THE EFFECTS OF SUPERHEATED STEAM PROCESSING CONDITIONS ON THE FUNCTIONALITY AND MICROFLORA OF OAT FLOUR INTENDED FOR USE IN INFANT FOOD FORMULATIONS

By

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A Thesis Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the Requirements of the Degree of

MASTER OF SCIENCE

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FACULTY OF GRADUATE STUDIES

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ABSTRACT

Superheated steam (SS) processing was evaluated as an alternative method for the heat treatment of oat groats (*Avena sativa*) particularly with respect to its ability to inactivate bacterial spores. The objectives of this research were to access the efficiency of a commercial kiln drying process to reduce the bioload of oat groats; to evaluate temperature (115°C, 121°C, 130°C, 145°C 160°C and 175°C), moisture (12 and 17%), inoculum level (10^4 and 10^7 cfu/g) and steam velocity (0.52 and 1.10m/s) with respect to SS performance and reduction of thermophilic spores in oat groats, and to examine the functional and chemical properties of oat flour after SS treatment. Of the 30 oat groat samples analyzed following kiln treatment, 26 (87%) contained thermophilic and flat sour spores. In contrast, only 11(37 %) of the 30 samples taken before kiln treatment contained spores. Overall, samples taken from the interior of the kiln at the cooling sections appeared to contain the highest numbers of thermophilic and flat sour spores.

Survivor curves for *Geobacillus stearothermophilus* ATCC 10149 spores in groats were plotted and *D* values were calculated. Increasing the temperature of SS from 115°C to 175°C resulted in a progressive increase in spore lethality. Temperatures of 160 and 175°C effectively reduced spore levels by approximately 5 $\log_{10}$ cfu/g, however, the groats were deemed unfit for further use based on color. At 130 and 145°C, maximum decreases in spore populations (usually less than 2 log) were observed at 5- 10 min of treatment; thereafter all survivor curves exhibited distinct tailing. Raising the superheated steam velocity from 0.52 to 1.10m/s had a minimal effect on spore lethality.

Also, tempering groats from 12 to 17% moisture content did not appear to improve the sporicidal effect.

The color change of oat groats following SS was most noticeable in samples treated at 160°C and 175°C; in this respect the groats appeared dark and exhibited a burnt appearance and odor. In all cases, neither an increase in the treatment times nor the flow rate had any noticeable effect on the pasting profile. Superheated steam processing successfully stabilized degradative enzymes, producing peroxidase negative samples at temperatures above 115°C. Under the processing conditions examined in this study, superheated steam processing (compared to conventional thermal treatment) was of limited value for the reduction of *Geobacillus stearothermophilus* spore viability. Further investigations of other steam velocities and temperature profiles may increase spore lethality and also produce oat groats with improved sensory and functional properties.

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1 INTRODUCTION

Cultivation of oats (*Avena sativa*, L) in Canada has a considerable economic influence on the agricultural industry. In particular, oat production in Manitoba has increased by approximately 122 % to 979,000 tonnes in 2006 compared to 440,700 tonnes in 2005 (Manitoba Agriculture, Food and Rural Initiatives, 2006).

Although oats have long been recognized as a highly nutritious food containing high amounts of protein and has traditionally been used as a food source for animals and humans (Webster, 2003) the increase in oat production is no doubt linked to various reported health benefits. For example, inclusion of oats in the human diet has been purported to lower blood cholesterol, regulate blood glucose and protect against colon tumor development (Moisture content Donald et al., 1992; Webster, 2003).

Conventional oat processing involves kiln drying, which is a critical heat treatment step necessary for both flavor (sensory properties) development and inactivation of fat-hydrolyzing enzymes which would otherwise contribute to oxidative rancidity. In addition, beneficial changes in the functional and nutritional qualities of oat products are enhanced (Dendy and Dobraszczyk, 2001; Webster, 2003). Once heat treated, the oats are milled into various products including cut groats, flakes and flour which are then supplied for human consumption: Oat flour, which is one of the major products produced, is used in a wide variety of foods including granola bars, hot cereals, extruded cereals, cookies, muffins, breads, meat extenders, beverages and baby food (Webster, 2003). In particular, the use of oat products in baby foods and formulations represents a lucrative market. However, quality control specifications by baby food

manufacturers including Gerber set limits on the number of total thermophilic and flat sour spores .Unfortunately, the temperatures employed during kiln drying rarely go above 100°C for any length of time (primarily to avoid total destruction of vitamins including thiamin or B_1). In order to supply oat products conforming to the microbiological specifications of baby food manufacturers, oat processors must re-evaluate their current processing methods and investigate alternative or emerging technologies.

Geobacillus (*Bacillus*) *stearothermophilus* is a thermophilic bacterium which produces one of the most heat resistant spores (Watanabe et al., 2003). This organism is found on or within plant food products including oats and is responsible for flat sour spoilage of low-acid (LA) canned foods. Presently, retorting using moist heat at 121°C or higher is the most common method used for spore destruction in LA canned foods (Russell, 1982). Unfortunately the relatively high temperature, moist environment and extended times can lead to a reduction in the nutritive value, color and taste of processed foods (Brown, 1994; Watanabe et al., 2003). Furthermore, this method is not applicable to all food products, particularly powders or flour based ingredients. Therefore alternative process (es) must be investigated for the destruction of heat resistant organisms which would otherwise reduce product quality.

Dry heat in the form of superheated steam (SS), which is steam having a temperature above the saturation temperature or boiling point is now being investigated as a replacement for hot air treatment traditionally used for oat drying. When water is heated at a given pressure and reaches its boiling point, it is referred to as saturated steam. If heated beyond the boiling point at the given pressure, the steam becomes

unsaturated or superheated. During processing the SS transfers some of its heat to the product and removes moisture via evaporation (van Deventer and Heijmans, 2001). There are several advantages for the use SS treatment of oats. An important one is cost-effectiveness. Since SS operates in a closed system, the exhaust steam can be collected, condensed and used in other parts of the system. Air pollution can also be reduced by collection of the condensate following separation and draining (van Deventer and Heijmans, 2001; Kudra and Mujumdar, 2002). SS drying produces an oxygen free environment, which can reduce product damage such as browning reactions and scorching. In addition, it may have the ability to inactivate thermophilic microorganisms because of its high heat capacity (Devahastin and Suvarnakuta, 2004).

One other major advantage for the application of SS to oat groats is the effect on oat starch granules. When starch is heated in the presence of water, gelatinization occurs. This event is marked by swelling of the granules and a rapid uptake of water leading to an in increase in viscosity (Kent and Evers, 1994). Since starch is important in modifying the textural qualities in many foods, it is possible that under appropriate operating conditions, SS can be manipulated to produce modified viscosities in oat flour preparations. Overall, optimizing SS processing conditions could be used to produce oat groats with low spore counts and improved physical and chemical properties especially useful in the manufacture of baby food formulations.

The objectives of the current research were three fold. The first objective was to assess the efficiency of a kiln drying process used in an oat mill plant with respect to microbiological decontamination. Secondly, an evaluation of SS treatment was performed with respect to the decontamination of oat groats challenged with *Geobacillus*

stearothermophilus spores. Finally, enzyme activity, color and functionality of oat groats following treatment with SS were analyzed in order to assess oat quality.

2 LITERATURE REVIEW

2.1 OATS

2.1.1 Nutritional composition

Among whole-grains, oats (*Avena sativa*) have numerous active components that easily make it one of the most nutritious cereals. It has the highest protein content (between 11-20%) and also an essential amino acid profile (Webster, 2003) that exceeds most other cereals. The lipid portion of oats comprises 5-9 % of the entire oat groat; it also possesses a favorable ratio of monounsaturated and polyunsaturated to saturated fatty acids, which increases its appeal in cholesterol-lowering diets (Shrickel, 1986; Webster, 2003). Numerous studies (Anderson et al., 1990, 1991, 1993; Klopfenstein, 1988; Woods et al., 1994; Davidson et al., 1991; Ripsin et al., 1992; Braaten et al., 1994, Webster, 2003) have reported on the health benefits of its soluble fibre fraction and ß-glucan content. These portions are associated with lowering blood cholesterol levels. Findings from these studies resulted in the United States Food and Drug Administration allowing food labels to display health claims linking oat soluble fiber to a reduced risk of coronary heart disease (Food and Drug Administration, 1997).

Oats also contain a variety of antioxidants such as aventhramides, which can function in health products to prevent degenerative changes and increase immune function (Moisture contentDonald et al., 1992). It should be noted that reported nutritional values for oats vary due to geographic differences. For example, Canadian, Australian, South American and European oat cultivars tend to be lower in protein, but higher in fat than those

cultivated in the United States (Webster, 2003). The proximate composition of oat grain from various sources is summarized in Table 1.

Table 1. Composition of oat grain: average values and ranges (%, dry basis)

Component	Average Value	Typical Range
Protein	15.2	11-20
Fat	7.6	5-9
Total dietary fiber	8.9	7-11
Starch	51.1	44-61
Moisture	10.0	9-14

Source: Welch, 1995a; Webster, 2003.

2.1.2 Structure

Figure 1 illustrates a cross sectional structural view of the oat kernel. When harvested, the oat kernel (caryopsis) is enclosed in a fibrous hull that is comprised of two tissue layers, a lemma and palea (Hareland and Manthey, 2003). These layers are left behind in the field as chaff (Webster, 2003). Trichome 'hairs' cover the surface of the kernel which is rounded at the back. Small indentations mark the location of the embryo. The other side of the kernel is referred to as the crease. During development, the groat is attached at its embryo end to the parent plant and receives nutrients via the vascular tissues that are found within the deepest point in the crease (Fulcher, 1986). Nutrient transport is the official function of this crease; however, it also serves as a reservoir for microorganisms.

Fulcher (1986) identified three distinct morphological structures on the oat kernel: the bran, the germ and the starchy endosperm. Most cereals tend to develop these structures, however, with the oat kernel, this separation is not clear. This may be due to the high fat content and soft texture of the kernel. The latter is evident in commercial oat bran, which characteristically has a large amount of the starchy endosperm remaining on the bran tissue.

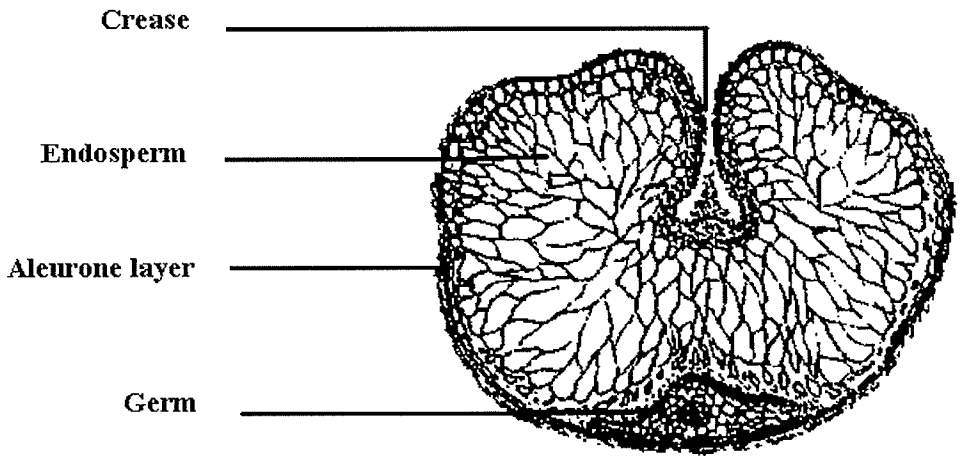

Figure 1. Cross sectional view of an oat kernel. Source: Webster 2003.

2.1.3 Production

In 2004-2005, a total of 3683 kilo tons (kt) of oats were produced in Canada (AAFC, 2006). The major worldwide producers in descending order are: Europe, including Russia (45%), Canada (14%), the United States (7%), Australia (5%), Asia (4%), South and Central Americas (24%) and Africa (1%) (Welch and Moisture contentConnell, 2001). Canada exports 50-70% of the world's oats, averaging 2 Mt from 1998 to 2002. US millers have expressed a preference for Canadian oats over US oats, because the

Canadian climate produces an oat that has a thinner hull which yields a higher quality final milled product (AAFC, 2002). On average, the US imports approximately 85% of the world trade (AAFC, 2004). In 2002, 98% of Manitoba's oat exports were shipped to the United States for the horse, milling and the pet food market (AAFC, 2004). Oat production in Manitoba increased 122.1% to 979,000 tons in 2006 compared to 440,700 tons in 2005 (Manitoba Agriculture, Food and Rural Initiatives, 2006). Exports are also anticipated to rise by 20% because of increased supplies, improved crop quality and a stronger US demand.

Can-Oat Milling Inc., located in the Canadian prairie provinces of Manitoba and Saskatchewan is the largest industrial supplier of oat products in the world, with the ability to process over 453 tons of finished product per day. Can-Oat Milling exports approximately 95% of its production to the U.S., Mexico, Central and South America, the Caribbean and Australia (Can-Oat Milling, 1998).

2.1.4 Utilization

Cultivated oats, *Avena sativa* L. has been utilized for many centuries as a food source for animals, by humans in desperate times and in medicinal applications (Webster, 2003). As a result of its availability and nutritional content, oats remained popular as an animal feed until the mid –1900s. It was not until the nineteenth century that oats became established as a breakfast staple due to advances in milling practices and increased

knowledge of its nutritional value. Recent studies on the ability of oats to lower serum lipid levels has urged the United States Food and Drug Agency to rule in favor of placing heart health claims on the labels of specific oat products (FDA, 1997).

2.1.5 Human consumption

Typical commercial oat products consist of rolled oats (whole-grain flakes), steel-cut groats, quick oat flakes, baby oat flakes, instant oats, oat bran, and oat flour. These products offer unique flavor and moisture retention characteristics, in addition to improving the nutritional quality of the end product (Welch, 1995b; Webster, 2003). Before being processed into oat flakes, groats are conditioned by steaming which softens them in preparation for flattening by rollers. Also, the heat and moisture of the steam completes enzyme inactivation, especially lipase which can contribute to product deterioration (Hutchinson et al., 1951, 1952; Sahasrabudhe, 1982; Urquhart et al., 1983). There are various forms of oat flakes on the market including rolled oats, steel-cut groats, quick oat flakes, baby oat flakes and instant oats. Oat flakes are used in cookies, granola and whole grain breads. They are also used as a thickening agent in soups, gravies and sauces or in meat products as an extender to improve the texture of the final product (Hoffman, 1995).

Oat bran is rich in soluble plant fiber and has twice the amount compared to rolled oats. Oat bran, unlike wheat bran which is a byproduct of the milling process, is formed when rolled oats or cleaned oat groats are ground and the resulting flour is separated into fractions. The oat bran fraction constitutes $\leq$50% of the starting material, while the remaining fractions are pooled to fine oat flour (Webster, 2003). Typical forms of

consumption are in hot or cold cereals, or prepared as porridge. Furthermore, it is used in breads, cookies and muffins (Hoffman, 1995).

Whole groats, steel-cut groats, or oat flakes can be further processed by passing them through a hammer mill yielding oat flour. Before milling the oats are cleaned, hulled, and steamed to inactivate enzymes (mainly lipoxygenase) and improve the product stability. Oat flour can is used in breads, cookies and breakfast cereals (Hoffman, 1995). It is also a major constituent of infant formulations possibly due to its high nutritional profile (Ranhotra and Gelroth, 1995). Additionally, it has a compatible flavor, negligible allergenicity, is easily available and economical. In many cases, this is an infant's first introduction to solid foods (Webster, 2003). Numerous oat-based baby foods can be found on the market and are normally retailed as a dry cereal.

2.1.6 Animal feed

The use of oats for livestock feed, especially farm horses has fallen over the past few years. This is mainly due to machinery replacing horses and mules (Baker, 1995). Also, animal nutrition research has demonstrated that oats have a lower energy content and are not regarded as efficient as corn for fattening animals. Additionally, feed manufacturers are able to make use of low-cost ingredients such as corn and soybean to supply high quality feed formulations (Webster, 2003).

2.2 Oat milling

2.2.1 The process

Figure 2 shows a typical milling flow diagram used to produce groats, cut groats, flakes, flour or bran. The general steps used in processing are cleaning, grading, hulling, groat separation, steaming, and flaking (Deane and Commers, 1986). The objective of the cleaning step is to remove straw, sticks, stones, dust, and foreign matter. As well, oat kernels that are not suitable for milling are removed at this point. The cleaning process includes the use of a milling separator that removes fine material and particles from the oat groats. In the milling separator, the top sieve deck which consists of a scalper reel removes straw and coarse objects from the oats. An aspiration screen is subsequently used to eliminate dust, loose hulls and light material. Additionally, a coarse top screen provides for the removal of other grains, sticks, seeds and larger stones. Following this step, the oats are passed through to a series of disk separators, which aid in separating oats from larger grains such as oilseeds or weed seeds. The operation of the disk separator is based on the difference in length between oat groats and unwanted products. Essentially, a cast iron disk outfitted with indented pockets catch the grains or the seeds. Smaller materials are picked up by the indents, while the larger ones are discarded, resulting in a clean oat flow.

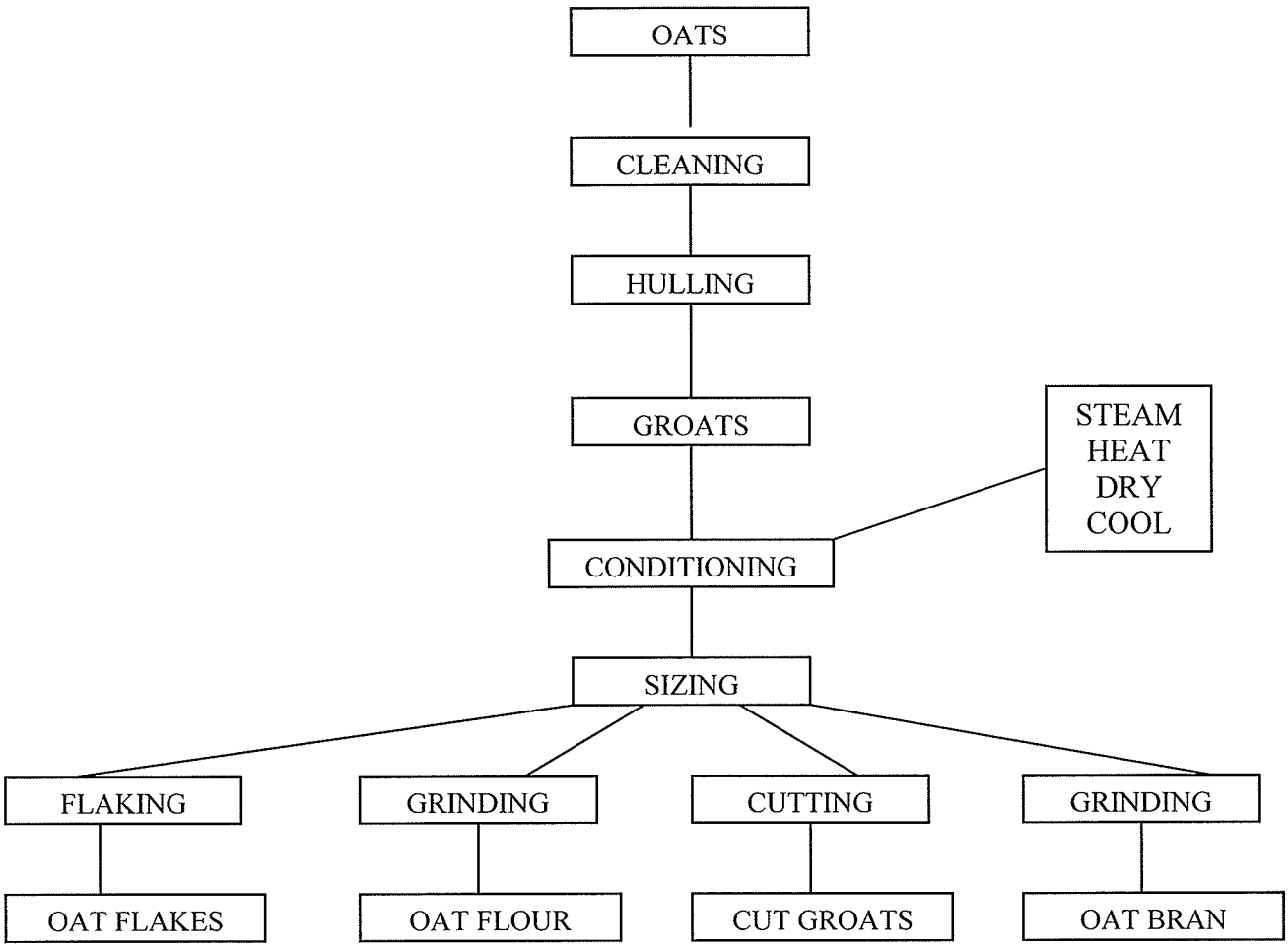

Figure 2. Flow diagram for oat milling process. Source: Can-Oat Milling Inc., Portage la Prairie, Manitoba, Canada.

In the grading step, the oats are separated into classes based on the width of the groat. Width graders or sizers divide the grains into different size classes based on the thickness of the grains. Thinner grains pass through round perforations while the oversized grains are removed. Further separation mechanisms such as gravity separators or paddy separators can be employed to eliminate any impurities such as hollow kernels or extraneous matter having the same length and width as the oat kernels. At this stage, the oats are ready to be hulled.

Hulling removes the hull from the groats inside the kernels and is usually performed using impact action and abrasion. Groats are separated from hulls, groat chips, fines and unhulled oats by aspiration. Unhulled oats are returned to the huller and the cleaned groats are further processed.

Kilning is a major processing step can occur before grading or after hulling. In the case of Can-Oat Milling, kiln drying follows hulling. Due to the high fat content of oats (5-9%; Webster, 2003), oxidative rancidity can occur within two to three days once grinding or flaking has occurred. Oats must therefore be kilned to inactivate the fat-hydrolyzing enzymes – primarily lipases, lipoxidases, and peroxidases – to prevent the formation of objectionable flavors (Hutchinson et al., 1951, 1952; Sahasrabudhe, 1982; Urquhart et al., 1983).

Of the oat enzymes, lipase has received the most attention because it catalyses the removal of free fatty acids from the major oat lipids which consists of triglycerides and partial glycerides. Youngs (1986) reported that the optimum pH for lipase activity was 7.4-7.5 at 35-39°C. However, during seed germination, the lipase activity increases (Ekstrand et al., 1993). Moisture content and time also have an effect on enzyme

activity. Lipase activity (69.5%) is primarily associated with the outer bran layer of the groat (Urquhart et al., 1983; Hareland and Manthey, 2003). Also, lipoxygenase (optimum pH 6.7), activity normally increases with storage, resulting in an increase in saturation of polyunsaturated free fatty acids with a preference for linoleic acid (Youngs, 1986). Heimann et al., (1973) found that lipoxygenase catalyzes the formation of hydroperoxides which are broken down to hydroxy acids by lipoperoxidases. These hydroxy acids are bitter tasting substances and are primarily responsible for rancidity in oat products. Currently, peroxidase, the most heat-stable enzyme in groats, is commonly used as an indicator of sufficiency for oat heat processing (Webster, 2003).

Figure 3 is a schematic of the kiln dryer used at Can-Oat Milling Inc., along with a profile for groat temperature, moisture and lipase activity (Ganssmann et al., 1995). The radiator kilns consist of heating and cooling sections placed on top of each other, with louvres and attachments for product feed. These openings also admit air and steam during treatment. Producers incorporate a steam conditioner after the feed section, which causes a rapid increase in oat temperature and moisture content. The steam conditioner which is found after the feed section raises the product temperature (~ 85°C) and moisture content rapidly from between 12-14% to approximately 17%. During the 1½ to 2 h process, the groats flow by the radiator sections where their temperature is elevated to 100°C. This time-temperature protocol results in enzyme inactivation and product stability. The oats are cooled to 20°C and the moisture content is dropped to approximately 10%. Conducting this step should successfully inactivate any of the heat-stable enzymes present and prolong the shelf-life of the product.

