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Pilot trials to determine the benefits of high pressure processing (HPP) for seafood in the UK

Report on phase 1 studies:

Confidential to:

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Executive Summary

Aside from irradiation, HPP is perhaps the most widely researched and commercially developed emerging non-thermal preservation technique for food processing. As currently used, HPP is an essentially non-thermal pasteurisation process in which a food is subjected to pressures in the region of 150 MPa to around 600 MPa (1500 to 6000 bar) and held at pressure for a time, generally under 10 minutes. A hold time of less than 5 minutes is recommended if this achieves the required processing objective.

As of September 2007, it was estimated that there were approximately 115 full scale industrial units in operation world wide. As of August 2006, around 60% of these vessels were in the USA, and around 20% were in Europe. There is only 1 commercial HPP plant in the UK: the Bare Fruit Products company in Belfast, manufacturing fruit smoothies under the 'Puro' brand-name. Spanish meat products are available in some UK retail outlets. Of the current world-wide HPP applications, almost 20% are for seafood processing. Current seafood applications include oyster shucking and lobster de-shelling. Whilst commercial products exist outside the UK there is limited independent public-domain 'know-how' in the UK regarding the pressure treatment of seafood. The main objective of this project is to redress this knowledge gap.

High pressure processing studies were carried out on 11 species of fish and shellfish in order to determine whether there were any potential processing benefits for the UK seafood processing industry. The species tested were: *Nephrops norvegicus*, mussels, oysters, crab, cold water prawns, lobster, warm water prawns, unsmoked salmon, squid, mackerel and cod.

In each case, a 20-run experimental design was employed and six of the 20 runs (from a single design block) were evaluated for sensory quality compared with a control sample. After processing, all 20 runs were evaluated for survival of TVCs, coliforms and pseudomonads. In some products, measurements of yield were carried out to determine

whether HPP offered any processing benefits, e.g. for peeling, picking or shucking of seafood.

Key findings for *Nephrops norvegicus*

Yield benefits as a result of HPP appear very promising; a yield increase of up to 3% was measured using 270 MPa, 1°C for 5 minutes. Further work is required to determine whether the apparent yield benefits seen with hand peeling are transferrable to commercial peeling operations. Pseudomonads were found to be very pressure sensitive in *Nephrops* which could prove useful for shelf life extension. Storage trials may form part of the second phase of the project to determine whether significant shelf-life extension can be achieved. Sensory results suggested that sour and astringent flavours could develop in the product along with 'rubbery' textures in some instances. Pressures of around 300 MPa gave the best sensory results.

Key findings for mussels and oysters

The main benefit from HPP treatment of mussels and oysters was the 'automatic' shucking that was achievable along with an increase in meat weight after processing. Muscle meat for example increased, on average, by 34%. This effect could also be utilised to introduce flavours into the product and to produce value-added products. Mussels were completely released from the shell at almost all conditions tested. Oysters tended to still have some loose attachment to the shell but could probably be separated by a simple vibrating belt. To prevent liquor loss post-process, it would be necessary to manually seal the product shut in some way, i.e. as per the 'gold-band' commercial oyster products. The effects of HPP on sensory quality appear to be favourable within the limits of the conditions tested. Samples were generally perceived to be plump, attractive and less chewy than the control. The degree of plumping in the case of oysters was related to the pressure and time applied and was therefore controllable.

Key findings for crab and lobster

Reductions in total viable counts were low in crab (<2 log reductions) but much higher in lobster (at least 4 log reductions in TVC); this could be due to practical limitations in terms of handling the product after picking, raw material variation or genuine differences in microbial resistance in the two substrates. The highest picking yield for crab was 53% found using a process of 250 MPa, 2.5 mins, 15°C or 300 MPa, 2.5 mins, 16°C, but no comparative picking yields could be taken on raw crabs. There was some run-to-run variability as to ease of picking of crab and larger scale studies are required to assess the yield benefits achievable using HPP compared with conventional processing. As a general though subjective point, it appeared that when removing the legs of crabs after processing, more meat could be removed from the purse. This finding, if transferrable to industrial operations, could be commercially significant because 'intact' purse meat commands a higher value than mechanically recovered purse meat. Yield on lobster claws was up to 23% higher than on cooked controls but this data did not take in to account cooking losses that could have occurred in the control sample. In general, pressures of greater than 270 MPa gave better results for tail yield in lobsters. Indications from the work are that overall picking yields from lobsters and crabs could be enhanced significantly because meat could be readily extracted even from the legs – gentle squeezing was sufficient to remove the meat from lobster legs. All treatments used pressure of between 200 and 300 MPa. Crab samples treated close to 300 MPa tended to score lower in terms of sensory quality and 'eggy' notes were sometimes reported. In many instances HPP treated lobster was considered to be higher quality than the control.

Key findings for cold and warm water prawns

Peeling yield for cold water prawns varied between 39% and 46% for the pressure treated samples but was on average 44%, which was the same as that of the control. A 2% increase in yield would be commercially significant, i.e. if 46% could be consistently achieved compared with 44% using normal production methods, but it is difficult to

determine whether these yield increases are genuine or are within the measurement errors inherent in the manual peeling process. Examination of the sample images from the trials would suggest that the very tip of the prawn was more frequently extracted compared with the control samples and therefore the yield increases could be genuine. In the case of warm water prawns peeling yields were enhanced substantially by HPP treatment, typically being 55-57% compared with 46% for the control. The highest yield was obtained using a process of 237 MPa at 11.1°C for 2.5 minutes and this pressure also gave the best sensory results. Cooked, pressure treated samples matched the controls for sensory quality. The tip of warm water prawns could be extracted fully intact in warm water prawns after pressure treatment. Larger-scale trials are required in order to give a more objective measure of peeling yield in a commercial context for both products. Larger trials are planned on warm water prawns in the second phase of the project to address this question more fully.

Key findings for salmon, squid, mackerel and cod

HPP was very effective for microbial inactivation with TVC, coliforms and pseudomonads being reduced to the limits of detection even with starting levels in the region of 10^5 cfu/g in some instances. However, pressure treatments in excess of 200 MPa generally resulted in fish products taking on a cooked appearance. This effect became increasingly noticeable as pressure increased. HPP is therefore unsuitable for use as a pasteurisation process if it is desirable to maintain the raw appearance of fish. However, it could still prove very useful for the processing of ready meals and other added-value products where a cooked appearance is not necessarily a problem. The cooked quality of pressure treated fish could match that of control samples but in some cases the product developed a very dense texture and was perceived to be of lower quality than controls. HPP could potentially be useful for enhancing marination using pressures below 200 MPa.

Future work

Five products have been short-listed for further work; these are: *Nephrops*, warm water prawns, crab, salmon and cod. Trials on crab, warm water prawns and *Nephrops* will focus on large scale picking/peeling trials to determine whether the yield benefits identified in phase 1 are transferrable to a commercial scale. Two commercial processors are participating in these studies. Trials on salmon and cod will focus on two areas: low pressure for marination and the production of ready-meals to compare product quality with that of conventional heat processed ready-meals.

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Contents

List of Figures

List of tables

1 Introduction

1.1 Basic principles of HPP

Aside from irradiation, HPP is perhaps the most widely researched and commercially developed emerging non-thermal preservation technique for food processing. As currently used, HPP is an essentially non-thermal pasteurisation process in which a food is subjected to pressures in the region of 150 MPa to around 600 MPa (1500 to 6000 bar) and held at pressure for a time, generally under 10 minutes. A hold time of less than 5 minutes is recommended if this achieves the required processing objective (Purroy 2007).

A small temperature rise is observed during pressurisation. This rise is typically around 3-4°C per 100 MPa of applied pressure for predominantly aqueous foods but varies depending on the product composition [see for example de Heij *et al* (2003); Ting *et al* (2002); Rasanayagam *et al* (2003)]. Although this is a small temperature rise, it can have significant implications on the overall lethality of the process, especially when pressure is combined with moderate heating. Consequently, an understanding of temperature variation within the vessel is important for commercial processing.

The majority of HPP products are packed and then filled into batch pressure vessels for treatment. The pressure medium is generally water. In the case of seafood applications, the product is generally brought into direct contact with the pressure medium. Commercial systems are designed to run with filtered sea water or 1% salt water in the main vessel (personal communications with NC Hyperbaric and Avure). In the case of NC Hyperbaric systems sea water can be used in the vessel but only mains water is connected to the pump intensifiers in order to extend pump seal life. Whether the product is packaged or not, the pressure applied can, when sufficiently high, inactivate vegetative microorganisms.

Since the process does not involve heating, the sensory and nutritional quality of products can be remarkably similar to their unprocessed counterpart. However, since HPP can affect the tertiary structure of proteins, colour and texture changes can and do occur in some cases. Enzyme resistance to HPP is product dependent and stabilising the product to enzymic changes must therefore be assessed on a case-by-case basis. Bacterial spores are very resistant to commercially achievable pressures. As a result, products that are currently on the market tend to be chilled and many are high acid or contain additional hurdles for microbial growth such as the presence of antimicrobial compounds.

1.2 Current commercial 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 is a selection of some of the products manufactured in Europe and the USA using high pressure processing. Fruit smoothies and Tapas products are now available in the UK.

Company	Product
Fresherized Foods (USA)	Range of guacamole, salsa dips and 'meal kits'
Motivatit Seafood (USA)	'Gold band' Oysters
Goose Point Oysters (USA)	Oysters
Hannah International (USA)	Hummous
Hormel Foods (USA)	Prepared ham products
Joey Oysters (USA)	Oysters
Oceans Choice (Canada)	Lobsters
Purdue Farms (USA)	Prepared poultry products
Shucks Maine Lobster (USA)	Lobster
Espuna (Spain)	Vacuum packed sliced ham, tapas
Pampryl (France)	Range of fruit juices
Frubaca (Portugal)	Apple juice
Espuna (imported into the UK)	Tapas
Bare Fruit Products (UK)	Fruit smoothies

Table 1. Examples of HPP products world-wide

As of September 2007, it was estimated that there were approximately 115 full scale industrial units in operation world wide (Purroy 2007). As of August 2006, around 60% of these vessels were in the USA, and around 20% were in Europe (Leadley 2007). As far as CCFRA is aware, there is only 1 commercial HPP plant in the UK: the Bare Fruit Products company in Belfast, manufacturing fruit smoothies under the 'Puro' brand-name. Spanish meat products are available in some UK retail outlets. Of the current world-wide HPP applications, almost 20% are for seafood processing.

1.3 Previous work on HPP effects on seafood

Whilst there are a growing number of seafood processors using HPP worldwide, there is very limited technical knowledge in the UK (in the public domain) regarding pressure processing of seafood. Much of the research carried out on high pressure processing of

seafood has focused on microbiological effects rather than functional benefits or quality changes. Often when quality parameters have been the subject of study, the hold times at pressure have been excessively long for commercial applications (i.e. greater than 5-10 minutes). Examples of work done to date are summarised in Table 2.

Reference	Species of fish	Pressure/time	Results
(Ramirez-Suarez & Morrissey 2006)	Albacore Tuna (Thunnus alalunga)	275 MPa and 310 MPa for 2,4, and 6 minutes	HPP treated sample had a slight increase in pH. Microbiological results showed that HPP can prolong the shelf life to > 22 days at 4°C. 310 MPa for 6 minutes resulted in the microorganisms not being able to recover during storage. HPP gave a higher gel hardness level than the controls in all the pressure treated samples. HPP resulted in significant colour change when compared to the control; the product became lighter and whiter as pressure and time increased.
(Ashie & Simpson 1996)	Bluefish and Sheephead	1000-3000 atmospheres for various time durations	The results showed that seafood enzymes were more sensitive to high pressure than the mammalian counterparts due to the environmental habitat. Colour changes were detected with the applied pressure; as the pressure increased the L* value of the fish increased, i.e. the fish became lighter. The b* values were reduced with pressure and time. Up to 2000 atmospheres for 10 minutes there was no significant difference in colour but above this the differences were significant.

Reference	Species of fish	Pressure/time	Results
(Hurtado, Montero, & Borderias 2006)	Octopus (Octopus vulgaris)	400 MPa for 15 min 400MPa in 3 5-min pulses at 7 and 40°C	Microbial counts were lower in the pressurized samples than in the controls, and the counts in the pulsed samples were generally lower than in the continuous processes for both temperatures. Production of Trimethylamine was greatly reduced in all pressurized samples. This was thought to be due to the decreased number of Gram negative bacteria which produce this compound. The levels of total volatile bases increased with storage in the control but remained constant at lower levels in the pressurized samples. The level of hardness in the controls decreased during storage whereas the levels of hardness in the pressurized samples remained stable throughout storage.
(Montero, Lopez-Caballero, & Perez-Mateos 2001)	Prawns (Penaeus japonicus)	400 MPa for 10 min at 7°C	Study compared pressurised sample and pressurised samples plus melanosis inhibitors. HPP appeared to increase melanosis but melanosis inhibitors were effective with and without HPP. Authors proposed a combination approach.

Reference	Species of fish	Pressure/time	Results
(Lopez-Caballero et al. 2000a)	Prawns (Penaeus japonicus)	200 and 400 MPa at 7°C for 10 mins	Study compared Vacuum packaging (VP) and VP HPP of prawns. Neither the VP nor HPP had an effect on the shear strength of the prawn muscle. Both methods decreased the levels of melanosis compared to the controls and increased the shelf life of the product. There were reports that the colour was affected in the HPP samples with a slight whitening of the product.
(He et al. 2002).	Oysters (Crassostr-ea gigas)	207 MPa for 2 min, 242 MPa for 0,1,2 min, 276 MPa for 0 and 1 min, 311 MPa for 0 min	Treatment of 242 MPa for 2 min caused 88% detachment of the adductor muscle. 310 MPa caused 100%; however, it was suggested that this would change with oyster type and season. HPP treatments extended the shelf life of the product in terms of aerobic and anaerobic plate counts, but coliforms increased above the controls after day 13 of storage.
(Koo et al. 2006)	Oysters (Crassostr-ea virginica and Crassost-rea gigas)	A range of pressure between 207 and 586 MPa and times between 0 and 6 min	Study into the inactivation of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in oysters. Both organisms were reduced to nondetectable levels after pressures of 586 MPa for 7-8 min.

Reference	Species of fish	Pressure/time	Results
(Lopez-Caballero et al. 2000b)	Oyster (Ostraea edulis)	400 MPa for 10 mins at 7°C or 400 MPa at 7°C for 5 mins	After high pressure treatment the oysters were more juicy and voluminous compared to the controls. The HPP treatment of oysters at 400MPa for 10 min at 7°C reduced the levels of target microorganisms.
(Calik et al. 2002)	Oysters (Crassost-rea gigas)	241 MPa for 1,60,120,240, 360, 480, 600 s. 276 MPa for 1, 60, 120, 180, 240, 300s. 310 MPa for 1,30,60,90,120, 180s. 345 MPa 1,10,20,30,40, 50, 60, 90, 120s	Study comparing the effect of pressure on *Vibrio parahaemolyticus* in pure culture and in oysters. *V.parahaemolyticus* was more barotolerant in the oysters compared to pure culture.
(Nagashi-ma et al. 1993)	Squid Mantle (Loligo bleekeri)	Up to 1000 MPa	Study on the gelation of squid mantle meat; this was formed at high pressure treatments above 600 MPa. Breaking strength values of pressurised samples were equivalent to thermally induced gels. But the breaking strain values were twice as high in the pressurised samples compared with the thermal induced samples. The colour of pressurised samples was white compared to yellow for the thermal induced gels.

Reference	Species of fish	Pressure/time	Results
(Paarup et al. 2002)	Squid Mantle (Todaropsis eblanae)	150, 200, 300 and 400 MPa for 15 min stored at 4°C	Pressures of between 200 and 400 extended the shelf life of squid; this altered the appearance of the sample from grey to a reddish colour.
(Linton, McClements, & Patterson 2003)	Mussels, prawns, scallops and oysters	300,400, 500 and 600 MPa for 2 min at 20°C and stored for up to 28 days at 2°C	The psychrotrophic counts in all the pressure treated samples were less than the controls and took longer to reach the cut off point in the shelf life test.
(Sequeira-Munoz et al. 2006)	Carp (Cyprinus carpio)	100, 140, 180 and 200 MPa at 4°C for 15 and 20 min	At levels of above 140 MPa the L* values of the fish increased with pressure and processing time. Values of a* and b* also increased as pressure increased with pressure and holding times above 140 MPa.
(Chevalier Bail, & Ghoul 2001)	Turbot (Scophthalmus maximus)	100,140, 180 and 200 MPa for 15 and 30 min at 4°C.	The samples which had been pressurised lost the transparent colour of raw fish and became lighter. Increased L* values with increased pressure and time.
(Amanatidou et al. 2000)	Fresh Atlantic Salmon	0,100,150, 200 MPa for 0,10,30, 60 min	Study measured the effectiveness of Modified Atmosphere and HPP on shelf-life of salmon. L* values of salmon increased with pressure treatment and time. Values of a* declined with increasing pressure and time and the product was unacceptable at 200MPa. The combined effect of HPP and MA increased the shelf life by at least 5 days compared to VP. Combination was more effective than either treatment alone.

Reference	Species of fish	Pressure/time	Results
(Fioretto et al. 2005)	Caviar	400 MPa for 15 min, 350MPa for 5 min in 3 cycles. 500 MPa for 15 min and 450 MPa for 5 min in 3 cycles	Inoculated caviar with *Staphylococcus aureus* and *Salmonella enteritidis*. Treatments of 500MPa /15 min and 450MPa/5 min x 3 cycles were successful in reducing levels of *S. aureus* and treatments of 400MPa/15 min and 350MPa/5 x 3 cycles were successful in reducing levels of *Salmonella enteritidis*.
(Matser et al. 2000)	Various species	100-1000 MPa for 5 min for colour trials. 100, 200 and 400 MPa for texture trials for 10 minutes	Most fish gave a change in colour at 150-200 MPa. Octopus retained its raw appearance until 400-800 MPa. Hardness increased as the pressure increased and product loss was increased at pressures of 200-400 MPa.

Table 2. Examples of existing work on HPP treatment of seafood

1.4 Equipment for pressure pasteurisation

There are two main types of HPP equipment available - batch and semi-continuous. Industrial food applications are almost exclusively batch. 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) is pumped into the vessel until the target processing pressure is reached and held at this pressure for the required time. 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 it is possible to

co-ordinate the process such that 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. Examples of vertical and horizontal batch pressure processing equipment for food use can be seen in Figure 1 and Figure 2.

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

Figure 2 A horizontal batch high pressure processing vessel (covers removed) from nc Hyperbaric (image courtesy of Carole Tonello, nc Hyperbaric)

There are a large number of equipment suppliers providing equipment on varying scales from laboratory through to full production scale models. A list of some of the larger manufacturers of high pressure processing equipment on an industrial scale can be found in Table 3. Market leaders are currently Avure Technologies and nc Hyperbaric. Both companies produce horizontal systems; Avure also produces vertical systems.

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
Kobelco (Kobe Steel Ltd) 9-12, Kita-Shinagawa-ku, Tokyo, 141-8688 Japan	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

Table 3 suppliers of commercial high pressure equipment for food processing

2 Objectives of the project and phase 1.

The seafood processing industry needs information on what high pressure processing can achieve and some typical treatment conditions. This project aims to provide this information, subject to the limitations of using laboratory scale equipment, and will allow industry to make informed choices on equipment purchases and test conditions. This project has the potential to identify new products and markets for the seafood industry and thus to improve profitability. In addition, the project could identify means of improving the safety of seafoods.

