EFFECTS OF COMBINED SHEAR AND THERMAL FORCES ON DESTRUCTION OF MICROORGANISMS

by

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To my parents,
for their memory

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ABSTRACT

To investigate the effectiveness of physical forces in destroying microorganisms a heat resistant ($D_{75}{}^{\circ}{}_{C}$=20 min) Gram-positive vegetative bacterium, *Microbacterium lacticum,* a Gram-negative vegetative bacterium, *Escherichia coli*, and vegetative cells and spores of Gram-positive bacterium, *Bacillus subtilis,* were subjected to high mechanical energies using gelatin and maize grits as carriers. A twin screw extruder, a piston capillary rheometer and a rotational rheometer were used.

When extruded with gelatin there was a strong correlation between the destruction of *M. lacticum* and the die wall shear stress and specific mechanical energy (SME). Within the limit of detection, no surviving *M. lacticum* could be detected in gelatin at the highest die wall shear stress of 409 kPa and a SME of 390 $kJ \cdot kg^{-1}$, giving at least 5.3 decimal reductions. There was no surviving *M. lacticum* in maize grits at 289 kPa die wall shear stress and 294 $kJ \cdot kg^{-1}$ SME, giving a 4.6 decimal reduction. The temperature at the extruder die remained below 61°C for all the extrusion experiments indicating that the bacterial destruction was due to combined shear and thermal forces rather than thermal forces alone. It was suggested that the thermal energy supplied during extrusion weakened the bacterial cell wall, making the cells susceptible to shear forces.

A maximum 3.2 decimal reduction in the number of spores of *B. subtilis* in maize grits was obtained at 595 kPa die wall shear stress and 844 $kJ \cdot kg^{-1}$ SME, below 43°C extruder die temperature. There was no statistically significant difference in the survival of *B. subtilis* PS346 and *B. subtilis* PS361, which is a heat sensitive strain due to lack of α- and β-SASP proteins, under the same extrusion conditions, suggesting that the main destruction mechanism was not heat. High reduction in the number of the viable spores suggested a possible "mechanical germination" inside the dynamic environment of the extruder.

A 4.2 logarithmic reduction in the number of *M. lacticum* in 30% (wwb) moisture content gelatin was observed in an unsheared sample in the piston capillary rheometer at 192 MPa and 60°C, showing that pressure could cause major destruction at high temperatures (60-75°C). No survival of *M. lacticum* was detected beyond 695 kPa shear stress and 64 MPa at 60°C, suggesting that an optimum combination of shear, thermal and pressure forces can cause an important reduction in the numbers of vegetative cells.

Shearing of the microorganisms in the rotational rheometer in gelatin showed that the shear resistance of the microorganisms were different. Although only three species of bacteria were tested, it appeared that Gram-positive bacteria were more resistant to shear forces than Gram-negative bacteria. The results suggested that the destruction

of the microorganisms at low shear forces (~3 kPa shear stress) was due to weakening of the bacterial cell wall at temperatures above 60°C. A maximum 1.4 logarithmic reduction in the number of *M. lacticum* was achieved after 4 min of shearing at 804 s^{-1} shear rate and 75°C. Based on the heat resistance data, thermal forces were not enough to cause significant destruction in the numbers of the microorganism, however, the temperature played a significant role by weakening the bacterial cell wall making it susceptible to shear forces. In this context, it is possible that there was a synergistic relationship between the shear and thermal forces. A shear D-value concept was introduced which was used to evaluate the shear resistance of microorganisms at different temperatures.

Starch conversion (determined by differential scanning calorimeter) due to low temperature extrusion of maize grit inoculated with *M. lacticum* and spores of *B. subtilis* showed that there is a positive correlation between the bacterial destruction and the starch conversion. Up to 94% starch conversion was obtained during low temperature extrusion of maize grit where the estimated degree of starch conversion due to heat alone was 3.8%.

The results suggested that if the shear forces can be optimally combined with thermal forces, an acceptable sterility can be achieved at significantly lower temperatures which would help to keep the quality of food products high.

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LIST OF ABBREVIATIONS AND SYMBOLS

$\dot{\gamma}_w$	: wall shear rate (s^{-1})
$\dot{\gamma}$	: shear rate (s^{-1})
ω	: angular velocity ($rad \cdot s^{-1}$)
ρ	: density ($kg \cdot m^{-3}$)
λ	: extensional viscosity (Pa·s)
σ	: shear stress (Pa)
η	: viscosity (Pa·s)
η_a	: apparent viscosity (Pa·s)
ε_e	: extensioanl strain rate (s^{-1})
σ_e	: extensioanl stress (Pa)
ΔP	: pressure drop
σ_w	: die wall shear stress (Pa)
η_w	: die wall viscosity (Pa·s)
$\dot{m}$	: gravimetric (mass) flow rate ($kg \cdot h^{-1}$)
$\bar{t}$	: mean residence time in the extruder
a_w	: water activitiy
BHI	: brain heart infusion
c^*	: coil overlap concentration
cfu	: colony forming units
DC	: degree of starch conversion (%)
DSC	: differential scanning calorimetry
$E(t)$	: exit age distribution
$F(t)$	: cumulative residence time distribution
K	: consistency index ($Ps \cdot s^n$)
K	: reaction rate constant (min^{-1})
MPa	: mega Pascal ($Pa \cdot 10^6$)
mPa·s	: milli Pascal second
N	: final number of microorganisms
N	: flow behaviour index
N_0	: initial number of microorganisms
P_0	: Bagley corrected pressure drop across the short die
Pa	: Pascal
Pa·s	: Pascal second
PBS	: phosphate buffered saline
P_L	: pressure drop across the long capillary die
Ps	: pressure drop across the short capillary die
Q	: volumetric flow rate ($m^3 \cdot h^{-1}$)
R	: radius

rpm	: revolution per minute
RVA	: rapid visco analyser
s^{-1}	: per second (measure of shear rate)
SME	: specific mechanical energy ($kJ \cdot kg^{-1}$)
T	: torque ($N \cdot m$)
t_d	: residence time in the extruder die
T_e	: endset temperature
T_o	: onset temperature
T_p	: peak gelatinisation temperature
VOR	: viscometry-ossilation-relaxation
w/v	: weight per volume
wwb	: wet weight basis
ΔH	: energy, enthalpy ($J \cdot g^{-1}$)
ΔT	: difference between two temperatures (T_e-T_o)

CHAPTER 1

GENERAL INTRODUCTION

1.1 THESIS OBJECTIVES AND OUTLINE

Thermal processing is the most common method used for the pasteurisation or sterilization of food products. Although heat, with its destructive effect on enzymes and microorganisms, ensures safety and long preservation, effects of heat on foods can be very detrimental. Some heat preservation processes cause significant reductions in nutritive value and drastic changes in organoleptic properties (Sala *et al.*, 1995). Thermal energy induces various chemical reactions in food materials and, in some cases, causes the formation of undesirable components (Hayakawa, 1996).

The availability of high quality preserved foods has always been important to consumers. Recently consumer demand has increased for the healthier food products that keep more of their original characteristics and are microbiologically safe and additive-free. Preservation technologies that prolong shelf life but do not have a detrimental effect on the quality attributes of the product are favoured. This market trend has stimulated the interest of food companies in developing some alternative methods of preservation. Nonthermal methods of food preservation are being developed to eliminate or at least minimize the quality degradation of foods that results from thermal processing. Foods can be processed nonthermally using high hydrostatic pressure, oscillating magnetic fields, high intensity electric fields, intense light pulses, irradiation, chemicals, biochemicals, and hurdle technology. Each nonthermal technology has specific applications in terms of the types of food that can be processed.

For example, high pressure, oscillating magnetic fields, antimicrobials, and hurdle technology are useful in processing both liquid and solid foods. Pulsed electric fields are more suitable for liquid foods and irradiation is useful for solid foods. Light pulses are more useful for surface pasteurisation, while irradiation and magnetic fields can be used to process pre-packed foods, reducing the risk of post-process contamination. It is clear that nonthermal technologies are not applicable to the processing of all kinds of foods. Each nonthermal technology has its merits and limitations. In many cases, (for example, in the inactivation of bacterial spores) it is necessary to use combined methods (Gustavo, 1998).

During nonthermal processing, the temperature of food is held below the temperatures normally used in thermal processing so that the quality degradation due to high temperatures is minimal. The vitamins, essential nutrients, and flavours are expected to undergo minimal or no changes during nonthermal processing. In addition, nonthermal processes utilize less energy than thermal processes.

The development of either a novel nonheat sterilization method such as application of ultra-high pressure, pulsed high voltage electric field or mild thermal treatments in combination with other techniques employing the "hurdle principle" would respond to consumer demand for improved food safety, and the shelf life with little destruction of nutrients (Hayashi, 1995, Hayakawa, 1996). The combination of heat with other methods enhances the lethal effect of heat on microorganisms and enzymes, and results in a lowering of the intensity of conventional heat treatments. This approach has lately revived the interest of scientists in what has become known as the 'preservation of foods by combined processes' (Sala *et al.*, 1995).

This study is designed to investigate the potential of using mechanical (shear) forces in combination with mild thermal energy for killing bacterial cells and spores as a method of food processing. The temperature necessary to achieve a useful bacterial kill can cause significant loss in the quality of the final product. If the same kill could be achieved using milder thermal processing combined with the mechanical (shear) energy input in a food processing equipment, e.g., in an extruder, thermal degradation of product quality could be reduced while minimising process energy requirements.

The approach employed was to extrude vegetative cells and spores by a twin-screw extruder and a piston capillary rheometer using gelatin and maize grits as carriers. The degree of starch conversion in maize grit extrudates were investigated by Rapid Visco Analysis (RVA) and Differential Scanning Calorimeter (DSC) allowing this to be compared with the bacterial destruction during low temperature extrusion. A rotational rheometer was also used in order to shear the low viscosity gelatin suspensions inoculated with vegetative cells. Other aspects of the study cover the viscous behaviour of gelatin and maize grits.

The outline of the thesis is as following:

The reminder of this chapter consists of a literature review covering extrusion as a decontamination process. Basic information about structures of microorganisms and their resistance to different environmental conditions, the structure of starch granules and the starch conversion during extrusion are also given in this chapter.

The materials and methods used in this study with the theory behind the measurement of residence time distribution, rheological measurements and RVA and DSC techniques are given in Chapter 2.

Chapter 3 investigates the destruction of vegetative cells in a gelatin carrier in a twin-screw extruder, a piston capillary rheometer and a rotational rheometer. Based on the rheological data collected from the rheometers and the extruder die, the flow behaviour of gelatin at low and high moisture levels was also investigated in Chapter 3.

The effect of extrusion shear forces on the destruction of *Microbacterium lacticum* and the spores of *Bacillus subtilis* in maize grits are investigated in Chapter 4. The rheological behaviour of maize grits was investigated based on the models proposed in the literature. The study of starch conversion by RVA and DSC measurements are also given in this chapter.

The potential of using combined shear and thermal forces as a method of pasteurisation and/or sterilisation in the food industry is discussed in Chapter 5. Further studies were suggested to confirm the results and to explore the different aspects of this work.

1.2 EXTRUSION COOKING AS A STERILIZATION PROCESS

In thermal processing, the time that the foods are exposed to pasteurisation or sterilisation temperatures is an important parameter for the quality of the final food product. It is important to choose an optimum time-temperature combination for the process in order to ensure the safety of the final product while maintaining the optimum quality of the food. It is well known that high temperature short time (HTST) or ultra high temperature (UHT) processing gives the best result for the sterilisation of many food stuffs since the loss of quality of the product is minimised due to the short processing times. A UHT process has been defined as the thermal treatment at temperatures in the range of 126.7 °C to 149°C with holding times of seconds to minutes (Brown and Ayres, 1975). Achieving these high temperatures in very short time is a process limitation for many foodstuffs such as semisolid and solid foods. To overcome this problem special equipment has been developed (e.g. scraped surface heat exchanger, rotating retorts). Extrusion cooking, described as a continuous high temperature (50-250°C), short time (30-120 sec) treatment under the conditions of high pressure (50-250 Bar), high shear and low hydration (10-30% water) (Bouveresse *et al.*,1982), provides an excellent opportunity to sterilise especially semi-solid foods, since the heat transfer coefficient of the process is about 1000 times greater than those of vessel cookers and reactors (Wiedmann, 1987). Wang (1993) described the extrusion cooking as a unique process, in that it combines several unit operations into one integrated step-heating, mechanical energy transfer, mixing cooking and shaping. The ability of replacing many batch processes into a continuous process makes extrusion cooking an economical process. The cooking time at high temperatures is a matter of seconds, which has a favourable effect in maintaining the properties of the ingredients and active substances, while giving higher rates for the destruction of microorganisms. Wiedmann (1987) illustrated (Figure 1.1) the desirable (destruction of microorganisms) and undesirable (thiamine loss and browning reactions) degradation processes during commercial sterilization, UHT sterilization and extrusion cooking. Figure 1.1 shows that high temperature extrusion is protecting the qualities of product while destroying the thermophilic spores. Wiedmann (1987) also reported that during high temperature extrusion, shear forces reinforce the efficiency of the process.

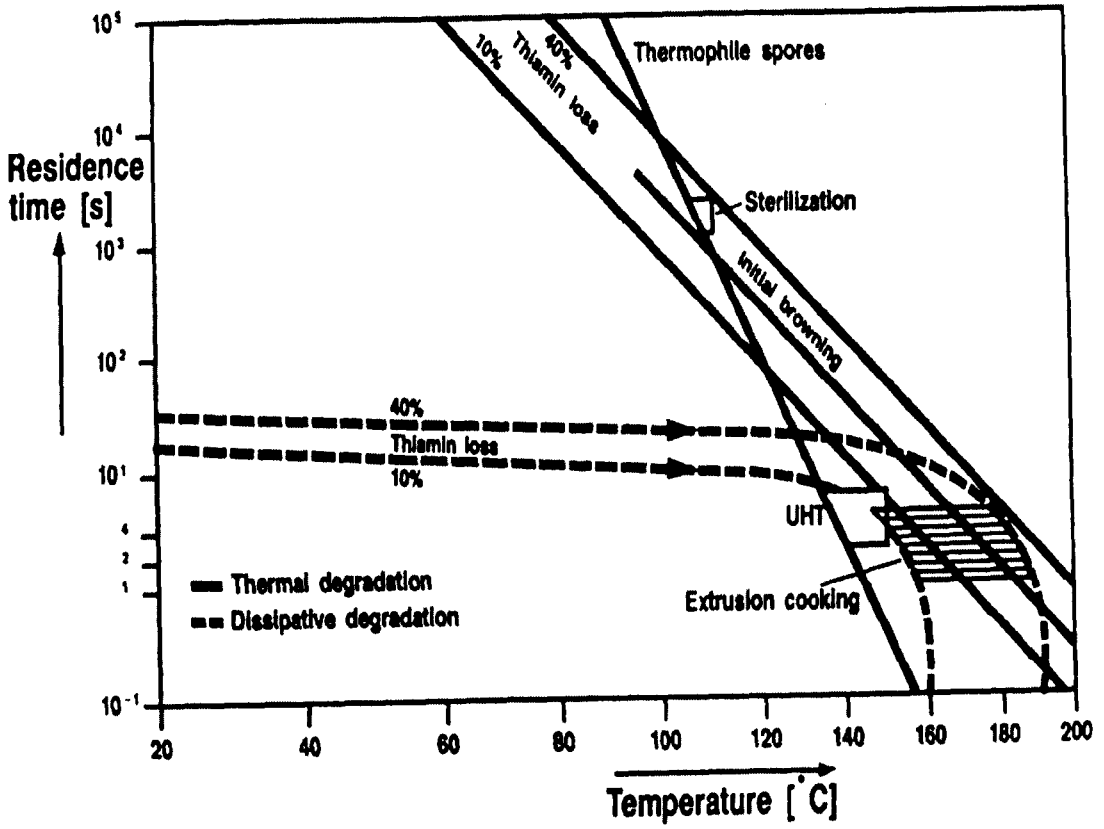

Figure 1.1 Destruction of microorganisms, thiamine loss and browning reactions in sterilization and high temperature extrusion processes. Adapted from Wiedmann, 1987.

Due to the many advantages of the extrusion process, this technology is becoming increasingly important in the food industry for transforming materials into intermediate or finished products (Mercier *et al.*, 1979; Guy, 1985; Colonna *et al.*, 1987; Camire *et al.*, 1990; Wang, 1993). As it becomes more and more popular, new products are being created and well known ones are copied and made more advantageously. Table 1.1 shows a number of well known food products for which conventional machines and equipment can be replaced by an extruder cooker.

Many researchers have investigated the sterilizing potential of extrusion cooking but in such studies, the effect of thermal forces under different processing parameters has been mainly investigated and the effect of shear forces on the destruction of microorganisms in extrusion processing has been of less concern.

Table 1.1 Production of extrusion cooked products to replace the conventional processes. Adapted from Wiedmann, 1987.

Product group	Product example	Conventional process
Modified cereal flours	Baby food	Drum dryer
Animal food	Pet food	Autoclave or oven
Dairy food	Caseinate reaction	Stirred tank reactor
Flavours	Roast flavours, Caramel	Roasting tank
Baked articles	Flat bread, biscuits	Baking oven
Breakfast cereals	Puffed rice, Cereal flakes	Bach cooker
Sweet articles	Fruit gums, liquorice, chocolate	Cooker and mogul Conch
Farinaceous food	Glass noodles, fish noodles	Cooker

Significant increase in survival of *Salmonella typhimurium* in spaghetti was observed in duplicate experiments when the extrusion temperature increased from 35°C to 55°C at a constant screw speed of 12 rpm. The explanation of this phenomenon was that at higher temperatures, the dough was less viscous and the cells experienced less shear forces. Inactivation of *S. typhimurium* increased when the screw speed increased from 12 rpm to 20 and 30 rpm at constant temperature of 35°C. Four times as many *S. typhimurium* cells survived at 12 rpm as compared with 20 and 30 rpm screw speed, supporting the idea of destructive effect of shear forces on *Salmonella typhimurium* (Walsh *et al.*, 1974).

By using a Brabender extruder, Kauffman and Hatch (1977) investigated the effect of shear forces on yeast cell viability in the extrusion cooking of a semi-moist dog food. They developed a semi empirical rate equation to relate yeast cell viability to shear rate, flow behaviour and residence time. They found that the viability of yeast cells could be reduced by 20-60% at 20 to 50 rpm screw speeds and a constant 40°C barrel temperature. The influence of thermal effects on yeast cells at this low temperature was considered negligible. They suggested that the combination of thermal and shear energy could be used to achieve the desired level of decimal reduction while preserving functional and nutritional properties of the food material with a minimum energy input. They explained that when optimally combined with shear forces, the heat weakens the

cell wall making it more susceptible to destruction by shear. The authors also drew attention to the point that smaller bacterial cells or spores should be less readily susceptible to viscous shear forces.

Using guar gum and maize grit as carriers, Melvin (1997) employed *Bacillus subtilis* as an indicator organism to examine the potential of extrusion as a decontamination process. Extrusion at 200°C with relatively short average residence times (~40 s) had a minor effect on spore survival in guar gum. Reductions in viable spore counts of more than 2 logs were observed during extrusion of guar at ambient temperature which was attributed to the higher viscosity and shear stress. The effect of extrusion temperature on spore inactivation in maize grits was different to that for guar since for maize the level of destruction was greater at higher temperatures. For example, at 180°C and a water content of 15%, a 4-5 log count reduction was observed compared with only a 1-log kill on extrusion at 20 °C. This discrepancy was attributed to differences in residence times and the flow properties of the two materials. Thermal killing of spores by exposure to maximum extrusion temperatures for maximum residence times in a static system did not account for the total reductions in counts, showing that both mechanical and thermal energy make important contributions to lethality. Although the precise killing mechanism was not determined, results suggested a possible germination in the extruder made spores susceptible to the heat and shear strains in the final zones of the barrel.

Using a single screw Brabender Plasticorder extruder with two electrically heated zones, Likimani *et al.* (1990a) investigated the effect of feed moisture, extrusion temperature, residence time, and shear strain on the destruction of *Bacillus globigii* spores during extrusion cooking of corn/soybean mixture. They applied a fractional factorial experiment with three variables at three levels. Screw speeds were 100, 120, 140 rpm and the feed moisture contents were 14, 18 and 22%. Higher temperatures increased lethality, whereas the influence of screw speed on spore destruction was minimized due to variation of residence time with moisture content and barrel wall temperature. Shear strains generated in the range of 5.503 and 5.703 in the extruder had a minor effect on spore destruction. No information was given in this study in terms of shear stress. They showed that lower feed moisture contents resulted in greater destruction of spores. This was explained as the effect of increased viscous dissipation of the mechanical energy into heat at lower feed moisture contents, thus raising the mass temperature. The authors

did not explain but, the increased viscosity at lower feed moisture contents could also increase the shear forces acting on spores within the dough. It could also be expected that the water activity (a_w) would be low at low moisture levels and the resistance of the spores to heat would be increased at low water contents, raising the question if the high kill was due to the temperature increase only.

Bouveresse *et al.* (1982) used a starch-protein-sucrose mix with 14 % water content to investigate the thermal destruction of *Bacillus stearothermophilus* spores in a BC-45 Creusot-Loire co-rotating twin screw extruder. A 3 ml sample of spore suspension containing $2.1x10^{11}$ spores of *B. stearothermophilus* was introduced within 1 sec to the inlet of the extruder operating at 78 rpm with 40 kg/h feed rate. The maximum residence time was 120 sec under these conditions. Spore destruction was calculated by dividing the total number of surviving spores in cumulated samples by the number of inoculated spores. Overall reduction ratios (N/N_0) were $2.9x10^{-5}$ and $7.9x10^{-8}$ at product temperatures of 165 and 182°C respectively, just before the die exit. The sterilization efficiency achieved at 182°C was concluded as effective enough, considering that the raw materials practically contain only about 5 spores/g i.e. at least 10^8 times less than the inoculated amount.

Van de Velde *et al.* (1984) compared the reduction in the number of *B. stearothermophilus* spores introduced as either a liquid or a freeze-dried inoculum. The screw speed was maintained at 80 rpm and the feed rate was 30 $kg.h^{-1}$. This resulted in a minimum residence time of 95±5 s. The temperature of food mix just before the die was 150°C or 165-168°C. The calculated spore reduction (N/N_0) was not significantly different for liquid and freeze-dried inocula (Table 1.2).

Table 1.2 Sterilizing effect of extrusion cooking on wet and dry spores of *B. stearothermophilus* adapted from van De Velde *et al.*, 1984.

Extrusion temperature	Overall spore reduction ratios (N/N_0)	
	Liquid inoculum (Wet spores)	Powder inoculum (Freeze-dried spores)
150°C	2.5×10^{-7}	3.6×10^{-7}
165-168°C	1.3×10^{-7}	3.6×10^{-7}

The results showed that there was no significant difference in the spore reduction ratios between 150°C and 165-168°C. Although the authors did not explain this, decreased viscosity (decreased shear forces and viscous dissipation energy) at high temperetures could be a possible explanation for this phenomenon.

Gry *et al.* (1984) have found that extrusion of pulverized natural spices (black pepper and paprika) at 90-150°C reduced the microbial load to less than 1000 g^{-1} with no adverse effect on aroma, taste or colour of the spices. The highest temperature, pressure, and shear stresses were obtained in the middle section of the extruder. The subsequent extruder sections were thus used for cooling to minimise the evaporation of volatile aroma components at the die outlet. Paprika was extruded under nitrogen to avoid unacceptable colour changes. Sensory evaluation by trained taste panels using triangular test indicated that no significant difference in taste (90 per cent level) could be found between extruded and non-extruded pepper or paprika. It was suggested that such a process might be used to replace treatment of spices with ethylene oxide or ionising radiation. Microbiological requirements for germinated spices state that the total content of microorganisms must be less than 10,000 per gram and the content of bacterial spores less than 1000 per gram, and this could be achieved by extrusion process.

Guzman-Tello and Cheftel (1987) investigated the assessment of the intensity of high temperature short time extrusion cooking by measuring the extent of thiamine destruction. Results indicated that thiamine destruction follows an apparent first order reaction and that the rate constant k of destruction, calculated from near isothermal extrusion experiments at 133, 142 or 152°C product temperature, follows an Arhenius relationship with an activation energy of 49.9 kJ/mol and a rate constant of 1.44×10^{4} s^{-1}. The pre-exponential constant k and activation energy E_a of batch process calculated according to the Arhenius relationship were $4.1 \times 10^{11} s^{-1}$ and 93.9 kJ/mol respectively. They suggested that wheat proteins or starch might exert a protective effect on thiamine. In order to investigate the influence of screw speed, extrusion experiments were carried out at screw speeds of 100, 125, 150 rpm. To keep a constant product temperature of 159-160°C the barrel temperature was reduced when the screw speed increased. The percentage destruction of thiamine increased markedly at higher screw speeds, despite a decrease in mean residence time as the screw speed increased. The possible explanation

for that was local product heating (hot spots) due to friction generated energy dissipation. The authors also suggested that it could be as a result of the shear forces generated at the reverse screw element.

Quequiner *et al.* (1989) developed an extrusion method for the pasteurisation of thermo-sensitive whey protein powder without any protein denaturation or texture modification. An industrial whey protein isolate (WPI) was experimentally inoculated with $5x10^5$ viable *Streptococcus thermophilus* cells per g, and extruded at 4-5% moisture content and 50 rpm screw speed in a Clextral BC-45 twin screw extruder without using a die. Under conditions of 143°C barrel temperature and 20 s minimum residence time there was 4.2 decimal reductions in the number of *S. thermophilus* cells. The shear forces under the extrusion condititions were low and the authors concluded that the observed effect was due to heat processing at low moisture contents.

Using the system mentioned before, Likimani *et al.* (1990b) examined the potential for injury of *B. globigii* spores during extrusion cooking. Recovery and enumeration of spores surviving the extrusion cooking process was conducted by using five culture media ranging from minimal to rich in nutrient composition. Three different temperature levels (100, 120 and 140°C) were used in extrusion experiments. Their results showed that extrusion processing at low barrel temperatures (80/100°C) resulted in injury to spores of *B. globigii*, whereas at higher temperatures spore injury was not detectable. The authors did not explain this finding. It could perhaps be the result of decreased viscosity, which leads to decreasing viscous shear forces. Injury at low temperature extrusion could be the result of mechanical or shear damage, rather than heat damage.

Likimani *et al.* (1990c) proposed a methodology for determining the destruction of bacterial spores in a Brabender single-screw plasticorder extruder under varying processing conditions. The proposed methodology evaluated destruction of *B. globigii* spores in an 18% moisture corn/soybean (70/30 % w/w) mixture. They measured average mass residence time at temperatures above 95°C. Considering that the extrusion residence times were less than 1 minute, they assumed that spore destruction below 95°C was negligible. D-value ("Extrusion D-value" or "Extrusion Death Rate" as proposed by the authors) for extrusion was calculated based on average time at mass

temperature >95°C. D-values ranged from 1.7 s at 115°C to 6.6 seconds at 100°C maximum mass temperatures, which resulted in a "Z-value" of 25.3°C, similar to that reported for the same strain with dry heat. Destruction of spores in the extruder was a function of mass temperature and residence time at constant moisture levels (18%) in a corn/soybean (70/30% w/w) mixture. Increasing mass temperature resulted in greater lethality while increasing screw speed, which reduced residence time, resulted in lower lethality.

Li *et al.* (1993) investigated the thermal inactivation and injury of *Clostridium sporogenes* spores during extrusion cooking, by using a mixture of mechanically deboned turkey (MDT) and white corn flour. An APV Baker MPF 50/25, 28.0 kW, co-rotating, intermeshing twin-screw extruder with 15:1 L/D (barrel length/diameter) was used. At 93.3°C and 115.6°C barrel wall temperatures just before the die exit, 2 and 5 logarithmic reductions in the number of *C. sporogenes* spores were obtained respectively. They concluded that there was a significant spore injury under both high and low temperature extrusion conditions. The overall bacteriological quality of the extruded MDT mixture with white corn flour was found to be excellent (no detectable spores) in both Brewer Anaerobic agar and nutrient agar.

1.3 PROPERTIES OF BACTERIAL CELLS AND SPORES

Resistance of bacterial cells and spores to different stress factors, such as physical stresses (heat, radiation, ultrasonic cavitation, electric field, desiccation, osmotic shock, freezing, decompression, uv light), chemical agents (e.g. detergents, solvents, antibiotics, acid and alkali), mechanical stresses (shear, high pressure, agitation) and biological stress (enzymes, autolysis), is related to the nature of the organism, cell wall composition, size, shape and growth phase of the organism. Understanding cell wall structure is important since the inactivation and/or injury of bacterial cells by shear forces appears to be related to damage to the bacterial wall structure. The structure of bacterial cells and spores and their resistance mechanisms to different stress factors will be discussed briefly in the following sections.

1.3.1 The bacterial cell wall

Bacterial cell wall structure has been reviewed by several researchers (Harrison, 1991, Pelczar *et al.*, 1993, Tortora *et al.*, 1994, , Middelberg, 1995) and will be summarised below.

The cell wall of microorganisms is a semi-rigid structure providing sufficient intrinsic strength to protect the cell from osmotic lysis. Additionally, it forms a biologically active boundary between the microorganism and its external environment. In a typical Gram-negative bacterium, such as *Escherichia coli*, the cell wall consists of an outer membrane and a peptidoglycan or murein layer, as shown in Figure 1.2. The wall provides mechanical strength to the cell, primarily because of the cross-linked peptidoglycan layer. The outer membrane consists of a lipid bilayer containing transmembrane proteins, phospholipids and lipopolysaccharide (LPS). It is anchored by binding to outer membrane proteins and by non-covalent cross bridging of adjacent LPS molecules with divalent cations such as Mg^{2+} and Ca^{2+}. In addition to the wall, a cytoplasmic membrane, composed primarily of phospholipids, maintains concentration gradients between the cell and its surroundings. This membrane possesses no mechanical strength (Middelberg, 1995).

Gram-positive bacteria such as *Bacillus* lack the outer membrane component, but in turn have a more dominant peptidoglycan structure (Harrison, 1991). These envelope structures are readily distinguished by their staining characteristics. The cytoplasmic membrane provides the major interactive barrier between the internal cell environment and the bulk media. Typically, the bilayer structure is approximately 4 nm wide (Harrison, 1991) and is comprised predominantly of phospholipid and protein. This biophysicochemical system actively maintains concentration gradients, houses transport systems and is involved in ATP generation. It does not provide any significant structural strength and is readily disrupted by osmotic shock in the absence of the structural component layers. The rigid peptidoglycan layer forms the basic structural framework of the cell envelope, providing its mechanical strength. The basic peptidoglycan structure, similar in all bacteria, is composed of linear polysaccharide chains of

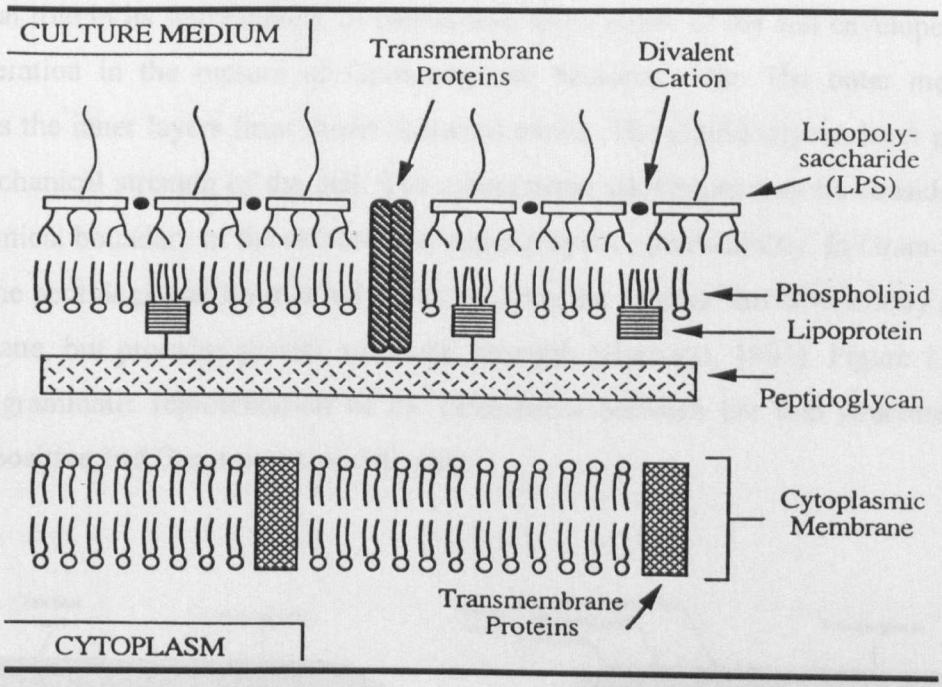

Figure 1.2 Simplified structure of the cell wall of *E. coli* showing its relationship with the cytoplasmic membrane. Adapted from Middelberg (1995).

alternating N-acetyl-D-glucosamine (NAG) and N-acetyl-muramic acid (NAM) residues joined by β-(1-4) glycosidic bonds. The chains are cross-linked by a tetrapeptide. The peptide branches of the parallel chains are further cross-linked. The resulting rigid grid structure acts as a single macromolecular network to provide the shape, tensile strength and osmotically protective nature of the cell envelope. Its strength is governed by the frequency of peptide chains and their cross linking. The peptidoglycan layer in Gram-negative bacteria is 1.5 to 2.0 nm in thickness and typically accounts for 10 to 20% of the cell envelope in terms of dry mass. It is spatially distinct from the cytoplasmic membrane and forms the major resistance to cell breakage. The cell envelope of Gram-positive bacteria is composed of 50-80% peptidoglycan, associated with teichoic acids, presenting a greater structural resistance to breakage. The outer membrane specific to Gram-negative bacteria is composed of protein, lipopolysaccharide and phospholipid. It separates the peptidoglycan layer from the bulk medium environment, thereby preventing their interaction. It is readily distinguished from the cytoplasmic membrane by the anisotropic nature of its bilayer configuration and its narrow spectrum of protein molecules. Divalent cations play an

essential role in its stabilization. In conclusion, three layers of the cell envelope require consideration in the rupture of Gram-negative bacterial cells. The outer membrane protects the inner layers from direct chemical attack. The peptidoglycan layer provides the mechanical strength of the cell. The cytoplasmic membrane may be considered the biochemical boundary of the cell and the major player in permeability. In Gram-positive cells, the peptidoglycan layer is not protected from the external environment by an outer membrane, but provides greater structural strength (Harrison, 1991). Figure 1.3 gives the diagrammatic representation of the differences between the fine structure of the Gram-positive and Gram-negative cell wall.

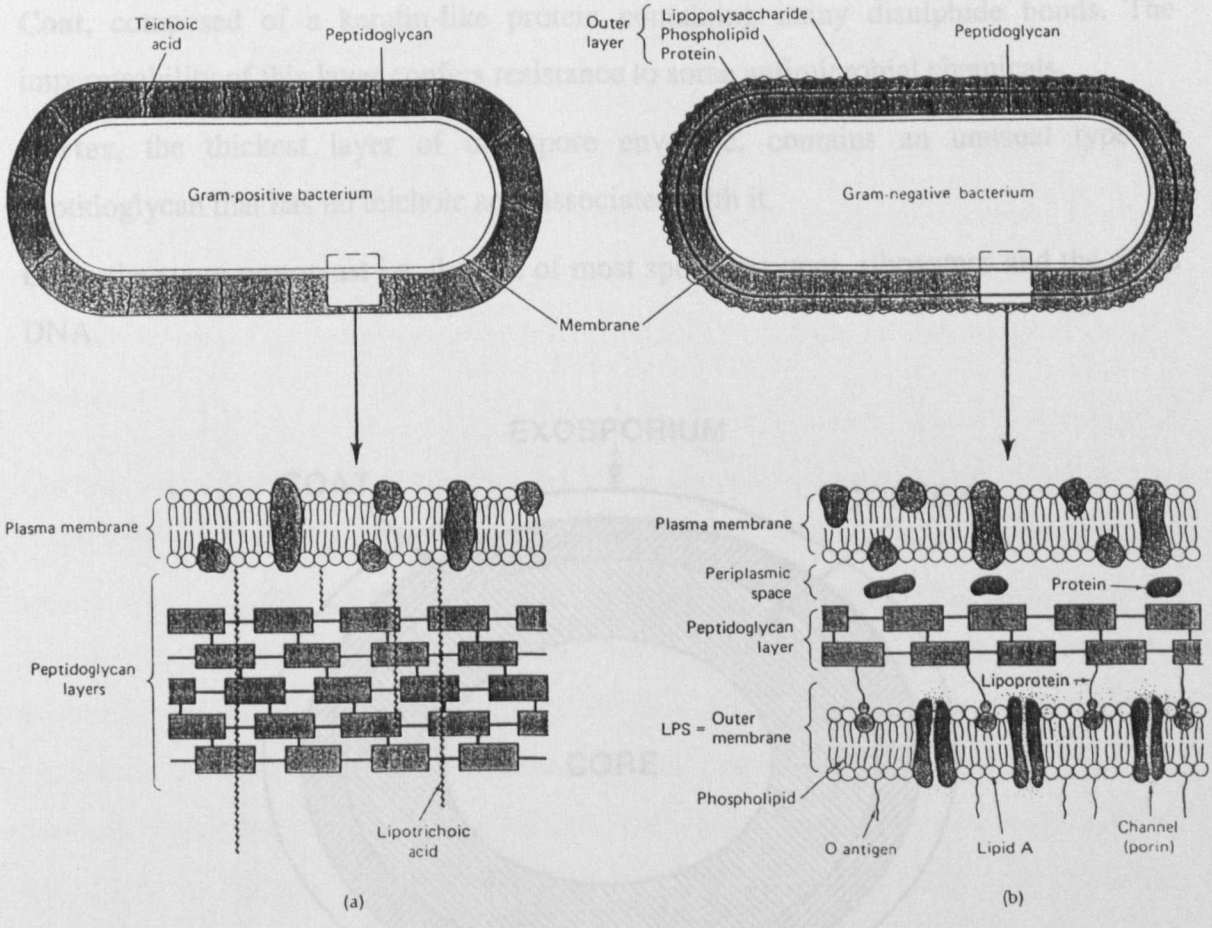

Figure 1.3 Diagrammatic representation of the differences between the fine structure of the Gram-positive cell wall (a) and Gram-negative cell wall (b) of bacteria. Adapted from Wistreich and Lechtman, 1988.

1.3.2 Bacterial spores

When essential nutrients (carbon, nitrogen or, more rarely, phosphorous) are depleted or when water is unavailable certain Gram-positive bacteria such as those of the genera *Clostridium* and *Bacillus*, form specialized "resting" cells called **endospores**. Unique to bacteria, endospores are highly durable dehydrated cells with thick walls and additional layers. Compared with vegetative cells, spores consist of several novel layers as shown schematically in Figure 1.4, and from the outside in, these are as following (Brooks *et al.*, 1991, Setlow, 1995):

Exosporium, present only in some spores; of unknown function.

Coat, composed of a keratin-like protein containing many disulphide bonds. The impermeability of this layer confers resistance to some antimicrobial chemicals.

Cortex, the thickest layer of the spore envelope, contains an unusual type of peptidoglycan that has no teichoic acid associated with it.

Core, the spore protoplast i.e. the site of most spore enzymes, ribosomes and the spore DNA.

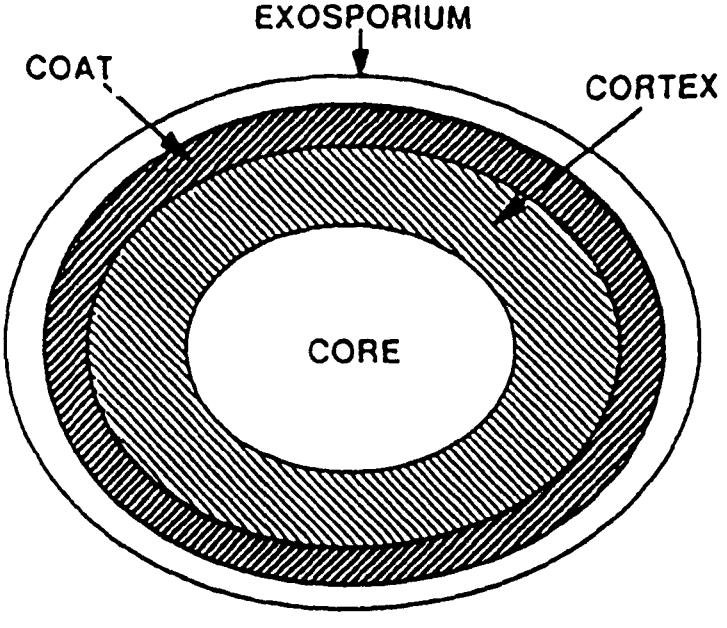

Figure 1.4 Schematic structure of the bacterial spore. The various layers of the mature spore are not drawn completely to scale. The spore's membranes are not shown. Adapted from Setlow, 1995.

Spores are formed internally in the bacterial cell. When released into the environment, they can survive extreme heat, lack of water, and exposure to many toxic chemicals and radiation (Nicholson and Setlow, 1990, Tortora *et al.*, 1995). Endospores can remain dormant for thousands of years. For example, 7500-year-old endospores of *Thermoactinomyces vulgaris* from the freezing muds of Elk Lake in Minnesota have germinated when rewarmed and placed in a nutrient medium (Tortora *et al.*, 1995). In order to survive for long periods of time, Setlow (1992) explained that spores need to meet the challenge of protecting their DNA from irreversible damage caused by chemical modifications such as alkalyation or oxidation, base removal by depurination, and damage caused by UV light. He stated that mechanisms must be operating to prevent accumulation of potentially lethal damage in spore DNA during long periods of dormancy. It was suggested that spores have evolved three distinct strategies for dealing with the potential damage of DNA. (i) The greatly decreased water content in the spore core retards and/or alters chemical reactions affecting DNA. (ii) The binding of spore DNA by a unique group of proteins, termed α/β-type small acid-soluble proteins (SASP), further slows and/or alters the DNA's activity. (iii) DNA repair processes act in the early minutes of spore germination to repair DNA damage accumulated during dormancy.

The process of endospore formation within a vegetative (parent) cell is known as **sporulation** or **sporogenesis**. It is not exactly clear what biochemical events trigger this process. In the first observable stage of sporulation, a newly replicated bacterial chromosome and a small portion of cytoplasm are isolated by ingrowth of the plasma membrane to form a spore septum. The spore septum becomes a double-layered membrane that surrounds the chromosome and cytoplasm. This structure, entirely enclosed within the original cell, is called a **forespore**. Thick layers of peptidoglycan are laid down between the two membrane layers. Then a thick spore coat of protein forms around the outer membrane. This coat is responsible for the resistance of endospores to many harsh chemicals.

The diameter of the endospore may be the same as, smaller than, or larger than the diameter of the vegetative cell. When the endospore matures, the vegetative cell wall dissolves (lyses), killing the mother cell, and the endospore is freed. Most of the water

present in the forespore cytoplasm is eliminated by the time sporulation is complete, and endospores do not carry out metabolic reactions. The highly dehydrated endospore core contains only DNA, small amounts of RNA, ribosomes, enzymes, and a few important small molecules. The latter include a strikingly large amount of organic acid called dipicolinic acid (found in the cytoplasm), which is accompanied by a large number of calcium ions. These cellular components are essential for dormancy and/or resuming metabolism later.

An endospore returns to its vegetative state by a process called **germination**. During germination, spores lose their characteristic resistance to heat, desiccation, pressure, vacuum, UV and ionising radiations, chemicals, including antibiotics, and extremes of pH. Germination can also generally occur at temperatures outside the limits for vegetative growth. Optimal germination for some mesophilic *Bacillus* species has been found as high as 50°C and slow germination of many strains occurs at 0°C. Spore germination generally occurs at lower a_w, than that required for vegetative growth. *Bacillus subtilis* spores have been shown to germinate at an $a_w < 0.8$ but only outgrew at $a_w > 0.9$ (Gould, 1969). Germination may be triggered by brief exposure to heat, by agents such as L-alanine for most species of *Bacillus* (Wuytack *et al.*, 2000), by mechanical forces acting on the spore (Pelczar *et al.*, 1993) or by exposure to moderate hydrostatic pressures (Sojka and Ludwing, 1994). Although some bacterial spores will germinate spontaneously when placed in a favourable environment (e.g. a nutritionally rich medium), others will not germinate unless first activated by some traumatic agent that damages the spore coat such as heat, abrasion, acidity or compounds containing free sulphydryl groups (Brooks *et al.*, 1991). During germination the endospore's enzymes break down most of the extra layers surrounding the endospore, water enters, and metabolism resumes.

Vegetative cells are killed by temperatures above 70°C, but most endospores can withstand 80°C for at least 10 minutes. For example, the endospores of *Clostridium botulinum*, the cause of the food poisoning called botulism, can resist boiling for several hours. Endospores of thermophilic (heat-loving) bacteria can survive in boiling water for 19 hours. Endospore-forming bacteria are a problem in the food industry because they are likely to survive processing, and if conditions for growth occur, some species can produce toxins and disease.

1.3.3 Heat inactivation of microorganisms

The preservation of foods by heat was first performed in France by Nicholas Appert around 1810 and this remained for a long time as an empirical practice until 1921 when W. D. Bigelow established a sound basis on which, still today, current methods of heat preservation rely (Throne, 1986).

Bigelow showed that every unit of heating time of a microbial population at a given temperature reduced the number of viable cells by a constant proportion. By plotting the log of the number of survivors as a function of heating times, a straight line is obtained. In this plot (survival curve), the minutes needed to reduce the number of viable cells to 1/10 (one log cycle) of its original value is now known as the **decimal reduction time**, or **D_t value**. When log D_t values are plotted vs. their corresponding heating temperatures the decimal reduction time curve is obtained. The numbers of degrees Celsius of temperature increase for the log D_t value to decrease by one log cycle is known as **z value**. The heat resistance of microorganisms is defined by these two parameters. Once a D_t value is known, the kinetics of death allow the prediction of the numbers of survivors after a given heating time. Furthermore, as the z value allows the calculation of the lethal effect of each temperature, the total lethal effect of any given process, including heating and cooling phases, can be estimated (Sala *et al.*, 1995).

The heat resistance data (D_t and z values) are very variable, being influenced by many factors. For example, the pH of the heating medium is one of the most important and one of the first to be known. But many others, such as the water activity (a_w), sporulation temperature and growth medium, composition of heating medium, incubation temperature and medium have been the subjects of investigation (Sala *et al.*, 1995).

Heat has been reported to damage different cell structures, including damage to cell membranes, ribosomes, DNA, RNA and enzymes. DNA is still considered the most likely lethal target molecule, but damage occurring at the same time in different molecules and/or other structures may also result in heat inactivation. Moist heat was reported to cause denaturation and coagulation of vital proteins such as enzymes, whereas dry heat causes oxidation of organic constituents of the cell, that is, it causes

them to burn slowly (Pelczar *et al.*, 1993). Dry heat treatments resulting in a high level of killing of wild type spores has been widely reported to cause a high percentage of various mutations (Russell, 1982). Dry heat is less effective in destroying bacteria than moist heat (Russell, 1982). Therefore in extrusion processing (low moisture content and low a_w), the resistance of bacterial cells and spores to destruction would be expected to be greater than with traditional moist heat treatments but not as resistant as under dry heat treatments.

Spore DNA is saturated with a novel group of small, acid-soluble proteins (SASP) of the α-, β- and γ- type, which are synthesised in parallel during sporulation and degraded in the first few minutes of germination. It is now recognised that binding of the α- and β- proteins is a key factor in spore DNA resistance to heat as well as desiccation, oxidising agents and UV radiation (Setlow, 1995).

1.3.4 Inactivation of microorganisms by high hydrostatic pressure

High pressure inactivation of vegetative cells and spores has been the subject of many studies recently. It has been reported that pressures between 300 and 600 MPa can inactivate the moulds, yeasts and most vegetative bacteria whereas bacterial spores can only be killed by very high pressures (>1000 MPa). Bacterial spores however, can often be stimulated to germinate by pressures of 50-300 MPa and germinated spores can then be killed by relatively mild heat or pressure treatments (Smelt, 1998). The mode of action of pressure on germination and killing of bacterial spores is still not well understood. It is generally accepted that high pressure kills microorganisms through the increased ionisation of water and acid molecules under the reduced volume conditions leading to protein denaturation (Earnshaw *et al.*, 1995). Pressure also causes integral and peripheral membrane proteins to become more detached from the plasma membrane when the membrane bilayer is sufficiently perturbed by pressure. Membrane damage, denaturation of enzymes and protein components of cells, and decrease of intracellular pH are counted among the major targets in Ultra High Pressure (UHP) inactivation (Hayashi, 1995, Smelt, 1998).

Pressure inactivation of spores has been attributed to pressure-induced germination with subsequent destruction of the germinated spores by the pressure and thermal forces. An

optimal hydrostatic pressure is needed in order to inactivate the spores successfully. Sojka and Ludwig (1994) showed that pressures between 600 and 1500 bar are best suited to initiate the germination of spores of *Bacillus subtilis*. Spores were pre-treated by applying 200-600 bar pressures at 40°C for 210 min. When a 5000 Bar pressure was applied to the pre-treated spores, the inactivation reached a plateau after 5-10 min indicating that a short time was enough to kill the germinated spores, and there were no further germination during the application of high (5000 Bar) pressure. Oscillatory pressurization was found to kill the spores more effectively than continuous pressurization (Sojka and Ludwig, 1994, Hayakava *et al.*, 1994).

In a typical experiment, egg white and yolk were coagulated by application of 620 and 400 MPa pressure respectively (Hayashi, 1995). It is interesting to know that egg shell was not crushed under these high pressures. This is due to the fact that hydrostatic pressure is instantaneously and uniformly transmitted to all parts of the egg's surface, and thus does not result in a significant deformation in their shape. Therefore, it is important to state that the destruction of microorganisms by shear and mechanical forces is different from the application of UHP. Shear stresses cause a change in the shape of the solid or liquid element and the magnitude of the stress required to destroy a structure would be much lower when compared to stresses used in UHP applications (Mitchell, 1997).

1.3.5 Mechanical breakage of microorganisms

There has been intensive research on the efficient and cost-effective microbial cell disruption for the production of commercial biotechnological products of intracellular derivation. Wimpenny (1967), Harrison (1991) and Middelberg (1995) have reviewed and compared the basic techniques for cell disruption. Among the many techniques used, disruptions of cells by mechanical methods involves either solid shear, e.g. bead milling, extrusion of frozen cells, or liquid shear e.g. high pressure homogenisation and mechanical agitation. Therefore, a brief summary of some of the mechanical methods will be given in the following sections.

1.3.5.1 High Pressure Homogeniser

Any system, which compresses a suspension of cells and releases it from a restricted gap causes shear forces that can break the cells (Wimpenny, 1967). High pressure homogenisers, one of the most widely known methods for large scale cell disruption, are a good example of this method. Brookman (1974) investigated the mechanism of cell disintegration in a high pressure homogeniser and found that 100% breakage of *Saccharomyces cerevisiae* was achieved in a single pass at 25 000 Psi (172.3 MPa).

Ease of disruption appeared to be related to the nature of the organism, cell wall composition, size, shape and growth phase. Keleman and Sharpe (1979) used a homogeniser (Stansted cell disrupter) to determine the relative forces required to disrupt a range of microbial cells. Distinctly different pressures were required to disrupt different micro-organisms. In order to achieve 50% disruption on a single pass, 15 MPa pressure was needed for *E. coli*, which is a rod shape Gram-negative bacterium (size 2-4x0.5 μm), whereas *Streptococcus faecalis* which is Gram-positive with coccal shape (1.0 μm diameter) required 150 MPa pressure for the same level of disruption. The pressure required for 50% disruption of *Saccharomyces cerevisiae*, an oval shaped yeast of 7-12x5-8 μm size, was 150 MPa. Theoretically, a bigger structure should be more susceptible to shear forces. However, the high pressures needed for disruption of *S. cerevisiae* may have been due to its thick cell wall structure. Cell wall thickness of *S. cerevisiae* (70 nm; Harrison, 1991) is an order of magnitude thicker than the cell wall thickness of *E. coli* (8.8±1.8 nm; Middelberg, 1995). Generally speaking, Gram-negative bacteria are easier to disrupt than Gram-positive bacteria and filamentous fungi, which in turn are easier to disrupt than unicellular yeasts (Wimpenny 1967, Harrison, 1991).

Ultramicroscopic studies of the disruption of Gram-negative bacteria by high pressure homogenisation have indicated two stages in the disruption process. In the initial stage, cell rupture is observed as discrete fractures in the electron dense peptidoglycan structure. The second phase involves the disintegration of the cell structure and the total liberation of its intracellular contents. Conditions of high velocity shear, sudden decompression with resultant cavitational stresses, turbulence and impingement are expected to exist within the process. Rapid pressure drop at the valve and the shear

generated between the valve faces due to the high velocity gradient (Brookman, 1974), impingement on a stationary plate and hydrodynamic cavitation (Harrison, 1991) are apparent as the significant causative mechanisms of cell rupture.

1.3.5.2 Solid Pressure Shear

A complete and very efficient cell disintegration has been achieved by pressing a frozen cell paste through a small slit or orifice under pressure. The X-press, Hughes press and Chaikoff press all operate on this principle. The solid shear and abrasive action of the ice crystals and plastic flow through the orifice are believed to be responsible for the cell rupture obtained (Wimpenny 1967, Harrison, 1991).

1.3.5.3 High speed bead mill

The bead mills, originally developed for the pigment and dye-stuff industry, provide grinding and dispersion by inter-particle collision and solid shear. The bead mill consists of either a vertical or a horizontal grinding chamber containing rotating discs or impellers mounted on a motor driven shaft. These accelerate the glass or plastic beads to supply a grinding action. Studies of cell rupture using high speed bead mills have been carried out on yeasts, and both Gram-positive and Gram-negative bacteria. This complex process is influenced by a wide range of parameters relating to the number and energy of impacts taking place, the energy transfer to the grinding elements, liquid shear, hydrodynamics and mixing. In addition, the residence time, nature and state of the cell envelope, and size of different organisms will affect the process (Harrison, 1991, Middelberg, 1995).

1.3.5.4 Mechanical agitation

Two types of large shearing forces can be obtained by mechanical agitation: rotational liquid shear and vibrational liquid shear. Rotational liquid shear can be generated in high speed blenders. In this case, the shear gradient at the blade edge is the product of the angular velocity of the blade edge and the radius of curvature of the edge of the blade. This method is ineffective for breaking most bacteria, as the shearing forces generated are not enough. Sufficient vibrational liquid shear can be obtained by a generator that mixes a liquid suspension containing small glass beads. The intense shear

fields occur around the rapidly accelerating glass beads, and this damages the cells (Wimpenny, 1967).

Disintegration of bacterial spores by agitation of the suspension with small inert particles was shown as a mechanical disintegration method by Curran and Evans (1941). Working on spores of different thermal resistance, they showed that a minimum 93.9% reduction was achieved after 5 hours of shaking with glass beads. Their results showed that there is no correlation between heat resistance of spores and mechanical destruction. They also used *E. coli* as an example of a vegetative cell and their result showed that there was no growth from *E. coli* shaken in a suspension for 5 hours. They stated that disintegration of bacterial spores or vegetative cells was due to the cumulative result of abrasion and repeated collisions between the particles and bacteria during the shaking process.

1.4 STARCH PROPERTIES AND MODIFICATION BY EXTRUSION

1.4.1 General features of starch granules

The structure of starch and the organization of starch granules have been extensively studied. All starches occur in nature as granules, each with its characteristic shape, size and submicroscopic structure, which depend on the botanical origin. Among the commercial starches, granules range in size from approximately 2 μm for rice to 100 μm for potato (Rickard *et al.*, 1991). The size of starch granules in maize grit was reported to be between 5-25 μm (Blanshard, 1987). Examination of maize grits used in this study by scanning electron microscopy (SEM) showed that the average starch granule size was 13 μm. The SEM of maize grit is shown in Figure 1.5.

Starch granules consist of two types of polysaccharides namely amylose (a linear polymer of D-glucose monomers) and amylopectin (a branched polymer). Most of the starches contain 20-30% amylose and 70-80% amylopectin. The amount of amylopectin can be as high as 99% in waxy maize starch (Manners, 1985).

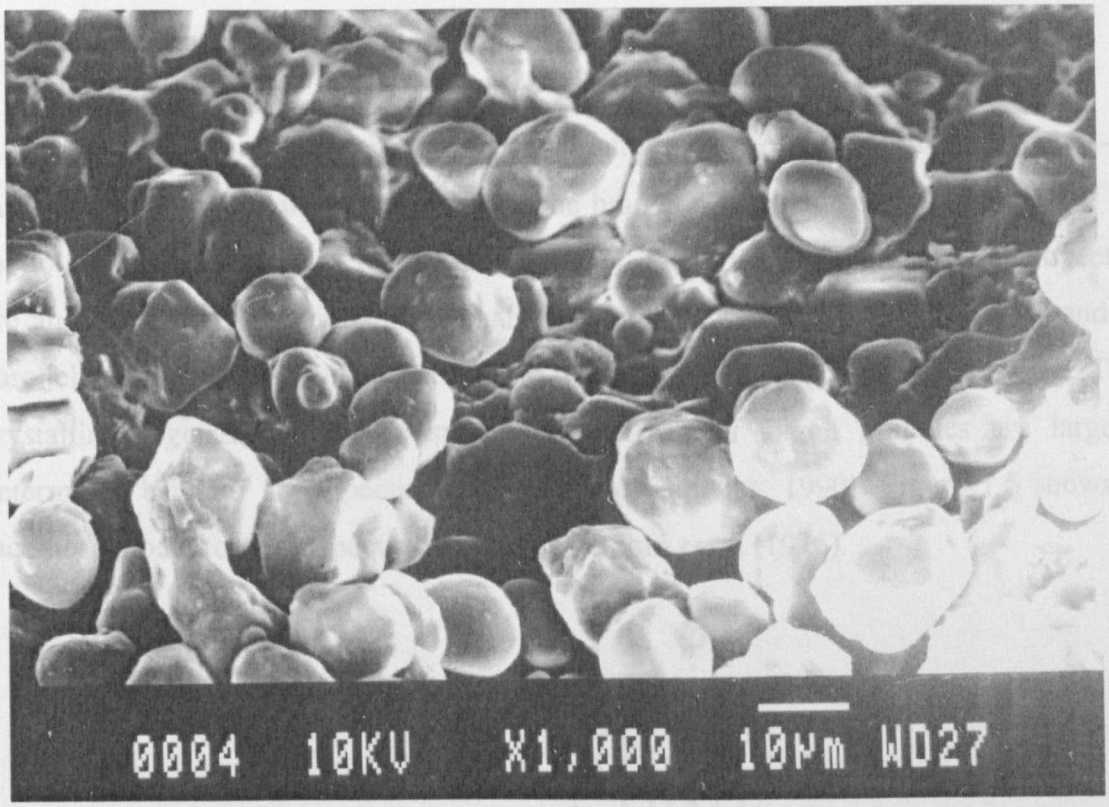

Figure 1.5 Scanning electron micrograph of maize grit

The starch granule is heterogeneous chemically in that it contains both amylose and amylopectin, and physically, because it has both amorphous and crystalline phases (Wang *et al.*, 1992). The principle crystalline compartment of starch is the external amylopectin chains. Only some parts of the amylose molecules may participate in the crystalline phase by chain folding. It is the presence of crystallites that makes the starch granule birefringent and have a distinct X-ray diffraction pattern (Billiaderis *et al.*, 1980).

