be an effective way to increase the TDF and SDF content of barley breads. Overall, it is apparent that the use of extruded flour would be better than the use of cooked flour. Waxy barley although preferred in breadmaking due to high TDF and SDF contents, yielded poor quality breads when used as a substitution for wheat flour. Nevertheless, this research had clearly demonstrated the use of extrusion cooking as an innovative approach to increase the use of waxy barley flour in bread-making without substantially deteriorating the bread quality.

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## 5. CONCLUSIONS AND FUTURE RECOMMENDATIONS

The substitution of wheat flour with barley flour reduced the loaf volume and altered the color and firmness/texture of the bread loaves. However, these changes were found to be dependent on the barley variety and flour pretreatments i.e. native, conventional pan cooking (at a fixed temperature and moisture) or extrusion cooking (under different temperatures and moistures). Cooking in either form (conventional pan or extrusion) of barley flour improved the baking functionality of waxy barley (Candle), while an opposite trend was noticed in the regular barley (Phoenix). Unlike the 15% native-Candle bread that had the highest firmness, the firmness of 15% cooked-Candle bread was close to control and the firmness of 15% HTHM-Candle bread was statistically similar to control. The present study has demonstrated that breads made with 15% cooked-Candle flour or 15% HTHM-Candle flour had acceptable physicochemical properties. However, extrusion cooking process, being highly adaptable, cost-effective and energy efficient technology, is most widely practiced in food industry. Hence, it seems the use of extruded flour to be highly practical than the use of cooked flour. Therefore, the addition of extruded-Candle flours, especially the HTHM-Candle flour, would be an effective way to increase the TDF and SDF content of barley breads.

It is noteworthy that the loaves of 30% HTHM-Candle breads did not collapse and were even comparable to its 5% substituted counterpart (Appendix 7). Production of such barley breads can be used as a useful vehicle to increase the SDF content in human diet. Waxy barley although preferred in bread-making due to the higher TDF and SDF contents, yielded poor quality breads upon substitution with wheat flour. This research had demonstrated clearly the use of extrusion cooking as an innovative approach to to increase the use of waxy barley flour in bread-making. The breads produced in this study were prepared using straight dough baking procedure. However, further research can be done to alter the baking formula in order to improve the barley bread quality, for example, to mask the effect of gluten dilution, vital wheat gluten may be added to the formula. Extruded barley flour at higher substitution levels (>15%) can be further investigated. Future studies can be done on the comparison of the baking functionality of extruded flours from different sources, such as oat and other barley varieties. Overall, the future of barley bread as a functional food seems to be bright.

