EFFECTS OF SUPERHEATED STEAM PROCESSING ON THE FUNCTIONAL PROPERTIES OF OAT GROAT, BRAN AND FLOUR, AND ON VIABILITY OF *GEOBACILLUS STEAROTHERMOPHILUS* SPORES

By

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A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

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DOCTOR OF PHILOSOPHY

Department of Biosystems Engineering University of Manitoba Winnipeg, Manitoba

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of

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Of

Doctor of Philosophy

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ABSTRACT

Oat grain (*Avena sativa*) used for human consumption is heat-treated to inactivate fatsplitting enzymes and prevent development of rancidity, obtain desirable flavour, and reduce microbial load. A typical conventional processing of oats involves conditioning with saturated (wet) steam followed by kiln drying. The latter is energy intensive and difficult to control. Additionally, there is a risk of grain cross-contamination with microorganisms present in the air used for cooling of the grain after the drying. In this work, superheated steam (SS) processing was evaluated as a method of heat treatment alternative to conventional processing of oats. The effects of SS on the physicochemical and functional properties of oat groats and/or flour and bran fractions were determined using: (a) mixed Canadian oat cultivars (typically used for commercial processing); (b) two oat cultivars varying in physical grain characteristics and composition (i.e. β -glucan content). Additionally, the viability of heat-resistant *Geobacillus stearothermophilus* ATCC 10149 spores challenged with SS was determined and modelled mathematically.

Oat groats processed with SS at temperatures of 110-130°C had acceptable moisture content, appearance, and exhibited cold paste (64°C) viscosity higher (by up to 1,200 cP) than that of conventionally processed oat grain. The use of SS at temperatures of 140-160°C for processing of groats resulted in even higher cold paste viscosity but also caused low final moisture content (4-7% wet basis, wb) and fast development of rancidity. Processing with SS was effective in the inactivation of peroxidase in groats in which post-processing moisture content was at 10% (wb) and less. The optimum conditions for SS processing of oat groats were: temperature of 110°C, a velocity of 1.00 m/s, and processing times of 10 and 14 min, both of which gave shelf stable groats with moisture content at the safe storage

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limit and cold paste viscosity significantly (P<0.05) higher than that of the conventionally processed oat groats.

The functional properties of oat groat, as well as flour and bran fractions obtained from groats of Furlong and HiFi cultivars processed with SS (at 110, 120, and 130°C) were similar or better than those obtained via the conventional process. Compared to the conventional heat treatment, the processing with SS significantly (P<0.05) decreased groat hardness but increased cold paste viscosity of flour and bran slurries. Generally, the effects of different heat treatments applied to groats on the functional properties of groats, flour, and bran fractions were augmented by the different chemical composition (mainly differences in the content and solubility of β -glucans) of the two oat genotypes tested. The size of groats (within each cultivar) significantly (P<0.05) affected groat hardness, but it appeared to have lesser influence on the physicochemical properties of flour and bran fractions. Among the four heat treatments examined (conventional and SS at 110, 120, and 130°C treatments), the SS treatment at 110°C appeared to have the most positive effects on the physicochemical properties (increased molecular weight and viscosity in solution) of β glucans isolated from the bran fractions of Furlong and HiFi. It appeared that the treatments of groats with SS at 120 and 130°C caused partial depolymerization of BG (lower molecular weights) thereby increasing their solubility, compared to the control heat treatment and the treatment with SS at 110°C. Subsequently, the apparent viscosities of aqueous solutions of BG obtained from samples treated with SS at 120 and 130°C were lower than those obtained from the samples control heat-treated and SS processed at 110°C.

Treatment of *Geobacillus stearothermophilus* spores with SS temperature of 105°C was initially (first 5 min of treatment) more effective than SS treatments at 130 and 145°C.

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Treatments with SS at very high temperatures (160 and 175°C) effectively reduced viability of *G. stearothermophilus* spores. An interruption of SS treatments at 105, 130, and 145°C by brief cooling intervals also effectively reduced the spore viability. The spore response to SS treatments at 105-175°C depended on inoculum size. A mean *z*-value of 25.4°C was calculated for the spores at low and high inoculum levels (3 and 6 log₁₀ colony forming units per gram, cfu/g, respectively) challenged with SS at 130-175°C.

Tailing was observed in survivor curves of *G. stearothermophilus* spores challenged with SS. The survivor curves were described best by the Weibull model, a non-linear regression model. The Weibull model was expanded by describing its parameters (α , β) as a function of SS temperature for the range of 130-175°C. This allowed accurate predictions of the spore survival ratios based on the SS temperature and processing time only (with the exception of predictions for spores at high inoculum level challenged with SS at 130°C). The Weibull model is recommended to describe and to model inactivation of *G. stearothermophilus* spores with SS at high temperatures (145-175°C).

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LIST OF ABBREVIATIONS

- AACC American Association of Cereal Chemists
- ATA alimentary toxic aleukia
- BA bile acids
- BG β-glucans
- CLA conjugated linoleic acid
- DON deoxynivalenol
- DSC differential scanning calorimetry
- FA fatty acids
- FDA Food and Drug Administration
- FFA free fatty acids
- GI gastro-intestinal
- HAD hot air drying
- HACCP hazard analysis and critical control point
- HPAEC high performance anion exchange chromatography
- HPSEC high performance size exclusion chromatography
- LDL low-density lipoprotein
- MALS multi angle light scattering
- MC moisture content
- NIV nivalenol
- RH relative humidity
- RTE ready to eat
- RVA rapid visco analysis
- SCFA short chain fatty acids
- SS superheated steam
- TL total lipids

1 INTRODUCTION

At one time oats were considered only as animal fodder and their human consumption was limited to areas in Scotland and Ireland (Webster 2002). Nowadays, oats are highly regarded as wholesome food beneficial to human health. Oat grain is rich in quality protein, unsaturated fatty acids, dietary fibre (especially its soluble fraction) minerals and vitamins, as well as phenolic compounds and other bioactive phytochemicals (Drzikova et al. 2005; Manthey et al. 1999; Sayar et al. 2006). In human consumption, this multifunctional food is used mainly as breakfast porridge (oatmeal), in various snack forms (granola bars), and in baby foods (Hoover et al. 2003; Sayar et al. 2006). Oat products are often used as ingredients or additives in bread and cookies production or as thickeners in sauces and gravies (Burnette et al. 1992). In 1997, the US Food and Drug Administration (FDA) ruled that "diets low in saturated fat and cholesterol that include soluble fibre from oats (β glucans) may reduce the risk of coronary heart disease" (Webster 2002). As a result of that, development of functional foods and food ingredients containing oat components has been explored (Bekers et al. 2001; Brennan and Cleary 2005).

Raw oat grain becomes rancid in a short period of time due to the presence of oil with high degree of unsaturation and active lipolytic enzymes. Uncontrolled activity of the enzymes shortens shelf life of oat products. The solution to that problem is hydro-thermal inactivation of the enzymes and this approach is commonly used in conventional processing of oats for human consumption (Liukkonen et al. 1993; Youngs 1986). The primary objective of conventional oat processing is therefore inactivation of the heat-labile lipolytic enzymes (Ganssmann and Vorwerck 1995). The secondary objectives are development of the characteristic "oaty/toasted" flavours and reduction of oat grain microbial

contamination. Conventional oat processing includes conditioning with saturated (wet) steam and kiln drying (Molteberg et al. 1995). The application of wet steam increases moisture content and temperature of oat grain for faster inactivation of the lipolytic enzymes. Kiln drying of the grain is therefore needed in order to bring the grain moisture content down to levels acceptable for storage. This step requires a major energy input. The kiln drying ends with a cooling step during which ambient air is drawn through the grain mass, introducing microorganisms present in the air. The kiln drying is difficult to control and not energy efficient.

A potential technology for oat grain is to use superheated steam (SS) processing instead of the kiln drying. Processing with SS has been proposed over a century ago as a drying method and is often referred to as SS drying (Elustondo et al. 2002; Schwartze and Brocker 2002). Superheated steam can be used to dry moist materials due to its capacity to absorb moisture as long as the SS temperature stays above the saturation point for water at a given pressure. It has been reported that SS drying rates are faster than those of hot air under certain conditions (Chow and Chung 1983; Tang and Cenkowski 2000). As a result, much lower energy consumption may be achieved when SS is used as a drying medium (Mujumdar and Huang 2007). Superheated steam drying technology has been accepted in paper, lumber, and sugar-beet industries either because of better quality of materials processed with SS or better energy efficiency compared to hot-air drying (Jensen 1992; Svensson 1985; Urbaniec and Malczewski 1997).

Changes other than drying may occur in food materials exposed to SS, including changes in structure, texture, colour, starch gelatinization, and protein denaturation (Tang and Cenkowski 2000). Temperatures commonly used in SS processing vary between 100

and 200°C (at atmospheric pressure). Processing of temperature-sensitive materials at temperatures below 100°C but at reduced pressure has also been utilized (Devahastin and Suvarnakuta 2004; Thomkapanich et al. 2007).

It is possible that SS drying/processing of oat grain could be much faster than the conventional processing. Moreover, SS processing could address the aforementionaed problematic issues experienced in the kiln drying. Additionally, it is possible that the SS, because of its high heat intensity, can be used to reduce load or eliminate vegetative cells and spores of microorganisms on oat grain. Mould spores of *Fusarium* potentially present on oats are of primary concern and their elimination is desirable.

Before SS processing technology can be implemented in the industrial oat processing, the effects of SS on oat grain quality needs to be evaluated and compared to that of conventionally processed grain. This research is going to expand knowledge on the effects of SS on the functional properties of oat groat, bran, and flour, as well as on viability of highly heat-resistant bacterial spores of *Geobacillus stearothermophilus*. Viability of the former spores exposed to SS at 115-175°C was examined earlier using the same SS processing equipment and the results indicated low efficacy of SS at 115 and 121°C in the reduction of the spores (Henry 2007). Therefore, in this research viability of *G. stearothermophilus* spores was evaluated at high temperatures of SS (130-175°C), as well as at very low temperature of SS (105°C) due to its closest similarity to saturated steam. The spores of *G. stearothermophilus* are commonly used as a biological indicator to validate moist heat sterilization due to their exceptional resistance to this type of heat (Brown 1994; Carlberg 2005; Spicher et al. 1999). Elimination of the more heat resistant *G.*

stearothermophilus spores (after exposure to SS) would assure inactivation of less heat resistant *Fusarium* spores which may contaminate oat groats.

The main objectives of this research were: (a) to develop a novel technique for processing of oat groats using SS at 110-160°C and a velocity of 0.35 and 1.00 m/s; (b) to determine the effects of SS (at optimum and near-optimum conditions determined in the previous study; Cenkowski et al. 2006b) and conventional processing on the physicochemical and functional properties of large and small oat groats of of two oat cultivars varying in BG content, as well as on bran and flour fractions obtained upon roller milling of the heat-treated groats; (c) to examine the effects of SS and conventional processing on the solubility, viscosity, and molecular features of β -glucans isolated from oat bran fraction; (d) to establish the effects of SS at 105 and 130-175°C on viability of heat-resistant *Geobacillus stearothermophilus* ATCC 10149 spores at two inoculum levels (3 and 6 log₁₀ cfu/g); (e) to assess the viability of *G. stearothermophilus* spores exposed to SS at 105, 130 and 145°C with intermittent cooling periods; and (f) to model inactivation of *G. stearothermophilus* spores exposed to superheated steam.

The thesis was written in a publication-manuscript format but more details were included in the thesis than in the publications. Chapter 5 has already been published in a peer-reviewed journal (Head et al. 2008) and Chapters 3 and 6 were submitted to peer-reviewed journals in February 2009.

2 LITERATURE REVIEW

2.1 Oats

Cultivated oats are cereal grains which belong to genus *Avena* L. within the grass family of *Gramineae* (Mustafa et al. 1998; Webster 2002). In the early classifications, the common cultivated oat was listed as one of hexaploid species: *Avena sativa* (hulled oats), *Avena byzantina*, and *Avena nud*a (hulless or naked oats) (Coffman 1961). In late 1970's, a newer classification included the two latter designations in the *Avena sativa* taxa, and they are now classified as *Avena sativa* var. *nuda* (Baum 1977; Webster 2002).

Before the nineteenth century oat crop was widely accepted as animal fodder and its human consumption was limited to areas in Scotland and Ireland (Webster 2002). In the famous quote regarding oat grain Samuel Johnson described oats as "a grain which in England is generally given to horses, but in Scotland supports the people." Equally famous response coming from the Scotsman Lord Elibank (reported by Sir Walter Scott) was "True, but where can you find such horses, where such men?" (Murphy and Hoffman 1992; Webster 2002).

Oats ranked seventh in the world cereal grain production in 2007 and 2008 crop years, accounting for 25,577,000 and 26,191,000 metric tonnes (mt), respectively (USDA 2009). In Canada, oats ranked as fourth primary crop produced in both 2007 and 2008 crop years (4,696,000 and 4,270,000 mt, respectively) (Statistics Canada 2009; USDA 2009). The prairie provinces of Manitoba, Saskatchewan, and Alberta produce most of Canada's oats (Hoover et al. 2003). Majority of oats produced in Canada is used for domestic livestock feed. Significant amount of that grain is also processed in Canada or exported (AAFC 2004, 2006). About 90% of oats exported from Canada are produced in Manitoba and

Saskatchewan (AAFC 2006). Oat consumption in Canada has fluctuated around 2,000,000 mt since the 2003 crop year (AAFC 2006, Statistics Canada 2008). Although oats consumption is lower than that of wheat and rice, it has an advantage in that it is consumed as a whole-grain, components of which may work synergistically in the reduction of risk of chronic diseases (Sayar et al. 2006).

2.1.1 Oats Morphology and Chemical Composition

Oat grain comprises the hull, pericarp, testa, aleurone, starchy endosperm, and embryo (Zhou et al. 1998a). The hull typically constitutes 250–300 g/kg of the kernel weight depending on cultivar (Mustafa et al. 1998, Webster 2002). Removal of hulls from oat caryopsis yields oat groats (MacArthur-Grant 1986). Pericarp, testa and aleurone account for 9%, whereas endosperm and embryo account for 63 and 3% of the grain, respectively (Zhou et al. 1998a). Oat kernel is covered by hairs – hollow single-celled projections of the pericarp – called trichomes (Fulcher 1986). The trichomes break free during grain threshing and handling causing eye, skin, and respiratory irritations (Burrows 2005). There is a large variation in the amount of hairs among oat cultivars, with hulless (naked) oats cultivars usually showing exceptional abundance of the hairs (Burrows et al. 2001). Breeding lines with trichomes only pronounced at the distal end of the groat have been developed via gene controlling (Burrows et al. 2001; Fulcher and Rooney Duke 2002).

The chemical composition of oats is somewhat different from other cereals. Oats have higher lipid content but lower carbohydrate content than wheat, barley and rice (Zhou et al. 1998a). Protein content of oat grain is similar to that of wheat but usually higher than amounts found in other cereals (Zhou et al. 1998a).

Oat carbohydrates

Oat carbohydrates are represented by following categories: starch, non-starch polysaccharides (structural polysaccharides), and free sugars and oligosaccharides (Webster 2002). Starch is the most abundant component in oats making up to 60% of the dry matter of the grain, and is found predominantly in the endosperm (Hoover et al. 2003; Makarski and Achremowicz 2002; Peterson 1992; Zhou et al. 1998a). Oat starch consists of linear amylose (18-34%) branched amylopectin (30-58%) and intermediate materials (Wang and White 1994a; Zhou et al. 1998a). The intermediate materials are also called anomalous amylose or anomalous amylopectin because they show similarities to the classical starch components (Hartunian Sowa and White 1992; Webster 2002). The presence of the intermediate materials is a subject of a debate in the scientific community. Some researchers claim that the intermediate material obtained during heating of oat starch dispersions is simply a contamination of the amylose fraction with native or hydrolyzed amylopectin due to co-precipitation (Tester and Karkalas 1996). As oat starch swells, amylose and amylopectin are co-leached from the granule while in most other cereal starches amylose is preferentially leached (Doublier et al. 1987; Paton 1987).

Granules of oat starch are weakly birefringent, polyhedral and irregular in shape, do not fall into discrete size distributions such as A and B types as in wheat and barley, and have high lipid content (Paton 1986; Youngs 1978; Zhou et al. 1998a). The individual granules show tendency to form aggregates (Makela and Laakso 1984; Stevenson et al. 2007). Individual starch granule size ranges between 3 and 12 μ m, whereas the size of granule aggregates varies from 30 to 60 μ m (Paton 1986; Peterson 1992; Wang and White 1994b). Oat starch can not be easily separated from other components of the grain (Zhou et al. 1998a). Discrete protein bodies are attached to oat starch granules but do not form a continuous protein matrix (Zhou et al. 1998a). Oat starch may contain between 1 and 3% of lipids, in contrast to the amount of up to 1.2% reported for starches of wheat and corn (MacArthur and D'Appolonia 1979a; Hartunian Sowa and White 1992; Zhou et al. 1998a). The presence of lipids within starch granules in the form of free fatty acids and lysophospholipids (phospholipids that lack one of their fatty acyl chains) has been confirmed, with the latter one being the dominant form (Gibinski et al. 1993; Morrison et al. 1993; Wang and White 1994b). The starch internal lipids reside in the cavity of the amylose helix or in the spaces between amylose and amylopectin and may affect starch properties during gelatinization (Zhou et al. 1998a). Oat starch content and physicochemical properties appear to be strongly affected by genotype and to a smaller degree by growing environment (Gudmundsson and Eliasson 1989; Hoover and Senanayake 1996; Rhymer et al. 2005).

Oat cell wall polysaccharides are primarily β -glucans (BG) and arabinoxylans (sometimes referred to as pentosans), with the former accounting for 85% of the wall (Miller et al. 1995). Other cell wall constituents present in small amounts are cellulose, glucomannan, phenolic acids, and amino acids (Miller et al. 1995; Miller and Fulcher 1995). The BG are not reviewed here because they are the subject of the next section of this literature review. Arabinoxylans (the major component of pentosans) are present in cell walls of oat kernel (up to 4%) and hull (about 29%) and consist mainly of arabinose and xylose (MacArthur-Grant 1986). Small quantities of mannose, galactose and uronic acid in oat arabinoxylan have also been found (Welch 1995). Structurally, arabinoxylans are linear chains of $(1\rightarrow 4)$ -linked- β -D-xylopyranosyl (Xylp) residues substituted with α -L-

arabinofuranosyl (Araf) residues as side branches (Izydorczyk and Biliaderis 1995; Li et al. 2005; Rattan et al. 1994). Arabinose is attached to the xylan backbone predominantly via α - $(1\rightarrow 3)$ but also via both α - $(1\rightarrow 3)$ and α - $(1\rightarrow 2)$ linkages (Holtekjolen et al. 2006; Izydorczyk and Biliaderis 1995). A unique feature of arabinoxylans is the presence of ferulic acid covalently linked to some of arabinose residues (Izydorczyk and Biliaderis 1995; Li et al. 2005). Ferulic acid may participate in covalent polysaccharidepolysaccharide or polysaccharide-protein interactions within the matrix of the cell walls (Izydorczyk and Biliaderis 1995). There is a great deal of structural heterogeneity within cereal arabinoxylans. Ratio of arabinose to xylose (Araf/Xylp), content of ferulic acid, substitution pattern of arabinose, and molecular size of arabinoxylans vary between grains of different cereals and depend on tissue localization within a grain (Izydorczyk and Biliaderis 1995). Ratio of arabinose to xylose, indicative of the degree of branching in arabinoxylans, is about 0.3-0.7 in oats, 0.5-0.8 in barley and about 1.2 in wheat (Henry 1985). The low values of Araf/Xylp in oats, as compared to wheat, show low-branched structure of arabinoxylans and may indicate their weaker solubility in water (Virkki et al. 2005). In fact, it has been reported earlier that oat arabinoxylans are insoluble in water but they are soluble in 2-4% alkaline aqueous solution (MacArthur-Grant 1986).

Free sugars and oligosaccharides are present in oats in quite low amounts, usually around 1% in oat flour and up to 3% in oat bran, while reducing sugars make less than 0.1% of oat kernel (Makarski and Achremowicz 2002; Peterson 1992; Zhou et al. 1998a). Sucrose (disaccharide) and raffinose (oligosaccharide) are two predominant fractions of simple sugars present in groats (MacArthur-Grant 1986; MacArthur and D'Appolonia 1979b; Welch 1995).

Oat proteins

Whole oat groat contains 11-20% (dry basis, db) of storage protein, with its major fraction (up to 75% of total protein) represented by the salt-soluble globulin (Peterson and Brinegar 1986; Webster 2002; Zarkadas et al. 1995). In other cereals (except rice) the major protein fraction is represented by alcohol-soluble prolamins whereas in oats prolamins (avenins) account for ~10% of total protein. Water or buffer-soluble albumins are also found in oat kernels and they represent primarily enzymes from germ and aleurone (Peterson 1992). Oat groat residual proteins – dilute alkali- or acid-soluble glutelins – represent less than 5-10% of total proteins (Robert et al. 1985). Protein concentration in oats has been shown to be affected by genotypic and environmental factors, with the former being slightly more dominant factor (Doehlert et al. 2001; Ozcan et al. 2006, Peterson et al. 2005).

Bran and endosperm contribute respectively 49 and 45% of total proteins, with the remainder present in germ (Youngs 1972). Although 30-35% of oat germ is represented by protein, the relatively small size of this fraction causes an overall small contribution of germ proteins to total kernel protein content (Webster 2002; Youngs 1972). Bran in oat cultivars with higher than average protein content tends to contribute more to the total protein pool (Peterson 1992). Endosperm proteins in mature oat groat appear microscopically as roughly spherical bodies about 0.3 to 5.0 µm in diameter (Peterson 1992). In contrast, endosperm proteins of wheat and barley appear as a relatively homogenous matrix in which the starch granules are embedded (Fulcher 1986).

Oat globulin is an oligomeric protein with structural properties similar to those of the 11S globulins of soybean and other legumes (Siu et al. 2002; Zhao et al. 2004). High content of globulin is probably accountable for the better protein nutritive value of oats when compared with wheat, barley, rye and corn (Fulcher 1986; Lockhart and Hurt 1986). It is due to the better balanced amino acid profile of globulins as opposed to prolamins, with the latter being low in lysine (Nnanna and Gupta 1996; Peterson and Brinegar 1986).

Oat protein contains all essential amino acids. Some of them, however, are present in low quantities. The limiting amino acids are lysine, methionine, threonine, and tryptophan, although the lysine content is somewhat higher than that in wheat and rye (Lapvetelainen and Aro 1994; Welch 1995). Also, oat lysine content does not decrease with the increase in groat total protein content, as it is in most cereals (Wu and Stringfellow 1995). A small decrease (5-8%) in lysine availability after hydro-thermal processing and flaking used in production of oat flakes has been observed (Horvatic and Guterman 1997).

In addition to complex protein, oat groats contain free amino acids such as asparagine (~440 μ g/g of groat), glutamic acid (~250 μ g/g), aspartic acid (~100 μ g/g), histidine (~80 μ g/g), proline (~70 μ g/g), arginine (~60 μ g/g), and valine (~40 μ g/g) (Mustafa et al. 2007; Welch 1995). The bran shows higher amino acid concentration than the endosperm (Mustafa et al. 2007). The free amino acids of groats may take part in the Maillard reaction during thermal processing and influence the colour of the final product.

The good nutritional quality of oat protein has been the driving force for research on production of oat protein concentrates (Guan and Yao 2008). Initially, oat flour or ground groats have been used as the source for protein extraction (Ma 1983; Ponnampalam et al. 1987). Recently oat bran has been considered for that purpose as removal of bran proteins

increases the amount of β -glucans left in the residue enhancing the value of the β -glucans concentrate (Guan and Yao 2008; Guan et al. 2007; Nnanna and Gupta 1996).

Use of oats in diets of persons with celiac disease has been debated for half a century (Thompson 2003). In celiac disease patients follow a gluten-free diet strictly avoiding the α -gliadin – protein fraction of gluten of wheat, rye, barley, and triticale. Certain sequences of amino acids found in the protein trigger inflammatory disorder of the small intestine (Burrows 2005; Peraaho et al. 2004). Oats lack the harmful amino acid sequences or the sequences occur in very small quantities and moderate oat consumption appears to be safe for adults with celiac disease. Most celiac disease organizations remain sceptical about the inclusion of oats in the gluten-free diet mainly due to the risk of contamination of commercial oat products with wheat, rye or barley. Exceptions are countries like Finland and United Kingdom, where Celiac Disease Societies permit use of oats in the gluten-free diet if they are not contaminated with the aforementioned grains (Peraaho et al. 2004; Thompson 2003).

Oat lipids

Lipid content of oats varies typically between 5 and 9% (db) of the groat but content as high as 18% has been found in experimental oat selections (Peterson and Wood 1997; Webster 2002). The lipid amount present in oat groats is the highest among cereal grains (Youngs 1986). In comparison, grains of wheat, barley, rice, and rye contain 2.1-3.8, 3.3-4.6, 0.8-3.1, and 2.0-3.5% of free lipids, respectively (Youngs 1986). The lipids of oat are distributed throughout the groat which is different from wheat and corn grain, where the lipids are concentrated in the germ (Price and Parsons 1979; Webster 2002). More than 50% of oat kernel total lipid is deposited in the endosperm, and about 90% of oat lipids are

present in endosperm and bran combined (Kianian et al. 1999; Peterson and Wood 1997, Webster 2002; Youngs 1986; Zhou et al. 1998b). The lipids present in native oat kernel are divided into three categories: non-starch, starch-surface and starch-internal lipids (Gibinski et al. 1993; Liukkonen and Laakso 1992).

Majority of oat lipids are triglycerides accounting for 32-56% of total lipids (TL) (Youngs 1986). Other lipid classes found in oats include phospholipids (5-26% of TL). glycolipids (7-12% of TL), sterols (0.1-9.3% of TL), and free fatty acids (2-11% of TL) (Webster 2002; Youngs 1986). The phospholipids (mainly phosphatidylcholine) and glycolipids (mainly galactolipids) are mainly covalently bound lipids and can be extracted with polar organic solvents (Aro et al. 2007; Webster 2002). Free or unbound lipids (triglycerides, free fatty acids, sterols) can be extracted from grain by non-polar solvents such as ethyl ether petroleum ether or hexane (Youngs 1986). The large variation (almost ten fold) in reported levels of oat sterol is caused mainly by differences in solvent extraction system used (Webster 2002). β-Sitosterol is the most abundant fraction (40-70%) of oat sterols, regardless of the extraction technique used (Jiang and Wang 2005; Maatta et al. 1999; Webster 2002). Other sterols found in oats have been identified as avenasterol (Γ^5 and v^7 -avenasterol), campesterol, and stigmasterol (Jiang and Wang 2005; Maatta et al. 1999; Webster 2002). It has been shown that addition of Γ^5 -avenasterol to heated sovbean oils slowed down the oils deterioration due to the sterol antioxidant properties (Kahlon 1989; White and Armstrong 1986). Food products i.e. margarines enriched with cereal phytosterols are available on the market in many countries (Kerckhoffs et al. 2002; McIntosh and Jacobs 2002). Such foods offer some protection against heart disease via a reduction in plasma cholesterol by the phytosterols (Kerckhoffs et al. 2002). Phytosterols

are more hydrophobic than cholesterol and therefore they show greater affinity for micells than cholesterol. Displacement of intestinal cholesterol from the micells by phytosterols reduces the intestinal absorption of cholesterol. This causes a compensatory increase in endogenous synthesis of cholesterol which results in a decrease of the pool of circulating cholesterol (Kerckhoffs et al. 2002). Phytosterols have also been linked to inhibition of development of chemically induced colon cancer in rodents (Maatta et al. 1999; McIntosh and Jacobs 2002).

Structural components of oat lipids, long chain fatty acids (FA), are represented mostly by palmitic (C16:0, 16-22%), oleic (C18:1, 28-40%), and linoleic (C18:2, 36-46%) acids (Erazo-Castrejon et al. 2001; Youngs 1978, 1986). Linoleic acid is also the major FA in oat glycolipids (Aro et al. 2007). Palmitic acid is a saturated FA whereas oleic and linoleic are unsaturated FA. These three fatty acids comprise about 95% of all FA of oats (Youngs 1986; Zhou et al. 1998b). The remaining 5% of FA is represented by myristic (C_{14:0}), stearic $(C_{18:0})$, and linolenic $(C_{18:3})$ acids (Kahlon 1989; Youngs 1986). Some of the fatty acids found in oats, such as linoleic acid, are essential for human nutrition. The unsaturated fatty acids (about 80% of all FA) are subject to oxidation which contributes to rancidity development during storage (Makarski and Achremowicz 2002; Youngs and Puskulcu 1976). Interesting relationship between oat lipid content and fatty acid composition was observed by Welch (1975) and by de la Roche et al. (1977). They discovered that an increase in the total lipid content was associated with a decrease in palmitic and linoleic acids and an increase in oleic acid. Additionally, increased lipid content of oat grain was correlated with an increase in the proportion of triglycerides and a decrease in the proportion of phospholipids. Distribution of FA in the three major oat lipid classes (triglycerides, glycolipids and phospholipids) may explain the aforementioned trend. There is more oleic but less palmitic acid in triglycerides than in glyco- and phospho-lipids. With increased lipid content of oats there is a relatively greater increase in the proportion of triglycerides over the glyco- and phospho-lipids. This results in greater amounts of oleic and lesser amounts of palmitic acids (de la Roche et al. 1977; Welch 1975; Youngs 1986). It is important to note that fatty acid composition is greatly influenced by genetic factors (over 70% of total variation) and to a lesser degree by growing environment (Zhou et al. 1998b). It is also expected that growing temperature affects the degree of unsaturation in oat lipids, i.e. greater degree of unsaturation in cooler climates (Youngs 1986).

Polyunsaturated fatty acids are not created in the human body in the course of metabolic changes and need to be included in the diet (Pijanowski et al. 1996a). Food products with high ratio of mono- and poly-unsaturated to saturated fatty acids are desirable for human nutrition. In oats this ratio is about 2.2:1 whereas recommended ratio is at least 1:1 (Lockhart and Hurt 1986; Webster 2002). The high level of polyunsaturated fatty acids in oats makes them nutritionally beneficial. It also adversely affects the flavour and quality of stored oats because the unsaturated fatty acids become substrates for lipolytic enzymes present in raw oats (de la Roche et al. 1977). First, glycerides are hydrolyzed by lipase with release of free fatty acids (Matlashewski et al. 1982). Then, lipoxygenase and lipoperoxidase convert the unsaturated fatty acids to hydroperoxides and hydroxy acids (Robinson et al. 1995; Youngs 1986). Lipoxygenase may also catalyze co-oxidation of lipid-soluble vitamins, such as vitamin E (Bryngelsson et al. 2002a; Robinson et al. 1995). The enzymatic action of lipase, lipoxygenase, and lipoperoxidase in unprocessed oats makes the grain vulnerable to a bitter taste and fast rancidity development during storage

(Ekstrand et al. 1992; Lehtinen et al. 2003). Hydro-thermal processes such as steaming, kiln drying or drum drying are performed in traditional oat processing to inactivate the fatfragmenting enzymes and to prolong shelf life of oat products (Deane and Commers 1986; Ganssmann and Vorwerck 1995). Majority of lipase (80%) is present in the outer bran layers of oat kernels and it has been reported that wet scrubbing or abrasion of mature oat grain results in a substantial loss of the enzyme (Martin and Peers 1953; Sahasrabudhe 1982; Urquhart et al. 1983).

Lipase activity in dry, un-germinated grains of most cereals is usually very low or nonexistent and increases dramatically after the start of germination (Peterson 1999; Urguhart et al. 1983). On the contrary, active lipase is found in mature un-germinated oat grains (Liukkonen et al. 1993; Urquhart et al. 1984). The rate of the hydrolysis is slow in dry intact grain and it may continue for several months (Heinio et al. 2002; Vahvaselka et al. 2006). Oat lipids are rapidly hydrolyzed by lipase when groats are crushed, milled or damaged because the lipase-rich outer layer is brought into contact with endosperm lipids and oil bodies in bran (Liukkonen et al. 1992a, 1993; Peterson 1999). The lipid hydrolysis reaction is instantaneous if groats are in contact with water (Vahvaselka et al. 2004; Youngs 1986). This occurs for example during wet fractionation of oat grain and results in substantial increase in free fatty acids levels (Liukkonen et al. 1995). Inhibition of lipase via adjustment of pH of oat flour aqueous suspensions from neutral to slightly alkaline (pH 8-9) solves the problem (Liukkonen et al. 1995). In fact, a protein-rich oat fraction obtained by such alkaline extraction of oat fiber has been shown to reduce oxidation rate of free micellar linoleic acid in aqueous suspension containing soybean lipoxygenase-1 (one of three isoenzymes of soybean lipoxygenases) (Robinson et al. 1995). Monomolecular form of linoleic acid is the preferred form of the substrate for lipoxygenase-1 thus reduction in concentration of linoleic acid in the monomolecular form reduces the oxidation rate. The proposed mechanism of this reduction probably involves trapping of the acid by cyclodextrin or stabilization of micelle structures (Lehtinen and Laakso 2000).

Oats high lipid level, considerable proportion of linoleic acid in total fatty acids and high endogenous lipase activity have caused interest in using oats as a substrate in production of conjugated linoleic acid (CLA) (Vahvaselka et al. 2004). Conjugated linoleic acid is the name of a group of positional and geometrical isomers (cis-9, cis-12) of linoleic acid. It is an essential fatty acid for humans and can be found in meat and dairy products such as milk, butter, yoghurt and cheese (Leger et al. 2007). Potential anticarcinogenic, antiatherogenic, immune-enhancing and weight-reducing properties of CLA have raised an interest in creating its supplement for human nutrition (Scimeca and Miller 2000). A novel microbial process for production of CLA has been utilized with the use of whole oat flour (Vahvaselka et al. 2004, 2006). Production of CLA from oats was done in two stages. Firstly, linoleic acid was released from lipids of non-heat treated oat flour slurries due to lipase action. Then, it was efficiently converted to CLA by resting (non-growing) cells of Propionibacterium freudenreichii ssp. shermanii via isomerization reaction (Vahvaselka et al. 2004). Excess water in oat slurries was required in order to obtain efficient enzyme action. The main concern with that was a possibility of growth of microorganisms other than the propionibacterium and possibly toxin formation. Microbial safety of the process was improved later by performing the lipid hydrolysis step at water activity (a_w) of 0.7 which created unfavourable conditions for proliferation of oat indigenous microflora without adverse effect on the progress of hydrolytic reaction (Vahvaselka et al. 2006).

Oat cultivars with high lipase activity can be utilized as natural bioreactors for hydrolysis of fats and oils to fatty acids and glycerol (Lee and Hammond 1990). Attempts of using moist oat kernels as lipase bioreactor for freeing of fatty acids from triglycerides have been reported (Lee and Hammond 1990; Parmar and Hammond 1994). Moistened oat groats were immersed in oils and the mix was gently agitated at 40°C. Lipase present in the outer layers of the groats released fatty acids to the oil phase whereas over 90% of glycerol released in the reaction accumulated inside the groats (Lee and Hammond 1990).

The high contents of lipids and unsaturated fatty acids make oats a capable dietary lipid supplement for dairy cows (Fearon et al. 1996). Milk fat composition is altered when a supply of fatty acids with 18 carbon atoms is delivered to the mammary gland. As a result of that milk fat having increased proportion of long chain unsaturated fatty acids is produced, which in turn produces a softer, easier to spread butter (Fearon et al. 1996). The presence of increased proportion of unsaturated FA in the milk fat could shorten its storage stability due to more rapid oxidation of the unsaturated FA. This issue has been addressed by Fearon et al. (1998) who compared the oxidative stability of the milk fats obtained from cows fed naked oats or barley based diets. The milk fat from cows offered diets based on oats had a significantly longer oxidation induction period than the milk fat obtained from cows fed the control (barley based) diet. A transfer of larger quantities or more diverse forms of antioxidants from the naked oat diets to the milk fat in comparison with the barley diets was proposed to be the reason for the better oxidative stability of milk fat coming from oat based diets. Content of α -tocopherol was similar in both diets and was excluded as being the only contributor to the better stability of milk fat originating from oat diet. A transfer of other antioxidant compounds (phenolics) from oat based diets but not the barley based diets to the milk fats was hypothesized (but not determined) to be the explanation for the observed differences in the oxidation induction period of the milk fats.

Oat phytochemicals

It is believed that free radicals trigger the initiation phase of degenerative diseases such as cancer, heart disease, arthritis (Miller et al. 2000). Antioxidant compounds of dietary origin may provide protection to the body from the chronic diseases and from aging (Adom and Liu 2002). The antioxidants function as free radical scavengers, complexers of prooxidant metals, reducing agents, and quenchers of singlet-oxygen formation (Adom and Liu 2002; Bryngelsson et al. 2002b; Emmons et al. 1999; Kahkonen et al. 1999). Also, high levels of endogenous antioxidants in foods contribute towards protection from rancidity and preservation of colour and taste (Skoglund et al. 2008). Oats are a good source of phytochemicals which exhibit antioxidant activity (Handelman et al. 1999; Oomah and Mazza 1999; Sun et al. 2006). Oat antioxidant properties when used in food packaging were proposed as early as the 1930's (Youngs 1986). Oat flour (marketed as Avenex and Aveno) was previously utilized commercially in prevention of oil rancidity in foods (Gray et al. 2000). Many of oat antioxidants belong to the group of phenolics, including free and bound phenolic acids, avenanthramides, and flavonoids (apigenin, luteolin, and tricin) (Bryngelsson et al. 2002b; Peterson 2001; Peterson et al. 2005). In addition to the phenolics, other entities showing antioxidant activity in oats are tocols (compounds with vitamin E activity), phytic acid, and as mentioned earlier the sterols (Peterson et al. 2001; Shahidi and Naczk 2004a).

Oats antioxidant activity is largely due to phenolic compounds as a high correlation between total phenolic content and antioxidant activity of oats has been indicated (Emmons
et al. 1999). Phenolic compounds are defined as substances with an aromatic ring bearing one or more hydroxyl groups, including their functional derivatives (Shahidi and Naczk 2004a). Ferulic acid is the major phenolic acid present in oats in free and bound forms, with the reported total content range of 75 to 300 mg/kg (Peterson 2001; Shahidi and Naczk 2004a; Webster 2002). The wide range reported reflects not only differences in samples (cultivar and environmental factors) but also differences in extraction methods used (Welch 1995). Other phenolic acids and their esters identified from oats are caffeic, *p*-coumaric, *p*hydroxybenzoic, vanillic, syringic, and sinapic (Webster 2002; Xing and White 1997). It is worth noting that ferulic, caffeic and *p*-coumaric acids inhibit low-density lipoprotein oxidation and may therefore reduce the risk of atherosclerosis (Dimberg et al. 2001; Emmons et al. 1999).

The major portion of phenolics in cereals (in oat grain ~75%) is insoluble bound through a covalent association with cell wall materials but the existence of free, soluble conjugated phenolics have also been reported (Adom and Liu 2002; Gray et al. 2002; Liu 2007). On the contrary, phytochemicals of fruits and vegetables are usually present in free or soluble conjugate forms as glycosides (Adom and Liu 2002; Zdunczyk et al. 2006). Grain cell wall materials are not easy to digest and may reach the colon undigested. Colonic bacteria ferment such materials releasing the bulk of bound phenolics which may be then absorbed, hence the health benefits of bound phenolics are retained (Adom and Liu 2002; Sidhu et al. 2007).

Phenolic compounds characteristic to oats are avenanthramides and avenalumic acids (Miller et al. 2000). Avenanthramides are *N*-cinnamoyl derivatives of anthranilic acid (Collins 1989; Emmons and Peterson 2001). Avenalumic acids are substituted bound phenolic acids which are the ethylenic homologues of p-coumaric, ferulic, and caffeic acids (Collins et al. 1991). Avenanthramides and avenalumic acids are present in oat grain and the hull (Peterson et al. 2002). Concentration of avenanthramides, ranging from 200 to 800 mg/kg of bran-rich fraction, has been shown to be significantly affected by genotype and growing environment (Collins 1986; Emmons and Peterson 2001). The most abundant avenanthramides are 2p, 2c, and 2f, where the 2 indicates 5-hydroxy-anthranilic acid substituted with: p-coumaric (p), caffeic (c), and ferulic (f) acids, respectively (Skoglund et al. 2008). The three avenanthramides are reportedly related to the fresh taste of oat products (Molteberg et al. 1996b). Stability of avenanthramides in thermally processed foods has been studied and it appears that these compounds retain their antioxidant activity after the application of heat (Dimberg et al. 2001).

Lipid-soluble tocols are entities that exhibit biological activity of vitamin E and are represented by four homologues each (α , β , γ , and δ) of tocopherol and tocotrienol (Aro et al. 2007; Peterson 2001). Oat grain contains 17-32 mg/kg of tocols which is less than the range reported for barley (42-80 mg/kg) (Peterson and Qureshi 1993). Tocotrienols are predominantly located in the endosperm, whereas tocopherols are concentrated in the germ (Peterson 1995). Genotypic and environmental factors have been shown to affect oat tocol concentration (Peterson and Qureshi 1993). The predominant tocols in oats are α tocotrienol and α -tocopherol (Peterson and Qureshi 1993; Welch 1995). The two α homologues contribute up to 90% of the grain total tocols but minor amounts of β -, γ tocopherols, and β -tocotrienols have also been detected (Shahidi and Naczk 2004b; Youngs 1986; Welch 1995). A strong correlation (R^2 =0.83) between oil concentration and tocotrienol (but not tocopherol) concentration has been found in oat genotypes with oil content ranging from 7 to 18% (Peterson and Wood 1997). Similarly, Swedish oat cultivars with 6-13% lipid content showed a similar trend in tocotrienol concentration (Bryngelsson et al. 2002b). Recent investigation of oil bodies isolated from oat aleurone and germ confirmed their intrinsic association with tocols (White et al. 2006). Tocols inhibit peroxidation of lipids in biological membranes by donating phenolic hydrogen atoms to free radicals. Thus they may serve as oily food stabilizers and a source of vitamin E in humans and livestock (Peterson et al. 2007). Also, tocotrienols are inhibitors of serum cholesterol biosynthesis in humans and experimental animals (Peterson et al. 2005).

Raw, green groats and flours show very high antioxidant activity which is reduced after a heat treatment (Emmons et al. 1999; Zadernowski et al. 1999). The reduction of antioxidant capacity after heat application indicates that the antioxidant capacity may be affected but is not completely destroyed. Caffeic, ferulic and p-coumaric acids are susceptible to thermal breakdown, with the caffeic acid being the most heat-sensitive (Huang and Zayas 1991). Apart from destruction of some of antioxidants, heat processing may change the ratio of various compounds due to thermal degradation (Dimberg et al. 1996). For example, vanillin and vanillic acid can be produced through thermal decomposition of ferulic acid whereas p-hydroxybenzaldehyde can be formed from pcoumaric acid (Dimberg et al. 1996). Effects of hydro-thermal processes, such as steaming and autoclaving, on the concentration of selected oat antioxidants have been investigated by Bryngelsson et al. (2002a). Steaming of oat groats did not affect levels of tocopherols and some of the avenanthramides. Autoclaving of grains caused an increase in the levels of tocopherols and tocotrienols but it caused a decrease in avenanthramides and elimination of caffeic acid.