Specifically, this project aims to provide:

- Platform knowledge about a new technology for the UK industry
- Information for the fish and seafood sector for the development of individual consortium projects
- Data on the extraction of edible meat and aiding shell removal/opening from shellfish
- Information on the potential to develop new high added-value products
- On-going demonstration and experimental facilities for use by the seafood sector

Phase 1 was specifically designed to be a screening trial, to identify commercial opportunities and to identify a short-list of 5 products for further exploratory work.

3 Materials and methods

3.1 General methods

Samples were delivered to CCFRA and stored at an appropriate temperature prior to testing (-18°C for frozen materials and 2-5°C for chilled or live materials). For each process run, samples were weighed into bags with sufficient reverse osmosis treated water to just cover the sample. Unless otherwise stated, the bags used were vacuum pouches provided by The Vacuum Pouch Company Ltd, UK. Product weights were recorded for each sample pack. The bags were heat sealed ready for processing; care was taken to expel as much air as was practical from the packs prior to sealing. Samples were placed into a high pressure processing vessel. Vessels used were a Stansted Fluid Power (UK) system having a working volume of approximately 5L and a maximum operating pressure of 400 MPa, or a 700 ml system manufactured by Engineered Pressure Systems International (Belgium). The pressure fluid in the Stansted system was a 30% w/w propylene glycol solution (Stansted Fluid Power, UK). The pressure fluid in the EPSI system was a 3% w/w MKU solution (an oil based corrosion inhibitor supplied by EPSI). The temperature of the vessels was not controlled but product temperatures before and after treatment were recorded wherever possible. Although product and fluid temperature could not be measured during processing, compression heating effects for predominantly aqueous materials are unlikely to have exceeded 3-4°C per 100 MPa of applied pressure (Leadley 2005). Compression heating of 100% propylene glycol has previously been reported to be 5.8 ± 0.6 per 100 MPa of applied pressure (Rasanayagam *et al.* 2003). Using a mixture model suggested by Rasanayagam *et al*. (2003) and reported to have given reasonable results for propylene glycol, an approximation for compression heating of the pressure fluid in the Stansted fluid is between 3.5 and 4.2°C per 100 MPa of applied pressure. Extensive temperature testing has previously been undertaken by CCFRA for the EPSI system and compression heating of the fluid is typically around 4°C per 100 MPa of applied pressure, but this is rapidly dissipated unless steps are taken to

retain the heat in the system. Compression heating of the MKU solution is marginally influenced by the initial temperature of the fluid (Leadley 2006).

Samples were pressure treated according to the experimental designs detailed in section 3.2. Peak pressure, come-up time and come-down time were recorded for each run. After treatment, samples were drained for 2 minutes and re-weighed. A sub-sample from each process was taken, weighed and handled as appropriate for each species (see methods section for individual species for details). In all cases, sub-samples were photographed using a digital imaging system (DigiEye, UK). Sub-samples, generally from the first block of each design, were subjected to sensory evaluation. For clarity, sensory reports for all species including the method used have been written as self-contained reports that are included as appendices to the main report. Similarly, to improve the clarity of the report, all images of the samples have been included as appendices rather than in the body of the report. Images have occasionally been included within the body of the report when illustrating a specific point of discussion.

Sub-samples from every pressure cycle were evaluated for total viable counts, pseudomonads and coliforms. Microbiological methods are detailed in section 3.3. Where possible, samples for microbiology were separately bagged prior to pressure treatment but this was not practical in all cases, e.g. for crab and lobster where hand picking of the sample was required.

3.1.1 Method detail specific to *Nephrops norvegicus*

Frozen, un-peeled tails of *Nephrops norvegicus* were supplied by Young's Seafood Ltd, delivered to CCFRA and stored at –18°C prior to starting high pressure experiments. For each pressure treatment, approximately 500 g of tails (actual weights were recorded) were placed into vacuum bags along with sufficient reverse osmosis treated water to just cover the sample. The initial temperature of tails prior to high pressure treatment was

0°C (s.d. = 0.86°C, n = 20). The temperature of the sample after processing was on average 0.3°C cooler than before treatment (s.d. = 0.4°C, n=20).

Each sample was pressure treated according to the experimental design as outlined in Table 5. Twenty tails were sub-sampled from each run and were weighed and hand-peeled. The weight of meat and shell recovered from the 20 sample sub-set was recorded for each process condition and yields were calculated.

3.1.2 Methods detail specific to mussels

Depurated rope grown mussels were supplied from Cornish Mussels Ltd. Samples were delivered live on the day of testing and stored chilled until use. Approximately 500g of mussels were weighed into a sterile stomacher bag (actual weight recorded) and sufficient reverse osmosis treated water was added to just cover the mussels. Samples were triple bagged as early experiments had shown that the sharp edges of the mussels made the bags prone to puncture. Commercially this would not pose a problem because the mussels would generally be brought into direct contact with the pressure fluid. The initial temperature of mussels prior to high pressure treatment was 11.4°C (s.d. = 2.2°C, n=20). The temperature of the sample after processing was on average 1.5°C warmer than before treatment (s.d. = 2.0°C, n=20).

Each sample was pressure treated according to the experimental design outlined in Table 5. After treatment, a sub-sample of 30 mussels was taken and weighed. The 30 mussels were examined to see how effective the process had been for shucking the meat from the shells. The weight of the meat and shells was recorded and yields were calculated.

3.1.3 Method detail specific to oysters

Oysters were supplied by Colchester Oyster Fisheries Ltd. Oysters were delivered the day before processing and were stored at <3°C overnight. For each run, 10 oysters were placed in stomacher bags and sufficient reverse osmosis treated water was added to cover

the samples. Oysters were triple bagged to protect against puncture. The initial temperature of oysters prior to high pressure treatment was 13.1°C (s.d. = 1.1°C, n=20). The temperature of the sample after processing was on average 1.7°C warmer than before treatment (s.d. = 1.0°C, n=19).

Each sample was pressure treated according to the experimental design as outlined in Table 5. After treatment, the oysters were drained and re-weighed. The oysters were examined to see how effective the process had been for shucking the meat from the shells. The weight of the meat and shells was recorded and yields were calculated.

3.1.4 Methods detail specific to crab

Crabs were delivered live to CCFRA on the day of processing. Live and cooked samples were supplied by the Blue Seafood Company Ltd and technical staff from the company were present on the day of the trials. Crabs were stored at <3°C. Live crabs were stunned prior to pressure treatment using a Crustastun humane crustacean stunner (Crustastun, UK). Crabs were weighed and filled (double bagged) into vacuum bags. Bags were then filled with sufficient reverse osmosis treated water to just cover each crab. The initial temperature of the crabs prior to high pressure treatment was 14.8°C (s.d. = 1.5°C, n=18). The temperature of the sample after processing was on average 1.6°C colder than before treatment (s.d. = 1.4°C, n=18).

Each sample was pressure treated according to experimental design as outlined in Table 5. After treatment, the crab was drained for 2 minutes and re-weighed. The claws of the crab were removed and weighed. The claws were then manually picked and subjectively assessed for ‘ease of picking’. The weight of meat and shell was recorded and yields were calculated. Meat was extracted from the legs of the crab for microbiological analysis. For practical reasons, extraction could not be carried out aseptically.

3.1.5 Method detail specific to cold water prawns

Cold water prawns were supplied frozen by Icelandic Export Centre Ltd and a representative from the company was present for the trials. The prawns were placed in a 3% w/w brine solution and thawed overnight in a chiller at <3°C in order to simulate commercial practice prior to peeling as advised by Lyons Seafoods Ltd. Approximately 500g of prawns (actual weights were recorded) were filled into vacuum bags and just covered with either chilled or ambient temperature reverse osmosis treated water. A separate bag of prawns weighing approximately 50g was also prepared, covered with reverse osmosis treated water and processed along with the main sample. This smaller sample was used for microbiological analysis to ensure that cross contamination risks were minimised. The initial temperature of the prawns prior to high pressure treatment was 8.6°C (s.d. = 5.4°C, n=18). The temperature of the sample after processing was on average 4.3°C warmer than before treatment (s.d. = 3.3°C, n=18).

Each sample was pressure treated according to experimental design as outlined in Table 5. After treatment, the prawns were drained for 2 minutes and re-weighed. A sub-sample of 30 prawns was hand-peeled. The meat and shell were weighed and yields were calculated.

3.1.6 Method detail specific to lobster

Lobsters were sourced from Clearwater Lobster Merchants. Staff from the Blue Seafood Company Ltd were present for the trials. The samples arrived live on the day of processing and were stored at <3°C prior to use. Lobsters were stunned prior to pressure treatment using a Crustastun humane crustacean stunner (Crustastun, UK). The lobsters were weighed, double bagged in vacuum pouches and covered with either chilled or ambient temperature reverse osmosis treated water. The mean initial temperature of the lobsters prior to high pressure treatment was 16.5°C (s.d. = 4.9°C, n=18). The

temperature of the sample after processing was on average 0.2°C warmer than before treatment (s.d. = 2.8°C, n=18).

Each sample was pressure treated according to the experimental design as outlined in Table 5. After treatment, the lobsters were drained for 2 minutes and re-weighed. The meat from one claw from each run was sub-sampled for microbiological enumeration. Handling of the meat was minimised as much as was practical but was not aseptic. The weight of the second claw was recorded; the claw was then manually picked. The weight of meat and shell was recorded and yields were calculated.

3.1.7 Method detail specific to warm water prawns

Raw frozen whole prawns were supplied by Lyons Seafoods Ltd. The prawns were thawed by soaking in a 3% w/w brine solution overnight at <3°C to simulate commercial practice prior to peeling. Approximately 500g of prawns (actual weights were recorded) were filled into vacuum packaging, double bagged and covered with reverse osmosis treated water at either ambient or chilled temperature. Each sample was pressure treated according to the experimental design as outlined in Table 5. After processing, samples were drained for 2 minutes and then re-weighed. The mean initial temperature of the prawns prior to high pressure treatment was 8.6°C (s.d. = 1.29°C, n=18). The temperature of the sample after processing was on average 3.0°C warmer than before treatment (s.d. = 3.9°C, n=17). A sub-sample of 10 prawns was taken, weighed, and manually peeled. The weight of meat and shell was taken and yields were calculated.

3.1.8 Method detail specific to salmon

Salmon trials were carried out at AFBI Belfast using a 35 litre HPP system manufactured by Avure (USA). Fresh farmed salmon (*Salmo salar)* was purchased from a local supplier. The salmon was provided vacuum packed; each pack was opened and the salmon was hand portioned before being re-vacuum packed. Temperature control was possible on the Avure system so a modified experimental design was employed as shown in Table 4.

Pressure (MPa)	Time (min)	Temperature (°C)
425	3	6
321	2	9
321	4	9
529	4	10
529	2	10
425	3	11
425	3	12
250	3	12
425	3	13
425	5	13
425	3	13
425	3	13
425	1	13
600	3	14
425	3	11
321	4	15
529	2	16
321	2	16
529	4	15
425	3	17

Table 4. Experimental design for salmon trials. Design values are those attained during the trials rather than target values

The compression fluid was water. Pack temperatures were not monitored but the vessel fluid temperature was measured throughout each run as was the come-up time and hold time. Separate samples of at least 50g of salmon were packed for each run for microbiological evaluation. After processing, samples were packed into insulated containers with glycol packs and transported back to CCFRA. Sensory evaluation of the

pressure treated samples took place the day after processing. Photography of the samples also took place the day after processing.

3.1.9 Method detail specific to squid

Squid was supplied by New Wave Seafoods (Fairford, Gloucestershire). Samples were supplied cleaned and size graded (30-35 squid weighing 10 kg). Squid tubes were packed for the sensory trials and photography; tentacles were packed for microbiological evaluation. All samples were vacuum packed the day before processing and were stored overnight at 2-5°C prior to pressure treatment. All trials were carried out on the ESPI high pressure system because pressures in excess of 400 MPa were required. The vessel wall temperature was set to 20°C but heat generated by continuous pumping of fluid around the vessel wall meant that the pressure transmission fluid was typically around 29°C. Each sample was pressure treated according to the experimental design as outlined in Table 6. The initial temperature of the pressure fluid prior to high pressure treatment was 28.9°C (s.d. = 1.8°C, n=19). The temperature of the fluid after processing was on average 0.4°C warmer than before treatment (s.d. = 1.4°C, n=16). The initial temperature of the squid was not recorded because the vacuum packed bags could not be pierced prior to HPP treatment, but samples were stored at 2-5°C and were brought out singularly for trials so the initial temperature is expected to be around 2-5°C. The average temperature of the squid after processing was 21.5°C (s.d. = 2.0°C, n=20).

3.1.10 Method detail specific to cod

Air-freighted Icelandic cod was supplied by Seachill Ltd (Grimsby, UK) and a member of staff from Seachill was present for the trials. The cod was four days from catch, having being caught on Friday 21st September, air-freighted to Grimsby on 22nd September and pressure treated on 25th September. The cod was portioned into 16-cm long pieces before being packed into vacuum bags. The samples were not vacuum-packed but care was taken to expel air from the pack prior to sealing. A separate portion was cut for microbiological evaluation. All samples were held on ice at all times during the experiments. After treatment the samples were transferred to a chiller operating at

2-5°C. All trials were carried out on the ESPI high pressure system because pressures in excess of 400 MPa were required. Each sample was pressure treated according to the experimental design as outlined in Table 6. The initial temperature of each sample was not recorded because the samples were vacuum packed but all samples were kept on ice until pressure treated. The temperature of the sample after treatment was typically 11.7°C (s.d. = 1.2°C, n=20).

3.1.11 Method detail specific to mackerel

Mackerel was supplied by New Wave Seafoods (Fairford, Gloucestershire). Samples for sensory evaluation were provided gutted and cleaned (approximate weight of each fish was 300g) and were hand-filleted by CCFRA staff. Each fillet was filled into vacuum packs and vacuum sealed. The packed samples were then stored overnight at 2-5°C before being pressure treated and subjected to sensory evaluation. The remaining samples, i.e. those not used in sensory evaluation, could not be treated due to a site flood. A second batch of mackerel was therefore purchased to complete the studies. Samples were provided by New Wave Seafoods and were provided as fillets. All 20 runs were completed on this new raw material for photography and microbiological studies. However, sensory evaluation was not repeated on these samples. Care should therefore be taken in inferring any relationships between the sensory evaluation and the microbiological data as different raw materials were used in each case. Each sample was pressure treated according to the experimental design as outlined in Table 6. The initial temperature of each sample was not recorded because the samples were vacuum packed, but all samples were stored at 2-5°C and brought out singularly for each pressure treatment. The temperature of the sample after treatment was typically 13.3°C (s.d. = 1.7°C, n=20).

3.2 Experimental design and statistical analysis

All experiments were based on a customised central composite design. Two basic designs were employed, one for fish using pressures between 200-600 MPa and one for

shellfish using 200-300 MPa. Times ranged between zero and five minutes. Temperature could not be controlled but was recorded before and after processing. The target values for pressure and time in the two designs are recorded in Table 5 and Table 6. The actual temperature, pressures and times achieved during processing were substituted for the target values of the design (established using Minitab version 15.0). There were five replicates of the centre point of the design. Where appropriate, an analysis of variance was carried out on the data and a quadratic surface response was fitted for each response variable.

Target pressure (MPa)	Target time (min)
250	2.5
280	1
220	1
250	2.5
220	4
280	4
250	5
250	2.5
250	2.5
250	0
250	2.5
300	2.5
250	2.5
200	2.5
220	4
250	2.5
280	1
220	1
250	2.5
280	4

Table 5. Experimental design used for shellfish experiments.

Target pressure (MPa)	Target time (min)
400	5
200	2.5
600	2.5
400	2.5
400	2.5
400	2.5
400	2.5
400	0
522	1
278	4
278	1
400	2.5
400	2.5
522	4
522	4
400	2.5
522	1
278	1
400	2.5
278	4

Table 6. Experimental design used for fish experiments

3.3 Microbiological methods

Methods used for microbiological enumeration were standard methods as recorded in the CCFRA Business Management manual (TES-MB-002 for aerobic plates counts, TES-MB-005 for coliforms and TES-MB-012 for pseudomonads). These methods are available on request.

3.4 Sensory evaluation methods

Unless otherwise stated, controls and runs 1-6 were subjected to sensory evaluation after processing using experienced assessors. Each assessor independently described the uncooked appearance and odour of the samples and awarded an overall quality grade for the raw sample using a 9-point scale. The sample was then cooked and the assessors described the appearance, odour, flavour and texture/mouthfeel and awarded a quality grade for the cooked assessment. The consensus scores were calculated and the individual comments combined. Detailed sensory methods and results are attached as self-contained reports in appendices 1-11. A short summary of the results is presented on a species-by-species basis in section 4.

4 Results

For clarity, results and discussion have been presented on a species-by-species basis. Sensory results are attached as separate self-contained reports in Appendices 1-11.

4.1 Nephrops

Microbial reductions obtained for HPP treatment of *Nephrops* are reported in Table 7. Yield data are reported in Table 8. Results for sensory evaluation are briefly summarised in section 4.1.3 but are fully reported in Appendix 1.

4.1.1 Microbiological results

Run no.	Pressure (MPa)	Initial product temp (°C)	Product temp after processing (°C)	Time (min)	TVC (per g)	TVC log reduction	Coliforms (per g)	Pseudomonads (per g)
Control 1 No Water	0.1	*	*	0	2.10E+03	0.0	<5	1.90E+03
Control 2 Water	0.1	*	*	0	1.00E+04	-0.7	<5	1.90E+03
1	269	-3.3	-3.1	2.5	*	*	*	*
2	297	0.4	-0.3	1	2.04E+02	1.0	<5	<50
3	239	0.2	0	1	1.10E+03	0.3	10	<50
4	268	0.5	-0.4	2.5	4.13E+02	0.7	5	<50
5	237	0.1	0.1	4	4.20E+03	-0.3	<5	<50
6	299	0.1	-0.1	4	3.31E+02	0.8	<5	<50
7	270	1	0.1	5	3.00E+02	0.8	<5	<50
8	268	0.7	-0.4	2.5	1.60E+03	0.1	<5	<50
9	267	0.1	-0.1	2.5	5.40E+03	-0.4	<5	<50
10	268	0.1	-0.2	0	7.10E+03	-0.5	<5	<50
11	269	-0.1	-0.1	2.5	5.00E+03	-0.4	<5	<50
12	323	-0.1	-0.7	2.5	3.40E+03	-0.2	<5	<50
13	268	0	-0.1	2.5	5.50E+01	1.6	<5	<50
14	219	0	-0.1	2.5	2.80E+03	-0.1	<5	<50
15	233	0.4	-0.2	4	7.54E+02	0.4	<5	<50
16	237	0.1	0.3	2.5	6.70E+03	-0.5	<5	<50
17	299	0.1	-0.1	1	9.09E+02	0.4	<5	<50
18	239	0.1	0.1	1	6.32E+02	0.5	<5	50
19	269	1	0.6	2.5	2.10E+03	0.0	45	50
20	298	0	0.5	4	2.00E+03	0.0	<5	50

Table 7. Nephrops microbial reductions

There was no evidence of statistically significant differences in Log TVC reductions as a function of pressure, temperature and time ($P>0.05$) within the design space tested. The maximum observed log reduction was 1.6 but this was not at the highest pressure as would perhaps have been expected. The narrow window of processing conditions and inherent variation in the raw material may both be factors explaining why variation in TVC reductions were relatively large and log reductions were small. Coliforms were absent in all samples including the controls. Pseudomonads were found to be very pressure sensitive in *Nephrops*, being reduced from 10^3 cfu/g to the limits of detection in 17 of the 20 runs.