6. APPENDIX

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Native Wheat $0.00 \pm 0.00^d$ Phoenix $0.00 \pm 0.00^d$ Candle $0.02 \pm 0.00^{cd}$						
0.00 0.00 0.02						
0.00	ء 0.00 <sup>d</sup>	0.02 ± 0.01°	$0.05 \pm 0.01^{d}$	$0.11 \pm 0.01^{d}$	$0.03 \pm 0.02^{abcd}$	$0.02 \pm 0.01^{b}$
0.02	е0.00 <sup>d</sup>	$0.02 \pm 0.00^{\circ}$	$0.15 \pm 0.00^{\circ}$	$0.08 \pm 0.00^{d}$	$0.02 \pm 0.00^{\text{hed}}$	$0.01 \pm 0.00^{b}$
	± 0.00 <sup>cd</sup>	0.07 ± 0.00°	$0.39 \pm 0.02^{a}$	$0.28 \pm 0.02^{a}$	$0.05 \pm 0.00^{ab}$	$0.06 \pm 0.00^{a}$
Extruded						
Phoenix						
100/30 0.02 ±	± 0.00 <sup>cd</sup>	$0.07 \pm 0.01^{de}$	$0.13 \pm 0.01^{\circ}$	0.07 ± 0.01 <sup>d</sup>	$0.03 \pm 0.00^{abcd}$	0.01 ± 0.00 <sup>b</sup>
100/50 0.14 ±	± 0,00 <sup>th</sup>	$0.38 \pm 0.01^{\rm b}$	$0.06 \pm 0.00^{d}$	$0.05 \pm 0.00^{d}$	$0.02 \pm 0.00^{cd}$	$0.01 \pm 0.00^{b}$
<b>130/30</b> 0.03 ±	± 0.00°	$0.07 \pm 0.00^{\circ}$	$0.05 \pm 0.00^{d}$	$0.06 \pm 0.00^{d}$	$0.02 \pm 0.00^{cd}$	0.01 ± 0.00 <sup>b</sup>
130/50 0.16 ±	± 0,01 <sup>a</sup>	0.44 ± 0.01 <sup>b</sup>	$0.06 \pm 0.00^{d}$	$0.07 \pm 0.00^{d}$	$0.02 \pm 0.00^{d}$	0.02 ± 0.00 <sup>b</sup>
Candle						
100/30 0.02 ± 0	± 0.00 <sup>cd</sup>	0.22 ± 0.01 <sup>cd</sup>	$0.25 \pm 0.00^{\text{b}}$	$0.19 \pm 0.01^{\circ}$	$0.03 \pm 0.00^{abcd}$	0.01 ± 0.00 <sup>b</sup>
100/50 0.09 ±	± 0.03 ±	$1.10 \pm 0.16^{a}$	$0.14 \pm 0.05^{\circ}$	$0.19 \pm 0.05^{\circ}$	$0.03 \pm 0.02^{abcd}$	0.01 ± 0.01 <sup>b</sup>
130/30 0.04 ±	± 0.00 ±	$0.31 \pm 0.02^{bc}$	$0.36 \pm 0.00^{a}$	$0.27 \pm 0.01^{ab}$	$0.06 \pm 0.01^{a}$	$0.02 \pm 0.00^{b}$
130/50 0.11 ±	± 0.01 <sup>b</sup>	$1.08 \pm 0.04^{a}$	$0.16 \pm 0.01^{\circ}$	$0.22 \pm 0.01^{\mathrm{hc}}$	$0.04 \pm 0.01^{abc}$	$0.04 \pm 0.01^{a}$

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Treatment	Substitution level (%)	Wheat	Phoenix	Candle
Native	0	$558 \pm 12^{a}$	······································	
	5		$488 \pm 31^{bcd}$	$475 \pm 24^{cde}$
	10		$426 \pm 23^{hij}$	$421 \pm 18^{hi}$
	15		$394 \pm 22^{jk}$	$340 \pm 13^{lm}$
Extruded				
temp/moist				
100/30	5		$427 \pm 15^{gh}$	$455 \pm 33^{ef}$
100/30	10		$323 \pm 06^{mn}$	$383 \pm 18^{k}$
100/30	15		263 ± 13°	$307 \pm 11^{n}$
100/50	5		$393 \pm 11^{jk}$	$446 \pm 19^{fg}$
100/50	10		$318 \pm 11^{mn}$	$415 \pm 06^{hij}$
100/50	15		258 ± 12°	$342 \pm 12^{\text{im}}$
130/30	5		$398 \pm 24^{ijk}$	$493 \pm 18^{bc}$
130/30	10		$308 \pm 24^{n}$	$427 \pm 20^{gh}$
130/30	15		$270 \pm 10^{\circ}$	$418\pm20^{hij}$
130/50	5		$410 \pm 18^{hij}$	507 ± 11 <sup>b</sup>
130/50	10		$352 \pm 31^{1}$	$457 \pm 11^{ef}$
130/50	15		$255 \pm 10^{\circ}$	$466 \pm 15^{def}$

Appendix 2a. Loaf volume of wheat and barley (native and extruded) breads<sup>1</sup>.

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<sup>1</sup> Expressed as mean  $\pm$  SD. <sup>a-o</sup> Values with different superscripts are significantly different (P<0.05). Analysis of variance of the results was performed using One Way ANOVA.

