Understanding the effects of high-pressure, high-temperature processing on the key quality parameters of green beans (*Phaseolus vulgaris)* with a view to assessing the potential quality benefits of the approach relative to conventional thermal processing

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Abstract

Studies were conducted to explore whether high pressure (up to 700 MPa) could be used in combination with elevated temperatures (up to 90°C initial temperature) to produce ambient stable green beans (*Phaseolus vulgaris*) with improved quality compared with conventionally heat processed samples. Colour changes, texture change and chlorophyll retention were explored at a range of pressures, temperatures and times using a surface response methodology. Texture changes were essentially related to temperature effects; higher temperatures resulted in a greater loss in texture. Significant improvements in texture retention were possible using High Pressure Sterilisation (HPS) but sample colour was negatively affected. Colour parameters were predicted primarily by time and pressure so deterioration in green vegetable quality for a commercially sterile products appears inevitable when using HPS. The use of ohmic heating as a pre-heating method greatly reduced cook values (T_{ref} = 100°C, z = 39C°) for colour degradation (down to 0.24, 0.12, 0.35 from 3.02, 2.50, 3.70 minutes for ohmically heating and water bath heated samples respectively) which yielded significant benefits in terms of colour retention of raw materials at the start of the HPS cycle; values of a^* and b^* for ohmically pre-heated samples were close to that of blanched beans.

Dedication

I would like to dedicate this thesis to my mam and dad for swimming against the tide and encouraging education and for not throttling me when it was no doubt deserved….

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List of definitions and/or abbreviations

(e.g. Roman, subscripts, dimensionless groups, Greek)

F_0 :	The lethal effect from a given thermal process, equivalent to the number of minutes at 121.1°C when assuming instantaneous heating and cooling (Department of Health 1994)
T:	Temperature (°C)
$T_{i:}$	Time (minutes)
'D' value:	Time (in minutes) at a given temperature to bring about a 10 fold reduction in a microbial population
'z' value:	The change in temperature (°C) required to bring about a 10 fold change in the 'D' value
z_p:	The change in temperature (°C) required to give a 10 fold change in the 'D' value at constant pressure
z_t:	The change in pressure (MPa) required to give a 10 fold change in the 'D' value at constant temperature
L^* :	Lightness parameter in CIE Lab colour space
a^* :	Colour parameter describing green to red in CIE Lab colour space
b^* :	Colour parameter describing blue to yellow in CIE Lab colour space
C^*:	Chroma (saturation) in CIE Lab colour space
ΔE:	Total colour change relative to a reference sample
P:	Pressure (MPa)
ρ:	Density ($kg.m^{-3}$)
C_p:	Specific heat capacity (J.kg.K)
α :	Thermal expansivity (1/K)
H:	Heckel Value
φ :	Relative density (ρ/ρ_{abs})
ρ_{abs}:	Absolute density after compression ($g.cm^3$)
P_f :	Peak Force (N)

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1 Chapter 1 – Introduction

1.1 Thermal processing for food preservation

Modern western consumers increasingly demand minimally processed, 'additive' free foods that are nutritionally sound, convenient, 'fresh-like' and yet have sufficient shelf-life to be compatible with modern weekly shopping habits. These demands have presented significant technical challenges for the food industry. Ambient stable foods are those which have a significant shelf-life without a requirement for chilling. Conventional processes for achieving ambient shelf stability include drying, aseptic processing and canning. The shelf-life of such foods varies quite widely depending upon the nature of the product. Taking canned products as an example, the shelf-life of canned fruit and vegetables can vary between around 9 months and 3 years (Holdsworth 1983). Handling, container construction and storage conditions can all influence the achievable shelf-life (Holdsworth 1983).

Conventional thermal preservation technologies for ambient stable 'canned' products have an excellent safety record (when applied correctly), are generally high throughput and can provide convenient, reasonable quality foods at a low cost (Mintel 2007). Canned fruits and vegetables are typically processed in a manner similar to that shown in Figure 1-1 (Holdsworth 1983).

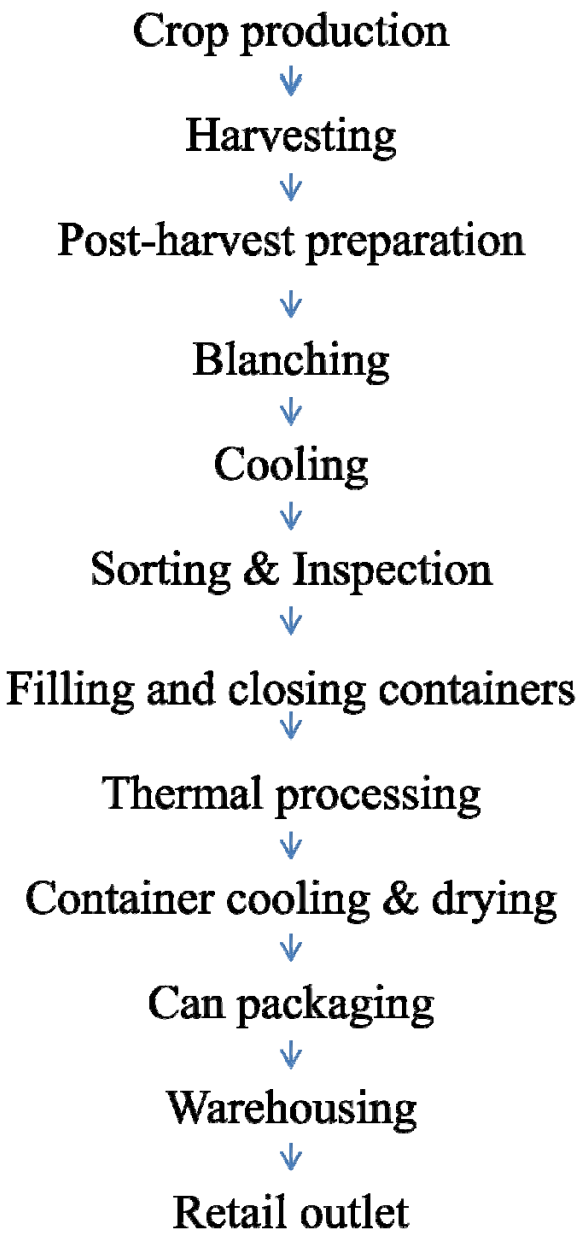

Figure 1-1. Flow sheet of main operations in a canning line (Holdsworth 1983).

Although canned foods are safe and affordable, such products are viewed as 'old fashioned' and of poor quality compared with chilled alternatives. For example, a recent Mintel market intelligence report noted that there was "Scepticism of consumers regarding the quality and healthiness of frozen and canned fruit and vegetables" and that "The frozen and canned fruit

and vegetable sectors continue to be plagued by the long-standing image that they are second best to fresh. Some 43% of consumers mainly buy fresh fruit and vegetables thinking that frozen and canned are less healthy and of poorer quality". (Mintel 2007). The loss of 'fresh-like' characteristics in heat processed foods is particularly problematic in fruits and vegetables where deleterious changes such as softening and discoloration can occur. Although there has been reasonable growth in the canned food market in recent years, this mature market struggles to compete with the chilled foods market. To take one example, the wet ambient soup market was valued at £260 million in 2005, up 11% from 2001. The fresh soup market grew by over 40% in the same time period (Mintel 2007).

Chilled heat processed foods such as cook-chilled ready meals (typically having a shelf-life of under 10 days) and Refrigerated Pasteurised Foods of Extended Durability (so called 'REPFEDs' typically having a chilled shelf life of 10-30 days) offer improved quality, in part, due to a much reduced thermal input. For example, a chilled product with less than 10 days shelf-life is typically subjected to a process equivalent to 70°C for 2 minutes, in order to bring about a 6 log reduction of *Listeria monocytogenes* (Betts & Betts 2009). A REPFED typically receives a process equivalent to 90°C for 10 minutes in order to bring about a 6 log reduction of psychrotrophic strains of *Clostridium botulinum* (Betts & Betts 2009). In either case the thermal input is significantly less than the minimum process of 121.1°C for 3 minutes (or equivalent) that is normally applied to canned foods (Department of Health 1994). Although the thermal process is less severe in chilled products like the one just described, the processes overall are energy intensive because of the need for chilled distribution and storage; they still

suffer from some quality degradation as a result of thermal damage and they are restricted to a relatively short shelf-life compared with heat sterilised products.

1.2 Calculation of thermal process lethality

Methods for the establishment of safe thermal processes for food production are complex and specialised. For detailed descriptions of typical approaches for process safety establishment the reader is referred to a range of publications from the Institute for Thermal Processing Specialists such as IFTPS (2004a) and IFTPS (2004b). In simple terms, when setting up an in-container pasteurisation or sterilisation process, in order to establish the required conditions for safety it is usual to carry out replicated measurements of the time temperature history of the product as it is heated inside a process vessel (e.g. a retort). Microbial inactivation as a result of heat treatment is a dynamic process; methods have been developed to integrate the lethal effect of non-isothermal heating (as is typically observed during commercial heat processing of foods) such that the overall effect of a time temperature profile on microbial inactivation can be determined. This integrated lethal effect of a time-temperature profile for sterilised products is established using Equation 1.1.

$$F_o = \int_0^t 10^{\frac{T-121.1}{10}}.dt \qquad (1.1)$$

Where :

F_o = the accumulated lethality of the measured temperature, with time, expressed in minutes at the reference temperature (121.1°C in the case of sterilised products).

T = the measured temperature of the product at time t in the time/temperature profile.

1.3 Emerging methods for food preservation

A number of alternative 'emerging' preservation processes have attracted research interest because such technologies may provide products having improved quality over those made using conventional thermal preservation and which address some of the limitations described previously. Many of these technologies remain very much in the research arena, some are on the brink of commercialisation and some (such as high pressure pasteurisation) are already in use, albeit on a relatively limited scale.

Some of the major emerging preservation technologies that are attracting research and commercial interest are briefly described below along with, where appropriate, details on current commercial applications. The list is not exhaustive, merely illustrative of the range of technologies that are coming to prominence. A comprehensive review of many of these technologies was published as a special supplement of the Journal of Food Science in 2000 and this review is recommended for readers interested in sources of information for a more in-depth discussions of various emerging technologies (IFT 2000). Although considered 'new' many of these technologies have been studied for decades and some, such as high pressure and irradiation, for 100 years or more.

1.3.1 Power ultrasound

Power ultrasound (typically in the range of 20kHz to 100kHz) has numerous non-preservation applications such as cutting, cleaning, emulsifying, de-gassing and foam breaking (Mason 1998;Patist & Bates 2008). It is not thought to be currently in use for microbiological inactivation in food production but there is evidence at laboratory scale demonstrating anti-microbial effects such as Patist & Bates (2008), Piyasena *et al*. (2003), Miles *et al*. (1995), Raso *et al*. (1998c), Ordonez *et al*. (1984) and Raso *et al*. (1998b). For the inactivation of microorganisms, it is usually suggested that ultrasound is combined with moderate heating and, in some cases, slightly elevated pressure; processes known respectively as thermosonication and manothermosonication. See for example Hurst *et al*. (1995), Sala *et al*. (1995) and Bermúdez-Aguirre & Barbosa-Cánovas (2008). Greater understanding is required relating to process scale up, inactivation mechanisms and food quality effects before power ultrasound can realistically be considered for preservation applications. It could prove very useful as a tool for augmenting conventional heating processes and is already being used for this purpose in some food processing facilities.

1.3.2 High Intensity Pulsed Light

Pulsed light is a surface preservation method in which a material is subjected to very short pulses (of the order of milliseconds) of broad-spectrum white light including wavelengths in the ultraviolet and near infrared range. The product is typically exposed to 1-20 pulses having an energy density in the range of 0.01 to 50 $J.cm^{-2}$ at the surface (IFT 2000). The main company that pioneered this method was Pure Pulse Technologies who claimed that their PureBright® system used a light spectrum closely resembling that of sunlight, but which was massively more intense than sunlight at the earth's surface (figures of 20,000 and 90,000 times

greater intensity have been quoted). More recently, a French based company, Claranor, has had some success in the sale of pulsed light systems for the food industry. For example, Nestlé are reported to be using a Claranor system for sterilising bottles caps and pulsed light is being used for the decontamination of salt water sprays for medicinal use (Genaux 2006). There are now claimed to be at least 10 Claranor commercial units in operation in Europe (Claranor 2010).

Pulsed light suffers from some of the same limitations affecting continuous ultraviolet light treatment, namely that areas of the treated surface that are in shadow are not effectively decontaminated. This problem can be alleviated by treatment chamber geometry and by the feed methods used to present the sample to the light source (personal communications with Claranor). On smooth surfaces such as stainless steel, vegetative bacterial cells can be reduced from 10^6 cfu/cm^2 to the limits of detection after only 1 pulse of 300-microsecond duration (Shaw *et al.* 2009). The level of microbial inactivation that can be achieved on food surfaces is however limited due to surface roughness effects. Trials at Campden BRI have determined that a 1-3 log reduction in total viable counts is typically achievable on food surfaces such as meat, cheese and fruit (Shaw *et al.* 2009) and similar results have been reported by other groups (Dunn *et al.* 1995;Ozer & Demirci 2006).

Potential applications for pulsed light in the food industry include the decontamination of packaging surfaces and of transmissive materials such as water and air. Applications for food are likely to include relatively short shelf-life products that are susceptible to mould growth such as baked goods. Complete surface pasteurisation is unlikely to be achievable on most

food surfaces but even a relatively modest reduction in microbial numbers could offer a significant shelf-life extension for very short-shelf life products.

1.3.3 Irradiation

Food irradiation is described by the UK Food Standards Agency as "a processing technique that exposes food to electron beams, X-rays or gamma rays, and produces a similar effect to pasteurisation, cooking or other forms of heat treatment, but with less effect on look and texture" (UK Food Standards Agency 2010). X-rays and radioactivity were discovered at the end of the 19th century and in the early years of the twentieth century, the possible application of these concepts to the preservation of food was already being investigated (Brennan *et al.* 1990). Irradiation of strawberries, for example, was investigated in Sweden in 1916; a paper on the elimination of *Trichinella spiralis* in pork with X-rays was published in 1921; and a patent for food preservation using ionising radiation was issued in France in 1930 (Brennan *et al.* 1990).

Three types of ionising radiation can be used for food irradiation – gamma rays, X-rays and electron beam radiation. Both gamma rays and X-rays are photons, whereas an electron beam is made up of β-particles. In all cases, the energy of the radiation source is limited/selected such that when the food is irradiated, electrons can be ejected from their orbits around molecular nuclei, but there is no interaction with the nuclei themselves, which would induce radioactivity (Grandison 2006;Woolston 2006).

Gamma rays are very short wavelengths at the ultraviolet end of the electromagnetic spectrum. They penetrate deeply into food, making them very useful for food irradiation.

The most widely used source of gamma rays is the radioactive isotope cobalt-60 (^{60}Co), which produces gamma radiation during its decomposition to the stable nickel-60, although caesium-137 (^{137}Cs) is also used (Leadley *et al.* 2002). Cobalt-60 has a half-life of 5.3 years and its radioactivity reduces by about 1% per month, so it must be replaced at suitable intervals.

X-rays, like Gamma rays, have very short wavelengths and good penetration depth in food materials. They are generated from machine sources, making them very controllable, but the conversion of electricity to X-rays is inefficient and expensive and hence this technology is less commonly used for food applications.

High energy electrons generated by an electron accelerator can also be used to irradiate food. However, application of this method is limited by the penetration depth relative to gamma or X-ray radiation. Electron beam irradiation has a maximum penetration depth of around 4 cm (Grandison 2006). This effectively limits the maximum sample thickness that can be treated is around 8 cm if irradiated from two sides. Electron beam irradiation therefore tends to be used on products that can be arranged in thin layers during the process.

Microbial inactivation is brought about by damage to nucleic acids by oxidative radicals originating from the radiolysis of water (Farkas 2006). In general, Gram negative microorganisms such as the common spoilage bacteria *Pseudomonas* and important pathogens such as *Salmonella* and *Escherichia coli* are more sensitive to ionising radiation than Gram positive bacteria but exceptions do occur (Farkas 1989). Different strains of the same organism can differ in their ability to survive irradiation. Pathogens such as *E.coli,*

Salmonella spp., *Listeria monocytogenes* and *Campylobacter* species are all relatively sensitive to irradiation, generally requiring doses of 0.5kGy or less to reduce the population by 90% in ground beef and poultry (Patterson 1990). A dose of 2.5kGy eliminates *Salmonella* from chicken carcasses and extends shelf life by 7 days at 3ºC (Patterson 1990). Food borne pathogens capable of growth at low temperatures, e.g. *Listeria* and *Aeromonas* species, are also sensitive to irradiation and will be eliminated by similar doses to those for *Salmonella*.

Most food spoilage bacteria are sensitive to radiation, although strains of the *Acinetobacter-Moraxella* have some resistance to radiation and may form a large proportion of the bacterial survivors on food irradiated with moderate doses. In experiments carried out on red meat and poultry, certain strains of this genus were shown to be even more resistant to radiation than spores, although their presence in such food is not significant from either a public health or a sensory perspective (Farkas 1989).

The radiation sensitivity of moulds is similar to that of vegetative bacteria. A dose of 2kGy can inhibit mould growth in strawberries, giving an extended shelf life of 14 days in chilled storage compared to 6 days without irradiation (Patterson 1990). Yeasts are more resistant, of a similar order to the most resistant bacteria, while viruses are very resistant to radiation and are unaffected by the maximum dose of 10kGy permitted for food irradiation.

Bacterial spores are capable of surviving irradiation, but are more sensitive to heat following the process. A dose in the region of 25-50kGy (known as Radappertisation), would be needed

to reduce all microorganisms to below the limits of detection but this is much higher than the doses typically used for food (which generally have an upper limit of 10 kGy). If the object of irradiation is the extension of shelf-life then the doses used will not destroy all bacterial spores. Care therefore needs to be taken with doses and subsequent storage to ensure that any pathogens capable of growth are controlled within the expected shelf life and storage conditions of the product.

The 'wholesomeness' of irradiation in terms of its effects on food toxicity, microbiological safety and nutritional status has perhaps been more widely investigated than any other novel preservation method, having been extensively studied for over 50 years (Farkas 2006). The FAO/IAEA/WHO expert committee on food irradiation concluded that "the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard…" and "introduces no special nutritional or microbiological hazards" (Brennan *et al.* 1990;Farkas 2006;Leadley *et al.* 2002).

In the same way in which the safety of irradiation has been extensively studied, there is an enormous body of research relating to the practical use of irradiation to extend the shelf-life of fruit, vegetables, meat and seafoods (Farkas 2006). The route to commercialisation of irradiation and the arguments for and against the technique have been widely debated. For example, critics such as "the food commission" argue that the wide spread use of irradiation would allow companies to relax hygiene standards (The Food Commission 2002) and that the technology offers limited benefits to consumers. Today, despite the availability of commercial systems and the proven efficacy of the process, industrial use is limited. The

Food Commission cites studies on attitudes to irradiation from EU consumer groups that suggest that there is little appetite for the technology (The Food Commission 2002). More recently, a review on public attitudes to emerging technologies concluded that irradiation "provokes responses of wariness and is a technology for which there is low understanding" (Lyndhurst 2009). There is evidence from some countries e.g. the USA that when consumers are provided with sufficient information they can be convinced of the benefits of irradiation but "on the whole attitudes towards novel food technologies in the USA and in Asian and developing countries seem to be more positive than they are in Europe. There are differences even amongst European attitudes though" (Lyndhurst 2009).

There are around fifteen facilities in the EU that are approved for food irradiation (Anon 2002). The exact amount of food irradiated per year in the EU is not certain, but an estimate for 2001 was around 22,000 Tonnes . Herbs, spices and poultry products account for the major proportion of this total.

Irradiation has been so comprehensively studied (Farkas 2006) that it can almost be considered as a 'conventional' non-thermal preservation method. Consumer confidence would appear to be the main barrier to irradiation being considered as a credible preservation method in the UK.

1.3.4 Pulsed Electric Field processing (PEF)

Pulsed electric field processing is a technique in which a food is pumped between paired electrodes and exposed to a pulsed high voltage field (typically 20-80 kV/cm for anti-microbial purposes) (IFT 2000). Treatment times are of the order of less than 1 second for

pasteurisation applications. This process reduces levels of microorganisms whilst minimising undesirable changes in the sensory properties of the food. Pasteurisation using PEF is ideally suited to pumpable homogenous products. Pasteurisation of foods having large particulates is not feasible because of the physical restrictions relating to the gap between electrodes. Products having a high salt content are also unsuitable for PEF treatment because their higher electrical conductivity reduces the resistance of the chamber and more energy is therefore required to reach the required electrical field strength for pasteurisation (IFT 2000). Examples of potential pasteurisation applications include the non-thermal treatment of various products such as fruit juices, liquid dairy products, beers and wines, egg products and tomato products (Floury *et al.* 2006;Hodgins *et al.* 2002;Jin & Zhang 1999;Kui *et al.* 2005;Martin *et al.* 1997;McDonald *et al.* 2000;Michalac *et al.* 2003;Min *et al.* 2003b;Min *et al.* 2003a;Min & Zhang 2003c;Wu *et al.* 2005;Zhang & Mittal 2005).

The main hurdle restricting commercialisation of PEF was, until recently, problems associated with scale-up of the equipment. The development of solid state switching systems in recent years has finally opened up the possibility of full scale-up to almost any throughput desired by the manufacturer. The work of a PEF consortium funded by the US 'DUST' programme (Dual Use of Science and Technology) led to the manufacture of a large scale processing unit that was installed and commissioned at the Ohio State University. The 'OSU-6' consists of 4 treatment chambers with a cooling system before and after each one to control the temperature of the product (Zhang 2002). A throughput of up to 2000 $L.hr^{-1}$ was achievable and the University successfully conducted trials on the pasteurisation of products such as orange juice, tomato juice, salsas and yoghurt products (Zhang 2002).

In 2002, the capital cost for a commercial PEF unit capable of processing 5000 $L.hr^{-1}$ was estimated to be around £460,000 (Zhang 2002). Depreciating the equipment over 5 years, the cost per litre of juice pasteurised using PEF was estimated to be around 0.02 £/L; this included all personnel, maintenance and utility costs. This was reported to be broadly in line with the costs associated with conventional thermal pasteurisation (Zhang 2002) but there has been some debate as to how these figure compare to a conventional thermal pasteurisation incorporating a regeneration step. More recently, a PEF installation at Stork Food & Dairy had an estimated investment cost of 2 million Euros and an estimated total cost of 1-2 Euro cents per litre of product (Toepfl 2007). A 65 kV/cm system with a 3mm chamber operating at up to 150 litres per hour had an estimated capital investment cost of 250,000 Euros (Toepfl 2007).

Large-scale equipment is now not only feasible but can be built to specification by companies such as Diversified Technologies Inc. (Massachusetts, USA, www.divtecs.com) and DIL (Germany, www.dil-ev.de). A number of European research facilities have laboratory scale equipment; pilot scale PEF equipment is available in research institutes including A&F (the Netherlands) and DIL (Germany).

In or around 2006 a US manufacturer, Genesis Juice in Eugene Oregon became the first manufacturer to sell foods pasteurised using pulsed electric fields. The company was believed to operate 3-4 shifts per week at a flow rate of 200 litres per hour (personal communication with Howard Zhang, USDA). The company appears to have now switched

from the use of PEF to high-pressure pasteurisation (http://www.genesisorganicjuice.com/faq.html , website last viewed April 2011).

There are at least 2 other commercial PEF plants in operation, one in Germany (Toepfl 2007) and one in Spain (personal communication with Professor Philip Richardson, Campden BRI, 2007 after a site visit to the plant). Both are applications for the permeabilisation of plant tissue for extraction purposes rather than for pasteurisation. Plant cells can be permeabilised at lower field strengths than are required for microbial inactivation. For example, Knorr & Angersbach demonstrated enhanced release of cell liquid from potato cubes at field strengths as low as 0.5 to 3 kV/cm (Knorr & Angersbach 1998). This potential for enhanced extraction using low field strengths makes the use of PEF as a processing aid a commercially realistic proposition (Heinz 2007;Toepfl 2007). There is thus growing interest in this use of PEF for enhancing extraction processes. Data presented by DIL, Germany suggested that PEF enhanced extraction yields could be better than current commercial practice using enzymes (Toepfl 2007).

1.3.5 Ohmic heating

Ohmic heating, where heat is generated by the passage of alternating electrical current (AC) through a body such as food, has been in use since the nineteenth century. Other names for this technology include resistance heating, direct resistance heating, Joule heating and Electroheating™ (Christian & Leadley 2006). Ohmic technology relies on the electrical resistance of the food to generate heat. Therefore if the electrical resistances of all components of the product are constant then the product heats uniformly. Particulate products can therefore be heated uniformly, overcoming the limitations of heat transfer in conventional HTST systems

such as plate, tubular or scraped surface heat exchangers. Products such as fruit juices and concentrates, shelf-stable milk, puddings, soups and liquid egg products can be heated rapidly, uniformly, and with reduced impact on the organoleptic properties of the product (Christian & Leadley 2006). Ohmic heating is not a new principle, several processes were patented for the use of electrical currents to heat pumpable liquids in the 19th Century and it was used for milk pasteurisation in the early 20th Century (De Alwis & Fryer 1990;Ruan *et al.* 2004). Although rapid volumetric heating offers quality benefits over conventional canning it is important to note that ohmic heating is still a thermal process and in this respect there are limits to what can be achieved in terms of preserving 'fresh-like' quality. The process is also difficult to operate without closely matching the physical properties of the different phases within the food. Having said this, ohmic heating is starting to be used more widely; the Italian Company Emmepiemme has installed at least 30 industrial ohmic heating plants worldwide. Applications include low and high acid products and even large particulate products such as peach halves (Municinó 2007).

1.3.6 Microwave and radio-frequency heating

Microwave and radio frequency heating are well-established thermal processing technologies that have found application in many process sectors. Commercial installations are common in the plastics, textiles, paper and board and wood (Decareau & Peterson 1986) and applications in the food industry are now increasingly common (Sumnu *et al.* 2005). The use of Radio Frequency processing for food thawing was commercialised in the 1960's and its use for food preservation was suggested in the early 1970's (Marra *et al.* 2009). Radio frequency has found wide-spread use as a post-baking drying process for cookies and snack foods, an application that began in the 1980s (Marra *et al.* 2009). Microwave processing has many

applications where pasteurisation or sterilisation is not necessarily the key objective. These include cooking, thawing, freeze drying, tempering, rendering, frying, blanching and drying (Sumnu *et al.* 2005). Food applications for *preservation* using microwave or radio-frequency heating are not however in widespread use.

In microwave and RF heating, as in ohmic heating, product heating occurs volumetrically. As a result, particulate heating is not dominated by conduction heating. This makes both techniques potentially attractive as rapid methods for thermal pasteurisation or sterilisation.

Microwave and radio frequency heating refers to the use of electromagnetic waves at particular frequencies used in order to generate heat in a food material. The frequency bands allocated for Industrial, Scientific and Medical (ISM) use are listed in Table 1-1. Of these frequencies, 896/915 MHz is generally used for microwave processing applications and 2450 MHz is used for domestic microwave ovens and some industrial process applications. Radio frequency heating utilises each of the frequencies outlined (although 40.68MHz is rarely used) depending on the application.

Nature	Frequency
Microwave	896 MHz (UK) or 915 MHz (US) 2450 MHz 5800 MHz 22125 MHz
Radio frequency	13.56 MHz 27.12 MHz 40.68 MHz

Table 1-1. Electromagnetic frequency allocations for ISM purposes (Mullin 1995)

The major advantage of microwave/RF heating is, as for ohmic heating, the rapid and volumetric temperature rise that can be achieved in foods. This results from two primary

heating mechanisms, a dielectric effect and an ionic heating effect (IFT 2000). Dielectric heating occurs when the water molecule, the principal constituent in most food materials, oscillates at the very high frequency of the microwave/RF field; such oscillations produce rapid heating. Ionic heating occurs when the oscillatory motion of ions in the food, under the influence of the microwave/RF fields, produces a heating effect. In food processing, the advantages of the rapid heating effects are amplified by the fact that both microwave and RF frequencies can penetrate the food to a depth of several cm (microwave) or tens of cm (RF), promoting a volumetric heating effect throughout the food. This can offers significant advantages over conventional thermal processing as high-temperature-short-time processes can minimise thermal damage to the food product but as has been noted for ohmic heating, microwave and RF are still in essence thermal processes with a limited capability to preserve 'fresh-like' quality.

Although microwave and radio-frequency heating offer potential quality and processing efficiency benefits there are disadvantages, most notably greater complexity with respect to process validation and high capital and operating costs. Unlike in conventional heating, it is less straight forward to predict the time-temperature history of different regions of a food product being heated in a microwave or radio-frequency field (Fellows 2009). As a food heats in a microwave field, the rate of microwave absorption increases leading to higher temperatures leading to greater absorption and so forth. This can lead to a phenomenon called 'runaway heating' (Fellows 2009). Whilst this phenomenon in itself can lead to practical challenges it is also more difficult to measure temperatures in a microwave or radio frequency field because conventional thermocouples cannot be used.

1.3.7 'Conventional' high pressure processing (HPP)

As currently used commercially, HPP is a non-thermal pasteurisation process in which a food is subjected to pressures in the region of 150 MPa to around 700 MPa (1500 to 7000 bar) and held at pressure for a time, generally under 5 minutes.

The extremely high pressures used for HPP can inactivate vegetative microorganisms, yeasts, moulds and certain enzymes (Simpson & Gilmour 1997) and a wealth of data are available to demonstrate its efficacy for this purpose in a wide range of food products (Table 1-2). The extent of microbiological inactivation is affected by many factors including; the intrinsic properties of the food such as its pH, water activity, fat content, protein content, mineral and sugar content (Black *et al.* 2007a;Hauben *et al.* 1998;Molina-Hoeppner *et al.* 2004;Oxen & Knorr 1993;Patterson *et al.* 1995;Rendueles *et al.* 2011); life cycle effects (organisms tend to be more sensitive to HPP in the exponential phase of growth) and the pressure, temperature and time combinations that are applied (Patterson *et al.* 2007;Rendueles *et al.* 2011).

Table 1-2, adapted from reviews by Patterson *et al*. (2007) and Rendueles *et al*. (2011), provides example studies demonstrating the efficacy of HPP as a preservation process against a wide range of microorganisms. Note the considerable variation in pressure resistance species-to-species and the also the effect of substrate on the level of inactivation achieved. Compare, for example, Table 1-2 rows 5 and 6 where a lower hold pressure (row 6) gave a higher level of reduction of *E.coli* O157:H7 because of the effect of the food substrate (orange juice rather than milk).

	Microorganism	Substrate	Treatment conditions: Pressure (MPa)/Temp(°C)/time (min)	Log_{10} reduction	Source
1	*Aeromonas hydrophila*	Ground pork	253/25/15	7	(Ellenberg & Hoover 1999)
2	*Bacillus cereus*	Skimmed milk	400/30/18	2.9-3.4	(McClements *et al.* 2001)
3	*Campylobacter jejuni*	Pork slurry	300/25/10	6	(Shigehisa *et al.* 1991)
4	*Escherichia coli* (ETEC)	Skimmed milk	600/20/15 700/20/15	3.44 >7	(Linton *et al.* 2001)
5	*E.coli* O157:H7	Skimmed milk	600/20/15 700/20/15	4.2-6.7 >7	(Linton *et al.* 2001)
6	*E.coli* O157:H7	Orange juice (pH3.4 – 3.9)	550/20/5	>7	(Linton *et al.* 1999)
7	*Listeria monocytogenes*	Raw poultry meat	375/20/30	6	(Patterson *et al.* 1995)
8	*Listeria monocytogenes*	Skimmed milk	400/30/24	4	(McClements *et al.* 2001)
9	*Pseudomonas fluorescens*	Skimmed milk	250/30/18	5	(McClements *et al.* 2001)
10	*Saccharomyces cerevisiae*	Pork slurry	300/20/10	2	(Shigehisa *et al.* 1991)
11	*Salmonella* Enteritidis	Orange juice	350/30/5	>8	(Bayindirli *et al.* 2006)
12	*Salmonella* Senftenberg 775W	Strained baby food	340/23/10	<2	(Metrick *et al.* 1989)
13	*Staphylococcus aureus*	Raw poultry meat	600/20/30	<4	(Patterson *et al.* 1995)
14	*Vibrio parahaemolyticus*	Oyster homogenate	300/10/2	>6	(Cook 2003)
15	*Yersinia enterocolitica*	Ground pork	304/25/15	7	(Ellenberg & Hoover 1999)
16	Norovirus	Oysters	400/5/5	4 log (PFU)	(Kingsley *et al.* 2006)

Table 1-2. Response of microorganisms to HPP. Adapted from Patterson *et al.* (2007) and Rendueles *et al.* (2011).

Because the process often does not involve significant heating, the sensory and nutritional quality of products can be remarkably similar to their unprocessed counterparts. The non-

thermal nature of high-pressure pasteurisation makes it an excellent candidate process for preserving the 'fresh-like' characteristics of foods. Unfortunately, bacterial spores are very resistant to commercially achievable pressures (see section 2.4). As a result, products that are currently on the market tend to be chilled and/or contain additional preservation hurdles such as pH or water activity control or other combinations of factors that have been demonstrated to prevent the growth of psychrotophic strains of *Clostridium botulinum* through microbiological challenge testing (personal experiences gained through working with the food industry on HPP product development on a confidential basis).

An additional challenge presented by HPP pasteurisation is that the response of enzymes to HPP is complex and substrate dependent. For example, HPP resistance of Pectin Methyl Esterase (PME) has variously been reported to be everything from "extremely resistant to moderately labile"(Katsaros *et al.* 2010) largely depending on the source of the enzyme. Many studies have explored the effects of HPP on PME inactivation in foods such as tomato products, citrus juices and various other fruits and vegetables (Basak & Ramaswamy 1996; Boulekou *et al.* 2010;Broeck *et al.* 2000;Cano *et al.* 1997;Goodner *et al.* 1998;Hernandez & Cano 1998;Katsaros *et al.* 2010;Nienaber & Shellhammer 2001;Parish 1998;Plaza *et al.* 2007;Porretta *et al.* 1995;Rodrigo *et al.* 2006;Stoforos *et al.* 2002;Van den Broeck *et al.* 1999).

Stabilising a food product microbiologically using HPP is, in many respects, simple compared with the challenges associated with preventing non-microbial spoilage due to residual enzyme activity because there is still a relatively high degree of uncertainty as to how enzymes present in a complex food recipe might respond to HPP.

To produce ambient stable products, high pressure must be combined with other preservation hurdles such as pH or water activity control. In order to *inactivate* bacterial spores, a number of approaches have been suggested including multiple pressure cycling (Hayakawa *et al.* 1994), pressure induced germination followed by pressurisation (Gould 1973;Sale *et al.* 1970) and the use of pressure in combination with heat (Wilson & Baker 1997). These approaches and others will be discussed in detail in chapter 2. It is the latter process of pressure in combination with heat that will be the focus of this thesis.

1.3.7.1 Current commercial HPP applications

Commercial pasteurisation using high pressure as a replacement for heating was not considered feasible until the late 1980's. Pioneering work in Japan led to the launch of the world's first high-pressure pasteurised food in 1990 - a jam product manufactured by the Meidi-ya Food Factory Co (Leadley & Williams 1996). Further high added-value products such as juices and dairy desserts appeared in Japan shortly afterwards. Since then, high pressure has been used as an alternative to heat pasteurisation by a number of companies throughout the world. Table 1-3 shows a selection of some of the products manufactured around the world using high pressure processing as of 2010. The list is not complete but serves to illustrate the range of products on offer. Much of the information in Table 1-3 has been compiled from company websites, personal communications with other researchers and contributions from an on-line high pressure food processing subject interest group available on the professional networking site "Linked-in" (www.linkedin.com).

Company	Product
Fresherized Foods (USA)	Guacamole and salsa dips, juices, 'meal kits'
Motivatit Seafood (USA)	Oysters
Clearwater (Canada)	Lobster
Donny Boy Fresh Food Co (AU)	Fruit juices, Coulis and chunky fruit
Odwalla (USA)	Fruit and vegetable juices, smoothies
MacLab (NZ)	Shucking of mussels for use in mussel powder production
Hannah International (USA)	Dips
Hormel Foods (USA)	Prepared ham products
Shucks Maine Lobster (USA)	De-shelled lobsters
Purdue Farms (USA)	Prepared poultry products
Espuna (Spain)	Vacuum packed sliced ham, tapas
Pampryl (France)	Range of fruit juices
Frubaca (Portugal)	Apple juice
New Image Natural Health (NZ)	"Col+" functional colostrums milk product

Table 1-3. Examples of high pressure products worldwide (2010)

NC Hyperbaric (a major supplier of HPP equipment) estimates that there are now approximately 145 full-scale units in operation worldwide with around half of these being located in the USA and around a quarter being in Europe. Of the 145 vessels, 12% are in use for juice and beverage production, 35% for vegetable products, 31% for meat products and 14% for shellfish production, the balance being other speciality applications (personal communication with NC Hyperbaric, 2010).

1.3.7.2 Equipment for pressure pasteurisation

A review of high pressure research papers quickly establishes that there are a large number of suppliers providing HPP equipment on varying scales from laboratory through to full production scale models. A list of some of the more commercially significant manufacturers of high pressure processing equipment for industrial processing can be found in Table 1-4. For commercial food processing, Avure Technologies and NC Hyperbaric dominate the market.

Avure Technologies, 23500 64th Avenue South, P.O. Box 97040, Kent, Washington 98064-9740, USA	nc Hyperbaric Condado de Treviño, 59 – Políg. Villalonguéjar 09001 BURGOS SPAIN
Engineered Pressure Systems International N.V. (EPSI) Walgoedstraat 19 B-9140 TEMSE Belgium.	Elmhurst Technologies Inc 60 Loudonville Road Albany, NY 12204-1513 USA
Stork Food & Dairy Systems B.V. Ketelstraat 2, 1021 JX Amsterdam, The Netherlands	Uhde GmbH Friedrich-Uhde-Strasse 15 44141 Dortmund Germany

Table 1-4. Significant suppliers of commercial HPP equipment for food processing (as of 2011)

There are two main types of HPP equipment available - batch and semi-continuous, but the market is dominated by batch systems and semi-continuous units have largely fallen out of use (personal communications with NC Hyperbaric and Avure Technologies). In a batch process the product is filled into bulk or individual primary packaging and loaded into the vessel. A liquid pressure medium (usually water in commercial systems) is then pumped via a low-pressure pump into the vessel until full. The use of a low pressure pump ensures that

filling occurs as rapidly as possible. It is only after the vessel is notionally 'full' that a high pressure pump is switched on and the vessel is 'topped-up' with water until the target processing pressure is reached. The product is held at this pressure for the required time. Pressure generation is through mechanical pressure exerted on the fluid and consequently transmitted to the product. There is usually a volume reduction of around 12% (resulting from the compressibility of water) as pressure is applied. This is reversed when the pressure is released (Tsiklis 1968). A small temperature rise is observed as a result of compression that is typically around 3-4°C per 100 MPa of applied pressure for predominantly aqueous foods but varies depending on the product composition (see chapter 2). Although this is a small temperature rise, it can have significant implications on the overall lethality of the process and can be positively harnessed for high pressure sterilisation applications as will be explored in this thesis.

Because of the volume reduction that is observed during pressurisation, flexible packaging is most suited to HPP but rigid packs with compression zones are a possible alternative (Mertens 1992;Mertens 1995). Pressure is transmitted isostatically in the system so there is no rupture or breakage of the packaging (Williams 1994). To reduce mechanical stresses on the packaging and to reduce the come up time to pressure, headspace (i.e. air space in the pack) is generally minimised and flood filling or vacuum packaging is recommended. Alternatively, packaging with a low profile and rounded edges is also feasible (personal communications with Avure Technologies and NC Hyperbaric). Min & Zhang (2007) provides a summary of available data on the response of packaging to high pressure pasteurisation.

In a semi-continuous system, the product is pumped directly into a treatment chamber and is separated from the pressure medium by a floating piston. As the pressure medium is introduced, the piston moves up to pressurise the product. By using a number of units with a parallel feed it is possible to co-ordinate the process such that, for example, one unit is emptying as another is filling and a third is pressurising. In this manner, a continuous stream of product can be supplied to a clean or aseptic filling system (Figure 1-2)

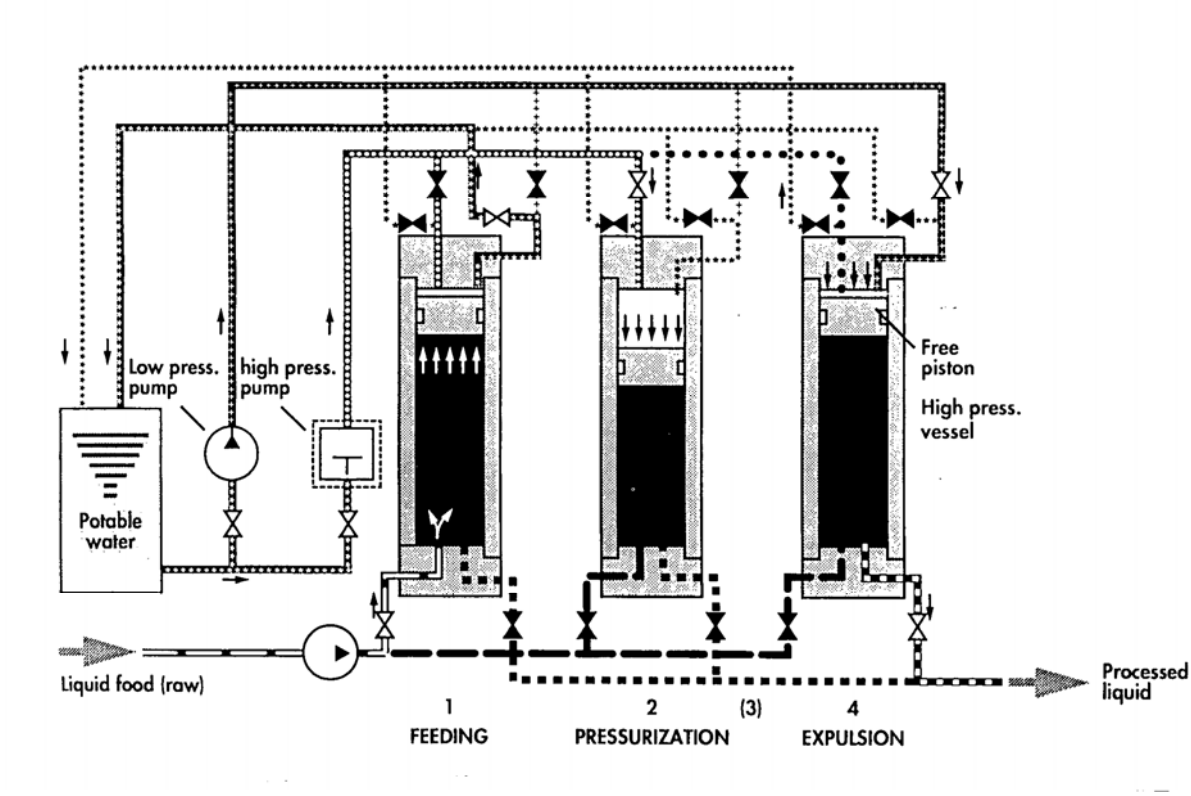

Figure 1-2. Example of semi-continuous HPP (FAO 2001)

Examples of batch vertical and horizontal pressure processing equipment for food use can be seen in Figure 1-3 and Figure 1-4. Batch systems have generally been favoured over the semi-continuous system because they enable the processing of larger particulates and offer greater flexibility for different product types.

Figure 1-3. A vertical batch high pressure processing vessel from Avure Pressure Systems (image courtesy of Nigel Rogers, Avure Technologies)

Figure 1-4. A horizontal batch high pressure processing vessel (covers removed) from nc Hyperbaric (image courtesy of Carole Tonello, nc Hyperbaric)

1.3.7.3 Process economics for HPP pasteurisation

Cost estimates for high pressure processing can vary quite widely because the costs are linked to the nature of the product itself and many processing factors come into play. Key factors that influence process economics include:

Fill ratio

The fill ratio (the ratio of product volume to vessel volume per cycle) must be maximised i.e. as much product as possible must be processed in each cycle. Fill ratio can be optimised by the selection of packaging that tessellates well in the process vessel i.e. without leaving excessive space. Fill ratio must be considered in any cost model and it is important to consider a realistically achievable figure. A fill ratio of around 50% is often cited as a conservative assumption. It should be noted that products are typically loaded into the vessel in baskets and this immediately reduces fill ratios substantially compared with loading the product directly into the vessel. There are however efficiency advantages in the use of baskets as they reduce loading and unloading times. The basket, if made from an insulating material, may reduce heat losses during the process, which will be advantageous in a thermal sterilisation process (Juliano *et al.* 2009), see Chapter 2.6 for details. For pumpable products, a semi-continuous system offers the best possible solution for maximising fill ratio because the product is filled directly into the processing chamber but as had been previously mentioned, semi-continuous systems are generally falling from favour because they lack flexibility relative to batch systems. The influence of fill ratio on processing costs (€/kg) is shown in Figure 1-5

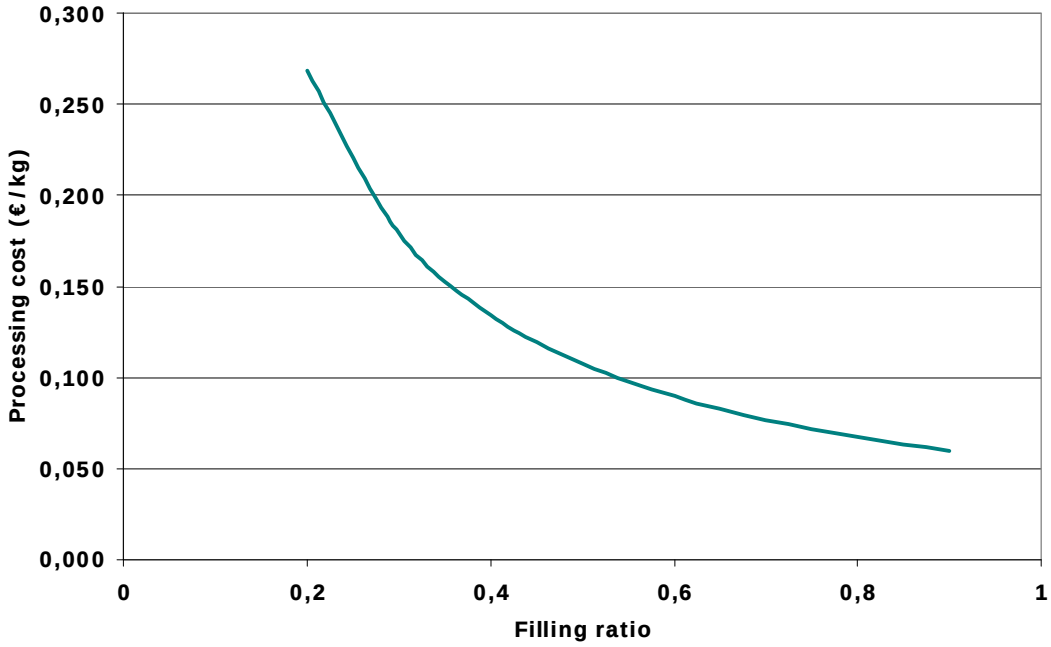

Figure 1-5. Fill ratio influence on processing costs per kilogram (Purroy 2007)

Process holding time and total cycle time

The hold time selected should be the minimum needed to ensure the target process is achieved. Advice from commercial equipment suppliers is that where possible hold times should be less than 5 minutes (Purroy 2007) because at holds times in excess of 5 minutes, the cost per kilogram for HPP processing starts to becomes uncompetitive with conventional preservation. Overall cycle times are affected by the hold time at pressure, the time needed to bring the vessel up to pressure and the time needed to decompress. If residual air is present in the pack, this must be compressed before the pressure in the vessel begins to increase. This time to compress entrained air extends the come up time and hence could increase the production costs per kilo of product. Come up times should be minimised by ensuring first that all air is removed from the vessel and then that the packaged food has been either vacuum

packed or flood filled with product to minimise air in the pack. Some high-pressure products use modified atmosphere packaging (MAP) for presentational, cosmetic/marketing purposes. MAP packaging has the disadvantage that it increases come up time to pressure, increases overall cycle time and thus reduces production throughput (Figure 1-6) for the reasons explained earlier when describing the influence of entrained air in the pack. As the percentage of gas in the pack increases, the come up time of the vessel increases and hence the overall cycle time increases. Furthermore, as the volume of gas increases the fill ratio of the vessel reduces because it becomes more difficult to efficiently fill the vessel with packs having large volumes of air. The increased come up time, along with the significant reduction in fill ratio leads to a substantial increase in the processing costs per kilo of product.

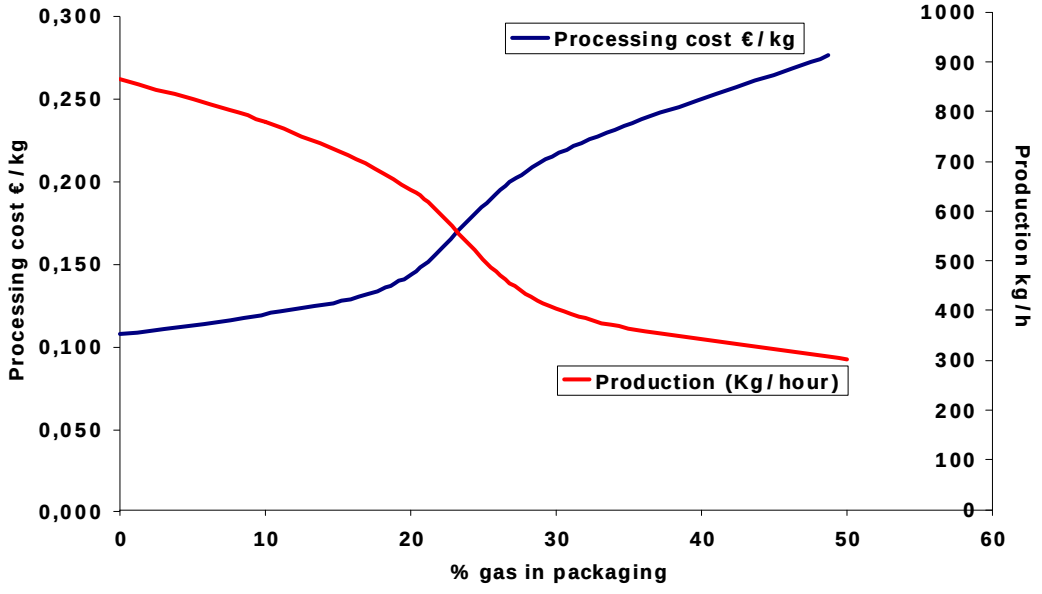

Figure 1-6. Effect of MAP packaging on processing costs (€/Kg) and throughput (Purroy 2007)

The influence of hold time on production costs and volumes is shown in Figure 1-7.

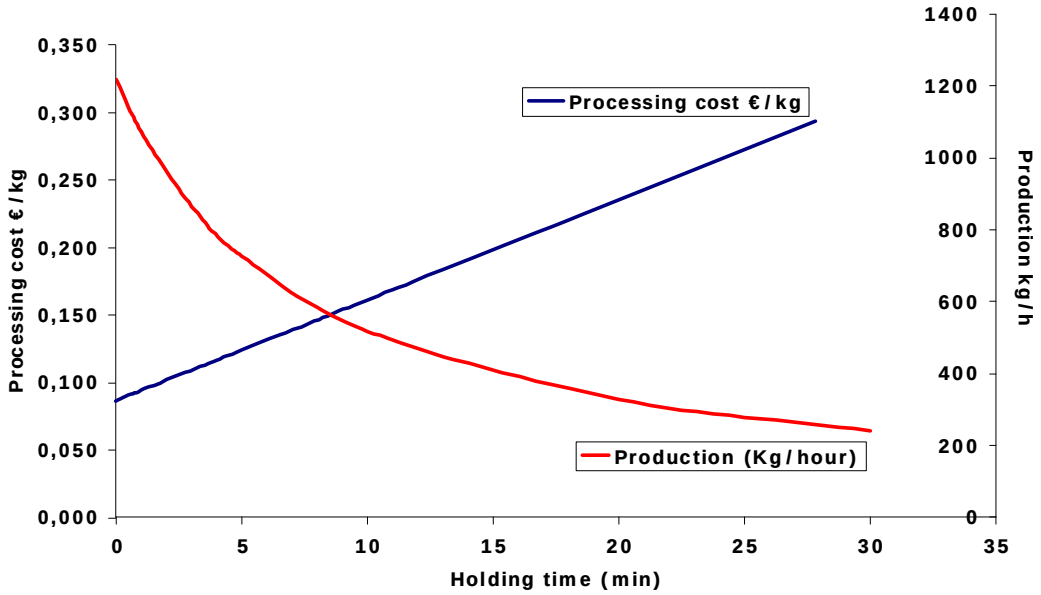

Figure 1-7. Processing costs (€/Kg) and volumes (Kg/hr) as influenced by hold time (Purroy 2007)

For most food pasteurisation applications (as distinct from the use of pressure for functional benefits e.g. for shellfish shucking) pressures used are of the order of 500-600 MPa; pressures of this magnitude are needed because of the resistance of bacterial pathogens - see for example IFT (2000). Processing and maintenance costs increase at these pressures (Figure 1-8) but cycle times can be reduced to commercially feasible times. For example, according to Avure technologies, processing at 400 MPa for 10 minutes is twice as expensive as processing at 800 MPa with zero hold time (Olsson 1995). The pressure applied should be the minimum required to achieve the desired level of lethality, balanced against the required throughput and hence the maximum cycle times that can be tolerated in practice.

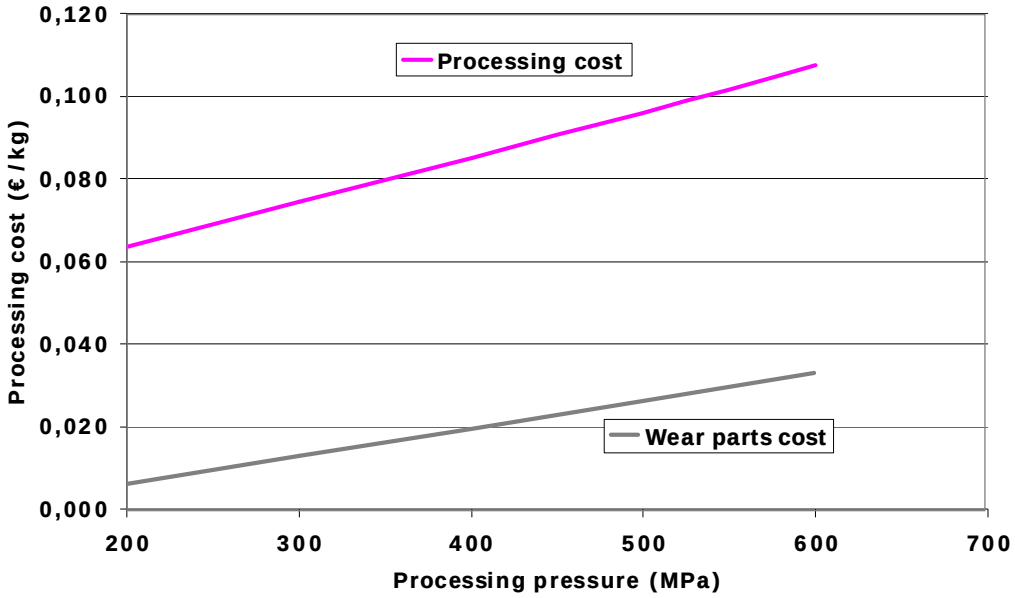

Figure 1-8. Processing costs as influenced by operating pressure (Purroy 2007). Note that the maximum operating pressure for NC Hyperbaric equipment is 600 MPa hence the absence of data for 700 MPa.

Various case studies have been developed for the costs of high-pressure pasteurisation. The Spanish meat processor Espuña, which has been manufacturing pressure treated ham since around 1996, estimated a processing on-cost of around 6 pence per kilogram for pressure processing (Grebol 2000). This was based on 5500 working hours per year using a 6 basket, 320 litre horizontal machine operating at 400 MPa. The hold time at pressure was 10 minutes and the complete cycle time of 15-16 minutes. The ham product was manufactured in exactly the same way as a conventional sliced ham but had an additional pressure pasteurisation step after packaging in order to inactivate *Listeria monocytogenes*. The achievable shelf-life was extended to the about 60 days but the stated shelf-life on pack was 30 days. This was a

commercial decision to ensure that at the declared shelf-life the product was still in good condition (Grebol 2000).

A very successful end-user of high-pressure technology has been Fresherised Foods in the USA (http://www.fresherizedfoods.com/, last viewed April 2011). Fresherised foods makes a range of pressure processed products including guacamole, salsa sauces and ready meals. Cost estimates based on the Fresherised foods guacamole process (Ting 2001) put the on-cost as a result of capital investment at around 2 pence per kilogram. This was based on a production throughput of 6664 tonnes of product per annum and capital cost for a 215 L Avure high pressure processor of 1.2 million pounds. The equipment was depreciated over 10 years in this cost model. Energy costs for a 215 L unit operating at 600 MPa at the throughputs achieved at the Fresherised Foods plant, were estimated to be 0.3 pence per kilogram.

More recently (Purroy 2007) estimated HPP pasteurisation processing costs to range between 4.3 to 16.5 €/cycle dependent on multiple factors as outlined previously. The NC Hyperbaric production cost models included vessel volume, fill ratio, cycle time, number of vessels and intensifiers, yearly hours of operation, investment costs, depreciation costs, energy and maintenance costs.

1.4 Summary of thesis content

This introductory chapter of the thesis has provided context for the interest in emerging preservation technologies as alternatives to conventional heat processing; specifically, consumer drivers for minimally processed products and the limitations of existing processes to address these drivers. A range of alternative emerging technologies was briefly reviewed and the current commercial status of high-pressure *pasteurisation* was outlined.

Thermal preservation is effective and produces reasonable quality food at an affordable price for consumers. However, thermal processing often causes deleterious food quality changes (notably colour and texture changes) in fruit and vegetables as will be demonstrated in Chapters 2, 4, 5 and 6. Consumer demand for minimally processed food products has driven research into alternative technologies that can preserve whilst causing minimal product changes. There are many such technologies under development. Of these, high pressure pasteurisation is now a relatively mature technology and can be used to inactivate vegetative organisms whilst often retaining 'fresh-like' product characteristics. The process is limited in capability because of the resistance of bacterial spores to high pressure. A number of approaches have been suggested in order to sterilise foods using high pressure (see chapter 2) but there is limited published data to show that theoretical quality benefits can be realised.

The remainder of the thesis will focus on the use of high pressure in combination with elevated temperatures in order to produce ambient shelf stable products. This process is commonly described as high pressure sterilisation (HPS), pressure assisted thermal

sterilisation (PATS) or High Pressure Thermal Processing (HPT). The term HPS will be used throughout the thesis but could be used interchangeably with HPT or PATS ; the latter term perhaps being the most representative of the latest thinking on the role of pressure in the combination process i.e as an aid to achieving peak temperature rapidly and uniformly. Specifically, the thesis will explore quality changes in vegetables and is focused primarily on green beans as a representative green vegetable that might be included in, for example, a prepared recipe dish. The central question being addressed by the thesis is whether high pressure can be used in combination with elevated temperatures to produce vegetable products with improved sensory properties compared with that achievable using conventional thermal processing. To date there is limited published data to support or refute this idea.

Chapter 2 will review published approaches for producing ambient shelf stable products using pressure, with a focus on its combination with heat, as well as exploring the available data on quality changes in foods at high pressure *sterilisation* conditions. It will highlight that data on food quality changes at high-pressure sterilisation conditions are limited and therefore demonstrates the need for objective data to be gathered that compares product quality from high pressure sterilisation processes with that achievable from conventional heating.

Chapter 3 describes the experimental equipment which has been used in this work. Approaches for assessing process uniformity at high-pressure-sterilisation conditions are discussed together with work to characterise the temperature variation within the test vessel. There are significant technical challenges in process validation at high pressure conditions.

Chapter 4 describes experiments in a high pressure vessel in which colour and quality changes in products processed at a single pressure/time/temperature combination were compared with those observed in conventional canning. It demonstrates that processed vegetable texture can be improved using HPS compared with conventional canning but will also show that the process can induce undesirable colour changes. The chapter provides data on real foods using a pressure/time/temperature combination that is much closer to commercially achievable conditions than much of the previously published data.

