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Extrusion Cooking of Barley Flour with and without Thermostable α-Amylase

by

Judy Sze-Mun Yeung 

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

in

Food Science and Technology

Department of Agricultural, Food and Nutritional Science

Edmonton, Alberta

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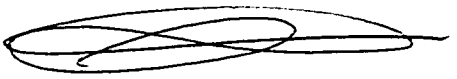

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Date: JAN 28, 2001

To my beloved Dad, Mom,

my sister, Sylvia and my brother, Samson.

Their love, dedication and insight enable me to succeed.

Abstract

Barley grains (waxy and regular) were pearled to different extents. The composition of pearling products (PF and PG) and the gel color of PG-flour were investigated. The PG-flour was then subjected to extrusion studied with and without α-amylase. Effects of extrusion conditions and α-amylase concentration on the degree of hydrolysis and dextrose equivalent, oligosaccharide composition, IDF, SDF and TDF of the extruded flours were studied. The study indicated that 32% of pearling is required to ensure the bright color of barley-based foods. Extrusion of barley flour with 4% α-amylase at 100°C and 50% moisture level lead to the maximum degree of hydrolysis. At each temperature and enzyme concentration, the saccharide composition (DP1-7), followed the order: DP2>DP6>DP3>DP5>DP7>DP4>DP1. Extrusion cooking increased the TDF and SDF contents of barley flours while IDF was decreased in CDC-Candle but increased in Phoenix. Overall, it was demonstrated that the dextrinized barley flour with different oligosaccharides and dietary fiber profile could be produced by extrusion processing.

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The author would like to express her most sincere thanks to her supervisor, Dr. Thava Vasanthan, for valuable support and guidance given at all stages in this study. The author would also like to express her gratitude to the other members of the committee. Sincere thank to Dr. Feral Temelli for her insightful detail in clarifying the thesis. The author appreciates the helpfulness of Dr. Ambikaipakan Senthilselvan for his suggestions in data analysis and reformatting statistical data, making it easier to understand. Thanks to Dr. Feral Temelli and Dr. Ambikaipakan Senthilselvan for making time to serve on the committee and all their good advice.

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List of Abbreviations

DE	Dextrose equivalent
DH	Degree of hydrolysis
DMH	Dimethyl hydrazine
DMSO	Dimethyl sulfoxide
DP	Degree of polymerization
F	Forward element
FAS	Fatty acid synthetase
HDL	High-density lipoprotein
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme A
HPLC	High performance liquid chromatography
HTST	High temperature short time
IDF	Insoluble dietary fiber
KB	Kneading block
KB/R	Reverse kneading block
LDL	Low-density lipoprotein
LH	Left hand
PF	Pearling flour
PG	Pearled grain
PUFA	Poly-unsaturated fatty acids
R	Reverse element
RH	Right hand
RS	Resistant starch
SCFA	Short chain fatty acids
SDF	Soluble dietary fiber
SK	Undercut element
SK-N	Transition element from undercut to others
TDF	Total dietary fiber

Chapter 1

Introduction and Thesis Objectives

Canada is the world second largest producer of barley with the production of 13.2 million metric tons (Food Agriculture Organization, 2000). Although majority of barley in Canada is grown for malting and brewing industries, only 20% is consumed by these industries. The remaining goes to animal feed and food industries, which is about 75% and 5% of total production, respectively.

In recent years, the demand for barley, especially hull-less varieties, has increased in non-malting food uses. The whole/pearled/milled grain can be processed into products like breakfast cereal, pasta, dextrin, miso, barley tea, etc. Results from several clinical and animal trials (Qureshi et al., 1980, McIntosh et al., 1993, Hawrysh, 1997) have suggested that regular intake of barley foods may benefit human health. Barley contains a soluble dietary fiber component, β-glucan, which can lower serum cholesterol levels, regulate blood glucose levels and improve immune function. Barley also contains tocotrienols, components of the vitamin E (tocols) family, which also have been postulated to reduce blood serum cholesterol (Hakkarainen et al., 1984, Wang et al., 1993).

For the above reason, this study focussed on the value-added processing of barley grains in order to enhance its food use. Pearling, an important primary processing in food-barley utilization, refers to the gradual removal of grain tissues (by abrasive action) starting from the outer grain tissues/layers, bran (i.e., pericarp, testa, aleurone and sub-aleurone layers) and germ. The removal of barley bran through pearling yields a bright white kernel that is ideal for various food applications. Furthermore, bran layers contain

the majority of barley lipids, which are composed of high amounts of unsaturated fatty acids (oleic, 18:1 and linoleic, 18:2 acid), and are highly prone to autooxidation and subsequent rancid odor development. Therefore, the storage stability and overall quality of pearled barley will be improved by removing of outer layers of the grain. Another important benefit of pearling is the removal of a variety of barley phenolic compounds and enzymes, such as polyphenoloxidase and peroxidase, along with the outer grain layers. This virtually eliminates the enzyme driven darkening of barley products.

Dextrinization is a process used to hydrolyze starch by acid or enzyme. The product is a mixture of mono-, di-, oligo- and polysaccharides, which is called dextrin. Dextrin can be used as a fat replacer, bulking agent, flavor and color encapsulating agent, etc.

High temperature short time (HTST) extrusion cooking has become one of the most popular new processes in food and feed industries. It is used extensively to produce products, such as snack foods, cereals, pasta, and pet foods. Due to many industrial advantages (ease of handling, low water use, low cost, etc), dextrinization by extrusion has been of interest to many researchers. Extrusion conditions, such as temperature, moisture and pressure/shear may change the content as well as the physicochemical and nutritional/physiological properties of barley flour components, including dietary fiber. However, little research has been done on dextrinization of barley flour by extrusion cooking.

The objectives of this thesis are:

1. To investigate the distribution of major grain components (i.e. starch, protein, lipid, β-glucan and ash) in a waxy (CDC-Candle) and a regular

(Phoenix) barley through a pearling study and to investigate the effect of varying degrees of grain pearling on the color characteristics of uncooked and cooked (gel) flour milled from pearled grain,

2. to study extrusion dextrinization of barley flour in order to investigate the effects of enzyme (α-amylase) addition level and extrusion conditions such as temperature and flour-moisture level on the hydrolysis of starch in barley flours by determining the "degree of starch hydrolysis (DH)", "dextrose equivalent (DE)" and mono-, di-, and oligosaccharide composition, and

3. to study the effect of extrusion cooking of native barley flour under various temperature/moisture combinations on the total, insoluble and soluble dietary fiber, β-glucan and resistant starch contents of barley flour.

1.1 References

Food Agriculture Organization, 2000: [http://apps.fao.org/cgi-bin/nph-db.pl?subset=agriculture]

Hakkarainen, R. V. J., Tyopponen, J. T., Hassan, S., Bengtsson, G., Lennart J. S. R. and Lindberg, P. O. 1984. Biopotency of vitamin E in barley. *Br J. Nutr.* 52:335-349.

Hawrysh, Z. 1997. Barley food products intervention trail for non-insulin-dependent (NIDDM) diabetic subjects. The Alberta Agricultural Research Institute, Edmonton, Alberta.

McIntosh, G. H., Jorgensen, L., Royle, P. 1993. The potential of an insoluble dietary fiber-rich source from barley to protect from DMH-induced intestinal tumors in rats. *Nutr. Cancer.* 19: 213-221.

Qureshi A. A., Burger, W. C., Prentice, N., Bird, H. R. and Sunde, M. L. 1980. Regulation of lipid metabolism in chicken liver by dietary cereals. *J. Nutr.* 110: 388-393.

Wang, L., Newman, R. K., Newman, C. W., Jackson, L. L., and Hofer, P. J. 1993. Tocotrienol and fatty acid composition of barley oil and their effects on lipid metabolism. *Plant Foods Hum. Nutr.* 43: 9-17.

Chapter 2

Literature Review

2.1 History of barley

Barley (*Hordeum vulgare*) is one of the major crops in the world. It is also one of the first ever cultivated grains, in fact, it was used as a form of currency in biblical times. The original area of cultivation has been reported (Nilan and Ullrich, 1996) to be in the fertile lands of Middle East. Archeological studies have found that barley was cultivated by about 8000 BC in Iran. Others found that barley in essentially the form that exists today was used at least 17,000 years ago in the Nile River Valley of Egypt. It is believed that barley was used in porridge, flat bread and fermented beverages as early as 6750 BC (Matz, 1991).

With the modern technologies, the total annual cereal production in the world has increased to about 2,062 million metric tons (Food and Agriculture Organization, 2000). Canada is the world's second largest producer of barley with the production of 13.2 million metric tons, which ranked after Germany, producing 13.3 million metric tons annually (Food and Agriculture Organization, 2000). Barley can be processed into products like breakfast cereals, barley tea, pasta, dextrin, miso, etc. However, in Canada, the use of barley as a food source for humans is limited (up to 5%), while up to 75% of the total barley production is used as feed for animals (cattle, pig, chicken, horse, etc) and 20% is used in the malting and brewing industry (Alberta Barley Commission, Calgary, AB, personal communication, 2000).

2.2 Types of barley

Barley is a diploid with seven pairs of chromosomes. Similar to any other grain, the chemical composition and the morphological characteristics of barley grain are determined by genotype and growing conditions. These characteristics directly influence the nutritional quality and functionality of this grain and its components.

Barley has three spikelets at each rachis node. All three spikelets are fertile for six-rowed barley but only the central spikelet is fertile for two-rowed barley (Duffus and Cochrane, 1996). Two-rowed barley is usually plump and has a greater uniformity of kernel size; thus it is more preferable for malting/brewing and other food processing (e.g. milling and rolling) purpose.

Barley grain can be hulled or hull-less. In the early 1970s, University of Saskatchewan researchers brought back the importance of hull-less strain when they were studying the nutritional quality of barley germ plasma (Bhatty et al., 1975). Western Canada, including British Columbia, Alberta, Manitoba and Saskatchewan, is the largest producer of hull-less two-rowed and six-rowed barley with approximately 138,620 ha and 92,280 ha, respectively, in 1999 (B. Fedak, personal communication, 2000). Alberta produced approximately 22,000 and 25,000 ha of hull-less two-rowed and six-rowed barley, respectively. Hull-less barley contains more protein, starch, total and soluble β-glucan compared to hulled barley (Bhatty, 1999).

2.3 Kernel structure

The cereal grain is one-seeded fruit called a caryopsis, in which the fruit coat is adherent to the seed (Fig. 2.1). Husk, pericap, testa, aleurone layer, embryo/germ and

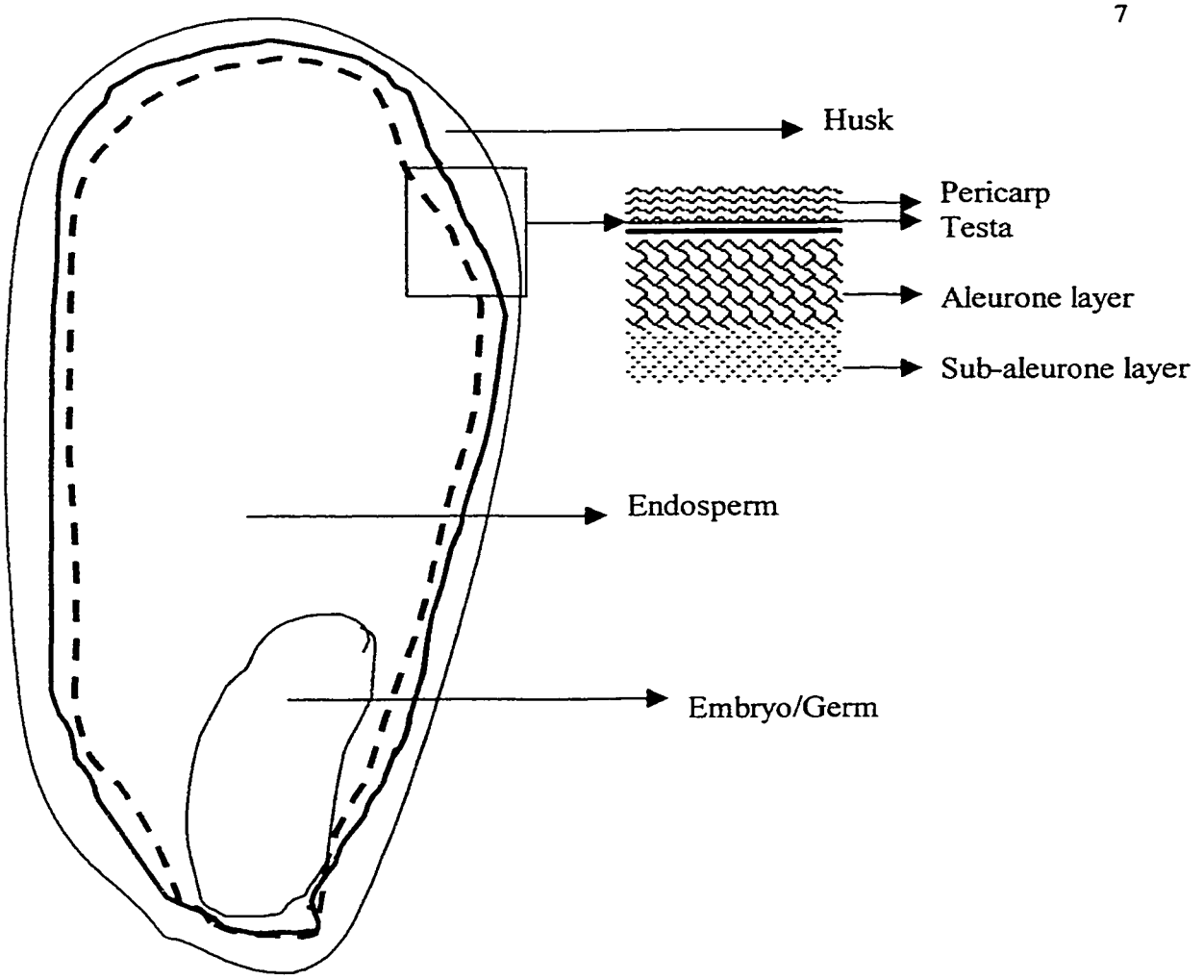


Figure 2.1: Diagram representing a longitudinal section cut of a barley caryopsis

endosperm are the major tissues of barley grain. Pearling/milling involves removing the bran layers (pericap, seed coat, aleurone layer and some sub-aleurone layer) and the germ from the seed, which can reduce the mineral and vitamin contents of the final pearled product.

2.3.1 Husk

Husk is the outermost part of hulled barley, which is 8 – 15% of the seed. It consists of 2 leaf-like structures, lemma and palea, which completely cover the grain. The husk contains significant amounts of cellulose, arabinoxylan, lignin, and minerals (phosphorus, potassium, magnesium, calcium, copper, iron, and silicon). It also contains polyphenols. Hull-less varieties, as the hull is less strongly attached and falls off during harvesting and threshing, contain lower amounts of fiber and minerals.

Two-rowed barley (plump and large kernels) contains lower husk content than six-rowed barley (lean and long kernels). The color of the grain depends on the pigments in caryopsis and palea. Caryopsis contains anthocyanin pigments or a black melanin-like compound. Anthocyanin displays red color in the pericarp and imparts a blue color to the aleurone layer.

2.3.2 Pericarp

Pericarp comprises 2% of the kernel weight and it attaches to husk by a "cementing" layer, which is present in significantly lower quantities in hull-less barleys. The pericap is also known as fruit coat and it consists of epidermis, hypodermis, cross cells and tube cells. It is composed mainly of cellulose, arabinoxylans, lignin, protein and other carbohydrates as minor components (MacGregor, 1998).

2.3.3 Testa

This thin tissue is bonded by two lipid layers and comprises 1 – 3% of total kernel weight. It contains celluloses, waxes and anthocyanogens (MacGregor, 1998). It is a semi-permeable membrane, which limits the movement of solutes to the interior grain and the two lipid layers limit the movement of water from the interior to the aleurone layers.

2.3.4 Aleurone

The aleurone layer separates the endosperm from the other grain tissues. It comprises 5 – 10% of total kernel weight. It consists of 3 layers of lining cells with a very rigid structure, which is hard to break down mechanically. Thiamin, riboflavin, niacin, nicotinic acid, pathothenic acid, tocotrienols (members of vitamin E family of compounds) and biotin are found in both aleurone and embryo tissue. The aleurone layer contains arabinoxylans, β-glucan, lipids in the form of triglycerides, protein, phytic acid/phytate, minerals, sucrose, and anthocyanogens (MacGregor, 1998).

2.3.5 Embryo/Germ

The germ or embryo comprises 2-4% of the kernel. The germ is high in lipid (13 –17%), protein and free amino acids (34%), sucrose (14%), raffinose (5 – 10%), arabinoxylan (8 – 10%), ash (5 – 10%), cellulose and some pectin. It also contains polymers of fructose, which is known as fructans or fructo-oligosaccharides.

2.3.6 Endosperm

The endosperm is the plant's major storage area for starch and protein, where starch is embedded in a protein matrix. It is the largest tissue in kernel, which comprises 75 – 80% of kernel. It is low in ash, oil and sugars. The endosperm cell walls are mainly

composed of β-glucan (70%) and the remainder is arabinoxylans, glucomannans, celluloses, proteins and phenolic constituents (Jadhav et al., 1998).

2.4 Composition

Similar to any other cereal grain, barley contains starch, protein, non-starch polysaccharides, and lipids as its major components (Table 2.1). Non-starch polysaccharides include cellulose, β-glucan and hemicellulose, which is also referred to as pentosans. Majority of the hemicelluloses in barley is arabinoxylans. The minor components are sugars, vitamins and minerals. The chemical composition and component functionality of barley grains differ with variety and between kernels of the same variety, due to genetic factors, growing conditions and analytical variations.

2.4.1 Starch

Composition and structure

Barley grain contains 52 - 72% starch (Table 2.1). Starch is a major component of cereal grains and it is a polymer of α-D-glucose. The glucose molecules are linked through glycosidic covalent bonds (α-1→4 and α-1→6 types). There are two types of starch molecules found in nature: amylose, a linear molecule and amylopectin, a branched molecule (Fig. 2.2 A and B). Depending on the amylose content of starch, barley has been classified as regular (22 - 26%), waxy (0 - 5%) and high amylose types (40 - 50%) (MacGregor and Fincher, 1996). Waxy starch is well suited to food applications due to its unique gel texture (gel produced by cooking in water and subsequent cooling), high water holding capacity and freeze-thaw stability.

Table 2.1: Chemical composition of barley

Component	% (w/w, dry matter basis)
Starch	52 – 72
Proteins:	9 – 14
Hordein	35 – 50
Globulins	20 – 30
Glutelins (Structural)	15 – 25
Albumins	3 – 5
Non-starch polysaccharides:	
Cellulose/Lignin	4 – 6
β-glucans	3 – 6
Arabinoxylans	4 – 7
Lipids	2 – 3
Ash	2 – 3
Sugars	0.6 – 3.7
Fructans	0.2 – 0.9

Source: MacGregor (1998)

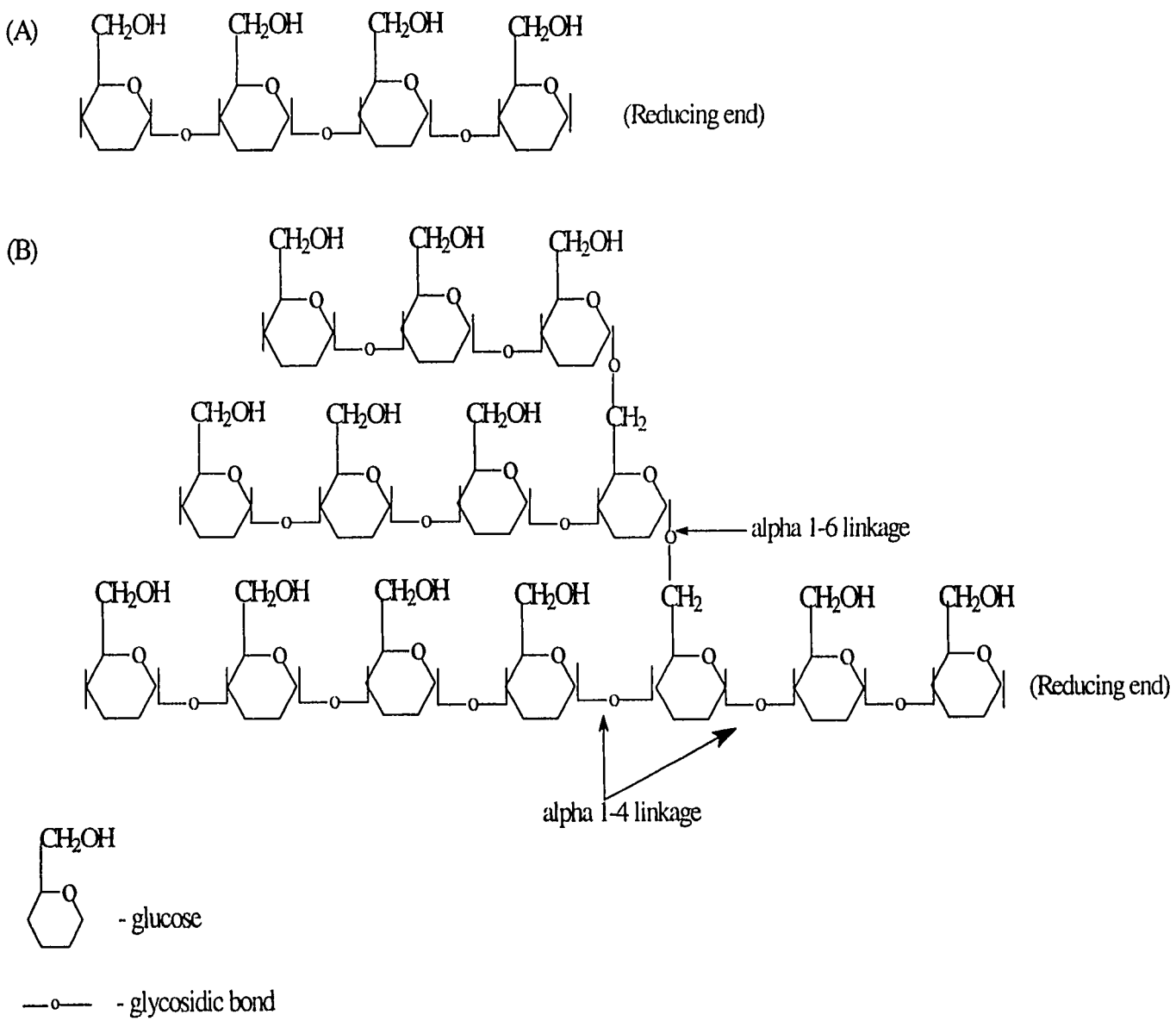


Figure 2.2: Structure of amylose (A) and amylopectin (B)

In plants, starch exists in a granular form (mostly spherical) where amylose and amylopectin are radially organized within the granule (Fig. 2.3). Barley starch consists of a mixture of lenticular large granules (15-25 μm in diameter) and irregularly shaped small granules (< 10 μm in diameter). Despite the fact that 80 - 90% of the total number of starch granules are small, it consists only 10 – 15% of total starch weight (Goering et al., 1973). Large granules compose 10 – 20% of total number of starch granules and 85 – 90% of total starch weight.

Swelling and gelatinization

When starch is suspended in cold water, starch granules absorb water and swell slowly. This swelling process is reversible. When the suspension is heated and temperature reaches close to 56°C to 62°C, starch granules swell rapidly and irreversibly. Amylose dissolves in water and leaks out from the granule. The viscosity of the starch paste is increased, birefringence and granule crystallinity are lost. This change is called gelatinization (Fig. 2.4). As starch is gelatinized, the molecules separate and become less compact, which makes it easier for enzymes to hydrolyze starch. The temperature at which maximum swelling occurs is referred to as the gelatinization point. If the starch paste is heated to a temperature higher than the gelatinization point, the starch granules burst. As a result, a sticky and thinner paste is formed.