Like in most cereal grains, oat antioxidants are contained predominantly in the bran and germ fractions (Miller et al. 2000). High concentration of phenolic compounds has been observed in the cell walls of the aleurone layer of oat kernel (Fulcher 1986). Oat is usually consumed as a whole-grain cereal hence retention of antioxidant-rich bran ensures the retention of potential health benefits (Chen et al. 2004; Peterson 2001). A production of oat fractions enriched in antioxidant active components via dry milling and sieving technology has been proposed (Gray et al. 2000). The increase in antioxidant activity of bran-rich fractions as compared to endosperm-rich fractions ranged between 60 and 257%, depending on the oat sample analyzed. Recently, a combination of extraction, air-classification, and microwave treatment has been investigated in production of oat bran concentrate was used as the starting material and a substantial enhancement of antioxidant activity as well as phenolics content (roughly doubled) was seen after the bran suspended in 50% ethanol was microwaved at 150°C for 10min.

Oat vitamins

Oats are a source of most water-soluble B vitamins and significant amounts of previously mentioned lipid-soluble vitamin E (Welch 1995). Water soluble thiamin, niacin, and folate exist in concentrations large enough to make a valuable dietary contribution (6-9, 8-15, and 30-60 mg/kg of oat kernel, respectively) (Lockhart and Hurt 1986; Webster 2002; Welch 1995). Other vitamins found in oat grain include riboflavin, pantothenic acid, pyridoxine, and biotin (Peterson 1992; Webster 2002). Outer bran layer and germ tissues are most probable locations of the B vitamins (Webster 2002). Inclusions of protein bodies in the aleurone layer of oats are the exclusive locations of niacin which is associated with

carbohydrate polymers and aromatic amines (Fulcher et al. 1981). Niacin location within the tough aleurone cell walls as well as its chemical associations with other components may render the niacin nutritionally unavailable for humans (Peterson 1992). This issue represent an area for future consideration.

Oat minerals

Minerals found in oat groats are the typical cereal grain minerals and include phosphorus, potassium, magnesium, calcium, sodium, manganese, iron, zinc, and copper (Peterson 1992; Webster 2002). Larger amounts of minerals are found in bran than in endosperm of groats (Peterson et al. 1975). Phosphorus is the most prevalent element in oat groat, with amounts of 290-550 mg/100g reported (Peterson et al. 1975; Skibniewska et al. 2002; Welch 1995). However, about 60-70% of it is present in the form of phytic acid (myo-inositol hexaphosphoric acid) (Garcia-Estepa et al. 1999; Webster 2002). Phytic acid has a strong ability to bind multivalent metal ions forming insoluble complexes (phytates) which lowers the absorption of phosphorus and phosphorus-bonded minerals (Garcia-Estepa et al. 1999; Skibniewska et al. 2002). Selenium, barium and boron in trace amounts have also been found in groats (Demirbas 2005; Peterson et al. 1975; Webster 2002). Concentration of trace elements in grain depends to some degree on fertilization techniques used (Srikumar and Ockerman 1991). It has been shown that inorganic fertilization was more efficient than manuring in increasing oat groat contents of copper, zinc and iron. The uptake of selenium and manganese, however, was more effective when manure was used rather than the inorganic fertilizer (Srikumar and Ockerman 1991).

High mineral content of oat bran and the fact that oats are often consumed as whole grains make them a significant source of minerals in human nutrition (Ekholm et al. 2003).

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On the other hand, the presence of anti-nutritional agents in oats such as previously mentioned phytic acid or phytate and polyphenols are known to reduce the bioavailability of certain minerals such as iron, calcium, magnesium, manganese and zinc (Greiner and Larsson Alminger 1999; Larsson and Sandberg 1995). It is important to note that phytate shows anticancerogenic effect via binding of metals like copper and non-heme iron, which can catalyze production of free radicals (McIntosh and Jacobs 2002; Plaami and Kumpulainen 1995). The phytate is mostly concentrated in the outer bran layers of grains and its amount can be reduced by activation of naturally occurring enzyme - phytase (Fredlund et al. 1997; Garcia-Estepa et al. 1999; McIntosh and Jacobs 2002). Phytase hydrolyzes phytate to myo-inositol and inorganic phosphates via intermediate myo-inositol phosphates (penta- to mono-phosphates), increasing mineral availability (Fredlund et al. 1997; Larsson and Sandberg 1992). The activity of phytase in oats is believed to be somewhat lower than in wheat and rye (Sandberg and Svanberg 1991). Additionally, heat treatment applied to commercial oat products decreases the already low phytase activity (Greiner and Larsson Alminger 1999). The problem of low phytase activity can be addressed by a minimal thermal processing and/or by use of additional processes (i.e. germination) (Wilhelmson et al. 2001). Germination process is sometimes used to improve texture (to soften) and flavour of cereals (Wilhelmson et al. 2001). A slow hydrolysis of starch, an increase in the lysine and tryptophan contents, and a slight decrease in prolamine content take place during germination (Peterson 1998; Wilhelmson et al. 2001; Wu 1983). Furthermore, germination has been proven to increase the activity of phytase in cereals (Larsson and Sandberg 1992; Peterson 1998; Sandberg and Svanberg 1991). Germinated oat groats with 79% reduction in phytate content were produced in a pilot-plant scale

process developed by Larsson and Sandberg (1995). Ekholm et al. 2003 investigated whether an addition of exogenous phytase and one of natural chelating agents (citric and malic acids, glucose) to diets containing oat bran would increase solubility of mineral elements to even greater degree. Out of the three chelating agents tested, citric acid was the most effective in increasing minerals solubility after the phytase treatment. The increase in the solubility depended on the mineral tested and was significant for Ca, Mg, Zn, and Mn, moderate for Fe, and negligible for K.

Degradation of β-glucans (BG) by endogenous β-glucanase and an increase in free fatty acids (FFA) due to germination have been observed in oats (Peterson 1998; Wilhelmson et al. 2001). BG breakdown in oat grain is not desirable if the grain is used for production of novel human foods in which the functional properties of β-glucans are of key importance. Reduction of molecular weight of BG due to the breakdown results in lowered viscosity of the polymers and may decline physiological effects in humans (Luhaloo et al. 1998). A short germination process (72 h at 15°C terminated with oven drying) suitable to obtain a whole grain oat product with retained BG content has been developed by Wilhelmson et al. (2001). In that process, 55-60% of BG was retained when compared with native oat and no substantial loss in molecular weight of the polymers was observed. Germination conditions (high moisture content and usually elevated temperatures) favour growth of microorganisms present on grain therefore it is of great importance to monitor the microbiological safety of products that underwent the germination process (Wilhelmson et al. 2001).

Another group of compounds in oats that deserve a brief mention are saponins. They contain a steroid or triterpenoid aglycone linked to one or more sugar chains (Webster

2002). Avenacoside A and B are two steroid saponins that have been isolated from oats (Tshesche et al. 1969; Tshesche and Lauren 1971). Oats saponins content ranges between 0.02 and 0.05% (db) of kernel and they are located mainly in the endosperm (Onning et al. 1993). In the plant saponins are supposed to have antibiotic effects (Onning et al. 1993). Bitter taste and enhancement of cell membrane permeability are some of properties associated with saponins (Onning et al. 1993; Webster 2002). The higher concentration of saponins in oat endosperm than in bran is somewhat surprising. The saponins supposed antibiotic effect would suggest that more saponins should be present in the outer parts of the kernel (Onning et al. 1993). Oat saponins physiological effects such as lowering of serum cholesterol, influencing carbohydrate digestion and intestine permeability in rats and gerbils have been investigated (Onning and Asp 1995a, b; Onning et al. 1996). The *in vitro* test results showed some increase in starch hydrolysis and improved rate of molecule transport through the intestine walls. The *in vivo* tests, however, did not confirm the positive findings and further research is needed to clarify the physiological effects of oat saponins.

2.1.2 Oat β-Glucans

Oat dietary fibre, represented by non-starch polysaccharides and lignins, is a mixture of soluble and insoluble fractions, with relatively high concentration of the soluble fraction as compared with other cereals (Manthey et al. 1999). The main components of the soluble fraction are β -glucans (BG). Oats groats contain 2-7% (db) of BG (Gajdosova et al. 2007; Roubroeks et al. 2000; Wood 2007). Some waxy (low amylose) barley genotypes may contain up to 10-11% of BG, rye may have up to 2%, and small amounts of BG (<0.5%) can be found in wheat (Bhatty 1993; Lee et al. 1997; Wood 2007). Content of total and soluble BG in oat is controlled by genes but is also affected by environmental factors such

as precipitation and soil nitrogen levels (Cervantes-Martinez et al. 2001; Kibite and Edney 1998; Welch et al. 1991). Genotype has generally stronger influence on BG content than environment, although genotype/environment interaction has been found to be significant in some cases (Givens et al. 2000; Lim et al. 1992; Miller and Fulcher 1994; Saastamoinen et al. 1992). The ratio of soluble to total content of BG, however, is not usually affected by genotype or by environment (Lee et al. 1997).

β-Glucans are found in the aleurone and endosperm cell walls of oat groat (Angelov et al. 2005; Luhaloo et al. 1998; Skendi et al. 2003). Distribution of BG in oat and barley kernels in cultivars of varying total BG content (3.7-6.4 and 2.8-11%, for oat and barley, respectively) was studied by Miller and Fulcher (1994). They reported that in oat cultivars of low BG content the subaleurone layer (the junction of the endosperm and the aleurone layer) was the primary area of BG deposition and as the total BG content of groat increased more BG was seen throughout the central endosperm. They concluded that differences in the endosperm cells size (smaller cells in high BG cultivar) and thickness of their walls (thicker walls in high BG cultivar) accounted for the different amounts of BG found in groats of these cultivars. Additionally, kernels of all oat cultivars had some of BG deposited in the depleted layer adjacent to the embryo. In comparison, barley kernels showed more uniform distribution of BG throughout the kernels regardless of the total BG concentration, with the highest levels found in the endosperm.

β-Glucans are linear polysaccharides composed of $(1\rightarrow 4)$ and $(1\rightarrow 3)$ -linked β-Dglucopyranosyl residues organized predominantly in a sequence of β- $(1\rightarrow 3)$ -linked cellotriosyl and cellotetraosyl units (Bell et al. 1999; Izydorczyk et al. 1998; Lazaridou and Biliaderis 2007). Chain links of the $(1\rightarrow 4)$ type comprise 70% of all chain links (Kim and

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Inglett 2006; Wood 2004). Additionally, there is an evidence of a minor amount of cellulose-like sequences with consecutive $(1\rightarrow 4)$ linkages longer than the tetraose type (Wood 2007). In oat, molar ratio of the cellotriosyl to tetraosyl units has been determined as 1.5-2.4 (Lazaridou and Biliaderis 2007; Wood 2004, 2007). In contrast, values of the molar ratio in other cereals range between 3.0-4.5, 1.8-3.5, and 1.9-3.0 for wheat, barley and rye, respectively (Lazaridou and Biliaderis 2007).

Molecular weight of oat BG polymers vary between 2.7×10^4 and 3×10^6 g/mol, with majority of values falling between 1 and 2×10^6 g/mol (Aman et al. 2004; Lazaridou and Biliaderis 2007; Roubroeks et al. 2000; Wood 2007). Differences in molecular weight of these polymers extracted from American and Swedish oat cultivars were reported (Ajithkumar et al. 2005). The Swedish cultivars contained BG of lower molecular weight than did cultivars of American origin (1.49 and 1.58 $\times 10^6$ g/mol, respectively). Molecular weight seemed to depend on the environmental factors more so than on the genotype . Another source of large variation in BG molecular weight maybe related to the isolation procedures and determination methods employed (Roubroeks et al. 2000).

Water solubility, viscosity and gel forming properties are displayed by BG and depend on the polymers molecular features such as distribution of cellulose-like sequences, linkage pattern, and molecular weight but also on concentration and temperature (Ajithkumar et al. 2005; Lazaridou and Biliaderis 2007; Rimsten et al. 2003).

2.1.3 Oat β-Glucans Health Effects

The health effects attributed to BG consumption in humans have been documented extensively (Whyte et al. 1992; Wood 2007). Physiological effects such as a reduction of serum total and low-density lipoprotein (LDL) cholesterol levels and a decrease in postprandial glucose and insulin levels following consumption of oat BG extract or oat bran have been reported (Anderson et al. 1991; Braaten et al. 1991; Karmally et al. 2005; Pomeroy et al. 2001; Queenan et al. 2007; Wood et al. 1994; Wu et al. 2006). In 1997 the US FDA approved a health claim related to oat bran as cholesterol-lowering food at a dose of 3 g of BG per day if it is a part of diet low in saturated fat and cholesterol (FDA 2001; Jenkins et al. 2002; Lovegrove et al. 2000).

Greater extent of the cholesterol-lowering effect has been found in subjects with hypercholesterolemia (Anderson et al. 1984; Brown et al. 1999; Maier et al. 2000). Similar physiological effects have been observed in rats and hamsters (Delaney et al. 2003; Kahlon et al. 1993; Lund et al. 1989; Rieckhoff et al. 1999; Yokoyama et al. 1998). The favourable reduction in the rise of blood glucose level that is normally observed after a meal helps in control of type II (non-insulin dependent) diabetes and lessens number and severity of complications arising from excess glucose in the blood (Pick et al. 1996; Wood et al. 1990). Other health benefits arising from BG consumption include: delay of intestinal transit and gastric emptying, increase of the fecal excretion of bile acids (BA), and promotion of the colon beneficial microflora growth (Angelov et al. 2005; Frank et al. 2004; Lia et al. 1995; Manthey et al. 1999; Queenan et al. 2007; Sudha et al. 2007). The delayed gastric emptying of ingested foods into the small intestine results in a sensation of fullness, often referred to as "stick to your ribs" property, and may help in weight control (Malkki and Virtanen 2001; Queenan et al. 2007; Webster 2002). Impediment of dietary cholesterol absorption as well as inhibition of bile acids reabsorption from the GI tract have been studied extensively (Peterson 1992; Roubroeks et al. 2000). Bile acids are acidic steroids synthesized from cholesterol in the liver (Kahlon and Chow 2000). There are several proposed mechanisms

of cholesterol level reduction and increased bile acids excretion from human gastrointestinal tract caused by oat BG (Brown et al. 1999; Sayar et al. 2005). The most commonly proposed hypothesis is direct binding of BA by BG and their subsequent excretion which diminishes BA reabsorption in terminal ileum (Dongowski 2007; Kahlon and Chow 2000; Kahlon and Woodruff 2003). This in turn results in increased synthesis of BA from the free cholesterol and a decrease in the total cholesterol body pool (Brown et al. 1999; Dongowski 2007; Kahlon and Chow 2000; Kerckhoffs et al. 2003; Queenan et al. 2007). Other suggested mechanisms of inhibition of the reabsorption of BA include: increased viscosity of intestinal contents (Dongowski 2007; Kerckhoffs et al. 2003; Sayar et al. 2005); inhibition of hepatic fatty acids synthesis by products of fermentation (Brown et al. 1999); and binding between BG and micelles formed from BA and fatty acids rather than the BA alone (Sayar et al. 2005). It has also been suggested that the BA binding by oat flour involves synergistic action of BG and lignin (Sayar et al. 2006). Fibre polysaccharides degraded in the gastrointestinal (GI) tract into shorter oligosaccharides which then serve as source of carbon for intestinal bacteria in colonic fermentation (Kontula et al. 1998). The major products of that fermentation are short-chain fatty acids (SCFA), namely acetate, propionate and butyrate (Karppinen et al. 2000; Lebet et al. 1998). The production of the SCFA results in a decrease in pH, which creates an environment preventing the growth of harmful bacteria and aids in absorption of minerals such as calcium and magnesium (Brassart and Schiffrin 2000; Sayar et al. 2007). It has been reported that oat BG concentrate fermented by simulated intestinal fermentation system which contained human fecal inoculum produced acetate and propionate concentrations similar to the concentrations produced by fibres like innulin and guar gum but increased amounts of butyrate (Queenan

et al. 2007). One of benefits of the increased butyrate production in the colon is the reduction of the risk of the organ's cancer development (Johansson et al. 1998; Kontula et al. 1998). Hydrogen – another product of colonic fermentation of fibre – results in undesirable gastrointestinal side effects such as flatulence and in some cases diarrhoea which is why some people remain unenthusiastic of oat consumption (Behall et al. 1998; Hallfrisch and Behall 2003).

Reports on the effect of oat BG on blood pressure in humans have been inconsistent. There have been some reports on the link between oat BG consumption and improvement (decrease) of the blood pressure in hyperinsulinemic patients medicated for essential hypertension (Pins et al. 2002). Other studies suggest that any cardio-protective benefits (i.e. blood pressure decrease) of regular dietary intake of BG may be a result of a combination of dietary factors including weight loss due to the consumption of fibre rich foods and should not be related only to the consumption of a single food component (i.e. BG) (Davy et al. 2002).

Another interesting health benefit of oat BG is the ability of activation or enhancement of defence mechanisms against bacterial and parasitic infections in animals and humans. It is believed that the BG immunostimulation happens primarily due to macrophage activation (Yun et al. 2003).

The effectiveness of BG physiological properties appears to be strongly related to the polymers viscosity which in turn is dependant on the molecular weight, structure and concentration (Beer et al. 1997; Brennan and Cleary 2005; Doublier and Wood 1995; Tietyen et al. 1995; Tosh et al. 2003; Wood et al. 1994). The molecular weight of oat BG itself does not appear to be the critical factor determining the physiological response when

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in human GI tract. The effect of consumption of yeast-leavened oat breads with varying BG molecular weights on human blood concentrations of lipids, insulin, and glucose was examined by Frank et al. (2004). The results of their investigation suggested that the molecular weight of BG present in oat bread consumed as a part of routine diet did not play an important physiological role in moderately hypercholesterolemic humans. In another study, on contrary, hypocholesterolemic effect of BG was enhanced in rats fed diets containing high molecular weight BG (intact oat bran) compared to the effect of diets containing low molecular weight BG (enzyme treated oat bran) (Tietyen et al. 1995). The decrease in the hypocholesterolemic potential of the β -glucan in the enzyme treated bran was attributed to a reduction in its viscosity as compared with the viscosity of the intact BG. It has also been suggested that the source of BG, the matrix of food product containing BG, and/or the product processing, rather than just the polymer molecular weight, may influence the cholesterol-lowering properties of BG from oat bran (Gallaher et al. 1999; Kerckhoffs et al. 2003; Romero et al. 1998). Findings of Kerckhoffs et al. (2003) showed that an orange juice containing BG preparations from oat bran had higher cholesterol-lowering potency than bread and cookies containing the same BG preparations.

There is no agreement in the scientific community on the amount of oat BG a person needs to ingest daily in order to lower blood cholesterol pool effectively. Some researchers and the US FDA recommend consumption of four servings per day, each containing at least 0.75 g of soluble fibre (total of 3 g/day) (Braaten et al. 1994; FDA 2001; Hallfrisch et al. 2003; Karmally et al. 2005). Other studies suggest that consumption of 3 g/day results in modest reductions of cholesterol and amounts higher than 3 g/day are needed for a significant hypocholesterolemic effect to occur (Lovegrove et al. 2000; Pomeroy et al.

2001; Ripsin et al. 1992). The positive physiological effects of BG seem to level off at about 10 g/day dose and further increase in consumed amount does not yield a linear response in lowering of serum cholesterol levels (Brown et al. 1999; Wood 2007). Dose response may differ among different subject groups and depend upon baseline level (Maier et al. 2000; Wood 2007). Finally, oat BG intake itself is merely one element of ones diet and presumably other dietary modifications, i.e. lower saturated fat and cholesterol intake and higher intake of antioxidants, should be incorporated in order to observe health benefits listed above (Brown et al. 1999; Whyte et al. 1992). To put the above mentioned dose amounts in perspective – the standard serving size for oatmeal is 40 g and should provide at least 4% db (or 3.6% wet basis, wb; based on 10% moisture content of rolled oats) BG soluble fibre according to the FDA recommendation (Webster 2002). This means that about 2.1 standard servings of oatmeal per day (88 g₃) would be necessary to assure consumption of 3 g of BG per day.

A high content of BG in human diet may be desirable due to its well documented health promoting effects whereas in animal feed low BG content may be preferable (Demirbas 2005; Welch et al. 1991). High BG content in feed increases viscosity in animal intestines and slows down digestibility reducing the feed nutritive value and efficiency (Holtekjolen et al. 2006). Additionally, sticky faeces in poultry fed high BG feed have been reported (Svihus et al. 1995).

2.1.4 Microflora of Oat Grain

Microflora present on grains in general consists of two groups of microorganisms. The first group is composed of microorganisms that normally occupy the plant. Examples include Gram-negative aerobic or facultative anaerobic rods *Erwinia (E. herbicola)*,

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Pseudomonas (P. herbicola), Xanthomonas, Flavobacterium, moulds *Cladosporium, Alternaria* and less frequently yeasts i.e. *Candida* (Ray 2004). Plant may also be infected with field fungus belonging to *Fusarium* genera. Microorganisms in the second group are the microorganisms that are deposited on grain due to contact with soil, air, water, insects, birds, and agricultural practices (i.e. use of untreated sewage). This group is much differentiated and organisms like Gram-negative Escherichia coli, Enterobacter, Grampositive Sarcina, Enterococcus, Bacillus (B. subtilis, B. cereus), Staphylococcus, and storage fungi *Penicillium* and *Aspergillus* may find their way to settle on grain (Wilhelmson et al. 2001). In the case of oats processing, ambient air used for cooling of the grain, may deposit even more microorganisms on it. Zhang et al. (1997) quantified microbial population present on oat groats and found a range between 22 and 1300 microorganisms

Contamination of oat grain with spores of *Fusarium* is a major health concern (De Nijs et al. 1996). The mould produces mycotoxins which may put adult consumer health at serious risk but may jeopardize an infant's life (Weidenborner et al. 1997). Mycotoxins are active secondary metabolites produced by fungal spores (De Nijs et al. 1996). Some of the mycotoxins are carcinogens, others cause toxicity of organs. Ingestion of food that is infested with moulds, and therefore may contain mycotoxins, causes mycotoxicosis in animals and humans (Pohland and Wood 1987; Tanaka et al. 1988). People consuming grain infested with toxigenic strains of *Fusarium* species may develop alimentary toxic aleukia (ATA) (Pomeranz et al. 1990; Ray 2001b). The most common mycotoxin produced by *Fusarium* moulds is deoxynivalenol (DON, vomitoxin). Other mycotoxins associated with *Fusarium* include nivalenol (NIV), T-2, HT-2 toxin, and zearalenone (De Nijs et al. 1996; Pohland and Wood 1987). Campbell et al. (2000) quantified levels of several mycotoxins in oats and barley crop samples from eastern Canada collected between 1991 and 1998. Oats contamination with DON was less frequent and less severe than that of barley. Nevertheless, 47% out of 73 oat samples contained DON at concentration (mean of the 8 years) of 0.33 mg/kg. Additionally, 15% out of 26 oats samples contained NIV (amount not quantified). A spectrum of *Fusarium* toxins has been found in infected grain of Finnish hulled and naked oat cultivars, and included: DON, NIV, HT-2 and T-2 toxins in amounts (as sum of all four) ranging from 70 to 413 μ g/kg (0.07-0.413 ppm) grain dry weight (Peltonen-Sainio et al. 2001).

Presence of *Fusarium* spores on oat groats used for production of flour utilized later as a thickener of infant formula could cause a serious health problems, including death. Therefore it is of tremendous importance that the oat flour used for production of infant foods has a superior quality. Additionally, oat grain infection with fungal pathogens causes groat discolouration which makes the groats unacceptable for food use. Species of *Fusarium*, *Microdochium* and *Alternaria* have been determined to be the primary cause of oat groat discolouration in a few oat cultivars in the United Kingdom between year 1997 and 2000 (Newton et al. 2003).

The US FDA has indicated a link between vomitoxin intake and outbreaks of acute gastrointestinal illness in humans (Giese 2000b). The DON level at 0.5 parts per million (ppm) was reported to cause problems. Advisory levels for the mycotoxin in food and feed were therefore set by the FDA (Giese 2000b). The limits of mycotoxins in foods considered "safe" for consumption have been changed by governing authorities many times over the last two decades. Before 1993 the level of concern was set at 2 ppm of DON in whole grain

but after 1993 the level of concern was decreased to 1 ppm of DON for finished flour products (Shaner 2003). In North America, amounts of DON present in food products are strictly regulated. Guidelines of 1 and 2 ppm of DON in un-cleaned soft wheat destined for use in non-staple foods for infants and adults, respectively, have been set by the Canadian government (Charmley et al. 1994).

The health risks associated with potential presence of *Fusarium* spores on grain put pressure on processors as they try to eliminate the fungal spores from the processed grain. Hot air, traditionally used to dry oats, is not capable of elimination of the fungal spores. There is also a risk of microbial cross-contamination of the grain while it is being cooled with ambient air at the end of the kiln drying. Superheated steam, due to its high heat capacity and processing temperatures usually higher than those used in hot-air drying, has a potential to largely reduce microbial load present on oat grain. Research in this area is needed to make such a conclusion.

2.2 Conventional Oat Processing

Raw oats become rancid in a relatively short period of time due to the presence of a relatively high content of lipids with a high degree of unsaturation and active lipolytic enzymes. Rancidity and off-flavours released due to the action of those enzymes render the grain unsuitable for human consumption. Therefore, the major step in oat processing is a hydro-thermal treatment, whose main purpose is to inactivate oat endogenous enzymes and prolong storage timeline of oat products (Ganssmann and Vorwerck 1995; Webster 2002).

2.2.1 Processing Steps

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General steps in the industrial processing of oats are: cleaning, drying and toasting of oats or groats (dehulled oats), dehulling, cutting, steaming and rolling to make flakes, and grinding to make flour and/or bran (Burnette et al. 1992). Usually the drying operation . precedes the dehulling stage because the dried hulls become brittle and therefore easy to remove from the groats (Lawrie 1999). Some processors, however, prefer to dry oat grain after the dehulling operation because it is believed that the raw groats are tougher and do not break up as much during the dehulling (Deane and Commers 1986).

The first step in oat processing is grain cleaning, with the use of several types of separators and aspirators (Lawrie 1999). Commonly used separators include: disc and indented cylinders, width sizer with slotted cylinders, gravity, and paddy separators (Deane and Commers 1986). During the cleaning operation, impurities such as sticks, stones, foreign seeds, and dirt are removed along with undesirable oat kernels. The latter include "double oats" - in which the hull also covers a secondary grain, "pin oats" - very thin with little or no groat, and "light oats" - of normal size but with little or no groat, which are then sold as an animal feed (Hoseney 1986). Cleaned and size graded oats are then heat-treated or dried.

The drying of oats can be done in a pan, rotary steam tube, or radiator column (kiln) dryer, with the latter one being currently the most popular (Webster 2002). The kiln dryer is a few storeys high rectangular-shaped column with several heating and one cooling section. Grain flows from the top (inlet) to the bottom (outlet) of the kiln, with the flow being controlled for a proper retention time and throughput (Webster 2002). Saturated steam is injected onto the grain at the kiln inlet, which increases grain moisture content from ~12-14% to ~17-18% and grain temperature from ambient to ~85°C. The increase in moisture

content of grain aids in more efficient inactivation of heat labile fat-splitting enzymes (Ganssmann and Vorwerck 1995; Youngs 1986). A matrix of steam radiators mounted across the dryer heats the grain further, up to ~102-105°C in the first heating section and to 88-98°C afterwards (Webster 2002). Air is pulled through the grain after each heating section in order to remove moisture. Oats temperature and moisture content decrease gradually as they reach the bottom of the kiln where they are cooled rapidly with ambient air drawn through the grain mass. There is a risk of cross-contamination of grain with microorganisms present in the air used for cooling. In total, oats retention time in the kiln is ~1.5-2.0 hours, and the final temperature and moisture content of grain at the bottom of the kiln are ~20°C and ~9.5%, respectively (Ganssmann and Vorwerck 1995).

As mentioned earlier, the main reason for carrying out the hydro-thermal processing of oats is the inactivation of majority of the fat-splitting enzymes to prevent development of rancidity during storage (Bryngelsson et al. 2002a). Final inactivation of the fat-splitting enzymes is achieved during steaming which is performed just before flaking of groats. In addition to the inactivation of enzymes, however, kilning develops the desirable characteristic "oat taste", brings about starch gelatinization to a certain degree, facilitates rolling of groats into flakes, and if done properly, helps in reduction of grain microflora (Bryngelsson et al. 2002a). Finally, the drying causes brittleness of the hulls (if performed before the dehulling operation) and allows their easier removal (Bryngelsson et al. 2002a; Deane and Commers 1986; Hoseney 1986; Lawrie 1999).

Oat dehulling (removal of hulls from oats) is performed in an impact or friction dehuller (Lawrie 1999). Oats are fed into a high-speed spinning rotor which then expels them onto the inside wall. The groats are freed from the hulls as they hit the inside wall of the dehuller. A rubber lining mounted to the inside wall of the machine helps in the separation and reduces groat breakage (Doehlert and McMullen 2001; Ganssmann and Vorwerck 1995; Hoseney 1986). The hulls are removed by aspiration and then used as a high fibre feed ingredient (Deane and Commers 1986). The oat groats remaining after the dehulling are about 75% of the total weight of oats (Lawrie 1999). After the dehulling, sound oat groats are subjected to polishing, aspiration, grading, and separation. A portion of that product remains as whole oat groats, whereas the remainder is cut into steel-cut oat groats (Lawrie 1999).

The steel-cutting operation is performed in rotary granulators, which cut the groats into 2-4 pieces (Burnette et al. 1992). Groats are fed into a rotating drum perforated with countersunk holes. As the groats align themselves in the holes and fall through them, they are being cut by stationary knives arranged around the outside of the drum (Webster 2002). The number of pieces produced per groat is controlled by adjustment of the rotation speed of the drum (Webster 2002). The steel-cut groats are made primarily for the subsequent production of quick and baby flakes or instant oats, depending on the size of the pieces used (Burnette et al. 1992; Lawrie 1999; Western and Graham 1961). Instant oats are produced from "instantized" steel-cut groats. A proprietary commercial process applied to groats before the cutting results in rapid cooking of the flakes made of the "instantized" steel-cut groats (Ganssmann and Vorwerck 1995; Webster 2002). Thickness of instant oat flakes is in the range of 0.279-0.457 mm (Webster 2002).

Flattening of whole groats between pressurized heated rolls produces rolled oats also called whole grain flakes (Ames and Rhymer 2003; Ganssmann and Vorwerck 1995). Flakes varying in thickness (0.508-0.914 mm) are produced by adjusting the gap between

the flaking rolls (Webster 2002). The thicker flakes maintain integrity for longer periods than the thinner flakes but they also require longer cooking period (Webster 2002). The flaking operation is preceded by a steaming operation during which groats are held in the steamer for 15-30 min at \sim 104°C (Webster 2002). The steaming brings about further starch gelatinization, makes the groats more flexible and helps to inactivate any remaining enzymes that cause rancidity (Bryngelsson et al. 2002a; Hoseney 1986). Flakes are then cooled and packaged for storage.

Grinding of whole or steel-cut groats, flakes, or middlings (fines, pieces knocked off groats during processing) results in whole oat flour, or a combination of low bran oat flour (endosperm rich fraction) and oat bran (bran-rich fraction) (Burnette et al. 1992; Lawrie 1999). Oat flour, unless otherwise specified, refers to whole-grain flour (Lawrie 1999). Hammer, pin, and roller mills are used for grinding (Deane and Commers 1986; Webster 2002). Ground material is directed onto a sieve with wire mesh for granulation control. Flour granulation depends on the starting material and the screens used in the mill (Ganssmann and Vorwerck 1995). Flour obtained from flakes tends to be finer than flour obtained from groats (Webster 2002). The high lipid content of oat grain makes it necessary to draw large quantity of air through the impact type mill to keep screens from plugging and to minimise heat build-up in the mill (Ganssmann and Vorwerck 1995; Webster 2002).

Oat bran is obtained by grinding of whole groats or rolled oats and separation of the resulting flour by sieving, bolting, or other suitable means (Burnette et al. 1992; Webster 2002). The soft texture and high fat content of oat groat do not permit the complete removal of the starchy endosperm from bran particles as it is in the case of wheat bran (Webster 2002). For that reason American Association of Cereal Chemists (AACC) defines oat bran

as: "... the food which is produced by grinding clean oat groats or rolled oats and separating the resulting oat flour by sieving bolting, and/or other suitable means into fractions such that the oat bran fraction is not more than 50% of the original starting material and has a total beta-glucan content of at least 5.5% (dry-weight basis) and a total dietary fiber content of at least 16.0% (dry-weight basis), and such that at least one-third of the total dietary fiber is soluble fiber..." (Anonymous 1989; Fulcher and Miller 1993). The bran fraction is enriched in the outer parts of the groat, especially subaleurone layer with thick cell walls rich in β -glucan (Aman et al. 2004). Wood et al. (1991b) reported an increase (by a factor of 1.5) of β -glucan concentration in oat bran (bran yield of 53%) in comparison to the concentration in whole groat. Design of traditional oat milling process and soft texture of oat kernel do not allow production of germ-rich fraction (Webster 2002).

2.2.2 Oats Products

Conventional oat products

The main traditional oat products include rolled oats, steel-cut groats, quick oat flakes, baby oat flakes, instant oats, oat bran, and oat flour (Webster 2002). The industrial use of whole oat groats is small in comparison with those of rolled oats and milled products (Burnette et al. 1992). Oat flakes are used for preparation of hot cereal, oatmeal, which is still very popular hot breakfast cereal in North America and United Kingdom (Webster 2002). They are also used for production of cold breakfast cereal and variety of ready to eat (RTE) products such as granola bars (Webster 2002). Bakery goods varying in texture can be produced by addition of varying the type oat flakes used, as larger flakes produce more uneven texture and smaller flakes make a smoother product (Webster 1986).

Oat products are often used as ingredients or additives in bread and cookies production or as thickeners in sauces, gravies and infant formulas (Burnette et al. 1992; Webster 2002). Oat bran is a common ingredient of breakfast cereals and nearly all flour-based products, including breads, muffins, crackers, pancakes, and cookies (Burnette et al. 1992; Sudha et al. 2007). Oat bran is often added to wheat breads, biscuits, muffins, cookies, and other bakery goods in order to increase dietary fiber intake among humans (Krishnan et al. 1987; Sosulski and Wu 1988; Sudha et al. 2007). The use of rigid bran particles, however, may disturb the formation of gluten-protein matrix in bread (Degutyte-Fomins et al. 2002). Sourdough fermentation has a potential in improving the structure of fibre-rich breads via hydration of bran particles (Degutyte-Fomins et al. 2002; Katina et al. 2005). Oat flour is sometimes added to wheat flour in bakery items due to oats excellent moisture retention properties (Burnette et al. 1992). Bread made of the mixture of wheat and oat flour stays fresher for longer periods of time. Water-binding capability of oat polysaccharides (Bglucans and arabinoxylans) contributes to the retained moistness and freshness of breads (Holtekjolen et al. 2006). Generally, as the amount of oat flour incorporated into wheat dough increases the resulting bread volume decreases (Oomah 1983). This happens because oat proteins do not possess the viscoelastic properties characteristic of wheat gluten (Flander et al. 2007; Oomah 1983). However, tasty bread with good texture and volume has been obtained by using whole grain oat flour and wheat flour at a ratio of 51:49, respectively, and supplemented with gluten (Flander et al. 2007). A portion of that bread (two slices ~30 g) has slightly higher amount of β -glucans (0.78 g) than the FDA required amount of 0.75 g per serving. Inclusion of oat crude oil or oil polar fraction into wheat breads, to replace or lessen the amount of normally used shortening, has been investigated

by Erazo-Castrejon et al. (2001). Bread formulations with added oat oil exhibited extended shelf life and increased loaf volume compared to breads made with shortening only.

Novel oat products

The findings of many human health benefits arising from oat BG intake and the FDA recommendations of consumption of at least 3 g of BG per day have stimulated research on creation of food products or additives that are rich in this fibre (Carriere and Inglett 2000; Lyly et al. 2003). Although the widely used milled oat products, oatmeal, flakes and flour remain the primary commercial materials, other functional preparations have been produced (Lee et al. 2005a). Oatrim, Nutrim, and Z-Trim (the last one obtained from oat hulls) are just some examples of patented novel products in which BG are the principal components of reported biological activity (Behall et al. 1998; Hallfrisch et al. 2003; Kim and Inglett 2006; Lee et al. 2005a). The suffix 'trim' is an acronym for 'technical research involving metabolism'.

Oatrim – one of the first oat functional ingredients became manufactured in 1991 and in 2003 the US FDA approved it for a heart healthy label claim (Lee et al. 2005a). It is prepared by α -amylase hydrolysis of oat starch in flour or bran to a specific level of amylodextrins without destroying its BG content (Inglett 1993; Lee et al. 2005a). Content of BG in Oatrim varies between 5 and 10% by weight but the polymers content as high as 24% by weight has also been reported (Carriere and Inglett 1999; Inglett and Newman 1994; Lee et al. 2005c). Reduction of plasma cholesterol levels in hypercholesterolemic hamsters was seen after dietary intake of Oatrim (Yokoyama et al. 1998). Similar results have been reported in human studies (Carriere and Inglett 2000; Xu et al. 2006).

Nutrim, which is a jet-cooked oat bran (or barley flour), is a hydrocolloidal extract produced via thermo-mechanical shear-based process rather than an amylase hydrolysis only (Carriere and Inglett 2000; Lee and Inglett 2006). During the wet extraction process bran fibre components are removed from cooked grain and processed flours. The reduction of cellulose components results in formation of hydrocolloids that can be used as substitutes for high fat components of foods such as frozen desserts, baked goods, meats and salad dressings (Carriere and Inglett 2000; Lee et al 2005c; Stevenson et al. 2007). As a result, substantial reductions of not only fat content but also energy intake, as well as increase in soluble fibre intake can be achieved (Hallfrisch and Behall 2003; Hallfrisch et al. 2003). Content of BG in some Nutrim products is higher than the content of Oatrim, but usually Nutrim contains 10% db of β -glucan (Carriere and Inglett 2000; Lee and Behall 2003; Hallfrisch et al. 2003).

Recently, new oat hydrocolloidal ingredients, called C-trims (C stands for calorie), have been prepared by steam jet-cooking and fractionation of oat bran (Lee et al. 2005b). The Ctrims contain anywhere between 15 and 50% of BG and can be used to control the texture and rheology of foods. Evaluation of C-trims potential use in cake and cookie baking was carried out by Lee et al. (2005a, b). It was concluded that a replacement of regular flour with up to 10% of C-trim20 (containing 20% BG) in cookie dough produced cookies with instrumental texture properties and sensory characteristics similar to those of control samples (Lee et al. 2005b). In the case of cake batters, replacement of about 20% of regular flour with the C-trim20 resulted in increased elastic properties of the batters and produced cakes with volume and textural properties similar to those of control cake. The incorporation of C-trim20 in cake batters resulted in cakes containing 1 g of BG per serving which would allow the use of FDA-recognized health claim on food labels (Lee et al. 2005a).

A product called Soytrim is obtained by blending of oat bran (or Nutrim) and soybean flour (Inglett et al. 2003). It has been shown that Soytrim can improve the nutritional value of some Asian foods by reducing saturated fat and increasing soluble fiber contents without negatively affecting their sensory characteristics (Inglett et al. 2003).

Oat starch but also non-starch polysaccharides are being considered for use in soups, sauces, and similar products because of their texture-controlling properties (Gibinski et al. 2006; Sikora et al. 2007). Texture of meat-based foods is often controlled by addition of large amounts of fat, which is not desirable due to current concerns about obesity and diseases related to high-fat diets of North Americans (Yang et al. 2007). Again, oat products have the potential to be used as fat-replacers and formulation stabilizers due to their good water binding properties (Tsai et al. 1998). Improved texture and flavour attributes of low fat pork sausages made with hydrated oatmeal or tofu at 10, 15, and 20% (w/w) addition levels have been demonstrated by Yang et al. (2007). Based on sensory evaluations the greatest overall acceptability of the low fat sausages was attained with the 15% addition level of either of the agents. Dawkins et al. (2001) was successful in obtaining acceptable goat and rabbit patties to which oat β -glucan (extracted from bran) or Oatrim were added in amounts ranging from 0.5 to 2.0%. These non-traditional sources of meat tend to be problematic in making sausages and patties due to their poor binding ability. The good hydration behaviour of the two oat products added to the patties made it possible to create a product with acceptable nutrient content and favourable sensory scores, especially at the 2% level of addition (Dawkins et al. 2001).

Incorporation of oat BG concentrate into low-fat white-brined cheese has been recently investigated (Volikakis et al. 2004). When compared to control (low-fat cheese without BG) the cheeses made with two levels of BG concentrate (0.7 and 1.4% w/w) had higher yield, extent of proteolysis, and levels of short chain fatty acids production during cheese ripening. However, the colour, flavour and overall impression scores of BG fortified cheeses were poorer than those of a typical (full-fat) white-brined cheese product.

Yoghurt-like products based on oat substrates (rolled oats, fiber concentrate, and wholegrain flour) fermented by lactic acid bacteria strains (Lactobacillus sp. and Bifidobacterium sp.) have been developed (Angelov et al. 2005, 2006; Bekers et al. 2001; Martensson et al. 2002). The BG included with oat medium used in such creations becomes a prebiotic for the probiotic bacterial cultures by stimulation of their growth (Jaskari et al. 1998). Recently developed oat-based medium which includes probiotic Bifidobacterium strains is an example of functional food products targeting the elderly population (Laine et al. 2003). In this population levels of bifidobacteria colonization of the intestinal microflora are reduced (Gavini et al. 2001). In order to serve their purpose, probiotics need to adhere well to intestinal mucus of the GI tract. Inclusion of Bifidobacterium probiotics in milk-based products is a common practice. The performance of these probiotics in milk, however, reduces the in vitro adhesion to human intestinal mucus (Ouwehand et al. 2001). Adhesion of thirteen strains of bifidobacteria to elderly human colonic tissue was not negatively influenced by the oat-based medium used and in case of two Bifidobacterium strains a significant increase of adhesion was observed (Laine et al. 2003).

Replacement of cow's milk fat in yoghurts with oat maltodextrin containing 5% of BG (obtained by enzymatic hydrolysis of ground oat grain) has been successfully attempted by

Domagala et al. (2006). Sensory quality of yoghurts made of non-fat milk with added either maltodextrin or milk fat was assessed and compared to that of yoghurt made of non-fat milk without additions (control). The yoghurts which contained maltodextrins or milk fat exhibited better sensory quality than the control youghurt. Moreover, no significant differences in sensory analysis, rheological properties, and most of the texture parameters (cohesiveness, gumminess, hardness, and adhesiveness) were found between yoghurts made with maltodextrin or milk fat.

The main challenge in creation of products enriched with BG is ensuring that the perceived sensory quality is acceptable to consumers (Lyly et al. 2003). Inclusion of high molecular weight BG in beverages may be preferred for enhanced physiological functionality but it may negatively influence the product viscosity. Inversely, low molecular weight BG may affect the sensory quality to a smaller degree than the high molecular weight polymer but the physiological efficacy may be compromised. Lyly et al. (2003) examined the effect of concentration and molecular weight of different oat BG preparations on the perceived sensory quality of a beverage prototype. The beverages made with high molecular weight BG were more viscous and had higher perceived thickness than the beverages made with low molecular weight preparations. Additionally, it was easier to add the low molecular weight BG preparations into a beverage in amounts sufficient for achieving the level recommended by the FDA for health benefits. Consumers' acceptability and factors affecting customers' willingness to use a novel beverage and ready-to-eat frozen soup containing oat BG were later determined by Lyly et al. (2007). The latter investigation revealed that sensory quality, especially the taste, influenced the willingness to consume the beverage and the soup stronger than the health claims associated with the inclusion of BG in these products.