Chapter 5 builds on the data provided in Chapter 4 through a further set of experiments which provides quality data in green beans, in terms of colour changes, texture change and chlorophyll retention, at a range of pressures, temperatures and times and provides insight into the relative influence of each factor on the resulting product quality. The chapter includes data on the fate of plant pigments at each process condition and theoretical conditions for 'optimum' product quality are identified.

Chapter 6 describes experiments relating to the influence of the pre-heating method as a pre-curser to a HPS treatment. The chapter provides data on colour and texture retention in green beans when pre-heated using conventional heating or ohmic heating along with the effects of pH modification and calcium chloride addition. The pre-treatment method could have important implications for the end product quality and the chapter provides insights as to how undesirable colour and texture changes in the pre-heating step of the process might be minimised. Chapter 7 provides an overall summary of the thesis and suggestions for further work.

2 Chapter 2 - Literature review on high pressure sterilisation

2.1 Introduction

Chapter 1 reviewed drivers for the interest in 'minimal processes', briefly outlined the limitations of 'conventional' thermal preservation technologies and then described key emerging technologies that were attracting research interest with a view to overcoming some of these limitations. The current commercial status of high pressure processing for pasteurisation was outlined: a reasonable number (>150) of commercial plants exist and products are available in several markets.

Chapter 2 will focus on published data relating to the use of high pressure for *sterilisation* applications and drivers for the interest in high pressure sterilisation will be outlined. Published data relating to high pressure sterilisation will be reviewed with a focus on quality effects at high pressure sterilisation conditions. All of the material reviewed in Chapter 2 has been done so to provide context for the data presented in chapters 3 - 6.

2.2 Quality changes in heat processed foods

Holdsworth (1997) in introducing the topic of the kinetics of food quality retention noted that when foods are heated in order to inactivate microorganisms, a series of chemical and physic-chemical reactions occur in the food. Some of these are wanted e.g. to induce a desired level of softening or to induce a cooked texture, and some are unwanted such as nutrient depletion or excessive colour and texture changes.

The kinetic factors 'D' and 'z', familiar to microbiologists and used to describe the inactivation kinetics of microorganisms have also been applied to describe the inactivation kinetics of various quality attributes of food products. The 'D' value or 'Decimal reduction time' can be defined as the heating time in minutes to give an inactivation of 90% of organisms (or measured response) or 10% survival (Katzin *et al.* 1942). The 'z' value is the temperature change necessary to alter the 'D' value by one log cycle i.e. by a factor of 10 (Holdsworth 1997). Holdsworth noted that 'for chemical constituent of foods the rates of degradation reactions are slower than for the destruction of microbial species' and presented these differences in general terms (Table 2-1).

Vulnerable factor	z-value (°C)
Bacterial spores	7-12
Vegetative cells	4-8
Vitamins	25-30
Proteins	15-37
Enzymes	10-50
Overall sensory quality	25-45
Texture and cooking	17-47
Colour degradation	17-57

Table 2-1. Some comparative z-values for heat-vulnerable constituents (Holdsworth, 1997)

The implication of this knowledge is that increasing process temperatures will have a much greater effect on the rate of microbial inactivation than on the rate of quality degradation. For example, a 10°C increase in temperature doubled thiamine destruction in meat but increased microbial inactivation by a factor of 10 (Greenwood *et al.* 1944). Similar findings have been observed for a wide range of quality related parameters. See for example, Hayakawa &

Timbers (1977), Van Loey *et al*. (1995) and Holdsworth (1985). Holdsworth (1997) includes a particularly comprehensive review of thermal effects on food quality.

2.3 Drivers for the interest in high pressure sterilisation

Hoover *et al*. (1989) noted that high pressure processing induces changes in the morphology, biochemical reactions, genetic mechanisms, cell membrane and wall of micro-organisms. The effective inactivation of vegetative micro-organisms using HPP has been well established but bacterial spores are known to be resistant to pressure (Balasubramanian & Balasubramaniam 2010) and this is a significant limitation for the technology (Black *et al.* 2007b). The pressure resistance of spores along with the variable response of enzymes to pressure processing, has generally limited commercial applications to chilled products (i.e. pasteurised rather than sterilised) that have additional preservation hurdles such as low pH, water activity control or the presence of curing agents where spore forming pathogens can be controlled.

Although there is clearly a wide spectrum of resistance among species at ambient temperatures, vegetative cells are generally inactivated at pressures of between 300 and 700 MPa (IFT 2000). Spore inactivation requires pressures far in excess of what is currently commercially achievable. For example, studies on the pressure resistance of spores of six *Bacillus* strains found that 40 minutes at 981 MPa (5 and 10°C) did not inactivate spores of *B.stearothermophilus* IAM12043, *B.sublilis* IAM12118 and *B.licheniformis* IAM13417 (Nakayama *et al.* 1996). Cheftel (1995) reported that some bacterial spores are resistant to pressures as high as 1000 MPa at room temperature (Balasubramanian & Balasubramanian 2010). A pressure treatment of 550 MPa at 37°C for 120 minutes resulted in no inactivation in a wild strain of *Bacillus subtilis* spores (Reineke *et al.* 2011).

For many manufacturers, high pressure will only become an attractive proposition when it can deliver ambient shelf stable products having significantly improved quality over conventional thermally sterilised products. The technology would then be offering something truly unique. There have been a number of published papers demonstrating some degree of spore inactivation using HPP with other preservation hurdles (see section 2.4) but relatively few claiming to result in commercial sterility. The most likely means, by which commercial sterility can be achieved in low acid foods using HPP in the short-term, is to combine pressure with elevated temperatures (see section 2.4).

Many published approaches for high pressure sterilisation utilise the fact that when pressure is applied to a foodstuff, a rapid temperature rise due to compression is observed (see section 2.6). Also, when the pressure is released, the product rapidly cools to close to its initial temperature. In the case of both heating and cooling, the effect is volumetric i.e. it occurs throughout the entire vessel and load. Rapid, volumetric heating and cooling after pressurisation could result in products with "improved quality over in-container heat sterilised semi-solid and viscous foods, particularly in large containers" (Juliano *et al.* 2009).

By pre-heating a product and then applying pressure, the compression heating in conjunction with the applied pressure could be sufficient to inactivate bacterial spores and produce an ambient shelf stable product. This process would essentially be a high-temperature short-time process with a possible additional contribution to lethality from the applied pressure. Recent

data however, suggests that it would be prudent to consider HPS purely as a thermal process until its effects on spores are fully understood (Bull *et al.* 2009;Knoerzer *et al.* 2010a).

What makes the HPS approach unique is that it could be applied equally well to a liquid or a solid food product. An additional benefit, as discussed, is that when the pressure is released, the product rapidly cools to close to its temperature at the start of the pressure cycle. High pressure in combination with elevated temperature potentially offers advantages over other volumetric heating techniques such as ohmic heating or microwave heating because it delivers rapid heating *and* cooling. This effect is illustrated in future chapters. In essence, since compression heating occurs throughout the load and the pressure is applied isostatically, the hold time at maximum temperature and pressure is minimised. The thermal degradation of the product should therefore be substantially diminished.

Intensive studies worldwide would suggest that there is commercial potential for the HPS process. In February 2009, the US National Center for Food Safety and Technology announced that the FDA had accepted a filing of a 'pressure assisted sterilisation process' after a seven-year multi-million dollar collaboration between Avure technologies (an equipment supplier), US Army Natick Soldier Research, Development and Engineering Centre (RDEC), Baxter Health Care, ConAgra Foods, Hormel Foods, General Mills, Basic American Foods, Unilever and Mars (NCFST 2009). Results from some of the outputs of this study are included in this Chapter - see for example Balasubramamian & Balasubramanian (2010) and Lau & Turek (2007). In the USA, before a low acid heat sterilised food can be placed on the market, documentation about the process and its efficacy must be submitted to

the FDA for pre-market approval (NCFST 2009). The US low acid canned food regulations (21 CFR 113) mention the requirement to demonstrate commercial sterility or 'essentially no risk' without defining the process requirement in order to achieve this (Koutchma *et al.* 2005). The successful filing of a PATS process (thought to be the first filing of its kind anywhere in the world) is therefore important because it demonstrates that the US regulatory authorities were satisfied with the microbiological efficacy of the approach.

Despite this positive development, a number of technical challenges still need to be addressed before pressure assisted thermal sterilisation finds widespread commercial use. These include issues such as:

- The need to develop strategies for the control and optimisation of temperature distribution within the processing vessel so as to ensure that products receive a consistent minimum process regardless of spatial position within the vessel.
- The establishment of routine methods for process establishment and control
- Optimisation of the pre-heating step of a high pressure sterilisation cycle to improve product quality
- More data on how quality parameters are affected by high pressure, high temperature combination processes.
- A greater understanding of the effects of HPS on bacterial spores.

This chapter will provide an overview of published approaches for achieving spore reduction using high pressure (whilst recognising that this is not the core topic of the thesis) and will

outline some of the technical challenges which still need to be addressed before the technique can be used for widespread commercial food production.

Whilst it is clear that establishing food safety is the single most important factor in the development of a new preservation process, there seems little point in committing substantial resources to demonstrating safety if the quality benefits of the technique do not give rise to products that are substantially better than foods prepared by conventional approaches. The focus of this chapter and the overall thesis then, relates to what food quality improvements (if any) may be achievable using high pressure in combination with elevated temperatures.

2.4 Spore inactivation using high pressure

This thesis is primarily concerned with food quality effects under HPS conditions so it is not the intention to provide a comprehensive review of spore inactivation in this section. Wilson *et al*. (2008) provided a review of recent work on HPS spore inactivation with a summary table of published studies which is provided in Appendix 1. The following section provides a selection of relevant studies on spore inactivation using HPS to provide context for the likely processing conditions that may need to be applied to produce commercially sterile products and the likely implications for product quality changes.

Work by Unilever research in the 1970's investigated the effects of temperature and pressure on vegetative cells and spores. This work clearly demonstrated the pressure resistance of bacterial spores and the likely need to use HPP in combination with other preservation hurdles (such as temperature, pH or water activity) for spore inactivation in low acid foods (Gould & Sale 1970;Gould 1973;Sale *et al.* 1970). A two stage pressure process was proposed, the first

to germinate spores and the second to inactivate these germinated spores. Germination was induced at around 50 MPa but retarded above 400 MPa. There appeared to be an optimum pressure for spore inactivation of between 200-400 MPa. Germination was also highly dependent on temperature and generally increased with an increase in temperature (Gould 1973). Hold times were generally quite long in these studies, for example in Sale *et al*. (1970) a range of spore types were treated at 1000 to 8000 atmospheres for 1 hour at 20°C. In Gould (1973) *Bacillus coagulans* spores were treated for 30 minutes at 2000 to 8000 atmospheres. Whilst these now classic studies contributed greatly to our understanding of HPP effects on spores, the hold times employed are unlikely to be relevant to commercial application where hold times are anticipated to be under 5 minutes in order to make the process commercially viable (see section 1.3.7.3)

A problem with a two-stage spore inactivation approach (and one noted by the authors) was that a sub-population of 'super dormant' spores always remained, which could not be induced to germinate. Gould (2006) on reflecting on his earlier work noted that he observed "great variability in pressure sensitivity of different spore types and…non-log-linear germination kinetics". The surviving fraction of *Bacillus coagulans* after being treated at 2000 to 8000 atmospheres, pH 8.0 for 30 minutes at temperatures between 25°C and 65°C (initial temperature before HPP) ranged between 4% and <0.00001% (Gould 1973). More recent attempts to inactivate spores using a two-stage approach have reported similar difficulties with sub-populations of spores that cannot be induced to germinate (Van-Opstal *et al.* 2004)

Efficient, reproducible and extensive inactivation of bacterial spores by high pressure is "likely only to be realised in combination with initial process temperatures that exceed 60°C" (Bull *et al.* 2009). A number of pressure/heat combination approaches have been suggested for achieving high pressure sterilisation, some of which have been patented. For example, Wilson & Baker (2003) working for US company Kal Kan reported on an approach utilising synergy between the compression heating that occurred in the product as it was pressurised, along with the lethality of the pressure process itself to produce an ambient stable product. Note that a number of Wilson & Baker patents pre-date this patent – see Wilson & Baker (1997; 1998; 1999; 2000; 2001) mainly for reasons of patenting in different regions of the world but the following section describes trials reported in a European patent by Wilson & Baker (2003).

The Kal Kan approach claimed to result in a commercially sterile product, in other words, that all organisms capable of growing under the intended storage conditions were reduced to acceptable levels, (Department of Health 1994). A spore reduction of 10 log cycles or more was said to be achievable for mesophilic, anaerobic and thermophilic spores (*B.subtilis*, *C.sporogenes* and *B.stearothermophilus*) using the Kal Kan approach. Furthermore, products manufactured using the process were said to be fresher in appearance compared with conventionally heat processed products. The process was said to be faster, more energy efficient and less detrimental to product quality than conventional retort processes. The suggested processing conditions for achieving commercial sterility were very wide ranging. As an example, the process could involve heating the product up to between 80 and 99°C, loading into the pressure chamber and pressurising to between 345 and 1035 MPa, but ideally

(according to the patent) to between 483 to 897 MPa. The pressure medium was pre-heated prior to pressure treatment. The process was said to be reliant on the combination of heat with pressure, i.e. neither the heating step nor the pressure treatment alone would be sufficient to stabilise the product for ambient storage. The patent claimed that the process was suitable for a wide range of product types including pet foods (high moisture and semi-moist), main meals, sauces, soups, stews, vegetables, beverages and juices. To sterilise a low acid food the authors claimed that the pre-pressurisation temperature could be between 75°C and 125°C but should ideally be in the range of 90°C to 100°C. The patent suggested a very large pressure range for use in combination with these temperatures; the applicable pressure range was cited at between 517 MPa and 1724 MPa but preferred pressures were over 620 MPa and even more preferably, over 862 MPa (a figure not currently achievable using factory scale equipment).

The additional lethality attained as a result of compression was claimed to be principally related to the peak temperature attained during pressurisation. The peak temperature (which is dependent on the initial temperature and the pressure applied – see Chapter 3 for practical examples and section 2.6 for theoretical aspects) ranged from around 100°C to 160°C in the studies conducted for the patent application. According to the authors, the peak temperature should ideally be in the range of 120°C to 140°C. The Kal Kal process is claimed to produce a commercially sterile low acid food and is claimed to achieve a spore reduction of over 10 log cycles.

The Kal Kan patents disclosed the results for a series of separate experiments. In one, 50g portions of a raw meat emulsion were weighed into plastic pouches and pre-heated to over 80°C prior to pressure treating at pressures of up to and greater than 827 MPa. A *Bacillus subtilis* spore strip was incorporated into the pouches prior to treatment. After treatment the products were stored at 4°C and then analysed for total aerobic and anaerobic counts, total aerobic and anaerobic spores, faecal streptococci, yeast and moulds, clostridia and *Bacillus subtilis* spore counts. Reductions of 3 to 7 log cycles were reported. Vegetative organisms were effectively eliminated but spores were resistant. The lethality of the process to bacterial spores was said to be enhanced when pre-pressurisation temperature was in excess of 80°C.

In the second example, 37 separate treatments in a phosphate buffer system were conducted, with varying pressure and pre-pressurisation temperature, and using 15 different chemical agents in conjunction with the pressure/temperature process. *Bacillus subtilis* spore strips were incorporated into each pouch used during testing. Pressure of 689 MPa and peak temperatures of 100°C for one minute were not sufficient to produce a 6-log reduction of *Bacillus subtilis* spores. Pressures in excess of 827 MPa for one minute in combination with a pre-pressurisation temperature of over 80°C were required to achieve 'total spore inactivation'. Sodium bicarbonate (2%), propionic acid (1%) or sodium chloride (≥5%) reduced the effectiveness of the treatment. Greater levels of inactivation were observed in the phosphate buffer system than had been found in the raw meat emulsion.

In the third example, a raw meat emulsion was weighed into plastic pouches and inoculated with a cocktail of spore forming organisms (*Clostridium sporogenes*, *Bacillus subtilis* and

Geobacillus stearothermophilus). Samples were pre-heated to either 75°C, 85°C or 95°C before pressure treatment at 620 MPa with varying hold times of up to 30 minutes. The results from these trials were somewhat inconclusive as seal failures were observed in the packs during storage. Growth was observed post process but this was attributed to post process leaker spoilage since the numbers of the inoculated spores in treated packs were below the limits of detection. The initial spore levels were 10^6 per gram. Results suggested that a pre-sterilsation temperature of 85°C with a hold of 1 minute at 620 MPa could be enough for a 6 log reduction of *Clostridium sporogenes*, *Bacillus subtilis* and *Geobacillus stearothermophilus*.

In a fourth example a raw meat emulsion was filled into plastic pouches ready for HPS trials. Trials were conducted with pre-pressurisation temperatures of between 85°C and 98°C followed by a pressure process of 620 MPa for between 1 and 30 minutes. After each process, samples were analysed for aerobic, anaerobic and thermophilic spores and an assessment of commercial sterility. Commercial sterility was said to be achieved with a pre-heat to 85°C followed by a pressure treatment of 30 minutes at 620 MPa or by a pre-heat to 98°C followed by a pressure treatment of 620 MPa for 5 minutes or over. In one of the trials the product was heated to 98°C but the pressure vessel was unable to reach the target pressure of 620 MPa, in fact the product was held for 30 minutes at 483 MPa and commercial sterility was still reported to be achieved.

It is perhaps unsurprising that a pre-heat to 98°C followed by a 5 minutes hold at 620 MPa (which resulted in a peak temperature of 129.4°C) yielded commercial sterility since an

examination of the time temperature profile from the patent data shows that the product achieved a thermal process equivalent to around 25 minutes at 121.1°C. Similarly, a pre-heat to 98°C followed by a 30 minute hold at 620 MPa (which resulted in a peak temperature of 116.1°C) gave a thermal lethality equivalent to almost 10 minutes at 121.1°C. In contrast, a pre-heat to 85°C followed by a 30 minute hold at 483 MPa only resulted in a thermal process equivalent to around 1 minute at 121.1°C. This reportedly resulted in commercial sterility despite the thermal contribution being far below that normally required in a conventional thermal process. For a brief discussion of the calculation of F_0 see Chapter 1.

The Wilson & Baker patent would appear to describe an elegant means of achieving a high temperature short time process for solid packs – something that is not achievable using conventional technologies. Processes such as ohmic heating or microwave can induce rapid volumetric heating but they are still limited by the need for conventional cooling. This combination pressure/heating process has the advantage that when the pressure is released the product cools down to close to its original pre-pressurisation temperature. The thermal contribution to lethality could *potentially* be lower than would be applied using conventional thermal processing (because of any lethality contribution from pressure) but recent studies suggest that this additional lethality from pressure is unlikely to be taken into account when establishing HPS processes in the short term (Bull *et al.* 2009). What is certain however is that exposure to elevated temperatures can be minimised because of the rapid volumetric heating and cooling effect. Since the compression heating occurs throughout the vessel, excessive thermal processing of pack edges, to ensure an adequate process at the centre of the pack, should be avoided. The Kal Kan process is also quite desirable from a process

economics viewpoint as it uses a single pressure pulse. De Heij *et al*. (2003) noted that the use of a single rather than multiple pressure pulse reduces maintenance costs and extends the working life of the pressure vessel.

A similar patented approach to the Kal Kan process was proposed by Meyer (2000a; 2000b; 2001; 2002; 2000c). The process again related to a low acid sterilisation process and utilised pre-heating followed by pressure treatment to harness the lethal effects of compression heating. The main difference between the two approaches is that the Meyer cycle utilises multiple pressure cycles with a hold at temperature between each pressure pulse. At least two pressure cycles are employed.

The Meyer patent claims that the Wilson & Baker approach of an 85°C pre-heat followed by a 30 minute hold at 620 MPa does not result in a 12 log reduction of *Clostridium botulinum* and further claims that a pre-heat to 98°C followed by a 5 minute hold at 620 MPa does not yield commercial sterility because sub lethal injury occurs and *Bacillus cereus* is able to recover and grow to unacceptable levels within one week of pressurisation. This statement, on first examination, seems highly unlikely given that the thermal contribution alone from this latter process is equivalent to around 25 minutes at 121.1°C. If applied correctly in a conventional thermal process this would readily inactivate 10^{12} *Clostridium botulinum* spores. As *Bacillus cereus* is a less heat resistant spore forming organism this would also be inactivated. The patent acknowledges that the thermal contribution alone should be sufficient to inactivate *Bacillus cereus* and postulates that the pressure causes spores to become more resistant to heat. Published data to support this idea is very limited (Bull *et al.* 2009).

According to the Meyer patent, the only process from the Wilson & Baker approach that yields commercial sterility is a pre-heat to 98°C followed by a 30 minute hold at 620 MPa and Meyer asserts (almost certainly correctly) that this process has too long a hold time to be of commercial significance for food production.

De Heij *et al.* (2003) investigated the likelihood of sub-lethal injury of *Bacillus subtilis* exposed to a single pressure pulse. Inoculated samples were prepared in a peptone and sodium chloride growth medium, filled into polyethylene pouches and then pressure treated (the conditions of the temperature/pressure process were not explicitly stated in the source material). After treatment, some pouches were enumerated for viable counts and the remainder were stored for 30 days at 37°C. The MPM (Most Probable Number) of surviving spores after 30 days storage "did not differ significantly from the plate counts immediately after treatment so there was no indication of recovery of spores during storage" (de Heij *et al.* 2003).

Meyer argued that heterogeneity in the response of spores to heat and pressure necessitated a double pressure pulse, the second pulse effectively inactivating organisms that survived the first due to natural dose response resistance. The preferred approach suggested by Meyer consisted of pre-heating the food to a predetermined initial temperature (above 70°C), subjecting the food to a first holding period at ultra high pressure (with claims covering a wide range of temperatures – see next paragraph) decompressing to atmospheric pressure and holding for between one second and five minutes.

This first cycle was followed by a second hold period at pressure, release of the pressure and final product cooling. The holding times and pressures in both pulses could be from <1 second to 200 minutes at 345-965 MPa or greater. Meyer suggested that the pressure chamber should be pre-heated to the same or greater temperature as that of the pre-heated food and most preferably should be at the final temperature that the product reaches after compression heating.

Although two pulses were considered sufficient to result in commercial sterility, the patent encompassed the use of a greater numbers of pulses. This was perhaps simply to 'ring-fence' a range of process conditions rather than a technical necessity. The hold-time at pressure and the pressure itself could be varied in the two pulses. In the examples set out within the patent, the target pressure in both cycles was the same but the second hold period was shorter than the first.

The products discussed within the patent were: a macaroni and cheese product, thin sliced New York steaks and short grain polished white rice blended with water (two parts water to one part rice). In an initial series of experiments, the patent attempted to establish that a single pulse approach as suggested by the Wilson & Baker was ineffective for the production of a commercially sterile product. Vacuum sealed pouches of the previously mentioned products, inoculated with around 10^5 cfu per gram of *Clostridium sporogenes* PA3679, were pre-heated to either 90, 95 or 98.9°C before being pressure treated using a single pulse of 413,

551 or 689 MPa for between 22 seconds and 20 minutes 23 seconds (dependent upon the pressure applied).

The results of these initial experiments prove difficult to interpret, as the data tables presented seem to only relate to one product but make no reference as to which product they are referring. This makes comparisons between the different products impossible. The results as presented are as follows. Two or three days after processing the inoculated samples were analysed for total viable counts. All but one process condition (551 MPa, 90°C preheat and 20 minute 23 second hold) showed no growth. After a week of storage, the samples were re-tested and under all conditions microbial counts were obtained and these were subsequently identified at *Bacillus cereus* spores. *Clostridium sporogenes* counts were obtained in all samples apart from process conditions of 98.9°C, 689 MPa and hold times of 66 seconds and above. As has been previously stated, it is unclear as to which specific product these data refer.

The Meyer cycle is potentially a very useful approach since it could reduce the overall cycle time required to achieve commercial sterility. However, multiple cycles are also likely to put greater stress on the vessel and seal components of the equipment, potentially shortening their operational life. Process validation also becomes even more complex for a multiple cycle process since the temperature of the product could be lower at the start of the second pulse than on the first pulse.

Andreas März has proposed a pressure/temperature combination process along similar lines to Wilson & Baker, (März 2002). A complete cycle consisted of heating the product to a pre-pressurisation temperature, holding the product at pressure for a specific time period and depressurising the system. Multiple cycles could be employed if desired. At face value this approach seems identical to the Wilson & Baker approach using a single cycle or the Meyer approach of using multiple cycles. However, an additional step was recommended prior to heating the product: subjecting the product to a predetermined level of oxygen for a time interval. It was claimed that this step increased the effectiveness of the process and allowed milder processing conditions than those proposed by Wilson & Baker or Meyer to be employed. The patent gave two example processes:

I. In the first, the product was pre-heated to 32°C and then treated at 6000 bar and 60°C for 300 minutes

II. In the second the product was pre-heated to 50°C and then pressure treated at 6000 bar for 180 minutes.

The vessel holding temperature is uncertain in these examples because in the text of the patent, 60°C is reported but in the tables of results, 90°C is reported. Suspensions of *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Clostridium sporogenes*, *Bacillus subtilis* and *Aspergillus niger* were packaged in a range of pack formats and exposed to the conditions outlined above. In the case of the first process, all organisms were reduced by over 6 log cycles in all pack formats with the exception of *Clostridium sporogenes* where, at best, a 2 log cycle reduction was obtained in all of the pack formats. In the case of the second example, all organisms, including *Clostridium sporogenes*, were reduced by over 6 log

cycles with the exception of sporogenes samples that were packed in 20 ml film containers. No explanation was offered as to why this package format resulted in a lower level of reduction. The patent discloses no information on methods to minimise temperature gradients within the vessel. With this limited data it is difficult to determine whether the packaging genuinely influenced the results with respect to sporogenes survival or whether the film samples were simply located in a cooler region of the vessel. The patent claims that the process is suitable for food, cosmetics and pharmaceuticals. It seems unlikely that the process could be used for food given the extended holding times employed because the process economics would not be favourable (see Figure 1-7). It could find applications in the pharmaceutical field where the products are very high value compared to food.

Matser *et al.* (2004) reported that total aerobic plate counts and spore counts in a range of food products could be reduced to less than the confidence threshold (log 1.0 to 1.4 depending on the product) after processing with two 900 MPa pulses with an initial temperature of 90°C). These results were reported for a range of products but were based on the natural microflora present, i.e. challenge testing was not reported. The results of this work are summarised in Table 2-2.

Product	Total count		Spore count	
	Initial	Post process	Initial	Post process
Green beans	6.1	<C.T.*	3.5	<C.T.
Spinach	8.2	<C.T.	3.5	<C.T.
Asparagus	6.9	<C.T.	4.7	<C.T.
Milk	4.4	<C.T.	0.7	<C.T.
Basil	4.8	<C.T.	3.9	<C.T.

Table 2-2: Total aerobic count and spore count before and after high-pressure sterilisation (two pulse process, initial temperature 90°C, pulse pressure 900 MPa). Matsers *et al.* (2004). * C.T.:Confidence threshold: log 1.0 – log 1.4 depending on product

Balasubramanian & Balasubramaniam (2010) studied the effects of pH, pressure, temperature and substrate on the inactivation of *Bacillus subtilis* ATCC6633 spores. Pressures were applied in the range of 690 to 827 MPa, targets for temperature *during the hold phase* (as distinct from initial temperatures before pressurisation) were 60, 65, 70 and 75°C. Note that this was the temperature of the pressure fluid in the vicinity of the sample.

Hold times varied up to a maximum of 10 minutes and pH was evaluated at 3 levels in citrate-phosphate buffer: pH 3, 5 and 7. Experiments were carried out using 3 substrates: citrate-phosphate buffer, tomato pureé (pH 4.05) and minced crab meat (pH 7.25). Surviving spores were enumerated within 30 minutes of completion of each process. Inactivation of the *B.subtilis* spores was found to increase with increasing process temperature, pressure and pressure-holding time.

In the phosphate buffer experiments, higher levels of spore inactivation were observed at lower pH values. For example, a maximum log reduction of 4 $\log_{10}$ cfu/ml was observed after a process of 690 MPa for 10 minutes 75°C at pH 3, whereas with the same processing conditions at pH 5 or 7, less than 4 log reduction was obtained. This suggests that pH can play a role in enhancing the lethal effect of a high pressure, high temperature process but Balasubramanian & Balasubramanian (2010) suggest that the effect is not major. However, work by Bull *et al*. (2009) using model materials closer to real food products suggested that pH was an important factor in enhancing the effect of a combined high pressure/high temperature process.

Balasubramanian & Balasubramanian (2010) did find that inactivation was reduced in the more complex food matrices; for example, a process of 827 MPa, 75°C for 5 minutes resulted in a 4 log reduction of spores in the tomato pureé (pH 4.05) whereas the same conditions applied to phosphate buffer at a pH of either 3 or 5 resulted in a log reduction of 7 and 6 $\log_{10}$ cfu/ml respectively. For crab-meat (pH 7.25), inactivation of *B.subtilis* spores was lower than in phosphate buffer at pH 7 (for the same process conditions) when the process temperature was less than 70°C but similar once the hold temperature was above 70°C. Inactivation of *B.subtilis* spores was reduced in the crab-meat compared with the tomato pureé. For example, with a pressure treatment of 827 MPa with a hold time of 5 minutes, the maximum log reduction in crab meat was around 3 (at 75°C) compared with around 4 in tomato pureé (again at 75°C).

For all pressure (680-827 MPa), pH (3,5,7) and temperature (60,65,70, 75°C) conditions tested, *B.subtilis* spore inactivation was less than 3 log units if the hold time was less than 3 minutes. The authors noted that Gao *et al*. (2006) reported that at pressures of 550-600 MPa with a process temperature of 87°C for between 10 and 20 minutes, a maximum log reduction of 6 $\log_{10}$ cfu/ml was obtained. Both studies suggest that if log reductions of *B.subtilis* spores of greater than 6 were required whilst utilising currently commercially achievable pressures then peak temperatures higher than 87°C, perhaps with extended hold times (beyond 3 minutes) are likely to be needed.

Koutchma *et al*. (2005) subjected *Clostridium sporogenes* PA3679 spores to a range of high pressure-high temperature combination processes to determine inactivation kinetics data. The trials for kinetic studies were conducted in phosphate buffer rather than in a real food matrix but some trials were also carried out using spore strips sandwiched between "commercial round scrambled egg patties".

Spores from *Clostridium sporogenes* PA3679 were selected for the trials because the authors noted that they were known to have a similar heat resistance to *Clostridium botulinum* and were often used as a surrogate in conventional thermal processing applications. Koutchma *et al*. (2005) noted that "most published data have dealt with the high pressure destruction of *Geobacillus*; *Clostridium* spores have been assumed to behave in a similar manner under high pressure". They went on to note "however recent studies on *Clostridium* have not confirmed this assumption". No reference is made to specific studies to which they are referring.

Koutchma *et al*. (2005) summarised previous studies on *Clostridium sporogenes* PA 3679 inactivation by pressure (Gola *et al.* 1996;Maggi *et al.* 1996;Mills *et al.* 1998;Rovere *et al.* 1996). Gola *et al*. (1996), Mills *et al*. (1996) and Maggi *et al.* (1996) all demonstrated *Clostridium sporogenes* PA3769 spores were highly resistant to pressure treatment without elevated temperatures. In studies by Maggi *et al*. (1996) the spores were not inactivated even by pressure as high as 1500 MPa when applied at 20°C for 5 minutes. These studies on *Clostridium sporogenes* PA3679 spores prior to Koutchma *et al*. (2005) all confirmed the need to combine pressure with elevated temperatures in order to inactivate the spores. For the

kinetic studies carried out by Koutchma *et al*. (2005), the conditions tested are shown in Table 2-3.

Pressure (MPa)	Initial temperature before pressurisation (°C)	Process temperature (°C)	Holding time (s)
600	67	91	0,60,120,180,240,300
	74	100	0,60,120,180,240
	82	108	0,30,60,90,120
700	62	91	0,60,120,180,240
	70	100	0,60,120,180,240
	78	108	0,30,60,90,120
800	58	91	0,60,120,180,240
	66	100	0,60,120,180,240
	74	108	0,30,60,90,120

Table 2-3. Experimental conditions used in kinetic studies on pressure-temperature inactivation of *Clostridium sporogenes* PA 3679 spores in phosphate buffer. (Koutchma *et al.* 2005).

Koutcham *et al.* (2005) observed log linear inactivation kinetics over the range of times, temperatures and pressures tested. Whilst time, pressure and temperature were all statistically significant in influencing the inactivation of *Clostridium sporogenes* PA 3679 spores it was temperature that was the most important factor. The 'D' values obtained from the studies are shown in Table 2-4.

	Pressure (MPa)	Temperature (°C)	D-values (s)
1	600	91	357.4
2	600	100	192.3
3	600	108	67.6
4	700	91	294.0
5	700	100	169.5
6	700	108	58.3
7	800	91	270.3
8	800	100	136.9
9	800	108	49.0

Table 2-4. D-values of *Clostridium Sporogenes* PA3679 after high pressure-high temperature treatments (Koutchma *et al.* 2005)

Note how changes in temperature had a greater influence on the 'D' value compared with changes in pressure, compare for example rows 1,4 and 7 of Table 2-4 where the temperature is constant but pressure increases, the 'D' value decreases as pressure increases but the changes are relatively modest. Contrast this with rows 1-3 where pressure is constant but temperature is changing – here is can be seen that the increase in temperature has a much more significant effect on the 'D' value.

The 'D' values obtained by Rovere *et al*. (1998) for *Clostridium Sporogenes* PA3679 were in good agreement with Koutchma *et al*. (2005) for a process at 800 MPa and 108°C (41.7 seconds compared with 49 seconds). Koutchma *et al*. (2005) reports that "heat treatment alone at 110°C yielded a 'D' value of 13.3 minutes in Rovere's study'. This suggests that pressure *in combination* with heating significantly enhances rates of inactivation compared with heat alone. However, Koutchma *et al.* (2005) found that the 'z_p' value for the organism (the change in temperature required to give a 10 fold change in the 'D' value at constant pressure) was 23.7°C at all pressures tested (600, 700 and 800 MPa) and that values for z_t (the change in pressure required to give a 10 fold change in the 'D' value at constant temperature) was 1500.7 MPa at all temperatures tested. These findings suggest that increasing pressure does not change the PA3679 spores sensitivity to heat and similarly, that increasing temperature does not increase the sensitivity of the spores to pressure (Figure 2-1 and Figure 2-2). In contrast to these findings, Patazca *et al*. (2006) found that *Geobacillus stearothermophilus* spores were less resistant to pressure at higher temperatures and that their pressure resistance was significantly lower than *C.sporogenes* PA3679.

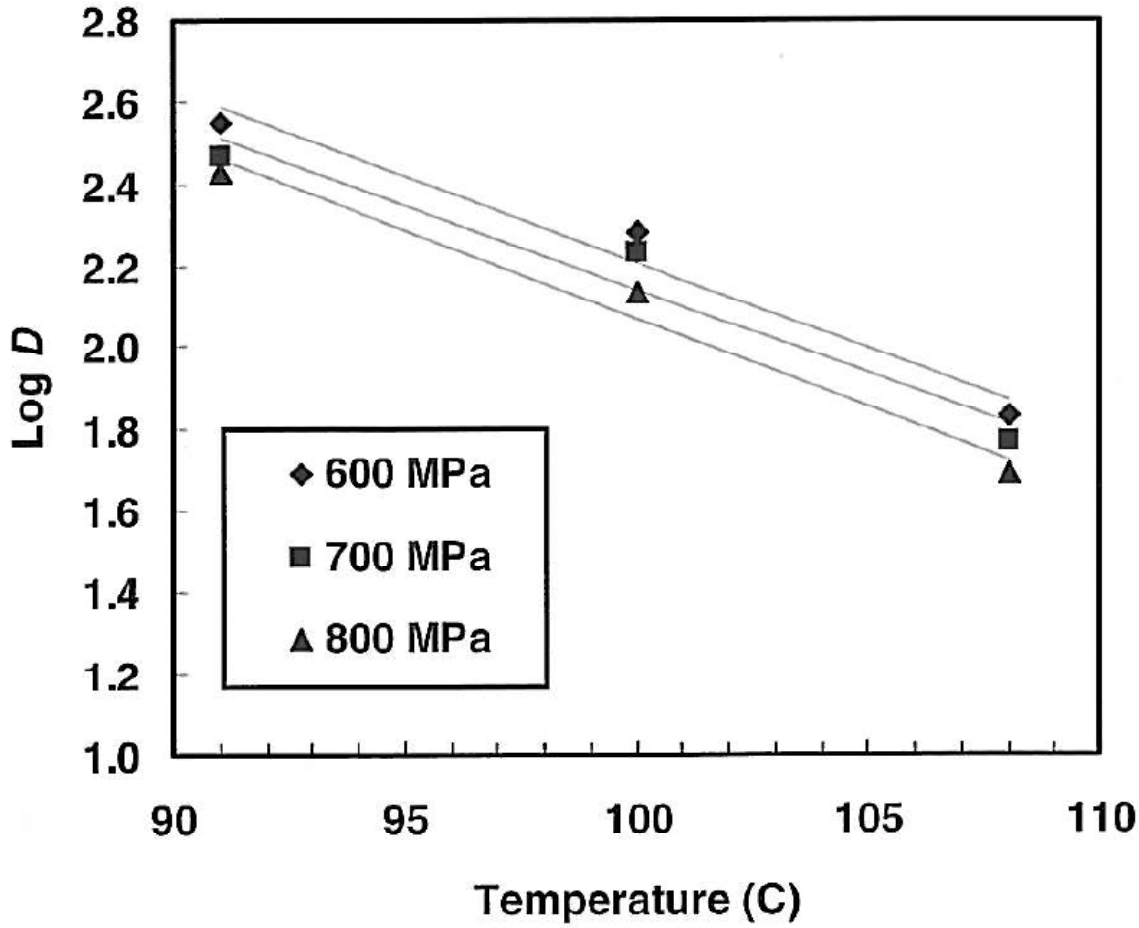

Figure 2-1. Temperature dependence of D-values for high pressure inactivation of Clostridium sporogenes PA3679 spores (Koutchma *et al.* 2005)

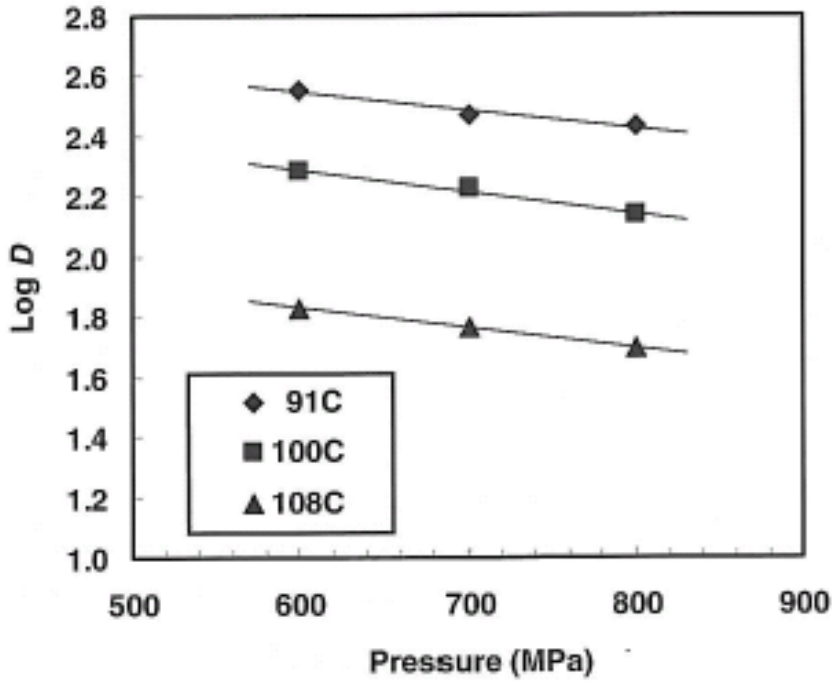

Figure 2-2. Pressure dependence of D-values for high pressure inactivation of Clostridium sporogenes PA 3679 spores (Koutchma *et al.* 2005)

This study by Koutchma *et al*. (2005) highlighted that PA3679 spores were considerably more resistant to HPS than *G.stearothermophilus*. For example, Koutchma *et al.* (2005) found a 'D' value for PA3679 spores of 19.2 minutes at 700 MPa and 121°C compared with 5.7 minutes for *G.stearothermophilus*. They concluded that a process of 2.24 minutes at 121°C at 600 or 700 MPa would be adequate to "destroy mesophilic and thermophilic spore-forming organisms" compared with a requirement of 27.5 minutes in a conventional retort process. Trials using spores strips in HPS treated egg patties established that a HPS process of 121°C for at least 3 minutes was sufficient to inactivate 6 logs of PA3679. Whilst PA3679 may be a better target organism to use that *G.stearothermophilus*, recent work suggests that the use of PA3679 as a surrogate for *Clostridium botulinum* under HPS conditions may risk overestimating the efficacy of the process (Bull *et al.* 2009).

Bull *et al*. (2009) reported on comprehensive studies compared the resistance of 5 proteolytic *Clostridium botulinum* strains (FRRB 2802, 2803, 2804, 2806 and 2807), and *Clostridium sporogenes* PA3679 when exposed to conventional thermal processing (100 to 110°C for a range of time points) or high pressure/high temperature combination processing (temperatures ranged between an initial sample temperature of 51.1°C and 103.1°C with a hold pressure of 600 MPa). The trials were carried out in 3 model food systems: a diluted bolognese sauce (30% w/w), a diluted cream sauce (50% w/w) and a rice water agar. Data presented by Bull *et al*. (2009) provides compelling evidence that PA3679 is *not* a good surrogate for *Clostridium botulinum* because it was frequently found to be less resistant to HPS in the model foods tested. Bull *et al*. (2009) also observed that a synergistic effect between high pressure and high temperature was not always observed and was strain and product dependent. They concluded that any HPS process for low acid foods "must be at least thermally equivalent to an F_0 of 2.8 minutes" in line with current industrial practice in the canned foods sector. Note that in practice this figure is rounded up to a minimum process of an F_0 of 3 minutes in order to provide a safety margin.

2.5 Published data on quality effects at high pressure sterilisation conditions

There is still surprisingly little published information on the food quality benefits that can be obtained from a combination pressure/heating process utilising commercially achievable processing conditions (Wilson *et al.* 2008). Patent claims in the Meyer patent (Meyer 2000c) are subjective – "Main meal entrees are 'unaffected' by the process", "vegetables have 'nearly raw' product quality", "potatoes have a 'freshly cooked' flavour", "eggs have a 'freshly cooked' flavour". Peer reviewed studies are limited. Matster *et al.* (2004) noted that the

effect of high pressure sterilisation on product quality was very much product dependent, particularly with respect to colour changes. They claimed that the colour of spinach was not changed as significantly by the process as, for example, green beans.

The texture of high pressure sterilised green beans has been shown to be significantly improved relative to conventional preservation methods (Krebbers *et al.* 2002). In this comparative study the quality effects of a range of preservation methods were investigated. The preservation methods studied were: high pressure pasteurisation, conventional thermal processing, freezing, and a double pulse sterilisation pressure treatment (after the Meyer patent).

This work is one of the few attempts to compare the quality benefits of a pressure sterilisation process with conventional preservation methods. As Krebbers noted, most studies have compared pressure pasteurisation quality changes relative to fresh products. In most cases it is true to say that there are differences between fresh products and pressure pasteurised products but the magnitude of this difference can be small relative to the difference between raw products and heat sterilised products. Green beans were subjected to treatments as outlined in Table 2-5.

Process	Conditions applied
HPP pasteurisation	500 MPa, ambient temperature, 60s
Pulsed high pressure sterilisation as per the Meyer process (designated "pHPP")	Pre-heated at 75°C for 2 minutes then processed at 75°C IT, 80s at 1000 MPa with second pulse of 1000 MPa after 30s at ambient pressure.
Retorted	4 min blanch at 90°C followed by 118°C for 30 minutes in 720ml glass jars.
Frozen (Blast)	Blanched as described previously, frozen at -20°C under forced air for 10 mins

Table 2-5. Processing conditions used in Krebbers *et al.* (2002)

Colour changes were measured immediately after each process and also after storage (1 month at 20°C for conventionally sterilised and pressure sterilised or 1 month at 6°C for raw and pressure pasteurised green beans). Immediately after processing, blanched and frozen beans had a more intense green colour compared with raw. Pressure processed green beans also had this more intense colour. Both conventionally sterilised and pressure sterilised beans exhibited the classic 'olive green' colour change typical of heat preserved beans and due to the conversion of labile chlorophylls a and b to yellow-olive coloured pheophytin, (Krebbers *et al.* 2002). The colour of heat sterilised and pressure sterilised beans was stable over the one month storage period. Raw, frozen and pressure pasteurised beans gradually deteriorated towards a pale yellow/green colour over the one month chilled storage period. Krebbers postulated that this was due to the residual activity of enzymes such as lipoxygenase, peroxidase or chlorophyllase. The texture of the beans was significantly affected by both blast freezing and conventional sterilisation. For example, the maximum force required to break raw beans was measured at around 39 N but immediately after freezing and thawing, the maximum force required was reduced to around 4 N. Immediately

after conventional sterilisation the maximum force required was around 1 N. In contrast, the firmness of pressure pasteurisation and pressure sterilised beans, whilst reduced, was much closer to the raw product. The maximum force required to break the beans was around 24 N and 23 N for pressure pasteurised and pressure sterilised beans respectively. The texture of pressure processed beans was largely retained after one month of storage (maximum forces being 25 and 20 N for pressure pasteurised and pressure sterilised respectively). It is important to note first that the peak temperature attained during processing was only around 100°C and that the pressures used for the sterilisation studies (1000 MPa) are far in excess of what is currently commercially achievable (700 MPa). The relevance of these points will be discussed in more detail in chapter 4 where comparable studies on colour and texture effects are reported using more commercially realistic operating conditions.

Ascorbic acid was relatively little affected by pressure pasteurisation with around 92% *retention* immediately after processing. In contrast, a conventional thermal process resulted in a *reduction* of around 90% of the original ascorbic acid content. Pressure sterilised beans retained around 76% of their original ascorbic acid content. However, with the exception of frozen beans, ascorbic acid content after 31 days of storage was reduced to less than 0.01% of the original level.

The effect of a double pulse (as described in Table 2-5 – 'pHPP') pressure sterilisation cycle on ascorbic acid levels was reported by Matser *et al.* (2004) for green beans, spinach and apple juice; the results are summarised in Figure 2-3. Note that ascorbic acid retention in high pressure sterilised samples (pHPP) was much closer to fresh and significantly better than the

retention observed in retorted products with the exception of apple juice (with added ascorbic acid) where ascorbic acid levels were higher in retorted products than in pressure sterilised samples.

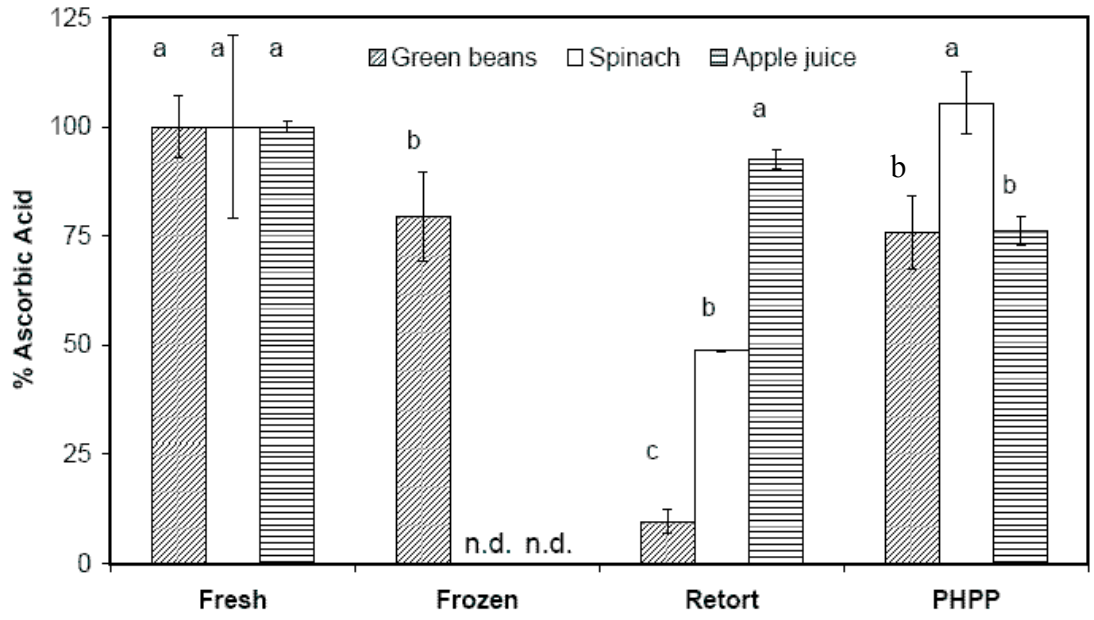

Figure 2-3: Ascorbic acid content (as a percentage of fresh) for frozen, heat sterilised and pressure sterilised green beans, spinach and apple juice (with added ascorbic acid).

Quality effects of high pressure sterilisation have also been studied for pulped tomato (Krebbers *et al.* 2003). Studies compared the impact of high pressure pasteurisation, high pressure sterilisation, conventional pasteurisation and conventional sterilisation (all conditions as described in Table 2-6) on key quality attributes of the product. The quality attributes studied were colour, viscosity, syneresis (separation of water from the paste), polygalacturonase (PG) activity, pectin methylesterase (PME) activity, lycopene content and sensory acceptability. The various preservation treatments had a significant impact on the

viscosity of the tomato pulp after processing. Table 2-6 summarises the effect of different treatments on syneresis and viscosity.

	Process type	Treatment	Viscosity (mPa*s, n=4)	Released serum moisture content (%)
1	Raw pulped tomato	-	548 [b]	17 [b]
2	Thermally pasteurised	4 min at 72°C	266 [d]	23 [c]
3	Thermally sterilised	20 min at 118°C	465 [c]	29 [d]
4	HPP pasteurised	2 min at 300 MPa, 20°C	531 [b]	19 [b]
5	HPP pasteurised	2 min at 500 MPa, 20°C	684 [a]	14 [b]
6	HPP pasteurised	2 min at 700 MPa, 20°C	767 [a]	14 [b]
7	HPP sterilised	2 x 30 s at 700 MPa and IT=80°C	261 [d]	21 [b]
8	HPP sterilised	1 x 30 s at 700 MPa and IT=90°C	288 [d]	5.2 [a]
9	HPP sterilised	2 x 30 s at 700 MPa and IT=90°C	322 [d]	19 [b]

Table 2-6: Viscosity (at a shear rate of 13.6 l/s) and syneresis values (released serum moisture in %) of different processing treatments (Krebbers *et al.* 2003). Mean values in the same column not sharing a common superscript (a-d) are significantly different (P<0.05)

The data shown in Table 2-6 show that a high pressure pasteurisation process of 300 MPa for 2 minutes at 20°C did not result in any significant change in viscosity of the tomato pulp compared with raw and that the released serum moisture was similar to raw (compare lines 1 and 4 in Table 2-6). Pasteurisation processes at higher pressures (Table 2-6 lines 4-6) resulted in a linear increase in viscosity with pressure (Krebbers *et al.* 2003). Conventional pasteurisation (Table 2-6 line 2) caused both a significant reduction in viscosity and a significant increase in serum moisture release. Interestingly, a conventional sterilisation

process (Table 2-6 line 3) caused very little reduction in viscosity but a significant increase in released serum moisture content. Unfortunately, conventional sterilisation also gave an orange/brown colour change in the pulp which resulted in a lower appreciation of the product from a trained sensory panel compared with all other treatments. Generally speaking, high-pressure sterilisation processes resulted in a reduction in viscosity that was similar to a conventional pasteurisation (compare Table 2-6 lines 2, 7,8 and 9). However, the water holding capacity of the pulp was better than that achievable with thermal pasteurisation; all sterilisation processes bar one resulted in released serum moisture contents that were not significantly different to the raw pulp (compare Table 2-6 lines 7-9 with line 1). The exception was a process of 30 seconds at 700 MPa and 90°C; this resulted in significantly less released serum moisture compared to the raw pulp. Lycopene content was largely unchanged by all processes except thermal sterilisation. Sensory panellists highly rated pressure pasteurised products (using pressure of up to 500 MPa) and product manufactured using 90°C and 700 MPa (single or double pulsed). All pressure-processed samples were preferred to thermal pasteurisation and sterilisation samples. Polygalacturonase (PG) was reduced to less than 2 % of the original activity by both conventional sterilisation and all high pressure sterilisation processes. Polygalacturonase was reduced by around 60% by pressure pasteurisation but increasing pressure did not improve inactivation, demonstrating pressure resistance (Krebbers *et al.* 2003). Pectin methyl esterase (PME) was reduced to less than 5% of its original activity by conventional sterilisation and by two of the three pressure sterilisation processes (1 or 2 pulses at 90°C and 700 MPa). In contrast, PME activity was doubled in thermal pasteurisation and increased by a factor of 5.5 to 6.5 by pressure pasteurisation.

Lau & Turek (2007) reported on trials comparing the quality of HPS treated products with retorted products. Tests were conducted on 5 food product types: chicken breast, salmon fillet, egg omelette, fried potato wedges and green beans. Although the results as presented in the report are somewhat lacking in hard data, they remain useful because they indicate potential quality benefits arising from HPS for a range of products and also because the samples were processed in larger scale machinery (35 litres), closer to what might be used industrially.

Chicken was sprinkled with a BBQ spice mix and griddled for 60 seconds prior to HPS. Salmon was soaked in a brine for 1 hour to improve the sensory properties as it is known that pressure treated fish products can become rubbery in texture after HPP (Lau & Turek 2007). Egg were whipped with citric acid, 23% canola oil and 0.2% xanthan gum prior to treatment. Potato wedges were cut, pan-fried, seasoned and packed ready for HPS. Green beans were trimmed, packed in brine with 1% sodium bicarbonate and 0.9% lactate "to provide better flavour, colour and texture". All food samples were packed in clear, high oxygen barrier retort pouches ready for either HPS treatment or traditional canning. All retorted samples were treated up to an F_0 of 6 minutes. HPS salmon and egg samples were pre-heated to 70°C in a water bath, the remaining products were pre-heated to an initial temperature of 80°C. The products were pre-heated to different initial temperatures based on fat content because compression heating in the salmon and egg samples was expected to be higher than in the potatoes, green beans and chicken (see section 2.6). Pre-heating to different initial temperatures was an attempt to ensure that the peak temperatures attained in the HPS cycle

were similar for all products. The HPS cycle employed was two pulses of 690 MPa each of 1 minute duration. The peak temperature attained for all products was approximately 106°C. Products were assessed by sensory evaluation, colour measurement and texture measurement.

Summary of sensory findings (Lau & Turek 2007)

HPS treated chicken was reported to retain the "flavour of fresh roast chicken" whereas canned chicken had a stewed flavour. Canned salmon had a loss of flaky texture and was of a faded pink colour that the panel associated with overcooking. In contrast HPS treated salmon was bright pink and reportedly had the appearance of freshly poached salmon. This is somewhat surprising because previous studies exploring the effects of high pressure pasteurisation on salmon have reported undesirable changes to product appearance and the development of undesirable 'rubbery' textures (Ashie & Simpson 1998;Dong *et al.* 2003;Hurtado *et al.* 2000;Leadley *et al.* 2008). Potato wedges had a "fresh boiled-like flavour" and "fried and spicy flavour notes" compared with canned samples that were reported as having "mild musty and overcooked notes". Retorted eggs had "a greenish brown hue with…metallic and sulfury notes" whereas as HPS treated eggs had "a good color, fresh hard boiled egg flavour, and a custard like firm texture". HPS treated green beans had a "discernible darker color than retorted beans" but were very crunchy "similar to lightly steamed green beans". HPS treated eggs had a very firm texture and had lost entrained air that was still observed in the canned samples.

Summary of colour evaluation (Lau & Turek 2007)

HPS chicken samples were "lighter and more yellow" than retorted products. HPS salmon was "lighter and more orange" than the retorted samples. Retorted potatoes were "darker and more brown" than HPS samples, retorted eggs were also darker than HPS eggs and were more yellow/green. HPS treated green beans were darker than retorted beans which is in agreement with the results reported in this thesis (see Figure 4-12). Lau & Turek (2007) suggested this might be a measurement artefact resulting from dark spaces between the beans causing inaccuracies in the results from the instrument being used for colour measurement. The results presented in Chapter 4 suggest this is not the case because the instrument used in the studies presented in this thesis does not suffer from the same measurement limitations mentioned by Lau & Turek (2007).

Summary of instrumental texture evaluation (Lau & Turek 2007)

Chicken samples had a similar resistance to cutting in both HPS and retorted products but the latter products "tended to fall apart parallel to the grain….resulting in less chew" in sensory evaluation. HPS treated salmon was firmer than retorted salmon which is reported to have fallen apart during texture measurement. Retorted and HPS potatoes had a similar peak resistance to cutting but HPS samples "showed less surface firmness and allowed greater penetration before fracturing than retorted potatoes". HPS treated egg had a "very firm texture" compared with retorted samples which were softer and cut more easily. HPS treated green beans had a "very firm texture" whereas retorted beans "cut easily and were very soft". Summary instrumental data is not provided in the work of Lau & Turek (2007) so the commentary is somewhat subjective. A graph is presented of a force distance profile for

retorted and HPS treated green beans which suggests that peak force required to cut the beans was approximately 5 and 45 Newtons respectively.

Key findings from the work (Lau & Turek 2007)

Lau & Turek (2007) acknowledged that they were uncertain as to whether the conditions employed would result in commercial sterility but noted that samples showed no evidence of spoilage after 3 months of ambient storage. They drew a series of tentative conclusions about the quality benefits achievable from high pressure sterilisation these included:

I. HPS produced fresher, less processed flavours in all products tested compared with retorted products
II. HPS produced less caramelisation/browning and generally yielded lighter colour products apart from in green beans where darkening was observed
III. Protein based foods tend to be firmer after HPS treatment and this can lead to rubbery textures in some products. Firming in egg was believed to be primarily due to loss of entrained air in HPS treated products
IV. HPS generally led to improved sensory attributes compared with retorted products
V. There was potential to optimise the texture of HPS foods by raw material selection and pre-treatment (this latter point being explored in Chapter 6)
VI. Quality will benefit from optimised pre-heating (see Chapter 6)
VII. Higher pressures and lower initial product temperatures at the start of the HPS cycle would enhance product quality.

A series of studies on the effects of HPS processing on the quality of scrambled egg patties for military ratio packs has been reported (Juliano *et al.* 2006a;Juliano *et al*. 2006b;Juliano *et al.* 2007). This particular product has historically been difficult to produce for ration packs using conventional retort technology due to the development of a green-gray discoloration, off-flavour development, and synerisis (Juliano *et al.* 2006b). Experiments were conducted to explore factors such as the effect of product formulation, manufacturing methods and pre-heating methods on texture and synerisis in scrambled egg patties. In egg patties formulated with xanthan gum (0.1 to 1.5% by weight) or cheese (20%) a reduction of at least 30% in hardness, cohesiveness and water loss was observed. However "all formulations experienced significant increased hardness, cohesiveness and resilience after HPS". The initial preparation method for the egg patties also influenced the final texture; patties formed with a reduced level of entrained air were softer in texture than patties produced with high levels of entrained air as this was subsequently displaced from the food matrix as has been previously reported by other authors (Juliano *et al.* 2006b;Yamazaki & Kinefuchi 2003). Faster pre-heating methods such as steam injection, whilst more efficient in terms of rate of pre-heating did not affect most of the measured texture parameters and synersis was not influenced by pre-heat method. Never-the-less, the authors noted that "During HPHT treatment.." (HPS) "the process of preheating is extremely important to ensure that the initial temperature throughout the food samples matches (or is higher than) the initial pressure system temperature" (Juliano *et al.* 2006b). Egg patties treated at >70°C and 675 MPa for 5 minutes had a good appearance but the "color, texture and flavour" were significantly altered and were unacceptable. This was particularly due to the dense structure obtained after treatment and as described by others (Juliano *et al.* 2006a;Lau & Turek 2007). Reformulation with xanthan gum, EDTA and flavours

provided improved retention of quality parameters after treatment at >70°C and 675 MPa for 5 minutes. The xanthan gum helped to soften the texture of the product and reduce synerisis (50-55%) and the EDTA prevented greening.