Gelatinization temperature of starch is generally presented in a range (e.g. 56°C to 62°C) and it is different for starch from different sources. This is due to the fact that crystalline regions of starch granules are slightly different in any given starch. They do not melt and become disordered at the same temperature. As a result, the granules of any given starch gelatinize at different temperatures. Gelatinization temperature is also

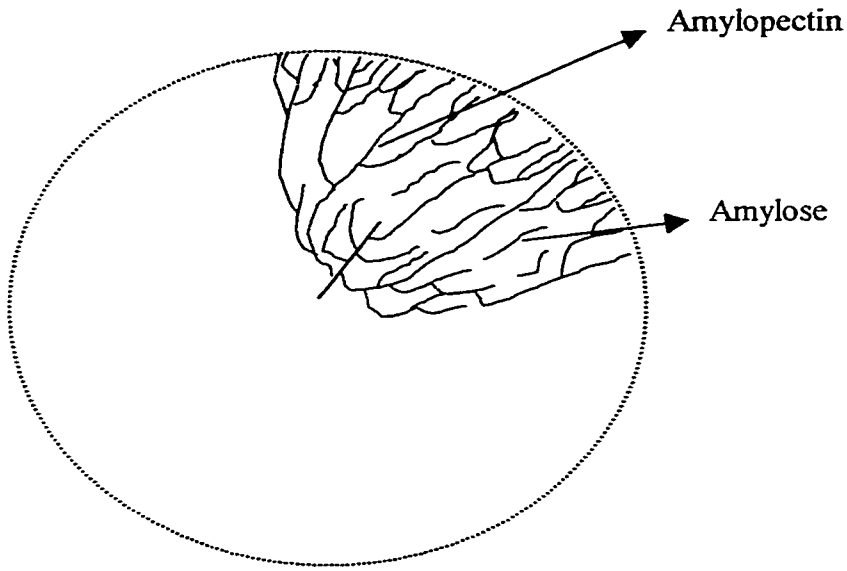


Figure 2.3: Diagram of a starch granule

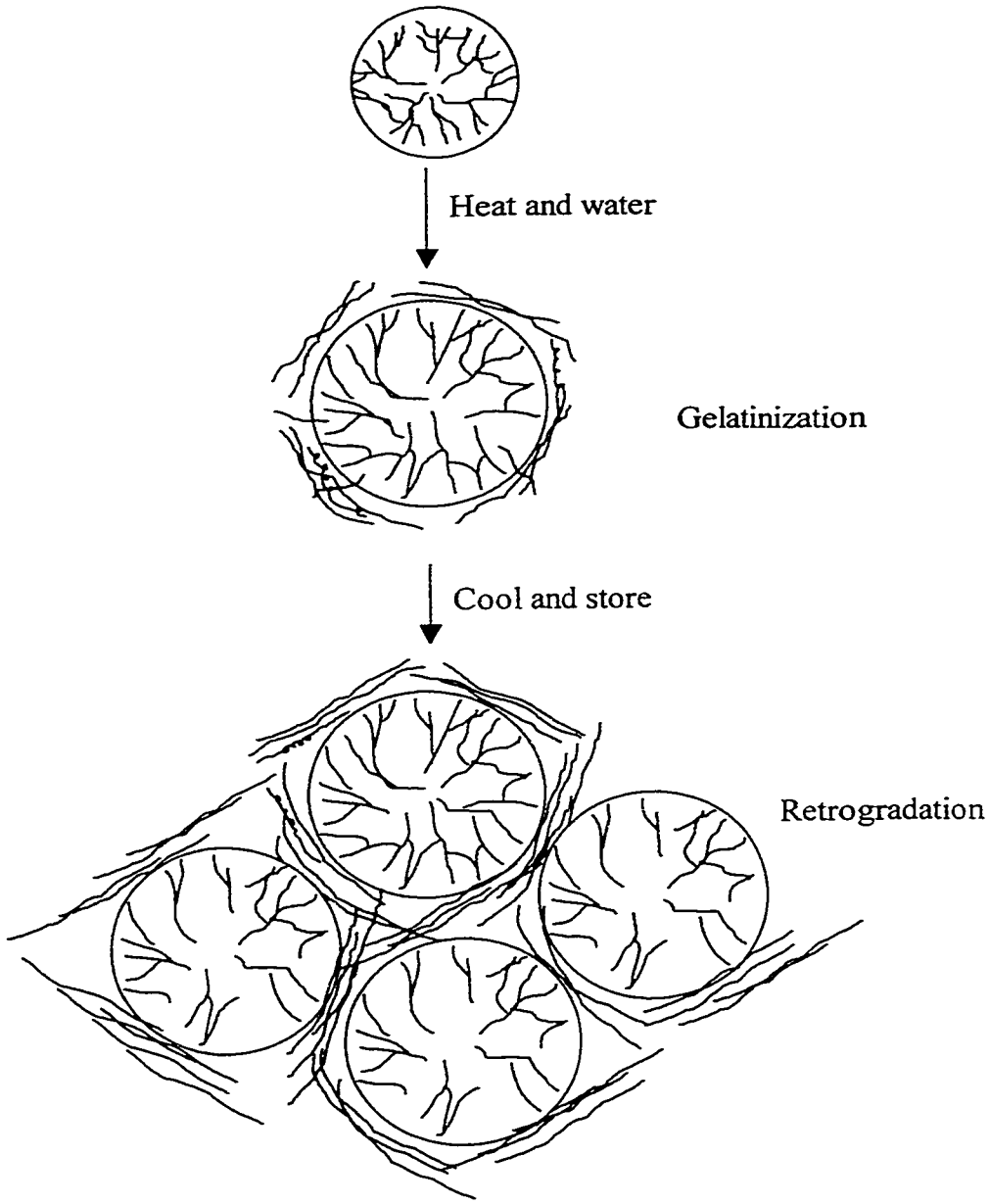


Figure 2.4: Diagram of starch gelatinization and retrogradation

influenced by the nature of the starch. High amylose and lipid content of starch seem to restrict swelling. High amylose and waxy starch have higher gelatinization temperature than regular starch (MacGregor and Fincher, 1996). Small granules gelatinize at a higher temperature than big granules.

Retrogradation

During cooling of the hot starch paste, amylose and the linear portions of amylopectin re-associate by hydrogen bonding to form a three-dimensional network, which traps water in a rigid structure (Fig. 2.4). This process is referred to as retrogradation.

Retrogradation mainly depends on the nature and percentage of amylose. A longer chain and higher amylose content result in a firmer gel structure. Re-association of amylose and linear portion of amylopectin is not likely to occur in waxy starch, because it contains up to 100% amylopectin and minimum amount of amylose. Thus, waxy starch paste remains as a thick paste during cooling. The opaqueness of the gel is also directly related to the amylose content.

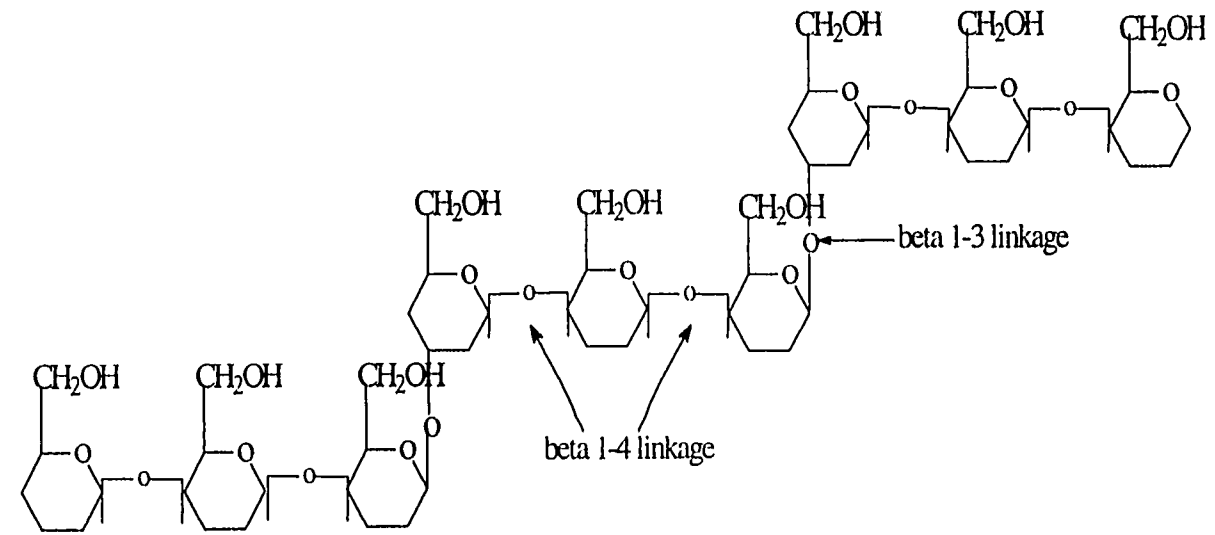
2.4.2 Protein

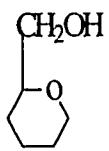
Barley contains 9-14% of protein (Table 2.1). It is concentrated at the endosperm (especially at the periphery) and the aleurone layer of the seed. All four major classes of protein [albumins (water – soluble proteins), globulins (salt – soluble proteins), prolamins (alcohol – soluble proteins) and glutelins (acid and alkali soluble proteins)] are present in barley grains, where prolamins make up the largest portion (Table 2.1). Prolamins are named differently in cereal grains. It is called hordein in barley, gliadin in wheat, and

avenin in oats, etc. The nutritional and functional qualities of barley proteins are relatively poor. Breeding research is underway to address this concern.

2.4.3 Dietary fiber

Barley is a good source of dietary fiber. Dietary fiber has two major classes, soluble and insoluble. Soluble dietary fiber includes β-glucan, arabinoxylans, fructans, etc. β-Glucan contains both β (1→4) and β (1→3) glycosidic linkages (Fig. 2.5) and it is the major structural component in the cell walls of barley grain endosperm. It forms a viscous and sticky solution when mixed with water (Dais and Perlin, 1982). Depending on the cultivar and production location/environment, β-glucan content in barley grain varies from 3 – 6% (w/w) (Table 2.1). The structure, physiochemical properties, method of extraction and purification of β-glucan have been investigated (Woodward et al., 1983; Klopfenstein and Hoseney, 1987; Woodward et al., 1988; Bhatty, 1992; Bhatty, 1993; Vasanthan and Bhatty, 1995; Yoon et al., 1995). Primary structural features of β-glucan are mainly composed of the glycosidic linkage sequence, the degree of polymerization (DP) of linear β (1→4) linked cellulose like segments (Fig. 2.6), and the glycosidic linkage profile. Glycosidic linkage profile refers to the percentage ratio between β (1→4) and β (1→3) linked glucosyl units, which is found to be 70% to 30% (Woodward et al., 1983). The physicochemical properties (i.e. viscosity, solubility, shear stability) of barley β-glucan mainly depend on the small differences in its primary structural features, instead of its degree of polymerization or overall asymmetrical conformation. Structural differences influence the ability of the β-glucan molecules to align into relatively stable molecular aggregates (Woodward et al., 1988; Gómez et al., 1997 a, b, c). Woodward and co-workers (1983) reported that the overall irregular conformation of β-glucan




- glucose

—o— - glycosidic bond

Figure 2.5: Structure of beta-glucan

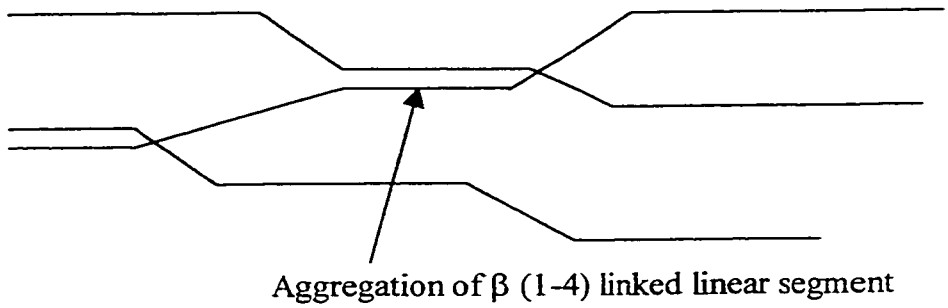
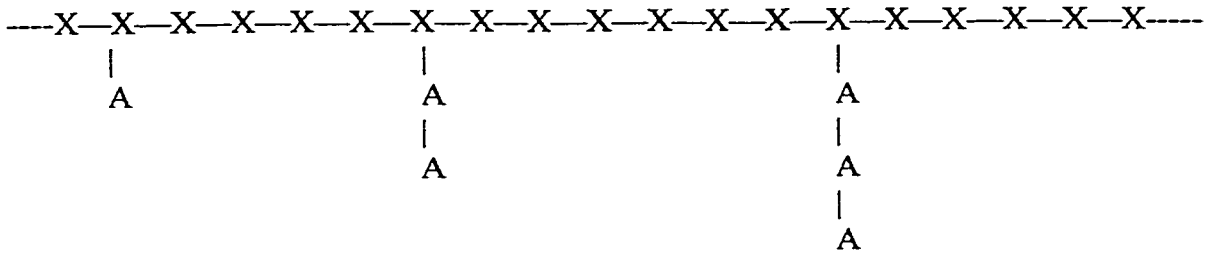


Figure 2.6: A schematic illustration of β-glucan linear segment aggregation

depends on the different spacing of β (1→3) linkages of the polymer. The authors further indicated that, higher percentages of β (1→3) linked β-glucosyl residues (high irregularity) in barley β-glucan chains reduce its tendency to aggregate, which in turn, enhances its solubility. High temperature is found to increase the aggregation potential of barley β-glucan and decrease its solubility (Gómez et al., 1997a). Study also showed that aggregation exits in both low- and high molecular weight β-glucan that relaxes upon shearing (Gómez et al., 1997c).

Arabinoxylans content in barley grain range from 4 – 7% (Table 2.1). It consists a β (1→4)-xylose polymer as a backbone, while some xylose units are substituted with arabinose residues (mono, di, or oligo) (Fig. 2.7). The degree and pattern of substitution determine the solubility and functionality of arabinoxylans. Arabinoxylan chains can be cross linked by phenolic acid, such as ferulic acid ((MacGregor, 1998). Degree of linkage also affects the solubility and solution properties of arabinoxylans. It is a minor component in endosperm cell walls but a major component in the cell walls of the aleurone and outer layers (seed coat, pericarp, etc) of the kernel. Similar to β-glucan, it can form a viscous solution in water.

Insoluble fiber fraction is composed of lignin, cellulose and resistant starch. Lignin cannot be digested by the human digestive system and is therefore classified as dietary fiber. Cellulose is a linear β (1→4)-D-glucan polymer, which is concentrated in the outer layer of barley grain. As a dietary fiber component, resistant starch cannot be digested in small intestine but it is fermented by microflora in colon. There are three types of resistant starch: physically inaccessible starch (RS1), resistant starch granules



X – xylose residue
A – arabinose residue
X—X – β (1→4) bond

Figure 2.7: Structure of arabinoxylan (Source: MacGregor, 1998)

(RS2) and retrograded starch (RS3). Processing raw food materials can destroy RS1 and RS2, but may develop RS3 by retrogradation.

2.4.4 Lipid

Lipid content of barley ranges between 2-3% (Table 2.1). Fatty acids in cereals occur in neutral lipids, glycolipids, and phospholipids. The predominant fatty acids are linoleic (55%), palmitic (21%), oleic (18%) and α-linolenic (6%) acids. The lipid is mainly distributed in the aleurone and germ. Lipid exits in either free or bound form in cereal grains. Bound lipid forms a complex with amylose (Morrison et al., 1993).

2.4.5 Minerals and vitamins

Ash content of barley ranges between 2 – 3% (Table 2.1). The predominant minerals are phosphorus, potassium, calcium and a small amount of magnesium, sulfur, sodium and other trace elements is present. Up to 85% of phosphorous in cereals and legumes occurs as phytic acid (Tsao et al., 1997). Phytate (salt of phytic acid) has the ability to bind minerals, such as iron, zinc, magnesium and calcium, which leads to unavailability of these minerals for utilization in the body. Ironically, its mineral binding ability has been investigated for antitumour and antiulcerogenic properties (McIntosh and Russell, 1988).

Barley contains all isomers (α, β, γ, and δ) of tocotrienols and tocopherols, which act as antioxidants and have cholesterol lowering effect (especially tocotrienols) (McIntosh and Russell, 1988; Wang et al., 1993a). Barley is also a source of B-complex vitamins, especially thiamine, pyridoxine, riboflavin and pantothenic acid. Barley also contains a significant amount of niacin. However, a part of the niacin is bound to protein, which makes it biologically unavailable.

2.5 Barley nutrition – dietary fiber and vitamin E

Research has indicated that regular consumption of barley products would benefit human health. Barley has been reported to have hypocholesterolemic effect in both animal and human studies (Newman et al., 1989, Martinez et al., 1992). High serum cholesterol has been recognized as one of the risk factors for coronary heart disease. Studies have also shown that barley consumption would improve the glycemic response in diabetic subjects (Harwrysh, 1997; Yokoyama et al., 1997; and Bourdon et al., 1999). A brief summary of some nutritional studies is presented below.

In a 28-days study (Newman et al., 1989), 14 men were put on barley and wheat diet. The results indicated that the barley diet did not show any significant cholesterol lowing effects on subjects with average or low cholesterol levels, but subjects with a high cholesterol level experienced a reduction in both total and low-density lipoprotein (LDL)-cholesterol levels. In an animal study (Wang et al., 1992), 96 male broiler chicks were fed with corn-soybean, barley or barley diet with β-glucanase. It was reported that barley β-glucan caused high viscosity in chick's small intestine and thereby interfering with the digestion of other nutrients, thus reduced plasma cholesterol and final body weights of chicks. The authors suggested that β-glucan could also decrease cholesterol by binding to bile acids. Glore et al. (1994) reviewed this subject and brought up several other mechanisms on how the soluble fiber could decrease cholesterol level. They reported that soluble fiber is fermented into short chain fatty acids (SCFA) (for example, acetate, propionate, and butyrate) by colonic bacteria and these SCFA inhibited hepatic cholesterol synthesis. Soluble fiber could also bind to fat and lead to its excretion. Such

excretion would suppress the absorption of fat soluble vitamins and some minerals (calcium, zinc and iron) (Toma and Curtis, 1986, Klopfenstein, 1988).

In a human study (Lupton et al., 1993), 44 men and women consumed cellulose or barley bran flour diet. Results showed that barley bran flour lowered serum cholesterol level as well as decreased gastrointestinal transit time by 8.02 h and increased daily fecal weight by 48.6 g in subjects. It was suggested that insoluble fiber diluted carcinogens in fecal bulk and shortened transit time in colon, thus it protected against colon cancer.

Beside dietary fiber, α-tocotrienol in barley, is believed to have hypocholesterolemic effect. Total concentration of vitamin E in barley varies from 55-65 mg/kg (db) up to 95 to 100 mg/kg (db) at harvest time (Hakkarainen et al., 1984). In an animal study (Wang et al., 1993a), 24 male broiler chicks were fed with barley oil, corn oil or margarine. Results showed that barley oil had a greater hypocholesterolemic effect than corn oil, while both α-tocotrienol and polyunsaturated fatty acids (PUFA) of barley might be responsible for the suppression of plasma total cholesterol and LDL concentration.

In another animal study (Qureshi et al., 1980), 20 white leghorn chicks were fed with corn, wheat, barley, oats or rye. Chicks on a barley diet showed a higher increase in enzymatic activities of acetyl-CoA carboxylase and fatty acid synthetase (FAS) and a greater decrease in the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (a rate-limiting enzyme in cholesterol and bile acid synthesis in liver). Since both biosynthesis pathways of cholesterol and fatty acid require acetyl-CoA as a common precursor, results indicated that acetyl-CoA was utilized to a greater extent by FAS

synthesis pathway, thereby limited its utilization in HMG-CoA reductase pathway. Thus, the cholesterol synthesis was being controlled and resulted in a reduction of serum cholesterol.

Barley diet may retard or even reverse the negative health condition of people who are suffering from diabetes. In a human study, 56 non-insulin-dependent diabetic subjects were involved in a 6 months dietary study (Hawrysh, 1997). There was a significant decrease in glycated hemoglobin values for the female subjects during the study. Male subjects experienced 24% and 23% decrease in mean fasting insulin values at 3 and 6 months, respectively. Results showed that 5 out of 33 oral hypoglycemics dependent subjects were able to decrease their dosage. Hawrysh (1997) also reported that the barley diet could increase high-density lipoprotein (HDL) levels while decreasing LDL/HDL ratio by 8.6% and 7.1% at 3 months and 6 months respectively compared to the initial level. In another human study, 5 volunteers, male and female subjects, were involved in a 3-weeks study period (Yokoyama et al., 1997). The subjects were required to consume barley and wheat pasta in order to test their blood glucose and blood insulin responses. The results indicated that barley pasta lowered the plasma insulin response, thus barley could have hypoglycemic effects. This conclusion agreed with that of Bourdon et al. (1999), who carried out a study with 11 healthy men and fed them β-glucan enriched pasta. Results suggested that carbohydrate was absorbed slower from the high fiber meal containing β-glucan with a slower response of insulin.

Barley also has anticarcinogenic effects. In an animal study, 55 Sprague-Dawley rats were fed cellulose, barley bran, wheat bran and spent barley grain (McIntosh et al., 1993). Results showed that spent barley grain was the most effective in lowering the

incidence of dimethyl hydrazine (DMH) induced tumors in rats. Insolubility of dietary fiber from barley could be the factor against intestinal cancer in rats. In another animal study (McIntosh et al., 1996), commercial barley bran mainly from the aleurone/sub-aleurone layer (13% dietary fiber), outer layer barley bran including the germ (25.5% dietary fiber) and spent barley grain bran (47.7% dietary fiber) were compared with wheat bran (44% dietary fiber) and cellulose (control, 98% dietary fiber). Results indicated that commercial barley and wheat bran were more effective than outer layer barley bran and spent barley grain bran in protection against DMH induced intestinal tumors in rats.

2.6 Barley processing

2.6.1 Pearling

Pearling is usually the initial step in barley flour milling. The process involves gradual removal of grain tissue starting from the outer layers (i.e. pericarp, seed coat, aleurone layers, sub-aleurone layer) and germ of grain by abrasive action. A pearler is generally composed of 6 – 8 abrasive carborundum or emery coated disks, which revolve at high speed (about 450 rpm) within a perforated cylinder or closed chamber (Leonard and Martin, 1963). It is usually a batch process, which is designed to obtain uniform removal of the grain outer layers. The process of pearling involves grain cleaning, conditioning/tempering (to about 15% moisture), pearling, screening/sifting, aspirating (to remove fine bran particle/spacs) and cooling (Fig. 2.8). Depending on the degree of pearling, products range from dehulled barley, pot barley (up to 15% pearl) and pearled barley (>15% pearl). The removed grain outer layers, called pearling flour (Fig. 2.8), is

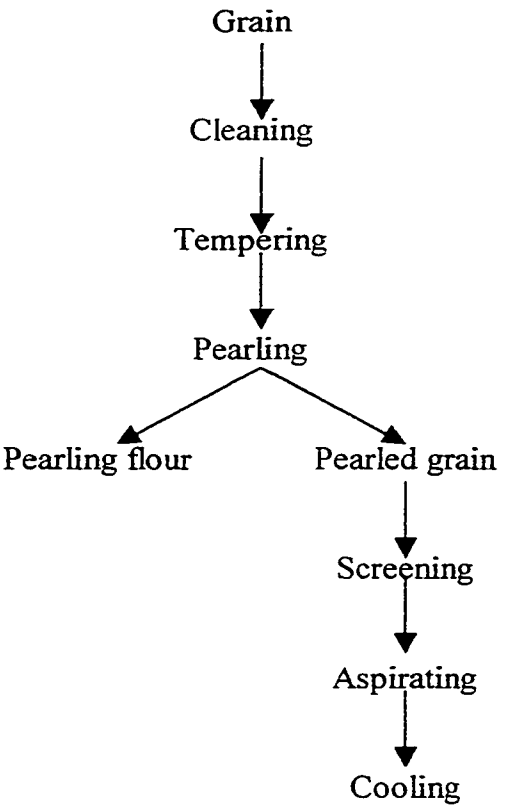


Figure 2.8: Flow chart showing pearling process of barley

usually sold as feed. Fiber content of these products differs. The insoluble fiber content of pearled grain gradually decreases upon pearling, while soluble fiber and β-glucan contents are somewhat increased (Pedersen et al., 1989). Research has shown that 30-40% pearling is the most desirable based on the balance between nutritional and physical properties (Marconi et al., 2000). The "degree of pearling" is a terminology, which needs some clarification as it's meaning differs with region. For instance, in North America, "30% pearled" means 30% pearling flour and 70% pearled grain. In Japan, where significant amounts of pearled barley is used in food products like rice extender, miso, etc, "70% pearled" means 70% pearled grain and 30% pearling flour.

2.6.2 Extrusion

Extrusion is a process of mixing, conveying, heating and forcing the material through a die (Fig. 2.9). Generally, there are two types of extruders, single screw and twin screw. Single screw extruder was developed in 1940's and mainly used to produce puffed snacks. Twin screw extruder was introduced to the food industry around 1970's. Compared to a single screw extruder, twin screw extruder has better conveying and mixing capabilities and provides interchangeable screw profile by combining different configured screw elements onto 2 parallel shafts (Harper, 1989).