1.4.2 The structure of amylose

Amylose is essentially a linear molecule consisting of up to several thousand glucose residues linked through α-(1→4) glycosidic bonds. In most starches one-third to two-thirds of the amylose fraction has secondary chains attached through occasional α-(1→6) branch points. Amylose has an average of 2-11 branch points per molecule, depending on source (Morrison and Karkalas, 1990).

1.4.4 The structure of amylopectin

Amylopectin is known as the branched structure of the starch linked by α-(1-6) linkages. It is a very large molecule, and the branch point linkages constitute 4-5% of the total linkages (Whistler and BeMiller, 1997). The branched regions of amylopectin are amorphous but the short linear chains beyond branch-points can form left-handed parallel-stranded double helices, similar to those described for crystalline amylose. The crystallinity, gelatinisation and swelling properties of starch granules are largely determined by their amylopectin (Morrison and Karkalas, 1990). Figure 1.6 shows a racemose model for amylopectin proposed by Robin *et al.* (1975).

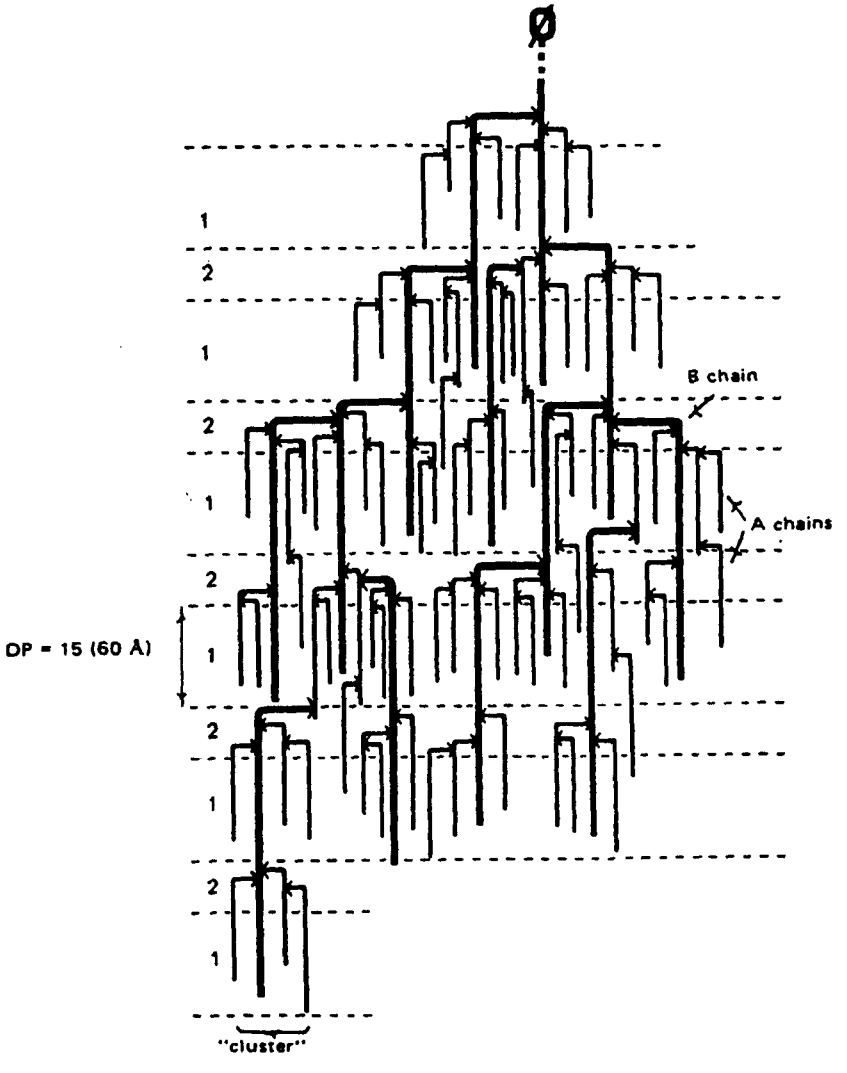

Figure 1.6 Racemose model for amylopectin proposed by Robin *et al.* (1975).

1.4.4 Gelatinisation of starch

Although the terms gelatinisation and cooking are not equivalent (Paton and Spratt, 1981), depending on the method used, often both refer to the same phenomena and are actually used interchangeably in the literature (Cai *et al.*, 1995). In water, starch molecules aggregate to form spherical granules with an average size about 400 times larger than the average size of single molecules. Starch granules dispersed in excess cold water exhibit a limited degree of swelling depending on the molecular organisation within the granule. The theory of the starch gelatinisation was given by Glicksman (1969) and Olkku (1978) as follows:

When starch granules are heated in the presence of an adequate amount of water a temperature is reached at which hydrogen bonding forces, which hold the starch granules together, weaken and the water is absorbed by the starch granule. The granules swell tangentially and loose their birefringence. Gelatinisation begins in the intracellular areas where the hydrogen bondings are weakest and spreads rapidly. As the temperature increases above the gelatinisation range, hydrogen bondings continue to be disrupted, water molecules become attached to hydroxyl groups and the granules continue to swell. As they swell, the swollen starch granules become increasingly susceptible to shear disintegration. The bonding forces in the granule also become weaker as heating is continued and the susceptibility of the granule to mechanical and thermal breakdown increases. When the granules have swollen to occupy the entire volume, some of the starch solubles, which had earlier diffused into the aqueous region, may diffuse back into the highly swollen granules. The system becomes a gel-like mass held together by associated bondings.

Gelatinisation is not a uniform process. Some granules will gelatinise at lower temperature than others, so the gelatinisation temperature is usually expressed as a range of temperatures. In general, the gelatinisation of the larger granules precedes the gelatinisation of the smaller ones (Olkku, 1978, Eliasson and Gudmundsson, 1996; Whistler and BeMiller, 1997).

Donovan (1979) suggested that the gelatinisation process takes place at a moisture

content higher than 61% (w/w), while at a lower moisture content the crystallites undergo the melting process upon continuous heating at significantly higher temperatures than those required for gelatinisation. Wang *et al.* (1992) measured the minimum water needed for starch gelatinisation by using a Differential Scanning Calorimeter (DSC), and confirmed that, regardless of the type or botanical origin of the starch, around 61% water is needed to obtain complete starch gelatinisation. Lower water content led to an increase in the temperature of the DSC endothermic peak. Below this critical water content the transition is generally called melting rather than gelatinisation.

Gelatinisation of starches can be followed by several methods including DSC (Donovan *et al.*, 1983, Wang *et al.*, 1989), X-ray diffraction (Vergnes *et al.*, 1987, Willet *et al.*, 1997), measurement of intrinsic viscosity (Diosady *et al.*, 1985, Vergnes *et al.*, 1987, Fujio *et al.*, 1995), measurement of molecular weight (Fujio *et al.*, 1995), measurement of particle size distribution (Zheng *et al.*, 1995), Gel Permeation Chromatography (GPC) (Davidson *et al.*, 1984, Vergnes *et al.*, 1987), Rapid Visco Analysis (RVA) (Whalen, 1999), water solubility and water absorption indices (Ollett *et al.*, 1990). In this study DSC and RVA were used for evaluation of starch conversion. The theories of the techniques used in this study are given in Materials and Methods (Section 2.6.1 and 2.6.2).

1.4.5 Starch conversion during extrusion cooking

A factor in addition to heat that has been used to alter the crystalline order and properties of starches is mechanical processing such as grinding and high pressure extrusion cooking (Harper, 1981, French, 1984). Chemical reactions can be initiated by supplying proper activation energies to the reactants. The form of the energies can be thermal, electrical, shear, or a combinations of these, depending on the chemistry involved. The majority of chemical reactions, however, are initiated by thermal energy. In the extrusion cooking of food materials, the chemical and physical changes that occur are effected not only by thermal energy but also by shear energy and possibly by pressure in the extruder. Thermal and shear energy are the key energies that would cause irreversible changes in the extruder (Wang *et al.*, 1992). Thermally converted (cooked) starch generally retains its granular shape and boundary and also some patterns

of birefringence (Wang *et al.*, 1989). In extrusion cooking of starch-containing materials, starch is heated under pressure, between 30 to 60 atmospheres at up to 40% moisture, to 250°C while it is sheared by the action of screws (Harper, 1981). At these temperatures and moisture contents, starch granules not only undergo both gelatinisation and melting, but the granules are also ruptured and broken into smaller pieces because of the high shear energy involved in the process.

Zheng *et al.* (1995) studied the changes in size and size distribution of starch granules under extrusion conditions by using a single screw extruder and an optical microscope coupled with an image analysis system. The mechanical energy input and material processing time were key factors influencing the degree of starch conversion. In a high shear extrusion process (40°C), the average granular size of extrudate was reduced from 12.4 μm for waxy maize starch to 1-2 μm, whereas in low shear extrusion (90°C), the granular size was reduced to 7μm. Fine particles (<0.5 μm) were observed in both extrusion processes. These varied between 76% under a high shear and long processing time conditions and 5% in a low shear and short-time process.

Nowadays, considerable interest has focused on the use of extruders to produce nutritious blended foods. Size reduction of starch granules in extrusion processes is a crucial factor to the final product quality because it plays an important role in the physical, rheological and sensory properties of the final products. For example, the granular sizes may affect the thermal conductivity, water diffusivity, heat capacity and bulk density of the extrudate. Granular size and size distribution also influence product sensory attributes. Therefore, granular size of the ingredients becomes a critical factor in the product design. For instance, when a product contains large particles the dispersibility of material in water will be rapid, but the resulting mixture is gritty. Conversely, when the particles are small, dispersibility of the material will be poor because of the tendency of fine particles to form clusters. However, the resulting mixture will be smoother (Zheng *et al.*, 1995).

The molecules in starch granules are linked together by bindings that vary in strength from relatively weak van der Waals forces to strong covalent chemical bonds (Zheng *et al.*, 1995). The binding energy of covalent bonds between most atoms in many biological materials is relatively strong, ranging in values from 30 to 230 kcal/mole. In

contrast, the intermolecular bond energy linked by non-covalent bonds and van der Waals forces tends to be weaker ranging in a value from 0.1 to 6.0 kcal/mole (Zubay, 1988). During extrusion cooking, mechanical energy is concentrated on some single bonds and causes bond elongation, elastic deformation, and/or even bond fracture. The damaged granules can then be broken by applying further shear forces. Higher shear energy must be supplied in order to obtain a product with finer powder (Zheng *et al.*, 1995).

Wang *et al.* (1991) investigated the effect of various water contents on gelatinisation and melting of Amioca, a waxy corn starch. Using a Perkin-Elmer DSC-4, they heated the Amioca-water mixtures with moisture contents of 0 to 99% (w/w), from 40°C to 210°C at a rate of 5°C/min. Based on the experimental data a model was developed to simulate conversion (gelatinisation and/or melting) of starch with different water contents. DSC-peak temperatures for Amioca-water mixtures increased from low seventies, for high moisture contents, to up to 230°C for bone-dry samples. Samples with higher than 60% (w/w) moisture content had a constant peak temperature of 71°C, showing that around 61% water content is required for complete gelatinisation upon heating. Lower water contents would allow only part of the starch to be gelatinised and the balance of the starch in the sample could melt if enough energy is applied. Wang *et al.* (1991) proposed that, below the critical water content, the converted starch would show two phases: gelatinised starch and melted starch, and that this would be the case for most starches after extrusion cooking.

Colonna *et al.* (1984) showed that in extrusion cooking of starch, the combined effect of shear together with heat and pressure are mainly responsible for starch conversion. They demonstrated that extrusion cooking led to a macromolecular degradation of amylose and amylopectin and considered that this was due to random chain splitting. It has also been suggested that the degradation at both granular and molecular levels occurred during extrusion of cereals (Guy, 1985). Extrusion destroys the organized crystalline structure either partly or completely, depending upon the amylose-amylopectin ratio and extrusion variables such as moisture content and shear. By using rheological and gel permeation chromatography methods, it has been shown that amylose and amylopectin are degraded by extrusion cooking into lower molecular weight materials (Colonna *et*

al., 1987). It was demonstrated that molecular degradation in the extruder is less extensive when lipids are present, suggesting that lipids may act as a lubricant in extrusion (Bhatnagar and Hanna, 1996). A similar result was reported by Wulansari (1999) in that waxy maize starch was modified by extrusion cooking with and without incorporation of gelatin. Addition of gelatin to starch did not reduce specific mechanical energy (SME), while the degree of starch conversion reduced significantly. The degree of conversion was determined by a DSC study of starch in the mixture, after removal of gelatin by collagens extraction and washing. At constant SME the degree of starch conversion decreased with the increasing levels of gelatin in the formulation. It was suggested that gelatin acts as a lubricant protecting the starch from being converted since more mechanical energy is dissipated in the gelatin phase than the starch phase.

The role of shear, temperature, moisture and composition are significant in the transformation of starch during extrusion (Harper, 1986). The amount of conversion in an extruder is interpreted in terms of a combination of the thermal and mechanical energy input. SME is a measure of the amount of mechanical energy going into the system and varies widely between extrusion processes. Since mechanical energy is converted into heat energy the separation of mechanical and thermal affects on starch conversion is not entirely straightforward. There have been some attempts to evaluate the relative importance of the shear and thermal energies for conversion of starch during extrusion cooking.

Davidson (1983) showed that mechanical stresses were important factors in the degradation of the amylopectin fraction of starch in a single screw extruder. Later, Davidson *et al.* (1984) developed a first order model, which defined the extent of mechanical degradation of the amylopectin component of wheat starch as a function of nominal shear stress and residence time in a single screw extruder. They drew attention to the difficulty of separating mechanical, oxidative and thermal effects on the mechanism of degradation of starch. They constructed their model on the assumption that the mechanical degradation was limited to material in a melted state and that the shear stress was the critical factor under the experimental conditions they employed.

Diosady *et al.* (1985) studied the degradation of wheat starch in a single screw extruder at 79-121°C temperature and 25-30% moisture content. At these temperatures, the

thermal degradation of starch was considered negligible and the degradation was mainly accredited to shear. In this study, the extent of molecular change was determined by solution viscometry.

Using a Creusot-Lorie BC 45 twin screw extruder, Owusu-Ansah *et al.* (1983) in their study of the effect of primary extrusion variables on the gelatinisation of cornstarch, reported a maximum gelatinisation of cornstarch at 100°C, when a high pressure building screw profile was used. SEM study of samples obtained from the extrusion process showed that there were no intact or partially disintegrated starch granules even at 100°C. However, maximum gelatinisation was observed at 170°C when a low pressure building screw profile was employed.

The study of the kinetics of phase transitition of starch in the presence of low moisture content, such as 15 to 40%, at which the extrusion cooking processes are mostly done, are limited. Wang *et al.* (1989) investigated the effect of thermal energy on gelatinisation and melting of Amioca, a waxy cornstarch, with limited water contents to determine the global order of reaction in an extrusion cooking environment. Phase transition of starch was determined by differential scanning calorimetry (DSC). The degree of conversion of starch was evaluated from DSC endotherm area, which is directly proportional to the mass of unconverted starch. The temperature dependence of the rate of conversion of corn starch followed the Arrhenius relation and the reaction was found to be zero order. To explain the combined effect of moisture content and the source of starch on thermal transition of starch, they developed a dimensionless parameter T/T_p where T is the operating temperature for the reaction and T_p is the peak gelatinisation temperature of the starch. Kinetic data available about different starches in the literature were plotted against the dimensionless parameter, T/T_p (Figure 1.7). The linear regression analysis of these data revealed the following equation to predict the reaction rate for the gelatinisation and melting of different starches at different water contents upon heating.

$$k = \frac{x}{74.97x^2 - 167.21x + 93.38} \qquad \textbf{Equation 1.1}$$

where k is the reaction rate constant of starch conversion, x is the dimensionless parameter, T/T_P. This equation was further developed to correlate T_p with the amount of

water for waxy corn starch:

$$T_p(°C) = 227.92 - 2.674W \qquad \textbf{Equation 1.2}$$

where W is the weight percentage of water in the Amioca-water system.

Based on this model, Figure 1.8 correlates the reaction time with the temperature for 50 and 100% conversion at 30% moisture content.

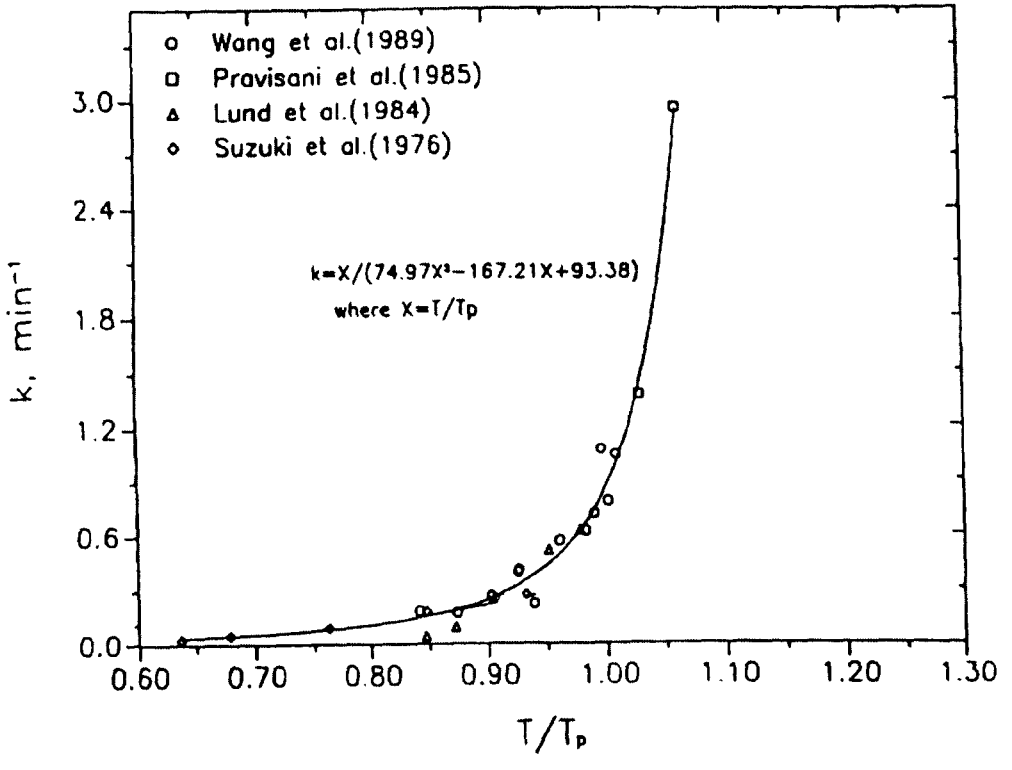

Figure 1.7 Correlation between rate constant of starch conversion k, and a dimensionless temperature parameter T/T_p. Adapted from Wang *et al.*, 1989.

Wang *et al.* (1992) studied the contribution of shear energy, in addition to thermal energy, to the process of gelatinisation and melting under extrusion conditions. They analysed the relative importance of shear vs. thermal energy on conversion (both gelatinisation and melting) of Amioca in an extrusion environment by calculating the expected effect for heat alone and comparing it with the total extent of conversion. In order to obtain an experimentally fitted reaction kinetic model, they used a Brabender laboratory-scale single screw extruder and a capillary rheometer (Instron Co., Model 3211) at various operating conditions. For the extrusion experiments, 50-200 rpm screw

speeds were combined with temperatures of 40-90°C and moisture contents of 30-35 %. For capillary rheometer experiments the temperatures were kept relatively low (21-50°C) in order to provide high shear energies to the samples. To facilitate starch conversion, material was fed several times through the capillary tube and the single screw extruder at a fixed temperature and shear rate. As in their previous studies, they determined the degree of starch conversion by using a DSC. When the operation temperature increased from 40 to 90°C, the conversion only increased 5-9%, whereas an increase of screw speed from 50 to 200 rpm, caused a change of shear stress from $2.2x10^4$ Pa to $3.38x10^4$ Pa at 70°C, increasing the conversion by ≈20-30%. As reported in earlier studies of extrusion cooking of starches below 100°C by Wang *et al.* (1989), the thermal effects on starch gelatinisation was insignificant. For example, in order to obtain 50% conversion at 80°C by thermal energy, 24 min of heating was required (Figure 1.8). This was more than an order of magnitude greater than the residence times in the extruder employed in their study.

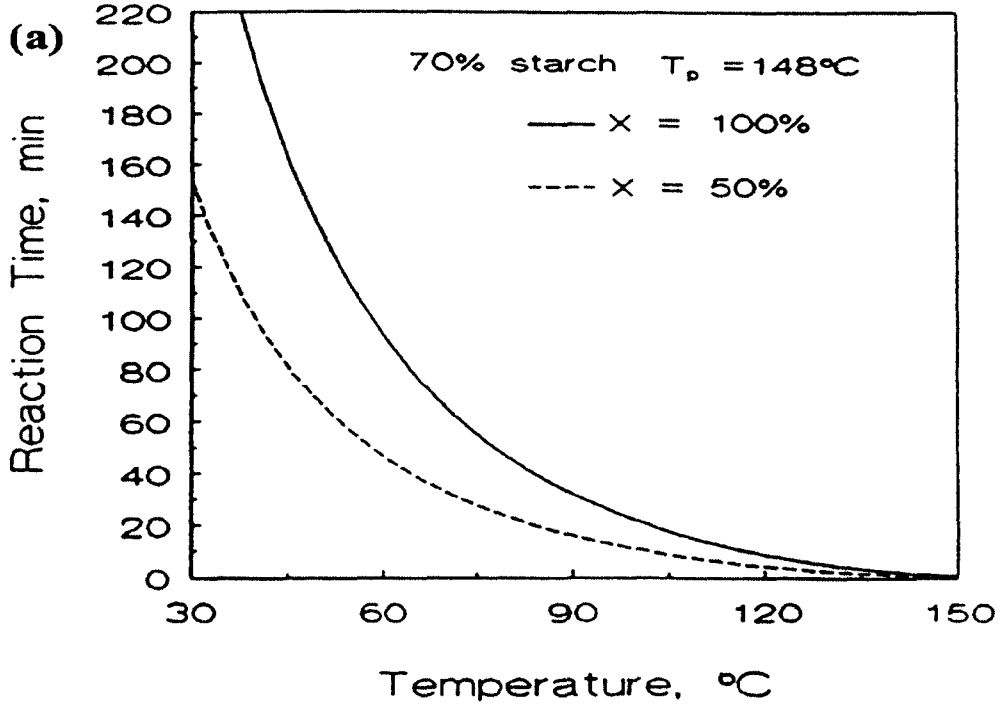

Figure 1.8 Reaction time required to obtain 50 and 100% conversion at different extruder operating temperature. Adapted from Zheng and Wang, 1994.

Their kinetic study on reaction rate constant of starch conversion suggested that shear energy not only causes starch conversion but that it also has a higher efficiency when compared with thermal energy. Their results showed that the shear activation energy reduced with increasing operation temperature and this was explained by the increase of internal energy with increasing of process temperature, which in turn increased the degree of freedom for molecular movements. Consequently, less additional energy was required to initiate shear reactions.

Minimum shear stresses above those at which the gelatinisation reaction could occur were investigated for single screw extrusion and capillary extrusion. Experimental critical shear stresses, defined as σ_{min}, were in the range of 1.0×10^4 Pa at 100°C to 1.9×10^6 Pa at 21°C. The temperature dependency of σ_{min} agreed with the temperature dependency of shear activation energy.

They investigated the possible temperature increase of the extrudate during extrusion by calculating the theoretical maximum temperature increase due to dissipation of mechanical energy and by measuring the temperature of the extrudate at the exit by means of an infrared pyrometer and a thermocouple. Their results suggested that the temperature increase through viscous dissipation was too low to cause any notable thermal cooking. They concluded that extruders can provide sufficient shear energy to convert starch efficiently and that the properties of the shear-cooked starches would be different to those thermally cooked.

In starch extrusion the crystalline part of the starch provides more restriction to water mobility than the amorphous part. In the extruder, starch can proceed to the melting zone with a non-uniform moisture distribution on the micro scale. If the temperature is sufficiently high, the starch may undergo glass transition and gelatinisation, producing a viscoelastic material. Although high temperatures may increase the water diffusivity of a material, the water diffusivity of gelatinised starch is less than the granule, due to the more porous structure of starch granules. This may lead to non-uniform water distribution and cause shear-induced starch conversion in the low moisture region, or thermal-induced starch conversion in high moisture region (Wang *et al.*, 1989).

An intrinsic viscosity study by Fujio *et al.* (1995) also supported the idea that more

molecular degradation occurred at lower moisture, leading to a reduction in molecular weight. Depolymerization of starch into small molecules may occur by heating alone, but shear treatment has been found to intensify depolymerization (Doublier *et al.*, 1986; Fujio *et al.*, 1995).

Using a capillary rheometer called Rheoplast with the facility to apply a well defined pre-shearing, Vergnes *et al.* (1987) correlated the degree of macromolecular degradation of maize starch with the intensity of thermo-mechanical treatment. This device was able to mimic the extrusion process better than a classical capillary rheometer because of its ability to pre-shear the material before the viscosity measurement in the capillary die. The degree of macromolecular change in starch structure was determined by x-ray diffraction patterns, gel permeation chromatography, water solubility and intrinsic viscosity measurements. Starch samples processed by the Rheoplast presented the same type of modifications as those obtained by extrusion cooking, including change of crystalline type from A- to E- and V-types, decrease of molecular weights as traced by GPC and intrinsic viscosity measurements.

These workers hypothesised that two successive steps occurred during the process, (i) the melting of starch in the Couette zone of the Rheoplast and (ii) the shearing of the molten material, which provided the macromolecular degradation by input of mechanical energy. Both thermal and mechanical energy are transmitted to the material in the Rheoplast. Initially, this energy contributed to the heating and melting of the material, i.e. disturbed the starch granule and transformed the starch into a homogenous jellified mass. Then mechanical energy provided to this melted material lead to product degradation by breaking bonds along macromolecular chains.

The intensity of the mechanical treatment is defined by melting time (t_f), rotation time (t_r), effective shearing time (Δt), piston rotation speed (Ω) and mechanical energy input ($\dot{W}$). The change in intrinsic viscosity (η) and water solubility (WS) depending on processing parameters is shown in Table 1.3.

Table 1.3 Change in intrinsic viscosity and water solubility of maize starch depending on process conditions in the Rheoplast (Vergnes *et al.*, 1987).

Processing conditions			**Computed results**			**Measured results**	
T (°C)	Ω (R/min)	t_r (s)	t_f (s)	Δt (s)	W (J/m^3)	$[\eta]$ (ml/g)	WS (%)
160	700	40	2.8	38.2	10.48×10^8	118	96
160	700	20	2.8	18.2	4.99×10^8	137	92
160	700	10	2.8	8.2	2.25×10^8	138	59
160	400	20	5.1	14.9	1.97×10^8	140	59
160	700	5	2.8	3.2	0.88×10^8	176	25
180	200	20	6.9	13.1	0.71×10^8	151	35
160	200	20	8.3	11.7	0.63×10^8	152	55
140	200	20	10.6	9.4	0.51×10^8	171	38

Similar results were obtained by Fujio *et al.* (1995), who used a flow tester to shear preheated molten potato, corn and wheat starch under varying pressures. The degradation of starch granules detected by gel filtration, molecular weight determination, viscosity measurements and glucose detection showed a significant contribution of shear energy to starch degradation.

Cai and Diosady (1993) studied the profile of wheat starch gelatinisation along a twin screw extruder channel and developed a first order model of starch gelatinisation in terms of temperature, shear stress and mean residence time in the cooking zone of the extruder. The regression analysis of their results showed that the starch gelatinisation in a twin screw extruder does not follow a simple reaction mechanism. The reaction follows a pseudo-second order rate law in the transition section followed by a pseudo-first order rate law. They suggested that the overall process could be approximated by a first order model since it gave good fit of experimental data for the complete cooking zone. They observed that the degree of gelatinisation is increased for all the combinations of temperature and moisture content, when the screw speed increased

from 200 rpm to 300 rpm. Low moisture systems had high viscosities, which resulted in high shear stress, leading to more extensive degradation. Similarly, high screw speeds generated high shear rates, also leading to more degradation of starch granules. However, the effect of temperature was complex. It had two simultaneous effects on degradation. It increased the degree of gelatinisation, thus making more degradation possible, but it also decreased the viscosity leading to a decrease in the mechanical degradation rate. For lower moisture content (25%), the extent of degradation increased with the temperature in the range of 100-120°C and decreased with temperature in the range of 140-160°C. For higher moisture content (30%), the extent of degradation increased with temperatures in the range of 100-160°C.

The influence of the shear and mechanical forces on the destruction of microorganisms and starch granules will be discussed in the following chapters. The term "mechanical forces" and "shear forces" were used interchangeably throughout the thesis, since these two terms were closely related in the extrusion process.

CHAPTER 2

MATERIALS AND METHODS

2.1 GENERAL MATERIALS AND METHODS

2.1.1 Extruder and Ancillary Equipment

A Clextral BC-21 twin screw, co-rotating, intermeshing extruder (Firminy, France) was used for the experiment. The 400 mm extruder barrel has a 16:1 length to diameter ratio and consists of four modules each 100 mm long. A slit die of 2 mm in height 20 mm in width and 14.5 mm in length was used. The schematic diagram of the extruder is shown in Figure 2.1.

A K-tron T20 volumetric feeder (Niederlenz, Schweiz, Switzerland) was used for feeding the material to the extruder. Sterile distilled water was continuously added using a DKM-Clextral reciprocating volumetric pump (type:TO/2, Firminy, France). The rate of addition was varied to give the desired die pressure. Both the feeder and the water pump were calibrated prior to the extrusion in order to avoid fluctuations during the operation. The last three temperature controlled sections of the extruder barrel were heated by electricity and cooled by a cooling system using cold water as the heat transfer medium. The inner barrel wall surface temperatures were measured by thermocouples inserted through the barrel wall. A transducer port located next to the feed port on the extruder barrel was modified to hold the hypodermic of a syringe. All inoculations were carried out from this point by using sterile syringes.

Barrel temperatures, back pressure, torque, screw speed and feed rate were recorded by a Clextral MEMOSYS computer. Collection of the samples was done after steady

state conditions were established, which was indicated by constant back-pressure, torque and barrel temperatures. The pressure and temperature at the entrance of the die was measured with a combined temperature and pressure sensor which was an integral part of the extruder. Because of a malfunction of this sensor, for some experiments, a Dynisco ER478 pressure transducer fitted to the die entrance was used for the pressure measurements. The temperature of the extrudate at the die exit (maximum temperature) was measured by carefully inserting a thermocouple insertion probe connected to a hand held digital thermometer (Digitron, 3202K) into the die.

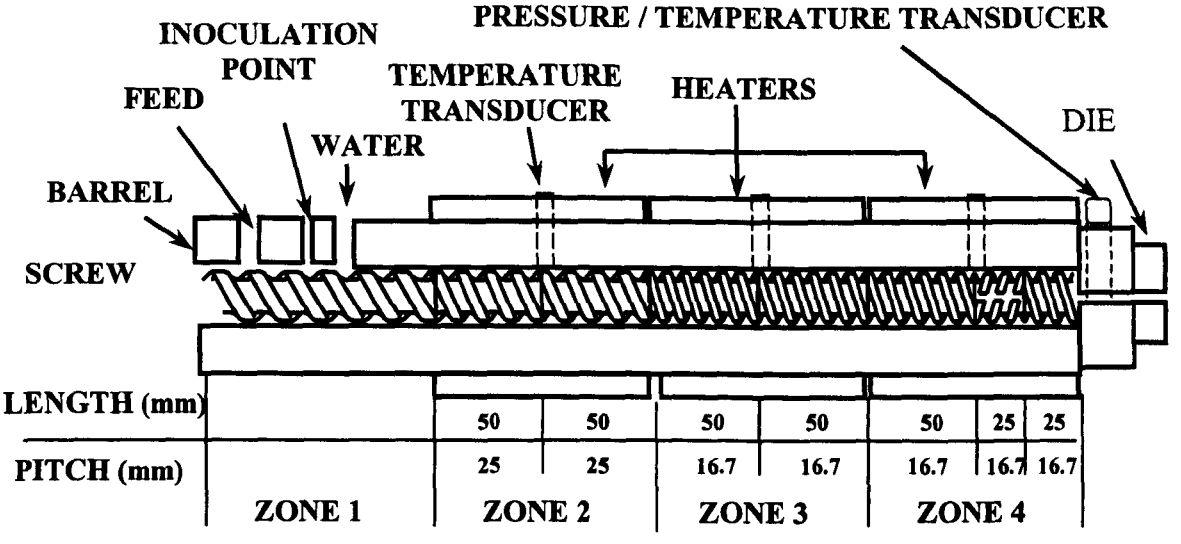

Figure 2.1 Schematic diagram of Clextral BC-21 twin screw extruder showing the inoculation point and screw profile.

The specific mechanical energy (SME) was calculated from the relationship:

$$SME(J \cdot kg^{-1}) = \frac{Tx2\omega}{T_F} = \frac{2\pi}{60}\frac{Tx2S}{T_F} \qquad \textbf{Equation 2.1}$$

where ω is the angular velocity (rad·s^{-1}),

T is the torque (N·m),

S is the screw speed (rpm),

F_T is the total feed rate: material feed rate + water feed rate (kg·hr^{-1}).

2.1.2 Other equipment

Other equipment used throughout the experiments is listed in Table 2.1.

Table 2.1 Equipment used throughout experiments.

Instrument	Brand/Model/Company
Analytical balance	Sartorius 1712, Germany.
Balance	Sartorius L420P^{+}, Germany.
Bohlin VOR rheometer	Bohlin instruments, Sweden.
Cyclotec mill	Model:1093, Foss Tecator, Sweden
DSC-7	Perkin Elmer, Norwalk, CT
Hunterlab Colorimeter	Hunter Associates Laboratory, USA
Infrared dryer	Sartorius Thermo Control YTC 01L, Germany.
Knifetec mill	Model:1095, Foss Tecator, Sweden
Ovens	Gallenkamp oven 300 plus series, Loughborough, UK.
Planetary mixer	Kenwood-Peerless, Peerless and Ericsson Ltd. Birmingham, UK
pH Meter	Jenway 3320, Fisher Scientific UK, Loughborough, UK.
Rapid Visco Analyser	RVA Series 4, Newport Scientific, NSW, Australia.
Sieves	Endecotts Test Sieve, Endecotts Ltd., London, UK.
Silverson mixer	IKA, Germany.
Spectrophotometer	LKB Biochrom, Ultraspec 4050, Surrey, UK.; Perkin-Elmer
Water baths	Grant Instruments (Cambridge) Ltd., Barrington, Cambridge, UK.
Weissenberg Rheogoniometer	Model: R-19
Whirlimixer	Fisons Scientific Equipment, Loughborough, UK.

2.1.3 Moisture determination

Extruded gelatin samples were cut into small pieces and dried at 70°C in a vacuum oven overnight. Powder gelatin samples were dried directly in the vacuum oven overnight.

The moisture contents of native and extruded maize grits samples were determined by oven drying at 105°C overnight. Extruded maize grits samples were ground in a Knifetec mill before drying. Unless otherwise stated, all the moisture contents are expressed as wet weight basis (wwb).

2.1.4 Carrier medium

Gelatin used as a carrier for extrusion experiments was supplied as a limed hide, 225 Bloom Kosher gelatin by Rousselot (France). The moisture content of the gelatin was approximately 13%. Gelatin was sieved through a 250 μm laboratory sieve for the heat resistance experiments and the experiments done with the rheometers.

Fine waxy maize grits were obtained from National Starch and Chemical Co., Manchester, UK, with approximately 12.0% (wwb) moisture content.

2.2 RESIDENCE TIME DISTRIBUTION ANALYSIS (RTD)

2.2.1 Theory

The RTD is indicative of the flow behaviour of the product passing through the extruder and provides a foundation for evaluating the extrusion. The RTD in an extruder is a useful means of determining optimal processing conditions for mixing, cooking and shearing reactions during the process (Lee and McCarthy, 1995). The average mean residence time of mass flow, degree of mixing and total strain exerted on the mass during its transition can be estimated from RTD functions (Lee and McCarthy, 1995, Fichtali and van de Voort, 1995). Under the extrusion conditions, complex physicochemical changes including transformations in textural properties as well as some nutritional loss occur in food materials. RTD function, with the knowledge of operating variables such as temperature, screw speed, screw configuration, and moisture content, provides the necessary information to predict

what fraction of material will undergo specific reactions (Lee and McCarthy, 1995). When working with microorganisms, the determination of mean residence time, which is estimated from RTD, is an important parameter for evaluation of death rate of the microorganism. Thus in this study the RTD function of different extrusion conditions has been determined.

The measurement of RTD in an extruder usually involves a stimulus response method (Ward *et al.*, 1996). A pulse of tracer is added to the feed port of the extruder and the concentration of this tracer in the extrudate is measured. A graphical plot of tracer concentration in the extrudate against time from addition to the feed port gives the RTD curve. Mathematical models have been used in the literature to analyse the RTD. The RTD of a material in an extruder is usually described by *E(t)* and *F(t)* plots (Altomare *et al.*, 1992, Peng *et al.*, 1994, Fichtali and van de Voort, 1995, Lee and McCarthy, 1995). The response of an extruder to a pulse inlet is given by an *E(t)* diagram, which represents the age distribution of material inside the extruder (Lee and McCarthy, 1995) and is given by:

$$E(t) = \frac{c(t)}{\int_0^\infty c(t)dt} \cong \frac{c(t)}{\sum_0^\infty c(t)\Delta t} \qquad \textbf{Equation 2.2}$$

Where *c(t)* is the tracer concentration appearing at the outlet at time *t* and Δt is the sampling time difference between the two subsequent samples. The *F(t)* plot is related to the *E(t)* plot and represents the cumulative distribution function in the exit stream at any time. *F(t)* is obtained by integrating the *E(t)* function.

$$F(t) = \int_0^t E(t)dt \cong \frac{\sum_0^t c(t)\Delta t}{\sum_0^\infty c(t)\Delta t} \qquad \textbf{Equation 2.3}$$

The mean residence time ($\bar{t}$), which represents the mean time that the material spent

in the extruder is given by:

$$\bar{t} = \int_0^t tE(t)dt \cong \frac{\sum_0^t tc(t)\Delta t}{\sum_0^\infty c(t)\Delta t} \qquad \textbf{Equation 2.4}$$

The maximum residence time was determined by the sampling time when no further die was detectable.

2.2.2 Experimental

The dye tracer method was used for RTD determination. For this purpose, the sodium salt of erythrosin B ($C_{20}H_6I_4O_5Na_2$) was used as a dye. Concentration of dye in extrudate samples determined by a spectrophotometer or a colorimeter was plotted as a function of time.

2.2.2.1 RTD of gelatin in extruder

LKB Biochrom Ultrospec 4050 Spectrophotometer was used for the determination of dye concentration in the extrudate samples.

2.2.2.1.1 Preparation of standard curve

The wavelength that gives maximum absorbance for erythrosine dye in 1.0% (wwb) gelatin solution was determined by scanning the absorbance of the solution between 515-545 nm. The maximum absorbance was found to be at 533 nm and this wavelength was used for the analysis.

Different concentrations of erythrosin dye in 1.0% (wwb) gelatin solutions were prepared. The absorbance of each solution with known concentration of dye was recorded against a reference solution (1.0% [wwb] gelatin solution without dye). A standard curve was obtained by plotting the absorbance readings against concentration.

2.2.2.1.2 Inoculation of the dye into the extruder and analysis of samples for determination of RTD

When steady state conditions were reached as indicated by a constant torque, pressure and product temperature, two samples were collected and sealed in polyethylene/aluminium (PE/Al) bags for use as reference samples. A sample (1 ml) of dye solution containing 30 mg of the dye was instantaneously introduced by a syringe into the extruder, from the inoculation point as shown in Figure 2.1. The extrudates were collected for every 60-sec as one continuous strand, over a period of 4 min following the introduction of the tracer. The samples were sealed in PE/Al bags and kept sealed until the time of analysis.

Each 60-sec sample strand was divided into 4 equal segments by length, and the weight of each segment was recorded. The average residence time associated with each segment was determined by taking the ratio of the cumulative weight corresponding to each segment to the total weight of the extruded strands collected over a 4 min period.

LKB Biochrom Ultrospec 4050 digital spectrophotometer was used to determine the amount of dye in each sample. For this purpose, gelatin samples were taken from the middle section of each segment and 1.0% (wwb) gelatin solutions were prepared from these samples. A 1.0% (wwb) reference solution was also prepared by using the reference sample. The absorbance of each sample solution was recorded against the reference solution. The average amount of dye in each sample was calculated by using the standard curve.

2.2.2.2 RTD of maize grits in extruder

Since it was not possible to extract the dye from extrudates of maize grits, the Hunter colorimeter was used for estimation of dye concentration in the samples. The collection of extrudates and preparation of the samples for the analysis remained the same as described previously for gelatin. The samples were ground by a coffee grinder and the intensity of the dye in each sample was measured as the red colour component (a-value) using a Hunterlab colorimeter. The following formula was

developed for the calculation of the die concentration in each sample.

$$C_i \cong W_{dye} \frac{c_i w_i}{\sum_{i=1}^{i=n} c_i w_i} \qquad \textbf{Equation 2.5}$$

Where, C_i is the dye concentration in i^{th} sample (mg dye per g sample),

W_{dye} is the total amount of the dye inoculated into the extruder (mg),

c_i is the redness value of the i^{th} sample,

w_i is the weight of i^{th} sample (g),

n is the total number of samples.

The rest of the calculations remained the same as for the determination of RTD for gelatin.

2.3 RHEOLOGICAL CHARACTERIZATION OF GELATIN AND MAIZE GRITS

The extruder die served as a slit die rheometer and the rheological characterisation of the gelatin and maize grits during extrusion were done according to the principles applied to the slit die rheometers. In addition the flow behaviour of gelatin was investigated by a rotational and a piston capillary rheometer.

2.3.1 Theory

It is outside the scope of this study to give detailed information about rheology. Only basic information will be given as a foundation to understand the use of equations for characterization of the materials used as carrier. This will also provide an understanding about the magnitude of shear forces acting on the test microorganisms during the experiments. Detailed information can be found elsewhere (Rha, 1978, Bourne, 1982, Howard, 1991, Borwankar, 1992, and Lapasin and Pricl, 1995).

Rheology can be defined as the study of deformation of a body under the influence of stresses. "Bodies" in this context can be either solids, liquids, or gases (Schramm, 1994). Ideal solids deform elastically. The energy required for the deformation is

fully recovered after the stresses are removed. Ideal fluids such as liquids and gases deform irreversibly, in other words they flow. The energy required for the deformation is dissipated within the fluid in the form of heat and cannot be recovered simply by removing the stresses. Most real solids can also deform irreversibly under the influence of forces of sufficient magnitude. The real bodies we encounter are neither ideal solids nor ideal fluids. Few liquids of technical or practical importance come close to ideal fluid behaviour. The vast majority of liquids simultaneously display fluid-like and solid-like behaviour. This phenomenon is known as **viscoelasticity**.

Isaac Newton (1642-1727) was one of the earliest researchers to study the flow of fluids. He was the first to express the basic law of viscometry by describing the flow behaviour for an ideal (Newtonian) liquid:

$$\eta = \frac{\sigma}{\dot{\gamma}} \qquad \textbf{Equation 2.6}$$

Where σ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}) and η is the viscosity (Pa.s). The measurement of viscosity of liquids requires the definitions of parameters which are involved in fluid flow. Some important definitions in viscometry are set out in the following:

Shear stress (σ), is the stress component applied tangential to the plane on which force acts. It is expressed in units of force per unit area (Pa). It is a force vector that possesses both magnitude and direction.

Strain, is a dimensionless quantity representing the relative deformation of a material. If the stress is normal to the sample surface, the material will experience **normal strain** (ε). On the other hand, when a material encounters a shear stress a **shear strain** (γ) is observed.

Shear rate ($\dot{\gamma}$) is the rate of change of shear strain. It can also be explained as the velocity gradient established in a fluid as a result of an applied shear stress. It is expressed in units of reciprocal seconds (s^{-1}).

Viscosity (η), is a measure of internal friction of a liquid or its tendency to resist flow. For a Newtonian liquids, the shear stress is directly proportional to the shear rate as defined by Equation 2.6 and the proportionality constant is the viscosity of the fluid.

Apparent viscosity (η_a), is the viscosity of a non-Newtonian fluid. Although defined by equation 2.6 it is not constant since it depends on the shear rate or shear stress employed in the measurement.

The various types of flow behaviour that can be observed have been discussed in detail in the literature (Rha, 1978, Bourne, 1982, Howard, 1991, Borwankar, 1992, Whorlow, 1992 and Schramm, 1994) and will be summarized as follows:

2.3.1.1 Newtonian Fluids

This type of flow obeys the Newton's law of viscosity and is characterized by a viscosity which is independent of the shear rate. Examples of Newtonian fluids are water, milk, low molecular weight liquid, solutions of low molecular weight solutes, very dilute polymer solutions and dilute emulsions (Rha, 1978).

2.3.1.2 Non-Newtonian fluids

Liquids that do not obey Newton's law of viscosity are known as non-Newtonian fluids. They outnumber the Newtonian fluids by far. Basically non-Newtonian materials can be time independent, where the viscosity changes as the shear rate is changed but the viscosity is independent of the time of shearing, or time dependent, where the viscosity changes with time under constant shear rate. In these types of flows, no proportional relationship exists between shear stress and shear rate as mentioned above, and the term apparent viscosity is widely used for the viscosity of non-Newtonian fluids.

2.3.1.2.1 Time independent

This includes pseudoplastic, Bingham plastic and dilatant flows. Pseudoplastic fluids show a decrease in viscosity as the shear rate increases. This is also known as shear thinning. Pseudoplastic behaviour is often due to the disruption of entanglements

between macromolecules with increasing shear rate, the orientation of asymmetric particles in the flow field (Launay *et al.*, 1986, Schramm, 1994) or disaggregation of particles due to shear forces (Schramm, 1994).

Dilatant or shear thickening fluids increase their viscosity with increasing shear rate. This type of fluid usually contains high concentrations of suspended solids. One interpretation for this type of behaviour is the break up of the layers of flowing particles at high shear rate resulting in increased interactions between layers.

Plastic or Bingham fluids describe pseudoplastic liquids that require a minimum shear stress known as "yield stress" to initiate the flow of material. Once this has been exceeded a linear (Bingham plasticity) or non-linear relationship between shear stress and shear rate can be obtained.

All the types of flow explained so far are shown on a single graph in Figure 2.4.

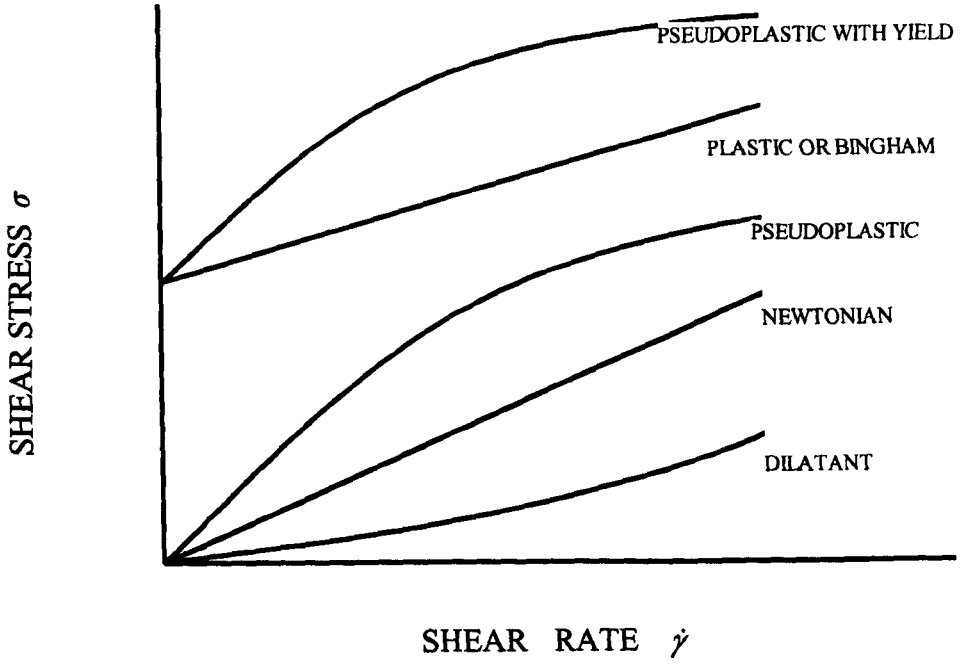

Figure 2.4 Shear stress versus shear rate plot for various types of flow.

2.3.1.2.2 Time dependent

In this type of flow, shear stress is a function both of the shear rate and the time for which the material is subjected to a shearing force. It is called ***thixotropy*** when the

apparent viscosity is decreased with time but the change is reversible. When the change (decrease) in viscosity is irreversible it is called ***shear thinning*** or ***work softening*** (Bourne, 1982).

Rheopectic describes the reversible increase in the apparent viscosity with the time of shearing. It is called ***shear thickening*** or ***work hardening*** if the increase in the apparent viscosity is irreversible.

A number of other equations, almost all of which are empirical in nature, have been described in the literature (Bourne, 1982) for characterisation of non-Newtonian fluids. These equations facilitate the handling of empirical data. It is important to appreciate that they are only valid in a limited shear rate range. The most common one is ***the power equation*** which is written as

$$\sigma = K \cdot \dot{\gamma}^n \qquad \textbf{Equation 2.7}$$

where σ is the shear stress,

K is the consistency index,

$\dot{\gamma}$ is the shear rate

n is the flow behaviour index.

Flow behaviour index n is a dimensionless number that indicates the closeness to Newtonian flow. For a Newtonian liquid n is 1; for a dilatant fluid n is greater than 1; and for a pseudoplastic fluid n is less than 1.

2.3.1.3 Equations applied for slit die, rotational and piston capillary rheometers

2.3.1.3.1 Slit die

The equations for calculation of apparent viscosity in a die are derived from a force balance on a cylindrical element inside the capillary die. The derivation of equations has been shown in the literature (Harper, 1981, Whorlow,1992).

In order to calculate the apparent viscosity, the wall shear stress and wall shear rate

in the die were calculated. The wall shear stress σ_w is determined by the pressure gradient (ΔP) along the length L of the die and is defined by the following equation, where the h is the gap thickness.

$$\sigma_w = \frac{h}{2} \cdot \frac{\Delta P}{\Delta L} \qquad \textbf{Equation 2.8}$$

The apparent shear rate ($\dot{\gamma}_{app}$), based on the average fluid velocity in the slit die, is defined by the following equation:

$$\dot{\gamma}_{app} = \frac{6Q}{wh^2} \qquad \textbf{Equation 2.9}$$

where Q is the volumetric flow rate and w is the slit width.

To account for the velocity profile in the die channel, the apparent shear rate is generally corrected to obtain the true wall shear rate. Rabinowitsch correction relates true wall shear rate to the apparent wall shear rate for power law fluids through the following equation for a slit die rheometer:

$$\dot{\gamma}_w = \frac{2n+1}{3n}\dot{\gamma}_{app} \qquad \textbf{Equation 2.10}$$

The apparent viscosity at the extruder die wall (η_w) is calculated according to the following equation:

$$\eta_w = \frac{\sigma_w}{\dot{\gamma}_w} \qquad \textbf{Equation 2.11}$$

2.3.1.3.2 Rotational rheometers

The Bohlin VOR rheometer used for shearing the test microorganisms in a gelatin carrier was equipped with cone and plate geometry. The shear stress (σ) for this geometry is given by:

$$\sigma = \frac{3T}{2\pi r^3} \qquad \textbf{Equation 2.12}$$

where T is the torque response of the fluid on measuring sensor and r is the radius of the cone and/or plate.

The shear rate for a cone angle θ and an angular velocity ω is given as below

$$\dot{\gamma} = \frac{\omega}{\tan\theta} \qquad \textbf{Equation 2.13}$$

2.3.1.3.3 Piston capillary rheometer

Apparent shear rate ($\dot{\gamma}_{app}$) and the die wall shear stress (σ_w) through a capillary die is given by the following equtions:

$$\dot{\gamma}_{app} = \frac{4Q}{\pi r^3} \qquad \textbf{Equation 2.14}$$

$$\sigma_w = \frac{\Delta P \cdot r}{2L} \qquad \textbf{Equation 2.15}$$

Where, r is the radius of the capillary die.

The Rosand RH-7 twin bore capillary rheometer allowed to apply Bagley and Rabinowitsch corrections.

The Bagley correction takes account of the pressure drops at entry to, and exit from, a capillary die. The correction is made by performing the tests using a capillary die in the left hand bore, and an orifice die of the same diameter in the right hand bore. Using this configuration of dies allows the analysis package to perform Bagley correction on the data. The pressure data from the two dies is used to extrapolate what the pressure reading would have been with a hypothetical zero length die. The pressure readings thus obtained are due entirely to the entry and exit pressure drops. The Bagley correction was applied according to the following formula

$$P_0 = P_S - \frac{P_L - P_S}{L_L - L_S} L_S \qquad \textbf{Equation 2.16}$$

Where, P_0 is the Bagley corrected pressure drop across the short die, P_S is the pressure drop through the short die, P_L is the pressure drop through the long die and L_L and L_S are the length of long and short dies respectively.

The Rabinowitsch correction takes account of the fact that the assumed parabolic velocity profile in the die is, with non-Newtonian fluids, actually more plug-like. The correction is applied to the observed shear rate, to give a 'true' shear rate on which subsequent calculations can be based. For a capillary die, the correction uses the value of the flow behaviour index n according to the following formula:

$$\dot{\gamma}_w = \left(\frac{3n+1}{4n}\right)\dot{\gamma} \qquad \textbf{Equation 2.17}$$

2.3.1.3.4 Extensional Behaviour

The flow of a fluid through a capillary die is characterized by shear behaviour. However, as material flows from the reservoir of a capillary rheometer into a die the streamlines converge and accelerate, producing a strong extensional flow. The flow into an orifice die, in particular, can usefully be used to deduce apparent extensional properties. The software of the Rosand RH-7 Capillary rheometer performs this analysis using widely accepted methods. Extensional stress (σ_e) is calculated according to the formula

$$\sigma_e = \frac{3(n+1)}{8}P_0 \qquad \textbf{Equation 2.18}$$

Extensional strain rate (ε) is given as

$$\varepsilon = \frac{4}{3}\frac{\eta}{n+1}\frac{\dot{\gamma}^2}{P_0} \qquad \textbf{Equation 2.19}$$

Dividing extensional stress by extensional strain rate gives the following extensional viscosity (λ) equation

$$\lambda = \frac{9}{32}\frac{(n+1)^2}{\eta}\frac{P_0^2}{\dot{\gamma}^2} \qquad \textbf{Equation 2.20}$$

2.3.2 Determination of volumetric flow rate

2.3.2.1 Volumetric flow rate of gelatin extrudate

In order to calculate the apparent shear rate at the extruder die, the volumetric flow rate was determined according to the following formula.

$$Q = \frac{\dot{m}}{\rho} \qquad \textbf{Equation 2.21}$$

where $\dot{m}$ is the gravimetric flow rate determined by collection of the extrudates for certain periods of time and ρ is the extrudate density.

In order to determine the density, the gelatin extrudates were coated with liquid paraffin after recording the weights of the samples. The coated samples were placed in to a 250 ml measuring cylinder and the water was added up to the 250 ml level. The extrudate was taken from the cylinder and the volume of the sample was calculated by subtracting the final volume of the water from 250 ml. The density, was calculated by dividing the weight of the sample to the volume of the sample.

2.3.2.2 Volumetric flow rate of maize grits extrudate

According to the moisture content of the extrudate, the density was linearly computed from the density of starch granule, ρ_s (1450 kg/m^3; Colonna *et al.*, 1989) and the density of the water, ρ_w, at the mean die temperature of extrusion. Equation 2.17 was applied for the calculation of the volumetric flow rate, where the gravimetric flow rate, $\dot{m}$, was estimated from total feed rate.

2.3.3 Determination of residence time of the material in the extruder die

The residence time of the material in the extruder die was calculated based on the volumetric flow rate (Q) and the die dimensions. If the A is the area of the die cross-section and l is the die length, the residence time in die, t_d, is given by:

$$t_d = \frac{A \cdot l}{Q}$$ **Equation 2.22**

2.4 TEST ORGANISMS

2.4.1 Microbacterium lacticum

JP2/1/1 strain of *Microbacterium lacticum,* which is a Gram positive, nonsporing heat resistant bacterium forming small round yellow colonies on BHI agar, was used as a test organism. This strain was originally isolated from pasteurised liquid whole egg (Payne *et al.*, 1979).

2.4.1.1 Growth and enumeration of the microorganism

For growth of liquid cultures of *M. lacticum* BHI broth (Oxoid), and for viable counts BHI agar plates were used (incubation time 48 to 72 h at 30°C). Phosphate Buffered Saline (PBS) pH 7.3 (Oxoid) was used as the dilution medium.