At Can-Oat Milling Inc., the oat groats are fed into the top of the kiln. Steam is used at the main inlet to rapidly increase the groat temperature and moisture content from 12 to ~ 17 %. Groat flow is restricted by a protocol that is designed to control the retention time and throughput. As the groats flow through the dryer, they are further subjected to additional heating when the grain comes into contact with the steam radiators. At these points, the groat temperature is approximately 100°C and lipase and peroxidase are completely inactivated. The temperature and the moisture content of the groat slowly drops as it continues through the dryer until it reaches the cooling sections (Webster, 2003; Ganssmann et al., 1995).

During kilning, desirable nutty, toasted flavors are developed in the oats. Additionally, kilning is instrumental in reducing the number of bacteria and fungi that contaminate the surface of the oat (Webster, 2003). Once kilned, the groats are cut, ground or flaked depending on end product use.

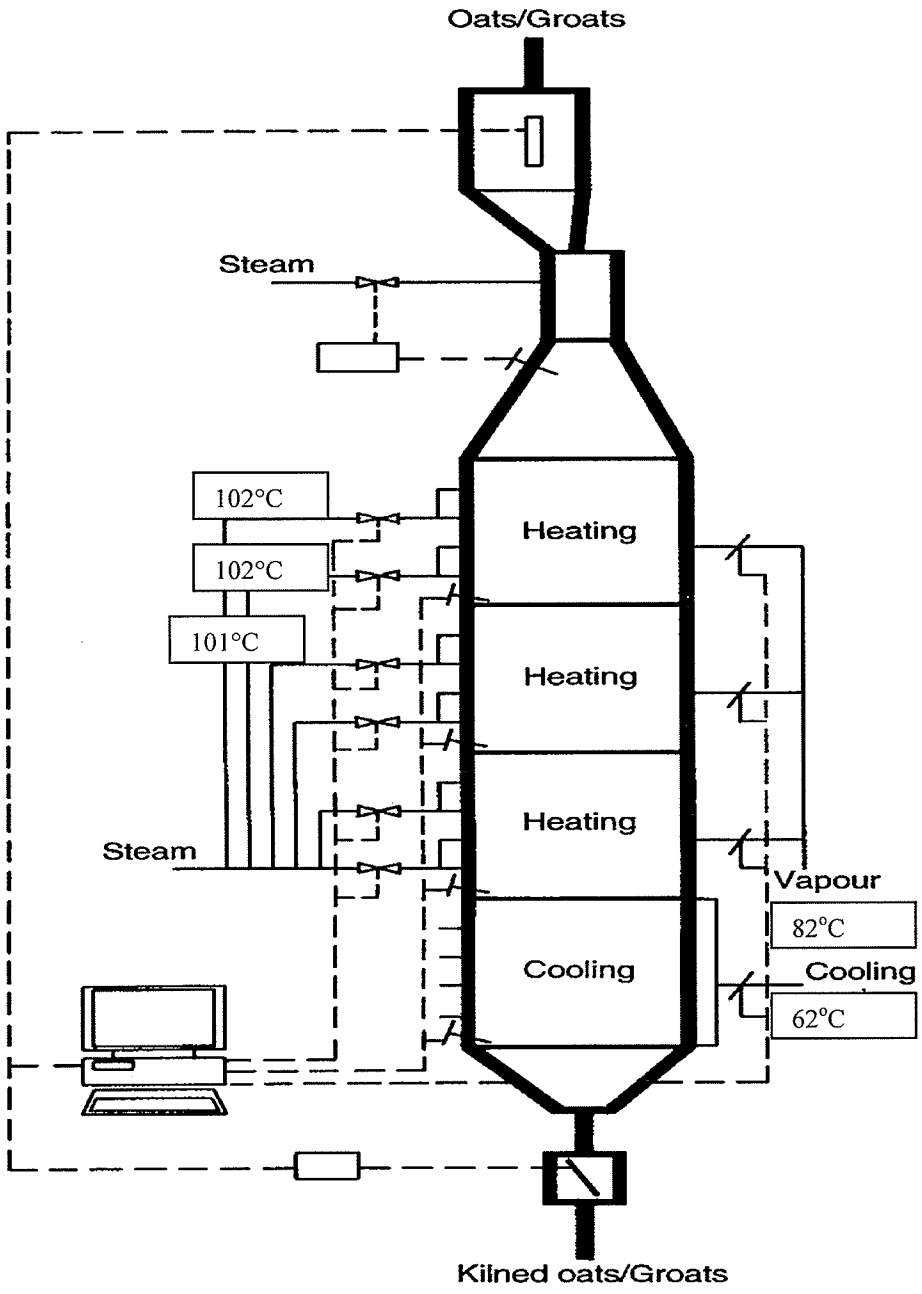

Figure 3. Schematic of kiln drying (includes oat temperature profile). Adapted from Can-Oat Milling Inc., Portage la Praire, Manitoba.

2.2.2 Oat microflora

The microbial flora of oats is expected to be similar to that of soil, and reflective of the storage environments and unit process operations (Jay, 1992). Although oats have a high protein and carbohydrate content, their relatively low moisture prohibits the growth of most microorganisms and controls insect infestations during proper storage. Oats should be stored at approximately 13% moisture or less, $a_w = 0.65$ at 3°C or less (Alberta Agriculture, Food and Rural Development, 2001). Definitive microbiological studies related to oat mill sanitation have not been reported, however, Thatcher *et al.,* (1953) examined the sanitation of several Canadian flourmills and its relationship to the microbial and insect matter content of flour. Finished flour samples from 50 mills contained total aerobic thermophilic spores and flat-sour spore counts ranging from 0 to 32 colony forming units (cfu) per gram. For unbleached samples, the average flat sour and total thermophilic aerobic spores spore count was 9.5 and 5 cfu/g, respectively. In bleached flour, the flat sour and thermophilic spore count averaged 7 and 12 cfu /g, respectively. The presence of thermophilic spores therefore appeared to be unaffected by the bleaching agent (chlorine-dioxide). Bleaching only had a slight effect in reducing the total viable count (2.4×10^4 cfu/g for bleached flour versus 5.9×10^4 cfu/g for unbleached flour). Similar results were noted for mold (unbleached and bleached flour samples contained 3.1 and 1.5×10^3 cfu/g, respectively (Thatcher et al., 1953). It was also reported that samples taken during the milling process contained spore counts similar to that of the finished product. This suggested that both the cereal and the equipment were sources of flat sour spores. Seiler (1986) compared the microbial counts between wheat and flour and reported that the bacterial counts in flour were about 100 times less than the

corresponding wheat (10^4 versus 10^6 cfu/g, respectively). It was also reported that microbial counts in both the wheat and flour decreased during storage; the death rate appeared to increase with an increase in moisture content and temperature.

2.3 Background

2.3.1 Bacterial spores

Bacterial spores have claimed the attention of researchers primarily because of their ability to resist a multiplicity of antimicrobial treatments and processing regimes commonly used in food manufacture. Accordingly, this resistance makes them extremely difficult to eradicate. Food preservation techniques, namely heat are designed to produce a final product that is relatively free of contamination and which has minimal changes in appearance, texture, flavor and nutritive properties. Spores which survive heat treatment can germinate and outgrow leading to the formation of vegetative cells that are responsible for food spoilage and or poisoning. The process of sporulation has been studied in detail for several members of the genus *Geobacillus* and it can be concluded that the process is basically the same for all sporeforming bacteria, (Palop et al., 1998). Typical stages involved in sporulation are described in Table 2.

Table 2. Stages in the sporulation process.

Stage	Characteristics
0-2	The logarithmic growth of the vegetative cell ends. Extracellular products such as amylase, proteases, nucleases and antibiotics begin to appear. Two nucleoids of the vegetative cell condense, forming a single axial chromatin filament. This stage ends once the septum starts to form at one pole of the cell.
3	Septation; the formation of the septum results in the synthesis of the forespore membrane and the compartmentalization of the DNA. Envelopment; the forespore is engulfed and becomes a distinct cell, bounded by two membranes within the mother cell.
4	Cortex formation. Peptidoglycan is laid down between the inner forespore membrane and the outer forespore membrane.
5	Spore coat synthesis; dipicloinc acid (DPA) is deposited, uptake of calcium; resistance to organic solvents develops; spore coat proteins are deposited on the outside of the developing spore.
6	Spore maturation; the coat material becomes dense; spore refractility increases and heat resistance develops.
7	Release of the mature spore by lysis of the mother cell.

Sources: Fitz-James & Young (1969), Russell (1982) and Nicholson & Setlow (1990)

Following lysis of the vegetative cell or sporangium, the spore is released and remains relatively metabolically dormant. At this stage the spore exhibits its greatest resistance. This dormant resistant (phase bright or retractile) spore can survive for exceptionally long periods of time in the absence of exogenous nutrients and or in marginal environments.

2.3.2 Sporulation

Commencement of sporulation can be viewed as the last chance for cell survival when surrounding conditions prevent normal cell growth. However, there must be certain nutrients and conditions present before this process begins. They are: temperature, pH, oxygen requirements, carbon and nitrogen sources, which can affect the rate and amount of spore formation along with the development of spore properties. Spore formation normally occurs at the end of the exponential growth phase.

2.3.2.1 Dormancy is a state whereby viable cells or organisms are typified by a lack of metabolic activity and development for an extended period. An extreme case where there is no metabolic activity is referred to as cryptobiosis. There have been many reports of instances of prolonged dormancy of spores. Although metabolically dormant, the spore is capable of monitoring the nutritional status of its environment. The dormant spore has three important roles, which include enhancement of survivability, disseminability, and coordination of development with favorable environmental conditions (Sussman, 1969). Factors that augment, maintain or induce dormancy are temperature; ionic environment, pH, and exchangeable cation load; water activity; oxygen and oxidation-reduction conditions; protein reagents; metabolic inhibitors, analogs and antibiotics. Ageing,

heating, reductants, low or high pH, or modified exchangeable cation load can transform a dormant spore into an activated state (Murrell, 1961; Lewis, 1969). Dormancy is a complex condition to express quantitatively, but according to Keynan and Evenchik (1969) the assumption can be made that dormancy is broken completely when the germination rate of the spore suspension is maximal. The process of activation ends the state of dormancy only temporarily, without ending cryptobiosis.

There are three separate processes that transform the dormant spore to the vegetative cell: activation, germination and outgrowth.

2.3.2.2 Activation of spores is the result of a treatment that does not itself start germination, but allows spores to germinate more rapidly or more completely or both. It breaks the dormancy period in spores, and is reversible. This stage is not considered to require metabolic activity but rather it depends on changes in macromolecular arrangements that are in response to time, temperature, ionic environment, solvents, reductants, humidity and adsorbed gases (Lewis, 1969).

The most common method used to activate spores is sub-lethal heat. Finley and Fields (1962) found that heating *Geobacillus stearothermophilus* spores at temperatures above 100°C resulted in activation. Alternatively, heating spores at temperatures below 100°C, induced dormancy. Work performed by Curran and Evans (1945), demonstrated that heat at sub-lethal temperatures (62-65°C) could also induce dormant spores to germinate. Powell and Hunter (1955) reported that heat activation must be performed in the presence of water. The magnitude of the response depended on the heating medium.

Activation is only one effect that heat can have on a bacterial spore. Other known effects are (Keynan and Evenchik, 1969):

i) Heat killing of spores

ii) Heat damage to some germination specific enzymes

iii) Heat-induced dormancy

iv) Quantitative population changes in heterogeneous spore suspensions

v) Altered and fixed changes in germination requirements as a result of heating

Agents that have been reported cause activation in bacterial spores include (Keynan and Evenchik, 1969):

i) Calcium dipicolinate (Ca-DPA)

ii) Low pH and reducing agents

iii) Ionizing radiation

iv) Various chemicals e.g. mercaptoethanol, thioglycollic acid, ethyl alcohol, dimethylformamide and dimethylsulphoxide

v) Ageing brought about by storage in suitable temperature and water environments.

Activated spores maintain most of the significant properties of the dormant state such as resistance to heat and radiation, non-stainability and refractility. However, activation causes quantifiable changes in the spore. The most identifiable of these changes are those that affect the rate of germination; changes in germination requirements, and changes in morphology, permeability and spore composition. The degree to which germination can be stimulated in non activated spores varies, with

some highly dormant non activated spores never germinating, even when grown on nutrient rich media. Once heat activated, these same spores will germinate normally and form colonies. In less dormant spores, some germination will occur even if they are not activated, though activation will increase the germination rate considerably. Activation may be the result of reversible changes in the tertiary structure of some spore macromolecules (Keynan and Evenchik, 1969). On a physiological level, changes in spore permeability have been reported. This is indicated by a number of substrates capable of being metabolized after activation, not before, showing an alteration in the spore permeability or an activation of an enzyme. Decreases in the L-alanine requirements for germination rates and the excretion of Ca-DPA and some amino acids during activation also have been observed. However, Gould and Hitchins (1963) noticed that exposing spores to mercaptoethanol induces activation and permits penetration of reducing agents such as lysozyme into the spore coat. Keynan et al. (1964) also proposed that the spore coat was the site of activation, correlating heat activation with denaturing of the proteins situated in the outer parts of the spore coat.

2.3.2.3 Germination encompasses rapid degradative changes in spores that are generally initiated by specific agents/germinants and is accompanied by well-defined cytological and chemical changes. Germination terminates dormancy and causes a loss of heat resistance. It has been described as an irreversible process that involves physical and chemical changes in the spore. Powell (1953) realized that at the start of germination, approximately 30% of the dry weight of the spore is excreted. It was also noted that the

material excreted from the spore during germination contains substances that are typical of the spore and which do not exist in vegetative cells. Powell (1953) also realized that the germination reaction occurred in conditions which did not permit growth and was therefore independent of it. Germination is accompanied by the following changes:

i) Loss of resistance to heat, radiation, desiccation, pressure, electric shock and chemical agents.

ii) Breaking of dormancy.

iii) Release of calcium and dipicolonic acid (DPA), a peptide that is found in spores, but not in vegetative cells.

iv) Changes in staining properties; decrease in refractive index and a decrease in dry weight.

v) A decrease in optical density (OD)

Numerous physical and chemical factors have long been identified as initiators of germination. Some of these metabolizable or nutrient initiators include amino acids (L-alanine is the most studied) and ribosides (Gould et al., 1968; Warren and Gould, 1968); sugars (Wolf and Thorley, 1957); non-metabolizable initiators such as ions (Levinson and Sevag, 1953); surfactants (Rode and Foster, 1960); chelators (Riemann and Ordal, 1961); environmental factors such as pH (Church et al., 1954); temperature (Knaysi, 1964) and ionic strength (Rode and Foster, 1960).

Antibacterial compounds such as preservatives including: phenols, parabens and sorbic acid are known also to inhibit germination (Gombas, 1983).

2.3.2.4 Outgrowth

Outgrowth is the transformation of a geminated spore into a vegetative cell and it occurs in a synchronous and orderly manner (Russell, 1982). Several morphological events become evident during this process. These include: swelling; elongation of the swelled spore and often emergence of the spore from the spore coat and septum formation or the first cell division (Setlow, 1984).

Chemical requirements necessary for spore outgrowth include: sulphur, phosphorous, nitrogen, carbon, amino acids and metal ions. Major physical requirements include temperature and pH (Strange and Hunter, 1969).

The macromolecular synthesis that takes place during the outgrowth process involves RNA synthesis, followed by protein synthesis, with DNA synthesis occurring afterward (Russell, 1982). Outgrowth can most easily be monitored by the changes in the optical density that follows germination since spores are transformed from phase bright to phase black (non retractile). Overall, outgrowth is considered a cellular process involving intracellular differentiation and is depicted as a series of distinct structural and functional alterations (LeeWing, 1980). Spores in the outgrowth phase are least resistant to heat and other antimicrobials.

2.4 *Geobacillus stearothermophilus*

Bacillus stearothermophilus has been transferred to the new type genus *Geobacillus stearothermophilus*. Nazina et al., (2001) proposed this transfer based on physiological characteristics, fatty acid analysis, DNA-DNA hybridization studies and 16 S rRNA gene sequence analysis. The genus *Bacillus* was dissected due to differences in phylogenetic

and phenotypic properties (Fritze, 2004). This resulted in the transfer of *Bacillus stearothermophilus* to the new genus, *Geobacillus*. *Geobacillus stearothermophilus* (Claus and Berkeley 1986; Nazina et al., 2001) are gram positive, thermophilic, facultative anaerobic rods, (0.9 to 1.0μ by 2.5 to 3.5μ) that produce oval spores (1.0 to 1.2μ by 1.5 to 2.2μ;) located terminally to sub terminally within sporangia. Colony morphology is not distinctive; size is variable and may appear translucent to opaque and smooth to rough. Optimum, maximum and minimum growth occurs at 50-65°C, 65-75°C, 30-45°C, respectively.

Geobacillus stearothermophilus is of particular importance in the food industry since it is the most heat resistant bacterium commonly encountered in processing and is usually the cause of flat sour spoilage in low acid canned food (pH above 4.5) including such foods as peas, corn, meat and marine products, and meat and vegetable mixtures. This kind of spoilage derives its name because the can ends remain flat, instead of the normal concavity that forms after thermal processing (Jay, 1992). Presence of flat sour bacteria in food results in lactic acid formation, leading to souring of the product. Due to its thermophilic nature, spores will not outgrow at temperatures below 43°C therefore proper product cooling especially following retorting but also during storage and distribution are crucial to preclude thermophilic spoilage.

Soil is a natural habitat for spores, especially those of the genus *Geobacillus* (Fields, 1970; Slepecky and Leadbetter, 1983). These organisms are resistant to desiccation and therefore are frequently found in air and dust (Ingram, 1969). Also, sporeformers can be commonly found on food-processing equipment where they can adapt, survive and grow (Ingram, 1969). Since most spores are soil borne they frequently contaminate the external

surfaces of vegetables, spices and grains. Additionally, cross-contamination occurs if the food process operation involves extensive chopping, mixing or handling.

2.5 Thermal inactivation and resistance

Bacterial spores are biochemically inert and are not in themselves responsible for food spoilage. However, following spore germination and outgrowth the emergence of vegetative cells and their ensuing metabolic activities often contribute to food spoilage and poisoning problems (Ingram, 1969; Gombas, 1983; Atrih and Foster, 2002). Prevention of food spoilage depends on the disruption/ inhibition to any part of the spore's lifecycle and includes treatments such heat (moist and dry); chemicals (NO_3, H_2O_2, Cl_2); radiation (ionizing and ultraviolet) and hydrostatic pressure (Ingram, 1969; Russell, 1982).

Spore resistance is due to spore core/protoplast dehydration and mineralization (Jay, 1992). Protoplast dehydration occurs during sporulation and is linked to the acquisition of heat resistance by the spore (Atrih and Foster, 2002). One theory involves the spore cortex, which is able to become hydrated and maintains core dehydration (Gould and Dring, 1975). Gould and Dring (1975) proposed an osmoregulatory hypothesis which predicts that the core must remain in osmotic balance with the surrounding cortex, allowing water to be drawn from the core, into the cortex, until the pressures are balanced. This will result in a dehydrated core (Gombas, 1983; Gould and Dring, 1975; Russell, 1982). The reduced core water content reduces the amount of water associated with spore proteins in the core. This in turn stabilizes the spore proteins

against thermal denaturation (Atrih and Foster, 2002). Spores also owe some of their thermal resistance to the presence of the spore coat that is multilayered and rigid due to cross-linked polypeptides. The coat also provides a permeability barrier to some compounds, thus slowing thermal transfer (Gombas, 1983; Fine and Gervais, 2005).

2.5.1 Moist heat

Moist heat or steam sterilization occurs under saturated conditions, when the relative humidity is 100%. In this method steam is used to transmit thermal energy to food until it reaches the same temperature of the steam. Overall, moist heat is more effective than dry heat because water molecules conduct heat better than air and also have greater penetration (Joslyn, 2001).

Bacterial spores accumulate minerals, which also contribute to spore stability and heat resistance (Atrih and Foster, 2002). The most common include: calcium, magnesium and manganese and are located in the core. An increased core mineralization is usually associated with decreased core water content, and this may contribute to enhanced heat resistance. Of these minerals, calcium has the ability to chelate with dipicolinic acid (DPA). Thermoresistance has been correlated to the presence of calcium (Halvorson, 1961; Gombas, 1983; Joslyn, 2001). Spores with low calcium levels are more sensitive to heat inactivation (Halvorson, 1961). Also, decreasing amounts of DPA in bacterial spores leads to reduced heat resistance (Gombas, 1983; Joslyn, 2001).

2.5.2 Dry heat sterilization

Compared to moist heat, dry-heat sterilization normally requires longer exposure times and or higher temperatures (Pflug et al., 1968, 2001). The diffusion and penetration of heat in dry systems is poor due to low heat transfer coefficients and heat capacities, which are commonly associated with conductive heating (Pflug et al., 1968, 2001). Despite these disadvantages, dry-heat sterilization can be used for heat stable, moisture sensitive or steam impermeable materials such as packaging materials and machinery (Brown, 1994; Joslyn, 2001). It does not cause corrosion or erosion of materials and can be used for products that have low water contents e.g., powders and oils (Joslyn, 2001). Table 3 shows the decimal reduction times (D-value; time required to reduce a given spore population by one log cycle or 90% at a specified temperature) of *Geobacillus stearothermophilus* spores when treated with dry (superheated steam) and moist heat. As observed the $D_{121°C}$ for moist and dry heat is 16 and 936 min, respectively.

Table 3. *D* -values of *Geobacillus stearothermophilus* spores under moist and dry heat.

Type of heat	Temperature (°C)	D-Value (min)
Moist	115	24 [1]
	120	16.7 [2]
	121.1	16 [3]
Dry	121	936 [4]
	160	0.35 [5]
	176	0.14 [4]

Sources: (1) Briggs, 1966; (2) Davies et al. 1977; (3) Brown, 1994; (4) Collier and Townsend, 1956; (5) Bruch et al. 1963

2.5.2.1. Factors influencing the sensitivity of spores to dry heat

According to Fox and Pflug (1968), the destruction of microorganisms by dry heat is different in order and also possibly different physiologically than moist heat. This can be noted by the larger D values for spores subjected to dry heat when compared to those in moist heat. Several factors have been shown to affect the dry heat resistance of bacterial spores including temperature; heat transfer system; spore carrier and recovery conditions (Fox and Pflug, 1968; Russell, 1982).

Temperature is possibly the most important variable and its effectiveness is normally a function of time (Pflug et al., 1968, 2001). Bond and Favero (1975) exposed spores of *Bacillus* sp to dry heat at 125 and 150°C and reported D values of 139 and 2.5 h , respectively. Angelotti et al. (1968), assessed the dry heat resistance of *Bacillus subitilis* spores heated from 105°C to 160°C on the surfaces of material (filter paper, stainless steel surface, stainless steel washers) or by encapsulating them in lucite and epoxy. Relatively high D values (D_{160} = 246 min) were observed for the encapsulated spores, while significantly lower D values (D_{160} = 2.6 min) were obtained for spores applied to surfaces. In this case, the spore resistance was influenced by the carrier, rather than the temperature.

2.5.2.2. Type of heat transfer system

Spore survival is affected by the heat transfer system e.g. superheated steam, hot air, infrared, conduction through metal or plastic since the kill rate is dependent on the rate of heat transfer to the sample. Fox and Pflug (1968) examined the effect of the flow rate of

air and nitrogen at various temperatures on the dry heat destruction rate of *Bacillus subtilis*. Their results showed that the effect of temperature on spore destruction was increased when the gas flow rate increased. It is possible that higher gas flow rates caused greater moisture loss, leading to spore destruction.

2.5.2.3. Effect of spore carrier

Spores dried on sand were found to be more resistant than those deposited on paper strips or glass tubes (Bruch et al., 1963). Angelotti et al. (1968) found that spores encased in a system were more resistant than the same organism applied to a surface. It was shown that the survival times of spores were higher when encapsulated in epoxy plastic ($D_{135\,°C}$= 114 min) compared to the surface of stainless steel strips ($D_{135°C}$ = 2.6 min). This difference was attributed to the varying water retention capacity of the carriers. The open surfaces would lose moisture quickly, causing the spores on the surface to become dehydrated. In enclosed systems, the reverse would be noticed, with water loss to the system occurring at a slower rate, causing the spores to be more resistant (Angelotti et al., 1968).