De Roeck *et al*. (2010) explored the effect of high pressure/high temperature combination processing on carrot-texture-degradation kinetics and compared the results to those obtained by conventional heating. Heat treated carrots were processed at 95-110°C for up to 10 minutes and HPS treated samples were processed at 95, 100 and 110°C at 600 MPa for up to 10 minutes.

De Roeck *et al*. (2010) found that texture degradation was very rapid at temperatures above 100°C and "both thermal and HP/HT treated carrots lost a considerable amount of hardness during the pre-process". However the rate of softening was slower in HPS treated samples compared with conventionally heat processed (by a factor of around 10).
De Roeck *et al*. (2010) postulated that HPS treatment decelerated "beta-eliminative degradation of the cell wall pectin" and since this reaction is "strongly dependent on the degree of pectin methyl esterification" the degree of methyl esterification (DM) of carrot pectin was evaluated after conventional-thermal and HPS treatments. The DM in HPS treated samples was lower than in conventionally heated carrot pectin and this reduction was thought to be the most likely reason for improved texture retention in HPS samples because methyl esters "are one of the main driving forces of the beta-elimination reaction" that leads to softening.

Nguyen *et al*. (2007) compared the texture, colour and carotene retention of HPS treated carrots with that of conventionally heat processed samples. Samples were treated at a range of temperatures (95-121°C) and pressures (500 and 700 MPa) for up to 15 minutes residence time at pressure. Thermally processed samples were treated at temperatures of 95, 105 and 121°C for up to 60 minutes with regular sampling throughout to determine changes in texture, colour and carotene retention. The overall colour change (expressed as ΔE – see equation 5.2) in both conventionally heat processed samples and HPS samples, increased with increasing hold temperature. At every hold temperature (95, 105 and 121°C) the overall colour change was lower in HPS samples compared with that observed in samples heated at atmospheric pressure. At a hold temperature of 105°C, overall colour change was smaller at 500 MPa than at 700 MPa indicating that pressure did play a role in modifying colour. However, at 121°C, overall colour change was similar at 500 and 700 MPa suggesting that the changes in ΔE were predominated by temperature effects.

Heat treated samples had a greater loss of texture compared with HPS samples. For example "samples treated at 121°C retained only 1.5% hardness after a 2 minute hold time" whereas at 121°C and 500 MPa there was a 16% retention and at 121°C and 700 MPa there was a 62% retention in texture. It is interesting that better texture retention was found at 700 MPa than at 500 MPa. Nguyen *et al*. (2007) proposed that this was due to the fact that the samples treated at 500 MPa had to be pre-heated to 86.1°C whereas as samples treated at 700 MPa were pre-heated to 73°C. This was necessary to ensure that the peak temperature attained in each run was similar (because the compression heating expected at 500 MPa would be less than the compression heating at 700 MPa).

2.6 Compression heating effects and their importance in HPS cycles

An understanding of compression heating effects on the product and pressure medium is critical for the correct implementation of a thermal/pressure combination process as they could have a significant impact on temperature gradients within the vessel. Heating as a result of pressurisation can be explained by gas laws (Wilson & Baker 2003; Rasanayagam 2003). Applying Equation 2.1 to solid and liquid materials, the temperature will rise when pressure is applied and decline when it is released.

$$\frac{dT}{dP} = \frac{T\alpha}{C_p}\rho \qquad (2.1)$$

Where T is temperature (K), P is pressure (Pa), ρ is density ($kg.m^{-3}$), Cp is the heat capacity of the substance at constant pressure (J/(kg.k) and α is the thermal expansivity (1/K). A mathematical treatment of the problem is given by Knoezer *et al*. (2007).

Rasanayagam *et al.* (2005) noted that Equation 2.1 is strictly only applicable to isentropic pressure changes. They also noted that Otero *et al*. (2000) used the equation above in an iterative method to calculate temperature rise of water for larger pressure differences and that problems are reportedly encountered with this approach when attempts are made to calculate volume under pressure since it cannot be taken as constant under these circumstances.

Compression heating can be quite marked even in relatively incompressible materials such as solid and liquid food products. Temperature rises due to compression could be in the region

of 3 to 9°C per 100 MPa of applied pressure, (de Heij *et al.* 2003) depending on the nature of the food material.

One of the main technical challenges facing any temperature/pressure combination process is to maximise the benefits of compression heating by minimising heat losses from the vessel (see section 2.7). Since the metal of the vessel wall does not significantly increase in temperature as a result of compression, temperature gradients will be set up as a result of compression heating of the pressure transmitting fluid and the food being treated. Material at the centre of the vessel is likely to be higher in temperature than material at the relatively cool surface of the vessel wall and at the interface with the vessel closures, (de Heij *et al.* 2003).

Non-uniformity due to pressure gradients is further complicated when the high pressure pump is started to introduce more of the pressure medium into the vessel, e.g. during the vessel come-up period. If cooler liquid enters the vessel, further temperature variation can be introduced. At present, major manufacturers of high pressure equipment such as Hydropac do not advocate pumping the pressure medium hot as it could reduce the fatigue life of high pressure pump components (personal communication with Hydropac, USA). Strategies for reducing heat losses are reported in section 2.7.

Data on compression heating effects in real food products is lacking in the public domain. Wilson & Baker (2003) stated that at 689 MPa, adiabatic heating of water increases the temperature approximately 20°C, whereas castor oil rises 40°C. They noted that a 27°C adiabatic temperature rise was observed in a model wet pet food at 620 MPa.

Hoogland *et al*. (2001) commented that compression heating temperature rises can be "well approximated" from Equation 2.1 but pointed out that since material properties (e.g. specific heat and density) are affected as a function of pressure and temperature, they must be measured in most food materials because published data is scant.

The compression heating effect that is achieved will depend on factors that include operating pressure, initial temperature, food composition, and the compression heating characteristics of the pressure medium. Compression heating effects of water are relatively well understood. However, relatively little data is available for real food products.

Rasanayagam *et al*. (2003) describe the development of a test rig to measure compression heating effects in soybean oil, olive oil, beef fat, chicken fat and salmon flesh. They used customised equipment developed in collaboration with Avure Technologies which used a 25 HP pump to pressurise the process vessel. Water was used as the pressure transmitting medium. The pump capacity was very high relative to the vessel volume such that the come-up time was less than 1 second, giving conditions close to a true adiabatic situation. The vessel was of a stainless steel construction with a volume of 54 ml containing a 20 ml sample holder consisting of a plastic syringe with movable plunger. The temperature of the sample was equilibrated prior to testing by immersion in a water bath set at the target initial temperature. Trials were carried out at between 150MPa and 600 MPa over a range of initial temperatures. A summary of data for compression heating studies in food products is summarised in Table 2-7.

Food substance	Initial Temp. (°C)	Process pressure (MPa)	Temperature increase (°C) per 100 MPa of applied pressure
Water[a]	20	N.R.	2.8
Water[a]	60	N.R.	3.8
Water[a]	80	N.R.	4.4
Steel[a]	20	N.R.	≈ 0
Chicken[a]	20	N.R.	2.9
Cheese (Gouda type) [a]	20	N.R.	3.4
Milkfat[a]	20	N.R.	8.5
Water[b]	25	N.R.	≈ 3
Mashed potato[b]	25	N.R.	≈ 3
Orange juice[b]	25	N.R.	≈ 3
Tomato salsa[b]	25	N.R.	≈ 3
2%-Fat milk[b]	25	N.R.	≈ 3
Salmon[b]	25	N.R.	≈ 3.2
Chicken fat[b]	25	N.R.	≈ 4.5
Beef fat[b]	25	N.R.	≈ 6.3
Olive oil[b]	25	N.R.	From 8.7 to <6.3*
Soy oil[b]	25	N.R.	From 9.1 to <6.2*
Water[c]	25	149	2.6 ± 0.1
Water[c]	25	298	2.7 ± 0.2
Water[c]	25	443	2.8 ± 0.1
Water[c]	25	592	2.9 ± 0.3
Salmon fish[c]	25	159	2.8 ± 0.3
Salmon fish[c]	25	321	2.9 ± 0.1
Salmon fish[c]	25	463	3.0 ± 0.2
Salmon fish[c]	25	611	3.0 ± 0.1
Extracted beef fat[c]	25	170	8.3 ± 0.7
Extracted beef fat[c]	25	342	6.5 ± 0.4
Extracted beef fat[c]	25	465	6.2 ± 0.4

Food substance	Initial Temp. (°C)	Process pressure (MPa)	Temperature increase (°C) per 100 MPa of applied pressure
Extracted beef fat[c]	25	613	6.3 ± 0.4
Crude beef fat[c]	25	162	4.1 ± 0.1
Crude beef fat[c]	25	315	4.4 ± 0.3
Crude beef fat[c]	25	463	4.4 ± 0.5
Crude beef fat[c]	25	605	4.4 ± 0.8
Linolenic acid[c]	25	170	9.0 ± 0.4
Linolenic acid[c]	25	340	7.8 ± 0.4
Linolenic acid[c]	25	490	7.1 ± 0.5
Linolenic acid[c]	25	602	5.9 ± 0.3
Soybean oil[c]	25	172	8.3 ± 0.7
Soybean oil[c]	25	312	7.5 ± 0.1
Soybean oil[c]	25	452	6.9 ± 0.3
Soybean oil[c]	25	617	6.3 ± 0.4
Olive oil[c]	25	173	8.7 ± 0.4
Olive oil[c]	25	325	7.3 ± 0.4
Olive oil[c]	25	455	6.9 ± 0.4
Olive oil[c]	25	501	7.2 ± 0.2
Propylene glycol[c]	25	164	5.8 ± 0.6
Propylene glycol[c]	25	325	5.3 ± 0.4
Propylene glycol[c]	25	470	5.1 ± 0.2
Propylene glycol[c]	25	548	5.1 ± 0.5
Ethanol[c]	25	144	10.6 ± 0.6
Ethanol[c]	25	325	5.4 ± 0.6
Ethanol[c]	25	457	5.5 ± 0.2
Ethanol[c]	25	540	6.8 ± 0.4

Table 2-7: Compression heating effects in a range of food products (de Heij *et al.* 2003)[a], (Ting *et al.* 2002)[b] (Rasanayagam *et al.* 2003)[c.] N.R. = not reported * Substances exhibited decreasing temperature as pressure increased

In Rasanayagam's work the temperature rise per 100 MPa of applied pressure was relatively consistent for water although it did rise slightly with increases in applied pressure (2.6 to 2.9°C for 149 MPa and 542 MPa respectively). Salmon flesh compression heating was similar to that of water, which is perhaps unsurprising given that water was the single biggest component of the sample (around 68%). The same is likely to hold true for many food materials.

The initial temperature of water has been shown to influence the degree of observed compression heating. For example, increasing the initial temperature from 25 to 60°C increased compression heating values from around 3 to 4°C per 100 MPa of applied pressure (Rasanayagam *et al.* 2003). Hoogland *et al*. (2001) reported a similar effect; a pressure treatment of 700 MPa resulted in a temperature rise of 2.8°C and 4.7°C per 100 MPa at an initial temperature of 20°C and 90°C respectively. A similar effect of initial temperature on compression heating can also be found in the results presented in Chapter 3 (see Figure 3-25).

Compression heating of oils did not seem to be significantly affected by increasing the initial temperature of the sample. Unsaturated fatty acids showed the highest degree of compression heating, followed by saturated fatty acid and then water. Rasanayagam *et al*. (2003) postulated that the relative 'bulkiness'of fat molecules compared to water meant that they would compress more on pressurisation and would therefore have higher compressibility values.

The influence of the compression medium on heating effects was reported by the National Center for Food Safety and Technology, (Balasubramanian & Balasubramaniam 2003). High pressure sterilisation studies were carried out using a pressure transmitting medium of either: varying concentrations of water-glycol mixes, or a 2% sodium benzoate solution. Increasing the ratio of glycol to water increased the maximum temperature attained by the pressure medium on compression. For example, the maximum temperature increase attained by a 75/25% mixture of water and glycol was approximately 22°C (with an initial temperature of 29.5°C). A 25/75% mixture of water and glycol resulted in a maximum temperature rise of around 27°C (with an initial temperature of 29.7°C). Another interesting point of note was that the time to reach maximum temperature increased with increasing concentration of glycol. A 75/25% water glycol mix reached maximum temperature 14 seconds (±2.6 seconds) after reaching target pressure. This time increased to 24.0 (± 5.7 seconds) and 36.0 (± 7.7 seconds) for 50/50 and 25/75 percent mixes of water/glycol respectively. This paper also raised an important point about how best to minimise temperature gradients during processing. To illustrate this point, using a 75/25 water/glycol mix and starting the process (827 MPa for 10 minutes) with the pressure fluid and vessel wall both at 70°C, the maximum temperature achieved was around 103°C. After a 10 minute hold a temperature drop of around 25°C occurred. If instead the vessel wall was at 70°C but the pressure fluid was at around 34°C (so that the peak temperature attained was closer to the vessel wall temperature) then the maximum temperature attained was around 73°C. The temperature drop over 10 minutes was then around 7°C (Balasubramanian & Balasubramaniam 2003). Although it would seem practical to maximise the temperature attained during compression heating, this could potentially result in very large temperature gradients across the vessel. The paper noted that for extended

holding periods it would be desirable to maximise the compression heating effect to account for the gradual decline in temperature during the hold period. The ideal solution is probably to maximise the compression heating effect and then minimise heat losses by, for example, the use of insulated liners (see section 2.7).

When 2% sodium benzoate was used as a pressure medium, inactivation levels of *Bacillus subtilis* spores were higher than when a glycol/water mix was used despite the sodium benzoate pressure medium exhibiting lower compression heating. This was attributed to the differing thermal properties of the compression media. The thermal diffusivity of the sodium benzoate solution was 1.5 times that of a 25/75 water glycol solution that exhibited the greatest degree of compression heating. The viscosity of the sodium benzoate solution was also lower than the 25/75 water/glycol solution. For commercial food sterilisation, water is likely to be the most suitable compression medium because it is non-toxic but also because it likely to exhibit similar compression heating characteristics to foods which are predominantly made up of water.

2.7 Optimising process uniformity and understanding compression heating effects

One of the challenges to be addressed for any temperature/pressure combination process such as that proposed by Wilson & Baker (1997) is temperature losses to the surroundings during the hold phase of the high pressure cycle. Heat losses from the vessel set up temperature gradients within the vessel and so the degree of lethality that is achieved is likely to vary and be dependent on the spatial location of the product within the vessel. Various strategies have therefore been proposed to minimise heat losses and improve temperature uniformity during HPS cycles.

In a patent by ATO and Unilever, a number of strategies are presented to minimise heat losses (van Schepdael *et al.* 2002). Suggested strategies include:

1. Use of a rapid rate of compression (>5 $MPa.s^{-1}$). This minimises the time available for heat transfer to occur between the product, compression medium and vessel wall. The use of an internal intensifier is one proposed method to achieve this. The disadvantage of this approach is that it reduces the working volume of the vessel which has an impact on process economics (see chapter 1).

2. The use of a vessel material with low heat transfer properties (<25 $W.m^{-1}.K^{-1}$) and a compression heating response that is similar to foods (1-8 K per 100 MPa). An alternative is the use of a liner inside the vessel having these properties. It is suggested that materials which are suitable for use as either a vessel material or as a liner include HDPE, PEEK, UHMWPE, PVC, EP or POM.

3. The use of a compression medium with similar, or higher, compression heating characteristics to the food. In most cases water will be the most appropriate pressure medium with the possible exception of very fatty foods where the compression heating of water may be too dissimilar to the heating characteristics of the food.

4. Avoiding heat losses from the food to the incoming pressure medium; one simple solution for this is to place the product inside an insulated container.

5. Applying a vessel temperature that is greater than the initial product temperature; this requires the use of approaches 1 and 2 above to prevent burning and product quality deterioration. In addition, there may be process limitations to the temperature at which the pressure vessel can be operated.

By applying one or more of these solutions, a more uniform temperature distribution could be achieved inside the pressure vessel. Because uniformity is improved, the patent claims that less severe temperature and pressure combinations can be used that result in similar levels of lethality as are attained by the process conditions stipulated by the Meyer and Kal Kan patents. Examples extracted from the patent are summarised in Table 2-8.

1	2
Optimised system giving equivalent lethality to 'non-optimised' system	**Non-optimised system**
60°C and two pulses of 950 MPa	70°C and 2 pulses of 960 MPa
90°C and two pulses of 600 MPa	90°C and 2 pulses of 750 MPa
90°C and 1 pulse of 910 MPa	90°C and 2 pulses of 750 MPa
105°C system temperature, product at 90°C, 1 pulse of 700 MPa for 150 seconds	90°C system temperature, product at 90°C, 2 x 60 second pulses of 700 MPa
105°C system temperature, product at 90°C, 1 pulse of 700 MPa for 150 seconds	90°C system temperature, product at 90°C, 1 x 300 second pulse of 700 MPa

Table 2-8 Summary of examples of 'optimised' pressure/temperature combination processes relative to 'non optimised'. Process conditions in column 1 are claimed to give equivalent spore inactivation to those of column 2 (van Schepdael *et al.* 2002)

Knoerzer *et al*. (2007) present a model for an Avure HPS system and show that the temperature changes seen in the vessel result from significant thermal losses (i) through the basket and the wall, and (ii) as a result of cold pressurising water ingress into the vessel. They show using simulation that a PTFE basket will reduce thermal losses and give much more uniform temperatures. The Knoerzer *et al*. (2007) model assumes the medium is water, for which compressibility data is available. Building on this work, the effect of different polymer materials and carrier optimisation on heat losses and temperature distribution have been reported in recent work by CSIRO (Knoerzer *et al.* 2010b;Knoerzer *et al.* 2010a;Knoerzer & Chapman 2011). Knoerzer *et al*. (2010a) note that during a HPS cycle the metal of the vessel will not heat under compression but that heating will occur in the food and pressure transmitting fluid which will lead to thermal gradients (Denys *et al.* 2000;Juliano *et al.* 2009;Knoerzer *et al.* 2007;Knoerzer *et al.* 2010a;Otero & Sanz 2003;Ting *et al.* 2002) with heat losses occurring towards the chamber walls. It is also noted that the incoming pressure fluid will also introduce temperature gradients and that in a vertical high pressure system the cold point in the vessel will often be at the fluid inlet. Insulated carriers made from polytetrafluoroethylene (PTFE) or polypropylene (PP) are reported by Knoerzer *et al.* (2010a) to be best suited for insulation properties in a high pressure vessel but a problem with using a liner is that it reduces the available volume for processing which in turn influences production costs (see Figure 1-5). Computational fluid dynamic modelling (CFD) was used to optimise the design of an insulated carrier for HPS such that it would have "maximum heat retention, temperature uniformity and usable volume". Knoerzer *et al.* (2010a) demonstrated by CFD modelling that a 5 mm thick PTFE carrier when used in a 600 MPa process with 90°C initial temperature actually provided good insulation and resulted in a more uniform temperature

distribution than a 70 mm thick carrier. Knoerzer *et al.* (2010a) explained that this was because the "carrier walls insulate the carrier contents from the cooler vessel walls, they themselves also act as heat sinks resulting in the formation of temperature gradients and heat loss". The use of CFD for carrier optimisation enabled the group to optimise carrier thickness for maximise temperature performance (where a 7mm thick carrier was optimal) or to optimise temperature performance and usable volume (where a 4mm carrier was identified as optimal). Another interesting proposal put forward in Knoerzer *et al.* (2010a) was the use of an "integrated temperature distributor" which was an equation to "evaluate the thermal process performance in an HPHT process through the vessel without requiring inputs from microbial kinetic parameters and only accounting for temperature and time data". Knoerzer *et al.* (2010b) also explored compression heating effects that might occur in the polymer used to manufacture the product carrier in a HPS cycle and concluded that "polymeric insulating plastics undergo significant compression heating" and that "internal heaters as available in some systems are therefore unnecessary if well designed polymeric baskets are use" since these will heat under pressure and effectively act as internal heaters.

2.8 Process validation of high pressure sterilisation processes

The validation of high pressure processes present significant technical challenges for the food manufacturer. The efficacy of a high pressure process is affected significantly by the composition of the food. For example high fat products are known to protect micro-organisms from pressure inactivation. Similarly, reduced water activity foods have been shown to confer baroprotection (Oxen & Knorr 1993). Various studies on spores have demonstrated that the food matrix influences the efficacy of the process (Bull *et al.* 2009;Margosch *et al.* 2004;Paredes-Sabja *et al.* 2007). It is generally recognised that pressure

pasteurisation processes must be established by challenge testing with either a specific pathogen of concern or an appropriate indicator organism with a similar pressure response to the target organism. Sizer *et al*. (2002) noted that "it is important to evaluate HPP microbial resistance in the actual foods rather than in traditional buffer solutions" and went on to comment that "each type of food must be investigated separately on a case-by-case basis until sufficient experience is gained to build a generalised model". Even when the effects of compression heating are not deliberately utilised in a process, for example in a pressure pasteurisation process, the magnitude of the temperature effect and its influence on process efficacy must be understood. This is particularly important when attempting to transfer lab scale results to full scale equipment. As Sizer *et al*. point out; many early pressure studies were carried out with no appreciation of compression heating or temperature distribution effects. If for example, an oil-based medium was used in lab studies, the lethality of the process could be higher than in a water-based full scale system, purely because of the pressure medium compression heating effects and/or heat losses from the vessel. Similarly, failure to characterise temperature distribution effects could lead to errors in the interpretation of results.

Sizer *et al*. (2002) proposed a number of possible routes to validate a high pressure/heating sterilisation process. These included:

1) considering HPP as a conventional thermal process;
2) demonstrating a 12-D process with biological validation using *C.botulinum* spores;
3) demonstrating a 12-D process using a surrogate organism.

4) demonstrating a 12-D process using established inactivation kinetics of *C.botulinum*

In the first approach, by understanding the compression heating effects in the food, process lethality could be calculated solely as resulting from the time temperature profile of the product. This approach would be essentially the same as that used in conventional thermal processing. However, for validation, measurement of both temperature distributions throughout the vessel and heat penetration inside the packed product is required. This approach, whilst undoubtedly of merit, would prove difficult in practice for routine factory use. For a temperature distribution study in a conventional batch steam retort, an absolute minimum of 3 probes per retort basket would generally be recommended and more would be advisable if at all practical, (Institute for Thermal Processing Specialists 1992). In a four basket retort, a minimum of 12 probes would therefore be required (plus an additional probe in the instrument pocket of the retort and two located in products). This, it must be stressed, would be a minimum requirement. Applying a similar approach to pressure vessels would presumably require a similar number of thermocouples to be fitted, which would be difficult to achieve in practice given the engineering challenges associated with the manufacture of thermocouple assemblies that will hold pressure at 700 MPa (see chapter 3 for more discussion of this). Grauwet *et al*. (2011) noted that direct measurement of temperature distribution was "technically too complex at pilot or industrial scale". However, recent developments in remote logging technologies that can operate at high pressures may greatly simplify temperature distribution studies for HPS in the future (Buckow & Agueeva 2006; Knoerzer *et al.* 2010c). Treating the process as a conventional thermal process would also take no account of pressure contribution to lethality as the process would be based purely on the

time/temperature profile of the product. However, this is probably a sensible approach in light of our growing understanding of how spores are inactivated in an HPS process in real foods (Bull *et al.* 2009).

In the case of the second approach, the method is feasible to establish that the process can achieve a target reduction of *Clostridium botulinum* spores but it is completely impractical for routine validation in a factory environment where inoculation with *Clostridium botulinum* would not be acceptable. A more practical solution for factory validation would be the use of an indicator organism or biological indicator with a similar pressure inactivation response to the most pressure resistant strain of *C.botulinum*. Sizer *et al*. (2002) suggested this as a third possible validation approach but this option is also not without problems. There is still debate as to what might be a satisfactory indicator organism and which strain of *C.botulinum* is most pressure resistant especially since resistance can vary depending on the food matrix (Bull *et al.* 2009).

The fourth option proposed is the use of an accurate kinetic model, ideally based on first order inactivation kinetics that would allow users to demonstrate a 6 log reduction under defined conditions and then extrapolate this to determine the time required for a 12 log reduction. Many studies have assumed first order inactivation kinetics for spore inactivation but do not account for lethality that may occur during the come up and come down phase of the HPS cycle (Bull *et al.* 2009). These effects could be significant in a commercial process whether the come up time is likely to be of the order of several minutes rather than the much shorter come up times that are typically employed in laboratory research. In the short term, kinetic

models are a useful tool for process validation but they are unlikely to replace the need for direct measurement of lethality of either the target pathogen or a suitable surrogate.

A fifth option, not proposed by Sizer *et al*. (2002) is the use of some other biological indicator to map temperature, pressure or lethality in a HPS process. Direct measurement of temperature and pressure at multiple points within a pressure processing vessel and within the food being treated has only recently become technically feasible and remains a significant challenge (Buckow & Agueeva 2006;Knoerzer *et al.* 2010c).

Pressure measurements for process control purposes are generally made using a strain gauge at a single point in the supply pipework to the vessel, i.e. it is external to the pressure vessel itself. An innovative approach for the indirect 'measurement' of pressure within a pressure vessel and within the food being treated has been suggested by Minerich & Labuza (2003b) and Minerich *et al*. (2003a). It was noted that "metal elements comprised of a face-centred cubic crystal lattice structure such as gold, silver, platinum and copper have nearly perfect plastic (ductile) and no brittle attributes whereas similar metals (nickel, aluminium, iron and lead) are less plastic and more brittle". The group selected powdered copper as a material which could be formed into tablets, hypothesising that, when pressure treated, it would compact in a reproducible manner that could be correlated to the pressure to which it was subjected. Tablets were produced in a Carver tablet press and vacuum sealed into packs. These were then placed in a 35 litre pressure vessel and treated at pressures in the range of 400 to 600 MPa for varying times and at a range of temperatures between 7 and 24°C. Plotting a Heckel value (Equation 2.2) against applied pressure yielded a straight line plot.

Heckel "studied the compression kinetics of powdered copper, nickel, tungsten and iron at high pressures to describe the relationship between the rate of change in density to pressure" (Heckel 1961a;Heckel 1961b;Minerich & Labuza 2003b) and developed the equation as shown in Equation 2.2.

$$H = L_n \frac{1}{(1 - \emptyset)} = KP + A \qquad (2.2)$$

Where H = Heckel Value

$\emptyset$ = relative density (ρ_0/ρ_{abs})

ρ_0 and ρ_{abs} = observed and absolute density ($g.cm^3$)

ρ_{abs} = 8.96 $g.cm^{-3}$ for copper

P = applied pressure (MPa)

K,A = constants (slope and projected intercept)

The density of the tablets, when expressed as Heckel values, could be correlated with pressure using the regression model shown in Equation 2.3

$$H = 0.00191P + 1.0534 \qquad (2.3)$$

This method potentially allows a user to place the copper tablets in both the pressure medium and the product and infer both the pressure achieved and any variation (which should, in theory, be zero) throughout the vessel. It is not known whether or not this method has found widespread use.

Good progress has been made regarding the study of chemical integrators to map temperature and pressure variation in a high pressure high temperature combination process but further work is needed (Grauwet *et al.* 2009;Grauwet *et al.* 2010a;Grauwet *et al.* 2010b;Grauwet *et al.* 2011;Plancken *et al.* 2008;Vervoort *et al.* 2011). Initial studies on α-amylase based indicators showed that these systems were not stable enough under HPS conditions to be viable as process integrators (Grauwet *et al.* 2011). Further studies have been conducted exploring the use of Ovomucoid - a commercially available trypsin inhibitor found in chicken egg white (Grauwet *et al.* 2011). A pressure-time-temperature-integrator (pTTI) was developed and tested by locating pTTIs in different spatial locations in a pilot scale HPS system and then exposing them to HPS conditions of 85°C (initial temperature) and 600 MPa for 2 and 5 minutes. They pTTI was able to withstand these process conditions (which are in a suitable operating range for the likely times, temperature and pressures that would be utilised in a HPS process) with residual activity remaining (an important characteristic for a process integrator of this time). Furthermore, spatial variation in temperature was detectable using the pTTI. Grauwet *et al*. (2011) also demonstrated that the inactivation kinetics of the pTTI could be engineered to specific HPS operating windows. This suggests that pTTIs of this nature could be valuable tools for process validation or optimisation. A greater understanding is needed of the HPS conditions necessary for process safety so that the pTTIs can be engineered to withstand the appropriate HPS conditions.

2.9 Process economics for high pressure sterilisation

Meyer *et al*. (2000) gave cost estimates for a high pressure sterilisation process based on a double pulse pressure treatment. The costs to modify an existing line and pressure sterilise

the product were calculated to be 0.055 £/kg (£:$ conversion from the original source). This was based on 5 vessels manufacturing around 61 million kg of product per year. Pressure equipment, buildings and ancilliary equipment were costed at £15.4 million. Labour was estimated at 0.011 £/kg, maintenance was 0.022 £/kg and the equipment was depreciated over 10 years (0.022 £/kg). This estimate also assumed a 690 MPa pressure treatment at 100°C with a 90 second pressurisation time and a 30 second depressurisation time. Each of the pressure vessels would be in operation 90% of the time and the fill ratio in the equipment would be 75%. According to Meyer, the cost estimate outlined above was arrived at independently by both Unilever and Basic American Foods using real operational data.

2.10 Summary of chapter, key conclusions and areas for further discussion

High pressure sterilisation has considerable potential as a preservation method but there are a number of technical and economic challenges that must be addressed before it can be adopted for commercial processing. The cost of processing, particularly in consideration of the throughputs that can be achieved, is a major obstacle for commercial uptake. This is particularly true of the UK where profit margins for food items are generally low. For the foreseeable future, the process could only be used for very high value products where a premium price could be charged.

Scale-up to industrial production could prove very difficult. The process relies on good temperature uniformity throughout the load, the pressure medium and the vessel itself. Maintaining a batch of product at an elevated temperature and still managing to fill the vessel, close it and come up to pressure without significant heat losses is likely to be extremely difficult to achieve in practice. A detailed understanding of vessel design and compression

heating effects in foods, pressure media and insulating carriers is needed in order to understand their impact of temperature gradients during HPS processing. Good progress has been made but further studies are needed.

Technically, there is growing evidence that the process can deliver a commercially sterile product. This assertion has been strengthened significantly by the recent US approval of Pressure Assisted Thermal Sterilisation (PATS) as an acceptable process for the production of low acid canned foods (NCFST 2009). Such a process has yet to be approved for food use in Europe. However, the exact processing conditions necessary to achieve commercial sterility are still not known with a high degree of certainty (Grauwet *et al.* 2011) but current thinking would suggest that HPS should in the first instance be considered as a thermal process with the use of pressure simply being a means to achieve a high-temperature-short time process (Bull *et al.* 2009).

It does appear that food quality can be improved by applying a HPS treatment rather than a conventional thermal sterilisation treatment (see section 2.5) but more data is needed. There is a particular need to understand quality effects on foods when they are processed at commercially meaningful time/temperature/pressure conditions.

Process validation remains a very challenging issue even if the process is considered purely thermal. Practically speaking, it will be very difficult to carry out temperature distribution studies in a high pressure vessel where the number of thermocouples that can be fitted is very limited. A wireless logger is an excellent solution but remains at a development stage. A

chemical time temperature integrator may be another useful solution and there has certainly been good progress made in this area but much work remains to be done. Work in Chapter 3 will highlight some of the practical difficulties of measuring temperature variation at high pressure, even in a laboratory system. The challenge becomes even greater at an industrial scale.

Despite the many years of research work that have now been conducted in the field of high pressure sterilisation there are still many areas where further research is required. A complete understanding of anti-microbial effects at high pressure and high temperature has yet to be achieved and even less is known about food quality effects resulting from the process. Chapters 4 and 5 of this thesis will present new data and insights into food quality effects under high pressure high temperature conditions attained using commercially achievable conditions.

3 Chapter 3 - Temperature distribution studies in a laboratory scale HPP system

3.1 Introduction

As discussed in Chapters 1 and 2, temperature is thought to play a critical role in the microbiological efficacy of a high pressure sterilisation process. It is clear that it would be extremely important, commercially, to have a good understanding of temperature variation (or some means of measuring lethality variation in lieu of temperature measurement) within the vessel. Experimentally, it is important to understand temperature variation in the system as a source of experimental error.

Accurate and reliable temperature measurement of product and process temperatures in a large scale vessel at high pressure is not simple using wired logging equipment and some authors believe it is not currently feasible. Vervoot *et al*. (2011) for example noted that "Direct measurement of the temperature distribution throughout the HP vessel and in-pack is not feasible with the measuring devices currently available". It is certainly true (as noted by Vervoot *et al*.) that measurement of product temperatures in sealed packs under HPS conditions is extremely challenging. Even environmental temperature measurement in small scale equipment can be problematic as will be discussed in this chapter.

This chapter summarises the findings from a series of temperature distribution studies that were carried out in a 700 ml laboratory scale high pressure vessel (EPSI, Temse, Belgium).

There are limited data published in the public domain regarding temperature distribution at elevated temperatures and pressures when using commercially relevant processing conditions. Grauwet *et al*. (2011) noted that there was "insufficient insight of the temperature distribution in a high pressure high temperature reactor".

3.2 Materials and methods

Temperature distribution studies were carried out in three stages, and a number of revisions were required in both equipment design and experimental protocols as practical experience was gained. Studies were carried out in a laboratory scale high pressure unit (Figure 3-1) manufactured by Engineered Pressure Systems International (Temse, Belgium). The vessel had an internal diameter of 70 mm and was 200 mm in length, having a working volume of approximately 700 ml. The temperature of the vessel wall was controlled by a heating/cooling water circulation loop contained within a grooved stainless steel liner in the vessel. The water for heating the vessel was pumped from a water bath, through the vessel wall liner and then re-circulated back into the water bath. Using this system the wall temperature could be controlled at any desired temperature within the range of 0 to 90°C. The pressure medium used for the trials consisted of water with 3% (v/v) of MKU (an oil based corrosion inhibitor).

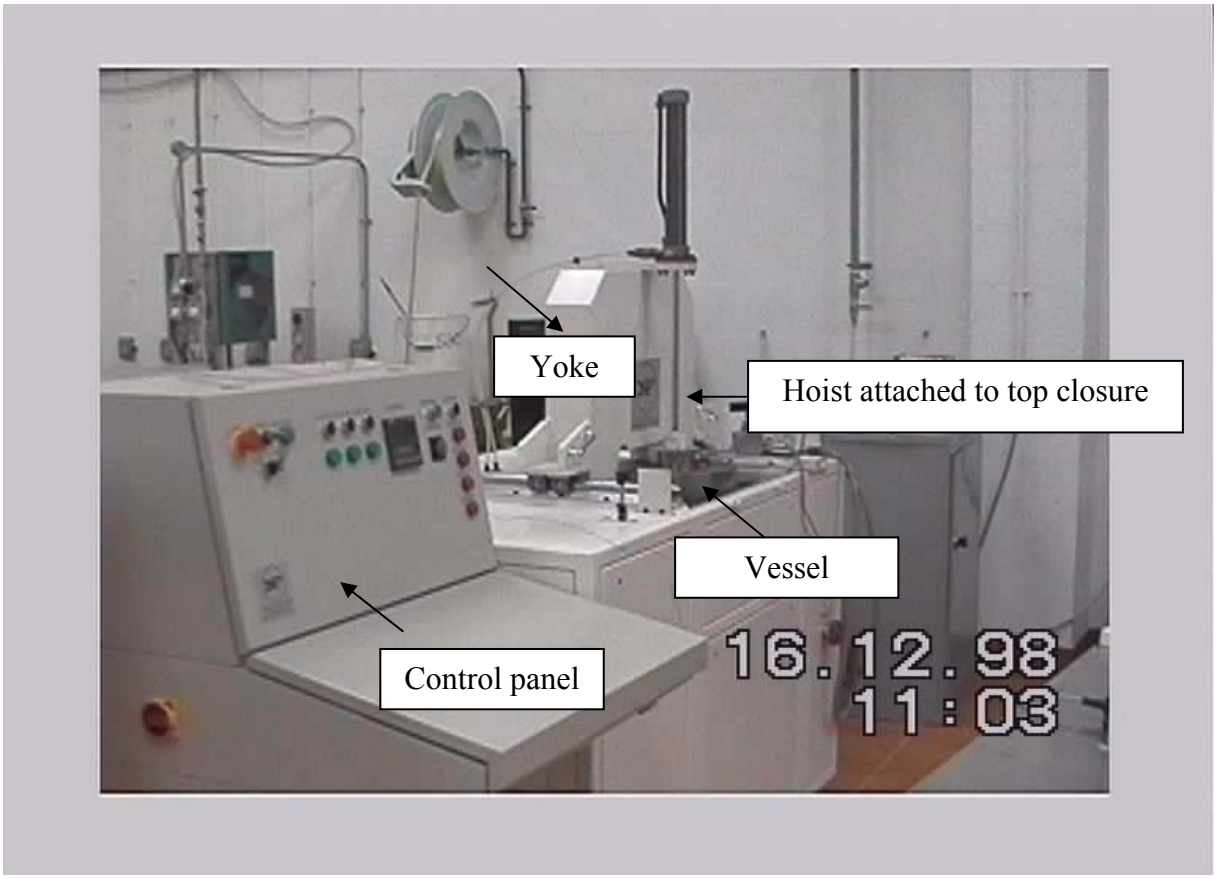

Figure 3-1. Laboratory scale HPP vessel used in the experiments

Prior to any experiments, all thermocouples used in the studies were calibrated by immersion in a circulating silicone oil bath at 90°C and 121°C using two reference standard instruments having known and traceable values of uncertainty. The uncertainty associated with the calibration did not exceed ± 0.25 °C. Temperature and pressure were recorded during the cycle using an MMS3000 data logger (RiL Instruments, Nottingham) set to record at 1 second intervals. Pressure readings were obtained by converting a voltage signal from the control system of the equipment. The datalogger was programmed to convert the input voltage signal into Mega-Pascal using a pre-determined calibration curve (data not presented). The control system consisted of a West 5010 industrial controller (Crawley, UK) connected to a Moore Industries SGT/XSGT Strain Gauge transmitter (Crawley, UK).

3.2.1 Stage 1: Scoping studies

The first stage of practical experiments consisted of initial exploratory work measuring temperature distribution in two areas at the base of the vessel (Figure 3-2). The experiments explored two pressures (500 and 700 MPa), two temperature (70°C and 90°C), two hold times (1 and 5 minutes) and the effect of the presence or absence of an insulated liner inserted into the vessel (Figure 3-3). The liner (where used) was a 10 mm thick, Polyetheretherketone (PEEK) cylinder measuring 150 mm in length and 70 mm in external-diameter. A floating lid (10 mm thick) was inserted into the liner to a depth of 38 mm giving the liner a working volume of approximately 200 cm^3. Figure 3-2 illustrates the position of the thermocouples and the dimensions of the PEEK liner. In trials where a liner was not used, the thermocouples remained in the same position in the vessel. Figure 3-3 show the PEEK liner used in the trials.

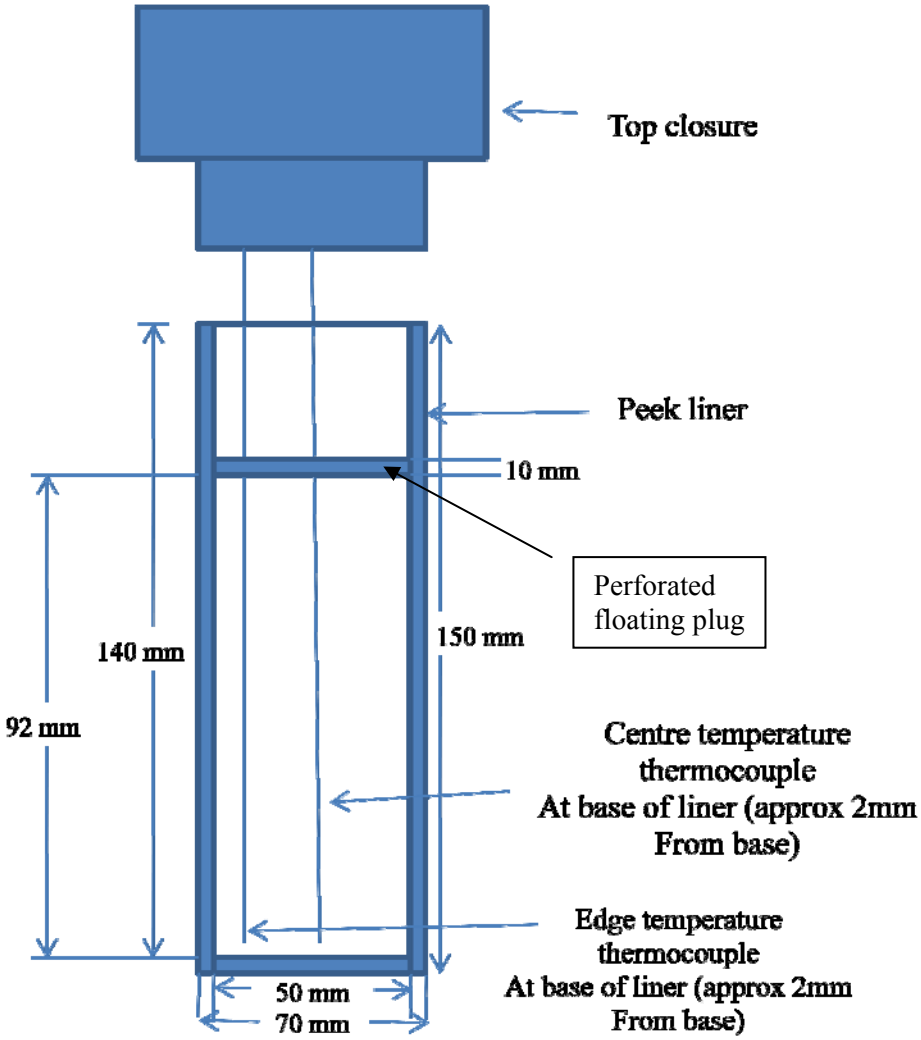

Figure 3-2. Initial positioning of thermocouples for stage 1 trials

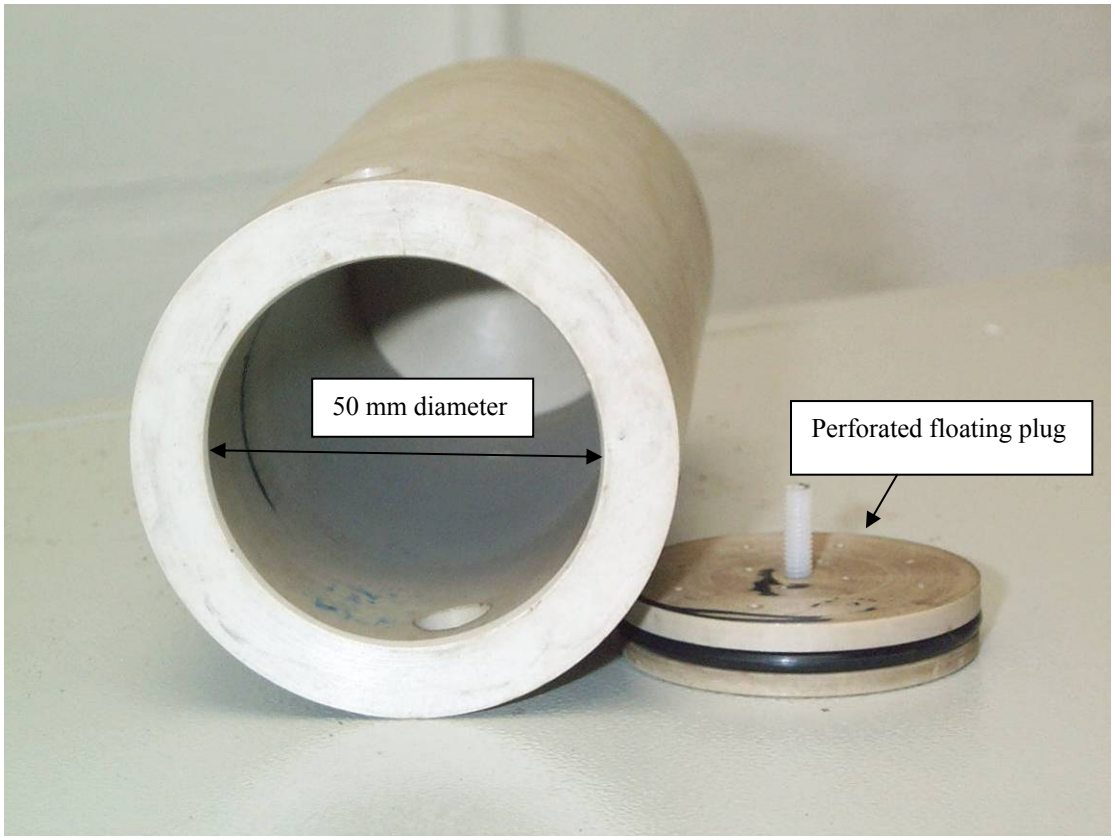

Figure 3-3. PEEK liner and floating lid used to insulate the vessel

A maximum treatment time of 5 minutes was selected on the basis that this was a realistic upper limit that could be employed for a commercial process (Purroy 2007). Similarly, 700 MPa was selected as a maximum pressure because it is at the upper limit of what is currently achievable on a commercial system (Leadley C.E. 2007). The temperatures and pressures were selected based on previously published operating conditions which could be employed for high pressure/temperature sterilisation purposes (Meyer *et al.* 2001;van Schepdael *et al.* 2002;Wilson & Baker 2003) see chapter 2 for details.

3.2.2 Stage 2 - multi-point temperature distribution studies

For the second stage of the studies, a thermocouple feed-through system was purchased from Engineered Pressure Systems International (EPSI, Temse, Belgium) which housed six thermocouples. The thermocouples were sealed into a plug (see Figure 3-4 and Figure 3-11) which was fitted in the top closure of the vessel and bolted in position with a securing plate on both the top and bottom of the closure.

Figure 3-4. Thermocouple feed-through used for temperature distribution studies

When the PEEK liner was fitted, the feed-through enabled temperature measurements to be made at three points in the top of the chamber and three points at the bottom. These locations (Figure 3-5) were categorised as 'top left', 'top centre', 'top right', 'bottom left', 'bottom centre' and 'bottom right'. Probes designated 'left' and 'right' were located close to the wall of the liner (within 5 mm), probes designated 'centre' were positioned along the central axis of the liner. The use of, essentially, rigid probes meant that precise location in the vessel was

not possible; instead the probes provided general insight into temperatures variation in the respective 'zones' of the chamber. The probes designated 'bottom' were approximately 2-5 mm from the base of the liner. Probes designated 'top' were approximately 6 mm from the base of the floating plug.

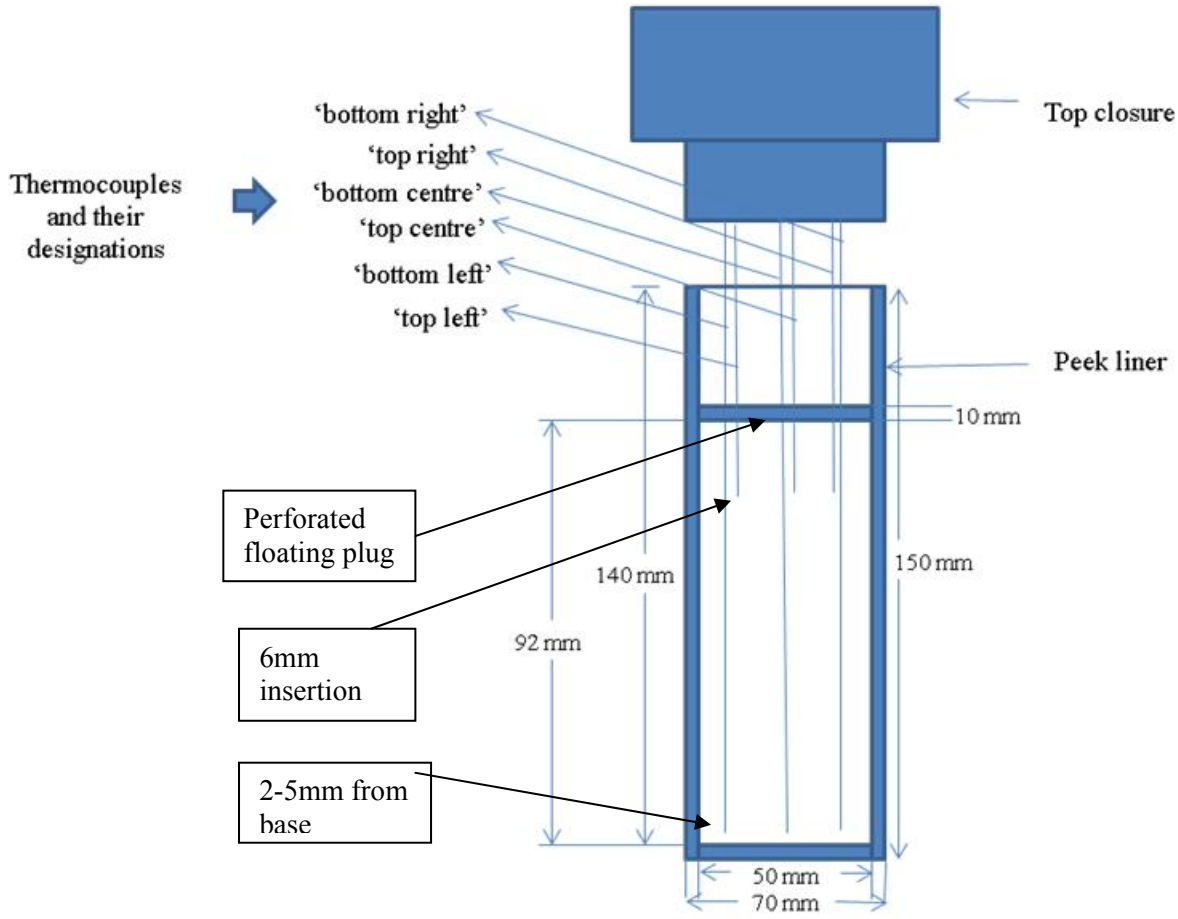

Figure 3-5. Location of thermocouples for multi-point temperature measurement (drawing not to scale)

Experiments were carried out at two pressures (500 and 700 MPa), two temperatures (70°C and 90°C) with a hold time of 5 minutes. Each experiment was carried out in triplicate. An Analysis Of Variance (ANOVA) was carried out on the resultant data in order to determine

the statistically significant factors affecting the temperature response observed in the vessel over time.

Stage 2 of the work identified the critical variables affecting the time temperature response within the vessel. Results from the top of the vessel were characterised by erratic spiking in the time temperature profile over the hold phase that suggested probe movement. During some runs, the probes were also slightly bent suggesting that the liner was moving in the vessel during the process. As the pressure medium entered the vessel at the bottom of the vessel, it appeared that the incoming liquid was pushing the liner upwards and bending the thermocouples indicated that a re-design of the liner was required (see 3.2.3).

3.2.3 Stage 3 – multi-point temperature distribution studies with modified liner

As a result of the findings in stage 2, two grooves, 2 mm deep by 5 mm wide were cut into the liner to allow the pressure medium to flow more readily up the sides of the liner in order to reduce liner movement. In addition, a retaining clip was constructed for the liner to ensure that its location within the vessel relative to the thermocouples was always consistent. In the finished layout (Figure 3-6), the bottom probes were almost touching the base of the liner (approximately 2-5m) and the top probes were around 16 mm from the base of the floating plug. Stage 3 experiments consisted of ten randomised temperature and pressure combinations within the range of 500-700 MPa and 70-90°C. Results were reported as time temperature plots and equivalent lethalities (F_o). An analysis was also made of the effects of initial temperature on the measured temperature rise per 100 MPa of applied pressure. The conditions tested were as shown in Table 3-1.

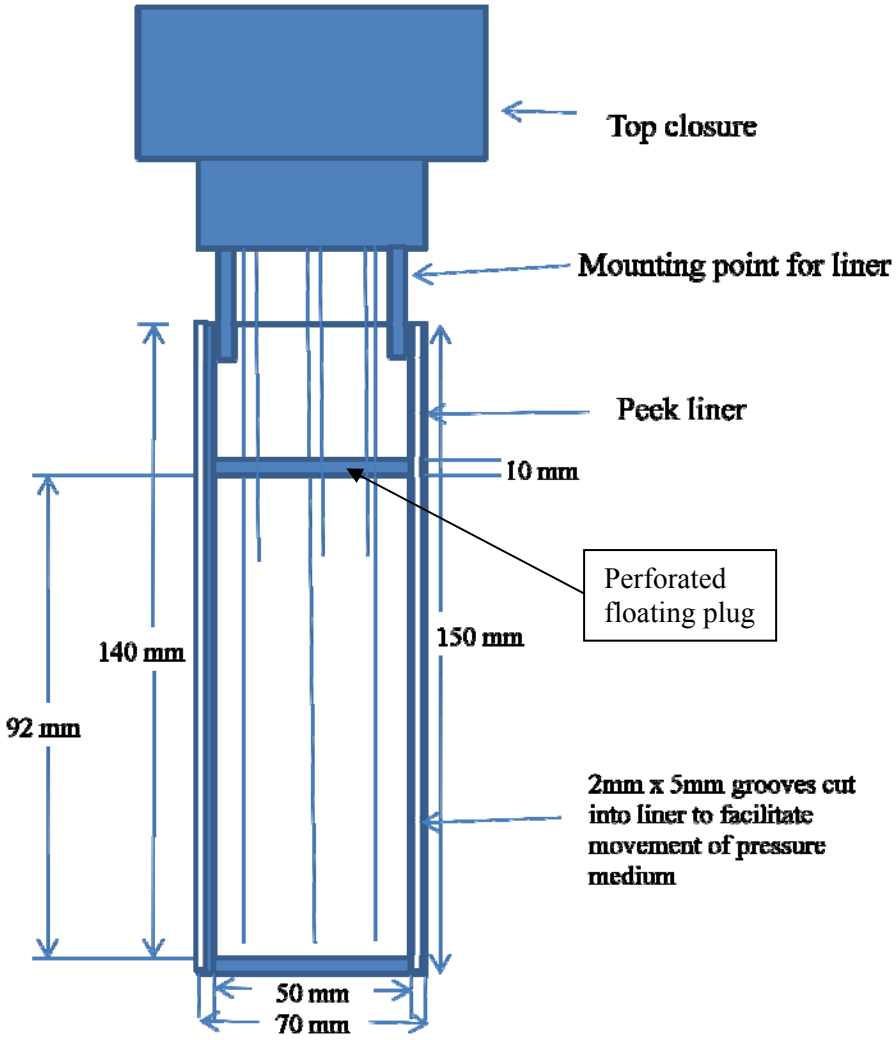

Figure 3-6. Finished experimental set up showing inclusion of mounting point for liner and grooves cut into the external surface of the liner. Drawing not to scale.

Pressure (MPa)	Initial Temperature (°C)
610	86
560	90
540	74
670	80
570	88
690	86
570	86
550	82
660	76
530	76

Table 3-1. Conditions used for stage 3 temperature distribution experiments

3.2.4 Pressure treatments

In all three stages, the methods employed for each pressure cycle were the same. The vessel wall was pre-heated to a target initial temperature for the experimental run using a circulation loop connected to a hot water bath. The PEEK liner was removed from the vessel and filled with pressure medium that had been pre-heated to close to the target initial temperature. The thermocouples were positioned as previously described (see Figure 3.2, 3.5 and 3.6) and the liner was then lowered into the high pressure vessel. The remaining vessel volume was filled with pressure medium from a central reservoir. The main pressure valve to the vessel was closed, a bleed valve on the top closure of the vessel was left open and the high pressure pump was started to ensure that all air was expelled from the vessel via the bleed valve. The high pressure pump was stopped, the bleed valve closed and the vessel temperature was allowed to equilibrate to the target initial temperature conditions. Once the temperature had stabilised, the high pressure pump was started and the vessel ramped up to the target pressure. Note that the temperature of the incoming fluid was not controlled and was at ambient temperature. The hold time for each cycle was set as required for each run. The high

pressure pump was automatically activated if the pressure dropped by more than 5 MPa from the target. At the end of the hold period the main pressure valve was opened. The come down time was not controlled between runs as it was not of interest for the distribution studies. The key phases of the PATS cycle are summarised in Figure 3-7 .

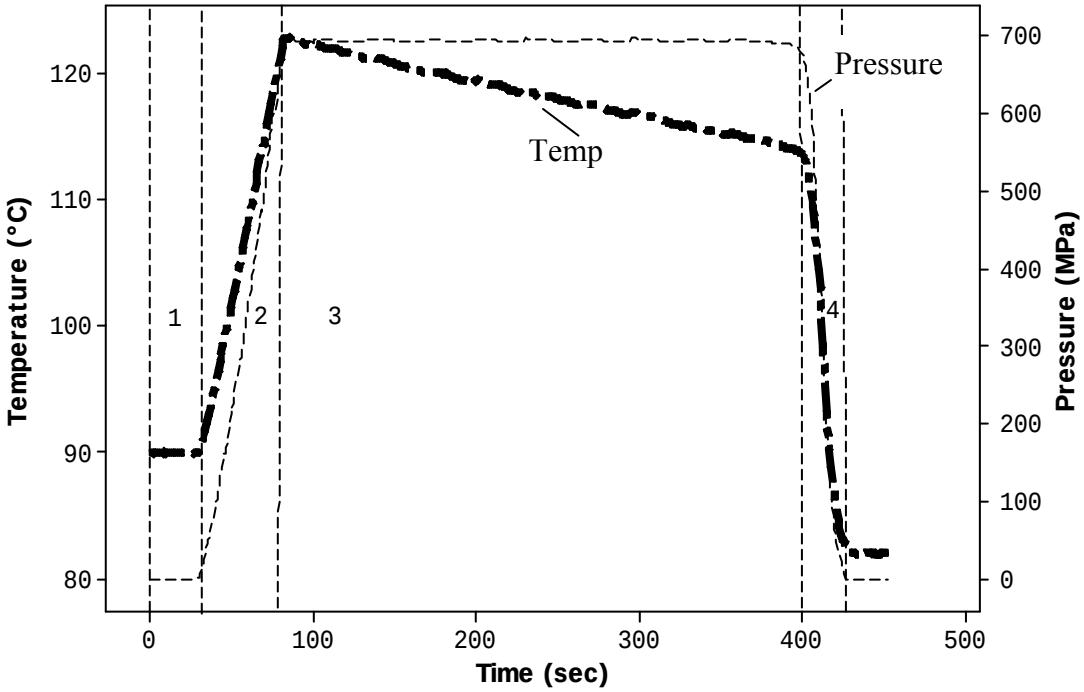

Figure 3-7. Stages in an example PATS process: 1 – temperature equilibration phase to reach initial temperature (IT) for trial, 2 – Come up time to pressure (CUT) accompanied by compression heating of pressure fluid, 3 – hold phase (with associated heat losses), 4 – come down time (CDT) as pressure is released at the end of the cycle with associated cooling of the compression fluid.

3.2.5 Lethality calculations

Time temperature profiles were obtained for each run, for each of the locations in the vessel and were also expressed as equivalent lethality values (F_o - minutes) with respect to Group I proteolytic *Clostridium botulinum* as described in section 1.2. It is important to note that the

calculated F_o values are only an expression of the *thermal* contribution to lethality and took no account of any lethality that could be attributed to the applied pressure. A sub F_o 3 process may not therefore automatically infer under processing in the case of a pressure/temperature combination process but as a conservative approach this should be assumed to be the case.

3.3 Results and discussion

3.3.1 Stage 1: Scoping studies

It became apparent early during experimentation that an insulated liner was essential to achieve any significant degree of compression heating during pressurisation. Figure 3-8 for example shows a run (initial temperature of 90°C, hold time of 5 minutes, hold pressure of 700 MPa) without the use of a liner and Figure 3-9 show the same processing conditions with the use of a PEEK liner. Without a liner, temperatures did not exceed 90°C (the initial temperature of the run) whereas when a liner was used, the peak temperature attained was 122.8°C. In all experiments conducted without an insulated liner, the temperature rise that was attained was, at best, only 5°C above the initial temperature of the pressure fluid. As a consequence of this finding, experiments carried out in the absence of a liner were halted. Temperature losses from the vessel were time dependent and the rate of temperature loss could be approximated by a linear fit over the 5 minute hold period. The effect of a one minute hold could therefore clearly be ascertained by studying the time temperature plot of a 5 minute hold. See Figure 3-9 as a typical example data-set.