Liquid cultures were produced in 1 litre flasks containing 200 ml of BHI broth inoculated by 1 ml of fresh culture produced from a single colony. The flasks were incubated overnight (approximately 18 h) in a shaker operating at 200 rpm at 30°C. Liquid cultures in small amounts were produced in universal tubes containing 10 ml of BHI broth inoculated with 20 µl of liquid inoculum produced from a single colony. The inoculated tubes were incubated at 30°C in a shaker operating at 200 rpm. The liquid culture contained an average of $1.0x10^9$-$1.0x10^{10}$ cfu·ml^{-1} after 18 h of incubation.

2.4.1.2 Growth curve

Two 1 litre flasks each containing 200 ml of BHI broth were inoculated with 1 ml of active liquid culture and the flasks were incubated at 30°C in a shaker operating at 200 rpm. The absorbance readings at 600 nm were taken at zero time and every 1-2 hour intervals thereafter. Samples taken at different stages of growth were diluted with sterile BHI broth in order to get absorbance readings below 0.50. The resultant

absorbances were calculated by multiplying the absorbance readings by the dilution factors.

2.4.1.3 Heat resistance tests

Heat resistance of *M. lacticum* was tested in gelatin and PBS. In order to test the heat resistance of the microorganism in gelatin, the liquid culture of the microorganism was mixed with gelatin to give a final moisture content of 65% (wwb). For this purpose, 3 g of gelatin with a known amount of moisture content was mixed with a calculated amount of culture broth diluted to 50% with PBS pH 7.3, in a sterile universal tube. This mix was kept in a 60°C water bath for 17 min in order to melt the gelatin. The pH of this mix was measured as 5.8-6.0 with a paper pH indicator. The mix was transferred into sterile universal tubes of known weight in 1-2 ml amounts by using sterile syringes. After recording the weight of tubes containing the mix, the tubes were immersed in a second water bath adjusted to the test temperature. A heating up time of 3 min was applied and then the first tube was taken from the water bath as the time zero sample. A dilution medium of 20 ml at 55°C was added to the tube and mixed with a Whirlimixer. The weights of the tubes with the dilution medium were recorded and serial dilutions were made for viable counting at 10-15 min intervals. Calculations were made on a weight basis in order to plot the survival curves.

In order to understand the effect of gelatin on the survival of *M. lacticum* a heat resistance test was also carried out in PBS at pH 5.9 in the absence of gelatin. Culture (3 ml) of *M. lacticum* in BHI broth was added to 15 ml of PBS at pH 5.8 in a sterile universal tube. The pH of this mix was measured as 5.9. A sample (1ml) of this mix was added to screw capped Eppendorf tubes and immersed in a 60°C water bath for 17 min before transferring to another water bath adjusted to the test temperature. As previously described, a 3 min heating up time was applied. The tubes were taken from the water bath at 10-15 min intervals, and serial dilutions were made for viable counts.

2.4.2 *Bacillus subtilis*

Vegetative cells and spores of *Bacillus subtilis* PS 346 and PS 361 derived from strain 168 (Fairhead *et al.*, 1993) carrying chloramphenicol resistance were used. Strain 346 (wild type, $\alpha^+\beta^+$) has the ability to produce α and β small acid soluble proteins (SASP). Strain PS 361 ($\alpha^-\beta^-$) is a mutant strain of PS 346 and lacks the ability to produce α and β-SASP (Mason and Setlow, 1986).

2.4.2.1 Vegetative cells of *Bacillus subtilis* PS 346

Liquid cultures were produced in sterile universal tubes containing 10 ml of BHI broth supplemented with 3μg/ml chloramphenicol. The tubes were inoculated with 20 μl of liquid inoculum produced from a single colony. The inoculated tubes were incubated at 30°C in a shaker operating at 200 rpm. For enumeration of the vegetative cells BHI agar plates were used (incubation time 15 h at 30°C).

2.4.2.2 Growth curve of *Bacillus subtilis*

Liquid cultures were grown in tests tubes in a shaker operating at 200 rpm at 30°C. Samples were taken every one hour intervals for the absorbance readings as explained for *M. lacticum*.

2.4.2.3 Spores of *Bacillus subtilis* PS 346 and PS 361

2.4.2.3.1 Induction of sporulation

Spores were produced through the exhaustion of nutrients by growth in 2xSG broth (Goldrick and Setlow, 1983). Strains were streaked out onto Brain Heart Infusion (BHI) agar and incubated overnight at 30°C. A colony of overnight culture was inoculated into a sterile test tube containing 10 ml of 2xSG broth supplemented with 3μg·ml^{-1} chloramphenicol. The test tube was incubated at 30°C in a rotary shaker operating at 200 rpm for 18 h. A sample (1 ml) of this culture was inoculated into a 2 litre baffle flask containing 250 ml 2xSG medium supplemented with 3μg·ml^{-1} chloramphenicol and incubated at 30°C in a rotary shaker operating at 250 rpm. Sporulation was followed using a phase contrast microscope. The culture was harvested when only small numbers of vegetative cells were present (typically 2-3

days). Subsequently the harvested spores were washed by repeated centrifugation/resuspension in sterile distilled water at 4°C (Bayliss and Waites, 1979). After the tenth wash the spore pellet was resuspended in sterile distilled water and stored at 4°C until required.

2.4.2.3.2 Viable spore count

The number of spores in the stock spore suspension was determined by plating the dilutions of spore suspension on nutrient agar. The colonies were enumerated after about 18 h of incubation at 30°C. The stock spore suspension contained average counts of 10^9 -10^{10} viable spores per ml.

2.4.3 Escherichia coli

Escherichia coli W3110 strain was used for the experiments conducted with Bohlin VOR rheometer. Liquid cultures were produced in sterile universal tubes containing 10 ml of BHI broth. The tubes were inoculated with 20 µl of liquid inoculum produced from a single colony. The inoculated tubes were incubated at 30°C in a shaker operating at 200 rpm. For enumeration of the vegetative cells BHI agar plates were used (incubation time 15 h at 30°C).

2.4.3.1 Growth curve of *E. coli*

The growth curve for *E. coli* was obtained by applying the same procedures as explained for *B. subtilis*.

2.4.4 Enumeration

The Miles-Misra (Miles *et al.*, 1938) counting method was employed for enumeration of all the test organisms. For preparation of suspensions for serial dilutions, about 1.5-2 g of samples were mixed with 18-20 ml of dilution medium in sterile universal tubes. The weight of sample and dilution medium used for each suspension was determined by an analytical balance. Surviving microorganisms were expressed as colony forming units (cfu) per gram of samples. The average of at least three countings were taken for calculation of the colony forming units for each

sample. The minimum recovery level was calculated based on observation of a single colony on the plate of undiluted suspension.

2.5 INACTIVATION OF MICROORGANISMS IN A GELATIN CARRIER

2.5.1 Inactivation of *M. lacticum* by low temperature extrusion

2.5.1.1 Inoculation of the microorganism into the extruder

At a steady state operation of the extruder two reference samples were taken into sterile stomacher bags and sealed. In order to avoid increasing the water content of the material by the liquid inoculum which, in turn, may reduce the local shear stress around the bacterial cell, the water pump was switched off momentarily and depending on the extrusion moisture level, 3-5 ml of culture containing 10^9-10^{10} cfu·ml^{-1} was injected by a sterile syringe within 5 s. The remainder of the inoculation culture was kept cool in an icebox for determination of the exact inoculation level. As soon as the inoculation of the microorganism was finished, the water pump was switched on. The samples were collected every 15 s from the beginning of injection until 4 min after the start of the operation. The collected samples were put into sterile stomacher bags, sealed and kept cool in the icebox until required for counting.

2.5.1.2 Sampling and enumeration of the surviving microorganisms after extrusion

For enumeration of viable cells after extrusion, samples (1g) were taken from the middle section of each extrudate into sterile universal tubes and about 19 ml of PBS was added to each tube. After melting the samples in a 60°C shaker water bath for 45 min, serial dilutions were made to obtain viable counts as explained in Section 2.1.6.

2.5.1.3 Validation of the sampling method for viable count

In order to validate the sampling method, 1 g samples taken from the middle section of each extruded sample and the remaining whole samples of extrudates were analysed for viable count for an experiment (experiment 3, Table 3.4). For whole sample analysis, sterile 1 litre Erlenmeyer flasks were used. According to sample weights 300-500 ml of PBS was added to the flasks and the samples were melted in a

60°C shaker water bath for 45 min. Serial dilutions were plated on BHI agar and incubated at 30°C for 48-72 h. The number of surviving microorganisms were calculated based on the weights of the samples and the dilution medium used.

2.5.1.4 Effect of enumeration method on *M. lacticum*

As a control, 200 µl of culture was added to a universal tube containing 1 g of reference sample of extrudate and following the addition of 19 ml of PBS, the tube was kept in the 60°C water bath for 45 min. The numbers of the microorganism in the inoculation culture and in heat-treated control sample was determined by viable counts. The reduction in the numbers of the microorganism in the control sample was considered when calculating the overall reduction.

2.5.2 Shear inactivation of *M. lacticum* in Rheometers

2.5.2.1 Preliminary experiments with Weissenberg rheogoniometer

A Weissenberg Rheogoniometer was used for shearing of the gelatin suspensions prepared with a liquid culture of *M. lacticum*. Cone and plate geometry with $2^{o}\ 0'\ 18''$ angle and 50 mm diameter was used to shear the samples.

2.5.2.1.1 Sample preparation and shearing the sample

Gelatin was sieved through a 250 micron mesh size sieve and the portion having particle sizes smaller than 250 micron was used for sample preparation. The desired moisture content of gelatin was achieved by using a liquid culture of *M. lacticum* in BHI broth as a liquid. According to the moisture level of gelatin, a calculated amount of liquid culture was added to 3 g of gelatin in a sterile universal tube, and mixed with a sterile spatula. The mixture was then heated in the 60°C water bath for 60 min in order to obtain a gelatin melt which was substantially free of air bubbles. A sample of this melt was transferred with a sterile syringe to the platen of the rheogoniometer, which was previously heated to 60°C. The remaining sample was kept in the 60°C water bath as a control. The heating chamber of the equipment was closed and the rotation speed was gradually increased to the desired value. The temperature of the sheared sample was monitored and recorded by means of a thermocouple mounted on the platen of the rheogoniometer. During the operation,

the temperature of the water bath was kept 2-4°C more than the platen temperature of the rheogoniometer. After operation the control sample was taken from the water bath and the sheared sample was recovered with a sterile spatula. The recovered sample and a sample taken with a sterile syringe from the control sample were added to sterile universal tubes of known weights. Dilution medium (15-20 ml) was added to each tube in the proportion of the sample weights and the tubes plunged into a 60°C water bath for 10 min in order to melt the samples. Serial dilutions were prepared from both tubes for viable counts. Calculations were made on a weight basis and the reduction in the number of the microorganism due to shear was obtained by subtracting the count reduction in the control sample due to heat from the overall count reduction in the sheared sample. The results were expressed as log reductions.

2.5.2.2 Bohlin VOR rheometer

A controlled shear rate rotational rheometer (Bohlin VOR) was used in order to shear the gelatin mix of *M. lacticum*. Cone and plate (CP 2.5/30) geometry was used with thermal cover to shear the samples. A digital thermometer (Digitron T202KC) was used to monitor the temperature of the sheared sample during the operation of the rheometer. As shown in Figure 2.2, in order to avoid any interference by thermocouple 2 to the flow pattern, the temperature of the sheared sample inoculated with the microorganism was monitored indirectly by thermocouple 1. Experiments done in the absence of the microorganism showed that the temperature of the sheared sample measured by thermocouple 2 was 1.5-1.7°C higher than the temperature measured by thermocouple 1. When quoting the temperature of the sheared sample this correction was taken into account.

2.5.2.2.1 Sample preparation and shearing of sample

Gelatin was sieved through a 250 micron mesh size sieve and the portion having particle sizes smaller than 250 micron was used for sample preparation. The gelatin mix of *M. lacticum* was prepared at 65% (wwb) moisture content level as explained for the heat resistance test in gelatin. The inoculation level of the bacterium in the mix was between $1x10^8$-$8x10^8$ cells per g of sample. A sample of the mix (~1.5 ml)

was added to a screw capped Eppendorf tube with a syringe under sterile conditions. This tube containing the sample was placed in a water bath adjusted to the plate temperature of the rheometer. After 3 min of heating up, 0.5 ml of the sample was

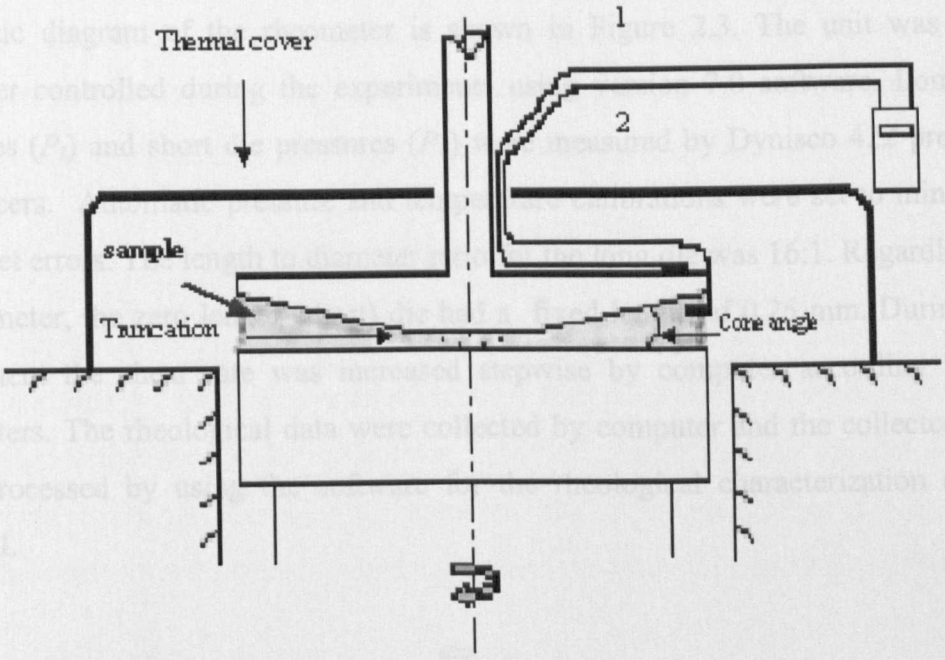

Figure 2.2 Schematic diagram of the measuring geometry of Bohlin VOR rheometer

placed on the plate of the rheometer. The remainder of the sample in the tube was used as control and the tube was submerged into the water bath after mounting a thermocouple to monitor the temperature of the control sample. The temperatures of the sheared sample and the control unsheared sample were recorded at the start of shearing and every min thereafter. At the beginning of shearing, the temperature of the sheared sample (monitored by thermocouple 1, Figure 2.2) and the temperature of the control sample was 3-5° C lower than the set operation temperature of the rheometer. During the operation the temperature of the samples increased gradually with the temperature of the control sample being kept higher (by 2-4°C) than the temperature measured by thermocouple 1 (Figure 2.2). This resulted in a higher (0.6-2.1°C) heat exposure of the control samples compared with the sheared samples. As soon as the shearing of the sample was finished the control sample was taken from the water bath and the sheared sample was recovered by a sterile spatula. The remainder of the process for determination of survival in the sheared and unsheared samples remained the same as explained for the Weissenberg experiments.

2.5.2.3 Extrusion capillary rheometer

A fully automated twin bore extrusion capillary rheometer (Rosand, model:RH7-2) with 280 mm barrel bore length and 15.0 mm barrel bore diameter was used. A schematic diagram of the rheometer is shown in Figure 2.3. The unit was fully computer controlled during the experiments using version 7.0 software. Long die pressures (P_l) and short die pressures (P_0) were measured by Dynisco 422 pressure transducers. Automatic pressure and temperature calibrations were set to minimize the offset errors. The length to diameter ratio for the long die was 16:1. Regardless of die diameter, the zero length (short) die had a fixed length of 0.25 mm. During the experiment the shear rate was increased stepwise by computer according to set parameters. The rheological data were collected by computer and the collected data were processed by using the software for the rheological characterization of the material.

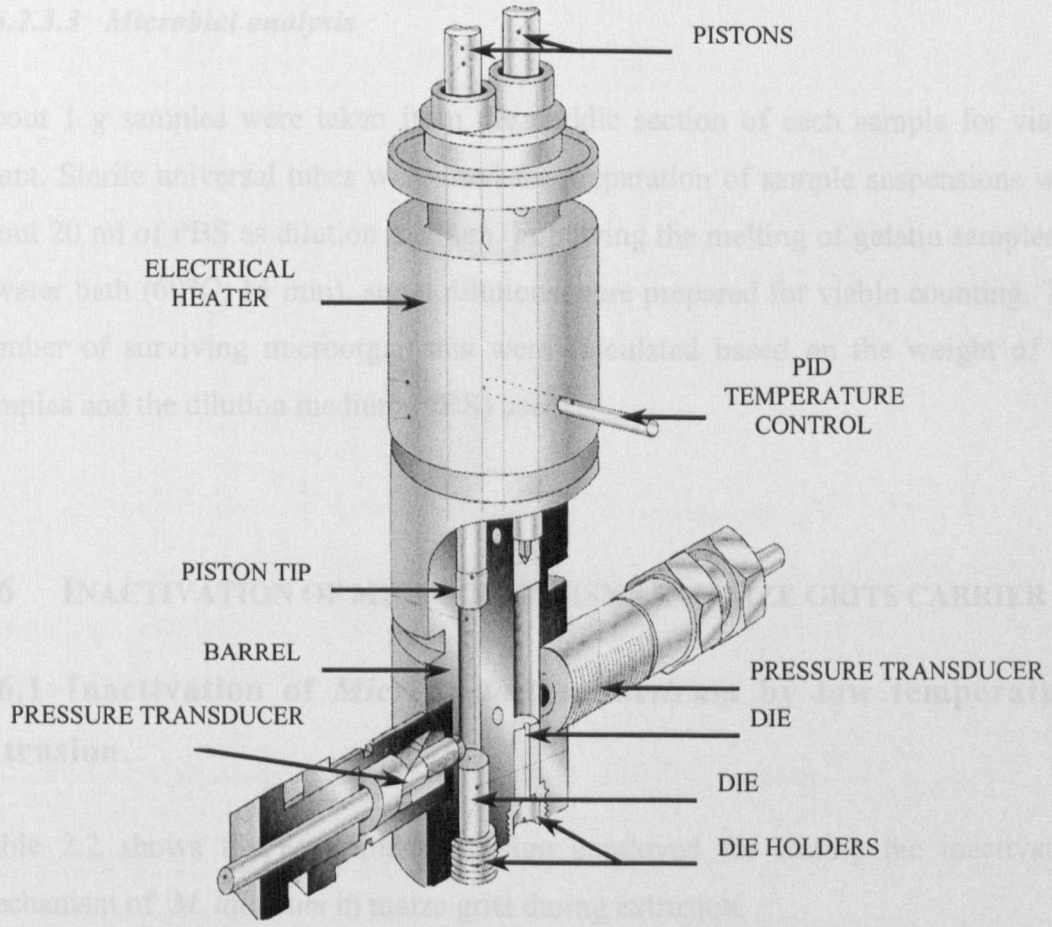

Figure 2.3 Schematic diagram of Rosand RH-7 Piston Capillary Rheometer.

2.5.2.3.1 Preparation of gelatin for the piston capillary rheometer experiments

Gelatin was sieved through a 425 μm laboratory sieve and was cooled by liquid nitrogen while mixing in a Kenwood-Peerless planetary mixer. A calculated amount of liquid culture, to give a final 30% (wwb) moisture content, was sprinkled by a syringe on gelatin while mixing. After mixing for about 10 min the mixture were taken into sterile plastic bags and kept at 4°C until the time of experiment.

2.5.2.3.2 Loading of inoculated gelatin in piston capillary rheometer

After reaching the set barrel temperature, the bores were filled with gelatin mix about 25 mm at a time, tamping between each charge. In order to allow the mix to reach an equilibrium temperature before extrusion, 7 min of preheating was applied under 1 MPa pressure. The samples were collected into sterile universal tubes at every stage of the shear rates and kept cold in an ice box until the time of microbial analysis.

2.5.2.3.3 Microbial analysis

About 1 g samples were taken from the middle section of each sample for viable count. Sterile universal tubes were used for preparation of sample suspensions with about 20 ml of PBS as dilution medium. Following the melting of gelatin samples in a water bath (60°C, 15 min), serial dilutions were prepared for viable counting. The number of surviving microorganisms were calculated based on the weight of the samples and the dilution medium (PBS) used.

2.6 INACTIVATION OF MICROORGANISMS IN MAIZE GRITS CARRIER

2.6.1 Inactivation of *Microbacterium lacticum* by low temperature extrusion.

Table 2.2 shows the experimental design employed for testing the inactivation mechanism of *M. lacticum* in maize grits during extrusion.

Table 2.2 The experimental design for inactivation of *M. lacticum* in maize grits.

Number of Experiment	Added Water (kg/h)	Screw speed (rpm)	Number of runs
1	0.355	80	4
2	0.355	120	4
3	0.355	160	3
4	0.533	80	5
5	0.533	120	5
6	0.533	160	4
7	0.710	80	5
8	0.710	120	5
9	0.710	160	5
10	0.888	80	3
11	0.888	120	4
12	0.888	160	3
13	1.065	80	3
14	1.065	120	4
15	1.065	160	4

2.6.1.1 Inoculation of Maize grits with *M. lacticum*

Maize grits were inoculated with *M. lacticum* as explained for gelatin in section 2.5.2.3.1. Different than inoculation of gelatin, maize grits were not sieved before the inoculation. The level of bacterial load after inoculation was between 10^6 - 10^7 viable cells per g of maize grits.

2.6.1.2 Extrusion of maize grits inoculated with *M. lacticum*

After establishing a steady state operation samples were collected into sterile PE/AL bags, sealed and cooled in an icebox. Maximum extrudate temperature at the exit of the extruder die was measured by a thermocouple and the die pressure measured by a

pressure transducer (Dynisco ER478) mounted on the extruder die. There was a 3-5 min time interval between each set of experiments in order to establish steady state operation conditions for the new settings. Since the maximum residence time inside the extruder barrel was around 3 min, this time gap also ensured the clearance of the material that belonged to the previous extruder settings.

2.6.1.3 Preparation of extrudates for microbial analysis

Cyclotec and Knifetec mills were used for grinding the extrudates for microbial analysis. Ground samples prepared by this way were also used for the analysis of starch conversion by Rapid Visco Analyser (RVA) and Differential Scanning Calorimetery (DSC).

2.6.1.3.1 Cylotec

A Cyclotec mill was used for grinding the samples in order to determine the degree of starch conversion by the RVA technique where the particle size was important. The Cylotec mill gives particle sizes below the sieve size used for grinding. In our case a sieve with 500 μm aperture was employed. Since the moisture content of the milled samples was high, no additional sieving was applied. Samples of extrudates (about 30 g) were frozen by liquid nitrogen and were fed into the Cylotec gradually. After milling, samples were kept cold in an icebox until the time of microbial analysis. The parts of the mills were soaked in Virkon (2%, w/v) for 10 min in order to prevent cross contamination between subsequent millings.

2.6.1.3.2 Knifetec

The Knifetec mill was used for grinding the samples for determination of starch conversion by DSC. Samples of the extrudates (about 30 g) were frozen by liquid nitrogen and milled for 10 s (5x2 s). After grinding, the samples were kept cold in an icebox until the time of microbial analysis. The parts of the mills were disinfected by Virkon (2%, w/v) in order to prevent cross contamination between subsequent millings.

2.6.1.4 Microbial analysis

Sample suspensions were prepared by using PBS as dilution medium. The suspensions were mixed by a vortex mixer for 3 min. The tubes were kept at 4°C for 2-3 min in order to allow the starch particles to settle. A sample of suspension (100 µl) was taken from the top of the tube and used for preparation of serial dilutions which were plated on BHI agar and incubated at 30°C for 2-3 days.

2.6.1.5 Determination of the effect of the milling process on viability of *M. lacticum*

A sample of maize grits mix with *M. lacticum* (30 g) was frozen by liquid nitrogen and milled by both Cylotec and Knifetec Mills. The milled samples were analysed for viable counts as explained previously. A sample of maize grits mixed with *M. lacticum* (not milled) was also analysed for viable count. The reduction in the number of viable cells due to the milling process was considered when calculating the reduction in the number of *M. lacticum* due to the extrusion process.

2.6.2 Inactivation of *Bacillus subtilis* spores by low temperature extrusion

In order to test the effect of low temperature extrusion on spores of *B. subtilis* only maize grits was used as a carrier medium.

2.6.2.1 Inoculation of Maize grits with spores

Maize grits were inoculated with spores of *B. subtilis* as explained for *M. lacticum* in section 2.6.1.1, but the final moisture content of the mix was adjusted to 15.0%. Compared to the mix of maize grits with *M. lacticum,* the lower level of final moisture content for the spore mix of maize grits was due to the risk of germination at higher water contents. After mixing for about 10 min the mixture were taken into sterile plastic bags and kept at 4°C until the time of experiment. The level of inoculation was between 10^6 - 10^7 viable spores per g of mixture.

2.6.2.2 Extrusion of maize grits inoculated with spores

The procedure was the same as employed for the *M. lacticum* mix of maize grits.

2.6.2.3 Preparation of extrudates for microbial analysis

Samples were prepared for microbial analysis as explained for *M. lacticum*.

2.6.2.4 Microbial analysis

The method for the microbial analysis of surviving spores remained the same as for the *M. lacticum* except that chilled sterile distilled water was used as dilution medium for preparation of sample suspensions. Serial dilutions were plated on nutrient agar and incubated at 30°C for overnight. The same dilution tubes were used for testing germination.

2.6.2.5 Test for germination control

In order to determine the number of germinated spores at certain stages of the extrusion process, a heat treatment of 65°C for 10 min was applied to the dilutions prepared for the viable counts. The difference in the number of the cfu between the heat-treated and non heat-treated dilutions was considered as the number of the germinated spores and this figure was used for the evaluation of the germination.

2.6.2.5.1 Germination outside the extruder

A possible germination of *B. subtilis* spores while the spore mix was in the extruder feeder at ambient temperatures was checked. For this purpose a sample was taken from the feeder outlet at the end of each set of experiments in order to determine the level of germinated spores. This figure was compared with the initial number of vegetative cells in the spore mix kept at 4°C.

2.6.2.5.2 Germination inside the extruder

To determine possible germination inside the extruder, the dilution tubes used for viable counts were plated on nutrient agar after a 10 min heat treatment at 65°C.

2.6.2.6 Determination of effect of milling process on spore viability

The procedure was the same as explained for *M. lacticum* (2.4.1.5).

2.7 CONVERSION OF STARCH BY EXTRUSION

2.7.1 Rapid Visco Analyser (RVA)

2.7.1.1 Theory

The Rapid Visco Analyser is an effective instrument used for determining the change in viscosity of starch, grain and flour during a temperature cycle. RVA also can be used to follow gelatinisation and pasting behaviour and to estimate the amount of starch conversion (Zeng *et al.* 1997, Whalen *et al.* 1997). It has been shown that the viscosity of a cooked sample correlates to the energy input in an extruder (Diosady *et al.*, 1985). The RVA empirically measures the viscosity of starch-water suspensions under controlled heating and shear conditions. The starch-water suspension is stirred at a uniform rate and heated to give a thick paste, held at any desired temperature for a specific time, and then cooled at a uniform rate. The torque required to drive the stirrer in the paste is recorded as a measure of the paste viscosity and is used to observe the physical changes in the starch granules. From the recorded viscosity trace, one or more parameters listed below may be used to characterize the properties of the sample.

Peak viscosity, maximum viscosity developed during the heating and/or high temperature holding portion of the test,

Peak time, time at which the peak viscosity occurred,

Pasting temperature, temperature where viscosity first increases by at least 2 RVA units over a 20 second period.

Peak temperature, temperature at which the peak viscosity occurred,

Holding strength, minimum viscosity after the peak, normally occurring around the commencement of sample cooling,

Breakdown viscosity, peak viscosity minus trough viscosity,

Final viscosity, viscosity at the end of the test,

Setback from Peak, final viscosity minus peak viscosity,

Setback from Trough, final viscosity minus trough viscosity.

These values can conveniently be determined using the software provided with the instrument.

The behaviour of starch from the RVA trace can be shown in Figure 2.5. At the beginning of the test, the temperature is below the critical gelatinisation temperature of the starch and the viscosity is low. As the temperature of the water is increased, the granules begin to swell and so increase the viscosity of the starch paste. The temperature at the onset of this rise in viscosity is known as the pasting temperature. The pasting temperature provides an indication of the minimum temperature required to cook the starch. Starch granules actually swell over a range of temperatures, indicating their heterogeneity of behaviour. This range is reflected in the steepness of the initial rise in viscosity in the pasting curve.

This process continues until the "peak viscosity" is reached. At this point when the majority of granules become swollen, a rapid increase in viscosity occurs. As the temperature increases further, the cohesive forces in the original granular structure become excessively weakened, and in consequence the observed viscosity collapses as the integrity of the granules is lost. The more soluble amylose leaches out into solution, followed at a slower rate in some cases by the amylopectin fraction. Granule rupture and subsequent polymer alignment due to the mechanical shear reduces the apparent viscosity of the paste. These combined processes that follow gelatinisation are known as pasting. It is common to measure the peak temperature and peak time that occur with the peak viscosity. Peak viscosity indicates the water-binding capacity of the starch and is often correlated with final product quality.

During the holding period of the test, the sample is subjected to a period of constant high temperature (usually 95°C) and mechanical shear stress. This will further disrupt the granules and amylose molecules will generally leach out into solution. This period is commonly accompanied by a breakdown in viscosity, to a holding strength, hot paste viscosity or trough. The rate of reduction depends on the

temperature and degree of mixing, or shear applied to the mixture and the nature of the material itself. The ability of a sample to withstand this heating and shear stress is an important factor for many processes. As the mixture cooled subsequently, re-association between starch molecules occurs to a greater or lesser degree. In sufficient concentration this causes the formation of a gel, and viscosity will normally increase to a final viscosity. This phase of the pasting curve is commonly referred to as the setback region, and involves re-ordering of the starch molecules. The setback has been correlated with texture of various products and is sometimes measured as the difference between final viscosity and peak viscosity, rather than holding strength. Final viscosity is the most commonly used parameter to define a particular sample's quality, as it indicates the ability of the material to form a viscous paste or gel after cooking (Whistler and BeMiller, 1997; Newport Scientific, 1998).

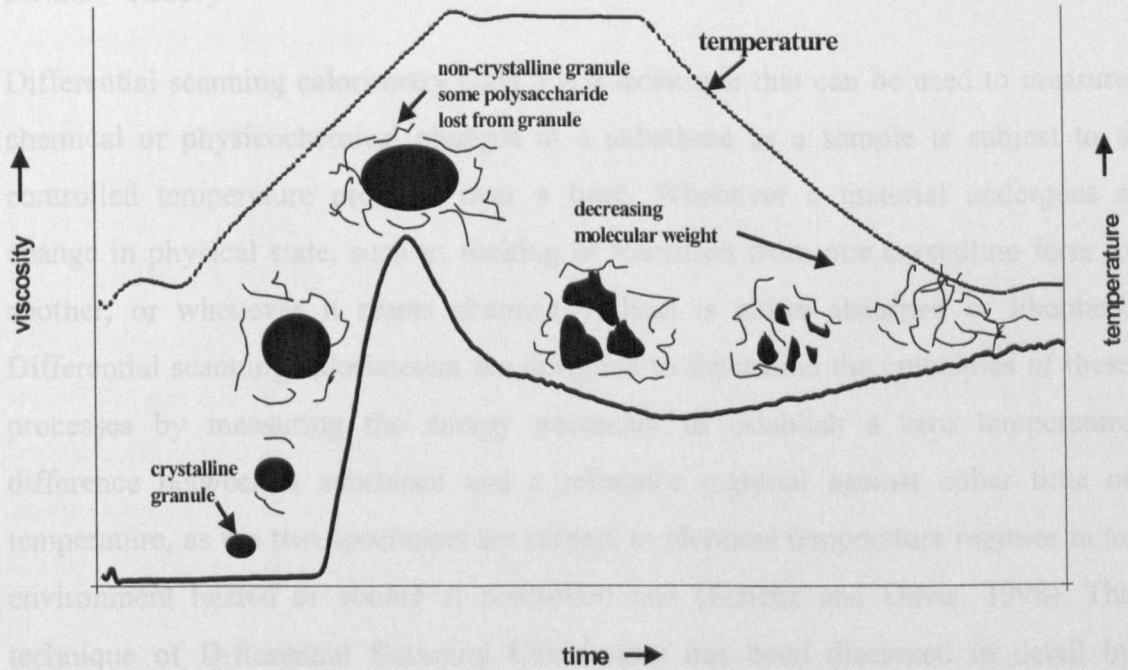

Figure 2.5 Representative RVA profile showing the typical swelling and disintegration that a granule undergoes during the cooking process in relation to viscosity. Adapted from Whistler and BeMiller (1997).

2.7.1.2 Experimental

RVA analysis was based on 3.50 g (dry weight basis) of samples plus 25.00 g of reverse osmosis (RO) water. The samples were prepared by milling with Cylotec and Knifetec as explained before. The paddle was placed into the canister containing the sample and RO water. The sample was then inserted into the RVA Series 4 (Newport Scientific, Australia), which was used along with the accompanying software (Thermocline). The stirring speed was 960 rpm for the first 10 seconds then at 160 rpm for the remainder of the test. The following profile was used throughout the study: idle at 25°C, 5 min at 25°C, ramp to 95°C at 13 min, hold at 95°C to 17 min, cool to 25°C at 25 min, hold at 25°C to 32 min.

2.7.2 Differential Scanning Calorimetry (DSC)

2.7.2.1 Theory

Differential scanning calorimetry (DSC) is a technique that can be used to measure chemical or physicochemical changes in a substance as a sample is subject to a controlled temperature program over a time. Whenever a material undergoes a change in physical state, such as melting or transition from one crystalline form to another, or whenever it reacts chemically, heat is either absorbed or liberated. Differential scanning calorimeters are designed to determine the enthalpies of these processes by measuring the energy necessary to establish a zero temperature difference between a substance and a reference material against either time or temperature, as the two specimens are subject to identical temperature regimes in an environment heated or cooled at controlled rate (Schenz and Davis, 1998). The technique of Differential Scanning Calorimetry has been discussed in detail by McNaughton and Mortimer (1975), Lund (1983), and Reid *et al.* (1993). In food science and industry, DSC is typically used for measuring melting temperatures, gelatinisation temperatures and enthalpies, crystallization rates, glass transition temperatures and starch conversion. Figure 2.6 shows the types of event measured by DSC.

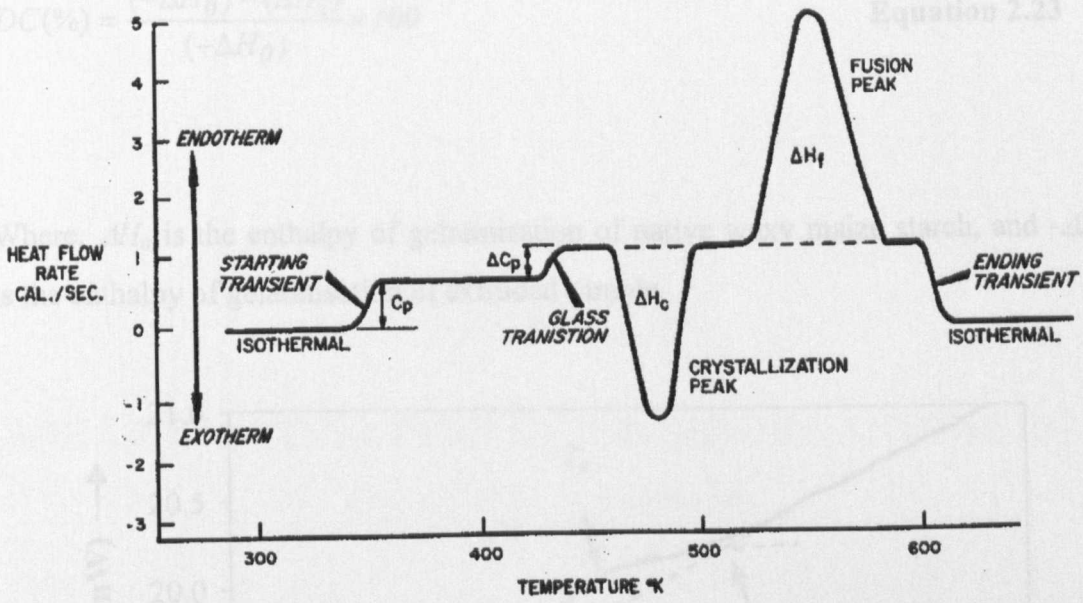

Figure 2.6 A set of thermal transitions that may appear when a sample is heated. Adapted from Nielsen, 1998.

2.7.2.2 Experimental

Differential Scanning Calorimetry was performed using a Perkin Elmer DSC-7 equipped with an intracooler and nitrogen gas as a flushing agent over the head. For calorimetric measurements a water:starch ratio of 3:1 was employed. Around 2.5 to 3 mg of the ground sample weighed in an aluminium pan and 3 times more water was added before the pan was hermetically sealed. The sample was equilibrated overnight before measurement in order to ensure the diffusion of the water into the granules. Two replicates of the samples were analysed under the same conditions.

Scanning of the samples was done from 0 to 95°C at a heating rate of 10°C/min. An empty sealed pan was used as a reference, and the temperature was calibrated using cyclohexane (T onset of 6.7°C) and indium (T onset of 156.78°C and Heat of fusion of 28.45 J/g). The gelatinisation temperature was differentiated into T_o (onset temperature), T_p (peak temperature), and T_e (endset temperature) (Figure 2.7).

Degree of conversion of starch was calculated based on the calculation by Wang *et al.* (1989):

$$DC(\%) = \frac{(-\Delta H_0) - (\Delta H_i)}{(-\Delta H_0)} \times 100$$ **Equation 2.23**

Where, ΔH_o is the enthalpy of gelatinisation of native waxy maize starch, and $-\Delta H_i$ is the enthalpy of gelatinisation of extruded sample.

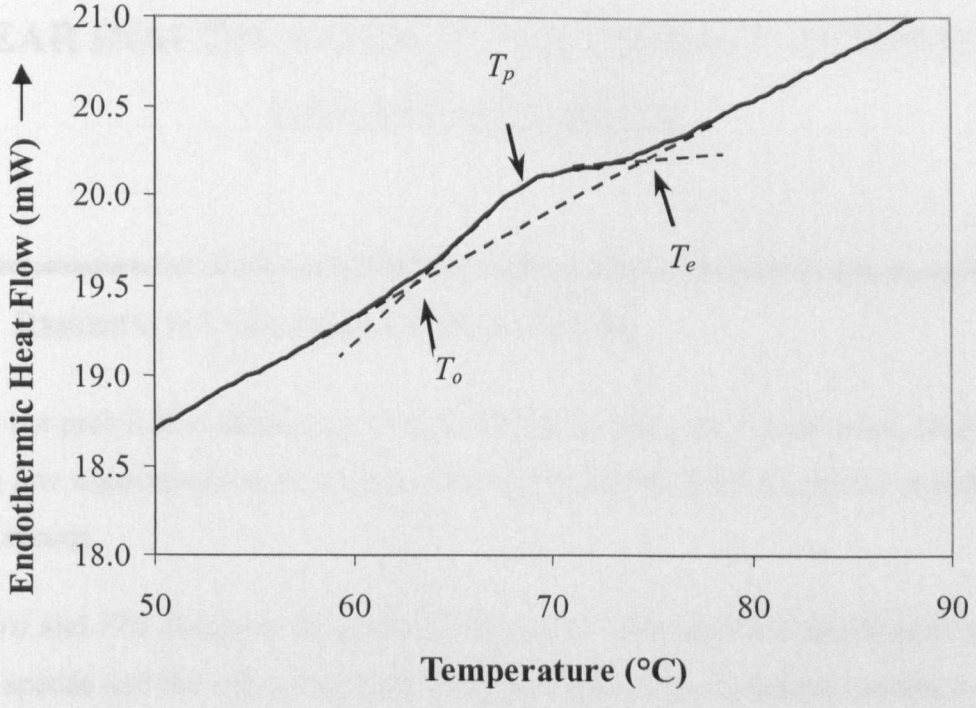

Figure 2.7 Gelatinisation peak of maize grits in DSC, differentiated into onset (T_o), peak (T_p), and endset (T_e).

CHAPTER 3

SHEAR INACTIVATION OF MICROORGANISMS IN A GELATIN CARRIER

3.1 RESIDENCE TIME DISTRIBUTION (RTD)

It was not practical to determine the RTD of gelatin for every experiment, therefore, only a few experiments were conducted to determine the RTD of gelatin at different screw speeds.

The *E(t)* and *F(t)* diagrams are given in Figure 3.1. *E(t)* becomes narrower at higher screw speeds and the mean residence time decreases. The minimum residence time, which is determined by the first detection of the dye in the samples, also decreases with increasing screw speed as is shown in Table 3.1. Table 3.1 also shows the mean residence times calculated from the concentration of dye in each sample as a function of sampling time. The details of the calculations are given in the Appendix.

Table 3.1 Mean and minimum residence times of gelatin extruded at different screw speeds in a Clextral BC 21 Extruder.

Screw speed (rpm)	Minimum Residence Time (s)	Maximum Residence Time (s)	Mean Residence Time (s)
120	30	170	58
200	24	160	53
300	20	155	49

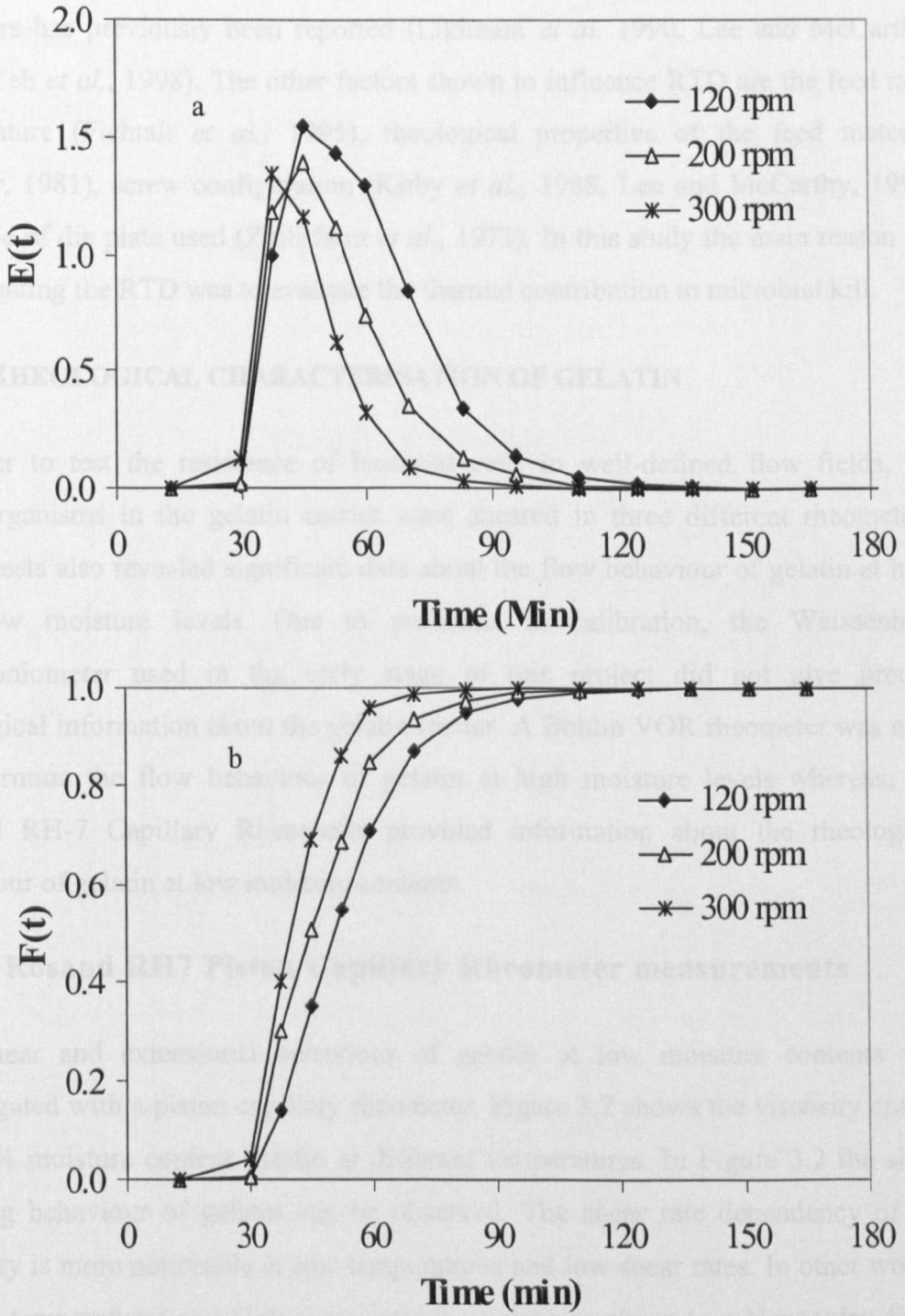

Figure 3.1 Distribution function for gelatin at different screw speeds determined using a marker dye. (a) Exit age distribution, *E(t)*, (b) Cumulative residence time distribution, *F(t)*. Extrusion parameters; 3kg/h feed rate, 0.750 kg/h water feed rate.

A decrease in mean residence time ($\bar{t}$) with increasing screw speed in twin-screw extruders has previously been reported (Likimani *et al.* 1990, Lee and McCarthy, 1995, Yeh *et al.*, 1998). The other factors shown to influence RTD are the feed rate, temperature (Fichtali *et al.*, 1995), rheological properties of the feed material (Herper, 1981), screw configuration (Kirby *et al.*, 1988, Lee and McCarthy, 1995) and type of die plate used (Zuilichem *et al.*, 1973). In this study the main reason for investigating the RTD was to evaluate the thermal contribution to microbial kill.

3.2 RHEOLOGICAL CHARACTERISATION OF GELATIN

In order to test the resistance of bacterial cells in well-defined flow fields, the microorganisms in the gelatin carrier were sheared in three different rheometers. These tests also revealed significant data about the flow behaviour of gelatin at high and low moisture levels. Due to problems in calibration, the Weissenberg Rheogoniometer used in the early stage of this project did not give precise rheological information about the gelatin carrier. A Bohlin VOR rheometer was used to determine the flow behaviour of gelatin at high moisture levels whereas, the Rosand RH-7 Capillary Rheometer provided information about the rheological behaviour of gelatin at low moisture contents.

3.2.1 Rosand RH7 Piston Capillary Rheometer measurements

The shear and extensional behaviour of gelatin at low moisture contents was investigated with a piston capillary rheometer. Figure 3.2 shows the viscosity curves for 30% moisture content gelatin at different temperatures. In Figure 3.2 the shear thinning behaviour of gelatin can be observed. The shear rate dependency of the viscosity is more noticeable at low temperatures and low shear rates. In other words, at high temperatures and high shear rates the gelatin is closer to a Newtonian fluid. The flow behaviour indices, determined by plotting the shear rates against the shear stresses on a logarithmic scale, with the slope giving the flow behaviour index (Figure 3.3), verified the shear thinning behaviour of gelatin at low moisture contents. Flow behaviour indices were 0.49 for 60°C, 0.66 for 70°C and 0.75 for

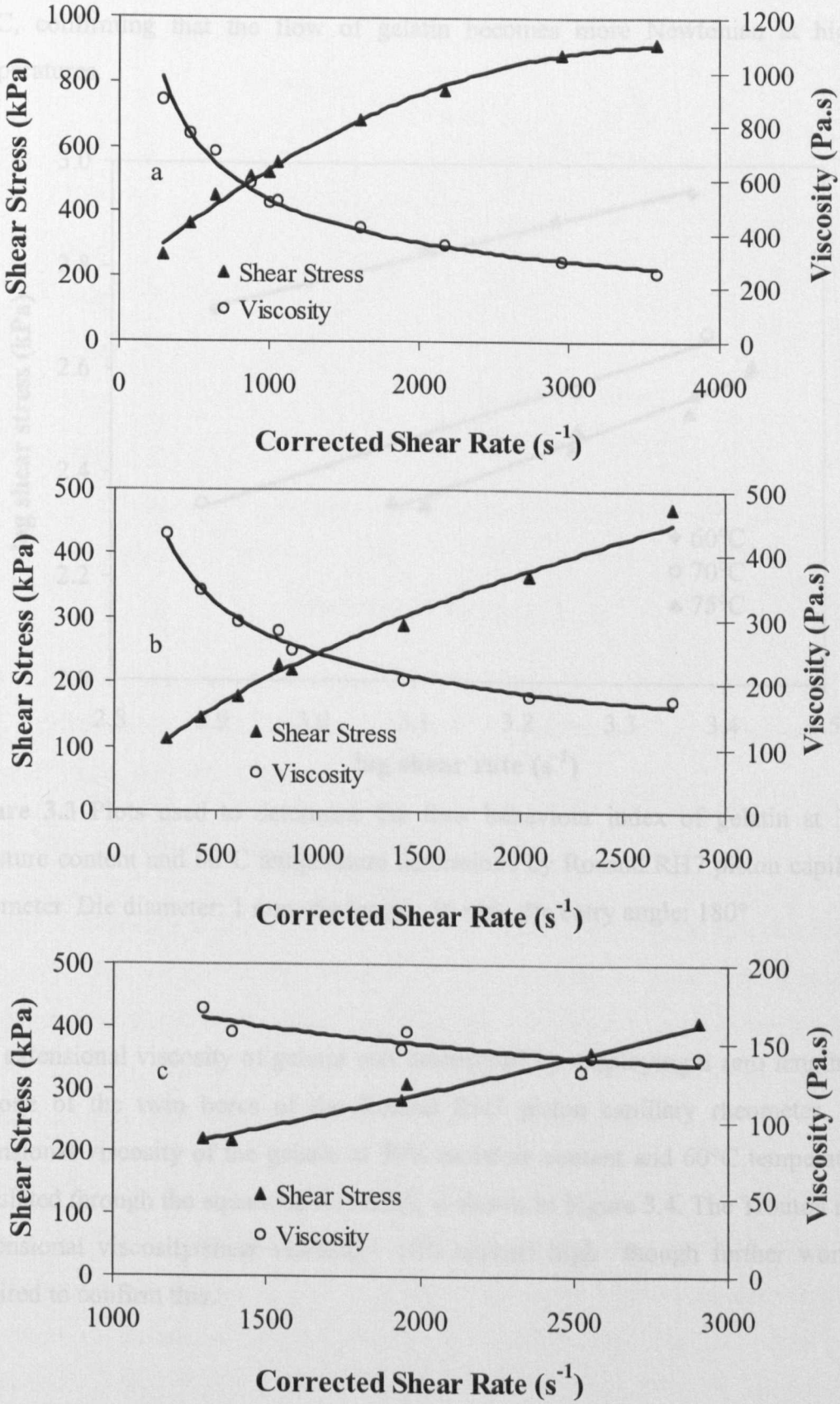

Figure 3.2 Viscosity and shear stress curves for gelatin at 30% moisture content at (a) 60°C, (b) 70°C and (c) 75°C. Data obtained by using capillary dies with 1.0 and 1.5 mm diameters and a 180°die entry angle.

75°C, confirming that the flow of gelatin becomes more Newtonian at higher temperatures.

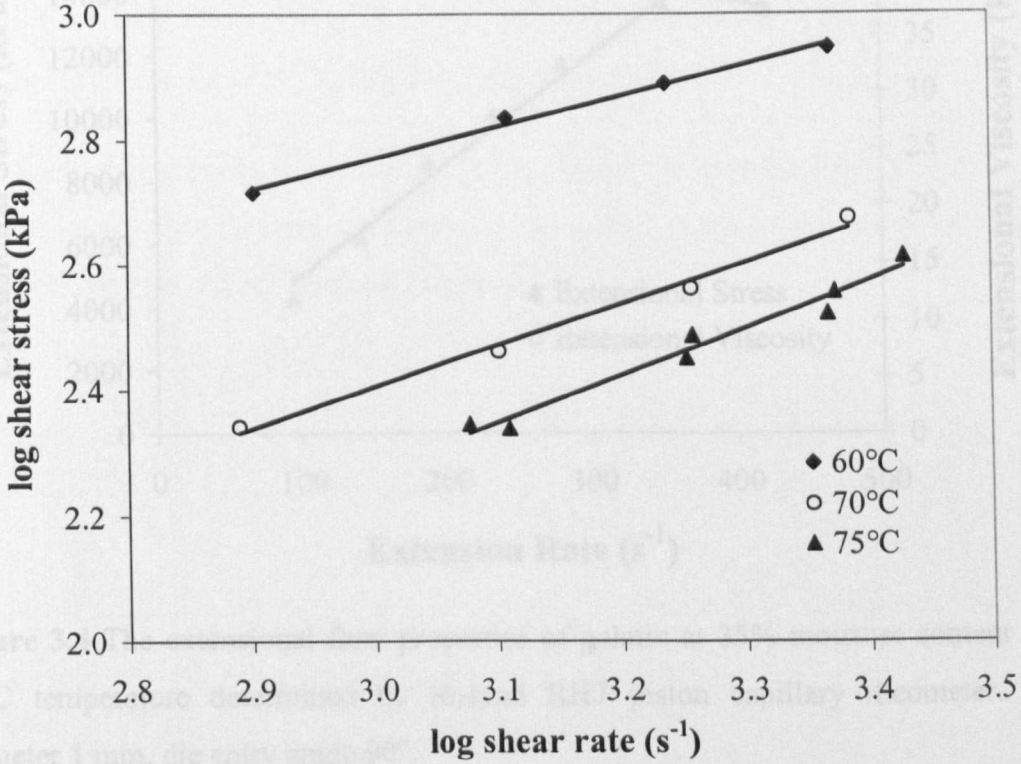

Figure 3.3 Plots used to determine the flow behaviour index of gelatin at 30% moisture content and 60°C temperature determined by Rosand RH7 piston capillary rheometer. Die diameter: 1 mm, die length: 16 mm, die entry angle: 180°

The extensional viscosity of gelatin was determined by employing a zero length die for one of the twin bores of the Rosand RH7 piston capillary rheometer. The extensional viscosity of the gelatin at 30% moisture content and 60°C temperature, calculated through the equations 2.18-2.20, is shown in Figure 3.4. The Trouton ratio (extensional viscosity/shear viscosity $\sim 10^2$) appears high though further work is required to confirm this.

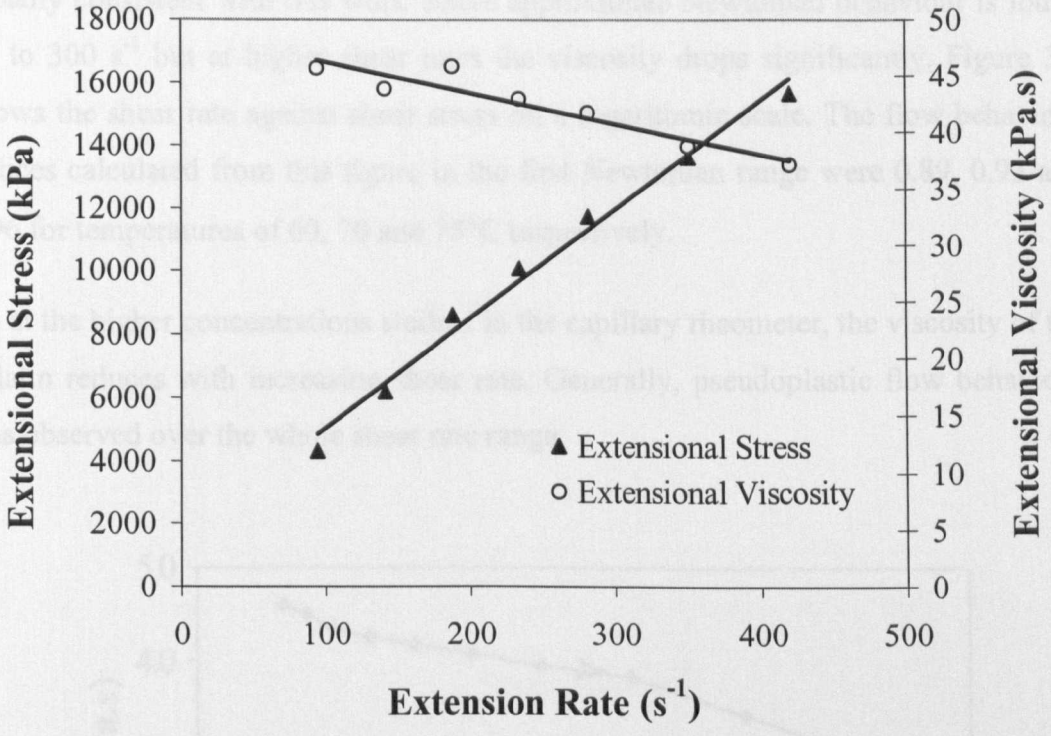

Figure 3.4 The extensional flow properties of gelatin at 35% moisture content and 60°C temperature determined by Rosand RH7 piston capillary rheometer. Die diameter 1 mm, die entry angle 90°.

3.2.2 Measurements using the Bohlin VOR

In order to understand the extent of shear forces involved during the shearing of microorganisms by the rotational rheometer, the viscosity of gelatin solutions was investigated in the Bohlin VOR. The rheology of gelatin solutions has been the subject of many studies and detailed information can be found in the literature (Stainsby, 1977, Leuenberger, 1991, Wulansari and Mitchell, 1998).

Figures 3.5-3.7 shows the viscosity curves for the 65% moisture content gelatin at 60, 65 and 70°C. For polymer solutions above the concentration where the molecules overlap (the c^* concentration) non-Newtonian behaviour frequently results from the disruption of entanglements. Newtonian behaviour is seen at low shear rates (maximum entanglement density) and high shear rates (no entanglements). Wulansari and Mitchell (1998) have reported Newtonian behaviour for gelatin when measured at 50°C up to 35% (wwb) concentration and maximum observed shear rate of 50 s^{-1}even though the coil overlap concentration (c*) was only about 10% . This is

broadly consistent with this work where approximate Newtonian behaviour is found up to 300 s^{-1} but at higher shear rates the viscosity drops significantly. Figure 3.8 shows the shear rate against shear stress on a logarithmic scale. The flow behaviour indices calculated from this figure in the first Newtonian range were 0.89, 0.93 and 0.96 for temperatures of 60, 70 and 75°C respectively.