2.5.2.4. Recovery conditions

Recovery conditions (incubation temperature and time, recovery media and diluent) have been examined in regards to their effect on spore heat resistance. Heat treated spores incubated at temperatures lower than the normal optimal growth temperatures for unheated spores seem to be more successful in producing a greater recovery of spores (Cook and Gilbert, 1968a, b, c; Roberts and Hitchins, 1969; Russell, 1982). Once spores

have been exposed to heat treatments, the incubation time necessary for germination increases, as a result, growth may be delayed (Russell, 1982). The use of trypticase soy agar or broth has been favored by many researchers as the recovery medium for spores subjected to dry heat destruction (Russell, 1982). There is still limited information on recovery and revival of spores following damage by dry heat (Russell, 1982).

2.5.2.5. Proposed mechanisms for spore inactivation by dry-heat

Relatively little is known of the precise inactivation mechanism of spores treated with dry heat; however it is generally agreed that they die due to oxidative reactions occurring within proteins. Since oxidation requires more energy than denaturation of proteins (lethality with moist heat) more energy is normally required for dry heat treatment.

2.5.2.6. Physical structure

Pflug and Schmidt (1969) stated that a physically smaller spore may be more resistant to dry heat. The initial size of the organism would be indicative of a tighter molecular structure, having greater resistance to the effects of denaturation or oxidation. As explained previously, the spore consists of a thick coat consisting of multilayers of polypeptides, which may serve as a barrier to the loss of moisture and minerals (Bond and Favero, 1975).

2.5.2.7. Water activity

The water activity of spores will change in relation to the relative humidity of the atmospheric environment unless the environment is controlled. A reduction in water activity is usually accompanied by an increase in the heat resistance. Angelotti et al. (1968), reported that *B. subtilis* spores with an a_w of 0.2 to 0.4 were more resistant (larger D values) than spores with greater or lesser a_w. Collier and Townsend (1956) showed that spores heated in superheated steam displayed a greater resistance than those heated in saturated steam. This is presumably due to the low water content of the heating system. Hoffman et al, (1968) determined the effect of cell moisture content on the thermal inactivation rate of bacterial spores exposed to temperatures between 108 and 192°C. Prior to the heat treatment, the spores were equilibrated to various relative humidity (RH) levels. Spores pre-equilibrated to lower RH levels were less resistant and vice versa. The results showed that the RH of the cells prior to heating adjusted the moisture content of the cell. This sequentially has an effect on microbial death.

2.6. Superheated Steam (SS) Drying

2.6.1. Background

Hausbrand (1924) introduced the idea of SS at the beginning of the 20th century, however, it was not until the 1950's that researchers examined the process more closely (Wenzel and White, 1951; Chu et al., 1953; Lane and Stern, 1956). Currently, commercial application of SS has been limited to drying industrial products such as paper products and sludge (Kudra and Mujumdar, 2002; Devahastin and Suvarnakuta, 2004).

In the food industry, SS is utilized in the Dole-Martin process. This system applies superheated steam at 222°C to pre-sterilize equipment and metal cans used in the production of low acid foods (pH>4.5) such as fish, meats and vegetables (Buchner, 1993; Brown, 1994). Overall acceptance of SS as a unit process operation is slow due to a lack of suitable equipment and insufficient knowledge of the process and its effect on product quality (van Deventer and Heijmans, 2001; Kudra and Mujumdar ,2002). Recently the system has been investigated to dry products such as spent grain (Tang and Cenkowski, 2001) potatoes (Tang and Cenkowski , 2000; Iyota et al., 2001) sugar beet pulp (Tang et al., 2000) wood (Kudra and Mujumdar ,2002) Asian noodles (Markowski et al., 2003) shrimp (Prachayawarakorn et al., 2002) lumber (Woods et al., 1994) wood pulp and paper (Douglas, 1994) coal (Potter and Beeby ,1994) and sludge (Francis and Di Bella, 1996).

Collier and Townsend (1956) conducted the first study on the resistance of bacterial spores to superheated steam. They indicated that all three sporeformers evaluated including *Bacillus stearothermophilus* were more resistant to SS than to moist heat. Also, *Bacillus polymyxa*, a mesophile which is easily destroyed by moist heat, exhibited resistance almost comparable to *B. stearothermophilus* when treated with SS. They concluded that systems using superheated steam as the sterilizing medium required temperatures 40-50°C higher than saturated steam systems to have a similar killing effect on bacterial spores. In 1999, Spicher et al. examined the resistance of *Bacillus subtilis* and *Bacillus stearothermophilus* spores to superheated steam. They also concluded that SS was less effective compared to moist heat as a sporicidal agent. Since then, there has been a paucity of information relative to its microbial efficacy.

2.6.2. Properties/drying ability of superheated steam

Superheated steam is steam that has a temperature above the saturation or boiling point. As water is heated at any specific pressure and reaches its boiling point, it is referred to as saturated steam. Once heated beyond the boiling point, the steam becomes unsaturated or superheated. At this point, the steam can transfer heat to the product that is being dried raising the product's temperature to the boiling temperature and transferring heat to the product. In contrast to saturated steam, a drop in temperature does not cause condensation of the steam as long as the temperature is higher than the saturation temperature. Any moisture that is evaporated does not need to be exhausted, but instead becomes part of the drying medium.

As with hot air drying, superheated steam drying has three separate phases. They are: initial heating period, constant-rate period, and the falling-rate period. In the first phase, the superheated steam transfers a portion of its heat to the product that is being treated. This is indicated by a rise in product temperature. There is an increase in moisture of the product at the beginning of the drying process (Bonazzi et al., 1996).

When the free water exhibits evaporation rates that are equal to superheated steam drying or with air drying at the same mass velocity, an inversion temperature occurs (Chu et al., 1953). This phenomenon takes place in the constant rate period of the process. Above the inversion temperature, superheated steam can dry faster than hot air; below this temperature, the drying rate is slower in superheated steam than in hot air.

During this period, the movement of moisture in the material is rapid, causing saturated conditions at the surface of the material. The internal resistance to moisture diffusion is

much less than the external resistance to water vapor removal from the products surface (Brooker et al., 1974). When the rate of moisture movement to the surface of the material is lower than the rate of evaporation, the outer surface will begin to dry, signaling the start of the next phase in the drying process, the falling rate period.

In this period, the drying rate decreases along with an increase in product temperature to that of the superheated steam. The drying rate continues to decrease until the moisture content of the product reaches the equilibrium moisture content and the drying process ends.

Numerous advantages for the operation of this process have been proven. Overall, it is very economical and energy-saving since SS operates under a closed-loop system therefore no energy is wasted and the exhausted steam can be recovered and reused in other processes. Also, due to a lack of oxygen in the SS system, product oxidation which can reduce quality (nutrient loss; enzymatic browning) is kept to a minimum. The absence of oxidation also prevents fires and explosions during drying. This process is also capable of recovering volatile organic compounds when drying products at a higher rate. Since the steam dryer is a closed system, there are no odor emissions, air pollution or other hazardous components; and any excess steam can be recycled and used as a heat source.

Superheated steam processing can also inactivate microorganisms such as bacterial spores (Spicher *et al.*, 1999). These authors found that *G. stearothermophilus* spores were more sensitive to superheated steam with an increase in degree of superheating. An increase in superheating by approximately 22°C (saturation steam temperature =120°C), required 60 minutes to produce a 50% decrease in the initial spore count of 10^5 cfu/g.

This increase in resistance also correlated to a decrease in the relative humidity of the treatment chamber. In a review of the application of drying of food products, Devahastin et al. (2004) reported that SS drying can assist in the destruction of microorganisms due to the high temperatures that can be attained.

In addition to added product benefits, superheated steam systems have higher heat transfer coefficients and increased drying rates that require less equipment and labor. However, here are several limitations to this process; the major drawback being that the system is new and more complex than hot-air dryers (Kumar and Mujumdar, 1990), therefore there is limited acceptance by the industry. In addition, the process produces a high temperature within the product, which creates problems for heat sensitive materials such as protein denaturation, browning reaction, discoloration, starch gelatinization and enzyme destruction. There is also the risk of air leakage and air infiltration when feeding and discharging products, thereby requiring more complex systems to be put into place (Mujumdar, 2000).

2.7. Processing and the Role of Oat Starch

Oat starch has been of little interest to cereal chemists, because in contrast to most other cereals, it cannot be easily separated from the other grain components (Zhou et al., 2000). But from the consumer's viewpoint, the pasting and gelatinization properties of the whole oat product are critical to its acceptance. There is a lack of published literature on the relationship between pasting and the quality of various end-products.

2.7.1. Pasting and Gelatinization Properties

Starch is the most abundant component of the oat grain and therefore plays a significant role in the quality of oat products. Gelatinization is an important part of the heat processing of starch-containing food systems that occurs once the starch-water slurry is heated. When heating continues, the starch granules absorb more water until they swell and eventually disrupt their crystalline structure. Doublier et al., (1987) reported that in oat starch pastes, amylose and amylopectin were co-leached from the granule. In most other cereal starches, the paste thickens as the amylose alone leaves the granules and become solubilized in the surrounding liquid.

Researchers have found that the response of some oat starch pastes to temperature during the cooling cycle is unique. There is a rapid development of cold viscosity that does not appear to be related to the tendency of the starch paste to retrograde. Pasting properties and characteristics such as swelling power and solubility are variable. Oat starch gels are more elastic, adhesive and translucent and show greater stability under storage conditions than pastes from unmodified wheat or corn starches (Paton 1979, 1986). In addition, oat starch pastes exhibit higher viscosities during the cooling cycle compared to wheat or corn, although oat starch granules when cooked are more sensitive to shear.

While heat-treating groats is necessary to improve and extend product quality and shelf life, color deterioration and changes in functionality of the product are difficult to circumvent.

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Oat groats

Oat groats were supplied by Can Oats Milling, Portage la Prairie, MB. Samples for microbiological evaluation were taken before (raw) and after kiln treatment (heated treated). Samples from the kiln walls were manually removed at the appropriate locations using sterile scrapers and placed into sterile bags. All samples were maintained at 4-6° C prior to analyses.

3.2 Microbial analysis of oat groats

Oat groat samples (25g) were aseptically added to sterilized 500ml blender jars containing 225 ml sterile 0.1% peptone (Difco, Becton Dickinson and Co., Sparks, MD). Samples were blended for 1 min and left undisturbed for another min to allow for particle settling. All serial dilutions were prepared using 0.1% peptone.

Evaluation of groats for yeast and mold was determined using potato dextrose agar (Difco) acidified to a pH of 3.5 with sterile 10% tartaric acid. Samples were also plated onto dichloran rose bengal chloramphenicol agar (Oxoid Ltd., Basingstoke, UK) supplemented with 100μg/ml chloramphenicol (Oxoid Ltd). Incubation was for 5 d at 20-22°C. Standard plate count was performed using plate count agar (Difco) with incubation at 32-35°C for 24-48 h. Coliforms were determined using violet red bile agar (overlay; Difco) with incubation at 35°C for 24 h. All samples were plated in duplicate and results expressed as $\log_{10}$ colony forming units (cfu) /g.

For thermophilic and flat sour spores, oat groats and kiln wall samples (22g) were blended in jars containing peptone water (0.1%, 198 ml). Portions of blended sample (20 ml) were pipetted into flasks containing 100 ml sterilized dextrose tryptone agar (Difco) with bromocresol purple (0.04%) and heated to approximately108 °C for 10 min (autoclave @ 5 psi). Following spore shocking the contents were allowed to cool to 45-47 °C and equally distributed among 5 petri plates. Once the medium solidified an additional layer (10-12 ml) of agar (2%, Difco) was overlaid onto the plates to discourage growth of spreading colonies. Incubation was at 55 °C for 48 h (APHA, 1992). Thermophiles were determined by counting all colonies and multiplying by 5. Flat sour bacteria were determined by counting all colonies surrounded by a yellow zone (acid production) and multiplying by 5. In both cases, counts are expressed as $\log_{10}$ cfu /10g.

3.2.1 Confirmation of isolates

Confirmation of *G. stearothermophilus* isolates from flat sour colonies was based on biochemical differentiation (API 50 CH; bioMèrieux, Hazelwood, MO) and phase contrast microscopy.

3.2.2 Spore production, harvesting and cleaning

Bacillus stearothermophilus ATCC 10149 now renamed *Geobacillus stearothermophilus* was used for spore production. The organism was initially cultured in tryptic soy broth (TSB, Difco) for 24 at 55 °C. Resultant growth (2-5 ml) was distributed over the surface of the sporulation medium (150 ml contained in 1-L Roux bottles or 150 ml in 750 ml canted neck tissue culture bottles (Corning Inc., Corning,

NY). The medium as described by Kim and Naylor (1966) consisted of (%, w/v): nutrient broth, 0.8 g ; yeast extract, 0.4 g; agar, 0.2 and was supplemented with $MnCl_2$ $4H_2O$ (10 µg/ml). The medium was adjusted to pH 7.2 with 1.0 N NaOH prior to sterilization. The inoculated medium was incubated for 1 week at 55 °C. Spore crops were dislodged from the agar surface using sterile glass beads (Fisher Scientific, Fair lawn, NJ) and harvested with sterile, cold distilled water. Spore suspensions were examined using phase microscopy (x 1000); dormant spores appeared phase bright.

Spore crops were pooled and centrifuged (x 2000g) at 1°C for 1 h and the resulting pellets re-suspended in equal amounts of sterile, cold distilled water. A 1 % lysozyme solution (Inovapure™ 300, Canadian Inovatech, Abbotsford, BC) was added at a rate of 1 ml/100ml to lyse vegetative bacteria and sporangia. The suspension was incubated overnight at 1 to 3 °C. Following incubation the spore suspensions were washed and centrifuged twice as previously stated and finally re-suspended in sterile, cold distilled water.

Spore suspensions were transferred to sterile, round bottom flasks (250 ml) shell frozen using liquid nitrogen and freeze-dried (10-146 MP-BA, Virtis Research Equipment, Gardiner, NY). After 3-5 d the dried spore powders were lightly crushed using a sterilized mortar and pestle, combined with sterile, washed sand (used as an inert carrier ;105-212µm particle size) and stored at 3-5 °C in sterile, airtight vials. Two sand-spore mixtures were prepared for investigation : 10^4 and 10^{6-7} cfu / g ; viable numbers were determined using direct plating (TSA, Difco) with incubation at 55 °C for 48 h.

3.3 Heat resistance of *G. stearothermophilus* spores in oat groats following conduction/ convection heating

A series of sterilized screw-capped test tubes (50 ml) containing 9 g of sterilized (121°C for 15 min) groats (12 or 17% moisture) mixed with 1 g of sand-spore mixture (c. 10^6 cfu /g) were heated to an internal temperature of 145 °C using an thermostatically controlled oil bath maintained at approximately 150°C (Blue M Electric Co. Blue Island, USA). At the end of each time interval (0, 10, 20, 30 and 40 min) triplicate tubes were removed and immersed into an ice-water bath for 2 min. Each sample was transferred to a blender jar containing 70 ml of cold, sterile peptone water. All sample test tubes were rinsed twice using 10 ml sterile peptone water. Following vortex mixing the contents were added to the appropriate blender jar. Survivors were determined by direct plating in duplicate using TSA (55 °C, 48 h). D-values were determined from the slopes of the straight line portions (following linear regression) of the survivor curves. Temperature and come-up times were monitored using a computer equipped with an Omega 6 software acquisition system (Omega Engineering Inc., CN, USA.) A thermocouple inserted through a hole in the cap (sealed using epoxy glue) of one of the test tubes was used to monitor the temperature.

3.4 Superheated steam processing system

The superheated steam dehydration system used in this study was developed in the Department of Biosystems Engineering at the University of Manitoba. The system consisted of a steam generator model MB9L (Sussman Electric Boilers, New York, NY) operating at 415 kPa, steam conveying pipelines, a drying chamber, steam superheaters and heating tapes, a hot-air supply system, a processing chamber and a data acquisition and control system (Tang, et al., 2000). Thermocouples T_1 and T_2 (Figure 4) were used to monitor the temperature below and above the sample in the processing chamber. Steam was conveyed through a series of pipes into a pressure reducing regulator (type 95l NPT body; Fisher Controls International Inc., Marshalltown, IA). Resulting steam pressure (101 kPa) equivalent to 1 bar or 1 atmosphere was passed through the processing chamber where the sample tray was located.

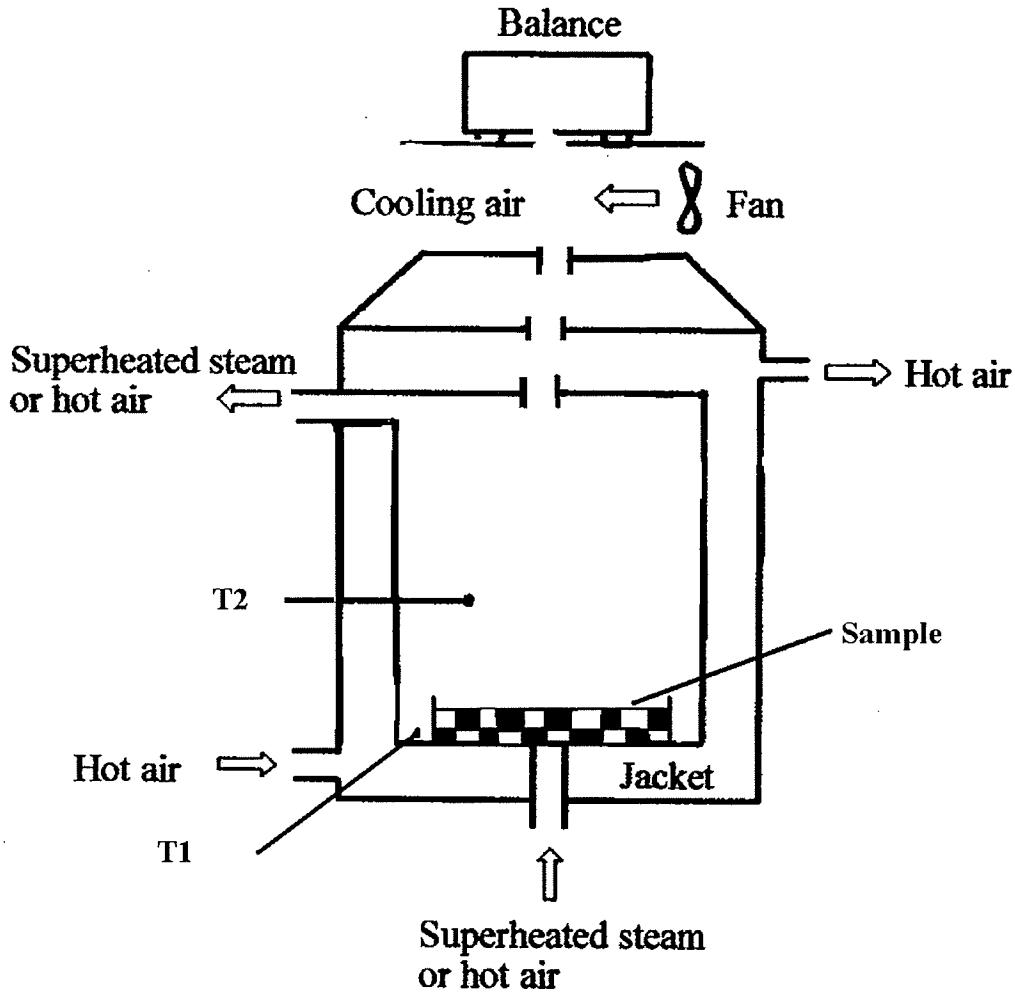

Figure 4. A simplified diagram of the drying chamber (adapted from Tang et al., 2000).

3.4.1 Superheated steam operating conditions

The chamber temperatures used during SS treatment of groats were 115, 121, 130, 145, 160, and 175°C ± 3°C. Steam temperatures were continuously monitored and recorded by thermocouples located inside the processing chamber and by a data acquisition system (Dept. Biosystems Engineering, University of Manitoba). Steam velocity (metres/second or m/s) was maintained at 0.52 ± 0.01m/s or 1.10 ± 0.01m/s. The steam flow rate was manually adjusted using a steam flow valve. Once the steam passed through the electrical superheaters (where the steam temperature was adjusted to the required value) it passed through the drying chamber, which housed the oat groat sample, and then on to the condenser. Steam velocity was determined based on the rate of collected condensate (collected in a drain pan and measured using a 100ml-graduated cylinder) and known steam properties (Tang and Cenkowski, 2000). The flow rate equation is shown below. Heating studies were also performed at 130 and 145°C with a steam velocity of 0.52 ± 0.01 m/s. Treatment times ranged from 10 to 40 min allowing for a come-up time of 30s. D-values were calculated using the reciprocal of the slope of the survivor curve.

Steam Flow Rate Equation:

$$\frac{W(g/h) \times V''(m^3kg^{-1}) \times 1/3600(s/h) \times 1/1000(g/kg)}{A(m^2)} = m/s$$

Where
W = collected water (g/h)
V" = specific volume of condensate (m^3kg^{-1})
A = intersection area of valve opening (m^2)

3.4.2 Superheated steam treatment of non-tempered (12 % moisture content) and tempered (17 % moisture content) oat groats

Oat groats (12 or 17 % moisture content; 9 g) and sand-spore mixture (1g, 10^4 or 10^7 cfu /g) were placed on a sample holder consisting of an aluminum ring with two wire mesh screens. The sample holder was subsequently placed in the processing chamber and exposed to SS at temperatures previously specified. Samples were heated for 30 s before actual timing was initiated (come-up time). All tests were performed in triplicate. Following treatment the sand spore mixture was transferred to dilution bottles using an alcohol flamed spatula. The wire mesh assembly was removed from the aluminum ring and sanitized in 95% ethanol between treatments.

3.5 Chemical analysis

3.5.1 Moisture determination of oat groats

Moisture content of the groat samples (10 g) was determined following heating at 130 °C for 22 h in a forced air oven (ASAE, 2000). All analyses were performed in duplicate. Groats following kiln drying (as supplied by the producer) had an initial moisture content of approximately 12%.

3.5.1.1 Tempering of oat groats

Groat samples were weighed into sterilized glass airtight containers and predetermined amounts of sterile distilled water were added to raise the moisture content up to 17%. Containers were shaken by hand every 10 min for the first hour. Tempering was performed overnight at 4°C. The required amount of tempering water was calculated using the following formula (AACC method 26-95, 2003):

$$\text{Weight of Water to add} = \frac{100 - \text{original moisture (\%)}}{100 - \text{desired moisture (\%)}} - 1 \times \text{weight of sample}$$

3.6 Enzyme analysis

3.6.1 Qualitative peroxidase analysis

SS treated samples were evaluated for peroxidase activity (AACC method 22-80, 2003). In this respect oat groats (10 g) were ground using a coffee grinder for 30 s and sifted using a # 20 screen sieve (850 μm opening) . Sample remaining on the screen was again ground, sifted and added to the original ground sample. Fifty ml of distilled, room temperature water was combined with 1 g of ground sample in a flask to which was added 2 ml of ascorbic acid (0.57 M), 3 ml of 2,6-dichloroindophenol sodium salt (0.003 M) and 0.1ml of hydrogen peroxide (4 ml of 30% H_2O_2 in 96 ml water). Following heating in a water bath at 38°C for 10 min with intermittent shaking, samples were observed for the absence of a blue color which indicated no peroxidase activity. All analyses were performed in duplicate.

3.7 Color analysis

Color measurements of SS treated groats and oat wholemeal flour were determined using a Minolta ChromaMeter 210 (Minotla Canada Inc., Mississauga, Ontario) equipped with a granular-materials attachment CR-A50. Colors were represented in "L", "a","b" values of the Hunter system. The L-value shows the lightness or darkness; "a" represents the redness (-a implies greenness) and "b" measures the yellowness (-b implies blueness). The L-value varies from 0 (black) to 100(white) while the *a*- and *b*- values represent the same spectrum range from -60 to 60. The colorimeter was standardized using a white ceramic plate. The total color difference was calculated from the means at each time interval using the following equation (Hunter Lab, 1996):

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

where:

ΔE = total color difference

ΔL, Δa, and Δb = difference between standard reading (raw sample at time 0) and sample readings for L-value, a-value and b-value at each time interval.