4.1.2 Yield/quality data

Run No.	Weight of tails before processing (g)	Weight of tails after processing (g)	Difference (g)	Weight of 20 tails (g)	Yield based on original weight (%)	Yield based on sum of meat and shell weights (%)
Control 1 No Water	501.1	501.1	0.0	150.4	65	66
Control 2 Water	504.2	*	*	158.0	54	71
1	501.4	*	*	*	*	68
2	500.4	567.7	67.3	154.4	62	72
3	500.4	563.5	63.1	115.3	68	71
4	503.5	642.2	138.7	117.2	65	69
5	500.9	550.6	49.7	139.9	68	71
6	501.7	620.0	118.3	148.1	69	73
7	502.4	505.1	2.8	131.1	74	72
8	502.6	551.2	48.6	135.0	69	73
9	499.5	543.3	43.8	130.4	69	71
10	504.8	649.3	144.5	162.3	62	70
11	502.4	514.6	12.2	110.9	66	74
12	502.4	509.7	7.3	116.5	66	71
13	503.1	507.3	4.2	113	72	73
14	503.2	413.7	-89.5	117.3	72	73
15	500.1	441.4	-58.7	135.5	71	72
16	501.6	499.0	-2.6	128.3	72	74
17	508.0	570.4	62.4	135.9	69	72
18	508.8	546.6	37.8	121.5	70	72
19	508.0	520.9	12.9	117.23	71	72
20	499.3	527.4	28.1	130.45	68	71

Table 8. Nephrops yield data

There was a statistically significant weight gain as a result of pressurisation ($P<0.05$) and the magnitude of the weight gain after pressure treatment was related to pressure applied ($P<0.1$), increasing pressure resulting in greater percentage weight gain. Percentage weight change could be predicted with reasonable accuracy using a quadratic response surface (Figure 3); however, pressure alone was a weak predictor of percentage weight change (Figure 4).

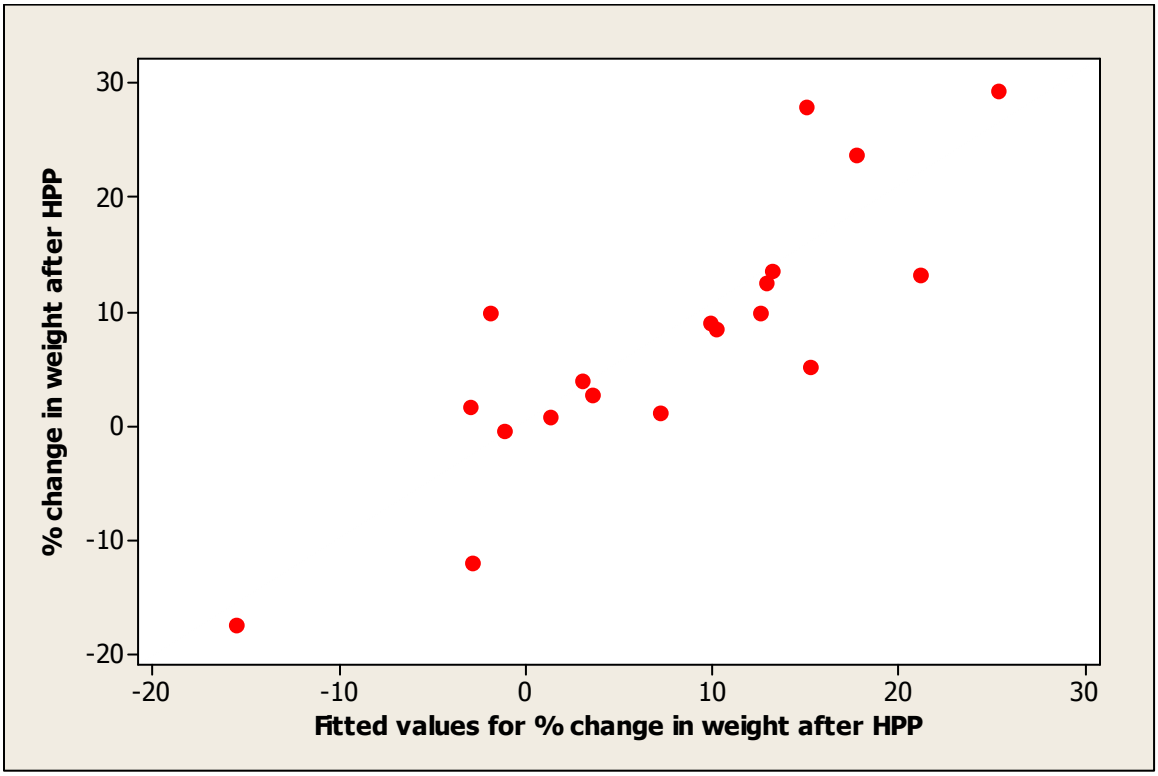

Figure 3. Nephrops predicted % weight change against measured % change after HPP

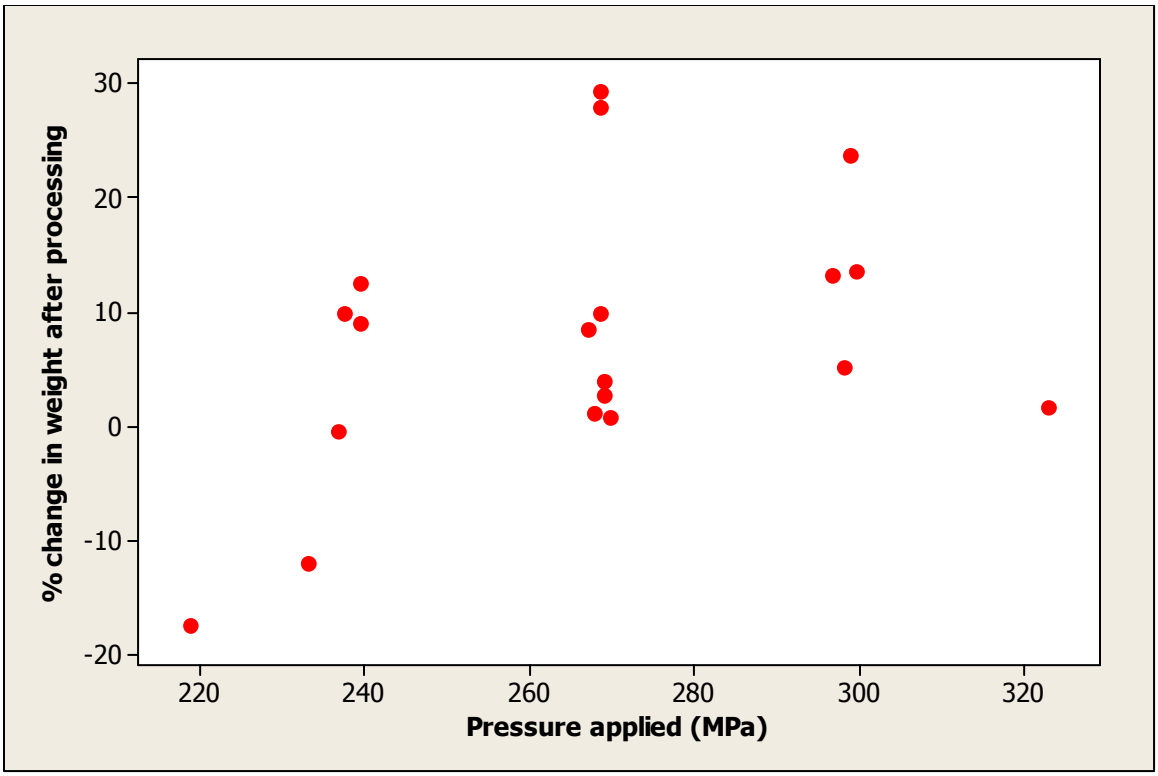

Figure 4. Nephrops % weight change against pressure applied

From a starting weight of around 500g *Nephrops* on average gained 36.4g after pressure treatment. The maximum measured yield resulting from hand-peeling of the *Nephrops* was 74% whereas in the control sample the yield was 65%. However, there were concerns that there was some bias being introduced into the yield measurements as staff became more proficient at peeling. For this reason yield measurements were repeated on the control samples and a yield of 71% was obtained. There was no statistically significant evidence ($P>0.05$) to demonstrate differences in peeling yield at different pressures and temperature within the design space; however, time was significant ($P<0.05$) with longer times generally appearing to give an increase in yield compared with untreated *Nephrops*; hold times of 5 minutes gave statistically significant differences in yield compared with the control. A three percent yield increase is substantial and of commercial interest. Further work in phase two of the project will generate more objective peeling yields by producing a large quantity of *Nephrops* and using commercial peeling equipment to obtain realistic commercial yields.

4.1.3 Brief summary of sensory results for *Nephrops*

Controls and runs 1-6 were subjected to sensory evaluation; for each evaluation 12 tails were assessed. Run 2 scored highest for uncooked quality and scored higher than the control, being brighter and having less translucency. After cooking, the Control was graded the highest followed by Runs 2 and 6. These were both slightly less bright, had lost the slight seawater odour present in the Control and were both slightly more gritty in texture. Run 6 also had a slightly astringent mouthfeel. All other samples tested were graded lower than Run 2 and Run 6 due to changes in appearance: dirty grey coloration, less bright and more shape loss; off odours and sour/acid flavour; more fibrous and gritty, astringent notes present and one described as rubbery/chewy.

Images of all pressure treated samples can be found in Appendix 12.

4.1.4 Key conclusions for Nephrops

Yield benefits as a result of HPP look very promising; a 3% yield increase is commercially significant and further work is therefore required to determine whether the apparent yield benefits seen with hand peeling are transferrable to commercial peeling operations. Since *Nephrops* are a commercially important species for the UK industry and the peeling yields are of interest, this species will be selected for further investigation in phase 2 of the work. Commercial peeling trials will be carried out on HPP treated *Nephrops*.

Regarding the microbiological results, pseudomonads were very pressure sensitive, which could prove useful for shelf life extension. However, total viable counts were not reduced to any significant degree. It is therefore difficult to postulate whether a meaningful shelf life extension could be obtained without knowing the make-up of the organisms that were present on the TVC plates. Storage trials may form part of the

second phase of the project where this question will be addressed more fully in certain species.

Sensory results for runs 1-6 suggested that sour and astringent flavours could develop in the product along with ‘rubbery’ textures in some instances. Interestingly, the two conditions which gave the best sensory results were at higher pressures, i.e. around 300 MPa. Since there was no clear optimum for microbiological inactivation and yield improvements but sensory quality was not seriously affected at the higher pressure treatments, it is proposed that phase 2 trials will involve larger scale peeling trials with a commercial processor using pressures of 300 MPa with a hold time of 5 minutes.

4.2 Mussels

Microbial reductions obtained for HPP treatment of mussels are reported in Table 9. Yield data are reported in Table 10. Results for sensory evaluation are briefly summarised in section 4.2.3 but are fully reported in Appendix 2.

4.2.1 Microbiological results

Run no.	Pressure (MPa)	Initial product temp. (°C)	Product temp. after process (°C)	Time (min)	TVC/g	TVC log reduction	Coliforms /g	Pseudomonads /g
Control 1 No Water	0.1 MPa	7	*	0	1.30E+04	0.0	<5	8.50E+03
Control 2 Water	0.1 MPa	16	*	0	1.00E+04	0.1	<5	7.20E+04
1	273	8.7	13.1	2.5	3.70E+03	0.5	<5	7.30E+03
2	299	13.2	13	1	3.60E+03	0.6	<5	2.00E+02
3	238	9.3	13.2	1	5.30E+03	0.4	<5	1.50E+02
4	272	9.2	12.2	2.5	2.80E+03	0.7	<5	<50
5	237	14	14.7	4	3.80E+03	0.5	<5	<50
6	299	13.6	11	4	5.00E+04	-0.6	<5	1.00E+02
7	272	8.9	10.7	5	4.00E+02	1.5	<5	1.50E+02
8	273	9.8	10.8	2.5	7.50E+02	1.2	<5	1.50E+02
9	274	7.4	12.6	2.5	7.13E+02	1.3	<5	4.50E+03
10	264	7.6	13.1	0	2.20E+03	0.8	<5	2.90E+03
11	271	13.1	13.4	2.5	7.80E+02	1.2	<5	2.50E+02
12	319	13.1	13.8	2.5	6.05E+02	1.3	<5	1.00E+02
13	273	13.1	13.1	2.5	4.32E+02	1.5	<5	<50
14	221	12.6	12.7	2.5	2.00E+03	0.8	<5	5.00E+01
15	237	12.7	13	4	9.04E+02	1.2	<5	9.50E+02
16	273	12.4	13	2.5	7.77E+02	1.2	<5	<50
17	*	12	13.4	1	1.41E+02	2.0	<5	<50
18	237	11.9	13.3	1	1.50E+03	0.9	<5	1.10E+03
19	273	13.1	14	2.5	8.60E+03	0.2	<5	<50
20	301	13.5	14.3	4	8.50E+02	1.2	<5	<50

Table 9. Mussels microbial reductions

The maximum measured TVC reduction in pressure treated mussels was 2 log cycles. Coliforms were absent in all samples including the controls. Pseudomonads were reduced from 10^3 cfu/g to the limits of detection in 7 of 20 conditions. There was no obvious pattern as to what conditions of pressure temperature and time gave optimal inactivation for TVCs or pseudomonads. As was the case for *Nephrops*, this could be due to the relatively small operating window used for processing, the natural raw material variation or a combination of these factors. Whilst the microbiological data is of interest, the primary aim of the trials was to investigate yield changes and shucking of the mussels using pressure.

4.2.2 Yield/quality data

Run no.	Weight before process (g)	Weight after process (g)	Difference (g)	Total weight of 30 mussels (g)	Weight of meat (g)	Yield based on total weight (%)	Yield based on sum of meat and shell weights (%)
Control 1 No Water	*	*	*	334.9	129.8	38.76	45.70
Control 2 Water	*	*	*	353.4	*	*	*
1	500.6	470.5	-30.1	339.5	192.4	56.67	58.06
2	505.0	475.4	-29.6	357.3	194	54.30	55.94
3	496.8	462	-34.8	313.9	174.4	55.56	56.88
4	510.0	467.8	-42.2	320.2	174.9	54.62	55.74
5	500.0	460.1	-39.9	359.5	196.3	54.60	56.10
6	504.0	490.6	-13.4	332.1	185.6	55.89	57.64
7	496.6	492.5	-4.1	341.2	184.6	54.10	57.29
8	503.0	502.4	-0.6	311.5	167.3	53.71	55.90
9	504.2	485.6	-18.6	354.4	194.9	54.99	56.67
10	499.2	486.9	-12.3	343.8	*	*	*
11	506.6	492.5	-14.1	333.4	174.2	52.25	53.97
12	495.2	466.7	-28.5	335.7	175.4	52.25	55.84
13	504.1	474.8	-29.3	324.3	173.7	53.56	55.66
14	510.5	*	*	321	168.5	52.49	54.27
15	510.4	*	*	*		*	*
16	500.0	441.4	-58.6	286.9	153	53.33	54.68
17	512.0	476.6	-35.4	*	105.1	*	55.58
18	503.3	478.1	-25.2	297.1	160.8	54.12	55.95
19	503.9	444.5	-59.4	309.6	162	52.33	54.00
20	516.0	456.6	-59.4	328.5	198.7	60.49	59.47

Table 10. Mussels yield data

The overall weight of the samples was reduced significantly after treatment ($P<0.05$), samples typically losing around 30g in weight after processing from a mean starting weight of 503g. Up to 12% weight loss was measured in some samples after treatment, which is thought to be due to liquor loss from the mussel once the shells opened and the pressure was released. This is presumably the reason that commercial HPP processors of shellfish shrink a band around the product to keep the shell closed after treatment.

Looking at the mussel meat in isolation, pressure treated samples on average gained in weight by 34%. This figure is based on typical weights of 30 mussels post treatment relative to the weight of 30 control mussels, i.e. it is not based on the pre-treatment weight of the actual mussels that were pressure treated. This direct measurement was not possible because a large sample was pressure treated and sub-samples were taken from this for shucking and yield assessment. This weight increase is therefore indicative only. The maximum recorded yield was 60% compared with a yield of 39% in the control sample. Most conditions resulted in a substantial weight gain in the meat relative to the controls (Table 10). Yield was always increased relative to the control (typically 50-60% yield compared with 39% in the control), but there were not statistically significant differences *between* treatments ($P>0.05$).

Mussels were readily shucked by HPP treatment with almost every treatment resulting in a high percentage of the samples being completely detached by the pressure treatment (Figure 5). In most cases, the shells of the mussels post process were free of visible meat contamination.

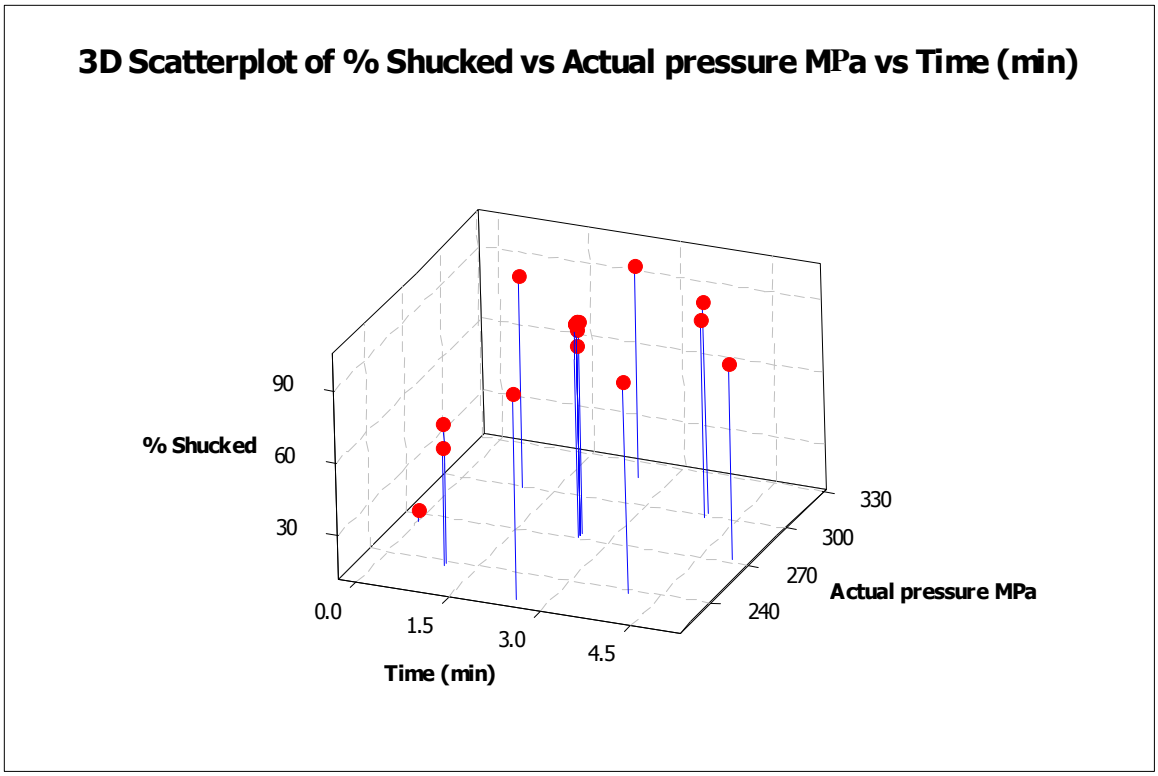

Figure 5. Shucking of mussels as a function of pressure and time

4.2.3 Brief summary of sensory results for mussels

Controls and runs 1-6 were assessed for sensory quality. For each evaluation 9 mussels were assessed. For the uncooked assessment all treated samples were consistently graded higher than the Control, with Run 5 and Run 6 graded the highest. All treated samples appeared plump and retained their shape much better than the Control. Run 5 and Run 6 were graded higher as they had a brighter appearance.