An oat-based bio-refinery for production of various value-added products including BG and protein concentrates, lactic acid, lipase-active powder, anti-irritant concentrate, and light and dark oat oils has been recently developed (Koutinas et al. 2007). Diverse processing techniques involved in the bio-reactor include but are not limited to pearling, air classification, solvent and alcoholic extraction, enzymatic hydrolysis, centrifugation, fermentation.

Non-food oat products

Oats soothing properties on various skin irritations resulting in itchiness have long been recognized in folk medicine (Murphy and Hoffman 1992). Whole oat flour and flakes have been used in cosmetics for many years (Burrows 2005). Oat antioxidants, including the avenanthramides are believed to be the "anti-itch" factor (Burrows 2005). Soaps containing colloidal oatmeal are widely available and are popular among individuals with sensitive skin. Oat β -glucans are added to surgical dressings in order to promote wound healing (Burrows 2005). Air classified fine oat flour or starch have been used as a base for cosmetics like eye shadow, or as replacement of talcum powder in body powders and corn starch in latex gloves (Burnette et al. 1992; Burrows 2005).

Recently, oat and rice processing wastes (hulls) have been evaluated as novel sources of high-quality amorphous silica (SiO₂) which can be used to prepare silicon compounds and siliceous materials (Zemnukhova et al. 2006). The silica yield was lower in oat hull than in rice hull (3-5 and 12-20% db, respectively) but both sources gave high-purity silica.

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2.3 Superheated Steam Processing

Superheated steam (SS) drying is one of novel methods being considered for replacement of hot-air drying. Although the principle of drying materials with SS has been known for over a century, only in the recent years its use is gaining interest (Mujumdar and Huang 2007). Superheated steam has been successfully used in drying of non-food related products such as paper, lumber, and coal (Douglas 1994; Jensen 1992; Svensson 1985).

In case of food and bio-products, the exposure to SS causes not only drying but may also bring about other changes such as starch gelatinization, enzyme destruction, protein denaturation, colour and texture changes, and deodorization (Devahastin and Suvarnakuta 2004; Tang and Cenkowski 2000, 2001). Therefore the term 'superheated steam processing' rather than just 'superheated steam drying' is often preferred when SS is used for heat treatment of foods.

2.3.1 What is Superheated Steam?

Superheated steam, also known as dry steam, is generated when additional sensible heat is delivered to saturated (wet) steam, raising the steam temperature above the corresponding saturation (boiling) point at a given pressure. Superheated steam can be used to dry moist materials due to its capacity to absorb moisture as long as the SS temperature stays above the saturation point for water. When SS comes in contact with a material it transfers heat to the product by loosing some of the SS sensible heat. The amount of heat lost by SS does not result in condensation of the steam, providing that the SS temperature is still higher than the saturation temperature at the processing pressure. This can be achieved by balancing the amount of SS used in processing with respect to the drying rates (van Deventer and Heijmans 2001). However, during the initial period of processing with SS, the temperature difference between the material and the SS is large enough to decrease the SS temperature below the saturation point and to cause a temporary condensation on the surface of the material (Iyota et al. 2001b; Pronyk et al. 2004; van Deventer and Heijmans 2001). Continuous delivery of heat energy from SS allows removal of that condensate at rates depending on steam temperature (i.e. higher rates at higher SS temperatures).

Moisture evaporated from the material is carried away by SS which makes the SS both the heat source and the drying medium (van Deventer and Heijmans 2001). Recycling of the drying medium can therefore be accomplished if additional sensible heat is added. That way energy consumption of SS drying/processing system can be minimised. Additionally, excess vapour resulting from the drying process is produced and can be used elsewhere in a processing plant. Aly (1999) reported on combining SS dryer and a water desalination unit in a milk powder producing plant.

Superheated steam processing at atmospheric pressure is not suitable for thermal treatment of heat-sensitive foods and bio-products (Pimpaporn et al. 2007). An alternative for processing of such products is to use SS at low-pressure (sub-atmospheric pressure) (Devahastin and Suvarnakuta 2004; Thomkapanich et al. 2007).

2.3.2 Benefits of Superheated Steam Processing

The SS processing/drying technique exhibits many potential benefits to the industry and to the consumer. The most often reported (Tang et al. 2000, 2005; van Deventer and Heijmans 2001) benefits include:

 improved energy efficiency – energy savings as high as 50-80% over use of hot air or flue gases;

- air-free environment no combustion or oxidative reactions yielding in enhanced quality of products and reduction of fire and explosion hazards;
- reduced environmental impact closed system design allows exhaust (condensate) reuse;
- concurrent blanching, sterilization, deodorization, and pasteurization as well as drying of food products;
- possibility of operating at pressures higher or lower than atmospheric.

Wide commercial acceptance of any novel technology has to be warranted by the benefits clearly outweighing the drawbacks. More research is therefore needed in the area of understanding the effects of SS on quality parameters (physical, chemical, microbial, and nutritional) of food products.

2.3.3 Foodstuffs Processed with Superheated Steam

Much of the research conducted on SS processing of foods has been focused on the drying aspect of the process (Elustondo et al. 2002; Iyota et al. 2001b; van Deventer and Heijmans 2001). Drying kinetics and modelling of the SS drying of foods and bio-products such as Asian noodles (Markowski et al. 2003), parsley (Martinello et al. 2003), shrimp (Prachayawarakorn et al. 2002), potato (Iyota et al. 2001a; Tang and Cenkowski 2000), rice (Soponronnarit et al. 2006), sugar-beet pulp (Tang et al. 2000), and distillers' and brewers' spent grain (Tang et al. 2005; Tang and Cenkowski 2001) have been reported. Some studies, however, also investigated various changes that occur in foods and bio-products as a result of SS processing. Some of these changes may be characteristic only to SS processing and may or may not be desirable from the point of view of a consumer.

Impingement SS drying/processing has been investigated as a potential replacement of the deep-fat frying of potato and tortilla chips with varying degree of success (Caixeta et al. 2002; Li et al. 1999; Pimpaporn et al. 2007). The deep-fat frying is often used in production of snacks such as potato chips and instant Asian noodles (Caixeta et al. 2002; Pronyk et al. 2008). Consumers growing concerns over increasing occurrences of obesity, however, force the food processors to find technologies that could lower caloric content of snack foods without sacrificing the sensory attributes commonly associated with them. Pimpaporn et al. (2007) examined effects of different pre-treatments on drying kinetics and quality of potato chips undergoing SS drying at pressure lower than the atmospheric. Blanching and freezing pre-treatments followed by SS processing at 90°C were found to be the most favourable conditions for making good quality chips.

Taechapairoj et al. (2004) characterized pasting and thermal properties of rice processed with SS at 150-170°C and 3.1 m/s in a fluidized-bed dryer at depths of 10-15 cm. Their work was undertaken to investigate feasibility of using SS processing to replace steaming and drying operations performed after soaking in conventional making of parboiled rice. Use of higher temperatures of SS and thinner bed depths caused greater extent of starch gelatinization, with the steam temperature having more profound effect. Faster and greater degree of starch gelatinization is desirable because it increases head rice yield. Additionally, higher processing temperatures and thinner bed depths caused darkening (yellowing) of the paddy kernels especially at 18% (db) moisture content of the kernels. Peak and breakdown viscosities of rice processed with SS were lower than those of raw rice. It is important that amount of paddy kernels with low degree of starch gelatinization (referred to as white belly) is limited. Less than 1.5% of paddy kernels processed in SS had the white belly and it was concluded that there is a potential for superheated steam processing in making parboiled rice.

Soponronnarit et al. (2006) further investigated the use of SS fluidized-bed dryer for production of parboiled rice on a pilot scale (100 kg/h capacity). They were able to produce parboiled rice in 4-5 min compared to 3-4 hours in conventional processing. Both technologies require soaking of paddy kernels before hydro-thermal treatment, and it was found that no white belly appeared when soaking time of 7-8 hours was used. Parboiled rice water adsorption, whiteness, and viscosity of rice flour, decreased when higher SS temperatures were used (a range of 120-160°C was tested). Hardness, however, increased with higher steam temperatures used. Rice processed with SS at 130°C showed similar pasting properties to those of rice obtained from conventional processing.

SS processing has been investigated for use in reduction of deoxynivalenol, a *Fusarium* mycotoxin, in naturally contaminated wheat kernels (Pronyk et al. 2006). Wheat kernels were exposed to SS temperature of 110, 135, 160, and 185°C, velocity of 0.65, 1.3, and 1.5 m/s, and processing times of 2-15 min. Velocity had no effect on DON reduction in samples. The two highest steam temperatures (160 and 185°C) were effective in significant reduction of DON, with up to 52% reduction achieved at 185°C and 6 min processing time.

2.4 Mathematical Modelling

Mathematical modelling is in other words application of mathematical expressions to describe a process. Mathematical models are only approximate representations of real-life experiments (Gould 1989; Ozilgen 1998; Whiting 1995). Models are usually formulated in a way that major process inputs generate major process outputs. Negligible inputs and

outputs are omitted in order to simplify models (Ozilgen 1998). Data obtained from an initial experiment, presented in tabular or graphical forms, are used to obtain a simplified model describing or explaining relations between inputs and outputs from the experiment, which could not be otherwise understood (Ozilgen 1998). Mathematical modelling of experimental work help in improvement of existing technologies and in development of new technologies, as the models are time and cost minimizing tools for better understanding of the actual processes (Glasscock and Hale 1994). Mathematical models of initial experimental work are also used to predict outputs of future experiments, providing that the prediction is done within the range of parameters used to obtain the models.

In model design, a question needs to be asked and then the question needs to be phrased in precise mathematical terms. In the process of the question formulation one needs to make a number of assumptions, define terms, make a list of variables with appropriate units, and add any known or assumed relations between variables (equations and inequalities) (Meerscchaert 1999). Mathematics is then used to solve the problem and the mathematical solution needs to be 'translated' back into a comprehensible answer to the original question (Meerschaert 1999). In modelling, non-dimensional or dimensionless parameters can be introduced for the analysis of a model. The dimensionless parameter is formed when a ratio of two characteristic (to a given system) values or quantities is taken, i.e. Reynolds ratio (Becker 1976).

2.4.1 Predictive Microbiology

In microbiology, models became valuable tools for making initial predictions of microbial behaviour in specified ranges and combinations of factors. Models do not completely replace microbial testing. They do, however, help in evaluation of potential
problems and in decision making processes that may arise in following situations: risk assessment of a presence of pathogens in foods after storage at specified conditions; identification of critical control points in developing an HACCP (hazard analysis and critical control point) program; evaluation of reformulations of food products, as well as, evaluation of unexpected out-of-process events (Cenkowski et al. 2002; Gould 1989; Whiting 1995).

Microbial models can be differentiated into growth models and inactivation/survival models (Peleg 1995). Within each category, models are classified as the primary, secondary, and tertiary level models (Whiting 1995). Models that describe how microbial numbers or other microbial responses change with time in a specified environment are called primary (Whiting and Buchanan 1994). Examples of primary models include: exponential growth rate during exponential growth phase, time to growth, Gompertz function (four parameter, asymmetrical, sigmoidal equation), and first-order thermal inactivation – the D-value (the time needed to eliminate 90% of given population at a constant temperature) (Gould 1989; Whiting 1995; Whiting and Buchanan 1994).

Secondary level models describe changes of the parameters of the primary models with respect to the changes in environmental conditions (i.e. temperature, pH, a_w , sodium chloride (NaCl) concentration). The *z*-value (the change in temperature needed to decrease the *D*-value of a particular micro-organism by 90%) is one of the secondary level models available. Other examples of secondary level models include: Arrhenius relationship (the logarithm of the rate vs. the reciprocal of the temperature; expressed in K), Belehradek model (square root model), and response surface equation (multiple polynomial regression equations) (Peleg 2002; Peleg et al. 2004; Whiting 1995).

There are different types of primary and secondary level models. They can be divided into: linear or non-linear; segregated or non-segregated (defined population of heterogeneous cells or averaged cells, respectively); structured or non-structured (multicomponent or single component, respectively). Furthermore, microbial models can be described as empirical (descriptive) or based on mechanistic and/or kinetic microbiological criteria, where analogies to well known processes are used (Ozilgen 1998; Whiting 1995). Modeling applications on tertiary level utilize primary and secondary models in the form of computer programs that can calculate microbial responses to changing conditions, display graphs, and compare responses of different microorganisms (Whiting 1995; Whiting and Buchanan 1994).

2.4.2 Modelling of Geobacillus stearothermophilus Spore Viability

Geobacillus (formerly *Bacillus*) *stearothermophilus* is a Gram-positive, thermophilic, spore-forming bacterium often associated with plant food products. The spores of this bacterium are very heat-resistant and usually survive canning and sterilization operations, where saturated steam (moist heat) is used. Flat-sour spoilage (formation of lactic acid, leading to souring of food product) of properly processed low acid canned foods may occur if the products are stored at elevated temperatures (Ng and Schaffner 1997). The spores of *G. stearothermophilus* are used as a biological indicator to validate moist heat sterilization because of their exceptional resistance to this type of heat (Brown 1994; Carlberg 2005; Spicher et al. 1999).

Behaviour of *Geobacillus stearothermophilus* spores in different environments has been mathematically modelled. Much of the modelling has been done on the growth characteristics. Ng and Schaffner (1997) modelled the effects of pH, temperature and

sodium chloride concentration on the growth kinetics of G. stearothermophilus in salty carrots medium. Their models can determine appropriate storage temperatures required for microbiologically safe shelf life of products with known pH and NaCl concentration. Ratkowsky et al. (1983) studied growth rates of several bacterial cultures, including G. stearothermophilus, throughout their entire biokinetic temperature range (30-80°C for G. stearothermophilus). The authors proposed a new, empirical non-linear regression model (Ratkowsky 2 model) suitable for modelling of the temperature dependence of bacterial growth. The new model fits better the experimental data (results for 30 bacterial strains reported) than the Arrhenius relationship. Effect of small inoculum size on time to spoilage by G. stearothermophilus was studied by Llaudes et al. (2001). A simplified Gompertz function was used to fit the experimental data. A single mathematical model describing the effects of temperature on microbial populations from growth to lethality was proposed by Peleg (1995). He also introduced a reduced version of that model sufficient to describe the destruction rate of bacterial spores. This equation demonstrated a good fit with published data on the heat destruction kinetics of G. stearothermophilus and Bacillus megaterium spores.

Inactivation of *G. stearothermophilus* spores under moist heat (92-110°C) at high pressure (5-7 x 10^8 Pa) was modeled by Rodriguez et al. (2004). The equations developed by them can be used to compare high-pressure processing treatments of different systems. Lambert (2003) proposed an empirical model based on a double Arrhenius function to mathematically model published thermal inactivation data sets. His model, the log R-fat model, uses chemical reaction kinetics approach to describe the inactivation curves.

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Heat stress and recovery of injured spores was modelled by Mafart and Leguerinel (1997) as a function of both the heat treatment intensity and the environmental conditions. They concluded that the recovery of spores is affected by two factors: the ε -factor and γ -factor. Both factors are independent of the heat treatment and the former reflects intrinsically the distance of incubation conditions from optimum conditions without previous heat treatment. The γ -factor reflects the degree of instability of spores due to the heat treatment intensity and incubation temperature affects the γ -factor according to the Arrhenius law.

Most of the research on modelling of the thermal destruction of *G. stearothermophilus* has been done on experimental data obtained from heat treatments where capillary tube technique was used (Abraham et al. 1990; Fernandez et al. 1996; Leontidis et al. 1999; Periago et al. 1998). In this technique the spore suspensions are injected into sterile capillary microhematocrit tubes which are then sealed. The capillary tubes are exposed to heat by plunging them into a stirred oil bath set at a desired temperature. Although some work on SS inactivation of *G. stearothermophilus* spores has been done (summarized in Chapter 5; Collier and Townsend 1956; Spicher et al. 1999, 2002), modelling of the SS effect on the spore viability has not been reported.

3 OPTIMIZATION OF SUPERHEATED STEAM PROCESSING OF OAT GROATS BY EVALUATION OF GROATS FUNCTIONAL PROPERTIES AND STORAGE STABILITY

3.1 Abstract

Superheated steam (SS) processing of oat groats with a lab-scale SS processing system was studied as a method of heat treatment alternative to conventional processing (conditioning with saturated steam followed by kiln drying) of oat groats. The study was divided into two parts. The objective of Part I was to determine optimum conditions of SS processing necessary to obtain groats with inactivated peroxidase, acceptable sensory characteristics, colour, and moisture content, as well as pasting properties comparable or unique to those obtained in conventional (commercial) processing. The objective of Part II was to determine storage stability of oat groats processed with the optimum SS processing conditions and conventionally during periods of ~180 and ~90 days (at 21 and 38°C, respectively). In Part I, raw or moisture tempered oat groats (~13 g) were processed with SS at varying temperatures (110-160°C), processing times (1-30 min), and velocities (0.35 and 1.00 m/s). Generally, cold paste (64°C) viscosity of samples processed with SS was higher than that of samples processed conventionally. The use of SS at high temperatures (140-160°C) for processing of groats resulted in high cold paste viscosity (3,800-5,600 cP) but also caused low final moisture content (4-7%). Oat groats processed with SS at lower temperatures (110-130°C) had acceptable moisture content and appearance, and exhibited reasonably high cold paste viscosity compared to groats processed conventionally. Superheated steam velocity of 1.00 m/s significantly (P<0.05) increased cold paste viscosity of oat slurry compared with that of 0.35 m/s (at the same SS temperature). Oat groats that were moisture tempered (to 14%) before SS treatment exhibited decreased cold paste

viscosity of slurry compared to that of groat that were processed with SS without moisture tempering. The optimum parameters of SS for processing of oat groats were found to be: temperature of 110°C, a velocity of 1.00 m/s, and two processing times of 10 and 14 min. In Part II, monitoring of hexanal and free fatty acid levels, moisture content, colour, and cold paste viscosity, as well as sensory panel evaluation of groats were carried out during storage. Groats processed with the optimum parameters of SS and conventionally remained shelf stable over the time periods tested. No substantial changes were noted in colour, cold paste viscosity, and content of free fatty acids of differently heat processed groats as storage time passed. Changes in moisture content of stored groats were noted and they reflected the seasonal changes in the humidity of the surroundings. At both storage temperatures, the amounts of hexanal released from groats processed either with SS or conventionally increased with the increase of storage time. However, the groats processed with SS released lesser amounts of hexanal than the groats processed conventionally. As the storage time progressed, groats processed with SS and conventionally became blander and it was increasingly more difficult for the sensory panellists to distinguish between the differently heat processed samples.

3.2 Introduction

Oat grain (*Avena sativa* L.) is a good source of high quality protein, unsaturated fatty acids, minerals and vitamins, phenolic compounds as well as dietary fibre, especially its soluble fraction (β -glucans) (Drzikova et al. 2005; Lapvetelainen et al. 2001; Liukkonen et al. 1992b; Sun et al. 2006). The main limiting factor for storage and handling of raw oats is degradation of oat lipids via enzymatic hydrolysis, followed by oxidation, which leads to development of rancidity and off-flavours (Ekstrand et al. 1992; Lehtinen et al. 2003). To

prevent the development of rancidity, oat grain is treated hydro-thermally to inactivate the lipolytic enzymes present in the grain (Ekstrand et al. 1992; Ganssmann and Vorwerck 1995). Typically, conventional oat processing involves conditioning with saturated (wet) steam followed by kiln drying (up to 100 min at 88-98°C) (Lawrie 1999; Molteberg et al. 1995). Kiln drying also develops the characteristic 'oaty' flavour, brings about starch gelatinization to a certain degree, and may cause a reduction of grain microflora (Bryngelsson et al. 2002a; Lawrie 1999). However, kiln drying is difficult to control and is not energy efficient.

Recently, an alternative drying method, in which superheated steam (SS) is utilized, has been considered for drying of food products (Uengkimbuan et al. 2006). Superheated steam is a type of unsaturated (dry) steam generated by addition of sensible heat to saturated (wet) steam. That addition of heat increases steam temperature above the corresponding saturation or boiling point at a given pressure. Superheated steam evaporates moisture from food products based on temperature difference between the SS and the saturation temperature of the moist product. Drying in SS can be accomplished in a closed loop as long as the evaporated moisture is condensed outside the loop and the pressure inside the loop is kept constant, with only supplemental heat added to keep the drying medium (steam) in the superheated state. In hot air drying, however, the moist air must be replaced with fresh air (Borquez et al. 2008) or the used air (saturated with moisture) needs to be dehumidified. Exposure of biological products to SS causes not only drying but also brings about changes such as starch gelatinization, enzyme destruction, protein denaturation, colour and texture changes, and deodorization (Devahastin and Suvarnakuta 2004; Tang and Cenkowski 2000, 2001). Therefore, the term 'SS processing' rather than just 'SS

drying' is often preferred when SS is used for heat treatment of foods. Advantages of SS processing over the kiln drying include: air-free environment (thus enhanced product quality), improved energy efficiency, higher drying rates, and reduced environmental impact when condensate is reused (Prachayawarakorn et al. 2004, 2006; Tang et al. 2005).

Oatmeal or oat flakes have been staple foods in human diet for a long time and there is a continuous demand for the desired 'oaty' and 'toasted' flavour in these products (Bryngelsson et al. 2002a). The fact that customers become accustomed to taste and aroma of so called 'traditional' (or specific) foods makes the decision of implementing new technologies into the existing processing methods more difficult (Cayot 2007). It is, therefore, important that oat groats processed with SS meet the adequate sensory characteristics demanded by customers. Sensory characteristics of oat products are influenced by the quality of raw material and the nature of heat processing used (Molteberg et al. 1996b). Severity of SS processing needs to be sufficient to inactivate lipolytic enzymes in oat groats and develop the characteristic toasted/oaty flavours, but mild enough to limit potential development of off-flavours due to lipid breakdown and oxidation.

Objectives of this study were to: (i) determine optimum conditions for SS processing of oat groats which would yield groats with functional properties comparable or unique to that of conventionally processed groats (Part I); (ii) determine storage stability (at 21 and 38°C) of oat groats processed with the optimum SS conditions and conventionally (PartII).

3.3 Materials and Methods Used in SS Processing Optimization Study (Part I)

3.3.1 Materials

Oat groats (de-hulled oats) from 2004 and 2005 growing seasons (mixed Canadian cultivars) were supplied by Can-Oat Milling (Portage la Prairie, MB, Canada). Groats were

stored at -18°C prior to SS processing. Additionally, oat groats of the same origin but processed commercially (conventionally) were supplied by Can-Oat Milling (Portage la Prairie) for comparison purposes.

3.3.2 Tempering of Oat Groats

Two methods were used to reach 14% (wb) moisture content of groats: (i) a predetermined amount of water was mixed with groats (ii) groats were exposed to wet steam. Tempering with water was done according to the AACC method 26-95 (AACC 2003). Water was added in small portions to a glass jar containing groats and the jar was tumbled for 60 seconds every ten minutes during the first hour of tempering. Tempering with wet steam was done in a vegetable steamer (VS-30 Combo II; Salton Canada, Dollard-des-Ormeaux, QC, Canada). A thin layer of groats was steamed to achieve 14% moisture content, groats were removed to cool for ~30 min at 23°C, and placed in a glass jar. The jars containing differently tempered groats were kept at 4°C overnight to ensure even distribution of the added moisture across individual groats.

3.3.3 Superheated Steam Processing

Oat groats (~13 g) were placed in a processing tray - an aluminium cup (inner diameter of 6.3 cm, outer diameter of 6.8 cm, and depth of 3 cm) with a wire mesh bottom (Fig. 3.1). The tray containing groats was placed in a processing chamber of SS processing system (Fig. 3.2) and exposed to SS. The main components of the processing system were: a 9 kW electric steam generator (model MB9L; Sussman Electric Boilers, New York, NY, USA), steam conveying pipelines, a pressure reducing regulator (type 95L NPT; Fisher Controls International Inc., Marshalltown, IA, USA), an electric superheater, a processing chamber, a hot-air supply system to the jacket of the chamber, and a data acquisition and control system.



Fig. 3. 1 Sample tray with a single layer of oat groats – top view.



Fig. 3. 2 Superheated steam processing system.

Briefly, pressurized saturated steam was produced by the steam generator. The SS was generated by reducing the pressure of saturated steam to 1 atmosphere. The SS was conveyed via pipelines through the steam superheater, where its temperature was adjusted to the desired level. The SS was then directed to the processing chamber (containing oat groats) and subsequently condensed outside of the chamber. During processing, hot air was forced through the air jacket surrounding the processing chamber to maintain adiabatic conditions in the chamber. The SS processing time ranged from 1 to 30 min, depending on the steam temperature (110-130°C with 5°C increments and 130-160°C with 10°C increments, and accuracy of $\pm 2^{\circ}$ C) and velocity used (0.35 and 1.00 \pm 0.02 m/s). Groats processed with SS were cooled at 23°C for 30-40 min and placed in paper bags used in commercial packaging of oat products (bags supplied by Can-Oat Milling, Portage la Prairie). The bags containing oat groats were placed in air-tight containers and stored at 4°C.

3.3.4 Moisture Content

Moisture content of ground oat groats was determined in duplicate at 130°C after one hour of drying in a forced air oven (737F, Fisher Scientific, Nepean, ON, Canada) according to the AACC method 44-15A (AACC 2003).

3.3.5 Pasting Properties

Oat groats were ground to pass 0.5 mm sieve using Retsch ZM 100 Ultra Centrifugal Grinding Mill (Retsch, Haan, Germany). Six grams of the resulting whole meal flour (corrected to 14% moisture content) was slurried with 25 mL of water and viscosity of the slurry was determined, in duplicate, with Rapid Visco Analyser, model RVA-4 (Newport Scientific Pty, Ltd., Warriewood, Australia). The slurry was heated from 30 to 64°C at a

rate of 6.8° C/min and then held at 64° C for 15 min with constant paddle stirring speed of 160 rpm, except for the first 10 s when a speed 960 rpm was used to disperse the sample (AACC Method 76-22). Viscosity of the slurry was recorded during the entire test but the final viscosity (at the 20th min of the test) was used to compare samples. Viscosity recorded during the test was measured in Rapid Viscoanalyser units, RVU, which were recalculated to centipoise units, cP (1 RVU \approx 12 cP), to express the apparent viscosity.

3.3.6 Sensory Evaluation

Sensory evaluation of oat groats processed with SS was conducted by experienced staff members of Can-Oat Milling Co. (Portage la Prairie). The expert panel compared the flavour of SS processed groats with the flavour of groats processed at their plant and provided feedback on the flavour (e.g. toasted, rancid) and acceptability of SS processed groats. This information was used to adjust processing parameters of SS for finding the optimum combination of SS parameters. The processed groats were stored for 3-4 weeks at ambient temperature to check for rancidity development.

3.3.7 Colour

Colour of oat groats was determined with a chromameter (CR-410, Konica Minolta, Tokyo, Japan) coupled with a circular sample holder (Attachment CR-A50, Konica Minolta). The groats were placed in the holder (50 mm diameter and 10 mm depth) ensuring that total volume of the holder was filled by the sample. A measurement was taken with the chromameter after which the groats were removed from the holder, placed back into the sample container, mixed, and this procedure was repeated with a new portion of groats. Measurements of the standard colour values (L, a, and b) were done in triplicate.

3.3.8 Peroxidase Analysis

Qualitative (negative/positive) analysis of peroxidase activity in oat groats was carried out according to the AACC method 22-80 (AACC 2003). Ground groats (1 g) were mixed with distilled water (50 mL), ascorbic acid solution (2 mL), 2,6-dichloroindophenol sodium salt (3 mL), and hydrogen peroxide solution (0.1 mL). The mixture was held in a water bath at 38°C and observation of blue colour formation was carried over a period of 10 min. No blue colour formation (indicative of peroxidase inactivity) after 10 min of the test was recorded as negative.

3.3.9 Statistical Analysis

Results were analyzed using a one-way analysis of variance (ANOVA) using SigmaStat (version 3.5, Systat Software Inc., Point Richmond, CA, USA). If differences among treatments (with steam temperature, velocity, processing time, and moisture tempering as factors) were found, then a pair-wise multiple comparison procedure using the Holm-Sidak method was used to determine which treatment means were significantly (P<0.05) different from each other.

3.4 Materials and Methods in Storage Stability Study (Part II)

3.4.1 Materials

Storage stability study was conducted on oat groats: (i) processed with the optimum SS conditions selected in Part I, (ii) conventionally processed at two plants (referred to as Plant A and Plant B). The SS parameters used for groats processing were: temperature of 110°C, a velocity of 1.00 m/s, and processing time of 10 and 14 min (referred to as SS-10 and SS-14, respectively).

3.4.2 Storage Conditions

Groats obtained from each processing method were divided in two portions. One portion of groats from each processing method was stored at room temperature $(21\pm1^{\circ}C)$ and the second portion was stored at elevated temperature $(38\pm1^{\circ}C)$. Storage at $38^{\circ}C$ was chosen to resemble the temperature often found in storage facilities in parts of North America and Mexico. Relative humidity (RH) of storage rooms was recorded but not controlled. Storage time of ~180 days was originally planned for groats stored at $21^{\circ}C$, whereas a shorter stoarage time (~90 days) was planned for groats stored at $38^{\circ}C$ because deteriorative reactions occur at faster rates at temperature higher than room temperature. Oat groats processed with SS and conventionally were stored in standard paper bags used in commercial packaging of oat products (bags supplied by Can-Oat Milling, Portage la Prairie). The paper bags filled with groats were placed in plastic containers (volume of 20 litres), allowing ~10 litres of headspace in each container.

Monitoring of moisture content, cold paste viscosity, colour, free fatty acids and hexanal contents, and sensory evaluation of stored oat groats was carried out throughout the storage periods according to the schedules given in Tables A.4 and A.5 (Appendix A) for storage at 21 and 38°C, respectively. Procedures for the measurement of moisture content and cold paste viscosity are described in Part I (sections 3.3.4 and 3.3.5, respectively). Due to limited sample size of the SS processed groats most analyses (except for pasting properties, colour and sensory evaluation) were performed in one replicate at scheduled time intervals.

3.4.3 Colour

The procedure of groat colour measurement followed that described in Part I (section 3.3.7). Additionally, a total change in groat colour during storage (expressed as ΔE value) was calculated with respect to the colour of groats recorded at the beginning of storage. The calculation of ΔE value is based on the following equation (Cenkowski and Sosulski 1998):

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

where, ΔE is total colour change, and ΔL , Δa , Δb are differences in the *L*, *a*, *b* values, respectively, at a particular interval of storage time and the beginning of storage.

3.4.4 Free Fatty Acids and Hexanal Contents

Determination of free fatty acids (FFA) content in fat extracted from oat groats was done according to the AOCS Ca 5a-40 official method and expressed in g/100g of fat (AOCS 1998). Fat was dissolved in ethyl alcohol (95%) and titrated with 0.25 N sodium hydroxide solution using phenolphthalein as an indicator, to a pale-pink endpoint. Hexanal determination was done according to the procedure reported earlier by Fritsch and Gale (1977). Amount of hexanal released from a sample was quantified via comparison of gas chromatographic signal to that of an added internal standard and was expressed in parts per million (ppm).

3.4.5 Sensory Evaluation

A sensory panel was organized and conducted by Can-Oat Milling (Portage la Prairie). Six panellists (4 men and 2 women) recruited among the staff, were trained in the Can-Oat Milling laboratory, to differentiate taste and identify: green (raw), overly toasted, rancid/bitter, and standard/bland oat flavours. The panellists evaluated oat groats without any knowledge as to the type of heat processing and storage conditions used. Four samples of oat groats (5 g each) were evaluated at each session by each panellist. The panellists were asked to smell each sample before tasting, the samples were requested to be chewed and moved around the mouth well, and finally swallowed. Water was used to cleanse the palate between the samples. A freshly kilned groat sample or freshly flaked flake sample, produced within 1-2 days of each sensory evaluation, was used for reference purposes. Vocabulary of sensory attributes (flavour descriptors) used in this study was modified from Heinio et al. (2002) and Molteberg et al. (1996a). Five flavour descriptors were chosen for monitoring: oaty (normal oat flavour), toasted (Maillard reaction products), raw (raw grasshay), bitter (bitter taste, caffeine), and rancid (old oil). Intensity of each descriptor was rated from 0 to 10 on an unstructured graphic scale (horizontal line), where the left side corresponded to 0 (no intensity) and the right side corresponded to 10 (highest intensity). Mean values and standard deviations of each descriptor, calculated over the ratings of all panellists at each session, were used for graphical expression of results.

3.5 Results and Discussion of SS Processing Optimization Study (Part I)

3.5.1 Pasting Properties

Cold paste (64°C) viscosity obtained for raw and heat-treated (with SS and conventionally) samples are listed in Tables A.1 and A.2 (Appendix A). In contrast with the oat groats harvested in 2004, the groats harvested in 2005 were characterized by higher viscosity of raw oat flour slurries (458±57 cP and 851±25 cP, respectively). Subsequently, higher cold paste viscosity of slurry was noted in samples obtained from heat processed (conventionally and with SS) groats of 2005 than 2004 crop. Generally, cold paste viscosity of samples processed with SS was higher than that of samples processed conventionally.

For example, groats exposed to SS at 1.00 m/s and 140°C for 5 min (groats were tempered with water to 14%) had substantially higher viscosity than groats processed conventionally on the same day (3979 ± 232 cP and 3174 ± 21 cP, respectively). A comparison of cold paste viscosity values obtained for samples processed for 5 min at two SS temperatures (115 and 140°C) and at different SS velocities is given in Table 3.1. At the same SS velocity, groats tempered (to 14% moisture content) before SS treatment exhibited significantly (P<0.05) lower cold paste viscosity than non-tempered groats, regardless of the tempering method used (saturated steam or water). Velocity and temperature of SS affected cold paste viscosity of ground oat groats. The use of SS at a velocity of 1.00 m/s significantly (P<0.05) increased cold paste viscosity of ground groats compared to the viscosity observed in groats processed with SS for the same duration of time but at low velocity (0.35 m/s; Table 3.1).

| SS Temperature (°C) | SS Velocity (m/s) | Tempering With | Viscosity (cP) |
|------------------------|----------------------|-----------------|-------------------|
| 115 | 0.35 | Water | 2836±211a |
| 115 | 0.35 | No Tempering | 3275±29b |
| 115 | 1.00 | Water | 3538±273b |
| 140 | 0.35 | Water | 3680±36a |
| 140 | 0.35 | Saturated Steam | 3624±202ab |
| 140 | 1.00 | Water | 3979±232b |
| 140 | 1.00 | No Tempering | 5212±147c |

Table 3.1 Cold paste viscosity^a (64°C) of oat groats^b (2004 crop) processed for 5 minutes with SS^c at 115 and 140°C at two velocities.

^aViscosity of slurry at the 20th min (the end) of the cold paste test (64°C); values are means of 2 replicates; values not significantly different (P>0.05) are indicated by the same letter (at each SS temperature). ^bGroats were ground to pass 0.5 mm sieve and resulting whole meal flour was used to prepare slurry. ^cSS – superheated steam.

Generally, processing of oat groats with SS temperature higher than 130°C resulted in cold paste viscosity between 3800 and 5600 cP, whereas conventional processing resulted in cold paste viscosity rarely exceeding 4200 cP (Fig. 3.3, Tables A.1-A.2 in Appendix A). At

SS temperatures higher than 130°C and at high steam velocity (1.00 m/s), higher values of cold paste viscosity were observed in groats processed for a short time (2.5 min) than in groats exposed to SS longer (5 min) (Fig. 3.3). At lower SS temperatures (110-130°C) and velocity of 0.35 m/s, a lesser effect of processing time on cold paste viscosity was noted (e.g. 3885±64 cP and 4034±57 cP after 5 and 10 min of processing with SS at 110°C and 0.35 m/s, respectively).



Fig. 3. 3 Effects of superheated steam (SS) temperature and processing time on cold paste viscosity (64°C) of whole meal flour obtained from oat groats (2004 crop) processed in SS at a velocity of 1.00 m/s. Symbols represent means of four replicates and bars represent their standard deviations.

3.5.2 Moisture Content

Typically moisture content of commercially processed oat groats varies between 9 and 10%. Moisture content of non-tempered oat groats processed with SS at 110-160°C and a velocity of 1.00 m/s are shown in Fig. 3.4. The use of SS at high temperatures (140-160°C) for groat processing caused very low final moisture content (4-7%) of groats even after a short processing time. As expected, a longer processing time was needed at lower SS

temperatures to reach moisture content of groats close to 9.5% (i.e. 10 and 14 min at 110°C,

3.5-4.0 min at 120°C, but only 1.0-1.2 min at 160°C).



Fig. 3. 4 Effect of superheated steam temperature and processing time on moisture content of oat groats processed with superheated steam at a velocity of 1.00 m/s. Symbols represent means of three replicates and bars represent their standard deviations.

3.5.3 Sensory Evaluation

Consumer acceptance of a food product depends largely on taste which is one of the most important sensory attributes (Lyly et al. 2007; Sampaio et al. 2004). Generally, oat groats processed with SS had a unique flavour compared to the conventionally processed groats. Intensity of toasted flavour was lower in groats processed with SS than in conventionally processed groats. Processing with SS at 110° C and 1.00 m/s for $\geq 10 \text{ min}$ resulted in groat flavour most similar (but still unique) to the conventionally processed material. Processing with SS at high temperatures (140-160°C) and a high velocity (1.00 m/s) had a detrimental effect on sensory characteristics of groats. A rancid flavour was

noted in these groats after 2 weeks of storage at ambient temperature. The rancidity may have been caused by extensive breakdown of oat lipids due to increased heat intensity of SS at temperatures higher than 130°C. Lehtinen et al. (2003) reported fast oxidation of oat lipids (especially polar lipids) due to a severe heat treatment (extrusion at 130°C) of oat bran. The authors suggested that the heat treatment induced disintegration of membrane structures and inactivated heat labile antioxidants present in oats, and recommended a use of less severe heat treatments if oat products are intended for long periods of storage. It is possible that heat processing of oat groats with SS at high temperatures may cause inactivation of oat antioxidants and shorten shelf life of the groats. Further research is needed to elucidate the effects of SS on oat antioxidants.

To reduce the deteriorative effect of increased heat intensity of SS at high temperature and velocity, a two-stage SS process with a combination of low and high steam velocities (0.35 and 1.00 m/s) was performed on groats processed at 115, 120, and 130°C. Duration of processing at each of the three temperatures was optimized to reach the final moisture content of groats close to 9.5%. Superheated steam velocity of 0.35 m/s was used in the initial period of processing (~5 min) to inactivate lipolytic enzymes and to develop the characteristic toasted/oaty flavour of groats. Then, the groats were exposed to SS at a velocity of 1.00 m/s for 2.25-2.50 min to quickly reduce moisture content. Among the three temperatures tested in the two-stage SS process, the temperatures of 120 and 130°C caused slight rancidity of groats (after 3 weeks of storage at ambient temperature), whereas the temperature of 115°C did not. The groats processed at 115°C in the two-stage treatment had less intense toasted flavour compared to the flavour of groats processed at 110°C in a onestage treatment (a velocity of 1.00 m/s and processing time of \geq 10 min). Furthermore, cold paste viscosity exhibited by groats processed in the two-stage treatment at 115° C did not differ significantly (P>0.05) from that exhibited by groats processed in the one-stage treatment at 110° C (Table 3.2).

Table 3. 2 Final moisture content (%, wb) and cold paste viscosity^a of oat groats^b processed conventionally and with superheated steam at 110 and 115°C and a velocity of 1.00 m/s for different durations of time.

| Processing Method | SS [°] Temperature (°C) | SS Velocity (m/s) | SS Processing Time (min) | Final Moisture Content (%) | Viscosity (cP) |
|----------------------|-------------------------------------|----------------------|-----------------------------|----------------------------------|-------------------|
| Conventional | • | - | _ | 9.4±0.1 | 4218±52a |
| With SS | 110 | 1.00 | 10 | 9.4±0.1 | 5300±60b |
| With SS | 110 | 1.00 | 14 | 9.4±0.2 | 5380±126b |
| With SS | 115 | 0.35 | 5 | 9.6±0.0 | 5286±69b |
| | | then 1.00 | 2.25 | | |

^aViscosity of slurry at the 20th min (the end) of the cold paste test (64°C); values are means of 2 replicates; values not significantly different (P>0.05) are indicated by the same letter.

^bGroats were ground to pass 0.5 mm sieve and resulting whole meal flour was used to prepare slurry. ^cSS – superheated steam.

3.5.4 Colour

Colour of heat processed oat groats is an important quality parameter and it may indicate changes in taste, texture, milling characteristics, and pasting properties of products obtained from the groats. Generally, after processing with SS, groats with final moisture content in the desired range (9-10%) were brighter, more yellow and red than the unprocessed groats (Fig. 3.5 and Table 3.3). These changes in the colour of groats were significant (P<0.05) after processing with SS at 120°C and higher (regardless of SS velocity) but they were not significant (P>0.05) after processing with SS at 120°C after processing with SS at low temperatures (for example 115° C and 0.35 m/s).



Fig. 3. 5 Effects of processing time on *L*, *a*, and *b* values of oat groats (2005 crop) processed with superheated steam at 120°C and a velocity of 1.00 m/s. Columns represent means of three replicates and error bars represent their standard deviations.

| SS ^b Temperature (°C) | SS Velocity (m/s) | L | а | b |
|-------------------------------------|----------------------|------------|-----------|------------|
| Unprocessed | _ | 42.8±0.4a | 3.5±0.1a | 9.4±0.3a |
| 115 | 0.35 | 44.0±1.9ab | 3.7±0.3a | 10.0±0.9ab |
| 115 | 1.00 | 46.0±0.1bc | 4.1±0.0b | 11.3±0.1bc |
| 140 | 0.35 | 46.0±0.9bc | 4.3±0.2bc | 12.3±0.5c |
| 140 | 1.00 | 46.9± 0.9c | 4.6±0.1c | 12.1±0.5c |

 Table 3. 3 Effects of superheated steam temperature and velocity on colour^a of oat groats tempered with water to 14% (wb) and exposed to superheated steam for 5 min.

^aL – brightness, a – redness, b – yellowness; values are means of 3 replicates; values not significantly different (P> 0.05) are indicated by the same letter.

^bSS – superheated steam.

Changes in the colour of oat groats due to duration of SS processing were not substantial, except for the initial period of exposure to SS (example in Fig. 3.5 but similar trends were seen at other SS temperatures). Tempering of oat groats to 14% moisture content before SS treatment did not significantly (P>0.05) affect the colour of groats compared to that of nontempered groats processed at the same SS temperature-velocity-time combinations (e.g after 5 min exposure to SS at 115°C and 0.35 m/s, the tempered groats exhibited L value of 44.0±1.9, but the non-tempered groats exhibited L value of 45.4±0.3). Oat groats processed conventioanlly appeared significantly (P<0.05) darker (lower L values) than oat groats processed with SS to final moisture content of 9-10% (example in Table 3.4).

| Processing Method | SS Temperature (°C) | SS Velocity (m/s) | SS Processing Time (min) | L | а | b |
|----------------------|---------------------------|-------------------------|--------------------------------|-----------|----------|-----------|
| Conventional | | - | | 49.6±0.7a | 4.9±0.1a | 14.6±0.3a |
| With SS | 110 | 1.00 | 10 | 52.9±0.3b | 5.2±0.1a | 15.5±0.2b |
| With SS | 110 | 1.00 | 14 | 52.6±0.5b | 5.1±0.2a | 15.5±0.2b |

Table 3. 4 Colour values^a of oat groats (2005 crop) processed conventionally (Plant A and B) and with SS^b at 110°C and a velocity of 1.00 m/s for 10 and 14 min.