3.7.1 Whole meal flour preparation

Whole meal samples were prepared by grinding (14,000 rpm) the groat samples using a Retsch ZM 100 grinder (Retsch GmbH 7 Co., KG, Haan, Germany) equipped with a 0.5 mm screen. Samples were store in re-sealable polyethylene bags at -40°C.

3.8 Functional analysis

3.8.1 Pasting characteristics of oat whole meal- standard cold pasting profile

For standard cold pasting evaluation, duplicate sub-samples of wholemeal flour were tested using a Rapid Visco Analyzer (RVA); series 4; (Newport Scientific Pty. Ltd., Warriewood Australia). Briefly, the method involved mixing 3 g of whole meal and 25 g of water (corrected to 14 % moisture content) over the following temperature-time profiles: 2 min at 25 °C; ramped up to 95 °C over 5 min; held at 95 °C for 3 min; ramped down to 25 °C over 5 min; held at 25 °C for 7 min resulting in a total run time of 22 min. During the run time, viscosity was recorded in RVA units (RVU) where 1 RVU is approximately equal to 12 centipoise. One or more of the following parameters were used to characterize the properties of the whole meal flour. Cold pasting: viscosity before heating (25 °C for 2 min); peak viscosity: highest viscosity attained by heating the sample to 95°C; trough: lowest viscosity attained during mixing of the sample held at 95°C. Breakdown was calculated by subtracting trough from peak values; final viscosity; viscosity at the end of testing; setback was calculated by subtracting trough from final; final peak; calculated by subtracting peak from final; shear thinning; breakdown expressed as percentage of peak viscosity. This method is referred to as Standard 1 Pasting Profile in AACC Method 76-21 (AACC 2003). An example of a typical RVA pasting curve showing the commonly measured parameters is shown in Figure 5.

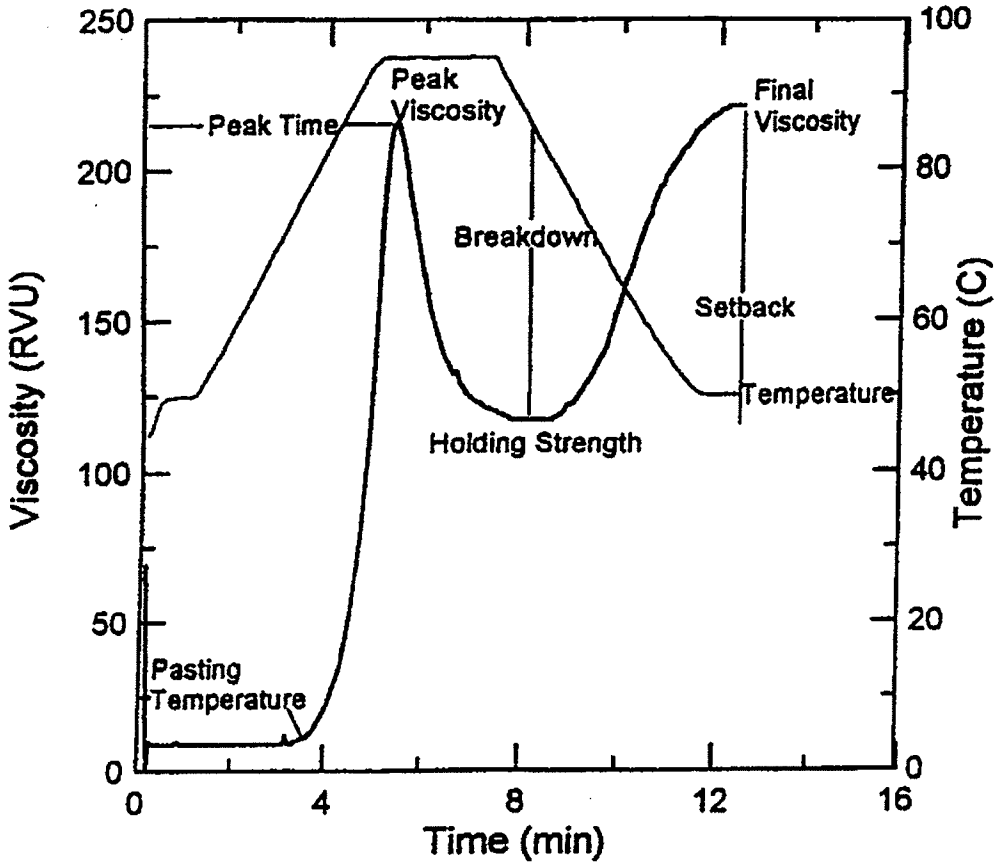

Figure 5 Example of RVA Pasting Curve for Oat Flour

3.9 Statistical Analysis

All data were analyzed using the Statistical Analysis Software (SAS) computer program, Version 8.2 (SAS Institute, Cary, NC). A multi-factorial analysis of variance (ANOVA) was performed on the samples treated with SS at 130 and 145°C at various times, velocities and moisture content. Mean differences were performed by Tukey's HSD test ($p<0.05$) on samples treated with SS at 115,121,160 and 175°C at an initial moisture content of 12 % and oat groats tempered to 17% moisture content.

4 RESULTS

4.1 Microbial analysis of commercial oat groats samples

Microbiological results for the oat groats taken before and after a 2 hour kiln treatment at 100°C are presented in Tables 4 and 5. Only 11 of the 30 samples (37 %) taken before kiln treatment contained thermophilic spores (range from 0 to 3.2 $\log_{10}$ cfu/ 10g). Ten of these samples were also positive for flat sour spores (range from 0 to 3.2 $\log_{10}$ cfu/ 10 g). In contrast, 26 of the 30 samples (86 %) analyzed following kiln treatment contained thermophilic spores (range from 0 to 3.6 $\log_{10}$ cfu/10g). Results for flat sour spores were identical.

The SPC for untreated samples ranged from $<10^1$ to 10^6 cfu/g with a mean value of 4.7 $\log_{10}$ cfu/g. Approximately 66% of the samples had values > 3 $\log_{10}$ cfu/g. Following treatment approximately 27 of the 30 samples or 90 % contained lower SPC results with an average of 2.7 $\log_{10}$ cfu/g. In this respect approximately 40% of the samples contained an SPC value > 3 $\log_{10}$ cfu/g.

Yeast and mold counts ranged from $<10^1$ to 10^6 cfu/g in untreated samples with a mean value of 3.2 $\log_{10}$ cfu/g. Approximately 16 or 53 % of the samples tested prior to treatment contained yeast and mold counts > 10^3 cfu/g. Following treatment the mean value was reduced to 2.1 $\log_{10\ cfu}$/g and only 5 or 16 % of the samples were $>10^3$ cfu/g.

Only 16 samples were assessed for coliforms all of which were positive. Values ranged from 10^1 to 10^6 cfu/g with a mean of 5.4 $\log_{10}$ cfu/g. Following treatment samples ranged from $< 10^1$ to 10^2 cfu/g with a mean of 2.3 $\log_{10}$ cfu/g.

Table 4. Microbiological analyses of oat groat samples before kiln treatment at 100°C. Kiln transit time = 2h.

Sample no.	Log_{10}cfu/g				
	Thermophilic[1]	Flat Sour[1]	Standard Plate Count[2]	Yeast & Molds[2]	Coliforms[2]
1	0	0	2.9	2.5	ND
3	3.2	3.2	2.5	2.5	ND
5	0	0	5.1	0	ND
7	2.4	2.4	2.5	0	ND
9	0	0	5.2	0	ND
11	2.7	2.7	0	0	ND
13	0	0	4.8	2.5	ND
15	2.4	2.4	0	2.5	ND
17	0	0	2.5	2.6	ND
19	1.7	1.7	2.5	2.5	ND
21	0	0	5.7	5.7	ND
23	0	0	5.9	4.1	ND
25	0	0	4.9	4.1	ND
27	0	0	5.4	5.2	ND
29	0	0	6.4	4.1	3.5
31	0	0	6	6.1	4.9
33	1.7	1.7	6	3.5	4.4
35	0	0	6.2	3.7	3.5
37	1.7	0	6.3	5.6	6
39	0	0	6	4.6	6.8
41	3	3	6.1	4.6	5.9
43	0	0	3.5	4.6	6.3
45	0	0	3.5	2.2	6.4
47	0	0	6.4	4.7	6.3
49	3.1	3.1	5.7	3.5	5.5
51	2.2	2.2	6	3.6	5.9
53	1.7	1.7	6	3.5	5.8
55	0	0	6.4	2.5	3.5
57	0	0	4.9	2.5	4.8
59	0	0	6	2.5	6.2
Std	1.2	1.2	1.9	1.7	1.1
Mean	0.9	0.8	4.7	3.2	5.4

[1]Results are means of one trial plated in quintuplicate ± standard deviations of means

[2]Results are means of one trial plated in quintuplicate ± standard deviations of means

[3] Not determined

Table 5. Microbiological analyses of oat groat samples after kiln treatment at 100°C. Kiln transit time = 2h.

Sample no.	Log_{10}cfu/g				
	Thermophilic[1]	Flat Sour[1]	Standard Plate Count[2]	Yeast & Molds[2]	Coliforms[2]
2	0	0	3.1	2.5	ND
4	2.4	2.4	0	3	ND
6	0	0	4.5	2.5	ND
8	2.3	2.3	2.5	0	ND
10	2.7	2.7	5.9	3.3	ND
12	2.4	2.4	0	0	ND
14	3.1	3.1	2.5	2.8	ND
16	2.5	2.5	0	2.5	ND
18	0	0	6	3.1	ND
20	2.2	2.2	2.5	2.5	ND
22	3.1	3.1	2.5	2.5	ND
24	3.5	3.5	2.7	2.5	ND
26	3.5	3.5	2.5	2.5	ND
28	3.6	3.6	2.5	2.5	ND
30	3	3	2.5	2.5	2.5
32	0	0	2.5	5.2	2.5
34	3.4	3.4	2.5	5.2	0
36	2.7	2.7	2.5	2.5	2.5
38	3.3	3.3	3.3	2.5	2.5
40	2.3	2.3	2.5	2.5	2.5
42	3.3	3.3	3.5	2.5	2.5
44	3.6	3.6	3	2.5	2.5
46	2.9	2.9	2.5	2.5	2.5
48	3.7	3.7	3.5	2.5	2.5
50	2.9	2.9	2.5	0	2.5
52	3	3	2.9	0	2.5
54	3.1	3.1	2.5	0	2.5
56	3.5	3.5	2.5	0	2.5
58	2.7	2.7	2.9	0	2.5
60	3	3	2.5	0	2.5
Std	1.12	1.12	1.30	1.45	0.63
Mean	2.6	2.6	2.7	2.1	2.3

[1]Results are means of one trial plated in quintuplicate ± standard deviations of means

[2]Results are means of one trial plated in quintuplicate ± standard deviations of means

[3] Not determined

4.1.1 Microbial analysis of kiln wall samples

Thermophilic and flat sour spore counts taken from samples lining the interior of the kiln are given in Table 6. Samples taken from the lower cooling sections (K5 and K6) appeared to contain higher numbers of both thermophilics and flat sour spores compared to the upper heating regions. Additionally, samples taken at the inlet of the kiln (K1) were negative for spore counts that are 0 per 10 g.

4.2 Isolate identification

Biochemical differentiation of spores recovered revealed the presence of *G. stearothermophilus*. Three isolates were identified (Appendices 1 and 2).

4.3 Superheated steam (SS) treatment of *G. stearothermophilus* spores in oat groats

4.3.1 Preliminary studies

Preliminary studies were performed using SS at 115, 121, 160 and 175°C. These temperatures were arbitrarily chosen in order to explore the effectiveness of SS with respect to spore decontamination in oat groats.

The survival curves for *G. stearothermophilus* ATCC 10149 heated at 115 and 121° C in oat groats (12% moisture content) and the corresponding D values are shown in Figure 6 and Table 7,

Table 6. Thermophilic and flat sour sporeformers in samples taken from the inside of the Can-Oat Milling kiln. See Figure 3 for a schematic of the kiln.

Samples	Spores (cfu/10g)[1]	
	Thermophilic	Flat sour
K1 (7th floor)[2]	ng[3]	ng[3]
K2 (6th floor)[4,5]	2.40	2.40
K3 (5th floor) [4,5]	2.30	2.30
K4 (5 1/2 floor)[4,5]	3.13	3.13
K5 (6 1/2 floor)[6]	TNTC[7]	TNTC[7]
K6 (cooling)[6]	TNTC[7]	TNTC[7]

[1 6] All results are the means of two trials, each performed in duplicate.

[2] 7th floor is the top floor of the kiln, where the raw groats enter the system.

[3] No growth occured

[4] The 6th, 5th and 5 1/2 floors are levels of the heating sections of the kiln.

[5] Temperatures at these sections are at 100°C.

[6] There are 3 cooling sections in the kiln, where no heating occurs, only air movement.

[7] Too numerous to count.

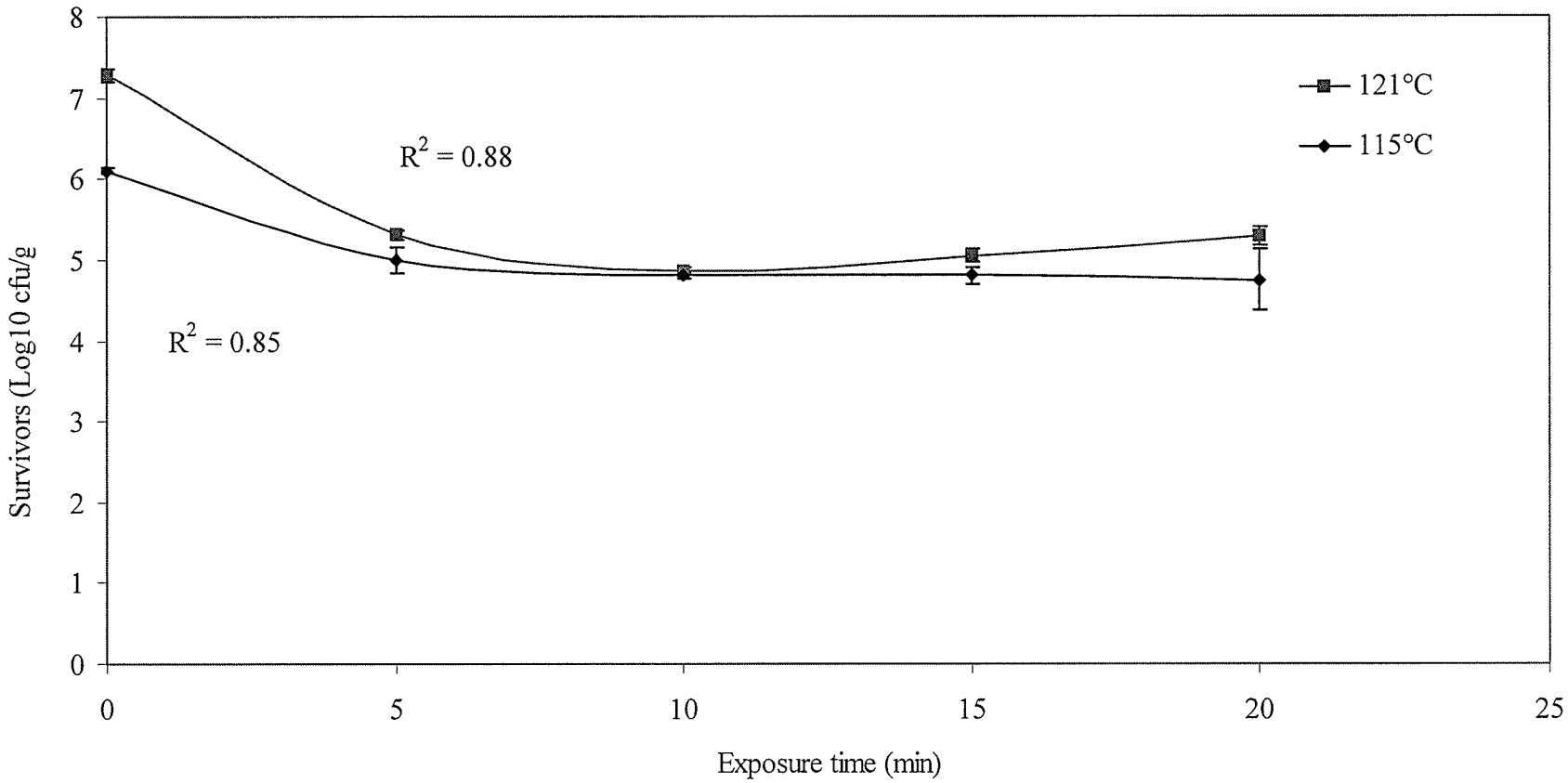

Figure 6. Survival of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats (12% moisture content) following treatment with superheated steam at 115°C and 121°C at a velocity of 1.10 m/s. Error bars represent the standard deviation of three trials each performed in duplicate (n=6). See Appendix 3.

Table 7. D values for *Geobacillus stearothermophilus* ATCC 10149 spores on oat groats (12% m.c) heated for 5 and 10 min with superheated steam (1.10m/s), at various temperatures.

Temperature (°C)	[1]D value (min) after treatment (min)	
	5	10
115	4.5	7.7
121	2.5	4
160	2.7	1.93
175	1.2	2

[1] Means of three trials, each performed in duplicate (n=6)

Table 8. D values for *Geobacillus stearothermophilus* ATCC 10149 spores on oat groats following treatment with superheated steam at 130°C and 145°C.

Temperature (°C)	*Treatment	*D value* (min) after treatment (min)	
		10	20
130	12% m.c; 0.52 m/s; 10^4	5.7	11.8
	17% m.c; 0.52 m/s; 10^4	4.7	9.3
	12% m.c; 1.10 m/s; 10^4	5.8	10.8
	17% m.c; 1.10 m/s; 10^4	5.5	10.6
	12% m.c; 0.52 m/s; 10^7	4.9	10.8
	17% m.c; 0.52 m/s; 10^7	6.6	11.1
	12% m.c; 1.10 m/s; 10^7	5.8	10.7
	17% m.c; 1.10 m/s; 10^7	5.2	12
145	12% m.c; 0.52 m/s; 10^4	5.4	10.9
	17% m.c; 0.52 m/s; 10^4	5.3	9.7
	12% m.c; 1.10 m/s; 10^4	4.5	8.4
	17% m.c; 1.10 m/s; 10^4	5.1	9
	12% m.c; 0.52 m/s; 10^7	4.8	9.3
	17% m.c; 0.52 m/s; 10^7	7.2	11.4
	12% m.c; 1.10 m/s; 10^7	4.8	9
	17% m.c; 1.10 m/s; 10^7	4.5	7.8

* Moisture content, velocity, inoculum level (cfu/g).

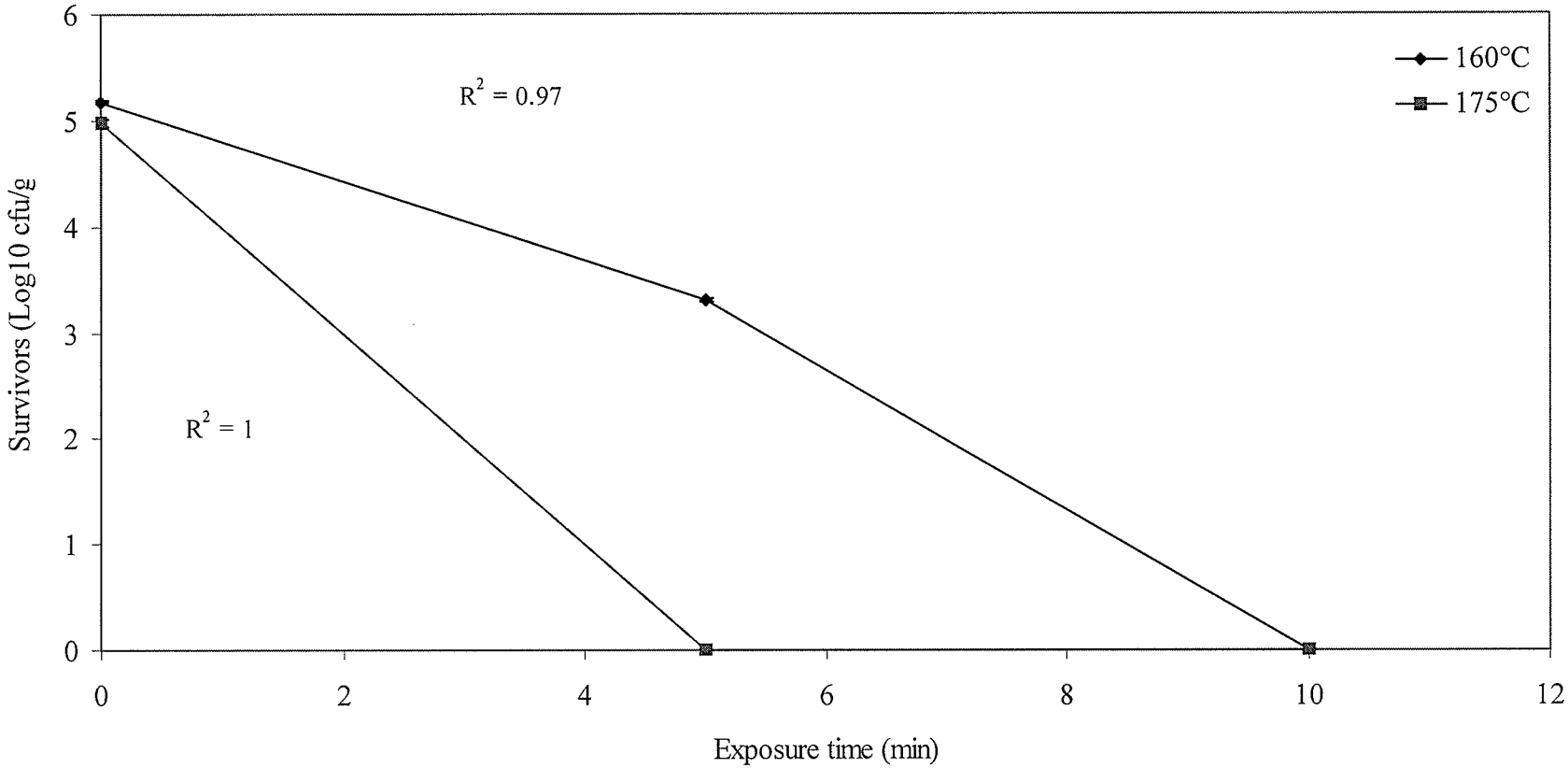

Figure 7. Survival of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats (12% moisture content) following treatment with superheated steam at 160°C and 175°C at a velocity of 1.10 m/s. Error bars represent the standard deviation of three trials each performed in duplicate (n=6). See Appendix 4.

respectively. Overall, the maximum rate of lethality at both temperatures occurred within 5 min of treatment. Thereafter both survivor curves exhibited a "tailing" appearance. Heating at 121°C resulted in an approximate 2.5 log maximum decrease in spores over the time course. D values for spores heated at 121 and 115°C and calculated at 5 min of treatment were 2.5 and 4.5 min, respectively.

Increasing the temperature to 160 and 175 °C was very effective in reducing spore levels (Figure 7). In both cases a 5 $\log_{10}$ cfu/g spore reduction was achieved at or within 10 min. $D_{160°C}$ and $D_{175°C}$ values were 2.7 and 1.2 min, respectively (Table7). Unfortunately at the latter temperatures the groats appeared crispy and burnt and were deemed unfit for further use.

4.3.2 Effect of a low inoculum application of *Geobacillus stearothermophilus* ATCC 10149 spores to oat groats heated at 130°C.