For the cooked assessment, again all treated samples were graded higher than the Control with the exception of Run 6 which was graded equally. Appearance was important, along with the texture. The treated samples retained their shape better, they were brighter and were all slightly less chewy than the Control. Run 2 was graded higher as it had retained its shape better than the other runs. Overall Run 2 appeared to be the treatment with the

most consistent effect on increasing sensory quality when compared to the Control sample.

Images of all pressure treated samples can be found in Appendix 13.

4.2.4 Key conclusions for mussels

Pseudomonads were again found to be relatively pressure sensitive, being reduced to the limits of detection in 7 of the 20 runs. This could prove useful for shelf life extension. The main benefit from HPP treatment of mussels, however, was the 'automatic' shucking that was achievable along with the increase in meat weight after processing. This effect could also be used to introduce flavours into the product and produce value-added products. Mussels were successfully shucked at almost all conditions tested. To prevent liquor loss from the mussels post-process, it would be necessary to manually seal the mussels shut in some way, i.e. as per the 'gold-band' commercial oyster products. The effects of HPP on sensory quality appear to be favourable within the limits of the conditions tested. Samples were perceived to be plump, attractive and less chewy than the control

Mussels will not be taken forward for phase 2 trials. The main reason for this is that phase 1 trials have proven the effectiveness of HPP for shucking within the processing range of 200-300 MPa and it is believed therefore that 'proof of principle' has been adequately assessed. There are microbiological benefits but automatic shucking is the key benefit and this has been shown to work very effectively without deleterious effects on sensory quality.

4.3 Oysters

Microbial reductions obtained for HPP treatment of oysters are reported in Table 11. Yield data are reported in Table 12. Results for sensory evaluation are briefly summarised in section 4.3.3 but are fully reported in Appendix 3.

4.3.1 Microbiological results

Run no.	Pressure (MPa)	Initial product temp. (°C)	Product temperature after processing (°C)	Time (min)	TVC/g	TVC log reduction	Coliforms per g	Pseudomonas per g
Control 1 No Water	0.1	*	*	0	5.09E+02	0.0	3.09E+02	6.00E+02
Control 2 Water	0.1	*	*	0	3.50E+02	0.2	8.00E+01	1.50E+02
1	273	13.6	12.1	2.5	9.27E+02	-0.3	2.00E+01	<50
2	304	13.7	10.1	1	3.50E+03	-0.8	1.50E+01	<50
3	240	12.4	10.2	1	1.50E+01	1.5	<5	<50
4	270	12.9	10.5	2.5	4.00E+01	1.1	<5	<50
5	238	11.9	10.6	4	3.50E+01	1.2	<5	5.00E+01
6	304	12.8	10.4	4	3.00E+01	1.2	<5	<50
7	273	11.9	9.4	5	7.00E+01	0.9	<5	<50
8	272	13.5	11	2.5	2.50E+01	1.3	<5	<50
9	274	11.3	11	2.5	<5	>2.0	<5	<50
10	269?	12.4	11.6	0	5.00E+00	2.0	<5	<50
11	272	12.9	11.9	2.5	1.25E+02	0.6	<5	<50
12	317	13	11.6	2.5	<5	>2.0	<5	<50
13	271	13.1	11.3	2.5	1.00E+01	1.7	<5	<50
14	219	13.4	*	2.5	3.50E+01	1.2	<5	<50
15	237	11.7	12.3	4	<5	>2.0	<5	<50
16	273	13.1	13	2.5	1.00E+01	1.7	<5	<50
17	302	15.3	12.4	1	4.50E+01	1.1	<5	<50
18	237	15.1	12.7	1	3.00E+01	1.2	<5	<50
19	270	15	12.1	2.5	<5	>2.0	<5	<50
20	304	13.8	12.5	4	<5	>2.0	<5	<50

Table 11. Oysters microbial reductions

Total viable counts were reduced from 10^2 cfu/g to the limits of detection in 5 of the 20 runs. Coliforms were reduced from 10^2 cfu/g to the limits of detection in 18 of the 20 runs. Pseudomonads were reduced from 10^2 cfu/g to the limits of detection in 19 of the 20 conditions. There was no obvious pattern as to what conditions of pressure, temperature and time gave optimal inactivation for TVCs. As was found for *Nephrops*, this could be due to the relatively small operating window used for processing, the natural raw material variation or a combination of these factors.

4.3.2 Yield/quality data

Run no.	Weight before process (g)	Weight after process (g)	Difference (g)	Meat weight from 10 oysters patted dry (g)	Yield (%) based on weight of meat (patted dry) over original weight of 10 oysters	Yield (%) based on weight of meat (patted dry) over sum of meat plus shells
Control 1 No Water	*	*	*	82	9	14
Control 2 Water	*	*	*	76	8	12
1	935.3	772.4	-162.9	131.3	17	18
2	981.7	839.2	-142.5	127	15	18
3	948.3	861.7	-86.6	100.1	12	16
4	923.9	759.3	-164.6	144.4	19	20
5	938.1	830.5	-107.6		*	*
6	894.3	785.8	-108.5	158.2	20	21
7	921.6	809.2	-112.4	164.6	20	21
8	935.7	786.1	-149.6	141.9	18	19
9	931.8	783.1	-148.7	134.99	17	18
10	923.8	801.8	-122.0	111.5	14	15
11	918.5	774.9	-143.6	142.2	18	19
12	981.2	862.0	-119.2	169.3	20	20
13	910.2	796.6	-113.6	154	19	21
14	905.4	740.1	-165.3	113.6	15	17
15	922.2	843.6	-78.6	148.6	18	20
16	919.4	771.8	-147.6	133.6	17	18
17	863.8	706.2	-157.6	117.6	17	18
18	923.4	811.0	-112.4	100.9	12	15
19	925.8	750.7	-175.1	134.7	18	19
20	881.0	730.6	-150.4	138.1	19	19

Table 12. Oysters yield data

The overall weight of all pressure treated samples was reduced after processing (Table 12) but as for mussels, this is thought to be due to liquor loss because the samples were not sealed prior to treatment. There was a lot of residual water on the control samples relative to the pressure treated so in order to get a consistent yield measurement, the meat of all samples was patted dry prior to weighing. Yield increases were significant in all pressure treated samples with yields being on average 16% in pressure treated samples compared with 9% for the controls. The weigh of meat from 10 oysters was on average 135g in pressure treated samples compared with 79g in the controls, i.e. an increase of around 71%. Anecdotal evidence would suggest that in some countries, e.g. the USA, this increase in volume is seen as a positive benefit because the oysters are larger and are viewed as more succulent. However, in other markets, e.g. Japan, the 'swelling' of the oyster is seen as a negative impact on the product (personal communication with NC Hyperbaric and Avure). Pressure and time both significantly ($P<0.05$) influenced the weight of the oysters after processing with time appearing to be particularly important in determining the final weight of the oyster, but a quadratic model was not appropriate to predict weight gain. Temperature was not significant ($P>0.05$). Pressure and time could both be manipulated to control the level of 'swelling' to match the requirements of the target market.

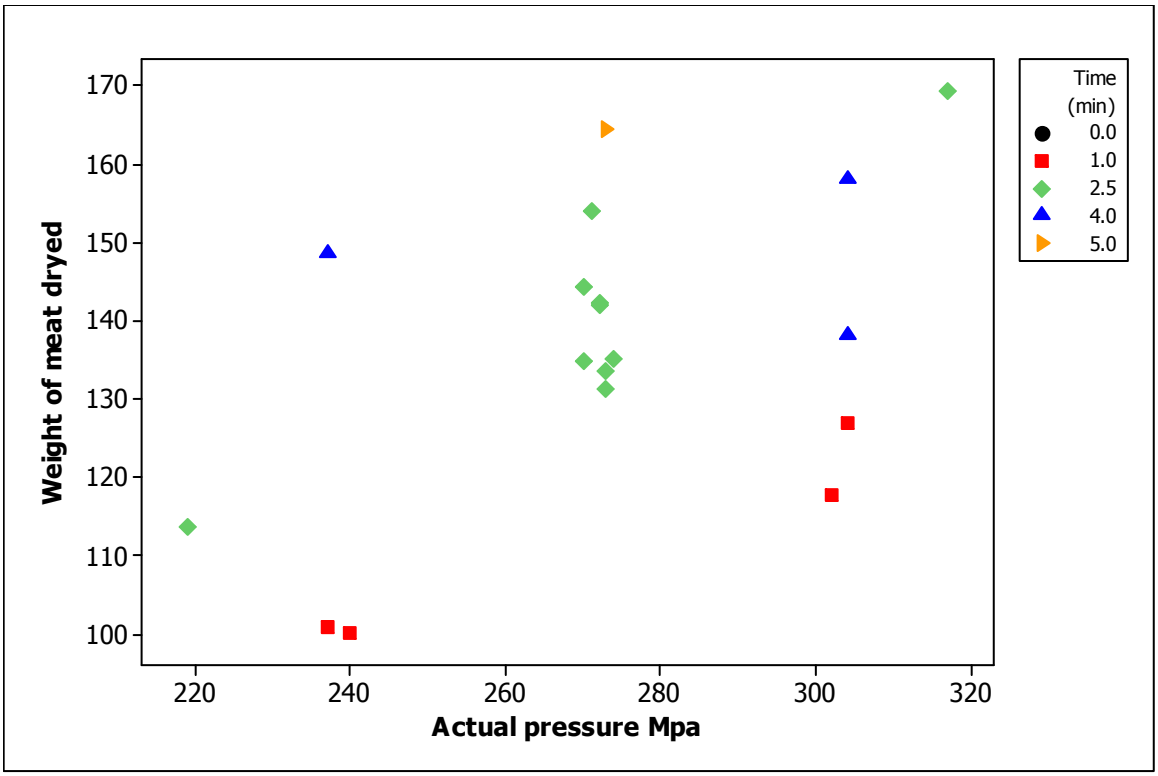

Figure 6. Influence of pressure and time on the weight of oysters after processing

Pressure and time both influenced the percentage of oysters shucked by a particular process (Figure 7) ($P<0.05$), but a quadratic surface response was not a suitable model for shucking. There was variation in '% shucked' even at the same processing conditions; this variation is not thought to be due to process variation but is thought to be due to the relatively subject assessment of 'shucked' compared with 'un-shucked'. In reality, there was a spectrum of attachment from 'firm' to completely detached. Only samples where there was complete detachment of the meat were considered to be 'shucked' in the visual assessment. Many of the conditions resulted in a level of detachment whereby it seemed likely that all of the meat could have been subsequently removed by, for example, a vibrating belt. As a general point, oysters were, in general, harder to shuck completely compared with mussels.

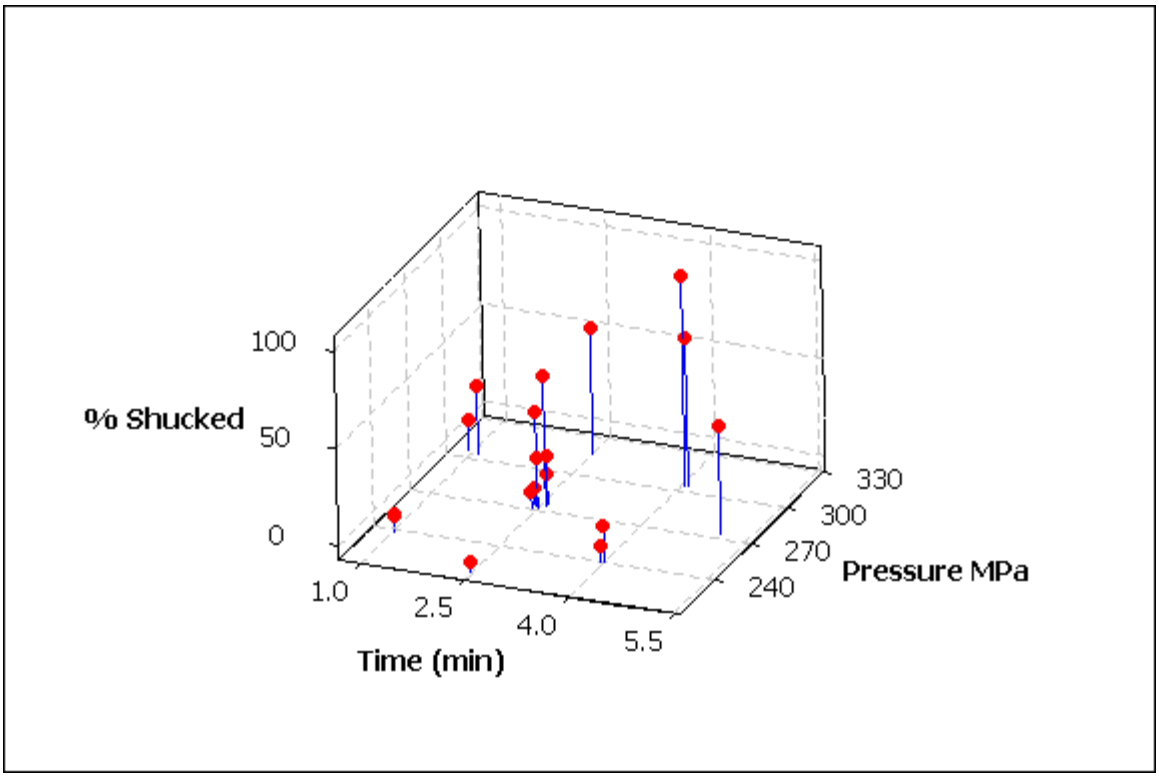

Figure 7. Influence of pressure and time on % oysters shucked

4.3.3 Brief summary of sensory results for oysters

Controls and runs 1-6 were assessed for sensory quality. For each evaluation eight oysters were assessed. For the uncooked assessment all the treated samples were graded higher than the Control, with Run 4 being graded the highest. All samples were brighter than the Control, very plump with little shape loss and all had a stronger fresh odour.

For the cooked assessment, Run 1, Run 5 and Run 6 were all graded higher than the Control as they were all plump with little shape loss and all had a stronger fresh odour. Run 1 was also described as brighter than the Control and less gritty. Run 4 was graded the lowest for the cooked assessment due to the presence of an off odour. Overall, Runs 1 and 6 appeared to be the treatments with the most consistent effect on increasing sensory quality when compared to the Control sample.

Images of all pressure treated samples can be found in Appendix 14.

4.3.4 Key conclusions for Oysters

Many of the conclusions made for mussels are applicable to oysters. Coliforms and Pseudomonads were found to be very pressure sensitive in oysters. This could prove useful for shelf life extension. The main benefit from HPP treatment, however, was the 'automatic' shucking that was achievable along with the increase in meat weight after processing. This effect could also be used to introduce flavours into the product and produce value-added products. Oysters could be successfully shucked using HPP but were generally more difficult to shuck compared with mussels in the sense that there was usually some level of light attachment after processing. As for mussels, to prevent liquor loss post-process, it would be necessary to manually seal the oyster shut in some way, e.g.. as per the 'gold-band' commercial oyster products. The effects of HPP on sensory quality appear to be favourable within the limits of the conditions tested. Samples were perceived to be plump, attractive and having 'fresh' odours.

Oysters will not be taken forward for phase 2 trials. The main reason for this is that phase 1 trials have proven the effectiveness of HPP for shucking within the processing range of 200-300 MPa and it is believed therefore that 'proof of principle' has been adequately assessed. There are microbiological benefits but automatic shucking is the key benefit and this has been shown to work very effectively without deleterious effects on sensory quality.

4.4 Crab

Microbial reductions obtained for HPP treatment of crab are reported in Table 13. Yield data are reported in Table 14. Results for sensory evaluation are briefly summarised in section 4.4.3 but are fully reported in Appendix 4.

4.4.1 Microbiological results

Run no.	Pressure (MPa)	Initial product temp. (°C)	Product temp. after processing (°C)	Time (min)	TVC (per g)	TVC log reduction	Coliforms (per g)	Pseudomonads (per g)
Control 1 Cooked	0. 1	*	*	0	8.0E+03	0.0	<5	2.00E+02
Control 2 Raw	0.1	*	*	0	7.1E+03	0.1	1.00E+01	<50
1	273	*	12.1	2.5	5.1E+03	0.2	<5	<50
2	301	14.3	13.3	1	1.5E+04	-0.3	<5	<50
3	238	10.4	12.8	1	9.2E+04	-1.1	1.20E+02	1.80E+03
4	272	*	13	2.5	1.5E+05	-1.3	7.50E+02	1.10E+03
5	237	13.9	12.3	4	4.8E+04	-0.8	2.45E+02	8.00E+02
6	*	14.6	12.3	4	4.0E+04	-0.7	1.18E+02	1.50E+03
7	270	16.2	12.8	5	1.4E+03	0.8	<5	<50
8	270	15	13	2.5	4.3E+04	-0.7	1.00E+01	<50
9	271	15.2	13.1	2.5	1.8E+03	0.6	<5	<50
10	268	16.1	14.5	0	2.5E+03	0.5	<5	<50
11	273	16.2	13.6	2.5	2.5E+03	0.5	<5	<50
12	319	16	13.7	2.5	1.9E+03	0.6	<5	<50
13	271	12.4	13.2	2.5	4.4E+03	0.3	3.00E+01	2.00E+02
14	221	15.2	13.2	2.5	3.7E+02	1.3	5.00E+00	2.50E+02
15	239	13.7	12.5	4	2.5E+03	0.5	<5	<50
16	271	15.2	12.8	2.5	3.4E+03	0.4	<5	3.80E+03
17	304	16.2	14.1	1	4.2E+03	0.3	2.04E+02	2.50E+03
18	239	14.8	13.1	1	5.2E+03	0.2	8.50E+01	3.00E+02
19	271	14.2	14	2.5	1.6E+03	0.7	<5	2.00E+02
20	301	15.9	13.2	4	6.3E+02	1.1	<5	<50

Table 13. Crab microbial reductions

The maximum reduction of TVCs in crab was 1.3 log cycles. This low level of inactivation could have been due to the method of handling the claws as it was not practical to pick the meat aseptically. However, it should also be noted that the pressures used in these studies were low for pasteurisation applications and it is well known that pressure resistance can vary considerably according to the food substrate. Coliforms were reduced from 10^1 cfu/g to the limits of detection in 11 of the 20 runs but were present at levels of up to 10^2 cfu/g in some of the treated samples. Pseudomonads were absent in the control and in 10 of the 20 conditions tested. They were present at up to 10^3 cfu/g in some samples. It seems likely that considerable variation in the microbiological counts in the raw material masked any differences between processing runs.

4.4.2 Yield/quality data

Run no.	Weight before drain (g)	Weight after drain (g)	Difference (g)	Weight of claws (g)	Claw yield based on original weight (%)	Claw yield based on sum of claw meat and shell weights (%)
Control 1 Cooked	*	*	*	*	*	*
Control 2 Raw	*	*	*	*	*	*
1	*	756.6	*	175.4	*	*
2	539.65	531.8	-7.9	130.3	43.82	47.50
3	669.42	672	2.6	141.5	48.69	51.69
4	592.92	600	7.1	147.1	45.55	50.76
5	661.37	673.4	12.0	153.6	51.24	54.20
6	548.54	537.9	-10.6	126.9	49.57	52.72
7	610.3	615	4.7	146.04	51.49	54.49
8	618.18	607.6	-10.6	142.5	53.12	56.70
9	581.43	579.18	-2.3	119.2	50.56	57.31
10	550.04	527.89	-22.1	127.25	48.64	51.20
11	632.3	619.9	-12.4	134.2	50.60	52.82
12	590.46	582.9	-7.6	133.42	52.98	55.00
13	667.37	662.8	-4.6	*	*	53.27
14	488.24	489.88	1.6	107	44.77	46.44
15	626.36	646	19.6	154.5	47.06	53.97
16	514.1	553	38.9	126.6	46.76	50.86
17	563.95	597.7	33.8	131.4	37.52	36.57
18	555.75	570.8	15.0	142.9	41.92	47.28
19	699.86	722.4	22.5	178.3	43.86	52.77
20	687.04	699.3	12.3	176.1	46.79	52.65

Table 14. Crab yields

There was no significant difference in the weight of the crab before and after processing ($P<0.05$). After pressure treatment the meat of the crabs could be hand-picked with varying degrees of success depending upon the processing conditions used. The complete contents of the body of the crab could be removed in many instances (see

Figure 8). The highest picking yield was 53%, found in runs 8 and 12 (250 MPa, 2.5 mins, 15°C and 300 MPa, 2.5 mins, 16°C respectively). Picking of raw crab for comparative yield values with picked pressure treated crabs was not possible. We do not have commercial yield data for picking of cooked crabs so we cannot assess whether a 53% picking yield in the claws is significant relative to conventional cooking and picking. This question will be addressed more fully in phase 2 trials.