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Moisture	Variety	Substitution	Temp	erature
		level (%)	100°C	130°C
30%	Phoenix	5	$427 \pm 15^{cd}$	$398 \pm 24^{efg}$
		10	$323 \pm 06^{ji}$	$308 \pm 24^{j}$
		15	$263 \pm 13^{k}$	$270 \pm 10^{k}$
	Candle	5	455 ± 33 <sup>b</sup>	$493 \pm 18^{a}$
		10	$383 \pm 18^{g}$	$427\pm20^{\rm cd}$
		15	$307 \pm 11^{j}$	$418 \pm 20^{de}$
50%	Phoenix	5	$393 \pm 11^{fg}$	$410 \pm 18^{def}$
		10	$318 \pm 11^{ji}$	$352 \pm 31^{h}$
		15	$258 \pm 12^{k}$	$255 \pm 10^{k}$
	Candle	5	$446 \pm 19^{bc}$	$507 \pm 11^{a}$
		10	$415 \pm 06^{def}$	$457 \pm 11^{b}$
		15	$342 \pm 12^{hi}$	466 ± 15 <sup>b</sup>

Appendix 2b. Loaf volume of extruded barley substituted breads<sup>1</sup>.

<sup>1</sup>Expressed as mean  $\pm$  SD. <sup>a-k</sup> Values with different superscripts are significantly different (P<0.05). Analysis of variance of the results was performed using multi-factorial  $(2 \times 2 \times 2 \times 3)$ 

ANOVA.

Treatment	Substitution	Wheat	Phoenix	Candle
	level (%)			
Native	0	$0.9 \pm 0.2^{rs}$		<u> </u>
	5		$1.2 \pm 0.3^{\circ - r}$	$1.1 \pm 0.1^{p-r}$
	10		$1.7 \pm 0.3^{i-m}$	$1.5 \pm 0.1^{1-0}$
	15		$1.8 \pm 0.2^{h-l}$	$2.3 \pm 0.2^{d-f}$
Extruded				
temp/moist				
100/30	5		$1.6 \pm 0.1^{j-n}$	$1.3 \pm 01^{n-q}$
100/30	10		$2.5 \pm 0.1^{de}$	$1.9 \pm 0.3^{g-k}$
100/30	15		3.7 ± 0.2 <sup>♭</sup>	$2.7\pm0.1^{d}$
100/50	5		$2.1 \pm 0.1^{fh}$	$1.4 \pm 0.1^{m-p}$
100/50	10		$2.6 \pm 0.1^{de}$	$1.4 \pm 0.1^{m-p}$
100/50	15		$4.3 \pm 0.2^{a}$	$2.1 \pm 0.1^{f-i}$
130/30	5		$1.9 \pm 0.3^{g-j}$	$1.1 \pm 0.2^{p-r}$
130/30	10		$3.1 \pm 0.1^{\circ}$	$1.5 \pm 0.0^{k-0}$
130/30	15		$4.3 \pm 0.2^{a}$	$1.4 \pm 0.2^{1-p}$
130/50	5		$1.6 \pm 0.1^{j-9}$	$1.0 \pm 0.0^{q-s}$
130/50	10		$2.2 \pm 0.3^{eg}$	$1.1 \pm 0.1^{p-r}$
130/50	15		$4.2 \pm 0.2^{a}$	$0.7 \pm 0.1^{s}$

Appendix 3a. Firmness<sup>1</sup> of wheat and barley (native and extruded) breads on day-1 of storage. \_

<sup>1</sup>Expressed as mean  $\pm$  SD. <sup>a-5</sup> Values with different superscripts are significantly different (P<0.05). Analysis of variance of the results was performed using One Way ANOVA.