Extrusion parameters include screw speed, feed moisture content, mass flow, barrel temperature, die hole diameter and screw configuration. All these parameters interact with each other, which makes the extrusion system complex. Screw speed is directly proportional to the flow rate and is controlled by the drive. Feed moisture content depends on the specific food application. For example, feed moisture content for pasta and puffed snacks are usually 32 and 11% respectively. In both single and twin

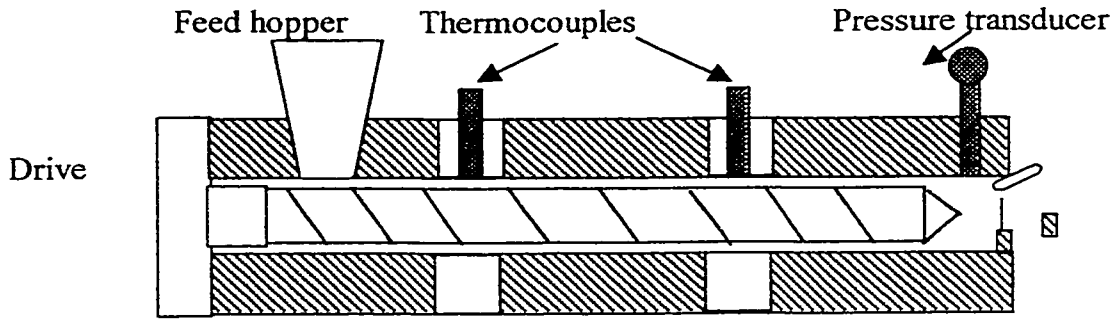


Figure 2.9: Cross-section of an extruder

screw extruders, the mass flow is not only influenced by screw speed, but also influenced by "drag" flow. The flow of material in the barrel instead of turning with the screw is called "drag", which is directly proportional to the screw speed (Harper, 1989). Barrel temperature is another important extrusion parameter, which is controlled by the heating device (heating/cooling jacket) and medium (electric, steam or oil). In order to extrude products with different shape, die hole design must be specified. Smaller die diameter induces pressure in the extruder barrel and the die design determines the shape of the product. Furthermore, screw configuration is designed for transferring, mixing and back flowing purposes. This concept mostly applies to twin screw extruder, where the configuration is built by combining different screw elements together. Convey elements (right hand screw) transfer material towards the die, which is expressed as pitch (mm)/length (mm) (e.g. 30/60) (Fig. 2.10). Kneading elements improve mixing, which is expressed as offset angle (°)/number of disc/length (mm) (e.g.45/5/30) (Fig. 2.11). Reverse elements (left hand screw) convey material away from the die and increase the pressure in the specific section and it is expressed as pitch (mm)/length (mm) (e.g.30/60 LH).

Single screw extruder can either consist of one continuous piece of screw or several pieces with various configured pieces combined together. The latter provides more flexibility and reduces the cost of replacing a worn section. Twin screw extruder is composed of two shafts of identical screw configuration and they rotate in either the same direction (co rotation) or different direction (counter rotation). Screw sections can also be non, partially, or fully intermeshing (Fig. 2.12). Intermeshing promotes drag flow. Intermeshing co-rotating twin screw extruder also provides self-wiping feature, which

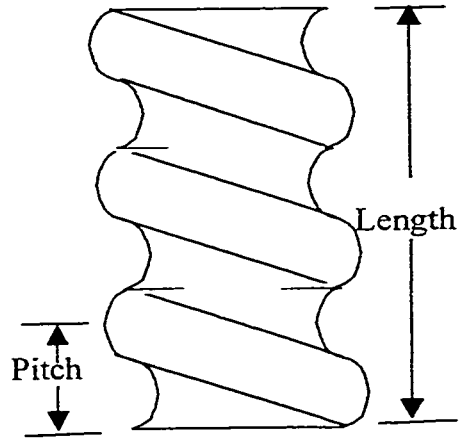


Figure 2.10: Diagram of a convey element

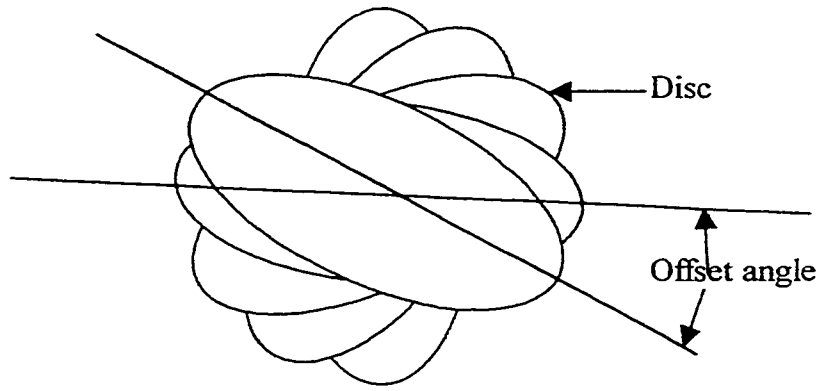


Figure 2.11: Diagram of a kneading element

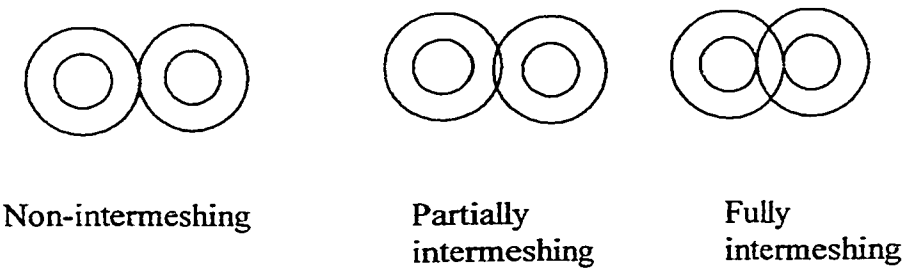


Figure 2.12: Cross-section of twin screws with different degrees of intermeshing

prevents buildup of material in the barrel. Twin screw extruder is designed with a sequence of unit operations: feeding, conveying, mixing, cooking, venting, cooling and pumping.

Feeding devices can be classified as volumetric feeding or gravimetric feeding (Harper, 1989). Volumetric feeding controls feed rate by volume and it is affected by the density of material. Gravimetric feeding controls feed rate by mass instead of volume. It is influenced by the amount of material in the hopper. Larger amount of material in the hopper may result in a higher feed rate.

Sometimes a preconditioner is attached to the extruder (Fig. 2.13). It is used to increase capacity, increase residence time and reduce mechanical power consumption for the extruder. It is an atmospheric or pressurized chamber, which acts as a pre-mixing/pre-heater by injecting water or live steam.

Extrusion has been introduced to the food industry over 50 years ago (Harper, 1989). However, applications are limited to production of snacks, pasta, breakfast cereals, pet foods, confectionery products etc. Researchers reported that high temperature extrusion cooking could inactivate native enzymes, such as α-amylase, lipase, lipoxygenase, peroxidase and urease, which caused food deterioration during storage (Linko et al., 1981). The inactivation of α-amylase and other enzymes allows products to retain their original desired quality and have longer shelf-life. Ironically, it was the observation that extrusion may not totally inactivate α-amylase that lead to the concept of using an extruder as a reactor for enzymatic liquefaction (Linko, 1989). Extrusion cooking is time efficient because it can be carried out in a continuous manner

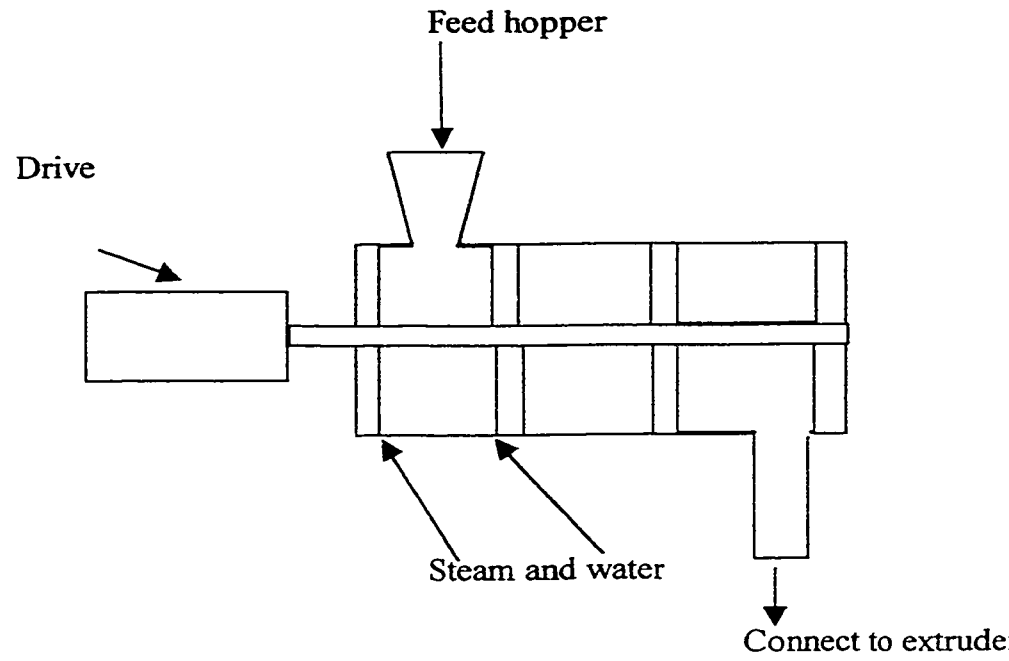


Figure 2.13: Diagram of a preconditioner

instead of conventional batch processing. The extruder also shows a good heat transfer with 95% efficiency, which made the process possible even in a short retention time.

Due to its flexibility, twin screw extruder is more commonly used for enzymatic liquefaction of starch (Harper, 1989). It has a higher conveying capability compared to a single screw extruder. It can also process a wider range of raw materials in terms of their particle size, moisture content, fat content etc. It can be configured for high or low shear applications with different modular designs, segmented screws and number of barrels.

Continuous enzymatic liquefaction of starch using extruder as a reactor

In an extruder, enzymatic liquefaction is carried out with thermal and mechanical energy. Under properly controlled operating conditions, pure starch or starch in flour can be hydrolyzed to different extents. The degree of hydrolysis can be calculated by the amount of sugar released (i.e. α-amylase releases maltose; glucoamylase releases glucose), and it can also expressed in terms of dextrose equivalent (DE), which represents the amount of reducing end. At high temperatures, the regular type of amylase (enzymes are proteins) are denatured and they lose their activity. However, the discovery of thermally stable α-amylase opened up more opportunities for high temperature extrusion liquefaction of starch. The operating conditions have to be optimized to ensure minimal enzyme inactivation and that the starch granules are gelatinized for enzyme susceptibility. Extrusion dextrinization of pre-gelatinized starch with thermally stable amylase can produce dextrins with up to DE 20 (Chouvel et al., 1983).

Higher degree of starch hydrolysis in an extruder is possible with glucoamylase. Unfortunately, it is difficult to carry out both liquefaction and saccharification parallelly, because the temperature of gelatinization would be too high and would inactivate

glucoamylase. The retention time is too short in an extruder. As temperatures for liquefaction and saccharification are different, it is hard to change the temperature from high (required for liquefaction) to low (required for saccharification). Thus, saccharification should be carried out as a separate unit operation as demonstrated by Linko et al. (1983a). The authors saccharified the liquefied extrudates with a shaker in an oven at 60°C with glucoamylase but without inactivating the thermostable α-amylase used during liquefaction. They obtained products with DE 94-95 in 24 h. Other research suggested that successful conversion could be obtained by introducing glucoamylase just before the die element, after the mass temperature had been decreased to 60 °C (Hakulin et al., 1983). This method would minimize cooling of dextrinized product and subsequent starch retrogradation before saccharification. The conversion was continued at 60 °C for 1 or 5 h and resulted in DE 75 and 94 products, respectively.

Hakulin et al. (1983) suggested that an increase in moisture content coupled with high temperature could result in complete starch gelatinization, thus a higher degree of starch hydrolysis. This is consistent with the study of Linko et al. (1983a), which demonstrated that higher DE could be obtained with increased water content. Usually, a minimum of 31% moisture level is required for starch gelatinization. However, under high temperature and high pressure extrusion cooking, starch gelatinization could occur at 20% moisture level (Linko et al., 1983b). This concept is similar to what was reported by Roussel et al. (1991). Their study suggested that extrusion at a low moisture content (30%) imparted higher shear stress and increased heat generation, which enhanced mechanical disruption of starch granules. The same study further suggested that a low

feed rate would lead to low filling of screw channels, thereby reducing the effectiveness of heat transfer.

Both starch gelatinization and enzyme activity are dependent on temperature and moisture. Starch gelatinization at 24-27% moisture increased rapidly with increasing temperature, but increased moderately under 18-21% moisture content (Chiang and Johnson, 1977). Research also indicated that an increase in temperature led to an increase in starch fragmentation (Theander and Westerlund, 1987). Such fragmentation would be preferable as it would enhance enzyme hydrolysis. However, if the extrusion temperature is higher than the optimal range for enzyme activity, the degree of hydrolysis will be decreased due to enzyme inactivation. Chouvel and co-workers (1983) showed that as temperature increased from 100 to 145 °C, DE decreased from 19.5 to 16.5.

In addition to temperature and moisture levels, pH, enzyme concentration, and die nozzle size are also important. Neutral pH has been shown to be more suitable as compared to acid or alkali conditions (Chouvel et al., 1983). Also equally important is the enzyme concentration, which should be proportional to the starch concentration. The higher the enzyme concentration, the higher the degree of hydrolysis until it reaches an optimum point. Increase in screw speed could cause incomplete starch gelatinization due to short retention time (Chiang and Johnson, 1977). Increased die nozzle size could decrease starch gelatinization due to a decrease in pressure and surface shear (Chiang and Johnson, 1977).

It is important that the enzyme used should be inactivated after hydrolysis in order to obtain a specific DE value. Linko et al. (1983b) studied the effect of 1% mercuric chloride and liquid nitrogen freezing on enzyme inactivation. The authors found both

treatments to be not effective. The liquid nitrogen was not effective because of incomplete freezing of extrudes. In addition, the active enzyme could be present internally and its activity could be restored after reconstituting dried extrudates in water. Studies have shown that thermostable α-amylase can be inactivated by using 0.2 M acetate buffer (pH 3.5) (Reinikainen et al., 1986) and by heating to above 155 °C (Linko, 1989).

Effects of extrusion on hyocholesterolemic activity and dietary fiber

Research showed that extrusion cooking did not alter the hypocholesterolemic effect of rice, oat and corn bran (Kahlon et al., 1998). The same research suggested that extrusion could improve the hypocholesterolemic activity of wheat bran. This concept was in agreement with Ralet et al. (1990), who illustrated that those mild extrusion conditions of wheat bran could increase arabinoxylan and β-glucan contents, decrease protein solubility and increase water absorption capacity. Wang and Klopfenstein (1993) reported that rats fed with extruded wheat, oat and barley had lower serum and liver cholesterol level compared to those fed with raw materials. Rats fed with extruded barley had the lowest cholesterol level.

Kahlon et al. (1998) found that extrusion under low energy decreased the total dietary fiber in rice, corn and wheat. However, under high energy, total dietary fiber of corn and wheat decreased, but increased in oat. There was no major change in the ratio of soluble to insoluble fiber for all varieties. Wang et al. (1993b) showed a decrease in total dietary fiber upon extrusion. Østergård et al. (1989) reported a slight increase in dietary fiber after extruding whole barley meal. Similar results were found by Björck et al. (1984). Nevertheless, other research showed no change in total dietary fiber upon

extrusion (Siljeström et al., 1986). Ralet et al. (1990) found no difference in total dietary fiber except under severe extrusion conditions. Previous studies found that extrusion cooking of various cereal grains and fractions could increase soluble dietary fiber (Björck et al., 1984, Siljeström et al., 1986, Shinnick et al., 1988, Aoe et al., 1989, Ralet et al., 1990, Wang et al., 1993b). Significant increases in glucose, maltose, maltotriose and maltotetraose were reported during extrusion cooking of wheat in a single screw extruder (Chiang and Johnson, 1977).

2.7 References:

Aoe, S., Nakaoka, M., Ido, K., Tamai, Y., Ohta, F., and Ayano, Y. 1989. Availability of dietary fiber in extruded wheat bran and apparent digestibility in rats of coexisting nutrients. *Cereal Chem.* 66: 252-256.

Bhatty, R. S. 1992. β-Glucan content and viscosities of barleys and their roller-milled flour and bran products. *Cereal chem.* 69(5): 469-471.

Bhatty, R. S. 1993. Extraction and enrichment of (1-3), (1-4)-β-glucan from barley and oat brans. *Cereal Chem.* 70(1): 73-77.

Bhatty, R. S. 1999. The potential of hull-less barley. *Cereal Chem.* 76(5): 589-599.

Bhatty, R. S., Berdahl, J. D., and Christison, G. I. 1975. Chemical composition and digestible energy of barley. *Can. J. Animal Sci.* 35: 759-764.

Björck, I., Nyman, M., and Asp, N. G. 1984. Extrusion cooking and dietary fiber: Effects on dietary fiber content and on degradation in the rat intestinal tract. *Cereal Chem.* 61: 174-179.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B., Schneeman, B. O. 1999. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with β-glucan. *Am. J. Clin. Nutr.* 69:55-63.

Chiang, B. Y. and Johnson, J. A. 1977. Gelatinization of starch in extruded products. *Cereal Chem.* 54(3): 436-443.

Chouvel, H., Chay, P. B., and Cheftel, J. C. 1983. Enzymatic hydrolysis of starch and cereal flours at intermediate moisture contents in continuous extrusion reactor. *Lebensm. Wiss-u. Technol.* 16: 346-353.

Dais, P. and Perlin, A. S. 1982. High-field C^{13}NMR spectroscopy of beta-D-glucans, amylopectin, and glucogen. *Carbohyd. Res.* 100: 103-116.

Duffus, C. M. and Cochrane, M. P. 1996. Formation of the barley grain – morphology, physiology, and biochemistry, in *Barley Chemistry and Technology*, A. W. MacGregor and R. S. Bhatty, (Ed.), pp. 31-72, American Association of Cereal Chemists, St. Paul, MN.

Food and Agriculture Organization, 2000: [http://apps.fao.org/cgi-bin/nph-db.pl?subset=agriculture]

Glore, S. R., Van Treeck, D., Knehans, A. W., and Guild, M. 1994. Soluble fiber and serum lipids: A literature review. *J. Am. Diet. Assoc.* 94: 425-436.

Goering, K. J., Fritts, D. H., and Eslick, R. F. 1973. A study of starch granule size and distribution in 29 barley varieties. *Starch.* 25:297-302.

Gómez, C., Navarro, A., Manzanares, A., Horta, A., and Carbonell, J. V. 1997a. Physical and structural properties of barley (1-3), (1-4)-beta-D-glucan. Part I. Determination of molecular weight and macromolecular radius by light scattering. *Carbohyd. Polym.* 32:7-15.

Gómez, C., Navarro, A., Manzanares, A., Horta, A., and Carbonell, J. V. 1997b. Physical and structural properties of barley (1-3), (1-4)-beta-D-glucan. Part II. Viscosity, chain stiffness and macromolecular dimensions. *Carbohyd. Polym.* 32:17-22.

Gómez, C., Navarro, A., Manzanares, A., Horta, A., and Carbonell, J. V. 1997c. Physical and structural properties of barley (1-3), (1-4)-beta-D-glucan. Part III. Formation of aggregates and analysed through its viscoelastic and flow behaviour. *Carbohyd. Polym.* 32:141-148.

Hakkarainen, R. V. J., Tyopponen, J. T., Hassan, S., Bengtsson, G., Lennart, J. S. R. and Lindberg, P. O. 1984. Biopotency of vitamin E in barley. *Br. J Nutr.* 52:335-349.

Hakulin, S., Linko, Y. Y., Linko, P., Seiler, K., and Seibel, W. 1983. Enzymatic convension of starch in twin-screw HTST-extruder. *Starch.* 35: 411-414.

Harper, J. M. 1989. Food extruders and their applications, in *Extrusion Cooking*, C. Mercier, P. Linko and J. M. Harper, (Ed.), pp. 1-15, American Association of Cereal Chemists, St. Paul, MN.

Hawrysh, Z. 1997. Barley food products intervention trial for non-insulin-dependent (NIDDM) diabetic subjects. The Alberta Agricultural Research Institute, Edmonton, Alberta.

Jadhav, S. J., Lutz, S. E., Ghorpade, V. M. and Salunkhe, D. K. 1998. Barley: Chemistry and value –added processing. *Crit. Rev. Food Sci.* 38: 123-171.

Kahlon, T. S., Edwards, R. H., and Chow, F. I. 1998. Effect of extrusion on hypocholesterolemic properties of rice, oat, corn and wheat bran diets in hamsters. *Cereal Chem.* 75(6): 897-903.

Klopfenstein, C. F. 1988. The role of cereal beta-glucans in nutrition and health. *Cereal Foods World.* 33: 865-869.

Klopfenstein, C. F. and Hoseney, R. C. 1987. Cholesterol-lowing effect of beta-glucan-enriched bread. *Nutr. Rep. Int.*, 36:1091-1098.

Leonard, W. H. and Martin, J. H. 1963. Barley, in *Cereal Crops*, pp. 478-543, The Macmillan Company, New York.

Linko, P. 1989. Extrusion cooking and bioconversions, in *Extrusion Cooking*, C. Mercier, P. Linko and J. M. Harper, (Ed.), pp. 235-245, American Association of Cereal Chemists, St. Paul, MN.

Linko, P., Colonna, P., and Mercier, C. 1981. High temperature short-time extrusion cooking, in *Advances in Cereal Science and Technology*, Y. Pomeranz, (Ed.), vol. 4, pp. 145-235, American Association of Cereal Chemists, St. Paul, MN.

Linko, P., Hakulin, S., and Linko, Y. Y. 1983a. Extrusion cooking of barley starch for the production of glucose syrup ethanol. *J. Cereal Sci.* 1: 275-284.

Linko, P., Linko, Y. Y., and Olkku, J. 1983b. Extrusion cooking and bioconversions. *J. Food Eng.* 2: 243-257.

Lupton, J. R., Morin, J. L., and Robinson, M. C. 1993. Barley bran flour accelerates gastrointestinal transit time. *J. Am. Diet. Assoc.* 93: 881-885.

MacGregor, S. 1998. Composition of barley related to food uses. Presented at International Food Barley Program, Canadian International Grains Institute in Winnipeg, Manitoba, Canada. October 19 to 22.

MacGregor A. W. and Fincher, G. B. 1996. Carbohydrates of the barley grain, in *Barley Chemistry and Technology*, A. W. MacGregor and R. S. Bhatty, (Ed.), pp. 73-130, American Association of Cereal Chemists, St. Paul, MN.

Marconi, E., Graziano, M., and Cubadda, R. 2000. Composition and utilization of barley pearling by-products for making functional pastas rich in dietary fiber and β-glucans. *Cereal Chem.* 77(2): 133-139.

Martinez, V. M., Newman, R. K., and Newman, C. W. 1992. Barley diets with different fat sources have hypocholesterolemic effects in chicks. *J. Nutr.* 122: 1070-1076.

Matz, S. 1991. *The Chemistry & Technology of Cereals as Food and Feed.* 2nd ed, pp. 135-136, Van Nostrand Reinhold/AVI, New York, NY.

McIntosh, G. H., Jorgensen, L., Royle, P. 1993. The potential of an insoluble dietary fiber-rich source from barley to protect from DMH-induced intestinal tumors in rats. *Nutr. Cancer.* 19: 213-221.

McIntosh, G. H., Le-Leu, R. K., Royle, P. J., Young, G. P. 1996. A comparative study of the influence of differing barley brans on DMH-induced intestinal tumors in male Sprague-Dawley rats. *J. Gastronenter Hepatol.* 11: 113-119.

McIntosh, G. H. and Russell, G. R. 1988. The role of barley in human nutrition, in *Alternative End Uses of Barley*, D. H. B. Sparrow, R. C. M. Lance and R. J. Henry, (Ed.), pp. 49-54, Royal Australian Chemical Institute, Parkville.

Morrison, W. R., Tester, R. F., Snape, C. E., Law, R., and Gidley, M. J. 1993. Swelling and gelatinization of cereal starches. IV. Some effects of lipid-complexed amylose and free amylose in waxy and normal barley starches. *Cereal Chem.* 70:385-391.

Newman, R. K., Lewis, S. E., Newman, C. W., Boik, R. J., and Ramage, R. T. 1989. Hypocholesterolemic effect of barley foods on healthy men. *Nutr. Rep. Int.* 39: 749-759.

Nilan, R. A. and Ullrich, S. E. 1996. Barley: Taxonomy, origin, distribution, production, genetics, and breeding, in *Barley Chemistry and Technology*, A. W. MacGregor and R. S. Bhatty, (Ed.), pp. 1-29, American Association of Cereal Chemists, St. Paul, MN.

Østergård, K., Björck, I., and Vainionpää, J. 1989. Effects of extrusion cooking on starch and dietary fibre in barley. *Food Chem.* 34: 215-227.

Pedersen, B., Bach Knudsen, K. E., and Eggum, B. O. 1989. Nutritive value of cereal products with emphasis on the effect of milling. *World Rev. Nutr. Diet.* 60:1-91.

Qureshi, A. A., Burger, W. C., Prentice, N., Bird, H. R., and Sunde, M. L.1980. Regulation of lipid metabolism in chicken liver by dietary cereals. *J. Nutr.* 110: 388-393.

Ralet, M. C., Thibault, J. F., and Della Valle, G. 1990. Influence of extrusion-cooking on the physico-chemical properties of wheat bran. *J. Cereal Sci.* 11: 249-259.

Reinikainen, P., Suortti, T., Olkku, J., Malkki, Y., and Linko, P. 1986. Extrusion cooking in enzymatic liquefaction of wheat starch. *Starch.* 38: 20-26.

Roussel, L., Vieille, A., Billet, I., and Cheftel, J. C. 1991. Sequential heat gelatinization and enzymatic hydrolysis of corn starch in an extrusion reactor optimization for a maximum dextrose equivalent. *Lebensm. Wiss-u. Technol.* 24: 449-458.

Shinnick, F. L., Longacre, M. J., Ink, S. L., and Marlett, J. A. 1988. Oat fiber: Composition versus physiological function in rats. *J. Nutr.* 118: 144-151.

Siljeström, M., Westerlunds, E., Björck, I., Holm, J., Asp, N. G., and Theander, O. 1986. The effects of various thermal processes on dietary fibre and starch content of whole grain wheat and white flour. *J. Cereal Sci.* 4: 315-323.