^aL – brightness, a – redness, b – yellowness; values are means of 3 replicates; values not significantly different (P>0.05) are indicated by the same letter.

^bSS – superheated steam.

3.5.5 Peroxidase Activity

The lipase enzyme is the main target of heat processing of oats because it hydrolyzes lipids with a release of free fatty acids and glycerol (Matlashewski et al. 1982). The fatty acids are then converted to hydroperoxides or hydroxy acids by another lipolytic enzyme - peroxidase (lipoperoxidase) (Robinson et al. 1995; Youngs 1986). Peroxidase, however, is more heat stable than lipase and for that reason peroxidase activity test is commonly used for monitoring of the adequate heating for lipase inactivation in oats (Cenkowski et al. 2006a). Qualitative analysis of peroxidase activity in oat groats treated with SS was, therefore, carried out to determine whether the lipolytic enzymes had been effectively inactivated during the SS treatment. Oat groats processed with SS (in one- and two-stage treatments) to final moisture content of 10% (wb) and less were peroxidase negative (Table A.3 in Appendix A).

3.6 Conclusions from SS Processing Optimization Study (Part I)

Generally, cold paste viscosity of whole flour obtained from oat groats processed with SS was higher (by up to 1200 cP) than that of groats processed conventionally. Moisture tempering (to 14%, wb) of oat groats before SS treatment did not affect their colour but it significantly (P<0.05) decreased cold paste viscosity of oat whole flour obtained from those groats. Groats processed with SS at a velocity of 1.00 m/s exhibited significantly (P<0.05) higher cold paste viscosity than groats processed with SS at 0.35 m/s (i.e. at 140°C and 5 min processing: 3538±273 cP at 1.00 m/s and 2836±211 cP at 0.35 m/s). Oat groats processed with SS at lower temperatures (110-130°C) had acceptable moisture content and appearance, and exhibited higher cold paste viscosity than groats processed conventionally.

The use of SS at high temperatures (140-160°C) further improved cold paste viscosity of whole flour slurries but also caused very low final moisture contents (4-7%). Additionally, the high SS temperatures triggered development of rancid flavour (noted after 2 weeks of storage at ambient temperature). Processing with SS proved to be effective in the inactivation of peroxidase in groats in which post-processing moisture content was at 10% (wb) and less.

Based on the results described above, the selected optimum SS processing parameters for oat groats were: temperature of 110°C, velocity of 1.00 m/s, and two processing times, 10 and 14 min. Processing with these parameters gave groats with inactivated peroxidase, sensory characteristics closest to the conventionally processed oat groats and the final moisture content close to 9.5%. Additionally, cold paste viscosity of whole flour slurry prepared from groats processed with the optimum SS parameters was significantly (P<0.05) higher than that of conventionally processed groats (5300 ± 60 cP and 5380 ± 126 cP versus 4218±52 cP, respectively). Oat groats processed with the optimum SS parameters appeared brighter than the groats processed conventionally (*L* value of 52.9 ± 0.3 and 52.6 ± 0.5 versus 49.6±0.7, respectively).

3.7 Results and Discussion of Storage Stability Study (Part II)

Storage stability of oat groats processed with the optimum parameters of SS, determined in Part I, was assessed at two storage temperatures; 21 and 38°C. For comparison purposes, the groats processed with SS were stored together with groats that were processed conventionally at two plants within the same time period as the SS processing.

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3.7.1 Pasting Properties and Moisture Content

Pasting properties of oat products are of key importance because these products are often used as thickening agents (Gibinski et al. 2006; Zhang et al. 1997). Stability of the pasting properties during storage is needed to ensure high technological value of oat products. In this study, cold paste (64°C) viscosity of all stored groat samples remained stable over the period of time originally planned for storage at 21°C (~180 days) and 38°C (~90 days) (Fig. 3.6). Samples processed conventionally in Plant B exhibited significantly (P<0.05) lower cold paste viscosity than the samples processed conventionally in Plant A and the samples processed with SS, but the viscosity also remained stable throughout the storage periods. The origin of the groats processed in Plant B (mainly cultivars grown in Saskatchewan) was different from the origin of the groats processed in Plant A and with SS (mainly cultivars grown in Manitoba), which may explain the differences in cold paste viscosities of those samples.

Moisture content of oat groats changed during storage at 21 and 38°C, and the changes are depicted in Fig. 3.7. Initial drop in the moisture content of stored groats was attributed to the equilibration process of the material with its surroundings. Then, the changes in the relative humidity (RH) of the surroundings were mirrored by the changes in moisture content (MC) of groats (low RH in the winter months and lower MC of samples at that time; higher RH during the spring and summer months expressed in higher MC of the samples).

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Fig. 3. 6 Effect of storage time on cold paste viscosity of whole flour obtained from oat groats processed conventionally (Plant A and B) and with superheated steam (SS) at 110°C and 1.00 m/s for 10 and 14 min (SS-10 and SS-14, respectively), and stored at 21 and 38°C. Columns represent means of two replicates and bars represent their standard deviations.





Fig. 3. 7 Effect of storage time on moisture content (%, wb) of oat groats processed conventionally (Plant A and B) and with superheated steam (SS) at 110°C and 1.00 m/s for 10 and 14 min (SS-10 and SS-14, respectively), and stored at 21 and 38°C.

3.7.2 Free Fatty Acids and Hexanal Contents

If not inactivated, lipase hydrolyzes lipids present in raw oat grain which results in a release of free fatty acids and glycerol (Matlashewski et al. 1982). Other lipolytic enzymes present in oat grain, lipoxygenase and lipoperoxidase, convert the unsaturated fatty acids to hydroperoxides and hydroxy acids (Jensen et al. 2005; Robinson et al. 1995; Youngs 1986). The hydroxyperoxides in turn are precursors for volatile secondary lipid oxidation products such as hexanal (Jensen et al. 2005; Molteberg et al. 1995). Thus, a measurement of the amount of FFA released from oats during storage provides information on potential development of hydrolytic rancidity. Consequently, a determination of hexanal amount released from oats during storage provides information on potential oxidative deterioration of oat lipids (Jensen and Risbo 2007). In this study, stable levels of FFA were observed in oat groats processed conventionally and with SS and stored at 21 and 38°C for ~180 and ~90 days, respectively (Fig. 3.8, left panel). The range of variation in FFA content due to storage time was small in each of the four samples (up to 0.37 ppm and up to 0.44 ppm at 21 and 38°C, respectively). However, at the same storage time, the range of variation in FFA content among the four groat samples was greater (up to 1.5 ppm and up to 1.25 ppm at 21 and 38°C, respectively), with the largest differences in FFA content noted between groats processed in Plant B and groat processed with SS. The small variation in FFA content due to storage time (noted in each of four groat samples), suggested that no hydrolytic rancidity had taken place during the storage periods and confirmed that oat lipase was completely inactivated during SS and conventional processing (as reported in Part I).

The effect of storage time on the levels of hexanal released from different heat processed oat groats stored at 21 and 38°C is shown in Fig. 3.8 (right panel). At both

storage temperatures, the amounts of hexanal released from groats processed either with SS or conventionally increased with the increase of storage time. However, the groats processed with SS released lesser amounts of hexanal than the groats processed conventionally. For example, during storage at 21°C the groats processed with SS released ~0.2 ppm of hexanal at the beginning of storage and up to ~0.8 ppm at the 168^{th} day of storage, whereas the groats processed conventionally released ~0.8 ppm of hexanal at the beginning of storage and up to 3.8 ppm at the 168th day. These results suggested that in the conventional processing oat groats were subjected to increased heat and/or oxidative stress (important for flavour development) than the SS processed groats. Thus, storage stability of the conventionally processed groats could be shortened due to the development of oxidative rancidity, if the groats were stored longer than the periods of time tested in this study. Although the groats processed conventionally were exposed to heat at lower temperature than the groats processed with SS, the duration of exposure to heat in conventional processing was much longer (up to 100 min) than in SS processing (10 and 14 min). It appeared, therefore, that the duration of exposure to heat, rather than the processing temperature alone, had a greater effect on triggering of the oxidation processes in groats. The fact that generally lesser amounts of hexanal were released during storage of the SS-10 groats than the SS-14 groats (Fig. 3.8, right panel) seemed to confirm that observation.



Fig. 3. 8 Effect of storage time on free fatty acids (left side) and hexanal (right side) contents in oat groats processed conventionally (Plant A and B) and with superheated steam (SS) at 110°C and 1.00 m/s for 10 and 14min (SS-10 and SS-14, respectively), and stored at 21 and 38°C.

In this study, the originally planned storage periods were ~180 and ~90 days for groats stored 21 and 38°C, respectively. At the end of those storage periods, stabilities of oat groats processed with SS and conventionally were good. Oat groats remaining after the originally planned storage periods were stored longer, until ~360 days (at 21°C) and ~170 days (at 38°C) and additional measurements of hexanal concentrations were performed. After ~360 days at 21°C, oat groats processed with SS exhibited lower hexanal levels than the groats processed conventionally (1.4-1.7 ppm and 6.9-7.4 ppm, respectively). Similarly, after ~170 days at 38°C, the groats processed with SS released lesser amounts of hexanal than the groats processed conventionally (~1.6 ppm and 7.7-9.1 ppm, respectively).

3.7.3 Colour and Sensory Evaluation

The colour of heat processed oat groats remained stable during storage at 21 and 38°C. The effect of storage time on brightness (L value) of stored groats is shown in Fig. 3.9. The changes in redness (a value) and yellowness (b value) of stored groats followed the same trend. Small fluctuations in the colour values were observed but they were attributed mostly to the heterogeneous nature of the grain material. Differences in the L, a, and b values over time can also be expressed as a single value, ΔE . The ΔE value defines the total change in colour (with respect to the colour at the beginning of storage) but does not provide specific information about how the colours differ (Cenkowski and Sosulski 1998). In this study, small values of ΔE were noted for groats stored at both temperatures, which confirmed the negligible effect of storage time (within the tested period) on the colour of heat processed oat groats (Table 3.5). Also, at the same storage temperature, groats processed with SS and conventionally exhibited similar changes in the colour over time.

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Table 3. 5 Total change in colour^a (ΔE) of oat groats stored at 21 and 38°C on the 83rd day of storage.

| Oat p · | | SSb | SS | SS | ΔΕ | |
|------------------|----------------------|------------|--------------------------|------------|---------|---------|
| Groat Processing | Temperature Velocity | Processing | Storage Temperature (°C) | | | |
| Sample | Method | (°C) | (m/s) | Time (min) | 21 | 38 |
| Plant A | Commercial | - | | - | 1.1±0.4 | 0.5±0.1 |
| Plant B | Commercial | - | - | - | 0.7±0.2 | 0.8±0.3 |
| SS-10 | With SS | 110 | 1.00 | 10 | 1.4±0.3 | 0.9±0.2 |
| SS-14 | With SS | 110 | 1.00 | 14 | 1.5±0.4 | 0.9±0.2 |

^aWith respect to the colour of groats at the beginning of storage.

^bSS – superheated steam.

Oat groats processed with SS had a flavour unique to that associated with the conventionally processed groats (as described in Part I). At the beginning of storage, less intense toasted and more intense raw flavours were noted in the SS processed groats compared to the conventionally processed groats. It appeared that, compared to the commercial processing, the two SS treatments (SS-10 and SS-14) caused less heat/oxidative stress in oat groats and decreased the extent of formation of the toasted flavour/aroma in the groats. The lesser heat stress and largely reduced availability of oxygen during SS processing may have minimized oat lipid breakdown and oxidation and protected oat natural antioxidants. Additionally, it is possible that during SS processing deodorization of groats occurred via removal of the volatile compounds responsible for the toasted flavour/aroma. As the storage time progressed, however, the flavour of all groat samples (SS and conventionally processed) became blander and the differences in the intensities of flavour descriptors became less pronounced (Figs. 3.10-3.12). Thus, it was increasingly more difficult for the panellists to distinguish between the differently heat processed samples.

At the beginning of storage, a slight bitterness was noted in the groats processed with SS and stored at 21 and 38°C (Figs. 3.10-3.11). The groats processed with SS also exhibited higher contents of FFA compared to the conventionally processed groats (although the difference was not significant; P>0.05), which may explain the perceived bitterness in the former. However, the intensity of the bitterness decreased with the increase of storage time, and after ~140 days at 21°C (~30 days at 38°C) similar intensities of bitterness were noted in groats processed with SS and conventionally.



Fig. 3. 10 Effect of storage time on the intensity of oaty, toasted, raw, and bitter flavours of oat groats processed conventionally (Plant A and B) and with superheated steam (SS) at 110°C and 1.00 m/s for 10 and 14 min (SS-10 and SS-14, respectively), and stored at 21°C. Columns represent means of at least 4 panellists' ratings and error bars represent their standard deviations.

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Fig. 3. 11 Effect of storage time on the intensity of oaty, toasted, raw, and bitter flavours of oat groats processed conventionally (Plant A and B) and with superheated steam (SS) at 110°C and 1.00 m/s for 10 and 14 min (SS-10 and SS-14, respectively), and stored at 38°C. Columns represent means of at least 4 panellists' ratings and error bars represent their standard deviations.


Fig. 3. 12 Effect of storage time on the intensity of rancid flavour of oat groats processed conventionally (Plant A and B) and with superheated steam (SS) at 110°C and 1.00 m/s for 10 and 14 min (SS-10 and SS-14, respectively), and stored at 21 and 38°C. Columns represent means of at least 4 panellists' ratings and error bars represent their standard deviations.

Generally, the intensities of rancid flavour noted in the groats processed conventionally or with SS and stored at 21 and 38°C were low (intensities did not exceed 1.2 on 0-10 scale; Fig. 3.12). However, the intensities of rancid flavour in the groats processed with SS were usually lower than those of the conventionally processed groats (except for the results of the session held on the 17th day of storage). With the increase of storage time, the sensory panel noted an increased intensity of rancid flavour in the conventionally processed groats stored at 38°C (Fig.3.12). This trend corresponded with the increase in the concentration of hexanal released from those groats as the storage time passed (Fig. 3.8, right panel). The panellists did not perceive an increased intensity of the rancid flavour in the conventionally processed groats but stored at 21°C (Fig. 3.12), even though the concentration of hexanal released from those groats was similar to that released from groats stored at 38°C (Fig. 3.8, right panel).

Flavour flaws of stored products (i.e. rancidity development) perceived by sensory panellists can sometimes be correlated with the results obtained from analytical methods. Fritsch and Gale (1977) examined development of rancidity in breakfast cereals (including oats) by simultaneous determination of hexanal content and sensory evaluations performed on the cereals stored at 21, 37, 45, and 55°C. An increase in the amount of released hexanal was noted prior to the onset of rancid odours detectable by humans, and whenever rancid odours were first noted by the panellists, the hexanal concentration in samples was already at the level of 5-10 ppm. In addition, regression analysis of sensory ratings (a nine point rating scale, where 1 was defined as fresh and 9 was defined as edible but deteriorated to a point where the product should not be sold) of oat cereal with the amounts of hexanal released from the oat cereal stored for 12 weeks at 37°C yielded a coefficient of correlation (R^2) of 0.99. In a study by Heinio et al. (2002) also a highly positive correlation (R^2 of 0.92) was calculated for rancid flavour intensities and hexanal amounts released from oats that have been processed (germinated and dried with hot air), crushed, and stored at 20°C for 12 months. Results from the aforementioned studies suggested that the determination of

hexanal concentration in stored products may be used as a measure of deterioration before the onset of rancid odours detectable by humans, providing that hexanal released from stored product is at 4-5 ppm or higher. In our study, the stored groats were not given an overall sensory rating similar to that reported by Fritsch and Gale (1977) but rather individual sensory attributes were assessed and were given a score. Since development of rancidity in oats is the major reason of their deterioration, the scores obtained for rancid flavour intensities were chosen to be regressed with the amounts of hexanal released from groats during storage. Table A.6 (Appendix A) lists the coefficients of correlation calculated for rancid flavour intensities and amounts of hexanal released from groats stored at 21 and 38°C (for ~170 days) following conventional and SS processing. Generally, the values of R^2 were low to intermediate, regardless of the heat processing method applied to groats before storage. However, a moderately positive correlation of the results (R^2 of 0.63-0.65) was obtained for the conventionally processed groats stored at 38°C, compared to those stored at 21°C. Coincidently, the groats stored at 38°C were characterized by higher scores for rancid flavour and they released more hexanal than the groats stored at 21°C (up to ~9 ppm and up to ~4 ppm of hexanal on the 168^{th} day of storage, respectively). The enhanced correlation of the sensory and chemical results for the groats processed conventionally and stored at 38°C confirmed the earlier suggestion that information on hexanal concentration in stored products is useful as a measure of deterioration (before rancid odours are detectable by humans) only when that concentration exceeds 4-5 ppm.

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3.8 Conclusions from Storage Stability Study (Part II)

The storage stability of oat groats processed conventionally and with optimum SS parameters (temperature of 110°C, a velocity of 1.00 m/s, and processing times of 10 and 14 min) and stored at 21 and 38°C was acceptable and exceeded the originally planned storage periods of ~180 and ~90 days, respectively. Cold paste (64°C) viscosity and colour of stored groats remained stable throughout the storage periods. Moisture content of stored groats reflected the changes occurring in the surroundings which were related to seasonal changes in the relative humidity of the air.

The content of free fatty acids in groats processed with SS and conventionally remained fairly stable during storage at both temperatures. Compared to processing with SS, the conventional processing caused increased levels of hexanal released from oat groats during storage. The concentrations of hexanal released from the SS processed groats did not exceed 2 ppm even after storage for ~360 days at 21°C or ~170 days at 38°C. The conventionally processed grain, however, released close to 7 ppm (at 21°C) and up to 9 ppm (at 38°C) of hexanal after storage for the same periods of time.

Sensory evaluation indicated that with the progression of storage time, groats processed with SS and conventionally became blander (loss of toasted and oaty flavour intensities), and it was increasingly more difficult for the panellists to distinguish between the different heat processed samples.

3.9 Overall Conclusions

It was proven that SS processing can be optimized to obtain shelf stable oat groats of unique paste viscosity, flavour and a brighter appearance than the conventionally processed groats. The optimum parameters for SS processing of oat groats were: temperature of 110°C, velocity of 1.00 m/s, and processing times of 10 and 14 min, both of which gave acceptable and shelf stable (at 21°C for ~180 days and at 38°C for ~90 days) products. Processing with SS may be used to obtain oat groats with modified functional properties (i.e. cold paste viscosity, colour), which can aid in the creation of new markets for oats. Additionally, processing with optimum SS conditions is several times shorter than the conventional processing (10 or 14 min at 110°C and up to 100 min at 88-98°C, respectively) which can allow for substantial savings of energy and cost.

4 EFFECTS OF CONVENTIONAL AND SUPERHEATED STEAM HEAT TREATMENTS ON THE FUNCTIONAL PROPERTIES OF OAT GROAT, BRAN AND FLOUR

4.1 Abstract

Superheated steam (SS) processing was evaluated as a method of heat treatment alternative to conventional processing of oat groats (conditioning with wet steam followed by kiln drying). Groats of two oat cultivars varying in β -glucan content and physical chracteristics were processed conventionally and with SS at 110, 120, and 130°C. The effects of the different heat treatments on the physical properties of whole oat groats (large and small) as well as on the physicochemical and functional properties of oat bran and flour fractions obtained upon roller milling of the heat-treated groats were assessed and compared. Additionally, an examination of the effects of SS and conventional processing on the solubility, viscosity, and molecular features of β -glucans isolated from oat bran fraction was carried out.

Compared to the conventional heat treatment, the SS processing significantly (P<0.05) decreased groat hardness but increased cold paste viscosity of flour and bran slurries (by altering the swelling properties of starch). Generally, the effects of different heat treatments applied to groats on the functional properties of groats, flour, and bran fractions were augmented by the different chemical composition (mainly differences in the content and solubility of β -glucans) of the two oat genotypes tested in this study. The size of groats (within each cultivar) significantly (P<0.05) affected groat hardness, but it appeared to have lesser influence on the physicochemical properties of flour and bran fractions. Among the four heat treatments examined, the SS-110 treatment appeared to have the most positive effects on the physicochemical properties (increased molecular weight and viscosity in

solution) of BG isolated from the bran fractions of Furlong and HiFi. It appeared that the treatments of groats with SS at 120 and 130°C caused partial depolymerization of BG (lower molecular weights) thereby increasing their solubilities, compared to the control heat treatment and the treatment with SS at 110°C. Subsequently, the apparent viscosities of aqueous solutions of BG obtained from samples treated with SS at 120 and 130°C were lower than those obtained from the samples control heat-treated and SS processed at 110°C.

4.2 Introduction

Oat grain (*Avena sativa* L.) has a high nutritional value and contains starch (~60%), protein (11-20%), dietary fibre (~10%), lipids (5-9%), minerals, vitamins, and other bioactive phytochemicals (Welch et al. 2000; Zhang et al. 1998a, b). The water soluble fraction of oat fibre – mixed linkage β -glucans (2-7%) is particularly important to human nutrition. β -Glucans (BG) have been implicated in the reduction of serum cholesterol level and thus they lower the risk for cardiovascular disease (Gajdosova et al. 2007; Givens et al. 2000; Skendi et al. 2003). The effectiveness of BG in the reduction of serum cholesterol level appears to be related to viscous properties of BG solutions which in turn are dependent on concentration and molecular weight of these polysaccharides (Brennan and Cleary 2005; Tosh et al. 2003).

During storage raw oats become rancid in a relatively short period of time due to the presence of a relatively high content of lipids with a high degree of unsaturation and active lipolytic enzymes. To prevent the rancidity development, the enzymes in oats are inactivated via hydro-thermal processing (Ekstrand et al. 1992; Ganssmann and Vorwerck 1995). Conventional oat processing includes conditioning with saturated (wet) steam and

kiln drying (Molteberg et al. 1995). Kiln drying develops the characteristic "oat taste", brings about starch gelatinization to a certain degree, and if done properly, helps in reduction of grain microflora (Bryngelsson et al. 2002a; Lawrie 1999). However, the kiln drying is difficult to control and not energy efficient. Additionally, there is a risk of grain cross-contamination with microorganisms present in the air used for cooling of the grain after the drying. Heat processing of oat grain may affect physicochemical properties of BG which in turn may alter viscous properties of BG (Zhang et al. 1998a).

Processing with superheated steam (SS) is potentially an alternative method for stabilizing oats. Superheated steam, also known as dry steam, is the water vapour which has been overheated to a temperature above the saturation (boiling) point at a given pressure. Superheated steam can be used to dry moist materials due to its ability to absorb moisture as long as the SS temperature stays above the saturation (condensation) point for water. High heat transfer capability of SS makes it suitable for rapid heating and processing. Also, the replacement of air with SS eliminates oxygen from the surroundings of the processed product reducing potential of oxidation. Exposure of biological products to SS causes not only drying but also brings about changes such as starch gelatinization, enzyme destruction, protein denaturation, colour and texture changes, and deodorization (Devahastin and Suvarnakuta 2004; Tang and Cenkowski 2000, 2001).

Effects of SS processing on selected functional properties of oat groats and their storage stability were investigated earlier (Chapter 3). In the earlier work, commercial samples of groats (mixed Canadian oat cultivars) were processed with SS and conventionally, and post-processing moisture content, colour, cold paste viscosity (64°C), and sensory characteristics, as well as storage stability of the groats were assessed. As a result of that

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work, optimum conditions for SS processing (temperature of 110°C, a velocity of 1.00 m/s) of oat groats needed for increased cold paste viscosity and longer storage stability than that observed in conventionally processed groats were established. It was also found that processing of oat groats with SS at 120 and 130°C increased cold paste viscosity even further, which may be desirable for certain end-product applications. This study was designed to assess and compare the effects of SS (at 110, 120 and 130°C) and conventional treatments applied to oat groats on the physicochemical and functional properties of whole groats and two milling fractions obtained upon roller milling of these groats. Two oat cultivars differing in physical grain characteristics and chemical composition were chosen to evaluate whether the effects of different heat treatments were augmented by different grain properties. Additionally, before heat treatments the groats of each cultivar were segregated into large (plump) and small (intermediate) groats to test the hypothesis that the heat treatment effects were influenced by groat size. The major objectives of this study were to: (i) process large and small groats of two oat cultivars varying in BG content with SS at different temperatures (110, 120, and 130°C) and conventionally, (ii) assess and compare the effect of SS and conventional processing on the physical properties of whole oat groats; (iii) determine the effects of SS and conventional processing on the physicochemical and functional properties of oat bran and flour fractions; and (iv) examine the effects of SS and conventional processing on the solubility, viscosity, and molecular features of BG isolated from oat bran fraction.

4.3 Materials and Methods

4.3.1 Materials

Two oat genotypes, Furlong and HiFi, were obtained from Can Oat Milling Co. (Portage la Prairie, MB, Canada). Both cultivars were grown in 2005 in the radius of 100 miles of Portage la Prairie. De-hulled grain was stored in polyethylene bags at -30°C before analyses. Oat groats were separated according to kernel size (i.e. width) by sieving over two slotted sieves (Dimo's Tool and Die Ltd./Labtronics[®], Winnipeg, MB, Canada) in series. Within each cultivar, groats retained on the top sieve were designated as plump (large), and groats retained on the bottom sieve were designated as intermediate (small). The top and bottom sieves used for separation of Furlong groats were: No. 6 (slots of 2.38 x 19.1 mm) and No. 4.5 (slots of 1.79 x 12.7 mm), respectively, whereas for separation of HiFi groats: No. 5.5 (slots of 2.18 x 19.1 mm) and 4.5 (slots of 1.79 x 12.7 mm), respectively. In the industry, oat groats referred to as plump are the ones retained on the sieve No. 5.5. Because Furlong groats were distinctively wider than HiFi groats, the top sieve with slots slightly wider (sieve No. 6) was used to separate the plump Furlong groats from the rest of the grain.

Chemicals of analytical grade were obtained either from Sigma-Aldrich (Oakville, ON, Canada) or from Fisher Scientific (Ottawa, ON, Canada).

4.3.2 Heat Treatments

Two types of heat treatment – conventional and with superheated steam (SS) – were performed on the plump and intermediate oat groats of each cultivar. The conventional heat treatment consisted of conditioning with wet steam followed by oven drying (referred to as control heat treatment). A flat layer of groats (~250 g) was exposed to saturated (wet) steam

for ~6 min in a vegetable steamer (VS-30 Combo II, Salton Canada, Dollard-des-Ormeaux, QC, Canada) to reach moisture content of 17-18%. After steaming, groats were kept at room temperature (23°C) for ~30 min and then exposed to hot air in a forced air oven (737F, Fisher Scientific, Nepean, ON, Canada) for 90 min. During the 90 min period, the hot air temperature was adjusted as follows: 102°C for 45 min, temperature drop to 82°C at a rate of 2°C/min, 82°C for 10 min, temperature drop to 60°C at a rate of 1.5°C/min, and 60°C for 10 min. Oat groats were then cooled at 23°C for 30-40 min, placed in an air-tight container, and stored at 4°C.

Superheated steam (SS) treatment experiments were performed with SS at a velocity of 1.00±0.02 m/s and temperatures of 110, 120 and 130°C (referred to as SS-110, SS-120, and SS-130, respectively). Raw oat groats (~13 g) contained in a processing tray - an aluminium cup (inner diameter of 6.3 cm and depth of 3 cm; Fig. 3.1 in Chapter 3) with a wire mesh bottom - were placed in the SS processing chamber (Fig. 3.2 in Chapter 3) and exposed to SS. Duration of SS processing at each temperature was optimized to reach the final moisture content of groats at 9-10% and was as follows: 11 min at 110°C, 4 min at 120°C, and 2.5 min at 130°C. Superheated steam-treated groats were then removed from the processing chamber, cooled at 23°C for 30-40 min, placed in an air-tight container, and stored at 4°C.

4.3.3 Dimensions and Hardness

One hundred oat groats were placed vertically with crease downwards directly onto a scanner (ScanMaker 4, Microtek Lab Inc., Fontana, CA, USA) glass and analyzed with Scan Wizard (version 2.6 Microtek Lab) to obtain the length and width of the groats. The groat hardness index was derived by analyzing groat samples (two portions of 300 kernels

each) with a single kernel characterization system (SKCS 4100, Perten Instruments Inc., Springfield, IL, USA). The SKCS instrument is normally used to measure wheat samples, which are much harder than oats. As a result, all hardness index values for oat groats were negative. Samples with higher values of hardness index (less negative) represented harder groats.

4.3.4 Scanning Electron Microscopy

Raw, control heat-treated, and treated with SS at 130°C plump oat groats were selected for this analysis. The kernels were fractured transversely. A small incision, only slightly beyond the aleurone layer, was made with a scalpel (No. 3 blade, Fisher Scientific) in the middle of the kernel, across the ventral crease. The scored kernels were broken by hand and half kernels were mounted, with the fractured surface facing up, onto aluminum stubs covered with Leit-C conductive carbon cement (Neubauer, Germany), and allowed to dry and set for 24 h. The mounted samples were placed in a Hummer VII (Anatech, Ltd., Hayward, CA, USA) sputter coater, coated with 50 nm of gold, and examined with a JEOL JSM-6400 scanning electron microscope at an accelerating voltage of 10 kV. Photographs were taken using Kodak TMAX 100 Black and White Professional 120 roll film.

4.3.5 Milling

Heat-processed oat groats (150 g in duplicate) were roller milled into flour and bran fractions using a modified Ross roll stand (Ross Machine and Mill Supply, Oklahoma City, OK, USA) equipped with 25-cm diameter rolls in conjunction with a rotary box sifter (Buhler Canada, Markham, ON, Canada) (Fig. 4.1). Two break passages (B1 and B2, respectively) were performed in a climate controlled room (21°C and 60% RH). The rolls in B1 and B2 were corrugated with 16 and 24 flutes (cuts) per 25.4 mm of roll surface, respectively. Roll corrugations were orientated dull-to-dull. The rolls were set at a differential speed (ratio of fast roll speed to slow roll speed) of 3:1 (slow roll speed of 185 rpm) in both break passages. The gap between the rolls was set at 0.2 mm and 0.08 mm in B1 and B2, respectively. Coarse material obtained in the first passage was reduced in the second passage. Flours from the first and second break passage (material smaller than 475 μ m) were combined and designated as oat flour. Material retained on the three sieves used in the B2 passage was combined and designated as bran.



Fig. 4. 1 The milling flow for oat groats. B1: first break rolls with 16 flutes per 25.4 mm of roll surface, B2: second break rolls with 24 flutes per 25.4 mm of roll surface. Numbers in blocks represent sieve apertures expressed in μm.

4.3.6 Colour

The colour of oat flour and bran was measured in triplicate using a chromameter (CR-410, Konica Minolta, Tokyo, Japan). The flour or bran sample was placed in a circular granular materials attachment (50 mm diameter and 10 mm depth; CR-A50, Konica Minolta) ensuring that the total volume of the attachment was filled. A measurement (L, a, b values) was taken and the sample was removed from the holder, mixed with the remaining material, and a new portion was placed in the holder for the next measurement.

4.3.7 Particle Size

Particle size distribution in flour and bran fractions was determined in duplicate using a laser diffraction sizer, Mastersizer 2000 (Malvern Instruments Ltd., Southborough, MA, USA) with Hydro 2000S and Scirocco 2000B attachments. Flour was suspended in anhydrous ethanol, whereas bran was dispersed in air immediately before the analysis. The size of particles (expressed as the diameter of equivalent sphere with the same volume) was determined based on the angle of the diffracted laser light as the particles passed in front of the laser beam. Particle diameter distributions were computed using the Mastersizer 2000 software (version 5.22, Malvern Instruments Ltd.) which compared sample's scattering pattern with the Mie theory diffraction model. The resulting distributions were volume based and expressed the volume proportion of particles with different diameters in the total volume of particles.

4.3.8 Chemical Composition

Moisture and ash contents were determined according to Approved Methods 44-15A and 08-01, respectively (AACC 2003). Protein content (N x 6.25) was determined by combustion nitrogen analysis (FP-248 Leco Dumas CAN analyzer, St. Joseph, USA).

calibrated with EDTA according to Approved Method 46-30 (AACC 2003). Starch content was determined by a total starch assay (AACC Approved Method 76-13) and β -glucans (BG) content was determined by a mixed-linkage BG assay (AACC Approved Method 32-23), both supplied by Megazyme International Ireland Ltd. (Wicklow, Ireland). Pentosans content was analyzed by the phloroglucinol colorimetric method (Douglas 1981). Soluble BG and pentosans in groat, flour, and bran were determined by extraction of the materials either with sodium phosphate buffer at pH \approx 7 (for BG) or water (for pentosans) at 40°C for 2 h and measurement of BG and pentosans content in the extracts. Extraction with the buffer was done at a ratio of 1:24 (solids to water) for groat and flour samples and 1:50 for bran samples. Extraction with water was done at a ratio of 1:30 (solids to water) for all samples. Solubility was expressed as the percentage of BG (or pentosans) present in the extract versus the total content of BG (or pentosans) present in each sample.

4.3.9 Water Retention Capacity

Samples of flour and bran (0.5 g) were suspended in water (10 mL), agitated in a rotary shaker overnight at 23°C and then centrifuged at 1000 rpm for 10 min. The weight of the remaining wet residue was recorded and the residue was dried at 120°C in a forced air oven (737F, Fisher Scientific) overnight and weighed again. Water retention capacity (WRC) was expressed as the amount of water retained in the residue per 1 gram of the dry residue.

4.3.10 Thermal Properties

A modulated differential scanning calorimeter, 2920 MDSC (TA Instruments, New Castle, DE, USA), controlled by TA Advantage software (version 1.1A, TA Instruments) was used to determine the thermal properties of oat flour, bran, and raw groats (whole meal). A hermetically sealed aluminium pan containing vacuum dried material (3.8-4.0 mg)

suspended in water (25% solids) was scanned from 20 to 120°C at 5°C/min with a temperature modulation amplitude of 0.75° C applied over a period of 60 s. An empty aluminium pan was used as a reference to balance the heat capacity of the sample pan. Onset, peak, and conclusion temperatures of endothermic transitions were measured in duplicate. Enthalpy change (ΔH) of each transition was calculated using Universal Analysis software (version 3.9A, TA Instruments).

4.3.11 Pasting Properties

Pasting viscosity of oat flour and bran slurries was determined in duplicate with a stirring and cooking viscometer - Rapid Visco Analyser (RVA-4, Newport Scientific Pty, Ltd., Warriewood, Australia) using two temperature profiles. The first profile involved holding flour slurry (3.5 g of flour corrected to 14% moisture content and 25 mL of water) at 50°C for 1 min, then heating from 50 to 95°C at a rate of 12.2°C/min, holding at 95°C for 2.5 min, cooling back to 50°C at a rate of 11.8°C/min, and holding at 50°C for 2 min. This profile is referred to as the general pasting profile in AACC Method 76-21 (AACC 2003). A constant paddle rotating speed of 160 rpm was used throughout the entire test (13 min) except for the first 10 s, when a speed of 960 rpm was used to disperse the sample. Peak viscosity, holding strength (trough), final viscosity, breakdown, and setback of the slurry were noted. For the second test, called cold pasting test (AACC Method 76-22), 6 g of material (corrected to 14% moisture content) was slurried with 25 mL of water. The slurry was heated from 30 to 64°C at a rate of 6.8°C/min and then held at 64°C for 15 min with constant paddle stirring speed of 160 rpm, except for the first 10 s when a speed 960 rpm was used to disperse the sample. Viscosity of the slurry was continuously recorded during the test but the final viscosity (at the 20th min of the test) was used to compare samples.

Viscosity of slurries recorded during both tests was measured in Rapid ViscoAnalyser units (RVU) which were later recalculated to centipoise units (cP; 1 RVU \approx 12 cP), to express the apparent viscosity of slurries.

4.3.12 Small Strain Rheology

Small strain dynamic rheological tests were performed on flour gels to determine viscoelastic properties of the gels. Aqueous suspension of flour (40% solids) was boiled for 15 min in a stainless steel mold (diameter of 7 cm, height 1 mm) and then cooled in ice water for 15 min. After cooling, the mold was opened, and a gel disc was carefully removed from the mold. A round gel sample (diameter of 25 mm) cut out from the gel disc was placed between serrated parallel plates (diameter of 25 mm) of the SR500 (Rheometrics, Scientific®, Piscataway, NJ, USA) dynamic stress rheometer, with the gap between the plates set at 0.95 mm. The edge of the sample was coated with mineral oil to prevent moisture loss during measurements. Frequency sweep from 0.03 to 7.5 Hz at a strain controlled at 0.43% was performed in duplicate at 23°C. Storage (elastic) and loss (viscous) moduli (G' and G'', respectively) of gels were measured. The value of 0.43% of the controlled strain was chosen based on preliminary stress sweeps were conducted to ensure that the frequencies (0.1, 1, and 10 Hz). The preliminary stress sweeps were conducted to ensure

4.3.13 Extraction and Purification of β-Glucans (BG)

Oat bran (50 g) obtained from plump groats was suspended in water (bran to water ratio of 1:12 and 1:20 for Furlong and HiFi, respectively) and heated at 45°C with continuous stirring for 30 min. The suspension was centrifuged (8000 rpm, 20 min) and the supernatant collected. The insoluble residue was re-extracted with 500 mL of water for 15 min. The supernatant was collected and combined with the supernatant from the first extraction, heated at 95°C for 10 min, and cooled on ice. Celite was added at a rate of 20 g/L and the suspension was mixed well and centrifuged. The supernatant was collected and mixed with Fuller's Earth at a rate of 20 g/L. The suspension was stirred for 20 min and then centrifuged. The supernatant was collected, filtered, and pH was adjusted to 6.5. Porcine pancreatic α -amylase and calcium chloride were added and the mixture was held at 35°C overnight. Standard iodine test was performed to check for presence of starch in the extract after the enzyme treatment (no starch detected). The mixture was boiled to inactivate the enzyme, cooled, centrifuged and filtered. Aqueous ethanol (95% v/v) was used to precipitate β -glucans. The precipitate was collected by decantation and centrifugation. The pellet was collected, dissolved in water by heating at ~80°C, and freeze-dried (Virtis 10-146-MP-BA, Gardiner, NY, USA). Protein content (as impurities) in BG isolates was determined using the Folin's reagent according to the method of Lowry et al. (1951).

4.3.14 Molecular Weight Determination

Weight-average molecular weight (M_w) of the isolated BG was determined by high performance size exclusion chromatography-multi angle light scattering, HPSEC-MALS. The system consisted of a Waters Alliance 2695, 2487 UV detector, controlled by Empower software (Waters Associates, Milford, MA), and a Wyatt DAWN Heleos light scattering detector, Optilab rEX RI detector, controlled by Astra software (Wyatt Technology, Santa Barbara, CA). The separation was performed on two columns in series, SB803HQ and SB806MHQ (300 x 7.8 mm; Shodex Showa Denko K.K. Tokyo, Japan) with a guard, OHPAKSB-G (50 x 6 mm; Shodex) maintained at 30°C. The samples (100 µL inject) were analyzed using an effluent of 0.075 M sodium nitrate buffer at a flow rate of 0.5 mL/min over 60 min. BG samples were dissolved with gentle heating in sodium nitrate buffer and filtered through Whatman 1.6 μ m GF/A filter.

4.3.15 Analysis of Oligosaccharides Released by Lichenase

Oat BG samples (5 mg) were dissolved in 2.5 mL of sodium phosphate buffer (20mM, pH 6.5) and digested with lichenase (4 U/mL, Megazyme) for 18 h at 40°C. After digestion, samples were heated to 95°C for 10 minutes to inactivate the enzyme, and then centrifuged (20,000 rpm, 20 min). Oligosaccharides released by lichenase were analyzed by high performance anion exchange chromatography (HPAEC). The system consisted of a Waters 625LC pump, 717plus WISP autosampler (Waters Associates), a Dionex CarboPac PA1 column (4 x 250 mm) with a PA1 guard column, and a Coulochem III (ESA, Chelmsford, MA) electrochemical detector equipped with the 5040 Analytical cell containing a gold target electrode. Eluents used for the analysis consisted of: 150 mM sodium hydroxide (eluent A) and 150 mM sodium hydroxide containing 300 mM sodium acetate (eluent B). Samples were eluted for 1 min with 65% of eluent A and 35% of eluent B, then from 1st to 9th min over a gradient to 50% of eluent B, and from 9th to 22nd min over a gradient to 100% of eluent B. From 22nd to 24th min a gradient was used to bring the eluents back to starting conditions (65% of eluent A and 35% of eluent B). The column was then allowed to equilibrate for 8 min giving a total run time of 32 min at a flow rate of 1 mL/min. Data was processed using Empower Software (Waters Associates). The oligosaccharide composition was calculated based on the % area of each peak (corresponding to particular oligosaccharide) in the total area of peaks for a given sample.

4.3.16 Rheological Measurements

Testing of flow behaviour of fresh aqueous BG solutions (1.0 and 1.4%, w/v) was performed on a controlled stress rotational rheometer (AR 2000, TA Instruments) equipped with concentric cylinder (diameter of cup and bob: 30 and 28 mm, respectively) and Peltier temperature control system (set at $22\pm0.01^{\circ}$ C). The flow behaviour was determined by measuring steady state shear viscosity over a range of shear rates (from 0.1 to 1500 1/s).

4.3.17 Statistical Analysis

Results for flour, bran, or groat within each cultivar/size group (for example Furlong/plump) were analyzed using one-way analysis of variance (ANOVA) using SigmaStat (version 3.5, Systat Software Inc., Point Richmond, CA, USA) with respect to the heat treatment effect (control heat-treated, SS-110, SS-120, and SS130). If differences among differently heat-treated samples were found within a cultivar/size group then a pairwise multiple comparison procedure using the Holm-Sidak method was used to determine which means differed significantly (P<0.05).

4.4 **Results and Discussion**

4.4.1 Chemical Composition of Raw Oat Groats

Whole groats of Furlong and HiFi cultivars were composed mostly of starch, protein and β -glucans (BG) with smaller amounts of ash and pentosans (Table 4.1). In both cultivars, the differences in composition of plump and intermediate groats were not statistically significant (P>0.05), except for the ash content of Furlong groats.

| Cultivar, Size | Sieve Slot Width (mm) | Starch | Protein ^b | Ash | Total β-Glucans | Water Soluble β-Glucans ^c | Total Pentosans | Water Soluble Pentosans ^d |
|----------------|--------------------------|-----------|----------------------|----------|--------------------|---|--------------------|---|
| Furlong Groat, | | | | | | | • | |
| plump | 2.38° | 63.8±0.4a | 13.7±0.1a | 2.1±0.0b | 4.0±0.0a | 2.5±0.1a (63.1) ^g | 2.0±0.1a | $0.1\pm0.0a$ (6.5) ^h |
| intermediate | 1.79 ^r | 64.1±0.2a | 13.5±0.1a | 2.0±0.0a | 3.9±0.1a | 2.7±0.1a (69.8) | 2.4±0.3a | 0.1±0.0a (6.0) |
| HiFi Groat, | | | | | | | | |
| plump | 2.18 ^e | 58.2±1.1a | 18.2±0.0a | 2.3±0.0a | 5.8±0.2a | 4.7±0.1a (81.1) | 2.4±0.0a | 0.2±0.0a (8.2) |
| intermediate | 1.79 ^f | 56.6±1.3a | 18.3±0.1a | 2.3±0.0a | 5.7±0.2a | 4.5±0.1a (79.1) | 2.2±0.1a | 0.2±0.0a (8.5) |

Table 4. 1 Chemical composition^a (%, db) of raw oat groats.