Run 8 gave the best picking yields and best visual appearance after processing (Figure 9). However, replicate processes at the same pressure and time gave variable results for picking (Figure 10). This variation could be due to ease of product picking, being highly susceptible to temperature changes (e.g. picking at 16.2°C or 12.4°C gave poor results compared with picking at 15°C) or it could be due to variation in the raw materials. It was not possible to identify statistically significant differences between claw yields between processing conditions.

As a general though subjective point, it appeared that when removing the legs of the crab after processing, more meat could be removed from the purse. This finding, if transferrable to industrial operations, could be commercially significant because 'intact' purse meat commands a higher value than mechanically recovered purse meat.

Figure 8. Body of crab removed after pressure treatment

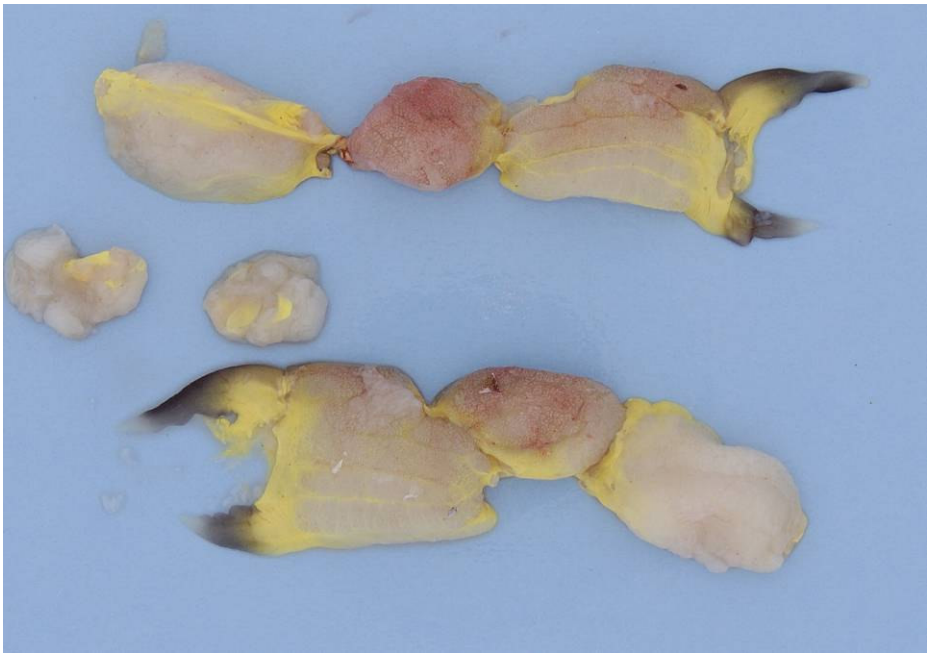

Figure 9. Run 8 hand-picked claw meat after treatment

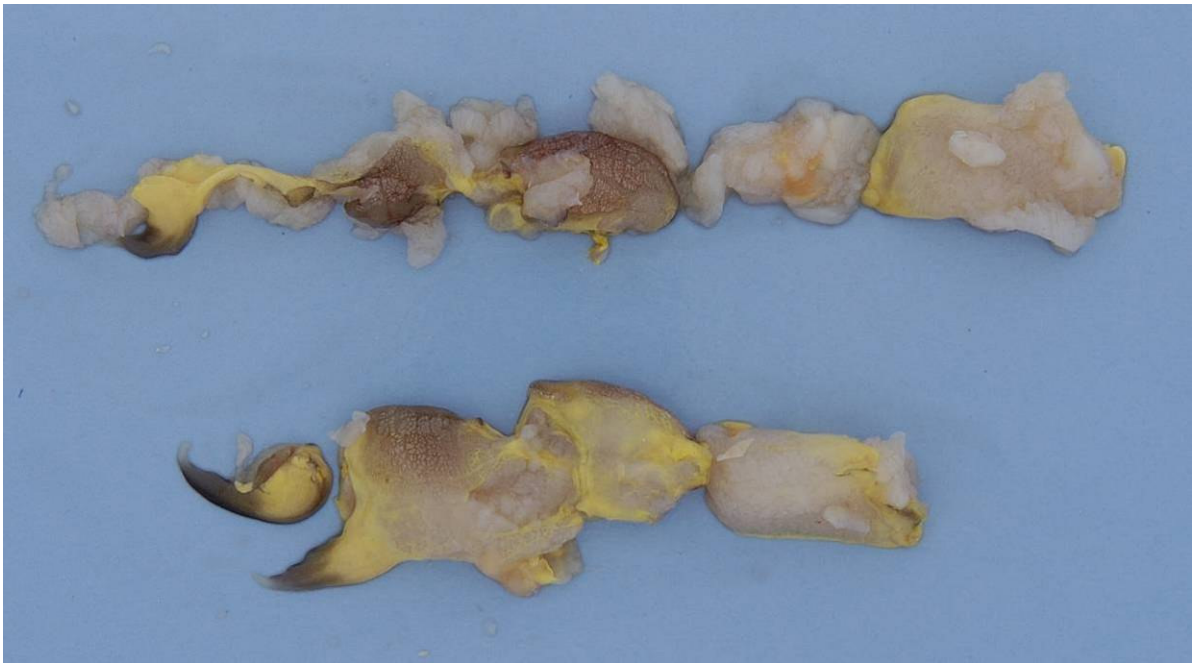

Figure 10. Run 11 hand-picked claw meat after treatment

4.4.3 Brief summary of sensory results for crab.

Controls and runs 1-6 were assessed for sensory quality. Two crab claws were assessed for each sample. For the uncooked assessment there was no Control sample for comparison. Run 1 and Run 4 were graded the highest, with less shape loss than the other samples. Run 2 was graded the lowest, with the most noticeable shape loss.

For the cooked assessment, none of the runs was graded as high as the Control. Run 3 had the highest grade, with a very fresh odour, and strong crab notes, with only slight loss of shape. Run 2 was graded the lowest, with marked loss of shape, and very slight bitter notes.

Images of all pressure treated samples can be found in Appendix 15.

4.4.4 Key conclusions for crab

Reductions in total viable counts were low; this could be due to practical limitations in terms of handling the product after picking but could also be due to raw material variation. The highest picking yield was 53%, found in runs 8 and 12 (250 MPa, 2.5 mins, 15°C and 300 MPa, 2.5 mins, 16°C respectively) but no comparative picking yields could be taken on raw crabs. There was some run-to-run variability as to ease of picking and larger scale studies are required to assess the yield benefits achievable using HPP.

As a general though subjective point, it appeared that when removing the legs of the crab after processing, more meat could be removed from the purse. This finding, if transferrable to industrial operations, could be commercially significant because 'intact' purse meat commands a higher value than mechanical recovered purse meat.

None of the samples from runs 1-6 matched the cooked sensory quality of the control but run 3 was still considered to be good quality. Interestingly, run 3 was the lowest pressure treatment of the 6 runs and also had a short hold time. The more severe treatments scored lower in terms of sensory quality and 'eggy' notes were sometimes reported.

Crab has been selected for further work in phase 2. A process of 250 MPa for 2.5 minutes is proposed for the studies as this gave the best picked appearance of the trial samples. A large batch (50-100 kg) of crabs will be pressure treated and then picked along-side conventionally processed crabs to determine whether there are significant yield benefits to be gained from using HPP.

4.5 Cold water prawns

Microbial reductions obtained for HPP treatment of cold water prawns are reported in Table 15. Yield data are reported in Table 16. Results for sensory evaluation are briefly summarised in section 4.5.3 and are fully reported in Appendix 5.

4.5.1 Microbiological results

Run No.	Pressure (MPa)	Initial product temp (°C)	Product temp after processing (°C)	Time (min)	TVC per g	TVC log reduction	Coliforms per g	Pseudomonas per g
Control Brined	0.1 MPa	*	*	*	1.60E+03	0.0	<5	<50
1	273	3.9	9.8	2.5	1.05E+02	1.2	<5	<50
2	302	11.3	12.7	1	5.50E+01	1.5	<5	<50
3	237	0.5	5.2	1	5.50E+01	1.5	<5	<50
4	270	6.6	10	2.5	4.00E+01	1.6	<5	<50
5	237	1.6	11	4	5.00E+00	2.5	<5	<50
6	298	*	8.7	4	6.00E+01	1.4	<5	<50
7	272	7	11.6	5	4.00E+01	1.6	<5	<50
8	270?	4.1	11.6	2.5	4.50E+01	1.6	<5	<50
9	270	6.3	12.4	2.5	1.50E+01	2.0	<5	<50
10	272	9	14.4	0	1.35E+02	1.1	<5	<50
11	270?	10.2	14.1	2.5	7.50E+01	1.3	<5	<50
12	321	10.1	14.8	2.5	6.50E+01	1.4	<5	<50
13	270	17.5	16.4	2.5	5.00E+01	1.5	<5	<50
14	223	*	17.1	2.5	1.15E+02	1.1	<5	<50
15	233	3.3	15.2	4	4.50E+01	1.6	<5	<50
16	246	17.1	17.3	2.5	3.00E+01	1.7	<5	<50
17	285	3.3	7.9	1	2.00E+01	1.9	<5	<50
18	237	17	16.3	1	5.00E+00	2.5	<5	<50
19	272	11	15.9	2.5	1.00E+01	2.2	<5	<50
20	299	15.1	17.3	4	5.50E+01	1.5	<5	<50

Table 15. Cold water prawns microbial reductions

The maximum reduction in total viable count was 2.5 log cycles. This was achieved in runs 5 and 18 using 237 MPa for 4 and 1 minute respectively. The initial product temperature for run 5 was 1.6°C compared with 17.1°C in run 18. Coliforms and pseudomonads were at non-detectable levels in the control and all treated samples. Log TVC reductions could not be predicted as a function of pressure, temperature and time using linear or quadratic terms ($P>0.05$). Again this could be due to variation in raw materials but is more likely to be due to the fact that pressure treatments were conducted over a very narrow pressure range.

4.5.2 Yield/quality data

Run No.	Weight before drain (g)	Weight after drain (g)	Difference (g)	Weight of 30 prawns (g)	Yield based on original weight (%)	Yield based on sum of meat and shell weights (%)
Control 1 Brined	*	*	*	157.2	43.96	45.61
1	596.26	465.7	130.6	156.4	44.05	45.96
2	576	433	143.0	153.7	45.28	46.74
3	543	358.5	184.5	*	*	45.79
4	545	451.6	93.4	139.2	45.26	46.43
5	563	369	194.0	145.6	45.95	48.03
6	460	383.6	76.4	148	42.91	43.52
7	456	351.1	104.9	164.3	44.00	45.13
8	437	328.9	108.1	156.5	45.11	46.45
9	604	371.6	232.4	138	45.22	45.92
10	558	496.8	61.2	153.1	43.96	45.29
11	458	382.4	75.6	139.7	45.38	45.38
12	536	496.9	39.1	161.7	44.84	44.84
13	536	541.8	-5.8	152.9	45.59	45.59
14	452	410.9	41.1	166.5	38.74	38.74
15	570	515.5	54.5	164.4	39.59	39.59
16	437	376.5	60.5	152.4	45.14	45.14
17	546	463.7	82.3	166.1	44.61	44.61
18	486	508.8	-22.8	167	44.79	44.79
19	611	568.9	42.1	164.6	43.32	43.32
20	677	587	90.0	161.8	45.36	45.36

Table 16. Cold water prawns yield data

Peeling yield varied between 39% and 46% for the pressure treated samples but was on average 44%, i.e. the same as the brined control. A 2% increase in yield would be commercially significant, i.e. if 46% could be consistently achieved compared with 44% using normal production methods, but it is difficult to determine whether these yield increases are genuine or are within the measurement errors inherent in the manual peeling process. Examination of the sample images from the trials would suggest that the very tip of the prawn was more frequently extracted compared with the control samples and therefore the yield increases could be genuine. Larger scale trials on commercial peeling equipment would be required in order to give a more objective measure of peeling benefits because the apparent yield increase is relatively small.

There was a statistically significant weight loss in cold water prawns after pressure treatment ($P<0.05$), the average weight across runs before processing being 532.4g and after being 443.1g.

4.5.3 Brief summary of sensory results for cold water prawns

Controls and runs 1-6 were assessed for sensory quality. For each evaluation approximately 60g of prawns were assessed. For the uncooked assessment none of the runs were graded higher than the Control. Run 1 was graded equal to the Control sample, and all other Runs were graded lower than the Control, with Run 6 achieving the lowest grade. This was due to less retention of the tip end, and more shape loss.

For the cooked assessment, the Control, Run 1 and Run 2 were graded the highest. The other runs were all graded as 'Just Acceptable' due to some eggy notes in the odour, and less prawn flavour.

Images of all pressure treated samples can be found in Appendix 16.

4.5.4 Key conclusions for cold water prawns

Total viable counts could be reduced by up to 2.5 log cycles but no conclusions could be drawn regarding the HPP sensitivity of coliforms and pseudomonds because they were absent in all samples including the controls. Average peeling yields across all 20 runs were no better than the control but yield increases of up to 2% were achieved in some runs. Large scale peeling trials would be required to determine whether this potential yield benefit was transferrable to commercial processing. The sensory quality of HPP treated prawns can match that of conventional processing.

Cold water prawns are not being taken forward to phase 2. The results of warm water prawns are likely to inform processors as to potential benefits for cold water prawns. A second reason for the omission of cold water prawns from phase 2 is that they are a very high volume, relatively low price product and are therefore less suitable for HPP processing compared with warm water prawns. This latter product is relatively low volume but high value so processing economics are likely to be more favourable. HPP processing can currently only achieve something in the region of 1-2 tonnes per hour per machine so is more likely to be able to match the required production volumes.

4.6 Lobster

Microbial reductions obtained for HPP treatment of lobster are reported in Table 17. Yield data are reported in Table 18. Results for sensory evaluation are briefly summarised in section 4.6.3 and fully reported in Appendix 6.

4.6.1 Microbiological results

Run no.	Pressure (MPa)	Initial product temp (°C)	Product temp after processing (°C)	Time (min)	TVC/g	TVC log reduction	Coliforms (per g)	Pseudomonas (per g)
Raw control	0.1MPa	*	*	0	1.30E+04	0.0	<5	<50
Cooked control	0.1 MPa	*	25.3	0	3.81E+02	1.5	<5	5.50E+02
1	273	17.8	18.1	2.5	5.00E+00	3.4	<5	<50
2	302	16.8	17	1	<5	>3.4	<5	<50
3	237	-0.5	4.4	1	1.00E+01	3.1	<5	<50
4	270	17.1	16.9	2.5	2.50E+01	2.7	<5	<50
5	237	14.6	19.1	4	5.00E+00	3.4	<5	<50
6	300	17	18.2	4	<5	>3.4	<5	<50
7	272	18.7	17.5	5	<5	>3.4	<5	<50
8	273	20	17.4	2.5	<5	>3.4	<5	<50
9	264	12.6	4.3	2.5	5.00E+00	3.4	<5	<50
10	250	18.6	18.9	0	<5	>3.4	<5	<50
11	270	18.7	18.5	2.5	4.00E+01	2.5	<5	<50
12	319	18.5	18.4	2.5	1.00E+01	3.1	<5	<50
13	269	19.3	19.6	2.5	6.00E+01	2.3	<5	<50
14	218	19.2	20.6	2.5	<5	>3.4	<5	<50
15	231	10.7	*	4	<5	>3.4	<5	<50
16	269	19.8	19.6	2.5	<5	>3.4	<5	<50
17	302	*	15.6	1	1.00E+02	2.1	<5	<50
18	238	19	19.9	1	5.00E+00	3.4	<5	<50
19	270	19.1	20.5	2.5	1.05E+02	2.1	<5	<50
20	304	*	18.7	4	1.50E+01	2.9	<5	<50

Table 17. Lobster microbial reductions

Total viable counts were reduced from 10^4 cfu/g to the limits of detection in 8 of the 20 process conditions but there were no clear optimum conditions. For example, the maximum recorded pressure did not result in the maximum level of inactivation as would typically be expected. Coliforms and pseudomonads were absent in all samples (including the control) apart from the cooked control where pseudomonads were found at 10^2 cfu/g. This is thought to be due to experimental error because a core temperature of 90°C was recorded when cooking the control lobster. As in the data for the previously reported species, differences between runs as a function of pressure, temperature and time appear to be being masked by raw material variation and the narrow range of pressure conditions tested.

4.6.2 Yield/quality data

Run no.	Weight before drain (g)	Weight after drain (g)	Difference (g)	Weight of claws(g)	Claw yield based on original weight of claws (%)	Claw yield based on sum of claw meat and shell weights (%)	Tail yield based on original weight of tail	Tail yield based on sum of tail meat and shell weights
Raw control	*	*	*	*	*	*	*	*
Cooked control	*	740	*	320	42.19	47.37	*	70.37
1	720.8	765.7	6.23	*	*	63.64	72.22	72.22
2	710.2	750	5.60	115	61.74	63.39	72.62	72.62
3	708.8	753	6.24	107	57.01	61.00	69.27	*
4	731.8	794	8.50	115	62.61	65.45	74.40	76.22
5	675.5	709.3	5.00	114	54.39	54.39	69.68	73.06
6	753	785	4.25	117	62.39	64.04	76.16	75.72
7	708	744	5.08	*	*	56.45	77.34	76.74
8	643	683	6.22	102	62.75	62.75	74.21	75.64
9	738.8	760.4	2.92	98	52.04	60.00	65.05	73.33
10	743.7	779.5	4.81	111	64.86	66.06	76.92	77.38
11	689.9	725	5.09	109	60.55	63.46	72.83	75.28
12	725	753	3.86	104	50.00	58.43	68.48	75.84
13	723.6	755	4.34	99	50.51	57.47	63.44	69.41
14	741.2	781	5.37	125	57.60	62.07	70.41	74.38
15	746.2	785	5.20	106	61.32	63.11	69.54	73.26
16	756.4	780.5	3.19	109	61.47	62.62	73.13	75.48
17	760.7	792	4.11	102	*	*	61.08	71.97
18	688	724	5.23	115	58.70	62.15	*	77.84
19	761.6	779	2.28	128	50.55	54.97	71.43	72.94
20	716.3	754	5.26	114	55.12	61.10	74.12	75.45

Table 18. Lobster yield data

Yield on lobster claws ranged between 50 and 65% and was on average 58% compared with a yield on a cooked control of 42%. Yield of lobster tails ranged between 61% and 71%; a control yield on cooked lobster tail was not taken. It was not possible to measure picking yield on raw lobsters. Differences between treated samples in tail and claw yield were not statistically significant (P>0.05) but tail and claw yields appeared to fall into two populations, yield increasing substantially at pressures of greater than 270 MPa, but some deviations from this general trend were observed (Figure 11 and Figure 12).