Moisture	Variety	Substitution	Тетр	erature
		level (%)	100°C	130°C
30%	Phoenix	5	$1.6 \pm 0.1^{hi}$	$1.9\pm0.3^{\mathrm{fgh}}$
		10	$2.5\pm0.1^{de}$	$3.1 \pm 0.1^{c}$
		15	$3.7 \pm 0.2^{b}$	$4.3 \pm 0.2^{a}$
	Candle	5	$1.3 \pm 01^{jkl}$	$1.1 \pm 0.2^{kl}$
		10	$1.9 \pm 0.3^{gh}$	$1.5 \pm 0.0^{ij}$
		15	$2.7\pm0.1^d$	$1.4\pm0.2^{ijk}$
50%	Phoenix	5	$2.1 \pm 0.1^{fg}$	$1.6 \pm 0.1^{hij}$
		10	$2.6 \pm 0.1^{d}$	$2.2\pm0.3^{\text{ef}}$
		15	$4.3 \pm 0.2^{a}$	$4.2 \pm 0.2^{a}$
	Candle	5	$1.4 \pm 0.1^{ijk}$	$1.0\pm0.0^{\text{lm}}$
		10	$1.4 \pm 0.1^{ijk}$	$1.1 \pm 0.1^{kl}$
		15	$2.1 \pm 0.1^{fg}$	$0.7 \pm 0.1^{m}$

Appendix 3b. Firmness<sup>1</sup> of breads made from extruded barley flour at day-1 of storage.

<sup>1</sup> Expressed as mean  $\pm$  SD. <sup>a-m</sup> Values with different superscripts are significantly different (P<0.05). Analysis of variance of the results was performed using multi-factorial  $(2 \times 2 \times 2 \times 3)$ ANOVA

Treatment	Phoenix	Candle
Native	9.1 ± 0.1	$12.7 \pm 1.0$
Pan-cooked	$12.7 \pm 0.3$	$0.9 \pm 0.4$
<b>Extruded</b> temp/moist		
100/30	$12.2\pm0.8$	$14.8 \pm 0.3$
100/50	$12.3 \pm 0.4$	$16.8 \pm 0.8$
130/30	$14 \pm 0.3$	$16.2 \pm 0.6$
130/50	$12.9 \pm 0.4$	$15.2 \pm 0.7$
<sup>1</sup> Expressed as mean	$n \pm SD.$	

Appendix 4. The water binding capacity (g) of barley flours<sup>1</sup>.