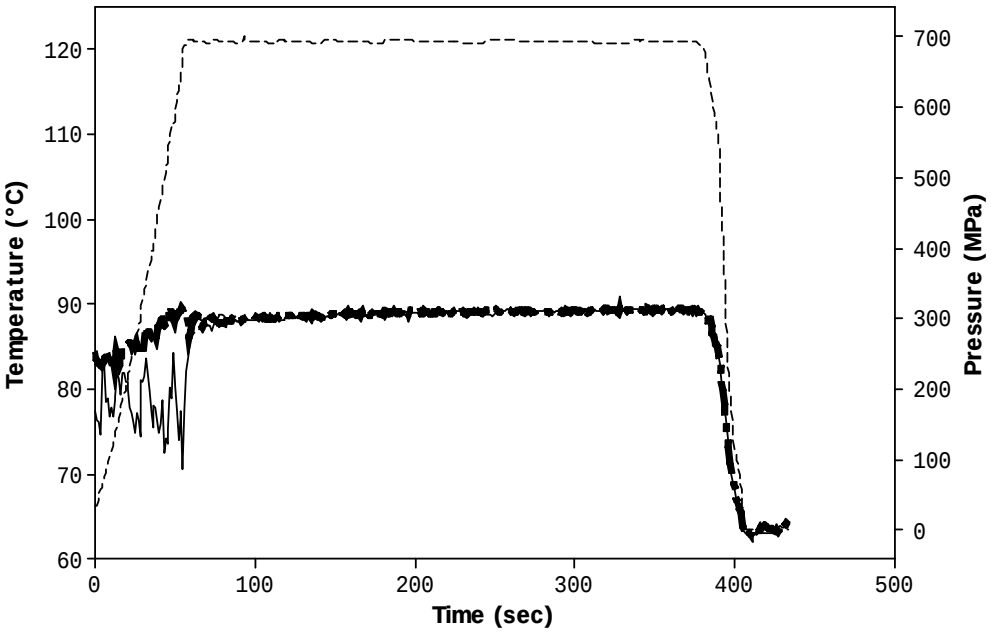

Figure 3-8. Temperature distribution at two points at the base of the vessel when liner was not in use. Key: — bottom centre; –-– Bottom left; --- Pressure

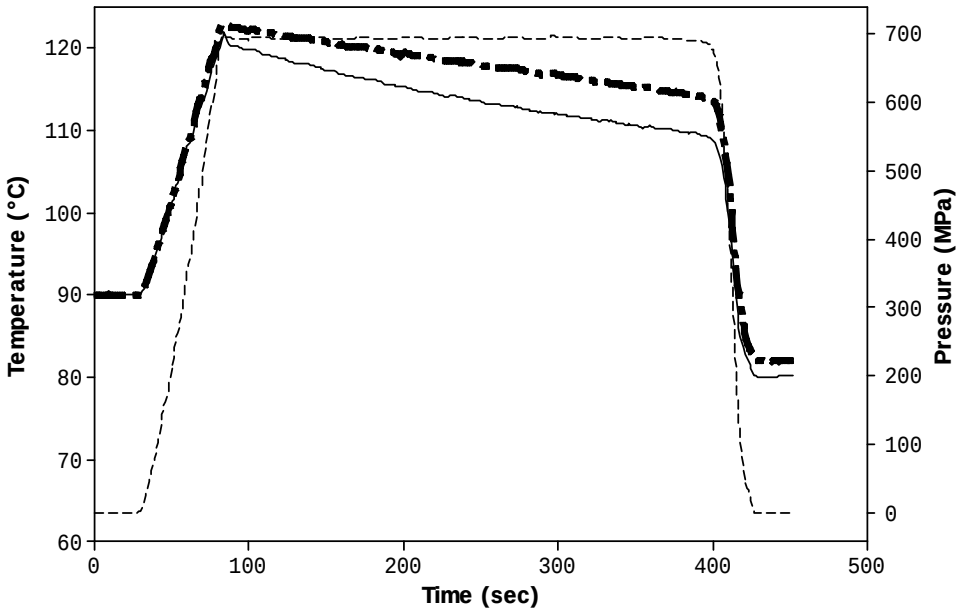

Figure 3-9. Temperature distribution at two points at the base of the vessel when liner was in use. Key: — bottom centre; –-– Bottom left; --- Pressure. Note that rate of temperature loss was approximately linear over a 5 minute hold period.

3.3.2 Stage 2 - multi-point temperature distribution studies

The first and second prototype designs for the thermocouple feed-through (designed by the supplier) failed on first use once the pressure in the vessel exceeded around 500 MPa. In both cases, the feed-through failed at the point where the thermocouples met the top closure plug. The probes were extruded out of the vessel against a securing plate on the top closure (Figure 3-10). This illustrates the significant technical challenge that surrounds temperature distribution measurement at high pressures and temperatures.

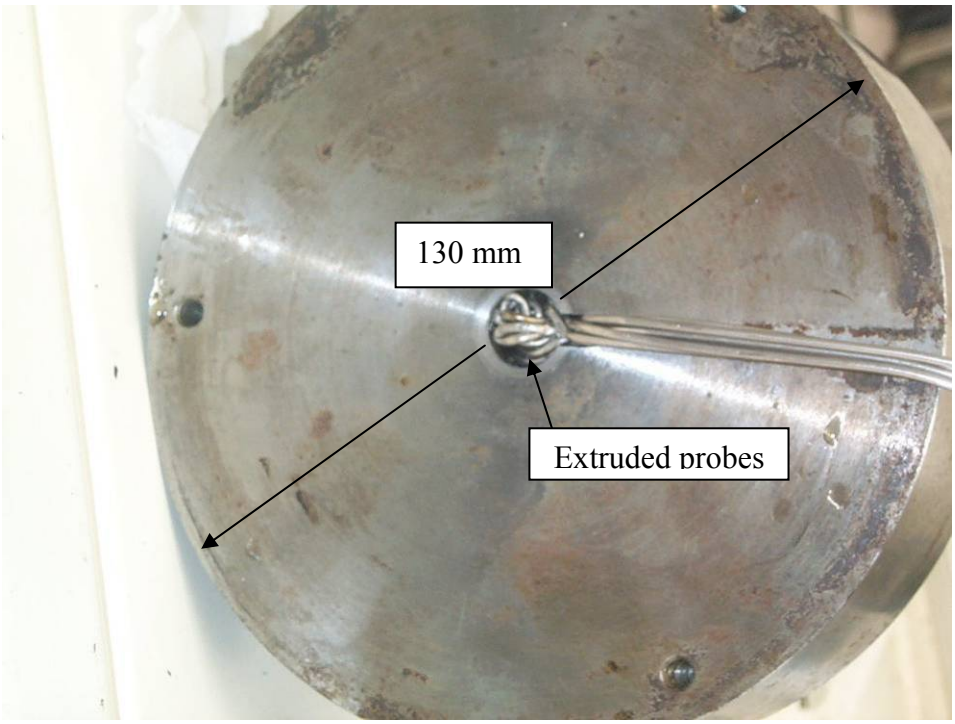

Figure 3-10. Image showing thermocouples extruded from the top of the vessel closure

The third design (Figure 3-11) was able to withstand a process of 700 MPa and was used in all subsequent distribution studies.

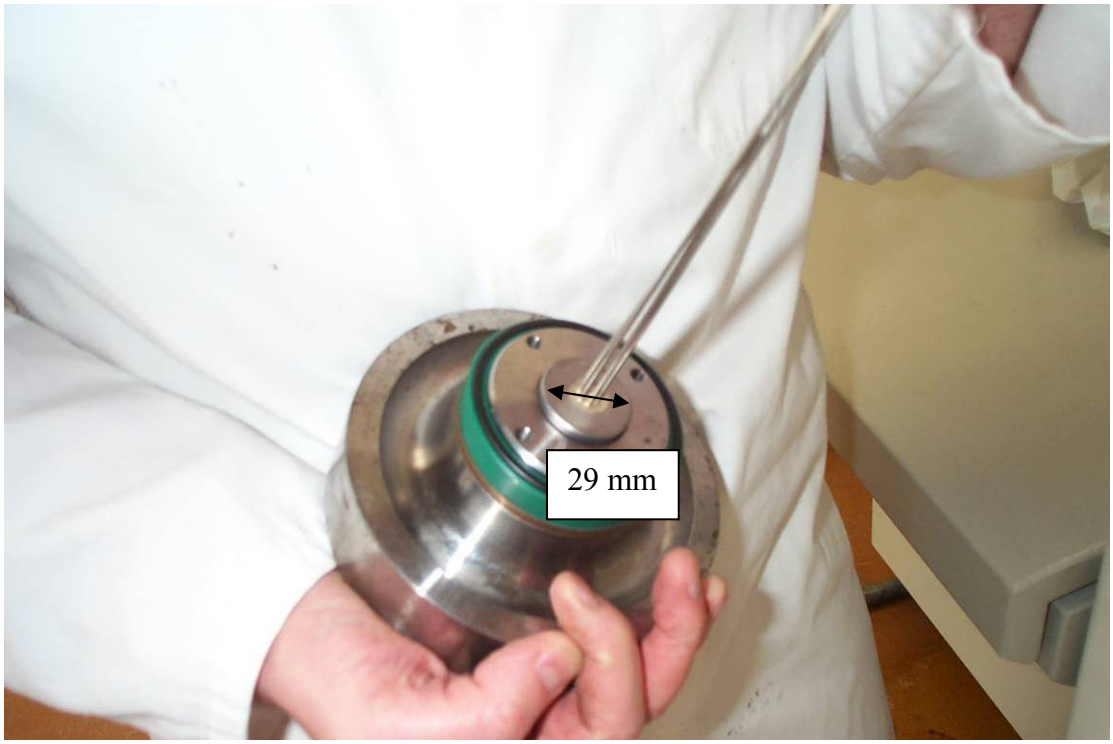

Figure 3-11. Final feed-through assembly used for experiments in stage 3. The feed-through is pictured in place on the top closure of the vessel. A securing plate screwed to the bottom of the closure completed the installation.

3.3.2.1 Characterisation of temperature and lethality distributions in stage 2 experiments

Time temperature traces for stage 2 experiments are shown in Figure 3-12 to Figure 3-15. A summary of the temperatures at each vessel location at key phases of the HPS cycle is given in Figure 3-16. The mean temperatures at the start of the process, start of hold and end of hold are presented in Table 3-2 along with the mean peak temperatures attained for each treatment condition.

Process	Initial temp (°C)	Temp. at start of hold (°C)	Temp. at end of hold (°C)	Mean peak temp. attained during HPS cycle (°C)
500 MPa, 70°C, 5 min hold	70.3 (1.3)	90.3 (2.1)	81.0 (3)	91.0 (1.7)
500 MPa, 90°C, 5 min hold	88.3 (1.7)	110.2 (1.7)	100.6 (2.0)	110.7 (1.5)
700 MPa, 70°C, 5 min hold	69.7 (1.5)	96.6 (2.5)	84.7 (2.0)	97.1 (2.1)
700 MPa, 90°C, 5 min hold	87.3 (0.6)	117.0 (3.2	102.5 (1.7)	118.4 (1.2)

Table 3-2. Mean temperatures attained during key phases of the HPS cycle. Note all figures have been rounded down as a conservative assumption; figure in brackets is the standard deviation.

The top right side of the vessel was consistently at a lower temperature than the other measured points in the vessel and the time temperature trace was often characterised by an erratic profile (See Figure 3-12 to Figure 3-15 for examples). At 500 MPa, 70°C the top right of the vessel was on average 3.3°C lower in temperature than the hottest part of the vessel (the top left). At 500 MPa, 90°C the top right of the vessel was on average 4.4°C lower in temperature than the hottest part of the vessel (the top centre). At 700 MPa, 70°C the top right of the vessel was on average 5.3°C lower in temperature than the hottest part of the vessel (the top left). At 700 MPa 90°C the top right of the vessel was on average 9.3°C lower in temperature than the hottest part of the vessel (top left). Repeat calibrations of the thermocouples suggested that this was a genuine effect rather than a thermocouple error.

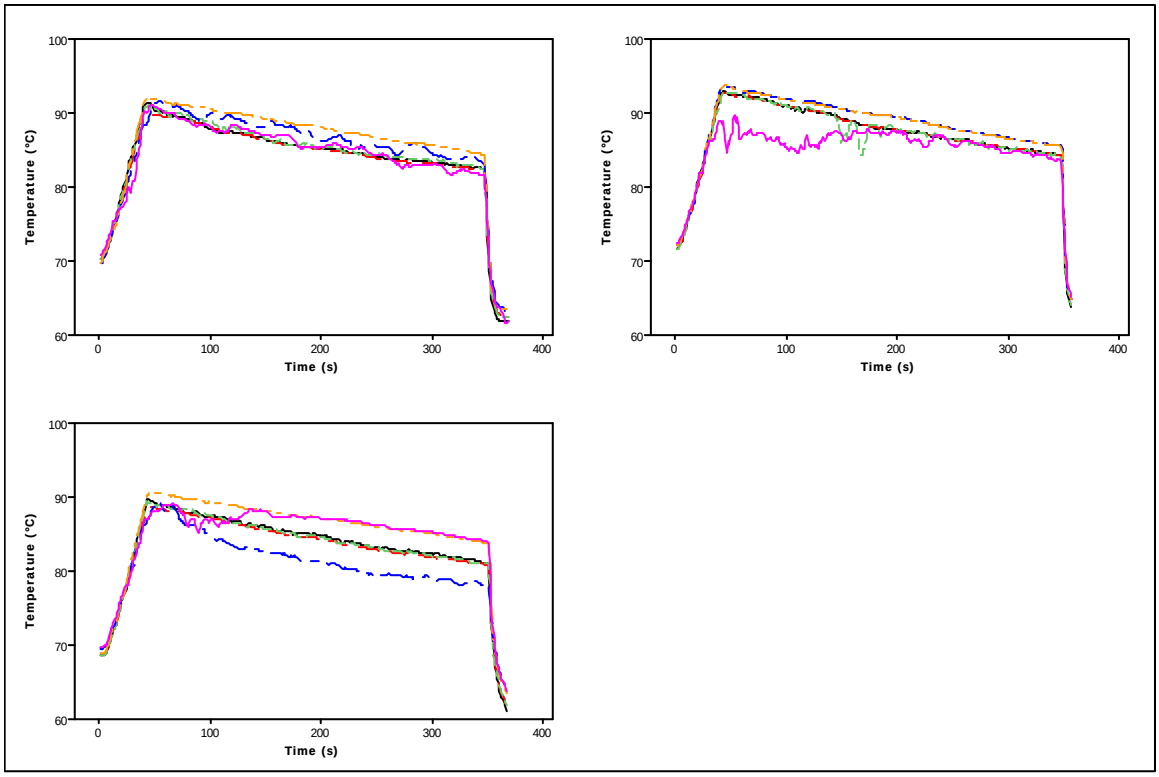

Figure 3-12. Stage 2 replicate distribution studies 500 MPa, 70°C target IT, 5 min hold. Bottom centre, bottom left, bottom right, top centre, top left, top right.

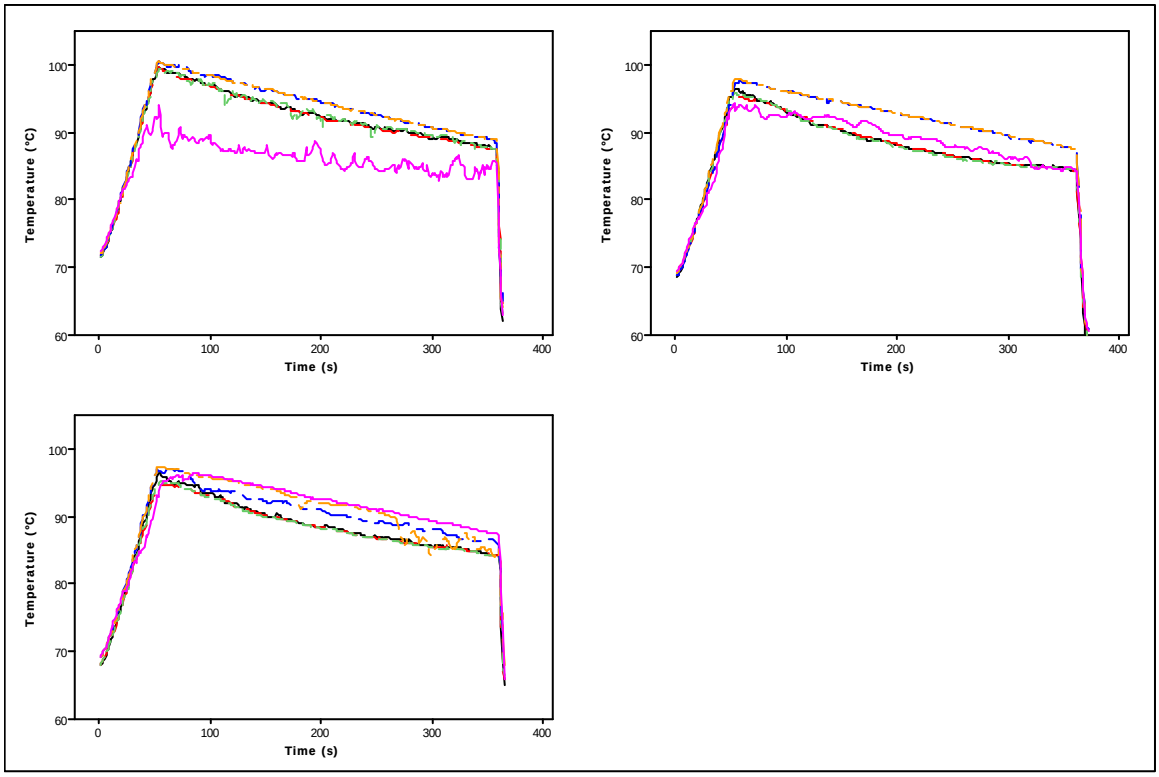

Figure 3-13. Stage 2 replicate distribution studies 700 MPa, 70°C target IT, 5 min hold. Bottom centre, bottom left, bottom right, top centre, top left, top right.

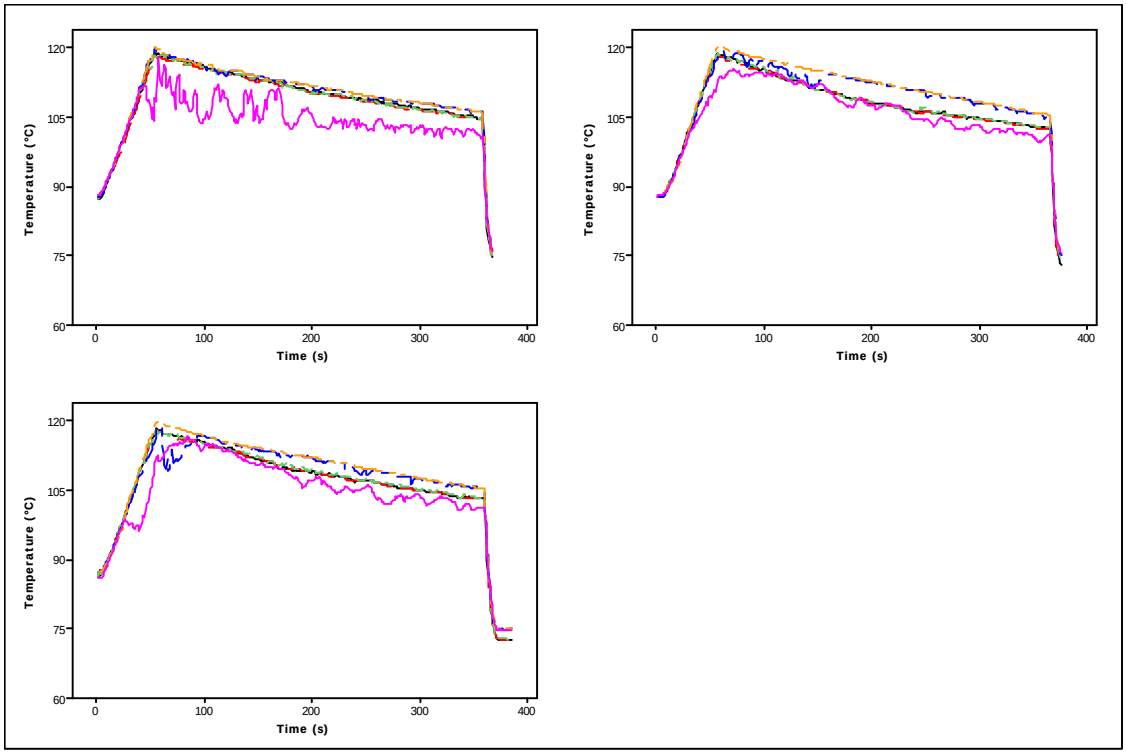

Figure 3-14. Stage 2 replicate distribution studies 700 MPa, 90°C target IT, 5 min hold. Bottom centre, bottom left, bottom right, top centre, top left, topright.

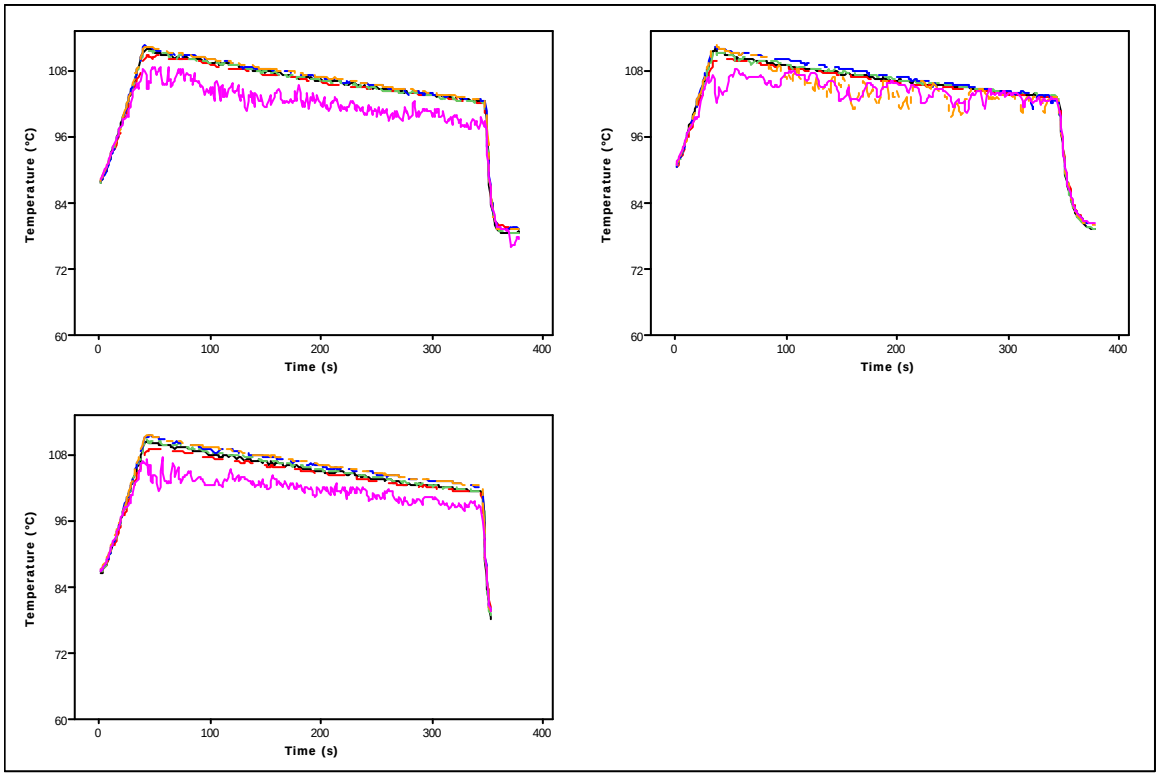

Figure 3-15. Stage 2 replicate distribution studies 500 MPa, 90°C target IT, 5 min hold. Bottom centre, bottom left, bottom right, top centre, top left, top right.

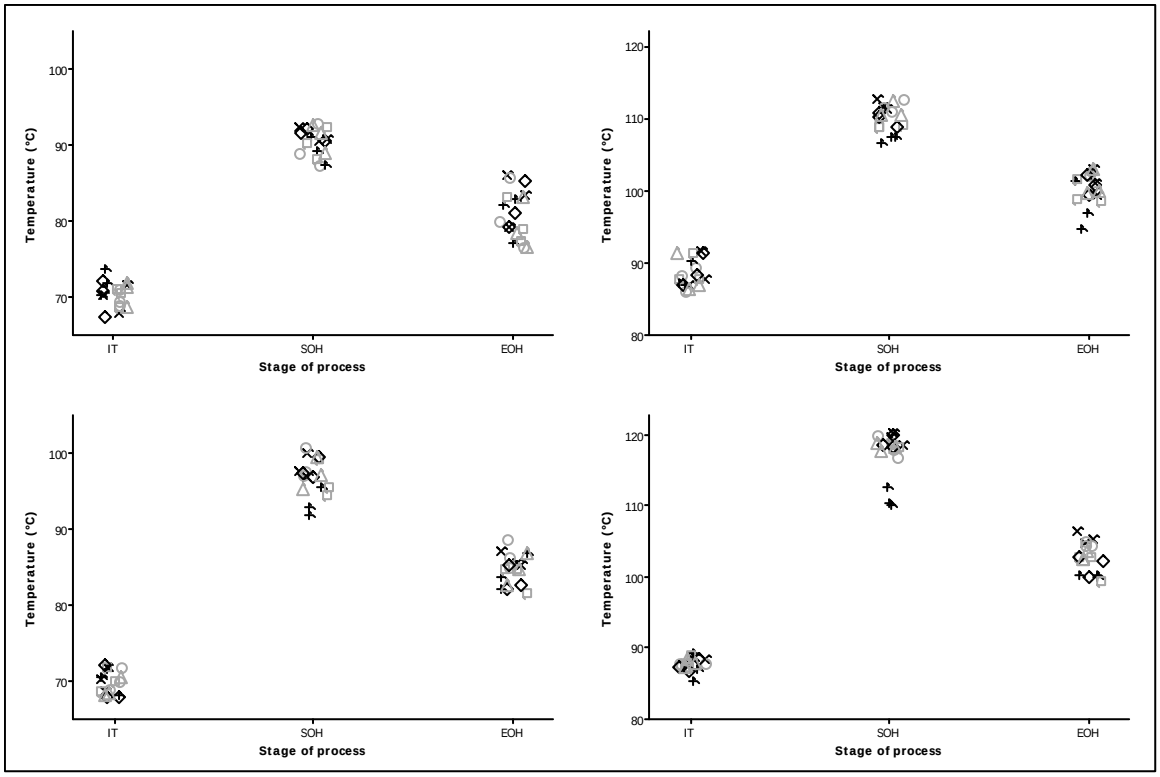

Figure 3-16. Temperature variation by spatial distribution at key stages of the HPS cycle. Stages: initial temperature (IT), start of hold (SOH), end of hold (EOH). Key: x top left; o top centre; + top right; □ bottom left; ◊ bottom centre; Δ bottom right. Note temperature distribution at each stage is fair but could be improved.

The erratic temperature profile at the top right of the vessel was possibly due to liner movement during the cycle which effectively resulted in the thermocouple moving in an out of the insulated liner during the cycle.

As discussed in Chapter 2, van Schepdael *et al*. (2002) proposed various solutions to minimise heat losses during a pressure assisted thermal process. The use of an insulating liner inside the vessel was one such solution. PEEK was used in these Campden BRI studies as an insulating liner because it was one of the materials suggested in van Schepdael *et al*. Typical thermal conductivity values for PEEK are in the region of 0.25 $W.m^{-1}.K^{-1}$ (Texloc 2010;

Boedeker 2010) but heat losses from the vessel were still substantial over a 5 minute hold period. In all of the runs, a gradual loss in temperature was observed at all measured points over the holding period with temperature losses of the order of 10-15°C (see Table 3-2). This is because the liner was not a perfect insulator and heat losses occurred to the vessel walls and to the top and bottom closures. The problem of heat losses may have been exacerbated by the use of a perforated floating plug on the top of the liner (see Figure 3-3 and Figure 3-5) to allow the thermocouples to be inserted into the liner since any cold pressure medium entering the vessel during the hold period could mix with pressure medium at the top of the liner via the perforated plug. Pre-heating of the incoming pressure medium was not possible because pumping at high temperatures would have substantially increased fatigue on the pump tie rods and it was possible that soft seals in the pump would fail (Personal communication with Engineered Pressure Systems International, 2005). It might have been possible to use alternative seals but those that were available at the time of the experiments had not been extensively tested under sterilisation conditions.

F_o lethality distributions at six different points in the vessel (see Figure 3-5 for probe locations) were calculated for each process condition used in phase 2 experiments using Equation 1.1 and are summarised in Figure 3-17 to Figure 3-20. In all cases the data are displayed as an interval plot with mean F_0 values derived from 3 replicate runs and sets of temperature measurements. Confidence intervals on the plots are quite large due to the relatively small numbers of replicates. As it is typically convention to express F_0 values in minutes this convention has been followed in the graphs even though the achieved F_0s are generally very small.

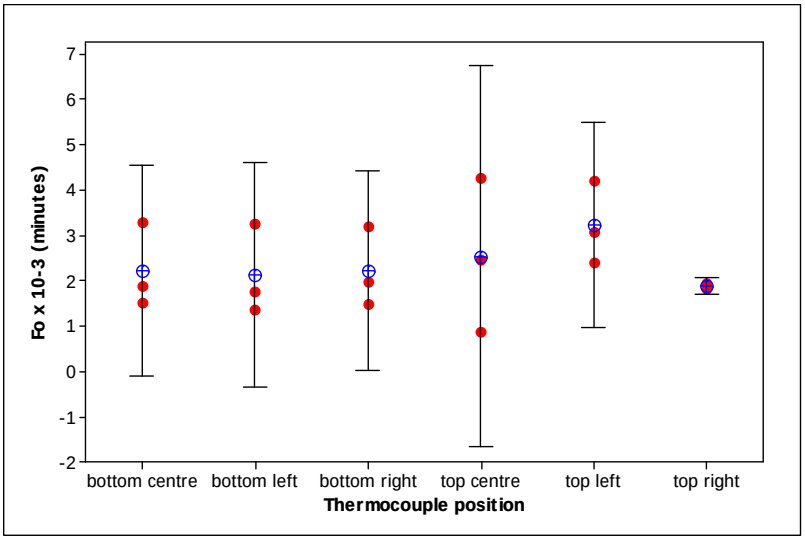

Figure 3-17. Distribution of F_0 at six different locations in the pressure vessel. Processing conditions: hold pressure 500 MPa, hold time 5 minutes, initial temperature 70°C. Note red circles are replicate runs, error bars represent 95% confidence interval for the population mean (indicated by the blue circle)

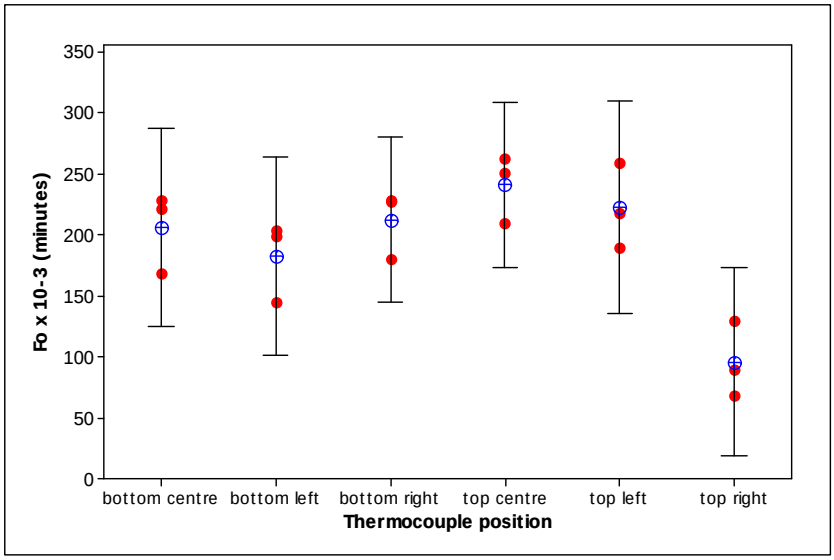

Figure 3-18. Distribution of F_0 at six different locations in the pressure vessel. Processing conditions: hold pressure 500 MPa, hold time 5 minutes, initial temperature 90°C. Note red circles are replicate runs, error bars represent 95% confidence interval for the population mean (indicated by the blue circle)

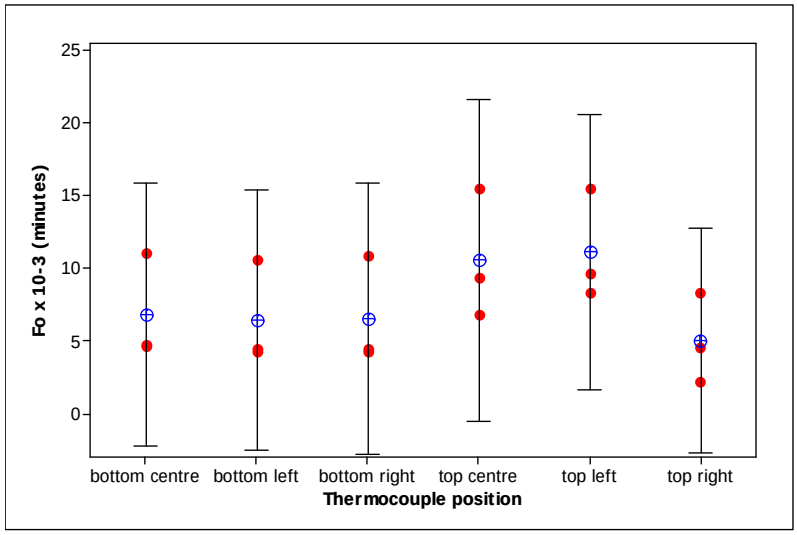

Figure 3-19. Distribution of F_0 at six different locations in the pressure vessel. Processing conditions: hold pressure 700 MPa, hold time 5 minutes, initial temperature 70°C. Note red circles are replicate runs, error bars represent 95% confidence interval for the population mean (indicated by the blue circle)

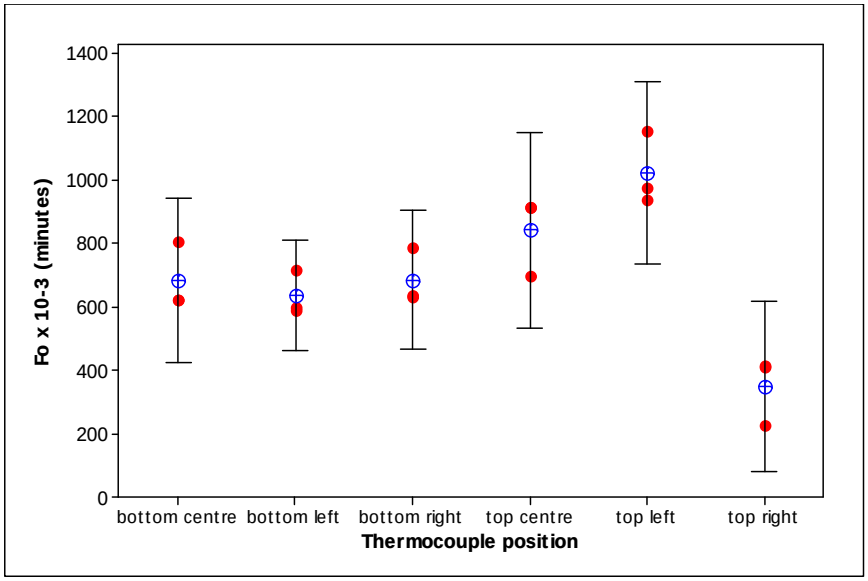

Figure 3-20. Distribution of F_0 at six different locations in the pressure vessel. Processing conditions: hold pressure 700 MPa, hold time 5 minutes, initial temperature 90°C. Note red circles are replicate runs, error bars represent 95% confidence interval for the population mean (indicated by the blue circle)

The accumulated F_o was small in all runs. Whilst the magnitude of the accumulated F_o value varied at different pressure/temperature combinations, the pattern of F_o distribution was relatively consistent. The base of the vessel was characterised by a relatively uniform distribution of F_o values (within a given run) whilst the top right side of the vessel generally gave lower values. This is unsurprising given the lower temperatures recorded in that region of the vessel (see Figure 3-16). The top left generally yielded slightly higher F_o values. Repeat calibrations of the thermocouples made inaccuracies in the thermocouples an unlikely explanation for the observed variation unless there was a direct effect of pressure on the thermocouple measurement. This is a possibility but limited published data in this area would suggest that the type K thermocouples used in this study are least susceptible to pressure related inaccuracies (Bloch & Chaisse 1967; Bundy 1961). A possible explanation for the lower F_o values on the top right side of the vessel was that the inlet for pressure medium entering the vessel was off-set to the right side at the base of the vessel. The PEEK liner had a solid base but a floating perforated lid. As pressure medium entered the vessel it could have been unevenly distributed between the right and left side of the liner and could have favoured passage up the right side. Cold pressure medium could therefore enter the top right side of the vessel first. Movement of the liner could also have resulted in the top thermocouples intermittently measuring outside the insulated region. This issue was addressed in the third stage of temperature distribution measurement.

The rate of change of temperature during both the come-up period and the hold-period of each pressure cycle was analysed at the six locations in the vessel for every run in the stage 2 trials. The temperature rise during the come up and temperature losses during the hold period could

be approximated by a straight line over the 5 minute hold times investigated in these experiments (see for example Figure 3-9). In consequence, the slope of the time-temperature plot during the come up phase, and the slope of the time-temperature plot during the hold phase was calculated for each experimental run by linear regression. These mean slopes were then compared to assess the effects of spatial position on the rate of change of temperature during the come-up phase and during the hold-phase (Figure 3-21and Figure 3-22). During the come up phase the top right side of the vessel rose in temperature at a slower rate compared with the other 5 locations in the vessel (Figure 3-21).

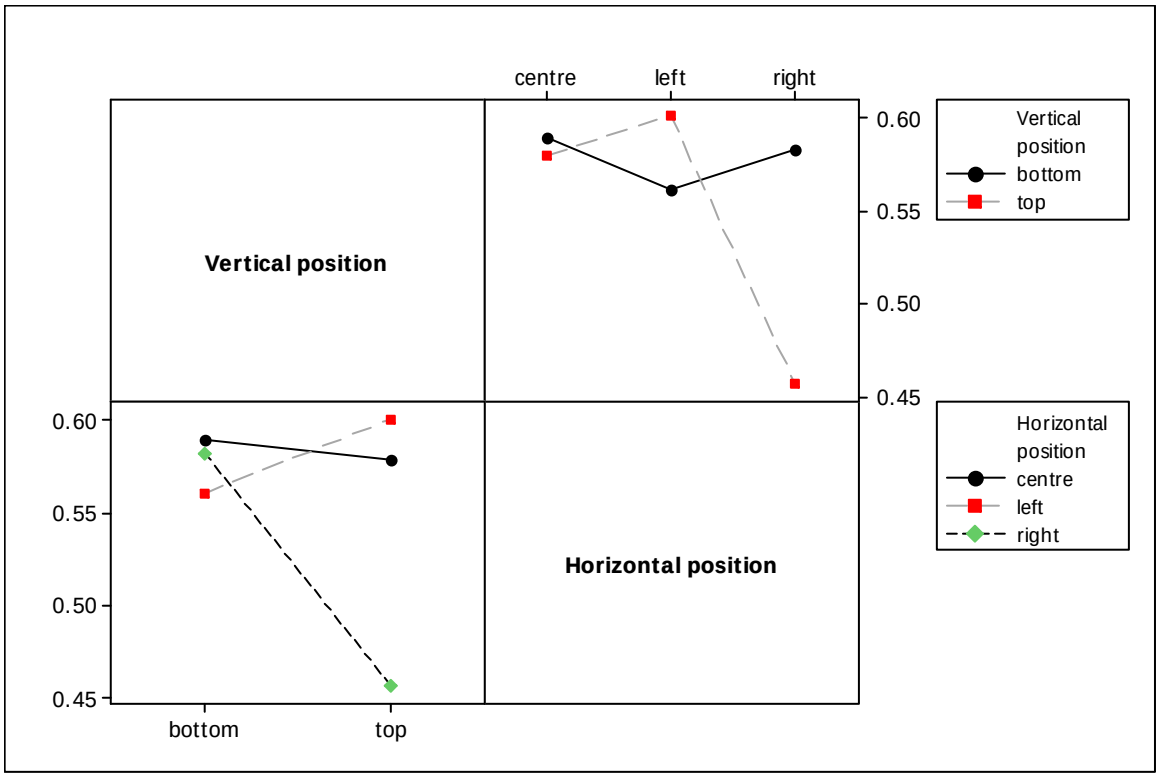

Figure 3-21. Rate of temperature rise (°C/s) during the come up period in the vessel as influenced by the six spatial positions measured during processing. "Vertical position" relates to whether the probe was at the top or bottom of the vessel. "Horizontal position" relates to whether the probe was on the left, centre or right hand side of the vessel.

Figure 3-21 shows the rate of change of temperature for all runs at the six measured locations during the come up period of every pressure cycle. The rate of change of temperature (i.e. the increase in temperature in °C/second) is reasonably consistent at all measured points except the top right of the vessel. Here the temperature rise per second is lower than in the rest of the vessel.

During the hold phase, the rate of cooling was also lower at the top right side of the vessel (Figure 3-22). This was perhaps because the temperature attained at the end of the come up period was lower than at the other 5 points and hence the temperature differential between the vessel wall and the pressure medium at that point was also lower thus reducing the rate of heat loss.

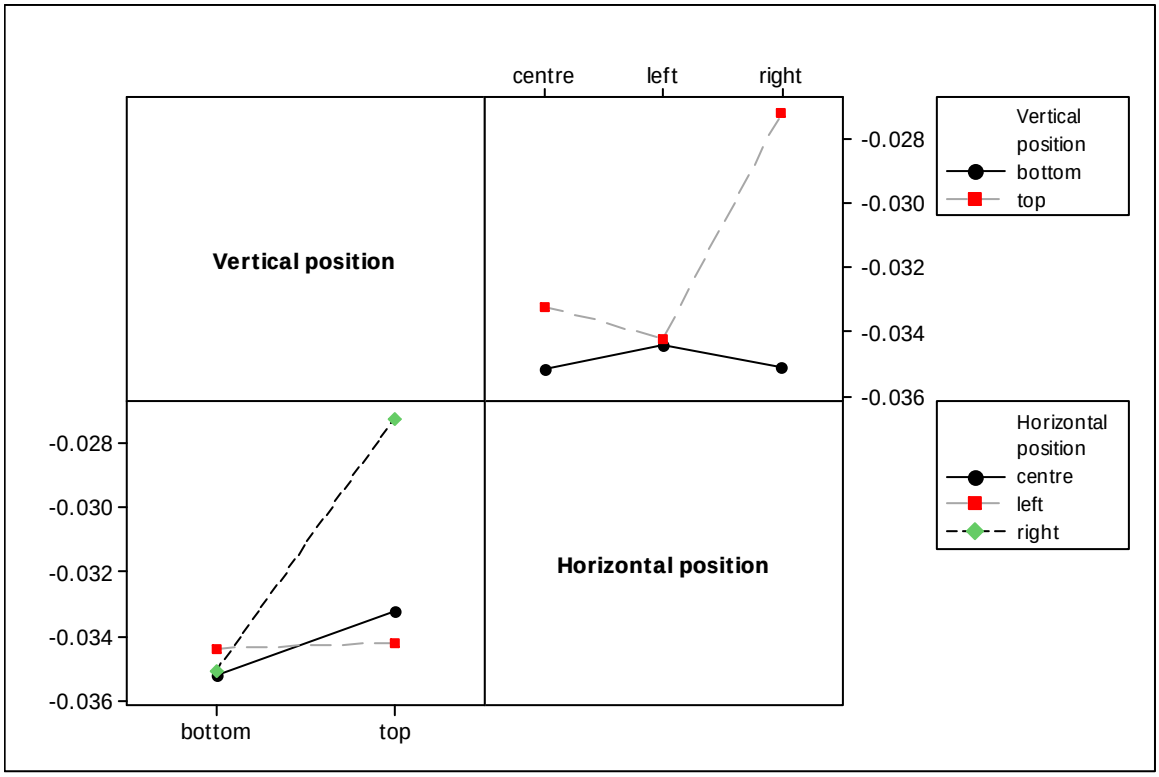

Figure 3-22. Rate of temperature loss (°C/s) during the hold period in the vessel as influenced by the six spatial positions measured during processing. "Vertical position" relates to whether the probe was at the top or bottom of the vessel. "Horizontal position" relates to whether the probe was on the left, centre or right hand side of the vessel.

There was statistically significant evidence ($P<0.05$) to suggest that the rate of change of temperature during the *come up* period was influenced by: the initial temperature; the interaction between horizontal and vertical position; the horizontal position in the vessel; the vertical position in the vessel; the target pressure/initial temperature interaction and the initial temperature/pressure/vertical location interaction.

During the hold period, there was statistically significant evidence ($P<0.05$) to suggest that the rate of change of temperature was influenced by: the target pressure; the target initial

temperature; the target pressure and initial temperature interaction; the vertical position; the vertical/horizontal position interaction and the horizontal position.

These findings essentially confirm the factors that would be expected to influence the rate of heating and cooling in the system. Higher initial temperatures lead to a higher peak temperature at the start of the hold phase. This results in the temperature differential between the vessel wall and the pressure fluid being higher (higher ΔT) and so the driving force for heat losses is higher. Higher processing pressures lead to a higher peak temperature at the start of the hold phase again leading to a greater temperature differential between the vessel wall and the pressure fluid; consequently leading to greater rates of heat loss during the hold phase. What was not known prior to conducting the trials was the nature of the temperature distribution in the vessel during HPS.

Temperature distribution was sub-optimal and the erratic time temperature traces observed during some runs, particularly at the top of the vessel, suggested that thermocouple movement was occurring. In addition, it was noted that during some runs the thermocouples were bent slightly when examined post process. This suggested that the liner was moving during the pressure treatment. The PEEK liner was therefore modified to try to improve temperature distribution and to minimise liner movement; a 2mm deep, 5mm wide groove was machined to allow the incoming pressure medium to more easily flow up the sides rather than lift the liner. A cradle was also constructed which mounted the liner to the top closure such that the thermocouples were consistently located in the same position relative to the liner.

A detailed characterisation of the temperature distribution during PATS would be an essential step in scheduling safe processing conditions for an industrial process. The equipment would also need to be designed a) to minimise heat losses and b) to optimise the uniformity of temperature distribution in the vessel. The experiments conducted in stage 2 characterised the system and identified cold points in the system . In addition, they identified the need to modify the liner to try to improve temperature distribution prior to studies on food (see Chapters 4-6).

3.3.3 Stage 3: Characterisation of temperature and lethality distributions with modified liner

After machining grooves into the PEEK liner, the time temperature profile in the vessel was much improved (smoother and more uniform - see Figure 3-23(a) and Figure 3-24 (a) for examples). However, non-uniformity was still observed; In general the top right and top centre of the vessel were found to be consistently higher in temperature than the other four points measured in the vessel. The bottom right side of the vessel was generally the coldest measured zone. The temperature range observed varied according to the HPS conditions employed but at the start of the hold phase was on average 2.8°C. Temperature variability in the vessel was reflected in the F_o values achieved at the six measured points in the vessel (Example plots of F_o distribution are shown in Figure 3-23 (b) and Figure 3-24 (b).

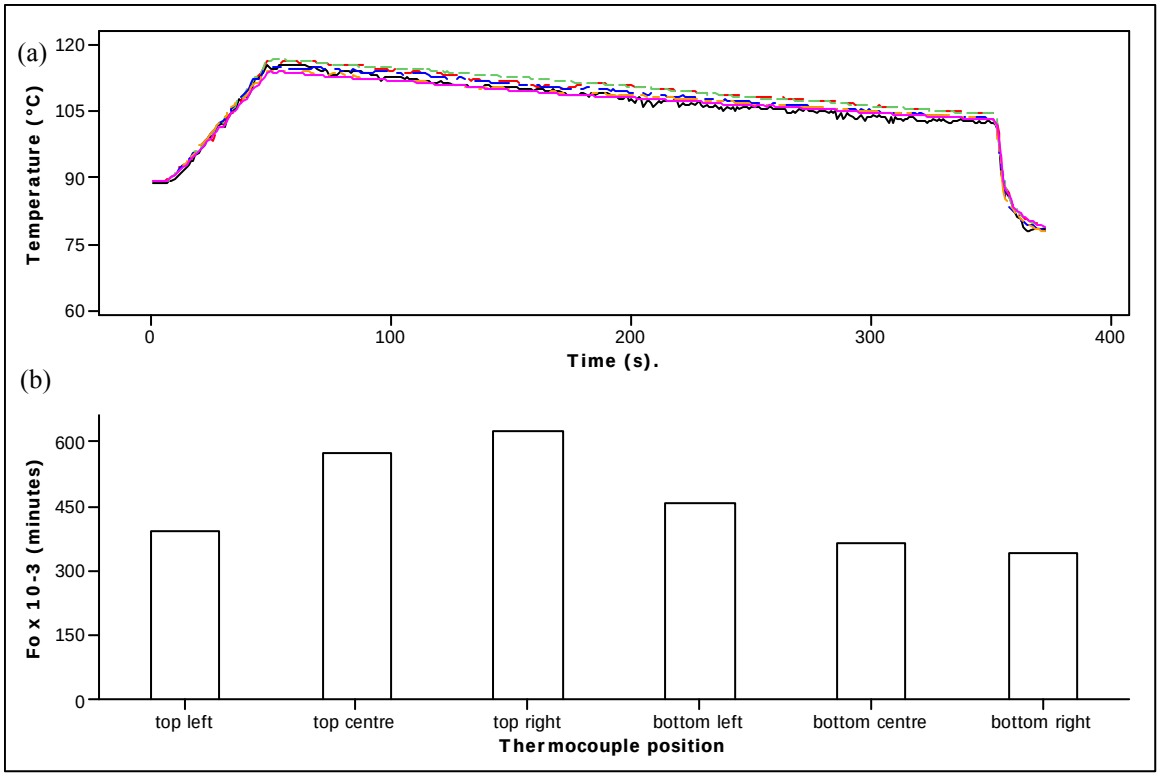

Figure 3-23. (a) Example temperature distribution plot for stage 3 experiments with (b) corresponding F_0 distribution. Pressure during hold phase 560 MPa, initial temperature 90°C, hold time 5 minutes.
Key top left, top centre, top right, bottom left, bottom centre, bottom right

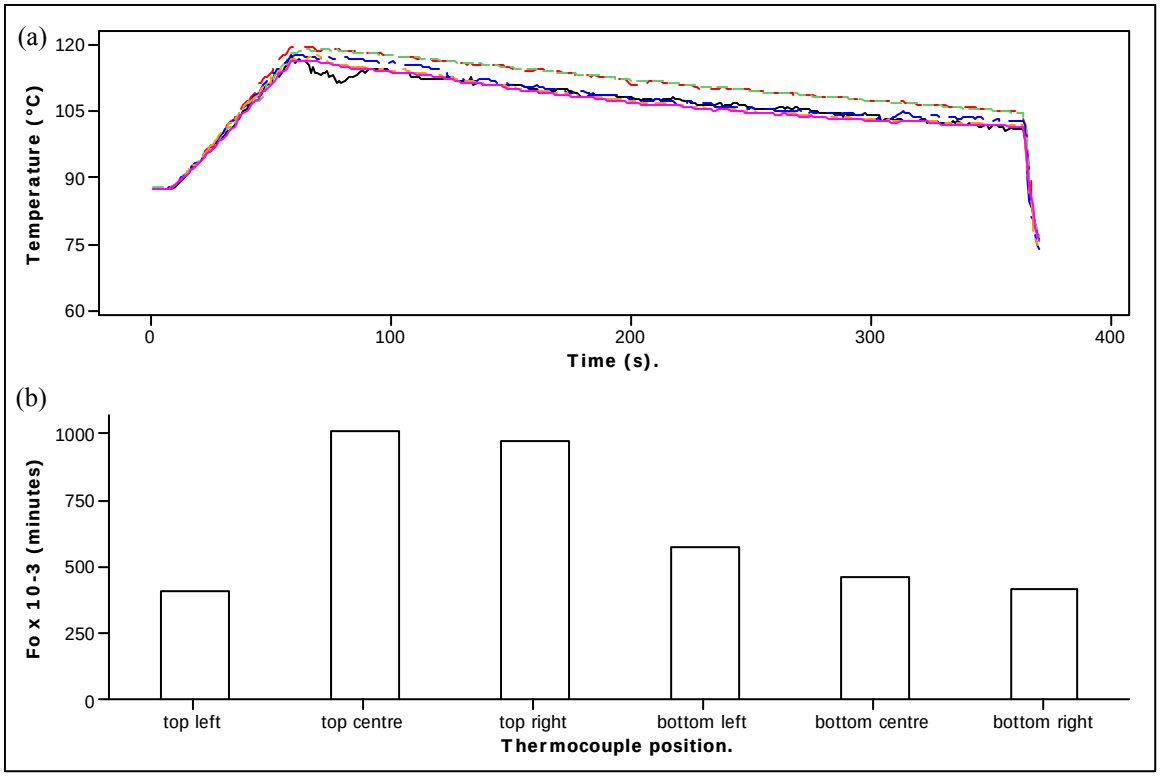

Figure 3-24. (a) Example temperature distribution plot for stage 3 experiments with (b) corresponding F_0 distribution. Pressure during hold phase 690 MPa, initial temperature 86°C, hold time 5 minutes.
Key top left, top centre, top right, bottom left, bottom centre, bottom right

The range of temperatures attained at the end of the "come up period" (See Figure 3-7) during each process is summarised in Table 3-3. The quoted 'lowest' and 'highest' temperatures represent the temperature measured at the coldest and hottest thermocouple position respectively for a given run.

Pressure (MPa)	Temp (°C)	Lowest probe temperature at end of come up (°C)	Location of probe	Highest probe temperature at end of come up (°C)	Location of probe	Range (°C)
530	76	95.8	Top left	98.1	Top centre	2.3
540	74	94.6	Bottom right	96.0	Bottom centre/bottom left	1.4
550	82	102.9	Bottom right	104.7	Top right	1.8
560	90	112.7	Bottom right	115.6	Top right	2.9
570	86	109.4	Bottom right	111.9	Top centre	2.5
570	88	110.7	Bottom right	113.8	Top right	3.1
610	86	110.9	Top left	114.7	Top centre	3.8
660	76	102.8	Top right	106.3	Top centre	3.5
670	80	105.9	Top left/bottom right	108.8	Top centre	2.9
690	86	115.0	Bottom right	118.8	Top centre	3.8

Table 3-3. Temperature range observed over the six thermocouples in each run at the end of the up period.

3.3.3.1 Temperature variation and heat losses during the hold phase of the process

The rate of temperature loss from the vessel during the hold phase varied between 1.1 to 4.1°C per minute depending on the pressure/temperature combination used and the spatial location within the vessel. In these experiments the vessel wall was maintained at the starting temperature of the pressure medium i.e. before pressurisation. Heat losses could be reduced by maintaining the vessel wall at the *final* expected temperature of the pressure medium. In stage 3 experiments, this approach would only have been of value for runs where the final temperature did not exceed 90°C since this was the maximum operational rating for the vessel wall. Heat losses for runs carried out at lower pressures were expected to be lower since the peak temperature attained during the run was lower and thus the temperature differential between the medium and the vessel wall was lower than in runs carried out at higher pressures.

Run ID	Thermocouple position	Mean hold temperature (°C)	Fo x 10^{-3}(minutes)
530 MPa 76°C	top left	93.8	10.89
	top centre	94.7	14.45
	top right	**91.6**	**8.15**
	bottom left	92.5	9.27
	bottom centre	92.5	9.15
	bottom right	92.8	9.97
540 MPa 74°C	top left	90.9	6.72
	top centre	93.5	11.51
	top right	93.5	11.37
	bottom left	91.1	7.24
	bottom centre	90.4	6.27
	bottom right	**90.1**	**5.71**
550 MPa 82°C	**top left**	**97.5**	**32.89**
	top centre	100.6	61.82
	top right	100.6	62.13
	bottom left	99.2	47.1
	bottom centre	98.4	37.97
	bottom right	98.2	35.77
560 MPa 90°C	**top left**	**108.0**	**390.36**
	top centre	110.0	570.37
	top right	110.3	623.19
	bottom left	109.0	458.14
	bottom centre	108.3	363.53
	bottom right	**108.0**	***338.87***
570 MPa 86°C	top left	104.8	170.47
	top centre	107.9	350.47
	top right	107.8	334.14
	bottom left	105.3	208.17
	bottom centre	104.3	163.26
	bottom right	**104.2**	**157.44**
570 MPa 88°C	top left	106.1	249.25
	top centre	108.9	430.48
	top right	109.0	440.94
	bottom left	106.1	238.91
	bottom centre	105.2	190.7

Run ID	Thermocouple position	Mean hold temperature (°C)	Fo x 10^{-3}(minutes)
	bottom right	**105.1**	**185**
610 MPa 86°C	top left	105.6	226.04
	top centre	108.4	428.01
	top right	108.4	421.24
	bottom left	105.7	251.97
	bottom centre	104.7	201.34
	bottom right	**104.4**	**180.2**
660 MPa 76°C	**top left**	**96.6**	36.85
	top centre	100.9	71.14
	top right	97.9	***36.15***
	bottom left	98.2	38.76
	bottom centre	97.9	38.34
	bottom right	98.3	41.22
670 MPa 80°C	top left	99.7	57.25
	top centre	102.2	106.27
	top right	102.6	102.7
	bottom left	100.4	64.9
	bottom centre	99.5	53.28
	bottom right	**99.2**	**49.38**
690 MPa 86°C	top left	111.7	402.58
	top centre	107.8	1007.65
	top right	111.7	966.96
	bottom left	108.6	571.48
	bottom centre	107.7	457.23
	bottom right	**107.4**	**409.95**

Table 3-4. Comparison of spatial variation in mean hold temperatures and accumulated lethality over a range of HPS conditions.

To determine the cold point of a conventional retort, it is common to evaluate time temperature traces by calculating the mean temperature during the hold phase (Smout & May 1997) and using this as an indicator of the coldest region of the retort. This is usually a sufficient indicator to identify the cold point because the retort temperature is usually stable

around a set point. In the case of the pressure/temperature combination processes explored in stage 2 and 3 experiments, substantial heat losses were shown to occur over the hold period. Because of these heat losses, mean temperatures over the hold period should only be used as an indicator for distribution evaluation purposes but should be treated with caution since the lowest average holding temperature does not automatically correlate to the area of lowest lethality over the hold period. This is illustrated in Table 3-4 in which the mean temperature over the hold period of the pressure cycle has been calculated along with the cumulative lethality (F_0) obtained for each complete HPS cycle. To take an example: results from a 660 MPa, 70°C process with a 5 minute hold period, identified the top left side of the vessel as having the lowest average hold temperature and thus, *potentially*, the area having the lowest area of lethality. However when lethality was calculated, the area of lowest lethality was actually the top right side of the vessel. Although the difference in lethalities in the two regions is marginal in this example, it illustrates that the use of mean hold temperatures alone to determine the worst performing region of the vessel is unwise. It seems likely therefore that temperature distribution studies for pressure vessels should be carried out in conjunction with heat distribution studies (i.e. mapping lethality throughout the vessel) to ensure that the worst case position in the vessel is correctly identified.

3.3.3.2 Influence of initial temperature on compression heating

The temperature rise in the compression medium per 100 MPa of applied pressure was calculated for a range of initial temperatures and plotted (see Figure 3-25). This plot is an interval plot showing the observed temperature rise per 100 MPa of applied pressure for a range of initial temperatures, along with a 95% confidence interval-estimate for the 'true' mean temperature rise per 100 MPa of applied pressure given a particular initial temperature.

Compression heating per 100 MPa of applied pressure was relatively consistent but there was statistically significant evidence ($P<0.05$) to suggest that it was affected by the initial temperature of the pressure medium (Figure 3-25). Higher initial temperatures yielded a slightly higher temperature rise per 100 MPa of applied pressure. The temperature rise per 100 MPa of applied pressure was on average 4.3°C but ranged between 3.8 to 4.9°C depending on the initial temperature of the pressure medium. Initial temperature alone however, was a relatively weak predictor of the observed temperature rise per 100 MPa (the regression fit having an adjusted R^2 of 35.6%).