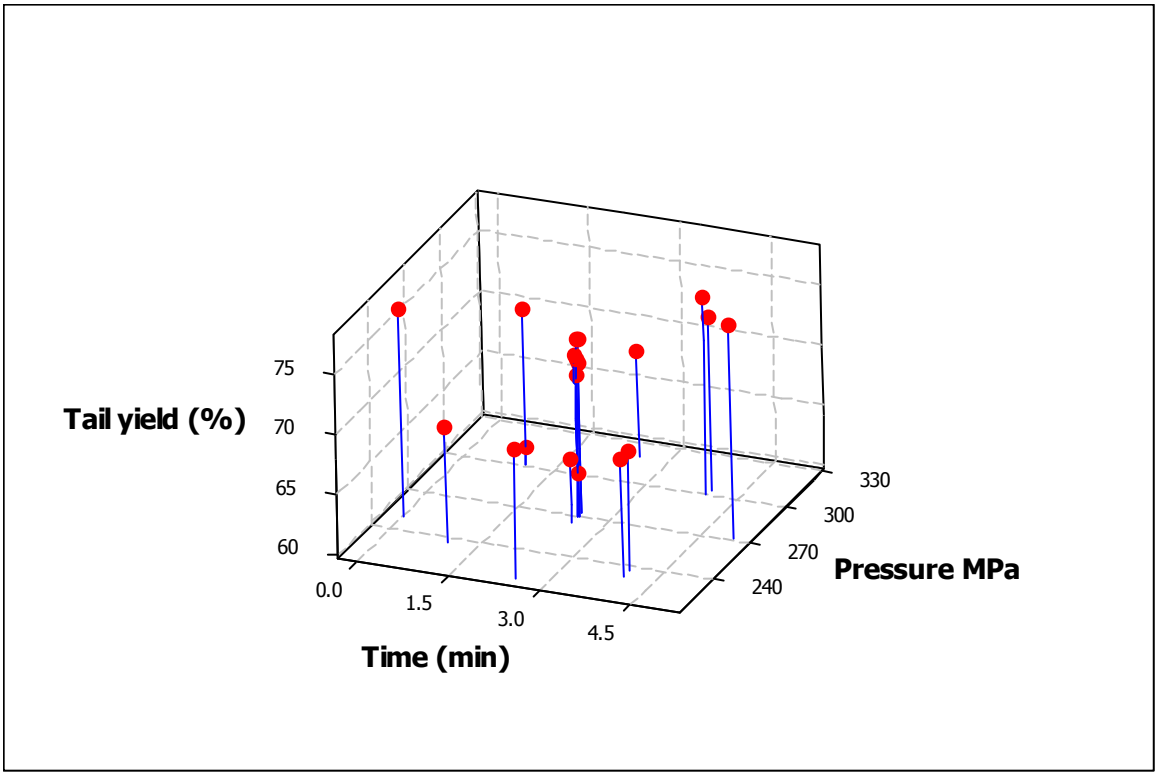

Figure 11. Yield on lobster tails as a function of pressure and time.

Meat yield from the lobster legs was explored in 3 of the 20 runs and compared with the cooked control. Whereas only 8% of the leg weight could be extracted as meat in the cooked control, a yield of 39-44% was achievable in the pressure treated samples. The leg meat could simply be squeezed out of the leg and this could probably be achieved mechanically in a commercial process. Complete extraction of the lobster was attempted for one run only (run 17) but was readily achieved. As was the case for crab, meat

extraction from all parts of the lobster was straightforward but some care was needed in order to extract the meat in whole pieces.

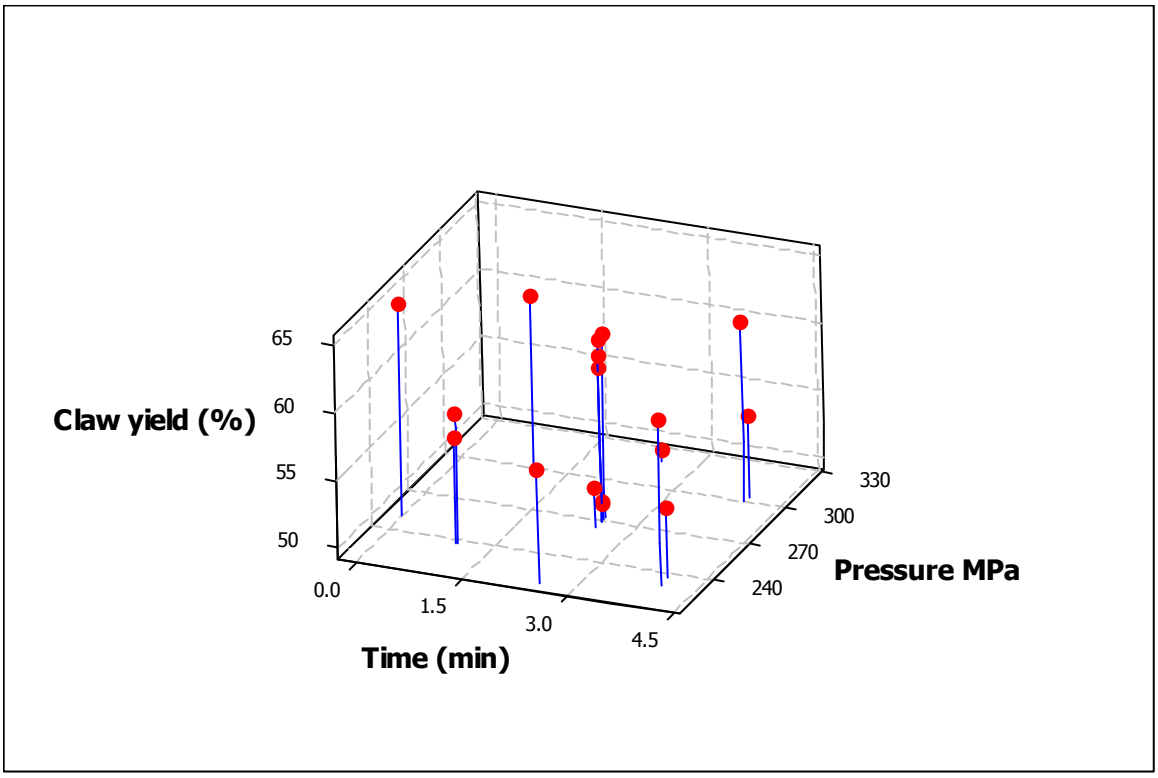

Figure 12. Yield on lobster claws as a function of pressure and time

4.6.3 Brief summary of sensory results for lobster

Controls and runs 1-6 were assessed for sensory quality. For the uncooked assessment there was no Control sample for comparison. Run 1 and Run 5 were graded the highest, with bright clean, white flesh and no loss of shape. Run 2, Run 3 and Run 6 were graded the lowest, with some grey tints in the flesh, and some loss of shape, which was most noticeable in Run 3, which showed complete breakdown.

For the cooked assessment, four runs were graded higher than the Control, which was found to be very fibrous, chewy and rubbery in texture. Runs 1 and 4 were graded the highest, with bright, clean flesh, slightly sweet fresh lobster flavour, and some

fibrousness. Run 2 and Run 5 were also graded higher than the Control, with moderately bright flesh, with only slight yellow or grey tints. Run 3 and Run 6 were graded the lowest, both with some sewage/skatole notes in the odour, and complete shape loss in Run 3.

Images of all pressure treated samples can be found in Appendix 17.

4.6.4 Key conclusions for lobster

Substantial reductions in total viable counts were suggested in some experimental runs, i.e. control samples had 10^4 cfu/ml and TVCs were reduced to non-detectable levels in some runs. There may of course be an element of sample-to-sample variation in terms of the initial microbial loading. Yield on lobster claws was up to 23% higher than cooked controls but this data did not take in to account cooking losses that could have occurred in the control sample. In general, pressures of greater than 270 MPa gave better results for tail yield. Indications from the work are that overall picking yields from lobsters could be enhanced significantly because meat could be readily extracted even from the legs – gentle squeezing was sufficient to remove the meat. Sensory evaluation by a trained panel suggested that in many instances HPP treated lobster was considered to be higher quality than the control. Although the results from the lobster work were positive and very interesting, this product is not being taken forward to phase 2. This is because the findings from crab are expected to be transferrable to lobster.

4.7 Warm water prawns

Microbial reductions obtained for HPP treatment of warm water prawns are reported in Table 19. Yield data are reported in Table 20. Results for sensory evaluation are briefly summarised in 4.7.3 and fully reported in Appendix 7.

4.7.1 Microbiological results

Run No.	Pressure (MPa)	Initial product temp (°C)	Product temp after processing (°C)	Time (min)	TVC/g	TVC log reduction	Coliforms (per g)	Pseudomonas (per g)
Control 1 Brined	0.1 MPa	-0.5	*	0	2.00E+03	0.0	1.50E+01	<50
1	273	8.3	14.3	2.5	6.72E+02	0.5	<5	<50
2	300	9.4	14.6	1	1.63E+02	1.1	<5	<50
3	237	8.9	3.3	1	3.68E+02	0.7	<5	<50
4	273	1.7	11.4	2.5	1.31E+02	1.2	<5	<50
5	239	11.8	13.5	4	1.05E+02	1.3	<5	<50
6	297	2.3	5.5	4	4.50E+01	1.6	<5	<50
7	271	*	13.3	5	1.45E+02	1.1	<5	<50
8	272	4.5	14.7	2.5	7.00E+01	1.5	<5	<50
9	264	7.8	5.2	2.5	1.77E+02	1.1	<5	<50
10	227	7.9	14.5	0	3.54E+02	0.8	<5	<50
11	236	11.1	13.9	2.5	4.86E+02	0.6	3.5	<50
12	315	10.8	14.6	2.5	1.00E+01	2.3	2.9	<50
13	268	10.3	16.3	2.5	1.10E+02	1.3	3.2	<50
14	219	*	15.2	2.5	3.20E+02	0.8	3.4	<50
15	233	10.2	6.4	4	2.45E+02	0.9	3.3	<50
16	273	12.6	16.1	2.5	2.36E+02	0.9	3.3	<50
17	300	11.1	12.9	1	2.54E+02	0.9	3.3	<50
18	238	12.8	16.5	1	2.63E+02	0.9	3.4	<50
19	272	14.1	16.4	2.5	1.25E+02	1.2	3.2	<50
20	299	16.2	*	4	8.00E+01	1.4	3.2	<50

Table 19. Warm water prawns microbial reductions

The maximum TVC reduction achieved in the trials was 2.3 log cycles. This was achieved in run 12 (321 MPa, 10.1°C, 2.5 minute hold time) which was also the highest pressure used in the trials. Log TVC reduction between runs could not be predicted using either a linear or quadratic model incorporating pressure, temperature and time ($P>0.05$). However, there was a statistically significant correlation between pressure and TVC log reduction ($P<0.05$) and, in plotting the data, a relationship between increasing pressure and log reductions was apparent (Figure 13). Coliforms were reduced from 10^1 cfu/g in the controls to the limits of detection in 18 of the 20 conditions. Pseudomonads were absent in all samples (including the control).

The focus of this particular set of trials was on 'peelability' of the prawns after pressure treatment and previous discussions with equipment suppliers (Avure and NC Hyperbaric) had established that 200-300 MPa was a suitable pressure range for enhancing peeling without unduly influencing product quality. Had the trials been conducted over a wider range of pressure conditions it is almost certain that increasing pressure would result in greater levels of log reductions as this has been established in many raw materials [see, for example (IFT 2000)].

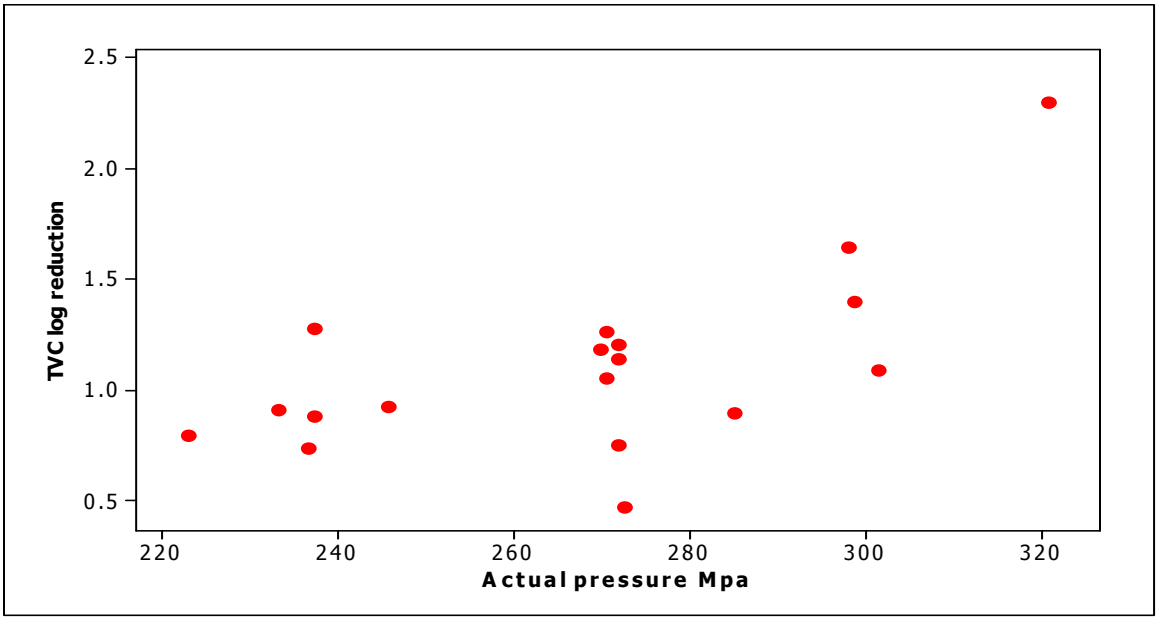

Figure 13. Influence of pressure on TVC log reductions.

4.7.2 Yield/quality data

Run No.	Weight before drain (g)	Weight after drain (g)	Difference (g)	Weight of 10 prawns(g)	Yield based on original weight (%)	Yield based on sum of meat and shell weights (%)
Control 1 Brined	*	*	*	211.1	49.03	53.41
1	561.2	514.3	46.9	191	55.81	56.94
2	571.3	583	-11.7	196.2	54.94	55.34
3	573.7	564.6	9.1	*	*	57.76
4	614.3	581.2	33.1	186.7	56.19	56.64
5	513.4	491.6	21.8	190.5	56.01	56.85
6	586.1	573.9	12.2	188.5	55.17	56.25
7	604.3	606.3	-2.0	191.3	54.73	*
8	591.1	596.3	-5.2	200.1	56.87	57.88
9	584.2	594.2	-10.0	195.3	55.97	56.63
10	*	572.1	*	197.1	56.93	57.72
11	606	601.6	4.4	192.3	56.99	57.47
12	577.1	590	-12.9	193.5	56.54	57.70
13	590.3	567	23.3	205.1	56.22	57.36
14	585.3	577.2	8.1	187	55.83	56.92
15	599.5	594.1	5.4	199.5	55.59	56.52
16	659.4	620.4	39.0	202.4	56.03	57.42
17	561.3	558.2	3.1	*	*	57.19
18	593.6	574.9	18.7	197.8	56.12	57.28
19	653.3	585.5	67.8	194.2	56.49	56.90
20	607.7	596.7	11.0	197.2	55.38	56.67

Table 20. Warm water prawns yield data

Peeling yields were increased substantially as a result of pressure treatment. Yield in the control samples was 49% compared with, on average, 56% in the pressure treated samples. The highest yield achieved was 57% (236 MPa, temperature of 11.1°C, hold time 2.5 minutes) but the differences between treatment conditions were very small (yield

ranging between 55-57%) and not could not be predicted as a function of pressure, temperature and time using linear or quadratic models (P>0.05).

Although subjective, in many cases it was noticeably easier to peel the prawns after pressure treatment compared with the controls. Most striking of all was the fact that even the very tip of the prawn could be removed by gently squeezing the tail (see Figure 14 and Figure 15 for a comparison between control samples and one HPP condition).

Figure 14 Control warm water prawns

Figure 15 Target pressure of 250 MPa, temperature of 10.2°C, hold time 2.5 minutes

4.7.3 Brief summary of sensory results for warm-water prawns

Controls and runs 1-6 were assessed for sensory quality. For each evaluation 8 warm water prawns were assessed. For the uncooked assessment Run 1 and Run 3 were both graded higher than the Control sample, both had better retention of the tip ends. Run 5 was graded equal to the Control, whereas Run 2, Run 4 and Run 6 were all graded lower. These were all less grey with green/yellow tints and they had more shape loss and less retained membrane.

For the cooked assessment the Control and Run 3 were graded the highest. The others all displayed shape loss and a 'woollier' appearance.

Images of all pressure treated samples can be found in Appendix 18.

4.7.4 Key conclusions for warm water prawns

Peeling yields were enhanced substantially by HPP treatment, typically being 55-57% compared with 46% for the control. Run 11 gave the highest yields (237 MPa, 11.1°C, 2.5 minutes) and a pressure of 237 MPa also gave the best sensory results from runs 1-6. Cooked, pressure treated samples matched the controls for sensory quality.

Since peeling yields benefits look very significant for this product it has been selected for further investigation in phase 2 of the project. A large quantity of prawns (50-100 kg) will be pressure treated, hand-peeled and compared with a control. A pressure close to 237 MPa with a 2.5 minute hold time is suggested as this treatment gave good yield, and sensory quality at 237 MPa appears to be acceptable. The temperature used for the trials will be decided after further discussions with industry and will also be subject to the temperature control limitations on the pressure system used for the work.

4.8 Salmon

Microbial reductions obtained for HPP treatment of salmon are reported in Table 21. Results for sensory evaluation are briefly summarised in 4.8.3 and fully reported in Appendix 8.

4.8.1 Microbiological results

Run No.	Pressure (MPa)	Time (min)	Temperature (°C)	TVC (per g)	TVC log reductions	Coliforms (per g)	Pseudomonads (per g)
Control 1	0.1	0	*	2.00E+05	0.0	1.70E+04	8.50E+02
Control 2	0.1	0	*	1.60E+05	0.1	1.20E+04	1.10E+05
1	425	3	6	245	2.9	<5	<50
2	321	2	9	1300	2.2	<5	<50
3	321	4	9	473	2.6	<5	<50
4	529	4	10	<5	4.6	<5	<50
5	529	2	10	10	4.3	<5	<50
6	425	3	11	291	2.8	<5	<50
7	425	3	12	305	2.8	<5	<50
8	250	3	12	3700	1.7	<5	<50
9	425	3	13	395	2.7	<5	<50
10	425	5	13	300	2.8	<5	<50
11	425	3	13	445	2.7	<5	<50
12	425	3	13	832	2.4	<5	<50
13	425	1	13	295	2.8	<5	<50
14	600	3	14	<5	4.6	<5	<50
15	425	3	11	527	2.6	<5	<50
16	321	4	15	2100	2.0	<5	<50
17	529	2	16	5	4.6	<5	<50
18	321	2	16	20000	1.0	<5	<50
19	529	4	15	<5	4.6	<5	<50
20	425	3	17	1200	2.2	<5	<50

Table 21. Salmon microbial reductions

Pressure significantly influenced the observed log TVC reductions ($P<0.05$) as can been seen in Figure 16. Pressures in the region of 200-300 MPa typically resulted in 1-2 log reductions; pressures around 400 MPa gave around a 3 log reduction; and pressures of 500-600 MPa reduced log TVC counts to the limits of detection. Temperature also significantly influenced log TVC reductions ($P<0.05$) but the magnitude of the effect was small relative to the influence of pressure. Hold time did not appear to significantly influence log TVC reductions ($P>0.05$) over the range of hold periods tested.