As at the higher concentrations studied in the capillary rheometer, the viscosity of the gelatin reduces with increasing shear rate. Generally, pseudoplastic flow behaviour was observed over the whole shear rate range.

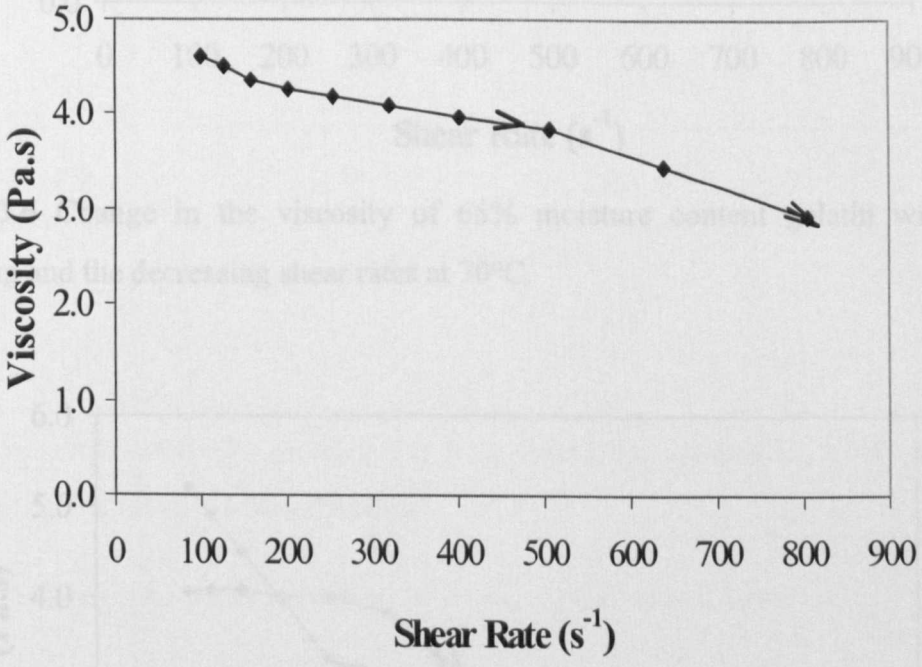

Figure 3.5 Change in the viscosity of 65% moisture content gelatin with the increase of shear rate at 60°C.

In Figure 3.6 and Figure 3.7 the viscosity of gelatin follows a different path, when the shear rate is decreased after it reaches the maximum shear rate, showing the time dependency of the gelatin solution under the experimental conditions. This could be due to some irreversible changes in the gelatin molecules as a result of shearing at high temperatures. The higher end viscosity of the gelatin solution could be due to cross linking but could also possibly be caused by loss of moisture during the experiment.

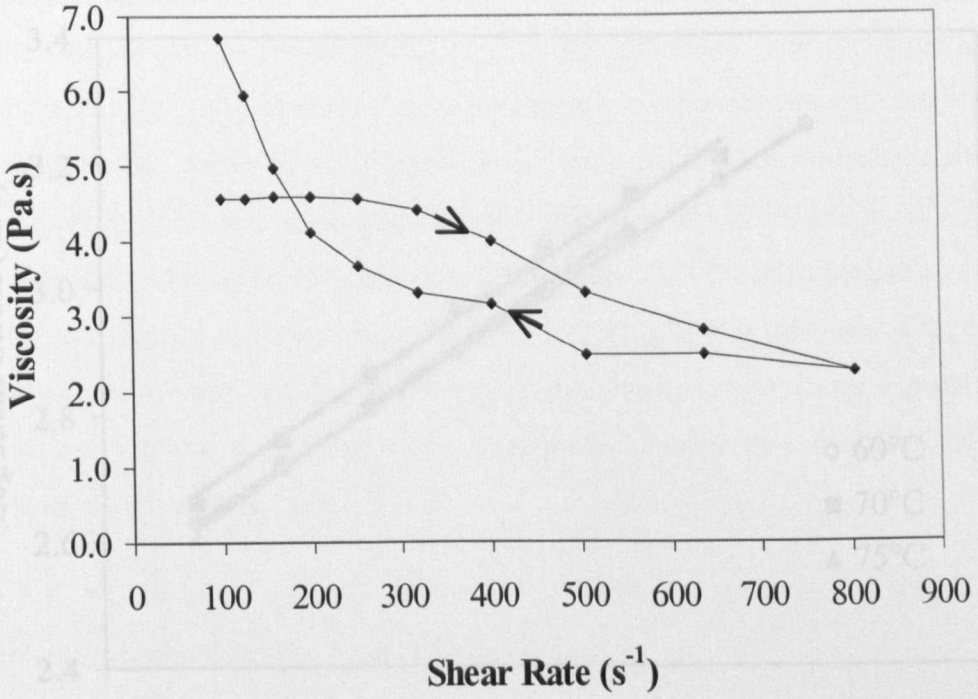

Figure 3.6 Change in the viscosity of 65% moisture content gelatin with the increasing and the decreasing shear rates at 70°C.

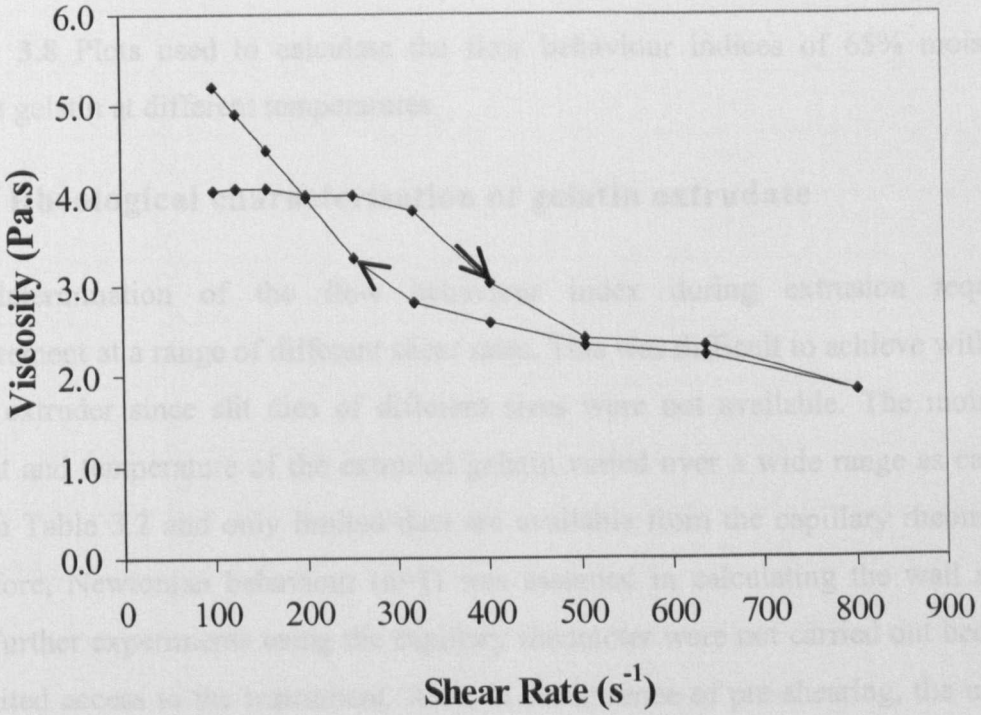

Figure 3.7 Change in the viscosity of 65% moisture content gelatin with the increasing and the decreasing shear rates at 75°C.

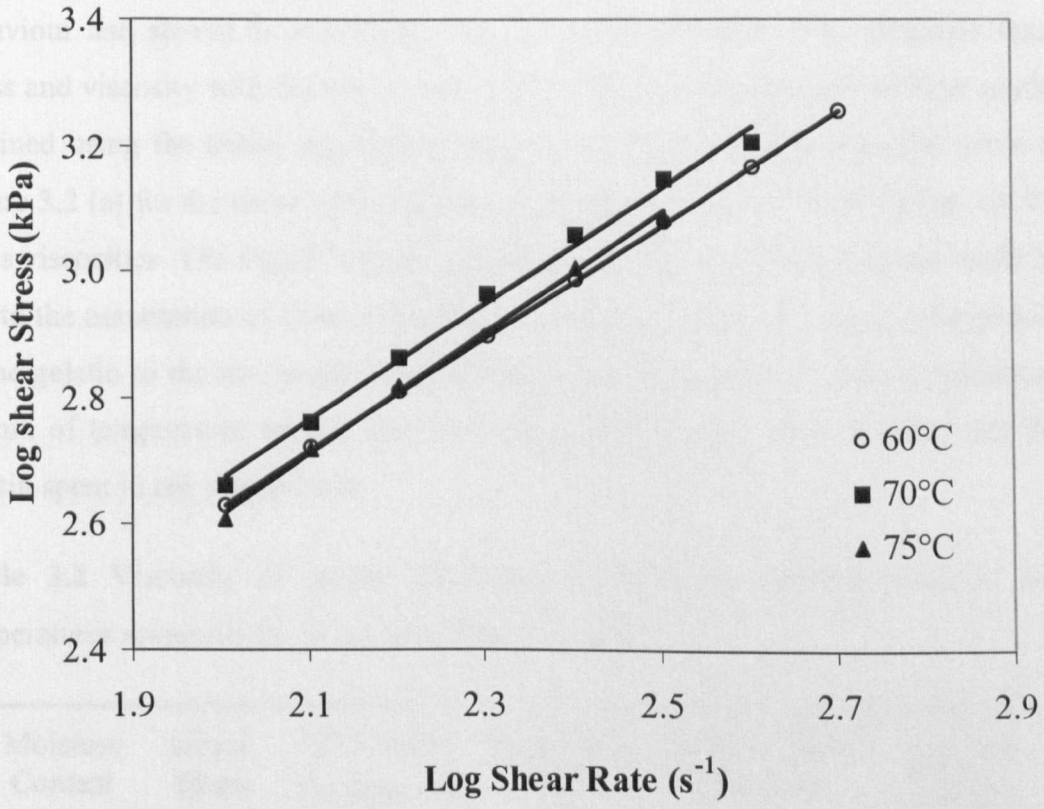

Figure 3.8 Plots used to calculate the flow behaviour indices of 65% moisture content gelatin at different temperatures.

3.2.3 Rheological characterisation of gelatin extrudate

The determination of the flow behaviour index during extrusion requires measurement at a range of different shear rates. This was difficult to achieve with the BC21 extruder since slit dies of different sizes were not available. The moisture content and temperature of the extruded gelatin varied over a wide range as can be seen in Table 3.2 and only limited data are available from the capillary rheometer. Therefore, Newtonian behaviour (n=1) was assumed in calculating the wall shear rate. Further experiments using the capillary rheometer were not carried out because of limited access to the instrument. Also, in the absence of pre-shearing, the use of the piston capillary rheometers for the rheological characterisation of the biopolymers relevant to extrusion has been questioned (Altamore *et al.*, 1992).

The shear rate and viscosity values calculated shown in Table 3.2 assume Newtonian behaviour and should be considered only as rough estimates. The increased shear stress and viscosity with decreasing moisture content is in agreement with the results obtained using the piston capillary rheometer. Extrapolating the viscosity curve in Figure 3.2 (a) for the shear rates attained in the extruder die (57-90 s^{-1}) gives 1.8-2.3 kPa.s viscosities. The slightly higher viscosities obtained in the extruder die could be due to the assumption of Newtonian behaviour and the fact that the time of exposure of the gelatin to the test temperatures during piston capillary rheometer experiments (7 min of temperature equilibrium) was significantly higher than the time that the gelatin spent in the extruder die.

Table 3.2 Viscosity of gelatin extrudates at different moisture contents and temperatures measured by the slit die of the extruder.

Moisture Content (%, wwb)	Screw Speed (rpm)	Maximum Temperature (°C)[a]	Wall Shear Stress (kPa)	Apparent Wall Shear Rate (s^{-1})	Apparent Viscosity (kPa.s)
45	100	50	48.6	67.5	0.7
45	100	44	69.4	75.0	0.9
31	200	50	173.6	56.4	3.1
25	140	60	277.8	67.5	4.1
27	100	50	302.1	80.3	3.8
24	120	51	340.3	59.0	5.8
25	100	61	319.4	71.4	4.5
27	250	68	381.9	54.8	7.0
19	120	73	409.7	56.6	7.2

[a] Maximum temperature measured at the end of the die.

3.3 INVESTIGATION OF THE INACTIVATION OF THE MICROORGANISMS BY COMBINED SHEAR AND THERMAL FORCES

3.3.1 Extrusion experiments

3.3.1.1 Growth curve of *M. lacticum*

The resistance of microorganisms to different stress conditions depends on many parameters. The pH of the heating media, water activity (a_w), growth medium, composition of the heating medium, age of the cells and incubation temperature have been reported as important factors effecting the heat resistance of microorganisms (Sala *et al.*, 1995). Microorganisms are likely to be more resistant to antimicrobial conditions at stationary phase than during exponential growth. Therefore, the growth curve of *M. lacticum* was determined. Figure 3.9 shows that the microorganism is reaching the stationary phase after 15 h. In our experiments, 18 h cultures were used throughout.

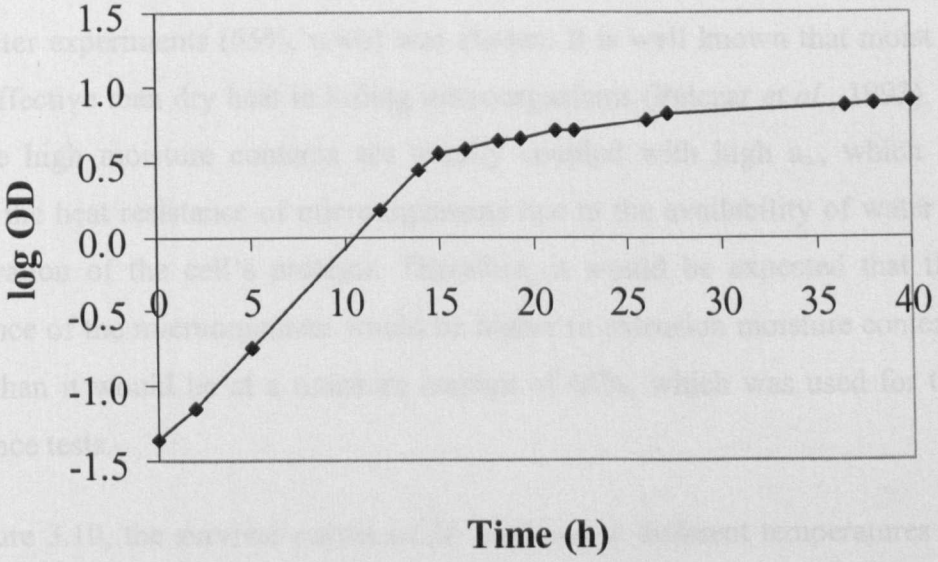

Figure 3.9 Growth curve of *M. lacticum* in BHI Broth at 30°C determined by OD readings at 600 nm.

3.3.1.2 Effect of enumeration method on *M. lacticum*

Recovery of surviving microorganisms required 45 min of heat treatment at 60°C in order to melt the extrudates and release the cells into the solution for serial dilutions. The effect of this heat treatment is deducted from the overall reduction for each extrusion experiment. The D-values shown in Table 3.3 show that the microorganism has high heat resistance. However, the cells injured during the extrusion process may not survive this extra heating process. No specific test was applied in this study to investigate the level of injury.

3.3.1.3 Heat resistance of *M. lacticum*

The heat resistance of the different strains of *M. lacticum* isolated from pasteurised egg was investigated by Payne *et al.* (1979). The JP 2/1/1 strain that is used here was found to be most resistant, having a D-value of approximately 9.5 min at 80°C in phosphate buffer at pH 7.1. Since the heat resistance of microorganisms is highly depend on the heating media it was necessary to test the heat resistance of the microorganism in gelatin. However, this could not be done in gelatin at extrusion moisture contents. Instead, the moisture content level for most of the rotational rheometer experiments (65%, wwb) was chosen. It is well known that moist heat is more effective than dry heat in killing microorganisms (Pelczar *et al.*, 1993). This is because high moisture contents are usually coupled with high a_w, which in turn reduce the heat resistance of microorganisms due to the availability of water for the denaturation of the cell's proteins. Therefore, it would be expected that the heat resistance of the microorganism would be higher in extrusion moisture contents (20-45%) than it would be at a moisture content of 65%, which was used for the heat resistance tests.

In Figure 3.10, the survival curves of *M. lacticum* at different temperatures in PBS and 65.0% moisture content gelatin showed that heating in gelatin increased the kill. At 60°C, 65°C and 70°C even in gelatin there was less than 1 log kill in 1 h, while at 75°C there was less than 1 log kill in 45 min in PBS and less than 3 log kills in 45 min in gelatin. Payne *et al.* (1979) reported that the microorganism has almost no kill at 75°C in PBS pH 7.1. Compared to the results of Payne *et al.* (1979), the heat

resistance of the microorganism was lower under our experimental conditions where the pH of the medium was lower (pH 5.8-6.0). In addition, the heating time of 17 min at 60°C for preparation of a homogeneous mix of microorganisms with gelatin prior to the heat resistance testing and heating up to the test temperature (for details see Section 2.1.5.1.3) also reduced the D-values.

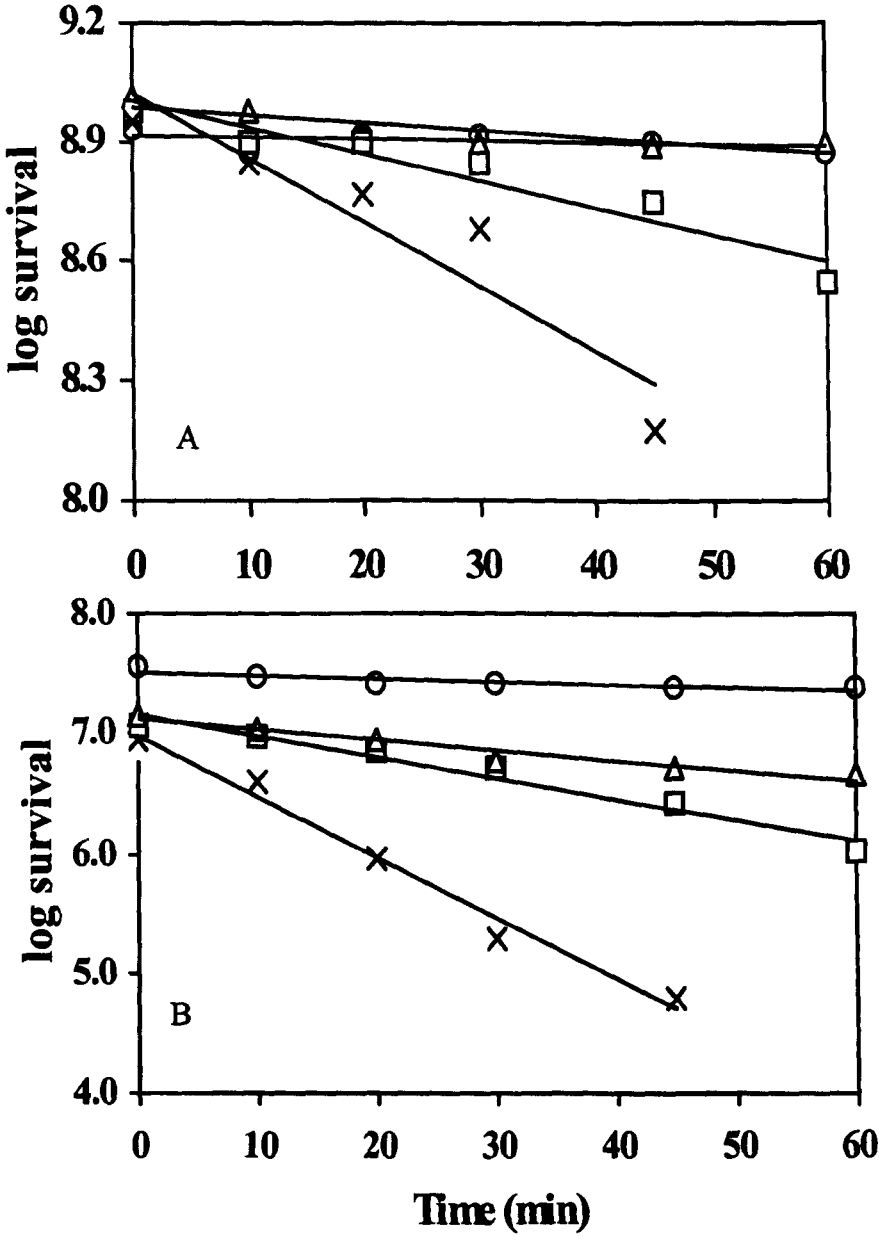

Figure 3.10 Survival curves of *M. lacticum* at different temperatures in PBS, pH 5.9 (A) and in gelatin (moisture content 65% [w/w]; pH 5.8-6.0) (B). (○ 60°C, (Δ) 65°C, (□)70°C, (×) 75°C. Different scales for log survival axis should be noted.

D-values evaluated from Figure 3.10 are shown in Table 3.3

Table 3.3 D-value of *M. lacticum* at different temperatures in 65.0% (w/w) gelatin solution (pH 5.8-6.0) and in PBS (pH 5.9).

Temperature (°C)	D-value in gelatin (h)	D-value in PBS (h)
60	6.3	16.3
65	1.9	8.1
70	1.0	2.9
75	0.3	1.0

3.3.1.4 Validation of the sampling

The results showed that there was no significant difference between the total count obtained by 1 g sample analysis and the whole sample analysis. For example, in the analysis done for the experiment where 0.9 log reduction was obtained (Table 3.4) the difference between the total counts obtained was less than 24% which resulted in only 0.1 log difference in terms of log reduction. In addition, RTD analysis showed that the 1 g sample taken from the middle section of each extrudate provided an accurate representation of the whole extrudate, since the calculations made based on this sampling method recovered more than 90% of the dye introduced into the extruder.

3.3.1.5 Effect of extrusion on *M. lacticum*

Table 3.4 shows the parameters of the extrusion experiments and the log reduction of *M. lacticum* for each experiment. The correlation coefficients between the logarithmic reduction of *M. lacticum* and the parameters of extrusion are shown in Table 3.5. The log reduction of *M. lacticum* shows a strong correlation with die wall shear stress, die pressure, viscosity and the maximum temperature, and a strong negative correlation with the moisture content. These relationships are self-consistent since a reduction in water content will result in an increase in viscosity and shear stress. The maximum temperature will increase with viscosity due to increased conversion of mechanical energy to heat.

Table 3.4 Parameters of the extrusion experiments showing the log reduction of *M. lacticum* for each experiment

Log Reduction	Screw Speed (rpm)	Extrudate Moisture Content (%, wwb)	Max. Temp. (°C)[a]	Die Pressure (Bar)	Temperatures in zone 1,2,3,4 (°C)[b]	SME (kJ/kg)	Wall Shear Stress (kPa)	Wall Shear Rate (s^{-1})	Die Wall Viscosity (kPa.s)
0.3	100	45	50	[c]	25-28-29-32	72.2	48.6	67.5	0.7
0.5	100	45	44	10	27-32-32-35	83.8	69.4	75.0	0.9
0.9	200	31	50	25	25-28-26-34	418.6	173.6	56.4	3.1
1.7	140	25	60	40	25-30-29-35	318.1	277.8	67.5	4.1
1.9	100	27	50	44	24-30-30-43	248.3	302.1	80.3	3.8
2.3	120	24	51	49	25-29-29-49	384.6	340.3	59.0	5.8
2.6	100	25	61	46	24-29-30-44	306.6	319.4	71.4	4.5
2.9	250	27	68	55	26-29-28-26	698.8	381.9	54.8	7.0
5.3[d]	120	19	73	59	25-30-30-51	389.7	409.7	56.6	7.2

[a] Maximum temperature measured at the end of the die.
[b] Measured temperature of the material at the barrel wall of the extruder in zones 1,2,3 and 4.
[c] Circular die with 3 mm diameter was used without a die pressure measurement.
[d] No residual microorganisms were detected.

Table 3.5 Correlation coefficients between the Log reduction of *M. lacticum* and extrusion Parameters

Parameter	Correlation Coefficient
Screw Speed (rpm)	0.33
Extrudate Moisture Content (%, wwb)	-0.82
Max.Temperature in the die end (°C)	0.86
Die Pressure (Bar)	0.85
SME (kJ/kg)	0.54
Wall Shear Stress (kPa)	0.87
Viscosity (kPa.s)	0.89

The correlation coefficient between log reduction of *M. lacticum* and SME was relatively low. This was because the correlation coefficients were produced over the data obtained at different screw speeds. If the other parameters remain the same, increasing the screw speed from 100 to 200 would double the SME value. Correlation coefficients determined over the extrusion data at 100 rpm only, showed a high correlation (0.99) between log reduction of *M. lacticum* and SME. High SME value due to high screw speed is associated with high shear rates inside the barrel and usually lower shear stresses at the die. Thus, the effect of the SME is not always straightforward and should be considered together with the other parameters of extrusion.

The relationship between the count reduction and the wall shear stress and the maximum die exit temperature are shown in Figure 3.11. The temperature profiles in the extruder barrel were in the range of 25 to 51°C for all the experiments and the temperature measured at the entrance to the die did not exceed 51°C for any experiment. The temperatures increased at the die due to high shear and pressure forces, resulting in higher temperature readings at the exit of the die. The maximum temperature readings at the die exit were between 44 and 73°C. Based on the die geometry, extrudate density and the mass flow rate of the material, the calculated average length of time that the material spent in the extruder die (Equation 2.22) was

1.7 s. Therefore it is unlikely that the temperature had any direct significant effect on the destruction of the microorganism. However, the high temperatures could make the cell wall and/or membrane of the bacterium more susceptible to physical forces and facilitate the destruction of the microorganism by shear and pressure forces.

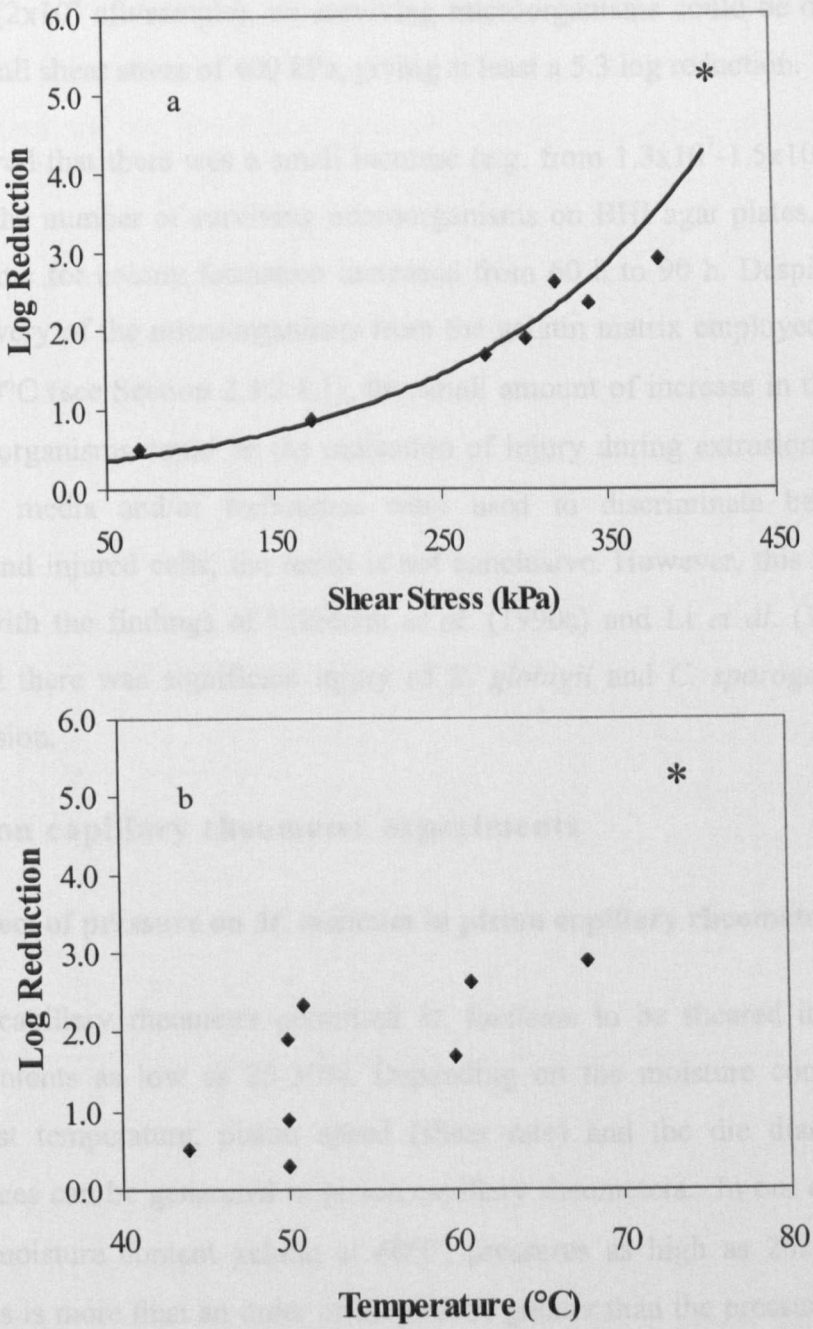

Figure 3.11 The dependence of log reduction of *M. lacticum* (a) on die wall shear stress, (b) maximum extrudate temperature measured at the die outlet. *No survival, log reduction was calculated based on minimum recovery level.

The strong non-linear relationship between the count reduction and the calculated die wall shear stress, the fact that the highest temperature recorded in the die end was below 75°C and the observation that substantial count reduction was obtained at lower temperatures suggest that physical forces around the reverse screw element and the die play a major part in the measured destruction of the cells. Within the limit of detection ($2x10^4$ cfu/sample), no surviving microorganisms could be detected at the highest wall shear stress of 409 kPa, giving at least a 5.3 log reduction.

It was observed that there was a small increase (e.g. from $1.3x10^7$-$1.5x10^7$ cfu/total samples) in the number of surviving microorganisms on BHI agar plates, when the incubation time for colony formation increased from 60 h to 90 h. Despite the fact that the recovery of the microorganisms from the gelatin matrix employed a 45 min heating at 60°C (see Section 2.3.2.1.1), the small amount of increase in the number of the microorganisms could be the indication of injury during extrusion. Since no resuscitation media and/or techniques were used to discriminate between the inactivated and injured cells, the result is not conclusive. However, this result is in agreement with the findings of Likimani *et al.* (1990a) and Li *et al.* (1993), who reported that there was significant injury of *B. globigii* and *C. sporogenes* spores during extrusion.

3.3.2 Piston capillary rheometer experiments

3.3.2.1 Effect of pressure on *M. lacticum* in piston capillary rheometer

The piston capillary rheometer permitted *M. lacticum* to be sheared in gelatin at moisture contents as low as 25-30%. Depending on the moisture content of the material, test temperature, piston speed (shear rate) and the die diameter, high pressure forces can be generated in piston capillary rheometers. In our experiments with 25% moisture content gelatin at 60°C, pressures as high as 202 MPa were created. This is more than an order of magnitude greater than the pressures involved in the extrusion process. The maximum die pressure generated in our extrusion experiments were 5.9 MPa (59 Bar) for the gelatin where the extrudate moisture content was 19%.

The analysis of the data revealed that the high pressures involved in piston capillary rheometer played an important role in destruction of *M. lacticum*. When the shearing of the material was complete, the remainder of the material was discharged from the bores of the rheometer after removing the dies. Microbiological analysis of a sample of these unsheared but pressured material (shearing in the bore of the rheometer is considered to be negligible) revealed significant reduction in the number of the bacteria. Table 3.6 shows the amount of bacterial reduction obtained in unsheared control samples and the maximum pressure reached in bores of the capillary rheometer for 25 and 30% moisture content of gelatin.

Table 3.6 The relationship between the logarithmic reduction of *M. lacticum* in unsheared control samples and the maximum pressure reached in the bores of the Rosand RH7 piston capillary rheometer at 60°C.

Moisture content (%)	Parameters	Exp. Set 1		Exp. Set 2		Exp. Set 3	
		Short die	Long die	Short die	Long die	Short die	Long die
25	Log reduction	3.0	n.s*	3.2	3.0	3.4	4.2
	Max. pressure (MPa)	67	202	25	66	51	192
30	Log reduction	2.6	2.2	2.3	2.3	-	-
	Max. pressure (MPa)	25	66	25	61	-	-

* n.s: no survival

Since the temperature employed (60°C) was not able to cause significant reductions in the number of the microorganisms shown in Table 3.6 ($D_{60°C}$ =6.25 h at 65% moisture content gelatin) and the sample was not sheared, it can be concluded that the pressure was responsible for the kill. Also, the bacteria would be expected to have a greather resistance to heat at low water contents of the experimental conditions (30%, wwb) than the 65% (wwb) moisture content employed for the heat resistance test, supporting that the temperatures employed during the pressurisation

of *M. lacticum* was insignificant on bacterial kill. The experiments were not designed to investigate the effect of pressure on the microorganism, but the outcome of the experiments is in agreement with the known effect of high pressures on microorganisms (see Section 1.3.4 for details). Most of the high pressure applications use pressures higher than 200 MPa for vegetative cells. In our experiments, relatively low pressures were involved. The significant bacterial destruction at these low pressures was probably due to the high temperatures involved in our experiments compared to most high pressure applications. Other than fluid foods (milk, fruit juice, etc.), most of the pressure applications are carried out by using an incompressible fluid (mostly water) to transfer the pressure onto the food material, which is packed in special plastic films. In our case, the microorganism was pressurised directly in gelatin matrix at low water contents, which could be considered as another factor in causing the higher bacterial kill.

Since the pressure in the bore of the capillary rheometer was changing with the change of piston speed, it is not possible to talk about a constant pressure value for a single experiment. In addition, the duration of the time that the microorganism was exposed to these maximum pressures were not the same for all the trials. Table 3.6 shows that the pressures in the range of 25-60 MPa have a similar effect on *M. lacticum* and only pressures higher than 190 MPa cause significant increase in destruction of the microorganism. Detailed experiments need to be conducted to give a clearer picture of the effect of pressure on *M. lacticum* in the piston capillary rheometer.

3.3.2.2 The effect of shear and extensional stresses on *M. lacticum*

Microbiological analysis of the samples extruded through the short (zero length) and long dies of the piston capillary rheometer showed that the extent of bacterial destruction was higher in the long die where the bacteria were exposed to high shear stresses. The extent of bacterial destruction in the short die where the shear forces were supposed to be negligible was limited. Figure 3.12 shows that a 2.5 logarithmic reduction in the number of *M. lacticum* was obtained by extruding the microorganism in 30% moisture content gelatin through the long die at 60°C.

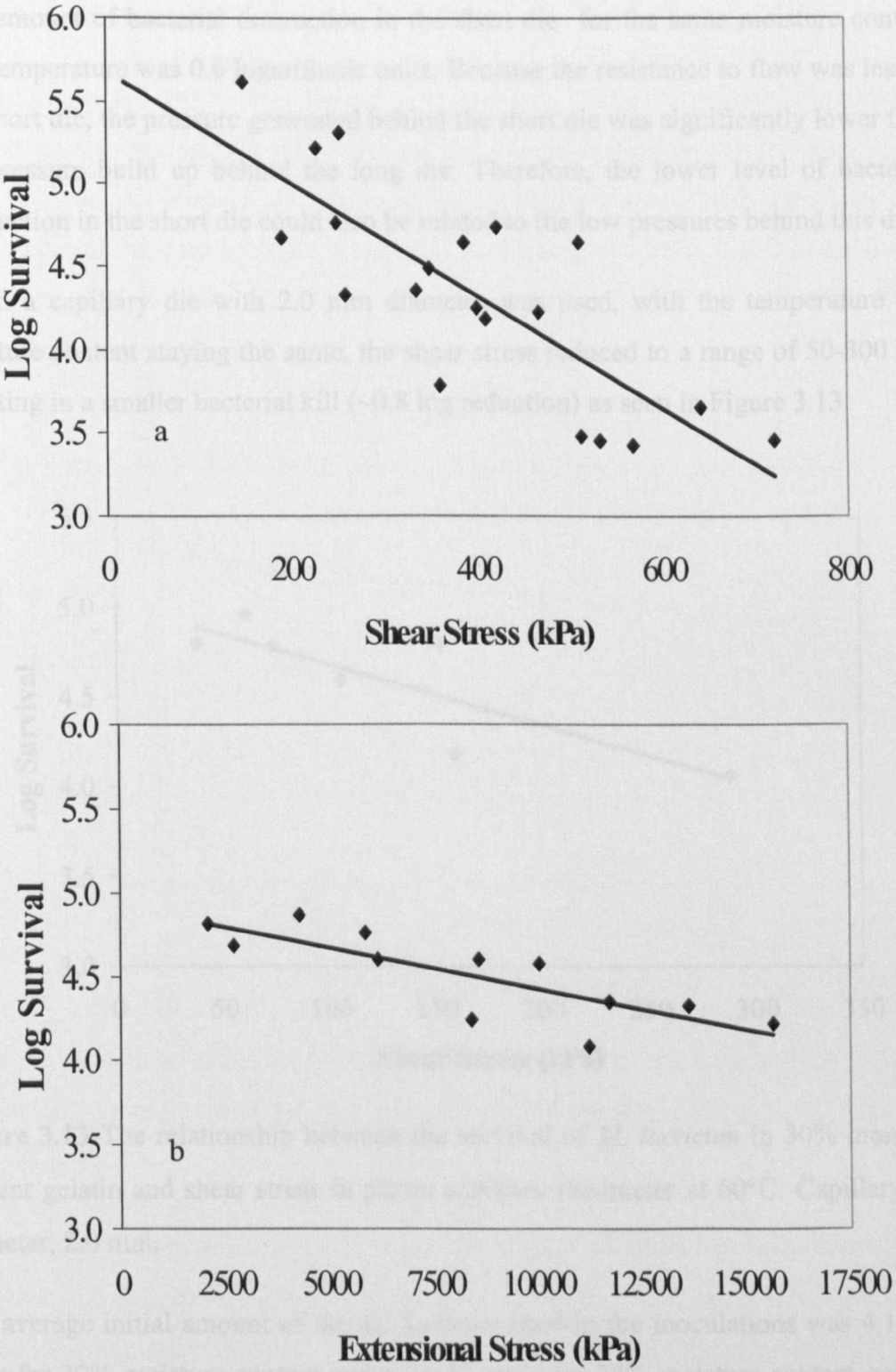

Figure 3.12 The relationship between the survival of *M. lacticum* in 30% moisture content gelatin and (a) shear stress in a long die, (b) extensional stress in a short die of the piston capillary rheometer at 60°C. Capillary die diameter, 1.0 mm.

The amount of bacterial destruction in the short die for the same moisture content and temperature was 0.6 logarithmic units. Because the resistance to flow was less in the short die, the pressure generated behind the short die was significantly lower than the pressure build up behind the long die. Therefore, the lower level of bacterial destruction in the short die could also be related to the low pressures behind this die.

When a capillary die with 2.0 mm diameter was used, with the temperature and moisture content staying the same, the shear stress reduced to a range of 50-300 kPa resulting in a smaller bacterial kill (~0.8 log reduction) as seen in Figure 3.13.

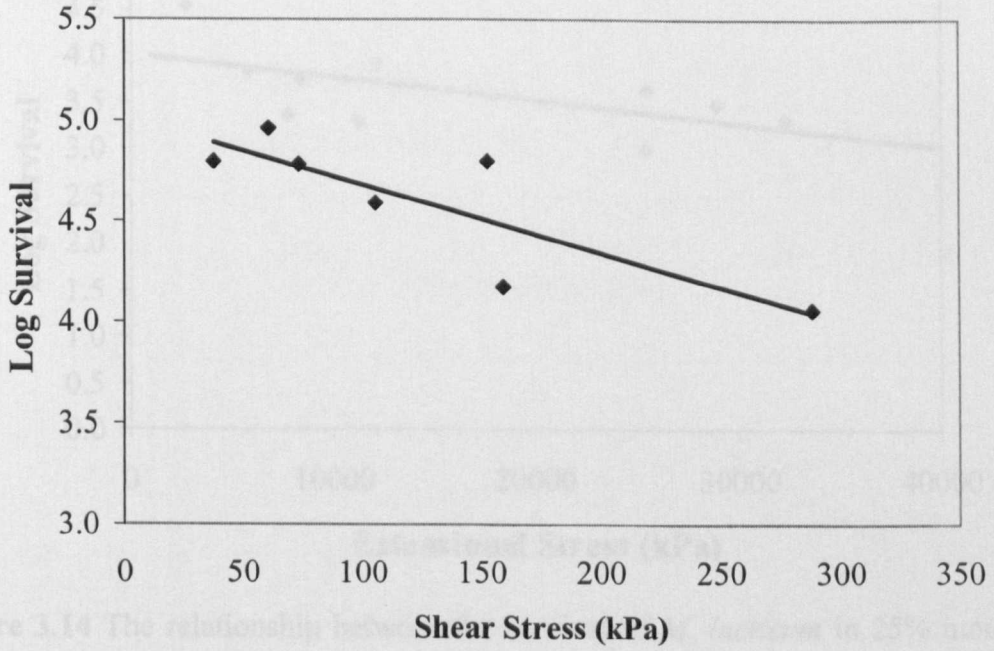

Figure 3.13 The relationship between the survival of *M. lacticum* in 30% moisture content gelatin and shear stress in piston capillary rheometer at 60°C. Capillary die diameter, 2.0 mm.

The average initial amount of the *M. lacticum* used in the inoculations was 4.1×10^6 cfu/g for 30% moisture content and 6.7×10^6 cfu/g for 25% moisture content gelatin. A 4.1 log survival (2.7 log reduction) at 165 kPa and a 3.5 log survival (3.3 log reduction) at 695 kPa shear stresses were determined from the sheared samples in the long die at 25% moisture content. Within the limit of detection (~3.6×10^2 per gram of gelatin), no survival was detected at higher shear stresses in the long die, demonstrating that the combination of shear, pressure and temperature forces can

give acceptable sterility to the material.

The relationship between the survival of *M. lacticum* after extrusion of 25% moisture content gelatin through the short die and the extensional stress is shown in Figure 3.14. The bacterial destruction due to the extensional stress was within the limit of 1 logarithmic unit for the extensional stresses in the range of 1000-33000 kPa showing that, compared to extensional stress, the shear stress was more effective in the destruction of *M. lacticum*.

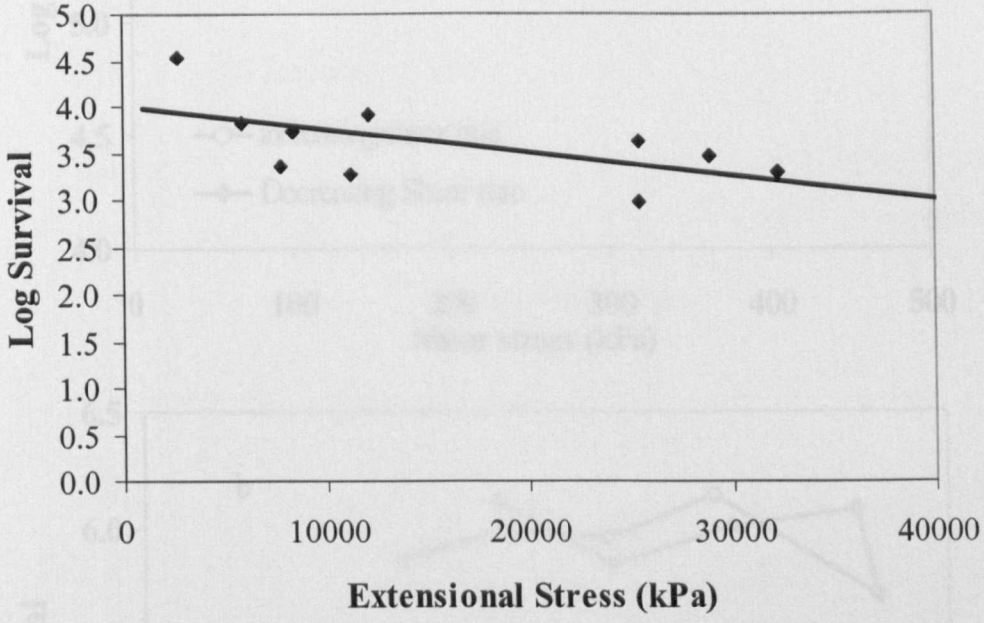

Figure 3.14 The relationship between the survival of *M. lacticum* in 25% moisture content gelatin and extensional stress in piston capillary rheometer at 60°C. Capillary die, 1.0 mm.

A set of experiments was designed where the shear rate (piston speed) was increased stepwise to a maximum value and then reduced to the initial value. The analysis of the samples against unsheared control samples showed that there was no significant bacterial destruction due to shearing of the microorganism in 30% moisture content gelatin through 1.0 and 1.5 mm dies at 70 and 75°C temperatures (Figure 3.15 and Figure 3.16). Considering that the initial amount of *M. lacticum* used in the inoculations was 1.2×10^7 cfu per g sample for this set of experiments, the significant reduction in the numbers of the microorganism due to the compression of the

microorganism in gelatin during equilibrium time at 70 and 75°C (7 min at 1 MPa) can be observed from Figure 3.15-16.

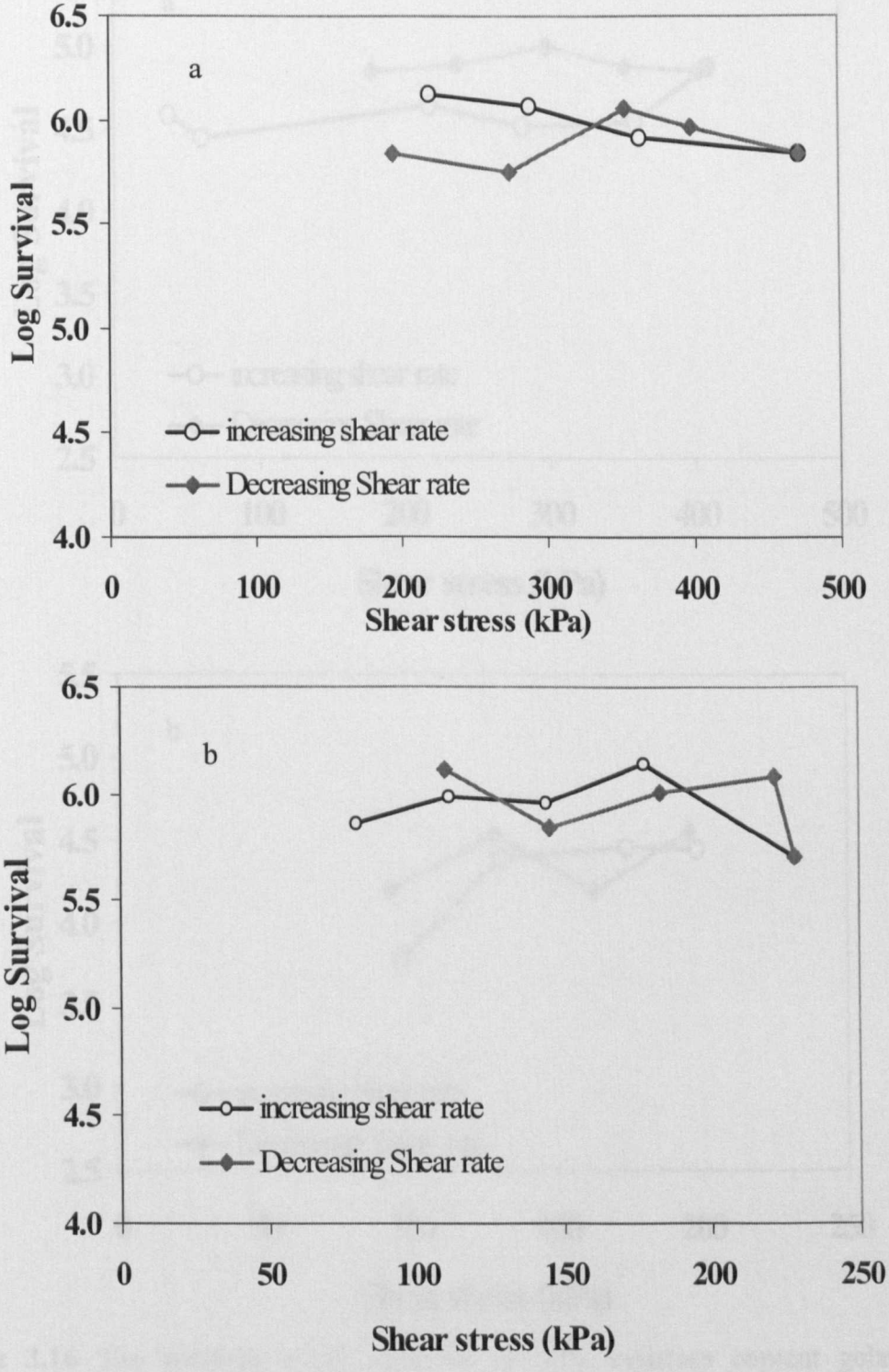

Figure 3.15 The survival of *M. lacticum* in 30% moisture content gelatin at increasing and decreasing shear stresses in piston capillary rheometer at 70°C. Capillary die (a) 1.0 mm, (b) 1.5 mm.

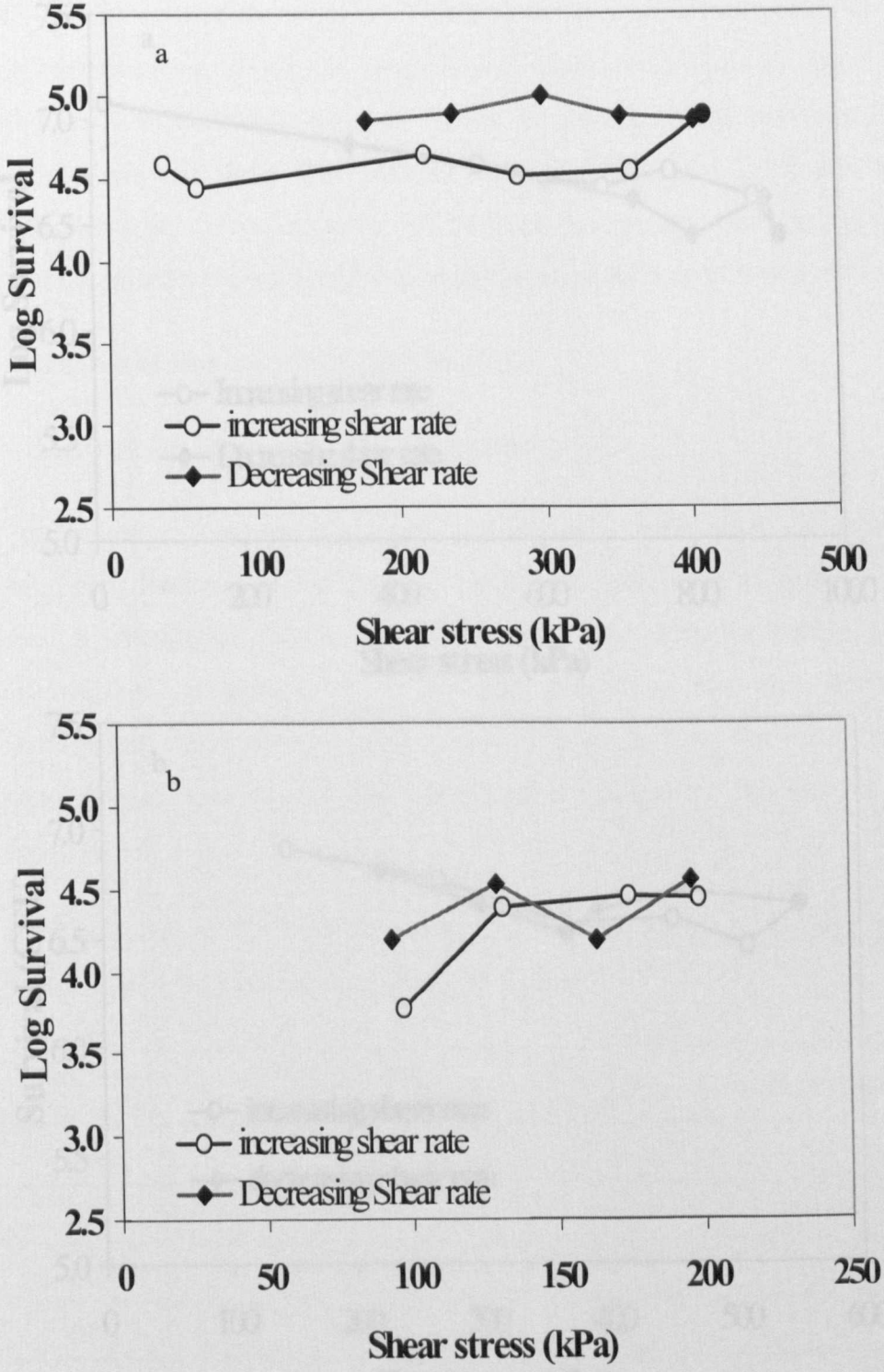

Figure 3.16 The survival of *M. lacticum* in 30% moisture content gelatin at increasing and decreasing shear stresses in piston capillary rheometer at 75°C. Capillary die (a) 1.0 mm, (b) 1.5 mm.

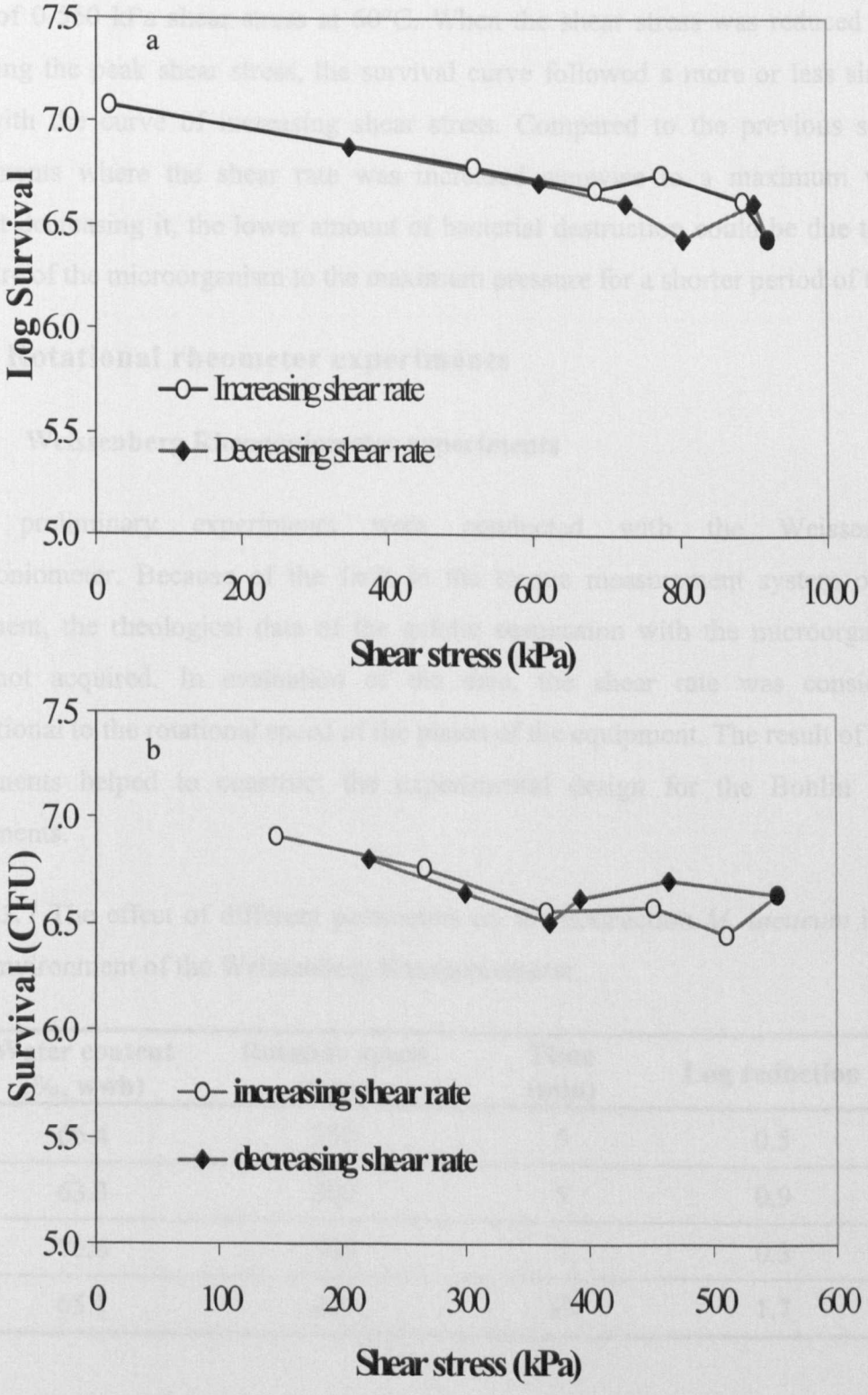

Figure 3.17 The survival of *M. lacticum* in 30% moisture content gelatin at increasing and decreasing shear stresses in piston capillary rheometer at 60°C. Capillary die (a) 1.0 mm, (b) 1.5 mm.

Figure 3.17 shows that there was less than 1 log reduction in the number of *M. lacticum* in the range of 0-918 kPa shear stress and about 0.5 log reduction in the range of 0-560 kPa shear stress at 60°C. When the shear stress was reduced after achieving the peak shear stress, the survival curve followed a more or less similar path with the curve of increasing shear stress. Compared to the previous set of experiments where the shear rate was increased stepwise to a maximum value without decreasing it, the lower amount of bacterial destruction could be due to the exposure of the microorganism to the maximum pressure for a shorter period of time.

3.3.3 Rotational rheometer experiments

3.3.3.1 Weissenberg Rheogoniometer experiments

Some preliminary experiments were conducted with the Weissenberg Rheogoniometer. Because of the fault in the torque measurement system of the equipment, the rheological data of the gelatin suspension with the microorganism were not acquired. In evaluation of the data, the shear rate was considered proportional to the rotational speed of the platen of the equipment. The result of these experiments helped to construct the experimental design for the Bohlin VOR experiments.

Table 3.7 The effect of different parameters on the destruction *M. lacticum* in the shear environment of the Weissenberg Rheogoniometer.