Theander, O. and Westerlund, E. 1987. Studies on chemical modifications in heat-processed starch and wheat flour. *Starch.* 39: 88-93.

Toma, R. B. and Curtis, D. J. 1986. Dietary fiber: Effect on mineral availability. *Food Techol.* 40(2): 111-116.

Tsao, G. T., Zheng, Y., Lu, J., Gong, C. S. 1997. Adsorption of heavy metal ions by immobilized phytic acid. *Appl. Biochem. Biotechnol.* 63-65:731-741.

Vasanthan, T. and Bhatty, R. S. 1995. Starch purification after pin-milling and air-classification of waxy, normal and high amylose barleys. *Cereal Chem.* 72:379-384.

Wang, L., Newman, R. K., Newman, C. W., and Hofer, P. J. 1992. Barley β-glucans alter intestinal viscosity and reduce plasma cholesterol concentrations in chicks. *J. Nutr.* 122: 2292-2297.

Wang, W. M. and Klopfenstein, C. F. 1993. Effect of twin-screw extrusion on the nutritional quality of wheat, barley, and oats. *Cereal Chem.* 70(6): 712-715.

Wang, L., Newman, R. K., Newman, C. W., Jackson, L. L., and Hofer, P. J. 1993a. Tocotrienol and fatty acid composition of barley oil and their effects on lipid metabolism. *Plant Foods Hum. Nutr.* 43: 9-17.

Wang, W. M., Klopfenstein, C. F., and Ponte, J. G., Jr. 1993b. Effects of twin-screw extrusion on the physical properties of dietary fiber and other components of whole wheat and wheat bran and on the baking quality of the wheat bran. *Cereal Chem.* 70(6): 707-711.

Woodward, J. R., Ficher, G. B. and Stone, B. A. 1983. Water-soluble (1-3), (1-4)-β-D-glucans from barley (*hordeum vulgare*) endosperm. II. Fine structure. *Carbohyd. Polym.* 3:207-225.

Woodward, J. R., Phillips, D. R. and Ficher, G. B. 1988. Water-soluble (1-3), (1-4)-β-D-glucans from barley (*hordeum vulgare*) endosperm. IV. Comparison of 40°C and 65°C soluble fractions. *Carbohyd. Polym.* 8:85-97.

Yokoyama, W. H., Hudson, C. A., Knuckles, B. E., Chiu, M. M., Sayre, R. N., Turnlund, J. R., Schneeman, B. O. 1997. Effect of barley β-glucans in durum wheat pasta on human glycemic response. *Cereal Chem.* 74: 293-296.

Yoon, S. H., Berglund, P. T. and Fastnaught, C. E. 1995. Evaluation of selected barley cultivars and their fractions for β-glucan enrichment and viscosity. *Cereal Chem.* 72:187-190.

Chapter 3

Pearling of Hull-less Barley: Product Composition and Gel-color of Pearled Barley Flours as Affected by the Degree of Pearling[1]

3.1 Introduction

Pearling, an important primary processing in food-barley utilization, refers to the gradual removal of grain tissues (by abrasive action) starting from the outer grain tissues/layers, bran (i.e., pericarp, testa, aleurone and sub-aleurone layers), and germ. The removal of barley bran through pearling yields a bright white kernel that is ideal for various food applications. The removed grain layers are called pearling flour (PF) and the remainder is called pearled grain (PG). The PF is usually sold as animal feed. Nevertheless, its use for producing various innovative food products such as high fiber (β-glucan rich) functional pasta has been studied (Marconi et al., 2000).

In barley, the starch and most of the storage proteins are generally confined to the endosperm cells, but several other storage and functional proteins (i.e., enzymes) exist in aleurone, sub-aleurone and germ tissues. The β-glucan is present mostly in the cell walls of endosperm, but small amounts exist in the cell walls of aleurone and sub-aleurone layers. β-Glucan in barley is more evenly distributed than in oat, where it is predominantly found in the sub-aleurone layers.

The type of component distribution pattern (uniform or gradient) in barley grain would influence the composition and functionality of the pearling products. Zheng et al. (2000) reported that the distribution of β-glucan within the grain of hull-less barley varies depending upon the variety. Gohl et al. (1977 and 1978) studied the distribution of

[1] A version of this study has been submitted for publication in *Journal of Agricultural and Food Chemistry*.

carbohydrates in barley grains harvested at early and late stages of maturity and reported that xylose, fructose and glucitol were found mostly in the outer layers. Trace amount of myoinositol was detected in all abrasive milling fractions. Sucrose was found in all abrasive milling fractions except in the bran. Stachyose and raffinose were concentrated in the center of the kernel. Glucose was found in all layers in increasing amounts towards the center of the kernel. The authors (Gohl et al.,1977 and 1978) further reported that protein was concentrated in the intermediate layers for early harvested barley grains and in 15-45% abrasion for late stage of maturity. Ash was found in the outer layers of kernel. Also, the lipid content of PF reached its highest concentrations in 10-25% and 15% of the abraded barley grains in early harvested and late harvested, respectively. Klamczynski et al. (1998) reported a significant increase in starch and total β-glucan contents in PG with progressive pearling. Bhatty (1997) demonstrated that the PF obtained by 30% pearling of barley grain constitutes the bran (pericap, testa, aleurone and sub-aleurone layers). Most of the lipids, protein and minerals (ash) are concentrated in the germ and bran. Bhatty and Rossnagel (1998) compared Canadian and Japanese barleys and reported that pearling to 55% decreased β-glucan, total dietary fiber, ash, and protein contents, but increased starch and soluble fiber contents. Klamczynski et al. (1998) reported that substantial amounts of barley grain protein and minerals (ash) are concentrated in PF.

Barley lipids contain relatively high amounts of unsaturated fatty acids, oleic (18:1) and linoleic (18:2) acids (Morrison, 1993), which are highly prone to autooxidation and subsequent rancid odor development. Therefore, the storage stability and overall quality of pearled barley will be improved by removing the outer layers of the grain. Another important benefit of pearling is the removal of a variety of barley phenolic compounds and

enzymes, such as polyphenoloxidase and peroxidase, along with the outer grain layers. This virtually eliminates the enzyme driven darkening of barley products. However, in terms of nutritional quality of pearled barley, the loss of tocols (vitamin E, a fat soluble component), protein, and valuable minerals along with the bran and germ is a negative effect brought about by pearling. Research showed that 20% PF contains high amounts of total tocols, α-tocopherol and α-tocotrienol (Wang et al., 1993; Peterson, 1994). It is clear from the literatures that there is a need to study the effect of pearling on flour gel color and also to study the grain components in the particular barley varieties in this study.

The objectives of the present study are: a) to determine the distribution of major components (i.e. starch, protein, lipid, β-glucan and ash) in waxy (CDC-Candle) and a regular (Phoenix) hull-less barley grains by a gradual layer-by-layer pearling of up to 80% (w/w) of the grain tissues and compositional analysis of pearling products namely PG and PF, and b) to study the effect of various degrees of grain pearling on the color characteristics of uncooked and cooked (gel) flour milled from PG.

3.2 Materials and Methods

Materials

Waxy barley grains (CDC-Candle) were provided by Mr. Jim Gray, Agricore, Calgary, AB. Regular barley grains (Phoenix) were obtained from Nakonechney Family Farms, Millet, AB. The analytical kits for starch and β-glucan determination were purchased from Megazyme International Ireland Ltd., Wicklow, Ireland. Other chemicals were obtained from Sigma Chemical Co., St. Louis, Missouri, USA.

Pearling and Milling

Barley grains were pearled at the University of Saskatchewan (Saskatoon, SK) using a "Satake" testing mill (model-TM05, Satake, Tokyo, Japan) (fitted with an abrasive roller and 1 mm screen). Grains (200 g) were pearled to varying degrees (10-80%, wet basis) (pearling time ranged from 13-56 min). At each degree of pearling, the PF and PG were collected. The PG were ground to flour in a Udy mill (0.5 mm screen).

Compositional Analysis

The protein, lipid, and ash contents of samples were determined according to the AOAC (1990) methods 979.09, 920.39, and 923.03, respectively.

Gel Preparation

Ten grams of flour (milled from PG) were mixed with 100 ml of water and then heated for 30 min in a water bath set at 96-100° C. The resulting paste was transferred into a Petri dish and stored for 1 and 72 h, at 4°C for it to set into a gel.

Color Measurements

A HunterLab Color Difference meter (model D52-2, Hunter Assoc. Laboratory, Fairfax, Virginia) was used to measure the Hunter ***L***, ***a*** and ***b*** values of the flours and gels.

Statistical Analysis

All experiments were carried out in triplicates. Analysis of variance of the results was performed using General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, 1989). Multiple comparison of the means was performed by least significant difference (LSD) test at α=0.05 level.

3.3 Results and Discussion

The compositions of whole barley grain and pearling products namely, PG and PF are given in Table 3.1, Table 3.2 and Figure 3.1.

3.3.1 Whole Grain

The starch content of regular (Phoenix) barley (63.62%, w/w) was ~5% higher than that of waxy (CDC-Candle) barley (58.50%, w/w). The β-glucan content of CDC-Candle barley (5.94%, w/w) was ~2% higher than that of Phoenix (3.83%, w/w). The differences in the contents of protein, fat, and ash between barley varieties were small (ranged from 0.1-1.2%).

3.3.2 Pearled Grain (PG) Composition

Regardless of the degree of pearling, PG from both Phoenix and CDC-Candle had high starch contents (~69-81% and ~64-79% for Phoenix and CDC-Candle, respectively) compared to the relatively lower starch contents in the corresponding whole grains (~64 and ~58% for Phoenix and CDC-Candle, respectively; Table 3.1 and Figure 3.1a). The starch content in PG from both varieties increased rapidly as the degree of pearling increased from 10 to 25% and gradually reached a plateau thereafter. This suggests that the tissue layers stripped initially due to pearling were composed mainly of non-starch materials and that starch is confined to the inner core of the grains. Furthermore, as the degree of pearling increases, a gradual decrease in the protein reserves of the grains was evident for both Phoenix and CDC-Candle (Table 3.1 and Figure 3.1b). This was indicative of a decreasing concentration gradient (from outer to inner tissue layers) for proteins across the grain.

The β-glucan content of pearled Phoenix grains (P-PG) ranged from ~3.7 to 4.5%, whereas that of the pearled CDC-Candle grains (C-PG) ranged from ~6.3 to 7.3% (Table 3.1

Table 3.1: Composition of regular (Phoenix) and waxy (CDC-Candle) barley grains and their pearled grains

Degree of Pearling (%)	Composition[a] (%, w/w, dry matter basis)				
	Starch	Protein	β-Glucan	Lipid	Ash
Phoenix					
Whole	63.62±1.15	13.51±0.50	3.83±0.22	2.13±0.14	1.99±0.12
10-12%[b]	68.87±0.22	12.82±0.13	4.26±0.12	1.51±0.07	1.67±0.05
23-25%	75.25±0.10	10.93±0.43	4.49±0.15	1.12±0.01	1.13±0.09
30-32%	76.57±0.36	10.40±0.22	3.87±0.13	1.05±0.03	0.95±0.10
48-50%	79.18±0.73	10.11±0.10	3.78±0.30	0.94±0.07	0.74±0.10
60-63%	80.49±0.60	9.30±0.23	3.70±0.18	1.17±0.04	0.63±0.14
78-80%	81.23±0.74	8.86±0.20	3.69±0.19	1.11±0.07	0.60±0.12
LSD (P<0.05)	0.45	0.24	0.17	0.10	0.11
CDC-Candle					
Whole	58.50±0.71	12.35±0.23	5.94±0.11	2.40±0.12	2.12±0.10
10-12%	64.13±1.10	11.93±0.45	6.28±0.15	1.89±0.10	1.58±0.07
23-25%	71.56±1.04	10.10±0.13	6.37±0.13	1.32±0.06	1.29±0.12
30-32%	73.65±0.37	10.21±0.55	6.49±0.23	1.19±0.08	1.18±0.09
48-50%	75.13±1.05	8.95±0.18	6.80±0.15	0.86±0.05	0.92±0.06
60-63%	77.89±0.65	7.01±0.69	7.01±0.18	0.81±0.07	0.74±0.13
78-80%	79.50±0.81	5.92±0.17	7.28±0.21	0.73±0.04	0.69±0.05
LSD (P<0.05)	0.93	0.46	0.18	0.11	0.10

[a] Values are means of three determinations ± standard deviation.
[b] Percent outer grain layers stripped and removed as PF leaving 88-90% of grain as PG.

Table 3.2: Composition of regular (Phoenix) and waxy (CDC-Candle) barley grains and their pearling flours

Degree of Pearling (%)	Composition[a] (%, w/w, dry matter basis)				
	Starch	Protein	β-Glucan	Lipid	Ash
Phoenix					
Whole	63.62±1.15	13.51±0.50	3.83±0.22	2.13±0.14	1.99±0.12
10-12%[b]	11.33±0.41	19.21±0.55	1.72±0.11	5.73±0.15	5.30±0.11
23-25%	28.90±0.65	23.86±0.47	1.92±0.28	5.33±0.10	4.43±0.12
30-32%	32.67±0.91	20.27±0.45	3.62±0.25	4.63±0.15	4.29±0.11
48-50%	48.67±0.58	16.23±0.38	3.90±0.12	3.22±0.18	3.30±0.21
60-63%	50.70±0.47	14.92±0.69	3.82±0.24	2.90±0.11	2.56±0.07
78-80%	59.10±0.34	14.12±0.46	3.97±0.20	2.34±0.15	2.41±0.14
LSD (P<0.05)	0.53	0.49	0.20	0.14	0.15
CDC-Candle					
Whole	58.50±0.71	12.35±0.23	5.94±0.11	2.40±0.12	2.12±0.10
10-12%	8.93±0.52	20.38±0.71	1.89±0.09	6.15±0.19	6.71±0.19
23-25%	22.73±0.20	22.71±0.60	4.56±0.10	5.75±0.20	4.77±0.15
30-32%	24.15±0.78	17.72±0.42	4.71±0.11	5.59±0.11	4.41±0.10
48-50%	41.65±0.73	16.14±0.75	5.04±0.08	4.08±0.14	3.27±0.17
60-63%	47.11±0.90	15.32±0.47	5.31±0.14	3.37±0.16	3.19±0.16
78-80%	53.64±0.62	13.95±0.63	5.49±0.20	2.80±0.13	2.35±0.17
LSD (P<0.05)	0.50	0.55	0.10	0.12	0.08

[a] Values are means of three determinations ± standard deviation.
[b] Percent outer grain layers stripped and removed as PF leaving 88-90% of grain as PG.

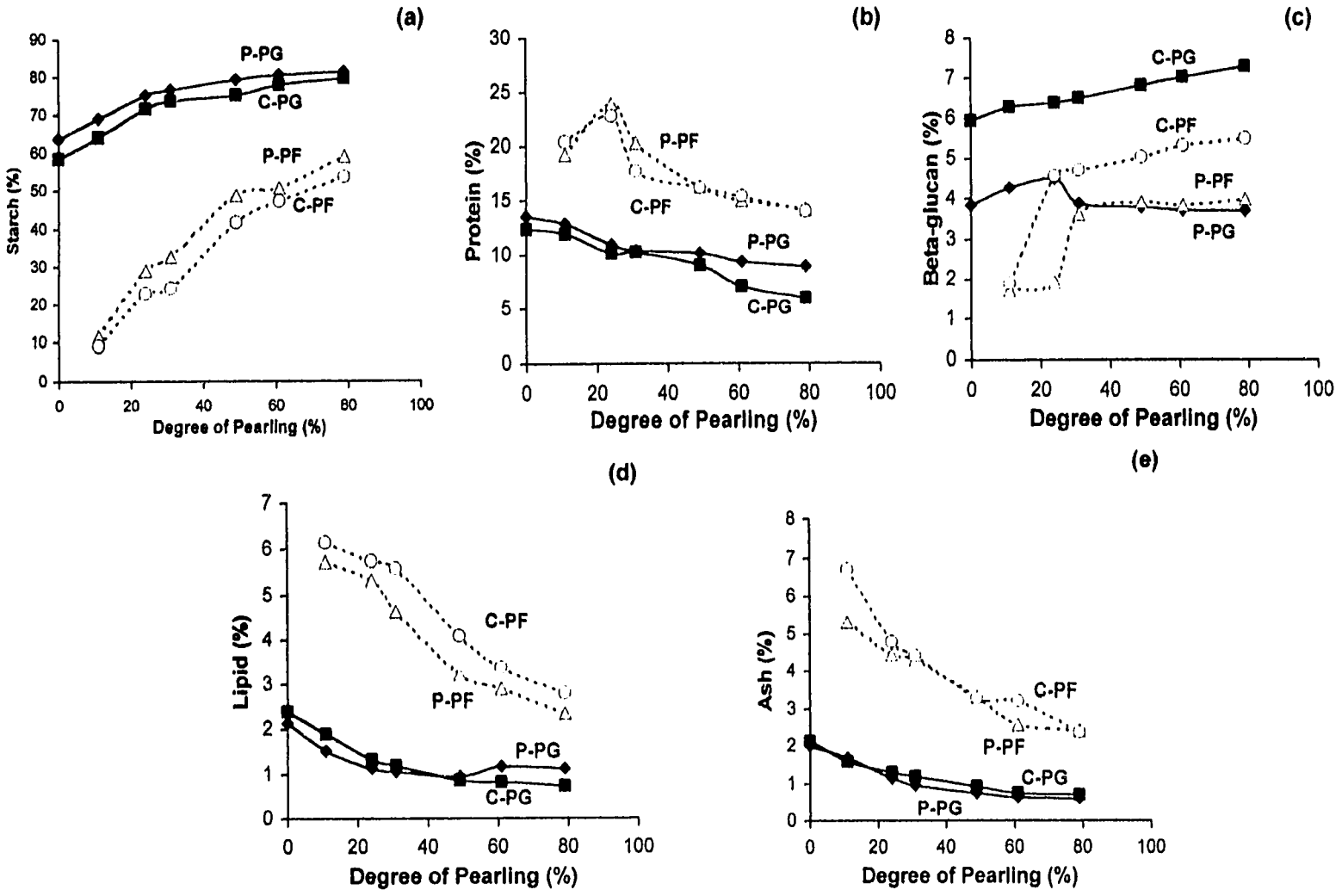


Figure 3.1: The starch, protein, beta-glucan, lipid and ash contents of the pearling products from CDC-Candle (C) and Phoenix (P) barley grains as affected by degree of pearling.
(Note: The whole grain component % values are considered for zero % degree of pearling.)

and Figure 3.1c). A sharp increase in the β-glucan content in P-PG up to a 25% of pearling followed by a gradual decrease suggests a concentration of β-glucan in the inner grain tissue layers, perhaps immediately beneath the sub-aleurone tissues. In contrast, there was a gradual increase in the β-glucan content of C-PG, indicating a positive concentration gradient towards the grain core. Zheng et al. (2000) also reported a similar trend for β-glucan in "low-β-glucan hull-less barley" (regular starch barleys) and concluded that the bulk of β-glucan was concentrated in the sub-aleurone layer and the endosperm located immediately beneath it. β-Glucan in "high-β-glucan hull-less barley" (waxy starch barleys) was reported to be localized at the inner endosperm (Zheng et al., 2000).

There were no substantial difference between the lipid contents of whole Phoenix and CDC-Candle grains. This resulted in similar lipid contents in PG of both varieties at the same degree of pearling. For both varieties, a continuous decrease in lipid content of PG as the degree of pearling increased suggests the existence of a negative concentration gradient towards the grain core (Table 3.1 and Figure 3.1d). Similar trends were observed for ash contents of PG from both barley varieties (Table 3.1 and Figure 3.1e). These findings are in agreement with those reported by Summer et al. (1985), Bhatty and Rossnagel (1998), Klamczynski et al. (1998) and Marconi et al. (2000).

3.3.3 Pearling Flour (PF) composition

For both varieties, starch and β-glucan contents of PF initially decreased with pearling of up to 10-12% (degree of pearling) and then increased through pearling of up to 78-80% (Table 3.2, Figure 3.1 a, c). This was due to the confinement of starch and β-glucan in the inner tissues of barley grain. Increase in the starch content of the PF after 10-12% of pearling was not gradual (Figure 3.1a). The increase occurred at two distinct stages, perhaps due to

the non-uniform distribution of starch in the aleurone layer and the endosperm. However, the pattern of increase in β-glucan content (Figure 3.1c) after 10-12% of pearling was found to be variety dependent. In CDC-Candle, a rapid increase up to 25% pearling and a gradual increase thereafter were observed. For Phoenix, a small increase existed up to 23% pearling, followed by a rapid increase up to 32% and a gradual, but small increase thereafter. This clearly indicates a nonuniform distribution of β-glucan across the endosperm and the possible concentration of β-glucan in the grain layers.

For both barleys, the protein content of PF (Figure 3.1b) initially increased up to 25% of pearling and decreased thereafter, suggesting high protein concentration in those layers. After 25% of pearling, the gradual decrease of protein content of Phoenix PF suggests that the protein distribution is uniform across the endosperm. However, in CDC-Candle, a sudden drop in protein content up to the degree of pearling 32% and a gradual decrease thereafter was observed. This suggests that the loss of protein along with the grain layers removed during 25-32% pearling, was minimal.

Both lipid and ash contents of barley PF investigated, increased up to 10-12% pearling and decreased thereafter, suggesting the concentration of these components in the outer layer stripped by 23-25% pearling. It is noteworthy to indicate the existence of a gradual, but a two-stage decrease in ash content after 12% pearling.

3.3.4 Gel Color

The gels were prepared from PG-flour of both varieties and photographed after refrigerated (4°C) storage for 1 h as well as 3 days. However, only the photographs for CDC-Candle gels are presented in Figure 3.2 since only a slight difference in the gel color between barley varieties was observed visually. The color of the gels prepared with whole grain flour

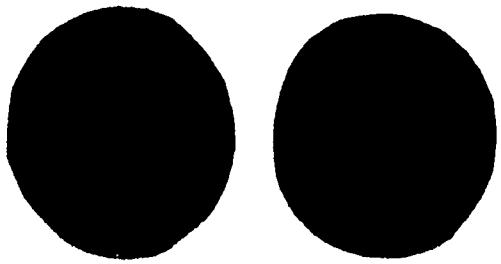
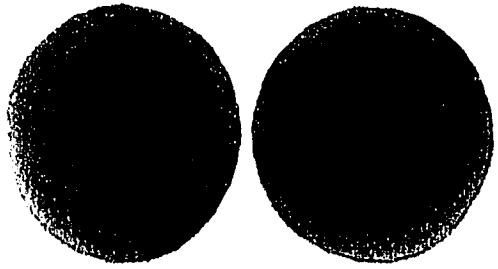
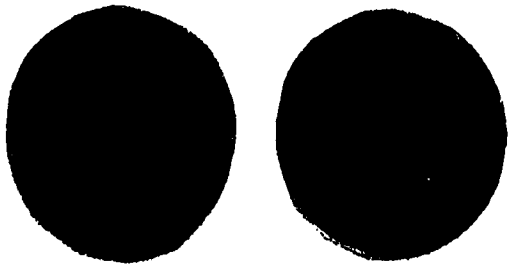
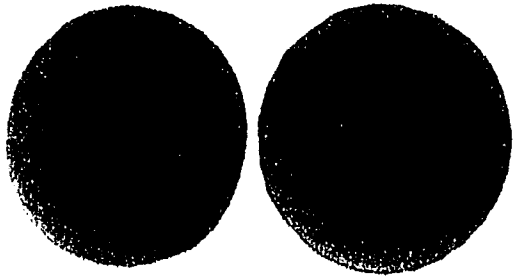
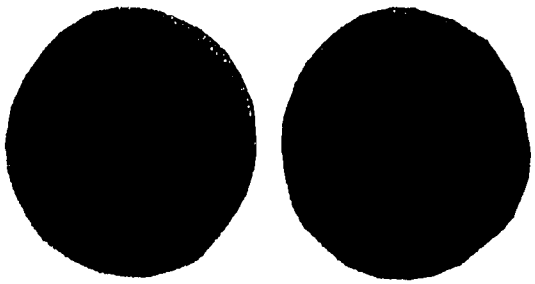
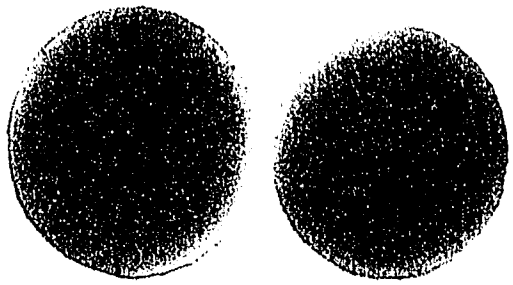
% Pearling	Gel Storage		% Pearling	Gel Storage	
	1 hour	3 days		1 hour	3 days
Whole			30-32%		
10-12%			48-50%		
20-25%			78-80%		

Figure 3.2: Color features of gels prepared with barley flours milled from whole and pearled grain

was darker (dark brown/black) than that of the flour milled from PG. The brightness of the PG-gels increased as the degree of pearling increased up to 32% and reached a plateau thereafter. Furthermore, it was evident that the stability of gel color was influenced by degree of pearling. The intensity of dark brown/black color of the gels prepared from whole grain flour increased during the 3 days storage based on personal subjective evaluation. Similar trends in the color intensities were observed for gels prepared with flour from 10-12% and 23-25% PG. However, the gel color was observed to be stable at a degree of 30-32% pearling and higher.