Survivor curves for spores heated at 130°C are presented in Figure 8. Based on the D values, 10^4 cfu/g spores treated at 130°C with a steam velocity of 0.52 m/s in groats containing 17% moisture content were less resistant compared to their counterparts heated in 12% moisture content groats (Figure 8, Table 8). In both samples, the major decrease in spore populations was observed at or within 10 min of treatment. Thereafter decreases in spore viability became less apparent. Examination of both survivor curves indicated an apparent tailing especially after 20 min.

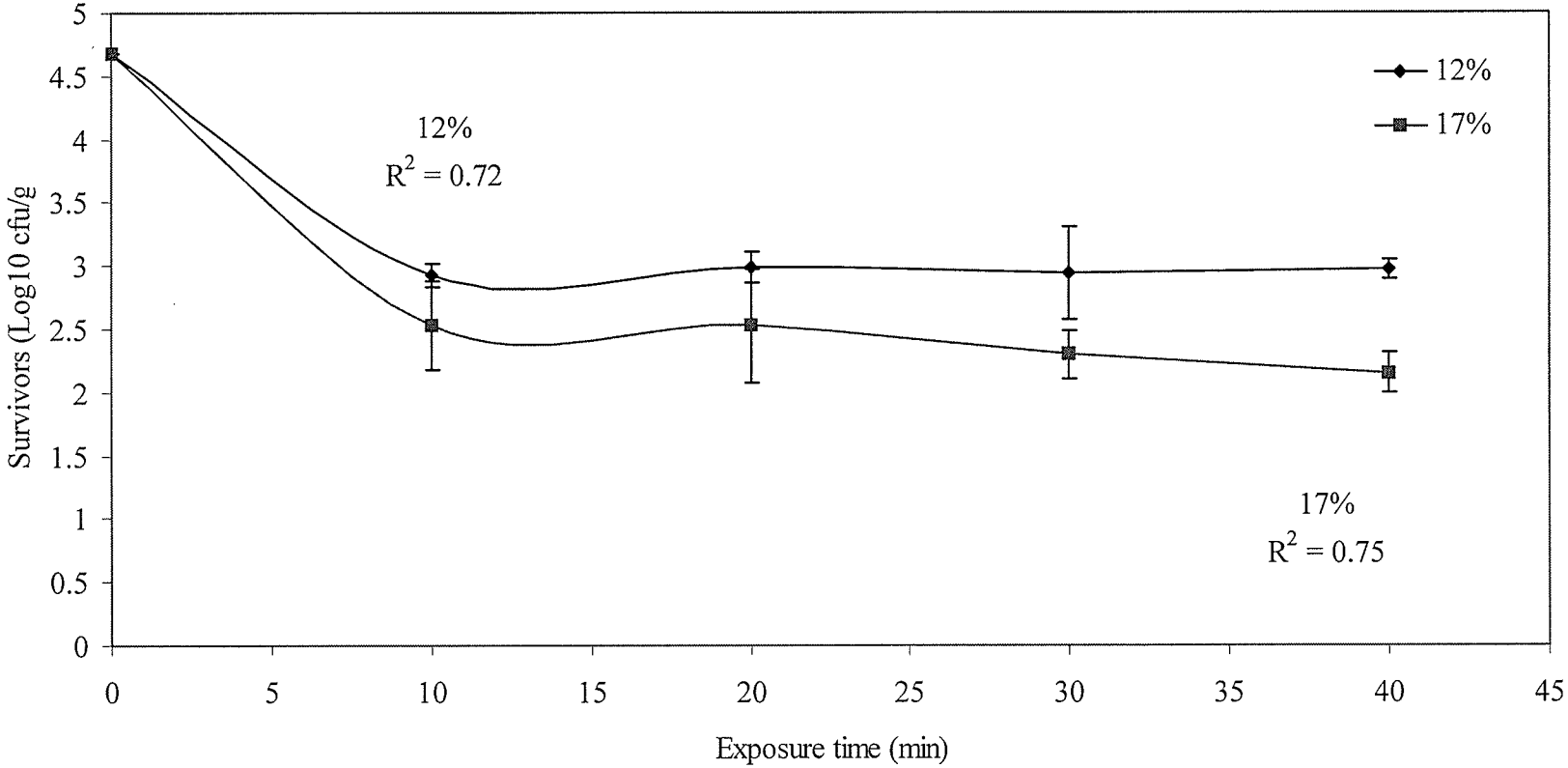

Figure 8. Survival of a 10^4 cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 130°C at a velocity of 0.52 m/s. Error bars represent the standard deviation of three trials each performed in duplicate (n=6). See Appendix 5.

Increasing the velocity of SS from 0.52 to 1.10 m/s appeared to have little effect on the resistance of spores heated at 130° C (Figure 9). D values calculated after 20 minutes for spores heated in 12% groats especially at 10 min (Table 8) indicated minimal effects due to an increase in steam velocity. Also, based on the D values (5.8 and 5.5 at 10 min for 12 and 17 % groats, respectively) and shape of the survivor curves, differences in spore resistance between the different moisture containing samples were not apparent.

4.3.3 Effect of a low inoculum application of *Geobacillus stearothermophilus* ATCC 10149 spores to oat groats heated at 145°C.

Increasing the SS temperature from 130° to 145°C also appeared to have minimal effects on spore (10^4cfu/g) viability (Figure 10). D values at 10 min (Table 8) were 5.4 and 5.3 min respectively for the 12 and 17% moisture content containing samples. Doubling the velocity rate of the SS treatment also appeared to have a minimal effect on spore resistance at 145°C (Table 8). In addition, spore lethality did not appear to differ between the different moisture content groats (Figure 11). Overall, spore populations were reduced by approximately 2 logs during the first 10 min of treatment at 145°C and in no case was the reduction higher than 2.5 logs.

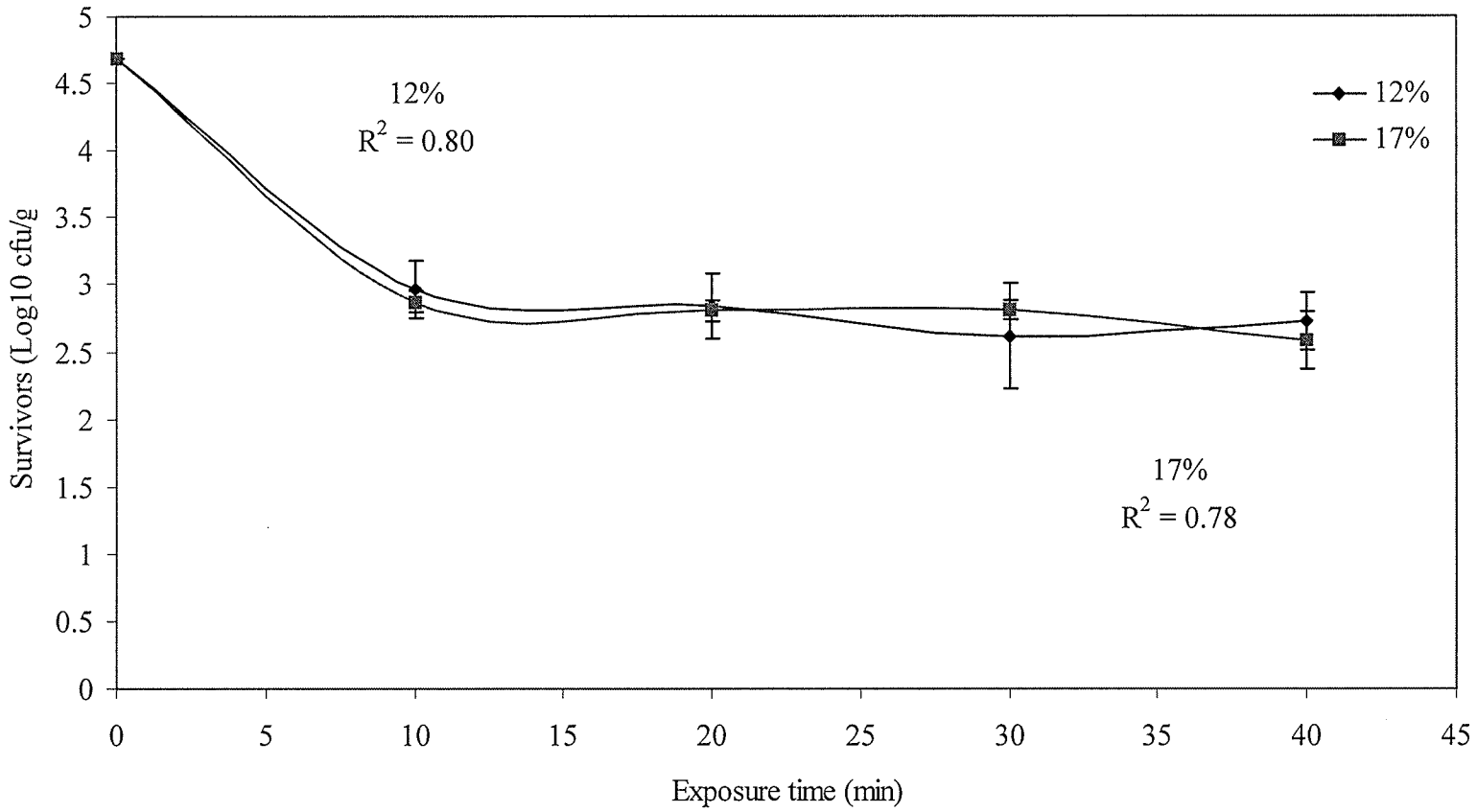

Figure 9. Survival of a 10^4cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 130°C at a velocity of 1.10 m/s. Error bars represent the standard deviation of three trials each performed in duplicate (n=6).See Appendix 6.

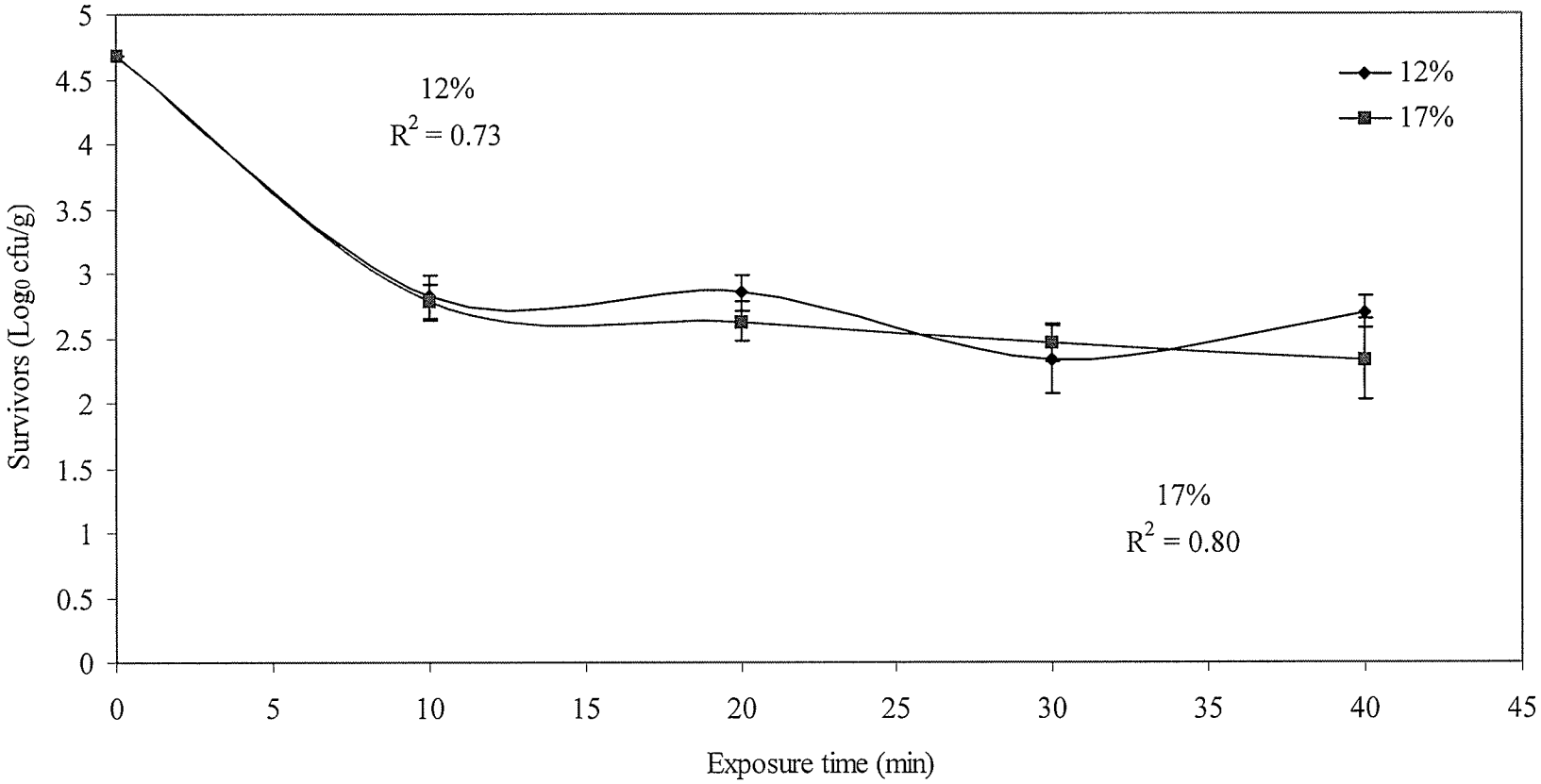

Figure 10. Survival of a 10^4 cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 145°C at a velocity of 0.52 m/s. Error bars represent the standard deviation of three trials each performed in duplicate (n=6). See Appendix 7.

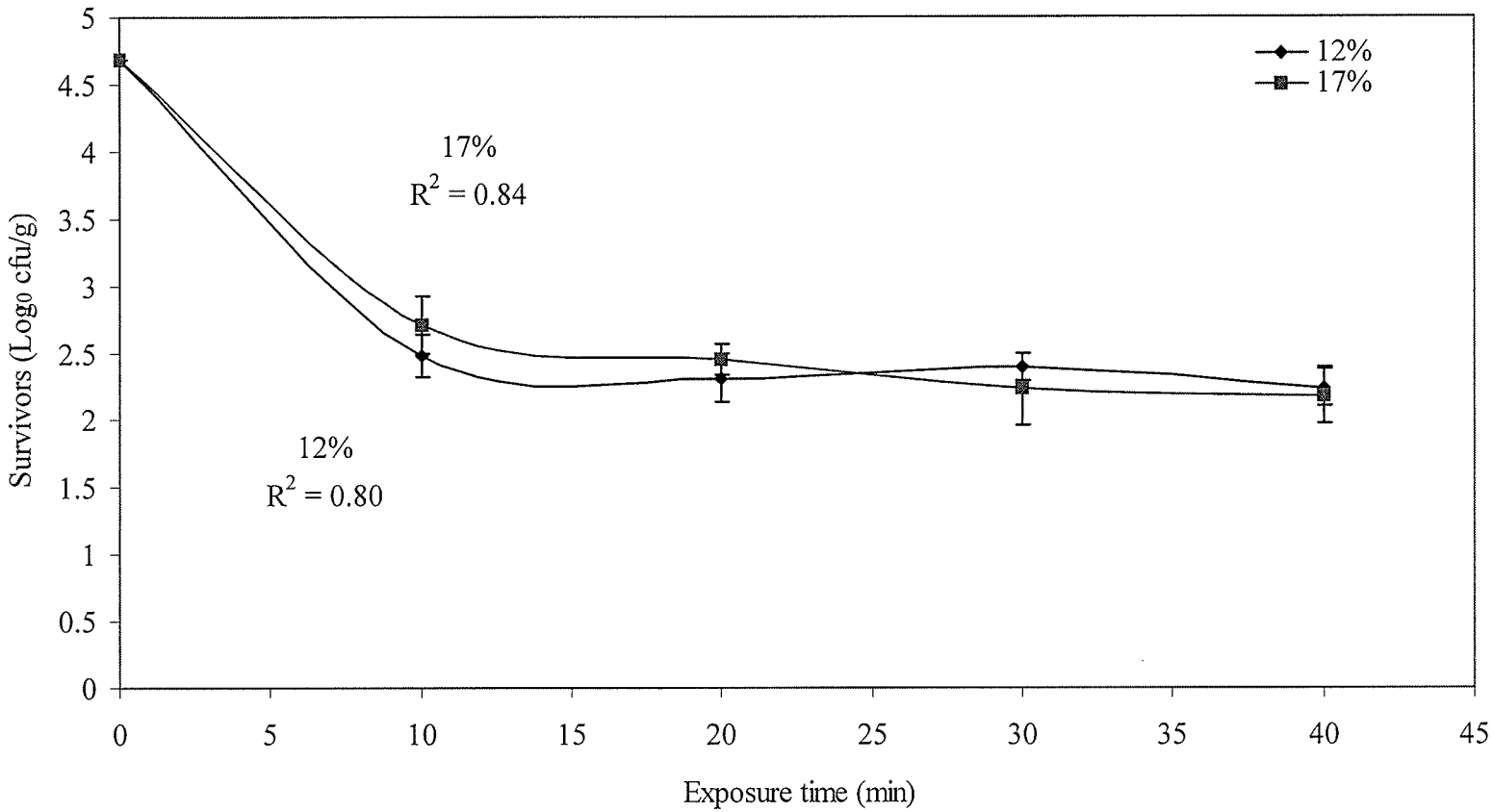

Figure 11. Survival of a 10^4 cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 145°C at a velocity of 1.10 m/s. Error bars represent the standard deviation of three trials each performed in duplicate (n=6). See Appendix 8.

4.3.4 Effect of a high inoculum application of *G. stearothermophilus* ATCC 10149 spores to oat groats heated at 130°C.

Spore survivor curves for groats inoculated with 10^7 cfu/g and heated at 130 °C with steam velocities of 0.52 and 1.10 m/s are given in Figures 12 and 13, respectively. Overall differences in the survivor curves based on groat moisture content appeared minimal even though D values obtained for the higher moisture content groats were lower (Table 8). Increasing the steam velocity also appeared to have a minimal effect on spore lethality.

4.3.5 Effect of a high inoculum application of *G. stearothermophilus* ATCC 10149 spores to oat groats heated at 145°C.

Survivor curves for spores treated at 145 °C with SS at 0.52 and 1.10 m/s in 12 and 17 % moisture content groats is illustrated in Figures 14 and 15, respectively. As previously observed, the highest reduction in spore viability was observed at or within the first 10 min of treatment. Compared to SS at 130° C, treatment at 145 °C especially at 1.10 m/s appeared as the most effective sporicidal treatment based on total spore reduction (Appendix 10). In this respect an approximate 3.5 $\log_{10}$ decrease was obtained at 40 min of heating.

The significance values for the factorial analysis of this study which consisted of: velocity (2 levels), inocula (2 levels), moisture (2 levels), temperature (2 levels) and time (4 levels) are given in Tables 9, 10 and 11 (Appendices 14 & 15). The moisture content of the groats appeared as the only main factor which did not significantly affect spore destruction.

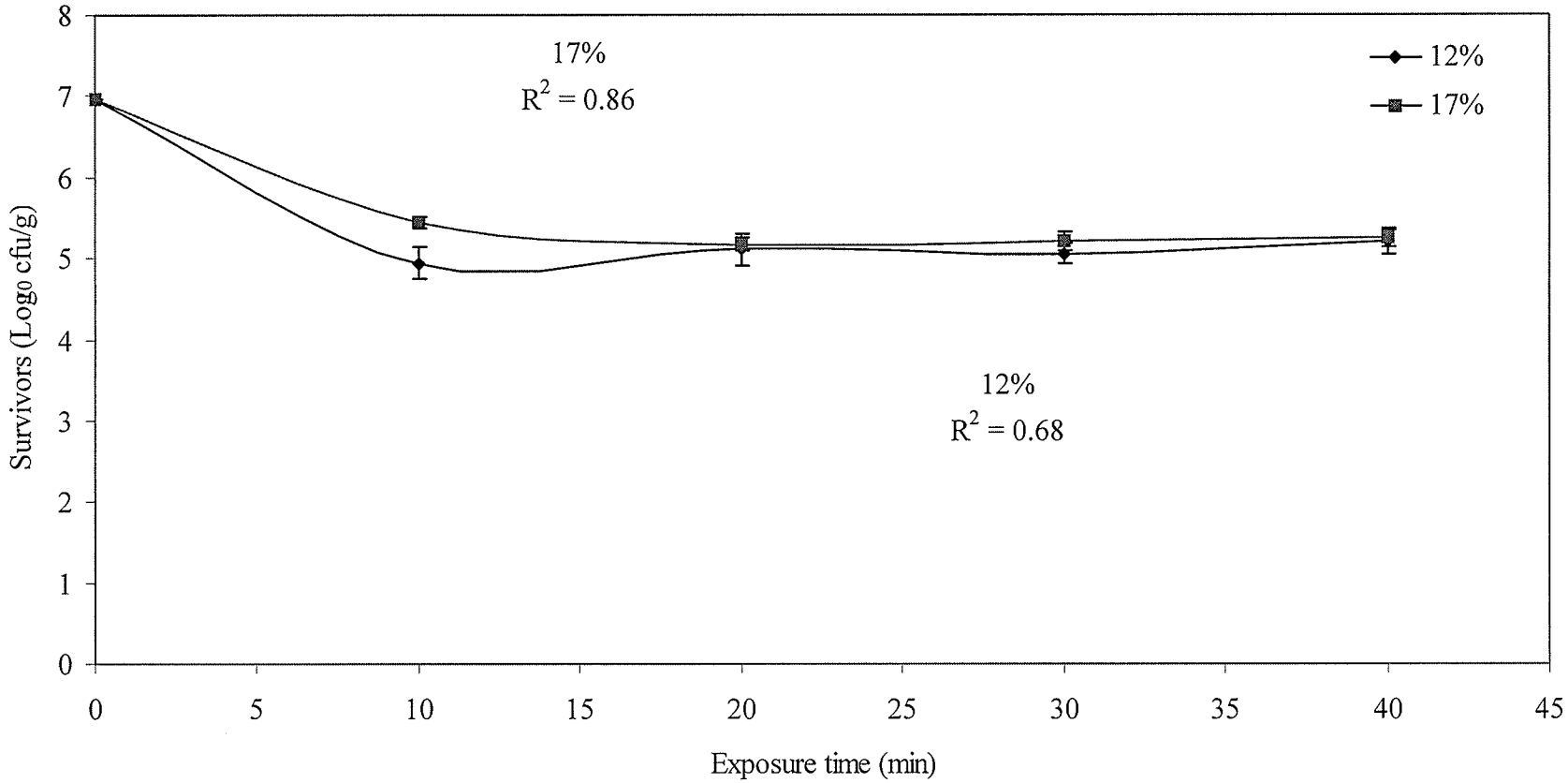

Figure 12. Survival of a 10^7 cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 130°C at a velocity of 0.52 m/s. Error bars represent the standard deviation of three trials each performed in duplicate (n=6). See Appendix 9.