The dependence of temperature rise per 100 MPa of pressure applied, on initial temperature is supported by data from previous studies (de Heij *et al.* 2003) and (Rasanayagam *et al.* 2003). De Heij *et al*. (2003) reported that in the case of water with an initial temperature of 20°C, the temperature rise per 100 MPa of pressure was 2.8°C. With an initial temperature of 80°C this temperature rise per 100 MPa of pressure rose to 4.4°C. Rasanayagam *et al*. (2003) reported that increasing the initial temperature of water from 25°C to 60°C increased compression heating values from around 3 to 4°C per 100 MPa of applied pressure. Reported values for compression heating per 100 MPa are likely to be system dependent since they are influenced by heat losses from the vessel which in turn are influenced by factors such as rate of come up and the selection of liner materials.

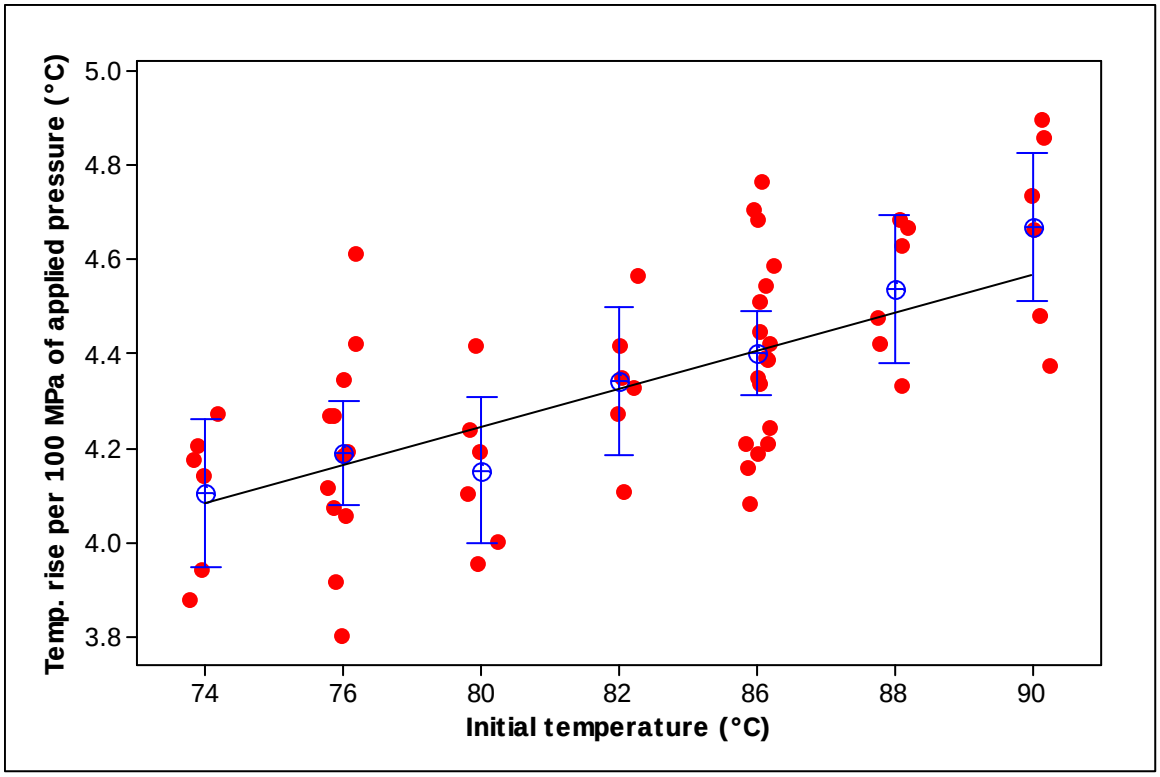

Figure 3-25. Temperature rise of the pressure medium per 100 MPa of applied pressure as influenced by the initial temperature of the pressure transmission fluid. Each red dot is the temperature rise measured per 100 MPa pressure from a single thermocouple during any given HPS cycle. Error bars represent the 95% confidence interval for the mean value of the population (indicated by a blue crossed circle)

3.4 Summary of chapter, key conclusions and areas for further discussion within the thesis

Existing Campden BRI equipment was modified by the inclusion of a PEEK liner and a re-designed thermocouple feedthrough on the top closure. Characterising temperature distribution within a high pressure vessel proved to be challenging. There were inherent difficulties in creating entry points for thermocouples at operating pressures of up to 700 MPa. On a commercial scale it could prove difficult to fit a sufficient number of thermocouples to a vessel in order to carry out a robust analysis of temperature distribution. Remote or wireless logging would be a much more practical solution.

Chemical or biological pressure/temperature integrators would be extremely valuable for process establishment but none are currently available that quite match the required duty for pressure sterilisation (see Chapter 2 for details). Minerich & Labuza (2003b) suggested the use of compressed copper tablets as pressure integrators but the tablets are not responsive to changes in temperature. They could therefore prove useful for pasteurisation pressure applications but not for sterilisation. Bauer & Knorr (2005) suggested the use of starch gelatinisation as a pressure time temperature integrator; this showed promise but it is likely that a great deal more practical evaluation would be required before it could be usefully applied as an analytical tool. Vervoort *et al*. (2011) proposed the use of xylanase from hyperthermophilic *Thermotoga maritima*as an indicator for temperature gradients in high pressure high temperature processing and this shows some potential. Similarly Grauwet *et al*. (2011) have demonstrated that ovomucoid based pTTIs have good potential for use in high pressure high temperature combination processing.

In the Campden BRI pressure equipment, temperature variation and heat losses within the vessel were substantial during the pressure hold period (losses amounted to between 1.1 and 4.1°C per minute depending on process conditions). There are a number of technical solutions available that could minimise heat losses – principally those outlined by van Schepael *et al*. (2002b) (see Chapter 2 for details). During these studies, there were practical restrictions that limited the methods that could be employed to minimise heat losses. In a custom designed system, temperature losses could be greatly reduced. Temperature variability is not in itself an insurmountable problem for the technology. However, it is fundamentally important that temperature variability can be accurately characterised, such that for any given run, the area of lowest lethality can be accurately predicted.

Temperature distribution in a pressure/temperature combination process is complex and highly dependent on the specific design features of the equipment being tested. Analysis of distribution data should take into account both worst case average temperatures and worst case heat distribution, as the two measures cannot be assumed to highlight the same 'cold spot' in the vessel. It should also be noted that heat distribution measurements assume an underlying mechanism of heat inactivation for the target microorganism that may not be hold true when pressure is also applied.

Within the context of this thesis, it was important to characterise temperature variation within the vessel as a precursor to conducting work on food quality effects.

4 Chapter 4 – Comparative studies on the effect of conventional canning technology and HPS processing on vegetable quality attributes

4.1 Introduction

For high pressure sterilisation to be commercially successful, the products must have better quality than those conventionally heat processed. This chapter thus examines the quality of products manufactured by the two methods. The primary aim of work was to evaluate the colour and texture of high pressure sterilised (HPS) green beans and to compare the results with those of raw beans, blanched beans and conventionally heat sterilised beans. Beans were selected as the raw material for the trials as a model material for various vegetables where it would be desirable to maintain the colour and texture of the product. Personal experience and technical discussions with clients in the food industry over a 15 year period have made it clear that the retention of 'fresh-cooked' attributes of vegetables in ambient stable products is a highly desirable but technically difficult objective for many manufacturers. Data demonstrating the quality of HPS treated vegetables compared with conventionally heat processed vegetables would therefore be of value to the food industry.

In the studies discussed in this chapter, a range of thermal processes were applied, from $F_0 = 1$ to 3 minutes. These studies differed from related work (Krebbers *et al.* 2002) discussed in previous chapters because: commercially achievable pressures were utilised; data was obtained after an extended ambient storage period; and HPS was compared with a range of thermal process severities. A secondary aim of the work was to evaluate the colour and texture of HPS treated carrots and to compare the results with heat sterilised (F_0 of 3 minutes)

samples. Experiments on carrots were not as detailed as those carried out for green beans and are therefore indicative only but are included here for completeness.

4.2 Materials and methods

4.2.1 Preparation of samples for testing

4.2.1.1 Green beans

Fresh green beans (*Phaseolus vulgaris*) were purchased from a local supplier (Drinkwater, Chipping Campden). A random sample of beans was selected for packing. The beans were trimmed by hand, cut in two and blanched by immersion in mains water at 74°C for 6 minutes.

Blanching, where used prior to canning, is typically undertaken to a) remove entrained air from the product in order to minimise oxygen in the container headspace; b) to modify tissue structure to ease packing and facilitate a greater weight-to-volume ratio; c) to reduce initial loading of microbial counts and to reduce the level of any contaminants such as pesticides; d) to inactivate spoilage enzymes, though this is of secondary importance in a canning operation since the product will subsequently receive a thermal treatment. Blanching does however serve to reduce enzyme activity prior to retorting which can be important if there are delays between filling, sealing and retorting; e) to help to standardise moisture levels that would otherwise vary due to water loss or uptake during retorting (Larousse & Brown 1997).

After blanching, the beans were removed from the hot water, cooled as rapidly as possible in mains water and drained. The beans were cut into slices of approximately 25 mm. Ten grams

of beans were randomly selected from the batch and filled into rectangular retortable foil laminate pouches (PETP/Aluminium/Orientated Nylon/Polypropylene) along with 10 ml of reverse osmosis filtered water. The pouches were vacuum sealed using a Multivac pouch sealer. The pouch dimensions were 140 mm x 40 mm.

Prepared beans were subjected to either canning experiments (F_0 1, 2 and 3 minutes), HPS, blanching, or blanching plus pre-heating to 86°C (i.e. to the point at which a pressure sterilisation cycle would usually be started). Since the experiments were conducted over a number of months, beans were purchased in 4 separate batches (designated "bean batch 1", "bean batch 2", "bean batch 3" and "bean batch 4" in section 4.3). Not all of the processing conditions described were applied in all bean batches and this is indicated in the data as appropriate.

4.2.1.2 Carrots

Fresh carrots were purchased from a local supplier (Drinkwater, Chipping Campden). The carrots were quartered by cutting lengthways down the central axis; each half was then cut in two. The four quarters were manually sliced, each slice being approximately 4 mm thick. Ten grams of carrots were randomly selected from the batch and filled into retortable foil laminate pouches (PETP/Aluminium/Orientated Nylon/Polypropylene) along with 10 ml of reverse osmosis filtered water. The pouches were vacuum-sealed using a Multivac pouch sealer. The pouch dimensions were 140 mm x 40 mm. Prepared carrots were subjected to either canning (F_0 = 3 minutes) or HPS. Since the experiments were conducted over a number of months, carrots were purchased in 2 separate batches designated "carrot batch 1" and "carrot batch 2" in section 4.3.

4.2.2 Canning experiments

Pouches, prepared as described in 4.2.1, were loaded into a pilot scale, single basket, Lagarde steam air retort (Lagarde, Malataverne, France). Time temperature measurements were taken at the geometric centre of the pouch, typically at 1 second intervals, throughout the retort cycle using either type K thermocouples (Omega, UK) attached to a MMS3000 data-logger (RiL Instruments, Nottingham UK) or type T thermocouples attached to a TM9616 data acquisition unit (cables and logger supplied by Ellab, UK). F_0 calculations were made in real time using either a laptop computer running Microsoft Excel (when using the MMS3000 logger and type K thermocouples) or using Valsuite software (Ellab UK, used in conjunction with the Ellab cables and TM9616). An overpressure retort cycle was selected, and the retort temperature was set to 115°C. Before selecting 115°C as the process temperature, higher retort temperatures were attempted but because the pouches were small with a very flat profile, the product heated too rapidly for accurate control of the target F_0. A temperature of 115°C was selected so that the product was at the hold temperature for a meaningful length of time before cooling commenced and target end of process F_0 values could be controlled with sufficient accuracy. Bean samples were heated to achieve a process equivalent to 121.1°C for 1, 2 or 3 minutes at the end of cooling; carrot samples were heated to achieve a process equivalent to 3 minutes at 121.1°C at the end of cooling. A finite difference model (CTemp version 6, Campden BRI) was used to estimate the accumulated lethality in heating and cooling in a conduction heated pack. These predictions were used as a guide for when to start cooling the retort such that the required lethality was attained at the end of the cooling period. Each process was carried out a minimum of two times with at least 3 pouches of product produced in each run so that all results were replicated at least 12 times.

Prior to any retorting or high-pressure experiments, all thermocouples used in the studies were calibrated by immersion in a well-mixed (circulating) silicone oil bath at 90°C and 121°C, using two reference standard instruments with known and traceable values of uncertainty. The uncertainty associated with the calibration did not exceed ± 0.25 °C.

4.2.3 High pressure sterilisation experiments

Studies were carried out in the EPSI laboratory scale high pressure unit described in Chapter 3, process control and thermocouple calibration were as described in section 3.2. Temperature was recorded during the cycle (in-pack and adjacent to the samples) using type K thermocouples (EPSI, Belgium) attached to an MMS3000 data logger (RiL instruments, UK). A thermocouple was inserted, through silicon sealant, into the beans at the geometric centre of the pouch and the silicone sealant was allowed to harden before starting temperature measurements. Two thermocouples were taped to the outside of the probed pouch and sandwiched between two additional pouches. Pressure readings were obtained by converting a voltage signal from the control system of the equipment (a West 5010 industrial controller connected to a Moore Industries SGT/XSGT strain gauge transmitter (Crawley, UK)) into the MMS3000 data-logger..

Pouches fitted with thermocouples as described above were filled into a PEEK liner (as described in Chapter 3, see also Figure 3-3) which was topped up with pressure medium at 86°C and the load was allowed to equilibrate. As the product was tightly packed into the insulating PEEK liner, this equilibration stage was quite long (around 60 minutes). Heating rates during the pre-heating cycle were typically of the order of 1.1°C per minute. This

equilibration step prior to starting the HPS runs is likely to have led to some over-processing of the samples compared to that which could be achieved in optimised equipment, but this was necessary to ensure that peak temperatures were high enough to be comparable with previously published conditions to yield commercial sterility (Meyer *et al*. 2001). To understand the influence of the pre-heating phase on colour and texture, some samples were analysed at the point at which the pressure cycle would be started. These samples are referred to as 'pre-treat' in the results.

The pressure process consisted of the pre-heat to 86°C followed by a 2 minute hold at 700 MPa, a one minute hold at ambient pressure and a second 2 minute hold at 700 MPa. A typical HPS cycle from the study is shown in Figure 4-1.

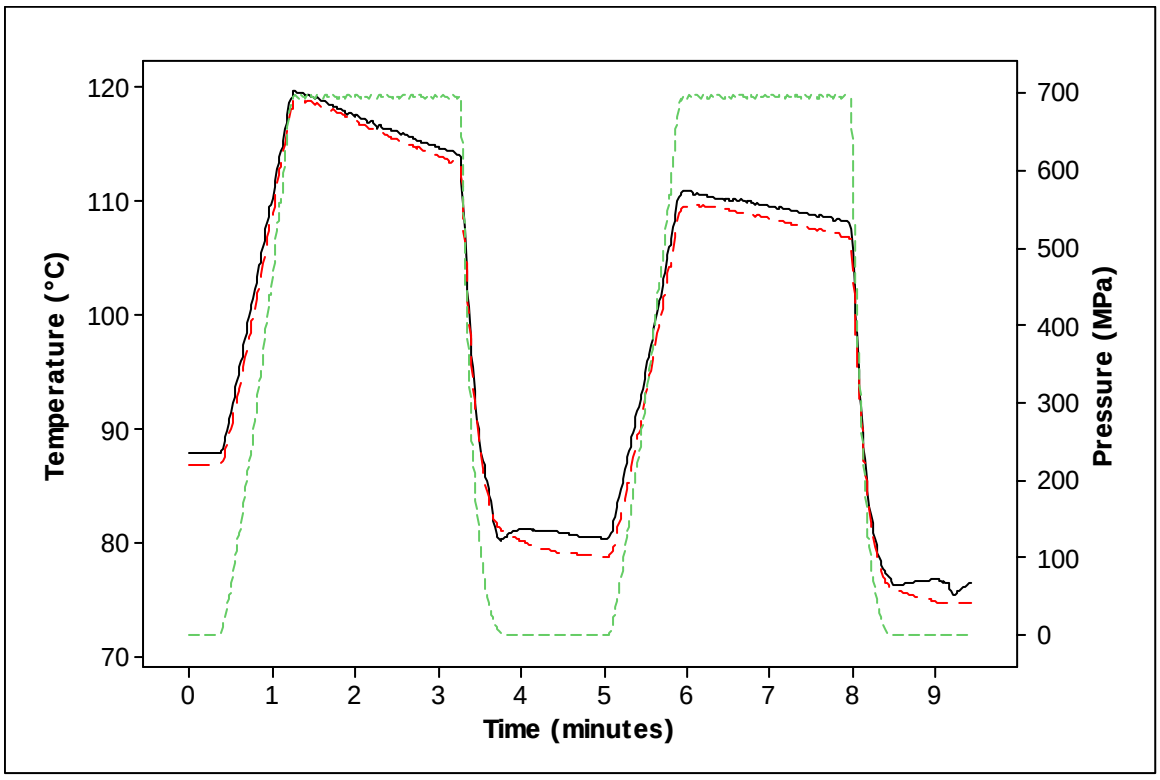

Figure 4-1. A typical HPS cycle used in the experiments. Key: HPS media temperature, Sample temperature, Pressure

On completion of the cycle, the samples were removed from the vessel and plunged into chilled water. HPS processes were carried out, as a minimum, in duplicate. In the case of green beans the time temperature profile within the high-pressure vessel resulted in a mean F_0 value of 0.7 minutes (note, this F_0 calculation only takes into account the thermal contribution to lethality that has occurred during HPS. Also, the kinetic parameters typically used for thermal sterilisation processes (e.g. a 'z' value of 10C° for *Clostridium botulinum)* may not be valid under HPS conditions – see Chapter 2). See Chapters 1 for further details on the lethal rate equation and its use. Trials on carrots were not carried out in duplicate (within carrot batches) and a lower initial temperature was used in the first batch (designated batch 1, where the initial temperature was 81°C). Total F_o values were 0.1 and 0.4 minutes for carrot batches 1 and 2 respectively.

4.2.4 Colour measurement

Before and after processing, colour measurements were made using a 'DigiEye' digital imaging system (DigiEye plc, Leicester, UK). This enabled a calibrated colour image of samples to be taken which could subsequently be measured using the CIE *L* a* b** uniform colour space system (ASTM, 2000; CIE, 1986). Diffuse D_{65} lighting was used to illuminate each sample as a digital image was taken. D_{65} lighting is a commonly used standard illuminant that according to the International Commission on Illumination (CIE) is "intended to represent average daylight and has a correlated colour temperature of approximately 6500 K" (Anon 2011) . Mean *L* a* b** measurements were reported for each set of samples from a processing run. For any given run, half of the processed pouches were retained and stored

for 7 months at ambient temperatures prior to being re-analysed for colour changes on storage.

A detailed discussion of the CIE $L^* a^* b^*$ scale and its interpretation has previously been published by Campden BRI (Whitworth 2006). For simple reference, the basic concept of the CIE $L^* a^* b^*$ colour space is summarised in Figure 4-2 (Whitworth 2006) and is outlined below:

- Increasing values of L^* indicate that the sample is becoming lighter; conversely, decreasingly values indicate that the sample is becoming darker.
- Increasing values of a^* indicate that the sample is becoming more red and less green, decreasing values indicate that the sample is becoming less red and more green.
- Increasing values of b^* indicate that the sample is becoming more yellow and less blue; decreasing values indicate that the sample is becoming more blue and less yellow.

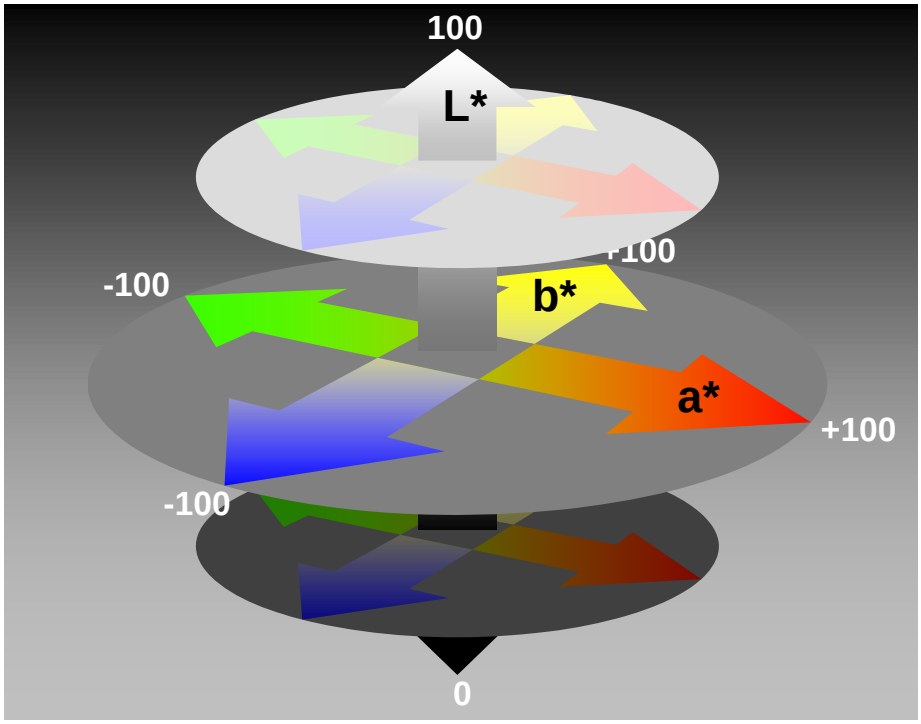

Figure 4-2. Basic concept of the CIE L* a* b* colour space (Whitworth 2006)

4.2.5 Texture measurement

According to Tunick (2011) food texture has been defined as "the sensory and functional manifestation of the structural, mechanical, and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetics". This broad definition opens up the potential for a wide range of techniques to assess food texture but perhaps the most prevalent form of texture analyser is one in which an instrument is used to measure the response of a sample to compression or tension. An example of this type of system (and the approach used in our experiments – a Stable Microsystems TA-XT2 analyser) is shown in Figure 4-3.

Figure 4-3. Stable Microsystems texture analyser

The basic stages of a texture measurement using this approach are as follows:

- A probe (selected according to the nature of the sample being measured) begins to move from a starting point towards the sample at a "pre-test" speed;
- When the probe registers a force equal to a pre-set trigger force, the speed changes from the pre-test speed to the "test" speed and the system starts to collect data;
- The probe continues to move into the sample at the test speed until the test is complete;
- When the test is finished, probe begins to move away from the sample at "post-test" speed;
- The probe stops once it is back to its original starting point.

The above represents a typical texture analysis cycle. A wide variety of probes and attachments are available for the TA-XT2 texture analyser depending on the sample being tested. For example, cylindrical probes from 2mm up to around 50 mm are available for puncture and penetration type testing (see 4.2.5.2). A blade set is available for texture analysis utilising cutting (see 4.2.5.1). Selection of the appropriate accessory for a particular

application requires specialist knowledge and experience; the TA-XT2 has over 60 accessories depending upon the application so the use of appropriate tooling is critical to a successful outcome. Details of the specific methods employed for trials on green beans and carrots are described in the following sections.

4.2.5.1 Bean texture analysis methods

Before and after processing, bean texture was determined by slicing force, using a Stable Micro Systems TA-XT2 texture analyser (Godalming, Surrey, UK) fitted with knife blade and inverted slotted base. A single piece of a bean was placed to span the slot and cut in half by the knife. The maximum force required and the total area under the force/distance curve were recorded during this cutting operation. Example plots are presented in section 4.3. For simplicity, peak force was used as the primary indicator of bean texture. The instrument settings were as shown in Table 4-1.

Test mode:	Measure force in compression
Option:	Return to start
Pre-test speed:	5 mm/s
Test speed:	5 mm/s
Post-test speed:	5 mm/s
Distance:	10mm
Trigger:	Auto, 10g

Table 4-1 Settings used for bean texture analysis

4.2.5.2 Carrots

Carrot slices were tested for texture by penetrometry, using a Stable Micro Systems (SMS) TA-XT2 texture analyser fitted with a cylindrical probe of 2 mm diameter. Preliminary tests compared the traces obtained both with the slice placed on a solid base, and the slice placed on a base having a 10mm diameter aperture through which the probe was allowed to pass after penetrating the sample. The former geometry was selected as the most appropriate test

method since sample thickness effects appeared less prominent using this option; the major peak on the trace corresponded to penetration of the top surface, as opposed to bulk failure of the sample piece. Separate tests were carried out on the outer and core regions of the slice, the latter typically giving a higher penetration force. The instrument settings used for the analysis were as shown in Table 4-2 below.

Test mode	Measure force in compression
Option	Return to start
Pre-test speed	2 mm/s
Test speed	2 mm/s
Post-test speed	5 mm/s
Distance	80% strain
Trigger	Auto, 5g

Table 4-2. Settings used for carrot texture analysis

4.2.6 Microbiological enumeration

Samples processed by the methods described in section 4.2.2 and 4.2.3 were stored for 7 months at ambient temperature. Samples were then enumerated for total aerobic and anaerobic pour plate counts, and total aerobic and anaerobic spore counts to determine how successful each process had been in controlling microbial survival and growth over storage. In samples where the thermal process was less than an F_0 of 3 minutes it was anticipated that some survival of micro-organisms might be observed after the storage period. It was not known whether survival and growth might occur in HPS treated samples because although the thermal aspects of the treatment were less than F_0 of 3 minutes (which would suggest that survival would be possible) it was not known what effect might be expected from the heat treatment in combination with pressure i.e. would the combination of pressure and heat be

sufficient to prevent growth and survival despite the thermal element of the process being sub-F_0 of 3 minutes.

Each sample was opened under aseptic conditions, one gram of each sample was weighed and a 10^{-1} dilution was prepared with maximum recovery diluent (MRD, Lab M). For aerobic and anaerobic plate counts, the samples were serially diluted in 9 ml MRD, and enumerated using Nutrient Agar (NA,Oxoid) incubated at 30°C for 48 hours or molten Eugon Agar (ES, Difco) + 0.1% starch (BDH) incubated anaerobically at 30°C for 5 days, respectively. The number of colonies on the plates was counted and calculated as aerobic and anaerobic plate counts per gram. These counts would indicate whether there were significant numbers of aerobic and/or anaerobic organisms present in the processed samples after treatment and storage.

For aerobic and anaerobic spore counts, the 10^{-1} dilution was placed in a sterile capped container and placed in a water-bath. A dummy tube of water with a thermometer was used to check the temperature of the water-bath. When the dummy tube reached 80°C, the timer was started for 10 minutes. The tubes were removed and cooled in iced water for 5 minutes and serial diluted in MRD (Lab M). The aerobic and anaerobic spore counts were then prepared and enumerated as described previously. These particular tests were undertaken to check whether spore forming organism had survived and were present in the processed samples after treatment and storage.

4.2.7 Statistical analysis

A General Linear Model (GLM) with Tukey's test (at the 5% significance level) was used to assess the effect of process, sample batch and months of storage on colour parameters and texture measurement values for 3 bean batches (as discussed in section 4.2.1.1) – "bean batch 1", "bean batch 2" and "bean batch 3" and 2 carrot batches "carrot batch 1" and "carrot batch 2". No storage data was available for the fourth bean batch ("bean batch 4") so this dataset was omitted from this particular analysis. All data analysis was conducted using Minitab version 14 (Minitab Inc, USA).

For green bean sample batch four (see above and section 4.2.1.1), Principal Component Analysis was used to reduce the three dimensional colour space to two dimensions (MINITAB 14.20). As a result, evidence of statistically significant differences between samples was tested by a one way ANOVA of L* + b* against process category with Tukey's test (at the 5% significance level). See section 4.3.1 for a discussion of the rationale for this approach. A one way ANOVA of mean peak forces was conducted with Tukey's test (at the 5% significance level) to assess the impact of increasing thermal processing severity on bean texture.

4.3 Results and discussion

4.3.1 Effect of processing on bean colour

4.3.1.1 Colour changes induced in green beans by high pressure sterilisation compared with those induced by thermal treatment to an F_0 of 3 minutes.

The effects of processing regime and storage on bean colour when treated with either a HPS process or when retorted at an F_0 of 3 minutes can be seen in Figure 4-4 to Figure 4-6 where *L*, a** and *b** values are reported for 3 sample batches after 0 and 7 months of storage. Mean values for L*, a* and b* for pressure sterilised and F_0 3 heat-treated beans are shown in Table 4-3 along with the effects of storage on colour changes.

		Month 0			Month 7		
		1	*2*	*3*	*4*	*5*	*6*
Sample	Bean batch	*L**	*a**	*b**	*L**	*a**	*b**
HPS	1	47.6 ± 2.0	9.1 ± 0.6	24.0 ± 3.0	46.7 ± 3.9	8.5 ± 0.7	21.7 ± 1.5
F_0 3	1	48.6 ± 2.5	5.4 ± 0.7	22.7 ± 3.0	46.7 ± 1.8	7.5 ± 0.6	18.9 ± 1.2
HPS	2	49.5 ± 1.3	7.5 ± 0.6	20.3 ± 1.7	45.6 ± 1.6	6.4 ± 0.5	19.2 ± 1.3
F_0 3	2	+	+	+	+	+	+
HPS	3	50.2 ± 3.0	10.0 ± 1.4	26.7 ± 4.3	48.4 ± 3.2	8.8 ± 1.3	24.5 ± 4.6
F_0 3	3	49.2 ± 1.9	6.9 ± 0.3	20.4 ± 1.5	45.3 ± 2.6	8.0 ± 0.5	22.1 ± 1.2
HPS	4	43.6 ± 2.6	7.8 ± 0.7	23.6 ± 2.5	+	+	+
F_0 3	4	46.3 ± 1.8	7.0 ± 0.8	27.3 ± 2.3	+	+	+

Table 4-3 Effect of treatment and storage on bean colour for F_0 3 and HPS treated beans. Note: + denotes that no sample measurements were taken. ± figure denotes 1 standard deviation from the mean

For bean batches 1, 2 and 3 there were only two processing conditions to analyse (i.e. HPS or F_0 3 treated), the data was therefore analysed by directly comparing *L*, a** and *b** values between batches and processes using ANOVA.

Differences in mean *L** values at month zero between F_0 3 and HPS samples were not significant (P>0.05) but there was some variation between bean batches (Column 1, Table 4-3). For example, the magnitude of the difference in L* between HPS treated and F_0 3 treated samples in "bean batch 4" was somewhat larger than was observed in any of the other bean batches. HPS samples in "bean batch 4" had a lower L* value indicating that the samples were darker in colour compared with the retorted samples. There was no significant darkening (P>0.05) of HPS or canned samples over storage but again there was some variation within bean batches (Column 1, Table 4-3).

Although a* values of all HPS samples were reduced on storage (Columns 2 and 5, Table 4.3), the change was generally not statistically significant (P>0.05). For F_0 3 samples, mean a* values increased on storage but there was batch-to-batch variability as to whether the effect was statistically significant. At month zero, a* values were significantly different (P<0.05) between HPS and canned samples (HPS samples were less green) but after the 7 months of storage there were no significant differences between samples from the two processes (P>0.05).

There was no statistically significant evidence (P>0.05) to suggest that *b** values of HPS or F_0 3 treated samples changed over the 7 month storage period. At month zero, *b** values of HPS and $F_0$3 treated sample were, in general, statistically significantly different (P<0.05) but there

was batch-to-batch variability with HPS samples being more yellow that F_0 3 treated samples in some batches but less yellow in others.

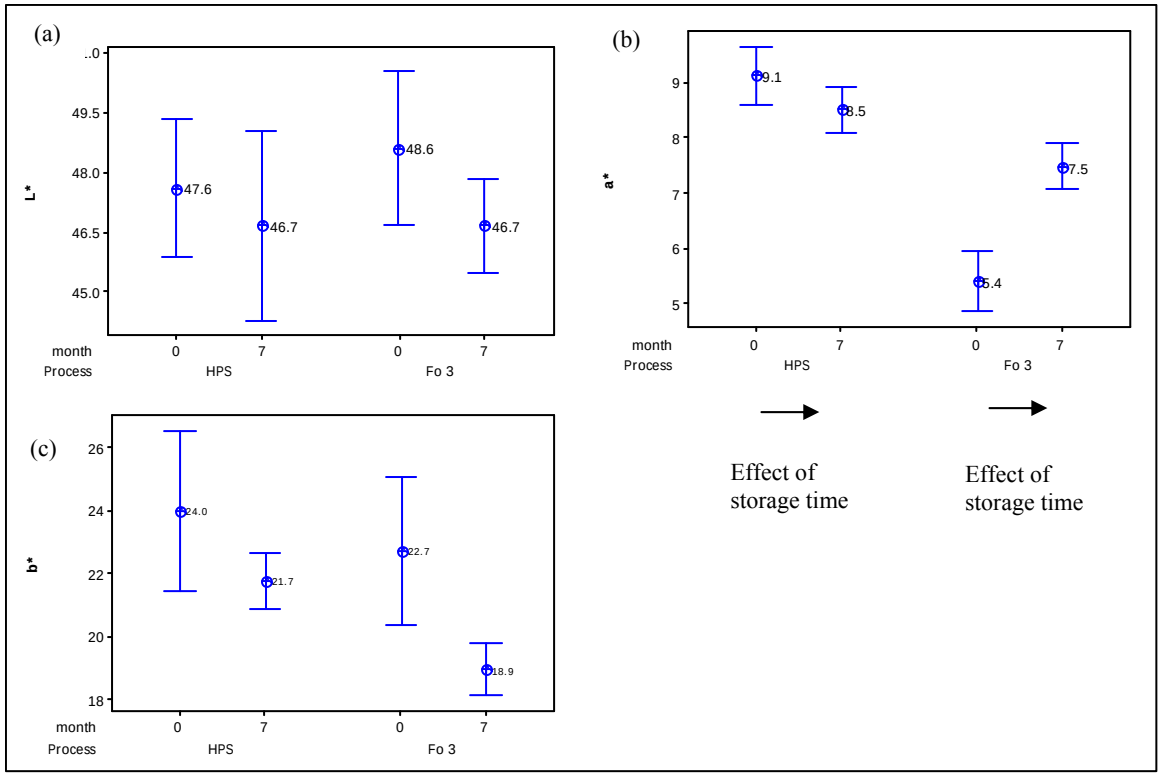

Figure 4-4 (a)-(c). Influence of processing regime and storage time on the colour of green beans. Data is for "bean batch 1" only. Plots show mean values of L* (a), a* (b) and b* (c) immediately after high pressure sterilisation or conventional retorting (F_0 of 3 minutes) and after 7 months of storage at ambient temperature. Error bars indicate the 95% confidence interval for the population mean.

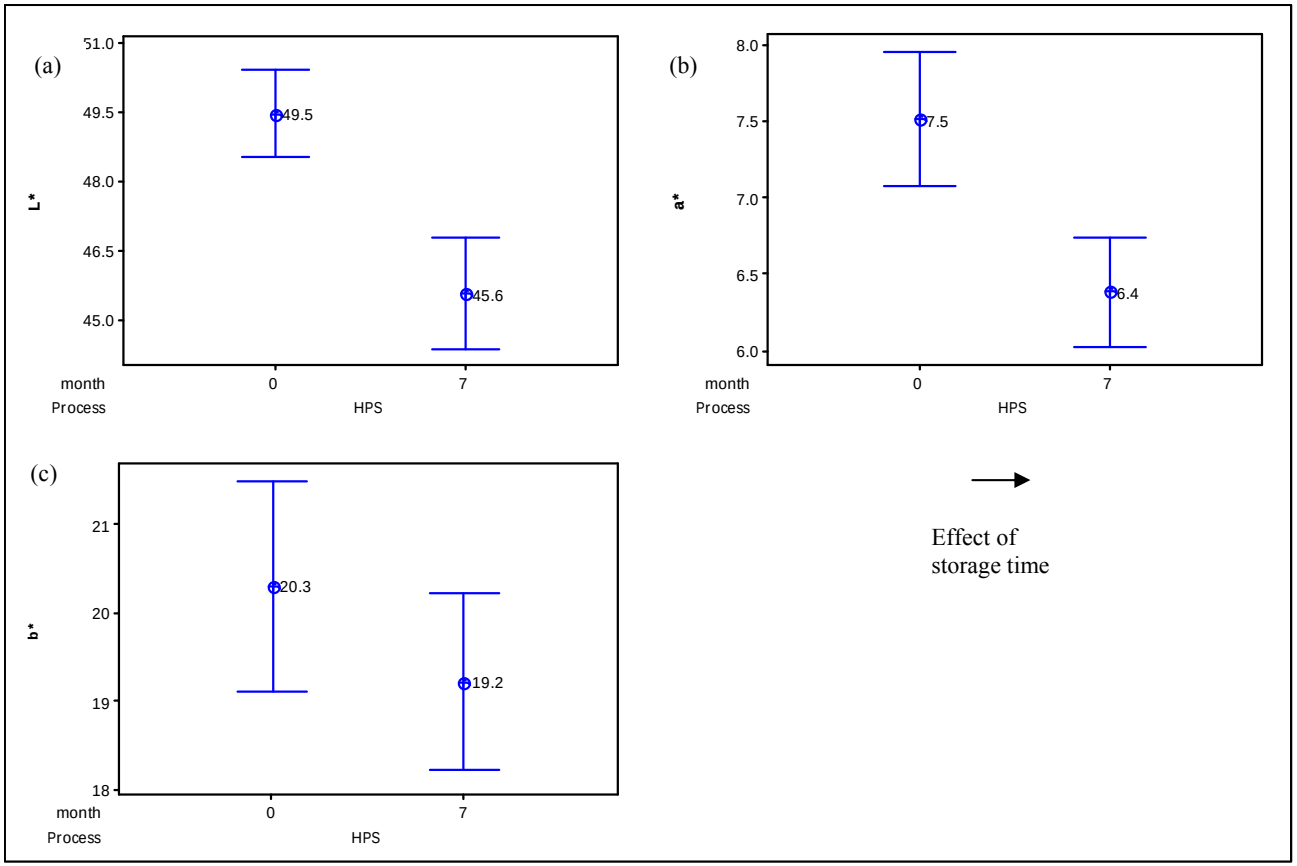

Figure 4-5. (a)-(c). Influence of processing regime and storage time on the colour of green beans. Data is for "bean batch 2" only. Plots show mean values of L* (a), a* (b) and b* (c) immediately after high pressure sterilisation and after 7 months of storage at ambient temperature. Error bars indicate the 95% confidence interval for the population mean.

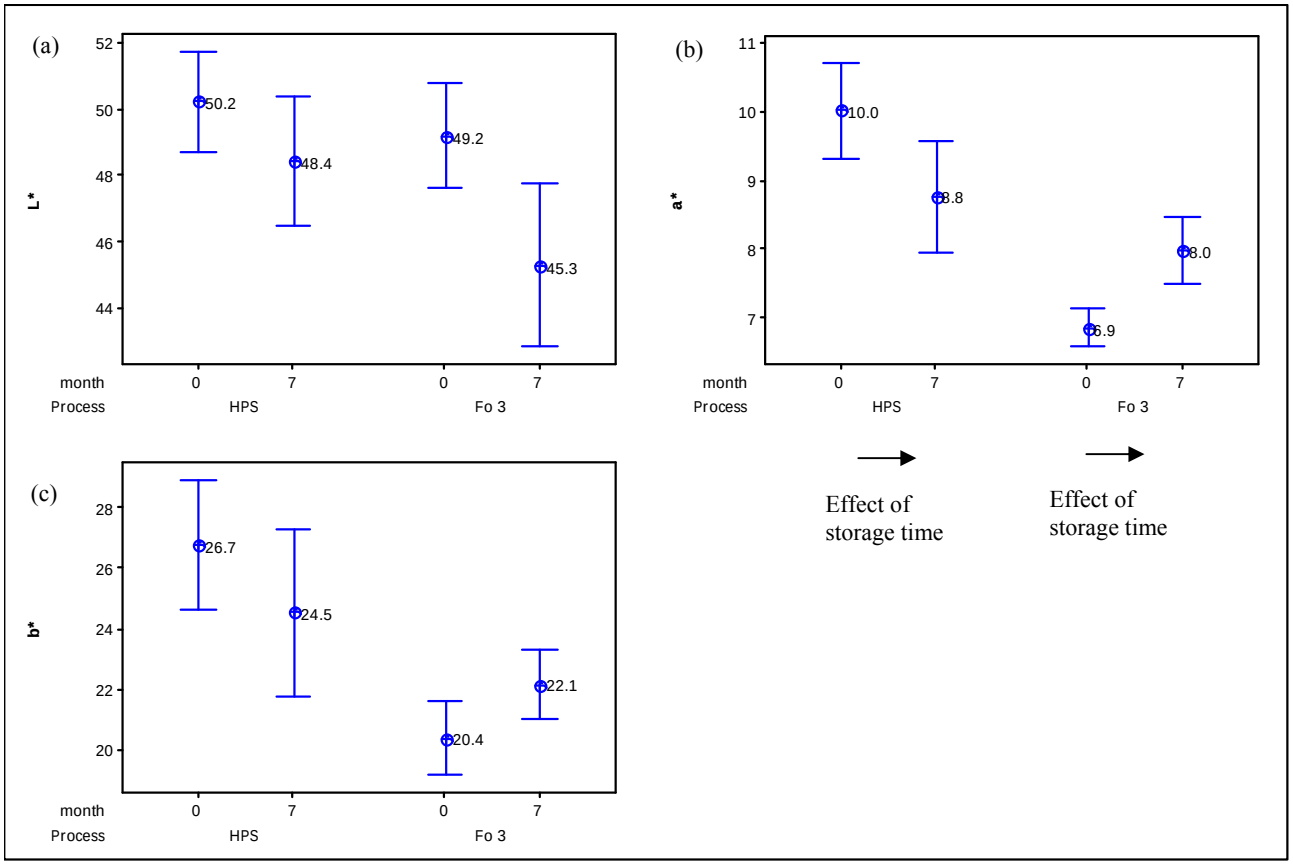

Figure 4-6(a)-(c). Influence of processing regime and storage time on the colour of green beans. Data is for "bean batch 3" only. Plots show mean values of L* (a), a* (b) and b* (c) immediately after high pressure sterilisation or conventional retorting (F_0 of 3 minutes) and after 7 months of storage at ambient temperature. Error bars indicate the 95% confidence interval for the population mean.

Figure 4-7 to Figure 4-11 are colour images of samples from bean batches 1-3, taken using the DigiEye digital imaging system. Colour measurements were taken immediately after processing and after 7 months of ambient storage. The reader should bear in mind that, whilst the images as taken by the DigiEye are calibrated, the printer used to generate this thesis is not calibrated so images are for indication only. True colour differences are best represented by the $L^* a^* b^*$ values of each sample. These values have been included in the figure legend and are summarised in Table 4-3.

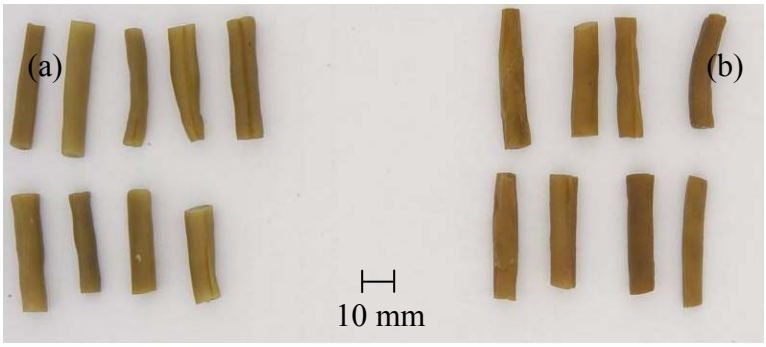

Figure 4-7 (a) and (b) Visual appearance of Bean Batch 1 samples a) retorted (F_0 of 3 mins) and b) HPS treated samples immediately after processing. Mean LAB values were a) 48.6, 5.4, 22.7 and b) 47.6, 9.1, 24.0

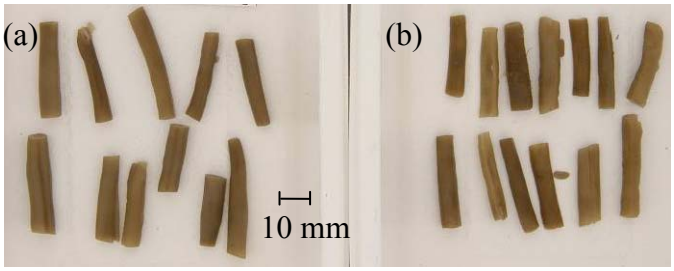

Figure 4-8 (a) and (b) Visual appearance of Bean Batch 1 samples a) retorted (F_0 of 3 mins) and b) HPS treated samples, after 7 months of storage. Mean LAB values were a) 46.7, 7.5, 18.9 and b) 46.7, 8.5, 21.7

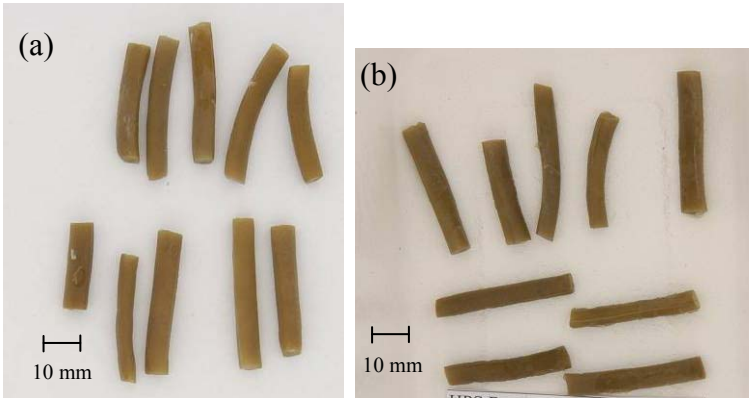

Figure 4-9 (a) and (b) Visual appearance of Bean Batch 2 samples a) HPS treated immediately after processing b) HPS treated samples after 7 months of storage. Mean LAB values were a) 49.5, 7.5, 20.3 and b) 45.6, 6.4, 19.2

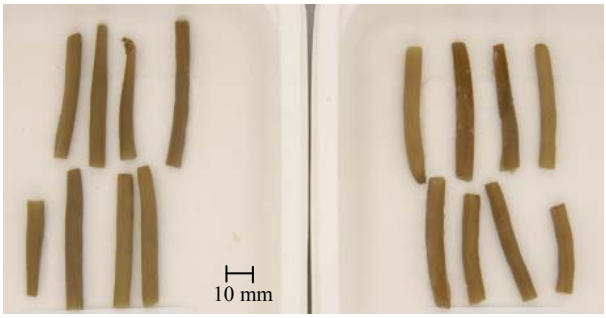

Figure 4-10 (a) and (b) Visual appearance of Bean Batch 3 samples a) retorted (F_0 of 3 mins) and b) HPS treated samples, immediately after processing. Mean LAB values were a) 49.2, 6.9, 20.4 and b) 50.2, 10.0, 26.7

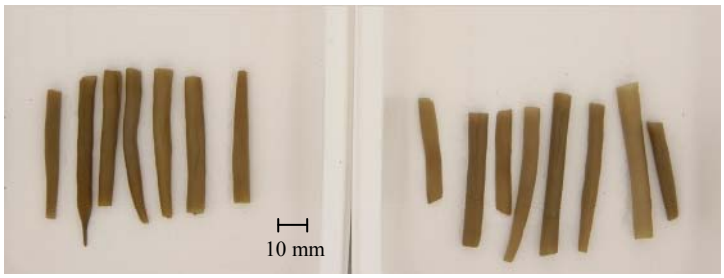

Figure 4-11 (a) and (b) Visual appearance of Bean Batch 3 samples a) retorted (F_0 of 3 mins) and b) HPS treated samples, after 7 months of storage. Mean LAB values were a) 45.3, 8.0, 22.1 and b) 48.4, 8.8, 24.5

4.3.1.2 Colour changes induced in beans by high pressure sterilisation compared with those induced by a range of thermal treatments (F_0 of 1, 2 and 3 minutes).

For bean batch 4, HPS samples were compared with a range of thermal processes (F_0 1 to 3 minutes, blanching and blanching plus pre-heating to the initial temperature for the HPS cycle) to assess the effects on the colour of the processed beans. Figure 4-12 show the effects of each treatment on *L*, a** and *b** values. Samples in Figure 4-12 are ordered in increasing severity of thermal process: 'Pre-treat' corresponds to samples that were blanched and pre-heated to 86°C in the high pressure vessel but no pressure process was applied. Graph shows mean values with error bars indicating a 95% confidence interval for the population mean.

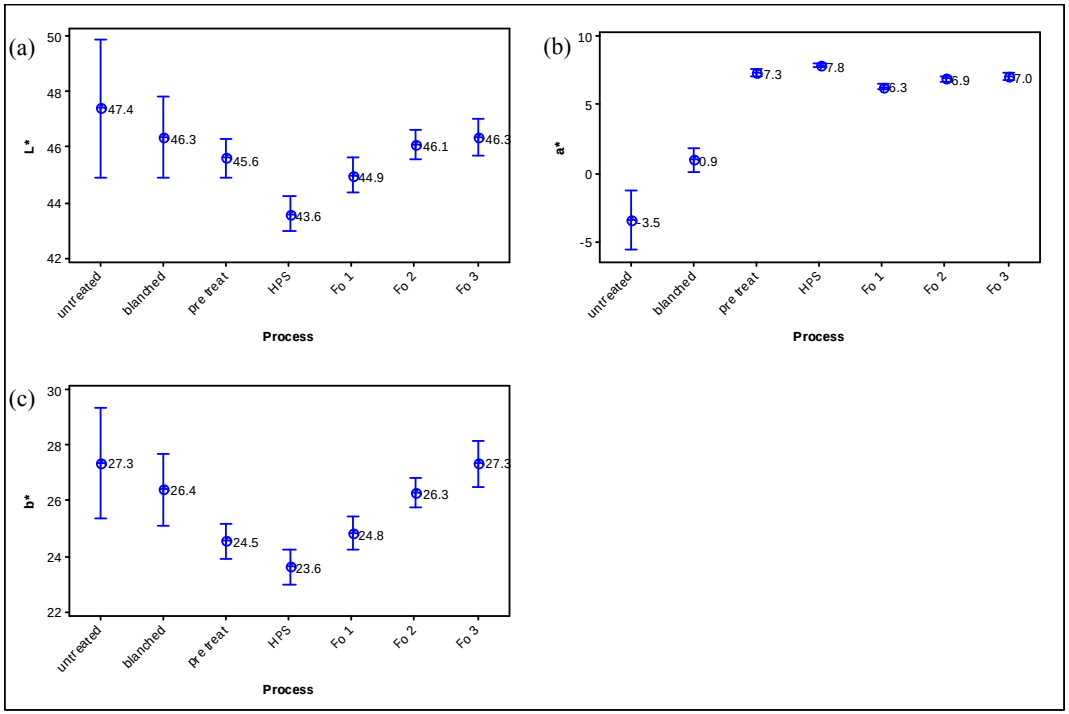

Figure 4-12(a)-(c). Influence of processing regime on the colour of green beans. Data is for "bean batch 4" only. Plots show mean values of L* (a), a* (b) and b* (c) of untreated beans, blanched beans, beans pre-heating prior to HPS, beans immediately after high pressure sterilisation or after a range of conventional retorting conditions (F_0 of 1-3 minutes). Error bars indicate the 95% confidence interval for the population mean.

Increasing severity of heat treatment initially resulted in a darkening of bean samples (Figure 4-12 a) as indicated by decreasing values of L*; the darkest samples being high pressure sterilised. As process severity increased beyond this point the samples became increasingly light with F_0 3 samples being similar in L* values to blanched beans. Figure 4-12 c shows that the values of *b** (for which increasing values indicate increasingly yellow samples) showed a very similar pattern; as thermal process severity increased, *b** initially decreased, with HPS being the least yellow; but as heating severity increased further, the samples became increasingly yellow. The values of a* (for which decreasing values indicate greener samples) were relatively similar for all processed samples that were more severe than blanching (Figure 4-12 b) , the largest mean difference between samples being only 1.5 units.

Figure 4-13 shows the Principal Component Analysis loading plot and score plot for the case of sample batch 4 in which principal component analysis (PCA) was used to simplify the three dimensional colour space into two dimensions for analysis purposes. Principal component one explained 61.8% of the data variation and was comprised of 0.677 *L** + 0.21 *a** + 0.705 *b**. Principal component two explained 33% of the variation and was comprised of 0.281*L** -0.960 *a** + 0.016 *b**. Principal component one was essentially comprised of equal loadings of *L** and *b** with only a small contribution from *a**. Principal component two was essentially *a** and there was little difference between samples with respect to this colour parameter in batch 4 (see Figure 4-12). An ANOVA of L* + b* mean differences by process category was therefore conducted to test for statistically significant differences in colour between processing regimes for bean batch 4.

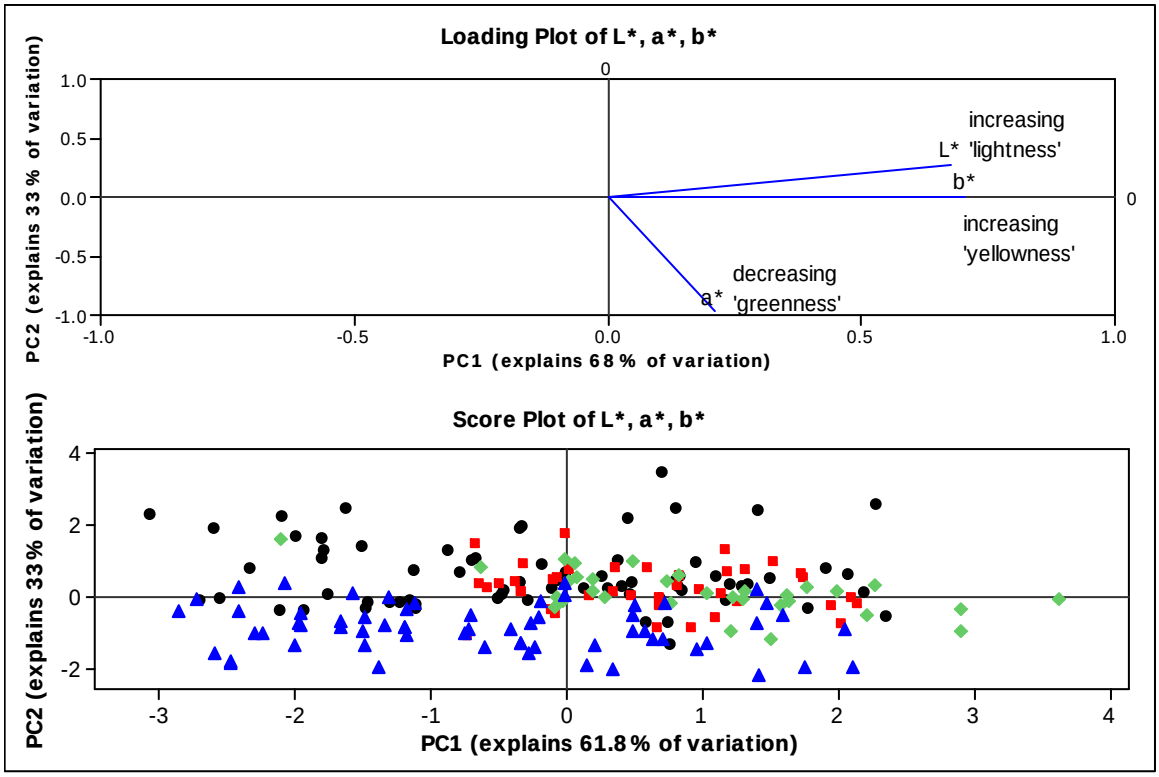

Figure 4-13. (a) Loading plot and (b) score plot from Principal Component Analysis on bean batch 4. Key: • $F_0$1, ■ $F_0$2, ♦ F_0 3, ▲HPS

Only HPS and F_0 processes 1-3 were included in this analysis because untreated and blanched beans were so markedly different from the retorted and HPS treated samples and their inclusion would have skewed the results . The mean values for $L^* + b^*$ for the different processing regimes are reported in Table 4-4.

Processing regime	Mean	Standard deviation	N
HPS	67.2[a]	4.81	62
F_0 1	69.7[b]	4.88	71
F_0 2	72.3[c]	3.06	40
F_0 3	73.6[c]	3.95	32

Table 4-4. Mean values for L* + b* for different heat processing regimes (F_0 1-3 minutes and HPS) for bean batch 4.

Note: samples sharing the same superscripted letter were not significantly different (P>0.05) from one another.

Table 4-4 shows that there was statistically significant evidence (P<0.05) to suggest that the mean value of $L^* + b^*$ for HPS was lower than that of all processing regimes. As noted in section 4.2.3, the thermal contribution to the HPS process was similar to that of the F_0 1 process but the mean $L^* + b^*$ values were different for the two processing regimes. This suggested that the colour shift observed in HPS samples was not due to thermal effects alone; there was some additional colour modification attributable to the HPS process. In practical terms, HPS samples were darker and less yellow compared with samples processed at F_0 1, 2 and 3. This effect was explored more fully in subsequent work (see Chapter 5).

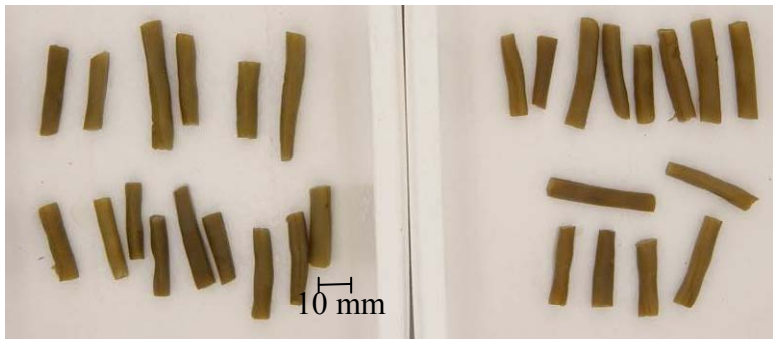

Figure 4-14 (a) and (b) Example of visual appearance of Bean Batch 4 samples a) retorted (F_0 of 3 mins) and b) HPS treated samples, immediately after treatment. Mean LAB values were a) 46.3, 7.0, 27.3 and b) 43.6, 7.8, 23.6

As previously noted (see section 2.5), Matser *et al.* (2004) observed that the effect of high pressure sterilisation on product quality was very much product dependent, particularly with respect to colour changes. Results from this thesis suggest that there is also batch-to-batch variability and it seems likely that variety trials will need to be conducted for selecting raw materials for pressure sterilisation in much the same way as has been done for many years for conventional sterilisation applications - see Mack (1939) for an early example "Variety trials – beans & beets".

In the Krebbers study (Krebbers *et al*. 2002) the effects on colour and texture of green beans were studied for a range of preservation methods including pressure pasteurisation, HPS, canning and freezing. Immediately after processing, blanched and frozen beans had a more intense green colour compared with raw. Pressure processed green beans were also reported to have had this more intense colour. As in the work described in this chapter, both conventionally sterilised and pressure sterilised beans exhibited the classic olive brown colour

change typical of heat preserved beans. The colour of heat sterilised and pressure sterilised beans was stable over a one month storage period whilst raw, frozen and pressure pasteurised beans gradually deteriorated towards a pale yellow/green colour over the one month chilled storage period. Krebbers postulated that this colour change was due to the residual activity of enzymes such as lipoxygenase, peroxidase or chlorophyllase. There was a reported increase in a^* on storage for canned samples but little change in HPS samples over storage. Our results (Table 4-3) also indicate that a^* values do not change significantly in HPS samples over storage but increases in a^* values in F_0 3 treated samples were batch dependent.

It should be noted that the storage period in the experiments by Krebbers *et al*. (2002) was only one month and the processing conditions used for HPS trials were considerably different to those used here. Samples were only pre-heated to 75°C and were pressure treated at 1000 MPa, a pressure treatment in excess of that of 700 MPa currently achievable on a commercial scale (personal communications with NC Hyperbaric, Avure and UDHE). The peak temperature attained by the samples in the Krebbers study was around 100°C, suggesting that greater heat losses were occurring than in the EPSI system used for these studies. Typically, the peak temperature attained by the product in the work described in this chapter was around 117°C.

Although the differences in colour that were observed between different processes and bean batches in our study were relatively small, they were perceptible to the human eye in almost all cases. Hunter and Harold (1987) noted that "in a side-by-side match, the eye can

distinguish approximately 10 million colours" and in the textiles industry, colour shifts of 0.8 CIE Lab units are visibly different.