The color parameters (Hunter ***L***, ***a*** and ***b***) of the flours and gels are given in Table 3.3. Hunter ***L***, ***a*** and ***b*** values of samples represent their brightness, redness and yellowness, respectively. The flours from both barley varieties had small differences in their color values. For a given variety, PG flours had a higher ***L*** and lower ***a*** and ***b*** values than those of the whole grain flours. However, there was a substantial difference between the color of flour and that of the gel. The ***L*** value of flour was higher and the ***a*** and ***b*** values were lower than those of corresponding gels. The brightness of the PG gels increased as the degree of pearling increased, while a small change was observed in ***a*** and ***b*** values. The ***L*** value of the gels prepared with flour from whole and PG (10-12% and 23-25%) decreased during the 3-day storage. A very small change in the ***L*** value of gels prepared with flour from PG, pearled to 30-32% or more, was observed.

3.4 Conclusions

Although the barley grain components, such as starch, protein, β-glucan, lipids, and ash, are distributed in various tissues (i.e., aleurone, germ, and endosperm) of grain, the

Table 3.3: The effect of pearling on the Hunter color values[a] of barley flour and gel

Pearling (%)	Flour[b]			Gel[c]					
				1 hour[d]			3 days[d]		
	L[e]	a[e]	b[e]	L	a	b	L	a	b
Phoenix									
Whole	87.7	1.4	10.4	55.9	2.7	13.1	55.0	2.3	11.0
10-12%	89.1	1.0	9.1	57.3	2.6	13.0	56.0	2.3	10.8
23-25%	90.9	0.7	6.9	59.2	2.6	12.6	58.1	2.2	10.1
30-32%	91.5	0.5	6.1	66.4	2.0	10.7	66.3	2.0	9.8
48-50%	92.9	0.4	5.4	66.8	2.0	10.7	66.5	2.0	9.7
78-80%	93.7	0.4	5.0	67.1	2.0	10.2	66.8	1.9	9.7
CDC-Candle									
Whole	87.0	1.3	10.5	50.0	2.7	11.8	46.7	4.0	11.2
10-12%	89.8	1.1	9.0	54.7	2.5	11.5	49.8	3.8	11.0
23-25%	91.6	0.6	6.7	58.9	2.1	10.8	55.3	3.3	10.4
30-32%	92.2	0.4	5.1	66.2	1.8	10.6	66.0	2.2	10.0
48-50%	93.0	0.4	4.6	66.5	1.8	10.6	66.0	1.9	9.9
78-80%	93.5	0.4	4.2	66.5	1.7	10.5	66.5	1.7	9.9

[a] Values are means of three determinations. Standard deviations are <3%.
[b] Barley flour is produced by Udy mill of PG.
[c] Aqueous slurry of flour (10%, w/v) cooked at 100° C for 0.5 h and stored at 4° C.
[d] Storage period at 4°C.
[e] Hunter L, a and b values of samples represent the brightness, redness and yellowness, respectively.

patterns of distribution of these components within a tissue differ widely depending upon the barley variety. Understanding this pattern of distribution through a gradual layer-by-layer grain pearling process will be useful in strategic selection of flour (PF) characteristics for industrial pearling operations. This may enable the production of barley flours rich in a particular grain component and also the optimization of flour functionality for different food/industrial applications. The visual and instrumental evaluation of the color of barley flour gels indicated that pearling of barley at least to a degree of 32% is required to ensure its bright color and stability in barley-based foods.

3.5 References

AOAC. 1990. *Official Methods of Analysis, 15th Ed.*, (methods 979.09, 920.39 and 923.03). Association of Official Analytical Chemists, Washington, D. C.

Bhatty, R. S. 1997. Milling of regular and waxy starch hull-less barleys for the production of bran and flour. *Cereal Chem.* 74: 693-699.

Bhatty, R. S. and Rossnagel, B. G. 1998. Comparison of pearled and unpearled Canadian and Japanese barleys. *Cereal Chem.* 75:15-21.

Gohl, B., Larsson, K., Nilsson, M., Theander, O. and Thomke, S. 1977. Distribution of carbohydrates in early harvested barley grain. *Cereal Chem.* 54: 690-698.

Gohl, B., Nilsson, M. and Thomke, S. 1978. Distribution of soluble carbohydrates in barley grain at late stage of maturity and relation to viscosity. *Cereal Chem.* 55: 341-347.

Klamczynski, A., Baik, B. K. and Czuchajowska, Z. 1998. Composition, microstructure, water imbibition, and thermal properties of abraded barley. *Cereal Chem.* 75: 677-685.

Leonard, W. H. and Martin, J. H. 1963. Barley. In *Cereal Crops*; pp. 478-543, The Macmillan Company, New York, NY.

Marconi, E., Graziano, M. and Cubadda, R. 2000. Composition and utilization of barley pearling by-products for making functional pastas rich in dietary fiber and β-glucans. *Cereal Chem.* 77: 133-139.

Morrison, W. R. 1993. Cereal starch granule development and composition, in *Seed Storage Compounds: Biosynthesis, Interactions and Manipulation,* P. R. Shewry and A. K. Stobart, (Ed.), pp. 175-190, Oxford Univ. Press, Oxford, UK.

Peterson, D. M. 1994. Barley tocols: Effects of milling, malting and mashing. *Cereal Chem.* 71: 42-44.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, Forth Edition Vol. 2, Cary, NC.

Summer, A. K., Gebre-Egziabher, A., Tyler, R. T. and Rossnagel, B. G. 1985. Composition and properties of pearled and fines fractions from hulled and hull-less barley. *Cereal Chem.* 62: 112-116.

Wang, L., Xue, Q., Newman, R. K. and Newman, C. W. 1993. Enrichment of tocopherols, tocotrienols and oil in barley fractions by milling and pearling. *Cereal Chem.* 70: 499-501.

Zheng, G. H., Rossnagel, B. G., Tyler, R. T. and Bhatty, R. S. 2000. Distribution of β-glucan in the grain of hull-less barley. *Cereal Chem.* 77: 140-144.

Chapter 4

Dextrinization of Starch in Barley Flours by Thermostable α-Amylase Using a Twin Screw Extruder as a Reactor[1]

4.1 Introduction

Twin screw extruders have been used as reactors for enzymatic liquefaction of starch, using thermal and mechanical energy, under precisely controlled operating conditions (Linko et al., 1983a; Linko et al., 1983b; Hakulin et al., 1983). The operating conditions have to be optimized for adequate starch gelatinization and for minimal enzyme inactivation during extrusion. Pure starch or flour can be hydrolyzed to different extents to produce a variety of dextrins that vary in their composition, physicochemical properties and functionality. The "degree of hydrolysis" (DH) is usually expressed in terms of dextrose equivalent (DE) of the product. Extrusion dextrinization of pre-gelatinized starch with thermally stable amylase has been shown to result in the formation of dextrins with DE of up to 20 (Chouvel et al., 1983). Higher degree of starch hydrolysis (DE 94-95) can be achieved in an extruder with subsequent saccharification using glucoamylase (Linko et al., 1983a).

The degree of starch gelatinization and enzyme activity during extrusion are influenced by moisture content, temperature, pH, enzyme concentration, screw speed and die nozzle size. The minimum moisture level required for starch gelatinization is about 31%. However, under high temperature and high pressure extrusion cooking, starch gelatinization could occur even at a moisture level of 20% (Linko et al., 1983b; Roussel et

[1] A version of this study has been submitted for publication in *Starch*.

al., 1991). High and moderate increases in gelatinization have been shown to occur when moisture levels during extrusion are within the range 24-27% and 18-21%, respectively (Chiang and Johnson, 1977). Roussel and co-workers (1991) showed that during extrusion of corn starch at 150°C and 100 rpm, water soluble dry solids reached 50% for a water content of 30%, but only 10% at 40% water. The above authors (Roussel et al., 1991) postulated that extrusion at a lower water content enhances starch gelatinization because higher shear stress in the reverse screw elements increases both heat generation and mechanical disruption of granules. Corn starch samples extruded with 20% water was shown to display a maximum *in vitro* digestibility (DE) of 21-22 at all barrel temperatures and screw speeds (150°C/175 rpm, 175°C/125 rpm, 200°C/150 rpm). This suggests that water content during extrusion exerts a predominant effect on the *in vitro* digestibility of gelatinized starch. The above authors (Roussel et al., 1991) also obtained DE of 21-22 at 30% moisture during extrusion. However, starch samples extruded at a lower screw speed (50 rpm) at low temperatures (100 and 125°C) did not display a DE of 21-22. Several authors (Davidson et al., 1984; Lawton et al., 1972; Roussel et al., 1991) have postulated that an increase in barrel temperature or screw speed can compensate for the lower friction developed at a higher water content.

Corn starch extruded with α-amylase at pH 5 to 7 was shown to display a substantial degree of hydrolysis (DE 19-22). However, samples extruded at pH 3 or 9 showed only marginal hydrolysis (Chouvel et al., 1983). The influence of enzyme/substrate ratio during liquifaction of corn starch with α-amylase showed a linear response between the extent of hydrolysis and the level of α-amylase. However, solubility

of the freeze-dried extrudates did not increase linearly with enzyme/substrate ratio (Chouvel et al., 1983). High screw speeds and large die nozzle size were shown to decrease starch gelatinization due to a decrease in residence time and to a decrease in pressure and surface shear, respectively (Chiang and Johnson, 1977). Roussel and co-workers (1991) showed that a high screw speed should be applied for high water content extrusion and a low screw speed for low water content. The heat generated during extrusion should be sufficient for gelatinization and then liquifaction, but should not cause thermal inactivation of α-amylase.

Inactivation of α-amylase at the end of liquifaction is of paramount importance in order to ensure a specific DE. Thermostable α-amylase has been shown to be readily inactivated by boiling in 0.2 M acetate buffer at pH 3.5 (Linko et al., 1983a; Reinikainen et al., 1986).

Little research has been done on dextrinized barley flour by extrusion. The objective of the present study was to investigate the effect of α-amylase concentration (0-4%, starch dry weight basis), extrusion temperature (90-140°C) and flour-moisture level (20-50%, flour dry wt basis) on the degree of starch hydrolysis (DH), dextrose equivalent (DE) and mono/di/oligo saccharide composition of dextrinized flours from two barley varieties (Phoenix and CDC-Candle). A preliminary study carried out in our laboratory indicated that the use of α-amylase at concentrations greater than 5% imparted an unacceptable dark brown color to the final product (dextrinized flour). Furthermore, the efficiency of enzyme action was found to decrease at temperatures below 80 °C (due to a low energy of activation) and beyond 150 °C (due to enzyme denaturation). Adjustment of

the flour moisture content to levels greater than 60%, result in a liquid product (dextrinized flour) that led to handling and processing problems. Thus, due to these limitations, the study had to be carried out within a narrow window of processing conditions given above.

4.2 Materials and Methods

Materials

CDC-Candle and Phoenix barley grains were obtained from Agricore, Calgary, AB and Nakonechney Family Farms, Millet, AB. The grains were pearled to remove 32% of outer grain layers using a "Satake" pearler (Model-TM05, Satake,Tokyo, Japan). The pearled grains (68% dry weight) were then pin-milled (Alpine Contraplex wide chamber mill Type A 250, Hosokawa Micron Systems, Summit, NJ) at the POS Pilot Plant (Saskatoon, SK) to obtain barley flour. Thermostable α-amylase (Termamyl 120L, Type S) from *Bacillus licheniformis* was obtained from Novo Nordisk Biochem. North American Inc. (Franklinton, NC, USA). The optimal conditions for this enzyme was pH 5-6, 90-100°C and 50-70 mg Ca^{2+}/kg. Standard solutions of glucose, fructose, sucrose, maltose and maltotriose were obtained from Sigma Chemical Co., St. Louis, MO, USA. Oligosaccharide standards (DP3 to DP7) were obtained from Supelco Bellefonte, PA, USA.

Chemical Composition

Moisture, protein, lipid and ash contents were determined by AOAC methods (1997). Total starch, β-glucan, soluble and insoluble dietary fiber content of barley flours

were determined using enzymatic assay kits and procedures outlined by Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland). Amylose content was determined by the method of Chrastil (1987).

Extrusion Processing

Barley flours were extruded at the Food Processing Development Centre, Alberta Agriculture, Food and Rural Development (AAFRD), Leduc, AB using a twin screw extruder (Werner and Pfleiderer ZSK 57W 50P, Stuttgart, Germany). The screw configuration (Table 4.1) and pasta die were kept constant throughout the processing. The feed rate, screw speed, and length-to-diameter (L/D) ratio of die nozzle were fixed at 50lb/h, 50 rpm and 20:1, respectively. The barrel temperature was varied from 90 to 140°C (90, 100, 120, and 140°C). The extrusion moisture levels were adjusted to 20, 35 and 50%. The α-amylase concentration was varied from 0%, 2% to 4% (starch dry weight basis). After extrusion, α-amylase activity in the dextrinized samples was inhibited by pH adjustment (<4.0) and heating to 95-100 °C for 6 minutes. Dextrinized samples were then kept on a tray at 75°C until dry. The dried samples were ground and screened through 60 mesh Tyler screen (Fisher Scientific Ltd., Nepean, ON).

Dextrose Equivalent (DE)

DE of dextrinized samples was determined using the method of Novo Nodisk Biochem North American Inc. (Franklinton, NC, USA). DE was calculated by the following equation:

$$DE = \frac{(T_{wb}-T_s)x500}{(T_{wb}-T_{dex})xWx\%DS}$$

Table 4.1: Screw configuration used in extrusion processing of barley flour [a]

Number of screws	Type of screw Element[b]	Screw Configuration[c]
1	F	30 / 30
2	SK	80 / 80
1	SK-N	80 / 40
2	F	80 / 80
1	KB	45 / 2 / 20
1	F	80 / 80
1	KB	45 / 5 / 20
2	F	80 / 40
1	KB	45 / 5 / 20
2	F	60 / 60
1	KB	45 / 5 / 40
1	KB/R	45 / 5 / 20
2	F	60 / 60
1	KB	45 / 5 / 40
1	KB/R	45 / 5 / 20
3	F	40 / 40
1	KB	45 / 5 / 40
1	F	40 / 40
1	R	40 / 20
1	F	40 / 40
6	F	30 / 30

a. Screw configuration is listed from the beginning of the barrel to the end
b. F: forward element; R: reverse element; SK: undercut element; SK-N: transition element from SK to other element; KB: kneading block; KB/R: reverse kneading block
c. For KB: offset angle (°)/number of discs/length (mm). For others: Pitch (mm)/length (mm)

T_{wb}: Titre of water blank (ml)

T_s: Titre of sample (ml)

T_{dex}: Titre of standard dextrose solution (ml)

W: Weight of sample (g)

%DS: Percentage of dry solid in sample

Degree of Hydrolysis (DH)

High Performance Liquid Chromatography (HPLC) Operating Parameters

DH of dextrinized sample was determined in triplicate by HPLC equipped with a Shimadzu Ezchrom Chromatography data processing system and a Supelcosil LC-NH2-5 μm column (Supelco, Bellefonte, PA, USA) of 25 cm length and 4.6 mm diameter. Eluent "A" was distilled water and eluent "B" was acetonitrile. The total run time was 40 minutes for each sample. The elution gradient was as follows: 1) A 10% and B 90% at the start; 2) A 35% and B 65% at 35 minutes; and 3) A 10% and B 90% at 37 minutes. A flow rate of 1.0 ml/min was maintained by a Varian 9010 Solvent Delivery System (Sunnyvale, CA, USA). A 500 ELSD (Evaporative Light-Scattering Detector) (Alltech, Mandel Scientific Co. Ltd., Guelph, ON), which evaporated solvent at 125°C was used for detecting maltose.

Sample Preparation for HPLC Analysis

Dextrinized sample (0.1 g) was extracted with 80% aqueous ethanol (1.0 ml) for 15h at 40°C. The extract was centrifuged at 5000 rpm for 10 minutes. A 50.0 μl aliquot of the supernatant was injected using a Hewlett Packard Series 1050 autosampler (Hewlett Packard, Mississauga, ON). Peak identification was achieved by using external standard

solutions of fructose, glucose, sucrose, maltose and maltotriose. Calibration curves were used to calculate the maltose concentration.

Generally, maltose is one of the main products of α-amylase hydrolysis. Therefore, DH was calculated by the following equation:

DH = [Maltose concentration in aliquot (mg/ml) x injection volume (ml) x volume adjustment factor (ml/ml)] / Weight of sample (mg)

Determination of Oligosaccharide Composition

HPLC Operating Parameters

The oligosaccharide profile of dextrinized samples was determined in triplicate by HPLC, using a Jordi Gel DVB Polyamine column (Bellingham, MA) of 250 mm length and 4.6 mm diameter. Eluent "A" was distilled water and eluent "B" was acetonitrile. The total run time was 30 minutes for each sample. The elution gradient was as follows: 1) A 10% and B 90% at the start; 2) A 40% and B 60% at 25 minutes; 3) A 0% and B 100% at 26 minutes; and 4) A 10% and B 90% at 30 minutes. A flow rate of 1.0 ml/min was maintained by a Varian 9010 Solvent Delivery System (Sunnyvale, CA, USA).

Sample Preparation for HPLC Analysis

Dextrinized sample (0.1 g) was extracted with 80% aqueous ethanol (1.0 ml) for 15h at 40°C. The solution was centrifuged at 5000 rpm for 10 minutes. A 10.0 µl aliquot of the supernatant was used for injection into the HPLC. Oligosaccharides were detected by using 500 ELSD (detector described above), which evaporated solvent at 125°C. Peak identification was achieved by using external standard solutions of glucose, maltose,

maltotriose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6) and maltoheptaose (DP7). Calibration was performed for each oligosaccharide standard.

Statistical Analysis

All experiments were carried out in triplicates. Analysis of variance of the results was performed using General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, 1989). 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 composition

The proximate composition of native barley flours (Phoenix and CDC-Candle) is shown in Table 4.2. There were minor differences in protein, lipid and ash contents of CDC-Candle and Phoenix flours. However, starch, insoluble dietary fiber (IDF), soluble dietary fiber (SDF) and β-glucan contents were considerably different. Compared to CDC-Candle, Phoenix had a higher starch and IDF, but lower β-glucan and SDF contents. Furthermore, β-glucan contents of CDC-Candle and Phoenix (6.5 and 3.9%, respectively) were higher than their SDF content (5.6 and 2.4%, respectively). This suggests that barley β-glucan consists of water-soluble and water-insoluble fractions.

4.3.2 Dextrinization

The degree of hydrolysis (DH) and dextrose equivalent (DE) of dextrinized barley flours under different combinations of processing conditions are presented in Table 4.3.

Table 4.2: Composition[a] (%, dry basis) of barley flour

Flour[b]	Starch	Protein	β-glucan	Lipid	Ash	Dietary Fiber	
						SDF	IDF
CDC-Candle	73.6±0.9	10.2±0.6	6.5±0.2	1.1±0.1	1.2±0.0	5.6±0.1	1.9±0.1
Phoenix	76.5±0.4	10.4±0.8	3.9±0.0	1.1±0.0	1.0±0.0	2.4±0.0	2.4±0.6

[a] Values are means of three determinations ± standard deviations.
[b] Barley flour produced by pin-milling of pearled (30-32%) grains.

Table 4.3: The Dextrose equivalent (DE) and Degree of Hydrolysis (DH) of Extruded Flours

Extrusion Conditions	DE		DH	
	Phoenix	CDC-Candle	Phoenix	CDC-Candle
90/0/20[a]	1.6 ± 0.1	1.8 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
90/0/35	2.6 ± 0.2	3.5 ± 0.0	0.3 ± 0.0	1.0 ± 0.0
90/0/50	4.9 ± 0.1	6.3 ± 0.5	0.8 ± 0.0	2.0 ± 0.1
90/2/20	10.6 ± 0.5	7.8 ± 0.4	4.7 ± 0.5	3.7 ± 0.2
90/2/35	14.4 ± 0.3	11.3 ± 0.3	5.1 ± 0.0	4.6 ± 0.5
90/2/50	19.4 ± 1.1	15.4 ± 1.0	10.4 ± 0.9	7.5 ± 0.5
90/4/20	16.5 ± 0.2	13.4 ± 1.2	7.1 ± 0.8	5.9 ± 0.3
90/4/35	18.4 ± 1.5	15.7 ± 0.8	10.1 ± 0.7	8.8 ± 0.7
90/4/50	22.2 ± 1.6	18.2 ± 0.9	18.2 ± 0.6	14.4 ± 0.5
100/0/20	2.1 ± 0.4	1.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
100/0/35	3.0 ± 0.1	2.3 ± 0.3	0.3 ± 0.0	0.7 ± 0.1
100/0/50	3.6 ± 0.3	3.4 ± 0.2	0.4 ± 0.0	1.5 ± 0.1
100/2/20	12.1 ± 0.5	13.4 ± 0.1	5.1 ± 0.4	4.6 ± 0.2
100/2/35	14.6 ± 0.2	13.2 ± 0.6	6.6 ± 0.6	6.6 ± 0.1
100/2/50	27.0 ± 0.5	15.6 ± 0.1	15.2 ± 2.6	11.0 ± 0.6
100/4/20	23.4 ± 1.5	19.5 ± 0.4	10.8 ± 1.0	8.1 ± 0.1
100/4/35	24.3 ± 1.4	21.0 ± 1.0	15.7 ± 1.2	12.3 ± 1.0
100/4/50	36.1 ± 0.7	24.9 ± 1.3	27.8 ± 5.0	21.8 ± 0.4
120/0/20	2.8 ± 0.1	3.0 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
120/0/35	3.1 ± 0.2	3.4 ± 0.2	0.1 ± 0.1	0.7 ± 0.1
120/0/50	4.1 ± 0.1	5.6 ± 0.3	0.2 ± 0.1	1.8 ± 0.2
120/2/20	8.6 ± 0.3	10.6 ± 0.3	2.4 ± 0.2	3.4 ± 0.0
120/2/35	14.9 ± 1.3	18.8 ± 0.5	3.7 ± 0.2	5.4 ± 0.1
120/2/50	22.3 ± 0.6	25.0 ± 0.5	7.3 ± 1.1	9.3 ± 0.2
120/4/20	17.3 ± 0.9	20.2 ± 1.4	3.8 ± 0.2	5.9 ± 1.0
120/4/35	20.1 ± 0.5	24.2 ± 0.5	4.2 ± 0.0	9.0 ± 0.5
120/4/50	26.9 ± 1.3	31.3 ± 1.5	10.0 ± 0.3	14.8 ± 0.2
140/0/20	3.1 ± 0.4	4.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
140/0/35	4.3 ± 0.1	4.7 ± 0.4	0.1 ± 0.0	0.1 ± 0.0
140/0/50	5.0 ± 0.3	5.1 ± 0.3	0.2 ± 0.0	0.3 ± 0.0
140/2/20	3.9 ± 0.4	6.9 ± 0.4	0.1 ± 0.0	0.7 ± 0.4
140/2/35	7.5 ± 0.4	10.3 ± 0.7	0.4 ± 0.0	3.2 ± 0.2
140/2/50	10.4 ± 0.6	16.2 ± 1.6	3.3 ± 0.3	5.7 ± 0.1
140/4/20	5.6 ± 0.1	9.4 ± 0.2	0.3 ± 0.0	2.2 ± 0.1
140/4/35	12.8 ± 1.0	18.8 ± 1.2	2.7 ± 0.2	5.3 ± 0.4
140/4/50	18.8 ± 1.3	23.0 ± 0.7	4.6 ± 0.1	8.0 ± 0.2

[a] The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour

Note: See Appendix 1a & b for statistical analysis.

4.3.2.1 Degree of Hydrolysis (DH)

Maltose was present in trace quantities (<0.005%) in native barley flours. However, native barley flours (Phoenix and CDC-Candle) extrusion processed (90°C and 20-50% moisture) without α-amylase addition, showed DH values in the range of 0.2-0.8% (w/w) and 0.2-2.0% (w/w), respectively (Table 4.3). At each extrusion temperature (90°C-140°C), DH increased with an increase in moisture content (20-50%) (Table 4.3). The decrease in DH values at temperatures exceeding 90°C, reflects inactivation of endogenous heat liable α- and β-amylase (Table 4.3), which are present, in trace quantities in Phoenix and CDC-Candle barley flours.

At a constant moisture level (20, 35, 50%) and α-amylase concentration (2 or 4%), the DH increased with an increase in temperature up to 100°C. Thereafter, hydrolysis decreased in both CDC-Candle and Phoenix barley flours. This suggests that the optimum temperature for processing is about 100°C. The extent of decrease in DH (>100°C) was higher in Phoenix. The decrease in DH at temperatures exceeding 100°C, suggests thermal inactivation of the added α-amylase.