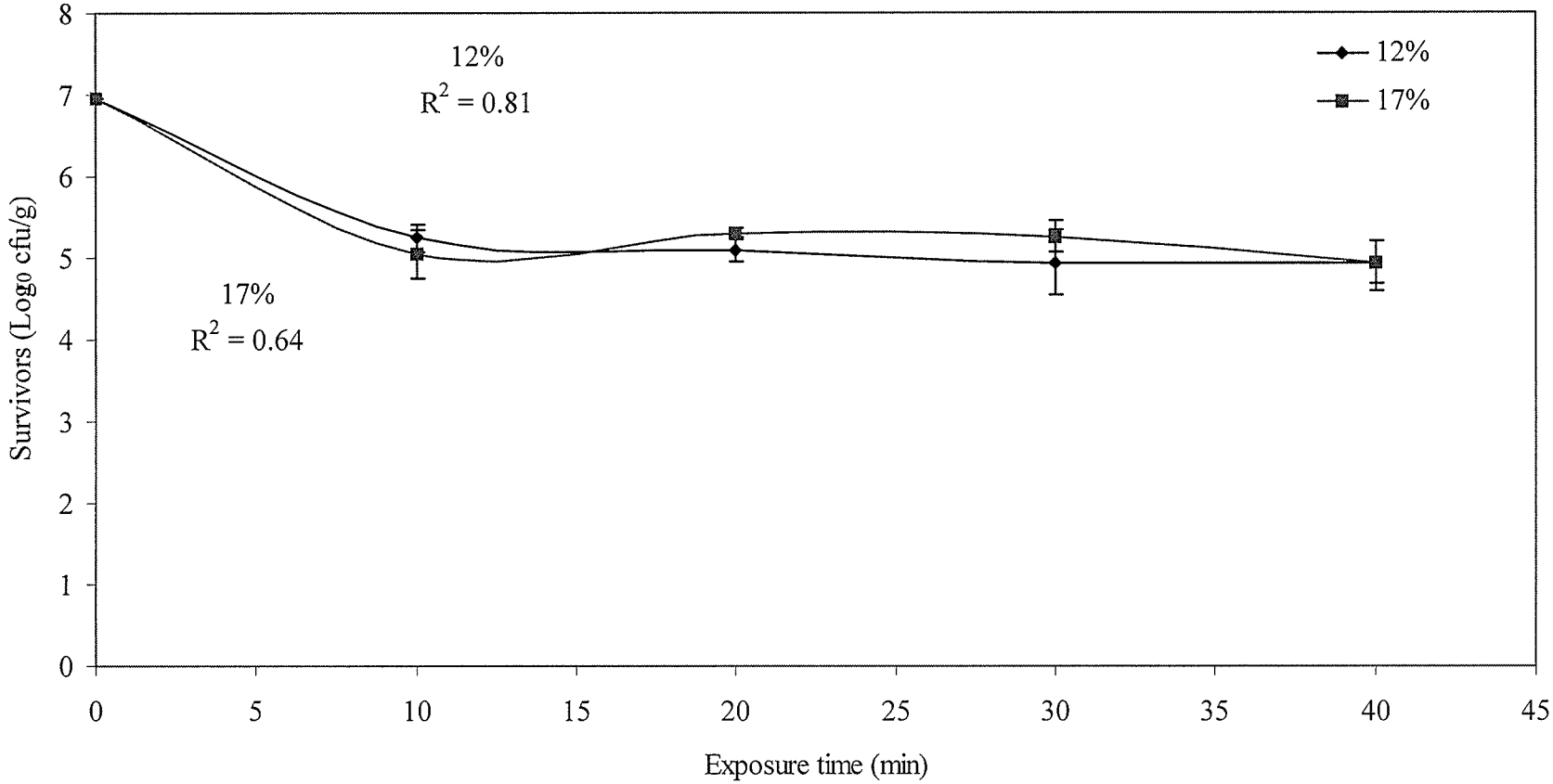

Figure 13. Survival of a 10^7 cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 130°C at a velocity of 1.10 m/s. Error bars represent the standard deviation of three trials each performed in duplicate (n=6). See Appendix 10.

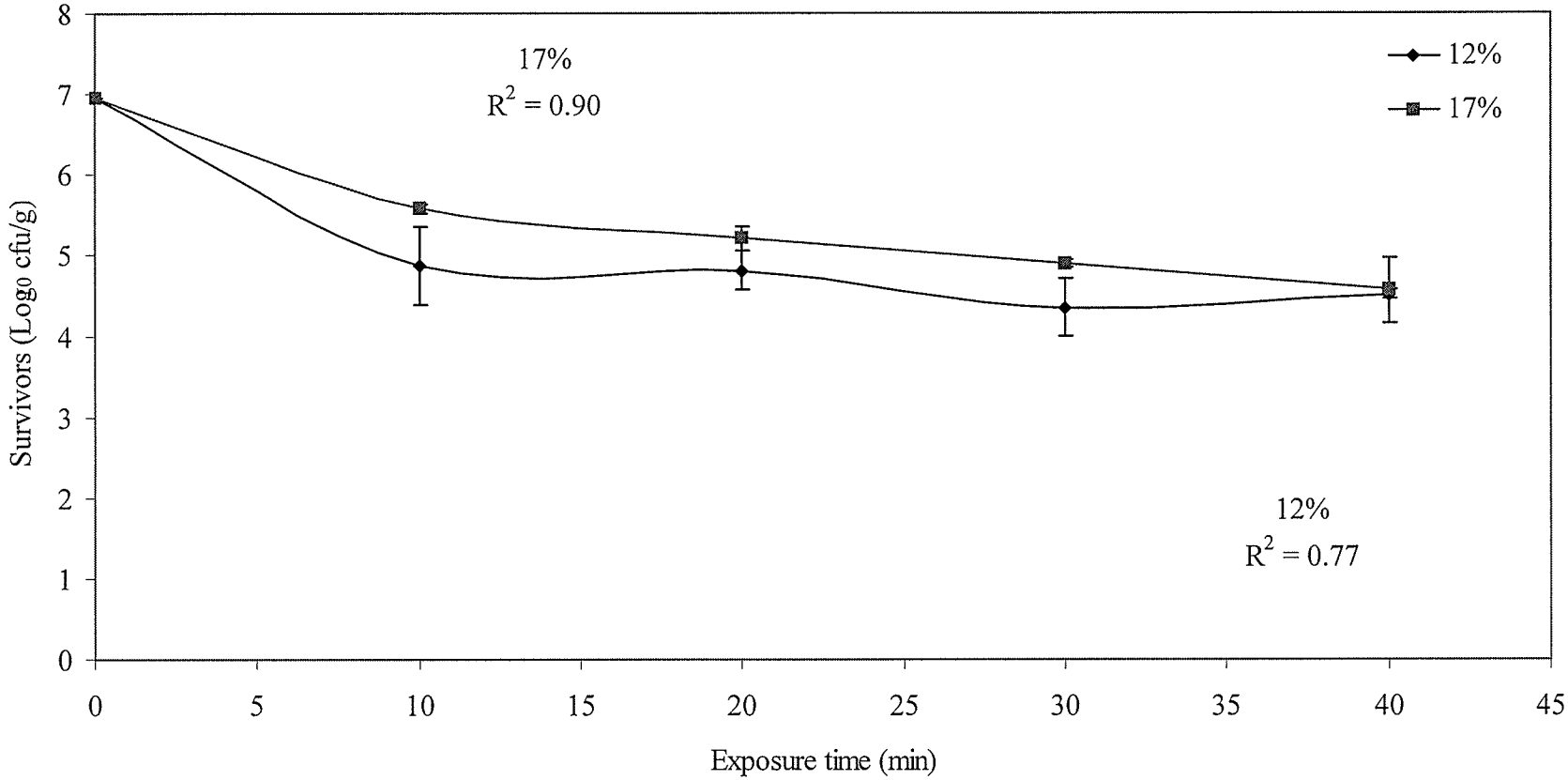

Figure 14. Survival of a 10^7 cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 145°C at a velocity of 0.52 m/s. Error bars represent the standard deviation of three trials each performed in duplicate (n=6). See Appendix 11.

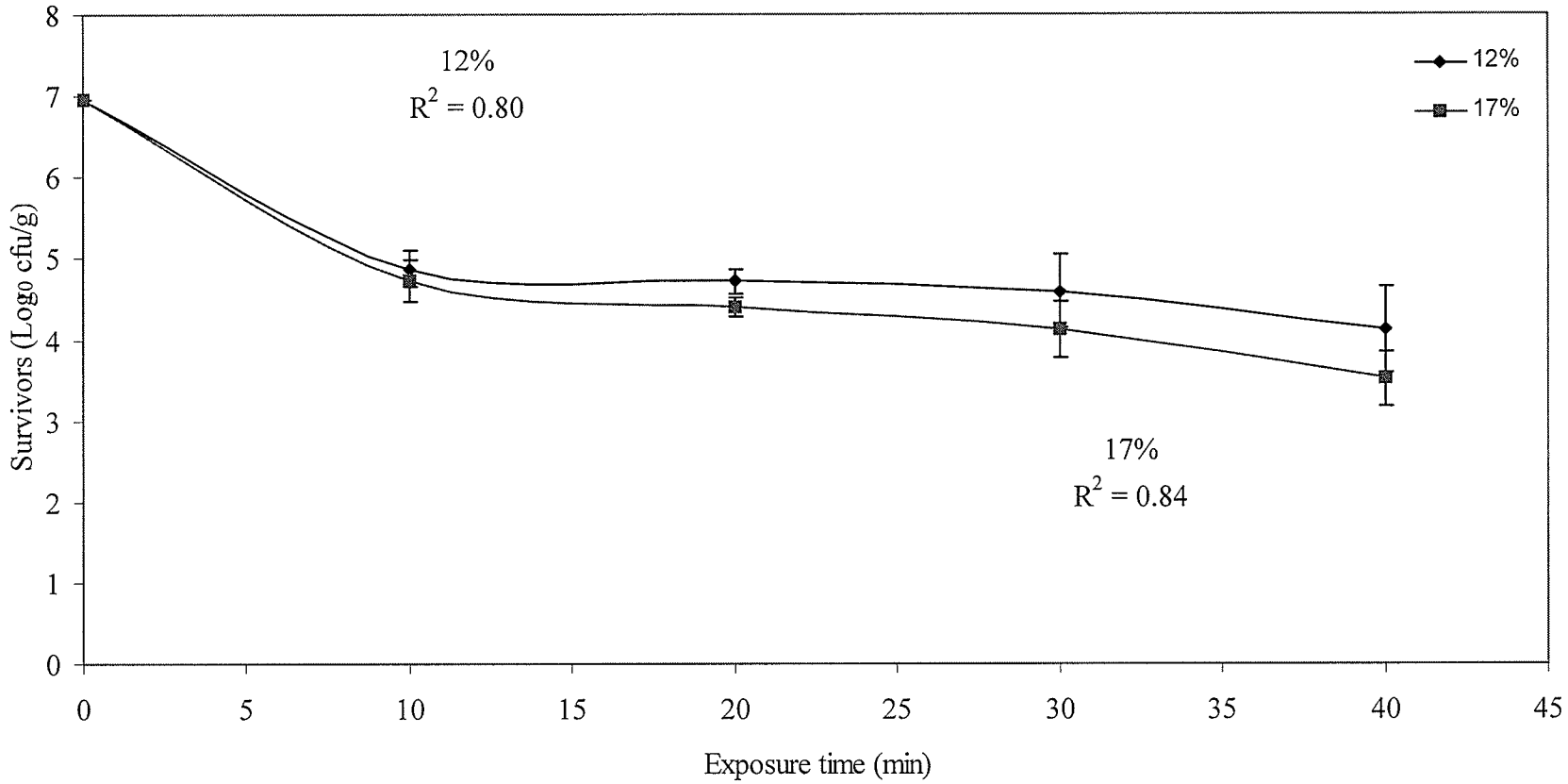

Figure 15. Survival of a 10^7cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 145°C at a velocity of 1.10 m/s. Error bars represent the standard deviation of three trials each performed in duplicate (n=6). See Appendix 12.

Table 9. Summary of treatment effects for oat groats treated with superheated steam.

Temperature and Inoculum level	Effect	P Value
130°C, low inoculum	Velocity	0.0371
	Moisture	<0.0001
	Time	<0.0001
	Vel*Moisture	<0.0001
	Vel*Time	0.6575
	Moisture*Time	0.0076
	Vel*Moisture*Time	0.0119
145°C, low inoculum	Velocity	<0.0001
	Moisture	0.4736
	Time	<0.0001
	Vel*Moisture	0.0193
	Vel*Time	0.0008
	Moisture*Time	0.1668
	Vel*Moisture*Time	0.0039
130°C, high inoculum	Velocity	0.0561
	Moisture	0.0013
	Time	<0.0001
	Vel*Moisture	0.1763
	Vel*Time	0.0159
	Moisture*Time	0.1749
	Vel*Moisture*Time	0.0004
145°C, high inoculum	Velocity	<0.0001
	Moisture	0.735
	Time	<0.0001
	Vel*Moisture	<0.0001
	Vel*Time	0.0005
	Moisture*Time	0.0166
	Vel*Moisture*Time	0.0217

Table 10. Summary of treatment effects for the 10^4 inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores on oat groats and exposed to superheated steam.

Main Effect	P Value
Time	<.0001
Moisture content	<.0001
Temperature	<.0001
Velocity	0.0182

2 Way Interactions	P Value
Time*Moisture content	0.0023
Time*Temperature	0.0011
Time*Velocity	**0.0981**
Moisture content*Temperature	<.0001
Moisture content*Velocity	<.0001
Temperature*Velocity	<.0001

3 Way Interactions	P Value
Time* Moisture content*Temperature	**0.2134**
Moisture content*Temperature*Velocity	0.0037
Time*Temperature*Velocity	0.0252
Time*Moisture content*Velocity	0.0163

4 Way Interactions	P Value
Time*Moisture content*Temperature*Velocity	0.0025

Table 11. Summary of treatment effects for the 10^{6-7} inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores on oat groats and exposed to superheated steam.

Main Effect	P Value
Time	<.0001
Moisture content	0.0339
Temperature	<.0001
Velocity	<.0001

2 Way Interactions	P Value
Time*Moisture content	0.0049
Time*Temperature	<.0001
Time*Velocity	<.0001
Moisture content*Temperature	**0.1172**
Moisture content*Velocity	<.0001
Temperature*Velocity	<.0001

3 Way Interactions	P Value
Time* Moisture content*Temperature	**0.1931**
Moisture content*Temperature*Velocity	<.0001
Time*Temperature*Velocity	**0.0796**
Time*Moisture content*Velocity	0.002

4 Way Interactions	P Value
Time*Moisture content*Temperature*Velocity	0.0125

4.4 Survival of *G. stearothermophilus* ATCC 10149 spores in oat goats heated in closed test tubes using an oil bath

The time course survival curves for spores (12 and 17 % moisture content) heated in groats at 145°C, in closed test tubes are presented in Figure 16. In both cases heating for 40 min reduced spore populations > 3 $\log_{10}$ cfu/g. Also, $D_{145°C}$ values for both moisture content samples (12.3 and 12.5mins respectively) appeared similar and the typical tailing, which was frequently observed in survivor curves obtained via SS, was noticeably absent.

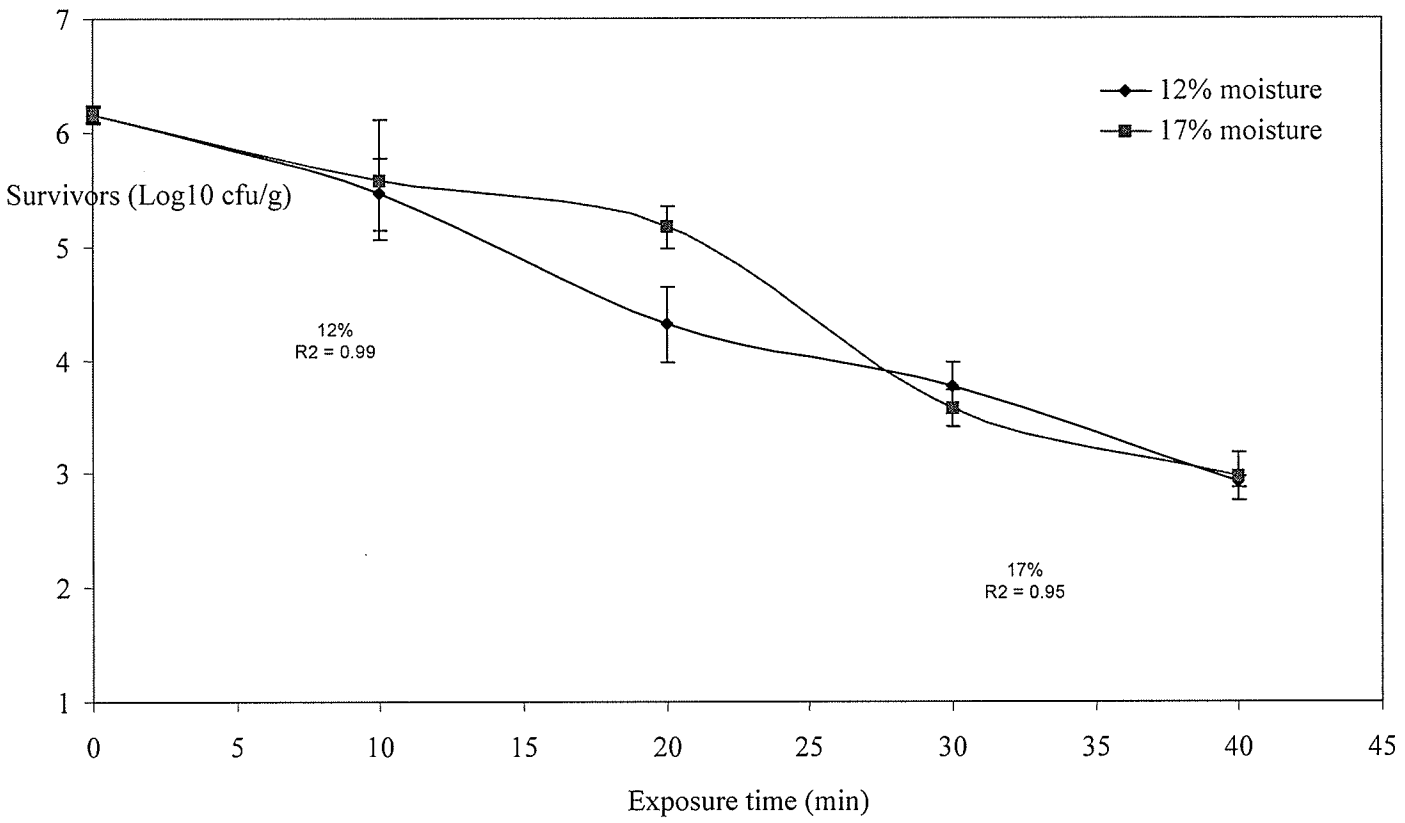

Figure 16 . The survival of*Geobacillus stearothermophilus* ATCC 10149 spores on oat groats following heating in an oil bath at 145oC. Oat groats moisture contents were at 12% and 17%. Error bars represent the standard deviation of three trials each performed in duplicate (n=6). See Appendix 13.

4.5. Color analysis of oat groats and whole meal oat flour treated with SS

All samples processed with SS exhibited some change in color and superficial appearance. Changes in color appeared localized and mainly occurred on the coat of the oat groat. Further examination of the whole meal indicated interior browning, especially at the higher treatment temperatures. At increasing temperatures, the surface of the oat groats became more brown (darker) and toasted in appearance and smell.

Overall, with increasing time of treatment at 115°C and 121°C (Table 12) the *a* and *b* color values for the groats and flour increased while the *L* values decreased.

Increasing the temperature to 160°C and 175°C (Table 13) resulted in similar results; however, the decrease in L values was considerably larger and more rapid. This in turn affected the total color difference (ΔE). Final color for groat and whole meal flour were darker than any of the previous samples; ΔE ranges for the groats and flour were 8.28 – 17.35 and 2.87 – 6.95, respectively.

Samples treated at 130°C (Table 13) and 145°C (Table 14) containing 12% moisture content had ΔE values that were higher than the 17% moisture content samples. Overall, tempered oat groats treated at 130°C at both velocity levels had the lowest ΔE (3.3-6.4) of all samples.

Table 12. Color readings for oat groats treatment with superheated steam at various temperatures. The color readings are given for the whole oat groats and wholemeal flour.

Temperature	Time	Oat Groats				Oat Wholemeal Flour			
		L[1]	a[1]	b[1]	ΔE	L[1]	a[1]	b[1]	ΔE
115°C	0	62.7 ± 0.1	4.3 ± 0	15.6 ± 0.2	0.0	58.1 ± 0.2	-0.16 ± 0	5.2 ± 0	0.0
	5	58.4 ± 0.1	4.9 ± 0.1	19.8 ± 1.5	6.0	56.8 ± 0	-0.26 ± 0	5.3 ± 0.1	1.3
	10	61.6 ± 0.5	5.3 ± 0.1	21.7 ± 0.5	6.3	57.1 ± 0.3	-0.29 ± 0	5.3 ± 0.1	1.0
	15	60.8 ± 1.4	5.3 ± 0.1	21.3 ± 1.0	6.1	57.2 ± 0	-0.27 ± 0	5.4 ± 0	0.9
	20	60.6 ± 1.6	5.4 ± 0	21.6 ± 0.2	6.5	57.1 ± 0	-0.28 ± 0	5.4 ± 0.1	1.0
121°C	0	62.7 ± 0.1	4.3 ± 0	15.6 ± 0.2	0.0	58.1 ± 0.2	-0.16 ± 0	5.2 ± 0	0.0
	5	58.1 ± 0.7	5.6 ± 0.1	20.4 ± 0.1	6.8	56.1 ± 0	-0.09 ± 0	5.1 ± 0	4.0
	10	59.3 ± 0.1	5.9 ± 0.1	21.6 ± 0.1	7.1	56.2 ± 0	-0.14 ± 0	5.2 ± 0	4.1
	15	58.9 ± 0.4	6 ± 0.1	21.5 ± 0.3	7.2	56.3 ± 0.2	-0.13 ± 0	5.3 ± 0.1	4.2
	20	58.5 ± 0.3	6.1 ± 0.2	21.4 ± 0.3	7.4	55.6 ± 0.3	-0.04 ± 0	5.5 ± 0	3.5
160°C	0	62.7 ± 0.1	4.3 ± 0.1	15.6 ± 0.2	0.0	58.1 ± 0.2	-0.16 ± 0	5.2 ± 0	0.0
	2.5	60.5 ± 1.0	7.3 ± 0.2	23 ± 0.1	8.3	55.8 ± 0.2	0.11 ± 0	6.9 ± 0.1	2.9
	5	58.4 ± 0.5	8.5 ± 0.2	22.5 ± 0.6	9.2	55.4 ± 0	0.52 ± 0	6.9 ± 0	3.3
	7.5	55.7 ± 0.1	9.6 ± 0.2	21.7 ± 0.4	10.7	54.8 ± 0.1	0.73 ± 0	7.1 ± 0.1	3.9
	10	53.9 ± 0.5	10.2 ± 0.2	21 ± 0.2	11.9	53.9 ± 0	1 ± 0	7.1 ± 0	4.8
175°C	0	62.7 ± 0.1	4.3 ± 0.1	15.6 ± 0.2	0.0	58.1 ± 0.2	-0.16 ± 0	5.2 ± 0	0.0
	2.5	53.4 ± 0.1	9.9 ± 0.3	20.3 ± 0.5	11.8	54 ± 0	0.9 ± 0	7.1 ± 0.1	4.6
	5	50.8 ± 0.1	9.9 ± 0.4	17.7 ± 0.4	13.3	52.6 ± 0	1.2 ± 0.1	7.3 ± 0.1	6.0
	7.5	46.2 ± 0.3	9.1 ± 0.4	14 ± 0.3	17.3	51.7 ± 0	1.3 ± 0	6.8 ± 0.1	6.8
	10	46 ± 0.5	8.3 ± 0.9	13.1 ± 0.8	17.4	51.5 ± 0	1.4 ± 0	6.7 ± 0.1	6.9

[1]Results are means of one trial performed in duplicate ± standard deviation of means.

Table 13. Color readings for oat groats following treatment with superheated steam at 130°C. The color readings are given for the whole oat groats and wholemeal flour.