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l'reatment	Treatment Substitution	_	L value			a value			b value	
	level (%)	Wheat	Phoenix	Candle	Wheat	Phoenix	Candle	Wheat	Phoenix	Candle
Native	0	44.3 ± 0.5 <sup>18</sup>			$18.1 \pm 0.3^{\mathrm{abc}}$			$32.8 \pm 0.2^{c-8}$		
	S		$41.6 \pm 1.2^{8}$	43.1 ± 3.7 <sup>fB</sup>		17.1 ± 0.4 <sup>ª-ſ</sup>	16.5 ± 0,9 <sup>a.f</sup>		$30.6 \pm 0.8^{8}$	$31.4 \pm 1.8^{fg}$
	10		47.1 ± 3.3 <sup>cfg</sup>	45.9 ± 3.3 <sup>f8</sup>		15.6 ± 0.8 <sup>a.h</sup>	$16.2 \pm 0.6^{a-b}$		33.2 ± 1.0 <sup>b-µ</sup>	32.5 ± 1.7 <sup>d-8</sup>
	15		50.2 ± 2.1 <sup>def</sup>	57.1 ± 3.3ª <sup>•d</sup>		$15.2 \pm 0.6^{n-1}$	$11.9 \pm 2.0^{8-1}$		$34.1 \pm 0.6^{n-8}$	33.4 ± 0.9 <sup>b-8</sup>
Extruded										
temp/moist										
100/30	S		47.8 ± 1.5 <sup>efg</sup>	48.0 ± 1.7 <sup>cfg</sup>		18.0 ± 0.1 <sup>abc</sup>	17.8 ± 0.1 <sup>abcd</sup>		34.7 ± 1.2 <sup>ª ℓ</sup>	35.1 ± 0.9 <sup>⊪·ſ</sup>
100/30	10		60.3 ± 6.3 <sup>ab</sup>	56.2 ± 2.3ª <sup>-d</sup>		12.6±3.8 <sup>b-1</sup>	15.5 ± 1.6 <sup>n-h</sup>		35.5 ± 1.7* €	37.3 ± 1.1 <sup>ª</sup>
100/30	15		63.7 ± 2.9 <sup>a</sup>	58.6±1.7 <sup>ahc</sup>		11.2 ± 1.7 <sup>i</sup>	13.7 ± 1.9 <sup>c-i</sup>		$34.3 \pm 0.8^{a-k}$	36.4 ± 1.5ª-d
100/50	S		47.8 ± 2.3 <sup>cfk</sup>	47.5 ± 2.7 <sup>ւի,</sup>		16.9 ± 0.8 <sup>n.f</sup>	17.7 ± 0.5" €		34.5 ± 1.0 <sup>n-f</sup>	34.8 ± 1.3 <sup>n-f</sup>
100/50	10		57.4 ± 4.2 <sup>a-d</sup>	51.3 ± 2.9 <sup>e-f</sup>		14.7 ± 3.3 <sup>c-i</sup>	17.3 ± 1.0ª ر		$36.8 \pm 1.8^{\rm ab}$	36.5 ± 0.9ªb
100/50	15		62.3 ± 5.1 <sup>ab</sup>	58.9±3.1 <sup>nbc</sup>		$12.0\pm3.2^{\rm hi}$	13.4 ± 2.4 <sup>6i</sup>		35.3 ± 2.3 <sup>₽-f</sup>	36.4 ± 1.3 <sup>nbc</sup>
130/30	S		49.6±3.3 <sup>def</sup>	45.1±1.1 <sup>6</sup> 8		17.6 ± 0.7 <sup>a-c</sup>	18.6±0.4 <sup>abc</sup>		35,9 ± 1,4ª-e	34.2 ± 1.2 <sup>∎-8</sup>
130/30	10		54.8±2.3 <sup>b-e</sup>	46.8±1.3 <sup>fg</sup>		15.9±1.3ª-h	$18.8 \pm 0.0^{\rm ab}$		$37.4 \pm 0.6^{\circ}$	35.8 ± 0.9**
130/30	15		58.9±2.8ªbc	47.6 ± 1.5 <sup>efk</sup>		13,9±1.8 <sup>d-1</sup>	18.4 ± 0.6 <sup>abc</sup>		36.5 ± 0.8 <sup>ab</sup>	35.9 ± 0,9ª⊷
130/50	S		43.4 ± 2.3 <sup>fb</sup>	43.5 ± 1.6 <sup>ft</sup>		18.5 ± 0.2 <sup>abc</sup>	$18.6\pm0.3^{\mathrm{abc}}$		32.6 ± 1.7°- <sup>8</sup>	32.8 ± 1.6°-8
130/50	10		50.2 ± 5.1 <sup>def</sup>	45.2 ± 1.9 <sup>f8</sup>		17.5 ± 0.5 <sup>a-c</sup>	18.9±0.3 <sup>ab</sup>		36,2 ± 2,8 <sup>∎-c</sup>	34.4 ± 1.7 <sup>a-8</sup>
130/50	15		56.6±1.1ª-d	47.0 ± 2.2 <sup>cfg</sup>		14.9 ± 0.8 <sup>b-i</sup>	$19.0 \pm 0.2^{a}$		37.3 ± 0.4ª	35.7 ± 1.5"*