At low extrusion temperatures (90 and 100°C), Phoenix was hydrolyzed to a greater extent than CDC-Candle. However, the above order was reversed at higher extrusion temperatures (120-140°C). This reversal in hydrolysis could be explained on the basis of differences in β-glucan content and starch swelling power (Table 4.2). Soluble β-glucan has been shown to have the capacity to form very viscous solutions. Furthermore, viscosity of barley flour slurries have been shown to be influenced by the content of soluble β-glucan, β-glucanase activity, and molecular weight of β-glucan (Izydorczyk et

al., 2000). Friction between swollen starch granules has also been shown to contribute to starch viscosity (Hoover and Vasanthan, 1994). This suggests that the viscosity of the CDC-Candle barley flour slurry could be higher than that of Phoenix, due to its higher β-glucan content and greater starch swelling power due to its low amylose content. Consequently, the rate of diffusion of α-amylase to the starch surface of granule would be greater in Phoenix due to the low viscosity of the flour slurry. This would then explain the difference in DH between CDC-Candle and Phoenix at low extrusion temperatures (Table 4.3). The difference in granular swelling between CDC-Candle and Phoenix would render starch granules of CDC-Candle more susceptible to shear at high extrusion temperatures. Consequently, granule fragmentation would be more pronounced in CDC-Candle. Fragmentation would render the starch contents more accessible to α-amylase hydrolysis. Furthermore, β-glucan fragmentation has also been shown to occur at high extrusion temperatures (Jiang and Vasanthan, 2000). The impact of β-glucan fragmentation on the extent of decrease in viscosity would be more pronounced in CDC-Candle due to its higher β-glucan content. Thus, both starch granule and β-glucan fragmentation contribute to the difference in DH between Phoenix and CDC-Candle at 120 and 140°C (Table 4.3).

4.3.2.2 Dextrose Equivalent (DE)

Phoenix and CDC-Candle flours extruded in the absence of α-amylase showed progressive increases in DE (Table 4.3) with an increase in moisture content (20-50%) and temperature (90-140°C). This reflects an increase in the number of reducing ends, resulting from fragmentation of starch and β-glucan chains due to mechanical shear. In the presence of α-amylase and at constant temperature, DE increased with an increase in

moisture content (Table 4.3). At constant moisture content, the maximum and minimum values of DE were obtained at 100°C and 140°C, respectively. Starch gelatinization has been shown to increase with an increase in moisture content. Furthermore, gelatinization increases accessibility of glycosidic linkages to hydrolysis by α-amylase. This would then explain the higher values of DE at 50% moisture. Partial inactivation of α-amylase at 120 and 140°C may have been the cause of the low DE values at these temperatures.

4.3.3 The mono-, di- and oligosaccharide composition of dextrinized flours

The influence of extrusion parameters on relative proportions of DP1 to DP7 oligosaccharides are presented in Table 4.4. At each temperature and enzyme concentration, the proportion of DP1 to DP7 at all moisture contents (20-50%), followed the order: DP2>DP6>DP3>DP5>DP7>DP4>DP1. At each temperature and moisture content, the proportion of DP1 to DP7 increased with enzyme concentration. At each enzyme concentration and moisture content, an increase in temperature from 90 to 100°C increased the proportion of DP1 to DP7. However, at 120 and 140°C, the proportion of DP1 to DP7, were much lower than that at 90°C (Table 4.4). At 90 and 100°C, the predominant oligosaccharide species were DP2, DP6 and DP3, whereas, at 120 and 140°C, these were DP2, DP6, DP5 and DP6, DP3, DP2, respectively. Roussel et al. (1991) showed that twin screw extrusion of raw corn starch at a high dry solids content and at a high thermostable α-amylase/starch ratio resulted in a high proportion of maltotriose, maltopentaose and maltohexaose (about 20% each) and a smaller proportion (about 10% each) of maltose, maltotetraose and maltoheptaose. Similar findings were also reported by Linko and co-workers (1980) and Chouvel and co-workers (1983) on raw

Table 4.4: Oligosaccharides profile (DP1-7) (%, w/w) of extruded barley flour

	DP1		DP2		DP3		DP4		DP5		DP6		DP7	
	Phoenix	Candle	Phoenix	Candle	Phoenix	Candle	Phoenix	Candle	Phoenix	Candle	Phoenix	Candle	Phoenix	Candle
90/0/20[a]	-[b]	-	0.2 ± 0.0	0.3 ± 0.0	-	-	-	-	-	-	-	-	-	-
90/0/35	-	-	0.3 ± 0.0	0.7 ± 0.1	-	-	-	-	-	-	-	-	-	-
90/0/50	-	-	0.8 ± 0.0	2.6 ± 0.1	-	-	-	-	-	-	-	-	-	-
90/2/20	0.5 ± 0.0	0.6 ± 0.1	4.9 ± 1 0	3.8 ± 0.6	3.2 ± 1.2	1.9 ± 0.5	1.5 ± 0.3	1.2 ± 0.2	3.9 ± 0.9	4.8 ± 0.7	4.7 ± 1.0	5.5 ± 0.7	2.4 ± 0.3	2.2 ± 0.1
90/2/35	0.4 ± 0.0	0.8 ± 0.1	5.3 ± 0 2	4.9 ± 0.6	4.6 ± 0.3	3.7 ± 0.4	2.1 ± 0.1	1.8 ± 0.1	4.8 ± 0.2	4.5 ± 0.3	5.9 ± 0.4	5.8 ± 0.2	2.4 ± 0.1	2.4 ± 0.2
90/2/50	0.7 ± 0.1	1.0 ± 0.0	10.5 ± 0.3	8.7 ± 0.6	5.9 ± 1.3	4.1 ± 0.3	3.0 ± 0.4	2.8 ± 0.1	5.6 ± 1.5	5.2 ± 0.3	7.3 ± 3.5	6.5 ± 0.4	5.7 ± 0.2	5.5 ± 0.1
90/4/20	0.3 ± 0.0	0.9 ± 0.0	7.9 ± 0.4	5.5 ± 1.2	3.6 ± 0.2	2.9 ± 0.3	1.4 ± 0.1	1.9 ± 0.1	4.2 ± 0.1	4.9 ± 0.9	5.3 ± 0.0	5.8 ± 0.9	5.5 ± 0.0	5.5 ± 0.2
90/4/35	0.8 ± 0.1	0.9 ± 0.1	11.2 ± 0.5	8.8 ± 0.8	5.2 ± 0.6	3.9 ± 0.5	2.5 ± 0.6	1.9 ± 0.1	5.8 ± 1.5	5.4 ± 0.4	6.2 ± 1.9	6.0 ± 0.4	5.6 ± 0.4	5.5 ± 0.0
90/4/50	0.8 ± 0.0	1.0 ± 0.0	19.5 ± 0.3	15.5 ± 0.4	7.6 ± 0.4	5.2 ± 0.2	4.3 ± 0.1	2.9 ± 0.0	6.1 ± 0.4	5.3 ± 0.2	8.8 ± 0.6	8.9 ± 0.2	6.0 ± 0.2	5.4 ± 0.1
100/0/20	-	-	0.2 ± 0.0	0.1 ± 0.0	-	-	-	-	-	-	-	-	-	-
100/0/35	-	0.3±0.0	0.3 ± 0.0	0.7 ± 0.0	-	-	-	-	-	-	-	-	-	-
100/0/50	-	0.5 ± 0.0	0.4 ± 0.0	2.0 ± 0.0	-	-	-	-	-	-	-	-	-	-
100/2/20	0.5 ± 0.1	0.4 ± 0.1	5.6 ± 0.5	4.8 ± 0.4	4.5 ± 0.6	3.4 ± 0.3	1.0 ± 0.0	1.5 ± 0.1	3.4 ± 0.5	3.4 ± 0.4	3.8 ± 0.0	5.4 ± 0.0	2.2 ± 0.1	2.6 ± 0.5
100/2/35	0.5 ± 0.0	0.6 ± 0.1	6.8 ± 0.2	6.7 ± 0.3	5.6 ± 0.3	4.1 ± 0.2	1.8 ± 0.1	1.9 ± 0.1	4.0 ± 0.2	4.1 ± 0.2	4.7 ± 0.5	5.6 ± 0.3	2.6 ± 0.1	2.1 ± 0.1
100/2/50	0.8 ± 0.1	1.0 ± 0.0	15.5 ± 1.5	11.7 ± 0.1	7.5 ± 0.0	6.2 ± 0.1	2.8 ± 0.1	3.3 ± 0.1	6.1 ± 0.3	6.2 ± 0.2	7.5 ± 0.3	7.2 ± 0.3	5.7 ± 0.1	5.6 ± 0.3
100/4/20	1.2 ± 0.0	1.2 ± 0.1	11.0 ± 0.3	8.4 ± 0.6	4.1 ± 0.3	3.6 ± 0.5	2.8 ± 0.1	3.0 ± 0.5	4.4 ± 0.9	4.7 ± 0.4	5.4 ± 0.3	6.0 ± 0.1	4.2 ± 0.1	5.6 ± 0.1
100/4/35	1.2 ± 0.1	1.9 ± 0.1	16.7 ± 0.1	12.3 ± 0.5	6.4 ± 0.5	5.4 ± 0.2	3.0 ± 0.3	3.2 ± 0.1	5.3 ± 0.5	4.7 ± 0.3	7.0 ± 0.2	7.1 ± 0.5	6.0 ± 0.0	6.6 ± 0.1
100/4/50	1.3 ± 0.1	2.0 ± 0.1	28.4 ± 0.5	25.5 ± 0.5	8.6 ± 0.5	7.1 ± 0.4	5.0 ± 0.2	5.4 ± 0.2	6.9 ± 0.6	6.8 ± 0.4	9.6 ± 0.1	9.4 ± 0.4	7.2 ± 0.0	8.6 ± 0.1
120/0/20	-	0.3 ± 0.0	-	0.2 ± 0.0	-	-	-	-	-	-	-	-	-	-
120/0/35	-	0.4 ± 0.0	0.3 ± 0.0	0.7 ± 0.1	-	-	-	-	-	-	-	-	-	-
120/0/50	-	0.4 ± 0.1	0.3 ± 0.0	1.6 ± 0.2	-	-	-	-	-	1.6 ± 0.5	-	3.8 ± 0.2	-	2.0 ± 0.2
120/2/20	0.3 ± 0.0	0.7 ± 0.0	2.8 ± 0.1	3.5 ± 0.7	3.2 ± 0.3	2.4 ± 0.6	1.7 ± 0.6	1.4 ± 0.2	2.8 ± 0.8	3.3 ± 0.5	4.9 ± 1.4	4.9 ± 0.7	3.3 ± 0.6	3.0 ± 0.2
120/2/35	0.5 ± 0.0	0.7 ± 0.0	3.8 ± 0.5	5.8 ± 0.3	4.7 ± 0.7	3.6 ± 0.3	2.2 ± 0.2	2.1 ± 0.1	3.5 ± 0.2	3.5 ± 0.2	5.7 ± 0.4	5.7 ± 0.3	3.4 ± 0.0	4.0 ± 0.1
120/2/50	0.5 ± 0.0	0.7 ± 0.0	8.0 ± 0.5	9.2 ± 0.3	5.6 ± 0.1	3.9 ± 0.3	2.6 ± 0.4	2.7 ± 0.2	5.9 ± 0.6	4.2 ± 0.2	7.8 ± 0.7	9.0 ± 1.1	5.7 ± 0.2	5.9 ± 0.1
120/4/20	0.0 ± 0.0	0.8 ± 0.1	4.2 ± 0.5	5.9 ± 0.3	4.8 ± 0.2	2.9 ± 0.3	1.7 ± 0.2	2.0 ± 0.1	4.2 ± 0.4	5.7 ± 0.3	5.6 ± 0.7	6.9 ± 0.3	5.5 ± 0.1	5.7 ± 0.0
120/4/35	0.7 ± 0.0	0.9 ± 0.1	4.7 ± 0.1	9.1 ± 0.6	5.9 ± 0.1	4.4 ± 0.5	2.4 ± 0.0	3.6 ± 0.2	5.0 ± 0.1	5.9 ± 0.3	6.6 ± 0.1	7.0 ± 0.5	5.7 ± 0.0	5.5 ± 0.1
120/4/50	0.9 ± 0.0	1.0 ± 0.0	12.5 ± 0.3	17.0 ± 0.2	6.1 ± 0.1	5.2 ± 0.2	3.2 ± 0.1	4.8 ± 0.4	6.6 ± 0.0	6.0 ± 0.8	8.2 ± 0.0	9.6 ± 0.6	5.5 ± 0.0	5.7 ± 0.0
140/0/20	-	-	-	0.3 ± 0.0	-	-	-	0.5 ± 0.0	-	-	-	-	-	-
140/0/35	-	-	0.1 ± 0.0	0.3 ± 0.0	-	-	-	0.6 ± 0.0	-	-	-	-	-	-
140/0/50	-	-	0.2 ± 0.0	0.7 ± 0.0	-	-	-	0.6 ± 0.0	-	-	-	-	-	-
140/2/20	-	0.6 ± 0.1	0.2 ± 0.0	0.9 ± 0.9	0.1 ± 0.0	2.1 ± 0.8	-	0.7 ± 0.4	0.9 ± 0.0	5.0 ± 1.0	1.5 ± 0.0	7.9 ± 0.9	-	3.4 ± 0.3
140/2/35	-	0.9 ± 0.1	0.6 ± 0.0	5.9 ± 0 .1	3.5 ± 0.0	5.0 ± 1.1	0.7 ± 0.0	2.6 ± 0.3	1.3 ± 0.0	5.7 ± 0.2	1.7 ± 0.0	8.3 ± 0.5	1.9 ± 0.1	4.3 ± 0.5
140/2/50	0.4 ± 0.0	1.1 ± 0.1	4.8 ± 0.6	8.4 ± 0.8	5.8 ± 0.8	8.3 ± 1.1	1.0 ± 0.3	4.3 ± 0.3	3.4 ± 0.6	7.2± 0.7	4.5 ± 1.3	12.0 ± 1.3	3.7 ± 0.7	6.6 ± 0.6
140/4/20	-	0.5 ± 0.1	0.5 ± 0.0	2.6 ± 0.4	0.7 ± 0.1	3.2 ± 0.6	0.7 ± 0.1	1.6 ± 0.3	1.4 ± 0.1	5.8 ± 0.4	3.1 ± 0.1	7.0 ± 0.8	2.1 ± 0.1	3.6 ± 0.5
140/4/35	0.3 ± 0.0	0.8 ± 0.1	2.7 ± 0.1	6.9 ± 0.9	4.1 ± 0.2	5.6 ± 0.8	2.0 ± 0.1	3.1 ± 0.4	2.9 ± 0.1	8.3 ± 0.9	4.9 ± 0.3	10.2 ± 0.7	3.6 ± 0.2	6.0 ± 0.2
140/4/50	0.4 ± 0.0	1.0 ± 0.1	5.8 ± 0.4	10.1 ± 0.9	6.5 ± 0.4	9.3 ± 0.9	2.7 ± 0.2	4.7 ± 0.4	5.0 ± 0.3	9.1 ± 1.2	6.8 ± 0.5	10.8 ± 1.4	3.6 ± 0.2	6.8 ± 0.4

[a]The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour
[b]Values are too low to be detected
Note: See Appendix 2 a, b, c, d, e, f and g for statistical analysis.

barley starch and pregelatinized corn starch, respectively.

4.4 Conclusions

The semi-dry extrusion dextrinization may be successfully used to produce malto-dextrin from barley flour with different dextrose equivalents and composition. The change in dextrose equivalent can be caused by enzyme hydrolysis or molecular (i.e. β-glucan and starch) fragmentation. The residence time can be increased by increasing the extruder barrel length, which will in turn increase the DH and DE of the final product.

4.5 References

AOAC. 1997. Official Methods of Analysis (method 925.10). Association of Official Analytical Chemists, Washington, DC.

Chiang, B. Y. and Johnson, J. A. 1977. Gelatinization of starch in extruded products. *Cereal Chem.* 54(3): 436-443.

Chouvel, H., Chay, P. B., and Cheftel, J. C. 1983. Enzymatic hydrolysis of starch and cereal flours at intermediate moisture contents in continuous extrusion reactor. *Lebensm. Wiss u. Technol.* 16: 346-353.

Chrastil, J. 1987. Improved colorimetric determination of amylose in starches or flours. *Carbohydr. Res.* 159: 154-158.

Davidson, V. J., Paton, D., Diosady, L. L. and Rubin, L. J., 1984. A model for mechanical degradation of wheat starch in a single-screw extruder. *J Food Sci.* 49: 1154-1157.

Hakulin, S., Linko, Y. Y., Linko, P., Seiler, K., and Seibel, W. 1983. Enzymatic conversion of starch in twin-screw HTST-extruder. *Starch.* 35: 411-414.

Hoover, R and Vasanthan, T. 1994. The flow properties of native, heat-moisture treated and annealed starches from wheat, oat, potato and lentil. *J. Food Biochem.* 18: 67-82.

Izydorczyk, M. S., Storsley, J., Labossiere, D., MacGregor, A. W., and Rossnagel, B. G. 2000. Variation in total and soluble β-glucan content in hullless barley: Effects of thermal, physical, and enzymic treatments. *J.Agri. Food Chem.* 48: 982-989.

Jiang, G. and Vasanthan, T. 2000. Effect of extrusion cooking on the primary structure and water solubility of β-glucans from regular and waxy barley. *Cereal Chem.* 77(3): 396-400.

Lawton, B. T., Henderson, G. A. and Derlatka, E. J. 1972. The effects of extruder variables on the gelatinisation of corn starch. *Can. J. Chem. Eng.* 50:168-172.

Linko, P., Hakulin, S., and Linko, Y. Y. 1983a. Extrusion cooking of barley starch for the production of glucose syrup ethanol. *J. Cereal Sci.* 1: 275-284.

Linko, P., Linko, Y. Y., and Olkku, J. 1983b. Extrusion cooking and bioconversions. *J. Food Eng.* 2: 243-257.

Linko, Y. Y., Vuorinen, H., Olkku, J. and Linko, P. 1980. Enzyme Engineering in Food Processing, in *Food Process Engineering.* P. Linko, and J. Larinkari, (Ed.), pp. 210-223, Applied Sci. Pub., London.

Reinikainen, P., Suortti, T., Olkku, J., Malkki, Y., and Linko, P. 1986. Extrusion cooking in enzymatic liquefaction of wheat starch. *Starch.* 38: 20-26.

Roussel, L., Vieille, A., Billet, I., and Cheftel, J. C. 1991. Sequential heat gelatinization and enzymatic hydrolysis of corn starch in an extrusion reactor optimization for a maximum dextrose equivalent. *Lebensm. Wiss-u. Technol.* 24: 449-458.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, Forth Edition Vol. 2, Cary, NC.

Chapter 5

Dietary Fiber Profile of Barley Flour as Affected by Extrusion Cooking[1]

5.1 Introduction

Traditionally, the non-digestible (resistant to digestion/hydrolysis by the alimentary enzymes of humans) constituents of plant cell walls, which consist of polysaccharides (cellulose, hemi-cellulose, mucilage, oligosaccharides, pectins), lignin and associated substances, such as waxes, cutin and suberin, have been termed as dietary fiber (Devries et al., 1999). According to the solubility in water, total dietary fiber (TDF) can be categorized into two groups, namely soluble (SDF) and insoluble (IDF) dietary fiber. SDF and IDF have been known to play different physiological roles in human health. The enzymatic-gravimetric methods have been commonly employed in the determination of TDF, SDF and IDF in foods (Caldwell and Nelson, 1999). The present study uses the procedure defined by Megazyme International Ireland Ltd., Wicklow, Ireland to determine the content of various dietary fiber fractions in extrusion cooked barley flour. This procedure, essentially, uses the protocols explained in AACC method 32-07 (1995) and AOAC method 991.43 (1995).

The cell wall or structural polysaccharides of barley endosperm consist primarily of β-glucan, arabinoxylan and cellulose. The cell walls of barley endosperm contain about 75% β-glucan and 20% arabinoxylan, whereas the aleurone cell walls contain 26% β-glucan and 71% arabinoxylan (Jadhav et al., 1998). The minor components are cellulose, glucomannan, and (1→3)-β-glucan (Jadhav et al., 1998). During the past two decades, barley β-glucan has received considerable research attention due to its health

[1] A version of this study has been submitted for publication in *Cereal Chemistry*.

benefits. Several studies have shown that barley β-glucan has significant blood cholesterol lowering effects (McIntosh et al., 1991; Martinez et al., 1992). β-Glucan in barley has also been known to increase the viscosity of intestinal fluid and thereby reduce the rate of sugar/starch absorption (Anderson et al., 1990), which is beneficial in the management of diabetes (Klopfenstein, 1988; Pick, 1994; Gosain, 1996).

Extrusion cooking is a popular food processing technique, especially for the production of fiber-rich products such as breakfast cereals, flat breads, dextrinized or cooked flour, etc. Due to its high content of TDF and a high proportion of SDF, an investigation into the use of barley flour in a variety of extruded products is of high importance from a nutritional point of view. Extrusion conditions, such as temperature, moisture and pressure/shear, may change the content as well as the physicochemical and nutritional/physiological properties of barley flour components, including dietary fiber. Little research has been done towards understanding the impact of extrusion cooking on the dietary fiber of barley flour. Ostergard et al. (1989) reported that dietary fiber content of barley increased upon extrusion cooking, accompanied by a decrease in total starch content. This is probably due to the formation of indigestible starch fragments. However, the increase in SDF did not account for the total decrease in starch. This is probably due to the newly formed indigestible starch fragments, which are too small to precipitate with 80% ethanol employed in the dietary fiber assay (Ostergard et al., 1989).

The objectives of the present study are to investigate the effect of extrusion cooking of barley flour (waxy and regular starch types) under different temperature/moisture combinations on the TDF, IDF, SDF, β-glucan and resistant starch

contents of barley flour. Furthermore, an attempt will be made to rationalize the changes in various dietary fiber fractions during extrusion cooking.

5.2 Materials and Methods

Materials

Waxy barley grains (CDC-Candle) were obtained from Agricore, Calgary, AB. Regular barley grains (Phoenix) were obtained from Nakonechney Family Farms, Millet, AB. The analytical kits for dietary fiber and β-glucan were purchased from Megazyme International Ireland Ltd., Wicklow, Ireland.

Pearling and Milling of Barley Grains and Extrusion Processing of Barley Flour

Pearling and milling of barley grains were carried out at the POS pilot plant, Saskatoon, SK. Grains were pearled to 30-32% in a "Satake" mill (Model-TM05, Satake, Toyko, Japan). The pearled grains were pin-milled (Alpine Contraplex wide chamber mill Type A 250, Hosokawa Micron Systems, Summit, NJ) into flour at 6,000 rpm and a feed rate of 150 kg/h. The flour was extruded in a twin screw extruder (Werner and Pfleiderer ZSK 57W 50P, Stuttgart, Germany). The barrel temperatures used in this study were 90°C, 100°C, 120°C and 140°C, while the moisture contents of 20, 35 and 50% (w/w, dry basis) were employed. The feed rate, screw speed and L/D ratio of die nozzle were set at 50 lb./hour, 50 rpm and 20:1, respectively. Extruded samples were dried for a week in a draft oven at 75°C, ground and screened (60 mesh). The extruder screw arrangement was according to an orthogonal design.

Chemical Analysis

The content of TDF, SDF and IDF in the native and extruded barley flour was measured by using the Megazyme total dietary fiber analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland). The contents of mixed linkage β-glucan in native and extruded barley flour were determined by using the Megazyme mixed-linkage β-glucan analysis kit. The resistant starch content was also analyzed according to the Megazyme procedure that involves a treatment step with dimethyl sulfoxide (DMSO). Kjeldhal procedure (AOAC, 1990, method 979.09) was employed to determine the total nitrogen content of the SDF, IDF and TDF.

The solubility of β-glucan in the native (at 100°C and 25°C) and extruded flours (at 25°C) was determined as follows. Flour (100 mg) was mixed with 10 ml of distilled water and subjected to continuous shaking for 2 h in a water bath maintained at 100°C or 25°C, followed by the centrifugation at 10,000 rpm for 5 min. β-Glucan content in a 0.1 ml aliquot of the supernatant was determined. The solubility was calculated as % of the total β-glucan content in the flour.

Statistical Analysis

All experiments were carried out in triplicates. Analysis of variance of the results was performed using General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, 1989). Multiple comparison of the means was performed by least significant difference (LSD) test at α=0.05 level.

5.3 Results and Discussion

The proximate composition of native barley flours is shown in Table 5.1. There were minor differences in protein, lipid and ash contents of CDC-Candle and Phoenix flours, whereas their starch, IDF, SDF and β-glucan contents were considerably different. Compared to CDC-Candle, Phoenix had higher starch and IDF but lower β-glucan and SDF contents. Furthermore, the β-glucan content of CDC-Candle and Phoenix (6.5 and 3.9%, respectively) were higher than SDF content (5.6 and 2.4%, respectively). This suggests that barley β-glucan contained water soluble as well as insoluble fractions. The water solubility of β-glucan in the native and extruded flours is given in Table 5.2. The solubility (determined at 100°C) of native β-glucan was 79% for CDC-Candle and 57% for Phoenix, which suggests that the SDF of native flour might contain a large amount of β-glucan (91% in CDC-Candle and 93% in Phoenix). The β-glucan in extruded flours had higher solubility (determined at 25°C) than that of its native counterparts (Table 5.2), where at each extrusion temperature, the solubility increased with an increase in the extrusion-moisture level. The solubility differences between native CDC-Candle and Phoenix β-glucans may be attributed to the variety-dependent molecular variations, while the enhanced solubility of extruded β-glucans may be attributed to the extrusion-induced molecular alterations (Jiang and Vasanthan, 2000).