Water content (%, wwb)	Rotation speed (rpm)	Time (min)	Log reduction
66.4	250	5	0.5
63.3	300	5	0.9
76.6	300	7	0.3
65.1	400	10	1.7

The maximum destruction rate was obtained at the highest rotation speed and operation time, whereas the minimum destruction rate was obtained at the highest moisture level.

The effect of operation time and the shear rate are shown in Figure 3.18 and Figure 3.19. Due to the difficulties in adjusting the gap between the cone and platen of the equipment precisely, the points on the graphs are scattered. These results cannot be considered conclusive. However, the overall trend in both curves shows that high shear rates (rotation speeds) and high operation times result in greater reduction in the number of *M. lacticum*. Further experiments conducted with the Bohlin VOR have confirmed these results.

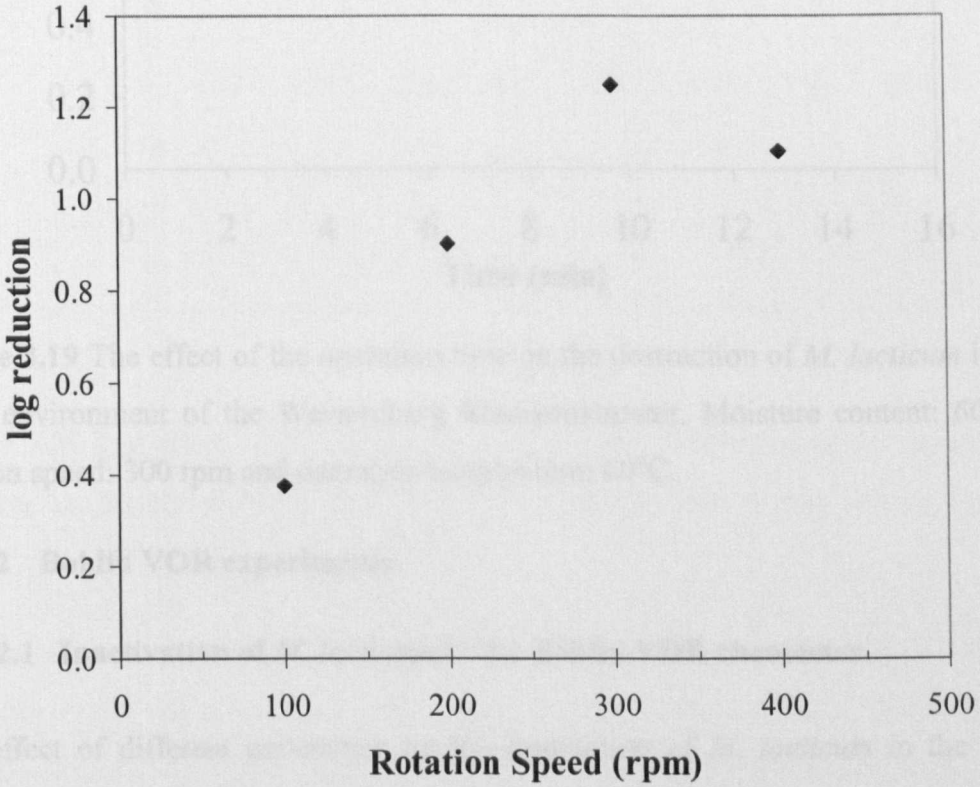

Figure 3.18 The effect of rotation speed on destruction of *M. lacticum* in the Weissenberg Rheogoniometer. Moisture content: 60.0%, operation time: 5 min and operation temperature: 60°C.

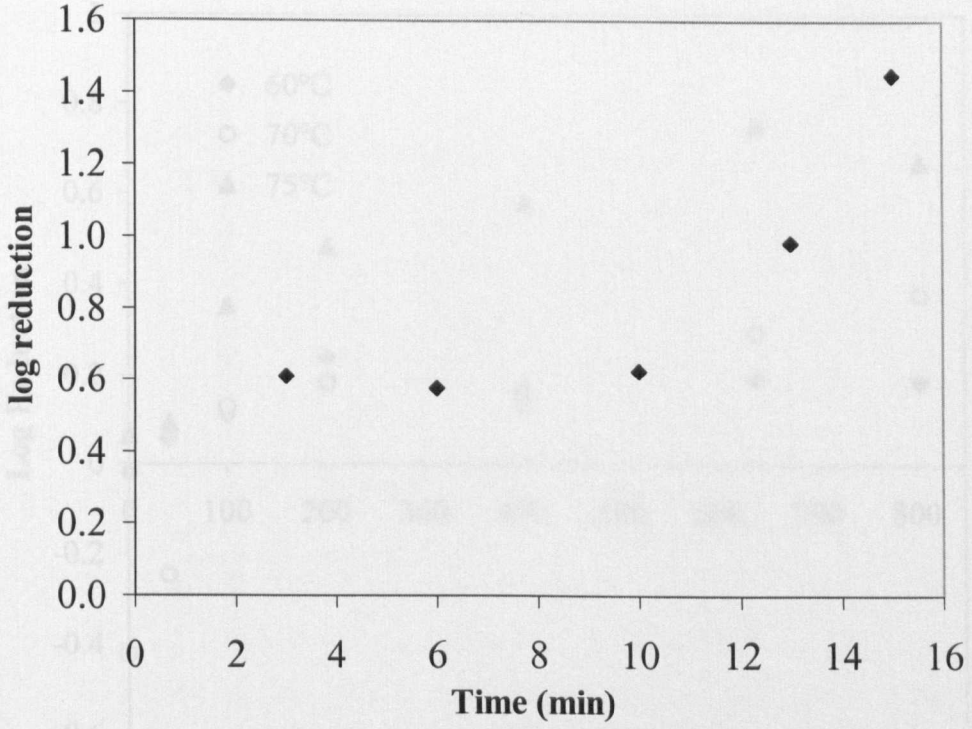

Figure 3.19 The effect of the operation time on the destruction of *M. lacticum* in the shear environment of the Weissenberg Rheogoniometer. Moisture content: 60.0%, rotation speed: 300 rpm and operation temperature: 60°C.

3.3.3.2 Bohlin VOR experiments

3.3.3.2.1 Inactivation of *M. lacticum* in the Bohlin VOR rheometer

The effect of different parameters on the destruction of *M. lacticum* in the shear environment of the Bohlin VOR was studied. In a preliminary experiment where the gelatin mix was kept in a 60°C water bath for 45 min before it was sheared in the rheometer at different temperatures and increasing shear rates, destruction of *M. lacticum* was increased by the increase of the temperature during shearing, as can be seen in Figure 3.20.

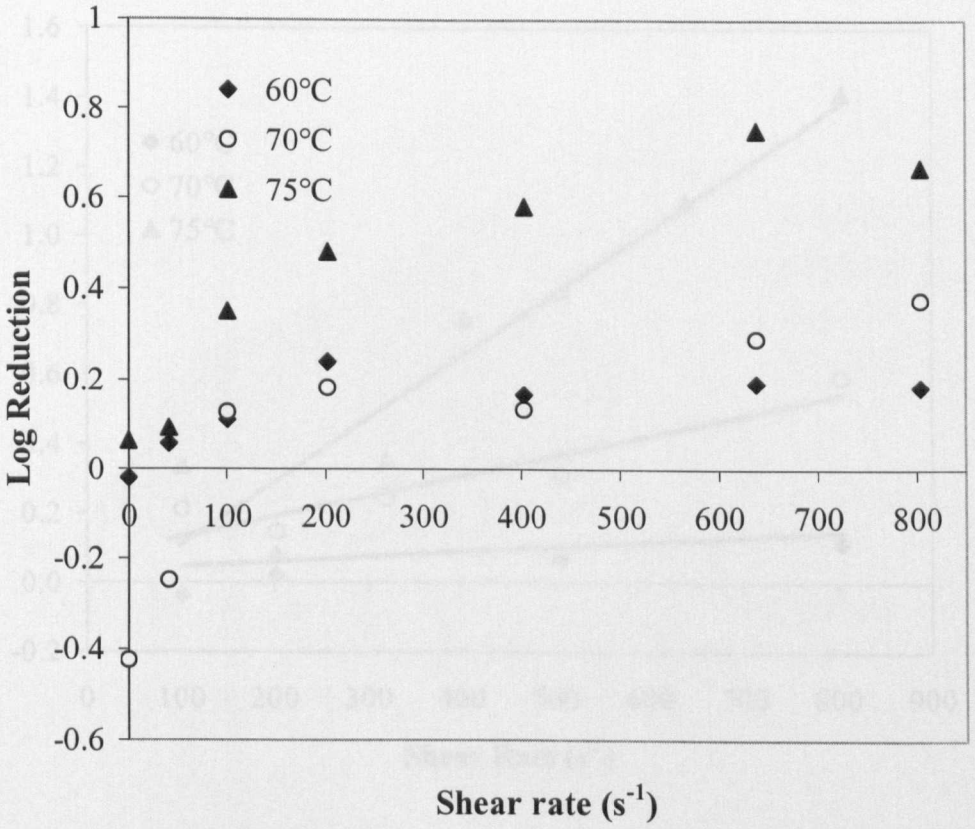

Figure 3.20 The effect of increasing shear rate on the destruction of *M. lacticum* at different temperatures. Water content: 65.0%, shearing time: 4 min, heating time of gelatin mix at 60°C: 45 min.

The negative values of bacterial destruction at shear rates close to zero (0.32 s^{-1}) were due to the slightly greater heat treatment of the control samples. Exposing the control samples to slightly higher temperatures was part of the experimental plan (see Section 2.3.2.2.1) in order to take in to account any effect of shear heating on bacterial destruction.

Figure 3.21 shows the effect of increasing shear rate at different temperatures on destruction of *M. lacticum*. In this experiment, the time of heating of the gelatin mix at 60°C was 17 min as explained in 2.3.2.2.1. Shearing of the microorganism, with gelatin at a 65% moisture content for 4 min at a shear stress of 2.8 kPa and 75°C temperature, resulted in a maximum kill of 1.4 log cycle (Figure 3.21), where the number of surviving microorganisms in the sheared sample was 5.2×10^6 cfu/g sample against 1.4×10^8 cfu/g sample in the non-sheared control.

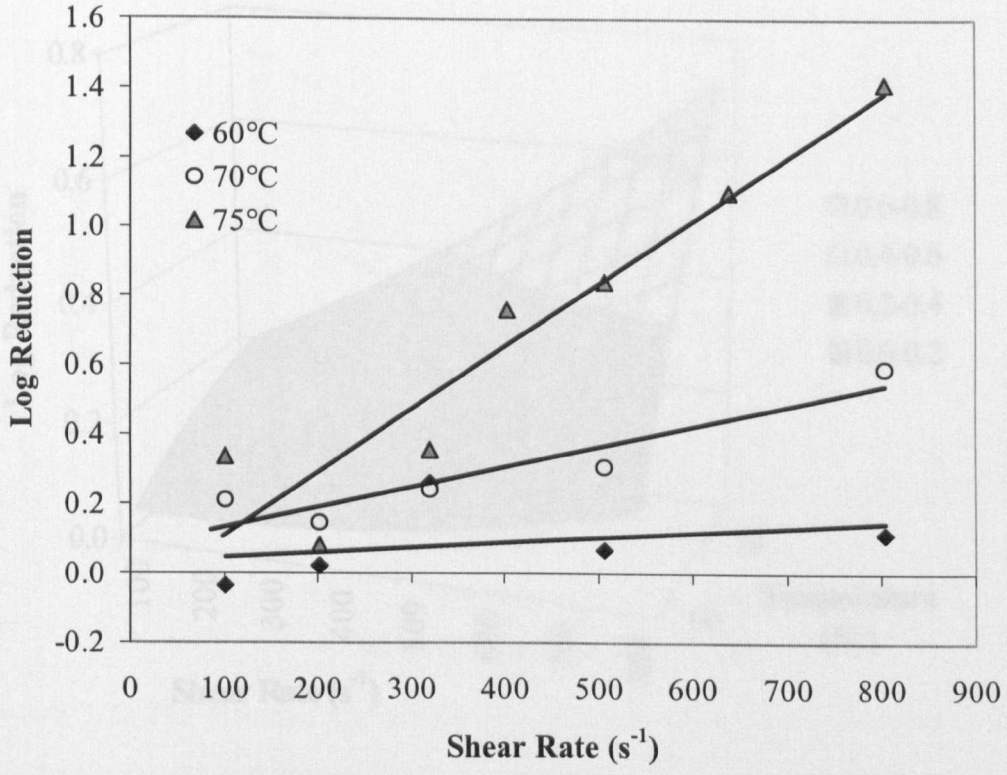

Figure 3.21 The effect of increasing shear rate on the destruction of *M. lacticum* at different temperatures. Moisture content: 65%, shearing time: 4 min.

A second order multi linear regression analysis revealed the response surface shown in Figure 3.22. In the range of 100-804 s^{-1} shear rate and 60-75°C temperature, the regression analysis approximates to a 0.8 log reduction at the highest shear rate and temperature. The regression coefficient for this estimation (0.67) can be considered reasonable, since the sample space was not large. The response surface clearly illustrates that bacterial destruction is increasing significantly with increasing temperature and shear rate. Considering that *M. lacticum* has a high heat resistance even at 75°C ($D_{75°C}$ =20 min) and the shearing time was only 4 min, the bacterial destruction can be mainly accredited to shear forces. As discussed previously, the significant role of the temperature could be due to the weakening of the bacterial cell wall as a result of the heat effect, which consequently made the cells more susceptible to the physical (shear) forces. Therefore, the high bacterial destruction at high temperature and shear rates could be explained by the coupling and/or synergistic affects of both thermal and shear forces.

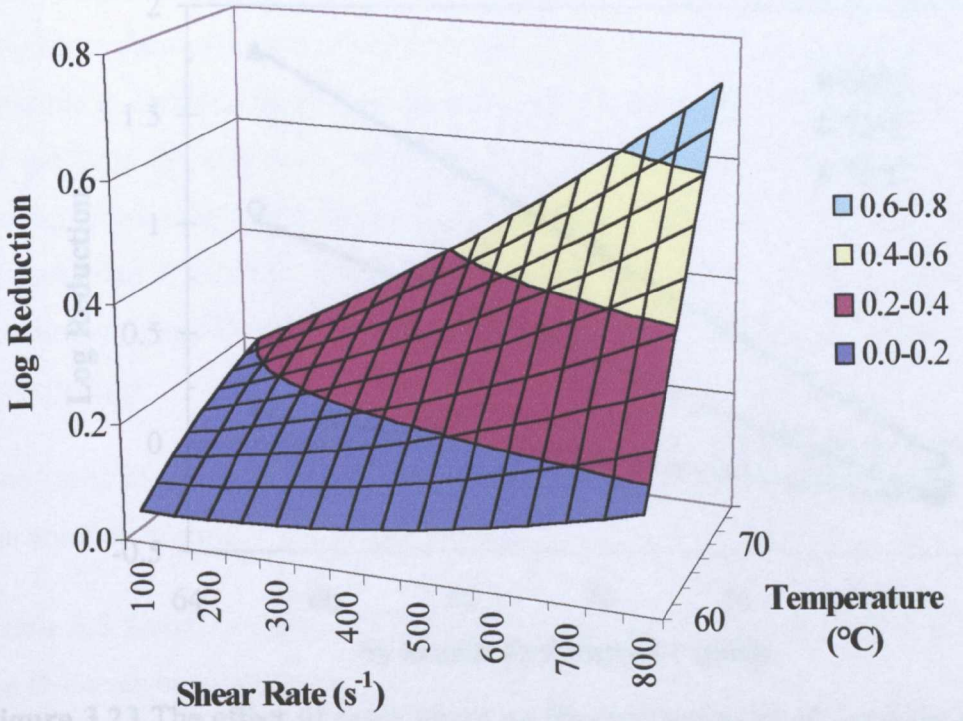

Figure 3.22 The response surface between the logarithmic reduction of *M. lacticum*, Temperature and Shear rate. Moisture content: 65%, shearing time: 4 min.

The effect of shear forces on the destruction of *M. lacticum* at different moisture contents and temperatures are shown in Figure 3.23. The increased bacterial destruction with increasing temperature and reducing moisture content was expected because of the synergistic effect of thermal and shear forces. Increased moisture content reduced the viscosity and hence the shear forces, resulting in a smaller reduction in the number of *M. lacticum*. The length of time of shearing also affected the death rate of the microorganism. Figure 3.24 shows the effect of operation time on the death rate of *M. lacticum* in the shear environment of the Bohlin VOR.

From Figure 3.24 it can be noted that the curves are intersecting on the y-axis at minus values. This can be explained by the fact that the control samples experienced higher temperatures throughout the shearing time. Therefore, up to a certain degree of shearing time, the reduction in the number of *M. lacticum* in the control samples was more than that in the sheared samples.

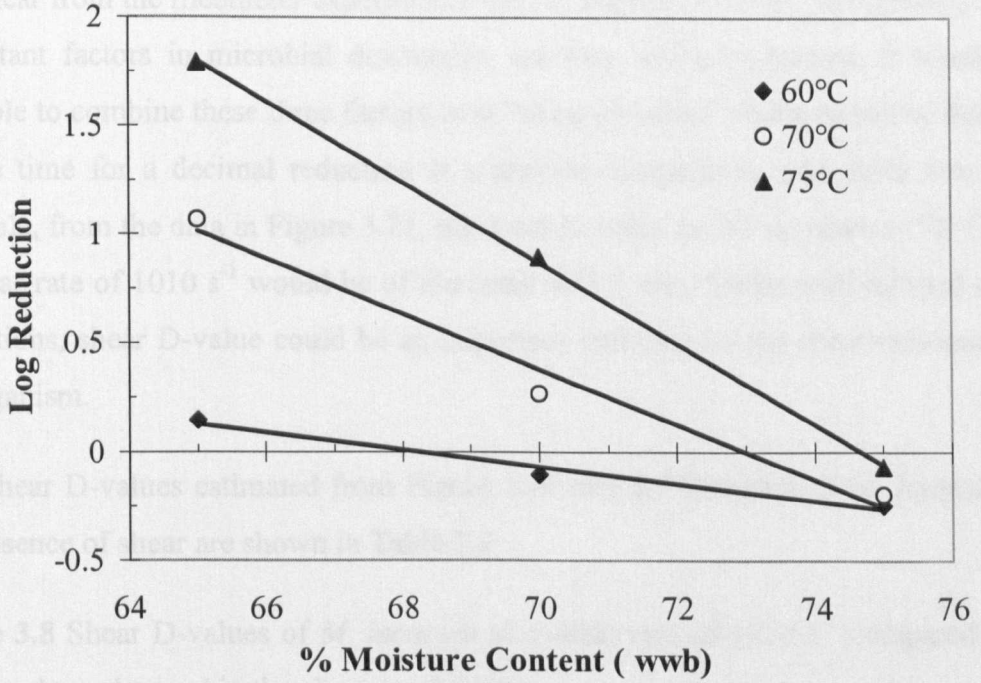

Figure 3.23 The effect of shear forces on the destruction of *M. lacticum* at different moisture contents and temperatures. Shear rate: 1010 s^{-1}, shearing time: 4 min.

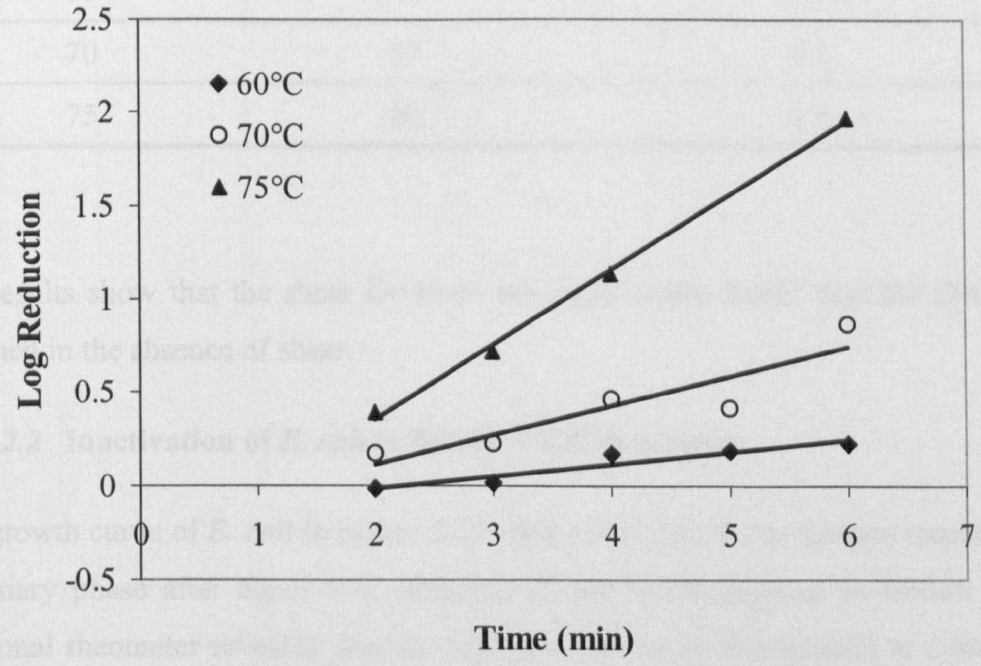

Figure 3.24 Dependence of log reduction of *M. lacticum* in 65% moisture content gelatin on the time of shearing at different temperatures. Shear rate: 1010 s^{-1}.

It is clear from the rheometer experiments that, in addition to shear rate (stress), other important factors in microbial destruction are time and temperature. It would be possible to combine these three factors in to "shear D-value" which would be defined as the time for a decimal reduction at a specific temperature and shear rate. For example, from the data in Figure 3.21, the shear D-value for *M. lacticum* at 75°C and at shear rate of 1010 s^{-1} would be of the order of 2.5 min. Under well-defined shear conditions, shear D-value could be an important indicator of the shear resistance of an organism.

The shear D-values estimated from Figure 3.24 and the D-values of *M. lacticum* in the absence of shear are shown in Table 3.8

Table 3.8 Shear D-values of *M. lacticum* at a shear rate of 1010 s^{-1} compared with the D-values obtained in the absence of shear.

Temperature (°C)	D-value (min)	Shear D-value (min)
60	375	15.4
70	57	6.3
75	20	2.5

The results show that the shear D-values are significantly lower than the D-values obtained in the absence of shear.

3.3.3.2.2 Inactivation of *E. coli* in Bohlin VOR rheometer

The growth curve of *E. coli* in Figure 3.25 shows that the microorganism reached the stationary phase after about 6 h. Shearing of the microorganism in Bohlin VOR rotational rheometer revealed that the resistance of the microorganism to combined shear and thermal stresses was not significantly influenced by the time of harvesting (Figure 3.26) In these experiments 8 h cultures were always used.

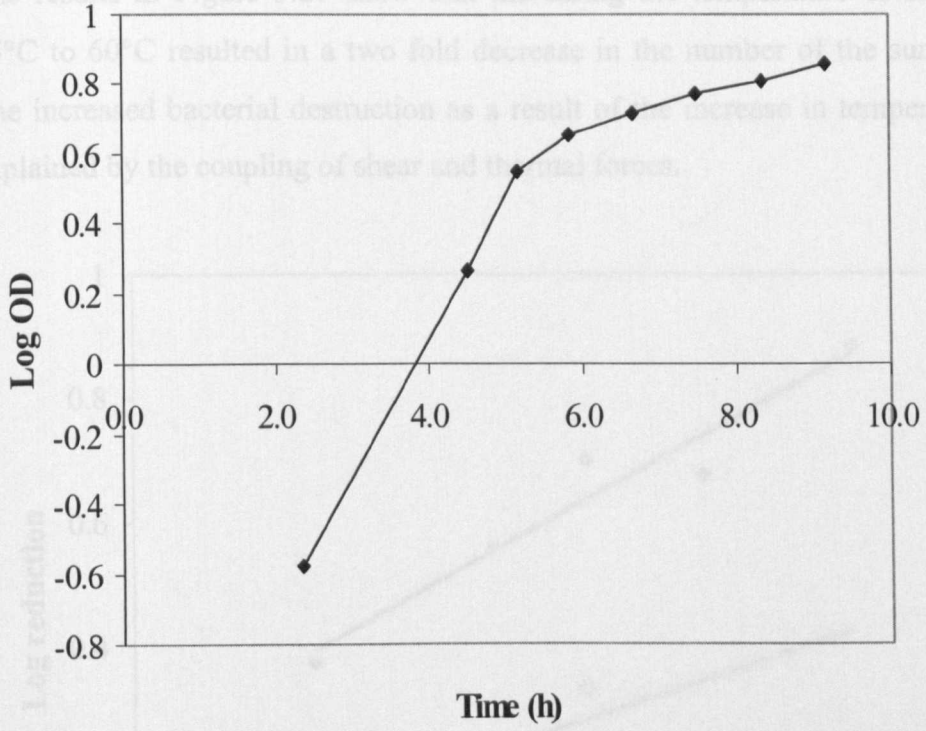

Figure 3.25 Growth curve of *E. coli* in BHI broth at 30°C determined by optical density (OD) readings.

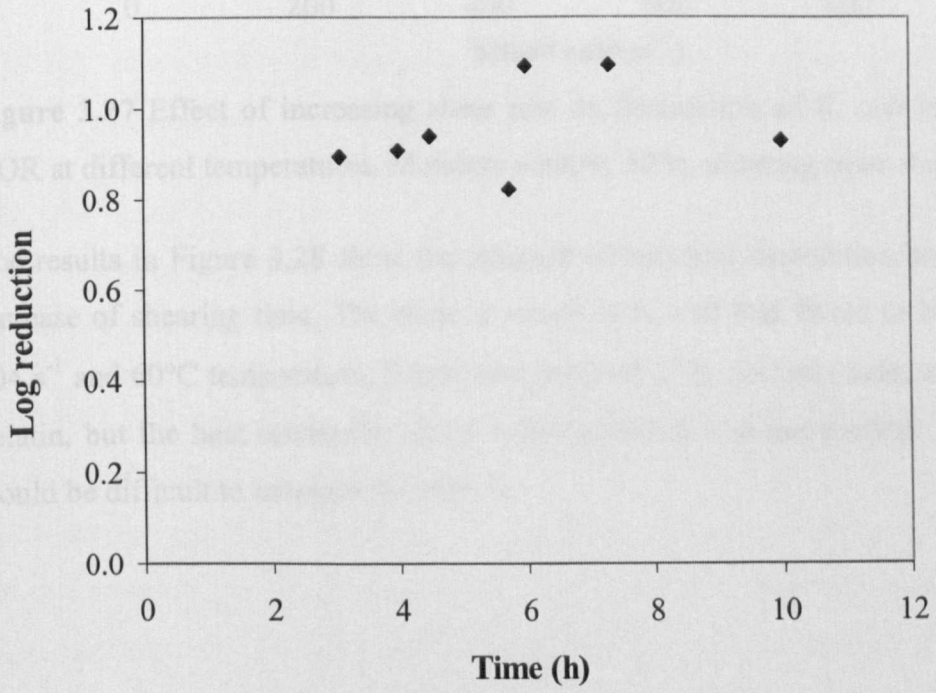

Figure 3.26 Resistance of *E. coli* harvested at different times to combined shear and thermal forces in Bohlin VOR rheometer. Temperature; 60°C, shear rate; 804 s^{-1}, shearing time: 4 min..

The results in Figure 3.27 show that increasing the temperature of shearing from 55°C to 60°C resulted in a two fold decrease in the number of the surviving cells. The increased bacterial destruction as a result of the increase in temperature can be explained by the coupling of shear and thermal forces.

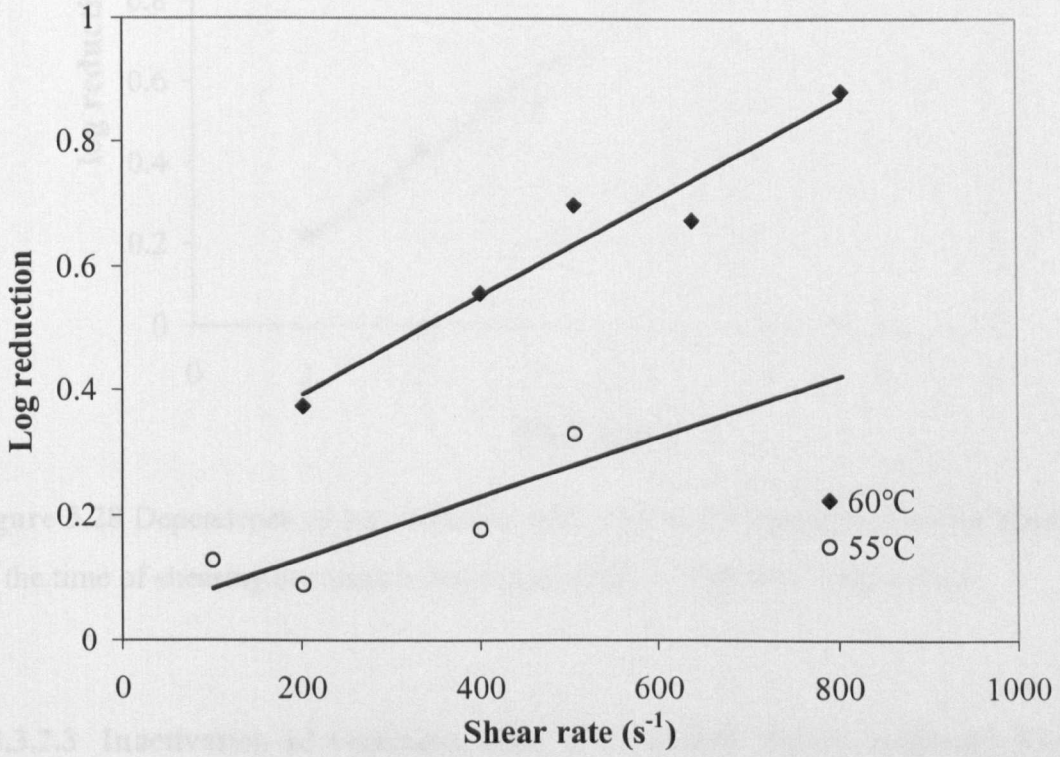

Figure 3.27 Effect of increasing shear rate on destruction of *E. coli* in the Bohlin VOR at different temperatures. Moisture content: 65%, shearing time: 4 min.

The results in Figure 3.28 show the increase of bacterial destruction because of the increase of shearing time. The shear *D-value* of *E. coli* was found to be 4.8 min at 804 s^{-1} and 60°C temperature. Significant survival of *E. coli* was detected at 64°C in gelatin, but the heat resistance of the microorganism was not studied. Therefore, it would be difficult to interpret the results.

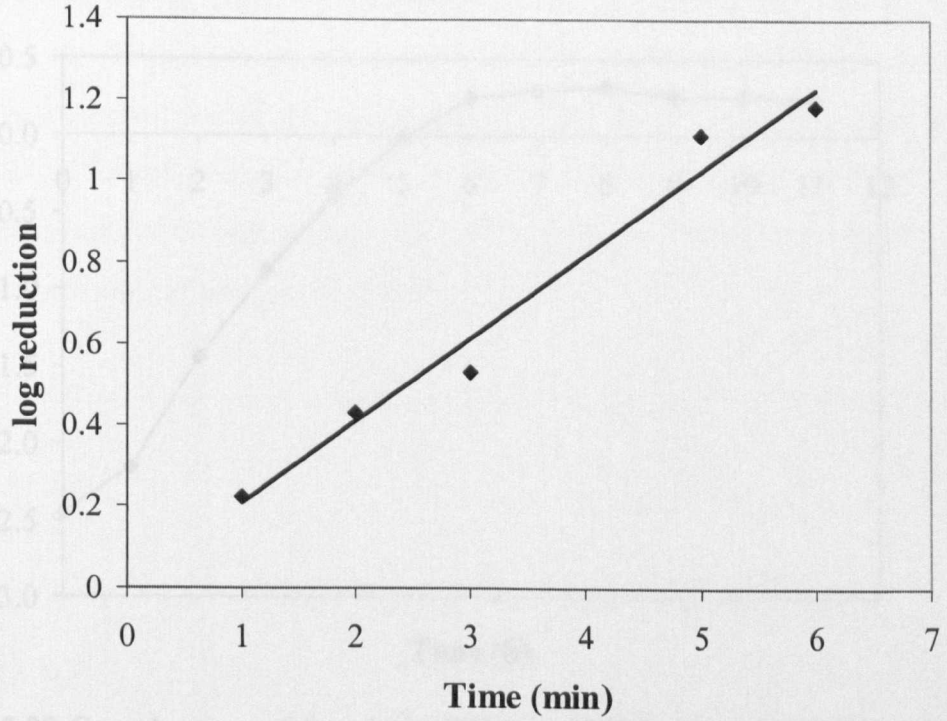

Figure 3.28 Dependence of log reduction of *E. coli* in 65% moisture content gelatin on the time of shearing at constant shear rate of 804 s^{-1} and 60°C temperature.

3.3.3.2.3 Inactivation of vegetative cells of *B. subtilis* PS346 in Bohlin VOR rheometer

The growth curve of *B. subtilis* PS346 is given in Figure 3.29. In order to test the shear resistance of the microorganism 8 h cultures were used where the microorganism was in its stationary phase.

Shearing of the vegetative cells of *B. subtilis* PS346 at 45 and 50°C temperatures, in the range of 0.32-1010 s^{-1} shear rate, did not give any significant bacterial kill against the unsheared control indicating that shear forces created by Bohlin VOR rheometer did not promote the killing of *B. subtilis* PS346 (Figure 3.30). In Figure 3.30, the negative values of logarithmic reduction in the numbers of *B. subtilis* PS346 at 55°C is because of the exposure of the control samples to slightly higher temperatures (see Section 2.3.2.2.1). It is a common practice to apply a 10 min heat treatment at 60°C

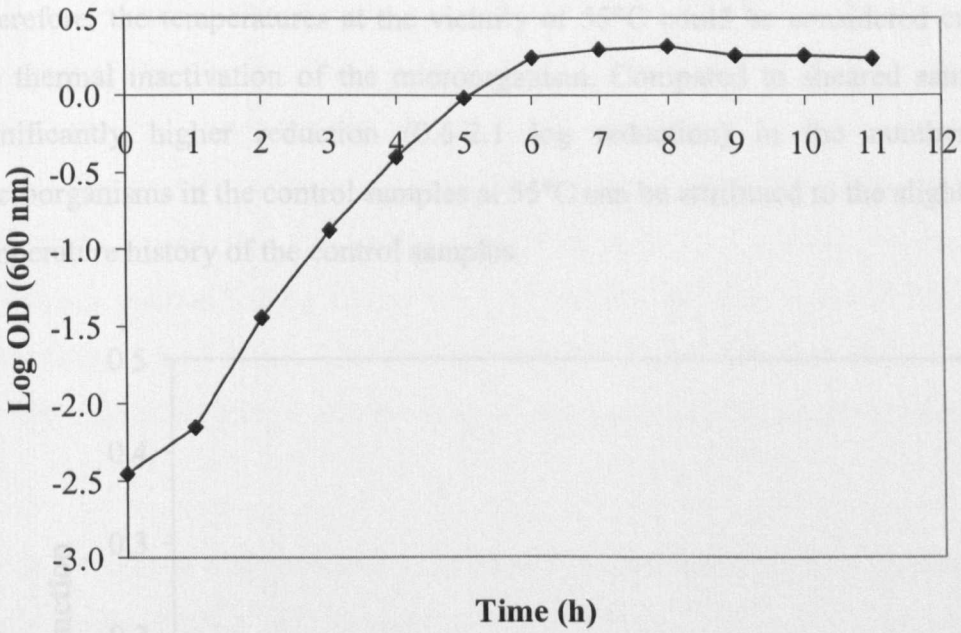

Figure 3.29 Growth curve of *B. subtilis* PS346 in BHI broth at 30°C determined by optical density (OD) readings.

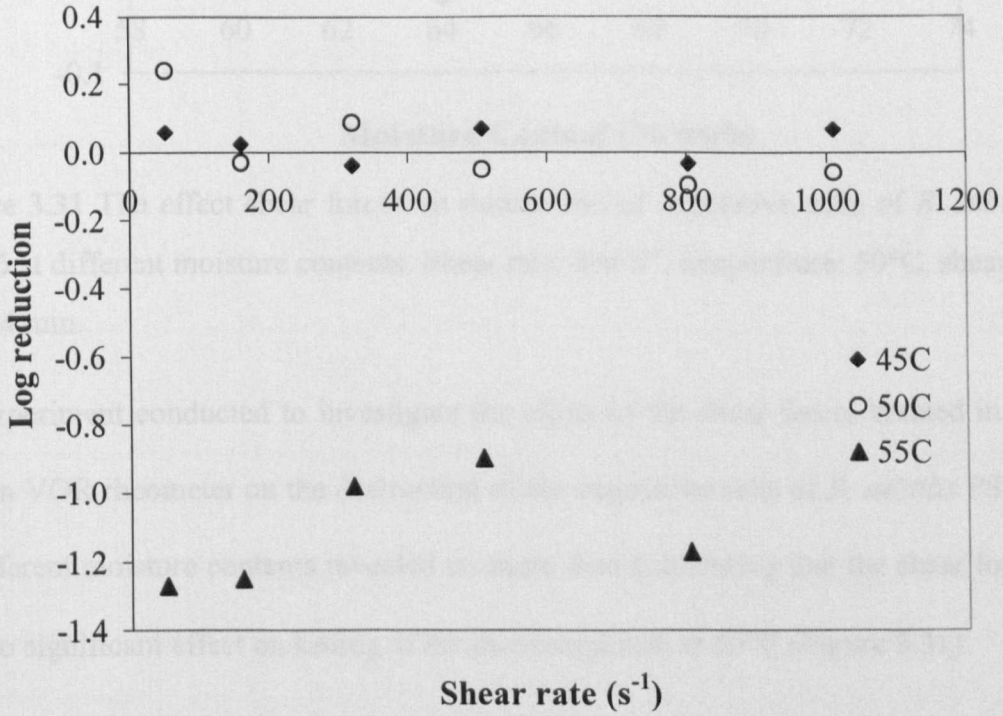

Figure 3.30 The effect of increasing shear rate on destruction of vegetative cells of *B. subtilis* PS346 at different temperatures. Water content: 65.0%, shearing time: 4 min.

to kill the vegetative cells of *B. subtilis* PS346 (Movahedi and Waites, 2000). Therefore, the temperatures at the vicinity of 55°C could be considered critical for the thermal inactivation of the microorganism. Compared to sheared samples, the significantly higher reduction (0.6-2.1 log reduction) in the number of the microorganisms in the control samples at 55°C can be attributed to the slightly higher temperature history of the control samples.

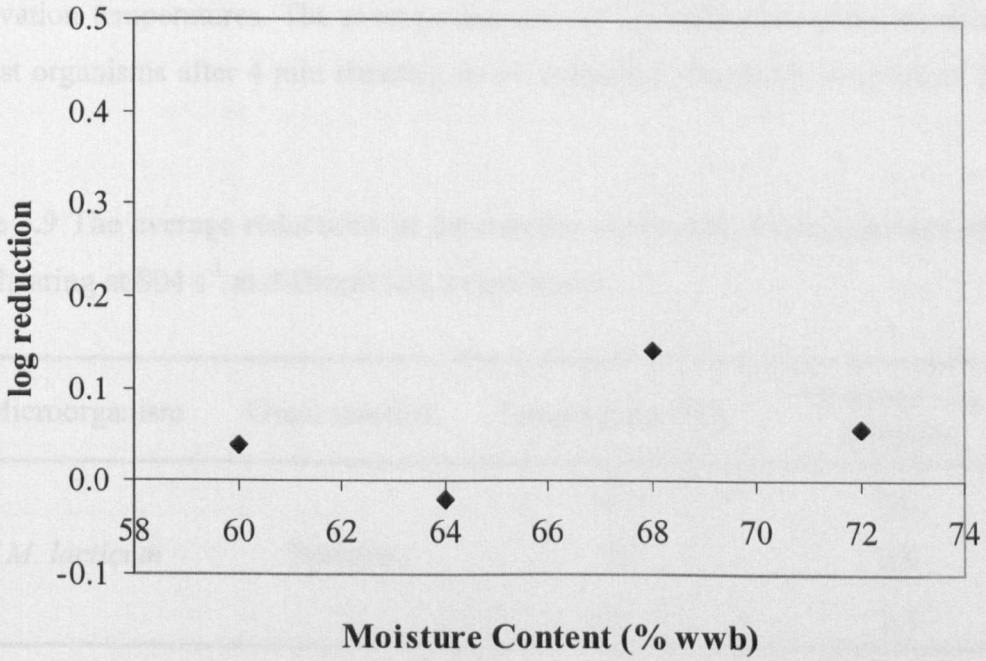

Figure 3.31 The effect shear forces on destruction of vegetative cells of *B. subtilis* PS346 at different moisture contents. Shear rate: 804 s^{-1}, temperature: 50°C, shearing time: 4 min.

An experiment conducted to investigate the effect of the shear forces created in the Bohlin VOR rheometer on the destruction of the vegetative cells of *B. subtilis* PS346 at different moisture contents revealed no more than confirming that the shear forces had no significant effect on killing of the microorganism at 50°C (Figure 3.31).

As discussed previously, the mechanical strength of the cell wall is closely related to the structure and the thickness of peptidoglycan layer and the frequency of peptide chains and their cross linking in the peptidoglycan structure. The peptidoglycan layer in Gram-negative bacteria is 1.5 to 2.0 nm in thickness and typically accounts for 10

to 20% of the cell wall in terms of dry mass. The cell wall of Gram-positive bacteria is composed of 50-80% peptidoglycan, associated with teichoic acids, presenting a greater structural resistance to breakage (See Section 1.3.1 for details). Therefore, it is not surprising to see that the shear resistances of the test microorganisms used in rotational rheometer experiments were not the same. The test temperatures in the rheometer for all the three microorganisms were low enough to prevent any significant thermal killing during the test period, but were close to their thermal inactivation temperatures. The average amounts of log reduction in the numbers of the test organisms after 4 min shearing in the rotational rheometer is given in Table 3.9.

Table 3.9 The average reductions in the number of the test microorganisms after 4 min shearing at 804 s^{-1} at different test temperatures.

Microorganism	Gram reaction	Temperature (°C)	Average Log reduction
M. lacticum	Positive	60	ns
		70	0.6
		75	1.4
B. subtilis	Positive	45	ns
		50	ns
		55	-0.8
E. coli	Negative	55	0.4
		60	0.9

ns: not significant

M. lacticum and *B. subtilis* are both Gram-positive microorganisms but their resistances to shear forces seems to be different under the test conditions applied. However, compared to the other microorganisms, *M. lacticum* was sheared at higher temperatures. The fact that there were no significant reduction in the number of *M. lacticum* at 60°C due to shearing, but an increased reduction at 70 and 75°C, could indicate that the peptidoglycan layer, which is responsible for the mechanical strength, is weakening beyond 60°C temperature. It was not possible to expose *B.*

subtilis to temperatures above 55°C due to the low heat resistance of the organism. It is possible to say that the peptidoglycan layer remained strong enough so that it was not damaged by the shear forces in the rotational rheometer. An order of 1 log reduction in the number of *E. coli* due to shearing at 60°C does not contradict with the above explanation, since *E. coli* is a Gram-negative bacterium containing less peptidoglycan in its cell wall.

Another factor that can determine the shear resistance of a microorganism could be the size and shape of the microorganism. All the three test microorganisms used in our experiments were rod shaped bacteria. Their size, as reported in the literature, is given in Table 3.10. By using the arguments analogous to those used to explain the breakup of emulsion droplets in a shear field (Akay, 1998), it could be suggested that the stress required for destruction scales with the dimensions of the microorganism.

Table 3.10 Size of the test microorganisms cited in different sources.

Microorganism	Diameter (μm)	Length (μm)	Reference
M. lacticum	0.4-0.8	1.0-4.0	a
E. coli	0.5-1.0	2.0-4.0	b
B. subtilis	0.5-2.5	1.2-10	a
Endospores of B. subtilis	0.8	1.5-1.8	c

a) Bergey's Manual of Systematic Bacteriology, 1986.
b) Pelczar *et al.*, 1993.
c) Bergey's Manual of Determinative Bacteriology, 1993.

In a shear field, it can be suggested that rod shape bacteria would be more susceptible to the shearing than cocci. On the other hand, in the shear field of the rotational rheometer at low viscosities, the rod shaped microorganisms can align themselves to the flow direction and consequently reduce the destructive effect of the shear forces. Alignment of a rod shaped microorganism in the flow direction would not be easy at high consistencies. Similarly, the alignment would not be possible, if the flow is turbulent and not fully developed. Therefore, the role of the shape of the microorganism in shear resistance could be more clearly observed in extrusion or piston capillary rheometers. However, no coccal shaped bacteria were employed in our experiments.

3.4 CONCLUSIONS

Viscosity measurements in the Bohlin VOR found that the gelatin showed almost Newtonian behaviour at 35% concentration at 70 and 75°C temperatures within the shear rate range of 100-302 s^{-1}. This result is consistent with the previous studies (Wulansari and Mitchell, 1998). At higher shear rates, the viscosity reduced significantly. Upon reducing the shear rate, the viscosity recovered to a higher viscosity than the original viscosity measured at the same shear rate of 101 s^{-1}, indicating irreversible changes in the molecular structure of gelatin during shearing at 70 and 75°C.

At low moisture contents, the measurements of gelatin by the piston capillary rheometer showed pseudoplastic behaviour. The flow behaviour index of gelatin increased with increasing temperature and moisture content as expected.

Measurement of the flow behaviour index of gelatin during extrusion was not possible since it was difficult to vary the shear rate. The wide range of moisture contents and temperatures of gelatin during extrusion did not allow the use of flow behaviour indices determined by piston capillary rheometer to calculate the apparent viscosity of gelatin at the extruder die. Therefore, the shear rate and the viscosity of the gelatin at the extruder die assumed Newtonian behaviour and should be considered only as estimates.

The extrusion results suggest that there is a strong correlation between shear stress and bacterial destruction. The results showed that the highest destruction occurred at the lowest moisture contents which gave rise to higher shear forces and temperatures. Being able to generate high shear stress below a temperature of 75°C by the manipulation of the water feed rate, the extrusion process could efficiently kill *M. lacticum* in gelatin carrier. Within the limit of detection ($2x10^4$ cfu/sample), no surviving *M. lacticum* could be detected at the highest wall shear stress of 409 kPa, giving at least a 5.3 log reduction.

The mean residence times of the microorganism in the extruder barrel was between 49-58 s and the exposure of *M. lacticum* to temperatures up to 73°C in the extruder

die was in the order of a second. Based on the available heat resistance data of *M. lacticum*, it can be concluded that the temperatures alone involved in the extrusion experiments should not have any significant effect on the destruction of the microorganism. However, the heat could make the cells more susceptible to physical forces, increasing the lethal effect of the shear forces.

Piston capillary rheometer experiments results suggest that pressure forces play an important role in destruction of *M. lacticum*. There were 2.2-4.2 log unit reductions in the number of *M. lacticum* in unsheared control samples due to pressure forces. The 60°C temperature employed in experiments was well below the destruction temperature of the microorganism. Therefore, it is unlikely that the temperature alone could be responsible for the bacterial kill. However, there is evidence that higher temperatures could increase the lethality of the pressure forces (Simpson and Gilmour, 1997; Arroyo *et al.*, 1997).

There was no survival at 25% moisture content and 60°C temperature at shear stresses higher than 695 kPa during extrusion through the capillary die of the piston capillary rheometer. Despite the high shear stresses (50-500 kPa) there was no significant bacterial destruction at temperatures of 70 and 75°C in the piston capillary rheometer. This could be due to the reduced viscosity because of the high shear rates and temperatures. The time of exposure to high pressures were also shorter in these experiments. Another factor could be the slip in the capillary die. At high temperatures, the gelatin mix gets a smoother plastic-like structure, which may increase the slip at the capillary die, preventing efficient shearing.

The effect of extensional stress on destruction of *M. lacticum* seems to be minor in the range of 1000-33000 kPa for a 30% moisture content gelatin at 60°C.

It can be stated that the bacterial destruction in the piston capillary rheometer is a function of composition of the material (mainly moisture content), shear, pressure and temperature forces.

It would not be possible to compare the lethal effects of shear stresses on microorganisms in the extruder die and the capillary die of the piston capillary rheometer, due to the shear and SME history in the extruder barrel (especially in the

high shear metering zone) before the microorganism reaches the extruder die. In addition, unlike the piston capillary rheometer experiments, the moisture contents and temperatures of extrusion experiments were in a wide range.

The maximum shear stress in the rheometer was about 3.0 kPa. This is more than two orders of magnitude lower than that required for the maximum detectable kill in the extruder. It is therefore not surprising that there is only a maximum kill of the order of 1 log reduction in the rotational rheometer compared with a 5 log reduction in the extruder. It is clear from the rheometer experiments that in addition to shear stress, time and temperature are important factors. Since the microorganisms are not rigid bodies, it can be assumed that the effect of the shear is not instantaneous but rather a function of time. The microorganism could be stretched and deformed by the prolonged time of shearing. A comparison between the "shear D-value" and the D-value for heat alone might provide some interesting information about the structure of microorganisms and their resistance to shear forces.

CHAPTER 4

SHEAR INACTIVATION OF MICROORGANISMS IN MAIZE GRITS CARRIER

4.1 RESIDENCE TIME DISTRIBUTION (RTD)

Residence time distributions of *M. lacticum* and the spores of *B. subtilis* were determined separately since the extruder screw speeds, moisture contents and temperature profiles employed were different for these two microorganisms. The E(t) and *F(t)* plots for maize grits under conditions used for *M. lacticum* at different screw speeds are given in Figure 4.1. Figure 4.2 shows the *E(t)* and *F(t)* plots for the *B. subtilis* conditions. The mean residence times calculated according to Equation 2.4 and the minimum residence time, which is determined by the first detection of the die in the samples for *M. lacticum* and *B. subtilis* are given in Table 4.1. Compared to gelatin, maize grits has higher mean and minimum residence times (see Table 3.1). This difference in residence times could be explained by the rheological properties of the materials (Harper, 1981), and higher feed rate employed for maize grits (4kg/h), compared to 3 k/h feed rate of gelatin feed rate (Fichtali *et al.*, 1995). As discussed in Chapter 3, the decrease in mean and minimum residence times with increasing screw speed has previously been shown by other researchers (Altomare *et al.*, 1992, Lee and McCarthy, 1995, Yeh *et al.*, 1998).

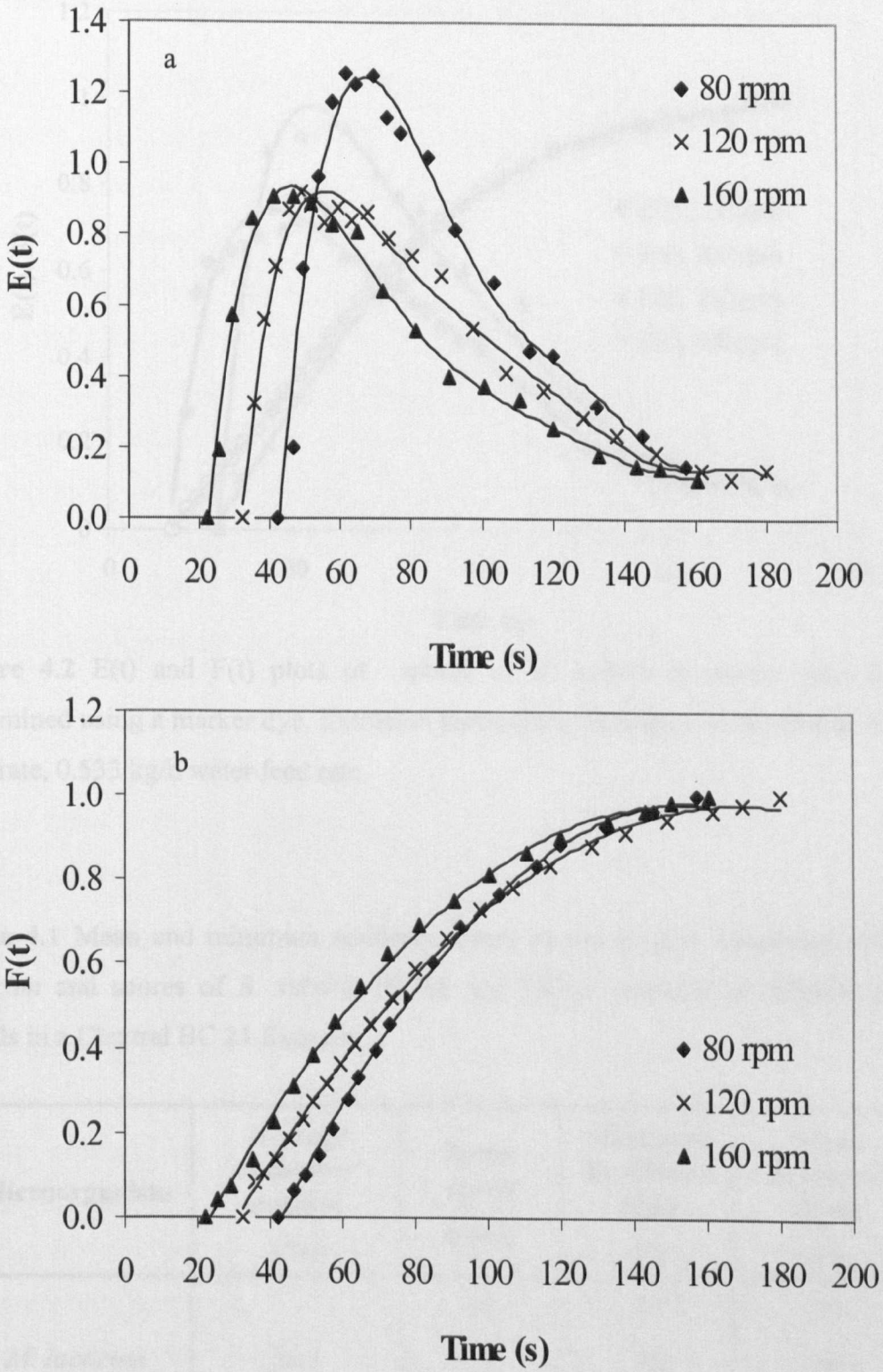

Figure 4.1 Distribution functions of *M. lacticum* in maize grits carrier determined using a marker dye. (a) exit age distribution, *E(t)* (b) Cumulative residence time distribution, *F(t)*. Extrusion parameters; 4 kg/h feed rate, 0.750 kg/h water feed rate.

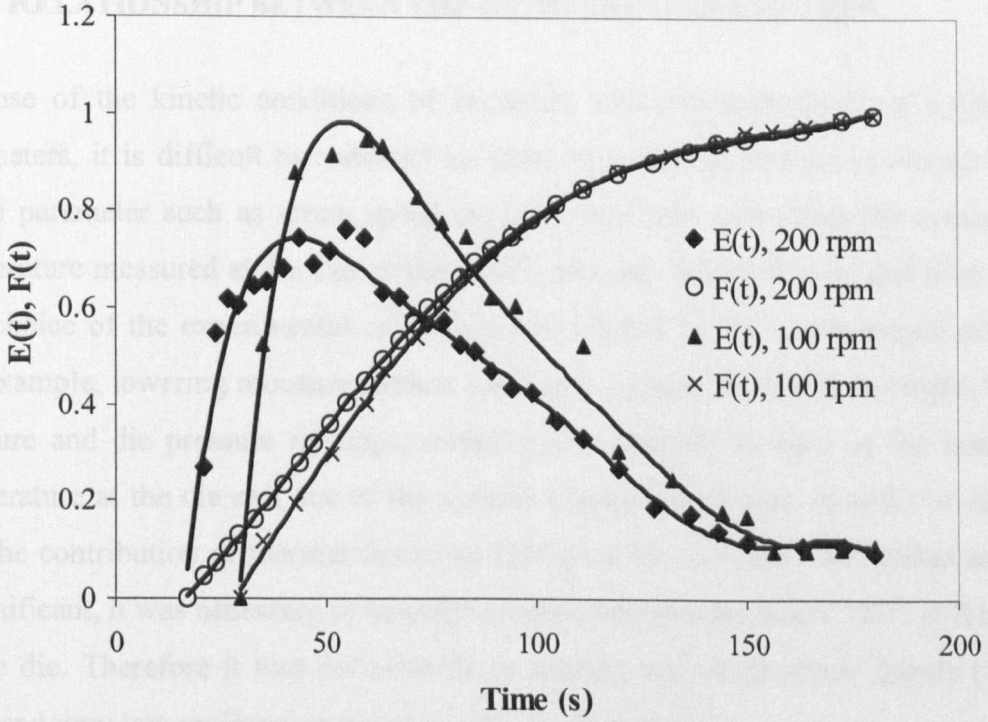

Figure 4.2 E(t) and F(t) plots of spores of *B. subtilis* in maize grits carrier determined using a marker dye. Extrusion parameters; indicated screw speeds, 4 kg/h feed rate, 0.533 kg/h water feed rate.

Table 4.1 Mean and minimum residence times of maize grits inoculated with *M. lacticum* and spores of *B. subtilis* PS346 and PS361 extruded at different screw speeds in a Clextral BC 21 Extruder.

Microorganism	Average moisture content (%)	Screw speed (rpm)	Minimum Residence Time (s)	Mean Residence Time (s)
M. lacticum	26.1	80	42	75
		120	32	69
		160	22	62
B. subtilis	23.6	100	30	77
		200	17	64

4.2 RELATIONSHIP BETWEEN THE EXTRUSION PARAMETERS

Because of the kinetic conditions of extrusion and interdependence of extrusion parameters, it is difficult to construct an ideal experimental design. A change in a single parameter such as screw speed or water feed rate will affect the maximum temperature measured at the exit of die, SME, pressure behind the die and flow rate. The choice of the experimental conditions was limited by the experimental set up. For example, lowering moisture content can cause excessive increase in torque, back pressure and die pressure readings, resulting in a marked increase in the material temperature at the die exit due to the viscous dissipation energy. In order to ensure that the contribution of thermal forces on killing of M. *lacticum* was limited and/or insignificant, it was necessary to keep the product temperature below 75°C at the exit of the die. Therefore it was not possible to employ very high screw speeds (>300 rpm) and very low moisture contents (< 23% in the barrel).

An understanding of relationships between different extrusion parameters would help to evaluate the results of extrusion experiments. The strong relationships between the interdependent extrusion variables also helped to correct an experimental error in die pressure readings.