Treatment	Time	Oat Groats				Oat Wholemeal Flour			
		L[1]	a[1]	b[1]	ΔE	L[1]	a[1]	b[1]	ΔE
130°C, 12% m.c, 0.52m/s	0	62.7 ± 0.1	4.3 ± 0	15.6 ± 0.2	0.0	58.1 ± 0.2	-0.16 ± 0	5.2 ± 0	0.0
	10	57.7 ± 0.5	5.6 ± 0.1	19.9 ± 0	6.7	56.4 ± 0.3	-0.31 ± 0	5.3 ± 0.1	1.7
	20	57.0 ± 0.7	5.6 ± 0	20.0 ± 0.3	7.3	56.7 ± 0	-0.39 ± 0	5.2 ± 0.1	1.4
	30	57.8 ± 0.2	5.8 ± 0	20.8 ± 0	7.3	56.5 ± 0.4	-0.31 ± 0	5.3 ± 0.1	1.6
	40	58.3 ± 0.5	5.9 ± 0.1	21.2 ± 0.6	7.3	56.4 ± 0.1	-0.27 ± 0	5.4 ± 0.1	1.7
130°C, 12% m.c, 1.10m/s	0	62.7 ± 0.1	4.3 ± 0	15.6 ± 0.2	0.0	58.1 ± 0.2	-0.16 ± 0	5.2 ± 0	0.0
	10	59.3 ± 0.2	5.7 ± 0.1	20.9 ± 0	6.5	56.6 ± 0.2	-0.36 ± 0.1	5.2 ± 0.1	1.5
	20	57.4 ± 0.6	6.0 ± 0	20.8 ± 0.1	7.6	56.2 ± 0.3	-0.24 ± 0.1	5.6 ± 0.1	1.9
	30	58.8 ± 0.4	6.2 ± 0.2	21.5 ± 0.2	7.3	56.3 ± 0	-0.21 ± 0	5.6 ± 0.1	1.8
	40	57.9 ± 0.5	5.9 ± 0	20.1 ± 0	6.8	56.7 ± 0	-0.30 ± 0	5.4 ± 0.1	1.4
130°C, 17% m.c, 0.52m/s	0	61.4 ± 0.4	4.8 ± 0.2	18.2 ± 0.3	0.0	59.1 ± 0.2	-0.74 ± 0	6.9 ± 0.1	0.0
	10	61.0 ± 0.5	5.6 ± 0.1	21.4 ± 0.3	3.3	56.3 ± 0.5	-0.24 ± 0.1	5.9 ± 0	3.0
	20	61.0 ± 0.3	6.2 ± 0	22.5 ± 0.2	4.5	56.2 ± 0.5	-0.17 ± 0.1	6.2 ± 0	3.0
	30	60.2 ± 0.2	6.9 ± 0.1	22.9 ± 0.2	5.3	56.1 ± 0.8	-0.07 ± 0.1	6.4 ± 0	3.1
	40	59.9 ± 0.2	7.0 ± 0	23.0 ± 0.1	5.5	56.4 ± 0.3	-0.01 ± 0	6.6 ± 0.1	2.8
130°C, 17% m.c, 1.10m/s	0	61.4 ± 0.4	4.8 ± 0.2	18.2 ± 0.3	0.0	59.1 ± 0.2	-0.74 ± 0	6.9 ± 0.1	0.0
	10	60.4 ± 0.3	6.3 ± 0.1	22.7 ± 0.3	4.8	56.8 ± 0.3	-0.26 ± 0.1	5.7 ± 0.2	2.6
	20	60.3 ± 0.2	6.9 ± 0	22.6 ± 0.2	5.0	56.7 ± 0	-0.13 ± 0.1	6.0 ± 0.1	2.6
	30	56.9 ± 4	7.4 ± 0.5	22.0 ± 2.3	6.4	56.0 ± 0.1	-0.14 ± 0	6.7 ± 0.1	3.2
	40	59.9 ± 0.1	6.6 ± 0	22.7 ± 0.1	5.1	56.5 ± 0.2	-0.10 ± 0.1	6.1 ± 0.1	2.8

[1]Results are means of one trial performed in duplicate ± standard deviations of means.

Table 14. Color readings for oat groats following treatment with superheated steam at 145°C The color readings are given for the whole oat groats and wholemeal flour.

Treatment	Time	Oat Groats				Oat Wholemeal Flour			
		L[1]	a[1]	b[1]	ΔE	L[1]	a[1]	b[1]	ΔE
145°C, 12% m.c, 0.52m/s	0	62.7 ± 0.1	4.3 ± 0	13.3 ± 0.3	0.0	58.1 ± 0.2	-0.16 ± 0	5.5 ± 0	0.0
	10	60.8 ± 0.4	7.1 ± 0	24.5 ± 0.2	11.7	55.5 ± 0.2	-0.08 ± 0	6.3 ± 0	2.7
	20	58.7 ± 0.1	8.3 ± 0	25.2 ± 0.2	13.2	54.9 ± 0.1	0.22 ± 0.1	6.7 ± 0	3.4
	30	56.1 ± 1.0	9.1 ± 0.2	24.4 ± 0.5	13.8	54.8 ± 0	0.44 ± 0	7.1 ± 0.1	3.7
	40	55.1 ± 0.3	10.2 ± 0	24.1 ± 0	14.5	54.1 ± 0.1	0.52 ± 0	6.9 ± 0.1	4.3
145°C, 12% m.c, 1.10m/s	0	62.7 ± 0.1	4.3 ± 0	13.3 ± 0.2	0.0	58.1 ± 0.2	-0.16 ± 0	5.5 ± 0	0.0
	10	58.2 ± 0.1	6.3 ± 0.1	22.2 ± 0.1	10.2	56.2 ± 0.2	-0.18 ± 0	6.0 ± 0.1	2.0
	20	58.8 ± 0.5	7.3 ± 0.1	23.3 ± 0.2	11.1	56.2 ± 0.1	-0.11 ± 0	6.2 ± 0.1	2.0
	30	57.3 ± 0.3	8.7 ± 0.4	24.6 ± 0.1	13.3	54.9 ± 0.2	0.41 ± 0.1	6.9 ± 0	3.5
	40	57.4 ± 0	9.7 ± 0.1	24.5 ± 0	13.5	54.6 ± 0.1	0.59 ± 0	6.9 ± 0	3.8
145°C, 17% m.c, 0.52m/s	0	61.4 ± 0.3	4.8 ± 0.2	18.2 ± 0.3	0.0	59.1 ± 0.2	-0.74 ± 0	6.9 ± 0.1	0.0
	10	59.5 ± 0.1	8.5 ± 0.5	23.7 ± 0.6	6.9	56.0 ± 0.3	0.16 ± 0.1	6.9 ± 0.1	3.2
	20	58.9 ± 0.2	8.2 ± 0.3	24.4 ± 0.2	7.5	55.8 ± 0.1	0.19 ± 0	7.2 ± 0	3.4
	30	58.6 ± 0.1	8.8 ± 0.1	24.8 ± 0.1	8.2	55.2 ± 0.1	0.46 ± 0	7.5 ± 0	4.1
	40	58.7 ± 0.3	9.5 ± 0.1	24.5 ± 0.2	8.3	54.4 ± 0.1	0.85 ± 0	7.5 ± 0	5.0
145°C, 17% m.c, 1.10m/s	0	61.4 ± 0.4	4.8 ± 0.2	18.2 ± 0.3	0.0	59.1 ± 0.2	-0.74 ± 0	6.9 ± 0.1	0.0
	10	61.3 ± 0.6	7.2 ± 0.1	24.2 ± 0	6.5	56.2 ± 0.1	0.18 ± 0.1	6.4 ± 0.1	3.1
	20	59.2 ± 0	8.6 ± 0.2	25.1 ± 0.3	8.2	55.6 ± 0.1	0.46 ± 0	6.9 ± 0	3.7
	30	56.3 ± 1.1	9.5 ± 0.3	24.6 ± 0.8	9.4	55.5 ± 0.4	0.65 ± 0.1	7.0 ± 0.2	3.9
	40	55.6 ± 0.5	10.3 ± 0.2	24.6 ± 0.3	10.2	54.8 ± 0	0.88 ± 0	7.2 ± 0.1	4.6

[1]Results are means of one trial performed in duplicate ± standard deviation of means.

4.6 Pasting characteristics of oat groats treated with superheated steam

As shown in Table 15, the hot peak values of groats treated at 115°C and 121°C were close to those of the raw and kilned oat groats (range from 202 to 240 RVU). At 160°C and 175°C the values decreased rapidly during treatment. For example, by 10 min the RVU values were about 50% of those obtained at 2.5 min. Hot peak values for groats treated at 130° C (Table 16) were similar to those at 115 and 121°C. Moisture content and velocity of treatment did not appear to have an obvious effect. At 145°C (Table 17), however, the hot peak values for all treatments were lower compared to those obtained at 20 min when heated at115 and 121°C.

Overall, the hot paste values for groats treated at 115°C, 121°C, 145°C, 160°C and 175°C all had relatively large standard deviations. Also, the hot paste values for superheated steam treated oats were approximately half of the hot paste values for the raw and kilned oat groats. The final viscosity values for all SS treated samples with the exception of 175°C were within a comparable range for raw and kilned oat groats (318-421 RVU, respectively). Increasing the treatment times at these temperatures (115°C, 121°C, 145°C, 160°C and 175°C) caused a decrease in values for all of the pasting characteristics. Standard pasting results, most similar to those of raw and kilned oat groats, were achieved at 130°C. At this temperature, tempering oat groats resulted a slight increase in the final viscosity (17%: 350 to 439 RVU versus 12%: 317 and 382 RVU).

Table 15. Means and standard deviation of standard pasting at three selected points (hot peak, hot paste, and final viscosity) for raw, kiln treated and superheated steam treated oat groats at 115°C, 121°, 160°C and 175°C at a velocity of 1.10m/s.

		Viscosity (RVU)		
Sample	Time (min)	Hot Peak[1]	Hot paste[1]	Final Viscosity[1]
Raw oat groats	0	199 ± 1	80 ± 1	318 ± 9
Kilned groats	120	235 ± 1	159 ± 2	421 ± 3
115°C, 12% m.c, 1.10m/s	5	202 ± 2	70 ± 90	353 ± 1
	10	217 ± 1	72 ± 92	365 ± 14
	15	213 ± 2	71 ± 90	350 ± 2
	20	215 ± 2	71 ± 91	368 ± 3
121°C, 12% m.c, 1.10m/s	5	234 ± 15	75 ± 95	367 ± 0
	10	238 ± 10	76 ± 95	368 ± 1
	15	240 ± 12	74 ± 94	366 ± 1
	20	239 ± 10	74 ± 94	370 ± 11
160°C, 12% m.c, 1.10m/s	2.5	156 ± 3	55 ± 69	372 ± 0
	5	121 ± 2	45 ± 56	351 ± 7
	7.5	96 ± 3	35 ± 41	317 ± 1
	10	85 ± 2	33 ± 37	301 ± 2
175°C, 12% m.c, 1.10m/s	2.5	92 ± 2	37 ± 44	317 ± 6
	5	64 ± 1	26 ± 28	242 ± 7
	7.5	45 ± 1	19 ± 19	154 ± 0
	10	42 ± 1	17 ± 15	141 ± 3

[1]Results are means of one trial performed in duplicate ± standard deviations of means

Table 16. Means and standard deviation of standard pasting at three selected points (peak, hot paste, and final viscosity) for raw, kiln treated and superheated steam treated oat groats at 130°C and at various velocities.

		Viscosity (RVU)		
Sample	Time (mins)	Hot Peak[1]	Hot paste[1]	Final Viscosity[1]
Raw oat groats	0	199 ± 1	80 ± 1	318 ± 9
Kilned groats	120	235 ± 1	159 ± 2	421 ± 3
130°C, 12% m.c, 0.52m/s	10	229 ± 1	145 ± 1	341 ± 2
	20	220 ± 6	136 ± 4	330 ± 6
	30	236 ± 2	145 ± 0	345 ± 0
	40	222 ± 1	138 ± 3	317 ± 24
130°C, 12% m.c, 1.10m/s	10	255 ± 5	155 ± 1	368 ± 2
	20	282 ± 6	161 ± 3	382 ± 9
	30	249 ± 0	146 ± 2	358 ± 6
	40	258 ± 3	151 ± 1	357 ± 7
130°C, 17% m.c, 0.52m/s	10	231 ± 3	150 ± 1	386 ± 9
	20	232 ± 1	148 ± 1	410 ± 26
	30	225 ± 3	145 ± 3	350 ± 16
	40	231 ± 1	150 ± 3	439 ± 16
130°C, 17% m.c, 1.10m/s	10	243 ± 3	151 ± 1	414 ± 25
	20	228 ± 6	145 ± 5	413 ± 12
	30	223 ± 2	142 ± 1	439 ± 9
	40	228 ± 10	146 ± 2	425 ± 29

[1]Results are means of one trial performed in duplicate ± standard deviations of means

Table 17. Means and standard deviation of standard pasting at three selected points (hot peak, hot paste, and final viscosity) for raw, kiln treated and superheated steam treated oat groats at 145°C and at various velocities.

		Viscosity (RVU)		
Sample	Time (mins)	Hot Peak[1]	Hot paste[1]	Final Viscosity[1]
Raw oat groats	0	199 ± 1	80 ± 1	318 ± 9
Kilned groats	120	235 ± 1	159 ± 2	421 ± 3
145°C, 12% m.c, 0.52m/s	10	217 ± 22	63 ± 81	351 ± 5
	20	167 ± 8	51 ± 65	338 ± 5
	30	150 ± 9	44 ± 54	332 ± 14
	40	141 ± 21	39 ± 46	327 ± 22
145°C, 12% m.c, 1.10m/s	10	198 ± 13	66 ± 85	356 ± 14
	20	183 ± 36	56 ± 70	342 ± 2
	30	146 ± 5	52 ± 65	322 ± 4
	40	116 ± 2	42 ± 51	304 ± 2
145°C, 17% m.c, 0.52m/s	10	217 ± 24	59 ± 74	383 ± 10
	20	171 ± 3	53 ± 65	368 ± 1
	30	150 ± 9	42 ± 51	369 ± 7
	40	128 ± 4	38 ± 43	336 ± 5
145°C, 17% m.c, 1.10m/s	10	203 ± 18	67 ± 86	373 ± 0
	20	176 ± 7	62 ± 80	377 ± 7
	30	147 ± 11	49 ± 61	361 ± 9
	40	141 ± 33	42 ± 51	344 ± 11

[1]Results are means of one trial performed in duplicate ± standard deviations of means

4.7 Peroxidase values of SS treated oat groats

As shown in Table 18, groats heated at all temperatures and processing conditions were sufficient to inactivate peroxidase with the exception of 115°C.

Table 18. Peroxidase activity of oat groats following treatment with superheated steam at various temperatures and velocities.

Temperature (°C)	Moisture content (% d.b)	Velocity (m/s)	Peroxidase activity
115	12	1.1	+
121	12	1.1	-
130	12	0.52	-
130	17	0.52	-
130	12	1.1	-
130	17	1.1	-
145	12	0.52	-
145	17	0.52	-
145	12	1.1	-
145	17	1.1	-
160	12	1.1	-
175	12	1.1	-

5.0 DISCUSSION

The ability of bacterial spores to germinate and outgrow in foods can contribute to their spoilage and in some cases to human and or animal food borne illness. Generally, spores produced by thermophiles exhibit the highest degree of heat resistance and are particularly important spoilage agents in low acid canned foods which have been heated above 100°C. Among sporeformers *Geobacillus* (formerly *Bacillus*) *stearothermophilus* which occurs naturally in soil, produces the most heat resistant spores. In many cases these spores survive heat treatment and germinate during prolonged cooling resulting in a type of spoilage referred to as flat sour (Richmond and Fields, 1966). For various commodity groups including sweeteners, starches and cereals the presence of spores (genus, species and number) is used as a sanitary indicator and acceptance of lots is based on numerical limits. For example, the maximum number of total thermophilic and thermophilic flat sour spores used for acceptance of raw cereals in the manufacture of baby food is 50 and 20 spores/10 g, respectively (APHA, 1992).

The first portion of this study focused on the microbiology of oat groats processed in a commercial facility. In this respect standard plate count (SPC), yeast and mold and coliform counts were performed as a means of assessing sanitation, handling, processing and storage of raw and kilned oat groats. Additionally, total thermophilic and flat sour spores were determined for reasons mentioned previously.

Information pertaining to the microbiology and growth of *Geobacillus stearothermophilus* in oats, groats and mills were not found, however, a few studies have examined this subject area relative to other grains. Bacterial counts for groats obtained in

this investigation were within the range reported by Mayou and Moberg (1992) for various cereal grains and cereal products. Mayou and Moberg (1992) examined various processing plants and found that samples obtained while performing routine quality control analyses contained SPC ranging from 10^2 to 10^6 cfu/g while molds were usually < $10^2 - 10^4$ cfu/g and coliform levels ranged from 10^2 to 10^4 cfu/g. Seiler (1986) reported that the microbial content of wheat (and therefore flour) was primarily dependent on the weather conditions encountered during growth and harvesting. The author also reported maximum levels of 10^7 and 10^8 cfu/g for SPC and mold counts, respectively in wheat during the growing season. Thatcher et al. (1953) assessed the sanitation of Canadian flour mills and its relationship to the microbial content of wheat flour. They reported average values (cfu/g) for milled, unbleached flour samples of 5.9×10^4 for SPC, 3×10^3 for mold, 9.5×10^1 for total thermophilic spores and 5.0×10^1 for flat sour spores. Generally, their results were similar to those of the raw and kilned oat groats obtained in the present study, although the level for thermophilic and flat sour spores was approximately 1 log greater for the kilned samples. Similarly, Richter et al. (1993) reported mean SPC and mold levels of 10^4 and 10^3 cfu/g, respectively for approximately 4,000 wheat flour samples obtained from commercial mills, railcars, bulk trucks and bags. Coliform levels of 150 MPN/g were also reported.

Ultimately, even though wheat and oat milling incorporate different processing methods, both products initially become contaminated from similar sources (soil and water) and should have similar microbiological profiles. Also, the exterior structure of oats including trichomes, similar to other cereals such as wheat (Jones et al., 1953; Seiler, 1986) are ideal locations for trapping microorganisms.

Initial results indicated that the kiln was a possible source for the survival of sporeformers during processing. A lower spore count from the heating sections of the kiln suggested that both the processing temperature (100°C) and the residence time (approx. 2 h) appeared sufficient to destroy most sporeformers that were present. In contrast, sporeformers seemed to survive and possibly grow in the cooling sections of the kiln. It is conceivable that microbial growth or a microbial reservoir in the kiln occurs as a biofilm either directly adjacent to the kiln walls or on adherent groat material. In either event spores and or groat material could be released from the biofilm contaminating groats during flow through (Flint et al., 2001). This poses additional problems since most microorganisms growing as a biofilm are more resistant to chemical cleaners and sanitizers. According to Flint et al. (2001) who examined the potential for *G. stearothermophilus* cells to form biofilms on stainless steel, attachment of spores was 10-100 times more likely if the surface was soiled. This may explain why higher numbers of spores were found on the interior wall of the kiln. Accumulation of groats, lipid material and trichomes on the kiln wall together with moisture condensation, would provide an excellent matrix for growth and or survival of microorganisms resulting in product contamination through the plant. Therefore, it is imperative that periodic, practical cleaning regimes be initiated to prevent the buildup and growth of spoilage bacteria in the kiln that would otherwise contribute to excessive cross contamination. At the very least kilns should be shut down periodically and the cooling sections be scaled to remove adherent wall material.

Superheated steam which possesses a relatively high heat capacity was investigated as an alternate treatment for kiln drying of groats especially for lots destined

for the baby food sector where standards for spore levels are strictly enforced. It was hypothesized that this alternate heat treatment could not only inactivate lipolytic enzymes and contribute to the desired roasted oat flavor but also have a sporicidal effect against thermophilic bacteria.

In this investigation temperature played an important role in the destruction of *G. stearothermophilus* spores. Spores treated with SS at lower temperatures (115 and 121°C) exhibited D-values of 7.7 and 4.4 min, respectively and are comparable to values reported by Alderton and Snell (1969).These researchers examined the dry-heat resistance of *G. stearothermophilus* spores at 115 and 120 °C, and reported D values of 10 and 5.88 min, respectively. Increasing the temperature of SS treatment in the present study to 160 and 175 °C resulted in a 5 $\log_{10}$ cfu/g spore reduction at or within 10 min and D values of 2.7 and 1.2 min, respectively. These values are comparable to those of Zmidzinska et al., (submitted 2006 for publication) who also investigated SS effects using *G. stearothermophilus* spores. The authors reported D values of 2.1 and 1.5 min, respectively. In comparison, Quast et al. (1977) reported a D value of 1.9 min for *G. stearothermophilus* spores when exposed to SS at 159 °C, however, treatment above 166°C resulted in no survivors. Overall, the decrease in D values with increasing temperatures was expected and confirms one of the basic tenets of microbial resistance to heat.

Although various sources of dry heat are sporicidal, not all are equal with respect to their lethality. For example, the use of hot air, hot inert gases, dry heat in a vacuum or superheated steam are not comparable methods in achieving dry heat sterilization (Spicher et al., 1999). In dry heating, either open or closed systems can be used. Open

systems allow spores to lose or gain water depending on external influences such as RH. In closed systems such as SS drying, however, water movement and availability of water to spores is controlled by the initial water content of the atmosphere in the enclosure/chamber and the volume of the chamber (Pflug et al., 1968, 2001). The latter system reduces the possibility for changes in the moisture content of spores resulting in slower destruction and as a result *D* values tend to be higher. In this respect, Fox and Pflug (1968) and Hoffman et al. (1968) reported that inactivation of cells or spores during dry heat treatment was strongly related to the loss of water and that the rate of inactivation appeared to be controlled by the limiting moisture content in the cell or spore. A possible basis for this inactivation could be the removal of bound water which is essential for maintaining the conformational (tertiary) structure of proteins (Soper and Davies 1971, 1973).

It was anticipated therefore that increasing the moisture content of the oat groats would result in a corresponding increase in spore inactivation. Also, it was expected that additional moisture would retard desiccation of the groats during treatment which could otherwise contribute to excessive roasting. However, increasing the moisture content of oat groats from 12 to 17 % did not seem to have a significant effect on reducing spore levels. Perhaps a greater increase in the moisture content of the groats would have resulted in a positive outcome.

In challenge studies, the ability of the spore carrier to retain moisture during treatment was also demonstrated to have a major effect on spore inactivation (Russell, 1982). For example, Bruch et al. (1963) reported that both mesophilic and thermophilic bacterial spores dried on sand were more resistant than those dried on paper or glass.

Differences in heat resistance of test materials were cited as unknown physical factors. Angelotti et al. (1968) reported that encapsulation of *Bacillus subtilis* spores in various solid materials resulted in a wide range in D values. The authors concluded that the ability of a spore carrier to maintain a certain moisture level (moisture retention) prior to and during heat treatment along with the heat penetration of the solids affected spore survival.

Increasing the velocity/flow rate of SS should result in an increase in spore lethality (Fox and Pflug, 1968). If the lethal effects of dry heat are due to oxidation (Pheil et al., 1967) or vaporization of cellular components or removal of bound water necessary to maintain protein integrity (Molin, 1977) then increasing the velocity of SS would be a positive move. At the very least increasing the velocity should increase the heat transfer rate to the target resulting in more rapid spore dehydration. In this respect Doyle and Ernst (1967) reported that static air at 121°C was about 50% less efficient as a sterilant compared to flowing air. In the current study the effect of velocity at 130 °C on lethality was not consistent and was perhaps more pronounced for samples treated at 145°C. At this temperature doubling the velocity of SS increased spore lethality as evidence by a decrease in D values. At the highest velocity (1.10m/s) a 3.5 $\log_{10}$ decrease in spores was observed for the $10^{6\text{-}7}$ inoculum sample. It is to be expected that the flow rate of the SS would affect the thermal resistance of the organism. A low flow rate should yield a higher D value and vice versa for a higher flow rate.

Spore density or inoculum size also has been demonstrated to affect the inactivation rate of bacterial spores (Vas and Proszt, 1957; Fox and Pflug, 1968; Molin and Östlund, 1976). Generally, the larger the inoculum, the greater the heat resistance.

For vegetative cells, it has been suggested that protein based protective substances are excreted during heating. In addition, with increasing population there is a greater probability of cells or spores having resistances much higher than the average. In this study, the influence of spore inoculum was not a consistent significant factor affecting lethality. Zmidzinska et al., (submitted 2006 for publication) reported that a lower spore inoculum (10^3 cfu/g) was more easily inactivated compared to a higher (10^6 cfu/g) inoculum but only at select temperatures (105 and 130°C). At ≥145°C the 10^3 inoculum exhibited higher D values compared to the 10^6 population. Although SS treatment of *G. stearothermopilus* spores was used in both cases, the latter study was performed using sand as a carrier while the present study utilized sand and oat groats as carriers. Since spores would be present on both sand and groats, it is possible that the effect of inoculum size in the present study was diminished by the chemical and physical composition of the food matrix or support medium where the spores were located (Fox and Pflug, 1968; Angelotti et al., 1968). For example, Bruch et al. (1963) reported that spore samples on sand were much more resistant compared to spores on paper or glass.