The dietary fiber (IDF, SDF and TDF) contents of the native and extruded barley flours are shown in Table 5.3. In general, the content of SDF and TDF increased upon extrusion cooking of both types of barley flours. The changes in the IDF content were found to be variety dependent. A minor decrease (compared to native flour) in IDF content of CDC-Candle barley was evident at all extrusion temperatures (Table 5.3). This

Table 5.1: Composition[a] (%, dry basis) of barley flour

Flour[b]	Starch	Protein	β-glucan	Lipid	Ash	Dietary Fiber	
						SDF	IDF
CDC-Candle	73.6±0.9	10.2±0.6	6.5±0.2	1.1±0.1	1.2±0.0	5.6±0.1	1.9±0.1
Phoenix	76.5±0.4	10.4±0.8	3.9±0.0	1.1±0.0	1.0±0.0	2.4±0.0	2.4±0.6

[a] Values are means of three determinations ± standard deviations.
[b] Barley flour produced by pin-milling of pearled (30-32%) grains.

Table 5.2. Solubility[a] of β-glucan in the native and extruded barley flours

Flour sample	Solubility (% of total β-glucan)	
CDC-Candle		
Native [b]	41.52±0.17	(79.36±0.35)[c]
Extruded		
90/20[d]	61.92±1.20	
90/35	62.90±0.92	
90/50	76.37±1.41	
100/20	86.68±1.65	
100/35	91.57±1.32	
100/50	94.10±0.81	
120/20	89.50±1.36	
120/35	93.57±0.94	
120/50	95.32±1.26	
140/20	74.94±1.15	
140/35	84.24±1.41	
140/50	89.55±1.62	
LSD (P<0.05)	01.52	
Phoenix		
Native	26.84±0.46	(56.70±1.29)[c]
Extruded		
90/20	26.97±1.01	
90/35	28.09±1.25	
90/50	39.33±0.16	
100/20	29.49±0.30	
100/35	32.62±1.27	
100/50	40.03±1.58	
120/20	31.56±1.51	
120/35	33.79±1.15	
120/50	40.96±1.07	
140/20	36.03±0.98	
140/35	38.90±0.30	
140/50	41.05±1.37	
LSD (P<0.05)	1.20	

[a] Solubility determined at 25°C. Values are means of three determinations ± SD.
[b] Barley flour produced by pin-milling of pearled (30-32%) grains.
[c] Solubility determined at 100°C.
[d] The temperature (°C)/moisture (%) used to produce extruded barley flour.

Table 5.3. The IDF, SDF and TDF contents[a] of native and extruded flours

	IDF (%, dry basis)	SDF (%, dry basis)	TDF (%, dry basis)
CDC-Candle			
Native [b]	1.89±0.07	5.63±0.11	7.52±0.09
Extruded			
90/20[c]	1.82±0.23	5.69±0.29	7.51±0.26
90/35	1.39±0.10	6.63±0.24	9.02±0.17
90/50	1.30±0.28	6.82±0.29	8.28±0.29
100/20	1.53±0.43	6.51±0.51	8.04±0.47
100/35	1.29±0.07	6.62±0.13	7.91±0.10
100/50	1.10±0.11	6.59±0.02	7.59±0.07
120/20	1.53±0.31	7.16±0.30	8.69±0.31
120/35	1.44±0.07	6.63±0.23	7.79±0.15
120/50	1.17±0.14	6.52±0.10	7.96±0.12
140/20	1.71±0.14	7.16±0.34	8.85±0.24
140/35	1.68±0.15	6.57±0.20	8.36±0.18
140/50	1.40±0.13	7.24±0.27	9.14±0.20
LSD (P<0.05)	0.15	0.20	0.18
Phoenix			
Native	2.43±0.57	2.35±0.03	4.78±0.30
Extruded			
90/20	5.56±0.24	3.63±0.19	9.19±0.22
90/35	6.42±0.23	3.59±0.31	10.01±0.27
90/50	6.43±0.22	3.80±0.10	10.23±0.16
100/20	5.51±0.24	3.46±0.24	8.96±0.24
100/35	4.74±0.21	3.06±0.06	7.79±0.14
100/50	5.90±0.06	3.45±0.24	9.36±0.15
120/20	5.08±0.09	3.22±0.21	8.30±0.15
120/35	5.57±0.42	3.41±0.15	8.83±0.29
120/50	5.59±0.43	3.61±0.25	9.19±0.34
140/20	4.19±0.32	3.21±0.45	7.39±0.39
140/35	4.77±0.49	3.03±0.12	7.75±0.31
140/50	4.47±0.45	3.54±0.23	8.00±0.34
LSD (P<0.05)	0.31	0.26	0.29

[a] Values are means of three determinations ± SD.
[b] Barley flour produced by pin-milling of pearled (30-32%) grains.
[c] The temperature (°C)/moisture (%) used to produce extruded barley flour.

suggests that the increase in TDF of CDC-Candle flour upon extrusion cooking was primarily due to the increase in SDF. This increase in SDF could be partially due to the transformation of some IDF into SDF during extrusion. In a study involving white and whole-meal wheat flour, Björck et al. (1984) have reported a slight increase in TDF with a substantial shift from IDF to SDF in the extruded white wheat flour. In the present study, the % increase in SDF in extrusion cooked CDC-Candle flour was considerably higher than the decrease observed in IDF (Table 5.3), suggesting the formation of additional SDF from non-dietary fiber components (i.e. starch) of native flour. Theander and Westerlund (1987) reported that highly reactive anhydro-compounds (i.e. 1,6-anhydrosaccharides) were generated during extrusion cooking and these compounds would react with starch or fragmented starch through trans-glycosidation reactions to form new branched glucans, which were resistant to amylase hydrolysis. In the present study, the formation of "indigestible glucans" might have contributed to the increase in SDF content of CDC-Candle flour.

Conversely, both IDF and SDF contributed to the increase in TDF content in extrusion cooked Phoenix flour with the contribution by IDF being prominent (Table 5.3). The substantial increase in IDF of the extruded Phoenix flour was unexpected. A number of mechanisms, have been suggested to explain the changes in dietary fiber profile (IDF, SDF and TDF) of processed grain flours. Fornalm et al. (1987) reported a decrease in the content of cellulose and lignin in extruded flours from barley and buckwheat. The occurrence of resistant starch (RS3, retrograded amylose) in thermally processed grains was reported by Englyst et al. (1983), Englyst and Cummings (1984), Ranhotra et al. (1989), and Englyst et al. (1992). Englyst et al. (1983, 1984) reported that

the resistant starch was insoluble in water and had properties similar to those of insoluble dietary fiber, but could be solubilized in 2M KOH or dimethyl sulphoxide (DMSO). Megazyme has developed a method to quantify RS3 in food samples and this methodology uses the difference in starch content before and after DMSO treatment to account for RS3. This protocol was used in this study to quantify RS3. Although there are contradictory reports on the presence of RS3 in TDF of extruded wheat flour (Björck et al., 1986; Siljeström et al., 1986), the data given in Table 5.4 suggests that the formation of RS3 might have been responsible for the increased content of IDF and TDF in extruded Phoenix flour. Regardless of the extrusion-moisture levels (i.e. 20% and 50%) the RS3 content of extruded Phoenix flour increased up to an extrusion temperature of 120°C and then showed a decrease at 140°C. For all temperatures employed, the RS3 formation was observed to be higher at 50% than at 20% moisture level. The RS3 content of both native and extruded CDC-Candle flour was little.

Forrest and Wainwright (1977) were able to extract a substance, which had a covalent association between a nitrogenous material and β-glucan from heated (65°C) barley endosperm cell walls. The crude nitrogen content of the IDF and SDF fractions (from the dietary fiber determination procedure) of native and extruded flour is shown in Table 5.5. The nitrogen content of both IDF and SDF fractions from extruded flours was generally higher than that of native flours. This suggests that the extrusion cooking has induced interactions between the nitrogenous compounds (i.e. protein) in barley flour and fiber. Data presented in Table 5.5 further suggest that IDF participated more in these interactions, especially at 100°C. However, no definite correlation was established between the nitrogen content of the fiber and extrusion conditions. It should be noted that

Table 5.4. Resistant starch (RS3) content[a] of native and selected extruded flours

Flour Sample		RS3 (%, w/w)
CDC-Candle		
Native[b]		0
Extruded	90/20[c]	0
	100/20	0
	120/20	0
	140/20	0
	90/50	0
	100/50	0
	120/50	0
	140/50	0.58±0.20
LSD (P<0.05)		–
Phoenix		
Native		0.83±0.15
Extruded	90/20	1.02±0.20
	100/20	1.08±0.15
	120/20	1.43±0.10
	140/20	1.10±0.21
	90/50	1.62±0.38
	100/50	1.75±0.25
	120/50	2.87±0.41
	140/50	1.94±0.33
LSD (P<0.05)		0.28

[a] Values are means of three determinations ± standard deviations.
[b] Barley flour produced by pin-milling of pearled (30-32%) grains.
[c] The temperature (°C)/moisture (%) used to produce extruded barley flour.

Table 5.5: Nitrogen content[a] of the insoluble and soluble dietary fiber

Flour sample	Insoluble Dietary Fiber	Soluble Dietary Fiber
CDC-Candle		
Native [b]	1.21±0.19	0.62±0.16
Extruded		
90/20[c]	1.05±0.18	1.27±0.11
90/35	1.27±0.19	1.00±0.27
90/50	1.21±0.26	0.89±0.23
100/20	1.75±0.28	0.87±0.06
100/35	1.88±0.19	1.03±0.08
100/50	2.37±0.36	0.85±0.05
120/20	1.40±0.16	0.60±0.19
120/35	1.59±0.19	1.13±0.11
120/50	2.01±0.33	0.86±0.12
140/20	0.89±0.03	0.61±0.08
140/35	1.09±0.14	1.14±0.18
140/50	1.33±0.07	0.70±0.05
LSD (P<0.05)	0.21	0.12
Phoenix		
Native [a]	1.29±0.31	1.09±0.10
Extruded		
90/20[b]	1.61±0.09	1.30±0.18
90/35	1.91±0.12	1.40±0.01
90/50	1.99±0.03	1.41±0.04
100/20	2.38±0.05	1.10±0.12
100/35	2.45±0.04	1.05±0.10
100/50	2.26±0.07	1.04±0.16
120/20	1.92±0.03	1.03±0.17
120/35	1.64±0.14	1.09±0.12
120/50	1.65±0.15	1.02±0.19
140/20	1.64±0.19	1.02±0.19
140/35	1.63±0.08	0.82±0.16
140/50	1.44±0.36	0.84±0.14
LSD (P<0.05)	0.14	0.15

[a] Values are means of three determinations ± standard deviations.
[b] Barley flour produced by pin-milling of pearled (30-32%) grains.
[c] The temperature (°C)/moisture (%) used to produce extruded barley flour.

such protein-fiber interactions would not have contributed to increased dietary fiber content of the flour (Table 5.3) because appropriate deductions were made for protein content (calculated from the crude nitrogen content during the quantification of IDF and SDF). However, the fiber-protein complexes present in extruded barley may resist digestion by enzymes in the human intestine and pass on to colon along with dietary fiber. The fate of this protein-fiber complex in the colon or its benefit to human health is yet to be determined.

5.4 Conclusions

Extrusion cooking increased the TDF of barley flours. The TDF increase in waxy-CDC-Candle barley was mainly due to an increase in SDF. For Phoenix, the increase in both IDF and SDF contributed to the increased TDF content. The change in dietary fiber profile during extrusion of barley flour may be attributed primarily to a shift from IDF to SDF, as well as the formation of RS3 and "enzyme resistant indigestible glucans" formed by trans-glycosidation.

5.5 References

AACC. 1995. Approved Methods of the American Association of Cereal Chemists (method 32-07). American Association of Cereal Chemists, Inc., St. Paul, MN.

AOAC. 1990. Official Methods of Analysis, 15th Ed (method 979.09). Association of Offical Analytical Chemists, Washington, D.C.

AOAC. 1995. Official Methods of Analysis, 16th Ed (method 991.43). Association of Offical Analytical Chemists, Washington, D.C.

Anderson, J. W., Deakins, D. A., Floore, T. L., Smith, B. M. and Whites, S. E. 1990. Dietary fiber and coronary heart disease. *Crit. Rev. Food Sci. Nutr.*, 29: 96-147.

Björck, I, Nyman, M. and Asp, N. G. 1984. Extrusion cooking and dietary fiber: effects on dietary fiber content and on degradation in the rat intestinal tract. *Cereal Chem.* 61:174-179.

Björck, I, Nyman, M., Pirkhed, D., Siljeström, M., Asp, N. G and Eggum, B. O. 1986. On the digestibility of starch in wheat bread. *J. Cereal Sci.* 4: 1-11.

Caldwell, E. F. and Nelsen, J. C. 1999. Development of an analytical reference standard for total, insoluble, and soluble dietary fiber. *Cereal Foods World.* 44: 360-362.

Devries, J. W., Prosky, L., Li, B. and Cho, S. 1999. A historical perspective on dietary fiber. *Cereal Foods World.* 44: 367-369.

Englyst, H. and Cummings, J. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* 109: 937-942.

Englyst, H. N., Kingman, S. M., and Cummings, J. H. 1992. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* 46(suppl.) S33.

Englyst, H., Anderson, V. and Cummings, J. 1983. Starch and non-starch polysaccharides in some cereal foods. *J. Sci. Food Agric.* 341: 1434-1440.

Fornalm, L. Soral-Smietana, M., Smietana, Z. and Szpenlowski, J. 1987. Chemical characteristics and physico-chemical properties of the extruded mixtures of cereal starches. *Starch/Stärke* 39: 75-78.

Forrest, I. S. and Wainwright, T. 1977. The mode of binding of β-glucans and pentosans in barley endosperm cell walls. *J. Inst. Brew.* 83: 279-286.

Gosain, K. 1996. Long-term effects of barley bread products on metabolic control of non-insulin dependent diabets mellitus. MSc thesis, University of Alberta, Edmonton, Canada.

Jadhav, S. J., Lutz, S. E., Ghorpade, V. M. and Salunkhe, D. K. 1998. Barley: Chemistry and value-added processing. *Crit. Rev. Food Sci.* 38: 123-171.

Jiang, G. S. and Vasanthan, T. 2000. Effect of extrusion cooking on the primary structure and water solubility of β-glucans from regular and waxy barley. *Cereal Chem.* 77: 396-400.

Klopfenstein, C. F. 1988. The role of cereal beta-glucans in nutrition and health. *Cereal Foods World.* 33(10): 865-869.

Østergård, K., Björck, I. and Vainionpää, J. 1989. Effects of extrusion cooking on starch and dietary fiber in barley. *Food Chem.* 34: 215-227.

Martinez, V. M., Newman, R. K., and Newman, C. K. 1992. Barley diets with different fat sources have hypocholesterolemic effects in chickens. *J. Nutr.* 122: 1070-1076.

McIntosh, G. H., Whyte, J., McArthur, R. and Nestel, P. J. 1991. Barley and wheat foods: Influence on plasma cholesterol concnetrations in hypercholesterolemic men. *Am. J. Clin. Nutr.* 53: 1205-1209.

Pick, M. 1994. Oat bran centration bread products: long-term effects on diabetic control, MSc Thesis, University of Alberta, Edmonton, Canada.

Ranhotra, G. S., Gelroth, J. A., and Leinen, S. D. 1999. Resistant starch in selected grain-based foods. *Cereal Foods World.* 5: 357-359.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, Forth Edition Vol. 2, Cary, NC.

Siljeström, M., Westerlund, E., Björck, I, Holm, J., Asp, N. G. and Theander, O. 1986. The effects of various thermal processes on dietary fiber and starch content of the whole grain wheat and white flour. *J. Cereal Sci.* 4: 315-323.

Theander, O. and Westerlund, E. 1987. Studies on chemical modifications in heat-processed starch and wheat flour. *Starch/Stärke* 39: 88-93.

Chapter 6

Conclusions and Recommendations

The pearling study indicated that the distribution of starch, protein, β-glucan, lipids, and ash within a barley grain differ widely with the variety. In both barley varieties (Phoenix and CDC-Candle), starch was confined to the inner core (i.e. endosperm) of the grains and 10-25% of pearling flour (PF) contained the highest concentration of protein. The β-glucan content of whole CDC-Candle barley grain was ~2% higher than that of Phoenix. Results showed that β-glucan in Phoenix was relatively higher in the inner grain layers, perhaps beneath the sub-aleurone tissues. In contrast, a positive concentration gradient of β-glucan towards the grain core was observed in CDC-Candle grain. In both types of barley, there was no substantial difference between their contents of lipid and ash, which were mostly concentrated in the outer layers of the grains. Understanding the grain component distribution through a gradual layer-by-layer grain pearling process will be useful in the production of barley flours with different characteristics for industrial uses.

The color of the gels prepared with whole grain flour was darker (dark brown/black) than that of the flour milled from pearled grain (PG). The brightness of the PG-gels increased as the degree of pearling increased up to 32% and remained unaffected thereafter. The visual and objective evaluations of the color of barley flour gels indicated that pearling of barley at least to a degree of 32% was required to ensure its bright color and stability in barley-based foods.

In the dextrinization study with PG-flour, at each extrusion temperature (90-140°C), degree of hydrolysis (DH) increased with moisture content from 20% to 50%.

At a constant moisture level, Phoenix and CDC-Candle flours extruded without thermostable α-amylase showed decrease in DH values at temperatures exceeding 90°C. This could be attributed to inactivation of starch hydrolyzing enzymes that are naturally present in barley flours, at higher extrusion temperatures. However, flour extruded in the presence of thermostable α-amylase (2 or 4%), the DH increased with an increase in temperature up to 100°C; thereafter, hydrolysis decreased in both CDC-Candle and Phoenix barley flours, which may be attributed to thermal inactivation of the added thermostable α-amylase. This suggests that 100°C is the optimum temperature for extrusion in the presence of thermostable α-amylase. Also, it was observed that at low extrusion temperatures (90 and 100°C), Phoenix was hydrolyzed to a greater extent than CDC-Candle. However, the above order was reversed at higher extrusion temperatures (120 and 140°C).

Phoenix and CDC-Candle flours when extruded without thermostable α-amylase showed progressive increases in dextrose equivalent (DE) with an increase in moisture content (20-50%) and temperature (90-140°C). This reflects an increase in the number of reducing ends, perhaps resulting from fragmentation of starch and β-glucan chains due to mechanical shear. In the presence of thermostable α-amylase, at constant extrusion temperature, DE increased (due to hydrolysis of starch) with an increase in moisture content. At each moisture level, the maximum and minimum values of DE were obtained at 100°C and 140°C, respectively.

At each temperature and enzyme concentration, the proportion of DP1 to DP7 at all moisture contents, followed the order: DP2>DP6>DP3>DP5>DP7>DP4>DP1. At each temperature and moisture content, the proportion of DP1 to DP7 increased with

enzyme concentration. At each enzyme concentration and moisture content, an increase in temperature from 90 to 100°C increased the proportion of DP1 to DP7. However, at 120 and 140°C, the proportion of DP1 to DP7, were much lower than that at 90°C. At 90 and 100°C, the predominant oligosaccharide species were DP2, DP6 and DP3, whereas, at 120 and 140°C, these were DP2, DP6, DP5 and DP6, DP3, DP2, respectively. The data from this research indicated that dextrinized barley flours with different saccharide composition can be produced by extrusion technology. This process could be used as another avenue to alter barley flour functionality for different food/industrial applications.

The contents of soluble dietary fiber (SDF) and total dietary fiber (TDF) increased upon extrusion cooking of both types of barley flours. The changes in the insoluble dietary fiber (IDF) content were found to be variety dependent. A minor decrease in IDF content of CDC-Candle barley was found, but an increase in IDF content of Phoenix was observed at all extrusion temperature. The increase in SDF in both barleys could be due to the transformation of some IDF into SDF during extrusion and the formation of additional SDF by trans-glycosidation. The increase in IDF in Phoenix flour could be due to the formation of retrograded amylose [resistant starch (RS3)] during extrusion cooking and subsequent cooling. For all temperatures employed, the RS3 formation was observed to be higher at 50% than at 20% moisture level.

Extrusion conditions, such as temperature, moisture, pressure/shear and their interactions may change the molecular structure as well as nutritional properties of barley flour components. Further research is warranted to understand the effect of extrusion processing of barley flours, and its health implications. Also, research is required to

understand the relationship between the properties of extruded and dextrinized barley flour products and their functionality/suitability for different food/industrial applications. These researches will further add value to barley flour and widen its usage.

Appendix

Appendix 1a: Dextrose equivalent (DE)[a] of extruded flours

Extrusion Conditions	Phoenix	CDC-Candle
90/0/20[b]	1.6 ± 0.1^{s}	1.8 ± 0.0^{qr}
90/0/35	$2.6 \pm 0.2^{q-s}$	$3.5 \pm 0.0^{m-p}$
90/0/50	4.9 ± 0.1^{mn}	6.3 ± 0.5^{kl}
90/2/20	10.6 ± 0.5^{k}	7.8 ± 0.4^{k}
90/2/35	14.4 ± 0.3^{i}	11.3 ± 0.3^{i}
90/2/50	19.4 ± 1.1^{ef}	15.4 ± 1.0^{g}
90/4/20	16.5 ± 0.2^{h}	13.4 ± 1.2^{h}
90/4/35	18.4 ± 1.5^{fg}	15.7 ± 0.8^{g}
90/4/50	22.2 ± 1.6^{d}	18.2 ± 0.9^{f}
100/0/20	2.1 ± 0.4^{rs}	1.1 ± 0.0^{r}
100/0/35	$3.0 \pm 0.1^{o-r}$	$2.3 \pm 0.3^{p-r}$
100/0/50	$3.6 \pm 0.3^{o-q}$	$3.4 \pm 0.2^{n-q}$
100/2/20	12.1 ± 0.5^{j}	13.4 ± 0.1^{h}
100/2/35	14.6 ± 0.2^{i}	13.2 ± 0.6^{h}
100/2/50	27.0 ± 0.5^{b}	15.6 ± 0.1^{g}
100/4/20	23.4 ± 1.5^{cd}	$19.5 \pm 0.4^{d-f}$
100/4/35	24.3 ± 1.4^{c}	21.0 ± 1.0^{d}
100/4/50	36.1 ± 0.7^{a}	24.9 ± 1.3^{b}
120/0/20	$2.8 \pm 0.1^{p-s}$	$3.0 \pm 0.1^{o-q}$
120/0/35	$3.1 \pm 0.2^{o-r}$	$3.4 \pm 0.2^{n-q}$
120/0/50	$4.1 \pm 0.1^{n-p}$	$5.6 \pm 0.3^{m-p}$
120/2/20	8.6 ± 0.3^{l}	10.6 ± 0.3^{ij}
120/2/35	14.9 ± 1.3^{i}	18.8 ± 0.5^{ef}
120/2/50	22.3 ± 0.6^{d}	25.0 ± 0.5^{b}
120/4/20	17.3 ± 0.9^{gh}	20.2 ± 1.4^{de}
120/4/35	20.1 ± 0.5^{e}	24.2 ± 0.5^{bc}
120/4/50	26.9 ± 1.3^{b}	31.3 ± 1.5^{a}
140/0/20	$3.1 \pm 0.4^{o-r}$	$4.1 \pm 0.2^{m-o}$
140/0/35	4.3 ± 0.1^{no}	4.7 ± 0.4^{mn}
140/0/50	5.0 ± 0.3^{mn}	5.1 ± 0.3^{lm}
140/2/20	$3.9 \pm 0.4^{n-p}$	6.9 ± 0.4^{k}
140/2/35	7.5 ± 0.4^{l}	10.3 ± 0.7^{ij}
140/2/50	10.4 ± 0.6^{k}	16.2 ± 1.6^{g}
140/4/20	5.6 ± 0.1^{m}	9.4 ± 0.2^{j}
140/4/35	12.8 ± 1.0^{j}	18.8 ± 1.2^{ef}
140/4/50	18.8 ± 1.3^{f}	23.0 ± 0.7^{c}

[a] Expressed as mean ± SD.

[b]The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour

[a-s] Values within the column with different superscripts are significantly different ($P<0.05$). Analysis of variance of the results was performed using multi-factorial (4 x 3 x 3) ANOVA.