4.2.1 Correction of the error in the die pressure readings

As explained in section 2.1.1, a Dynisco ER478 pressure transducer fitted to the die entrance was used for the die pressure measurements. There was an error in die pressure readings for a set of experiments (trial 3). This error showed up when torques were plotted against die pressures as shown in Figure 4.3 (a). The pressure readings for trial 3 were also not consistent with the back pressure readings of the extruder's built in transducer. Excluding the experimental points of trial 3, there was a positive linear correlation ($r^2 = 0.91$) between the die pressure and torque readings of Clextral MEMOSYS computer which was an integral part of the extrusion. This linear relationship was used to correct the die pressure readings for trial 3. Figure 4.3 (b) shows the relationship between the torque and die pressure after the correction.

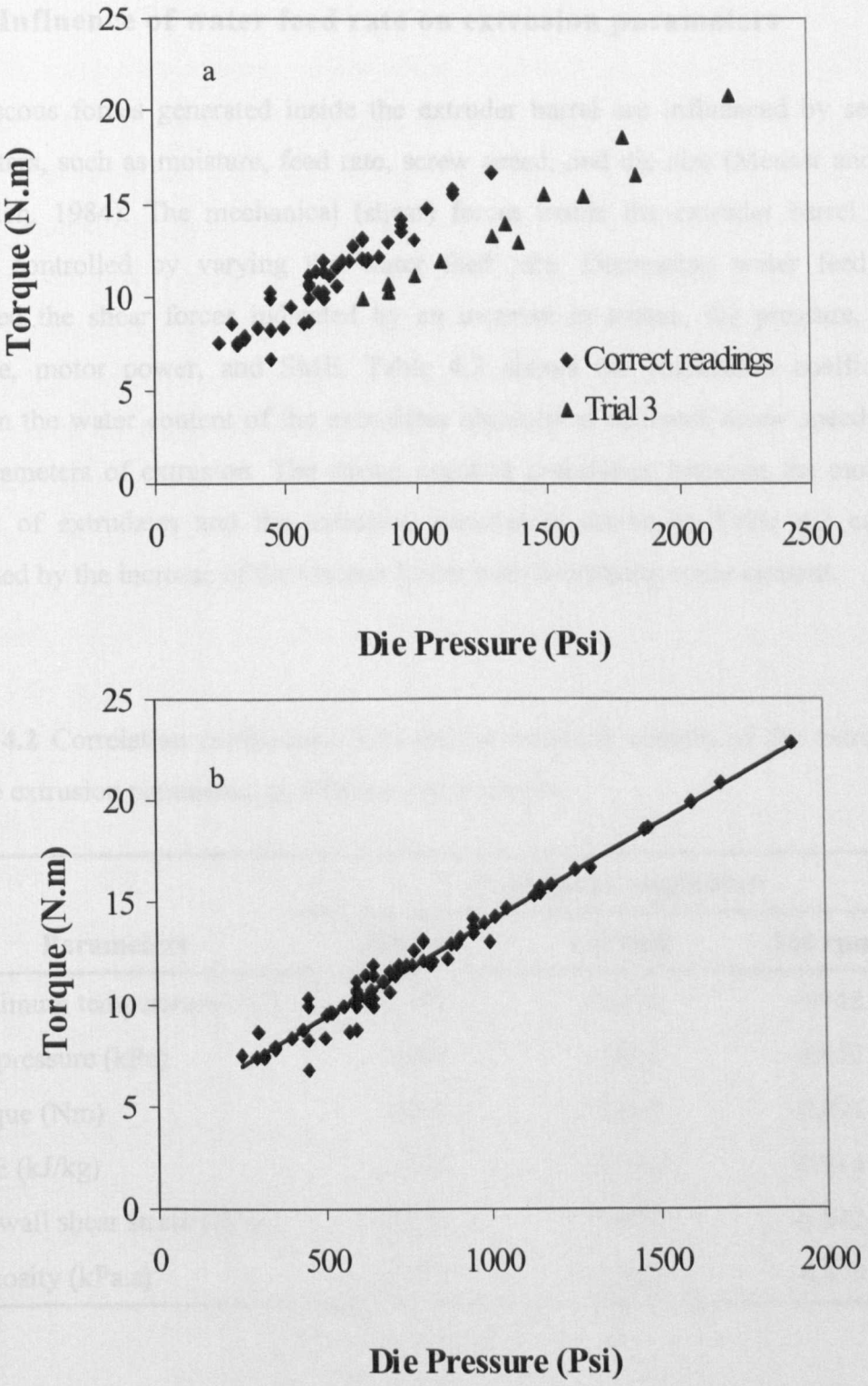

Figure 4.3 Correlation between torque and die pressure readings (a) showing the error in die pressure readings in trial 3, (b) after correction.

4.2.2 Influence of water feed rate on extrusion parameters

The viscous forces generated inside the extruder barrel are influenced by several parameters, such as moisture, feed rate, screw speed, and die size (Meuser and van Lengerich, 1984). The mechanical (shear) forces inside the extruder barrel were mainly controlled by varying the water feed rate. Decreasing water feed rate increased the shear forces indicated by an increase in torque, die pressure, back pressure, motor power, and SME. Table 4.2 shows the correlation coefficients between the water content of the extrudates obtained at different screw speeds and the parameters of extrusion. The strong negative correlation between the moisture content of extrudates and the extrusion parameters shown in Table 4.2 can be explained by the increase of the viscous forces with decreasing water content.

Table 4.2 Correlation coefficients between the moisture content of the extrudates and the extrusion parameters at different screw speeds.

	Correlation coefficients		
Parameters	**80 rpm**	**120 rpm**	**160 rpm**
Maximum temperature (°C)	-0.797	-0.962	-0.942
Die pressure (kPa)	-0.870	-0.892	-0.842
Torque (Nm)	-0.878	-0.867	-0.891
SME (kJ/kg)	-0.874	-0.910	-0.914
Die wall shear stress (kPa)	-0.870	-0.892	-0.842
Viscosity (kPa.s)	-0.895	-0.927	-0.873

4.2.3 Relationship between the shear stress and SME

SME is an important parameter for evaluation of thermo-mechanical treatment of the material during extrusion. Degradation of starch granules during extrusion is a well-known phenomenon and its link with thermo-mechanical treatment as expressed by SME has been reported by many researchers (Meuser and van Lengerich, 1984,

Davidson *et al.*, 1984, Vergnes *et al.*, 1987, Della Valle, 1995, Willett *et al.*, 1996). The SME parameter combines screw speed, total feed and torque in one equation (Equation 2.1). Among these parameters, the screw speed is related to the shear rate inside the extruder barrel whereas motor torque provides a direct indication of the energy absorbed by the material due to the shear exerted by the extruder screw and die orifice (Fichtali *et al.*, 1995). The design of the die is important in controlling the extruder performance and extrudate quality (Akdogan, 1996, Bouzaza, 1996). The size and the length of the extruder die rules the pressure build up behind the die, which in turn, influences the shear stress generated in the extruder barrel and the die. The shear stress calculated from the pressure drop across the die is an important parameter that indicates the severity of shear forces exerted on the material while it passes thorough the extruder die. Meuser and van Lengerich (1984) showed a relationship between die hole diameter, product moisture content, temperature and SME. They suggested that by manipulating one of these variables within certain limits, a desired level of SME can be obtained.

Figure 4.4 shows the change in die wall shear stress and SME with the variation of the moisture content of extrudates. Increased screw speed increases the SME while reducing the shear stress. Similar relationship can be seen in Figure 4.5. The decrease of shear stress with increasing screw speed can be explained by the reduced degree of fill in the extruder barrel (constant feed rate), which in turn reduced the pressure behind the die. In addition, increasing the screw speed increases the temperature of the material and thus reduces the viscosity. As a result the torque and the pressure generated at the die decreases. Davidson (1992) showed a similar correlation between SME and shear stress measured for extruded starch with a capillary viscometer.

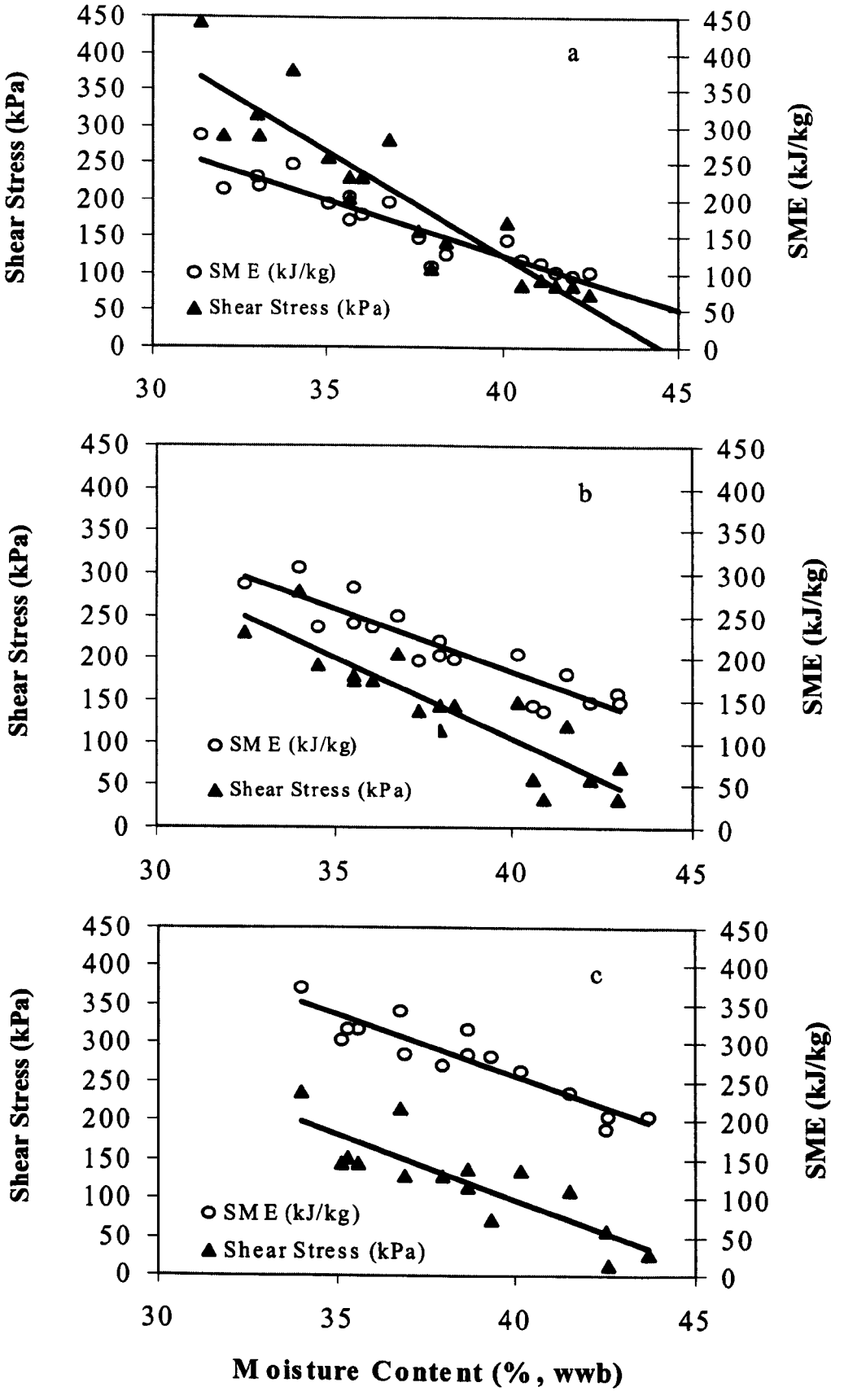

Figure 4.4 Variation of SME and die wall shear stress with the moisture content of extrudates at (a) 80 rpm, (b) 120 rpm and (c) 160 rpm screw speeds of extrusion.

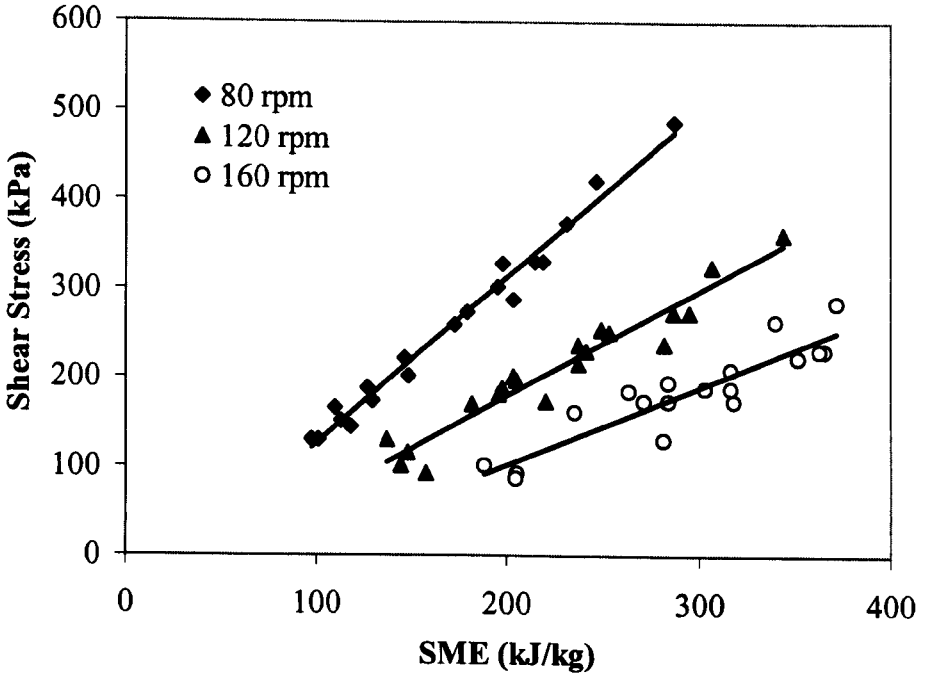

Figure 4.5 Relationship between SME and die wall shear stress at different screw speeds.

4.3 RHEOLOGICAL CHARACTERIZATION OF MAIZE GRITS EXTRUDATES

The control of the quality of end products and the choice of the optimum operation conditions require a better knowledge of the rheological properties of the material being processed (Della Valle *et al.*, 1996). There has been substantial research about the viscous properties of molten starch during extrusion (Harper, 1981, Vergnes and Villemaire, 1987, Senouci and Smith, 1988, Ollett *et al.*, 1990, Davidson, 1992, Battacharya and Padmanabhan, 1992, Altomare at *et al.*, 1992, Della Valle *et al.*, 1996), although the viscous behaviour of maize grits at the temperatures used in this work have not been extensively studied. Ollett *et al.* (1990) reported that extrusion of maize grits in the temperature range of 90-150°C totally disrupted the starch granules when the SME was larger than 115 kWh/t (414 kJ/kg). Della Valle *et al.* (1996) showed that during the extrusion of potato starch at temperatures between 120-210°C (temperature before the die) the potato starch was in a molten state beyond a critical SME of 120-150 kWh/t (432 kJ/kg-540 kJ/kg). In this study, DSC results showed that the crystalline structure of the maize grits was not completely destroyed (Figure

4.12) when the maize grits was extruded at high moisture contents (31-44%) and low temperatures (75°C maximum temperature at the exit of die for *M. lacticum*). Since there is a close relationship between the molecular integrity of the material and the viscous behaviour, the applicability of the proposed models for the estimation of the viscosity of maize grits under the conditions of our experiments will be discussed.

Viscous behaviour of dough and starch melt during extrusion obeys a power law model (Harper, 1981, Vergnes and Villemaire, 1987, Senouci and Smith, 1988, Ollett *et al.,* 1990, Davidson, 1992, Battacharya and Padmanabhan, 1992, Altomare *et al.*, 1992, Della Valle *et al.*, 1996) of type:

$$\eta = K \cdot \dot{\gamma}_w^{n-1} \qquad \textbf{Equation 4.1}$$

where η is the apparent viscosity, K is the consistency index, $\dot{\gamma}_w$ is the die wall shear rate and n is the flow behaviour index.

The wall shear stress (σ_w) and the apparent shear rate ($\dot{\gamma}_{app}$) were calculated according to the Equations 2.8.-2.9. The Rabinowitsch correction (Equation 2.10) was applied to obtain the true wall shear rate. The values of flow behaviour index (n) were calculated according to models proposed by Vergnes and Villemaire, (1987) and Della Valle *et al.* (1996) as discussed below.

The flow behaviour of maize grits was determined in a Rosand RH-7 piston capillary rheometer and the result is shown in Figure 4.6. Piston driven capillary rheometers are often used to characterize the flow of materials inside the extruders. However, as discussed in Chapter 3, the determination of the rheological properties of biological (food) materials in this way is questioned because of the change in the molecular structure of the food materials as a result of the mixing and the shearing action of the screws in the extruder barrel (Altomare *et al.*, 1992). Because of this problem some attempt has been made to determine the online measurement of the flow properties of the material during extrusion (Altomare *et al.*, 1992, Battacharya and Padmanabhan, 1992, Della Valle *et al.*, 1996). Based on their online measurements, Vergnes and Villemaire, (1987) and Della Valle *et al.* (1996) proposed the following model for the estimation of flow behaviour index for maize starch.

$$n = n_o + \alpha_1 T + \alpha_2 X + \alpha_3 SME + \alpha_{12} TX + \alpha_{13} T \cdot SME + \alpha_{23} X \cdot SME \qquad \textbf{Equation 4.2}$$

where, T is the temperature (°C), X is the moisture content between 0-1.0, and SME is in kWh/ton. n_0, and α_1 to α_{23}, were calculated by multilinear regression and the values are reported in Table 4.3.

The same researchers suggested the following model for the estimation of the consistency index for maize starch.

$$K = K_0 \exp\left[\frac{E}{R}\frac{1}{T_a} - \alpha(X) - \beta(SME)\right] \qquad \textbf{Equation 4.3}$$

where T_a is the absolute temperature of the melt (K). The values of the coefficients K_0, β, E/R, and α are given in Table 4.3.

Table 4.3 Values of the coefficients in the rheological models for molten waxy maize starch.

Model	Della Valle *et al.* (1996)	Vergnes and Villemaire (1987)
n_0	-1.02	0
α	26.1	10.6
α_1 (°C)$^{-1}$	7.2×10^{-3}	6.54×10^{-4}
α_2	2.54	0.112
α_3 (kWh/t)$^{-1}$	5.6×10^{-4}	-
α_{12} (°C)$^{-1}$	Ns	7.28×10^{-3}
α_{13} (°CkWh/t)$^{-1}$	Ns	-
α_{23} (kWh/t)$^{-1}$	Ns	-
β (kWh/t)$^{-1}$	5.5×10^{-3}	0.89×10^{-9} [a]
K_0 (Pa.s^n)	1.36×10^{-4}	7.36
E/R (K)	9700	4250

[a] The unit was given as $(J/m^3)^{-1}$ for model used by Vergnes and Villemaire (1987).
ns: no significant effect at the 5% confidence interval.

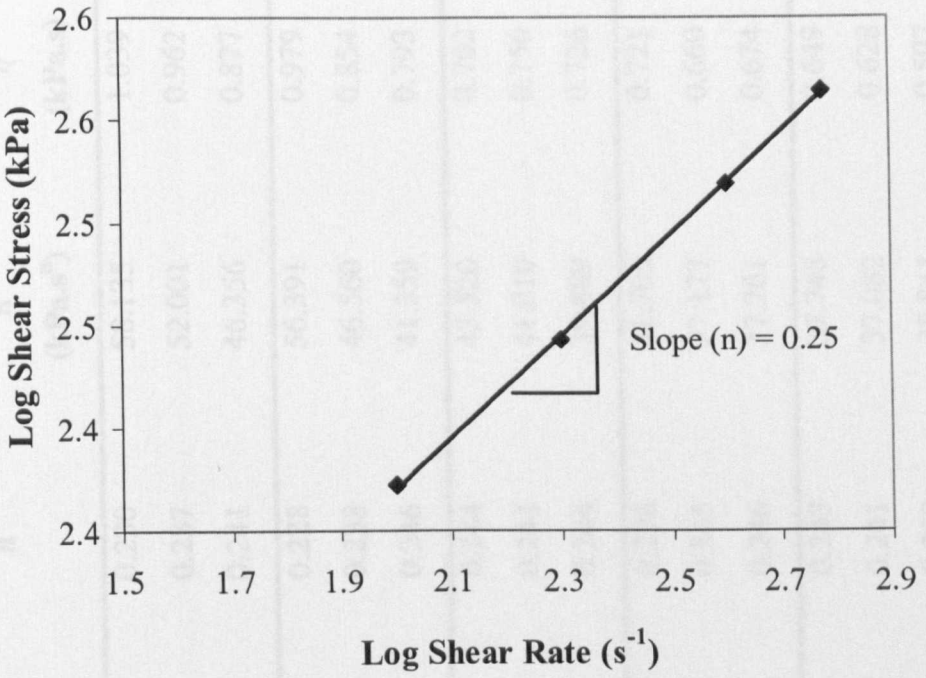

Figure 4.6 Flow behaviour index of maize grits determined using a Rosand RH-7 piston capillary rheometer. Moisture content 30%, temperature at the die 88°C.

Due to the unavailability of an online rheological measurement, the flow behaviour index of maize grits during extrusion was approximated by the models suggested by Vergnes and Villemaire (1987) and Della Valle *et al.* (1996). Tables 4.4 and 4.5 show the values of parameters for the rheological characterisation of maize grits estimated according to the above models. The average values of extrusion parameters for these experiments are given in Table 4.6. Analysis of variance (ANOVA) revealed that the flow behaviour index calculated according to coefficients proposed by Vergnes and Villemaire (average = 0.253, n=58, $P<0.05$) was significantly lower than the flow behaviour index calculated based on the coefficients suggested by Della Valle *et al.* (1996) (average = 0.419). The flow behaviour indices estimated based on Vergnes and Villemaire's model were in the range of 0.24 to 0.27 showing only a minor increase with increasing screw speed, and the increase of extrudate moisture content did not have significant effect on the flow behaviour index. The flow behaviour indices estimated according to the model suggested by Della Valle *et al.* were in the range of 0.34-0.48 and increased with increasing screw speed and extrudate moisture content.

Table 4.4 Estimation of the viscosity of maize grit during low temperature extrusion at different screw speeds and moisture contents according to model proposed by Vergnes and Villemaire (1987). For extrusion parameters see Table.4.6.

Screw speed (rpm)	Water feed rate (kg/h)	Average MC of extrudate (%)	σ_w (kPa)	$\dot{\gamma}_w$ (s^{-1})	n	K ($kPa.s^n$)	η (kPa.s)
80	0.355	31.0	380.5	186.3	0.230	58.135	1.039
120	0.355	32.5	289.4	186.3	0.237	52.001	0.962
160	0.355	32.8	227.1	186.5	0.241	46.356	0.877
80	0.533	35.2	308.5	190.2	0.228	56.391	0.979
120	0.533	35.0	248.8	190.2	0.238	46.560	0.854
160	0.533	35.2	216.4	190.2	0.246	41.359	0.793
80	0.710	37.3	210.9	200.7	0.244	43.520	0.762
120	0.710	37.7	199.1	199.6	0.244	41.010	0.750
160	0.710	37.8	194.8	199.7	0.244	39.889	0.726
80	0.888	40.6	164.6	205.8	0.238	41.709	0.721
120	0.888	42.1	129.8	206.1	0.245	37.177	0.660
160	0.888	40.5	157.0	205.2	0.246	37.261	0.674
80	1.065	41.5	187.7	214.3	0.243	37.748	0.649
120	1.065	42.5	132.3	216.9	0.241	37.082	0.628
160	1.065	43.5	109.9	217.3	0.239	35.841	0.597

Table 4.5 Estimation of the viscosity of maize grit during low temperature extrusion at different screw speeds and moisture contents according to model proposed by Della valle *et al.*, 1996. For extrusion parameters see Table.4.6.

Screw speed (rpm)	Water feed rate (kg/h)	Average MC of extrudate (%)	σ_w (kPa)	$\dot{\gamma}_w$ (s^{-1})	n	K ($kPa.s^n$)	η (kPa.s)
80	0.355	31.0	380.5	186.3	0.303	10.968	1.145
120	0.355	32.5	289.4	186.3	0.326	10.858	0.991
160	0.355	32.8	227.1	186.5	0.352	10.743	0.854
80	0.533	35.2	308.5	190.2	0.328	10.938	1.121
120	0.533	35.0	248.8	190.2	0.359	10.747	0.841
160	0.533	35.2	216.4	190.2	0.389	10.630	0.743
80	0.710	37.3	210.9	200.7	0.411	10.616	0.774
120	0.710	37.7	199.1	199.6	0.399	10.621	0.708
160	0.710	37.8	194.8	199.7	0.414	10.586	0.712
80	0.888	40.6	164.6	205.8	0.416	10.638	0.726
120	0.888	42.1	129.8	206.1	0.445	10.513	0.626
160	0.888	40.5	157.0	205.2	0.442	10.525	0.661
80	1.065	41.5	187.7	214.3	0.441	10.536	0.625
120	1.065	42.5	132.3	216.9	0.446	10.520	0.616
160	1.065	43.5	109.9	217.3	0.461	10.482	0.589

Table 4.6 Average values of the main extrusion parameters for experiments on the extrusion of maize grits inoculated with *M. lacticum*.

Screw Speed (rpm)	Water Feed (kg/h)	Feed (kg/h)	Moisture Content (%,wwb)	Maximum Temp. (°C)	Die Temp. (°C)	Die Pressure (kPa)	Torque (Nm)	SME (kj/kg)
80	0.355	4.0	32.4	69.6	59.5	9125.7	17.2	238.1
120	0.355	4.0	32.2	73.0	60.8	6941.0	14.2	294.8
160	0.355	4.0	32.7	74.9	60.0	5446.9	13.0	360.1
80	0.533	3.9	35.3	62.0	54.8	7398.3	14.6	199.8
120	0.533	3.9	35.1	67.5	56.2	5966.5	12.7	261.1
160	0.533	4.0	35.0	70.7	58.0	5190.0	12.3	327.1
80	0.710	4.0	37.7	58.7	54.3	5286.9	11.4	145.9
120	0.710	3.9	37.7	63.6	55.4	4773.7	10.8	213.5
160	0.710	3.9	37.8	64.2	54.4	4672.0	11.4	299.6
80	0.888	3.8	40.8	54.8	51.3	3864.9	9.5	119.8
120	0.888	3.8	41.2	56.6	52.0	3111.6	8.8	169.2
160	0.888	3.7	39.7	60.8	53.0	3765.3	10.3	272.7
80	1.065	3.6	43.5	56.3	53.3	2987.7	8.1	109.8
120	1.065	3.8	41.9	54.7	50.3	3171.8	8.4	153.9
160	1.065	3.8	42.6	53.9	48.3	2635.3	8.5	208.3

The flow behaviour indices estimated according to Vergnes and Villemaire's model were practically the same as the value we measured (0.25) by using the piston capillary rheometer (Figure 4.6). However, this comparison cannot be conclusive since there were only limited data from the capillary rheometer.

Figure 4.7 shows the change in the viscosity of maize grits as measured in the extruder die with increasing screw speed and moisture content. Decrease of die wall viscosity with increasing screw speed can be observed more markedly at low water feed rates of 0.355 kg/h and 0.533 kg/h where the shear forces would be expected to have greater effect on degradation of starch granules. A similar phenomenon can be observed from Figure 4.8 where the die wall viscosities are plotted against water feed rate for extrusion at different screw speeds. The decrease in viscosity with the increase of moisture content has already been reported (Colonna *et al.*, 1989, Harper, 1981, Altomare *et al.*, 1992, Della Valle *et al.*, 1996) and is in agreement with the expected role of water as a plasticizer. It could be expected that the material would have lower viscosities at low screw speeds due to the relatively longer residence times. However, at high screw speeds the material experiences higher shear rates inside the extruder barrel. According to Figure 4.7-8, it can be suggested that the relative effect of higher shear rates is more influential on degradation of the maize grits than the effect of higher (7-13 s, in terms of mean residence time) reactions times at low screw speeds. The reduction of viscosity with increasing shear rate was reported by Della Valle *et al.* (1996) for potato and maize starch, and by Altomare *et al.* (1992) for rice starch.

The viscosity difference for the different screw speeds is marked at low moisture contents whereas at high moisture contents the difference in viscosities due to extrusion screw speed is insignificant. The noticeable reduction in the viscosity of maize grits at high screw speeds (Figure 4.7-8) can be explained by the increased degradation of starch granules due to high shear forces. As discussed in Section 1.3 it is well established that substantial depolymerisation (degradation) of starch takes place in the extruder due to high shear forces. Reduction in the viscosity due to macromolecular degradation during the extrusion of different starches has been reported by many authors (Vergnes and Villemaire, 1987, Ollett *et al.*, 1990,

Battacharya and Padmanabhan, 1992, Vergnes *et al.*, 1993 and Dalle Valle *et al.*, 1996).

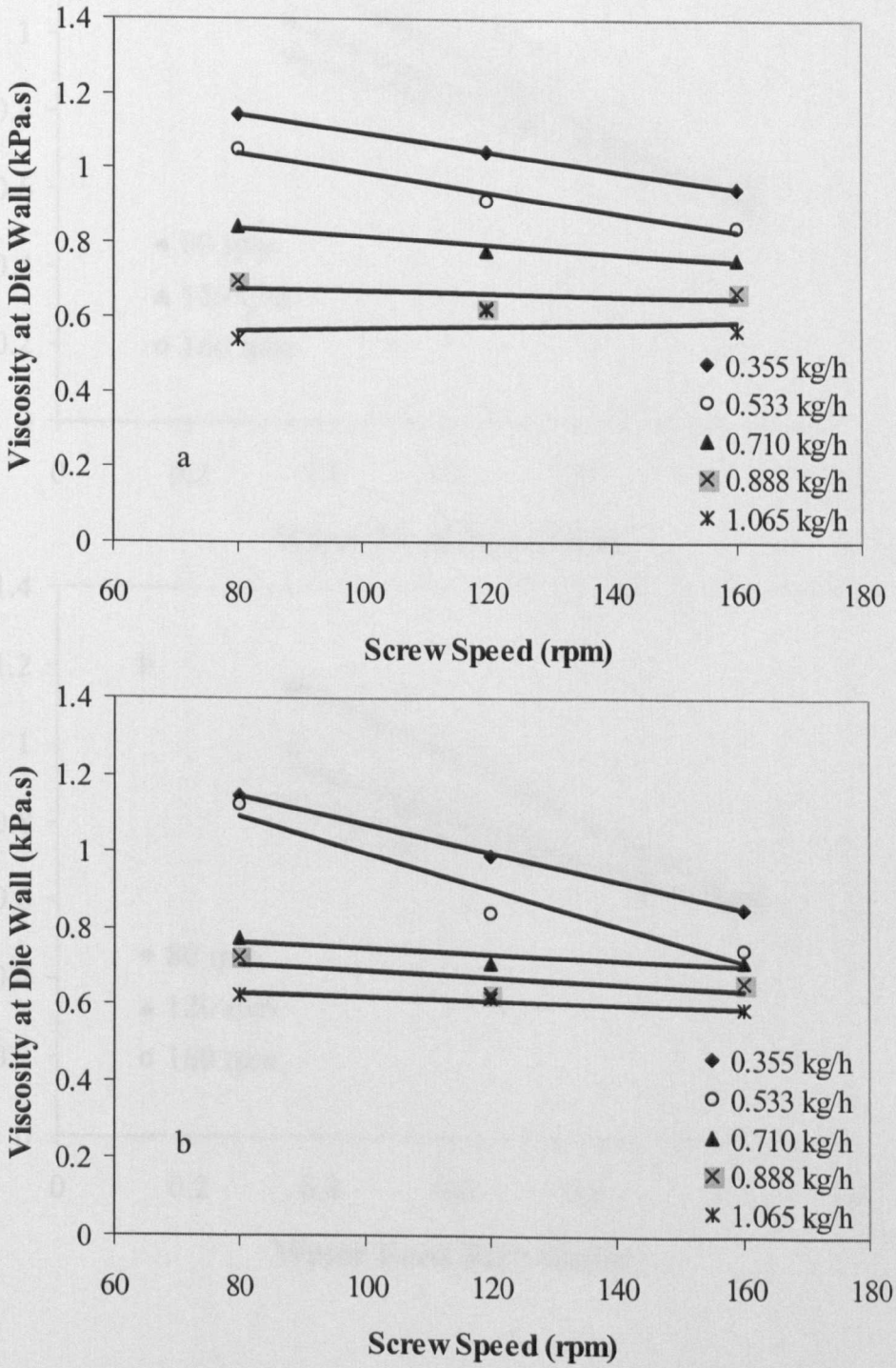

Figure 4.7 Change in the viscosity of maize grits as measured in the extruder die with the change in screw speed and moisture content as given by the water feed rates. (a) Vergnes and Villemaire's model (1987), (b) model of Della Valle *et al.*, 1996. For the extrusion parameters see Table 4.6.

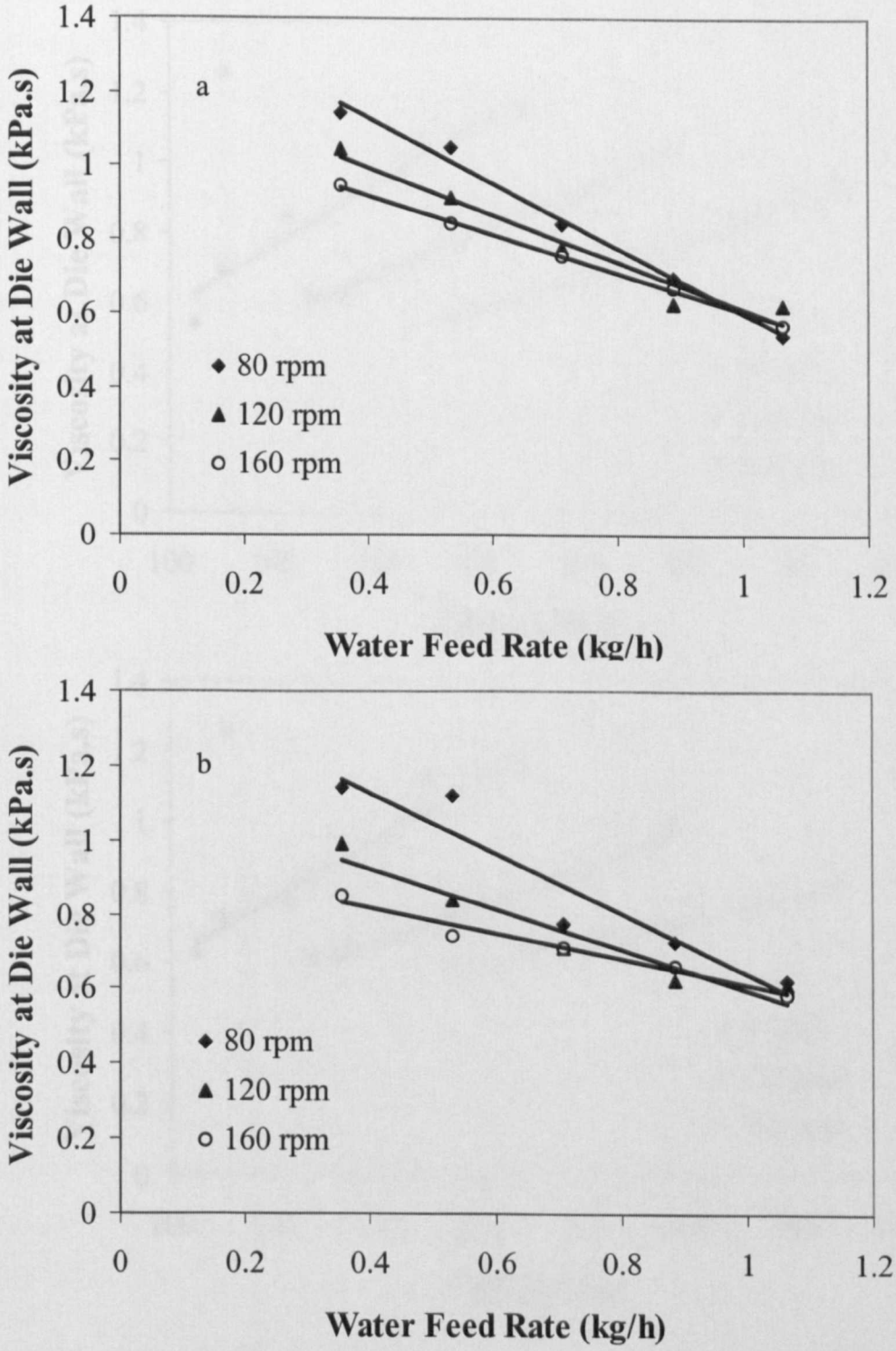

Figure 4.8 Relationship between the water feed rate of extrusion and the viscosity of maize grits calculated according to (a) Vergnes and Villemaire's model (1987) (b) model of Della Valle *et al.,* 1996. For the extrusion parameters see Table 4.6.

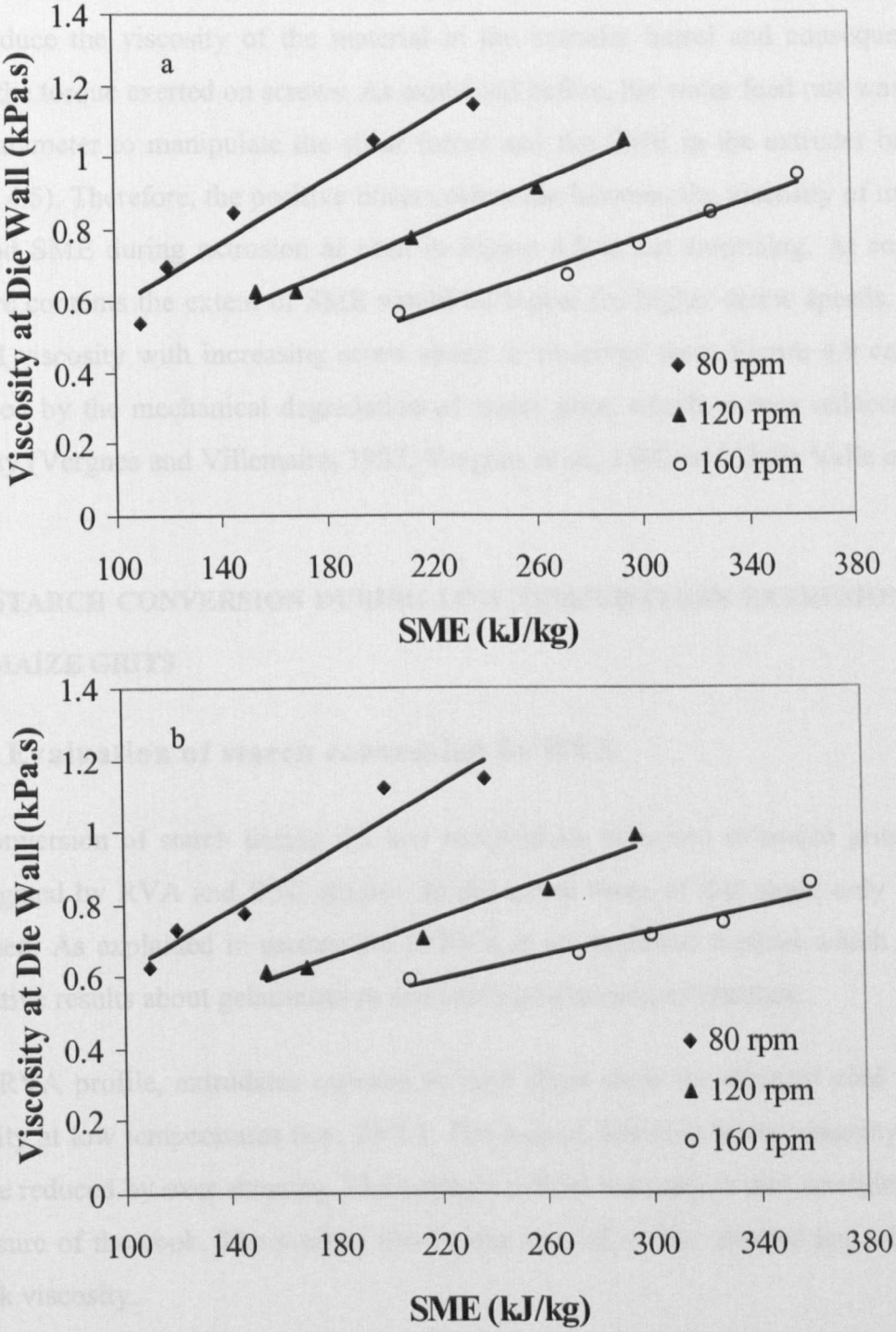

Figure 4.9 Relationship between the SME and the viscosity of maize grits calculated according to (a) Vergnes and Villemaire's model (1987) (b) model of Della Valle *et al.*, 1996. For the extrusion parameters see Table 4.6.

SME is a function of torque, screw speed, and the total feed rate. High water feed rates reduce the viscosity of the material in the extruder barrel and consequently reduce the torque exerted on screws. As explained before, the water feed rate was the main parameter to manipulate the shear forces and the SME in the extruder barrel (Figure 4.5). Therefore, the positive linear correlation between the viscosity of maize grits and SME during extrusion as seen in Figure 4.9 is not surprising. At certain moisture contents the extent of SME would be higher for higher screw speeds. The reduced viscosity with increasing screw speed as observed from Figure 4.9 can be explained by the mechanical degradation of maize grits, which in turn reduces the viscosity (Vergnes and Villemaire, 1987, Vergnes *et al.*, 1993 and Della Valle *et al.*, 1996).

4.4 STARCH CONVERSION DURING LOW TEMPERATURE EXTRUSION OF MAIZE GRITS

4.4.1 Evaluation of starch conversion by RVA

The conversion of starch during the low temperature extrusion of maize grits was investigated by RVA and DSC studies. In the initial stage of this study only RVA was used. As explained in section 2.6.1, RVA is an empirical method which gives qualitative results about gelatinisation and pasting behaviour of starches.

In an RVA profile, extrudates exposed to high shear show an elevated cold water viscosity at low temperatures (i.e., 25°C). The area of this cold water viscosity peak may be reduced by over shearing. The setback or final viscosity is also considered as a measure of the cook. The product that is less cooked or less sheared has a higher setback viscosity.

The RVA profile for maize grits extruded with *B. subtilis* PS361 and *M. lacticum* at different screw speeds and moisture contents are given in Figure 4.10 and Figure 4.11, respectively. The condition of extrusion was more severe for the *Bacillus subtilis* than the conditions for *M. lacticum*. During the extrusion of *B. subtilis*, the SME was in the range of 210- 845 $kJ \cdot kg^{-1}$ and the maximum temperature at the exit of die was in the range of 46-96°C, whereas for *M. lacticum*, these values were 102-

398 $kJ \cdot kg^{-1}$ and 49-74.9°C, respectively. The higher mechanical energy input and high shear forces created by high screw speeds and low moisture contents, resulted in more starch conversion as indicated by increased cold viscosity and reduced setback viscosity. The native maize starch had higher peak viscosity since the starch was not converted. Extrusion of *B. subtilis* PS361 in high shear and SME environment resulted in an increase in cold viscosity of maize starch as can be seen from Figure 4.10. There was no observable increase in cold viscosity in the RVA profile of extruded maize starch inoculated with *M. lacticum*. For all the extruded samples, the overall viscosity profile was lower than the viscosity profile of raw control, indicating that the starch was substantially cooked (converted) during extrusion at low temperatures.

The difference in setback viscosities were less marked for the extruded samples of maize grits inoculated with *M. lacticum* than for the extrudates of maize grits inoculated with *B. subtilis* PS361, because of the extent of mechanical and shear energies involved during extrusion. There was no observable peak viscosity for the extrudate that experienced 845 $kJ \cdot kg^{-1}$ SME during extrusion of *B. subtilis* PS361, indicating that the maize starch was completely converted.

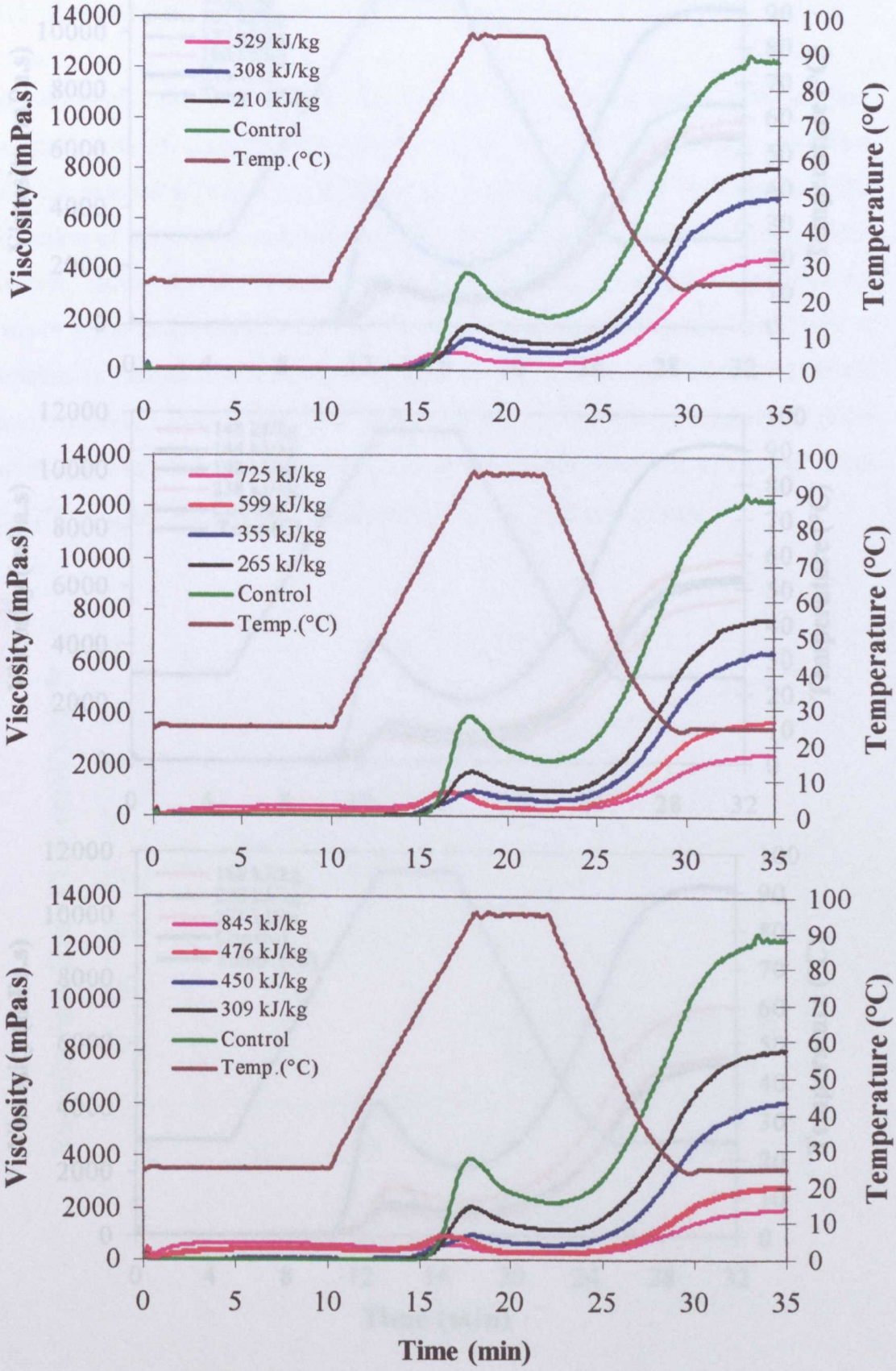

Figure 4.10 The RVA profile for maize starch following the extrusion with *B. subtilis* PS361 at different moisture contents and screw speeds (a) 100 rpm, (b) 150 rpm and (c) 200 rpm. The labels show SME in kJ/kg.

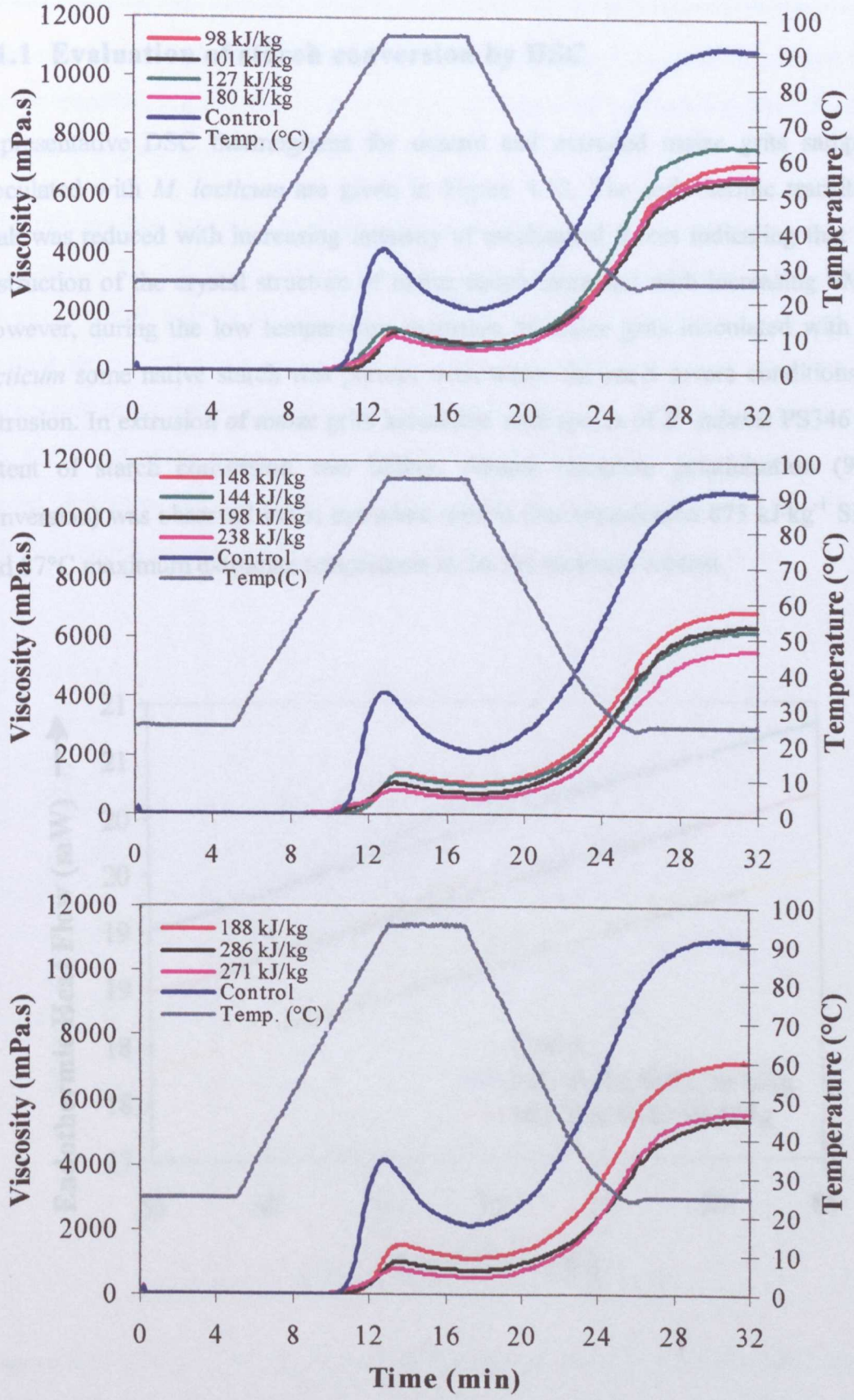

Figure4.11 The RVA profile for maize starch following the extrusion with *M. lacticum* at different moisture contents and screw speeds (a) 80 rpm, (b) 120 rpm and (c) 160 rpm. The labels show SME in kJ/kg.

4.1.1 Evaluation of starch conversion by DSC

Representative DSC thermograms for control and extruded maize grits samples inoculated with *M. lacticum* are given in Figure 4.12. The endothermic transition peak was reduced with increasing intensity of mechanical forces indicating that the destruction of the crystal structure of maize starch increased with increasing SME. However, during the low temperature extrusion of maize grits inoculated with *M. lacticum* some native starch was present even under the most severe conditions of extrusion. In extrusion of maize grits inoculated with spores of *B. subtilis* PS346 the extent of starch conversion was higher. Almost complete gelatinisation (94% conversion) was observed in the extrudate sample that experienced 873 $kJ \cdot kg^{-1}$ SME and 87°C maximum extrudate temperature at 24.5% moisture content.

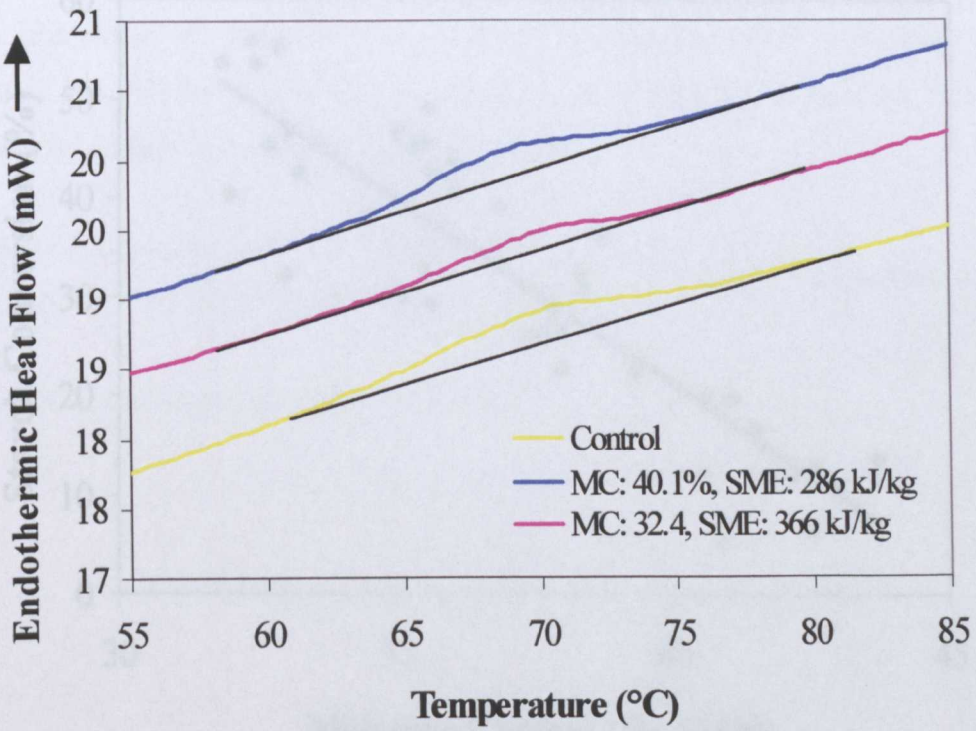

Figure 4.12 DSC thermograms of native and extruded waxy maize starch inoculated with *M. lacticum*. Screw speed; 160 rpm, die temperatures; 53-60°C, moisture contents and SME as shown in the legends.

Since the water feed rate was the key parameter to control the shear forces and SME in the extruder, degree of starch conversion increased with decreasing water feed rate as seen in Figure 4.13.

The relationship between the degree of starch conversion as calculated according to Equation 2.23 and SME during the extrusion of maize grits inoculated with *M. lacticum* and *B. subtilis* PS346 is shown in Figure 4.14. The Figure shows that for a certain degree of starch conversion, the amount of SME required increases with increasing screw speed. This can be explained by the opposite effects of screw speed on SME and shear stress. As explained previously (Figure 4.5), increasing screw speed increases the SME while reducing the shear stress. Therefore, the effect of SME on starch conversion should be considered together with shear stress.

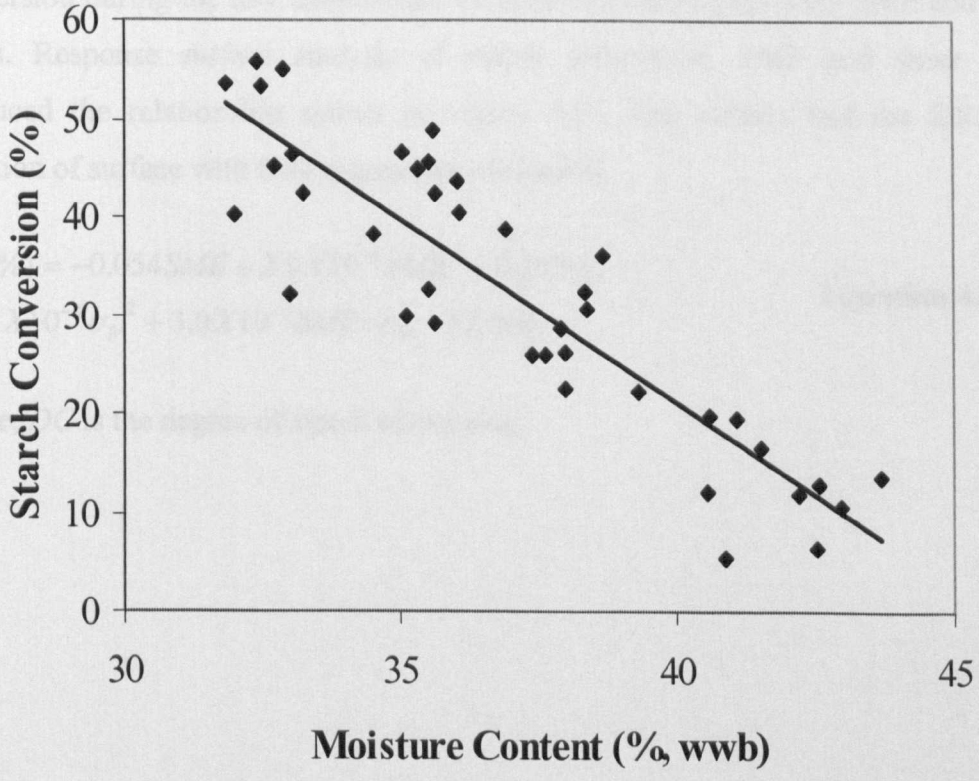

Figure 4.13 The relationship between the degree of starch conversion determined by DSC studies and the moisture content of maize grits extrudates inoculated with *M. lacticum*.