Usually survivor curves are linear in shape and indicate a logarithmic order of death corresponding to first-order kinetics, however, deviations such as tailing and the presence of 'shoulders' have been noted (Stumbo, 1973) and could indicate a mix of two species or strains having different heat sensitivities. In the present study, all survivor curves prepared using SS treatment data were typical in that they were biphasic. In this regard a sharp decline in numbers was observed within the first 5-10 min of treatment thereafter the survivor curves exhibited "tailing" for the duration of treatment. Discounting the presence of two species or strains, some researchers believe that

progressive dehydration of spores in an open system during treatment can also result in tailing (Brown, 1994; Cerf, 1977; Ingram, 1969; Vas and Proszt, 1957). For example, Angelotti et al. (1968) reported that spores containing intermediate moisture (0.2 to 0.4 a_W) were much more resistant to dry heat compared to spores having 0.9 a_W. In this study biphasic survivor curves could have resulted from differences in heat resistance between spores located on sand and groats due to the rate at which moisture was reduced during treatment. For example, if spores located on the surface of groats contained a higher moisture content (perhaps due to diffusion of water from the groat to the spore) compared to those on sand, they could become more heat sensitive. Alternatively, if sand particles heat up faster than groats, by virtue of their surface area and reduced moisture content, then spores located on their surfaces could be inactivated first. At the beginning of SS treatment, the steam is in close contact with the sample. Since the sample has a lower temperature than the steam, condensation will result (Prachayawarakorn et al., 2004; Pronyk et al., 2004). The condensate can mimic moist heating conditions in the system which ostensibly results in greater spore destruction. Overall, in this investigation the highest reduction in spores occurred within the first 5-10 min of treatment, thereafter the effects of SS appeared to be minimal.

Interestingly, the survival curves for *G. stearothermophilus* spores heated in closed capped test tubes using an oil bath did not show any tailing. Russell (1971) stated that under different treatment conditions, the same strain can give different time-survivor curves. For example, Roberts and Ingram (1965) reported that *C. botulinum* type E heated in open tubes similar to SS treatment generated curves with tailing. However, when spores from the same population were heated by total immersion using a closed system,

similar to the studies using an oil bath, tailing was absent. The presence or absence of tailing in survivor curves between open and closed systems may therefore be due to the rate of moisture loss from the surface of the spores (spore desiccation) during heating and the water vapor equilibrium within the tube.

Moist heat or saturated steam is far more effective in destroying bacterial spores compared to dry heat (Brown, 1994). At any given temperature more energy per molecule of saturated steam is available compared to dry air; therefore steam provides greater heat transfer through the sample. Since spores are permeable, a rapid diffusion of steam would result in their complete saturation and ultimately death (Savage, 1959; Doyle and Ernst, 1967) due primarily to protein denaturation.

When oat groats were heated at 145°C in a closed system (test tubes), spore populations were reduced by > 3 $\log_{10}$ cfu/g. However, at 145°C using SS, maximum spore reduction ranged from 2.0 to 2.5 $\log_{10}$ cfu/g. This reduction does not appear to be as appreciable as expected. Collier and Townsend (1956) performed the first published study on the resistance of *G. stearothermophilus* spores to superheated steam and saturated steam. They reported that at 121°C under saturated steam, a D-value of 1.9 min was achieved, while for superheated steam, 936 min was necessary.

Visual appeal is an important factor in the process of selecting and consuming a food. In oat processing this is particularly important because the color profile of the oat groat is used as a sign of proper roasting which ultimately influences flavor and texture (Cenkowski et al., 2006). Superheated steam processing is known to produce browning or discoloration in temperature sensitive food products (Prachayawarakorn et al., 2004). All samples processed with SS during this study exhibited some change in color and

superficial appearance. The changes in color occurred on the seed coat of the groats as was expected since it would come in contact with the heat source. From this investigation, SS treatment of groat samples containing 12% moisture content and treated at 130 and 145°C appeared to exhibit higher color differences (ΔE) than the 17 % moisture content samples. It is possible that additional moisture in tempered samples (17 % moisture content) reduced the roasting or darkening effect, partially due to caramelization of sugars (Nielsen et al., 2002). At 160 and 175°C all samples, regardless of moisture content, appeared burnt , and ΔE values were also the highest. Overall, tempered oat groats treated at 130°C at both velocity levels exhibited the lowest ΔE (3.3-6.4) of all samples. Further trials and comparison to commercial oats will be needed to confirm that these parameters are indeed suitable for commercialization.

Oat flour and in general all oat products intended for human consumption must be peroxidase negative at the end of heat treatment (Webster, 1986; 2003). Peroxidase activity is generally used to monitor the adequacy of heat treatment required to inactivate various endogenous lipolytic enzymes including lipase, peroxidase and lipoxygenase. All SS treated samples with the exception of those treated at 115°C (for 20 min) gave negative peroxidase results. Typical industry holding times for oat groats during kiln drying at 100°C are between 90-120 min. If processing at this temperature is desired, then an additional steaming step following the superheated steam process may need to implemented (Webster, 2003). Inactivation of lipase is dependent on moisture content. Generally an increase in moisture facilitates lipase inactivation by heating.

Starch pasting and gelatinization are important properties in the processing of any oat end-product undergoing heat treatment in the presence of water. The pasting profile

determined by the Rapid Visco Analyzer (RVA) corresponds to the viscosity of the starch during the heating cycle, which shows the events occurring in the starch granules during heating. Previous studies have found that pre-treatment of oat grains with heat noticeably affects the viscosity of oat flour slurries made from the treated grain, resulting in a decrease in viscosity (Doehlert et al., 1997). These researchers also found that roasting (dry heat) affected the ability of ß-glucans (a major contributor to oat flour viscosity) to bind/interact with water and each other, with ß-glucans extracted from raw or roasted flours not differing significantly in size or amount, thus causing a decrease in viscosity. On the other hand, they discovered that steaming (moist heat) oat grains produced highly viscous flour slurries that increased in viscosity over time. Other researchers also found that oat samples treated with moist heat had greater viscosities compared to those treated with dry heat. Their moist heat treated samples gained moisture due to steam freely penetrating the oat samples, but they were unable to define a clear mechanism by which the dry heat and moist heat affected the oat grain (Zhang et al., 1997). From this study, the kiln treated oat grains (combination of moist and dry heat treatment, obtained from Can-Oat Milling) had viscosities that were higher than most of the superheated steam treated oat grains, which was in agreement with the literature. The raw groat viscosities were lower than the viscosities for all superheated steam treatment temperatures, except for those treated at 160°C and 175°C. These results are not in agreement with published results of Doehlert et al, (1997), which indicate that raw groats had higher viscosities than the roasted/dry heat treated oat grains. The difference in these findings is most likely due to the nature of the heat treatments. While the previous studies used a convection oven at

104°C to carry out the roasting/dry heating, the heating mechanism is quite different from that of SS processing.

Another factor to consider is the moisture penetration in the oat grain during heat treatment. This affects the slurry viscosity because water is needed for transfer of heat and in this case, for the gelatinization of starch. A lack of water inhibits the breaking open and binding of starch to hydrogen binding sites resulting in lower viscosities (Doehlert et al., 1997) as was observed in the SS processed samples. In the present study, treatment with SS resulted in decreased moisture content in oat groats at all treatment levels. Viscosity was also affected by the steam temperature.

A superheated steam investigation of tortilla chips performed by Li et al. (1999) found that at higher steam temperatures, the moisture content was lower, additionally; chips treated at higher steam temperatures and higher flow rates gelatinized faster and had an increased ability to reabsorb water. In our investigation, this effect was most noticeable at the higher steam temperatures (160°C and 175°C).

CONCLUSIONS

In this study, *Geobacillus stearothermophilus* spores and oat groats were subjected to various levels of SS heat treatment. Spores were evaluated for their survivability, while oat groat functionality was examined. The following results were obtained in this investigation:

1. There is a high potential for groats to become contaminated with thermophilic and flat sour spores while undergoing kiln heat treatment. Analysis of kiln treated oat groats revealed that prior to kiln treatment, 37% of the groat samples tested positive for spores and of the samples examined after kiln treatment, 86% contained thermophilic and flat sour spores.

2. Superheated steam treatment at 175°C (10 mins) delivered the best reduction in *Geobacillus stearothermophilus* spores with a resulting D-value of 1.2mins.

3. Increasing the temperature of superheated steam from 115°C (20 mins) to 175°C (10 mins) resulted in a progressive increase in spore lethality. Nevertheless, changes in processing conditions such as steam velocity and oat moisture content had a minimal effect on spore lethality.

4. A closed system, utilizing moist heat, proved to be slightly more effective than superheated steam in regards to spore lethality. Spore populations heated at 145°C in the closed system were reduced by > 3 $\log_{10}$ cfu/g, while at 145°C using superheated steam, maximum spore reduction ranged from 2 to 2.5 $\log_{10}$ cfu/g.

5. The peroxidase enzymes contained in the oat groats were effectively inactivated in all but one of the superheated treatment temperatures. At 115°C (20 mins), the samples were peroxidase positive indicating that the lipolytic enzymes in the oat groats were not deactivated by that processing temperature and condition.

6. At 160°C (10 mins) and 175°C (10 mins), superheated steam processing had the most noticeable effect of all processing temperatures on oat groat color. The oat groats appeared dark and exhibited a burnt appearance and odor. Also, at all temperatures, increasing the treatment times and the steam flow rate did not have any noticeable effect on the pasting profile.

7. In this study, superheated steam processing (compared to conventional thermal treatment) was of limited value for the reduction of *Geobacillus stearothermophilus* spore viability. Alterations to the steam velocity and treatment time could be further explored to improve superheated steam lethality.

RECOMMENDATIONS FOR FUTURE RESEARCH

1. Examine the effects of carriers such as sand or glass on spore lethality.

2. Increase oat groat moisture content to facilitate lethality, perhaps, by combining vacuum drying following treatment.

3. Further investigate the biphasic phenomenon that was observed during heating of the oat groats by superheated steam.

4. Agitation of the sample during heat processing.

5. Evaluation of the superheat steam treated oat groat's nutrient content.

6. Conduct shelf-life studies on the superheated steam processed oat groats, to determine the storability of treated product.

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APPENDICES

Appendix 1. Characteristics of 3 bacterial isolates taken from oat groat samples.

Morphological and Biochemical characteristics	Isolates		
	BI-22	BI-26	BI-28
Rod shaped cells	+	+	+
Smooth opaque colonies	+	+	+
Gram stain	+	+	+
Endospores produced	+	+	+
Spore shape	ellipsodial	ellipsodial	ellipsodial
Catalase	+	+	+
Growth at 55°C	+	+	+
Growth at 65°C	+	+	+
Acid from glucose	+	+	+

Appendix 2 . Results of supplementary biochemical tests performed on the three oat groat isolates.

API 50CH Tests	Isolates		
	BI-22	BI-26	BI-28
Glycerol	+	+	+
Erythritol	+	-	+
D-Arabinose	+	-	-
L-Arabinose	-	-	-
Ribose	+	+	+
D-Xylose	+	-	+
L-Xylose	+	-	+
Adonitol	-	-	+
ß Methyl-xyloside	-	-	-
Galactose	+	+	+
D-Glucose	+	+	+
D-Fructose	+	+	+
D-Mannose	+	+	+
L-Sorbose	+	-	-
Rhamnose	-	-	+
Dulcitol	-	-	-
Inositol	-	-	-
Mannitol	+	-	-
Sorbitol	+	+	+
Methyl-D-mannoside	-	-	-
Methyl-D-glucoside	+	+	+
N Acetyl glucosamine	+	-	-

Appendix 2 (ctd). Results of supplementary biochemical tests performed on the three oat groat isolates.

API 50CH Tests	Isolates		
	BI-22	BI-26	BI-28
Amygdaline	-	-	-
Arbutine	-	-	+
Esculine	+	+	+
Salicine	+	-	+
Cellobiose	-	-	-
Maltose	+	+	+
Lactose	-	-	-
Melibiose	-	-	-
Saccharose	+	+	+
Trehalose	+	+	+
Inuline	-	-	-
Melezitose	-	-	-
d-Raffinose	+	-	-
Amidon	-	-	-
Glycogen	-	-	-
Xylitol	+	-	-
ß Gentiobiose	-	-	-
D-Turanose	+	+	+
D-Lyxose	-	+	-
D-Tagatose	+	-	+
D-Fucose	-	-	-
L-Fucose	-	-	-
D-Arabitol	+	+	-
L-Arabitol	-	-	-
Gluconate	-	-	-
2 ceto-gluconate	-	-	-
5 ceto-gluconate	-	-	-

Appendix 3. Survival of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats following treatment with superheated steam at 115°C and 121°C at a velocity of $1.10ms^{-1}$.

Treatment time (min)	Log 10 cfu/g			
	115°C		121°C	
	Survivors	Difference	Survivors	Difference
0	6.10 ± 0.09^{a}	0	7.28 ± 0.04^{a}	0
5	4.99 ± 0.07^{b}	1.11	5.31 ± 0.15^{b}	1.97
10	4.81 ± 0.04^{c}	1.29	4.87 ± 0.05^{c}	2.41
15	4.77 ± 0.09^{c}	1.33	$5.05 \pm 0.10^{b, c}$	2.23
20	4.72 ± 0.14^{c}	1.38	5.29 ± 0.38^{b}	1.99

[1]Values represent the means of three trials each performed in duplicate (n=6) ± S.D.

[2]Log_{10} cfu/g at t = 0 - log_{10} cfu/g at $t = t_1$.

Results followed by the same superscript for each time period at each temperature are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test.

Appendix 4. Survival of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats (12% m.c) following treatment with superheated steam at 160°C and 175°C at a velocity of 1.10m/s.

Treatment time (min)	Log $_{10}$ cfu/g			
	160°C		175°C	
	Survivors[1]	Difference[2]	Survivors[1]	Difference[2]
0	5.18 ± 0.01^{a}	0	4.98 ± 0.04^{a}	0
5	3.31 ± 0^{b}	1.87	0^{b}	4.98
10	0^{c}	5.18	0^{b}	4.98

[1]Values represent the means of three trials each performed in duplicate (n=6) ± S.D.

[2]Log $_{10}$ cfu/g at t = 0 - log $_{10}$ cfu/g at t = t_1.

Results followed by the same superscript for each time period at each temperature are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test.

Appendix 5. Survival of a 10^4 cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 130°C at a velocity of 0.52m/s.

Treatment time (min)	Log_{10} cfu/g			
	12% m.c dry basis		17% m.c dry basis	
	Survivors[1]	Difference[2]	Survivors[1]	Difference[2]
(Control) 0	4.68 ± 0.10^a	0	4.68 ± 0.10^a	0
10	2.93 ± 0.09^b	1.75	2.53 ± 0.35^b	2.15
20	2.99 ± 0.12^b	1.69	2.53 ± 0.45^b	2.15
30	2.94 ± 0.37^b	1.74	2.3 ± 0.19^b	2.38
40	2.97 ± 0.08^b	1.71	2.15 ± 0.16^b	2.53

[1]Values represent the means of three trials each performed in duplicate (n=9) ± S.D.

[2]Log_{10} cfu/g at t = 0 - log_{10} cfu/g at t = t_1.

Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test.

Appendix 6. Survival of a 10^4 cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 130°C at a velocity of 1.10m/s.

Treatment time (min)	Log $_{10}$ cfu/g			
	12% m.c dry basis		17% m.c dry basis	
	Survivors[1]	Difference[2]	Survivors[1]	Difference[2]
(Control) 0	4.68 ± 0.10[a]	0	4.68 ± 0.10a	0
10	2.96 ± 0.21[b]	1.72	2.87 ± 0.08[b]	1.81
20	2.83 ± 0.24[b]	1.85	2.80 ± 0.08[b]	1.88
30	2.61 ± 0.39[b]	2.07	2.81 ± 0.07[b]	1.87
40	2.72 ± 0.21[b]	1.96	2.58 ± 0.21[c]	2.1

[1]Values represent the means of three trials each performed in duplicate (n=9) ± S.D.

[2]Log $_{10}$ cfu/g at t = 0 - log $_{10}$ cfu/g at t = t_1.

Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test.

Appendix 7. Survival of a 10^4 cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 145°C at a velocity of 0.52m/s.

Treatment time (min)	Log $_{10}$ cfu/g			
	12% m.c dry basis		17% m.c dry basis	
	Survivors[1]	Difference[2]	Survivors[1]	Difference[2]
(Control) 0	4.68 ± 0.10^{a}	0	4.68 ± 0.10^{a}	0
10	2.82 ± 0.17^{b}	1.86	2.78 ± 0.14^{b}	1.9
20	2.85 ± 0.14^{b}	1.83	2.63 ± 0.15^{bc}	2.05
30	2.34 ± 0.27^{c}	2.34	2.46 ± 0.14^{c}	2.22
40	2.70 ± 0.12^{b}	1.98	2.34 ± 0.31^{c}	2.34

[1]Values represent the means of three trials each performed in duplicate (n=9) ± S.D.

[2]Log $_{10}$ cfu/g at t = 0 - log $_{10}$ cfu/g at t = t_1.

Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test.

Appendix 8. Survival of a 10^4 cfu/g inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 145°C at a velocity of 1.10m/s.

Treatment time (min)	Log $_{10}$ cfu/g			
	12% m.c dry basis		17% m.c dry basis	
	Survivors[1]	Difference[2]	Survivors[1]	Difference[2]
(Control) 0	4.68 ± 0.10^{a}	0	4.68 ± 0.10^{a}	0
10	2.48 ± 0.16^{b}	2.2	2.71 ± 0.21^{b}	1.97
20	2.31 ± 0.18^{bc}	2.37	2.45 ± 0.12^{bc}	2.23
30	2.39 ± 0.10^{b}	2.29	2.23 ± 0.27^{c}	2.45
40	2.24 ± 0.14^{c}	2.44	2.18 ± 0.21^{c}	2.5

[1]Values represent the means of three trials each performed in duplicate (n=9) ± S.D.

[2]Log $_{10}$ cfu/g at t = 0 - log $_{10}$ cfu/g at t = t_1.

Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test.

Appendix 9. Survival of a 10^7 inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 130°C at a velocity of 0.52m/s.

Treatment time (min)	Log_{10} cfu/g			
	12% m.c dry basis		17% m.c dry basis	
	Survivors[1]	Difference[2]	Survivors[1]	Difference[2]
(Control) 0	6.96 ± 0.10^{a}	0	6.96 ± 0.10^{a}	0
10	4.94 ± 0.20^{c}	2.02	5.45 ± 0.07^{b}	1.51
20	5.11 ± 0.19^{bc}	1.85	5.17 ± 0.08^{c}	1.79
30	5.06 ± 0.12^{bc}	1.9	5.21 ± 0.12^{c}	1.75
40	5.2 ± 0.14^{b}	1.76	5.26 ± 0.11^{c}	1.7

[1]Values represent the means of three trials each performed in duplicate (n=9) ± S.D.

[2]Log_{10} cfu/g at t = 0 - log_{10} cfu/g at $t = t_1$.

Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test.

Appendix 10. Survival of a 10^7 inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores in oat groats at moisture contents of 12% and 17% following treatment with superheated steam at 130°C at a velocity of 1.10m/s.

Treatment time (min)	Log $_{10}$ cfu/g			
	12% m.c dry basis		17% m.c dry basis	
	Survivors[1]	Difference[2]	Survivors[1]	Difference[2]
(Control) 0	6.96 ± 0.10^a	0	6.96 ± 0.10^a	0
10	5.24 ± 0.18^b	1.72	5.05 ± 0.30^b	1.91
20	5.09 ± 0.13^b	1.87	5.30 ± 0.06^b	1.66
30	4.94 ± 0.39^b	2.02	5.26 ± 0.20^b	1.7
40	4.94 ± 0.26^b	2.02	4.93 ± 0.34^b	2.03

[1]Values represent the means of three trials each performed in duplicate (n=9) ± S.D.

[2]Log $_{10}$ cfu/g at t = 0 - log $_{10}$ cfu/g at t = t_1.

Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Tukey's Standardized Range Test.

Appendix 13. Survival of *Geobacillus stearothermophilus* ATCC 10149 spores on oat groats (12% and 17% m.c.) following heating in closed test tubes at 145°C.

Treatment time (min)	Log $_{10}$ cfu/g			
	12% m.c dry basis		17% m.c dry basis	
	Survivors[1]	Difference[2]	Survivors[1]	Difference[2]
(Control) 0	6.16 ± 0.06[a]	0	6.16 ± 0.08[a]	0
10	5.46 ± 0.31[b]	0.7	5.58 ± 0.52[b]	0.58
20	4.31 ± 0.33[c]	1.85	5.17 ± 0.19[b]	0.99
30	3.77 ± 0.21[d]	2.39	3.57 ± 0.16[c]	2.59
40	2.92 ± 0.04[e]	3.24	2.97 ± 0.21[d]	3.19

[1]Values represent the means of three trials each performed in duplicate (n=6) ± S.D.

[2]Log $_{10}$ cfu/g at t = 0 - log $_{10}$ cfu/g at t = t_1.

[3] Results followed by the same superscript for each time period are not significantly different ($p \leq 0.05$) according to Tukey' Standardized Range Test.

Appendix 14. Summary of treatment effects for the 10^4 inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores on oat groats and exposed to superheated steam.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Time	4	168.5775725	42.1443931	1096.5	<.0001
Moisture content	1	1.0179037	1.0179037	26.48	<.0001
Temperature	1	2.0701837	2.0701837	53.86	<.0001
Velocity	1	0.2178037	0.2178037	5.67	0.0182
Time*Moisture content	4	0.6634775	0.1658694	4.32	0.0023
Time*Temperature	4	0.7348475	0.1837119	4.78	0.0011
Time*Velocity	4	0.3052692	0.0763173	1.99	0.0981
Moisture content*Temperature	1	0.6923004	0.6923004	18.01	<.0001
Moisture content*Velocity	1	1.3485004	1.3485004	35.08	<.0001
Temperature*Velocity	1	1.2369704	1.2369704	32.18	<.0001
Time* Moisture content*Temperature	4	0.2256558	0.056414	1.47	0.2134
Moisture content*Temperature*Velocity	1	0.3322704	0.3322704	8.64	0.0037
Time*Temperature*Velocity	4	0.4375108	0.1093777	2.85	0.0252
Time*Moisture content*Velocity	4	0.4788642	0.119716	3.11	0.0163
Time*Moisture content*Temperature*Velocity	4	0.6535358	0.163384	4.25	0.0025

Appendix 15. Summary of treatment effects for the 10^7 inoculum of *Geobacillus stearothermophilus* ATCC 10149 spores on oat groats and exposed to superheated steam.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Time	4	172.1885358	43.047134	784.55	<.0001
Moisture content	1	0.2502604	0.2502604	4.56	0.0339
Temperature	1	10.3293504	10.3293504	188.26	<.0001
Velocity	1	2.7928838	2.7928838	50.9	<.0001
Time*Moisture content	4	0.8450958	0.211274	3.85	0.0049
Time*Temperature	4	6.3667725	1.5916931	29.01	<.0001
Time*Velocity	4	1.6273642	0.406841	7.41	<.0001
Moisture content*Temperature	1	0.1358504	0.1358504	2.48	0.1172
Moisture content*Velocity	1	2.0553504	2.0553504	37.46	<.0001
Temperature*Velocity	1	1.3545038	1.3545038	24.69	<.0001
Time* Moisture content*Temperature	4	0.3371058	0.0842765	1.54	0.1931
Moisture content*Temperature*Velocity	1	1.1578704	1.1578704	21.1	<.0001
Time*Temperature*Velocity	4	0.4654442	0.116361	2.12	0.0796
Time*Moisture content*Velocity	4	0.9630308	0.2407577	4.39	0.002
Time*Moisture content*Temperature*Velocity	4	0.7196442	0.179911	3.28	0.0125