Values are means of 2 replicates; values in the same column, within each cultivar, followed by the same letter do not differ significantly (P>0.05). ^bN x 6.25.

°Groats were extracted at 40°C for 2 h (groats to water ratio 1:24, w/v). β -Glucans were determined in the extract. ^dGroats were extracted at 40°C for 2 h (groats to water ratio 1:30, w/v). Pentosans were determined in the extract.

Groats were retained on slotted sieve No. 6 (Furlong) or No. 5.5 (HiFi).

^fGroats passed through slotted sieve No. 6 (Furlong) or No. 5.5 (HiFi) and were retained on slotted sieve No. 4.5 (both Furlong and HiFi).

^gSolubility expressed as percentage of total β-glucans.

^hSolubility expressed as percentage of total pentosans.

Substantial differences in the composition of groats between the two cultivars were observed. The groats of Furlong contained a higher amount of starch but lower amounts of protein and BG than HiFi. The contents of ash and pentosans, however, were similar. The solubility of BG in water at 40°C was lower for Furlong than HiFi (63.1-69.8% and 79.1-81.1%, respectively). The content of soluble pentosans was low in both cultivars (0.1% and 0.2% for Furlong and HiFi groats, respectively). It has been reported that variations in the content of BG in oats are affected by genetic and environmental factors (Lee et al. 1997; Manthey et al. 1999; Saastamoinen et al. 1992). The HiFi genotype was developed specifically to contain more BG or soluble fibre in the groat than other cultivars (McMullen et al. 2005).

4.4.2 Physical Characteristics of Oat Groats

Appearance of oat groats

Scanning electron micrographs were taken to illustrate the appearance of cross-section of oat groats before and after heat treatments (Figs. 4.2–4.3). Among groats treated with SS, only those treated with SS at 130°C were assessed as they represented the harshest treatment. As opposed to raw groats, the heat-treated groats appeared to have cracks around the ventral crease (Fig. 4.2). The cracks/openings observed in the groats treated with SS appeared larger than those observed in the control heat-treated groats. The fractures were especially visible in the groats of Furlong.

Generally, the endosperm of Furlong groats appeared less compact than that of HiFi (Fig. 4.2). The groats of HiFi fractured along the cell walls whereas those of Furlong fractured across the cells exposing the cells contents (especially visible in the sub-aleurone layer; Fig. 4.3). Also, the sub-aleurone layer of HiFi groats appeared to have thicker cell walls than that of Furlong groats (Fig 4.3). It has been reported earlier that the thickened cell walls of the sub-aleurone layer in oat grain contain relatively large amounts of BG (Fulcher 1986; Luhaloo et al. 1998; Wood et al. 1991b).



Fig. 4. 2 Scanning electron micrographs of plump oat groats (magnification x 30).





Dimensions and hardness of oat groats

The dimensions and hardness index of oat groats before and after different heat treatments are given in Table 4.2. The heat-treated groats exhibited slightly smaller dimensions than the raw groats, probably due to 3.0-3.6% loss of moisture after the heat treatments (Table 4.2). Among the differently heat-treated groats, those processed with SS at 120°C and 130°C exhibited greater width than the groats which were processed with SS at 110°C or control heat-treated. Additionally, for Furlong cultivar, the groats processed with SS at 130°C were significantly (P<0.05) wider than the groats processed with SS at 120°C. The effects of processing with SS at 120 and 130°C on the width of HiFi groats were similar but not statistically significant. Within each cultivar/groat size group, no significant (P>0.05) differences were noted in the length of groats exposed to different heat treatments. Similar trends in the changes of groat dimensions due to different heat treatments applied were observed for plump and intermediate groats.

For both cultivars, the different heat treatments caused a significant (P<0.05) decrease of hardness of groats compared to the hardness of raw groat (Table 4.2). The SS processed groats exhibited values of hardness index significantly (P<0.05) lower than those of control heat-treated groats. Moreover, an increase of SS temperature caused a significant (P<0.05) decrease of groat hardness. However, the magnitude of variation in the hardness of groats due to the different heat treatments was smaller than the magnitude of variation due to the genotype, i.e. groats of Furlong were softer than groats of HiFi. Also, within each cultivar, plump groats were softer than intermediate groats.

| Cultivar Size / Treatment | Final Moisture | Dimer | | |
|-----------------------------------|----------------|------------------|-------------------|-------------------|
| | Content (%) | Length (mm) | Width (mm) | Hardness Index |
| Groat Furlong, plump | | | | |
| Raw | 13.2 ± 0.1 | 9.15±0.79b | 3.22±0.15b | -39.6±0.8a |
| Control Heat-Treated ^b | $9.9{\pm}0.0$ | 8.83±0.69a | 3.07±0.14a | -57.5 ± 1.4 b |
| SS-110 ^c | 9.8±0.1 | 8.88±0.66ab | 3.08±0.13a | $-60.0\pm0.8c$ |
| SS-120 | 9.9±0.1 | 8.95±0.78ab | 3.19 ± 0.15 b | -65 7+1 7d |
| SS-130 | 9.9±0.0 | 8.93±0.84ab | 3.28±0.15c | -65.3±1.1e |
| Groat Furlong, intermediate | | | | |
| Raw | 13.1 ± 0.1 | 7.97±0.92b | $3.04\pm0.18b$ | -33 5+0 42 |
| Control Heat-Treated | 9.8±0.1 | 7.58±0.84a | $2.90\pm0.15a$ | -44.1+0.2b |
| SS-110 | 9.6±0.0 | 7.47±0.85a | $2.91\pm0.17a$ | -49 7+0 3c |
| SS-120 | 9.6±0.2 | 7.67±0.89ab | $2.96\pm0.18a$ | -52 8+0 6d |
| SS-130 | 9.5±0.1 | 7.80±0.80ab | 3.07±0.17b | -53.9±1.9e |
| Groat HiFi, plump | | | | |
| Raw | 12.7 ± 0.1 | 8.42±0.79b | $2.91 \pm 0.16b$ | -27 8+0 8a |
| Control Heat-Treated | 9.7±0.1 | 7.93±0.79a | 2.79±0.18a | -34 8+0 9h |
| SS-110 | 9.7±0.2 | 8.05±0.73a | 2.79±0.18a | -41.8+1.30 |
| SS-120 | 9.8 ± 0.1 | 8.23±0.80ab | 2.85±0.18ab | -43.9±0.8d |
| SS-130 | 9.7±0.0 | 7.98±0.87a | 2.91±0.20b | -45.2±0.0e |
| Groat HiFi, intermediate | | | | |
| Raw | 12.7±0.1 | $7.72 \pm 0.93a$ | 2 77+0 21b | -15 0+0 /2 |
| Control Heat-Treated | 9.7±0.1 | $7.47 \pm 0.90a$ | 2.67 ± 0.210 | -15.5-10.4a |
| SS-110 | 9.6±0.1 | 7.67±0.86a | $2.70\pm0.19ab$ | -20.0-1.00 |
| SS-120 | 9.5±0.2 | 7.58±0.89a | $2.69\pm0.18a$ | -33 3+1 0d |
| SS-130 | 9.4±0.1 | $7.43 \pm 0.84a$ | $2.74\pm0.19ab$ | -33 5+0 8e |

Table 4. 2 Effects of heat treatments on the final moisture content^a, dimensions^a and hardness^a of oat groats.

SS-130 9.4 ± 0.1 $1.43\pm0.84a$ $2.74\pm0.19ab$ $-33.5\pm0.8e$ ^aValues are means of 2 replicates (moisture content), 100 replicates (length, width) and 600 replicates (hardness); values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05). b Control heat treatment – conditioning with wet steam followed by oven drying.^cSS-110, 120, 130 – processing with superheated steam (SS) at 110, 120, and 130°C, respectively. $-33.5\pm0.8e$

The differences in hardness of groats of Furlong and HiFi could be ascribed to differences in content of BG in the two genotypes (i.e. groats of HiFi contained more BG than groats of Furlong). β -Glucans are major structural components of cell walls in oat, thus increased proportion of β -glucans in groat increases the thickness of cell walls. Thicker cell walls throughout the kernel, in turn, render the kernel harder. In fact, a close positive correlation of kernel hardness and content of BG has been found for oats (Doehlert and McMullen 2000; Engleson and Fulcher 2002) and barley (Gamlath et al. 2008). However, the differences in groat hardness observed among the different heat-treated groats of same cultivar can not be attributed to differences in chemical composition. The observations of greater extent of crack/fracture formation in groats combined with the findings of greater groat width after application of SS heat treatment could explain the decreased hardness of SS-treated groats, compared to that of control heat-treated groats (within same cultivar). In fact, the differences in groat hardness due to heat treatments applied agreed with the observations of cracks/fissures formation (Fig. 4.2) and closely corresponded to the differences in the width of groats (indirectly confirming the formation of fractures; Table 4.2). Both, surface cracks and internal fissures have been shown to weaken the structure and integrity of grain and thus to reduce groat hardness (Gates et al. 2008; Siebenmorgen et al. 2005). Drying/heat processing of grain is a major cause of kernel fissuring (Cnossen et al. 2003; Siebenmorgen et al. 2005). Stress cracking in cereals such as rice during drying has been explained via the glass transition concept (Cnossen and Siebenmorgen 2000). At a given moisture content (MC), as kernel temperature passes through the glass transition temperature (Tg), the starch (the major component of cereals) changes its state from a "glassy" into a "rubbery". At either of these states the thermo-physical properties of kernels

differ (e.g. thermal expansion coefficient and specific volume increase above Tg). During drying, a rapid loss of moisture from the surface of grain induces MC gradient from the surface to the center of a kernel. If a sufficient MC gradient within a kernel is present, a significant portion of the kernel periphery will transition into the "rubbery" state while the center will remain in the "glassy" state, thus causing differences in intrakernel material property. The differences in material property, if large enough, will in turn cause differential stresses within the kernel causing kernel fissures/cracks. In this study, the groats processed with SS were exposed to greater heat intensity and higher temperatures than the control heat-treated groats. It appeared that the more severe SS heat conditions possibly caused more extreme MC gradients across groat and may have caused formation of increased number of fissures in groats, or the formed fissures had larger size. Further work needs to be done to determine the exact mechanism of fracture formation in oat groats exposed to SS and its effect on groat hardness, and no early conclusions should be drawn. However, providing that the glass transition concept is the correct explanation of the formation of fractures in heat-treated groats, it could also help to explain why the large (plump) groats were softer than the small (intermediate) groats within each cultivar. Assuming that heat penetration into the center of small groats occurs faster than into the center of large groats, the resulting MC gradient in the small groats will be lesser than that in the large groats. The lesser MC gradient will result in lesser differential stresses within the small groats and cause less kernel fissures/cracks.

Hardness of groats is expected to influence power consumption during milling operation (Kilborn et al. 1982). The fact that the groats processed with SS were softer than the control heat-treated groats may have its benefits on an industrial scale, as the softer groats require

less energy to grind them. In this study, a measurement of milling energy of differently heat-treated oat groats was not performed due to the inadequate sample size used for milling.

4.4.3 Milling Yields and Physicochemical Characteristics of Oat Bran and Flour Fractions

Milling yields of oat bran and flour fractions

Roller milling along with hammer milling are commonly used by oat processors to obtain bran- and endosperm- rich fractions, referred to as oat bran and flour, respectively (Deane and Commers 1986; Doehlert and Moore 1997; Wang et al. 2007). In this study, a series of roller mills and sifters were used to obtain bran and flour fractions (Fig. 4.1). The milling protocol was optimized to obtain bran fraction complying with the definition of oat bran given by the American Association of Cereal Chemists (AACC 1989). By that definition: "oat bran is the food which is produced by grinding clean oat groats or rolled oats and separating the resulting oat flour by sieving bolting, and/or other suitable means into fractions such that the oat bran fraction is not more than 50% of the original starting material and has a total β -glucans content of at least 5.5% (dry weight basis) and a total dietary fibre content of at least 16.0% (dry weight basis), and such that at least one-third of the total dietary fibre is soluble fibre." (Fulcher and Miller 1993).

The yields of bran fraction obtained by roller milling of differently heat-treated Furlong groats ranged from 38.2 to 40.0% and from 38.8 to 40.6% for plump and intermediate groats, respectively (Table 4.3). Plump and intermediate groats of the HiFi yielded from 49.0 to 50.0% and from 49.5 to 52.7% of bran, respectively. Only HiFi intermediate groats processed with SS yielded bran fraction exceeding the 50% benchmark of the starting

material mentioned in the AACC bran definition. The yields of bran fraction obtained from Furlong groats were similar to those reported by Doehlert and McMullen (2000) who examined the milling characteristics of various oat genotypes grown in North Dakota. The yields of bran fraction reported by them varied from \sim 33 to \sim 43%.

| Cultivar Size / | Millin | Recovery | |
|-----------------------------------|-----------|---|---------------------|
| Treatment [–] | (% | | |
| ireautient | Flour | Bran | (%) |
| Furlong, plump | | 0 07 0 00 00 00 00 00 00 00 00 00 00 00 | 6 |
| Control Heat-Treated ^c | 60.8±1.6a | 38.2±1.2a | 99.0±0.4a |
| SS-110 ^d | 57.8±0.0a | 40.0±0.4a | 97.8±0.4a |
| SS-120 | 58.6±1.5a | 39.5±0.5a | 98.1±1.0a |
| SS-130 | 59.4±0.2a | 39.5±0.3a | 98.9±0.1a |
| Furlong, intermediate | | | |
| Control Heat-Treated | 59.7±0.6a | 39.8±0.2a | 99.4±0.8a |
| SS-110 | 57.6±0.6a | 40.2±0.2a | 97.8±0.4a |
| SS-120 | 58.2±2.5a | 40.6±2.1a | 98.8±0.4a |
| SS-130 | 58.4±2.4a | 38.8±1.7a | 97.2±0.7a |
| HiFi, plump | | | |
| Control Heat-Treated | 49.9±1.2a | 49.0±0 9a | 98 9+0 3a |
| SS-110 | 47.0±0.9a | $49.2\pm0.4a$ | $96.9 \pm 0.3 a$ |
| SS-120 | 46.2±1.0a | 50.0±0.2a | $96.1\pm1.2a$ |
| SS-130 | 47.2±2.3a | 49.3±1.4a | $96.5\pm0.9a$ |
| HiFi, intermediate | | |) 010 <u>-</u> 019u |
| Control Heat-Treated | 48.7±0.1a | 49.5±0.4a | 98.2±0.2a |
| SS-110 | 46.1±1.6a | 51.4±0.1b | 97.5±1.4a |
| SS-120 | 44.4±1.6a | 52.1±0.0c | 96.5±1.6a |
| SS-130 | 44.3±0.0a | 52.7±0.1d | 97.0±0.1a |

 Table 4. 3 Milling yields^a and recovery^b of flour and bran obtained by roller milling of heat-treated oat groats.

^aAll values are means of 2 replicates; values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05).

^bRecovery is represented as % of weight (Flour + Bran) recovered after milling of groats.

^cControl heat treatment – conditioning with wet steam followed by oven drying.

^dSS-110, 120, 130 - processing with superheated steam (SS) at 110, 120 and 130°C, respectively.

The HiFi groats yielded about 10% more bran and concomitantly less flour than the Furlong groats (Table 4.3). In addition, the intermediate groats of HiFi gave about 2% more bran and concomitantly less flour than the plump groats (Table 4.3). The observed differences in bran yields of Furlong and HiFi were most likely caused by substantially different content of BG in groats and groat hardness. Groats of HiFi contained more BG, exhibited thicker cell walls of sub-aleurone layer, and were harder than groats of Furlong (Tables 4.1-4.2, Fig. 4.3). It has been reported that higher yield of oat bran was associated with higher content of BG in oat groat (Doehlert and McMullen 2000; Gates and Dobraszczyk 2004). Furthermore, reports on positive correlation of grain BG content and hardness with bran yield can be found for oats (Engleson and Fulcher 2002) and barley (Gamlath et al. 2008).

The different heat treatments applied to groats did not significantly (P>0.05) affect the milling yield and recovery of flour and bran fractions, except for the yield of bran obtained from the intermediate HiFi groats. The control heat-treated intermediate HiFi groats yielded the lowest amount of bran (49.5%) whereas the SS-130 treated groats yielded the highest amount of bran (52.7%).

Colour of oat bran and flour fractions

Bran fractions of Furlong were slightly darker and more yellow than bran fractions of HiFi, whereas flour fractions of both cultivars exhibited very similar colour. Different heat treatments applied to oat groats before milling had very similar effects on the colour of bran and flour fractions in each cultivar. Flour and bran fractions obtained from groats treated with SS were generally only slightly brighter (higher L values) than those obtained from the control heat-treated groats (Table 4.4).

| Cultivar, Size / | L | | а | | b | |
|-----------------------------------|----------------|---------------|--------------------------|---------------|-------------------------------------|--------------------------|
| | Bran | Flour | Bran | Flour | Bran | Flour |
| Furlong, plump | | | ····· | | | : |
| Control Heat-Treated ^b | 71.4±0.4a | 81.6±0.2a | 2.1±0.0a | 0.0±0.0ab | 10.7±0.1a | 8.0±0.1b |
| SS-110 ^c | 72.3±0.1b | 82.5±0.2b | 2.1±0.0a | $-0.1\pm0.1a$ | 10.7±0.1a | 75+02a |
| SS-120 | 72.1±0.2b | 82.8±0.2b | 2.1±0.0a | 0.0±0.0ab | $10.6\pm0.0a$ | 8.0+0.1b |
| SS-130 | 72.3±0.1b | 82.5±0.2b | 2.1±0.0a | 0.1±0.0b | 10.6±0.0a | 7.5±0.1a |
| Furlong, intermediate | | | | | | |
| Control Heat-Treated | 70.9±0.2a | 81.9±0.2a | 2.3±0.0a | $0.0\pm0.0a$ | 10 7+0 0a | 9.0+0.1c |
| SS-110 | 71.2±0.2ab | 82.4±0.1b | 2.2±0.0a | $0.0\pm0.0a$ | 10.7 ± 0.00 | 8 6+0 1b |
| SS-120 | 71.5±0.1b | 82.8±0.1c | 2.3±0.0a | $0.0\pm0.0a$ | $10.8\pm0.0b$ | 8.0 ± 0.10 8.2+0.1a |
| SS-130 | 71.6±0.1b | 82.4±0.1b | 2.3±0.0a | 0.1±0.0b | 10.7±0.0a | 8.1±0.1a |
| HiFi, plump | | | | | | |
| Control Heat-Treated | 74.1±0.1a | 82.2±0.2a | $1.5 \pm 0.0a$ | 0 1+0 0b | 10 3+0 0a | 7.6+0.0b |
| SS-110 | 74.8±0.2b | 82.8±0.2b | $1.4\pm0.0a$ | -0.2 ± 0.02 | $10.3\pm0.0a$ 10.4+0.0b | 7.0±0.00 7.6±0.1b |
| SS-120 | 74.7±0.2b | $82.9\pm0.1b$ | $1.4\pm0.0a$ | $-0.2\pm0.0a$ | 10.4 ± 0.00 10.2 ± 0.00 | 7.0 ± 0.10 |
| SS-130 | 74.8±0.2b | 82.6±0.2ab | 1.4±0.0a | -0.2±0.0a | 10.2±0.0a | 7.2±0.1a |
| HiFi, intermediate | | | | | | |
| Control Heat-Treated | 74 0+0 6a | 82 4+0 2a | 1 5+0 1a | 0.0+0.02 | 10.2 0.1 - | 0.0.0.1 |
| SS-110 | 74.4+0.2ab | 82.9±0.1b | $1.5\pm0.1a$ | $0.0\pm0.0a$ | $10.5\pm0.1a$ | $8.0\pm0.1c$ |
| SS-120 | $74.9\pm0.2a0$ | 82.9 ± 0.10 | $1.5\pm0.0a$ | $0.0\pm0.0a$ | 10.3 ± 0.00 | 7.7±0.0b |
| SS-130 | 75.2 ± 0.1 h | 82.7±0.1ab | $1.3\pm0.0a$ 1 4+0 0a | 0.1 ± 0.00 | $10.4 \pm 0.1 ab$ 10.3 \pm 0.1 a | $7.0\pm0.0ab$ |

Table 4. 4 Effects of heat treatments on colour values^a of oat bran and flour obtained by roller milling of oat groats.

 $\frac{\text{SS-130}}{\text{aValues are means of 3 replicates; } L - \text{brightness, } a - \text{redness, } b - \text{yellowness; values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05).}$ $b^{\text{b}}\text{Control heat treatment - conditioning with wet steam followed by oven dried.}$ $c^{\text{SS-110, 120, 130 - processing with superheated steam (SS) at 110, 120, and 130^{\circ}\text{C}, respectively.}$

a

An increase in SS temperature appeared to have little effect on the degree of brightness, and in most cases the differences in brightness of samples treated with different SS temperatures were not statistically significant (P>0.05). Generally, the different heat treatments applied to oat groats had very little effect on the redness (*a*) and yellowness (*b*) values of bran and flour fractions (Table 4.4). Only flour fractions obtained from SS-120 and SS-130 processed groats were significantly (P<0.05) less yellow than flour fractions obtained from SS-110 and control heat-treated groats (except for flour fractions obtained from Furlong plump groats). Within the same cultivar, the colour parameters of flour and bran fractions obtained from groats of different size (plump or intermediate) did not differ substantially (Table 4.4).

Colour changes of heat processed grain might indicate changes in taste, texture, milling characteristics, pasting properties, and shelf life stability of grain products (Cenkowski et al. 2006a; Giese 2000a).

Particle size of oat bran and flour fractions

The effects of the control and SS heat treatments on the distribution of particle diameter in bran and flour fractions obtained by milling of intermediate HiFi groats are shown in Fig. 4.4. The profiles of particle diameter distribution in flour and bran fractions obtained from plump HiFi groats as well as from plump and intermediate Furlong groats were similar (Figs. B.1-B.3 in Appendix B).

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A

Fig. 4. 4 Particle diameter distribution of oat bran (A) and flour (B) obtained by roller milling of heat-treated HiFi intermediate groats. Control heat treatment – conditioning with wet steam followed by oven drying; SS-110, 120, 130 – processing with superheated steam (SS) at 110, 120, and 130°C, respectively.

The bran fraction exhibited a bi-modal distribution of particle diameter with the main population of particles ranging from 120 to 1700 μ m. The minor population of particles smaller than 100 μ m most likely represents some free and compact starch granules that detach easily from the larger bran particles during handling. It has been reported that individual oat starch granules range from 3 to 12 μ m whereas granule aggregates range from 20 to 150 μ m (Fulcher 1986; Paton 1986). The flour fraction was characterized by a multi-modal distribution of particles with the main populations of particles with diameters ranging from 2.7 to 12.3 μ m, from 12.3 to 64 μ m, and from 64 and 600 μ m. A minor population of very small particles with diameters between 0.4 and 2.7 μ m represents an artifact resulting from the use of an internal algorithm of the Mastersizer instrument software, utilized in calculations of particle diameter.

The distribution of particle diameters exhibiting more than one mode can be characterized by the median particle diameter, $D_{0.5}$. The $D_{0.5}$ divides distribution of particle diameters into two equal halves (50% of particles is smaller and 50% of particles is larger than the median particle diameter) and indicates a general trend of the distribution towards either fine or coarse size. The different heat treatments applied to oat groats did not significantly (P>0.05) affect the median particle diameter of flour fractions (Table 4.5). However, the median diameter of bran particles obtained from the control heat-treated groats was generally significantly (P<0.05) smaller than that of bran particles obtained from the SS processed groats. An increase in the SS temperature had no additional effect on the value of the median particle diameter of bran fractions. Within each cultivar, bran particles obtained from the plump kernels were slightly smaller (exhibited smaller values of $D_{0.5}$) than those obtained from the intermediate kernels (Table 4.5).

| Cultivar, Size / | D _{0.5} (μm) | | | | |
|-----------------------------------|-----------------------|--------|--|--|--|
| Treatment | Bran | Flour | | | |
| Furlong, plump | | | | | |
| Control Heat-Treated ^b | 405±1a | 58±4a | | | |
| SS-110 [°] | 438±4b | 45±1a | | | |
| SS-120 | 436±3b | 52±7a | | | |
| SS-130 | 433±3b | 50±3a | | | |
| Furlong, intermediate | | | | | |
| Control Heat-Treated | 459±4a | 52±4a | | | |
| SS-110 | 454±5a | 46±8a | | | |
| SS-120 | 459±5a | 45±4a | | | |
| SS-130 | 458±4a | 53±1a | | | |
| HiFi, plump | | | | | |
| Control Heat-Treated | 421±5a | 58±11a | | | |
| SS-110 | 443±3b | 51±4a | | | |
| SS-120 | 437±4b | 65±0a | | | |
| SS-130 | 440±1b | 61±6a | | | |
| HiFi, intermediate | | | | | |
| Control Heat-Treated | 427±3a | 52±6a | | | |
| SS-110 | 455±2b | 50±2a | | | |
| SS-120 | 452±6ab | 58±6a | | | |
| SS-130 | 445±9ab | 55±7a | | | |

Table 4. 5 Median particle diameter^a ($D_{0.5}$) of oat bran and flour fractions obtained by rollermilling of heat-treated oat groats.

 ${}^{a}D_{0.5}$ – particle diameter which divides the distribution into two equal halves; values are means of 2 replicates; values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05).

^bControl heat treatment – conditioning with wet steam followed by oven drying.

^cSS-110, 120, 130 - processing with superheated steam (SS) at 110, 120, and 130°C, respectively.

It has been reported that hardness of groats may affect the particle size of the resulting milling fractions with harder kernels breaking into larger particles than the softer kernels (Doehlert and McMullen 2000). In this study, the intermediate groats were generally harder than the plump groats within each cultivar (Table 4.2), which may explain why the bran fractions originating from the intermediate groats were coarser than those originating from the plump groats (Table 4.5). Within each cultivar, the differences in hardness of the differently heat-treated groats, however, could not explain the differences in the $D_{0.5}$ values
observed in bran fractions obtained from those groats. Namely, the control heat-treated groats were significantly (P<0.05) harder than the SS processed groats (Table 4.2) but the $D_{0.5}$ values of bran fractions obtained from the control heat-treated groats were significantly lower from those of bran fractions obtained from the SS processed groats (Table 4.5). It appears, therefore, that conventional and SS heat treatments not only differently affected the overall hardness of groats (probably due to varying extent of fracture formation), but also possibly affected physical structure of cell walls, which influenced the particle size of bran fractions.

The particle size of milling fractions may affect their functional properties such as hydration capacity, viscosity of slurry, and bread-baking quality (Zhang et al. 1997, 1998b). Doehlert et al. (1997a) and Zhang et al. (1997) reported that finely ground oat flour generated much higher viscosity in slurry and yielded more BG during extraction than medium or coarsely ground flour. This behaviour was explained by a greater surface area and therefore faster hydration of particles in the finely ground than in the coarsely ground flour, when compared at the same concentration levels.

Chemical composition of oat bran and flour fractions

The chemical composition of oat bran and flour fractions obtained from the differently heat-treated groats is shown in Tables 4.6 and 4.7, respectively. As expected, the effects of heat treatments applied to groats on the chemical composition of bran and flour fractions of both cultivars were marginal. Contents of starch, protein, ash, total BG and pentosans were not significantly (P>0.05) different among bran and flour fractions obtained from the differently heat-treated groats of each cultivar. However, substantial differences in BG content were observed between Furlong and HiFi milling fractions (Tables 4.6-4.7).

| Cultivar, Size / Treatment | Starch | Protein ^b | Ash | Total β-Glucans | Total Pentosans |
|-----------------------------------|-----------|----------------------|-------------|--------------------|--------------------|
| Bran - Furlong, plump | | | 5 W. 4. | | |
| Control Heat-Treated ^c | 49.9±0.3a | 17.8±0.3a | 3.4±0.0a | 7.8±0.1a | $3.6 \pm 0.1a$ |
| $SS-110^{d}$ | 50.2±0.2a | 17.7±0.0a | 3.4±0.0a | 7.6±0.1a | 3.4±0.2a |
| SS-120 | 49.4±0.3a | 17.8±0.1a | 3.4±0.0a | 7.7±0.1a | 3.7±0.1a |
| SS-130 | 49.6±0.1a | 17.8±0.1a | 3.5±0.0a | 7.7±0.0a | 3.7±0.2a |
| Bran - Furlong, intermediate | | | | | |
| Control Heat-Treated | 48.8±1.3a | 17.3±0.1a | 3.3±0.0a | 7.5±0.1a | 3.9±0.1a |
| SS-110 | 49.2±0.1a | 17.2±0.0a | 3.4±0.0a | 7.7±0.2a | 4.1±0.1a |
| SS-120 | 48.8±0.1a | 17.5±0.3a | 3.4±0.0a | 7.6±0.1a | 3.9±0.3a |
| SS-130 | 48.2±0.1a | 17.5±0.0a | 3.5±0.0a | 7.9±0.2a | 3.7±0.2a |
| Bran - HiFi, plump | | | | | |
| Control Heat-Treated | 45.9±0.1a | 22.0±0.0a | 3.3±0.0a | $9.9 \pm 0.2a$ | $3.4\pm0.1a$ |
| SS-110 | 45.6±0.3a | 22.3±0.0ab | 3.3±0.0a | $9.8 \pm 0.1a$ | $3.2\pm0.1a$ |
| SS-120 | 45.3±0.4a | 22.3±0.0b | 3.3±0.0a | 9.8±0.2a | $3.1\pm0.4a$ |
| SS-130 | 45.3±0.1a | 22.3±0.1ab | 3.4±0.0a | 9.8±0.2a | $3.0\pm0.0a$ |
| Bran - HiFi, intermediate | | | | | |
| Control Heat-Treated | 45.8±0.2a | 21.6±0.0a | 3.3±0.0a | 10.1±0.0a | 3.2±0.1a |
| SS-110 | 46.2±0.1a | 21.3±0.1a | 3.3±0.0a | 9.9±0.2a | 3.4±0.0a |
| SS-120 | 45.7±0.5a | 21.4±0.1a | 3.2±0.0a | 10.0±0.0a | 3.2±0.2a |
| SS-130 | 46.1±0.0a | $21.3 \pm 0.1a$ | $32\pm0.0a$ | $9.8 \pm 0.1a$ | $32+01_{2}$ |

Table 4. 6 Chemical composition^a (%, db) of oat bran obtained by roller milling of heattreated groats.

^aValues are means of 2 replicates; values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05). ^bN x 6.25

^cControl heat treatment – conditioning with wet steamed followed by oven drying.

^dSS-110, 120, 130 – processing with superheated steam (SS) at 110, 120 and 130°C, respectively.

| Cultivar, Size / Treatment | Starch | Protein ^b | Ash | Total β-Glucans | Total Pentosans |
|-----------------------------------|-----------|----------------------|---|--------------------|--------------------|
| Flour - Furlong, plump | | | 4.04.0 A.04.0 | | |
| Control Heat-Treated ^c | 76.0±0.0a | 10.6±0.2a | 1.1±0.0a | $1.8 \pm 0.0 b$ | $0.8 \pm 0.1a$ |
| SS-110 ^d | 76.4±0.0a | 10.5±0.0a | 1.1±0.0a | 1.6±0.0a | $0.7\pm0.1a$ |
| SS-120 | 76.3±0.1a | 10.4±0.2a | 1.1±0.0a | 1.6±0.0a | $0.8 \pm 0.0a$ |
| SS-130 | 76.3±0.4a | 10.5±0.1a | 1.1±0.0a | 1.6±0.0a | $0.7 \pm 0.0a$ |
| Flour - Furlong, intermediate | | | | | |
| Control Heat-Treated | 76.7±0.3a | 10.3±0.1a | 1.0±0.0a | 1.6±0.1a | $0.7 \pm 0.0a$ |
| SS-110 | 77.1±0.7a | 10.1±0.0a | 1.0±0.0a | 1.6±0.0a | $0.8 \pm 0.1a$ |
| SS-120 | 77.4±0.9a | 10.1±0.0a | 0.9±0.0a | 1.6±0.1a | $0.8 \pm 0.1a$ |
| SS-130 | 76.7±0.3a | 10.2±0.0a | 1.0±0.0a | 1.6±0.0a | 0.9±0.1a |
| Flour - HiFi, plump | | | | | |
| Control Heat-Treated | 71.9±1.4a | 13.1±0.0a | 1.3±0.0a | $2.2\pm0.1a$ | 1 0+0 0a |
| SS-110 | 71.0±0.2a | 13.0±0.0a | $1.3 \pm 0.0a$ | $2.1\pm0.0a$ | $1.0\pm0.0a$ |
| SS-120 | 71.9±0.1a | 13.2±0.0a | 1.3±0.0a | $2.1\pm0.0a$ | 1.0+0.2a |
| SS-130 | 71.0±0.2a | 13.1±0.0a | 1.3±0.0a | $2.2\pm0.0a$ | $1.1\pm0.0a$ |
| Flour - HiFi, intermediate | | | | | 1=0.04 |
| Control Heat-Treated | 71.3±0.6a | 13.0±0.0a | 1.3±0.0b | 2.3±0.0a | $0.9 \pm 0.2a$ |
| SS-110 | 72.0±0.9a | 12.9±0.2a | 1.2±0.0b | 2.1±0.1a | $1.0\pm0.0a$ |
| SS-120 | 72.3±0.3a | 12.6±0.1a | 1.2±0.0b | 2.2±0.1a | $0.9 \pm 0.0a$ |
| SS-130 | 71.4±0.1a | 12.8±0.1a | 1.0±0.0a | 2.2±0.1a | $0.9\pm0.0a$ |

| Table 4. 7 Chemical | composition ^a (| (%, db) of • | oat flour | obtained | by roller | milling o | of heat- |
|---------------------|----------------------------|--------------|-----------|----------|-----------|-----------|----------|
| treated gr | oats. | | | | 2 | 0 | |

^aValues are means of 2 replicates; values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05). ^bN x 6.25

^cControl heat treatment – conditioning with wet steam followed by oven drying.

^dSS-110, 120, 130 - processing with superheated steam (SS) at 110, 120 and 130°C, respectively.

The HiFi bran fractions exhibited much higher (by up to 2.5%) content of total BG than the Furlong bran fractions. The flour fractions of HiFi also exhibited higher (by up to 0.5%) content of BG than the flour fractions of Furlong. The chemical composition of bran fractions obtained in this study generally agreed with that reported by Luhaloo et al. (1998) who examined chemical composition of ten commercial oat bran fractions collected from Australia and Northern Europe. They reported that starch, protein, and ash contents varied from 40 to 61%, 10 to 21%, and 2.0 to 4.1% of bran, respectively, whereas contents of BG and pentosans varied from 4.7 to 8.3% and 2.3 to 4.7% of bran, respectively.

Water retention capacity of oat bran and flour fractions

It has been reported that hydration properties of fibre preparations may influence processing (e.g. dough mixing) as well as texture of foods, and that the hydration potential may be influenced by heat processing, chemical treatments, particle size, and physical structure of fibre particles (Auffret et al. 1994; Paton and Lenz 1993). Water retention depends on the surface area of particles but also on the surface properties such as porosity of particles, their swelling and solubility. The hydration properties of oat bran and flour fractions obtained from Furlong and HiFi groats were assessed by examining the water retention capacity (WRC) and expressed as the amount of water held by the fractions after mixing with water (overnight) followed by centrifugation (Fig. 4.5).



Fig. 4. 5 Water retention capacity of flour and bran fractions obtained by roller milling of heat-treated Furlong and HiFi groats. Control heat treatment – conditioning with wet steam followed by oven drying; SS-110, 120, 130 – processing with superheated steam (SS) at 110, 120, and 130°C, respectively. Columns with the same letter, within each group, do not differ significantly (P>0.05).

Within each cultivar, bran and flour fractions obtained from the large (plump) and the small (intermediate) groats exhibited similar WRC values. For flour fractions, no substantial differences in WRC were noted either due to the heat treatments applied before milling or due to the genotype. Among bran fractions, the differences in WRC due to the different heat treatments were also small but more pronounced than those noted in flour fractions. Bran fractions obtained from the control heat-treated oat groats of both cultivars retained more water than those obtained from the SS processed groats, although the differences were not always statistically significant. The differences in the WRC of bran samples could be partially explained by the differences in the particle size and the corresponding total surface area of those fractions. For both cultivars, the D_{0.5} values for bran fractions made from the control heat-treated groats were lower (indicating finer particles) than those for bran fractions made from the SS processed groats (except for bran fractions obtained from Furlong intermediate groats). Thus, the bran particles from the control heat-treated samples exhibited larger total surface area and potentially adsorbed and retained more water than the bran particles from the SS processed samples. Genotype had a substantial effect on WRC of bran fractions (Furlong bran fractions retained more water than HiFi bran fractions) which could be ascribed to different content of BG (bran of Furlong contained less BG than bran of HiFi). β-Glucans are the main components of cell walls and higher proportion of these polysaccharides may increase the thickness of the cell walls. The thicker and more complex cell walls restrict water penetration thus may decrease WRC of bran. In fact, lower uptake of water during steeping has been correlated with increased content of BG in cell walls of barley grain (Gamlath et al. 2008).

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Thermal properties of oat bran and flour fractions

Thermal properties of oat starch play an important role in the end-product quality (e.g. texture) because they affect pasting behaviour of starch suspensions exposed to heat (Hoover et al. 2003; Rhymer et al. 2005). The DSC thermograms obtained for flour and bran fractions of Furlong and HiFi cultivars revealed two prominent transitions (peaks) at ~60°C and ~90°C, corresponding to starch gelatinization and melting of amylose-lipid complex, respectively (Fig. 4.6 and Figs. B.4-B.6 in Appendix B). The temperatures (T_o – onset, T_p – peak, T_c – conclusion) and enthalpy changes (ΔH) associated with the two transitions observed in flour and bran fractions obtained in this study are summarized in Tables 4.8 and 4.9, respectively. The temperature and enthalpy of gelatinization of flour fractions obtained from different heat-treated groats were not significantly (P>0.05) different (except for flour fractions obtained from Furlong intermediate groats) (Fig. 4.6, Table 4.8). For bran fractions of both cultivars, the T_p of gelatinization of samples obtained from the SS processed groats was approximately 1°C lower than that of samples obtained from the control heat-treated groats (Table 4.9). The transition temperatures and ΔH of the second peak observed in flour and bran fractions obtained from differently heat-treated groats were not significantly (P<0.05) different and showed no general trend due to heat treatments applied (Tables 4.8-4.9). It should also be noted that differences in transition temperatures of starch gelatinization were noted for the two oat cultivars tested, with Furlong samples exhibiting the $T_{\rm o}$, $T_{\rm p}$ and $T_{\rm c}$ values 2-3°C lower than those of HiFi samples.



Fig. 4. 6 Differential scanning calorimetry thermograms of flour (A) and bran (B) obtained by milling of heat-treated Furlong intermediate groats. Control heat treatment – conditioning with wet steam followed by oven drying; SS-110, 120, 130 – processing with superheated steam (SS) at 110, 120, and 130°C, respectively.

| | | Starch Gel | atinization | · | Malti | | T 10 | 1 |
|-----------------------------------|-------------------|---------------------------|-------------------|--------------------|-------------------|--------------------|--------------------------------------|----------------------------------|
| Cultivar, Size / | | | | | | ig of Amylo | se-Lipid Com | plex |
| Treatment | | ¹ _p | | ΔH | T_{o} | T_{p} | T_{c} | ΔH |
| | (°C) ^b | (°C) ⁶ | (°C) ⁰ | (J/g) ^c | (°C) ^b | (°C) ^b | $(^{o}C)^{b}$ | $(J/g)^{c}$ |
| Flour - Furlong, plump | | | | | | | | |
| Control Heat-Treated ^d | 51.1±0.4a | 58.7±0.2a | 71.9±0.8a | 8.9±0.1a | 80.0±1.3a | 92.2±0.1a | 101.6±1.0a | 1.9±0.5a |
| SS-110 ^e | 50.9±0.1a | 58.3±0.2a | 71.8±0.5a | 9.7±0.2a | 78.1±2.3a | 92.3±0.1a | 101.5±0.0a | $2.2\pm0.4a$ |
| SS-120 | 50.7±0.1a | 58.3±0.0a | 70.9±1.2a | 9.8±0.4a | 76.8±1.1a | $92.0\pm0.1a$ | $102.3 \pm 1.6a$ | 33+15a |
| SS-130 | 50.8±0.2a | 58.3±0.2a | 70.7±0.6a | 9.3±0.7a | 80.5±3.2a | 93 2±0 6a | $102.2 \pm 1.0a$ $102.7 \pm 1.3a$ | $2.5 \pm 1.3a$ |
| Flour - Furlong, intermediate | | | | | | 951 <u>2</u> —0.0u | 102.7±1.54 | 2.5-1.54 |
| Control Heat-Treated | 51.2±0.1a | 58.9±0.0c | 71.4±0.0a | 8.2±0.1a | 80.3±1.5a | 92.1±0.5a | 101.3±0.0a | $1.8 \pm 0.0a$ |
| SS-110 | 50.6±0.3a | 58.5±0.2bc | 71.1±0.4a | 9.2±0.1a | 80.3±0.7a | 92.7±0.4a | $101.8 \pm 0.4a$ | $1.9\pm0.3a$ |
| SS-120 | 50.4±0.2a | 57.7±0.1a | 68.8±1.4a | 8.0±0.3a | 80.5±3.8a | 91.9±0.9a | $101.6 \pm 1.2a$ | $1.6\pm0.6a$ |
| SS-130 | 50.5±0.3a | 58.0±0.1ab | 69.7±1.4a | 8.5±1.2a | 77.9±1.2a | 91.7±0.4a | 102.1±0.9a | $2.6 \pm 0.4a$ |
| | | | | | | | | |
| Flour - HiFi, plump | | | | | | | | |
| Control Heat-Treated | 53.1±0.5a | 60.4±0.1a | 71.8±1.5a | 9.5±2.3a | 81.4±2.0a | 92.4±0.3a | $100.5 \pm 0.4a$ | $1.3 \pm 0.4a$ |
| SS-110 | 53.2±0.0a | 60.6±0.0a | 72.4±0.2a | 10.1±0.4a | 80.4±0.5a | $93.0\pm0.0a$ | $100.7\pm1.6a$ | $1.3 \pm 0.1a$ 1.3 $\pm 0.3a$ |
| SS-120 | 53.2±0.1a | 60.4±0.1a | 72.4±0.1a | 9.6±1.1a | 84.9±5.0a | 94.3±1.1a | $100.7\pm0.4a$ | 0.8+0.5a |
| SS-130 | 52.5±0.1a | 60.4±0.2a | 73.5±0.1a | $10.9 \pm 0.3a$ | 81.7±0.0a | 92.6±1.1a | 101.0+0.2a | $1.2\pm0.2a$ |
| Flour - HiFi, intermediate | | | | | 0111-0104 | 92.0-1.1u | 101.0=0.24 | 1.2-0.24 |
| Control Heat-Treated | 53.1±0.1a | 60.9±0.2a | 74.7±0.5a | 10.6±0.4a | 80.9±0.9a | 91.8±0.7a | 101.0±0.2a | $1.2 \pm 0.1a$ |
| SS-110 | 53.0±0.2a | 60.8±0.1a | 73.3±0.1a | 11.1±0.1a | 81.4±0.8a | 92.5±0.6a | 100.9±0.2a | $1.3 \pm 0.1a$ |
| SS-120 | 53.2±0.2a | 61.0±0.2a | 74.8±2.4a | 11.3±0.2a | 81.1±1.1a | 92.2±0.5a | $101.0\pm0.4a$ | $1.2\pm0.3a$ |
| SS-130 | 53.1±0.2a | 60.8±0.4a | 72.7±0.4a | 10.8±0.3a | 82.0±1.4a | 92.0±1.0a | 101.0±1.1a | $1.2\pm0.2a$ |

Table 4. 8 Thermal properties of oat flour^a obtained by roller milling of heat-treated oat groats.