In order to understand the relative effects of thermal and shear energies on starch conversion, Figure 1.7 was extrapolated to estimate the effect of thermal energy alone on starch conversion. This extrapolation showed that 3 min exposure of starch with 30% moisture content to a temperature of 75°C resulted in only a 5.5% conversion. During the extrusion of maize grits inoculated with *M. lacticum* the temperature of the extruder barrel remained always below 60°C and the maximum temperature of the product at the die exit was below 75°C for all the experiments. Considering that the maximum residence time of maize grits inside the extruder barrel was les than 3 min, it can be concluded that the contribution of the thermal energy on starch conversion was negligible. Multilinear analysis performed on data also showed that t-observed value for temperature was smaller than t-critical ($1.04<1.69$, $\alpha=0.05$) indicating that the temperature was not a significant factor for estimation of starch conversion. The important parameters for predicting the starch conversion during the low temperature extrusion of maize grits were SME and shear stress. Response surface analysis of starch conversion, SME and shear stress produced the relationship shown in Figure 4.15. The surface had the following equation of surface with 0.84 regression coefficient.

$$DC(\%) = -0.054SME + 2.9X10^{-4}SME^2 + 0.355\sigma_W - 5.2X10^{-4}\sigma_W{}^2 + 3.0X10^{-5}SME \cdot \sigma_W - 15.969$$

Equation 4.4

where, DC is the degree of starch conversion.

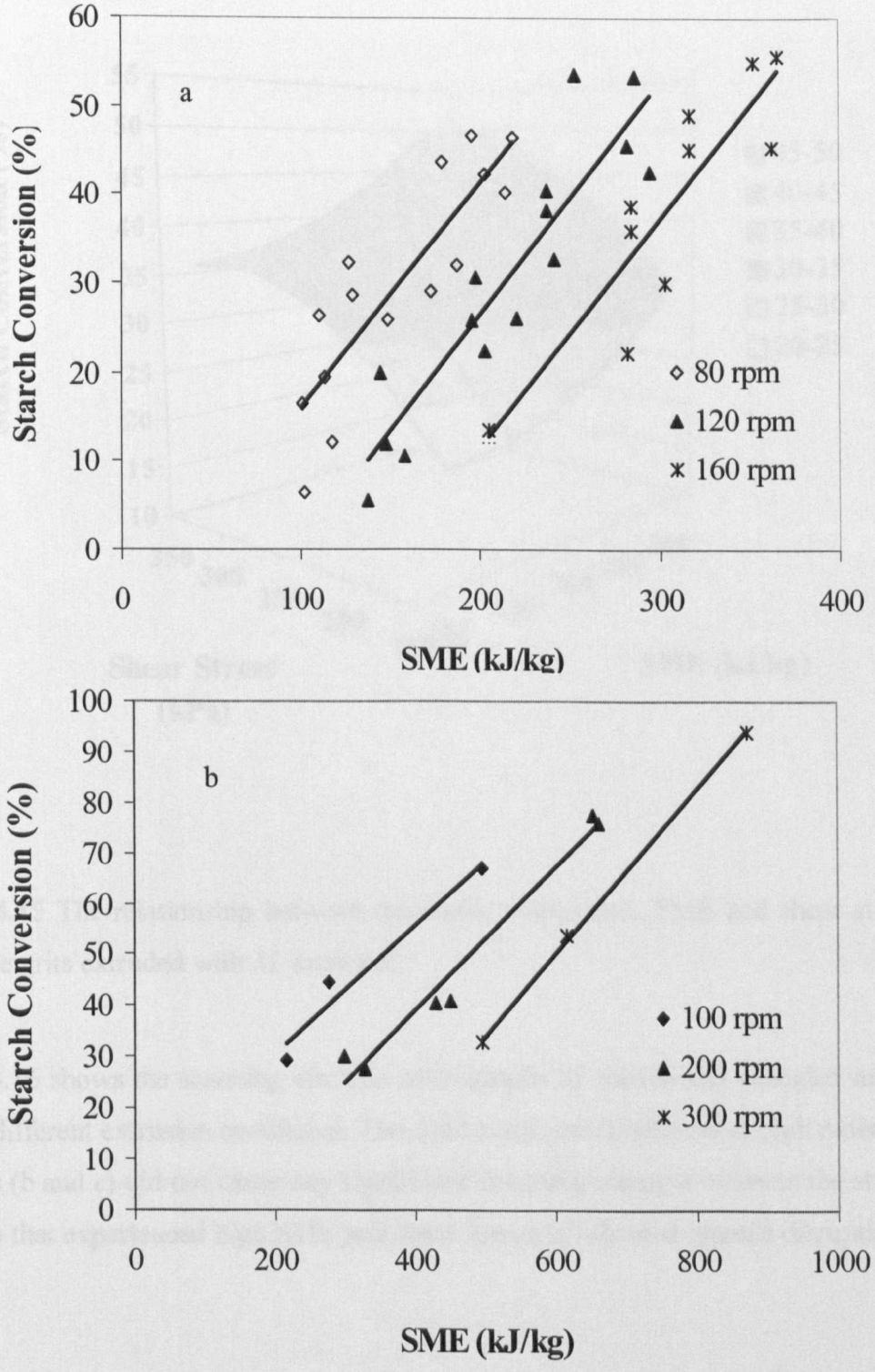

Figure 4.14 The relationship between the degree of starch conversion and SME during extrusion of (a) maize grits inoculated with *M. lacticum* (b) maize grits inoculated with spores of *B. subtilis* PS346.

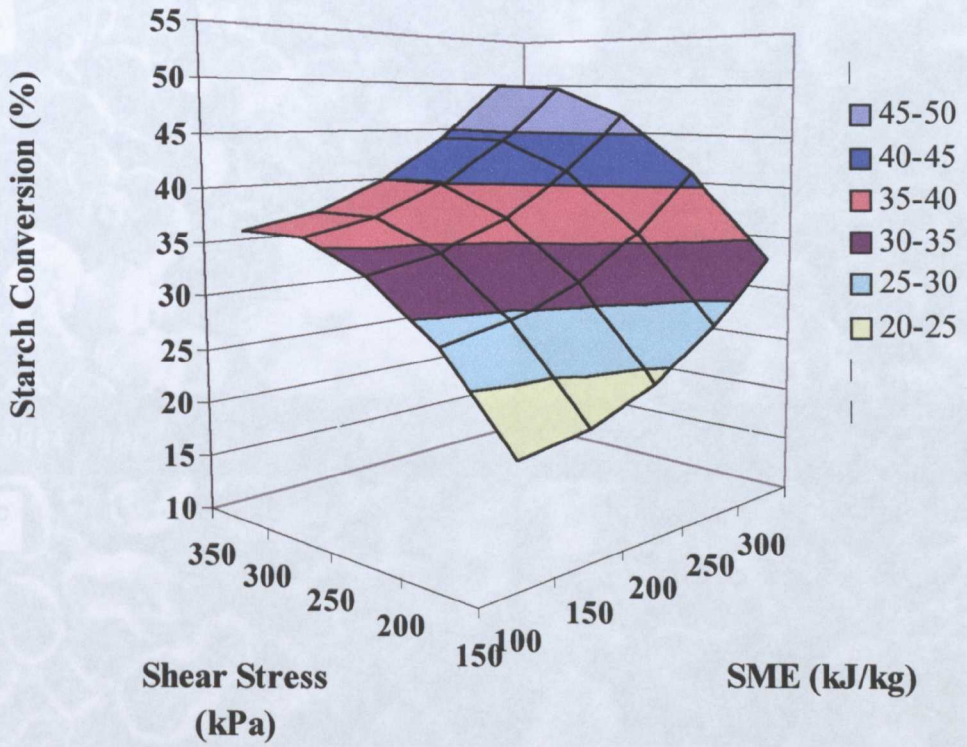

Figure 4.15 The relationship between the starch conversion, SME and shear stress for maize grits extruded with *M. lacticum.*

Figure 4.16 shows the scanning electron micrographs of native and extruded maize grits at different extrusion conditions. The mild extrusion conditions at high moisture contents (b and c) did not cause any significant structural changes whereas the starch granules that experienced high SME and shear forces (d) showed granule disruption.

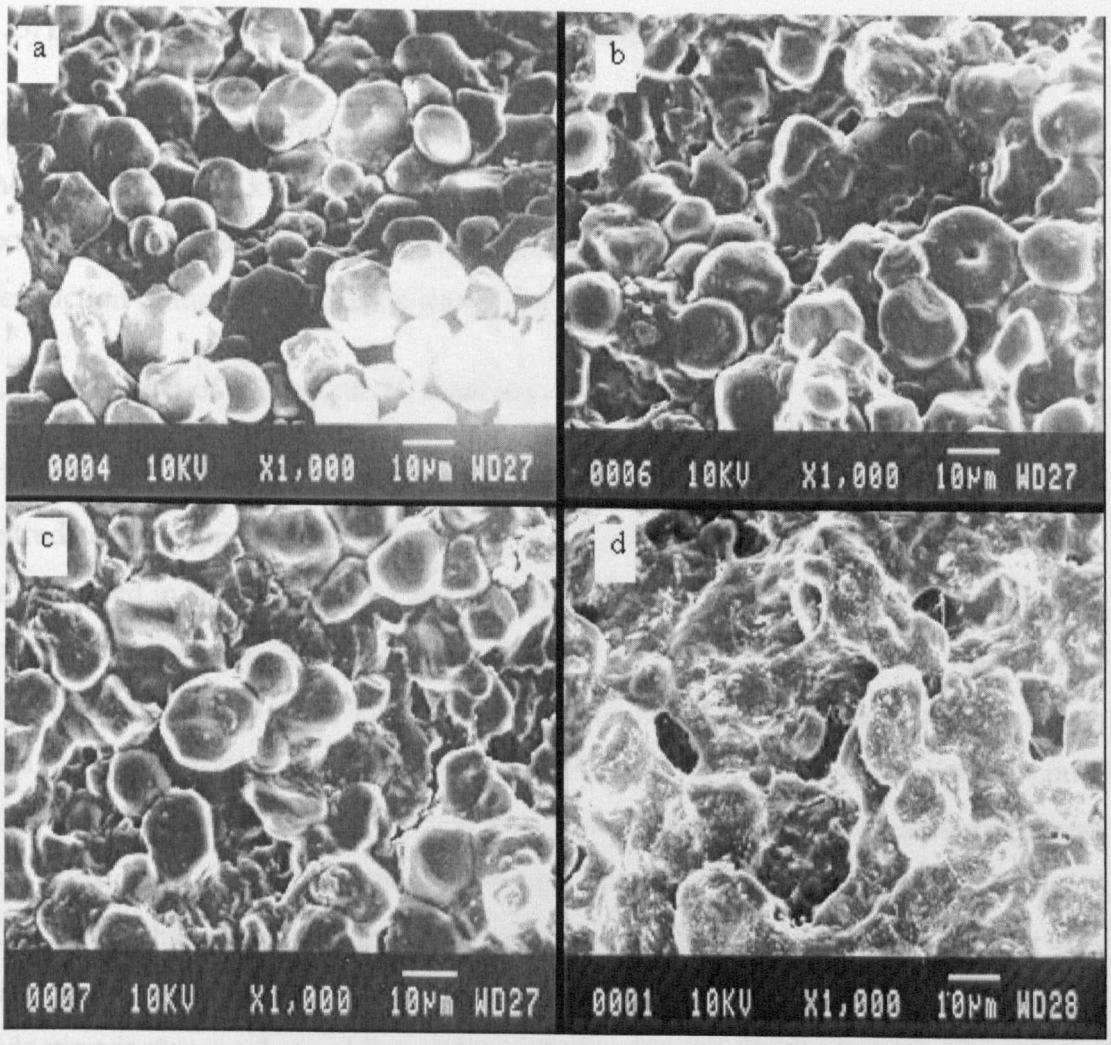

Figure 4.16 Scanning electron micrographs of maize grits extruded at different extrusion conditions. (a) Raw maize grits (control), (b) 80 rpm, 1.065 kg/h water feed rate, 53°C maximum temperature (c) 160 rpm, 1.065 kg/h water feed rate, 55°C maximum temperature, (d) 80 rpm, 0.355 kg/h water feed rate, 74°C maximum temperature.

4.5 MICROBIAL INACTIVATION DURING EXTRUSION

4.5.1 Inactivation of *M. lacticum* by extrusion

M. lacticum and spores of *B. subtilis* were used as test organisms in order to examine the mechanism of killing of spores and vegetative cells during low temperature extrusion of maize grits. Relative contribution of shear and thermal energies on destruction of the test organisms was of particular interest.

4.5.1.1 Effect of milling on *M. lacticum*

The recovery of *M. lacticum* from maize grits extrudates involved freezing of the extrudates by liquid nitrogen followed by grinding with Cyclotec and Knifetec mills as described in section 2.4.1.3.1 and 2.3.1.3.2. The effect of the freezing and grinding processes during the recovery of the microorganism was investigated. Figure 4.17 shows a reduction of about 0.6 logarithmic units after 10 s (5 x 2 s) of milling, which was the length of time employed for the grinding of the samples in the Knifetec mill. This reduction in the number of survivors due to the nature of the recovery process was considered when calculating the degree of kill in the extrusion process. Figure 4.17 shows that there is a rapid decrease in the number of survivors in the first 40 s of the milling process. Further increasing the milling time did not result in significant reduction in the numbers of the microorganism. This could be explained by the fact that the temperature of the frozen samples in the mill increased with increasing milling time until it reached a temperature where the samples were not frozen any more. The bacterial cell wall is supposed to be more fragile at freezing temperatures, making it more sensitive to the shear forces generated by the high-speed blade of the Knifetec. Wimpenny (1967) reported that large shearing forces could be obtained by mechanical agitation. In mechanical agitation, the shear gradients at the blade edge were considered as the product of the angular velocity of the blade and the radius of the curvature of the edge of the blade. Converti *et al.* (1996) reported the lethal effect of shear stress generated by mechanical mixing on *Saccharomyces cerevisiae* during fermentation of the dilute starch hydrolysate solution.

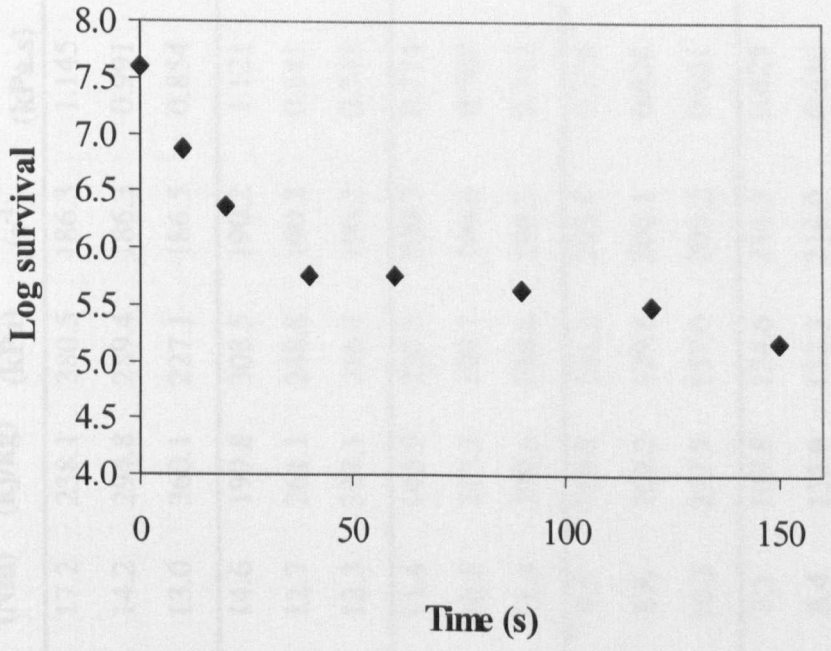

Figure 4.17 Effect of grinding time on survival of *M. lacticum* in frozen extrudate of maize grit extrudates in the Knifetec mill.

4.5.1.2 Investigation of the inactivation mechanism of *M. lacticum* during extrusion

As explained in Section 2.4.1 the experimental design employed 3-5 replications for each extrusion condition. Table 4.7 shows the average values of logarithmic reduction of *M. lacticum* and the parameters of the extrusion. The correlation between the logarithmic reduction and the extrusion parameters at different screw speed is given in Table 4.8. When the correlation is extended to include the parameters of all the screw speeds, the correlation coefficients for die pressure, torque, SME, and shear stress reduced notably. This is because these parameters are intrinsically related to screw speed. Therefore, the data are categorised according to screw speeds and analysed separately for each screw speed.

Table 4.7 Logaritmic reduction in the number of *M. lacticum* during the extrusion of maize grit and the parameters of extrusion. The values are the average of 3-5 replications.

Screw Speed (rpm)	Water Feed (kg/h)	Log Reduction	Feed (kg/h)	Moisture Content (%,wwb)	Maximum Temp. (°C)	Die Temp. (°C)	Die Pressure (kPa)	Torque (Nm)	SME (kj/kg)	σ_w (kPa)	$\dot{\gamma}_w$ (s^{-1})	$\eta_w{}^a$ (kPa.s)
80	0.355	3.61	4.0	32.4	69.6	59.5	9125.7	17.2	238.1	380.5	186.3	1.145
120	0.355	4.61*	4.0	32.2	73.0	60.8	6941.0	14.2	294.8	289.4	186.3	0.991
160	0.355	4.61*	4.0	32.7	74.9	60.0	5446.9	13.0	360.1	227.1	186.5	0.854
80	0.533	2.81	3.9	35.3	62.0	54.8	7398.3	14.6	199.8	308.5	190.2	1.121
120	0.533	3.35	3.9	35.1	67.5	56.2	5966.5	12.7	261.1	248.8	190.2	0.841
160	0.533	3.68	4.0	35.0	70.7	58.0	5190.0	12.3	327.1	216.4	190.2	0.743
80	0.710	0.98	4.0	37.7	58.7	54.3	5286.9	11.4	145.9	220.5	200.7	0.774
120	0.710	1.88	3.9	37.7	63.6	55.4	4773.7	10.8	213.5	199.1	199.6	0.708
160	0.710	2.62	3.9	37.8	64.2	54.4	4672.0	11.4	299.6	194.8	199.7	0.712
80	0.888	0.38	3.8	40.8	54.8	51.3	3864.9	9.5	119.8	161.2	205.8	0.726
120	0.888	0.50	3.8	41.2	56.6	52.0	3111.6	8.8	169.2	129.8	206.1	0.626
160	0.888	0.57	3.7	39.7	60.8	53.0	3765.3	10.3	272.7	157.0	205.2	0.661
80	1.065	0.82	3.6	43.5	56.3	53.3	2987.7	8.1	109.8	124.6	214.3	0.625
120	1.065	0.40	3.8	41.9	54.7	50.3	3171.8	8.4	153.9	132.3	216.9	0.616
160	1.065	0.37	3.8	42.6	53.9	48.3	2635.3	8.5	208.3	109.9	217.3	0.589

* **No survival. The values were calculated based on the mimimum recovery level.**

[a] **Calculated according to model proposed by Della valle *et al.*, 1996.**

Table 4.8 Correlation coefficients between the logarithmic reduction of *M. lacticum* and the parameters of extrusion at different screw speeds.

Parameters	*80 rpm*	*120 rpm*	*160 rpm*	*Overall*
Starch Conversion (%)	0.94	0.99	0.96	0.95
Moisture Content (%, wwb)	-0.99	-0.97	-0.98	-0.96
Maximum Temperature (°C)	0.96	0.95	0.97	0.95
Die Pressure (kPa)	0.98	0.98	0.97	0.82
Torque (N.m)	0.99	0.98	0.96	0.87
SME (kJ/kg)	0.99	0.98	0.94	0.73
Shear Stress (kPa)	0.98	0.98	0.97	0.82
Shear Rate (s^{-1})	-0.99	-0.93	-0.90	-0.92
Apparent Viscosity (kPa.s)	1.00	0.98	0.98	0.89

Strong negative correlations were observed between the bacterial destruction and the moisture content and shear rate. As explained previously, reduced water content increases the pressure forces, causing an increase in viscous forces (shear stress, apparent viscosity) and the SME. The negative correlation between the shear rate and the bacterial destruction can be explained by the fact that the water feed rate is positively correlated with the volumetric flow rate which dictates the shear rate (Equation 2.9). Thus, high shear rates can indirectly be considered as indication of high moisture contents.

The high correlation coefficient between the maximum temperature of the extrudate and the logarithmic reduction is due to the fact that the increased shear forces and mechanical energy increases the product temperature because of the viscous dissipation energy. However the exposure of the microorganism to a maximum temperature of 75°C for the order of a second (1.7 s maximum) is supposed to have virtually no thermal killing effect on the microorganism. Therefore, this high correlation between the bacterial destruction and the maximum extrudate temperature should be considered as the indication of high shear and mechanical energies. Nevertheless, it could be expected that temperatures close to the destruction

temperatures of the microorganism would make the cells more susceptible to shear forces. A possible synergistic effect between the temperature and shear forces was discussed previously. Zheng and Wang (1994) showed the shear activation energy is reduced with increasing temperature during the extrusion of maize starch at low temperatures. They suggested that the temperature increased the internal energy, which caused an increase in the degree of freedom for molecular movements. Consequently, less additional energy was required to initiate shear reactions for destruction of the starch granules. The same interpretation can be extended to the killing of microorganisms in an extrusion environment, where the shear and thermal forces are combined.

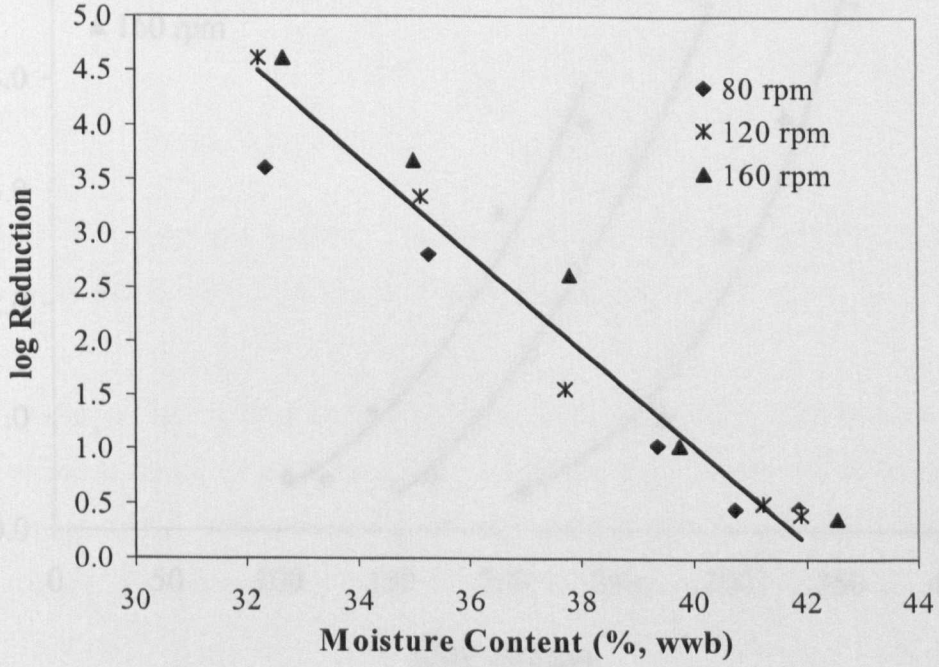

Figure 4.18 Relationship between the logarithmic reduction of *M. lacticum* and the moisture content at different screw speeds of extrusion.

The relationship between the extrusion moisture content and the bacterial destruction in Figure 4.18 shows that logarithmic reduction is reducing with increasing water content. This was expected since the water feed rate was the main parameter for controlling mechanical and shear forces during extrusion. There was no observable

difference in the bacterial destruction due to the screw speed, and all the data points could be represented by a single curve.

Figure 4.19 shows how the logarithmic reduction changes with SME at different screw speeds. The bacterial destruction at low screw speeds of extrusion was limited (maximum 3.6 log reduction), whereas no survival was observed at high screw speeds (120 and 160 rpm) at 0.355 kg/h water feed rate, giving at least 4.6 logarithmic reduction (minimum recovery level, 6.3×10^2 cfu per g sample).

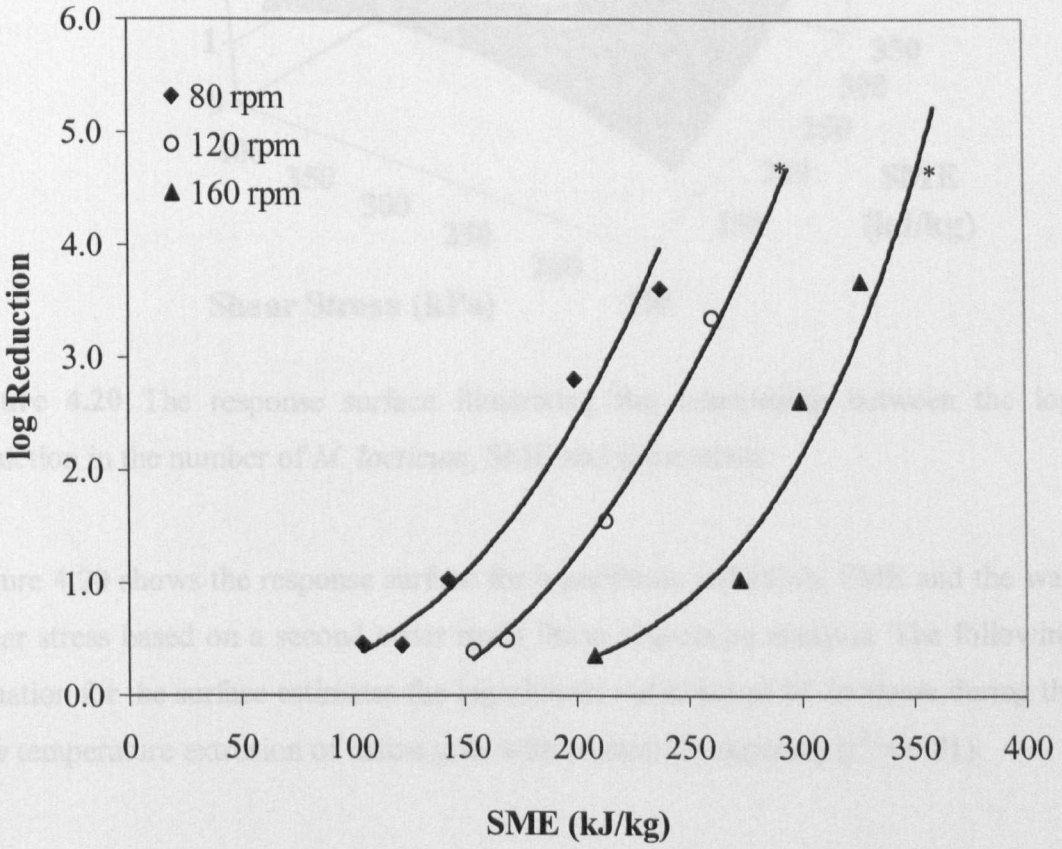

Figure 4.19 The relationship between the logarithmic reduction of *M. lacticum* and SME at different screw speeds of extrusion. * no survival, log reduction calculated based on minimum recovery level.

When the analogy of destruction of starch granules at low temperature and high shear environment is applied to the destruction of the microorganism in the extruder, it is not surprising that the bacterial destruction is increasing with increasing input of SME and shear stress.

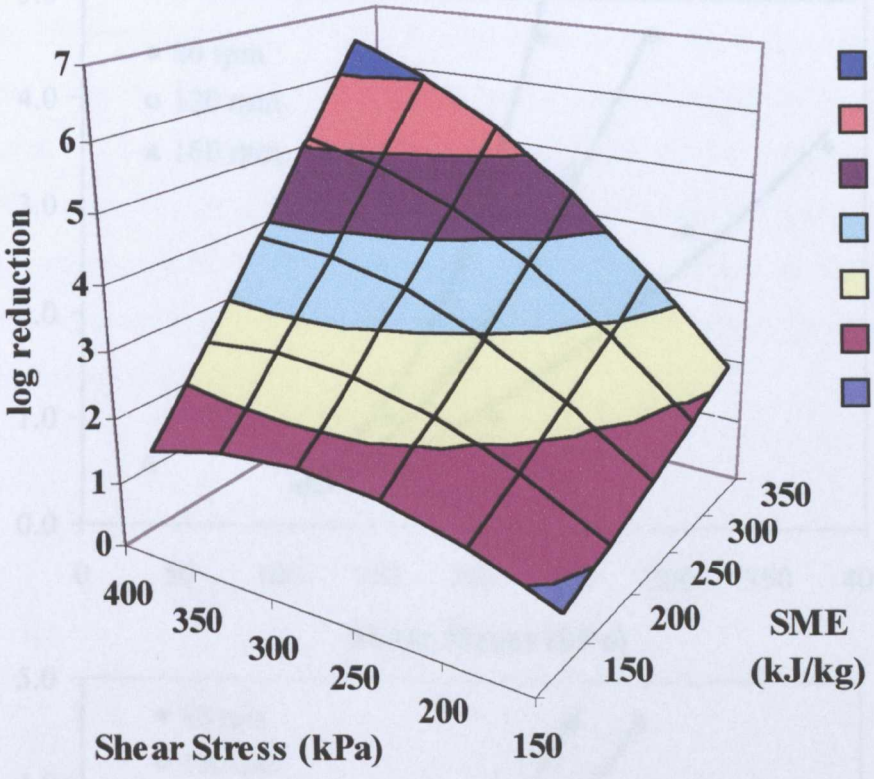

Figure 4.20 The response surface illustrating the relationship between the log reduction in the number of *M. lacticum*, SME and shear stress.

Figure 4.20 shows the response surface for logarithmic reduction, SME and the wall shear stress based on a second order multi linear regression analysis. The following equation for the surface estimates the logarithmic reduction of *M. lacticum* during the low temperature extrusion of maize grits with reasonable accuracy ($r^2 = 0.81$).

$$\log N_0/N = -8.56x10^{-3} SME + 1.0x10^{-5} SME^2 + 0.012\sigma_w - 4.0x10^{-5}\sigma_w{}^2 + 8.0x10^{-5} SME\sigma_w - 0.69$$

Equation 4.5

where *log N_0/N* is the logarithmic reduction.

According to Figure 4.20 the combination of high shear stress and SME, which is achieved at low moisture contents and high screw speeds, gives the highest bacterial destruction.

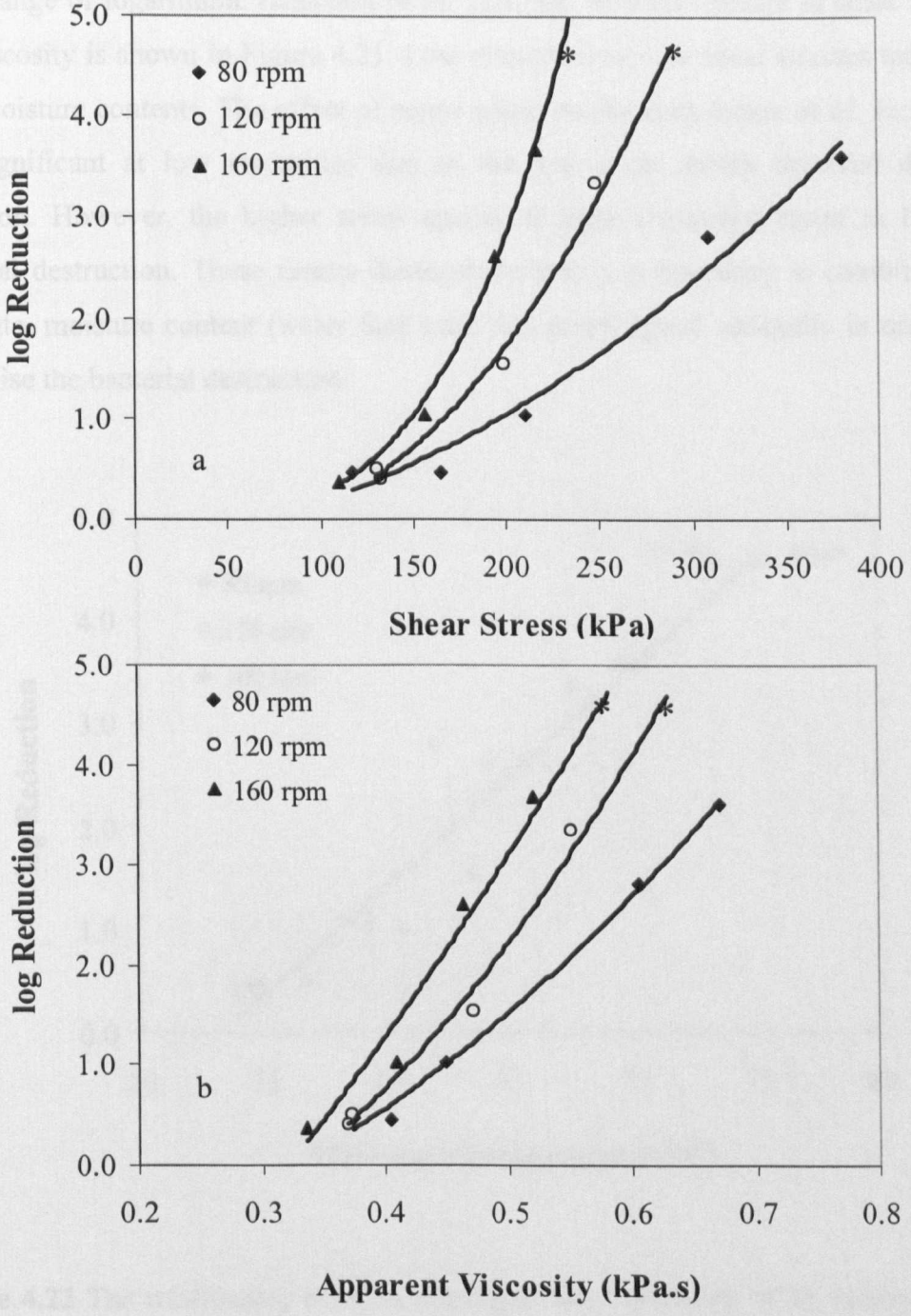

Figure 4.21 The relationship between the logarithmic reduction of *M. lacticum* and (a) shear stress and (b) viscosity measured at the die wall, at different screw speeds of extrusion. * No survival, log reduction calculated based on minimum recovery level.

The change of logarithmic reduction of *M. lacticum* with the change in shear stress and viscosity is shown in Figure 4.21. Low viscosities or low shear stresses indicate high moisture contents. The effect of screw speed on the destruction of *M. lacticum* is insignificant at low viscosities due to the low shear forces involved during extrusion. However, the higher screw speeds at high viscosities result in higher bacterial destruction. These results demonstrate that it is necessary to combine the feed rate, moisture content (water feed rate) and screw speed optimally in order to maximise the bacterial destruction.

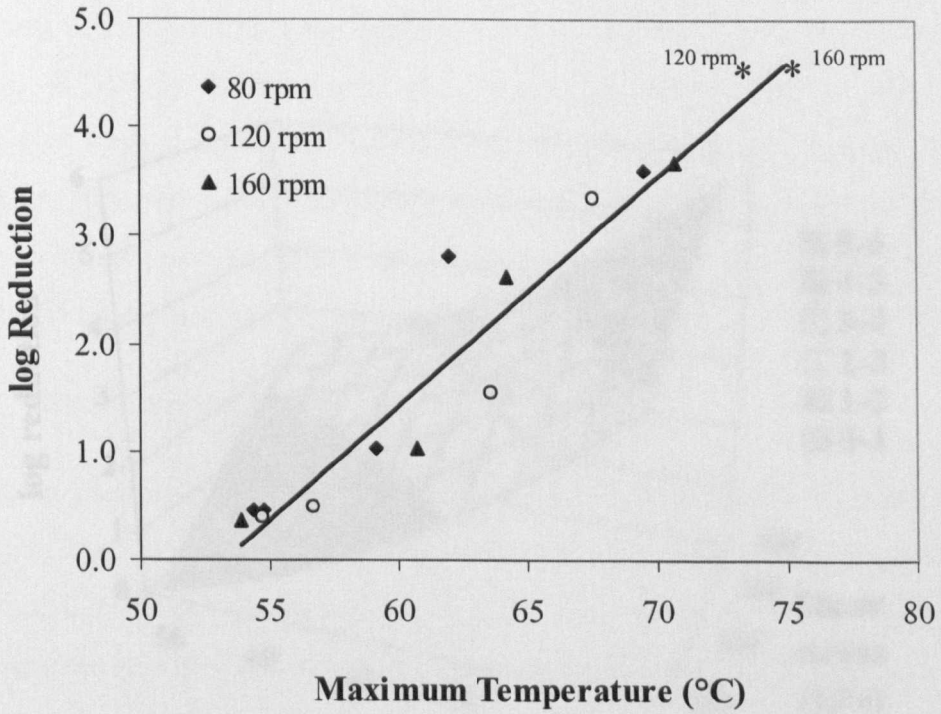

Figure 4.22 The relationship between the logarithmic reduction of *M. lacticum* and maximum extrudate temperature at different screw speeds of extrusion. * no survival, log reduction calculated based on minimum recovery level.

Figure 4.22 shows that the relationship between the bacterial destruction and maximum extrudate temperature measured at the exit of die is not significantly affected by the screw speed. This is because the extrusion temperatures were always well below the destruction temperature of the microorganism. The positive correlation between bacterial destruction and the maximum extrudate temperature

indicates the positive correlation between the mechanical (shear) forces and the bacterial destruction.

The response surface for bacterial destruction with shear stress and temperature based on a second order multilinear regression analysis is given in Figure 4.23. The following equation of surface is relating the temperature and shear stress to the logarithmic reduction (r^2= 0.85).

$$\log N_0 / N = -0.619T + 6.40x10^{-3}T^2 + 19.05x10^{-3}\sigma_w - 1.29x10^{-5}{\sigma_w}^2 - 9.12x10^{-5}T\sigma_w + 13.483$$

Equation 4.6

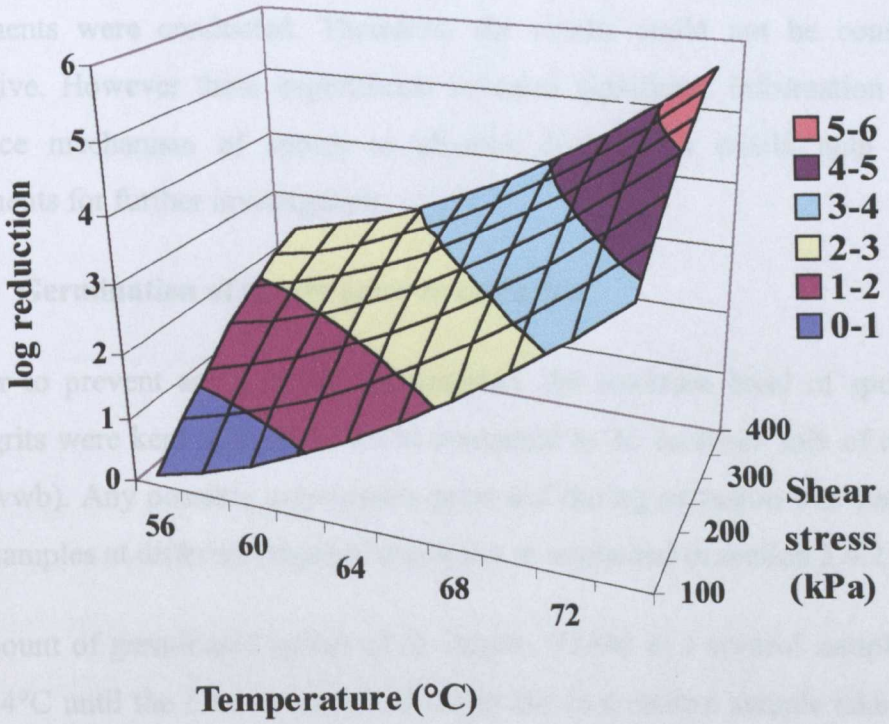

Figure 4.23 The response surface for the logarithmic reduction in the number of *M. lacticum* and the SME and Shear stress.

In order to correlate the temperature, SME and the wall shear stress with bacterial destruction, a first order multilinear regression analysis was conducted. The regression coefficient for the following equation, estimating the logarithmic reduction, was 0.85.

$$LogN/N_0 = -0.857MC + 0.102T + 4.4x10^{-3}SME + 3.5x10^{-3}\sigma_w - 2.792$$ **Equation 4.7**

where, MC is the moisture content.

The applicability of the models developed for the estimation of the logarithmic reductions in the number of *M. lacticum* needs to be tested with further experiments.

4.5.2 Inactivation of spores of *B. subtilis* by extrusion

In order to investigate the relative influence of thermal and shear energies on the destruction of spores, wild type spores of *B. subtilis* PS346 and the heat sensitive strain of *B. subtilis* PS361 were used as test organisms. Only a few sets of experiments were conducted. Therefore, the results could not be considered as conclusive. However these experiments revealed significant information about the resistance mechanism of spores to physical forces and would help to design experiments for further investigation.

4.5.2.1 Germination of spores prior to extrusion

In order to prevent any possible germination, the moisture level of spore mix of maize grits were kept low (15%, wwb) compared to *M. lacticum* mix of maize grits (30%, wwb). Any possible germination prior and during extrusion was controlled by taking samples at different stages of extrusion as explained in section 2.4.2.5.

The amount of germinated spores of *B. subtilis* PS346 in a control sample that was kept at 4°C until the time of microbial count and in a control sample taken from the feeder of the extruder after extrusion process were 25.6% and 27.2% respectively, indicating that there was no significant germination after the preparation of spore mix with maize grits until the time of extrusion.

The affect of grinding on germination of spores in Knifetec mill was also evaluated as explained in section 2.4.2.5. The results showed that there was no significant germination due to the grinding process. For example, a 5.5% germination due to the grinding process was considered to be within the limits of experimental error.

Table 4.9 The average amount of germinated spores of *B. subtilis* PS346 in extruded samples.

Screw Speed (rpm)	Water Feed (kg/h)	Germination (%)
100	0.533	38.6
	1.065	4.1
	1.420	-
150	0.533	14.5
	1.065	66.0
	1.420	-
200	0.533	53.0
	1.065	43.0
	1.420	59.4
300	0.533	20.0
	1.065	29.1
	1.420	37.7

The average amount of germinated spores of *B. subtilis* PS346 in the extruded samples are given in Table 4.9. The results show that the average amounts of germinated spores are higher in extruded samples compared to the un-extruded samples, suggesting that not all the germinated spores were killed by the subsequent shearing and higher temperatures in the reverse screw element and in the extruder die. This could also be due to the difference in the degree of germination. The surviving germinated spores could mostly be at their initial phase of germination (phase darkening), where their resistance to thermal and shear forces would be higher than vegetative cells, at the time passing through the extruder die.

4.5.2.2 Investigation of the inactivation mechanism of *B. subtilis* spores during extrusion

In order to estimate the thermal energy contribution on the destruction of spores during extrusion, the heat resistance of the spores needs to be known. The heat resistance of the spores used in our experiments are given in the literature as in Table 4.10. In a study at Nottingham, Melvin (1997) has determined the heat resistance of the spores of *B. subtilis* PS346 over a range of temperatures and moisture contents that is comparable to the extrusion environment. The heat resistance of the spores of *B. subtilis* PS346 tested in static environment is given in Table 4.11.

Table 4.10 Heat resistance *of B. subtilis* spores as cited in the literature.

Strain	Heat treatment	D value (min)	Reference
PS346	Dry heat at 120°C	30	A
PS346	Wet heat at 90°C	30	B
PS361	Dry and wet heat at 90°C	3	A, B

A Setlow and Setlow (1995)
B Fairhead *et al.* (1993)

Table 4.11 Survival of spores *B. subtilis* PS346 in maize grits exposed to different temperatures. Adapted from Melvin, 1997.

Temperature (°C)	Time (s)	Moisture (%, wwb)	Survival
40	50	15	100
180	40	15	100
180	50	15	92

The influence of shear and mechanical energy input on destruction of the spores was studied by varying the water feed rate. The mean values for spore destruction and the parameters of extrusion for two sets of experiments for wild type spores of *Bacillus subtilis* PS346 are given in Table 4.12.

Table 4.12 Mean values of spore destruction and the parameters of extrusion for wild type spores of *Bacillus subtilis* PS346.

Screw Speed (rpm)	Water Feed (kg/h)	Average MC of extrudate (%)	Log Reduction	Moisture Content (%,wwb)	Maximum Temp. (°C)	Die Temp. (°C)	SME (kJ/kg)	σ_w (kPa)	η_w (kPa.s)
100	0.533	23.0	0.84	23.0	86.0	55.0	450.8	676.1	1.54
	1.065	31.5	1.33	31.5	66.4	53.0	232.2	331.7	1.15
	1.420	35.8	1.64	35.8	55.4	49.0	193.4	280.7	0.98
150	0.533	22.8	0.86	22.8	95.0	70.0	496.5	460.4	1.30
	1.065	30.9	1.08	30.9	75.5	59.0	319.3	292.7	0.99
	1.420	35.5	2.13	35.5	61.2	52.0	231.6	196.9	0.89
200	0.533	23.9	1.88	23.5	90.8	56.0	630.4	432.0	1.35
	1.065	31.8	1.51	31.6	74.2	49.5	479.3	346.6	0.97
	1.420	36.2	1.58	36.1	58.9	47.5	322.7	211.9	0.89
300	0.533	24.5	3.24	24.5	86.5	55.3	873.2	388.6	1.37
	1.065	31.8	2.14	31.8	70.5	47.0	616.3	277.7	1.03
	1.420	36.2	2.18	36.2	62.0	53.0	496.6	220.8	0.83

The temperature of the barrel in all 4 zones was set to 25°C. However, because of mechanical energy input the temperature of the maize grits extrudate reached up to 95°C at the exit of the die depending on the water content during extrusion. Exposure of wild-type spores up to 180°C for 40-50 s (~ mean extrusion residence time) did not produce any significant reduction in the number of spores in a static environment as shown in Table 4.11. Considering a 30 min D value of the wild type spores of *B. subtilis* PS346 at 90°C, it could be concluded that the thermal energy has no significant affect on spore destruction since the exposure time of the spores to these maximum temperatures were only of the order of a second (0.8-1.7 s).

Figure 4.24 shows the relationship between the main extrusion parameters and the logarithmic reduction in the number of the spores of *B. subtilis* PS346. The mode of relationship for the extrusion screw speeds of 100 and 150 rpm is different from the extrusion screw speeds of 200 and 300 rpm. At low screw speeds of extrusion, decreasing SME, shear stress, and temperature as a consequence of increased water content, increases the spore destruction. This result seems to be anomalous since, it would be expected that the kill rate of spores would be higher at high shear and SME. However similar results were obtained by Melvin (1997). She has reported that the number of surviving wild-type spores of *B. subtilis* PS346 after extruding in maize grits at a maximum barrel temperature of 120 was 0.5, 0.007 and 0.05% at water contents of 23, 33 and 41 %, respectively. The higher survival at low moisture contents was explained by the relatively shorter mean residence times at low water contents (50 s mean residence time for 23% compared to 59 s for 41% moisture content). It was also suggested that the heat resistance of spores would be greater at lower water contents.

As discussed previously, it has long been known that mechanical forces acting on the spores (Gould, 1969, Pelczar *et al.*, 1993) or exposure of spores to moderate hydrostatic pressures (Sojka and Ludwing, 1994) can initiate germination. Gould (1969) reported germination-like changes (phase darkening, loss of heat resistance etc.) collectively termed "mechanical germination" during a variety of routine microbiological procedures. Some bacterial spores can germinate spontaneously when placed in a favorable environment (e.g. a nutritionally rich medium) and some spores would not germinate unless first activated by some traumatic agent that

damages the spore coat such as heat, abrasion, acidity and compounds containing free sulphydryl groups. (Brooks *et al.*, 1991). It is also known that bacterial spores, can be stimulated to germinate by pressures of 50-300 MPa and germinated spores can then be killed by relatively mild heat or pressure treatments (Smelt, 1998). Therefore, it can be suggested that mild pressures, temperatures and mechanical forces generated inside the extruder at relatively high moisture contents enhance the germination of spores and the germinated spores are subsequently inactivated by the heat and shear stresses generated in the reverse screw element and extruder die region.

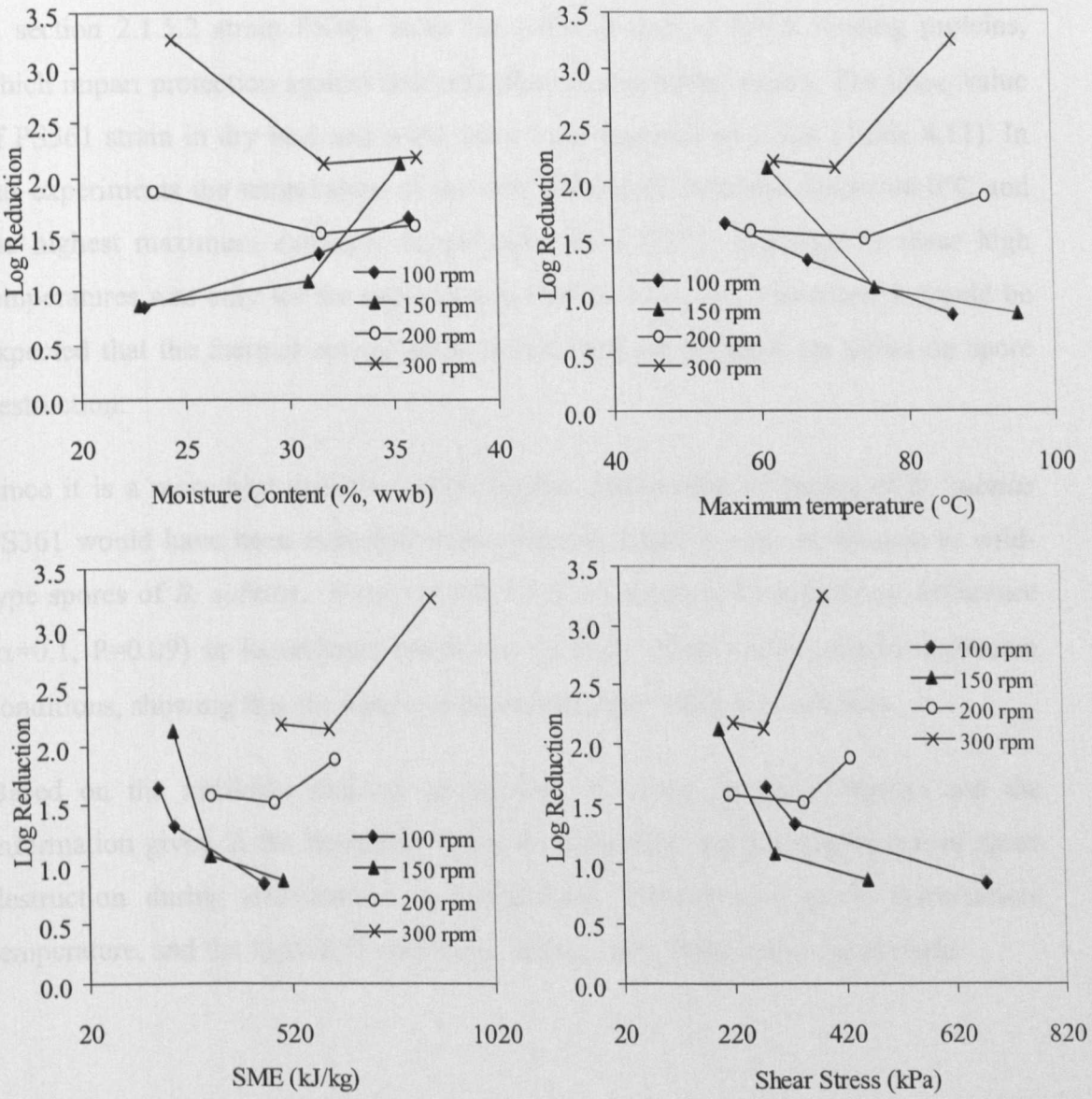

Figure 4.24 The relationship between the destruction of spores of *B. subtilis* PS346 and the main extrusion parameters.

From Figure 4.24 it can be seen that the direction of the relationship is changing beyond 200 rpm screw speed, the correlation between the main extrusion parameters and the spore destruction becoming similar to the response of *M. lacticum* to the same extrusion parameters. The increased spore destruction at high screw speeds could be due to the increased mechanical germination. The increase of temperature with increasing screw speed could intensify the killing of spores following germination.

Table 4.13. shows the relationship between the main extrusion parameters and the logarithmic reduction in the number of the spores of *B. subtilis* PS361. As described in section 2.1.5.2 strain PS361 lacks the SASP-α and -β DNA binding proteins, which impart protection against heat and other antimicrobial agents. The $D_{90°C}$ value of PS361 strain in dry heat and water have been reported as 3 min (Table 4.11). In our experiments the temperature of the extruder barrel remained below 46.0°C and the highest maximum extrudate temperature was 95.7°C. Exposure to these high temperatures was only for the order of a second (0.8 - 1.7 s). Therefore, it would be expected that the thermal energy alone would have an insignificant affect on spore destruction.

Since it is a more heat sensitive strain, higher inactivation of spores of *B. subtilis* PS361 would have been expected when extruded under similar conditions to wild-type spores of *B. subtilis*. However there was no statistically significant difference (α=0.1, P=0.09) in logarithmic reductions of these strains under similar extrusion conditions, showing that the main mechanism of destruction was not heat.

Based on the available data on destruction of spores during extrusion and the information given in the literature, it can be suggested that the mechanism of spore destruction during extrusion is a compromise between the spore germination, temperature, and the high SME and shear stresses generated inside the extruder.

Table 4.13 Mean values of spore destruction and the parameters of extrusion for *Bacillus subtilis* PS361

Screw Speed (rpm)	Water Feed (kg/h)	Average MC of extrudate (%)	Log Reduction	Moisture Content (%wwb)	Maximum Temp. (°C)	Die Temp. (°C)	SME (kJ/kg)	σ_w (kPa)	η_w (kPa.s)
100	0.533	22.8	0.90	22.8	77.0	42	528.9	816.8	1.54
	1.065	30.2	1.64	30.2	57.5	42	308.1	484.4	1.38
	1.420	34.3	2.75	34.3	52.0	43	210.1	316.7	1.11
150	0.355	20.3	2.59	20.3	87.0	46	724.6	700.0	1.50
	0.533	23.6	1.26	23.6	81.7	44	598.8	583.2	1.28
	1.065	30.2	2.07	30.2	66.4	44	355.0	340.6	1.06
	1.420	34.3	1.87	34.3	55.0	42	265.0	244.8	0.99
200	0.355	20.4	3.20	20.4	95.7	43	844.9	595.2	1.16
	0.533	24.4	2.15	24.4	77.0	43	1057.9	292.7	1.37
	1.065	29.6	2.08	29.6	68.3	44	449.6	316.7	1.06
	1.420	37.2	1.88	37.2	46.0	36	308.8	196.9	0.98

4.5.3 Relationship between bacterial destruction and the starch conversion

There was no distinguishable effect of screw speed on the relationship between destruction of *M. lacticum* and starch conversion, and all the data points could be represented by a single curve (Figure 4.25). The exponential relationship between the bacterial destruction and the starch conversion indicates that the microorganism is affected more by the shear and thermal forces in the high shear region. Microorganisms are not as rigid as starch granules and their cell wall, which is responsible for the mechanical strength, are made mainly of phospholipids and proteins. As discussed previously, the main structure responsible for the mechanical strength of a cell wall is the peptidoglycan layer. At ambient temperatures, the resistance of a microorganism to shear forces would be expected to be more than the resistance of a starch granule due to the smaller size of a microorganism (1-4 μm length and 0.4-0.8 μm of diameter for *M. lacticum* compared to starch granules having 5-25 μm diameter) and the elastic nature of the cell structure. However, at temperatures as high as 60°C the peptidoglycan layer of microorganisms weakens and liquefies. Tthis makes the cell susceptible to rupture and/or breakage by shear forces. On the other hand, the effect of a 60°C temperature for a 3 min of maximum residence time in the extruder would have virtually no effect on conversion of starch (Zheng and Wang, 1994). Therefore, compared to the starch conversion, bacterial destruction is occurring at a higher rate at high shear conditions of extrusion.

The relationship between the destruction of spores of *B. subtilis* PS346 and starch conversion is shown in Figure 4.26. The relationship between spore destruction and the starch conversion was not as strong as it was observed for *M. lacticum*. This could be explained by the complex mechanism of spore destruction due to the involvement of mechanical germination. It would be expected that the vegetative cells and spores would have a different reaction to thermal and shear forces. The discrepancies in Figure 4.26 can be explained by the coexistence of ungerminated dormant (resistant) spores and the germinated spores during extrusion.

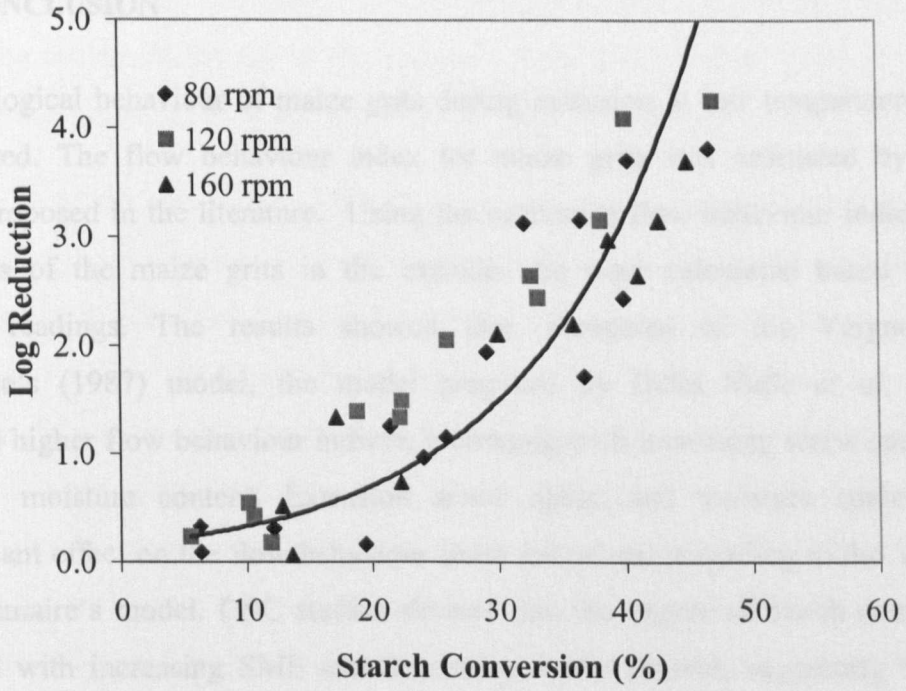

Figure 4.25 Relationship between the destruction of *M. lacticum* and starch conversion determined by DSC studies.