The classical mechanism for degradation of chlorophylls a and b to pheophytin is the substitution of magnesium in the porphyrin ring of the chlorophyll molecule with hydrogen (Clydesdale & Francis 1976); degradation of chlorophyll is therefore proportional to hydrogen ion concentration (Clydesdale & Francis 1976). Various options have been proposed to retain the bright green colour of heat treated chlorophyll-containing vegetables but most have had limited success. The use of alkalising agents has been proposed, for example, to reduce hydrogen ion concentration and thus minimise pheophytin formation (Clydesdale & Francis, 1976). The "Blair process" suggested the use of calcium or magnesium hydroxides and this was reported to give good results in terms of colour retention immediately after processing but the benefits are said to have been lost on storage (Clydesdale & Francis 1976). Some of these approaches could be worth re-visiting in the context of pressure sterilisation processes in an attempt to optimise colour retention. Studies at HPP pasteurisation conditions suggested that pH modification to alkaline conditions (6.5 to 8) showed potential as a method of maintaining a positive green colour in pressure treated coriander (Dai *et al.* 2008). Increasing pH helped to decrease colour changes and to maintain the chlorophyll content of the herb.

4.3.2 Effects of processing on carrot colour

The effects of processing regime and storage on the colour of carrots can be seen in Figure 4-15 and Figure 4-16 where *L*, a** and *b** values are reported for 2 sample batches that were either pressure processed or heat treated to F_0 3. All graphs show mean values and 95% confidence intervals for the population mean.

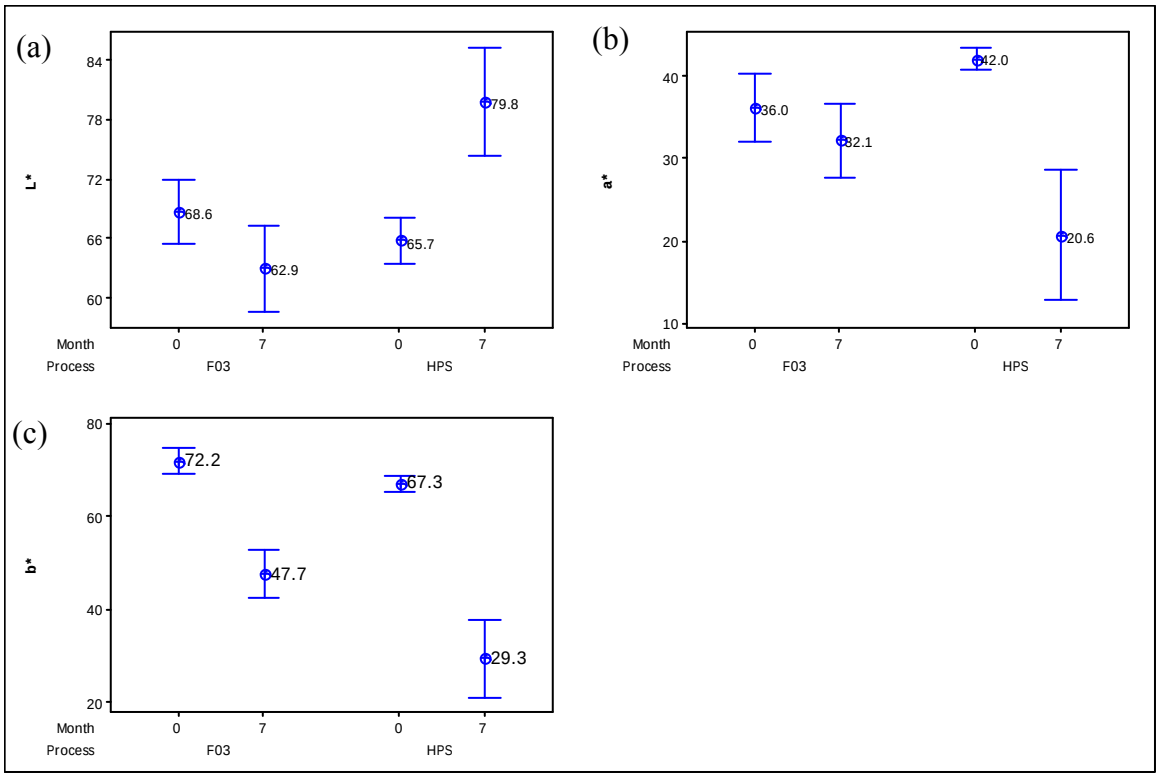

Figure 4-15. (a)-(c). Influence of processing regime and storage time on the colour of carrots. Data is for "carrot batch 1" only. Plots show mean values of L* (a), a* (b) and b* (c) immediately after high pressure sterilisation or conventional retorting (F_0 of 3 minutes) and after 7 months of storage. Error bars indicate the 95% confidence interval for the population mean.

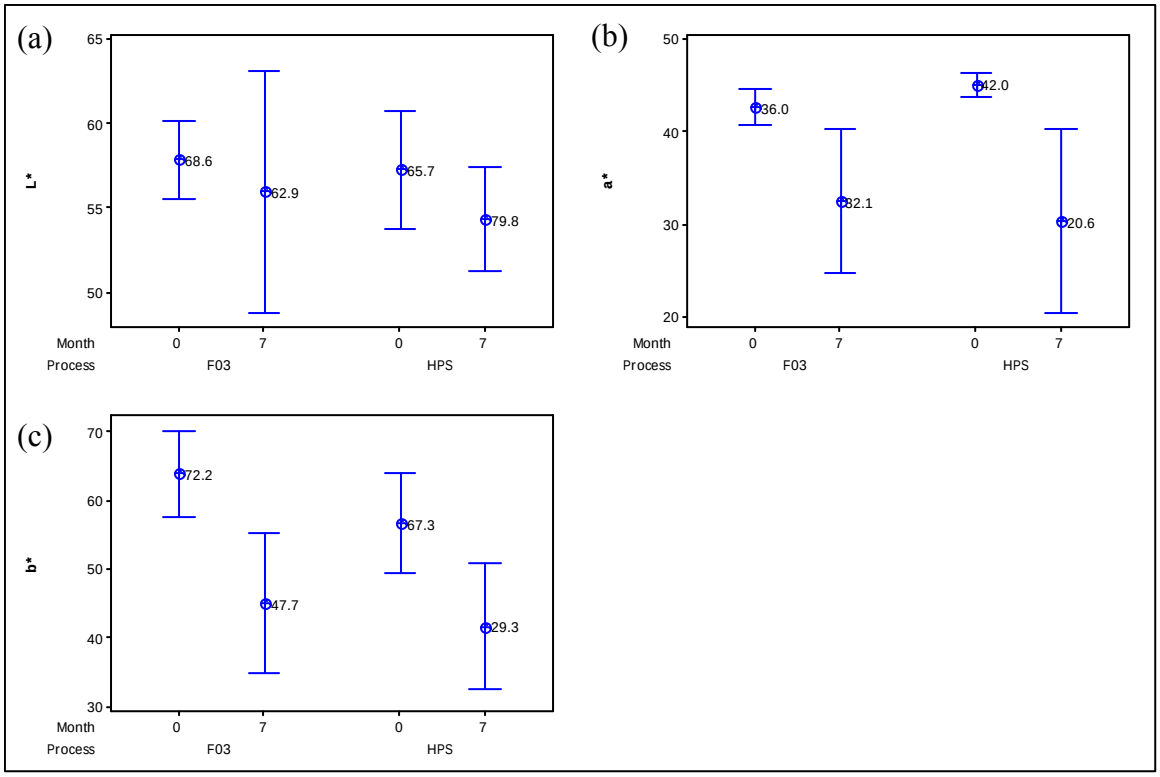

Figure 4-16. (a)-(c). Influence of processing regime and storage time on the colour of carrots. Data is for "carrot batch 2" only. Plots show mean values of L* (a), a* (b) and b* (c) immediately after high pressure sterilisation or conventional retorting (F_0 of 3 minutes) and after 7 months of storage. Error bars indicate the 95% confidence interval for the population mean.

Mean and standard deviations for colour changes in the processed carrots are reported as *L*, a** and *b** values for both batches in Table 4-5. Values of L*, a* and b* for HPS and canned carrots from both batches were not significantly different from one another (P>0.05). However, it should be noted that one of the assumptions of the GLM used for the data analysis was broken because HPS and canned samples did not appear to have equal variances (Fisher 1935). For this reason the quoted 'p' values for carrot samples should be interpreted with caution.

After 7 months of storage *L** values had not changed significantly in any canned samples or in HPS batch 2 (p>0.05), but were significantly higher than at month 0 in HPS treated samples from batch 1 (p<0.05), indicating that this batch of HPS treated carrots became lighter on storage. After 7 month's storage the values of *L** for canned and HPS treated samples were not significantly different from one another in batch 2 (p>0.05) but again were significantly higher in batch 1 (p<0.05).

			Month 0			Month 7		
	1	2	3	4	5	6	7	8
1	Sample	Batch	*L**	*a**	*b**	*L**	*a**	*b**
2	HPS	1	65.7 ± 2.9	42.0 ± 1.7	67.3 ± 2.2	79.8 ± 4.4	20.6 ± 6.3	29.3 ± 6.7
3	F_0 3	1	68.6 ± 4.1	36.0 ± 5.4	72.2 ± 3.6	62.9 ± 5.7	32.1 ± 5.8	47.7 ± 6.6
4	HPS	2	57.3 ± 1.4	45.1 ± 0.5	56.6 ± 3.0	54.3 ± 1.2	30.4 ± 4.0	41.5 ± 3.7
5	F_0 3	2	57.9 ± 1.5	42.7 ± 1.2	63.8 ± 3.9	56.0 ± 4.5	32.4 ± 4.9	44.9 ± 6.4

Table 4-5. Effect of treatment and storage on colour for F_0 3 and HPS treated carrots. *Note*: quoted ± figure represents one standard deviation from the mean

Values of *a** for canned and HPS treated samples were not significantly different at month zero in either sample batch (p>0.05). After 7 months of storage, *a** values had not changed

significantly in canned samples from either carrot batch but had decreased significantly in HPS samples from both carrot batches ($p<0.05$). For example, a* in HPS treated samples from batch one decreased from 42.0 at month 0 to 20.6 after 7 months storage and a* in HPS treated samples from batch two decreased from 45.1 at month 0 to 30.4 at month 7. This reduction in *a** indicates that the HPS samples were less red after storage. After 7 months of storage, the *a** values from carrot batch one for retorted carrots (a* = 32.1) and HPS (a* = 20.6) treated samples were significantly different from one another ($p<0.05$) (being lower in HPS treated samples) however they were not significantly different in carrot batch 2 ($p>0.05$) where the a* value of retorted carrots was 32.4 compared with 30.4 in the HPS treated samples.

Values of *b** for canned and HPS treated samples were not significantly different from one another at month zero in both batch samples ($p>0.05$). After 7 months of storage, *b** values had changed significantly ($p<0.05$) in both canned and HPS treated samples, *b** decreasing on storage in all cases; this indicates that all samples were less yellow than at month 0. For example, for carrot batch 1, b* values from retorted carrots reduced from 72.2 to 47.7 over storage and b* values from HPS treated carrots reduced from 67.3 to 29.3. After 7 months of storage, *b** values of canned and HPS treated samples were significantly different from one another in carrot batch 1 (being much lower in HPS treated samples) but were not significantly different in carrot batch 2.

The colour change on storage in HPS treated samples from batch 1 was dramatic, the carrots essentially being bleached. Figure 4-17 and Figure 4-18 show the significant change in appearance of HPS treated carrots from batch 1 after 7 months of storage.

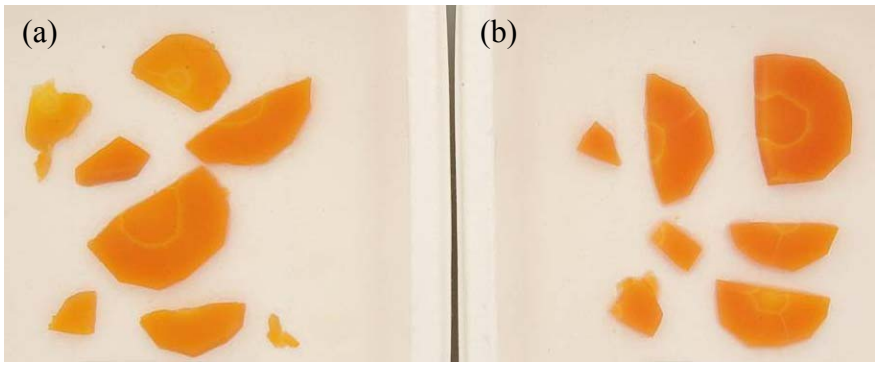

Figure 4-17 (a) - (b). Visual appearance of carrot batch 1 samples a) retorted (F_0 of 3 mins) and b) HPS treated samples, immediately after processing. Mean LAB values were a) 68.6, 36.0, 72.2 and b) 65.7, 42.0, 67.3

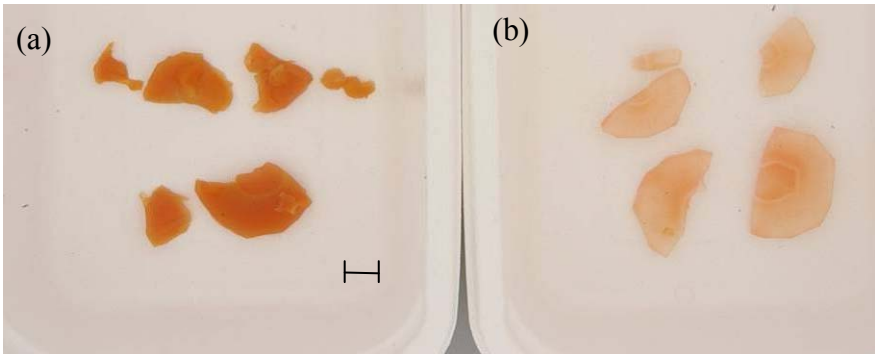

Figure 4-18. (a) - (b). Visual appearance of carrot batch 1 samples a) retorted (F_0 of 3 mins) and b) HPS treated samples, after 7 months ambient storage. Mean LAB values were a) 62.9, 32.1, 47.7 and b) 79.8, 20.6, 29.3. Note the bleaching of HPS treated carrots

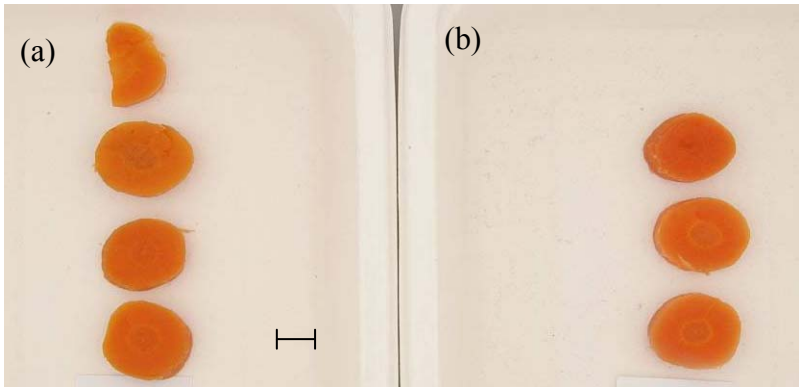

Figure 4-19 (a) - (b). Visual appearance of carrot batch 2 samples a) retorted (F_0 of 3 mins) and b) HPS treated samples, immediately after processing. Mean LAB values were a) 57.9, 42.7, 63.8 and b) 57.3, 45.1, 56.6

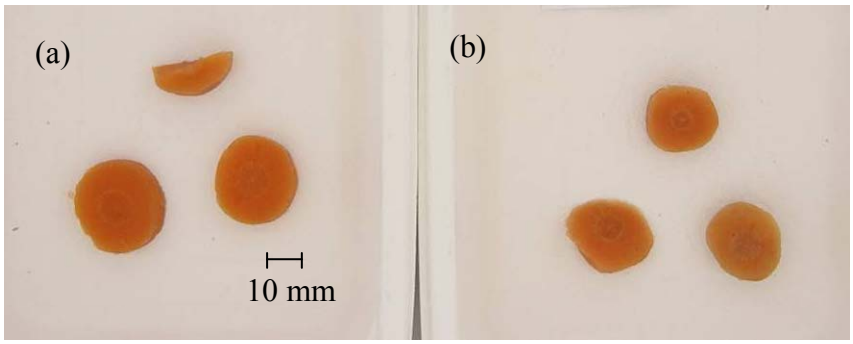

Figure 4-20 (a) – (b) Visual appearance of carrot batch 2 samples a) retorted (F_0 of 3 mins) and b) HPS treated samples, after 7 months ambient storage. Mean LAB values were a) 56.0, 32.4, 44.9 and b) 54.3, 30.4, 41.5. Note that colour of HPS treated samples remained stable over storage.

It is possible that the bleaching in HPS treated samples from batch 1 was due to residual enzyme activity; the enzyme surviving the milder thermal process applied under HPS conditions. Krebbers *et al*. (2002) noted that enzymes such as lipoxygenase and peroxidase can "induce negative changes in colour and flavour of vegetables" during storage. A blanch prior to HPS treatment could potentially resolve this problem but has not been explored within this set of experiments. Carrot samples were not blanched, because it was not normal

practice to do so in a large UK cannery (personal communication, 2005). Adams (1982) studied the effects of a range of blanching conditions on the subsequent quality of frozen vegetables and concluded that "omitting a hot water blanch completely, gave as high a quality product as did blanching for various times". The thermal stability of Lipoxygenase at atmospheric pressure is dependent upon the enzyme source and the food medium and can range from 40 to 130°C (Ludikhuyze *et al.* 2001). The threshold pressures for inactivation of lipoxygenase have been typically characterised between 400 and 600 MPa (Ludikhuyze *et al.* 2001) but varied depending upon the enzyme source and the substrate in which the enzyme was being characterised. The studies reported by Ludikhuyze *et al*. (Ludikhuyze *et al.* 2001) had not specifically characterised lipoxygenase in carrots.

Batch 1 samples were HPS treated with an initial temperature of 81°C and achieved a peak temperature of 109.7°C; this resulted in an F_0 of only 0.1 minutes. Batch 2 samples were HPS treated with an initial temperature of 85.5°C and reached a peak temperature of 116.2°C with a resulting F_0 of 0.4 minutes. This slight increase in initial temperature (and, perhaps more significantly, the increase in peak temperature attained as a result of pressurisation) was, it would appear, sufficient to stabilise the samples from batch 2.

4.3.3 Effects of processing on bean texture

An example force/distance plot for conventionally heat treated (F_0 =3 minute) and HPS treated beans is shown in Figure 4-21. Note the considerably higher peak force required to cut through the HPS treated beans compared with the conventionally retorted beans (approximately 6 N compared with 3 N in this example). The traces also suggest that the HPS sample had a better retention of structure as indicated by the high peak forces in the 5 to 7.5 mm distance into the sample; in contrast the canned sample had a marked peak of 3 Newtons (5-6 mm into the sample) followed by a rapid drop of in the force required to traverse through the rest of the sample. The HPS sample also had a second peak once the blade had a) cut through the sample surface, b) traversed through the sample and then c) cut through the bottom surface of the bean (around 8 mm into the sample). The canned sample exhibited a similar but much reduced peak (around 1 Newton compared with around 4 Newtons) at around the same distance into the sample.

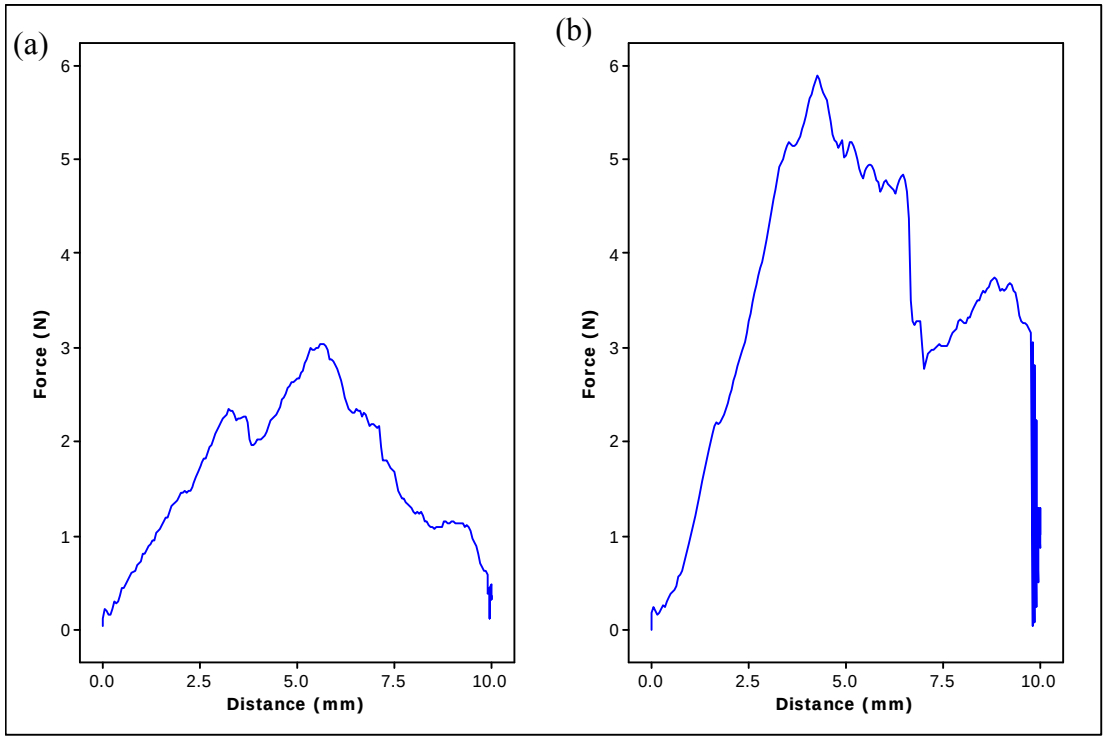

Figure 4-21. Example force/distance plot (a) canned (F_0 = 3 minutes) and (b) HPS treated beans

The effect of processing and storage on the texture of green beans across all batches is summarised in Figure 4-22 (a) and (b).

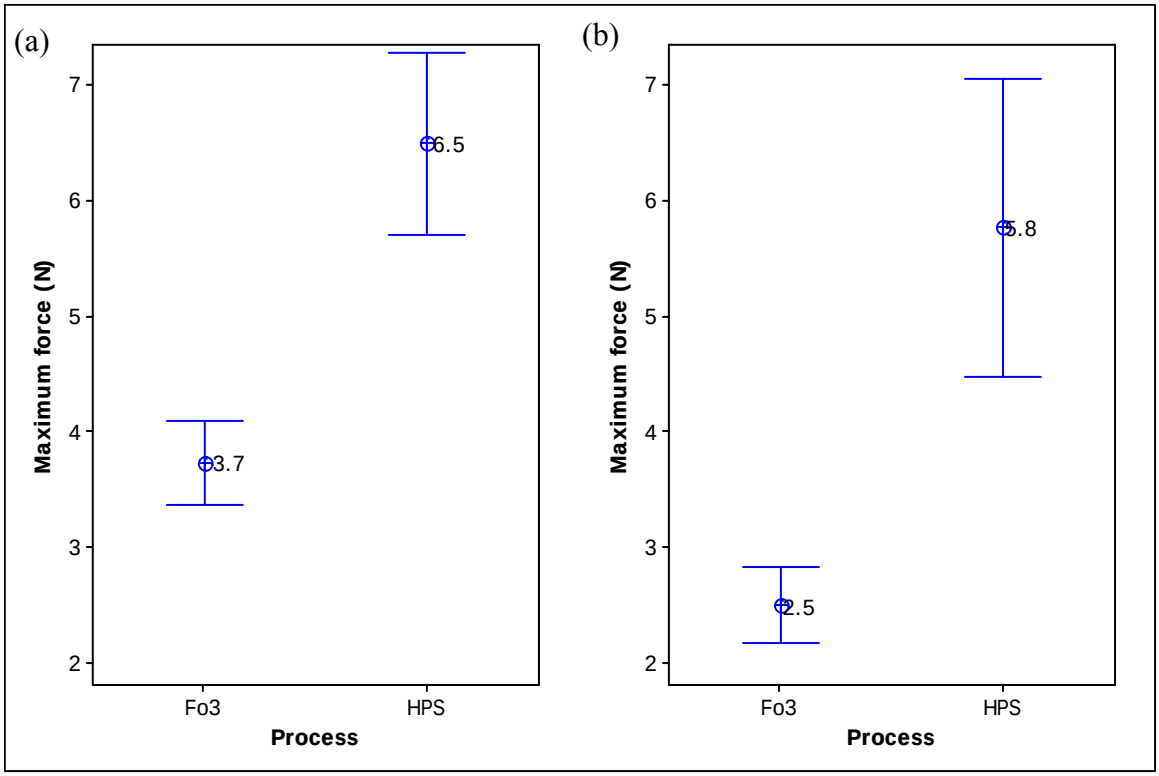

Figure 4-22 (a)-(b). Mean peak force required to cut retorted beans (F_0 = 3 minutes) or HPS treated beans after (a) 0 months of storage (b) 7 months of storage. Error bars denote 95% confidence interval for estimate of the population mean

HPS samples were significantly more firm than canned samples, both immediately after processing and after 7 months of storage ($P<0.05$). There was some softening of the samples over the storage period but, across bean batches, the pressure treated samples were approximately twice as firm as F_0 3 treated samples, even after storage. This is encouraging because it is relatively simple to improve the texture of food samples immediately after processing (for example by applying a minimal thermal process) but it is difficult to maintain these initial quality improvements over an extended ambient shelf life because quality deterioration such as significant softening can occur. An analysis of variance demonstrated

that bean batch, process within bean batch (i.e. F_0 3 and HPS) and storage period (within bean batch and process type) all significantly affected peak force required to cut the samples ($p<0.05$ in all cases).

The effect of increasing the severity of thermal processing on peak forces required to cut through the bean samples is shown in Table 4-6 for a single bean batch ("bean batch 4").

Processing regime	Mean peak force to cut sample	S.D	N
HPS	5.37^{a}	1.97	36
$F_0 = 1$	4.75^{a}	1.79	83
$F_0 = 2$	4.60^{ab}	1.52	37
$F_0 = 3$	3.63^{b}	0.97	29

Table 4-6. Influence of processing regime (retorting to F_0 1,2 or 3 minutes or HPS) on mean peak force required to cut bean samples.Results are for bean batch 4 only. *Note*: samples sharing the same superscripted letter were not significantly different ($p>0.05$) from one another.

Raw beans, blanched beans and pre-heated-only samples had mean peak forces of 29.5, 26.2 and 25.8 N respectively, so substantial softening occurred in all processed samples relative to raw, blanched and pre-treated beans. Although there was an apparent trend for peak forces to decrease as thermal processing severity increased, there was no statistically significant evidence to show that HPS treated samples were more firm than F_0 1 or F_0 2 samples ($P>0.05$). This suggested that the significantly improved firmness of the beans relative to F_0 3 samples ($P<0.05$) was primarily related to the reduced thermal process applied, rather than

any firming action of the pressure itself. The influence of temperature and pressure on bean texture is explored more fully in chapter 5.

In the study of Krebbers *et al*. (2002) up to 60% of the original firmness of the raw sample was retained post process compared with only 3% retained after conventional sterilisation. This is a much greater level of texture retention than was found in our studies. However, as has been previously explained, the peak sample temperature attained in the Krebbers study was only around 100°C compared with 117°C in our studies and the softening process is thought to be 'temperature dependent β-elimination of pectin' (Krebbers *et al*. 2002).

When raw, retorted (F_0 of 2 minutes) and HPS treated samples were sectioned and stained using ruthenium red (to stain pectin) there were clear differences between samples (Figure 4-23 to Figure 4-25). When sectioning HPS samples (Figure 4-25) it was noted that cuticles under the epidermal layer had a tendency to shear off and seemed more brittle. Staining of the cell wall in the collenchyma layer (underneath the epidermal layer) also suggested that the cell walls of pressure treated samples appeared more swollen (contrast Figure 4-24 with Figure 4-25). This swelling of the collenchymas layer could potentially be a contributing factor to the observed increase in firmness. The staining of HPS treated samples also appeared to be much more dense compared with canned samples which could be due to physical changes to the pectin, again potentially contributing to the observed increase in firmness.

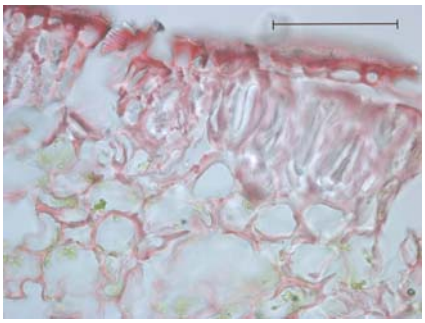

Figure 4-23. Cross section of pod from raw bean sample stained with ruthenium red (x 40 objective). Scale indication is 100 µm

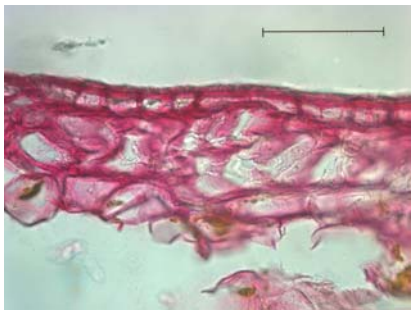

Figure 4-24. Cross section of pod from F_0 2 treated bean sample stained with ruthenium red (x 40 objective). Scale indication is 100 µm

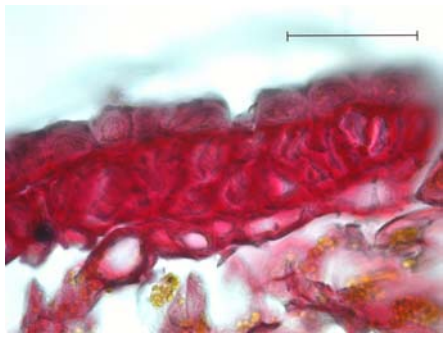

Figure 4-25. Cross section of pod from HPS treated bean sample stained with ruthenium red (x 40 objective). Scale indication is 100 µm. Note the extensive staining and swelling of cells in the collenchyma layer (underneath the epidermal layer) compared with the raw bean and $F_0$2 treated sample.

4.3.3.1 Effect of processing on carrot texture

The effect of processing regime on carrot texture is reported in Figure 4-26.

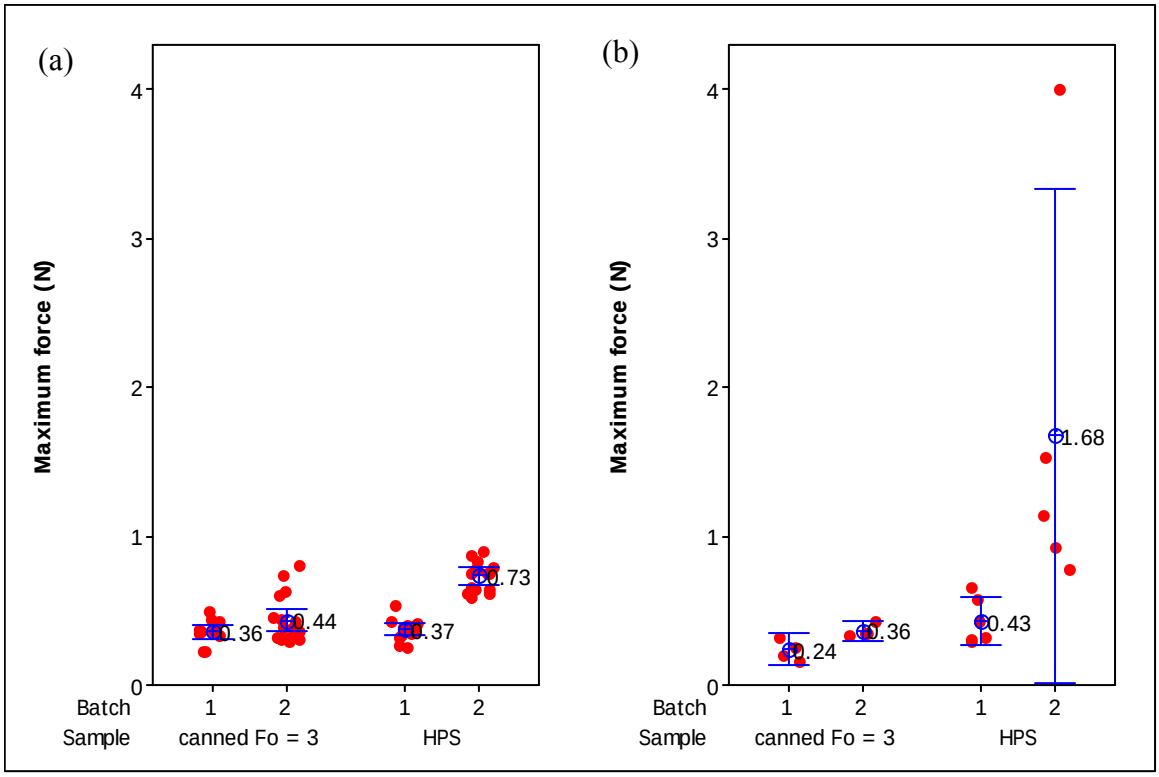

Figure 4-26. Mean peak force required to cut retorted carrots (F_0 = 3 minutes) or HPS treated beans after (a) 0 months of storage (b) 7 months of storage. Data presented is for 2 carrot batches (batches 1 and 2). Error bars denote 95% confidence interval for estimate of the population mean. Individual measurements have also been added to better illustrate the differences in texture between processes; because of the small number of replicates, the confidence intervals on the data for HPS batch 2 after 7 months are very large.

For batch 1, there were no significant differences ($P<0.05$) between the texture of HPS and retorted samples (F_0 of 3 minutes) immediately after processing, but after 7 months of storage HPS samples were significantly more firm ($p<0.05$) than the retorted samples (peak force of 0.36 N compared with 0.24N for retorted products). HPS treated samples appeared to retain

their texture on storage, whereas canned samples appeared to soften. In reality, the observed softening of the canned carrots was not statistically significant ($P>0.05$). For batch 2, HPS treated carrots were significantly more firm than the retorted samples, both immediately after processing and after 7 months of storage ($p<0.05$ and $p=0.093$ respectively). The wide spread on the 95% confidence interval for the mean texture of HPS treated carrots after storage was primarily due to a single outlier that was very firm; this skewed the results. This outlier was included in the data because, after reviewing, there was no obvious reason to believe that it was not a genuine measurement.

The experiments on carrot samples were not carried out in the same level of detail as was the case for the green bean experiments. The results were based on very small sample sizes and should therefore be treated as indicative only. Nevertheless, these results are also encouraging in that they suggest that high pressure sterilised vegetable products could have improved texture retention compared with conventionally retorted products. It should also be noted that these trials compared HPS with products retorted to a total F_0 of 3 minutes. Personal experience with the canning industry would suggest that few processors are processing to an F_0 at the *end of heating* of 3 minutes and frequently process to well beyond this target as both a safety margin and to control thermophilic spoilage. Personal experience with the canning industry would suggest that F_0 of around 6 minutes at the *end of heating* is fairly typical industrial practice. This suggests that the texture improvements seen with HPS could be even more significant when compared with commercially available products. However, commercial canneries could also be using intervention techniques such as the

addition of calcium chloride as a firming agent. It is not known whether such intervention techniques, when applied to HPS treated samples, would yield any further improvements in texture.

4.3.4 Microbiological measurements

HPS and canned (F_0 3) samples from bean batches 1, 2 and 3 and both carrot batches were enumerated for total aerobic and anaerobic plate counts and total aerobic and anaerobic spore counts after 7 months of storage. All samples except a single F_0 3 bean sample (which demonstrated post-process leaker spoilage) had no detectable counts (<10 cfu/g).

Whilst the absence of detectable counts after processing cannot assure safety from a process establishment perspective (challenge testing with pathogens or a suitable indicator organism would be required to demonstrate a known level of microbial reduction) the data does at least support the idea that pressure in combination with heat could potentially be used to commercially manufacture ambient stable low acid foods.

4.4 Summary of chapter, key conclusions and areas for further discussion within the thesis

As discussed in Chapter 2, there is a growing body of evidence to show that high pressure can be used in combination with heat to inactivate bacterial spores. There is however, limited independent data to demonstrate whether there are actually tangible quality benefits that would make it worthwhile for manufacturers to use the process commercially. This chapter has clearly demonstrated improvements in texture retention in the case of green beans when

using HPS compared with a conventional thermal process of up to F_0 3 minutes, but the case is less compelling for carrot samples. As mentioned previously, few commercial canneries would be operating at F_0 3 minutes, so softening of vegetables is likely to be even greater in a commercial context. However, commercial canneries could also be using intervention techniques such as the addition of calcium chloride as a firming agent.

Data from our studies on green beans would suggest that texture improvements appear largely due to the reduced thermal process applied in HPS treatments compared with conventional canning. It seems likely that greater levels of texture retention could be achieved if peak temperatures during the cycle could be reduced. This could potentially be achieved with the use of higher pressures than are currently commercially achievable as suggested by the results of Krebbers *et al*. (2002). What is less certain at the present time, is what combinations of pressure and initial temperature can yield true commercial sterility.

In the case of green beans, the colour of HPS treated samples was not an improvement over traditionally canned samples and in some respects was less desirable as the HPS samples were darker. HPS carrot samples were initially a deeper orange compared with F_0 3 samples but the bleaching effect observed on storage suggests that either pre-blanching or assuring peak temperatures during HPS of greater than 109.7°C may be required to adequately control spoilage enzymes. As was noted for texture, it may be possible to improve colour retention with the use of higher pressures and reduced peak temperatures, giving due consideration to enzyme inactivation.

With respect to the efficacy of HPS for microbiological inactivation, this chapter provides supporting results but was in no way intended to be conclusive. Validating the microbiological safety of pressure sterilised samples is complex (see Chapter 2). For process establishment, a detailed understanding of temperature distribution within a commercial scale high pressure vessel is also required and it is very difficult to map temperatures with a meaningful number of thermocouples at such high operating pressures as was demonstrated in Chapter 2.

Chapter 5 builds on the work of Chapter 4 by exploring a range of pressures, temperatures and hold times to assess the impact on colour, texture. In addition, attempts were made to explore the effects of pressure assisted thermal sterilisation on chlorophylls. In particular, Chapter 5 provides insights in to the relative importance of controlling pressure, temperature and time as a means of retaining (or otherwise) key product quality parameters.

5 Chapter 5 – Use of surface response methodology to explore the effects of time, temperature and pressure on product quality

5.1 Introduction

The primary aim of the work described in chapter 5 was to explore the effects of *a range* of pressures, temperatures and times on key product quality parameters (primarily colour and texture) in processed green beans. Quality effects resulting from such a wide range of process conditions have not been previously reported in the public domain for real vegetable products. A secondary objective from the work was to compare and contrast the temperature distribution profiles obtained at each process condition.

Green beans were packed into retortable pouches (see section 4.2)and then subjected to a range of pressure/temperature/time combinations. Pressures ranged between 298 to 700 MPa, initial temperatures ranged between 52.4°C to 90°C and hold times varied between 0 minutes (in which the vessel was de-compressed as soon as it had reached the target pressure) and 6.6 minutes. Nineteen process conditions were explored in total. After processing, the colour, texture and chlorophyll levels were measured in the processed beans and compared with those of control samples. A quadratic response surface regression was attempted to try to develop a predictive model for each of the measured response variables i.e. in order to predict what texture and colour might result from a given combination of pressure, temperature and time. These models were used to estimate the best conditions for optimum retention of colour and texture. The bench-mark product for this optimisation exercise was blanched beans i.e. the

best conditions were considered to be those that resulted in product quality closest to that of blanched beans.

Theoretical ‘optimised’ conditions to give product quality close to that of blanched beans were identified; conditions in excess of the ‘optimised’ conditions would be likely to result in greater texture losses and colour changes. Detrimental colour changes at elevated temperature and pressure were demonstrated in Chapter 4 using a single pressure/temperature/time combination that was compared to the results obtained using a range of conventional thermal process severities.

In chapter 4, texture retention was shown to be improved by a HPS process relative to traditional thermal processing and the data suggested that texture changes were almost exclusively related to temperature effects. Chapter 5 provides deeper insights into the effects of pressure, temperature and time on the retention (or otherwise) on the quality of green beans and supports the idea that texture changes in beans at high pressure sterilisation conditions are predominantly due to temperature effects.

5.2 Materials and methods

5.2.1 Preparation of samples for processing

Beans were sourced and prepared as described in the materials and methods section of chapter 4 (see page 141). All beans used in the study were from a single source and date of purchase. The prepared beans were subjected to a range of time, temperature and pressure conditions within the range of 52.4-90˚C, 298-700 MPa and 0 to 6.6 minutes. Samples having a hold time of 0 minutes were brought up to the target hold pressure and the pressure was immediately released. Full details of the experimental design can be found in section 5.2.6.

5.2.2 High pressure sterilisation experiments

Studies were carried out in the EPSI laboratory scale high pressure unit; process control and thermocouple calibration were as described in section 3.2. Probe location, temperature monitoring and pre-process temperature equilibration were as described in section 4.2.3. A typical pressure-time-temperature profile from the experiments is given in Figure 5-1**.** In contrast to the process cycle used in Chapter 4, a single pressure pulse was used rather than a double pulse. This was done mainly to reduce experimental variability that could occur in the equilibration step between pulses using the 2 pulse approach. On completion of the cycle, the samples were removed from the vessel and plunged into chilled water. As can be seen in Figure 5-1, the application of pressure resulted in a rapid rise in product temperature but heat losses occurred over the holding period as described in section 3.3.2.1. One means of reducing heat losses further would be to set the temperature of the vessel wall at the peak

temperature attained by the sample. The experimental vessel had a maximum wall temperature of 90°C so this was not possible for runs were peak temperatures were in excess of 90°C. For consistency, the vessel wall temperature was therefore always set close to the starting temperature of the sample.

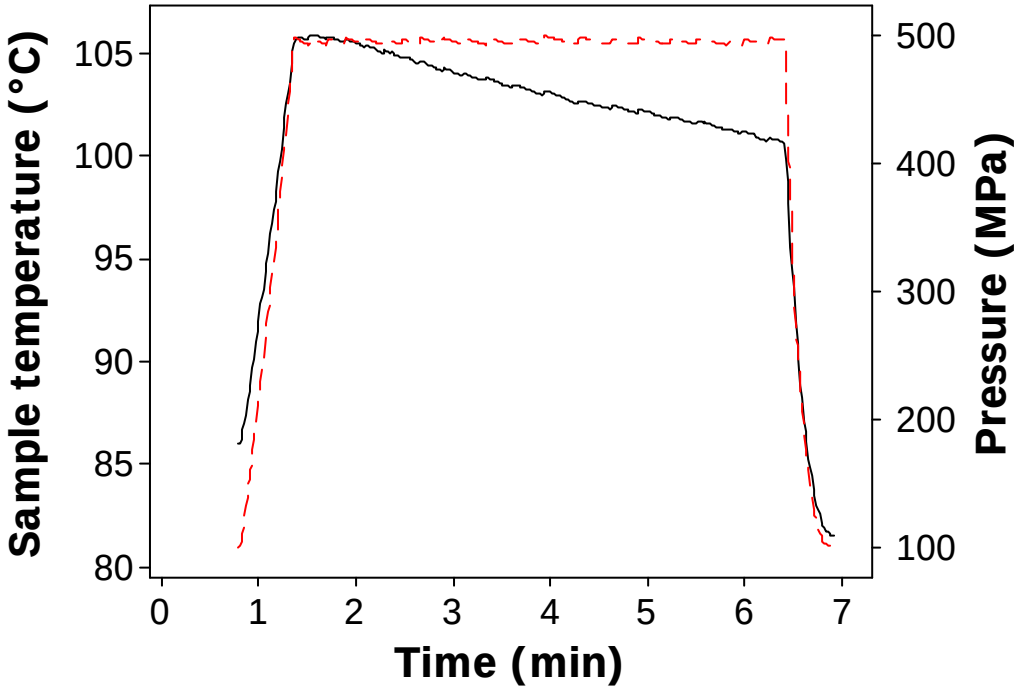

Figure 5-1: Typical time-temperature-pressure cycle utilised in surface response experiments. .....: vessel pressure,___Product temperature.

5.2.3 Colour measurement

Before and after processing, colour measurements were made using a 'DigiEye' digital imaging system (DigiEye plc, Leicester, UK) as described in section 4.2.4. Mean measurements were reported for *L**, *a**, and *b**, *C** and *ΔE* for each set of beans from a processing run. Chroma *C** (a transformation of the a* and b* measurements) was calculated from Equation 5.1 (Whitworth 2006). Similarly, *ΔE* (a measure of total colour change relative to a reference standard) was derived using Equations 5.2 to 5.5. The reference standard used was the *L*, a*, b** values obtained for raw untreated beans.

$$C^* = \sqrt{a^{*2} + b^{*2}} \qquad (5\text{-}1)$$

$$\Delta E = \sqrt{[(\Delta L *)^2 + (\Delta a *)^2 + (\Delta b *)^2]} \qquad (5\text{-}2)$$

Where:

$$\Delta \boldsymbol{L}^* = \boldsymbol{L}^*_{\ \boldsymbol{control}} - \boldsymbol{L}^*_{\ \boldsymbol{treated}} \qquad (5\text{-}3)$$

and

$$\Delta \boldsymbol{a} = \boldsymbol{a}^*_{\ \boldsymbol{control}} - \boldsymbol{a}^*_{\ \boldsymbol{treated}} \qquad (5\text{-}4)$$

and

$$\Delta \boldsymbol{b}^* = \boldsymbol{b}^*_{\ \boldsymbol{control}} - \boldsymbol{b}_{\boldsymbol{treated}} \qquad (5\text{-}5)$$

5.2.4 Texture measurement

Before and after processing, bean texture was determined by slicing force as described in section 4.2.5.1.

5.2.5 Determination of chlorophyll

Separation of *chlorophyll-a* and *chlorophyll-b* was achieved after processing by using reversed phase HPLC with gradient elution techniques and detection in the visible region (Langer 1976;Matus *et al.* 1981;Ohmacht 1979). Samples were extracted in ice-cold acetone and filtered prior to analysis. Gradient elution consisted of a 25 cm x 4.6 mm CS SIL C18 column and a linear gradient programme running from 100% solvent A to 100% solvent B over 20 minutes at a flow rate of 1 ml per minute. Solvent A was 75% methanol/0.001M phosphate buffer (pH 7), solvent B was ethyl acetate and detection was at 430 nm. The purpose of determining chlorophyll levels was to see if they could be related to the visible changes in product colour that were expected as a result of the knowledge gained in Chapter 4.

5.2.6 Experimental design and statistical analysis

The experimental design was based upon a central composite surface response design created using MINITAB version 14.0 which established non-correlated target conditions of pressure, pressure and hold-time. After completion of the experiments, the target values were substituted for those actually achieved during each process. The final experimental design space spanned temperatures between 52.4˚ and 90˚C, pressures between 298 and 700 MPa and hold times between 0 and 6.6 minutes. This design was selected in order to ensure that a wide range of processing conditions were explored and also so that the resulting data could potentially be used to construct predictive models.

There was no significant degree of correlation between the factors (the pressure-temperature correlation was 0.39, the time-temperature correlation was 0.93 and the time-pressure correlation was 0.49) indicating that the integrity of the original design was maintained and no confounding factors were introduced. There were 5 replicates of the centre point of the design (75°C, 550 MPa, 2.5 minute hold time). In total 19 experiments were carried out. The full list of experimental conditions is shown in Table 5-1. An analysis of variance was carried out on the resultant data. Residual errors deviated from normality for some responses (L*, a*, b*, C*, and ΔE) so associated 'p' values for the various regression terms are not quoted. These 'p' values were not required for the interpretation of the data as presented.

Run number	Initial temperature (°C)	Pressure (MPa)	Time (min)
1	61.6	400	5
2	61.3	400	0
3	87.7	700	0
4	74.8	550	2.5
5	82.1	550	2.5
6	90.0	400	0
7	72.5	700	2.5
8	73.6	550	2.5
9	73.4	550	6.6
10	52.4	550	2.5
11	69.9	550	2.5
12	73.3	298	2.5
13	59.6	700	0
14	61.4	700	5
15	72.7	550	0
16	85.9	400	5
17	88.0	700	5
18	75.0	550	2.5
19	73.8	550	2.5

Table 5-1. Experimental conditions used in the central composite surface response design

Spearman's rank correlation coefficients and their associated probabilities were calculated to check for correlations between: the colour measurement parameters L*, a* and b*, C*, ΔE; chlorophyll levels; and levels of two unidentified breakdown peaks identified in from the HPLC analysis termed products X and Y. This test was carried out using the statistical software package 'R' (R Development Core Team 2007). Optimisation of colour and texture was conducted using the response optimisation tools available in MINITAB version 15.0. The target values for colour and texture optimisation were based on data for blanched beans. Blanched beans were used as a 'idealised' target as it was considered unrealistic to assume that product quality could be close to raw beans given the temperatures and pressures used in the trials.

5.3 Results and discussion

5.3.1 Regression models

Regression models were attempted for *L*, a*, b*, C*,* ΔE, peak force, *Chlorophyll-a*, *Chlorophyll-b* and two unknown HPLC peaks termed Product X and Product Y (see section 5.3.4). It was postulated that products X and Y were pheophytins i.e. the typical breakdown products of chlorophyll as a result of heating (Clydesdale & Francis 1976) but this was not confirmed experimentally. Table 5-2 includes a summary of the R^2 values obtained for each regression model. The sequential sums of squares were used to assess the relative influence of the linear, quadratic and interaction terms in the overall model. The relative influence of the factors in the design (temperature, time and pressure) could then be estimated from this analysis. Note, the larger the number (Table 5-2), the more significant that factor in explaining the observed data. For example, for *a**, 96.7% of the observed variation is explained by the regression model (which includes linear terms, squared terms and interactions. However, 83.5% of the observed variation in the data is explained by linear and quadratic terms (Table 5-2, column 4) suggesting that *interactions* between pressure temperature and time are far less important than the linear and quadratic terms in the model. Using the same analysis it can be seen that the linear term for time explains substantially more of the observed variation in *a** than does temperature or pressure. These data are presented in Table 5-2 and the practical significance of this analysis is discussed in the sections that follow. The coefficients for each regression equation are reported in Table 5-3 and a worked example of use of the coefficients for modelling purposes is shown in section 5.3.3.1.

Factors	Terms			Interaction terms		
	Linear	Quadratic	Overall	Temperature	Pressure	Time
a* - total R^2 = 96.7%						
1	2	3	4	5	6	7
Temperature	11.3	0.9	12.1	*	2.4	8.2
Pressure	4.7	12.6	17.3	*	*	2.6
Time	46.7	7.3	54.1	*	*	*
Overall	62.7	20.7	83.5		13.2	
b* – total R^2 = 85.1%						
Temperature	12.1	7.0	19.1	*	0.0	5.9
Pressure	44.0	0.5	44.5	*	*	0.0
Time	8.0	7.4	15.4	*	*	*
overall	64.1	14.9	79.1		6.0	
C - total R^2 = 81.1%*						
Temperature	19.8	8.0	27.8	*	0.3	4.0
Pressure	42.2	0.0	42.2	*	*	0.3
Time	1.2	5.2	6.4	*	*	*
overall	63.2	13.2	76.5		4.7	
ΔE – total R^2 = 97.2%						
Temperature	7.1	0.2	7.3	*	2.7	9.0
Pressure	8.2	12.4	20.6	*	*	2.9
Time	45.7	8.8	54.6	*	*	*
overall	61.0	21.5	82.5		14.6	
Peak Force – total R^2 = 91.5%						
Temperature	64.3	7.7	72.0	*	0.2	9.3
Pressure	1.3	0.0	1.3	*	*	1.2
Time	0.7	6.8	7.4	*	*	*
overall	66.2	14.6	80.8		10.7	
Chlorophyll a – total R^2 = 78.4%						
Temperature	17.5	0.7	18.2	*	3.0	11.6
Pressure	5.4	1.3	6.7	*	*	2.9
Time	16.9	19.1	36.0	*	*	*
overall	39.7	21.2	60.9		17.5	
Chlorophyll b – total R^2 = 85.8%						
1	2	3	4	5	6	7
Temperature	29.1	0.5	29.7	*	0.5	7.5
Pressure	3.9	1.1	5.0	*	*	3.3
Time	25.3	14.5	39.8	*	*	*
overall	58.3	16.2	74.5		11.3	
Product X – total R^2 = 78.5%						
Temperature	0.2	0.2	0.4	*	6.8	12.3
Pressure	1.9	32.7	34.6	*	*	5.3
Time	17.6	1.6	19.3	*	*	*
overall	19.7	34.5	54.2		24.3	
Product Y – total R^2 = 73.0%						
Temperature	0.2	1.3	1.6	*	0.8	28.6
Pressure	0.2	22.9	23.1	*	*	12.6
Time	4.4	2.1	6.5	*	*	*
overall	4.9	26.3	31.2		41.9	

Table 5-2. Goodness of fit (R^2) for each regression and a break down of the relative influence of each of the design factors in the regression equation.Note each number in the table is a proportion of the total R^2 value.

1	2	3	4	5	6	7	8	9	10
Term	a*	b*	C*	ΔE	Peak force (g)	Chlorophyll a (μ-volts)	Chlorophyll b (μ-volts)	X (μ-volts)	Y (μ-volts)
Constant	-50.64	41.49	29.59	-48.85	-21290	224841.00	6546.00	-228786.00	698454.00
Temperature	0.70	-0.29	-0.1213	0.8545	518.6	-2104.00	678.00	18669.00	2964.00
Pressure	0.11	0.00	0.02672	0.11357	25.67	-322.90	-18.08	-1568.00	-2941.00
Time	3.36	-1.66	-1.024	3.6918	960.3	-29956.00	-4974.00	22851.00	-312.00
Temp*Temp	0.00	0.00	0.001546	-0.00271	-3.385	-0.41	-7.60	-87.47	-49.42
Pressure*Pressure	0.00	0.00	-1.4E-05	-4.2E-05	-0.01265	0.12	0.02	1.92	1.80
Time*Time	0.01	0.10	0.1039	0.00449	71.3	992.50	486.20	1752.00	1661.00
Temperature*Time	-0.02	0.01	0.00952	-0.02485	-11.861	244.54	45.97	-734.50	-683.70
Temperature*Pressure	0.00	0.00	-0.00019	-0.00077	-0.1339	2.25	-0.05	-7.20	11.17
Pressure*Time	0.00	0.00	-0.00081	-0.00327	-1.163	8.84	-4.89	61.34	93.52

Table 5-3. Regression coefficients for each response.

5.3.2 Effect of pressure, temperature and hold time on processed bean colour

Values for *L** could not be fitted (see Figure 5-2) with a sufficient degree of accuracy (having an R^2 of only 57.7%) so further predictive modelling was not undertaken using this parameter. Values of *L** varied very little between processing conditions (mean L* across all runs was 51.2, s.d.=0.9, n=19) and were reasonably close to the *L** value of the raw beans (51.8) though generally a little darker (Figure 5-2).

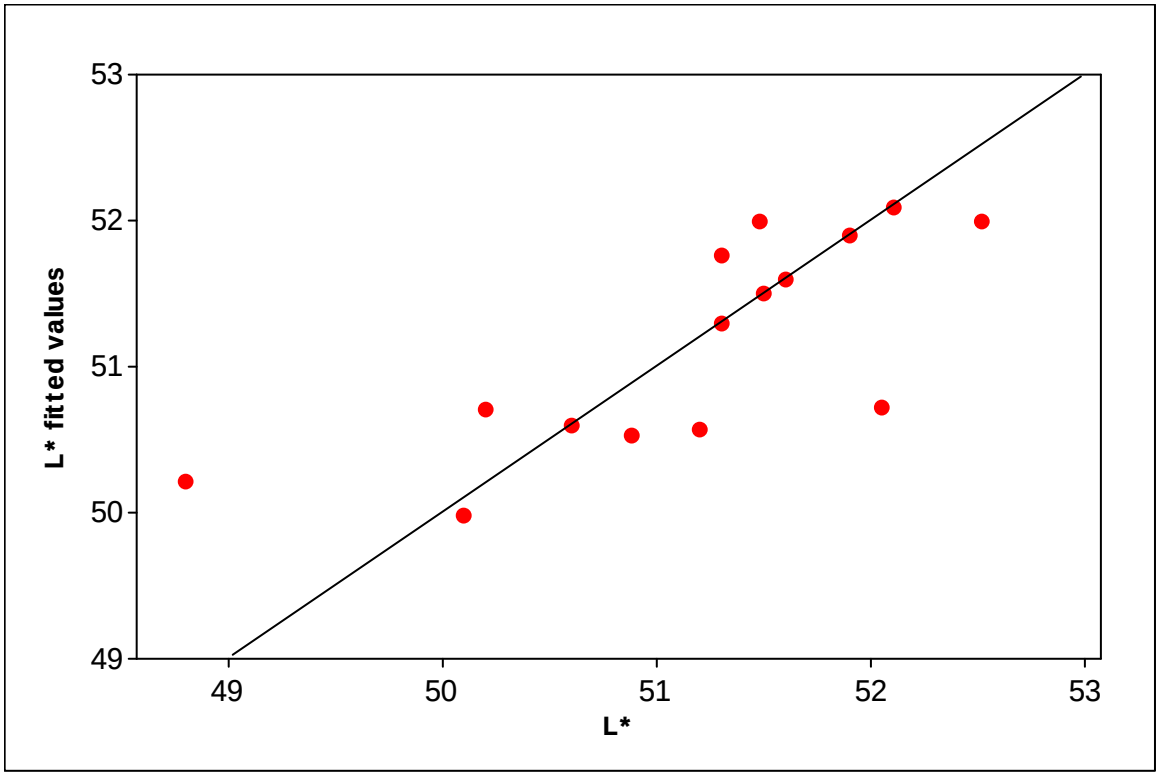

Figure 5-2. Plot of observed values of L* versus surface response, fitted values of L* for a range of temperature, pressure and time processing combinations. The line is not a regression fit but represents an perfect fit between the fitted and measured value i.e. L* fitted = L*.

A good correlation was established between the measured *a** values for each run and the predicted values of the regression equation ($R^2 = 96.7\%$, $R^2_{Adj} = 90.7\%$) i.e. 96.7% of the

observed variation could be explained by the quadratic model. Although there was clearly a good fit between observed and predicted values of a^* the R^2 value was somewhat inflated due to a single data point some way outside the grouping of most of the data set. Even removing this data-point, there was still a good match between observed and predicted values (Figure 5-3).

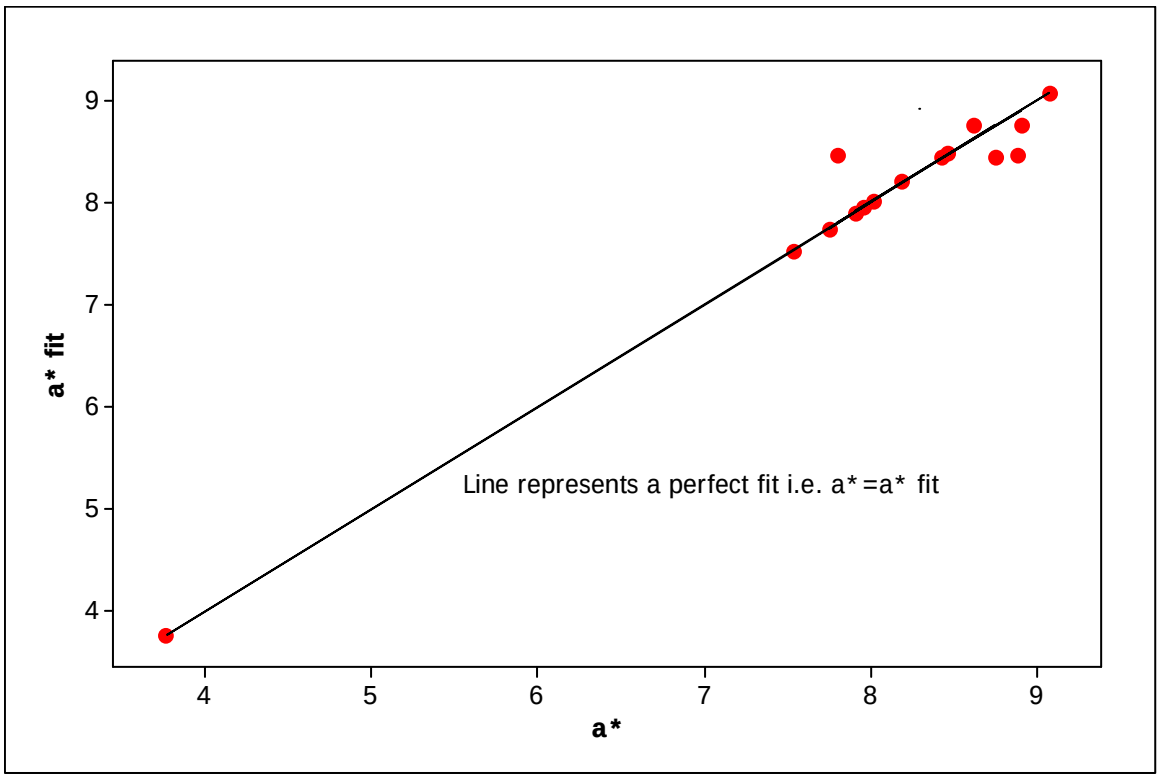

Figure 5-3. Plot of observed values of a* versus surface response, fitted values of a* for a range of temperature, pressure and time processing combinations. Note that even omitting the single point lying some way outside the main dataset (a* of 3.8) there is still a good fit between the observed and predicted values of a*.