Aoisture	Flour	Moisture Flour Substitution		L value	a	a value	b vi	b value
		level (%)	100°C	130°C	100°C	130°C	100°C	130°C
30%	Phoenix	S	47.8 ± 1.5 <sup>ef</sup>	49.6±3.3 <sup>def</sup>	18.0±0.1 <sup>abc</sup>	17.6±0.7 <sup>a.c</sup>	34.7 ± 1.2 <sup>abc</sup>	35.9 ± 1.4 <sup>ªbc</sup>
		10	$60.3 \pm 6.3^{ab}$	$54.8 \pm 2.3^{b-c}$	$12.6 \pm 3.8^{fg}$	$15.9 \pm 1.3^{a-f}$	$35.5 \pm 1.7^{abc}$	$37.4 \pm 0.6^{8}$
		15	63.7 ± 2.9 <sup>a</sup>	$58.9 \pm 2.8^{abc}$	11.2 ± 1.7 <sup>8</sup>	13.9 ± 1.8°*	$34.3 \pm 0.8^{abc}$ $36.5 \pm 0.8^{a}$	$36.5 \pm 0.8^{a}$
	Candle	S	48.0 ± 1.7 <sup>cf</sup>	45.1 ± 1.1 <sup>f</sup>	17.8±0.1 <sup>ª-d</sup>	18.6±0.4 <sup>ªb</sup>	$35.1 \pm 0.9^{\text{abc}}$	34.2 ± 1.2 <sup>abc</sup>
		10	$56.2 \pm 2.3^{a-d}$	$46.8 \pm 1.3^{f}$	15.5 ± 1.6 <sup>a-f</sup>	$18.8 \pm 0.0^{ab}$		$35.8 \pm 0.9^{\text{abc}}$
		15	58.6±1.7 <sup>abc</sup>	47.6±1.5 <sup>cf</sup>	$13.7 \pm 1.9^{d-8}$	18.4±0.6 <sup>#b</sup>	~	$35.9\pm0.9^{\mathrm{abc}}$
50%	Phoenix	S	47.8 ± 2.3 <sup>cf</sup>	43.4 ± 2.3 <sup>f</sup>	16.9±0.8 <sup>a-c</sup>	$18.5 \pm 0.2^{ab}$	$34.5 \pm 1.0^{abc}$	32.6±1.7°
		10	$57.4 \pm 4.2^{a-d}$	50.2 ± 5.1 <sup>def</sup>	$14.7 \pm 3.3^{h-B}$	$17.5 \pm 0.5^{n-c}$	$36.8 \pm 1.8^{a}$	$36.2 \pm 2.8^{abc}$
		15	$62.3 \pm 5.1^{ab}$	56.6 ± 1.1 <sup>ª-d</sup>	$12.0 \pm 3.2^{fg}$	14,9±0.8 <sup>a-g</sup>	8	$37.3 \pm 0.4^{B}$
	Candle	5	47.5 ± 2.7 <sup>cf</sup>	43.5 ± 1.6 <sup>r</sup>	17.7 ± 0.5 <sup>n-d</sup>	$18.6 \pm 0.3^{\rm ab}$	$34.8 \pm 1.3^{nbc}$	$32.8 \pm 1.6^{\rm bc}$
		10	$51.3 \pm 2.9^{c-f}$	$45.2 \pm 1.9^{f}$	$17.3 \pm 1.0^{a-c}$	$18.9 \pm 0.3^{n}$	$36.5 \pm 0.9^{a}$	$34.4 \pm 1.7^{abc}$
		15	$58.9 \pm 3.1^{abc}$	47.0 ± 2.2 <sup>cf</sup>	$13.4 \pm 2.4^{\rm efg}$	$19.0 \pm 0.2^{a}$	~	357 + 1 5ªbc

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Analysis of variance of the results was performed using multi-factorial  $(2 \times 2 \times 2 \times 3)$  ANOVA.