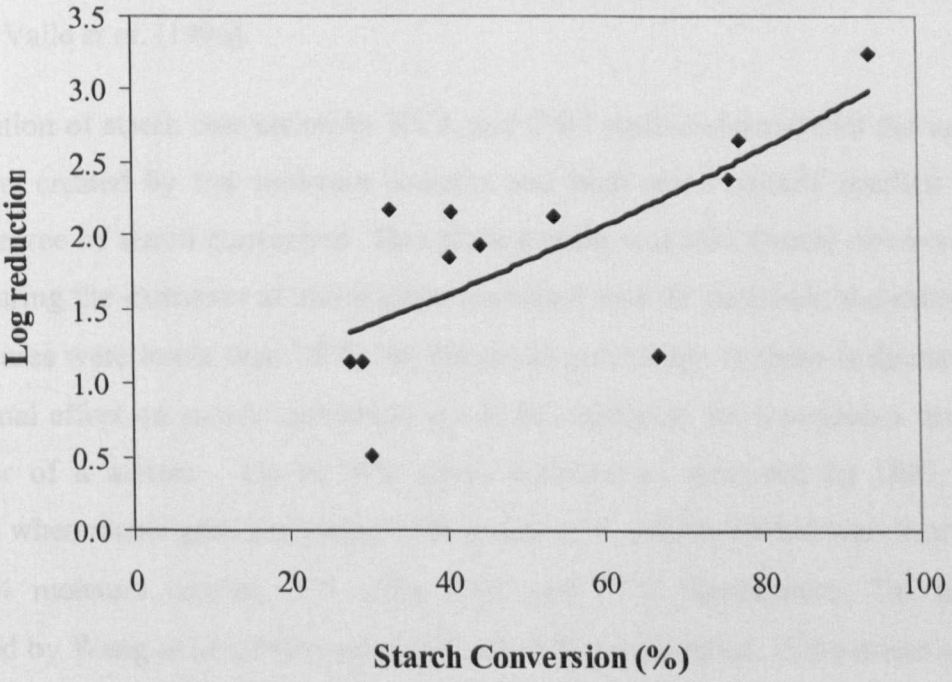

Figure 4.26 Relationship between the destruction of spores of *B. subtilis* PS346 and the starch conversion determined by DSC studies.

4.6 CONCLUSION

The rheological behaviour of maize grits during extrusion at low temperatures was investigated. The flow behaviour index for maize grits was estimated by using models proposed in the literature. Using the calculated flow behaviour indices, the viscosities of the maize grits in the extruder die were calculated based on die pressure readings. The results showed that, compared to the Vergnes and Villemaire's (1987) model, the model proposed by Della Valle *et al.* (1996) estimated higher flow behaviour indices, increasing with increasing screw speed and extrusion moisture content. Extrusion screw speed and moisture content had insignificant effect on the flow behaviour index calculated according to the Vergnes and Villemaire's model. DSC studies showed that the degree of starch conversion increased with increasing SME and decreasing water content, suggesting that the viscosity was influenced by screw speed and extrusion moisture content. Therefore, the model suggested by Della Valle *et al.* (1996) seems to be more realistic for calculation of the flow behaviour index of maize grits during extrusion at low temperatures. The measured viscosities of maize grits by piston capillary rheometer (0.36-1.30 kPa.s) were similar to those obtained by employing the model proposed by Della Valle *et al.* (1996).

Investigation of starch conversion by RVA and DSC studies showed that the severe conditions created by low moisture contents and high screw speeds resulted in a higher degree of starch conversion. This phenomenon was also clearly observed by SEM. During the extrusion of maize grits inoculated with *M. lacticum,* the extrusion temperatures were lower than 75°C. As discussed previously, at these temperatures, the thermal effect on starch conversion would be negligible for a residence time of the order of a minute. Up to 94% starch conversion, measured by DSC, was obtained when maize grits inoculated with spores of *B. subtilis* PS346 were extruded at 24.5% moisture content, 873 kJ/kg SME and 87°C temperature. The model suggested by Wang *et al.* (1989) estimates only 3.8% conversion, if the maize starch is exposed to a 71°C temperature (mean die temperature) for 3 min (maximum residence time). Considering that the material experienced these high temperatures for only few seconds at the extruder die region, the conversion due to heat alone can

be taken as negligible. However, as suggested by Zheng and Wang (1994), the temperature could have increased the degree of molecular disorder by increasing the internal energy, making the starch granules more susceptible to the shear forces.

Investigation of the correlation coefficients between the logarithmic reduction of *M. lacticum* and the parameters of extrusion showed that the correlation coefficients were high (≥ 0.89) for SME, maximum temperature and shear stress. The correlation coefficients between logarithmic reduction and the moisture content and shear rate were strongly negative. The negative correlation between the logarithmic reduction and the moisture content can be explained by the decreased shear and mechanical forces inside the extruder as more water was added during extrusion. The shear rate and the moisture content of the material are positively correlated because increasing the moisture content increases the volumetric flow rate, which determines the shear rate in the extruder die. Therefore, the negative correlation between the logarithmic reduction and the shear rate is in agreement with the known effect of moisture content on bacterial destruction.

Screw speed played an important role on destruction of *M. lacticum* by influencing the SME, shear stress, and the viscosity of the material. Increasing the screw speed increases the SME, resulting in more degradation of starch granules, which in turn reduces the shear stress and the viscosity. Due to this phenomenon, the influence of screw speed on destruction of *M. lacticum* was evident at low moisture contents and insignificant at high moisture contents (>38%, wwb). This was probably due to the fact that, at high moisture contents, the viscosity of the material was not high enough to apply high shear stresses on the microorganism.

The maximum temperature of the extrudate at the exit of the die was 75°C. Therefore, it is suggested that the high levels of the destruction of *M. lacticum* (complete destruction at 120 and 160 rpm at 0.355 $kg.h^{-1}$ water feed rate) was due to the coupling of thermal and shear forces, where temperature weakened the bacterial cell wall for efficient destruction of the cells by shear forces.

The results showed that the SME and shear stress, which are mainly influenced by moisture content and screw speed, are important parameters determining the

destruction of *M. lacticum* during extrusion in maize grits. No survival was observed at the combination of high SME and shear stress, which was achieved by high screw speeds at low moisture contents.

Using maize grits as carrier, significant reduction in the number of both wild type and mutant spores of *B. subtilis* were obtained by extrusion. A maximum 3.2 logarithmic reduction in the number of *B. subtilis* PS346 and *B. subtilis* PS361 were obtained at high SME (845-873 kJ/kg) and shear stresses (388-595 kPa). However, significant reductions were observed at mild conditions of extrusion (low SME, low shear stress, low temperatures, high moisture content) suggesting that mechanical germination as defined by Gould (1969), has occurred prior to inactivation by shear and heat. Under similar extrusion conditions, the reductions in the number of *B. subtilis* PS361, the heat sensitive strain due to the lack of SASP-α and -β DNA binding proteins, were not statistically different from that of the heat resistant strain PS346, indicating that the main mechanism of destruction was not heat. Considering that the maximum extrusion temperatures were less than 100°C and the exposure to these maximum temperatures in the extruder die was in the order of a second, it can be concluded that the effect of thermal forces on spores was insignificant. However, at these temperatures the germinated spores can easily be killed. The results suggested that the mechanism of spore destruction during high-shear low-temperature extrusion took place in two stages. In first stage, the spores were germinated by the stimulating effect of shear, mild temperature (40-70°C) and mild pressures (mechanical germination) in the extruder barrel. In the second stage, the germinated spores were inactivated by the high shear forces generated in the reverse screw element and the extruder die.

There was a strong nonlinear correlation between the destruction of *M. lacticum* and starch conversion determined by DSC studies. Comparing the degree of starch conversion (maximum ~60%) with up to 6 logarithmic reduction in the number of *M. lacticum*, it can be concluded that the destruction of microorganism during extrusion was far more efficient than the destruction of starch granules. This can probably be explained by the relative heat resistance of starch granules and *M. lacticum*, which would have important impact on the effectiveness of shear forces.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Detailed discussion of the findings has been given within each results chapter. A more general discussion about the potential of using combined shear and thermal forces in destruction of the microorganisms will be given in this chapter. Future work that could be carried out to confirm the results and to explore the different aspects of the project will also be discussed.

Currently, extrusion cooking is the only operation that can combine high physical and thermal energies for the processing of semi-solid foods. However, the nature of the extrusion process makes it difficult to evaluate the effect of these two energies separately on destruction of spores and/or vegetative cells. This is partly because these two energies are interdependent and vary from process to process. Another issue with extrusion cooking is that not all the material undergoes the same time, temperature and mechanical (shear) energy (Harper, 1981, Likimani *et al.*, 1990c). For example, the existence of hot spots in the materials that cannot be measured by conventional temperature measurement systems has been reported by many researchers (Harper, 1981, Guzmantello and Cheftel, 1987). The conversion of mechanical (shear) energy into heat was another problem making it difficult to investigate the relative effectiveness of shear and thermal energies on destruction of the microorganisms. We were conscious of the problems with temperature control and mechanical heating due to extrusion or shearing in the rheometers. Over-estimation of the shear effect on microorganism was avoided by working well below the thermal destruction conditions (time-temperature combinations) where

significant destruction of the test organisms would be expected. For example, in order to eliminate effects of thermal forces on *M. lacticum* during extrusion, the barrel temperatures were kept low for all the zones and the maximum temperature at the end of the barrel (die entrance) was only 61°C even under the most severe shear heating conditions. The exposure of the microorganism to temperatures as high as 75°C due to viscous dissipation energy in the extruder die was only the order of a second. Heat resistance data for *M. lacticum* suggests that under the extrusion conditions the effect of thermal energy alone on bacterial destruction was insignificant. It is appreciated that the above argument does not entirely eliminate the idea that there could be localised hot spots which were responsible for thermal destruction. However, further support for the view that physical forces play a major role comes from the results with the rotational rheometer. For vegetative cells of *B. subtilis* the non-sheared thermal control which was maintained 2°C above the shearing temperature of 55°C showed more destruction than was the case for microorganisms in the sheared sample. This suggests that in this environment the control undergoes greater thermal destruction than the sheared sample. It is therefore very difficult to explain the increased destruction of *M. lacticum* and *E. coli* in the rheometer under shear in terms of localised thermal effects.

The analysis of the data for correlation coefficients, and the linear and non-linear multivariate analysis showed that shear stress, SME, temperature and moisture content were primarily important parameters in destruction of *M. lacticum*. Based on these analyses, models were proposed for estimation of destruction of *M. lacticum* in maize grits carrier during low temperature extrusion. The repeatability of the extrusion experiments with the maize grits carrier was reasonably good and more than 50 data points were employed for the derivations of the models. However, the repeatability of experiments with the gelatin carrier was less good and the experimental procedures did not allow the collection of enough data for the development of a model for the gelatin carrier during extrusion. However, the results showed that substantial count reduction can be achieved in both gelatin and maize grits, if the extrusion parameters are chosen to combine moderate temperatures with high shear stresses and SME. As discussed previously, a temperature that is not enough to inactivate a microorganism on its own, can act synergistically on

destruction of microorganisms when combined with physical forces. It was suggested that this was due to the weakening of bacterial cell wall by the heat, which in turn made the cells susceptible to shear forces.

Physical forces can be subdivided into shear stresses, extensional stresses and pressure. It is of interest to determine the relative importance of the shear and extensional stresses. Investigation of the effect of shear and extensional stresses on destruction of *M. lacticum* in gelatin carrier in the piston capillary rheometer showed that the extensional stresses generated in the short die of the capillary rheometer could cause microbial destruction. Extensional stresses are generated as the material flows from the reservoir of a capillary rheometer and/or extruder barrel into a capillary or slit die due to the converging and accelerating streamlines. In the short die of the extruder the shear flow is virtually zero and thus, theoretically, the stress is purely extensional. However, both shear and extensional stresses occurs in the long die. Therefore, when evaluating the effect of shear stresses on the destruction of microorganisms the extensional (elongational) stresses that will be present should also be considered.

The pressures involved in extrusion experiments were less than 10 MPa (as measured at the extruder die) for all the extrusion experiments. The pressure in the piston capillary rheometer were often much higher and had a significant effect on the destruction of *M. lacticum* at temperatures in the range of 60 to 75°C. A 4.2 logarithmic reduction in the number of *M. lacticum* were obtained by 192 MPa pressure at 60°C. Compared to most high pressure applications, the high level of bacterial kill at this moderate pressure was probably due to the effect of temperature on the bacterial cell wall, as discussed in previous chapters. For the same reason, pressures as low as 51 MPa could cause more than 2 logarithmic reduction in the number of the microorganism. During the course of increasing the shear rate (shear stress) stepwise in the piston capillary rheometer, a 3.3 logarithmic reduction in the number of *M. lacticum* was obtained at a pressure of 64 MPa and a shear stress of 695 kPa at 60°C and 25% moisture content. When the shear stress was increased to 1075 kPa in the next step, which resulted in 97 MPa pressure, no survival was detected. Although the experiment was not originally designed to investigate the

effect of pressure forces on destruction of the microorganism, the results showed that complete destruction of the microorganism is possible if the shear, thermal and pressure forces combined optimally. Since the experiments with the piston capillary rheometer were conducted in a different laboratory (Rosand Precision, Stourbridge), it was not possible to carry out further experiments to explore the possibility of combining pressure forces with temperature and shear forces. It is evident that it would be interesting to further investigate the possibility of combining shear, thermal and pressure forces in the piston capillary rheometer for efficient destruction of the microorganisms which may provide fundamental information for designing a sterilisation process. Sojka and Ludwig (1994) reported that the moderate pressures can initiate the germination of *B. subtilis* spores and the pressures between 60-150 MPa are best suited for germination. The possibility of creating moderate pressures in the piston capillary rheometer gives an excellent opportunity to search for the optimum combination of process parameters for the inactivation of spores.

The shear heating during the shearing of the microorganisms in the rotational rheometer was taken into account by the experimental setup as explained in Section 2.3.2.2. The exposure of control samples to 2-4°C higher temperatures during the course of shearing eliminated the overestimation of the bacterial destruction by shear forces by ensuring that the control samples always experienced slightly higher temperatures than sheared samples. When the shear forces were less or the time of shearing was not long enough to compensate for the bacterial destruction due to slightly higher temperatures in control samples, the calculated bacterial destruction in sheared samples (against control samples) had negative values. As mentioned earlier, this phenomenon was clearly observed during the shearing of vegetative cells of *B. subtilis* in the shear rate range of 50 to 1010 s^{-1} for 4 min at 55°C. As discussed previously, the microorganism was resistant to shear forces probably because of its cell wall structure (Gram-positive) but was susceptible to thermal destruction since the temperature was in the vicinity of the thermal destruction of the organism. Therefore, the logarithmic reduction of the microorganism remained negative for the whole shear rate range, showing that the experimental method employed could not overestimate the shear killing of the microorganisms. In contrast for *M. lacticum,* the D-value on shearing was substantially lower than the D-value of the microorganism

determined in the absence of shearing. The "shear D-value" can be an important parameter for estimation of the shear resistance of the microorganisms for a possible sterilisation process that relies on shear forces. It can also be a useful parameter for optimisation of fermentation processes where the shear forces are involved due to mixing and agitation.

The significant contribution of mechanical energy to starch conversion during low temperature extrusion has long been known. In this work, up to 58% conversion of maize starch was obtained by a single pass through the twin-screw extruder during the extrusion of *M. lacticum* below 75°C at average moisture content of 32%. When the extruder temperature was set to the ambient temperature (25°C) for all the barrel zones for the extrusion of *B. subtilis* spores, up to 94% conversion occurred at 24.5% moisture content. Due to the conversion of mechanical energy into heat, the product temperature measured at the barrel wall increased gradually, reaching about 55°C at the entrance of the die and 87°C at the die exit. However, as discussed previously, the thermal affect on starch conversion at these temperatures were insignificant for the mean residence time in the extruder. In agreement with previous work, it was concluded that starch conversion was due to mechanical (shear) forces. However as suggested for microorganisms, the role of thermal forces on overall starch conversion could be synergistic rather than additive. As explained by Zheng and Wang (1994), thermal energy supplied during extrusion will increase the internal energy of starch granules, increasing the degree of freedom for molecular movements. Subsequently the starch granules will be more susceptible to shear forces and less additional energy will be needed to initiate the shear destruction of the granules.

Up to 3.2 logarithmic reductions were obtained in the number of the spores of *B. subtilis* PS346 and the heat sensitive strain PS361. The temperatures employed were low enough to eliminate possible thermal destruction of spores. Based on the available heat resistance data of the spores, a maximum 96°C temperature treatment of the spores for few seconds in the extruder die would have no significant effect on spore destruction. In addition, there was no statistically significant difference between the destruction of wild and mutant type of spores under similar extrusion conditions, suggesting that the spore destruction achieved was not due to heat.

Several authors have suggested that the bacterial spores are too small to be disrupted by shear forces in a single screw extruder (Kauffman and Hatch, 1977, Likimani *et al.*, 1990b). Although not enough experiments were carried out to draw strong conclusions about the destruction mechanism of the spores during low temperature extrusion, we think that the germination of spores in the dynamic environment of the extruder could be the main reason for the high level of spore destruction. As discussed previously (see Section 1.3.2-3) there is considerable evidence that the spores can germinate under physical forces. Likimani *et al.* (1990c) observed injury of *B. globigii* spores at low temperature extrusion, whereas there was no injury at high temperatures. This observation also supports the idea of so-called mechanical germination. Further work should concentrate on extending residence times of the spores in the extruder by employing a screw profile which uses more mixing and reverse screw elements. Such a screw profile will not only extend the residence time but will also provide high mechanical and abrasion forces which can stimulate germination.

Compared to starch granules, the level of destruction achieved for *M. lacticum* and spores of *B. subtilis* was much higher. For example, if we express the bacterial destruction in terms of percentage by using the approach employed for calculation of the starch conversion (Equation 2.21), a 4.6 logarithmic reduction in the number of *M. lacticum* (see Table 4.7) would correspond to 99.998%. However, the degree of starch conversion for the same experiment was below 60%. Taking into consideration that the size of starch granules (average ~13 μm) are an order of magnitude greater than *M. lacticum* (0.4-0.8 x 1.0-4.0 μm) and the assumption that the effect of shear forces on a rod shape particle would be more destructive than that of a spherical particle, it can be concluded that the starch granules were much more resistant to the shear and thermal forces than *M. lacticum* under the experimental conditions employed. Although it could be argued that a small amount of "damage" is sufficient to inactivate a microorganism whereas more severe disruption is required to initiate the starch gelatinisation, the most probable reason for the difference between the destruction of starch granules and microorganims could be the different resistance of the particles to thermal forces. As explained earlier, temperatures above 60°C would be enough to weaken the bacterial cell wall, making

it susceptible to shear forces, whereas the same temperatures would have no significant effect on starch granules within the maximum residence times (3 min) of extrusion process. This outcome may have an important impact on the process of decontamination of some food ingredients (gums, thickening agents, spices etc.) by extrusion, where the extensive transformation of the materials is not desirable. Knowing the material properties and the properties of the target organisms (such as heat sensitivity, gram reaction, spore or vegetative cells), an optimum design of parameters can be chosen for extrusion in order to get the maximum bacterial destruction while keeping the change in the material properties at the minimum level.

The overall results showed that the utilization of shear and pressure forces in combination with thermal forces has a potential to be used as a process of decontamination and/or sterilisation in the food industry. It is evident that more investigation is needed to confirm the results and to explore the different aspects of this work.

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APPENDIX

Table A.1. Summary of calculations for determination of E(t) and F(t) diagrams for extrusion of gelatin at 120 rpm. Extrusion parameters; 3kg·h^{-1}, feed rate, 0.750 kg·h^{-1} water feed rate, 2 mm slit die, 30°C die temperature, 83 Bar back pressure.

Sample weight (g)	Sample mean time t (s)	Δt	Absorbance	Dye/Sample (mg).	Dye/g sample C(t) (mg)	C(t).Δt	t.C(t).Δt	Exit age E(t)=C(t)/∑ C(t).Δt	F(t)
25.8	13	13	0.000	0.00	0.00	0.00	0.00	0.00	0.00
8.1	30	17	0.004	0.03	0.00	0.07	1.94	0.00	0.00
7.1	37	8	0.513	3.50	0.49	3.76	140.63	0.02	0.14
8.0	45	8	0.798	6.12	0.77	5.79	260.40	0.03	0.34
7.3	53	8	0.850	5.99	0.82	6.25	329.34	0.03	0.56
6.6	60	7	0.670	4.26	0.64	4.49	267.78	0.02	0.72
14.6	70	11	0.436	6.10	0.42	4.44	311.65	0.02	0.88
10.7	83	13	0.173	1.78	0.17	2.10	174.26	0.01	0.95
14.8	96	13	0.068	0.97	0.07	0.84	79.83	0.00	0.98
14.8	110	15	0.023	0.33	0.02	0.33	36.09	0.00	0.99
13.4	125	14	0.011	0.14	0.01	0.15	18.52	0.00	0.99
12.6	137	13	0.005	0.06	0.01	0.06	8.57	0.00	0.99
15.4	151	14	0.002	0.03	0.00	0.03	4.07	0.00	1.00
12.7	166	14	0.001	0.01	0.00	0.01	2.23	0.00	1.00
17.1	180	15	0	0.00	0.00	0.00	0.00	0.00	1.00
Sum=189.0				Sum=29.3		Sum=28.3	Sum=1635.3		

Mean Residence Time $(\bar{t}) = \sum_0^\infty t.C(t).\Delta t / \sum_0^\infty C(t).\Delta t \cong 1635.32/28.30 \cong 58$ sec

Table A.2. Summary of calculations for determination of E(t) and F(t) diagrams for extrusion of gelatin at 200 rpm. Extrusion parameters; 3kg·h^{-1}, feed rate, 0.750 kg·h^{-1} water feed rate, 2 mm slit die plate, 36°C die temperature, 27 Bar back pressure.

Sample weight (g)	Sample mean time t (sec)	Δt	Absorbance	Dye/Sample (mg).	Dye/g sample C(t) (mg)	C(t).Δt	t.C(t).Δt	Exit age E(t)=C(t)/Σ C(t).Δt	F(t)
17.7	9	9	0.000	0.00	0.00	0.00	0.00	0.00	0.00
14.0	25	16	0.011	0.15	0.01	0.17	4.12	0.00	0.01
8.8	36	11	0.650	5.49	0.62	7.12	256.63	0.02	0.26
6.2	44	8	0.830	4.98	0.80	6.00	261.25	0.03	0.48
9.3	51	8	0.690	6.13	0.66	5.14	263.54	0.02	0.66
13.1	63	11	0.470	5.93	0.45	5.05	315.81	0.02	0.84
12.0	75	13	0.213	2.45	0.20	2.57	192.57	0.01	0.93
16.2	89	14	0.079	1.23	0.08	1.07	95.14	0.00	0.97
19.2	107	18	0.034	0.63	0.03	0.58	61.79	0.00	0.99
15.6	124	17	0.008	0.12	0.01	0.13	16.64	0.00	1.00
16.2	140	16	0.005	0.08	0.00	0.08	10.72	0.00	1.00
17.0	157	17	0.003	0.05	0.00	0.05	7.51	0.00	1.00
14.1	172	16	0.000	0.00	0.00	0.00	0.00	0.00	1.00
Sum=179.4				Sum=27.2		Sum=27.9	Sum=1485.7		

$$\text{Mean Residence Time } (\bar{t}) = \sum_0^\infty t.C(t).\Delta t \,/\, \sum_0^\infty C(t).\Delta t \cong 1485.7/27.9 \cong 53 \text{ sec}$$

Table A.3. Summary of calculations for determination of E(t) and F(t) diagrams for extrusion of gelatin at 300 rpm. Extrusion parameters; 3kg·h^{-1}, feed rate, 0.750 kg·h^{-1} water feed rate, 2 mm slit die, 37°C die temperature, 17 Bar back pressure.

Sample weight (g)	Sample mean time t (sec)	Δt	Absorbance	Dye/Sample (mg).	Dye/g sample C(t) (mg)	C(t).Δt	t.C(t).Δt	Exit age E(t)=C(t)/∑ C(t).Δt	F(θ)
15.5	8	8	0.000	0.00	0.00	0.00	0.00	0.00	0.000
14.6	23	15	0.075	1.07	0.07	1.09	24.63	0.00	0.040
11.0	35	13	0.790	8.53	0.77	9.81	346.46	0.03	0.397
13.2	47	12	0.679	8.79	0.66	7.98	377.91	0.02	0.687
14.1	61	14	0.362	4.98	0.35	4.79	291.37	0.01	0.861
15.5	76	15	0.186	2.81	0.18	2.66	200.93	0.01	0.958
14.9	91	15	0.053	0.77	0.05	0.78	70.52	0.00	0.987
19.1	107	17	0.015	0.28	0.01	0.25	26.47	0.00	0.996
14.0	124	16	0.005	0.07	0.00	0.08	9.92	0.00	0.998
13.9	138	14	0.002	0.03	0.00	0.03	3.73	0.00	0.999
14.7	152	14	0.001	0.01	0.00	0.01	2.11	0.00	1.000
15.2	167	15	0.000	0.00	0.00	0.00	0.00	0.00	1.000
Sum=175.7				Sum=27.3		Sum=27.5	Sum=1354.0		

$$\text{Mean Residence Time } (\bar{t}) = \sum_0^\infty t.C(t).\Delta t \,/\, \sum_0^\infty C(t).\Delta t \cong 1354.04/27.47 \cong 49 \text{ sec}$$

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Effects of Combined Shear and Thermal Forces on Destruction of *Microbacterium lacticum*

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A twin-screw extruder and a rotational rheometer were used to generate shear forces in concentrated gelatin inoculated with a heat-resistant isolate of a vegetative bacterial species, *Microbacterium lacticum*. Shear forces in the extruder were mainly controlled by varying the water feed rate. The water content of the extrudates changed between 19 and 45% (wet weight basis). Higher shear forces generated at low water contents and the calculated die wall shear stress correlated strongly with bacterial destruction. No surviving microorganisms could be detected at the highest wall shear stress of 409 kPa, giving log reduction of 5.3 (minimum detection level, 2×10^4 CFU/sample). The mean residence time of the microorganism in the extruder was 49 to 58 s, and the maximum temperature measured in the end of the die was 73°C. The $D_{75°C}$ of the microorganism in gelatin at 65% water content was 20 min. It is concluded that the physical forces generated in the reverse screw element and the extruder die rather than heat played a major part in cell destruction. In a rotational rheometer, after shearing of a mix of microorganisms with gelatin at 65% (wt/wt) moisture content for 4 min at a shear stress of 2.8 kPa and a temperature of 75°C, the number of surviving microorganisms in the sheared sample was 5.2×10^6 CFU/g of sample compared with 1.4×10^8 CFU/g of sample in the nonsheared control. The relative effectiveness of physical forces in the killing of bacteria and destruction of starch granules is discussed.

There have been extensive studies on the use of extrusion processing to reduce microbial counts in food ingredients (2, 4, 7, 10, 16, 17). Likimani et al. (12) proposed a methodology for determining the destruction of bacterial spores in a Brabender single-screw Plasticorder extruder under varying processing conditions. The *D* value ("extrusion *D* value" or "extrusion death rate" as proposed by the authors) for extrusion was calculated on the basis of average time at a mass temperature of >95°C. They stated that destruction of spores in the extruder was a function of mass temperature and residence time at constant moisture levels (18%) in a corn-soybean (70%/30% [wt/wt]) mixture. Increasing mass temperature resulted in greater lethality, while increasing screw speed, which reduced residence time, resulted in lesser lethality.

Using the same system as mentioned above, Likimani and Sofos (11) examined the potential for injury of *Bacillus globigii* spores during extrusion cooking. Their results showed that extrusion processing at low barrel temperatures (80 to 100°C) resulted in injury to spores of *B. globigii*, whereas at higher temperatures spore injury was not detectable.

In an extrusion process, both mechanical and thermal energy are applied to the material. When the process is applied to starch-containing systems, it is clearly recognized that both forms of energy can play an important role in starch conversion (18). In this context, starch conversion refers to the loss of ordered crystalline regions in the granule and more severe damage including polysaccharide degradation and loss of granule integrity (5). Attempts have been made to deconvolute the influence of the two forms of energy by calculating the expected effect for heat alone and comparing it with the total extent of conversion (19). Studies using a capillary rheometer have allowed an estimate of the shear forces required to destroy the ordered structure within the granule. The amount of residual order in this case can be measured by differential scanning calorimetry. It has been shown that, for waxy maize starch, granule destruction by mechanical forces can occur at much lower temperatures than those required to gelatinize or melt the starch granule under conditions where no mechanical energy is applied. The minimum shear stress required to induce starch conversion decreases strongly with increasing temperature (23).

In this paper, we present some results on the application of a similar approach to the destruction of microorganisms by extrusion. The objective of the work was to quantify the shear stress required to promote microbial killing under thermal conditions which were mild compared with those required to kill the microorganism. There were two longer-term objectives of the study. Firstly, if the relationship between physical forces and heat in the destruction of microorganisms could be better understood, then it might be possible to devise processes where a combination of the two energy forms could be used to kill microorganisms more efficiently and at lower temperatures than would be the case with heat alone. Secondly, the susceptibility of microorganisms to physical forces might provide a useful way of differentiating between microorganisms and possibly even fractionating them by removal of the more susceptible in a mixture by the application of physical forces.

The approach employed was to extrude the microorganisms in a gelatin carrier through a narrow-slit die. *Microbacterium lacticum*, isolated from pasteurized liquid whole egg, was selected, as it has been reported to have a high thermal stability (14). Gelatin, when plasticized by low amounts of water, will form a melt at temperatures below that required to inactivate this organism, and subsequent enumeration is facilitated by the way in which gelatin can be solubilized at relatively low temperatures.

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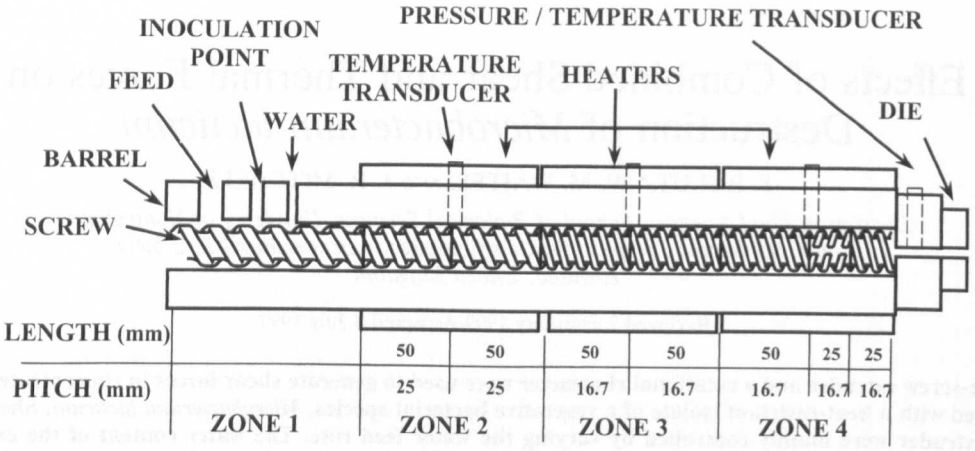

FIG. 1. Schematic diagram of Clextral BC-21 twin-screw extruder showing screw configuration and inoculation point.

The result of the extrusion experiments was supported by using a rheometer equipped with cone and plate geometry to shear samples inoculated with *M. lacticum* at lower viscosities.

MATERIALS AND METHODS

Microorganism growth and enumeration. The JP2/1/1 strain of *M. lacticum*, which is a gram-positive, nonsporing heat-resistant bacterium forming small round yellow colonies, was used as a test organism. This strain was originally isolated from pasteurized liquid whole egg (14).

Brain heart infusion (BHI) broth (Oxoid) was used for growth of liquid cultures, and BHI agar plates were used for viable counts. Phosphate-buffered saline (PBS), pH 7.3 (Oxoid), was used as the dilution medium.

Sterile universal tubes containing 10 ml of BHI broth were inoculated with 20 μl of liquid inoculum produced from a single colony taken from BHI agar. The inoculated tubes were incubated at 30°C in a shaker operating at 200 rpm. The growth curve of the microorganism obtained by reading the optical density of the liquid culture at 600 nm showed that the microorganism reached the stationary phase after about 15 h. In our experiments, 18-h cultures were used for inoculation into the extruder and preparation of the gelatin mix with the microorganism for shearing in the rheometer.

For enumeration, the Miles-Misra (13) counting method was applied, and the BHI plates were incubated at 30°C for 48 to 72 h.

Carrier medium. As the carrier medium, for extrusion experiments limed hide 225 bloom gelatin (Rousselot) was used without any treatment. For the heat resistance experiments and the experiments done with the rheometer, gelatin sieved through a 250-μm-pore-size laboratory sieve was used.

Moisture determination. Extruded gelatin samples were cut into small pieces and dried at 70°C in a vacuum oven overnight. Powdered gelatin samples were dried directly in the vacuum oven overnight.

Heat resistance tests. The heat resistance of the microorganism was tested in gelatin and PBS. In order to test the heat resistance of the microorganism in gelatin, the liquid culture of the microorganism was mixed with gelatin to give a final moisture content of 65% (wt/wt). For this purpose, 3 g of gelatin with a known amount of moisture content was mixed with a calculated amount of culture broth diluted to 50% with PBS (pH 7.3) in a sterile universal tube. This mix was kept in a 60°C water bath for 17 min in order to melt the gelatin. The pH of this mix was measured as 5.8 to 6.0 with a paper pH indicator. The mix was transferred into sterile universal tubes of known weight in 1- to 2-ml amounts by using sterile syringes. After the weight of tubes containing the mix was recorded, the tubes were immersed in a second water bath adjusted to the test temperature. A heating-up time of 3 min was applied, and then the first tube was taken from the water bath as the time zero sample. A dilution medium of 20 ml at 55°C was added to the tube and mixed with a WhirliMixer (Fisons). The weights of the tubes with the dilution medium were recorded, and serial dilutions were made for viable counting at 10- to 15-min intervals. Calculations were made on a weight basis in order to plot the survival curves.

In order to understand the effect of gelatin on the survival of *M. lacticum*, a heat resistance test was also carried out in PBS at pH 5.9 in the absence of gelatin. A culture (3 ml) of *M. lacticum* in BHI broth was added to 15 ml of PBS at pH 5.8 in a sterile universal tube. The pH of this mix was measured as 5.9. A sample (1 ml) of this mix was added to screw-cap Eppendorf tubes and immersed in a 60°C water bath for 17 min before being transferred to another water bath adjusted to the test temperature. As previously, a 3-min heating-up time was applied. Samples were taken from the water bath at 10- to 15-min intervals, and serial dilutions were made for viable counts.

Extrusion. Extrusion was carried out in a Clextral BC-21 self-wiping and corotating twin-screw extruder (Firminy, France). The 400-mm extruder barrel has a 16:1 length-to-diameter ratio and consists of four modules each 100 mm long. The temperatures of the last three modules are controlled by electrical heating and water-cooling systems. A slit die of 2 mm in height, 20 mm in width, and 14.5 mm in length was used. A transducer port located next to the feed port on the extruder barrel was modified to hold the hypodermic needle of a syringe. All inoculations were carried out from this port by using sterile syringes. A schematic diagram of the extruder showing the screw configuration and the inoculation point is given in Fig. 1. The set heater temperatures in the last three zones of the extruder barrel varied in order to achieve stable extrusion but never exceeded 45°C. The solid gelatin was added in the form of granules to the feed port at an addition rate of 3 $kg \cdot h^{-1}$ with a volumetric feeder (K-TRON, type T20). Distilled water was continuously added with a piston pump (DKM-Clextral, type TO/2). The rate of addition was varied to give the desired die pressure. Both the feeder and the water pump were calibrated prior to the extrusion in order to avoid fluctuations during the operation. The torque was continuously monitored, and the pressure and the temperature immediately before the die were measured with a combined pressure-temperature transducer. The temperature of the extrudate at the die exit (maximum temperature) was measured by carefully inserting a thermocouple insertion probe connected to a handheld digital thermometer (Digitron, 3202K) into the die. The specific mechanical energy (SME) was calculated from the following relationship:

$$\text{SME} = \frac{2 \times \text{screw speed (rad} \cdot \text{s}^{-1}) \times \text{torque (N} \cdot \text{m)}}{\text{total feed (kg} \cdot \text{h}^{-1})}$$

Residence time distribution analysis. The dye tracer method was used for the residence time distribution analysis. For this purpose, erythrosin B ($C_{20}H_6I_4O_5Na_2$) (Sigma) was used as a tracer. The mean, minimum, and maximum residence times of the material inside the extruder were determined as explained in the literature (6, 9, 15, 20).

Inoculation of the microorganism into the extruder. At a steady-state operation of the extruder, two blank samples were put into sterile stomacher bags and sealed. In order to avoid increasing the water content of the material by liquid inoculum, which in turn may reduce the local shear stress around the bacterial cell, the water pump was switched off from the control panel momentarily, and depending on extrusion moisture level, 3 to 5 ml of culture containing 1×10^9 to 8×10^9 CFU/ml was injected by a sterile syringe within 5 s. The remainder of the inoculation culture was kept in ice for determination of the exact inoculation level. As soon as the inoculation of the microorganism was finished, the water pump was switched on. The samples were collected every 15 s from the beginning of injection until 4 min after the start of the operation. Samples were put into sterile stomacher bags, sealed, and kept in ice until counted.

Sampling and enumeration of the surviving microorganisms after extrusion. For enumeration of viable cells after extrusion, samples (1 g) were removed from the middle section of each extrudate into sterile universal tubes and 19 ml of PBS was added to each tube. After the samples were melted in a 60°C shaker water bath for 45 min, serial dilutions were made and the Miles-Misra (13) counting method was applied for viable counts.

As a control, 200 μl of culture was added to a universal tube containing 1 g of blank sample, and following the addition of 19 ml of PBS, the tube was kept in the 60°C water bath for 45 min. The numbers of microorganisms in the inoculation culture and in the heat-treated control sample were determined by viable counts. The reduction in the numbers of the microorganisms in the control sample was considered when calculating the overall reduction.

In order to validate the sampling method, 1-g samples taken from the middle

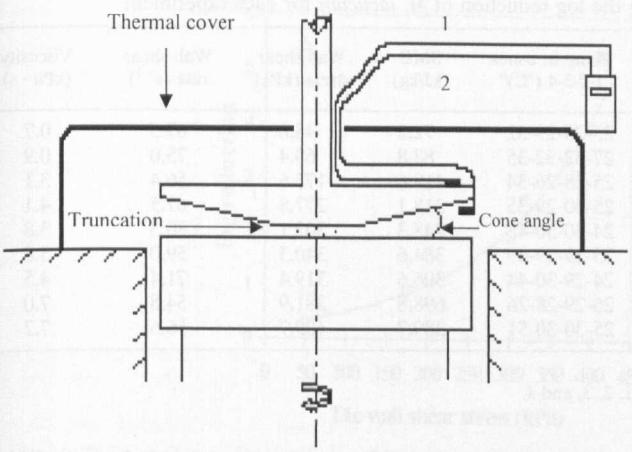

FIG. 2. Measurement geometry and thermocouple positions for Bohlin VOR rheometer.

section of each extruded sample and the remaining whole samples of extrudates were analyzed for viable count. For whole-sample analysis, sterile 1-liter Erlenmeyer flasks were used. According to sample weights, 300 to 500 ml of PBS was added to the flasks and the samples were melted as explained above. The weight basis was used to calculate the number of surviving cells.

Experiments with the rotational rheometer. A controlled-shear-rate rotational rheometer (Bohlin VOR) with cone and plate geometry (2.5° cone angle, 30-mm cone diameter) was used for shearing of the gelatin mix of *M. lacticum*. A thermal cover, as shown in Fig. 2, was used in order to prevent the heat loss during shearing of the samples. A digital thermometer (Digitron T202KC) was used to monitor the temperature of the sheared sample during the operation of the rheometer. As shown in Fig. 2, in order to avoid any interference by thermocouple 2 with the flow pattern, the temperature of the sheared sample inoculated with the microorganism was monitored indirectly by thermocouple 1. Experiments done in the absence of the microorganism showed that the temperature of the sheared sample measured by thermocouple 2 was 1.5 to 1.7°C higher than the temperature measured by thermocouple 1. When quoting the temperature of the sheared sample, this correction was taken into account.

The gelatin mix of *M. lacticum* was prepared at 65% (wt/wt) moisture content level as explained for the heat resistance test in gelatin. The inoculation level of the bacterium in the mix was between 1×10^8 and 8×10^8 cells/g of sample. A sample of the mix (1.5 ml) was added to a screw-cap Eppendorf tube with a syringe under sterile conditions. This tube containing the sample was placed in a water bath adjusted to the plate temperature of the rheometer. After 3 min of heating up, 0.5 ml of sample was removed to the plate of the rheometer. The remainder of the sample in the tube was used as a control, and the tube was submerged in the water bath after a thermocouple to monitor the temperature of the control sample was mounted. The temperatures of the sheared sample and the control unsheared sample were recorded at the start of shearing and every minute thereafter. At the beginning of shearing, the temperature of the sheared sample (monitored by thermocouple 1 [Fig. 2]) and the temperature of the control sample were 3 to 5°C lower than the set operation temperature of the rheometer. During the operation, the temperature of the samples increased gradually. During the operation time of the rheometer, the temperature of the control sample was kept higher (by 2 to 4°C) than the temperature measured by thermocouple 1 (Fig. 2). This resulted in a higher (0.6 to 2.1°C) heat exposure of the control samples than of the sheared samples. As soon as the shearing of the sample was finished, the control sample was taken from the water bath and the sheared sample was recovered with a sterile spatula. The recovered sample and a sample taken with a sterile syringe from the control sample were added to sterile universal tubes of known weights. Dilution medium (15 to 20 ml) was added to each tube in the proportion of the sample weights, and the tubes were plunged into a 60°C water bath for 10 min in order to melt the samples. Serial dilutions were prepared from both tubes for viable counts. Calculations were made on a weight basis, and the reduction in the numbers of microorganisms due to shear was obtained by subtracting the count reduction in the control sample due to heat from the overall count reduction in the sheared sample. The results were expressed in terms of log reduction.

TABLE 1. *D* value of *M. lacticum* at different temperatures in PBS (pH 5.9) and in 65% (wt/wt)-moisture-content gelatin (pH 5.8 to 6.0)

Temp (°C)	*D* value (h) in:	
	PBS	Gelatin
60	16.3	6.7
65	8.1	2.0
70	2.7	1.0
75	1.0	0.3

RESULTS

Heat resistance tests. Survival curves of *M. lacticum* at different temperatures in PBS and gelatin (Fig. 3) showed that heating in gelatin increased the killing but that at 60, 65, and 70°C even in gelatin there was less than 1 log kill in 1 h, while at 75°C there was less than 1 log kill in 45 min in PBS and less than 3 log kills in 45 min in gelatin. Payne et al. (14) reported that the microorganism shows almost no killing at 75°C in PBS, pH 7.1. They also found that the $D_{80°C}$ value of the microorganism was 9.5 min in the same medium. Compared to these *D* values, our lower *D* values in PBS (Table 1) are probably due to the lower pH of the medium employed. In addition, the heating time of 17 min at 60°C and heating-up time at the test temperature also reduced the *D* values.

Sampling for extrusion experiments. The whole-sample analysis showed that there was no significant difference between the total count obtained by 1-g-sample analysis and that obtained by the whole-sample analysis. For example, in the analysis done for the experiment where 0.9 log reduction was

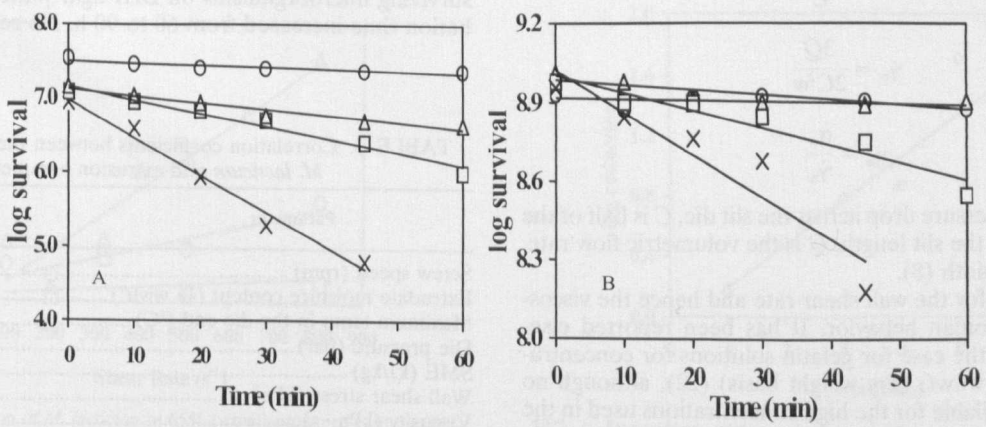

FIG. 3. Survival curves of *M. lacticum* at different temperatures in gelatin (moisture content, 65% [wt/wt]; pH 5.8 to 6.0) (A) and PBS (pH 5.9) (B). ○, 60°C; △, 65°C; □, 70°C; ×, 75°C.

TABLE 2. Parameters of the extrusion experiments showing the log reduction of *M. lacticum* for each experiment

Log reduction	Screw speed (rpm)	Extrudate moisture content (% wwb[e])	Maximum temp (°C)[a]	Die pressure (bar)	Temp in zones 1-2-3-4 (°C)[b]	SME (kJ/kg)	Wall shear stress (kPa)	Wall shear rate (s^{-1})	Viscosity (kPa · s)
0.3	100	45	50	—[c]	25-28-29-32	72.2	48.6	67.5	0.7
0.5	100	45	44	10	27-32-32-35	83.8	69.4	75.0	0.9
0.9	200	31	50	25	25-28-26-34	418.6	173.6	56.4	3.1
1.7	140	25	60	40	25-30-29-35	318.1	277.8	67.5	4.1
1.9	100	27	50	44	24-30-30-43	248.3	302.1	80.3	3.8
2.3	120	24	51	49	25-29-29-49	384.6	340.3	59.0	5.8
2.6	100	25	61	46	24-29-30-44	306.6	319.4	71.4	4.5
2.9	250	27	68	55	26-29-28-26	698.8	381.9	54.8	7.0
5.3[d]	120	19	73	59	25-30-30-51	389.7	409.7	56.6	7.2

[a] Maximum temperature measured at the end of the die.
[b] Measured temperature of the material at the barrel wall of the extruder in zones 1, 2, 3, and 4.
[c] Circular die with 3-mm diameter was used without a die pressure measurement.
[d] No residual microorganisms were detected.
[e] wwb, wet weight basis.

obtained (Table 2) the difference between the total counts obtained was less than 24%, which resulted in only 0.1 log difference in terms of log reduction. Also, residence time distribution analysis showed that the 1-g sample taken from the middle section of each extrudate provided an accurate representation of the whole extrudate and that the calculations made based on this sampling method recovered more than 90% of the dye introduced to the extruder.

Residence time distribution analysis. The calculated mean residence time was 58, 52, and 49 s for screw speeds of 120, 200, and 300 rpm, respectively. The maximum residence time ranged from 155 to 170 s, increasing with decreasing screw speed. The increase in residence time with decreasing screw speed has been previously shown for twin-screw extruders (9, 15).

Although the correlation between die wall shear stress and killing suggests that bacterial destruction occurs within the extruder die, it also seems possible that some killing will occur within the reverse screw element before the die. Residence time measurements made by using radioactive tracers (3) show that the material spends a major proportion of the total residence time in this zone, whereas in our experiments the mean residence time in the die, calculated from the die dimensions and throughput, was 1.3 s.

Microbial inactivation during extrusion. The wall shear stress (τ_w), the wall shear rate ($\dot{\gamma}_w$), and the apparent viscosity at the die wall (η_w) were estimated from the expressions

$$\tau_w = \frac{\Delta PC}{L}$$

$$\dot{\gamma}_w = \frac{3Q}{2C^2w}$$

$$\eta_w = \frac{\sigma_w}{\dot{\gamma}_w}$$

where ΔP is the pressure drop across the slit die, C is half of the slit thickness, L is the slit length, Q is the volumetric flow rate, and w is the slit width (8).

The expression for the wall shear rate and hence the viscosity assumes Newtonian behavior. It has been reported elsewhere that this is the case for gelatin solutions for concentrations up to 29% (wt/wt) (dry weight basis) (22), although no information is available for the high concentrations used in the extruder.

The log reduction of *M. lacticum* (Tables 2 and 3) shows a strong correlation with die wall shear stress, viscosity, and maximum temperature and a strong negative correlation with the moisture content. These relationships are self-consistent since a reduction in water content will result in an increase in viscosity and shear stress. The maximum temperature will increase with viscosity due to increased conversion of mechanical energy to heat. Interestingly, the correlation coefficient between log reduction of *M. lacticum* and SME was relatively low. The torque on the screws and hence the SME were related to the consistency of the product over the whole screw length. This relatively low correlation suggests that the conditions at the die and possibly at the end of the screws (reverse screw element) are most important for bacterial destruction.

The relationship between count reduction and the wall shear stress and the maximum die exit temperature are shown in Fig. 4. The temperature measured at the entrance to the die did not exceed 50°C for any experiment. The strong nonlinear relationship between the count reduction and the calculated die wall shear stress, the fact that the highest temperature recorded in the die end was below 75°C, and the observation that substantial count reduction was obtained at lower temperatures suggest that physical forces around the reverse screw element and the die play a major part in the measured destruction of the cells. Within the limit of detection (2×10^4 CFU/sample), no surviving microorganisms could be detected at the highest wall shear stress of 409 kPa, giving at least 5.3 log reduction.

It was observed that there was little increase (e.g., from 1.3×10^7 to 1.5×10^7 CFU/total samples) in the number of surviving microorganisms on BHI agar plates, when the incubation time increased from 60 to 90 h. No resuscitation media

TABLE 3. Correlation coefficients between the log reduction of *M. lacticum* and extrusion parameters

Parameter	Correlation coefficient
Screw speed (rpm)	0.33
Extrudate moisture content (% wwb[a])	−0.82
Maximum temp in the die end (°C)	0.86
Die pressure (bar)	0.85
SME (kJ/kg)	0.54
Wall shear stress (kPa)	0.87
Viscosity (kPa · s)	0.89

[a] wwb, wet weight basis.

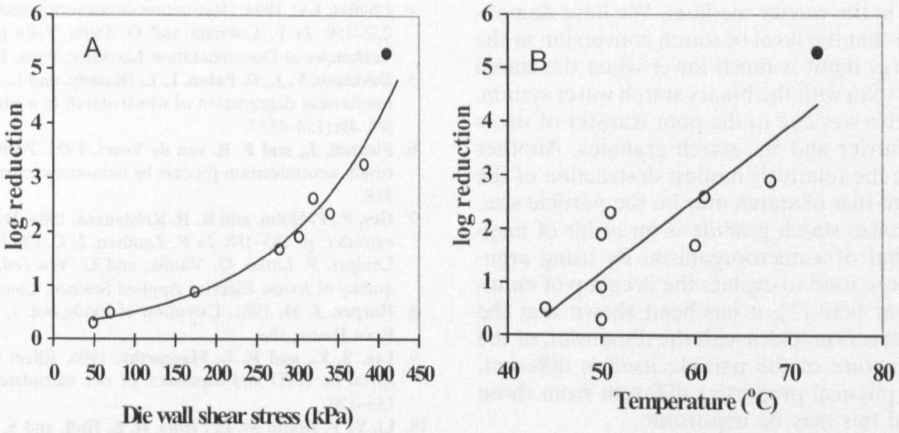

FIG. 4. The dependence of log reduction of *M. lacticum* on die wall shear stress (A) and maximum extrudate temperature measured at the die outlet (B). (Refer to Table 2 for the other parameters of each experiment.) ●, no residual microorganisms detected.

and/or techniques were used to discriminate between the inactivated and the injured cells.

Because the temperature and the shear stress distribution within an extruder are difficult to define, further experiments were carried out with a rheometer equipped with cone and plate geometry where the shear stress and shear rate throughout the sample should be constant. Because of mechanical limitations, lower sample viscosities than would be possible within an extruder had to be used.

Rheometer experiments. In the rheometer, the count reduction of *M. lacticum* relative to the control increased with increasing shear rate and temperature (Fig. 5). Shearing of the microorganism with gelatin at 65% (wt/wt) moisture content for 4 min at a shear stress of 2.8 kPa and a temperature of 75°C resulted in a maximum killing of 1.4 log cycle (Fig. 5), where the number of surviving microorganisms in the sheared sample was 5.2×10^6 CFU/g of sample compared with 1.4×10^8 CFU/g of sample in the nonsheared control.

Figure 6 displays the count reduction with time at a temperature of 75°C and a shear rate of 804 s^{-1}. The curve in this figure intersects the *y* axis at a negative value, probably because the control samples experienced slightly higher temperatures than the sheared samples.

DISCUSSION

The extrusion results suggest that bacterial destruction takes place within the reverse screw element and the extruder die region where the highest shear forces are generated. The flow patterns in the die are complex since the die is relatively short and fully developed flow does not occur. This and the fact that the wall shear stress was calculated only from an entrance pressure mean that the calculated stress should be regarded only as an estimate. Nevertheless, it is of interest to compare the values found with those required to "kill" a starch granule. "Total destruction," defined as no viable microorganisms above the limit of detection in this study, occurred at a wall shear stress on the order of 400 kPa. Zheng and Wang (23), using a combination of extrusion and capillary rheometer, found that the minimum shear stress required to convert starch ranged from 10^1 to 10^3 kPa, the value decreasing as the temperature increased. In their case, a mean shear stress taken as two-thirds of the wall shear stress was used. At a temperature of 50°C, the shear stress for a rate constant of starch granule destruction approaching the value required for complete destruction for a 1-s pass through the die was 600 kPa. However, at the moisture content employed, the peak differential scanning calorimetry temperature for the melting endotherm was 148°C.

It therefore appears that at the same shear stress, temperatures closer to the temperature required for thermal destruction of ordered structures are required for a microorganism compared with a starch granule. There are three possible reasons for the greater stability of microorganisms compared with the starch granule. A factor of significance may be the coupling

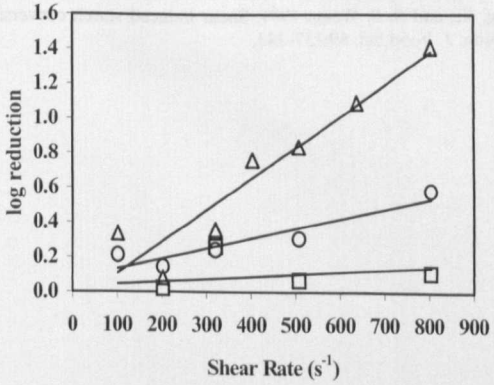

FIG. 5. Log reduction of *M. lacticum* in 65% (wt/wt)-moisture-content gelatin compared to that in the unsheared control, at increasing shear rates at 60°C (□), 70°C (○), and 75°C (△). Shearing time was 4 min.

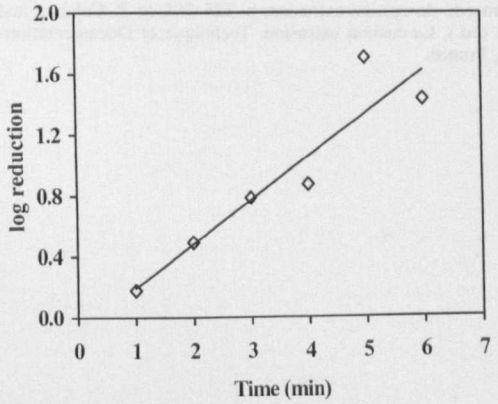

FIG. 6. Dependence of log reduction of *M. lacticum* in 65% (wt/wt)-moisture-content gelatin on the time of shearing in the rheometer at 75°C and a shear rate of 804 s^{-1}.

between the particles in the carrier medium. We have demonstrated elsewhere (21) that the level of starch conversion at the same mechanical energy input is much lower when the starch is in a gelatin medium than with the binary starch water system. It was suggested that this was due to the poor transfer of stress between the gelatin carrier and the starch granules. Another factor that can explain the relatively modest destruction of the bacteria compared with that of starch may be the particle size. The linear size of a maize starch granule is an order of magnitude greater than that of a microorganism. By using arguments analogous to those used to explain the breakup of emulsion droplets in a shear field (1), it has been shown that the stress required for destruction scales with the dimension of the particle. Thirdly, the nature of the particle itself is different. Microorganisms have physical properties different from those of starch granules, and this may be important.

The maximum shear stress in the rheometer was about 3.0 kPa. This is more than 2 orders of magnitude lower than that required for the maximum detectable killing in the extruder. It is therefore not surprising that there is a maximum killing on the order of only 1 log reduction in the rheometer compared with a 5 log reduction in the extruder. It is clear from the rheometer experiments that, in addition to shear stress, important factors are time and temperature. It would be possible to combine these three factors into "shear *D* value," which would be defined as the time for a decimal reduction at a specific temperature and shear rate. For example, from the data in Fig. 6 the shear *D* value for *M. lacticum* at 75°C and a shear rate of 804 s^{-1} would be on the order of 3.5 min. A comparison between the shear *D* value and the *D* value for heat alone might provide some interesting information about the structure of microorganisms. Finally, we consider that it would be interesting to compare the results with a different carrier medium and determine whether elongational stresses have a greater effect than shear stresses.

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PUBLICATIONS

The following publication and the oral presentations have been based on the work described in this thesis.

PUBLICATIONS

Bulut, S., Waites, W.M. and Mitchell, J.R. (1999). Effects of combined shear and thermal forces on destruction of *Microbacterium lacticum*. *Applied and Environmental Microbiology*, **65**, 4464-4469.

ORAL PRESENTATIONS

1) Bulut, S. Destruction of microorganisms by combined shear and thermal forces. *Food Micro'99*, 13-17 September 1999, Veldhoven, Netherlands.

2) Bulut, S. Effect of combined shear and thermal forces on destruction of microorganisms. 10th World Food Science and Technology Symposium, 4-8 October, 1999, Sydney, Austalia.