Linear and quadratic terms explained a good proportion of the observed variation in the data (R^2 of 54.1%) i.e. a significant proportion of the observed fit of 96.7% indicating that interaction terms were of relatively lower importance. *Pressure*, t*emperature* and *time* all played a role in influencing the observed changes in *a**, but the *hold time* was the most influential predictor of changes in *a** value in the model. Samples (according to *a** measurements) became less green and increasingly red with longer hold times at elevated temperature and pressure. However, in practical terms, the colour variation described by *a** and observed between almost all runs appeared to be quite small. For example, Figure 5-4 shows blanched beans and three HPS processes where the three HPS processes had similar pressures and initial temperatures but had varying lengths of hold time; there appeared to be little practical difference in green/red colouration between the samples.

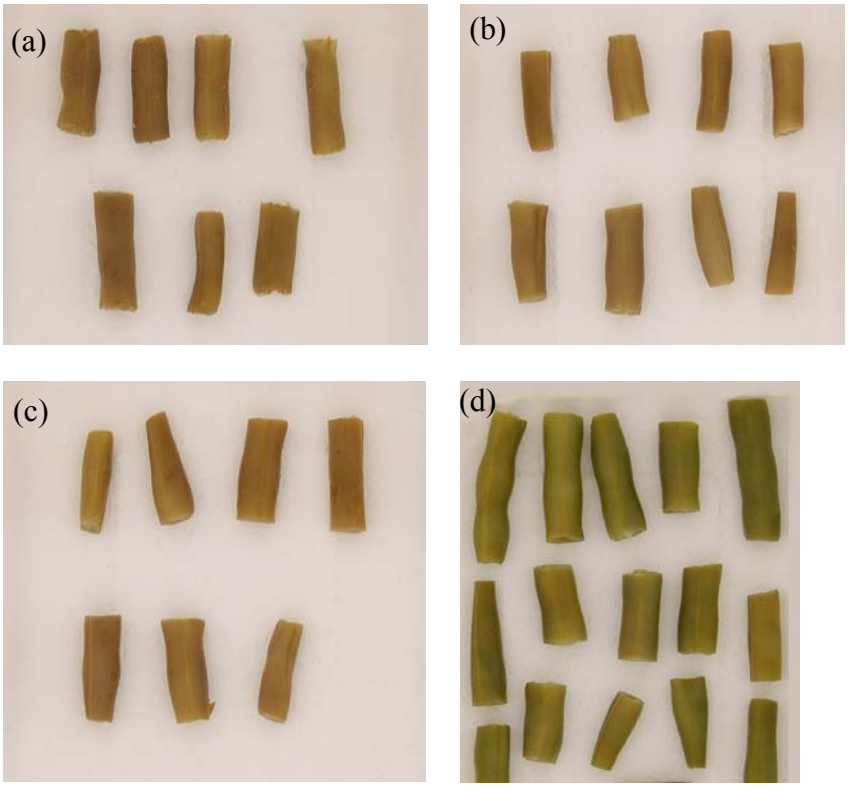

Figure 5-4 (a)-(d) Colour change in green beans as influenced by hold time at a given pressure and temperature (a) 72.7°C Initial temperature, 550 MPa pressure cycle, 0 minutes hold time (b) 73.6°C, 550 MPa, 2.5 minute hold (c) 73.4°C, 550 MPa, 6.6 minute hold (d) blanched beans (74°C for 6 minutes). Note scale bar not included because images have been cropped and re-sized for presentation purposes and scaling information has been lost.

In Chapter 4, only limited changes in a* were found between the different thermal processes applied. Contour plots predicting *a** values resulting from high pressure sterilisation are shown in Figure 5-5. For each pair of factors the value of the third factor is held at the mid-point of the design. An identical approach has been used for all other contour plots presented.

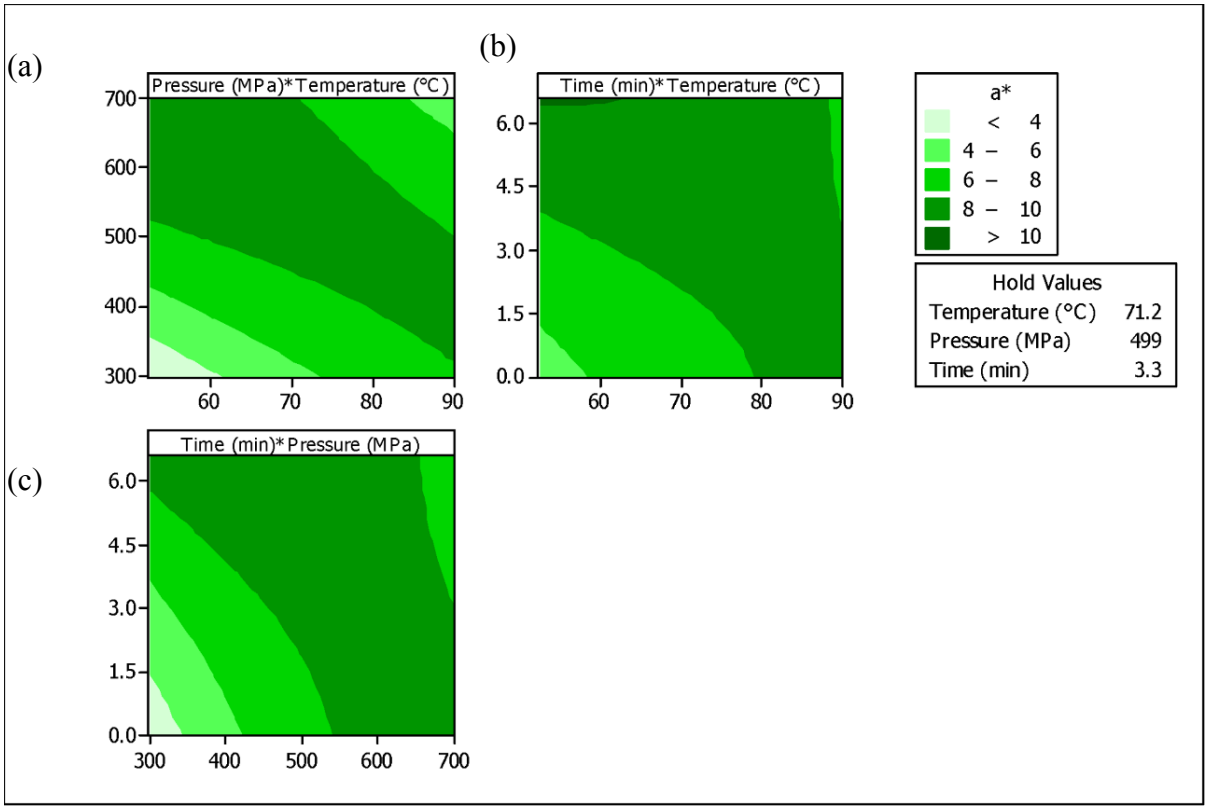

Figure 5-5 a-c. Contour plot for predicted changes in a* with changing pressure, temperature and time. Note that for each plot (a) to (c) the third factor has been set at the mid-point of the design of the experiment. For example (a) plots pressure and temperature variation assuming that time is fixed at 3.3 minutes (see 'hold values' in legend)

The contour plot (Figure 5-5a) suggests that as temperature increases, samples become less green and increasingly red. For example, between 60 and around 72°C, *a** values are between 4 and 6 but as the temperature increases to between 72 and 90°C the *a** value increase to 6-8 indicating decreasing green colouration and increasing red colouration. Similarly, as pressure increases, samples become less green and increasingly red. Figure 5-5 (b) also suggests that samples become less green with increasing temperature and suggest that temperature need to be kept at less than around 80°C with hold times of less than around 4 minutes in order to minimise changes in *a** (and keep *a** values at <8). Figure 5-5 (c) suggest that pressures need to be kept at less than around 550 MPa and hold times at less than 6 minutes in order to minimise changes in *a** (and keep *a** values at <8). The conditions typically cited to achieve commercial sterility using HPS (Meyer *et al.* 2000) are generally likely to be much higher than 80°C and 550 MPa and so undesirable changes in *a** can be expected.

Values for *b** could be predicted reasonably well (R^2 = 85.1%, R^2_{Adj} = 58.1%). *Temperature*, *pressure* and *time* all played a role in the observed changes in *b** but the observed variation was primarily predicted by the influence of *pressure* (explaining 44% of the variation) with hold time and temperature being of lesser importance. The match between observed and predicted values of *b** is shown in Figure 5-6.

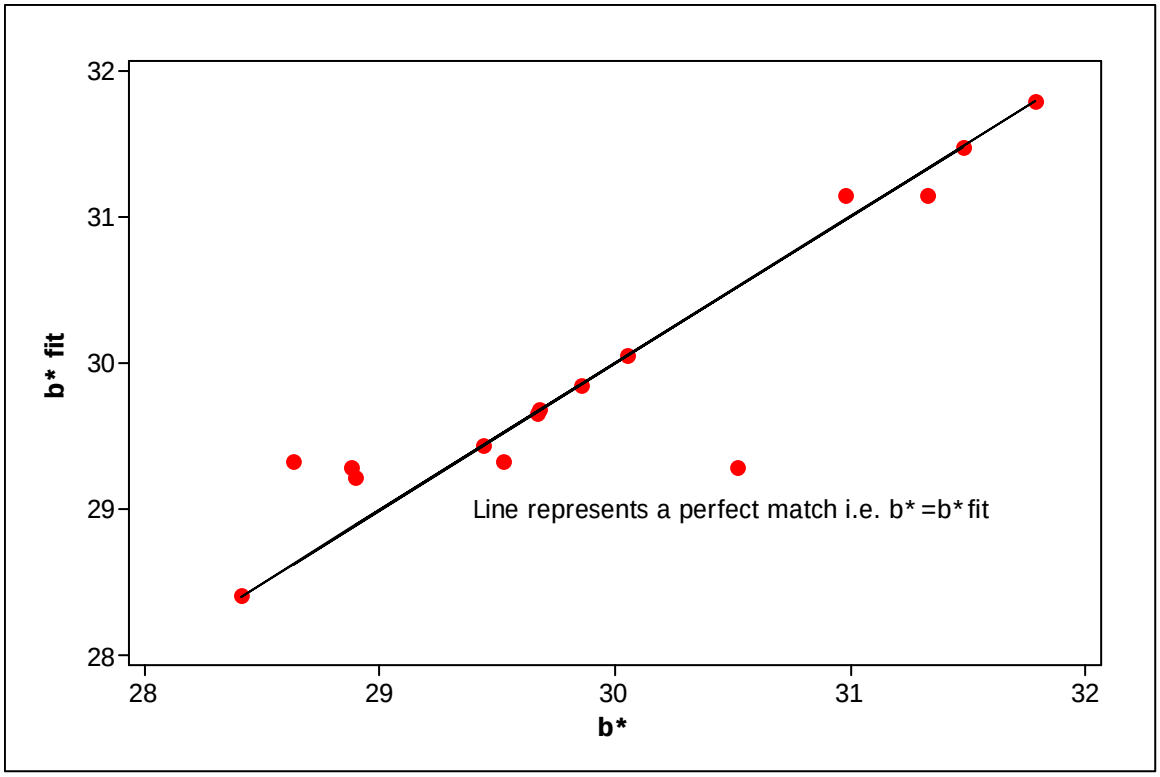

Figure 5-6. Plot of observed values of b* versus surface response, fitted values of b* for a range of temperature, pressure and time processing combinations. Note: the line is not a regression fit but represents an perfect fit between the fitted and measured values i.e. b* fitted = b*.

Contour plots predicting *b** as a function of pressure, temperature and time are shown in Figure 5-7. Increasing pressure to levels above around 550 MPa led to a decrease in *b** i.e. the samples became less yellow and more blue (Figure 5-7 a and c) If the hold time at a given temperature exceeded around 1.5 minutes then there was also a decrease in b* indicating that the samples became more blue and less yellow (Figure 5-7 b).

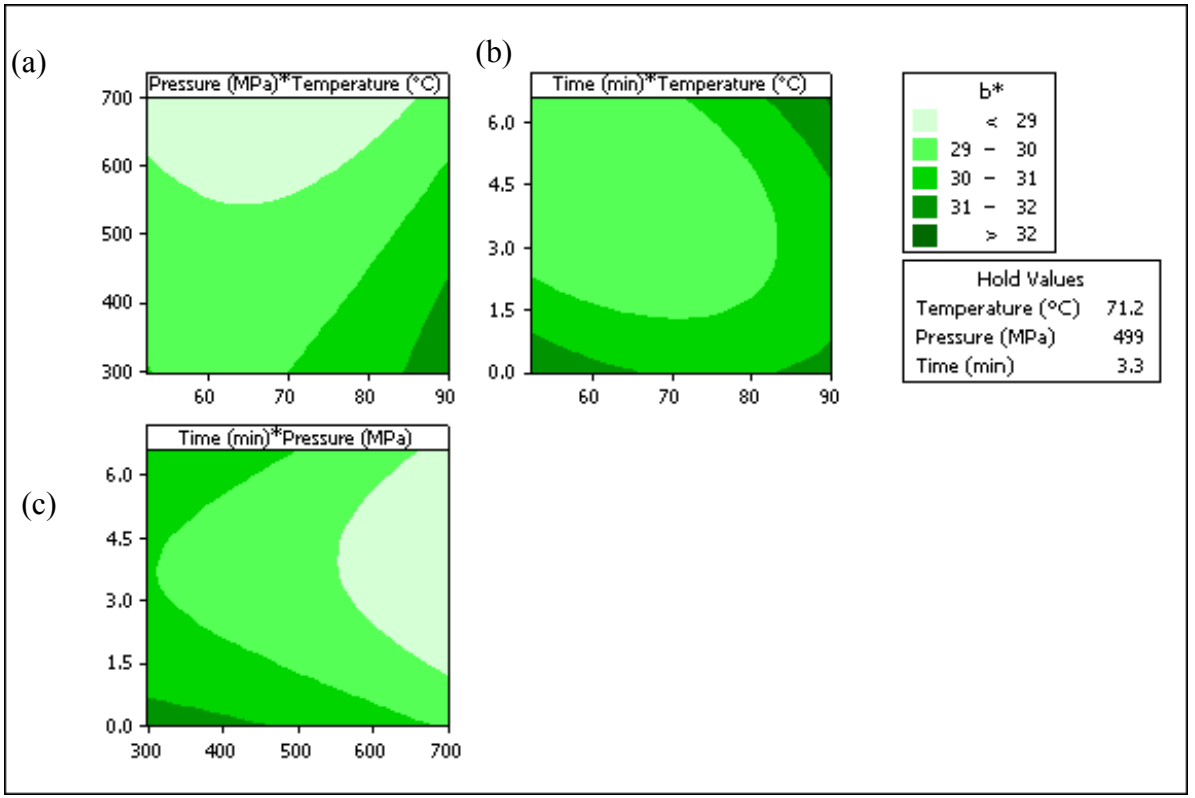

Figure 5-7 a-c. Contour plot for *b as influenced by pressure, temperature and time. Note that for each plot (a) to (c) the third factor has been set at the mid-point of the design of the experiment. For example (a) plots pressure and temperature variation assuming that time is fixed at 3.3 minutes (see 'hold values' in legend)**

Values for Chroma (C^*) could be predicted reasonably well (R^2 = 81.1%, R^2_{Adj} = 47.1%). Linear terms were most important in the model (R^2 = 63%) with pressure being the most important predictor of C^* changes followed by temperature. The match between observed and predicted values of C^* is shown in Figure 5-8. Contour plots predicting C^* as a result of high pressure sterilisation are shown in Figure 5-9.

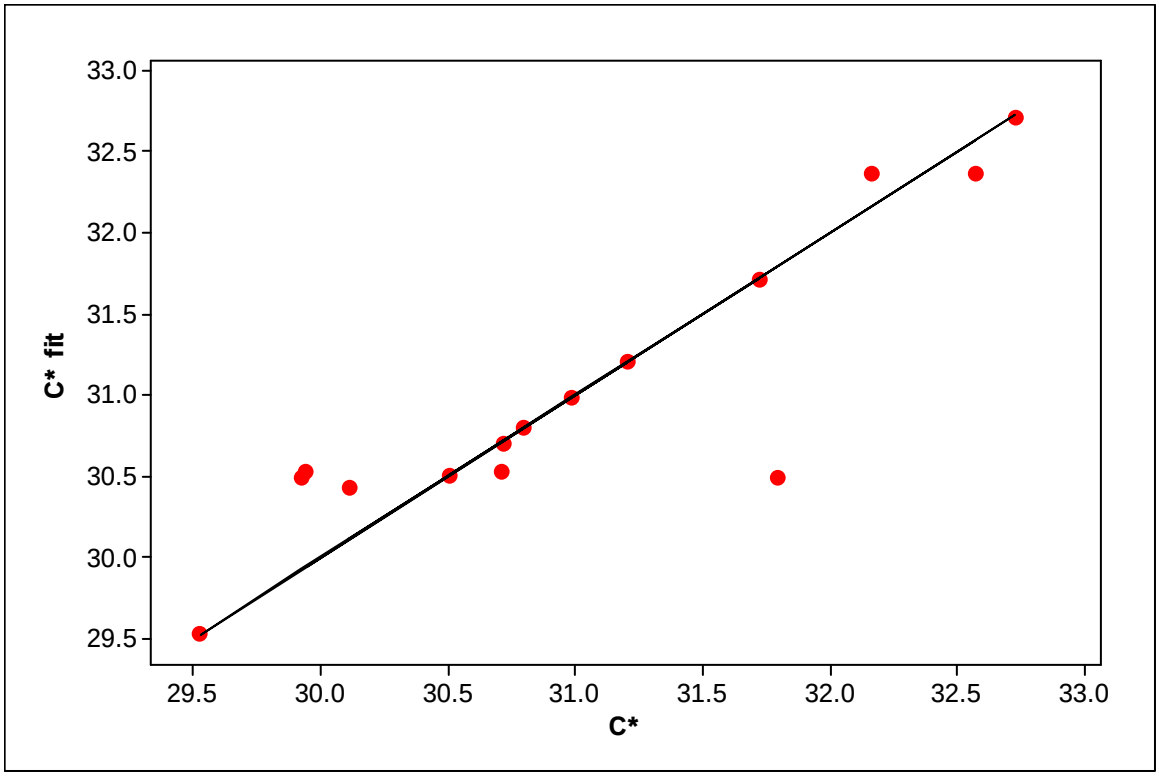

Figure 5-8. Plot of observed values of C* versus surface response, fitted values of C* for a range of temperature, pressure and time processing combinations. The line is not a regression fit but represents an perfect fit between the fitted and measured values i.e. C* fitted = C*.

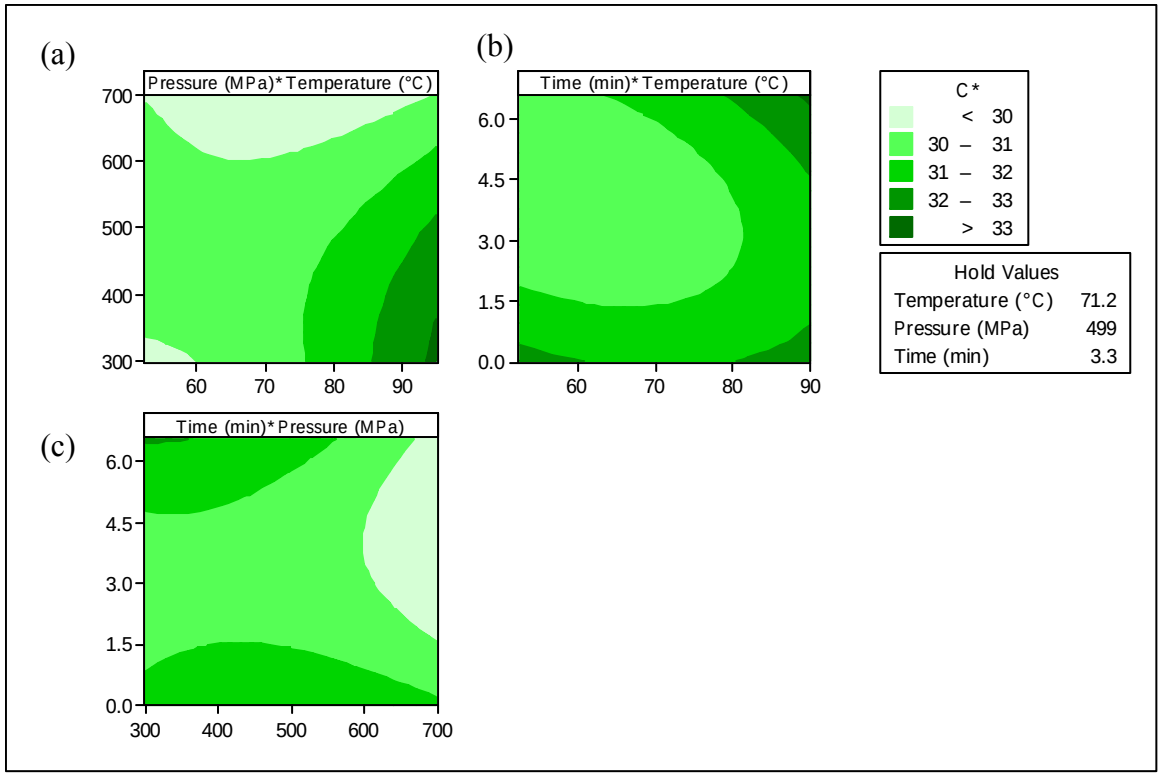

Figure 5-9 a-c. Contour plot for *C as influenced by pressure, temperature and time. Note that for each plot (a) to (c) the third factor has been set at the mid-point of the design of the experiment. For example (a) plots pressure and temperature variation assuming that time is fixed at 3.3 minutes (see 'hold values' in legend)**

Chroma changed very little when temperatures were maintained below around 75° and when pressures were maintained below around 600 MPa (Figure 5-9 a). Above 75°C, Chroma increased indicating that the intensity of colour of the samples increased (Figure 5-9a). However, above 600 MPa Chroma decreased Figure 5-9a). Hold times in excess of 1.5 minutes at elevated temperatures resulted in a reduction in Chroma indicating a reduction in intensity of colour Figure 5-9b). With holds times of between 1.5 and 4.5 minutes and at pressures between 300 and 600 MPa there appeared to be little change induced in Chroma Figure 5-9c).

Total colour change ΔE could again be predicted well (R^2 = 97.1%, $R2_{adj}$ = 92.0%) although as was the case for a^*, the correlation coefficient was inflated due to a single data point located some way from the main data set. A plot of observed versus fitted values for ΔE is shown at Figure 5-10. *Time* at elevated temperature and pressure was the most important determinant of total colour change describing over half of the observed variation. Contour plots predicting ΔE as a result of high pressure sterilisation are shown in Figure 5-12.

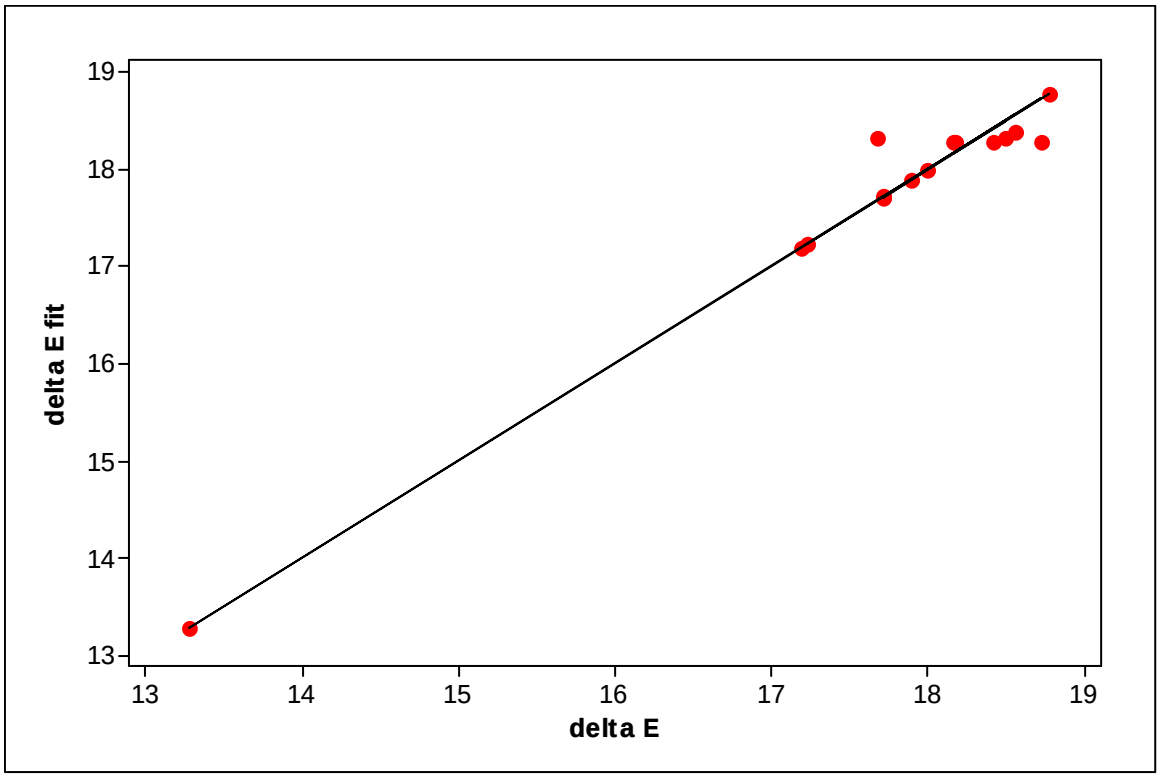

Figure 5-10. Plot of observed values of ΔE versus surface response, fitted values of ΔE for a range of temperature, pressure and time processing combinations. The line is not a regression fit but represents an perfect fit between the fitted and measured values i.e. ΔE fitted = ΔE.

The colour parameter of ΔE in the context of these experiments is perhaps the most useful measure of colour changes in the samples as it relates directly to the colour shift that has occurred *relative* to the blanched bean samples. Since the underlying assumption in this chapter is that the target 'ideal' is produce samples that are as close as possible to blanched beans, samples having the lowest values of ΔE can be considered optimal with respect to colour retention. As can be seen in Figure 5-12 a, values of ΔE increased with both increasing temperature and increasing pressure. It was only at very low temperatures (<60°C)

and pressure (<400 MPa) that changes in ΔE were minimised. The effects of pressure and hold time can be seen in Figure 5-12c; ΔE increased with increasing pressure and with increasing hold time at a given pressure. Only at temperatures less than 60°C and with very short hold times (virtually instantaneous de-compression) were changes in ΔE minimised (Figure 5-12 c). Since even the pre-heat conditions for HPS are likely to exceed 70°C (with currently achievable pressure conditions) it seems unlikely that substantive retention of desirable bright green colours will be achievable in products such as green beans. As discussed previously, samples from most of the conditions tested for HPS appeared to have a very similar appearance in terms of colour (see Figure 5-4 for example). As predicted from Figure 5-12, only runs having very short hold periods, very low initial temperatures and very low hold pressures showed any potential for colour retention (Figure 5-11).

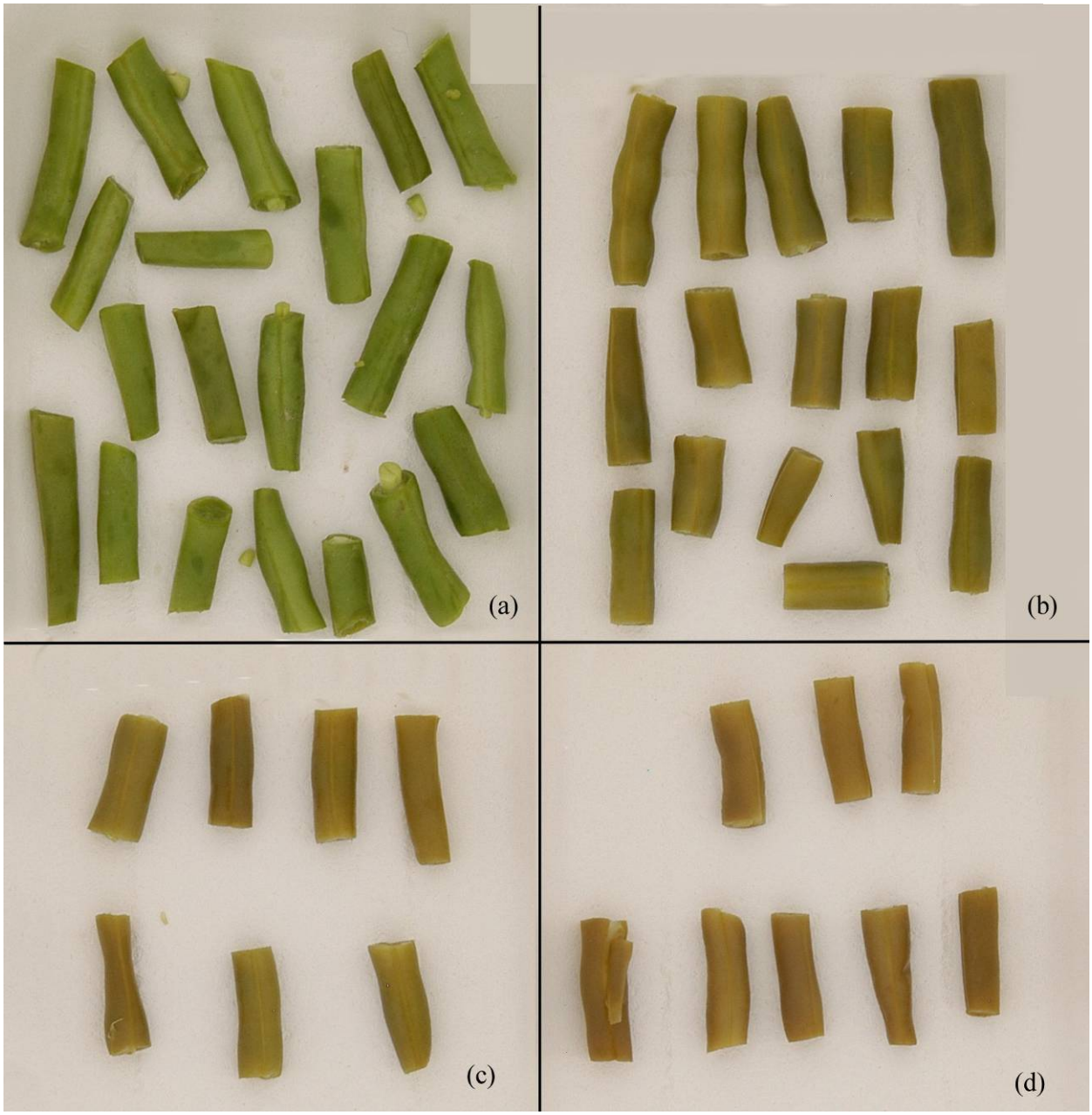

Figure 5-11. Examples of bean appearance after processing: (a) untreated control, (b) blanched only, (c) 61.3°C, 400 MPa, 0 minute hold time, (d) 87.7°C, 700 MPa, 0 minute hold time.

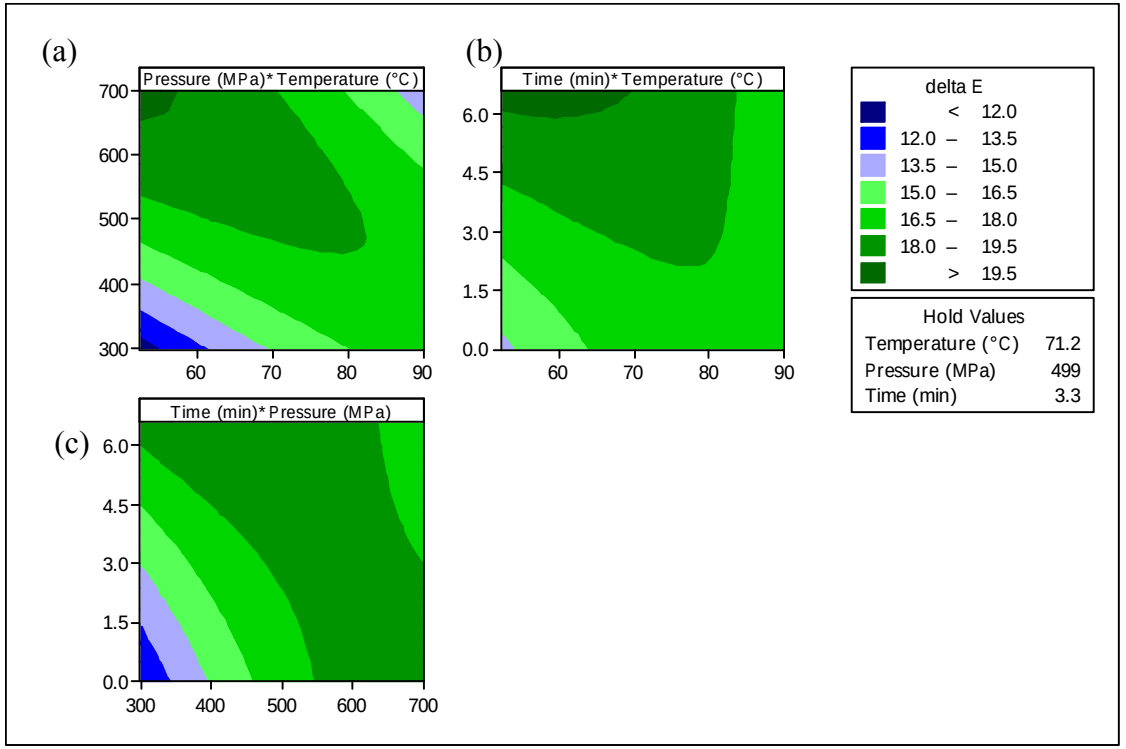

Figure 5-12 a-c. Contour plot for ΔE as influenced by pressure, temperature and time. Note that for each plot (a) to (c) the missing factor has been set at the mid-point of the design of the experiment. For example (a) plots pressure and temperature variation assuming that time is fixed at 3.3 minutes (see 'hold figure' in legend)

5.3.3 *Effects of processing on texture*

A good correlation was established between the measured peak forces for each experimental run and the predicted values of the regression equation (R^2 = 91.5%, R^2_{Adj} = 76.2%) i.e. 91.5% of the observed variation could be explained by the quadratic model (Figure 5-13). *Temperature* was by far the most important factors in determining peak force, accounting for 72% of the observed variation.

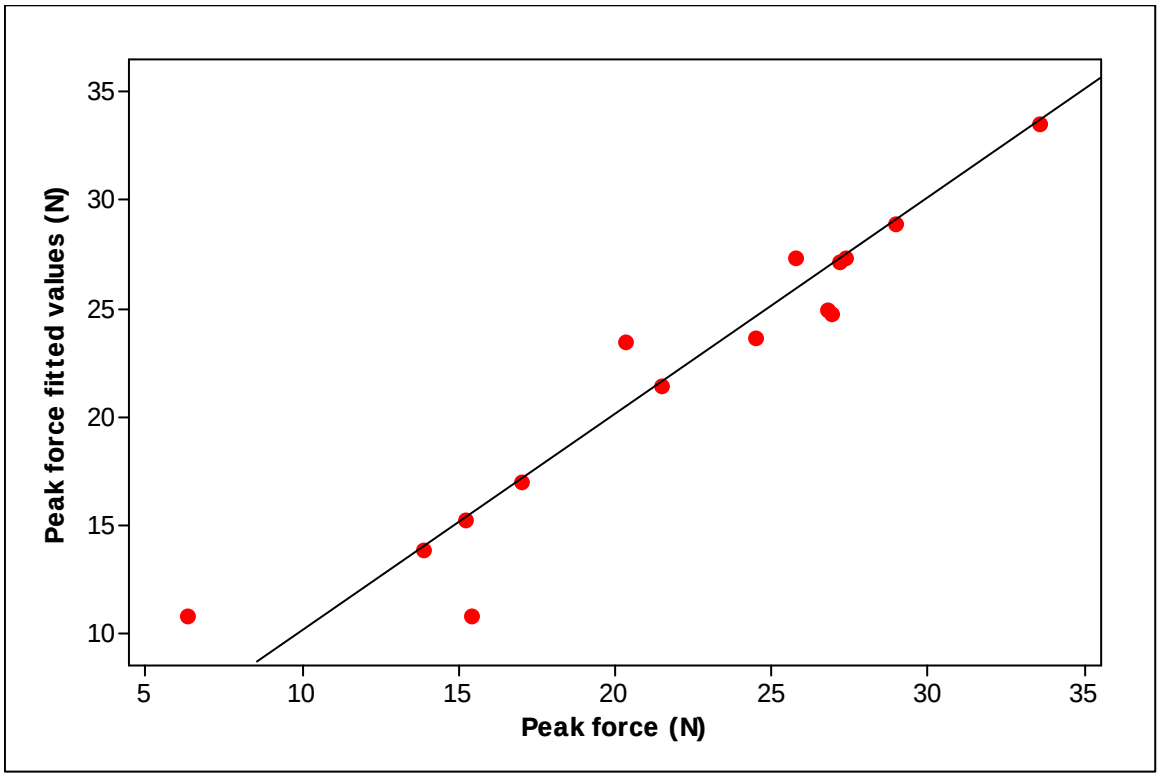

Figure 5-13 Plot of observed values of peak force versus surface response, fitted values for peak force for a range of temperature, pressure and time processing combinations. The reference line represents a perfect fit i.e Peak force predicted = Peak force measured.

Contour plots predicting peak force as a result of HPS treatments are shown in Figure 5-14. Changes in peak force appear to be largely related to the temperature to which the beans were exposed (Figure 5-14a). As temperature increased, the peak force needed to cut the beans reduced. This result is as expected given the results obtained in previous experiments (See 4.3.3) where softening of the treated beans was related to the degree of thermal process severity. Changes in texture seemed to be very pressure insensitive having a large region in the pressure processing space where texture did not seem to be affected (Figure 5-14c). This is an encouraging finding since a key potential advantage of the HPS approach is the minimised time at elevated pressure.

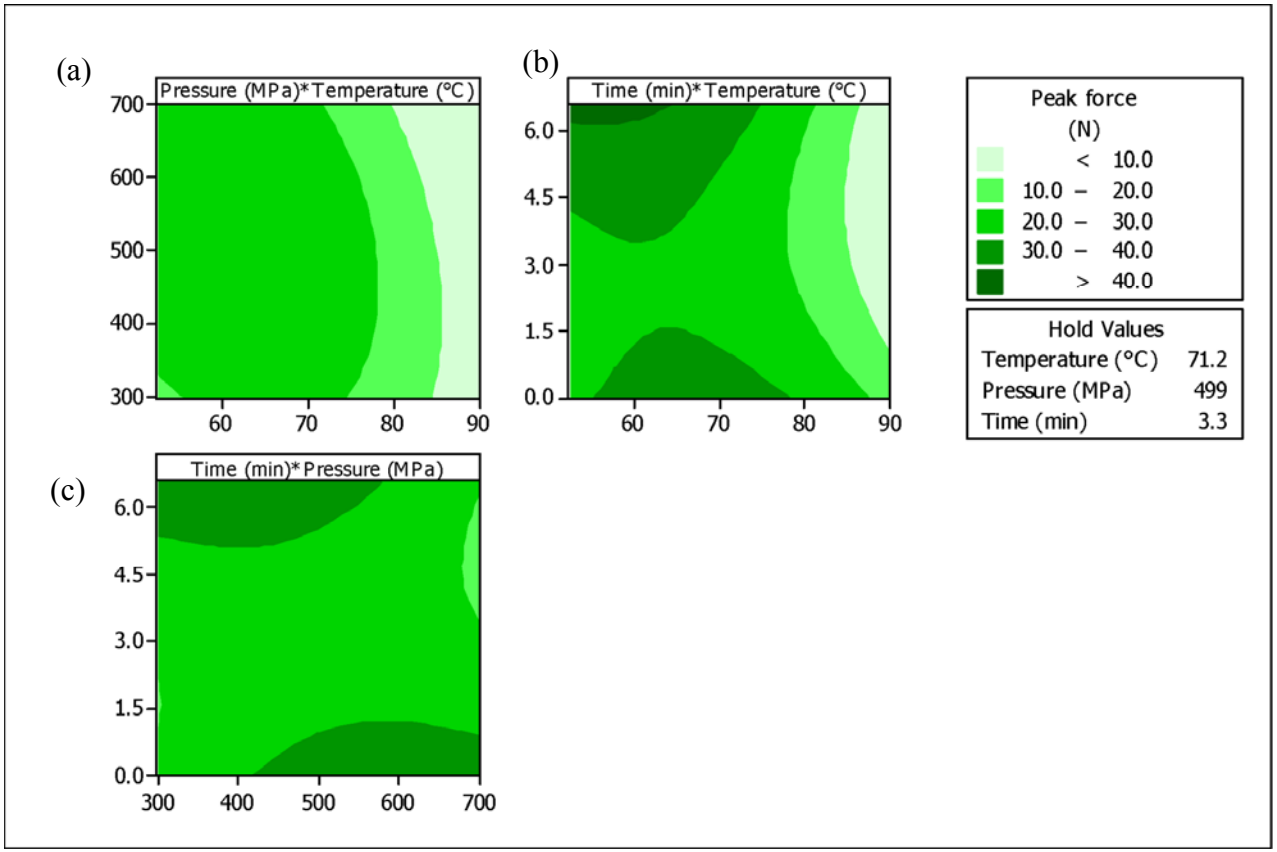

Figure 5-14 a-c. Contour plot for *Peak force* as influenced by pressure, temperature and time. Note that for each plot (a) to (c) the missing factor has been set at the mid-point of the design of the experiment. For example (a) plots pressure and temperature variation assuming that time is fixed at 3.3 minutes (see 'hold figure' in legend)

Figure 5-15 shows actual mean peak force required to cut the beans for all of the experimental runs. The runs have been ordered by initial temperature to illustrate the effects of temperature. Initial temperatures in excess of 80°C caused significant softening of the beans.

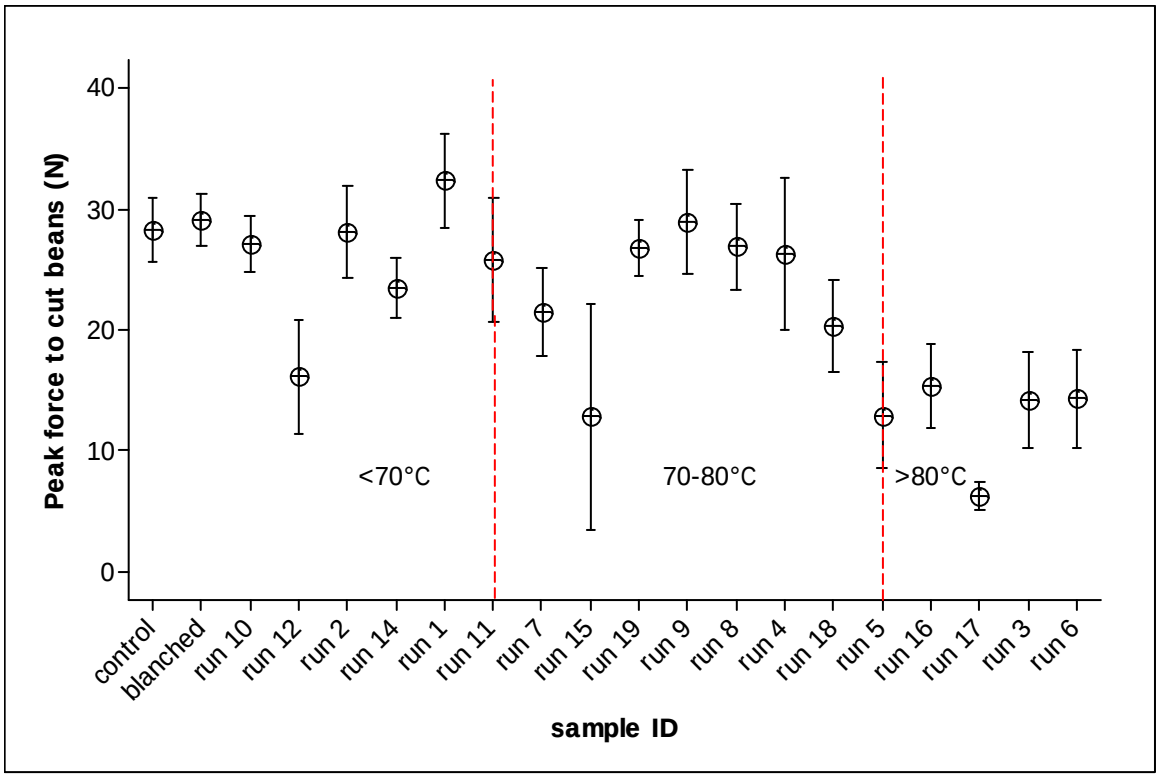

Figure 5-15. Mean peak forces required to cut beans after a range of HPS treatments. Note samples are ordered with increasing initial temperature to illustrate temperature effects on bean texture. Red lines split the plot into 3 temperature regions. Error bars indicate 95% confidence interval for the population mean.

5.3.3.1 Worked example of use of the regression coefficients to predict texture changes

The peak force required to cut HPS treated beans is described by the terms reported in Table 5-3. (column 1) and their coefficients (column 6). Inputting these terms into Equation 5.6 gives:

$$P_f = 518.6T + 25.67P + 960.3T_i - 3.39T^2 - 0.013P^2 + 71.3T_i^2 - 11.86TT_i - 0.13TP - 0.16PT_i - 21290 \quad (5.6)$$

Where T = Temperature (°C)

P = Pressure (MPa)

T_i = Time (minutes)

P_f = Peak force expressed in grams (divide by 100 to convert to Newtons)

The predicted peak force when processing at P = 500 MPa, for T_i = 2.5 minutes with an initial temperature of T = 69.9°C would therefore be 2740g divided by 100 = 27.4 Newtons. The actual measured mean peak force for these conditions was 25.8 Newtons.

5.3.4 Effects of processing on chlorophyll levels

All pressure/temperature/time combinations reduced levels of *chlorophyll-a* and *chlorophyll-b* to very low levels making accurate quantification impossible. Example HPLC chromatograms for (a) raw beans and (b) HPS treated samples (400 MPa, 0 hold time and 61.3°C initial temperature) are shown in Figure 5-16. Along with the substantial reduction in Chlorophyll a and b levels two new peaks termed X and Y were repeatedly observed in the Chromatograms of HPS treated samples. These peaks were thought to be pheophytins but this was not confirmed experimentally.

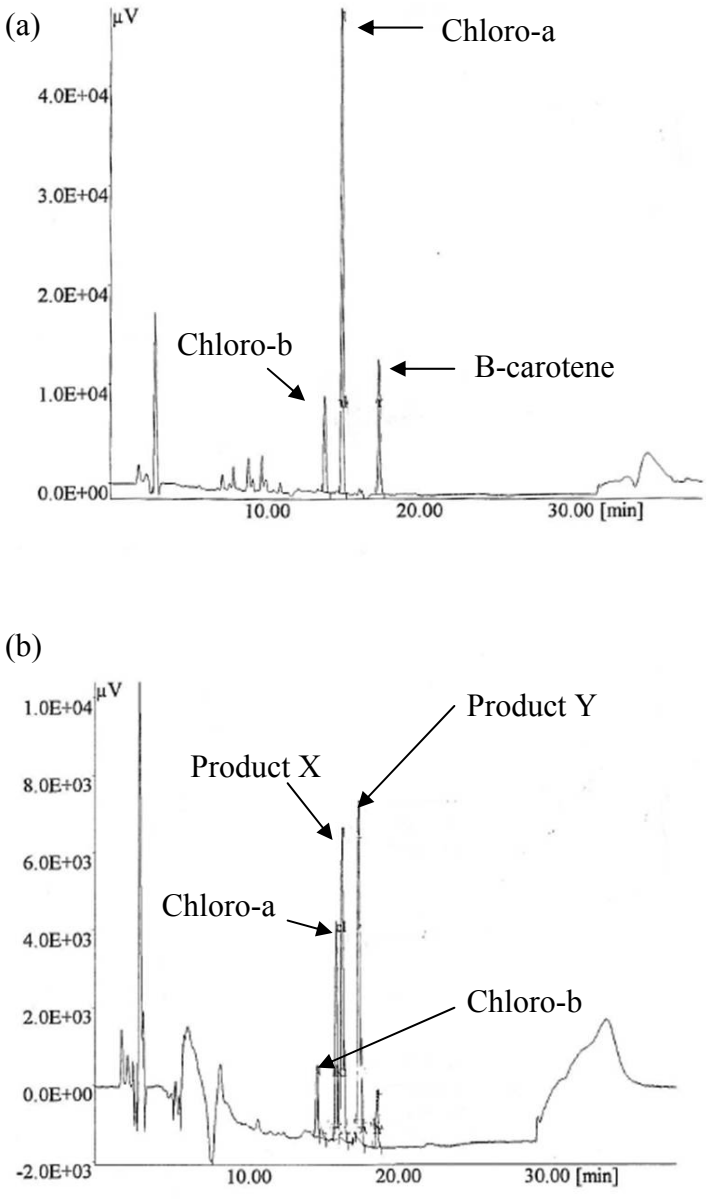

Figure 5-16 Chromatograms for (a) raw beans (b) HPS beans 61.3°C initial temperature, 400 MPa, hold time 0 (instantaneous decompression)

Despite the low residual levels of chlorophylls, the use of surface response methodology resulted in a reasonable correlation between predicted and measured peak areas for *chlorophyll-a* and *chlorophyll-b* and for the assumed breakdown products X and Y for the various process combinations (R^2 of 78.4, 85.8, 78.5 and 73.0% respectively). *Chlorophyll-a* breakdown was primarily predicted by changes in *temperature*, *time* and the *interaction* of temperature and time (Table 5-2) rather than by pressure. Similarly, the breakdown of *chlorophyll-b* was essentially related to time/temperature effects rather than pressure effects. Interestingly, changes in pressure explained a significant proportion of the observed variation for breakdown products X and Y (34.6 and 23.1% respectively). This could suggest that products X and Y were not simply direct break-down products of *chlorophyll-a* and *chlorophyll-b*. This is supported by the fact that there was not a significant correlation between *chlorophyll-a* or *chlorophyll-b* and breakdown products X and Y (Table 5-4). The only significant correlation ($P<0.05$) was that between *chlorophyll-a* and *chlorophyll-b*.

	a*	b*	Chlorophyll a	Chlorophyll b	Product X
Spearman rank correlation coefficients					
b*	-0.07	*	*	*	*
Chlorophyll a	0.09	0.17	*	*	*
Chlorophyll b	-0.35	-0.05	**0.78**	*	*
Product X	0.21	-0.21	-0.11	-0.23	*
Product Y	-0.13	-0.29	0.04	0.00	**0.86**
Probability of correlation occurring by chance					
b*	0.79	*	*	*	*
Chlorophyll a	0.76	0.56	*	*	*
Chlorophyll b	0.20	0.87	**0.00**	*	*
Product X	0.45	0.46	0.67	0.35	*
Product Y	0.65	0.30	0.87	0.99	**0.00**

Table 5-4. Spearman's rank correlation coefficients and associated probabilities. Figures in bold are significantly correlated (P<0.05)

It was hoped that the experiments described in this chapter would provide some insights into how Chlorophyll was degraded under various combinations of high temperature and pressure. Unfortunately, all of the conditions tested resulted in levels of Chlorophyll a and b that were too low to quantify.

5.3.5 Optimisation of colour and texture

Using optimisation tools available in Minitab 14, the various predictive models derived from the experimental data (Table 5-3) were used to predict the effects of variations in pressure, temperature and time on *a*, b** and peak force. Pressure, temperature and time were varied in an iterative process in order to find conditions were the predicted values of *a*, b** and peak force for HPS treated samples were as close as possible to the respective values measured in blanched beans (*a** = 1, *b** = 31.5 and peak force = 29.1 N). The optimised conditions found to give product colour and texture close to that of blanched beans are shown in Table 5-5 along with the desirability values for each response. Desirability values close to 1 indicate that the solution is close to ideal. Also included are the target and predicted achieved values for peak force, *a** and *b** at the optimised processing regime. As can be seen, product quality similar to that of blanched beans (in terms of colour and texture) could theoretically be achieved under these conditions.

	Target value	Optimised value	Individual desirability
Temperature (°C)	*	62.6	*
Pressure (MPa)	*	415.8	*
Time (min)	*	0	*
a*	1.0	4.5	0.78
b*	31.5	31.3	0.99
Peak Force (N)	29.1	29.1	0.99

Table 5-5. Optimised colour and texture of pressure sterilised beans with the associated processing conditions and individual desirability values. Composite desirability was 0.93.

It is important to recognise that the process conditions specified in Table 5-5 (an initial temperature of 62.6°C, pressure of 415.8 MPa with instant decompression (hold time of 0 minutes) are much lower than the suggested requirements for full sterilisation e.g. (Meyer 2000b); (Wilson & Baker 1997). This process could therefore produce the desired quality results (although still with fairly significant changes in *a**) but would not achieve the necessary level of microbiological inactivation (see section 2.4).

In the studies discussed in this chapter, samples processed at conditions close to the optimum for quality retention (e.g. 61.6°C initial temperature, 400 MPa for 5 minutes) were not ambient stable when stored and in fact all runs where peak temperatures did not exceed 105°C resulted in visible spoilage (i.e. blown packs). This observation is in agreement with findings by Meyer *et al*. (2000; 2001).

In Chapter 4 it was demonstrated that colour changes in pressure sterilised green beans were negative when samples were processed under HPS conditions likely to yield commercial

sterility (see also Leadley, Tucker & Fryer (2007)). However, clear benefits in texture retention were apparent when compared with conventional canning.

Due to the nature of the surface response methodology used in the current study, it was not possible to predict quality changes under conditions outside the design space. However, since texture changes appear to be primarily related to temperature effects it seems likely that better texture retention could be achieved by the use of much higher pressures than are currently commercially attainable along with a reduced initial temperature to ensure peak temperatures are minimised. This is supported by data from Krebbers *et al.* (2002) who demonstrated excellent texture retention in green beans when operating with a starting temperature of 75°C (peak temperatures of around 100°C) and a pressure of 1000 MPa. This supports the data from Chapter 4 which suggested that improved texture retention was essentially related to the reduced thermal impact of the high pressure sterilisation process.

5.3.6 Temperature distribution effects in the HPS conditions studied

A summary of results for the temperature distribution studies that were carried out in conjunction with studies on colour, texture and chlorophyll retention are reported in Table 5-6. The top of the vessel was consistently higher in temperature than the bottom of the vessel with the top right and top centre of the vessel generally found to be hot-spots during each HPS cycle (Table 5-6). See Figure 3-5 for thermocouple locations and descriptors. This finding is in agreement with the temperature distribution studies described in Chapter 3 and was generally found to hold true across the full spectrum of HPS conditions used in these

experiments. However, the mean range of temperatures for all distribution tests carried out in "stage 3 trials" (described in section 3.3.3) was 2.8°C (s.d = 0.8, n=10) whereas for the HPS trials described in this chapter, the mean range across all runs was considerably higher at 4.9°C with a wider spread in the range of temperatures measured for each run (s.d. = 3.5, n=17). Distribution studies in Chapter 3 were carried out across a fairly narrow range of initial temperatures (16°C) whereas the studies in Chapter 5 were conducted across a wider range of initial temperatures (38°C). In the distribution studies from chapter 3 there was no correlation between the initial temperatures used and the range of temperatures seen during runs ($p>0.05$) whereas for the trials carried out in Chapter 5, the wider range of temperatures observed across runs was correlated with the initial temperatures used in the experiments ($p<0.05$). Generally speaking, temperature distribution was worse in runs starting at a lower initial temperature (Figure 5-17).

Run No.	Pressure (MPa)	Initial temp. (°C)	Lowest probe temp at end of come up (°C)	Location of probe	Highest probe temperature at end of come up (°C)	Location of probe	Range (°C)
1	400	61.6	78.3	Product	83.1	Top right	4.8
2	400	61.3	77.6	Product	84.8	Top centre	7.2
3	700	87.7	*	*	*	*	*
4	550	74.8	99.8	Bottom right and product	101.5	Top centre	1.6
5	550	82.1	104.3	Top left	107.9	Top centre	3.6
6	400	90.0	106.0	Top left	110.6	Top right	4.7
7	700	72.5	101.1	Bottom right	104.2	Top centre	3.1
8	550	73.6	96.5	Bottom left	99.1	Top right	2.6
9	550	73.4	94.7	Top left	99.5	Top right	4.7
10	550	52.4	74.5	Product	89.9	Top right	15.4
11	550	69.9	92.4	Top right and product	93.8	Top left	1.3
12	298	73.3	87.0	Bottom left	90.2	Top right	3.2
13	700	59.6	86.6	Product	97.6	Top centre	11

Run No.	Pressure (MPa)	Initial temp. (°C)	Lowest probe temp at end of come up (°C)	Location of probe	Highest probe temperature at end of come up (°C)	Location of probe	Range (°C)
14	700	61.4	88.0	Product	93.4	Top right	5.4
15	550	72.7	96.1	Product	99.4	Top right	3.3
16	400	85.9	105.6	Bottom right	108.5	Top right	2.8
17	700	88.0	*	*	*	*	*
18	550	75.0	95.4	Top centre	101.0	Top right	5.5
19	550	73.8	96.9	Product	100.5	Top right	3.5

Table 5-6. Summary of temperature distribution during HPS cycles used for the trials. Note * denotes data not recorded.

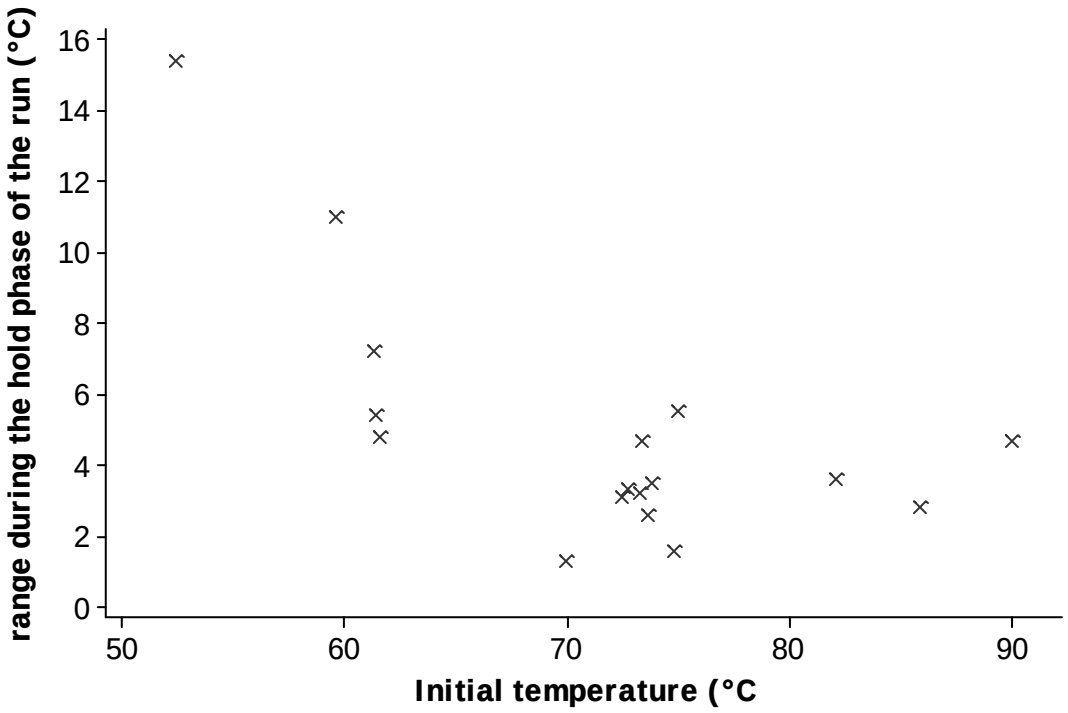

Figure 5-17. Range of temperatures seen during the hold phase of each HPS experiment for a given initial temperature at the start of the run. Note the poorer temperature distribution at lower initial temperatures.