^aFlour suspension (25% solids); values are means of 2 replicates; values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05).

 ${}^{b}T_{o}$, T_{p} and T_{c} indicate the temperature of onset, peak and conclusion of each phase transition, respectively. ${}^{c}\Delta H$ indicates enthalpy of each phase transition and is expressed on the basis of starch content (%, db).

^dControl heat treatment – conditioning with wet steam followed by oven drying.

°SS-110, 120, 130 - processing with superheated steam (SS) at 110, 120 and 130°C, respectively.

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| Cultivar Size / | | Starch Gel | atinization | | Melting of Amylose-Lipid Complex | | | | |
|-----------------------------------|-----------|------------|-------------|-------------|----------------------------------|-------------------|-------------------|--------------|--|
| Treatment | T_{o} | T_{p} | T_{c} | ΔH | T_{o} | $T_{\rm p}$ | T_{c} | ΔH | |
| •••••• | (°C)° | (°C)" | (°C)° | $(J/g)^{c}$ | (°C) ^o | (°C) ^b | (°C) ^b | $(J/g)^{c}$ | |
| Bran - Furlong, plump | | | | | | | | | |
| Control Heat-Treated ^d | 51.6±0.0a | 60.0±0.3b | 73.3±0.4b | 8.4±0.2a | 78.5±0.6a | 91.1±0.1a | 100.3±0.3a | 2.0±0.1a | |
| SS-110 ^e | 51.1±0.2a | 59.1±0.1a | 71.3±0.4ab | 9.6±0.2ab | 78.1±1.3a | 91.3±0.3a | 101.1±0.6a | 2.4±0.9a | |
| SS-120 | 50.4±0.5a | 59.1±0.2a | 70.8±0.3a | 10.5±0.3b | 77.6±1.1a | 90.2±0.2ab | 100.4±0.4a | 2.2±0.1a | |
| SS-130 | 50.2±0.2a | 59.0±0.2a | 71.4±0.6ab | 10.0±0.5b | 79.3±0.2a | 89.6±0.4b | 99.7±0.7a | 1.7±0.2a | |
| Bran - Furlong, intermediate | | | | | | | | | |
| Control Heat-Treated | 51.8±0.6b | 60.5±0.2b | 73.3±0.3b | 9.5±1.1a | 80.1±2.3a | 90.6±0.8a | 99.9±0.1a | 1.9±0.4a | |
| SS-110 | 50.4±0.1a | 59.1±0.2a | 72.0±0.8ab | 10.2±0.3a | 78.1±0.0a | 90.1±0.1a | 100.5±0.3a | 2.2±0.1a | |
| SS-120 | 50.3±0.1a | 59.2±0.2a | 71.1±0.2a | 9.9±0.5a | 80.0±3.1a | 89.9±0.3a | 100.1±0.1a | 3.1±1.2a | |
| SS-130 | 49.9±0.0a | 58.7±0.2a | 70.8±0.3a | 10.4±0.2a | 78.2±0.7a | 90.3±0.4a | 100.0±0.4a | 2.2±0.0a | |
| | | | | | | | | | |
| Bran - HiFi, plump | | | | | | , | | | |
| Control Heat-Treated | 53.7±0.1a | 61.8±0.0b | 74.0±0.2a | 9.9±0.4a | 80.0±1.4a | 90.3±0.4a | 98.8±0.3a | 1.4±0.0a | |
| SS-110 | 52.2±0.4a | 60.7±0.4a | 72.9±0.7a | 10.1±1.5a | 81.4±1.6a | 88.7±0.5a | 98.7±0.3a | 0.8±0.5a | |
| SS-120 | 52.2±0.4a | 60.8±0.2ab | 72.6±0.1a | 10.9±0.1a | 80.9±0.2a | 89.8±1.2a | 98.8±0.6a | 1.2±0.1a | |
| SS-130 | 52.1±0.5a | 61.0±0.1ab | 73.1±0.0a | 11.0±1.1a | 80.0±0.0a | 90.4±0.1a | 98.7±0.1a | 1.4±0.2a | |
| Bran - HiFi, intermediate | | | | | | | | | |
| Control Heat-Treated | 53.6±0.0a | 62.3±0.2b | 74.4±0.3a | 10.9±0.1a | 80.5±1.6a | 90.4±0.0a | 98.8±0.0a | 1.3±0.1a | |
| SS-110 | 53.3±0.2a | 61.9±0.0ab | 73.5±0.5a | 10.6±0.0a | 82.3±2.0a | 88.3±0.9a | 98.5±0.3a | 0.8±0.6a | |
| SS-120 | 53.3±0.2a | 61.6±0.0a | 74.2±1.1a | 11.3±0.7a | 79.6±0.2a | 90.3±0.0a | 99.3±0.3a | 1.5±0.0a | |
| SS-130 | 53.0±0.2a | 61.5±0.1a | 73.4±0.1a | 11.8±0.5a | 80.3±0.8a | $89.5 \pm 0.6a$ | $99.1 \pm 0.0a$ | $1.4\pm0.2a$ | |

Table 4. 9 Thermal properties of oat bran^a obtained by roller milling of heat-treated oat groats.

^aBran suspension (25% solids); values are means of 2 replicates; values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05). ^b T_{o} , T_{p} and T_{c} indicate the temperature of onset, peak and conclusion of each phase transition, respectively. ^c ΔH indicates enthalpy of each phase transition and is expressed on the basis of starch content (% db).

^dControl heat treatment – conditioning with wet steam followed by oven drying.

^eSS-110, 120, 130 - processing with superheated steam (SS) at 110, 120 and 130°C, respectively.

It appeared that similar amount of starch was gelatinized after the control heat treatment and the SS processing at different temperatures (similar values of ΔH for flour and bran fractions obtained from differently heat-treated groats in Tables 4.8-4.9). For each cultivar, groat size (large versus small) did not appear to augment the effects of different heat treatments on the transition temperatures and ΔH exhibited by bran and flour fractions.

The effects of heat treatments typically used in commercial processing (steam conditioning and kiln drying) on oat groat thermal properties were examined by Oomah (1987) who reported that drying and steam conditioning of oat groats induced only partial gelatinization of starch (26 and 24%, respectively) and increased starch transition temperatures (T_0 and T_p) by up to 2°C (with respect to the results obtained for raw groats).

4.4.4 Pasting Viscosity and Gelation of Oat Bran and Flour Fractions

Pasting viscosity of oat bran and flour fractions

General pasting curves for flour fractions (slurried with water) obtained from differently heat-treated plump Furlong groats are shown in Fig. 4.7. The pasting curves for flour fractions obtained from intermediate Furlong as well as plump and intermediate HiFi groats followed a similar trend. The key pasting parameters; peak viscosity, final viscosity, trough, breakdown, and setback) obtained from the general pasting test for flour fractions are summarized in Table 4.10. The pasting parameters of flour fractions in each cultivar/size group generally did not vary substantially due to the different heat treatments applied before milling. Within each cultivar, similar values of pasting parameters were observed in flour originating from plump and intermediate groats.



Fig. 4. 7 Rapid Visco Analyzer pasting curves for oat flour slurries following the general pasting test. Flours were obtained by roller milling of heat-treated Furlong plump oat groats. Control heat treatment – conditioning with wet steam followed by oven drying; SS-110, 120, 130 – processing with superheated steam (SS) at 110, 120, and 130°C, respectively.

| Cultivar, Size / Treatment | Peak Viscosity (cP) | Trough (cP) | Breakdown (cP) | Final Viscosity (cP) | Setback (cP) |
|-----------------------------------|---------------------------|--|-------------------|----------------------------|-----------------|
| Flour - Furlong, plump | | ······································ | | | |
| Control Heat-Treated ^b | 3028±38a | 1972±37a | 1056±1a | 3567±135a | 1595±98a |
| SS-110 ^c | 2990±16a | 1949±8a | 1041±8a | 3506±32a | 1558±23a |
| SS-120 | 3000±41a | 1958±6a | 1042±35a | 3660±29a | 1703±35a |
| SS-130 | 2913±29a | 1889±28a | 1023±1a | 3475±96a | 1586±69a |
| Flour - Furlong, intermediate | | | | | |
| Control Heat-Treated | 3107±11c | 2003±15a | 1104±4b | 3723±20b | 1721±5a |
| SS-110 | 3020±19ab | 2013±18a | 1006±1a | 3615±10a | $1602 \pm 8a$ |
| SS-120 | 3048±3b | 2016±3a | 1032±6a | 3726±1b | $1710\pm4a$ |
| SS-130 | 2993±6a | 1975±16a | 1018±21a | 3638±35a | 1662±50a |
| Flour - HiFi, plump | | | | | |
| Control Heat-Treated | 3212±31b | 2003±19b | 1209±12b | 3429±47b | 1426±66a |
| SS-110 | 2961±13a | 1910±28a | 1052±42a | 3267±5a | 1357±23a |
| SS-120 | 2949±1a | 1910±11a | 1040±12a | 3300±30ab | $1391 \pm 41a$ |
| SS-130 | 3006±1a | 1895±1a | 1111±3a | 3284±37ab | $1389 \pm 38a$ |
| Flour - HiFi, intermediate | | | | 0201-0740 | 1507-504 |
| Control Heat-Treated | 3155±20a | 2061±4a | 1093±17a | 3557±2a | 1496±1a |
| SS-110 | 3149±14a | 2018±32a | 1131±18a | $3424\pm61a$ | 1406+28a |
| SS-120 | 3135±16a | $2051 \pm 32a$ | $1084 \pm 16a$ | 3440+7a | 1389+392 |
| SS-130 | 3107±6a | 2019±3a | $1088 \pm 3a$ | $3439\pm21a$ | 1420+18a |

Table 4. 10 Effects of heat treatments on pasting properties^a of oat flours obtained by roller milling of oat groats.

 $\frac{\text{SS-130}}{\text{*Obtained from the general pasting test; values are means of 2 replicates; values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05).$ *Control heat treatment – conditioning with wet steam followed by oven drying.*SS-110, 120, 130 – processing with superheated steam (SS) at 110, 120 and 130°C, respectively.

The changes in viscosity of flour slurry during the general pasting test reflect the molecular events occurring in starch granules due to gelatinization, and may be affected by the thermal properties of starch. From the DSC analysis of the Furlong and HiFi flour fractions, it was determined that generally gelatinization temperature of starch present in the flour fractions was similar in the differently heat-treated samples (within each cultivar/size group; Table 4.8). It appeared, therefore, that different heat treatments applied to oat groats before milling had a similar effect on the thermal as well as pasting properties (i.e. gelatinization) of oat starches.

During heating of aqueous suspensions of starch-containing materials, the complete gelatinization of starch is preceded by swelling of starch granules. A measurement of pasting viscosity at temperature close to the gelatinization temperature of oat starch, but not high enough to cause complete gelatinization allows to analyze the swelling properties of starch granules before their dissolution. Cold paste viscosity test performed at 64°C was used to determine the effects of different heat treatments on the swelling properties of starch present in oat flour and bran fractions. Cold pasting curves for flour fractions obtained from differently heat-treated large (plump) HiFi groats are shown in Fig. 4.8. The cold pasting curves for flour and bran fractions obtained from intermediate HiFi as well as plump and intermediate Furlong groats followed a similar trend. For both cultivars, substantial differences in cold paste viscosity of flour and bran fractions obtained upon milling of differently heat-treated groats were noted (Figs. 4.8-4.9 and Table B.2 in Appendix B). Flour and bran fractions obtained from the control heat-treated groats exhibited significantly (P<0.05) lower cold paste viscosity than their counterparts obtained from SS processed groats (except for bran fractions obtained from HiFi intermediate

groats). In most cases, an increase in the cold paste viscosity was observed with increasing temperature of SS applied to the groats, although the differences were not always significant (P>0.05). Within each cultivar, the values of cold paste viscosity of flour and bran fractions obtained from the plump groats were similar to those noted in flour and bran fractions obtained from the intermediate groats. The milling fractions of HiFi exhibited much greater cold paste viscosity than those of Furlong (Fig. 4.9). The differences in the cold paste viscosity between these two cultivars were most probably due to the differences in BG content (Tables 4.6-4.7). It has been reported that polymers like BG, which have high water-binding capacity, compete for water with starch during pasting and increase viscosity of slurry (Colleoni-Sirghie et al. 2004). Zhou et al. (2000) also reported that BG contributed largely to pasting properties of whole oat flour.



Fig. 4. 8 Rapid Visco Analyzer pasting profiles for oat flour slurries following the cold paste test (64°C). Flours were obtained by roller milling of heat-treated HiFi plump oat groats. Control heat treatment – conditioning with wet steam followed by oven drying; SS-110, 120, 130 – processing with superheated steam (SS) at 110, 120, and 130°C, respectively.



Fig. 4. 9 Effects of heat treatments on final cold paste viscosity (64°C) of flour (A) and bran (B) obtained upon roller milling of Furlong and HiFi oat groats. Control heat treatment – conditioning with wet steam followed by oven drying; SS-110, 120, 130 – processing with superheated steam (SS) at 110, 120, and 130°C, respectively. Columns with the same letter, within each group, do not differ significantly (P>0.05).

B

A

Gelation properties of oat flour fractions

Oat flour is often used to alter rheological properties of foods because of the ability of oat starch paste to form gels upon cooling (Shim and Mulvaney 1999). Gels are viscoelastic materials, that is, they exhibit fluid-like (viscous) flow as well as a solid-like (elastic) response when force is applied. Hydro-thermal treatments involved in oat processing may alter viscoelastic properties of gels prepared from oat flour. Small strain oscillatory testing was used to evaluate the effects of different heat treatments applied to oat groats on the viscoelastic properties of gels prepared from oat flour fractions. The mechanical spectra of gels prepared from flour fractions of Furlong and HiFi plump groats are shown in Fig. 4.10. The mechanical spectra of gels prepared from flour fractions of intermediate groats of the two cultivars are shown in Appendix B (Fig. B.7). All gels (40% solids) exhibited much higher values of elastic (G') than viscous (G") modulus which indicated strong elastic behaviour of the gels. Both moduli were essentially independent on the frequency of applied shear. It has been reported that the independence of G' and G" moduli on frequency of applied shear indicates a strong gel and a presence of cross-links in the network structure of the gel (Shim and Mulvaney 1999). For both cultivars tested in this study, gels made of flour fractions originating from groats processed with SS at 110°C exhibited the lowest values of storage and loss moduli (Fig. 4.10 and Table 4.11) and in some cases that decrease was significant (P<0.05). An increase in SS temperature to 120 and 130°C caused the values of G' and G" to increase and either match or exceed the values obtained for gels made of flour originating from the control heat-treated groats. Generally the gels made of flour fractions originating from plump groats exhibited slightly higher values of G' and G" moduli than those originating from intermediate groats of both cultivars (Table 4.11).

However, the loss tangent, tan δ (G"/G', indicating elasticity of a gel) calculated for these gels were not significantly (P>0.05) affected by the heat treatments or groat size in each cultivar indicating similar elasticity of all tested gels.



Fig. 4. 10 Mechanical spectra of gels (40% solids) made from Furlong (A) and HiFi (B) flour obtained upon milling of heat-treated plump groats. Control heat treatment – conditioning with wet steam followed by oven drying; SS-110, 120, 130 – processing with superheated steam (SS) at 110, 120, and 130°C, respectively.

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| Cultivar, Size / | Storage Modulus, G' | Loss Modulus, G" | tan δ |
|-----------------------------------|---------------------------------------|------------------|------------|
| Treatment | (kPa) | (kPa) | (G"/G') |
| Flour - Furlong, plump | · · · · · · · · · · · · · · · · · · · | | |
| Control Heat-Treated ^d | 15.81±0.02b | 2.30±0.08b | 0.15±0.01a |
| SS-110 ^e | 9.27±0.16a | 1.47±0.02a | 0.16±0.00a |
| SS-120 | 15.57±0.16b | 2.31±0.17b | 0.15±0.01a |
| SS-130 | $16.78 \pm 0.10c$ | 2.44±0.13b | 0.15±0.01a |
| Flour - Furlong, intermediate | | | |
| Control Heat-Treated | 14.27±0.28b | 1.86±0.08a | 0.13±0.01a |
| SS-110 | 9.36±0.05a | 1.52±0.00a | 0.16±0.00a |
| SS-120 | 13.71±0.10c | 2.01±0.06a | 0.15±0.01a |
| SS-130 | 16.47±0.21d | 2.68±0.22b | 0.16±0.02a |
| | | | |
| Flour - HiFi, plump | | | |
| Control Heat-Treated | 14.45±0.04ab | 2.48±0.35a | 0.17±0.02a |
| SS-110 | 13.10±0.87a | 2.14±0.29a | 0.16±0.01a |
| SS-120 | 16.31±0.28b | 2.71±0.10a | 0.17±0.00a |
| SS-130 | 16.03±0.48b | 2.43±0.08a | 0.15±0.00a |
| Flour - HiFi, intermediate | | | |
| Control Heat-Treated | 12.01±0.84a | 2.06±0.10a | 0.17±0.02a |
| SS-110 | 10.40±0.20a | 1.93±0.07a | 0.19±0.00a |
| SS-120 | 13.61±0.31a | 2.05±0.14a | 0.15±0.01a |
| SS-130 | 13.10±1.07a | 2.13±0.08a | 0.16±0.01a |

Table 4. 11 Effects of heat treatments on the storage^a (G') and loss^a (G") moduli and their ratio^a of gels^b prepared from oat flour^c.

^aValues are means of 2 replicates and were recorded at 0.75 Hz; values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05). ^bGels were prepared from flour and water at ratio of 40:60 (w/w).

^cFlour was obtained by roller milling of heat-treated oat groats.

^dControl heat treatment – conditioning with wet steam followed by oven drying.

^eSS-110, 120, 130 - processing with superheated steam (SS) at 110, 120 and 130°C, respectively.

4.4.5 Characteristics of Non-Starch Polysaccharides of Oat Bran and Flour Fractions

β-Glucans and pentosans (the latter composed mostly of arabinoxylans) are the major non-starch polysaccharides (NSP) of oat grain and are found in the cell walls of the endosperm and the aleurone layer of oat groat. The junction of the endosperm and the aleurone layer, called subaleurone layer, exhibits thick cell walls particularly rich in BG (Miller and Fulcher 1994). Resistance of the subaleurone layer to milling attrition results in

formation of coarse particles (bran) enriched in BG. There is an ongoing interest in oat BG, caused by findings of a link between consumption of oat BG and the reduction of serum cholesterol levels in humans (Ripsin et al. 1992; Wood 2004, 2007). The positive physiological effects of BG are linked to their viscosity building properties in the intestines, leading to a decreased absorption of cholesterol (Malkki and Virtanen 2001). The viscosity building properties of BG are affected by their solubility/extractability, which in turn is affected by molecular weight and concentration of BG (i.e. content of soluble BG) (Cui and Wood 2000; Sayar et al. 2005; Skendi et al. 2003). Relatively little is known about physiological effects of oat pentosans (arabinoxylans) upon human consumption. However, it has been reported that water soluble arabinoxylans of wheat and rye exhibited positive effects on colonic health by promoting the proliferation of probiotic bacteria (Bifidobacterium species) (Crittenden et al. 2002; Hopkins et al. 2003). It is anticipated, therefore, that consumption of oat pentosans would have similar physiological effects. It is important to note that the viscosity building properties of oat NSP are not only beneficial to human health, but are also important for food applications.

Effect of heat treatments on the solubility of NSP

The viscosity building properties of NSP present in oat flour and bran fractions obtained in this study, were assessed by measuring the content of soluble BG and pentosans (Fig. 4.11) and their solubility (expressed as percentage of the total BG content; Table 4.12) in water at 40°C. The content of soluble BG and their solubility in flour and bran fractions of both cultivars were significantly (P<0.05) affected by the different heat treatments applied to groats before milling. For both cultivars, the lowest and the highest amounts of soluble BG were found in samples treated with SS at 110 and 130°C, respectively (Fig. 4.11).



Fig. 4. 11 Content of water soluble β-glucans and pentosans in flour and bran obtained by roller milling of Furlong and HiFi groats. Control heat treatment – conditioning with wet steam followed by oven drying; SS-110, 120, 130 – processing with superheated steam (SS) at 110, 120, and 130°C, respectively. Columns with the same letter, within each group, do not differ significantly (P>0.05).

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| Cultivar, Size / | Solubility o | Solubility of β -Glucans | | of Pentosans |
|-----------------------------------|--------------|--------------------------------|-----------------|-------------------|
| Treatment | (| %) | (0 | %) |
| | Bran | Flour | Bran | Flour |
| Furlong, plump | | | | |
| Control Heat-Treated ^b | 22.3±0.4b | 29.4±0.0a | 3.1±0.3a | 14.7±1.5a |
| SS-110 ^c | 20.7±0.5a | 28.5±0.7a | 2.8±0.3a | 15.7±1.9a |
| SS-120 | 29.1±0.7c | 36.9±5.7ab | 2.9±0.1a | 15.3±0.6a |
| SS-130 | 33.9±0.2d | 37.9±1.3b | 3.3±0.3a | $17.8 \pm 0.8a$ |
| Furlong, intermediate | | | | |
| Control Heat-Treated | 23.7±0.5b | 30.6±2.0a | 2.2±0.3ab | 13.7±1.3a |
| SS-110 | 21.1±0.8a | 29.1±0.6a | 2.0±0.1ab | 11.1±1.3a |
| SS-120 | 29.6±0.6c | 34.4±1.7ab | 1.8±0.1a | 11.7±1.3a |
| SS-130 | 33.3±1.1d | 40.1±0.6b | 2.5±0.2b | 11.7±1.1a |
| HiFi, plump | | | | |
| Control Heat-Treated | 39.9±1.0ab | 42.0±5.2ab | $4.1\pm0.2a$ | 61+02a |
| SS-110 | 37.5±0.8a | 39.2±1.9a | $3.8\pm0.3a$ | $5.0\pm0.6a$ |
| SS-120 | 44.1±1.7b | 46.9±2.4b | 4.6±0.6ab | $6.4\pm1.5a$ |
| SS-130 | 52.7±2.3c | 47.7±0.6b | 5.1 ± 0.1 b | 6 4+1 0a |
| HiFi, intermediate | | | | 0. 1–1.0 u |
| Control Heat-Treated | 39.6±0.9a | 41.6±0.5a | 4.5±0.2a | 9.2±2.0a |
| SS-110 | 36.8±0.8a | 39.2±2.1a | 4.0±0.4a | 8.8±0.3a |
| SS-120 | 45.8±1.2b | 45.6±1.9ab | 4.3±0.4a | 8.9±1.1a |
| SS-130 | 48.1±0.6b | 49.3±1.1b | 4.9±0.2a | 9.3±0.3a |

Table 4. 12 Solubility^a of β -glucans and pentosans in bran and flour obtained by roller milling of heat-treated oat groats.

^aExpressed as percentage of total β -glucans and total pentosans, respectively; values are means of 2 replicates; values in the same column, within each cultivar/size group, followed by the same letter do not differ significantly (P>0.05).

^bControl heat treatment – conditioning with wet steam followed by oven drying.

°SS-110, 120, 130 - processing with superheated steam (SS) at 110, 120, and 130°C, respectively.

The magnitude of variation in the amounts of soluble BG found in the differently heattreated samples was smaller than the magnitude of variation due to the genotype. The bran fractions obtained from Furlong groats exhibited much lower content of soluble BG (1.6-2.6%) than the bran fractions obtained from HiFi groats (3.7-5.1%).

The solubility of BG exhibited by flour fractions ranged from 29 to 40% (Furlong) and 39 to 49% (HiFi), whereas in bran fractions ranged from 21 to 34% (Furlong) and from 37

to 53% (HiFi) (Table 4.12). Although the solubility of BG exhibited by flour fractions of each cultivar was greater than that of bran fractions, the amount of soluble BG was much greater in the bran than in the flour fractions (Fig. 4.11). It has been reported that more BG can be extracted from fine than coarse flour (Zhang et al. 1997). Thus finer particles of flour fractions yielded more BG during extraction than coarser particles of bran fractions. For both cultivars, bran and flour fractions originating from the plump and intermediate groats exhibited similar solubility of BG. The different heat treatments applied to oat groats before milling significantly (P<0.05) affected the solubility of BG in bran and flour fractions. The SS-120 and SS-130 treatments significantly increased, whereas the SS-110 treatment decreased, the solubility of BG, compared to the control heat treatment (Table 4.12). Although significant effects of the different heat treatments on the solubility of BG were seen in flour and bran fractions, it should be noted that the genotype also affected the solubility substantially, i.e. bran and flour fractions of HiFi exhibited greater solubility of BG than their counterparts in Furlong.

For both cultivars, the content of soluble pentosans in bran and flour fractions of both cultivars was low (0.07-0.16% and 0.06-0.12%, respectively) and generally not significantly (P>0.05) affected by the different heat treatments applied to groats (except for bran fractions obtained from Furlong intermediate and HiFi plump groats; Fig. 4.11). Solubility of pentosans in the milling fractions of both cultivars was not substantially affected by the different heat treatments applied to groats before milling (except for bran fractions of Furlong intermediate and HiFi plump; Table 4.12). However, generally, the SS-110 treatment applied to groats before milling appeared to decrease the amount of soluble pentosans and their solubility in flour and bran fractions.

There are many facors that contribute to the solubility of NSP in oat, including genotype, cell wall architecture, molecular characteristics of NSP (molecular weight, DP3/DP4 ratio), associations of NSP with the rest of cell wall components, heat treatment history, particle size, presence of enzymes, extraction conditions (temperature, pH, duration) and solvents used (Izydorczyk and Dexter 2008; Lazaridou et al. 2007). Heat processing of oat grain appears to reduce the solubility of BG compared to that of raw oat grain (Lazaridou et al. 2007). Luhaloo et al. (1998) reported 30-43% solubility of BG extracted (at 38°C) from commercial oat bran collected from Australia and Northern Europe, whereas Beer et al. (1997) reported ~65% solubility of BG from a single extraction of raw oat grain with hot water.

Molecular structure and weight of oat β *-glucans*

To determine the effects of different heat treatments applied to groats before milling on the physicochemical properties of BG, the polymers were isolated from bran fractions originating from plump groats of both cultivars and analyzed. The BG preparations contained no starch and only very small amounts of protein; 3.5-4.9% and 2.0-3.1% (db) in Furlong and HiFi, respectively. It was reported that a small amount of protein present in BG isolates has a negligible influence on the viscosity of BG solutions (Bhatty et al. 1991; Colleoni-Sirghie et al. 2003b). The molecular structure of isolated BG was determined via analysis of oligosaccharides released from BG after lichenase digestion. Lichenase, $(1\rightarrow 3)(1\rightarrow 4)$ - β -D-glucan hydrolase, cleaves specifically the β - $(1\rightarrow 4)$ -linkage of the 3-Osubstituted β -D-glucopyranosyl residues in BG. Due to that action, oligosaccharides with different degree of polymerization (DP) are obtained, with 3-O- β -cellobiosyl-D-glucose (trisaccharide unit; DP3) and 3-O- β -D-cellotriosyl-D-glucose (tetrasaccharide unit; DP4)

accounting for 90-95% of the total oligosaccharides, and longer oligosaccharides (DP>5) accounting for 5-10% of the total oligosaccharides (Izydorczyk and Dexter 2008). The molar ratio of oligosaccharides with the DP3 and 4 (DP3/DP4) constitutes the fingerprint of the structure of BG from various cereals. Typical values of the molar ratio of DP3/DP4 reported for oat, barley and wheat are: 1.8-2.4, 2.5-3.3 and 3.5-4.5, respectively (Wood et al. 1991a; Wang et al. 2003). In this study, BG isolated from Furlong and HiFi bran fractions were degraded predominantly to tri- and tetra-saccharides as a result of lichenase digestion (Table 4.13). The tri- and tetra-saccharides accounted for 91.7-92.2% and 91.4-92.3% of the total oligomers released from BG of Furlong and HiFi, respectively. The remaining ~8% of the oligosaccharides released from BG were represented mostly by those with DP of 5, 6 and 9 (2.9-3.1%, 1.9-2.2% and 1.2-1.4%, respectively). The oligosaccharides with DP of 10-17 were present in very small quantities (< 1%). β -Glucans from bran fractions of Furlong exhibited only slightly higher molar ratio of tri- to tetrasaccharides than BG from bran fractions of HiFi (Table 4.13). Also, within each cultivar, only slight differences in the molar ratio of tri- to tetra-saccharides of BG extracted from differently heat-treated samples were observed. Johansson et al. (2007) also reported that heat processing such as cooking, drying, and baking did not affect the molecular structure of oat BG. Colleoni-Sirghie et al. (2003a) reported that differences in the molar ratio of DP3 to DP4 in BG extracted from oat genotypes with high and normal BG content were very small and not significant (P>0.05).

| β-Glucans Source | DP3 ^a | DP4 ^a | DP5 ^a | DP6 ^a | DP7 ^a | DP8 ^a | DP9 ^a | DP(10-17) ^a | DP3 + DP4 | DP3/DP4 ^b |
|-----------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------------|-----------|----------------------|
| Bran - Furlong, plump | | | | | | | | | | |
| Control Heat-Treated ^c | 56.92 | 35.05 | 2.94 | 1.88 | 0.47 | 0.64 | 1.33 | 0.77 | 92.0 | 2.14 |
| $SS-110^{d}$ | 56.68 | 35.45 | 2.90 | 1.88 | 0.48 | 0.68 | 1.28 | 0.64 | 92.1 | 2.11 |
| SS-120 | 56.34 | 35.35 | 3.02 | 1.96 | 0.50 | 0.69 | 1.35 | 0.80 | 91.7 | 2.11 |
| SS-130 | 56.54 | 35.66 | 2.86 | 1.88 | 0.47 | 0.65 | 1.26 | 0.67 | 92.2 | 2.09 |
| Bran - HiFi, plump | | | | | | | | | | |
| Control Heat-Treated | 56.41 | 35.86 | 2.90 | 2.02 | 0.47 | 0.71 | 1.17 | 0.45 | 92.3 | 2.08 |
| SS-110 | -56.47 | 35.84 | 2.87 | 2.01 | 0.45 | 0.72 | 1.20 | 0.45 | 92.3 | 2.08 |
| SS-120 | 55.87 | 35.50 | 3.14 | 2.18 | 0.49 | 0.79 | 1.39 | 0.63 | 91.4 | 2.08 |
| SS-130 | 55.84 | 35.72 | 3.11 | 2.16 | 0.50 | 0.77 | 1.33 | 0.54 | 91.6 | 2.07 |

Table 4. 13 Structural features of β -glucans isolated from oat bran.

^aPeak area % from the chromatograms of the lichenase digests of β -glucans; DP: degree of polymerisation; DP3: 3-O- β -cellobiosyl-D-glucose; DP4: 3-O- β -cellotriosyl-D-glucose; DP5 through DP9 and DP(10-17): cellulose-like oligosaccharides with DP between 5 and 17. ^bMolar ratio.

^cControl heat treatment – conditioning with wet steam followed by oven drying. ^dSS-110, 120, 130 – processing with superheated steam (SS) at 110, 120, and 130°C, respectively.

Weight average molecular weights, M_w , of the BG isolates were determined using a HPSEC-MALS system to gain further insight into the molecular characteristics of these polysaccharides. Generally, β -glucans isolated from the bran fractions of HiFi cultivar exhibited higher M_w than BG isolated from the bran fractions of Furlong cultivar (Table 4.14).

| B-Glucans Source | M _w |
|-----------------------------------|----------------|
| | (g/mol) |
| Bran, Furlong plump | |
| Control Heat-Treated ^b | 485,000 |
| $SS-110^{\circ}$ | 584,100 |
| SS-120 | 316,300 |
| SS-130 | 283,700 |
| Bran, HiFi plump | |
| Control Heat-Treated | 1,170,000 |
| SS-110 | 1,627,000 |
| SS-120 | 860,500 |
| SS-130 | 755,600 |

Table 4. 14 Molecular weight^a (M_w) of β -glucans isolated from oat bran.

^aWeight-average molecular weight.

^bControl heat treatment – conditioning with wet steam followed by oven drying.

^cSS-110, 120, 130 - processing with superheated steam (SS) at 110, 120, and 130^oC, respectively.

The different heat treatments applied to oat groats before milling substantially affected the M_w of BG extracted from bran fractions. The β -glucans extracted from bran of the SS-110 treated groats showed higher M_w than BG from bran of the control heat-treated groats, and substantially higher M_w than BG from bran fractions of the SS-120 and SS-130 treated groats (Table 4.14). It appeared that the treatments of groats with SS at 120 and 130°C caused partial depolymerization of BG which lowered their M_w thereby increasing their solubilities (Table 4.14 and 4.12). The depolymerization of BG is often caused by the action of endogenous β -glucanase which, if not inactivated, may hydrolyze BG thus decreasing their molecular weight (Lazaridou et al. 2007). Inadequate inactivation of β -glucanase during certain heat treatments of barley and oat has been reported (Doehlert et al. 1997b; Izydorczyk et al. 2000; Jaskari et al. 1995). The active ßglucanase rapidly depolymerized BG and decreased viscosity of flour or bran slurries at 25-30°C. In this study, no decline in viscosity of flour or bran slurries was observed during the cold paste test (30-64°C), thus it seems unlikely that the β -glucanase was still active in the bran fractions of Furlong and HiFi after the different heat treatments applied to oat groats upon milling. It is likely that the SS treatment at 120 and 130°C affected the microstructure of cell walls or the interactions of the NSP with each other and the rest of cell wall components to a greater degree than the control or SS-110 treatments. As a result, soluble BG of decreased M_w could be extracted from the SS-120 and SS-130 treated samples. Further work is needed to elucidate the mechanism of BG depolymerization after SS processing at 120 and 130°C. Zhang et al. (1998a) reported that $M_{\rm w}$ of BG extracted form steamed or roasted oat grain was equivalent to $M_{\rm w}$ of BG extracted from raw grain. They suggested that steaming and roasting affected physical structure of BG polymers; roasting increased whereas steaming decreased the polymer chains propensity to form associations but did not affect their M_w compared to raw BG.

It has been reported that the solubility of β -glucans appears to increase with decreased molecular weight of BG (Izydorczyk and Dexter 2008). In this study, the trends observed for M_w of BG due to different heat treatments (within each cultivar; Table 4.14) could explain the observed differences in BG solubility of the differently heat-treated samples

(Table 4.12), e.g. the SS-110 treated samples exhibited the lowest solubility of BG but the highest M_w of this polymer.

Rheological properties of β -glucans

Figure 4.12 shows the apparent viscosity of aqueous solutions of BG (1.0 and 1.4% w/w, 22°C) extracted from bran fractions of control heat-treated and SS treated groats. The apparent viscosity of BG solutions was shear rate dependant and showed shear thinning behaviour, with increasing shear rates. The onset of shear thinning depended on the molecular weight and concentration of BG. Within each cultivar, the onset of shear thinning shifted towards lower shear rates with increasing molecular weight of BG. These results are in agreement with others (Lazaridou et al. 2003; Papageorgiou et al. 2005). In both cultivars, the highest apparent viscosities were observed for BG extracted from bran of groats processed with SS at 110°C, followed by those extracted from bran of groats which were control heat-treated and processed with SS at 120 and 130°C. The differences in the viscous properties of BG solutions corresponded to the differences in M_w of these polymers as affected by their origin (cultivar) and heat treatments. It was reported by Colleoni-Sirghie et al. (2003b) that BG extracted from oat genotypes with high BG content (up to 7.8%) exhibited higher M_w than BG extracted from oat genotypes with normal BG content (4.4%). Thus at the same concentration, solution viscosity of BG extracted from oat genotypes with high BG content was significantly greater than that of BG extracted from genotypes with normal BG content.



Fig. 4. 12 Apparent viscosity of solutions (1.0% and 1.4%, w/w; at 22°C) of BG isolated from bran fractions of the control heat-treated (conditioned with wet steam and oven dried) and superheated steam (SS) processed (at 110, 120 and 130°C) groats.

Zhang et al. (1998a) examined the effects of wet steaming and roasting (in a convection oven at 105°C) on rheological properties of BG extracted form oat grain. They reported that reduced amount of BG could be extracted from the steamed grain, compared to roasted grain, but the extracts from steamed grain had greater viscosity than the extracts from the roasted grain. The researchers hypothesized that steaming may disrupt polymer-polymer binding and replace it with polymer-water binding which would result in a more linear BG chain configuration, generating greater viscosity of BG in solution.

Both, molecular structure and weight of BG play a crucial role in the polymers conformation, solubility and viscosity (Johansson et al. 2004; Skendi et al. 2003; Wood 1993). The viscosity-building properties of BG are believed to play a key role in the reduction of serum cholesterol levels in humans (Colleoni-Sirghie et al. 2004), as well as influence consistency of foods which contain BG (Bhatty 1993; Lazaridou et al. 2003). In order to maintain the health promoting properties of oat BG, heat processing of oats should be controlled to avoid reduction of M_w and viscosity of BG (Luhaloo et al. 1998; Tosh et al. 2003). Among the four heat treatments examined in this study, the SS-110 treatment appeared to have the most positive effects on the physicochemical properties (highest M_w and apparent viscosity in solution) of BG isolated from the bran fractions of Furlong and HiFi.

It is likely that the increased solubility of BG in samples processed with SS at 120 and 130°C, and increased viscosity of solubilized BG in samples processed with SS at 110°C contributed to the increased cold paste viscosity of the flour and bran fractions obtained from these SS processed samples (compared to control heat-treated samples). Further research is needed to elucidate whether the increased cold paste viscosity of oat slurry after SS processing (compared to that of control heat-treated grain) is caused only by increased swelling of starch or it is also affected by the increased solubility of BG (or increased viscosity of the solubilized BG).

4.5 Conclusions

Assessment and comparison of the effects of superheated steam (at 110, 120 and 130°C) and conventional (control) heat treatments applied to oat groats on the physicochemical and functional properties of whole groats and their flour and bran fractions were made. Two oat cultivars differing in physical grain characteristics and chemical composition were chosen to evaluate whether the effects of different heat treatments were augmented by different grain properties. Additionally, before heat treatments the groats of each cultivar were segregated into large (plump) and small (intermediate) groats to test the hypothesis that the heat treatment effects were influenced by groat size.

Both types of heat treatment (SS and control) induced formation of cracks/fissures in groats and caused decreased groat hardness as well as increased transverse width, compared to raw groats. The extent of those changes, however, was greater due to the SS processing than due to the control heat treatment, and generally the hardness index of groats decreased with the increase of SS temperature. Additionally, groat size (large versus small) and genotype affected the hardness of differently heat-treated groats, i.e. the large groats of both cultivars were generally softer than their small counterparts and groats containing less β -glucan (Furlong) were softer than those with higher β -glucan content (HiFi).

Milling yields of flour and bran fractions obtained upon roller milling of groats of both cultivars were not significantly (P>0.05) affected by the different heat treatments applied to those groats (except for HiFi intermediate groats), but they differed substantially between the two cultivars (the HiFi groats yielded 10% more bran and concomitantly less flour than the Furlong groats). Similar values of the median particle diameter (D_{0.5}) were noted for flour fractions obtained from the differently heat-treated groats. Different heat treatments applied to groats before milling, groat size, or genotype did not substantially affect the water retention capacity (WRC) of flour fractions. The D_{0.5} values of bran fractions obtained from the SS processed groats were significantly (P<0.05) higher than those for samples obtained from the control heat-treated groats (except for bran fractions obtained from Furlong intermediate groats). The increase in SS temperature, however, had no additional effect on the median particle size of bran fractions. Only slight differences were observed in the $D_{0.5}$ values of bran fractions obtained from plump and small groats (within each cultivar). Within each cultivar, the finer particles of bran fractions obtained from the control heat-treated groats (compared to those obtained from the SS-treated groats) exhibited slightly increased WRC. Genotype had a substantial effect on the WRC of bran fractions (bran fractions of Furlong retained more water than bran fractions of HiFi), whereas groat size (within each cultivar) did not influence the WRC of bran fractions obtained from differently heattreated groats. Both SS (at 110, 120 and 130°C) and control heat treatments applied to oat groats before milling had similar effects on the colour of flour and bran fractions of each cultivar. Differences in the colour of bran fractions due to genotype were greater than the

differences due the different heat treatments, i.e. bran fractions of Furlong were slightly darker and more yellow than those of HiFi.

Generally, it appeared that different heat treatments (SS or control) applied to oat groats before milling had a similar effect on the thermal properties of flour and bran fractions of Furlong and HiFi. Only the peak temperature of starch gelatinization measured for bran fractions of the SS processed groats was approximately 1°C lower than that of samples obtained from the control heat-treated groats. However, it should be noted that differences in transition temperatures of starch gelatinization in flour and bran fractions noted for the two oat cultivars were larger than the differences due to different heat treatments applied (with Furlong samples exhibiting the T_0 , T_p and T_c values 2-3°C lower than those of HiFi samples). For each cultivar, groat size (large versus small) did not appear to augment the effects of different heat treatments on the transition temperatures and ΔH exhibited by bran and flour fractions. The pasting properties (i.e. gelatinization of starch) and elasticity of gels made of flour fractions appeared to be similar in differently heat-treated samples of each cultivar, with groat size and genotype having only small effects on these properties. The swelling properties of starch (assessed in cold paste viscosity test at 64°C), however, appeared to be significantly affected by different heat treatments applied to groats and genotype, but not the groat size. Compared to the control heat treatment, the SS treatments significantly increased the cold paste viscosity of milling fractions of HiFi and Furlong. In most cases, an increase in the cold paste viscosity was observed with increasing temperature of SS applied to the groats, although the differences were not always significant (P>0.05). The milling fractions of

HiFi exhibited much greater cold paste viscosity than those of Furlong (most probably due to the differences in BG content between the two cultivars).

Since it is postulated that viscosity-building properties of oat BG play a vital role in the physiological activity of these polysaccharides, it is important to ensure that thermal processing applied to oat groats does not have negative effects on these properties. In this study, variations in weight average molecular weight (M_w) and solubility of BG were observed among BG extracted from bran fractions of differently heat-treated Furlong and HiFi groats. Among the four heat treatments examined, the SS-110 treatment appeared to have the most positive effects on the physicochemical properties (increased M_w and viscosity in solution) of BG isolated from the bran fractions of Furlong and HiFi. It appeared that the treatments of groats with SS at 120 and 130°C caused partial depolymerization of BG (lower M_w) thereby increasing their solubilities compared to the control heat treatment and the treatment with SS at 110°C. Subsequently, the apparent viscosities of aqueous solutions of BG obtained from samples treated with SS at 120 and 130°C were lower than those obtained from the samples control heat-treated and SS processed at 110°C. Additionally, the genotype substantially affected the viscositybuilding properties of oat BG. Solubility of BG was lower in flour and bran fractions of Furlong than in flour and bran fractions of HiFi. Also, molecular weights of BG isolated from Furlong bran fractions were lower than those of BG isolated from HiFi bran fractions. Subsequently, the apparent viscosity of aqueous solutions of BG isolated from Furlong bran fractions were lower than those of BG isolated from HiFi bran fractions.