Appendix 1b: Degree of hydrolysis (DH)[a] of extruded flours

Extrusion Conditions	Phoenix	CDC-Candle
90/0/20[b]	0.2 ± 0.0^{l}	0.2 ± 0.0^{t}
90/0/35	0.3 ± 0.0^{kl}	$1.0 \pm 0.0^{r-t}$
90/0/50	$0.8 \pm 0.0^{i-l}$	$2.0 \pm 0.1^{p-r}$
90/2/20	$4.7 \pm 0.5^{e-h}$	$3.7 \pm 0.2^{l-n}$
90/2/35	$5.1 \pm 0.0^{e-h}$	$4.6 \pm 0.5^{k-m}$
90/2/50	10.4 ± 0.9^{cd}	7.5 ± 0.5^{gh}
90/4/20	$7.1 \pm 0.8^{d-f}$	5.9 ± 0.3^{ij}
90/4/35	10.1 ± 0.7^{cd}	8.8 ± 0.7^{ef}
90/4/50	18.2 ± 0.6^{b}	14.4 ± 0.5^{b}
100/0/20	0.1 ± 0.0^{l}	0.1 ± 0.1^{t}
100/0/35	0.3 ± 0.0^{kl}	0.7 ± 0.1^{st}
100/0/50	$0.4 \pm 0.0^{j-l}$	$1.5 \pm 0.1^{q-s}$
100/2/20	$5.1 \pm 0.4^{e-h}$	4.6 ± 0.2^{kl}
100/2/35	$6.6 \pm 0.6^{e-g}$	6.6 ± 0.1^{hi}
100/2/50	15.2 ± 2.6^{b}	11.0 ± 0.6^{d}
100/4/20	10.8 ± 1.0^{c}	8.1 ± 0.1^{fg}
100/4/35	15.7 ± 1.2^{b}	12.3 ± 1.0^{c}
100/4/50	27.8 ± 5.0^{a}	21.8 ± 0.4^{a}
120/0/20	0.0 ± 0.0^{l}	0.1 ± 0.0^{t}
120/0/35	0.1 ± 0.1^{l}	0.7 ± 0.1^{st}
120/0/50	0.2 ± 0.1^{l}	$1.8 \pm 0.2^{q-s}$
120/2/20	$2.4 \pm 0.2^{h-l}$	$3.4 \pm 0.0^{m-o}$
120/2/35	$3.7 \pm 0.2^{g-k}$	$5.4 \pm 0.1^{i-k}$
120/2/50	7.3 ± 1.1^{de}	9.3 ± 0.2^{c}
120/4/20	$3.8 \pm 0.2^{f-j}$	5.9 ± 1.0^{ij}
120/4/35	$4.2 \pm 0.0^{e-i}$	9.0 ± 0.5^{ef}
120/4/50	10.0 ± 0.3^{cd}	14.8 ± 0.2^{b}
140/0/20	0.0 ± 0.0^{l}	0.0 ± 0.0^{t}
140/0/35	0.1 ± 0.0^{l}	0.1 ± 0.0^{t}
140/0/50	0.2 ± 0.0^{l}	0.3 ± 0.0^{t}
140/2/20	0.1 ± 0.0^{l}	0.7 ± 0.4^{st}
140/2/35	$0.4 \pm 0.0^{j-l}$	$3.2 \pm 0.2^{n-p}$
140/2/50	$3.3 \pm 0.3^{g-l}$	$5.7 \pm 0.1^{i-k}$
140/4/20	0.3 ± 0.0^{kl}	$2.2 \pm 0.1^{o-q}$
140/4/35	$2.7 \pm 0.2^{h-l}$	5.3 ± 0.4^{jk}
140/4/50	$4.6 \pm 0.1^{e-h}$	8.0 ± 0.2^{fg}

[a] Expressed as mean ± SD.

[b]The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour

[a-t] Values within the column with different superscripts are significantly different ($P<0.05$). Analysis of variance of the results was performed using multi-factorial (4 x 3 x 3) ANOVA.

Appendix 2a: DP1 (%, w/w)[a] of extruded flours

Extrusion Conditions	Phoenix	CDC-Candle
90/0/20[b]	-	-
90/0/35	-	-
90/0/50	-	-
90/2/20	0.5 ± 0.0^{e}	$0.6 \pm 0.1^{h-k}$
90/2/35	0.4 ± 0.0^{fg}	$0.8 \pm 0.1^{d-g}$
90/2/50	0.7 ± 0.1^{d}	$1.0 \pm 0.0^{b-d}$
90/4/20	0.3 ± 0.0^{h}	$0.9 \pm 0.0^{c-e}$
90/4/35	0.8 ± 0.1^{cd}	$0.9 \pm 0.1^{c-f}$
90/4/50	0.8 ± 0.0^{cd}	1.0 ± 0.0^{cd}
100/0/20	-	-
100/0/35	-	0.3 ± 0.0^{m}
100/0/50	-	$0.5 \pm 0.0^{g-k}$
100/2/20	0.5 ± 0.1^{ef}	$0.4 \pm 0.1^{k-m}$
100/2/35	0.5 ± 0.0^{ef}	$0.6 \pm 0.1^{h-l}$
100/2/50	0.8 ± 0.1^{cd}	$1.0 \pm 0.0^{b-c}$
100/4/20	1.2 ± 0.0^{b}	1.2 ± 0.1^{b}
100/4/35	1.2 ± 0.1^{b}	1.9 ± 0.1^{a}
100/4/50	1.3 ± 0.1^{a}	2.0 ± 0.1^{a}
120/0/20	-	0.3 ± 0.0^{m}
120/0/35	-	0.4 ± 0.0^{m}
120/0/50	-	0.4 ± 0.1^{lm}
120/2/20	0.3 ± 0.0^{gh}	$0.7 \pm 0.0^{e-i}$
120/2/35	0.5 ± 0.0^{e}	$0.7 \pm 0.0^{g-j}$
120/2/50	0.5 ± 0.0^{ef}	$0.7 \pm 0.0^{g-j}$
120/4/20	0.0 ± 0.0^{i}	$0.8 \pm 0.1^{d-h}$
120/4/35	0.7 ± 0.0^{d}	$0.9 \pm 0.1^{d-g}$
120/4/50	0.9 ± 0.0^{c}	$1.0 \pm 0.0^{b-d}$
140/0/20	-	-
140/0/35	-	-
140/0/50	-	-
140/2/20	-	$0.6 \pm 0.1^{g-k}$
140/2/35	-	0.9 ± 0.1^{cd}
140/2/50	0.4 ± 0.0^{gh}	1.1 ± 0.1^{bc}
140/4/20	-	$0.5 \pm 0.1^{g-k}$
140/4/35	0.3 ± 0.0^{gh}	$0.8 \pm 0.1^{d-g}$
140/4/50	0.4 ± 0.0^{fg}	$1.0 \pm 0.1^{b-d}$

[a] Expressed as mean ± SD.

[b]The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour

[a-m] Values within the column with different superscripts are significantly different ($P<0.05$). Analysis of variance of the results was performed using multi-factorial (4 x 3 x 3) ANOVA.

Appendix 2b: DP2 (%, w/w)[a] of extruded flours

Extrusion Conditions	Phoenix	CDC-Candle
90/0/20[b]	0.2 ± 0.0^{l}	0.3 ± 0.0^{o}
90/0/35	0.3 ± 0.0^{l}	0.7 ± 0.1^{no}
90/0/50	0.8 ± 0.0^{l}	$2.6 \pm 0.1^{k-m}$
90/2/20	$4.9 \pm 1\ 0^{h-j}$	3.8 ± 0.6^{jk}
90/2/35	$5.3 \pm 0\ 2^{hi}$	4.9 ± 0.6^{ij}
90/2/50	10.5 ± 0.3^{e}	8.7 ± 0.6^{ef}
90/4/20	7.9 ± 0.4^{f}	5.5 ± 1.2^{hi}
90/4/35	11.2 ± 0.5^{de}	8.8 ± 0.8^{ef}
90/4/50	19.5 ± 0.3^{b}	15.5 ± 0.4^{b}
100/0/20	0.2 ± 0.0^{l}	0.1 ± 0.0^{o}
100/0/35	0.3 ± 0.0^{l}	0.7 ± 0.0^{no}
100/0/50	0.4 ± 0.0^{l}	2.0 ± 0.0^{lm}
100/2/20	5.6 ± 0.5^{gh}	4.8 ± 0.4^{ij}
100/2/35	6.8 ± 0.2^{fg}	6.7 ± 0.3^{h}
100/2/50	15.5 ± 1.5^{c}	11.7 ± 0.1^{cd}
100/4/20	11.0 ± 0.3^{e}	8.4 ± 0.6^{fg}
100/4/35	16.7 ± 0.1^{c}	12.3 ± 0.5^{c}
100/4/50	28.4 ± 0.5^{a}	25.5 ± 0.5^{a}
120/0/20	-	0.2 ± 0.0^{o}
120/0/35	0.3 ± 0.0^{l}	0.7 ± 0.1^{no}
120/0/50	0.3 ± 0.0^{l}	$1.6 \pm 0.2^{m-o}$
120/2/20	2.8 ± 0.1^{k}	$3.5 \pm 0.7^{j-l}$
120/2/35	3.8 ± 0.5^{jk}	5.8 ± 0.3^{hi}
120/2/50	8.0 ± 0.5^{f}	9.2 ± 0.3^{ef}
120/4/20	4.2 ± 0.5^{ij}	5.9 ± 0.3^{hi}
120/4/35	$4.7 \pm 0.1^{h-j}$	9.1 ± 0.6^{ef}
120/4/50	12.5 ± 0.3^{d}	17.0 ± 0.2^{b}
140/0/20	-	0.3 ± 0.0^{o}
140/0/35	0.1 ± 0.0^{l}	0.3 ± 0.0^{o}
140/0/50	0.2 ± 0.0^{l}	0.7 ± 0.0^{no}
140/2/20	0.2 ± 0.0^{l}	0.9 ± 0.9^{no}
140/2/35	0.6 ± 0.0^{l}	$5.9 \pm 0\ .1^{hi}$
140/2/50	$4.8 \pm 0.6^{h-j}$	8.4 ± 0.8^{fg}
140/4/20	0.5 ± 0.0^{l}	$2.6 \pm 0.4^{k-m}$
140/4/35	2.7 ± 0.1^{k}	6.9 ± 0.9^{gh}
140/4/50	5.8 ± 0.4^{gh}	10.1 ± 0.9^{de}

[a] Expressed as mean ± SD.

[b]The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour

[a-o] Values within the column with different superscripts are significantly different ($P<0.05$). Analysis of variance of the results was performed using multi-factorial (4 x 3 x 3) ANOVA.

Appendix 2c: DP3 (%, w/w)[a] of extruded flours

Extrusion Conditions	Phoenix	CDC-Candle
90/0/20[b]	-	-
90/0/35	-	-
90/0/50	-	-
90/2/20	3.2 ± 1.2^{i}	1.9 ± 0.5^{l}
90/2/35	$4.6 \pm 0.3^{e-h}$	$3.7 \pm 0.4^{g-j}$
90/2/50	$5.9 \pm 1.3^{c-e}$	$4.1 \pm 0.3^{e-i}$
90/4/20	3.6 ± 0.2^{hi}	$2.9 \pm 0.3^{i-l}$
90/4/35	$5.2 \pm 0.6^{c-g}$	$3.9 \pm 0.5^{f-j}$
90/4/50	7.6 ± 0.4^{ab}	$5.2 \pm 0.2^{d-f}$
100/0/20	-	-
100/0/35	-	-
100/0/50	-	-
100/2/20	$4.5 \pm 0.6^{f-i}$	$3.4 \pm 0.3^{g-k}$
100/2/35	$5.6 \pm 0.3^{c-f}$	$4.1 \pm 0.2^{e-i}$
100/2/50	7.5 ± 0.0^{ab}	6.2 ± 0.1^{cd}
100/4/20	$4.1 \pm 0.3^{g-i}$	$3.6 \pm 0.5^{g-j}$
100/4/35	6.4 ± 0.5^{bc}	5.4 ± 0.2^{de}
100/4/50	8.6 ± 0.5^{a}	7.1 ± 0.4^{bc}
120/0/20	-	-
120/0/35	-	-
120/0/50	-	-
120/2/20	3.2 ± 0.3^{i}	$2.4 \pm 0.6^{j-l}$
120/2/35	$4.7 \pm 0.7^{e-h}$	$3.6 \pm 0.3^{g-k}$
120/2/50	$5.6 \pm 0.1^{c-f}$	$3.9 \pm 0.3^{f-j}$
120/4/20	$4.8 \pm 0.2^{d-h}$	$2.9 \pm 0.3^{g-k}$
120/4/35	$5.9 \pm 0.1^{c-e}$	$4.4 \pm 0.5^{e-h}$
120/4/50	6.1 ± 0.1^{cd}	$5.2 \pm 0.2^{d-f}$
140/0/20	-	-
140/0/35	-	-
140/0/50	-	-
140/2/20	0.1 ± 0.0^{j}	2.1 ± 0.8^{kl}
140/2/35	3.5 ± 0.0^{hi}	$5.0 \pm 1.1^{d-g}$
140/2/50	$5.8 \pm 0.8^{c-f}$	8.3 ± 1.1^{ab}
140/4/20	0.7 ± 0.1^{j}	$3.2 \pm 0.6^{g-k}$
140/4/35	$4.1 \pm 0.2^{g-i}$	5.6 ± 0.8^{de}
140/4/50	6.5 ± 0.4^{bc}	9.3 ± 0.9^{a}

[a] Expressed as mean ± SD.

[b]The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour

[a-l] Values within the column with different superscripts are significantly different ($P<0.05$). Analysis of variance of the results was performed using multi-factorial (4 x 3 x 3) ANOVA.

Appendix 2d: DP4 (%, w/w)[a] of extruded flours

Extrusion Conditions	Phoenix	CDC-Candle
90/0/20[b]	-	-
90/0/35	-	-
90/0/50	-	-
90/2/20	$1.5 \pm 0.3^{h-j}$	1.2 ± 0.2^{jk}
90/2/35	$2.1 \pm 0.1^{e-h}$	$1.8 \pm 0.1^{h-j}$
90/2/50	3.0 ± 0.4^{bc}	$2.8 \pm 0.1^{d-f}$
90/4/20	1.4 ± 0.1^{ij}	1.9 ± 0.1^{hi}
90/4/35	$2.5 \pm 0.6^{c-f}$	$1.9 \pm 0.1^{h-j}$
90/4/50	4.3 ± 0.1^{a}	2.9 ± 0.0^{de}
100/0/20	-	-
100/0/35	-	-
100/0/50	-	-
100/2/20	1.0 ± 0.0^{jk}	1.5 ± 0.1^{ij}
100/2/35	$1.8 \pm 0.1^{g-i}$	$1.9 \pm 0.1^{g-i}$
100/2/50	$2.8 \pm 0.1^{b-d}$	3.3 ± 0.1^{cd}
100/4/20	$2.8 \pm 0.1^{b-e}$	$3.0 \pm 0.5^{c-e}$
100/4/35	3.0 ± 0.3^{bc}	3.2 ± 0.1^{cd}
100/4/50	5.0 ± 0.2^{a}	5.4 ± 0.2^{a}
120/0/20	-	-
120/0/35	-	-
120/0/50	-	-
120/2/20	$1.7 \pm 0.6^{h-j}$	1.4 ± 0.2^{ij}
120/2/35	$2.2 \pm 0.2^{d-h}$	$2.1 \pm 0.1^{f-h}$
120/2/50	$2.6 \pm 0.4^{b-f}$	$2.7 \pm 0.2^{d-f}$
120/4/20	$1.7 \pm 0.2^{h-j}$	$2.0 \pm 0.1^{g-i}$
120/4/35	$2.4 \pm 0.0^{c-g}$	3.6 ± 0.2^{c}
120/4/50	3.2 ± 0.1^{b}	4.8 ± 0.4^{ab}
140/0/20	-	0.5 ± 0.0^{lm}
140/0/35	-	$0.6 \pm 0.0^{k-m}$
140/0/50	-	$0.6 \pm 0.0^{k-m}$
140/2/20	-	0.7 ± 0.4^{kl}
140/2/35	0.7 ± 0.0^{k}	$2.6 \pm 0.3^{e-g}$
140/2/50	1.0 ± 0.3^{jk}	4.3 ± 0.3^{b}
140/4/20	0.7 ± 0.1^{kl}	$1.6 \pm 0.3^{h-j}$
140/4/35	$2.0 \pm 0.1^{f-i}$	$3.1 \pm 0.4^{c-e}$
140/4/50	$2.7 \pm 0.2^{b-e}$	4.7 ± 0.4^{b}

[a] Expressed as mean ± SD.

[b]The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour

[a-m] Values within the column with different superscripts are significantly different ($P<0.05$). Analysis of variance of the results was performed using multi-factorial (4 x 3 x 3) ANOVA.

Appendix 2e: DP5 (%, w/w)[a] of extruded flours

Extrusion Conditions	Phoenix	CDC-Candle
90/0/20[b]	-	-
90/0/35	-	-
90/0/50	-	-
90/2/20	$3.9 \pm 0.9^{g-j}$	$4.8 \pm 0.7^{e-j}$
90/2/35	$4.8 \pm 0.2^{c-i}$	$4.5 \pm 0.3^{f-k}$
90/2/50	$5.6 \pm 1.5^{a-f}$	$5.2 \pm 0.3^{e-h}$
90/4/20	$4.2 \pm 0.1^{e-j}$	$4.9 \pm 0.9^{e-j}$
90/4/35	$5.8 \pm 1.5^{a-e}$	$5.4 \pm 0.4^{d-h}$
90/4/50	$6.1 \pm 0.4^{a-c}$	$5.3 \pm 0.2^{d-h}$
100/0/20	-	-
100/0/35	-	-
100/0/50	-	-
100/2/20	3.4 ± 0.5^{ij}	3.4 ± 0.4^{jk}
100/2/35	$4.0 \pm 0.2^{f-j}$	$4.1 \pm 0.2^{h-k}$
100/2/50	$6.1 \pm 0.3^{a-c}$	$6.2 \pm 0.2^{c-e}$
100/4/20	$4.4 \pm 0.9^{d-j}$	$4.7 \pm 0.4^{e-k}$
100/4/35	$5.3 \pm 0.5^{b-g}$	$4.7 \pm 0.3^{e-k}$
100/4/50	6.9 ± 0.6^{a}	$6.8 \pm 0.4^{b-d}$
120/0/20	-	-
120/0/35	-	-
120/0/50	-	1.6 ± 0.5^{l}
120/2/20	2.8 ± 0.8^{jk}	3.3 ± 0.5^{k}
120/2/35	$3.5 \pm 0.2^{h-j}$	$3.5 \pm 0.2^{i-k}$
120/2/50	$5.9 \pm 0.6^{a-d}$	$4.2 \pm 0.2^{g-k}$
120/4/20	$4.2 \pm 0.4^{e-j}$	$5.7 \pm 0.3^{d-g}$
120/4/35	$5.0 \pm 0.1^{b-h}$	$5.9 \pm 0.3^{d-f}$
120/4/50	6.6 ± 0.0^{ab}	$6.0 \pm 0.8^{c-f}$
140/0/20	-	-
140/0/35	-	-
140/0/50	-	-
140/2/20	0.9 ± 0.0^{l}	$5.0 \pm 1.0^{e-i}$
140/2/35	1.3 ± 0.0^{kl}	$5.7 \pm 0.2^{c-g}$
140/2/50	3.4 ± 0.6^{ij}	7.2 ± 0.7^{bc}
140/4/20	1.4 ± 0.1^{kl}	$5.8 \pm 0.4^{c-f}$
140/4/35	2.9 ± 0.1^{jk}	8.3 ± 0.9^{ab}
140/4/50	$5.0 \pm 0.3^{c-i}$	9.1 ± 1.2^{a}

[a] Expressed as mean ± SD.

[b] The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour

[a-l] Values within the column with different superscripts are significantly different ($P<0.05$). Analysis of variance of the results was performed using multi-factorial (4 x 3 x 3) ANOVA.

Appendix 2f: DP6 (%, w/w)[a] of extruded flours

Extrusion Conditions	Phoenix	CDC-Candle
90/0/20[b]	-	-
90/0/35	-	-
90/0/50	-	-
90/2/20	$4.7 \pm 1.0^{f-i}$	$5.5 \pm 0.7^{h-k}$
90/2/35	$5.9 \pm 0.4^{c-h}$	$5.8 \pm 0.2^{h-j}$
90/2/50	$7.3 \pm 3.5^{a-f}$	$6.5 \pm 0.4^{g-j}$
90/4/20	$5.3 \pm 0.0^{d-i}$	$5.8 \pm 0.9^{h-j}$
90/4/35	$6.2 \pm 1.9^{b-h}$	$6.0 \pm 0.4^{h-j}$
90/4/50	8.8 ± 0.6^{ab}	$8.9 \pm 0.2^{c-f}$
100/0/20	-	-
100/0/35	-	-
100/0/50	-	-
100/2/20	$3.8 \pm 0.0^{h-j}$	$5.4 \pm 0.0^{i-k}$
100/2/35	$4.7 \pm 0.5^{f-i}$	$5.6 \pm 0.3^{h-j}$
100/2/50	$7.5 \pm 0.3^{a-e}$	$7.2 \pm 0.3^{c-h}$
100/4/20	$5.4 \pm 0.3^{d-i}$	$6.0 \pm 0.1^{h-j}$
100/4/35	$7.0 \pm 0.2^{a-g}$	$7.1 \pm 0.5^{f-i}$
100/4/50	9.6 ± 0.1^{a}	$9.4 \pm 0.4^{b-d}$
120/0/20	-	-
120/0/35	-	-
120/0/50	-	3.8 ± 0.2^{k}
120/2/20	$4.9 \pm 1.4^{c-i}$	4.9 ± 0.7^{k}
120/2/35	$5.7 \pm 0.4^{c-h}$	$5.7 \pm 0.3^{h-j}$
120/2/50	$7.8 \pm 0.7^{a-d}$	$9.0 \pm 1.1^{c-e}$
120/4/20	$5.6 \pm 0.7^{d-i}$	$6.9 \pm 0.3^{g-i}$
120/4/35	$6.6 \pm 0.1^{b-g}$	$7.0 \pm 0.5^{g-i}$
120/4/50	$8.2 \pm 0.0^{a-c}$	$9.6 \pm 0.6^{b-d}$
140/0/20	-	-
140/0/35	-	-
140/0/50	-	-
140/2/20	1.5 ± 0.0^{jk}	$7.9 \pm 0.9^{d-g}$
140/2/35	1.7 ± 0.0^{jk}	$8.3 \pm 0.5^{d-g}$
140/2/50	$4.5 \pm 1.3^{g-i}$	12.0 ± 1.3^{a}
140/4/20	3.1 ± 0.1^{ij}	$7.0 \pm 0.8^{g-i}$
140/4/35	$4.9 \pm 0.3^{f-i}$	$10.2 \pm 0.7^{a-c}$
140/4/50	$6.8 \pm 0.5^{b-g}$	10.8 ± 1.4^{ab}

[a] Expressed as mean ± SD.

[b]The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour

[a-k] Values within the column with different superscripts are significantly different ($P<0.05$). Analysis of variance of the results was performed using multi-factorial (4 x 3 x 3) ANOVA.

Appendix 2g: DP7 (%, w/w)[a] of extruded flours

Extrusion Conditions	Phoenix	CDC-Candle
90/0/20[b]	-	-
90/0/35	-	-
90/0/50	-	-
90/2/20	2.4 ± 0.3^{ef}	2.2 ± 0.1^{i}
90/2/35	2.4 ± 0.1^{ef}	2.4 ± 0.2^{hi}
90/2/50	5.7 ± 0.2^{b}	5.5 ± 0.1^{d}
90/4/20	5.5 ± 0.0^{b}	5.5 ± 0.2^{d}
90/4/35	5.6 ± 0.4^{b}	5.5 ± 0.0^{d}
90/4/50	6.0 ± 0.2^{b}	5.4 ± 0.1^{d}
100/0/20	-	-
100/0/35	-	-
100/0/50	-	-
100/2/20	2.2 ± 0.1^{ef}	2.6 ± 0.5^{hi}
100/2/35	2.6 ± 0.1^{e}	2.1 ± 0.1^{i}
100/2/50	5.7 ± 0.1^{b}	5.6 ± 0.3^{d}
100/4/20	4.2 ± 0.1^{c}	5.6 ± 0.1^{d}
100/4/35	6.0 ± 0.0^{b}	6.6 ± 0.1^{bc}
100/4/50	7.2 ± 0.0^{a}	8.6 ± 0.1^{a}
120/0/20	-	-
120/0/35	-	-
120/0/50	-	2.0 ± 0.2^{i}
120/2/20	3.3 ± 0.6^{d}	3.0 ± 0.2^{gh}
120/2/35	3.4 ± 0.0^{d}	4.0 ± 0.1^{ef}
120/2/50	5.7 ± 0.2^{b}	5.9 ± 0.1^{d}
120/4/20	5.5 ± 0.1^{b}	5.7 ± 0.0^{d}
120/4/35	5.7 ± 0.0^{b}	5.5 ± 0.1^{d}
120/4/50	5.5 ± 0.0^{b}	5.7 ± 0.0^{d}
140/0/20	-	-
140/0/35	-	-
140/0/50	-	-
140/2/20	-	3.4 ± 0.3^{fg}
140/2/35	1.9 ± 0.1^{f}	4.3 ± 0.5^{e}
140/2/50	3.7 ± 0.7^{cd}	6.6 ± 0.6^{bc}
140/4/20	2.1 ± 0.1^{ef}	3.6 ± 0.5^{fg}
140/4/35	3.6 ± 0.2^{cd}	6.0 ± 0.2^{cd}
140/4/50	3.6 ± 0.2^{cd}	6.8 ± 0.4^{b}

[a] Expressed as mean ± SD.

[b]The temperature (°C)/concentration of thermo-stable α-amylase (%, starch basis)/moisture (%) combination used to produce extruded barley flour

[a-i] Values within the column with different superscripts are significantly different ($P<0.05$). Analysis of variance of the results was performed using multi-factorial (4 x 3 x 3) ANOVA.