Freatment	Treatment Substitution L value L value		L value			a value			b value	
	level (%)	Wheat	Phoenix	Candle	Wheat	Phoenix	Candle	Wheat	Phoenix	Candle
Native	0	79.2 ± 0, l <sup>a</sup>			-0.4 ± 0.0'			17.4 ± 0.1 <sup>印</sup>		
	5		75.6 ± 0.6 <sup>klm</sup>	75.9 ± 1.1 <sup>h-l</sup>		$0.7 \pm 0.2^{\text{lm}}$	$0.7 \pm 0.1^{\text{trano}}$		19,0 ± 0,4 <sup>cd</sup>	19.3 ± 0.4 <sup>bc</sup>
	10		$75.6 \pm 0.7^{klm}$	75.9 ± 0.5 <sup>j-m</sup>		$1.0 \pm 0.1^{\text{hijk}}$	1,0 ± 0,1 <sup>⊌hi</sup>		$19.3 \pm 0.4^{hc}$	19.7 ± 0.3 <sup>a</sup>
	15		75.1 ± 0.5 <sup>lm</sup>	$74.9 \pm 0.7^{m}$		1.4 ± 0.1 <sup>bcd</sup>	1.3 ± 0.1 <sup>cde</sup>		$19.8 \pm 0.3^{abc}$	$20.4 \pm 0.5^{8}$
Extruded										
temp/moist										
100/30	S		79.1 ± 0.2ªb	78.9 ± 0.3 <sup>abcd</sup>		0.5 ± 0.1 <sup>opq</sup>	$0.5 \pm 0.2^{nop}$		18.1 + 0.1 <sup>def</sup>	181+01 <sup>cf</sup>
100/30	10		78.0±0.6 <sup>ª-f</sup>	77.8 ± 0.4 <sup>d-f</sup>		$0.9\pm0.1^{hij}$	$0.7 \pm 0.1^{hm}$		$17.5 + 0.5^{\text{elbh}}$	179+03tib
100/30	15		77.3 ± 0.9 <sup>t-j</sup>	77.2 ± 0.4 <sup>6j</sup>		1.2 ± 0.1 <sup>def</sup>	1.1±0.1 <sup>clyh</sup>		$17.3 \pm 0.4^{\text{Bh}}$	17.7 ± 0.4° <sup>th</sup>
100/50	Ś			78.6±0.7ªbcdo		0.7 ± 0.1 <sup>lmn</sup>	0.3 ± 0.1 <sup>pq</sup>		18.2 ± 0.1 <sup>def</sup>	18.1±0.2 <sup>ef8</sup>
100/50	10		77.9 ± 0.4 <sup>b-f</sup>	78.6 ± 0.2 <sup>abcde</sup>		$1.2\pm0.1^{ m elbh}$	$0.7 \pm 0.1^{\text{Im}}$		17.7 ± 0.4°的	18.1 ± 0.1 <sup>def</sup>
100/50	15		76.6±0.6 <sup>h-k</sup>	76.7 ± 0.5 <sup>&amp; k</sup>		1.6±0.0 <sup>nb</sup>	1.2 ± 0.1 <sup>cfg</sup>		$17.5 \pm 0.1^{\text{eft}^{1}}$	18.2 ± 0.4 <sup>def</sup>
130/30	5		79.0 ± 0.4 <sup>ªbc</sup>	79.1 ± 0.1 <sup>ªb</sup>		0.8 ± 0.0 <sup>klm</sup>	0.3 ± 0.1 <sup>4</sup>		18.1 + 0.3 <sup>cB</sup>	178+01 efbh
130/30	10		77.9 ± 0.3°-8	78.2 ± 0.2 <sup>a.f</sup>		1.2 ± 0.0 <sup>efµh</sup>	$0.7 \pm 0.1^{hm}$		18.1 ± 0.4 <sup>ef</sup>	18.1 + 0.3 <sup>def</sup>
130/30	15		77.5 ± 0.4 <sup>⊷l</sup>	77.5 ± 0.2 <sup>⊷i</sup>		$1.5\pm0.0^{bcd}$	1.0 ± 0.0 <sup>ຢlili</sup>		17.2 ± 0.2 <sup>h</sup>	18.1 ± 0.0 <sup>ef</sup>
130/50	5		78.0 ± 0.4 <sup>b-f</sup>	78.0 ± 0.1 <sup>b-f</sup>		0.9 ± 0.1 <sup>ijkl</sup>	0.3 ± 0.1 <sup>q</sup>		18.2 ± 0.1 <sup>de</sup>	18.0 + 0.1 teleh
130/50	10		77.5 ± 0.3 <sup>°-i</sup>	77.6 ± 0.1 <sup>e-h</sup>		$1.5 \pm 0.0^{bc}$	0.8 ± 0.1 <sup>jklm</sup>		17.6 ± 0.1 <sup>e的</sup>	18.1 ± 0.2 <sup>ef</sup>
130/50	15		130/50 15 76.3 ± 0.0 <sup>1-m</sup>	76.2 ± 0.1 <sup>j.m</sup>		$1.7 \pm 0.0^{a}$	$0.8 \pm 0.1^{ijklm}$		17.7 ± 0.0 <sup>clbh</sup>	$17.8 \pm 0.0^{\text{ebt}}$

Appendix 7. Cross-sectional views of breads, showing the effect HTHM- barley (Candle) flour substitution.

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