This study demonstrates that SS can be successfully utilized in oat processing. The functional properties of oat groat, as well as flour and bran fractions obtained from groats

of Furlong and HiFi cultivars processed with SS were similar or better than those obtained via the conventional process. Generally, the effects of different heat treatments applied to groats on the functional properties of groats, flour, and bran fractions were augmented by the different chemical composition (mainly differences in the content and solubility of β -glucans) of the two oat genotypes tested in this study. The size of groats (within each cultivar) significantly (P<0.05) affected groat hardness, but it appeared to have lesser influence on the physicochemical properties of flour and bran fractions.

5 EFFECTS OF SUPERHEATED STEAM ON *GEOBACILLUS* STEAROTHERMOPHILUS SPORE VIABILITY

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5.1 Abstract

Aims: To examine the effect of superheated steam (SS) treatment on *Geobacillus stearothermophilus* ATCC 10149 spores.

Methods and Results: Two inoculum levels of spores of *G. stearothermophilus* were mixed with sterile sand and exposed to SS at 105 to 175° C. The decimal reduction time (*D*-value) and the thermal resistance constant (*z*-value) were calculated. The effect of cooling of spores between periods of exposure to SS was also examined. A mean *z*-value of 25.4°C was calculated for both inoculum levels for SS processing temperatures between 130 and 175° C.

Conclusions: Spore response to SS treatment depends on inoculum size. SS treatment may be effective for reduction in viability of thermally resistant bacterial spores provided treatments are separated by intermittent cooling periods.

Significance and Impact of the Study: There is a need for technologies that require short thermal processing times to eliminate bacterial spores in foods. The SS processing technique has the potential to reduce microbial load and to modify food texture with less energy in comparison to commonly used hot air treatment. This work provides information on the effect of SS processing parameters on viability of *G*.

stearothermophilus spores.

Keywords: superheated steam, *Geobacillus stearothermophilus*, spores, thermal resistance, *D*-value, *z*-value.
5.2 Introduction

Bacterial spores, including those of *Geobacillus stearothermophilus*, are frequent contaminants of raw food and can affect product shelf life after traditional hot air drying (HAD) because of their thermal resistance (Devahastin and Suvarnakuta 2004).

Superheated steam (SS) drying is an emerging technology that has the potential to replace commonly used hot air treatment (Pronyk et al. 2004; Tang and Cenkowski 2001; Tang et al. 2005). The high heat intensity of SS may result in reductions of bacterial numbers in foods and can be used to modify food properties (Devahastin and Suvarnakuta 2004; Markowski et al. 2003; Cenkowski et al. 2006b). Superheated steam occurs when saturated steam is heated to a temperature above the saturation or boiling point corresponding to its pressure. During processing, the SS gives up some of its sensible heat to a material being processed. If the SS temperature stays above its saturation point, the amount of heat lost by the SS does not result in condensation. However, during the initial period, when the processed material is below the saturation temperature of the steam, condensation may occur (Pronyk et al. 2004). The SS processing technique has many benefits over HAD including: it excludes oxygen which can enhance product quality, it intrinsically has improved energy efficiency, it yields accelerated drying, and has reduced environmental impact through condensate reuse (Tang and Cenkowski 2001; Tang et al. 2005). Disadvantages of the SS processing technique include: high capital cost, complexity of the equipment, and a requirement that treated products be resistant to high temperatures. A number of products have been tested in SS processing/drying systems including: Asian noodles (Markowski et al. 2003), brewers' and distillers' spent

grains (Tang et al. 2005), sugar-beet pulp (Tang et al. 2000), potatoes (Tang and Cenkowski 2000), and potato chips (Caixeta et al. 2002).

Geobacillus (formerly *Bacillus*) *stearothermophilus* is a Gram-positive, thermophilic, spore-forming bacterium often associated with plant food products. The spores of this bacterium are very heat resistant and usually survive canning and sterilization operations, where saturated steam (moist heat) is used. Flat-sour spoilage of properly processed low acid canned foods may occur if the products are stored at elevated temperatures (Ng and Schaffner 1997). The spores of *G. stearothermophilus* are often used as a biological indicator to validate moist heat sterilization because of their exceptional resistance to this type of heat (Brown 1994; Carlberg 2005; Spicher et al. 1999).

The thermal resistance of spores depends on whether moist (steam) or dry (hot air) heat is used (Brown 1994). Moisture increases the germicidal effects of heat (Carlberg 2005). Due to the high heat resistance of spores potentially present in food products, severe or long thermal processing conditions are required to achieve commercial sterility. This can result in reduction of nutrients, colour, flavour, and texture characteristics of the products. If the extent of thermal treatment could be reduced, products with higher quality and with less overall energy consumption could be generated. Foods have been pre-treated to decrease spore heat resistance with varied results, for example: acidification (Fernandez et al. 1995; Periago et al. 1998; Rodrigo et al. 1997, 1999), oil addition (Rodrigo et al. 1999), or NaCl addition (Cook and Gilbert 1969; Periago et al. 1998) have been examined. Also, addition of monoglycerides (Kimsey and Adams 1981) or free fatty acids (Tremoulet et al. 2002) in the heating medium reportedly increases the effectiveness of thermal treatments against *G. stearothermophilus* spores.

From a restricted number of studies, it appears that G. stearothermophilus spores were more thermally resistant to SS than to moist (saturated) steam. Spicher et al. (1999) found that spore resistance (expressed as time needed to destroy 50% of initial population) increased rapidly with a greater degree of steam superheating, and reached a maximum (4.1 times more resistant) at 22°C of superheating (with a saturation temperature of 120°C). Further increases in superheating resulted in decreased spore resistance, but this did not change as rapidly as it had prior to reaching its maximum. Collier and Townsend (1956) compared spores of three bacterial species (including G. stearothermophilus 1518) for resistance to SS (138-204°C) and to saturated steam (93-138°C). While they found that spore resistance was higher towards SS treatment than to saturated steam, they further found that lowering the spore inoculum level (two fold) increased the apparent resistance to SS treatment. In the present study, spores of G. stearothermophilus were chosen as an example of highly heat resistant, non-pathogenic species that may be present in foods. It should, however, be noted that there are other bacterial spores (i.e. Bacillus subtilis var. globigii) that are more sensitive to SS and these have been suggested to be better markers for sterilization with SS. Also, there may be differences in heat resistance of strains within one bacterial species in response to SS conditions (Spicher et al. 1999, 2002).

Thermal inactivation of spores can also be achieved with a fractional (double- or triple-stage) heat treatment commonly referred to as tyndallization (Gould 2006; Valero and Salmeron 2003). During classic tyndallization, spores are exposed to heat (65-85°C for 30 min) three times with 24 h holding periods in between heat challenges (Pijanowski et al. 1996b). That way spores are allowed to germinate and their elimination is achieved

during the next heating period (Cho et al. 1999). Modifications of the tyndallization method to improve spore lethality (Brown et al.1979; Cho et al. 1999) have been made by using anaerobic conditions during the holding period, shorter heating times (i.e. 10 or 15 min), and shorter holding periods between heat exposures (i.e. only 20 min).

The aims of this research were: (a) to determine the thermal resistance (z-values) of G. stearothermophilus spores at 3 and 6 \log_{10} colony forming units per gram (cfu/g) during treatment with SS up to 175°C; and (b) to determine the effect of SS treatment with intermittent cooling on the reduction of spores.

5.3 Materials and Methods

5.3.1 Microorganism and Maintenance

G. stearothermophilus ATCC 10149 (Charm Sciences Inc., Malden, MA, USA) was maintained on tryptic soy agar (TSA) slants (Difco Laboratories, Detroit, MI, USA) following growth on TSA (24-36 h; 55°C). Slants were maintained at 4°C for subsequent use.

5.3.2 Spore Production

G. stearothermophilus spores were produced according to the method of Kim and Naylor (1966) with an addition of a freeze-drying step. The resultant spore crop was frozen in liquid nitrogen and freeze-dried using a Viritis 10-146MP-BA vacuum freezer (Viritis Inc., Gardiner, NY, USA). More detailed information on spore production is given in Appendix C. The dried spore material was lightly ground into a powder using a sterile mortar and pestle and mixed in a sterilized jar by hand shaking with sterilized, washed sand (commercially available decorative white sand) having a particle size of 105

to 212 μ m. The spore concentration of the mixture per gram was targeted so that 3 or 6 \log_{10} cfu/g could be inoculated and was estimated by serial dilution in 0.1% peptone (Difco) followed by direct plate counting on TSA (55°C for 24-36 h). All spore-sand mixtures were kept at 4°C before use.

5.3.3 Equipment

A SS processing system developed in the Department of Biosystems Engineering at the University of Manitoba (Tang et al. 2005) was used in experiments. The main components of the system (Fig. 5.1) were: a 9 kW electric steam generator, steam conveying pipelines, a 2 kW electric superheater, a processing chamber, a hot-air supply system to the jacket of the chamber maintaining adiabatic conditions for the processing chamber, and a data acquisition and control system.



Fig. 5. 1 Superheated steam (SS) processing system.

The sample tray consisted of two squares of Spectra nylon mesh (20 µm mesh opening; 14% open area; 55 µm thickness) (Spectrum Laboratories Inc., Rancho

Dominguez, CA, USA) housed between two tight fitting aluminum rings (inner diameter 6.3 cm; outer diameter 7.5 cm; Fig. 5.2). A layer of spore-sand mix (1 g) was placed between the two mesh squares in the sample tray. The sample tray was then placed in the processing chamber and exposed to SS for up to 30 s come-up time (depending on the operating temperature) before actual timing of the treatment commenced. The velocity of SS passing through the processing chamber was adjusted to 0.35±0.02 m/s and the steam temperature and processing time were varied during tests.



Fig. 5. 2 Sample tray with a sample mix placed between two mesh squares – top view.

5.3.4 Superheated Steam Treatment of Spore-Sand Mix

The SS temperatures applied to the spore-sand mix ranged from 130 to $175\pm2^{\circ}$ C in 15°C increments. The high temperatures of SS (130-175°C) were chosen because results of the earlier study on viability of *G. stearothermophilus* spores exposed to SS at 115-175°C (using the same SS processing equipment) indicated low efficacy of SS at 115 and 121°C in the reduction of spores (Henry 2007). Tests were also performed at 105°C in order to compare the lethal effects of 5°C superheat with those of 30 to 75°C. The

processing times ranged from 0.5 min to 480 min, depending on the SS temperature. Processing time at each SS temperature was long enough to achieve at least one log cycle reduction in the number of spores and to allow calculation of the *D*-value.

Spore treatment with SS was also performed using intermittent cooling periods as outlined in Table 5.1.

Table 5. 1 Superheated steam processing protocols (A and B) used for challenge of G.

 stearothermophilus spores.

| SS [*] (min) _ | Cooling (mi | Period n) | SS (min) | Cooling (mi | Period n) | SS (min) |
|----------------------------|--|--|--|--|--|---|
| _ | 25°C | 4°C | | 25°C | 4°C | _ |
| 5 | 5 | 10 | 5 | | | |
| 5 | 5 | 10 | 5 | 5 | 10 | 5 |
| | SS [*] (min) _ 5 5 | SS* Cooling (min) (mi 25°C 5 5 5 5 5 5 | $\begin{array}{ccc} SS^{*} & Cooling Period \\ (min) & (min) \\ \hline 25^{\circ}C & 4^{\circ}C \\ \hline 5 & 5 & 10 \\ 5 & 5 & 10 \\ \end{array}$ | $\begin{array}{cccc} SS^{*} & Cooling Period & SS \\ (min) & (min) & (min) \\ \hline 25^{\circ}C & 4^{\circ}C \\ \hline 5 & 5 & 10 & 5 \\ 5 & 5 & 10 & 5 \\ \end{array}$ | $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ |

Superheated steam processing time.

In Protocol A a spore-sand sample was exposed to SS for 5 min, cooled for 15 min, and exposed to SS for another 5 min. Three SS temperatures were used i.e., 105, 130, and 145° C. During cooling the sample tray was initially placed at 25° C (to reduce condensate formation from moisture in the air) for 5 min and then it was transferred to 4° C for 10 min. Protocol B consisted of 3 x 5 min exposures to SS with two 15 min cooling periods in between. Each experiment was conducted in triplicate.

5.3.5 Spore Survivors

Surviving *G. stearothermophilus* spores following treatment with SS were determined using serial dilution in 0.1% peptone (Difco) followed by duplicated spread plating on TSA medium, incubation at 55°C for 24-36 h and enumeration (Swanson et al. 1992). Results were reported as log_{10} cfu/g of spore – sand mix and were the averages of the two plates. The results were then used to calculate *D*- and *z*-values (Ray 2001c).

5.3.6 Microscopic Observation of SS-Treated Spores

Untreated control and spore-sand mixtures treated 5 min with SS at 130°C were cooled 5 min at 25°C, refrigerated 10 min at 4°C, and within 5 min were inoculated on microscope slides coated with sterile nutrient agar (Difco). After applying cover slips, the slides were placed in lidded sterile Petri dishes containing moistened filter paper. Slides were held at 55°C for 1.5 h without interruption and then were examined at 15min intervals with phase contrast optics (magnification x 1000) to assess spore germination by photomicrography (Carl Zeiss MicroImaging Inc., Thornwood, NY, USA).

5.3.7 Moisture Content Changes as a Result of SS Processing

Samples of uninoculated clean sand (1 g) were treated with SS at 105, 130, 145, 160 or 175°C from 5 to 30 s in 5 s increments and from 1 to 5 min in 1 min increments of processing time. Differences in mass of the samples before and after treatment at each sampling period were determined.

5.3.8 Statistical Analysis

All experiments were conducted in triplicate. For each replicate, the number of survivors (\log_{10} cfu/g) was analyzed with JMP IN Ver.4 software (SAS Institute Inc., Cary, NC, USA). One way Anova tests and means comparisons (Student t-test) were performed in order to determine the statistical significance (P<0.05) of the results obtained at different processing temperatures, times and inoculum levels. The results from experiments following Protocol A and B (at 105, 130, and 145°C) were compared one to another and to the results obtained from the experiments with continuous SS processing for the same duration of exposure to SS (10 and 15 min).

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5.4 Results

Survival ratios (survivors after treatment compared to initial number, log_{10} N/N_o) of spores exposed to continuous SS treatment were plotted against treatment time and are given in Figs. 5.3 and 5.4 (for 3 and 6 log_{10} cfu/g inoculum levels, respectively). Data points in the resulting survivor curves represent the averages of three experiments with vertical bars as standard deviations. Where error bars are not visible, the standard deviation of means was smaller than the symbol size.

A steeper negative slope of the survivor curve at its beginning was observed at every temperature of SS treatment and at both inoculum levels (Figs. 5.3 and 5.4). In the tested range, the highest reduction of *G. stearothermophilus* spores was achieved with SS temperatures of 160 and 175° C at the high inoculum and with 175° C at the low inoculum.



Fig. 5. 3 Survivor curves for *G. stearothermophilus* spores (3 log₁₀ cfu/g inoculum level) challenged with superheated steam at temperatures from 105 to 175°C. Symbols represent means of three trials. Vertical bars represent standard deviations of the means.



Fig. 5. 4 Survivor curves for *G. stearothermophilus* spores (6 log₁₀ cfu/g inoculum level) challenged with superheated steam at temperatures from 105 to 175°C. Symbols represent means of three trials. Vertical bars represent standard deviations of the means.

With the low inoculum, treatment at 160°C was initially (≤ 15 min) only slightly more effective than 105°C (Fig. 5.3). Similarities in spore resistance at 3 log₁₀ cfu/g at 130 and 145°C (at ≤ 15 min) were also seen at the higher inoculum, but at 105°C as well as at 130 and 145°C (Fig. 5.4). At the high inoculum, at 5 min exposure to SS between 105 and 145°C numbers of surviving spores present were not significantly different (P>0.05). However, treatment for 5 min at 160°C yielded a significantly greater reduction than obtained at 105 to 145°C. In contrast, with the lower inoculum at 5 min exposure to SS, there was no difference in spore survival at 105, 130 and 160°C. Among the different treatments at the lower inoculum, heating for 5 min at 145°C was the least effective for reducing spore viability. *D*-values were calculated and these represented the time needed to destroy 90% of the microbial population at a specific temperature (Table 5.2). *D*-values were obtained from the slope of the regression line fitting the entire survivor plots at each temperature with one exception. When spores at the high inoculum were processed with SS at 130° C for up to 480 min, a one \log_{10} cycle reduction in spore survivors was not achieved. Therefore, the *D*-value for that treatment was estimated from the data obtained for the first 60 min of processing. Otherwise, the standard approach to *D*-value calculation was used to allow data comparison with other work and facilitate its use by industry.

Table 5. 2 *D*-values of *G. stearothermophilus* spores at inoculum levels of 3 log₁₀ and 6 log₁₀ cfu/g challenged with superheated steam at different temperatures.

| SS* | <i>D</i> -value (min) | | | |
|---------------|-------------------------------|------------------------------|--|--|
| Temperature - | Inoculum | Inoculum | | |
| (°C) | $(3 \log_{10} \text{ cfu/g})$ | $(6 \log_{10} \text{cfu/g})$ | | |
| 105 | 23.5 | 93.3 | | |
| 130 | 65.9 | 101.7 | | |
| 145 | 63.0 | 29.0 | | |
| 160 | 9.3 | 2.1 | | |
| 175 | 2.2 | 1.5 | | |

* Superheated steam.

From the *D*-values, *z*-values for the two inoculum levels were calculated (where the *z*-value represents the change in processing temperature needed to achieve 90% decrease in the *D*-value). The *z*-values were determined from regression lines obtained by plotting log *D*-values versus corresponding processing temperatures (Fig. 5.5). Experiments conducted at 105° C were not included in calculations of the *z*-values because the interference caused by condensation necessitated use of a slightly different processing procedure. The *z*-values found were 28.4 and 22.4°C for the 3 and 6 log₁₀ cfu/g inoculum

levels, respectively, and a mean value of 25.4°C was calculated for the two populations of spores.



Fig. 5. 5 Thermal death time curves of *G. stearothermophilus* spores challenged with superheated steam at temperatures from 130 to 175°C. Spore inoculum level: low - 3 log₁₀ cfu/g, high - 6 log₁₀ cfu/g.

Results from SS processing with intermittent cooling intervals (Table 5.1) are shown as a percent reduction in viable spores (Table 5.3). A comparison of mean recoveries between the A and B protocols for the same SS temperatures at both inoculum levels indicated that significantly (P<0.05) greater reductions occurred when the number of cooling intervals was increased (except at 130°C at the high inoculum level). The percent reduction of spores obtained following Protocol A and B were compared to the results from experiments where continuous exposure to SS for the same duration of time (10 and 15 min, respectively) was used (Tables 5.4 and 5.5). The means of the percent reduction of spores obtained following Protocol A for 10 min exposure to SS were significantly (P<0.05) greater than for 10 min continuous SS processing for both inocula at all temperatures (except 105° C with the low inoculum). The reductions during Protocol B were also significantly (P<0.05) larger than those following continuous SS processing for the same 15 min SS exposure at all temperatures at both inoculum levels.

 Table 5. 3 Percent reduction of viable G. stearothermophilus spores challenged with superheated steam following Protocol A and B at different temperatures.

| \mathbf{SS}^{*} | Percent Reduction of Spores [†] | | | | | |
|-------------------|--|-----------------------|------------------------------|-----------------------|--|--|
| Temperature | Inocu | ılum | Inoculum | | | |
| (°C) | $(3 \log_{10} \text{cfu/g})$ | | $(6 \log_{10} \text{cfu/g})$ | | | |
| | Protocol A | Protocol B | Protocol A | Protocol B | | |
| 105 | 94.7±1.9 ^{ª‡} | 98.4±0.4 ^b | 77.9±0.9 ^{a‡} | 80.9±1.3 ^b | | |
| 130 | 88.5±1.3 ^a | 92.9±1.4 ^b | 84.0±3.8 ^a | $89.0{\pm}1.7^{a}$ | | |
| 145 | 85.9±0.3 ^a | 93.7±0.1 ^b | 84.9±0.8 ^a | 95.6 ± 1.3^{b} | | |

Superheated steam.

[†] The comparison was between Protocol A vs Protocol B but within the same inoculation level.

[‡] The same letters in each row indicate no significant difference (P>0.05) between sample means of adjacent columns.

[§] Values represent means of triplicate analyses with their standard deviations.

Table 5. 4 Percent reduction of *G. stearothermophilus* spores at 3 log₁₀ cfu/g challenged with SS at different temperatures for 10 or 15 min compared to results from Protocol A or B.

| SS* | Percent Reduction of Spores [†] (From 3 log ₁₀ cfu/g) | | | | | |
|------|---|-------------------------|------------------------|-------------------------|--|--|
| (°C) | Protocol A | Continuous SS 10 min | Protocol B | Continuous SS 15 min | | |
| 105 | 94.7±1.9 ^{a‡} | 94.9±0.3 ^a | 98.4±0.4 ^{a‡} | 96.4±0.9 ^b | | |
| 130 | 88.5±1.3 ^a | 82.0±3.8 ^b | 92.9±1.4 ^a | 87.3±0.7 ^b | | |
| 145 | 85.9±0.3ª | 76.7±3.6 ^b | 93.7±0.1 ^a | 83.7±3.3 ^b | | |

* Superheated steam.

[†] The statistical comparison was between Protocol A vs 10 min continuous SS processing and Protocol B vs 15 min continuous SS processing.

[‡] The same letters in each row indicate no significant difference (P>0.05) between sample means of adjacent column.

[§] Values represent means of triplicate analyses with their standard deviations.

| Table 5. 5 | Percent reduction of G. stearothermophilus spores at 6 log ₁₀ cfu/g challenged |
|------------|---|
| | with SS at different temperatures for 10 or 15 min compared to results from |
| | Protocol A or B. |

| SS [*] | Percent Reduction of Spores [†] (From $6 \log_{10} \text{cfu/g}$) | | | | | |
|-----------------|---|-------------------------|------------------------|-------------------------|--|--|
| (°C) | Protocol A | Continuous SS 10 min | Protocol B | Continuous SS 15 min | | |
| 105 | 77.9±0.9 ^{a‡} | 64.1±4.4 ^b | 80.9±1.3 ^{a‡} | 60.5±1.9 ^b | | |
| 130 | 84.0 ± 3.8^{a} | 70.3 ± 2.9^{b} | $89.0{\pm}1.7^{a}$ | 65.5±4.4 ^b | | |
| 145 | 84.9 ± 0.8^{a} | 44.4±20.3 ^b | 95.6±1.3 ^a | 71.2±2.2 ^b | | |

* Superheated steam.

[†] The statistical comparison was between Protocol A vs 10 min continuous SS processing and Protocol B vs 15 min continuous SS processing.

[‡] The same letters in each row indicate no significant difference (P>0.05) between sample means of adjacent column.

[§] Values represent means of triplicate analyses with their standard deviations.

Microscopic observation of spores indicated that those treated with SS at 130°C for 5 min germinated 25-30 min faster than spores not treated with SS. Different phases of spore germination are depicted in Fig. 5.6 a and b.



Fig. 5. 6 Germination phases of *G. stearothermophilus* spores treated with superheated steam at 130°C and 0.35 m/s velocity for 5 min (1000 x magnification).

Experiments done on sand samples exposed to SS at various temperatures showed that initial steam condensation existed on samples. The lower the SS temperature used the longer was the time needed to remove the condensate. At 105°C, 4.7 min were necessary for condensate to evaporate from the surface of the sand but this was reduced to 1.9 min at 130°C, 0.75 min at 145°C, and 0.28 min at 160°C. At 175°C the condensation was absent from samples after the first 5 s of treatment.

5.5 Discussion

When a z-value for *G. stearothermophilus* spore destruction by SS treatment was calculated for treatment at 130 to 175° C, the value of 25.4° C obtained was similar to those reported for conventional steam treatment (z in the range of $13-31^{\circ}$ C). The mean z-value found was also close to the z-value of 26° C for *G. stearothermophilus* spores exposed to superheated steam reported by Collier and Townsend (1956).

Very high SS temperatures (160 and 175° C at high inoculum level and 175° C at low inoculum level) proved to be effective in reducing *G. stearothermophilus* spores. Use of such treatment temperatures, however, may negatively influence important properties of some food products, including their storage stability, and consumer acceptance (Cenkowski et al. 2006b).

At 105°C, the first 5 min of SS processing would resemble moist heat treatment due to the initial condensate formation (sample temperature was at the saturation point) and yielded substantial spore lethality (Carlberg 2005). Additionally, temperature in the SS processing chamber varied by 2°C, and when the fluctuation in the atmospheric pressure was added, this led to moments of condensation. For example, if the atmospheric pressure from one experiment to another changed from 100 to 102 kPa (which is common at the University laboratory used) and the temperature controller allowed the temperature to decrease to 103°C, condensation would be observed until the controller activated the heaters and increased the chamber temperature. Due to heat inertia this could take several sec, and the same sample could experience 3 to 4 cycles within a 20 min experiment. Therefore, it was likely that it was condensate formation at the lower temperature SS treatments that rendered spores more thermally sensitive.

The present results show that inoculum level affected spore response to SS. This observation is very important from the process application perspective, as low spore levels may alter the efficacy of SS treatments. At 105 and 130°C SS, spores at the lower inoculum level were eliminated faster than those at the high inoculum. The opposite was observed at SS temperatures of 145, 160 and 175°C. It is not uncommon that low spore populations do not respond the same as high spore populations to stress (Llaudes et al. 2001). Caipo et al. (2002) studied the effects of inoculum size on Bacillus megaterium spore germination. Their results indicated that spore interactions in more concentrated suspensions appeared responsible for causing more rapid germination. This may have been involved in spore response to SS treatment because the spores germinated and then were inactivated. This is consistent with the present observation of faster elimination of spores at the high inoculum processed at 145, 160 and 175°C with SS. A similar effect of inoculum size on thermal resistance of G. stearothermophilus spores challenged with SS was observed by Collier and Townsend (1956), where the lower spore inoculum level exhibited increased apparent thermal resistance to SS treatment at 160-190°C.

The greater effectiveness of intermittent than continuous SS treatments can be explained by condensate formation between SS challenges. Samples challenged with SS according to protocols A and B were exposed to initial condensate formation 2 or 3 times, respectively. This may explain the greater spore elimination during intermittent than during continuous SS treatment (where condensate formation occurred only once). Also, sublethal heat treatment, among other factors, causes dormant bacterial spores (phase bright) to be activated and germination to begin (phase grey and dark) ending in formation of an emerged cell. One of the first indications related to initiation of germination is the loss of spore heat resistance (Ray 2001a). Initiation of spore germination following the first SS exposure would increase spore susceptibility during subsequent thermal challenge and may explain the greater effectiveness of intermittent than continuous SS treatments. This phenomenon is commonly referred to as the tyndallization effect. Cho et al. (1999) reported greater effectiveness of spore inactivation when tyndallization-like processing was used. In their study, spores of Bacillus subtilis ATCC 6633 were exposed to single- and double-stage conventional or ohmic heating. The dual heating resulted in more effective inactivation of spores regardless of the heating method used (conventional or ohmic). Brown et al. (1979) investigated effects of a tyndallization-like process (double-stage heating separated by a period of anaerobic incubation) on sporeforming organisms (Bacillus cereus and Bacillus subtilis) in milk and cream. However, reductions following the two-stage process did not improve spore lethality, and they attributed the lack of success to different rates of germination of the different bacterial species as well as variability in response within a single species.

5.6 Conclusions

When a z-value for G. stearothermophilus spore destruction by SS treatment was calculated for treatment at 130 to 175°C, the value of 25.4°C obtained was similar to those reported for conventional steam treatment (z-value in the range of 13-31°C). The concentration of spores in the inoculum affected the response of spores to SS. When SS treatments were interrupted by brief cooling intervals, the number of spores killed was significantly increased. It was evident that the higher dry temperatures (130 and 145°C) achievable by SS treatments were not as effective against spores as lower temperatures (105°C) in the presence of moisture as condensate. Superheated steam treatment may be effective for reduction in viability of thermally resistant bacterial spores provided treatments are separated by intermittent cooling periods or very high SS temperatures (160-175°C) are used. The use of high SS temperatures, however, may be limited to products that are not thermally sensitive as their properties and storage stability may be compromised; for example it was noted previously (Chapter 3, section 3.5.3) that oat groats processed with SS at temperatures higher than 130°C became rancid within 2-3 weeks of storage at room temperature.

6 MODELLING OF INACTIVATION OF *GEOBACILLUS* STEAROTHERMOPHILUS SPORES EXPOSED TO SUPERHEATED STEAM

6.1 Abstract

Spores of Geobacillus stearothermophilus at two inoculum levels (3 and 6 log10 colony forming units per gram, cfu/g) were challenged with superheated steam (SS) at a velocity of 0.35 m/s and temperature of 130, 145, 160, and 175°C. Tailing was observed in the survivor curves (plots of survival ratios, $\log_{10}(\frac{N}{N_o})$ against treatment time) as a monotonic upward concavity. Therefore, non-linear regression models; the Weibull and Kamau models, instead of the traditional first order kinetics model were used to describe the survivor curves. The Weibull model was successfully fitted to all survivor curves obtained in this study, whereas the Kamau model fitted only some. Good fit of the Weibull model to spore survivor curves and high correlation between the predicted and the experimental values of spore survival ratios were observed for SS treatment at 145-175°C at both inoculum levels, and for SS at 130°C at low inoculum level. The Weibull model was expanded by describing its parameters (α , β) as a function of SS temperature for the range of 130-175°C. This allowed accurate predictions of the spore survival ratios based on the SS temperature and processing time only (with the exception of predictions for spores at high inoculum level challenged with SS at 130°C). The Weibull model is recommended to describe and to model the inactivation of G. stearothermophilus spores in SS at high temperatures (145-175°C).

6.2 Introduction

Thermal inactivation of bacterial vegetative cells and spores has been traditionally presumed to follow the first order kinetics model:

$$\log_{10}\left(\frac{N}{N_o}\right) = -kt \tag{6.1}$$

where N_0 is the initial number of microorganisms (cfu/g), N is the number of microorganisms at time t (cfu/g), t is the treatment time (s or min), and k is a rate constant (1/s or 1/min) (Peleg 2002; van Boekel 2002). From this equation the classic *D*-value (time necessary for 1 log₁₀ reduction in the number of microorganisms) can be obtained by calculating the reciprocal of k (1/k = D). This simple first order model is widely accepted within food industry because of its simplicity and usefulness for calculation of treatment times needed to achieve commercial sterility (van Boekel 2002).

According to the first order kinetics model presented in a semi-log plot, the ratio of microorganisms surviving a heat treatment is expected to linearly depend on the duration of the treatment. However, it has been shown that in reality the ratio of surviving microorganisms is not always linearly correlated with treatment duration, and deviations such as shoulders, tails (biphasic curves), upward or downward concavity occur in survivor curves (Bialka et al. 2008; Juneja and Marks 2005; Shull et al. 1963). Heat destruction of bacterial spores is now believed to be dependent on the strain, treatment duration and temperature (Tremoulet et al. 2002), as well as on the presence of sub-populations of spores with different heat resistance arising from the spore physiological state (i.e activated, dormant, or so-called "super dormant" spores) (Iciek et al. 2006; Sapru et al. 1993). In spore inactivation, the shoulders occurring in survivor curves have been mainly associated with the activation phenomenon, whereas tails have been

considered to be the indication of resistance heterogeneity within the population of spores (Manas and Pagan 2005; Xiong et al. 1999). Simultaneously occurring phenomena of spore activation and inactivation may result in non-linearity of survivor curves obtained for spores (van Boekel 2002). A number of models have been proposed to describe the non-linearity in survivor curves; logistic (i.e. Kamau model), vitalistic, as well as Weibull distribution models (Guan et al. 2006; Kamau et al. 1990; Xiong et al. 1999). The latter models are simple (only two parameters determine the curve path) and versatile as they can accurately fit either straight, concave downwards or upwards survival curves (Manas and Pagan 2005).

In the previous study (Chapter 5) the survivor curves obtained for *Geobacillus stearothermophilus* spores challenged with superheated steam (SS) at 130-175°C and a velocity of 0.35 m/s showed non-linear dependence on the treatment duration. Therefore, the objectives of this work were: (i) to apply the Weibull and the Kamau models to the experimental data obtained from the aforementioned study; (ii) to assess the adequacy of fit of these models and recommend the best model describing the inactivation of *G. stearothermophilus* spores exposed to SS.

6.3 Materials and Methods

6.3.1 Materials

Spores of *Geobacillus stearothermophilus* ATCC 10149 were produced as described earlier (Chapter 5: sections 5.3.1 and 5.3.2, and Appendix C.1) and mixed with sterile sand. Spore concentration per gram of the mix was targeted so that 3 or 6 \log_{10} cfu/g could be inoculated for treatment with SS.

6.3.2 Superheated Steam Processing and Enumeration of Surviving Spores

Spore-sand mix was exposed to SS at a velocity of 0.35 ± 0.02 m/s and temperature of 130, 145, 160, and $175\pm2^{\circ}$ C for different durations of time. Each experiment was conducted in triplicate. The equipment and methodology of the SS challenge were described earlier (Chapter 5, sections 5.3.3 and 5.3.4). Surviving spores following exposure to SS were determined using serial dilution in 0.1% peptone (Difco Laboratories, Detroit, MI, USA) followed by duplicated spread plating on tryptic soy agar medium, incubation at 55°C for 24-36 h, and enumeration (Swanson et al. 1992). Results (reported as log_{10} cfu/g of the spore-sand mix) were used to calculate survival ratio, log_{10} ($\frac{N}{N_o}$), of spores at each processing time for construction of survivor curves.

6.3.3 Data Analysis and Model Evaluation

Weibull model

The Weibull model takes into account the biological variation within a population of microorganisms with respect to thermal inactivation. As a result, the model is a statistical model of distribution of different inactivation times (van Boekel 2002) and has a cumulative form of:

$$\log_{10}\left(\frac{N}{N_{a}}\right) = -\frac{1}{2.303} \left(\frac{t}{\alpha}\right)^{\beta} \tag{6.2}$$

where N_o is the initial number of microorganisms (cfu/g), N is the number of microorganisms at time t (cfu/g), t is the treatment time (s or min), α is the characteristic time or scale parameter (s or min), and β is the shape parameter (non-dimensional). In a semi-log plot the Weibull distribution corresponds to a concave upward survivor curve when β <1, concave downward curve if β >1, and is linear if β =1. The first order kinetics approach (straight line in a semi-log plot) is therefore a special case of the Weibull

model. Although the Weibull model is empirical, a link can be made with physiological effects due to inactivating agents (i.e. heat) acting on microorganisms. For example, when $\beta < 1$, the remaining organisms have the ability to adapt to the applied stress and it is more difficult to inactivate them, whereas when $\beta > 1$, the remaining organisms become increasingly more damaged.

Historically, the Weibull model has been used in failure engineering to describe time to failure in electronic and mechanical systems (van Boekel 2002). The 90% percentile of the failure time distribution – called reliable life, t_R , can be calculated from the parameters α and β in the Weibull model as follows:

$$t_{\rm R} = \alpha(2.303)^{\frac{1}{\beta}} \tag{6.3}$$

where α and β are the scale and shape parameters, respectively (van Boekel 2002). The t_R is analogous to the classic *D*-value when one log₁₀ reduction is considered (Manas and Pagan 2005).

Kamau model

The Kamau model is a logistic model that can describe non-linear survivor curves and has a general form of:

$$\log_{10}\left(\frac{N}{N_{a}}\right) = \log_{10} 2 - \log_{10} \left[1 + \exp(kt)\right]$$
(6.4)

where, N_0 is the initial number of microorganisms (cfu/g), N is the number of microorganisms at time t (cfu/g), t is the treatment time (s or min), exp is the base of the natural logarithm (exp ≈ 2.71), and

$$k = 4[(dN/dt)_{max}]/N_o$$
(6.5)

The differential $(dN/dt)_{max}$ is the slope of survivor curve at the point of inflection (maximum killing rate), and k/4 is the maximum specific killing rate (Kamau et al. 1990).

Survivor curves having two distinct killing phases (biphasic curves) can be analyzed with the Kamau model in a two-term exponential form of equation 6.4:

$$\log_{10}\left(\frac{N}{N_{o}}\right) = \log_{10}\left[\frac{2n}{1 + \exp(k_{1}t)} + \frac{2(1-n)}{1 + \exp(k_{2}t)}\right]$$
(6.6)

where n and (1-n) represent two fractions (sub-populations) of organisms differing in heat resistance, and k_1 and k_2 are the specific killing rates for the two sub-populations, respectively (Kamau et al. 1990). The above form of the model assumes exponential killing of both sub-populations but at different, independent rates.

Model evaluation

Sigma Stat (version 3.5, Systat Software Inc., Point Richmond, CA, USA) was used for non-linear regression analysis and for determination of the parameters of the Weibull and Kamau models. The models were constructed using averages from two experimental data sets, and the third data set was used for model validation. Adequacy of the goodness of fit of each model to the experimental data was determined by the adjusted correlation coefficient (R^2) and standard error of estimate (root mean square error, RMSE). The best model describing *G. stearothermophilus* spore inactivation in SS was selected as the one with the highest value of R^2 and the lowest value of RMSE. Additionally, the pattern of residuals (the difference between observed and predicted values of $\log_{10}(\frac{N}{N_e})$) calculated for predictions of each model was examined. Models were validated against an experimental third data set of $\log_{10}(\frac{N}{N_e})$. High values of correlation coefficient (R^2) of that regression indicated high accuracy of the model.

6.4 **Results and Discussion**

Figures 6.1-6.3 show the fit of the Weibull and Kamau models (Eqs. 6.2 and 6.6, respectively) to the survivor curves obtained for *G. stearothermophilus* spores at two inoculum levels challenged with SS of different temperatures. The Weibull model fitted all survivor curves obtained for the spores at both inoculum levels (Figs. 6.1-6.2), whereas the Kamau model fitted only the survivor curves obtained for 130, 145, and 160°C at high inoculum, and for 160°C at low inoculum (Fig. 6.3). It is possible that the lack of success in fitting the Kamau model to all experimental data was caused by the fact that this model was proposed for describing inactivation of bacterial vegetative cells (*Listeria monocytogenes* and *Staphylococcus aureus*) and may not entirely apply to spore inactivation kinetics.

For each model, the residuals (difference between the observed and predicted values) of the survival ratios, $\log_{10} \left(\frac{N}{N_o}\right)$ of spores were calculated for each SS temperature and plotted against treatment time (Figs. D.1-D.3 in Appendix D). There were no significant patterns of the residuals, and generally the spread of the residuals was random (especially for SS at 160 and 175°C) indicating a good fit of each model to the experimental data. The parameters of goodness of fit (RMSE and R^2) of the Weibull and Kamau models are summarized in Table 6.1. The RMSE measures the average deviation between the observed and fitted (predicted) values, thus smaller values of RMSE indicate a better fit of a model (Buzrul and Alpas 2004, 2007). Generally, smaller values of RMSE were obtained for survival ratios of spores at low inoculum level than those at high inoculum level. Values of the adjusted coefficient of correlation (R^2) approaching 1 indicate a good fit of a model to the experimental data.



Fig. 6. 1 Fit of the Weibull model (Eq. 6.2) to survivor curves obtained for *G. stearothermophillus* spores (at 3 log₁₀ cfu/g, i.e. low inoculum level) challenged with superheated steam at 130-175°C.



Fig. 6. 2 Fit of the Weibull model (Eq. 6.2) to survivor curves obtained for *G. stearothermophillus* spores (at 6 log₁₀ cfu/g, i.e. high inoculum level) challenged with superheated steam at 130-175°C.



Fig. 6. 3 Fit of the Kamau model (Eq. 6.6) to survivor curves obtained for *G. stearothermophillus* spores (at 3 and 6 log₁₀ cfu/g, i.e. low and high inoculum levels, respectively) challenged with superheated steam at 130-160°C.

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SS^a Temperature Inoculum Level R^{2c} Model RMSE^b $(\log_{10} \text{cfu/g})$ $(^{\circ}C)$ Weibull 0.11 0.93 3 _d Kamau 130 Weibull 0.12 0.70 6 Kamau 0.13 0.64 Weibull 0.10 0.94 3 Kamau 145 Weibull 0.14 0.96 6 Kamau 0.14 0.96 0.98 Weibull 0.08 3 0.99 Kamau 0.03 160 Weibull 0.09 0.99 6 Kamau 0.99 0.11 Weibull 0.10 0.94 3 Kamau 175

Table 6. 1 Goodness of fit parameters of the Weibull and Kamau models (Eqs. 6.2 and 6.6,respectively) predicting survival ratios of G. stearothermophilus sporeschallenged with superheated steam.

^aSS – superheated steam.

^bRMSE – root mean square error (standard error of estimation).

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 ${}^{c}R^{2}$ – adjusted coefficient of correlation.

^d Model did not fit the experimental results.

Very high values of the R^2 were obtained for the Weibull model applied to the survival ratios obtained at low inoculum at all SS temperatures (R^2 of 0.93-0.99). At high inoculum level, the R^2 of the Weibull model applied to experimental data for SS at 145-175°C were also very high (0.95-0.99), but for SS at 130°C the R^2 value decreased to 0.70, indicating poorer fit of the Weibull model.

Weibull

Kamau

0.12

0.95

Both Weibull and Kamau models were validated and the correlation coefficient (R^2) and slope of the regression line were determined (example shown in Fig. 6.4 and results summarized in Table D.1 in Appendix D). When data for spore inactivation in SS treatment at 130°C was analyzed, the Weibull model provided more accurate predictions (indicated by R^2 and slope approaching 1) of survival ratios at low inoculum level than at high inoculum level, whereas a reversed trend was noted for predictions at 145°C (Table D.1). For higher SS temperatures (160 and 175°C) similar predictions of the survival ratio were observed at both inoculum levels. Also, similar predictions of survival ratio were obtained from the Kamau and the Weibull models fitted to the survivor curves for the same SS treatment temperature (at the same inoculum level).



Fig. 6. 4 Correlation between experimentally determined and predicted (Eq. 6.2) values of survival ratio obtained for *Geobacillus stearothermophilus* spores (at 3 log₁₀ cfu/g) challenged with superheated steam at 130°C.

Parameters of the Weibull and Kamau models obtained for the spore survival ratios at each SS temperature and inoculum level are summarized in Tables 6.2 and 6.3, respectively. In most instances, the value of the shape parameter (β) in the Weibull model was less than 1 which could indicate that the spores became increasingly adapted to the heat as SS treatment progressed, or activation and inactivation phenomena occurred simultaneously but at different rates during SS treatment causing the concave upward shape of the survivor curves. More research is needed to elucidate the physiological changes occurring in the spores exposed to SS.

| SS ^b Temperature (°C) | Inoculum Level (log ₁₀ cfu/g) | α | β | t _R ° | D-value ^d |
|-------------------------------------|---|-------|------|------------------|----------------------|
| 130 | 3 | 1.90 | 0.35 | 20.78 | 65.9 |
| 130 | 6 | 5.88 | 0.21 | 316.53 | 101.7 |
| | | | | | |
| 145 | 3 | 3.82 | 0.39 | 31.05 | 63.0 |
| 145 | 6 | 13.25 | 1.08 | 28.60 | 29.0 |
| | | 1 50 | 0.61 | 6.05 | 0.2 |
| 160 | 3 | 1.59 | 0.61 | 6.25 | 9.3 |
| 160 | 6 | 1.56 | 1.29 | 2.97 | 2.1 |
| | | | | | |
| . 175 | 3 | 0.005 | 0.18 | 0.44 | 2.2 |
| 175 | 6 | 0.35 | 0.72 | 1.13 | 1.5 |

Table 6. 2 The parameters^a of the Weibull model (Eq. 6.2) used to obtain survival ratios ofG. stearothermophilus spores challenged with superheated steam.