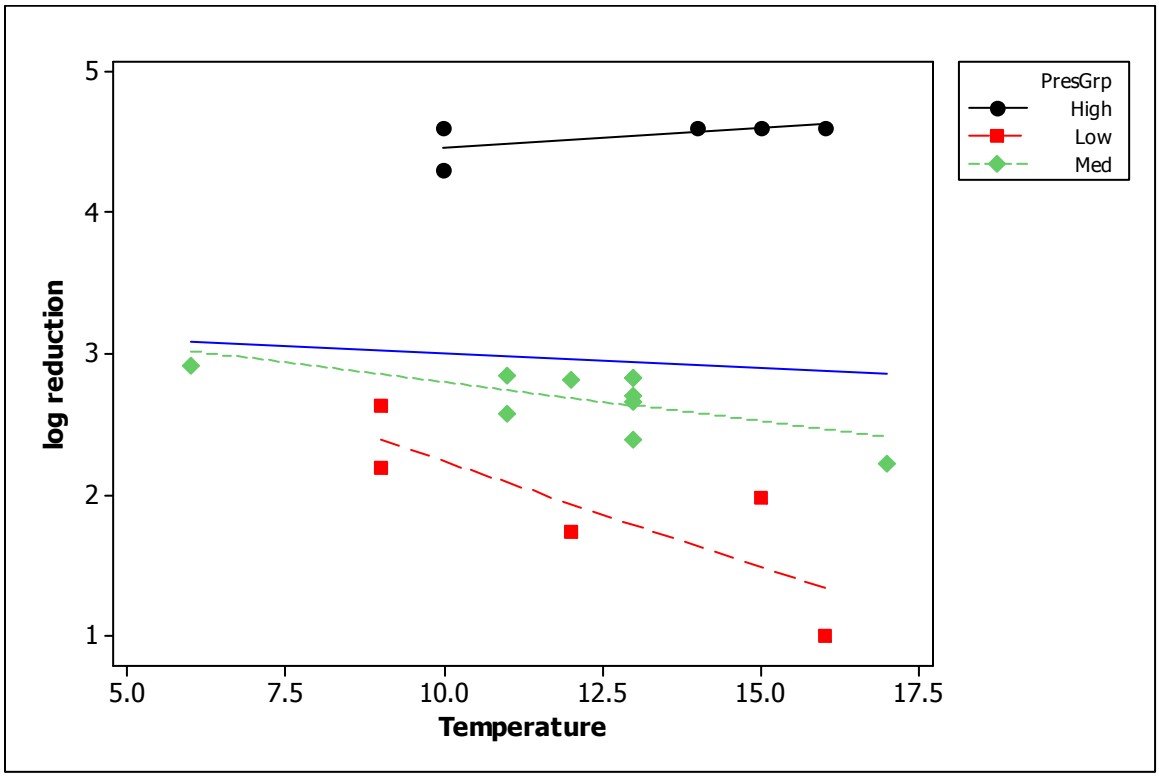

Figure 16 Influence of pressure and temperature on TVC log reduction

Coliforms were reduced from 10^4 cfu/g in the controls to the limits of detection in all 20 runs. Similarly, pseudomonads were reduced from between 10^2 and 10^5 cfu/g to the limits of detection in all 20 runs. A quadratic surface response was not an appropriate model for the response of log TVC reduction to pressure, temperature and time.

4.8.2 Yield/quality data

Colour changes as a result of processing were evident with the product taking on a cooked appearance in all treatments over 250 MPa. Differences in L* could be predicted reasonably well with a quadratic response surface and the vast majority of the observed variation was attributable to changes in pressure (R^2 66.5%, P = 0.058). Changes in a* and b* as a result of processing could be predicted very well using a quadratic surface response (R^2 = 89.7 and 92.3 respectively) with both parameters being influenced by pressure (P<0.05) and temperature (P<0.01). Time was not found to significantly influence a* and b* values (P>0.05). Whilst temperature had a significant effect on *a** and *b** values, pressure was by far the most important determinant of colour shift. Figure 17 shows a* and b* measurements for all 20 runs with each data point indicating the pressure applied during the run. There is good grouping of the data points according to the pressure applied. As pressure increases a* and b* measurements decline.

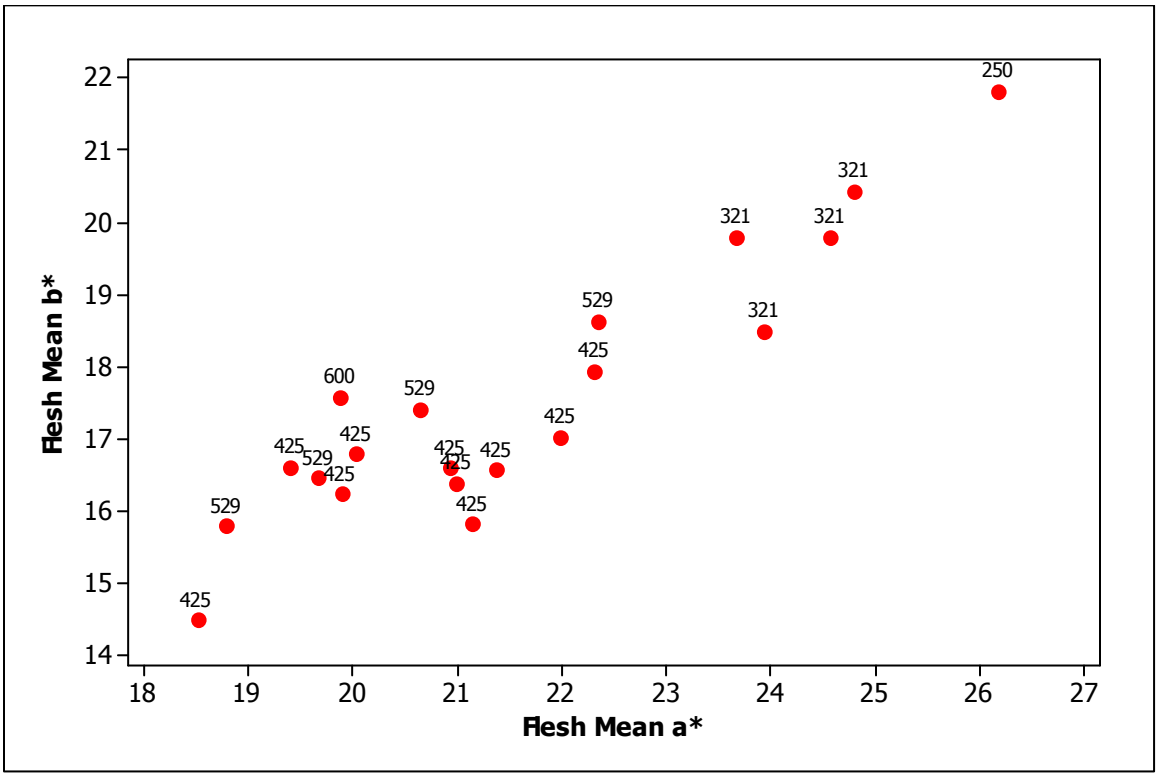

Figure 17. Influence of pressure on a and b* colour measurements in unsmoked salmon*

This uniform and predictable shift in a* *and* b* can be described by the parameter C* which is calculated from the *a** and *b** values using Equation 1.

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

Equation 1

Values of C* could be predicted very well using a quadratic surface response model (Figure 18). As pressure increases, the value of C* decreases; samples took on an increasingly pink, cooked appearance.

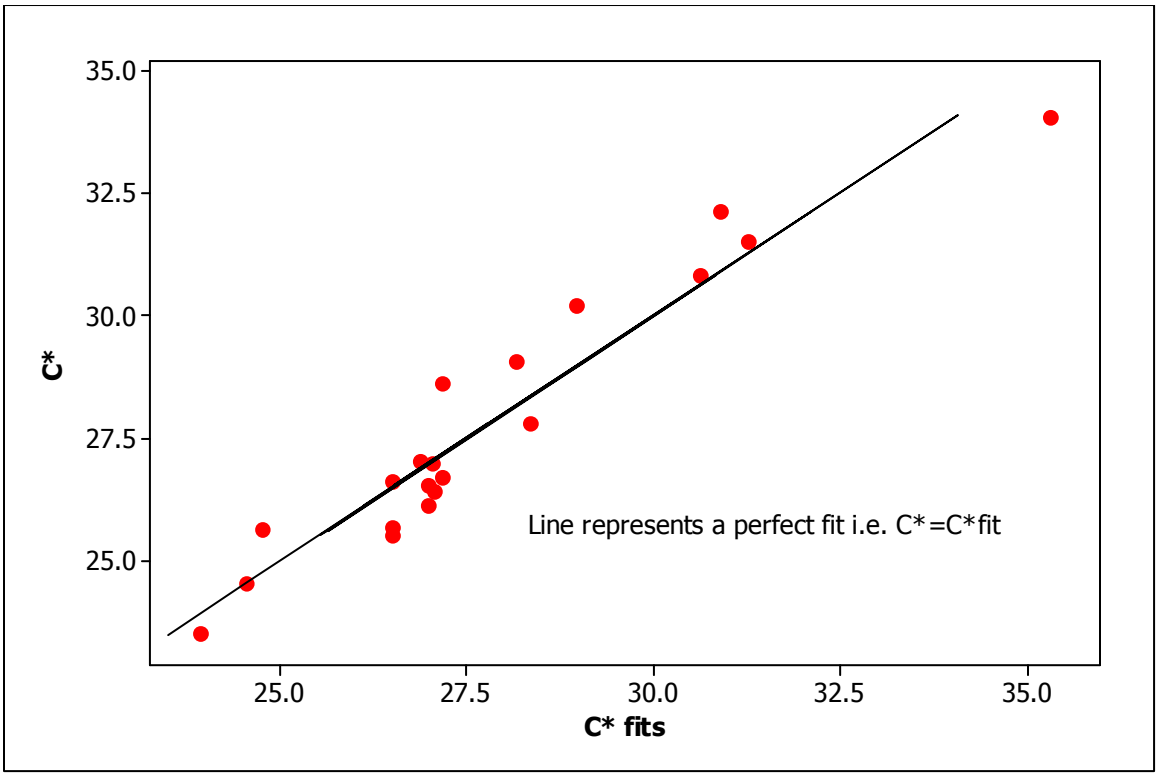

Figure 18. Predicted versus actual C values for pressure treated salmon*

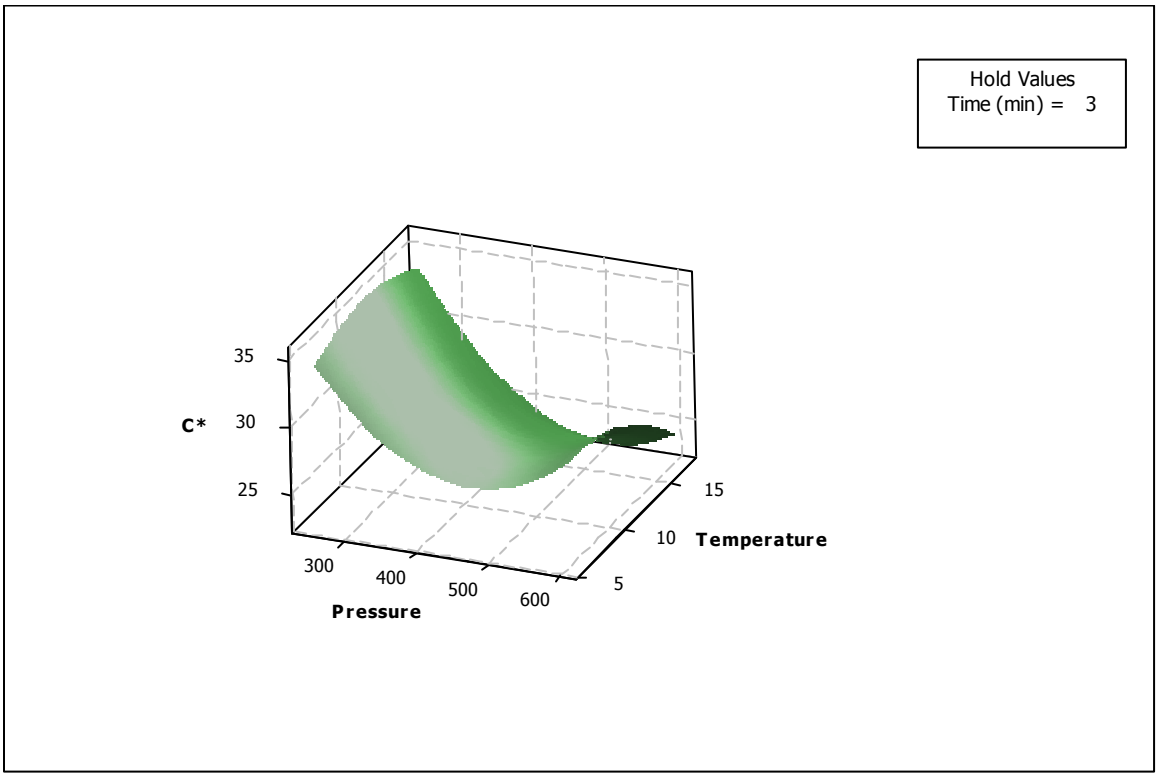

Figure 19. Influence of temperature and pressure on the value of C in unsmoked salmon*

4.8.3 Brief summary of sensory results for salmon

A control and runs 1, 8, 10, 11, 14 and 20 were assessed for sensory quality. For each evaluation one fillet was assessed. For the uncooked assessment none of the samples were graded as high as the Control, which was bright and moist. Run 20 was graded the highest, with dense, moderately bright flesh. Run 1 and Run 11 were graded the lowest, with very dense, compressed flesh, and no defined flake structure.

For the cooked assessment none of the samples were graded as high as the Control, which was bright and moist, with a very fresh odour. Run 8 and Run 20 were graded the highest, with moderately bright flesh, and expected balanced flavour. Run 1, Run 10 and Run 14 were graded the lowest, being pale in colour, and with a very fibrous, tough texture.

Images of all pressure treated samples can be found in Appendix 19.

4.8.4 Key conclusions for salmon

Total viable counts, pseudomonads and coliforms could be reduced to the limits of detection in salmon using HPP. It is very likely that HPP could be used to inactivate pathogenic organisms and therefore to effectively pasteurise the product but this would need to be confirmed by challenge test. However, the pressures required for pasteurisation would lead to an undesirable 'cooked' appearance in the product. HPP is therefore unsuitable for the pasteurisation of salmon if it is desirable for the product to still appear raw after processing. It could still have applications, however, for use as a pasteurisation step, e.g. in a fish based ready meal, if it were not critical for the fish to appear raw. There may also be applications for using low pressures (<250 MPa) to assist with marinating (as is done to produce flavoured oysters in the USA). This particular application will be explored further for salmon in phase 2 of the project.

4.9 Squid

Microbial reductions obtained for HPP treatment of squid are reported in Table 22. Weight changes after processing are reported in Table 23. Methods and results for sensory evaluation are briefly summarised in section 4.9.3 and fully reported in Appendix 9.

4.9.1 Microbiological results

Run number	Pressure (MPa)	Time (Min)	Initial temperature of Pressure fluid (°C)	Product temperature after processing (°C)	TVC per g	Log TVC reduction	Coliforms per g	Pseudomonads per g
Control	0.1	0	*	*	270000	*	120	<10
1	400	5	29.2	21	50	3.7	<5	<10
2	200	2.5	28	21.2	24000	1.1	1100	<10
3	600	2.5	29.9	18.7	10	4.4	<5	<10
4	*400*	*2.5*	*28.4*	*19.4*	*1100*	*2.4*	*<5*	*<10*
5	400	2.5	28.6	22.8	341	2.9	<5	<10
6	400	2.5	28.8	21.3	30	4.0	<5	<10
7	400	2.5	28.2	22.2	50	3.7	<5	<10
8	400	0	28.1	21.4	2300	2.1	90	<10
9	522	1	27.4	18.8	5	4.7	<5	<10
10	278	4	28.3	24.4	600	2.7	10	<10
11	278	1	29.1	19.5	2400	2.1	<5	<10
12	400	2.5	29.7	21.6	25	4.0	<5	<10
13	400	2.5	*	25	25	4.0	<5	<10
14	522	4	26	18.7	<5	4.7	<5	<10
15	522	4	28.8	19.6	15	4.3	<5	<10
16	400	2.5	25.7	23.8	80	3.5	<5	<10
17	522	1	30.1	24.6	<5	4.7	<5	<10
18	278	1	29.6	21	15000	1.3	<5	<10
19	400	2.5	32.2	20.7	<5	4.7	<5	<10
20	278	4	33.4	23.8	1000	2.4	<5	<10

Table 22. Squid microbial reductions

Pressure was found to significantly influence log TVC reduction (P<0.05) with increasing levels of microbial inactivation found with increasing pressure (Figure 20). Temperature and time were not significant (P<0.05). A quadratic surface response model could not be used to accurately model inactivation as a function of pressure, temperature and time.

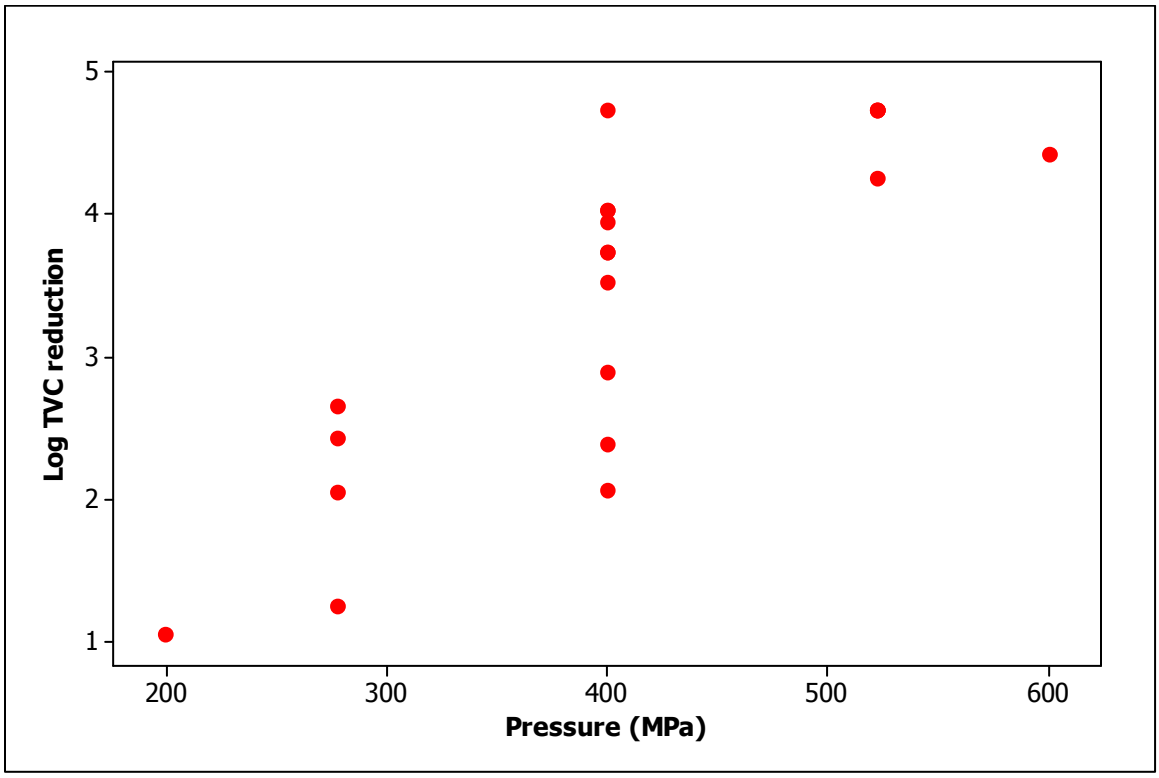

Figure 20. Influence of pressure on log TVC reduction in squid

Coliforms were reduced from 10^2 per g in the control sample to the limits of detection in 19 of the 20 conditions tested. Pseudomonads were absent in all samples including the control. TVCs could be reduced from 10^5 cfu/g in the controls to the limits of detection in some processing conditions, e.g. 522 MPa for 1 minute at 30.1°C.