The coldest point in the system was not always the same run to run but was frequently found to be the probe measuring the product temperature. This might have been due to a spatial effect but it could also be due to product composition, since the product is predominantly aqueous and the pressure medium contains some oil, you might expect the compression heating effect to be lower in the beans than in the pressure fluid.

5.4 Conclusions for chapter 5

Surface response methodology was a useful technique for determining the relative importance of where pressure, temperature and time exerted an influence on the observed variations in colour and texture as a result of processing. In essence, texture changes were essentially related to the temperature used for each process, higher temperatures resulted in a greater loss in texture. Since pressure had little effect on texture, it is conceivable that by minimising peak temperatures and using very high pressures, very good texture retention could be achievable. This is supported by the work reported in Chapter 4 and by that of Krebbers *et al*. (2002); the use of lower peak temperatures and higher pressures resulted in better texture retention in green beans. However, to truly realise the potential benefits of this approach there is an inherent assumption that pressures and temperatures can be modified in such a way as to deliver 'equivalent' lethalities i.e. that a low temperature high-pressure process could deliver the same microbiological lethality as a high-temperature low-pressure process. This assumption has been called into question in recent years (Bull *et al.* 2009). In addition, commercially achievable pressures are currently limited to 700 MPa which means that peak temperature by necessity must currently be high in order to assure commercial sterility.

Colour parameters changes were predicted primarily by time and pressure so it seems likely that in the case of green beans, deterioration in quality for a commercially sterile product is inevitable when using a pressure-time-temperature process. However, it should be noted that colour changes have previously been found to be very much product dependent as noted by Matser *et al.* (2004); here the spectrum of colour retention ranged from complete retention of raw colour through to colour equivalent to that achievable by conventional preservation.

Data relating to quality changes resulting from pressure sterilisation remains limited because the focus of much of the research effort to date has understandably related to demonstrating the microbiological efficacy of the process. Quality benefits (or otherwise) derived from HPS are certainly worthy of further study however because if the differences between HPS and conventional heat treatments are not sufficiently large it would prove difficult to make the case for commercial investment in the technology.

In contrast to high pressure pasteurisation, high pressure sterilisation is, in development terms, relatively untested (at least with reference to data available in the public domain) both in terms of microbiological efficacy and product quality enhancement. It seems likely that it will be some years before there are main-stream commercial applications of PATS but it is planned that US military rations will contain PATS processed ambient stable products in the near future (personal communication with US Natick soldier research, development & engineering center, IFT 2009)

6 Effect of pre-heating methods of product quality at the start of the HPS cycle

6.1 Introduction

Experiments reported in Chapter 4 began to explore the effects of the pre-heating step on quality attributes prior to a HPS cycle (see for example Figure 4-12). In Chapter 5 the focus of the work was on quality changes attributable to the HPS cycle after pre-heating. In Chapter 6, experiments are reported that explored the pre-heating phase of a high pressure sterilisation cycle to provide insights into how the pre-heat phase might be optimised such that the raw materials at the start of the HPS cycle were in the best possible condition. Experiments were carried out comparing different methods of pre-heating (ohmic-heating and water-bath heating) with the addition of a firming agent (Calcium chloride) and a pH modifier (Sodium Bicarbonate).

Calcium chloride (E509) and Sodium Bicarbonate (E500) are legally classified in the EU as miscellaneous food additives (according to the miscellaneous food additives regulations, 1995) and are generally permitted *quantum satis*; meaning that they "can be used in accordance with good manufacturing practice at a level not higher than is necessary to achieve the intended purpose and provided that such use does not mislead the consumer"(Campden BRI 2008). As with all additives covered by this legislation, there are certain foods in which miscellaneous additive usage is restricted to only specific ingredients. For example, canned and bottled fruit and vegetables have a restricted list of 22 miscellaneous food additives which includes Calcium Chloride but not sodium carbonates (Campden BRI 2008).

Sodium bicarbonate was used in these experiments as a pH modifier to produce bean samples that were more alkaline than untreated bean products (pH 6.4 rather than 5.7). This factor was included to see whether a more alkaline pH would give better retention of the green colouration of the beans after the pre-heat cycle as has been previously proposed, for example, in the "Blair Process" (Clydesdale & Francis 1976); see section 4.3.1.2.

Calcium plays an important role in plant tissues because it is involved in processes such as delaying senescence, increasing tissue firmness and preventing enzymic browning (Balla & Farkas 2006). It is typically found in fruit and vegetables at a level of approximately 3-300 mg/100g of fresh material (Duckworth 1979). The addition of calcium chloride to heat processed foods is expected to increase firmness because of the interaction between "calcium ions with pectic polysaccharides in the middle lamellae and parenchyma cell walls" (Balla & Farkas 2006).

Firming agents based on calcium salts were once in common usage for the processing of fruit & vegetables in order to improve their texture (Lopez 1987) but UK supermarkets are increasingly demanding 'additive-free' recipe formulations – see for example the Tesco kitchen cupboard promise "All Tesco Chilled Prepared Meals are made with only ingredients you'd find in your kitchen cupboard" (Tesco website 2011). The food industry has responded to retailer demands by developing additive free products wherever possible.

6.2 Materials and methods

6.2.1 Preparation of green beans prior to pre-heat trials

Green beans from a single raw material batch were obtained from a local supplier (Drinkwaters, Chipping Campden). Four batches of beans (each of 1.2 kg) were produced from this single raw material. The beans were washed in mains water and trimmed ready for blanching. Mains water (9 litres) was added to a steam jacketed pan; the temperature of the pan was monitored using a hand-held thermocouple (Comark, Hitchin UK) until a temperature of 85°C was achieved at which point, individual batches of beans were added to the pan along with a level of calcium chloride and/or sodium bicarbonate as required according to Table 6-1.

Sodium bicarbonate was added as an alkalising agent as required so that studies could be conducted at two pH levels – 5.7 (the pH of the untreated beans) and 6.4 to explore the effect of pH on colour retention during a potential pre-heat phase of high pressure sterilisation. An upper pH level of 6.4 was settled upon because informal tasting of blanched samples at a range of pH levels suggested that this was the highest level of alkalinity that could be achieved before the presence of sodium bicarbonate became detectable and unpleasant.

After heating the water and adding the beans, the temperature of the water in the pan was brought back up to 74°C and then held for 6 minutes. After blanching, the beans were cooled in a sink of cold mains water, rinsed and drained for 2 minutes. The beans were cut into 2.5 cm lengths using a rotary bean cutter. After the blanch, four replicate samples of 50g per batch were assessed for peak force required to cut the beans (see section 4.2.5). Four replicate

samples of 100g per batch were retained for use in ohmic pre-heating heating trials (see section 6.2.2) and four replicate samples of 100g per batch were retained for pre-heating trials using a conventional water-bath (see section 6.2.3). Sample of blanched beans were retained for colour measurement immediately after blanching (see section 6.2.5). A further sample of beans was retained in order to measure the pH of each batch after blanching using a calibrated pH meter (Mettler MP220, Leicestershire).

Batch number	Level of sodium bicarbonate addition (%w/v)	Level of Calcium Chloride addition (%w/v)
1	0	0.1
2	0	0.25
3	6.6	0.1
4	6.6	0.25

Table 6-1. Levels of sodium bicarbonate and calcium chloride added to each of four batches of beans prior during blanching.

6.2.2 Pre-heating trials using ohmic heating

Four replicate runs were performed in a small batch ohmic heating unit (supplied by C-Tech Inovation, Chester) – see Figure 6-1 and Figure 6-2. The unit could deliver up to 10 kW of power and required a 40 amp, 240 volt, 50 Hz supply. The unit was designed to heat products having a starting conductivity of between 0.05 to 2.0 S/m. The electrodes used were 90mm wide by 95 mm high.

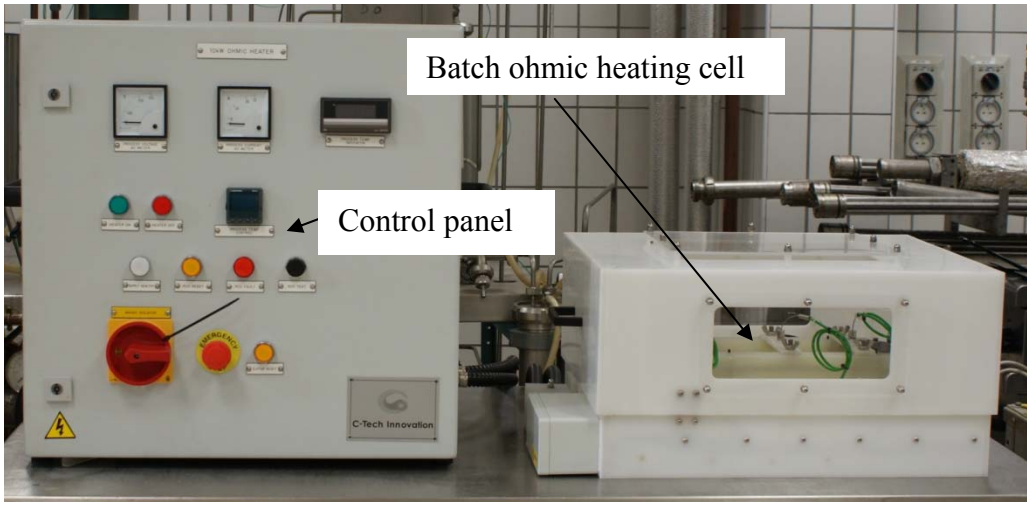

Figure 6-1. Batch ohmic heating unit used in the ohmic heating pre-heating studies.

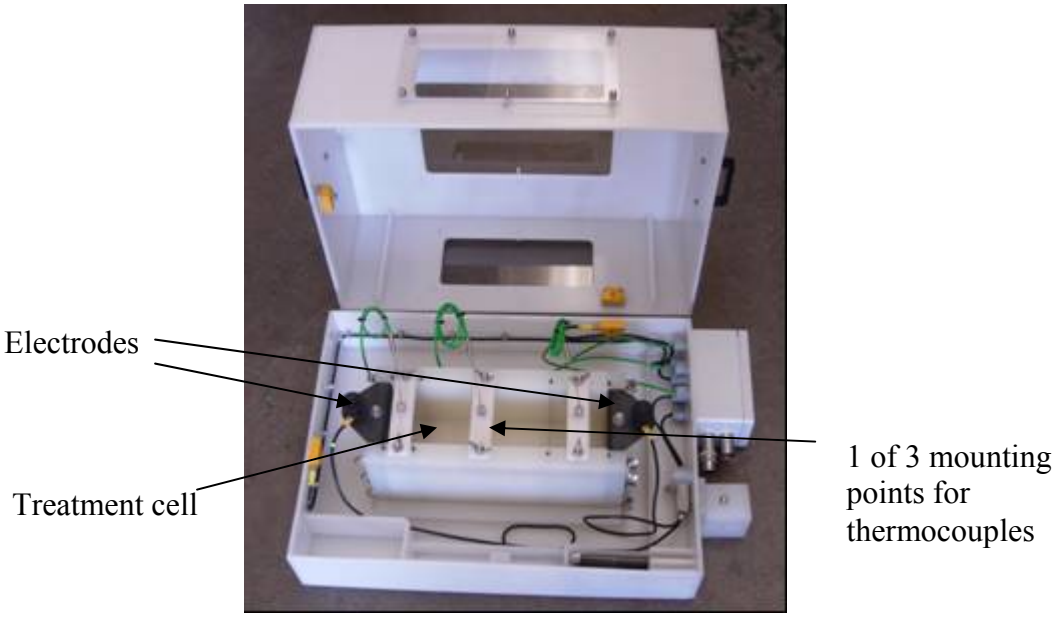

Figure 6-2. View of ohmic heating cell

The ohmic heating cell had a maximum working capacity of approximately 750 ml. Blanched, prepared beans (100g) were placed in the ohmic heating unit which was then filled with brine solution (5.6g salt per litre of water).

A single bean was placed on a thermocouple in order to monitor the product temperature during the ohmic heating cycle. The ohmic heater was operated until a product temperature of 86°C ± 1°C was reached. After heating, the ohmic unit was switched-off; the product was drained and finally rinsed in mains water for 2 minutes. Bean samples (15) were used for colour measurements; the balance being used for texture measurement.

6.2.3 Pre-heating trials using a water-bath

Blanched beans (100g samples) were packed into five pouches (The vacuum pouch company Ltd., Bury) and 750 ml of brine solution (5.6g of salt per litre prepared in an identical fashion to the brine used in the ohmic studies) was added to each pouch before heat sealing. One of the five bags was fitted with a flexible packaging gland (Ellab A/S, Norwich) and a thermocouple (type K) was threaded through the gland in order to record the time temperature history of the batch using a squirrel datalogger (Grant Instruments, Cambridgeshire). The water-bath was set at 90°C; bags were placed in the bath and the product temperature was monitored until a temperature of 86°C ±1°C was reached. After heating, the bags were removed from the water-bath and placed in iced water. The heated and cooled product was then drained from the pouch and bean samples (15) were used for colour measurements; the balance being used for texture measurement.

6.2.4 Texture measurement method

Before and after pre-heating trials, bean texture was determined by slicing force as described in section 4.2.5.1. Peak forces to cut the beans samples were recorded for each of the pre-heating methods (raw, blanched, ohmic pre-heat and water-bath pre-heat with varying levels of sodium bicarbonate and calcium chloride – as per Table 6-1).

6.2.5 Digieye colour measurement method

Colour measurements were taken with a 'Digieye' digital imaging system (DigiEye plc, Leicester, UK) as described in section 4.2.4. Values of *L*, a*, b** were recorded for each pre-heating method (blanched, ohmic pre-heat and water-bath pre-heat with varying levels of sodium bicarbonate and calcium chloride – as per Table 6-1).

6.2.6 Statistical analysis

A General Linear Model with Tukey's test (at the 5% significance level) was used to assess the effect of pH, calcium addition within treatments, and pre-treatment applied (raw, blanched, water-bath pre-heated to 86°C or ohmically pre-heated to 86°C) on colour parameters and texture measurement values. All data analysis was conducted using Minitab version 14 (Minitab Inc, USA).

6.3 Results and discussion

6.3.1 Effect of pre-heating method on bean texture

The mean forces required to cut bean samples after the various treatments were applied, are summarised in Table 6-2. The pH of the samples had no significant effect on bean texture after processing ($p>0.05$). The thermal treatments applied had a significant effect on bean texture ($p<0.05$). Table 6-2 shows the significantly different texture of the raw, untreated beans compared with all of the thermal treatments. When raw beans were omitted from the data analysis there was no significant difference ($p>0.05$) between the different pre-heating treatments applied (blanching, ohmic heating or water-bath heating) regardless of the level of calcium chloride addition.

Treatment	Calcium addition (% w/v) to blanch water	N	Mean peak force required to cut bean samples (N)
Blanch	0.10	160	28.04[a]
Blanch	0.00	20	27.93[a]
Ohmic	0.10	160	27.55[a]
Waterbath	0.10	160	27.43[a]
Waterbath	0.25	160	27.39[a]
Ohmic	0.25	160	27.27[a]
Blanch	0.25	160	26.84[a]
Raw	0.00	20	23.21[b]

Table 6-2 Mean peak force required to cut beans after a range of pre-heating steps. Mean values having the same letter subscript are not significantly different from one another.

Boxplots of the mean peak force required to cut beans after pre-treatment with the methods outlined in Table 6-1 are shown in Figure 6-3.

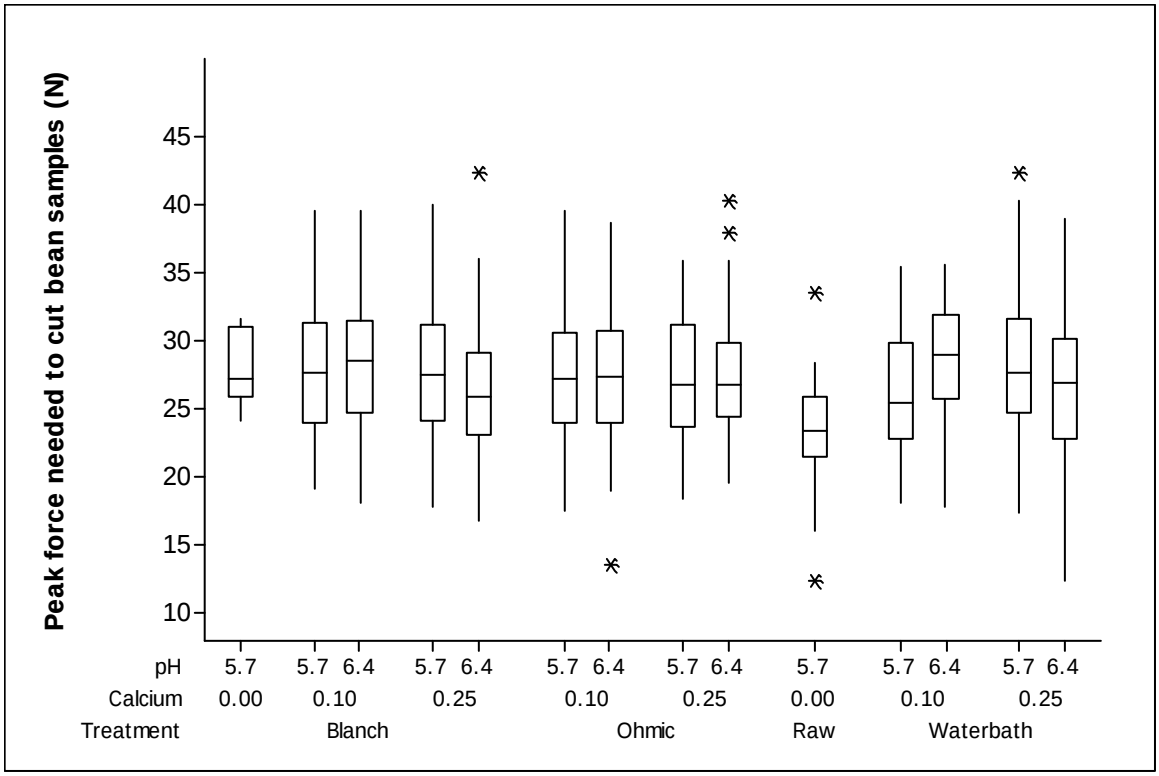

Figure 6-3. Mean peak force required to cut through bean samples after: blanching; blanching and ohmic heating to 86°C; blanching and heating in a water-bath to 86°C.

As can be seen from Table 6-2 and Figure 6-3, the peak force required to cut the beans was very similar across all treatments regardless of; the pre-heating method applied; the pH of the beans; or the level of Calcium Chloride added to the blanch water. Processed bean samples were in fact significantly more firm than the raw beans (typically 26-28 N peak force was required to cut heat treated beans compared with around 23 N for raw beans). This represented an increase in firmness of around 16-20%. Blanched beans from the trials in Chapter 5 were also marginally firmer than the raw beans (29.1 N versus 28.3 N) but the difference was not statistically significant ($p>0.05$). Peak force to cut blanched beans from the trials in Chapter 4 was around 12% lower than in raw beans (29.5 N for the control and 26.2 N for the blanched samples).

Beans blanched without calcium chloride addition were not significantly less firm than samples blanched in varying levels of calcium chloride ($p>0.05$). The water supply in Chipping Campden is hard (Calcium Carbonate levels of 271 mg per litre – www.thameswater.co.uk last viewed 6th July 2011). Blanching in hard water can increase the firmness of vegetable products because calcium ions in the water can react with pectin in the cell membranes of the vegetables to produce a firmer product as previously described (see section 6.1). This could explain why the calcium chloride addition yielded little benefit compared with beans blanched in mains water alone. The use of hard water along with the addition of calcium chloride could also explain why heat treated products were all consistently more firm than the raw beans.

As discussed, samples pre-heated to 86°C were firmer than raw beans (16-20%) regardless of the method employed whereas samples that were pre-heated to 86°C for HPS in the work

described in Chapter 4 had a loss in firmness of around 12% of the starting firmness. This suggests that optimisation of the pre-heating step before HPS could offer some benefits in terms of texture retention – if the texture of the material at the start of the HPS cycle is firmer then it is reasonable to expect that the final texture of the product after the HPS cycle would also be improved.

6.3.2 Heating profiles and cook values from water-bath and ohmic pre-heating.

Van Loey *et al*. (1995) derived 'z' values for key quality parameters for peas and white beans and noted that the 'z' value for colour loss in peas was of the order of 25 to 33 C°. Hayakawa & Timbers (1977) determined a 'z' value of 39 C° for 'colour destruction' in green beans. The 'z' value determined by Hayakawa & Timbers was used to calculate cook values for ohmically pre-heated beans and water-bath pre-heated beans. These cook values were calculated up to the point where the product reached 86°C. The reference temperature used for the cook value calculations was 100°C. Example time temperature traces for ohmically pre-heated and water-bath pre-heated products are shown in Figure 6-4.

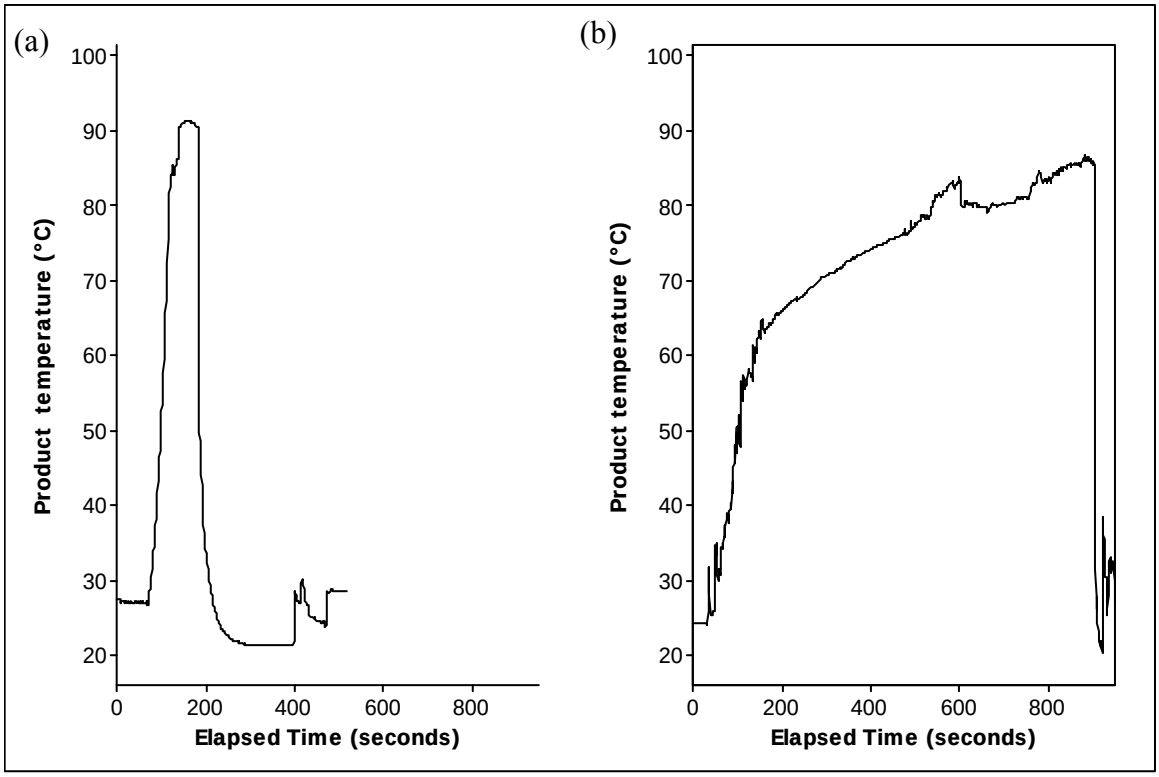

Figure 6-4. Comparison of heating rates when pre-heating samples prior to HPS using (a) ohmic heating; (b) water-bath heating

As expected, ohmically heated samples heated to the set point much more rapidly (Figure 6-4) and in consequence the cook values attained were much lower than in the water-bath-heated samples. Mean, minimum and maximum cook values for all (a) ohmically heated and (b) water bath heated products were (a) 0.24, 0.12, 0.35 and (b) 3.02, 2.50, 3.70 respectively. As expected, the significantly reduced cook value for ohmic heating led to much improved colour retention (Figure 6-8).

6.3.3 Effect of pre-heating method on bean colour

The effect of pre-heating method; level of Calcium Chloride addition; and pH on the colour of beans after blanching and pre-heating to 86°C in preparation for a high pressure sterilisation process is shown in Figure 6-5 to Figure 6-7.

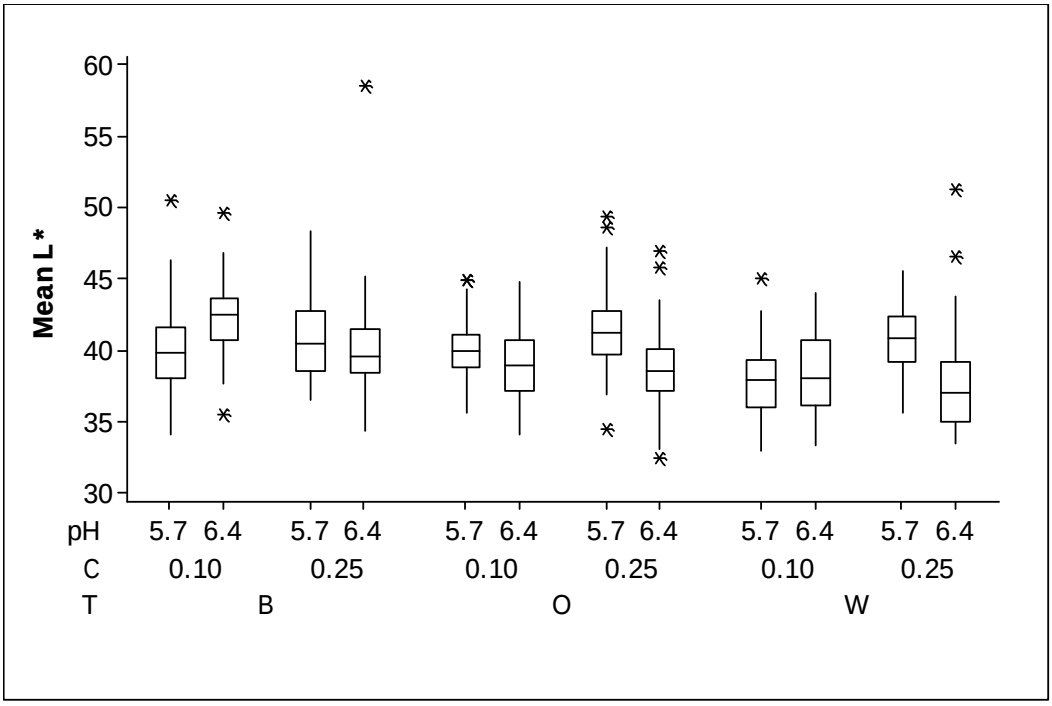

Figure 6-5. Influence of Calcium Chloride levels (C - % w/v), pH, and treatment (B = blanched; O = blanching followed by ohmic pre-heat to 86°C; W = blanching followed by water-bath heating to 86°C on L* of treated beans

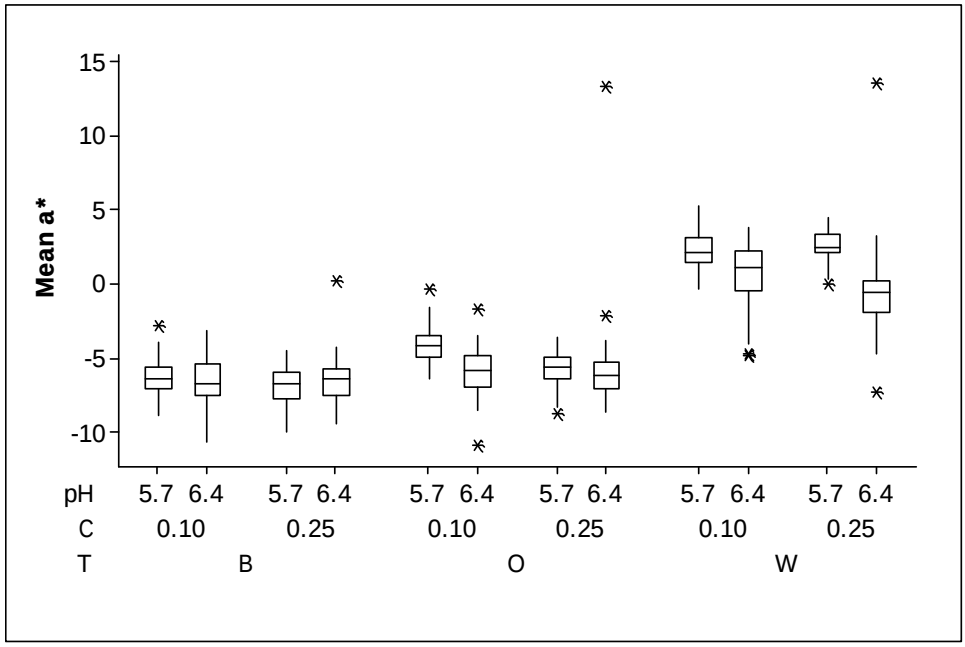

Figure 6-6. Influence of Calcium Chloride levels (C - % w/v), pH, and treatment (B = blanched; O = blanching followed by ohmic pre-heat to 86°C; W = blanching followed by water-bath heating to 86°C on a* of treated beans

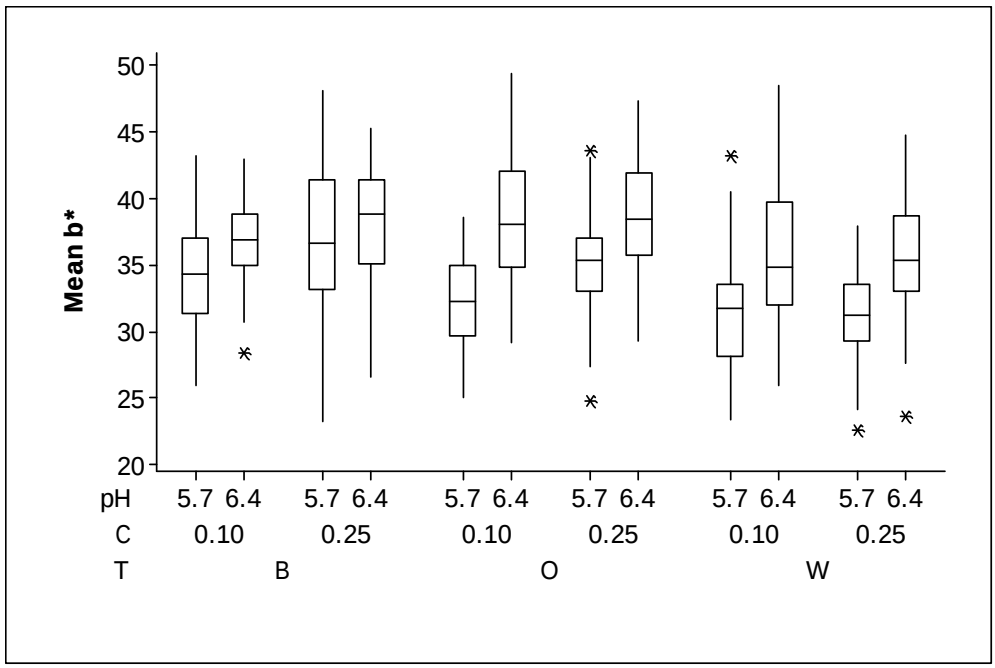

Figure 6-7. Influence of Calcium Chloride levels (C - % w/v), pH, and treatment (B = blanched; O = blanching followed by ohmic pre-heat to 86°C; W = blanching followed by water-bath heating to 86°C on b* of treated beans

Whilst differences in the mean values of L^* were *statistically* different ($p<0.05$) across heat treatments (blanched; blanched then ohmically heated; blanched then heated in a water bath) they were of little *practical* significance since the mean L* values for the 3 treatments were 40.7, 39.7 and 38.6 respectively i.e. the practical magnitude of the differences was very small. Similarly, pH modification resulted in *statistically* significant differences in L^* across batches but was of little practical significance (pH 5.7 samples were, on average $L^* = 40.0$ compared with $L^* = 39.3$ for pH 6.4 samples. Varying calcium chloride level did not affect values of L^* across treatments ($p>0.05$).

Both statistically significant ($p<0.05$) and *practically* significant differences in a^* were observed as a result of the different thermal pre-treatments. The mean values of a^* for blanched-water-bath pre-heated, blanched-ohmic pre-heated and blanched-only were 1.2, -5.3 and -6.6 i.e. ohmically pre-heated samples had a better retention of green colouration and were similar in appearance to blanched beans. Figure 6-8 illustrates the difference very clearly. Water-bath pre-heated samples (Figure 6-8 c) had the characteristic colour change to olive green typically seen in heat processed vegetables whereas the ohmically pre-heated beans (Figure 6-8 a) retain a bright green colour closer to the colour of blanched beans.

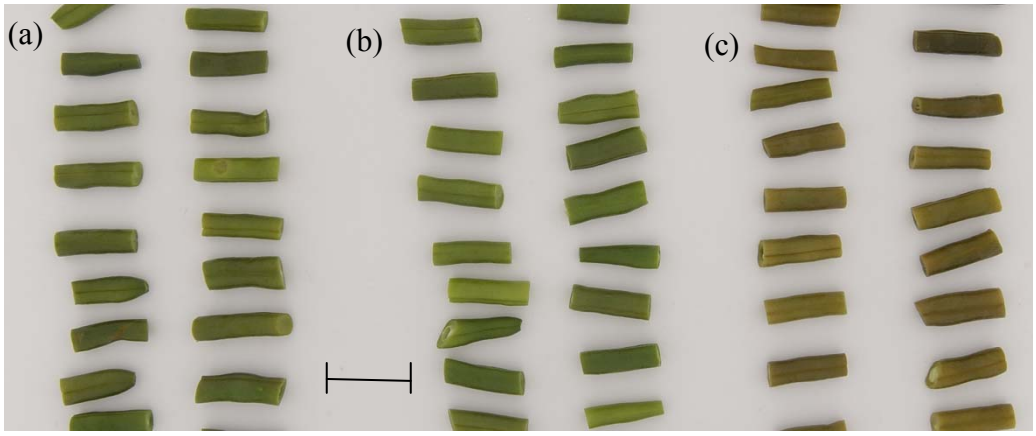

Figure 6-8. Example image showing the effect of thermal pre-treatment on the colour of green beans. Sample pH was 5.7, Calcium Chloride brine addition was at 0.25% (w/v). Images from left to right are (a) blanched-then ohmically heated to 86°C, (b) blanched at 74°C for 6 minutes, (c) blanched-then water-bath heated to 86°C. Scale bar indicates approximately 2.5 cm.

Values of *b** for blanched and blanched-ohmically pre-heated samples were not significantly different from one another ($p>0.05$) but water-bath heated samples were significantly different ($p<0.05$). Water-bath heated beans had a downward shift in b* indicating that they were becoming more blue and less yellow; *b** values for blanched; blanched-and-ohmically-pre-heated; and blanched-and-water-bath-pre-heated being 36.5, 36.2 and 33.6 respectively.

The pH of the product also significantly affected *b** values ($p<0.05$) with pH 5.7 samples having *b** values of 33.6 on average compared with 37.2 for samples at pH 6.4. The more alkaline samples were more yellow in colour compared with untreated samples. Figure 6-9 (a) to (d) shows the effect of calcium chloride levels and pH on product colour for blanched-ohmically-pre-heated products. Samples at pH 6.4 had a brighter green appearance though the difference was quite subtle in comparison to the changes induced by the different heat processing regimes.

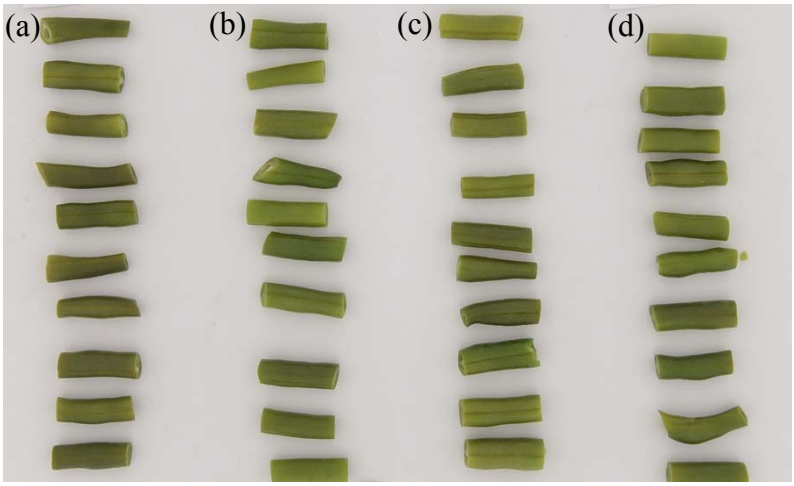

Figure 6-9 (a) – (d). Influence of calcium chloride levels and pH on bean colour. (a) pH 5.7, CaCl2 = 0.1% w/v, (b) pH 5.7, CaCl2 = 0.25% w/v, (c) pH 6.4, CaCl2 = 0.1% w/v, (d) pH 6.4, CaCl2 = 0.25 % w/v. Mean L*, a*, b* values were (a) 39.9, -4.0, 32.4, (b) 41.2, -5.6, 35.1, (c) 39.0, -5.9, 38.6, (d) 41.2, -5.6, 35.1

6.4 Summary

Results from studies described in Chapter 6 demonstrated that beans were relatively resilient to texture losses as a result of the pre-heating method and there was little benefit in employing a rapid heating method if the goal was solely to retain product texture. However, samples pre-heated to 86°C were firmer than raw beans (16-20%) regardless of the method employed whereas samples that were pre-heated to 86°C for HPS in the work described in Chapter 4 had a loss in firmness of around 12% of the starting firmness. This variability does suggest that there is some, albeit perhaps limited, scope for optimisation of the pre-heating step before HPS for texture retention. If the texture of the material at the start of a HPS cycle is optimised and as firm as possible then it is reasonable to expect that the final texture of the product after the HPS cycle would also be improved.

The use of ohmic heating as a pre-heating method greatly reduced cook values for colour degradation (down to 0.24, 0.12, 0.35 from 3.02, 2.50, 3.70 for ohmically heating and water bath heated samples respectively). These reduced cook values yielded significant benefits in terms of the retention of a bright green colouration. Values of a^* and b^* for ohmically pre-heated samples were close to that of blanched beans indicating that a rapid volumetric heating method such as ohmic or microwave could yield significant benefits to the overall quality of HPS treated products by minimising undesirable changes prior to pressure sterilisation.

The pre-heating method employed in Chapters 4 and 5 – i.e. filling into the vessel, adding hot water and then allowing equilibration to occur is far from optimal at either a lab or commercial scale because the pouches would be densely packed into an insulated vessel and

would therefore have a relatively slow heating rate. Heating the packs loose with a high ratio of heat transfer fluid to product (as in the water-bath studies described here) or even heating products and then hot filling would be more efficient in terms of optimising the pre-heat step but both options would be considerably more complex in terms of managing the overall HPS cycle and our studies indicate that water bath pre-heating would still lead to substantial losses in product quality.

If un-packaged product were volumetrically heated (e.g. with ohmic or microwave heating) up to the starting temperature for the HPS cycle then this product would have to be filled into pouches and loaded into the vessel without an appreciable loss of temperature which would be logistically complex. Despite this additional complexity, it is likely to be worthwhile to design a system of this nature if it is desirable to optimise product quality at the start of the HPS cycle.

7 Conclusion and recommendations

Chapter one of the thesis provided context for the interest in emerging preservation technologies as alternatives to conventional heat processing; specifically, consumer drivers for minimally processed products and the limitations of existing processes to address these drivers. A range of alternative emerging technologies was briefly reviewed and the current commercial status of high-pressure *pasteurisation* was outlined.

Thermal preservation is effective and produces reasonable quality food at an affordable price for consumers. However, thermal processing often causes deleterious food quality changes (notably colour and texture changes) in fruit and vegetables as was demonstrated in Chapters 2, 4, 5 and 6. Consumer demand for minimally processed food products has driven research into alternative technologies that can preserve whilst causing minimal product changes. There are many such technologies under development. Of these, high pressure pasteurisation is now a relatively mature technology and can be used to inactivate vegetative organisms whilst often retaining 'fresh-like' product characteristics. The process is limited in capability because of the resistance of bacterial spores to high pressure. A number of approaches have been suggested in order to sterilise foods using high pressure (see chapter 2) but there is still limited published data to show that theoretical quality benefits can be realised.

The remainder of the thesis focused on the use of high pressure in combination with elevated temperatures in order to produce ambient shelf stable products. This process is commonly described as high pressure sterilisation (HPS) or pressure assisted thermal sterilisation

(PATS). Specifically, the thesis explored quality changes in vegetables and focused primarily on green beans as a representative green vegetable that might be included in, for example, a prepared recipe dish. The central question being addressed by the thesis was whether high pressure could be used in combination with elevated temperatures to produce vegetable products with improved sensory properties compared with that achievable using conventional thermal processing.

Chapter 2 reviewed published approaches for producing ambient shelf stable products using pressure, with a focus on its combination with heat, as well as exploring the available data on quality changes in foods at high pressure *sterilisation* conditions. It highlighted that data on food quality changes at high-pressure sterilisation conditions are still limited and demonstrated the need for objective data to be gathered comparing product quality from high pressure sterilisation processes with that achievable from conventional heating.

Chapter 3 described the experimental equipment used in the studies. Existing Campden BRI equipment was modified by the inclusion of a PEEK liner and a re-designed thermocouple feedthrough on the top closure. Characterising temperature distribution within a high pressure vessel proved to be challenging. There were inherent difficulties in creating entry points for thermocouples at operating pressures of up to 700 MPa. On a commercial scale it could prove difficult to fit a sufficient number of thermocouples to a vessel in order to carry out a robust analysis of temperature distribution. Remote or wireless logging would be a much more practical solution.

Chemical or biological pressure/temperature integrators would be extremely valuable for process establishment but none are currently available that quite match the required duty for pressure sterilisation. Minerich & Labuza (2003b) suggested the use of compressed copper tablets as pressure integrators but the tablets are not responsive to changes in temperature. They could therefore prove useful for pasteurisation pressure applications but not for sterilisation. Bauer & Knorr (2005) suggested the use of starch gelatinisation as a pressure time temperature integrator; this showed promise but it is likely that a great deal more practical evaluation would be required before it could be usefully applied as an analytical tool. Vervoort *et al*. (2011) proposed the use of xylanase from hyperthermophilic *Thermotoga maritima*as an indicator for temperature gradients in high pressure high temperature processing and this shows some potential.

In the Campden BRI pressure equipment, temperature variation and heat losses within the vessel were substantial during the pressure hold period (Heat losses amounted to between 1.1 and 4.1°C per minute depending on process conditions). There are a number of technical solutions available that could minimise heat losses – principally those outlined by van Schepael *et al*. (2002b). During these studies, there were practical restrictions that limited the methods that could be employed to minimise heat losses. In a custom designed system, temperature losses could be greatly reduced. Temperature variability is not in itself an insurmountable problem for the technology. However, it is fundamentally important that temperature variability can be accurately characterised, such that for any given run, the area of lowest lethality can be accurately predicted.

Temperature distribution in a pressure/temperature combination process is complex and highly dependent on the specific design features of the equipment being tested. Analysis of

distribution data should take into account both worst case average temperatures and worst case heat distribution, as the two measures can not be assumed to highlight the same 'cold spot' in the vessel. It should also be noted that heat distribution measurements assume an underlying mechanism of heat inactivation for the target microorganism that may not hold true when pressure is also applied.

Chapter 4 described experiments in a high pressure vessel in which colour and quality changes in products processed at a single pressure/time/temperature combination were compared with those observed in conventional canning. The experiments clearly demonstrated that improvements in texture retention were possible in the case of green beans processed using HPS compared with a conventional thermal process of up to F_0 3 minutes, but the case was less compelling for improvements in carrot samples.

Data from our studies on green beans would suggest that texture improvements appear to be largely due to the reduced thermal process applied in HPS treatments compared with conventional canning. It seems likely that greater levels of texture retention could be achieved if peak temperatures during the cycle could be reduced. This could potentially be achieved with the use of higher pressures than are currently commercially achievable as suggested by the results of Krebbers *et al*. (2002).

In the case of green beans, the colour of HPS treated samples was not improved compared with traditionally canned samples and in some respects was less desirable as the HPS samples were darker. HPS carrot samples were initially a deeper orange compared with F_0 3 samples but a bleaching effect observed on storage that suggested that either pre-blanching or assuring

peak temperatures during HPS of greater than 109.7°C might be required to adequately control spoilage enzymes. It might be possible to improve colour retention with the use of higher pressures and reduced peak temperatures, giving due consideration to enzyme inactivation and the requirements for microbiological stability.

With respect to the efficacy of HPS for microbiological inactivation, Chapter 4 provided supporting results but was in no way intended to be conclusive. Validating the microbiological safety of pressure sterilised samples is complex as it is likely to require challenge testing on a product by product basis until such time as reliable models are available that accurately predict the inactivation of *Clostridium botulinum* on a worst case basis. For process establishment, a detailed understanding of temperature distribution within a commercial scale high pressure vessel is also required and it is very difficult to map temperatures with a meaningful number of thermocouples at such high operating pressures as was demonstrated in Chapter 2.

Chapter 5 built on the data provided in Chapter 4 through a further set of experiments which provided quality data in green beans, in terms of colour changes, texture change and chlorophyll retention, at a range of pressures, temperatures and times and provided insight into the relative influence of each factor on the resulting product quality. Surface response methodology was a useful technique for determining the relative importance of where pressure, temperature and time exerted an influence on the observed variations in colour and texture as a result of processing. In essence, texture changes were essentially related to the temperature used for each process, higher temperatures resulted in a greater loss in texture. Since pressure had little effect on texture, it is conceivable that by minimising peak

temperatures and using very high pressures, very good texture retention could be achievable. This conclusion was supported by the work reported in Chapter 4 and by that of Krebbers *et al*. (2002); the use of lower peak temperatures and higher pressures resulted in better texture retention in green beans. However, to truly realise the potential benefits of this approach there is an inherent assumption that pressures and temperatures can be modified in such a way as to deliver 'equivalent' lethalities i.e. that a low temperature high-pressure could deliver the same microbiological lethality as a high-temperature low-pressure process. This has far from been comprehensively demonstrated for spore inactivation. In addition, commercially achievable pressures are currently limited to 600 MPa which means that peak temperature by necessity must currently be high in order to assure commercial sterility.

Colour parameters changes were predicted primarily by time and pressure so it seems likely that in the case of green beans, a deterioration in quality for a commercially sterile product is inevitable when using a pressure-time-temperature process. However, it should be noted that colour changes have previously been found to be very much product dependent as noted by Matser *et al.* (2004); here the spectrum of colour retention ranged from complete retention of raw colour through to colour equivalent to that achievable by conventional preservation.

Chapter 6 described experiments exploring the influence of the pre-heating method as a pre-curser to a HPS treatment. The chapter provided data on colour and texture retention in green beans when pre-heated using conventional heating or ohmic heating along with the effects of pH modification and calcium chloride addition.

Results from studies described in Chapter 6 demonstrated that beans were relatively resilient to texture losses as a result of the pre-heating method and there was little benefit in employing a rapid heating method if the goal was solely to retain product texture. However, samples pre-heated to 86°C were firmer than raw beans (16-20%) regardless of the method employed whereas samples that were pre-heated to 86°C for HPS in the work described in Chapter 4 had a loss in firmness of around 12% of the starting firmness. This variability does suggest that there is some, albeit perhaps limited, scope for optimisation of the pre-heating step before HPS for texture retention. If the texture of the material at the start of a HPS cycle is optimised and as firm as possible then it is reasonable to expect that the final texture of the product after the HPS cycle would also be improved.

The use of ohmic heating as a pre-heating method greatly reduced cook values for colour degradation (down to 0.24, 0.12, 0.35 from 3.02, 2.50, 3.70 for ohmically heating and water bath heated samples respectively). These reduced cook values yielded significant benefits in terms of the retention of a bright green colouration. Values of a^* and b^* for ohmically pre-heated samples were close to that of blanched beans indicating that a rapid volumetric heating method such as ohmic or microwave could yield significant benefits to the overall quality of HPS treated products by minimising undesirable changes prior to pressure treatment.

The pre-heating method employed in Chapters 4 and 5 – i.e. filling into the vessel, adding hot water and then allowing equilibration to occur is far from optimal at either a lab or commercial scale because the pouches would be densely packed into an insulated vessel and would therefore have a relatively slow heating rate. Heating the packs loose with a high ratio of heat transfer fluid to product (as in the water-bath studies described here) or even heating

products and then hot filling would be more efficient in terms of optimising the pre-heat step but both options would be considerably more complex in terms of managing the overall HPS cycle and our studies indicate that water bath pre-heating would still lead to substantial losses in product quality.

If un-packaged product were volumetrically heated (e.g. with ohmic or microwave heating) up to the starting temperature for the HPS cycle then this product would have to be filled into pouches and loaded into the vessel without an appreciable loss of temperature which would be logistically complex. Despite this additional complexity, it is likely to be worthwhile to design a system of this nature if it is desirable to optimise product quality at the start of the HPS cycle.

High Pressure Sterilisation does offer the potential to improve the quality of ambient stable products but accurate, controlled and reproducible production cycles will be complex to achieve on a commercial scale. Considerable quality losses can occur at the pre-heat stage and a focus on optimisation of this step could yield considerable benefits.

Our present understanding of microbial inactivation using HPS suggests that the thermal effects are critical to the overall efficacy of the process but it may one day be possible to achieve an equivalent degree of lethality using milder heating in combination with much higher pressures than are currently commercially achievable. Until such time as this effect has been comprehensively demonstrated the food industry is likely to be restricted to processing at a maximum pressure of 600 MPa and attaining a thermal process equivalent to at least 121.°C for 3 minutes. This raises an important question as to whether the quality

improvements attainable are *sufficiently good* to warrant the additional processing costs and complexity. The industry may be better served by focusing on process optimisation of existing thermal processes where considerable over process is routinely employed the purpose of ensuring an additional safety margin. In addition, the need for consistency of raw material supplies has led to the wide-spread use of Individually Quick Frozen (IQF) ingredients which have often already been reduced in quality compared with fresh raw materials. Reduced quality ingredients which are then further heat-processed leads to even greater quality losses. It would be an extremely interesting exercise to focus on exactly what quality improvements could be attained using an optimised conventional thermal process with high quality raw materials and to compare these products to an optimised HPS cycle using currently achievable pressures.

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Appendix 1 – review of published spore inactivation studies using HPS conditions adapted from Wilson *et al*. (2008)

Organism	Matrix	Pressure fluid	Pressure (MPa)	Processing Temp (°C)	IT of pressure fluid (°C)	Peak temp of pressure fluid (°C)	Final temp of pressure fluid (°C)	Hold time (mins)	Log reduction	Ref
Clostridium botulinum type A	Phosphate buffer pH 7.0	50% glycol water fluid	<689	75	N/A	N/A	N/A	20	<2	Reddy *et al*. (2003)
C.botulinum type A	Phosphate buffer pH 7.0	50% glycol water fluid	827	75	60.3±1.1	92.4±2.3	74.3±0.5	20	3.0	Reddy *et al*. (2003)
C.botulinum type E Alaska	Phosphate buffer pH 7.0	2% sodium benzoate sol^{n}	827	<35	12.9±0.4	43.0±0.2	36.2±0.4	5	<1.5	Reddy *et al*. (1999)
C.botulinum type E Alaska	Phosphate buffer pH 7.0	2% sodium benzoate sol^{n}	827	40	16.7±0.8	46.3±0.8	39.7±0.9	10	5	Reddy *et al*. (1999)
C.botulinum type E Alaska	Phosphate buffer pH 7.0	2% sodium benzoate sol^{n}	827	55	35.5	62.9	54.0	5	5	Reddy *et al*. (1999)
C.botulinum type E Alaska	Phosphate buffer pH 7.0	2% sodium benzoate sol^{n}	689	50	N/A	N/A	N/A	5	<1.5	Reddy *et al*. (1999)
C.botulinum type E Alaska	Phosphate buffer pH 7.0	2% sodium benzoate sol^{n}	827	35	12.9±0.4	43.0±0.8	39.7±0.9	5	<1.5	Reddy *et al*. (1999)

Organism	Matrix	Pressure fluid	Pressure (MPa)	Processing Temp (°C)	IT of pressure fluid (°C)	Peak temp of pressure fluid (°C)	Final temp of pressure fluid (°C)	Hold time (mins)	Log reduction	Ref
C.botulinum type E Alaska	Phosphate buffer pH 7.0	2% sodium benzoate sol^{n}	827	50	28.9±0.4	57.0±1.1	49.1±1.1	5	5	Reddy *et al*. (1999)
C.botulinum type E Beluga	Phosphate buffer pH 7.0	2% sodium benzoate sol^{n}	827	40	16.7±0.8	46.3±0.8	39.7±0.9	5	<1.5	Reddy *et al*. (1999)
C.botulinum type E Beluga	Phosphate buffer pH 7.0	2% sodium benzoate sol^{n}	827	60	36.8±0.5	67.2±0.2	59.0±0.4	5	5	Reddy *et al* (1999)
C.sporogenes NCIMB 8053	Distilled water	10% Dromus oil in water emulsion	400	60	N/A	N/A	N/A	30	<1	Mills *et al*. (1998)
C.sporogenes NCIMB 8053	Distilled water	10% Dromus oil in water emulsion	Sequential 60 and 400 MPa cycle	60	N/A	N/A	N/A	90	<1	Mills *et al*. (1998)
C.sporogenes PA 3679	McIlvaine citrate phosphate buffer pH 4.0	2% hydraulic fluid solution (hydrolubric 142)	404	45	N/A	N/A	N/A	15	2	Stewart *et al*. (2000)
C.sporogenes PA	McIlvaine	2%	404	45	N/A	N/A	N/A	15	1	Stewart *et*

Organism	Matrix	Pressure fluid	Pressure (MPa)	Processing Temp (°C)	IT of pressure fluid (°C)	Peak temp of pressure fluid (°C)	Final temp of pressure fluid (°C)	Hold time (mins)	Log reduction	Ref
3679	citrate phosphate buffer pH 5.0	hydraulic fluid solution (hydrolubric 142)								*al*. (2000)
C.sporogenes PA 3679	Mcllvaine citrate phosphate buffer pH 4.0	2% hydraulic fluid solution (hydrolubric 142)	404	70	N/A	N/A	N/A	15	6	Stewart *et al*. (2000)
C.sporogenes PA 3679	Mcllvaine citrate phosphate buffer pH 4.0	2% hydraulic fluid solution (hydrolubric 142)	404	70	N/A	N/A	N/A	15	4	Stewart *et al*. (2000)
C.sporogenes PA 3679	Mcllvaine citrate phosphate buffer pH 7.0	2% hydraulic fluid solution (hydrolubric 142)	404	70	N/A	N/A	N/A	15	<0.5	Stewart *et al*. (2000)
C.sporogenes	Chicken	Mineral oil	689	80	N/A	100	N/A	5	5	Crawford *et*

Organism	Matrix	Pressure fluid	Pressure (MPa)	Processing Temp (°C)	IT of pressure fluid (°C)	Peak temp of pressure fluid (°C)	Final temp of pressure fluid (°C)	Hold time (mins)	Log reduction	Ref
ATCC 7955	breast meat									*al*. (1996)
C.sporogenes ATCC 7955	Citric acid buffer pH6.5	5% hydrolubric 120-B in water	650	55	N/A	N/A	N/A	15	0.4±0.0	Paredes-Sabja *et al*. (2007)
C.sporogenes ATCC 7955	Citric acid buffer pH6.5	5% hydrolubric 120-B in water	650	75	N/A	N/A	N/A	15	1.8±0.7	Paredes-Sabja *et al*. (2007)
C.sporogenes ATCC 3584	Citric acid buffer pH6.5	5% hydrolubric 120-B in water	650	55	N/A	N/A	N/A	15	0.6±0.2	Paredes-Sabja *et al*. (2007)
C.sporogenes ATCC 3584	Citric acid buffer pH6.5	5% hydrolubric 120-B in water	650	75	N/A	N/A	N/A	15	2.5±1.0	Paredes-Sabja *et al*. (2007)
C.sporogenes ATCC 7955	Citric acid buffer pH4.75	5% hydrolubric 120-B in water	650	55	N/A	N/A	N/A	15	0.8±0.1	Paredes-Sabja *et al*. (2007)
C.sporogenes ATCC 7955	Citric acid	5% hydrolubric	650	75	N/A	N/A	N/A	15	5.7±0.2	Paredes-Sabja *et al*.

Organism	Matrix	Pressure fluid	Pressure (MPa)	Processing Temp (°C)	IT of pressure fluid (°C)	Peak temp of pressure fluid (°C)	Final temp of pressure fluid (°C)	Hold time (mins)	Log reduction	Ref
	buffer pH4.75	120-B in water								(2007)
C.sporogenes ATCC 3584	Citric acid buffer pH4.75	5% hydrolubric 120-B in water	650	55	N/A	N/A	N/A	15	0.8±0.1	Paredes-Sabja *et al.* (2007)
C.sporogenes ATCC 3584	Citric acid buffer pH4.75	5% hydrolubric 120-B in water	650	75	N/A	N/A	N/A	15	6.0±0.0	Paredes-Sabja *et al*. (2007)
Bacillus cereus ATCC 14579	McIlvane Citrate phosphate buffer pH 7.0	5% mobile Hydrasol 78 in water	690	40	N/A	N/A	N/A	2	8	Raso *et al*. (1998a)
Bacillus subtilis As 1.1731	Milk buffer pH 7.0	Bis (2-ethylhexyl) sebacate	479	46	87	N/A	N/A	14	6	Gao & Jiang (2005)
C.botulinum type B	Mashed carrot	80% ethanol 20% rhizinus oil mixture	600 MPa ramp at 2 MPa/s	80	80	100	80	70	5	Margosch *et al*. (2004)
C.botulinum type	Mashed	80%	600 MPa	80	80	116	80	6	5	Margosch

Organism	Matrix	Pressure fluid	Pressure (MPa)	Processing Temp (°C)	IT of pressure fluid (°C)	Peak temp of pressure fluid (°C)	Final temp of pressure fluid (°C)	Hold time (mins)	Log reduction	Ref
A	carrot	ethanol 20% rhizinus oil mixture	ramp at 6 MPa/s							*et al*. (2004)
C.botulinum type B	Mashed carrot	80% ethanol 20% rhizinus oil mixture	800	80	80	116	80	4	2.3	Margosch *et al*. (2004)
C.botulinum type A	Crabmeat	50% glycol water fluid	827	75	62.0±3.2	95.6±4.4	76.1±1.0	15	3.2	Reddy *et al*. (2003)
C.botulinum type A	Crabmeat	50% glycol water fluid	827	75	60.3±1.1	92.4±2.3	74.3±0.5	20	2.3	Reddy *et al*. (2003)
Bacillus Amyloliquefaciens	Mashed carrot	80% ethanol 20% rhizinus oil mixture	800	70	N/A	N/A	N/A	64	2.1	Margosch *et al*. (2004)
Bacillus Amyloliquefaciens	Mashed carrot	80% ethanol 20% rhizinus oil mixture	800	80	N/A	N/A	N/A	4	1.15	Margosch *et al*. (2004)
Bacillus	Egg pattie	Propylene	700	105	67	N/A	N/A	3	5	Rajan *et al*.

Organism	Matrix	Pressure fluid	Pressure (MPa)	Processing Temp (°C)	IT of pressure fluid (°C)	Peak temp of pressure fluid (°C)	Final temp of pressure fluid (°C)	Hold time (mins)	Log reduction	Ref
Amyloliquefaciens	mince	Glycol								(2006)
Bacillus Amyloliquefaciens	Egg pattie mince	Propylene Glycol	700	110	73	N/A	N/A	3	7	Rajan *et al*. (2006)
Bacillus Amyloliquefaciens	Egg pattie mince	Propylene Glycol	700	121	84	N/A	N/A	3	nd	Rajan *et al*. (2006)