 $a\alpha$ - scale parameter (min), β - shape parameter (non-dimensional).

^bSS – superheated steam.

 ${}^{c}t_{R}$ – reliable life (min) calculated from $t_{R}=\alpha(2.303)^{1/\beta}$, and analogous to the *D*-value for the first log reduction. ^d*D*-values (min) were obtained by fitting the first order kinetics model to the inactivation data (values reported in Chapter 5).

| Table 6.3 The parameters ⁴ | of the Kamau | model (Eq. 6.6 | 6) used to | obtain survival | ratios of |
|---------------------------------------|------------------|-----------------|------------|-----------------|-----------|
| G. stearothermo | ophilus spores c | challenged with | h superhea | ited steam. | |

| SS | ^o Temperature (°C) | Inoculum Level (log ₁₀ cfu/g) | k ₁ | k ₂ | n |
|----|-------------------------------|---|----------------|----------------|------------|
| | 130 | 3 | _c | - | - |
| | 130 | 6 | 0.011 | 0.660 | 0.314 |
| | 145 145 | 3 | _ 0.092 | - 4.605 | - 0.761 |
| | 1(0 | | 0.099 | 0.554 | 0.051 |
| | 160 | 5 | 0.088 | 0.334 | 1 168 |
| | 160 | 0 | 0.980 | 0.805 | 1.100 |
| | 175 | 3 | - | - | - |
| | 175 | 6 | - | - | - |

 k_1 – specific killing rate for organisms in 'n' sub-population; k_2 – specific killing rate for organisms in '1-n' sub-population; n – fraction (sub-population) of organisms having heat resistance different from that of sub-population '1-n'

population '1-n'. ^bSS – superheated steam.

^cModel did not fit the experimental results.

The α and β parameters in the Weibull model can be used to calculate the reliable life (t_R), the time in which one log₁₀ reduction in the number of surviving organisms will occur. The reliable life calculated for the first log₁₀ reduction of an organism is analogous to the classic *D*-value often used in food industry for calculations of processing time, even though the two values have a different meaning (t_R has a probabilistic interpretation while *D*-value is the reciprocal of a first order rate constant) (Buzrul 2007). The values of t_R obtained from the parameters of the Weibull model fitted to *G. stearothermophillus* spore inactivation data, as well as the *D*-values obtained from the first order kinetics model applied to the same inactivation data (reported in Chapter 5) are listed in Table 6.2. For spores at high inoculum level, the values of the t_R and the *D*-values were similar (except for SS treatment at 130°C). However, the t_R values obtained for spores at low inoculum level were much lower than the classic *D*-values. For spores at low inoculum level, over-processing could be avoided if the Weibull model was used rather than the classical first order kinetics model to predict processing time needed for one log₁₀ reduction of spores.

From the regression results for the Weibull model it was noted that generally the α parameter had a decreasing trend, and the β parameter had tendency to increase in their value with SS temperature (Table 6.2 and Fig. D.4 in Appendix D). The k₁ and n parameters in the Kamau model also appeared to depend on the SS temperature (Fig. D.5 in Appendix D). However in the case of the Kamau model, the regression analysis was based only on the three available points obtained only for data at one inoculum level, which may not be reliable enough to ensure whether the observed trend was factual or accidental.

The Weibull model fitted all survivor curves for both inoculum levels of *G*. *stearothermophilus* spores treated with SS and was effective at predicting the spore survival

ratios for each SS temperature. Thus, the Weibull model was considered for investigation if survival of spores exposed to SS could be modelled when only SS temperature and processing time were known. Different values of parameters of the Weibull model were obtained for each SS temperature when the model was applied to the experimental data. Therefore, a single unifying equation for the Weibull model was needed. The α and β parameters were expressed as a linear function of SS temperature:

$$\alpha = a_2 - a_1 T \tag{6.7}$$

$$\beta = b_2 - b_1 T \tag{6.8}$$

where, a_1 , a_2 , b_1 , and b_2 are coefficients, and T is SS temperature (°C). These relationships (Eqs. 6.7-6.8) of the model parameters were, therefore, inserted into the original equation of the Weibull model (Eq. 6.2) to obtain an expanded Weibull model equation:

$$\log_{10}\left(\frac{N}{N_{a}}\right) = -\frac{1}{2.303} \left(\frac{t}{(a_{2}-a_{1}T)}\right)^{(b_{2}-b_{1}T)}$$
(6.9)

Non-linear regression of the expanded Weibull model (Eq. 6.9) was performed (Table 6.4) and the survival ratios of *G. stearothermophilus* spores at each inoculum level were predicted based solely on SS temperature and treatment time (Figs. 6.5-6.6).

| Inoculum Level (log ₁₀ cfu/g) | Parameter | Coefficient | Regression Value | RMSE ^a | R ^{2b} |
|---|-----------|----------------|---------------------|-------------------|-----------------|
| 3 | α | aı | 0.0334 | | |
| | | a ₂ | 5.8816 | 0.214 | 0.58 |
| | β | b_1 | 0.00002 | 0.21 | |
| | | b ₂ | 0.3027 | | |
| 6 | α | aı | 0.0189 | 0 472 | 0.76 |
| | | a ₂ | 4.358 | | |
| | β | b_1 | -0.0501 | 0.475 | 0.70 |
| | | b ₂ | -6.853 | | |

Table 6. 4 Regression results for the expanded Weibull model (Eq. 6.9).

^aRMSE – root mean square error (standard error of estimation).

 ${}^{b}R^{2}$ – adjusted coefficient of correlation.



Fig. 6. 5 Comparison of the predicted results based on the expanded Weibull model (Eq. 6.9) and measured values of survival ratio for *G. stearothermophilus* spores at low inoculum level (3 log₁₀ cfu/g) challenged with superheated steam at 130-175°C.

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Fig. 6. 6 Comparison of the predicted results based on the expanded Weibull model (Eq. 6.9) and measured values of survival ratio for *G. stearothermophilus* spores at high inoculum level (6 log₁₀ cfu/g) challenged with superheated steam at 130-175°C.

The residuals of the $\log_{10} \left(\frac{N}{N_o}\right)$ predictions (using the expanded Weibull model) were plotted against treatment time for all SS temperatures at each inoculum level (Figs. D.6-D.7 in Appendix D). An examination of those plots indicated a good fit of the expanded Weibull model (Eq. 6.9) to the experimental data for spores at low inoculum level treated with SS at 130 and 175°C and a slightly poorer fit to the data for spores treated with SS at 145°C (overprediction at processing time <60 min) and 160°C (underprediction at processing time >5 min). The inactivation of spores at high inoculum level treated with SS at 160 and 175°C was described by the expanded Weibull model fairly well (Fig. 6.6). However, an overprediction of the $\log_{10} \left(\frac{N}{N_o}\right)$ was seen for spores treated with SS at 145°C (at processing time <60 min) and underprediction of the survival ratios was seen for spores treated with SS at 130°C (at processing time >5 min).

The results of the validation of the expanded Weibull model obtained for all SS treatments are summarized in Table D.2 (Appendix D). In general, the expanded Weibull model provided more accurate predictions of *G. stearothermophillus* spores survival ratios at low inoculum level than at high inoculum level. Specifically, high values of the R^2 (0.82-0.97) obtained for all SS treatments of spores at low inoculum level indicated accurate predictions of the survival ratios. The inactivation of spores at high inoculum level was accurately predicted for SS treatments at 145 and 160°C (R^2 of 0.82 and 0.99, respectively), less accurately predicted for SS at 175°C (R^2 of 0.70) and inaccurate predictions were obtained for SS at 130°C (R^2 of 0.17) (Table D.2).
6.5 Conclusions

The Weibull model successfully fitted all survivor curves obtained for *G. stearothermophilus* spores treated with SS, whereas the Kamau model did not fit the curves obtained for SS treatment at 175°C at high inoculum level and the curves obtained for SS treatment at 130, 145, and 175°C at low inoculum level. Good fit of the Weibull model to survivor curves and high correlation between the predicted and the experimental values of survival ratio were observed in all SS temperatures at both inoculum levels (with the exception of data for spores at high inoculum level challenged with SS at 130°C).

The Weibull model was expanded by describing its parameters (α , β) as a function of SS temperature for the range of 130-175°C. This allowed accurate predictions of the spore survival ratios based on the SS temperature and processing time only (with the exception of predictions for spores at high inoculum level challenged with SS at 130°C). A further improvement of the Weibull model could be attempted by including the effects of SS velocity on the model parameters.

The Weibull model is recommended to describe and to model inactivation of G. stearothermophilus spores with SS at high temperatures (145-175°C).

7 GENERAL CONCLUSIONS

This research demonstrated that superheated steam can be successfully utilized in oat processing, and that functional properties of oat groats, flour, and bran vary depending on the processing conditions, i.e. temperature, velocity, and processing time used. In addition, the inactivation of highly heat-resistant *Geobacillus stearothermophilus* spores subjected to superheated steam was achieved at certain processing conditions/protocols. Application of the Weibull model, expanded to include mathematical relationships of the model parameters with the temperature of SS treatment, was successfully achieved. The general conclusions of this work are:

Oat groats processed with SS at 110-130°C had acceptable moisture content and appearance, and exhibited cold paste (64°C) viscosity higher than that of groats processed conventionally. The use of SS at 140-160°C further increased cold paste viscosity but also caused very low final moisture content (4-7%, wb) of groats and fast development of rancidity. Groats processed with SS at a velocity of 1.00 m/s exhibited significantly (P<0.05) higher cold paste viscosity than groats processed with SS at 0.35 m/s (i.e. at 140°C and 5 min processing 3538±273 cP and 2836±211 cP, respectively). Processing with SS proved to be effective in the inactivation of peroxidase in groats in which post-processing moisture content was in the desired range of 9-10% (wb).

The optimum conditions for SS processing of oat groats were: temperature of 110°C, a velocity of 1.00 m/s, and two processing times (10 and 14 min). These parameters gave groats with inactivated peroxidase, sensory characteristics closest to the conventionally processed groats and the final moisture content close to 9.5% (wb). Additionally, cold paste viscosity of whole flour slurry prepared from the groats processed with the two optimum SS

conditions was significantly (P<0.05) higher than cold paste viscosity of the conventionally processed groats (5300±60 cP and 5380±126 cP versus 4218±52 cP, respectively).

Compared to conventional (control) heat treatment, the SS processing at 110, 120 and 130° C caused decreased hardness and increased transverse width of oat groats of two cultivars (Furlong and HiFi). Additionally, groat size (large and small) and genotype affected the hardness of differently heat-treated groats, i.e. the large groats of both cultivars were generally softer than their small counterparts, and groats containing less β -glucan (Furlong) were softer than those with higher β -glucan content (HiFi).

Milling yields of flour and bran fractions obtained upon roller milling of groats of both cultivars were not significantly (P>0.05) affected by the different heat treatments applied to those groats (except for HiFi intermediate groats), but the yields differed substantially between the two cultivars (the HiFi groats yielded 10% more bran and concomitantly less flour than the Furlong groats). Both SS and control heat treatments applied to oat groats before milling had similar effects on the colour of flour and bran fractions of each cultivar.

The effects of different heat treatments applied to oat groats before milling on the thermal properties of flour and bran fractions were lesser than the effect of oat genotype. Additionally, groat size (large versus small in each cultivar) did not appear to augment the effects of different heat treatments on the thermal properties exhibited by bran and flour fractions. The pasting properties (i.e. gelatinization of starch) and elasticity of gels made of flour fractions appeared to be similar in differently heat-treated samples of each cultivar, with groat size and genotype having only small effects on these properties. The swelling properties of starch (assessed in cold paste viscosity test), however, appeared to be significantly affected by different heat treatments applied to groats and genotype, but not

the groat size. Compared to the control heat treatment, the SS treatments significantly increased the cold paste viscosity of milling fractions of HiFi and Furlong. In most cases, an increase in the cold paste viscosity was observed with increasing temperature of SS applied to the groats, although the differences were not always significant (P>0.05). The milling fractions of HiFi exhibited much greater cold paste viscosity than those of Furlong (most probably due to the differences in BG content between the two cultivars).

Solubility, molecular weight and apparent viscosity of oat BG varied depending on the type of heat treatment (conventional or SS) used. Compared to conventional heat treatment, the SS treatment at 110°C appeared to have the most beneficial effect on viscosity-building properties of BG (increased molecular weight and apparent viscosity), whereas the SS treatments at 120 and 130°C appeared to cause depolymerization of BG, which lowered their molecular weights but increased their solubility. Additionally, the genotype substantially affected the viscosity-building properties of oat BG. Solubility of BG was lower in flour and bran fractions of Furlong than in flour and bran fractions of HiFi. Also, molecular weights of BG isolated from Furlong bran fractions were lower than those of BG isolated from HiFi bran fractions. Subsequently, the apparent viscosity of aqueous solutions of BG isolated from Furlong bran fractions were lower than those of BG isolated from HiFi bran fractions.

Spores of *Geobacillus stearothermophilus* ATCC 10149 were used in this work to represent heat-resistant microflora often found on oat grain and other foodstuffs. The spore response to SS treatments at 105-175°C depended on inoculum size. A mean *z*-value of 25.4°C was calculated for both inoculum levels for SS treatment temperatures between 130 and 175°C. The spores were inactivated much faster at SS temperatures of 160 and 175°C,

or when SS treatments at 105, 130 and 145°C were interrupted by periods of cooling (intermittent SS treatment). However, it was also found that oat groats processed with SS at high temperatures (\geq 130°C) became rancid after 2-3 weeks of storage at ambient temperature. Superheated steam at 160-175°C could be used to sterilize working surfaces or food products that are less sensitive to heat than oats (i.e. with lesser content of lipids than oats).

Tailing was observed in survivor curves of *G. stearothermophilus* spores exposed to SS. Therefore, non-linear regression models (the Weibull and Kamau models) were used to describe the survivor curves. The Weibull model successfully fitted all survivor curves obtained for *G. stearothermophilus* spores treated with SS, whereas the Kamau model did not fit the curves obtained for SS treatment at 175°C at high inoculum level and the curves obtained for SS treatment at 130, 145, and 175°C at low inoculum level. When the Weibull model was used, a high correlation (R^2 of 0.93 or better) was observed between the experimental and the predicted values of the survival ratio for all SS temperatures at both inoculum levels (with the exception of data for spores at high inoculum level challenged with SS at 130°C where R^2 was 0.7).

The Weibull model was expanded by describing its parameters (α , β) as a function of SS temperature for the range of 130-175°C. This allowed accurate predictions of the spore survival ratios based on the SS temperature and processing time only (with the exception of predictions for spores at high inoculum level challenged with SS at 130°C). The Weibull model is recommended to describe and to model the survival ratio of *G. stearothermophilus* spores challenged with SS at high temperatures (145-175°C).

8 RECOMMENDATIONS FOR FUTURE RESEARCH

The results of this research indicated that processing of oat groats with SS at temperatures higher than 110° C caused partial depolymerization of β -glucans. It would be beneficial to elucidate whether oat starch was also partially depolymerized due to exposure to SS. Moreover, the effects of SS processing on the physicochemical properties of oat β -glucans and starch could differ for oats of the same origin (cultivar) but grown in different environments and years, and should be assessed.

The exact mechanism of fracture formation in oat groats due to exposure to SS needs to be determined. Additionally, the effect of SS processing on the structure of cell walls throughout oat groat should be determined, as it would possibly help to explain the reasons of the greater degree of depolymerization of β -glucans with the increase of SS temperature. Also, knowledge of the effects of SS processing on retention of vitamins and antioxidant activities in oat products would be useful.

It was found that oat groats processed for 10 or 14 min with SS at 110°C and a velocity of 1.00 m/s (the optimum temperature and velocity of SS) were shelf stable and exhibited desired functional properties (i.e. higher viscosity of cold paste and brighter colour than the conventionally processed groats). The optimum temperature and velocity of SS were those at the extreme experimental conditions tested in this research. It would be beneficial to determine the effects of SS at temperature below 110°C and velocity above 1.00 m/s on the functional properties of oat groats.

The finding of increased lethality of *Geobacillus stearothermophilus* spores challenged with SS at 105°C compared to the lethalities at 130 and 145°C (inoculum of $3 \log_{10} \text{cfu/g}$) indicated that formation of condensate at the lower temperature of SS rendered the spores

more thermally sensitive. Therefore, a further investigation of the sporicidal effect of SS when moisture is added (e.g. by modifying pressure of the SS) should be carried out. Additionally, the sporicidal effect of SS could be altered if the spores were inoculated onto a biological material such as oat groats. Thus, the sporicidal effect of SS should be determined when spores are present on oat groats.

In this work the SS velocity of 0.35 m/s was used for treatment of spores, whereas the velocity of 1.00 m/s was found to be optimum for SS processing of oat groats. Therefore, the sporicidal effect of SS at 1.00 m/s (perhaps combined with the intermittent treatment) should also be determined. Also, the use of SS velocities other than 0.35 m/s for treatment of *G. stearothermophilus* spores at SS temperatures of 145-175°C could help in further improvement of the expanded Weibull model.

More research is needed to elucidate the physiological changes occurring in bacterial spores exposed to SS, which would help in understanding why tailing was observed in most survivor curves of *G. stearothermophilus* challenged with SS.

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APPENDIX A

| | ~~ | | | |
|-----------------|--------------------------|------------------|-----------------|---------------------------------|
| Tempering | SS | SS | \mathbf{SS} | Viscosity |
| Method | Temperature | Velocity | Processing Time | (oP) |
| | (°C) | (m/s) | (min) | (01) |
| | | | | |
| | | <u>2004 Crop</u> | | |
| Water | 130 | 0.35 | 5 | 4393 ± 64 (n ^c =2) |
| Water | 130 | 0.35 | 7.5 | 4094 ± 17 (n=2) |
| Water | 130 | 0.35 | 10 | $5317 \pm 102 (n=2)$ |
| Water | 125 | 0.35 | 5 | 4230 ± 71 (n=2) |
| Water | 125 | 0.35 | 7.5 | 4367 ± 34 (n=2) |
| Water | 125 | 0.35 | 10 | 4221 ± 15 (n=2) |
| Water | 120 | 0.35 | 5 | 4122 ± 26 (n=2) |
| Water | 120 | 0.35 | 7.5 | 4356 ± 71 (n=2) |
| Water | 120 | 0.35 | 10 | $4198 \pm 10 (n=2)$ |
| Water | 115 | 0.35 | 7.5 | $4199 \pm 9 (n=2)$ |
| Water | 115 | 0.35 | 10 | $3963 \pm 18 (n=2)$ |
| Water | 110 | 0.35 | 5 | $3885\pm64 (n=2)$ |
| Water | 110 | 0.35 | 7.5 | $4406\pm84 (n=2)$ |
| Water | 110 | 0.35 | 10 | 4034 ± 57 (n=2) |
| Water | 110 | 0.35 | 15 | 4331 ± 49 (n=2) |
| Water | 110 | 0.35 | 20 | 4170 ± 141 (n=2) |
| Water | 110 | 0.35 | 30 | 4190 ± 21 (n=2) |
| Water | 150 | 1.00 | 2.5 | 3670 ± 32 (n=4) |
| Water | 140 | 1.00 | 5 | 3979 ± 232 (n=4) |
| Water | 140 | 0.35 | 5 | 3680 ± 36 (n=4) |
| Water | 115 | 1.00 | 5 | 3538±273 (n=6) |
| Water | 115 | 0.35 | 5 | $2836\pm211 (n=6)$ |
| Water | 115 | 0.35 | 2.5 | 3423 ± 222 (n=6) |
| Saturated Steam | Unprocessed ^d | - | - | 4691 ± 124 (n=4) |
| Water | Unprocessed | - | - | 448±23 (n=2) |
| - | 160 | 1.00 | 2.5 | 5069±339 (n=4) |
| - | 160 | 1.00 | 5 | $3833\pm267 (n=4)$ |
| | 140 | 1.00 | 2.5 | 5598 ± 684 (n=4) |
| - | 140 | 1.00 | 5 | 5212 ± 147 (n=4) |
| - | 150 | 1.00 | 2.5 | 5309 ± 118 (n=4) |
| - | 150 | 1.00 | 5 | 4533 ± 153 (n=4) |
| Water | 150 | 1.00 | 5 | 3633±292 (n=4) |
| Saturated Steam | 140 | 0.35 | 5 | 3624 ± 202 (n=4) |
| Water | Unprocessed | - | - | 516±32 (n=2) |

Table A. 1 Cold paste viscosity^a (64°C) of oat flour slurry obtained by grinding of raw or moisture tempered^b oat groats processed in superheated steam at various temperatures, velocities and processing times.

| | · | | | | |
|---|-------|-------------|------------------|------|--------------------|
| | - | 150 | 1.00 | 5 | 4596±68 (n=4) |
| | - | Unprocessed | - | - | 466±12 (n=2) |
| W | 'ater | 120 | 1.00 | 2.5 | 2546±33 (n=2) |
| W | 'ater | 130 | 1.00 | 2.5 | 2736±62 (n=2) |
| W | 'ater | 140 | 1.00 | 1 | 3067±16 (n=2) |
| W | ater | Unprocessed | - | - | 626±20 (n=2) |
| | - | 115 | 0.35 | 5 | 3275 ± 29 (n=4) |
| | - | 115 | 0.35 | 5 | 2972 ± 369 (n=4) |
| | | | then 1.00 | 2.5 | |
| | - | 115 | 0.35 | 5 | 3299±69 (n=3) |
| | | | then 1.00 | 2.5 | |
| | - | Unprocessed | - | - | 458±57 (n=2) |
| | | | <u>2005 Crop</u> | | |
| | - | Unprocessed | - | - | 851±25 (n=2) |
| | - | 130 | 0.35 | 5 | 5256±143 (n=4) |
| | | | then 1.00 | 2.5 | |
| | - | 120 | 0.35 | 5 | 5102±133 (n=4) |
| | | | then 1.00 | 2.5 | |
| | - | 115 | 0.35 | 5 | 5064±75 (n=4) |
| | | | then 1.00 | 2.5 | |
| | - | Unprocessed | - | - | 1086±49 (n=2) |
| | - | 110 | 1.00 | 10 | 5300±60 (n=4) |
| | - | 110 | 1.00 | 14 | 5380±126 (n=4) |
| | - | 115 | 0.35 | 5 | 5286 ± 69 (n=4) |
| | | | then 1.00 | 2.25 | |
| | | | | | |

^aViscosity of flour slurry obtained from the cold paste (64°C) test at the 20th min (the end) of test. ^bTempering with water or saturated steam up to 14% (wb). ^cn = number of replicates.

| | | _ |
|---|---|--|
| Groats Obtained From | Date | Viscosity (cP) |
| | <u>2004 Crop</u> | |
| Plant B: Bottom ^b of Kiln Plant A: Bottom of Kiln Plant A: WOG ^d , Plant A: WOG Plant B: WOG Plant A: Bottom of Kiln Plant A: Top ^e of Kiln Plant A: Bottom of Kiln | May 26, 2005 May 26, 2005 August 3, 2005 September 1, 2005 September 3, 2005 September 3, 2005 September 19, 2005 September 21, 2005 | $\begin{array}{c} 4116 \pm 35 \ (n^{c} = 2) \\ 4140 \pm 52 \ (n = 2) \\ 3174 \pm 21 \ (n = 2) \\ 3695 \pm 98 \ (n = 2) \\ 3965 \pm 14 \ (n = 2) \\ 3628 \pm 23 \ (n = 2) \\ 458 \pm 57 \ (n = 2) \\ 4469 \pm 91 \ (n = 2) \end{array}$ |
| Mi | <u>x of 2004 and 2005 Crop</u> | |
| Plant A: Top of Kiln | September 21, 2005 | 806±1 (n=2) |
| | <u>2005 Crop</u> | |
| Plant A: Top of Kiln Plant A: Bottom of Kiln Plant A: Bottom of Kiln | September 28, 2005 October 13, 2005 November 18, 2005 | 851±25 (n=2) 4244±26 (n=2) 4218±52 (n=2) |
| ^a Viscosity of flour slurry obtained from the ^b ·Bottom of kiln' indicates groats after kil ^c n = number of replicates. ^d WOG = whole oat groats. | te cold paste (64°C) test at the 20 th min (th n processing. | e end) of test. |

Table A. 2 Cold paste viscosity^a (64°C) of oat flour slurry obtained by grinding of oatgroats processed in kiln at two processing plants (A and B) on different days.

"Top of kiln' indicates groats before kiln processing (raw).

| SS ^a Temperature (°C) | SS Velocity (m/s) | Processing Time (min) | Final Moisture Content (%, wb) | Tempering ^b (to 14%, wb) | Peroxidase Activity |
|--|----------------------|--------------------------|--------------------------------------|--|------------------------|
| - | - | Unprocessed | 12.5 | No | + |
| - | - | Unprocessed | 14.0 | Yes | + |
| 110 | 1.00 | 10 | 9.4 | No | |
| 110 | 1.00 | 14 | 9.4 | No | <u> </u> |
| 115 | 1.00 | 5 | 10.3 | Yes | _ |
| 120 | 1.00 | 2.5 | 10.7 | Yes | + |
| 130 | 1.00 | 2.25 | 9.1 | Yes | _/+ |
| 140 | 1.00 | 2.5 | 7.1 | No | |
| 140 | 1.00 | 5 | 5.9 | No | |
| 140 | 1.00 | 1 | 10.3 | Yes | _ |
| 140 | 1.00 | 5 | 6.1 | Yes | |
| 150 | 1.00 | 2.5 | 5.5 | No | _ |
| 150 | 1.00 | 5 | 4.6 | No | |
| 160 | 1.00 | 2.5 | 5.0 | No | _ |
| 160 | 1.00 | 5 | 4.0 | No | _ |
| 115° | 0.35 | 5 | | 110 | |
| | then 1.00 | 2.25 | 9.6 | No | |
| 115° | 0.35 | 5 | 2.0 | 110 | |
| | then 1.00 | 2.5 | 96 | No | _ |
| 120° | 0.35 | 5 | 2.0 | 110 | |
| | then 1.00 | 2.5 | 8.8 | No | |
| 130° | 0.35 | 5 | 0.0 | 110 | — |
| | then 1.00 | 2.5 | 7.8 | No | **** |

Table A. 3 Final moisture content and peroxidase activity of oat groats following processing with superheated steam at various temperatures, velocities, and processing times.

^aSS – superheated steam. ^bTempering with water. ^cTwo-stage process, i.e. groats were processed with SS at 0.35 m/s for 5 min and then with SS at 1.00 m/s for 2.25 or 2.5 min.

| | | | | | | We | ek | | | | | · · · · · · · · · · · · · · · · · · · |
|------------------|--------------|---|---|---|---|----|----|----|----|----|----|---------------------------------------|
| Test | Start (0) | 2 | 4 | 8 | 9 | 12 | 13 | 16 | 17 | 20 | 24 | 26 |
| Sensory | x | x | x | | x | | х | x | | x | x | |
| RVA ^a | x | x | x | | x | | х | | х | x | | х |
| Moisture | x | x | x | | x | | х | | х | x | | х |
| Hexanal | х | x | x | x | | | | | | | х | |
| FFA ^b | х | x | x | x | | | | | | | x | |
| Colour | x | | х | X | | х | | x | | x | x | |

Table A. 4 Schedule of testing for oat groats stored at 21°C.

^aRVA = Rapid Visco Analysis.

 b FFA = Free Fatty Acids.

| Table A. 5 | Schedule | of testing | for oat | groats | stored at 38°C. |
|------------|----------|-------------|---------|--------|-----------------|
| ~ | ~~~~~~~ | 01 00000000 | 101 000 | D. 0 | |

| | | | | | | ۰ | Week | | | | | |
|------------------|--------------|---|---|---|---|----|------|---|---|----|----|----|
| Test | Start (0) | 1 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 12 | 13 |
| Sensory | x | x | x | х | х | ·x | х | | | х | х | х |
| RVA ^a | x | x | х | | х | | | х | | | х | |
| Moisture | x | x | х | | x | | | х | | | x | |
| Hexanal | x | x | x | | x | | | х | | | х | |
| FFA ^b | x | x | x | | x | | | х | | | х | |
| Colour | x | | | x | | x | x | x | x | x | х | |

^aRVA = Rapid Visco Analysis.

^bFFA = Free Fatty Acids.

Table A. 6 Coefficients of correlation (R2) between sensory scores for rancid flavoura and amounts of hexanal released from oat groats stored at 21 and 38oC for ~170 days.Schedule of testing for oat groats stored at 38°C.

| Oat | Drocosing | SS^{b} | SS | SS | 1 | 2 ² | | |
|---------|------------|-------------|----------|------------|--------------------------|----------------|--|--|
| Groat | Processing | Temperature | Velocity | Processing | Storage Temperature (°C) | | | |
| Sample | Wiethou | (°C) | (m/s) | Time (min) | 21 | 38 | | |
| Plant A | Commercial | - | - | - | LC° | 0.63 | | |
| Plant B | Commercial | - | - | - | 0.45 | 0.65 | | |
| SS-10 | With SS | 110 | 1.00 | 10 | LC | -0.21 | | |
| SS-14 | With SS | 110 | 1.00 | 14 | LC | 0.26 | | |

^aWith the exclusion of data obtained on the 17th day of storage at both temperatures.

^bSS – superheated steam.

^cLC - low correlation ($R^2 < 0.2$).

APPENDIX B

Formulas used for calculation of the amount of flour and water for preparation of gel discs (40% solids):

S = ((100 - 10%) * 4 g) / (100 - MC)W = 6 g + (4 g - S)

where: S is the corrected weight of flour (g), W is the corrected amount of water (mL), MC is the actual moisture content of the flour (%).

Formulas used for calculation of the amount of flour, bran or whole meal and water for preparation of slurries:

S = ((100 - 14%) * X) / (100 - MC)W = 25 + (X - S)

where:

S, W and MC are as above,

X is the weight of a sample before correction for MC:

3.5 g of flour (for general pasting test)

6 g of flour, bran or whole meal (for cold paste test).



Α

B

Fig. B. 1 Cumulative particle size distribution of bran (A) and flour (B) obtained by roller milling of HiFi plump groats. Control heat treatment – oat groats wet steamed and oven dried; SS-110, 120, 130 – groats processed with superheated steam (SS) at 110, 120, and 130°C, respectively.

Cumulative Distribution (Undersize %) Bran Furlong, plump **Control Heat-Treated** SS-110 SS-120 SS-130 0 0.1 1 10 100 1000 10000 Particle Diameter (µm) 100 Flour Cumulative Distribution (Undersize %) Furlong, plump 0 0.1 1 10 100 1000 10000 Particle Diameter (µm)

Α

B

Fig. B. 2 Cumulative particle size distribution of bran (A) and flour (B) obtained by roller milling of Furlong plump groats. Control heat treatment – oat groats wet steamed and oven dried; SS-110, 120, 130 – groats processed with superheated steam (SS) at 110, 120, and 130°C, respectively.



A

B

Fig. B. 3 Cumulative particle size distribution of bran (A) and flour (B) obtained by roller milling of Furlong intermediate groats. Control heat treatment – oat groats wet steamed and oven dried; SS-110, 120, 130 – groats processed with superheated steam (SS) at 110, 120, and 130°C, respectively.

| Table B. 1 Thermal prope | rties of raw oat groats ^a . |
|---------------------------------|--|
|---------------------------------|--|

| Cultivor Sizo | | Starch Gela | atinization | | Melting of Amylose-Lipid Complex | | | |
|-----------------|---------------------------------------|-------------------------|---------------------------------------|----------------------|---------------------------------------|---------------------|---------------------------------------|----------------------|
| Cultival, Size | $T_{\rm o} (^{\rm o}{\rm C})^{\rm b}$ | $T_{p} (^{\circ}C)^{b}$ | $T_{\rm c} (^{\rm o}{\rm C})^{\rm b}$ | $\Delta H (J/g)^{c}$ | $T_{\rm o} (^{\rm o}{\rm C})^{\rm b}$ | $T_{p} (^{o}C)^{b}$ | $T_{\rm c} (^{\rm o}{\rm C})^{\rm b}$ | $\Delta H (J/g)^{c}$ |
| Groat - Furlong | | | | | | | | |
| plump | 49.5±0.0a | 57.9±0.2a | 71.3±0.9a | 11.3±0.7a | 77.9±0.5a | 91.2±0.1b | 101.4±0.3a | 2.7±0.1a |
| intermediate | 49.5±0.1a | 58.6±0.2a | 71.4±0.9a | 10.6±0.3a | 77.0±1.0a | 90.5±0.2a | 101.5±0.2a | 2.8±0.2a |
| Groat - HiFi | | | | | | | | |
| plump | 51.9±0.6a | 60.4±0.5a | 73.1±0.9a | 12.4±0.4a | 77.9±1.8a | 92.3±1.4a | 101.5±1.3a | 2.14±0.5a |
| intermediate | 52.6±0.2a | 61.3±0.2a | 73.6±0.1a | 12.6±0.3a | 79.6±0.1a | 91.2±0.5a | 101.2±0.0a | 2.14±0.1a |

^a25:75 ratio of whole meal to water; values are means of 2 replicates; values in the same column, within each group, followed by the same letter do not differ significantly (P>0.05). ^b T_{o} , T_{p} and T_{c} indicate the temperature of onset, peak and conclusion of each phase transition, respectively. ^c ΔH indicates enthalpy of each phase transition and is expressed on the basis of starch content (% db).



Fig. B. 4 Differential scanning calorimetry thermograms of flour (A) and bran (B) obtained by milling of heat-treated HiFi intermediate groats. Control heat treatment – oat groats wet steamed and oven dried; SS-110, 120, 130 – groats processed with superheated steam (SS) at 110, 120, and 130°C, respectively.



Fig. B. 5 Differential scanning calorimetry thermograms of flour (A) and bran (B) obtained by milling of heat-treated HiFi plump groats. Control heat treatment – oat groats wet steamed and oven dried; SS-110, 120, 130 – groats processed with superheated steam (SS) at 110, 120, and 130°C, respectively.



Fig. B. 6 Differential scanning calorimetry thermograms of flour (A) and bran (B) obtained by milling of heat-treated Furlong plump groats. Control heat treatment – oat groats wet steamed and oven dried; SS-110, 120, 130 – groats processed with superheated steam (SS) at 110, 120, and 130°C, respectively.

| Cultivor Size / | Viscosity a | t 5 th minute | Viscosity at | t 10 th minute | Viscosity at 15 th minute | | |
|-----------------------------------|-------------|--------------------------|--------------|---------------------------|--------------------------------------|-----------|--|
| Treatment | (c) | P) | (c | P) | (| (cP) | |
| | Bran | Flour | Bran | Flour | Bran | Flour | |
| Furlong, plump | | | | | | | |
| Control Heat-Treated ^b | 686±32a | 300±16a | 3504±97a | 2013±5a | 4497a | 2339±23a | |
| $SS-110^{\circ}$ | 848±19b | 373±13b | 4318±87b | 2433±82b | 5489Ъ | 2826±81b | |
| SS-120 | 814±23b | 365±16b | 4365±30b | 2295±16b | 5627b | 2689±13b | |
| SS-130 | 899±28b | 371±1b | 4625±42c | 2285±71b | 5921c | 2740±88b | |
| Furlong, intermediate | | | | | | | |
| Control Heat-Treated | 708±18a | 288±5a | 3543±50a | 1893±21a | 4511a | 2180±23a | |
| SS-110 | 843±16b | 360±6b | 4153±36b | 2233±47b | 5348b | 2591±54b | |
| SS-120 | 836±14b | 352±23b | 4397±25c | 2187±22b | 5649c | 2561±24b | |
| SS-130 | 915±26c | 359±13b | 4683±90d | 2254±36b | 5971d | 2651±44b | |
| HiFi, plump | | | | | | | |
| Control Heat-Treated | 2455±17a | 218±10a | 13470±202a | 3034±35a | 15560a | 3510±30a | |
| SS-110 | 2358±10b | 242±9a | 14664±94b | 3272±30b | 16674b | 3811±28b | |
| SS-120 | 2528±40c | 260±12a | 14838±291b | 3329±36b | 16827b | 3942±39c | |
| SS-130 | 2707±27d | 263±7a | 15252±186b | 3395±24b | 17126b | 4096±0d | |
| HiFi, intermediate | | | | | | | |
| Control Heat-Treated | 2210±36a | 175±1a | 12996±3a | 2961±52a | 15380a | 3458±70a | |
| SS-110 | 2087±23a | 183±4ab | 14381±23b | 3235±62a | 16518b | 3771±73a | |
| SS-120 | 2111±74a | 193±6ab | 14253±149b | 3125±36a | 16298ab | 3694±24a | |
| SS-130 | 2125±45a | 211±10b | 14461±100b | 3186±136a | 16295ab | 3765±152a | |

Table B. 2 Cold paste (64°C) viscosity^a of oat bran and flour at the 5th, 10th, and 15th minute of the cold paste test.

^aValues are means of 2 replicates; values in the same column, within each group, followed by the same letter do not differ significantly (P>0.05). ^bControl heat treatment – oat groats wet steamed and oven dried ^cSS-110, 120, 130 – groats processed with superheated steam (SS) at 110, 120 and 130°C, respectively.



Fig. B. 7 Mechanical spectra of gels (40:60, w/w) made from Furlong (A) and HiFi (B) flour obtained by milling of heat-treated intermediate groats. Control heat treatment – oat groats wet steamed and oven dried; SS-110, 120, 130 – groats processed with superheated steam (SS) at 110, 120, and 130°C, respectively.



Fig. B. 8 HPSEC-MALS elution patterns obtained for β-glucans isolated from HiFi (A) and Furlong (B) bran. Control heat treatment – oat groats wet steamed and oven dried; SS-110, 120, 130 – groats processed with superheated steam (SS) at 110, 120, and 130°C, respectively.

APPENDIX C

Appendix C. 1 Production of *G. stearothermophilus* spores

G. stearothermophilus spores were produced according to the method of Kim and Naylor (1966). A loopful of slant culture was streaked onto TSA and incubated at 55°C for 24-36 h. Isolated colonies were inoculated into sterile test tubes containing tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, USA) and the test tubes were incubated at 55°C for 24 h. Resultant broth cultures (5 mL) were inoculated into sterile, 600 mL (150 cm²) plastic cell culture flasks with canted necks (model # 430823 Corning Inc., Corning, NY, USA) containing 150 mL of sporulation medium which consisted of: 0.8% (w/v) nutrient broth (Difco), 0.4% (w/v) yeast extract (Difco), 10 mg/kg MnCl₂·4H₂O, 2% agar (Difco) and was adjusted to pH 7.2 using 1N NaOH. The inoculated flasks were incubated at 55°C for up to 7 days and spore development was assessed using phase contrast optics (1000 x) (MC-100 photomicroscope, Carl Zeiss, Thornwood, NY, USA) at 24 h intervals. Resultant spore crops, harvested from the agar surfaces with the aid of sterile glass beads and 10-15 mL of 4°C sterile distilled water (SDW), were pooled into 50 mL sterile centrifuge tubes. The spore suspensions were centrifuged at 1°C for 1 h at 2000 \times g using a Sorvall RC2-B centrifuge (Sorvall, Du Pont Co., Wilmington, DE, USA). Supernatants were discarded and pellets were washed with 150-200 mL of SDW, and re-suspended in 4°C SDW to their original volume. A 1% (w/v) lysozyme solution (Inovapure[™] 300, Canadian Inovatech, Abbotsford, BC, Canada) was added to the re-suspended spore pellets at a ratio of 1:100 (v/v). Following vigorous mixing, overnight incubation (4°C) and centrifugation (1 h, 2000 \times g, 1°C), the spore pellets were washed with 150-200 mL 4°C SDW. The lysozyme treatment, centrifugation, and washing steps were repeated at least 3

times in order to ensure liberation of endospores, lysis of vegetative cells and removal of cellular debris. The purity of the spore suspension was checked microscopically. Spore pellets were finally re-suspended in 4°C SDW to their original volume, frozen in liquid nitrogen and freeze-dried using a Viritis 10-146MP-BA vacuum freezer (Viritis Inc., Gardiner, NY, USA).

APPENDIX D

Table D. 1 Validation of the Weibull and Kamau models (Eqs. 6.2 and 6.6, respectively) –results of linear regression of the experimental and predicted values of survivalratios of G. stearothermophilus spores challenged with superheated steam.

| SS ^a Temperature (°C) | Inoculum Level (log ₁₀ cfu/g) | Model | Slope | R^{2b} |
|-------------------------------------|---|---------|----------------|----------|
| | 2 | Weibull | 1.05 | 0.96 |
| 130 | 5 | Kamau | _ ^c | - |
| 150 | 6 | Weibull | 0.84 | 0.74 |
| | 0 | Kamau | 0.82 | 0.76 |
| 145 | 2 | Weibull | 0.75 | 0.86 |
| | | Kamau | - | - |
| | 6 | Weibull | 1.12 | 0.97 |
| | 0 | Kamau | 1.09 | 0.97 |
| | 3 | Weibull | 1.10 | ≈1.00 |
| 160 | 2 | Kamau | 1.11 | 0.99 |
| 100 | 6 | Weibull | 0.99 | 1.00 |
| | 0 | Kamau | 1.00 | 1.00 |
| | 3 | Weibull | 1.00 | 0.96 |
| 175 | 5 | Kamau | - | - |
| 175 | 6 | Weibull | 1.10 | 0.96 |
| | 0 | Kamau | - | _ |

^aSS – superheated steam.

 ${}^{b}R^{2}$ – correlation coefficient of the regression line.

^c Model did not fit the experimental results.



Processing Time (min)





Fig. D. 2 Residuals of survival ratios obtained for the Weibull model (Eq. 6.2) predictions at high inoculum level ($6 \log_{10} \text{cfu/g}$).



▲ 130°C High Inoculum × 145°C High Inoculum 0 160°C High Inoculum ■ 160°C Low Inoculum

Fig. D. 3 Residuals of survival ratios obtained for the Kamau model (Eq. 6.6) predictions at low and high inoculum levels (3 and 6 log₁₀ cfu/g, respectively).



Fig. D. 4 Effects of superheated steam temperature on α and β parameters in the Weibull model (Eq. 6.2).



Fig. D. 5 Effects of superheated steam temperature on k₁, k₂, and n parameters in the Kamau model (Eq. 6.6).



Processing Time (min)

Fig. D. 6 Residuals of survival ratios obtained for the expanded Weibull model (Eq. 6.9) predictions at low inoculum level ($3 \log_{10} \text{ cfu/g}$).



Fig. D. 7 Residuals of survival ratios obtained for the expanded Weibull model (Eq. 6.9) predictions at high inoculum level ($6 \log_{10} \text{cfu/g}$).
| SS ^a Temperature (°C) | Inoculum Level (log ₁₀ cfu/g) | Slope | R^{2b} |
|-------------------------------------|---|-------|----------|
| 130 | 3 | 0.93 | 0.97 |
| | 6 | 0.15 | 0.17 |
| 145 | 3 | 0.78 | 0.82 |
| | 6 | 0.82 | 0.82 |
| 160 | 3 | 0.78 | 0.97 |
| | 6 | 0.93 | ≈1.00 |
| 175 | 3 | 1.09 | 0.97 |
| | 6 | 1.28 | 0.70 |

Table D. 2 Validation of the expanded Weibull model (Eq. 6.9) – results of linear regression of the experimental and predicted values of survival ratios of G. stearothermophilus spores challenged with superheated steam.

^aSS – superheated steam. ^b R^2 – correlation coefficient of the regression line.