4.9.2 Yield/quality data

Run number	Pressure (MPa)	Time (Min)	Weight before HPP (g)	Weight after HPP (g)	% change
Control	0.1	0	164.1	164.1	*
1	400	5	160.3	132.6	-17
2	200	2.5	169.2	149.9	-11
3	600	2.5	180.9	155.9	-14
4	400	2.5	190.8	160.0	-16
5	400	2.5	113.9	93.1	-18
6	400	2.5	131.3	108.8	-17
7	400	2.5	113.9	111.6	-2
8	400	0	158	145.9	-8
9	522	1	165.5	165.1	0
10	278	4	129.8	124.4	-4
11	278	1	149	149.4	0
12	400	2.5	157.4	157.2	0
13	400	2.5	179.2	171.1	-5
14	522	4	155.1	154.8	0
15	522	4	128.4	128	0
16	400	2.5	157	156.6	0
17	522	1	158.6	158.7	0
18	278	1	132.5	132.1	0
19	400	2.5	179.2	178	-1
20	278	4	172.4	172.3	0

Table 23. Squid yield data

The weight of each squid tube was recorded before and after processing but runs 1-6 were handled more, e.g. for photography prior to sensory evaluation, and this is thought to be the reason for the bigger weight losses post process in the first 6 samples. The weight of the squid after processing was slightly reduced and was statistically significantly different from the weight before processing ($P<0.05$).

4.9.3 Brief summary of sensory results for squid

A control and runs 1-6 were assessed for sensory quality. For the uncooked assessment none of the samples were graded as high as the Control, which was very bright and moist, with the expected milky white colour. The Run 6 sample was graded the highest, being moderately bright and moist, with very little loss of shape. The Run 2 sample was graded the lowest, with a denser, slightly dirty flesh, and slight loss of shape.

For the cooked assessment none of the samples were graded as high as the Control, which was moderately bright and moist, with very little loss of shape, and a balanced salty and sweet flavour. Run 1 and Run 2 were graded the highest, having a similar bright and moist appearance to that of the control, but with more bitterness in the flavour. Runs 3, 5 and 6 were graded the lowest. Runs 3 and 5 were badly cleaned internally, and had some protein cook-out present, a moderately bitter flavour, and extremely fibrous texture. Run 6 also had an extremely fibrous texture, but a cleaner appearance, and was also very bitter.

Images of all pressure treated samples can be found in Appendix 20.

4.9.4 Key conclusions for squid

HPP reduced TVCs, pseudomonads and coliforms to the limits of detection at some pressure/time/temperature combinations and it seems likely that it could be applied for pasteurisation of squid (this would need to be confirmed by challenge testing). However, the sensory quality of the squid was not as good as the control (although still considered to be of fairly good quality). HPP also seemed to increase the perceived bitterness of the sample relative to the control. In conclusion, HPP gave good reductions in microbiological loading and hence would almost certainly extend shelf life but there were no major benefits in terms of quality improvements. This product has not been selected for further work in phase 2.

4.10 Mackerel

Microbial reductions obtained for HPP treatment of mackerel are reported in Table 24. Weight changes before and after processing are reported in Table 25. Results for sensory evaluation are briefly summarised in section 4.10.3 and are fully reported in Appendix 10

4.10.1 Microbiological results

Run No.	Pressure (MPa)	Product temp after processing (°C)	Time (min)	TVC	TVC log reduction	Coliforms	Pseudomonads
Control	0.1	*	0	4.40E +04	*	<5	2.10E+04
1	400	11.9	5.0	<5	>3.94	<5	<50
2	200	15.8	2.5	<5	>3.94	<5	<50
3	600	14.4	2.5	<5	>3.94	<5	900
4	400	12.3	2.5	80	2.74	<5	1500
5	400	11.5	2.5	<5	>3.94	<5	<50
6	400	14.5	2.5	<5	>3.94	600	<50
7	400	13.2	2.5	<5	>3.94	<5	<50
8	400	10	0.0	<5	>3.94	<5	<50
9	522	15.2	1.0	<5	>3.94	<5	<50
10	278	12.9	4.0	<5	>3.94	<5	<50
11	278	13	1.0	<5	>3.94	<5	<50
12	400	12.3	2.5	<5	>3.94	<5	<50
13	400	13.1	2.5	<5	>3.94	<5	<50
14	522	10.6	4.0	<5	>3.94	<5	<50
15	522	11.2	4.0	<5	>3.94	<5	<50
16	400	13.5	2.5	<5	>3.94	<5	<50
17	522	15.7	1.0	<5	>3.94	<5	<50
18	278	15.1	1.0	<5	>3.94	<5	3000
19	400	14.5	2.5	<5	>3.94	<5	<50
20	278	15	4.0	<5	>3.94	<5	<50

Table 24. Mackerel microbial reductions

Total viable counts were reduced from 10^4 cfu/g in the control samples to the limits of detection in 19 of the 20 conditions tested. Coliforms were non-detectable in all but one of the treatment conditions including the control. Pseudomonads were reduced from 10^4 cfu/g in the control to the limits of detection in 17 of the 20 conditions tested. There

was no clear pattern explaining why pseudomonads survived in 3 runs and this could simply be due to sample-to-sample variation or experimental error.

4.10.2 Yield/quality data

Run Number	Blocks	Product temp after processing	Time (min)	Weight before processing	Weight after processing	% change
Control			0			
1	400	11.9	5.0	102.6	103.5	1
2	200	15.8	2.5	93.8	93.9	0
3	600	14.4	2.5	108.7	110	1
4	400	12.3	2.5	102.6	104.1	1
5	400	11.5	2.5	98.2	100	2
6	400	14.5	2.5	110	111.3	1
7	400	13.2	2.5	98.9	97.9	-1
8	400	10	0.0	93.6	95.8	2
9	522	15.2	1.0	89.9	89.9	0
10	278	12.9	4.0	99.8	99.8	0
11	278	13	1.0	102.9	103.2	0
12	400	12.3	2.5	99.2	99.2	0
13	400	13.1	2.5	107.9	109.8	2
14	522	10.6	4.0	97.4	96.1	-1
15	522	11.2	4.0	113.4	114.4	1
16	400	13.5	2.5	100.9	101.3	0
17	522	15.7	1.0	87	87.4	0
18	278	15.1	1.0	83.7	84.3	1
19	400	14.5	2.5	109.7	109.5	0
20	278	15	4.0	106.7	107.2	0

Table 25. Mackerel yield data

There was a statistically significant difference ($P<0.05$) between mackerel fillet weights before and after pressure treatment but in practical terms this difference was very small (average weights before and after processing differed by only 0.6g).

Significant colour changes were apparent in pressure treated mackerel; as pressure increased, the samples took on an increasingly ‘cooked’ appearance. The only sample with a visual appearance close to raw was run 2 processed at 200 MPa.

4.10.3 Brief summary of sensory results for mackerel

Controls and runs 1-6 were assessed for sensory quality. For the uncooked assessment only the Run 2 sample was graded as high as the control, being bright and moist, with very little loss of shape. Run 3 and Run 4 were graded the lowest, with very dense flesh, and little defined flake structure.

For the cooked assessment none of the samples were graded as high as the Control, which was bright and moist, with a well-balanced, typically oily flavour. Runs 1, 4 and 5 were graded the highest, with moderately bright flesh, and expected balanced flavour, with only slight acidity. Runs 2 and 3 were graded the lowest, being less bright, with some loss of shape. The Run 2 sample had a dry, open appearance, and the Run 3 sample was very dense and compressed.

Images of all pressure treated samples can be found in Appendix 21.

4.10.4 Key conclusions for mackerel

The process was very successful in terms of microbiological inactivation with TVCs and pseudomonads being reduced dramatically as a result of pressure treatment. However, above 200 MPa the process caused very significant colour changes and general changes in visual appearance; the product appeared compressed with a loss of defined flakes. HPP is unlikely to be suitable for use as a pasteurisation process where it is desirable that the product still appears raw. As was the case for salmon, HPP could still be useful for the non-thermal pasteurisation of ready meals or as a processing aid for marination. Mackerel is not being taken forward for further evaluation in phase 2. Cod and salmon are being investigated further and data from these trials is likely to give a good indication as to the likely effects on other fish species.

4.11 Cod

Microbial reductions obtained for HPP treatment of cod are reported in Table 26. Yield data are reported in Table 27. Results for sensory evaluation are briefly summarised in section 4.11.3 and are fully reported in Appendix 11.

4.11.1 Microbiological results

Run No.	Pressure (MPa)	Product temp after processing	Time (min)	TVC	TVC log reduction	Coliforms (per g)	Pseudomonads (per g)
Control			0	94000		5	110000
1	400	13	5	95	3.0	<5	<50
2	200	12.8	2.5	3500	1.4	<5	400
3	600	10.9	2.5	<5	>4.3	<5	<50
4	400	12.4	2.5	80	3.1	<5	<50
5	400	11.3	2.5	150	2.8	<5	<50
6	400	11	2.5	125	2.9	<5	<50
7	400	12	2.5	110	2.9	<5	<50
8	400	11.6	0	1800	1.7	<5	<50
9	522	11	1	55	3.2	<5	<50
10	278	8.1	4	904	2.0	<5	<50
11	278	13.1	1	450	2.3	<5	<50
12	400	11.4	2.5	259	2.6	<5	<50
13	400	12.6	2.5	105	3.0	<5	<50
14	522	10.7	4	<5	>4.3	<5	<50
15	522	10.7	4	15	3.8	<5	<50
16	400	12.2	2.5	218	2.6	<5	<50
17	522	12.2	1	45	3.3	<5	<50
18	278	11.3	1	3300	1.5	<5	350
19	400	12.3	2.5	150	2.8	<5	<50
20	278	13.6	4	1600	1.8	<5	<50

Table 26. Cod microbial reductions

Total viable count log reductions were significantly influenced by pressure and hold time ($P<0.05$ in both cases) but pressure was the more important factor in determining the level of microbial inactivation achieved (Figure 21). TVCs were reduced from 10^4 cfu/g

in the controls to varying levels depending on pressure and time; in runs 3 and 14 total viable counts were reduced to the limits of detection (>4.3 logs reduction).

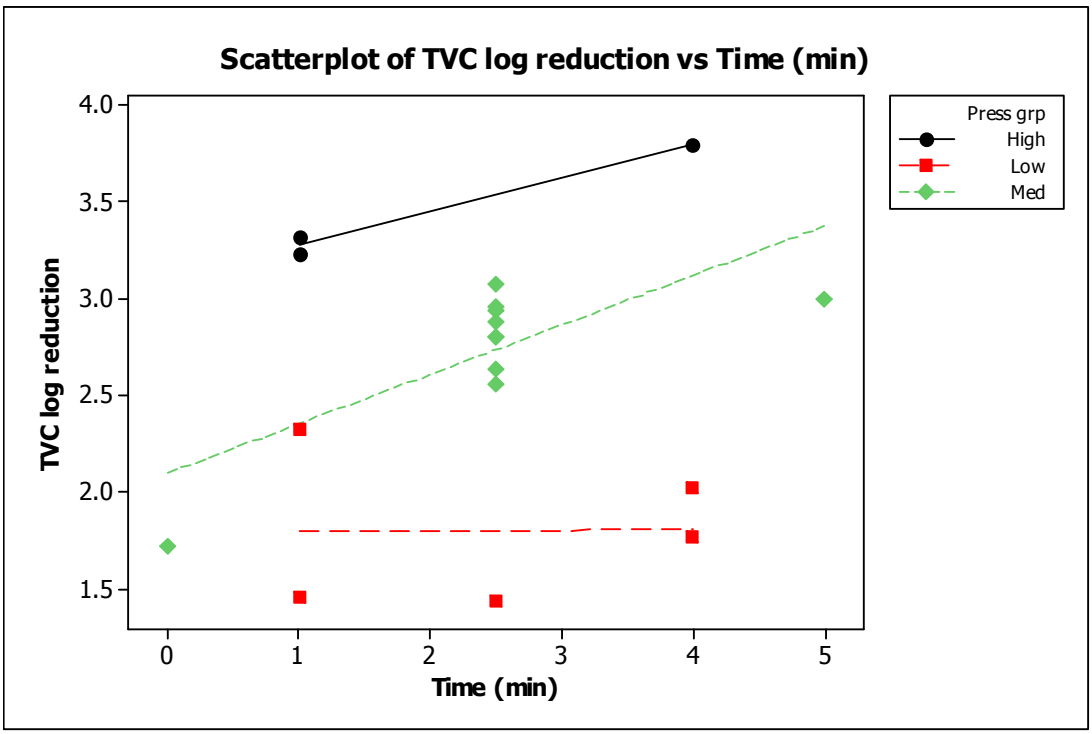

Figure 21. Influence of pressure and time on log TVC reduction in cod.

Coliforms were at very low levels in the control sample (5 cfu/g) but were reduced to the limits of detection in all pressure treated samples. Pseudomonads were reduced from 10^5 cfu/g to the limits of detection in all process runs. The weights of the fillets before and after processing were not statistically significantly different (P>0.05).

4.11.2 Yield/quality data

Run No.	Pressure (MPa)	Product temp after processing (°C)	Time (min)	Weight before processing (g)	Weight after processing (g)	% change
Control	0,1	*	0	*	*	*
1	400	13	5	107.8	105.94	-1.7
2	200	12.8	2.5	103.15	99.98	-3.1
3	600	10.9	2.5	123	121.43	-1.3
4	400	12.4	2.5	130.47	130.75	0.2
5	400	11.3	2.5	133.54	130	-2.7
6	400	11	2.5	136.65	136.58	-0.1
7	400	12	2.5	140.37	140.4	0.0
8	400	11.6	0	103.54	103.47	-0.1
9	522	11	1	121.29	120.7	-0.5
10	278	8.1	4	154.39	154.4	0.0
11	278	13.1	1	85.5	86.22	0.8
12	400	11.4	2.5		135.07	
13	400	12.6	2.5	123.78	123.3	-0.4
14	522	10.7	4	125.4	125.4	0.0
15	522	10.7	4	125.95	125.9	0.0
16	400	12.2	2.5	126.71	126.7	0.0
17	522	12.2	1		139.8	
18	278	11.3	1	152.04	152.9	0.6
19	400	12.3	2.5	123.89	123.8	-0.1
20	278	13.6	4	138.5	138.78	0.2

Table 27. Cod yield data

As was found for mackerel and salmon, pressure treatment of cod resulted in a cooked appearance. Only 1 sample maintained a raw appearance – run 2 with a pressure of 200 MPa. HPP can undoubtedly be a very efficient pasteurisation process but a 'cooked' appearance was a common theme in all of the fish species at pressures of greater than 200 MPa. This makes it unlikely that high pressure could be used as a pasteurisation process for raw fish. However, there may still be opportunities for using high pressure to process fish in recipe dishes or to use high pressure as a means of rapidly marinating fish products. This is an area of interest to some of the equipment manufacturers (personal communication with Avure) and will be explored more fully in phase 2 experiments.

4.11.3 Brief summary of sensory results for cod

A control and runs 1-6 were assessed for sensory quality. For the uncooked assessment, none of the samples were graded as high as the Control, which was bright and moist, with good retention of fillet shape. Runs 3, 5 and 6 were graded the lowest, with moderate loss of brightness and little defined flake structure.

For the cooked assessment, only Run 3 was graded as high as the control, being moist with well defined flakes, and a well-balanced flavour and soft, moist texture. Runs 5 and 6 were graded the lowest, having a drier appearance. Run 5 also had a firm, dry, fibrous texture and Run 6 had acidic and bitter notes in the flavour.

Images of all pressure treated samples can be found in Appendix 22.

4.11.4 Key conclusions for cod

As was found for salmon and mackerel, HPP was very efficient with respect to microbiological inactivation, but a cooked appearance was induced in all samples treated over 200 MPa, making HPP unsuitable for pasteurisation of raw cod were it desirable to maintain a raw appearance. Interestingly, of the six runs tested for sensory quality, run 3 treated at 600 MPa was the only sample to be of comparable quality to the control. This suggests that HPP could be used to pasteurise cod in products where the colour change was not a problem and the sensory quality of the product would not be compromised. It would be interesting to compare the quality of a pressure treated ready meal, subsequently cooked by a consumer, with that of a heat processed ready meal that was subsequently cooked by the consumer. This may be explored further in phase 2 of the project.

5 Conclusions

Key findings from the work to date can be summarised as follows:

- HPP is an effective method for the inactivation of a range of microorganisms provided the pressure applied is sufficiently high.
- At the pressures used for shucking/picking applications, reductions in TVCs varied from species to species, but even at pressures of 200-300 MPa substantial reductions in pseudomonads and coliforms could be achieved, which could offer useful shelf-life extension. It should be noted however that no attempts were made to detect sub-lethal injury and recovery of the organisms and this is known to sometimes occur with HPP at the lower end of the pressure spectrum.
- At pressures in excess of around 200 MPa, all fish species began to take on a cooked appearance and this effect was more pronounced as pressure increased. This means that HPP is unlikely to be suitable for pasteurisation of wet fish and is probably best used to produce multi-component pasteurised ready-meals where a cooked appearance in the fish is not necessarily a concern. It may be possible to use pressures less than 200 MPa to enhance marination and to flavour products. Both of these applications are worthy of further exploration and may be studied in phase 2 of the project
- The cooked quality of HPP treated fish can match that of untreated controls which once again suggests that HPP could be used for ready-meal processing without compromising on product quality. It may be that by only fully cooking the product once (on consumption) the eating quality may be improved compared with a 'twice-cooked' product (once during manufacture and once by the consumer). This may be explored in phase 2 of the project.
- Substantial yield benefits may be possible for all shellfish. This was particularly apparent for mussels and oysters. The sensory quality of these products was generally considered to be improved by the HPP process. Care must be taken however to ensure that yield increases do not change the product eating quality

too dramatically. The results of these trials suggest that the amount of water uptake could be manipulated by varying the pressure and hold time applied.

- Substantial yield benefits look achievable for warm water prawns and possibly *Nephrops* but larger scale peeling trials are required in both cases to confirm that these apparent benefits are transferrable to a commercial scale operation. Larger scale peeling trials (50-100 kg) batches will form part of the work programme for phase 2 of the project.
- Lobsters and crabs can be picked raw after pressure treatment, offering the potential for completely new added-value products, i.e. picked, raw, ready-to-cook meat. Meat can be readily extracted even from difficult areas such as the legs; in some cases the meat can simply be squeezed out of the leg.
- The project has been very successful in meeting the core objectives as described in section 2. A great deal of practical knowledge has been developed to assist the industry in making informed choices about the use of HPP for their products. Clear benefits have been identified with respect to yield, microbiological inactivation and sensory quality but at the same time some of the limitations of the technology have been highlighted. A number of opportunities have been identified for the development of added value products and some of these ideas may be explored more fully in the second phase of the project. The high pressure facilities and expertise developed between CCFRA and Norconserv is now available for seafood processors to use on a confidential basis and a number of companies are already in discussions with CCFRA about conducting specific trials for their particular product lines.

6 Future work

Five products have been selected for further work in phase 2 of the project. The short-list of products is as follows:

- Crab
- Warm water prawns
- *Nephrops*
- Salmon
- Cod

Trials on crab, warm water prawns and *Nephrops* will focus on large scale picking/peeling trials to determine whether the yield benefits identified in phase 1 are transferrable to a commercial scale. Two commercial processors are participating in these studies. Trials on salmon and cod will focus on two areas: low pressure for marination, and the production of ready-meals, to compare product quality with that of conventional heat processed ready-meals.

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