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

Report on phase 2 studies:

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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 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 generally 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. It is thought that there is only one 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 HPP manufacturing for seafood products exists 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 was to redress this knowledge gap.

In the first phase of the project, 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 seafood products tested were Nephrops, mussels, oysters, crab, cold water prawns, lobster, warm water prawns, unsmoked salmon, squid, mackerel and cod. The results from the first phase of the project can be found in report reference FMT/REP/95900/1.

Five products were short-listed for further work; these were Nephrops, warm water prawns, crab, salmon and cod. Trials on crab, warm water prawns and Nephrops focused on large scale picking/peeling trials to determine whether product yield benefits

identified in phase 1 were transferrable to larger scale processing. Trials on salmon and cod focused on pasteurisation and shelf-life evaluation.

Significiant yield increases were observed in warm water prawns that were peeled after HPP treatment (a 3.7% increase compared with a control) and the sensory quality of the product was close to that of the untreated control. Only modest yield increases were observed in *Nephrops norvegicus* (0.9% increase over the controls); the quality of HPP samples were again close to that of the control, but textural differences were observed. It may be possible to improve yields and product quality in both products by process optimisation.

Large yield increases were observed in the picking of brown crab. Brown meat yield was 23% in the HPP treated sample compared with 18% in the control. Similarly, white meat yield was 12.9% compared with 8.3% in the control. However, the product quality was poor in this series of trials and there was excessive water uptake, particularly in the brown meat. Again it is important to note that, with more detailed trials for process optimisation, yield improvements with better product quality are expected to be achievable.

HPP was very successful for the inactivation of spoilage organisms in salmon and cod. This latter product was held chilled for 11 days and aerobic plate counts, pseudomonad counts and coliform counts were at, or close to, non-detectable levels for the duration of storage. Non microbial spoilage was observed in the cod and more detailed work is required to control this issue.

Trials were carried out with salmon to assess whether pre-treatment with CO_2 prior to HPP treatment offered any benefits in terms of microbial reduction or product quality. Some improvements in microbial kill were observed using a pre-treatment of CO_2 and texture changes were lessened by a CO_2 pre-treatment but the benefits in both cases were probably too small to make the process commercially viable.

Acknowledgements

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1 Introduction

1.1 Introduction to HPP technology

The report for the first phase of this project (FMT/REP/95900/1) reviewed many fundamental aspects of high pressure processing including:

- Basic principles
- Current commercial applications
- Previously published research in HPP for seafood
- Equipment availability and types

This information will therefore not be repeated in this report but a concise summary of HPP fundamentals can be found below in order that this report can be read as a stand-alone document.

1.2 Basic principles of HPP

As currently used, HPP is non-thermal pasteurisation process in which foods are subjected to pressures in the region of 150 to around 600 MPa (1500 to 6000 bar) and held at pressure for a time, generally under 10 minutes. These pressures can be put into context by considering that two 5-tonne elephants stood on a 5 pence piece would generate approximately 400 MPa of pressure. Products treated at these pressures are not crushed because water is used as the pressure transmitting medium and the applied pressure is uniformly distributed throughout the load (not in a uni-directional manner as would be the case in our elephant example above). Even delicate products such as grapes can be treated without crushing occurring.

The majority of HPP products are packed and then filled into batch pressure vessels for treatment, using water as the pressure transmitting medium. In the case of seafood

applications, the product is generally brought into direct contact with the water. 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 whilst often (not always) having minimal effects on product quality. 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.

Whilst the use of HPP for the shelf-life extension of seafoods is of interest, there is also a great deal of interest in the use of HPP to achieve desirable processing benefits; examples include automatic shellfish shucking, lobster shell removal and yield improvements.

2 Objectives of the project and phase 2.

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

The first phase of the project 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. The aim of the second phase of the project was to provide further data at more commercially realistic processing volumes regarding peeling/picking yield benefits and to provide data on possible shelf life extensions that could be achievable in fish species.

3 Materials and methods

3.1 Nephrops norvegicus

Frozen, un-peeled tails of *Nephrops norvegicus* were supplied by an industrial partner. The tails were tempered to -3°C in Scotland and were transported by chilled courier to an English site (detail withheld for confidentiality) where a 55-litre HPP vessel was available for trials. The equipment was similar to that shown in Figure 1 (supplied by NC Hyperbaric, Spain) but photographs of the actual vessel used were not permitted at the trial site.

Figure 1. A horizontal batch high pressure processing vessel
(picture courtesy of NC Hyperbaric, Spain)

In total, approximately 100 kg of Nephrops were used for the trials. The temperature of the samples on arrival was between 0 and 1°C. Around half of the batch was filled into baskets for use in the HPP vessel as shown in Figure 2. The remainder of the product was left in bags and transported back to Scotland for peeling as controls (again using controlled temperature vehicle).

The HPP equipment used mains water as the pressure medium. The temperature of the water could not be controlled but before each run the vessel water tank was drained and refilled to ensure that the temperature was as low as possible. The Nephrops were processed in three batches; in all cases the samples were treated at 300 MPa for 5 minutes. The pressure vessel was loaded with two baskets per cycle. The come up time to pressure was approximately 2 minutes 10 seconds. The weight of Nephrops before and after processing was recorded along with the initial water temperature and the product temperature immediately after processing.

Figure 2. Nephrops samples loaded into baskets prior to pressure treatment

After processing, the samples were re-weighed and packed for transportation to Scotland for peeling. Samples were sent by chilled courier; they arrived at the Scottish site on the day of pressure processing and were chill stored overnight at the factory before peeling.

Peeling was carried out in a semi-automatic process. The layout of the peeling operation was as illustrated in Figure 3 and Figure 4.

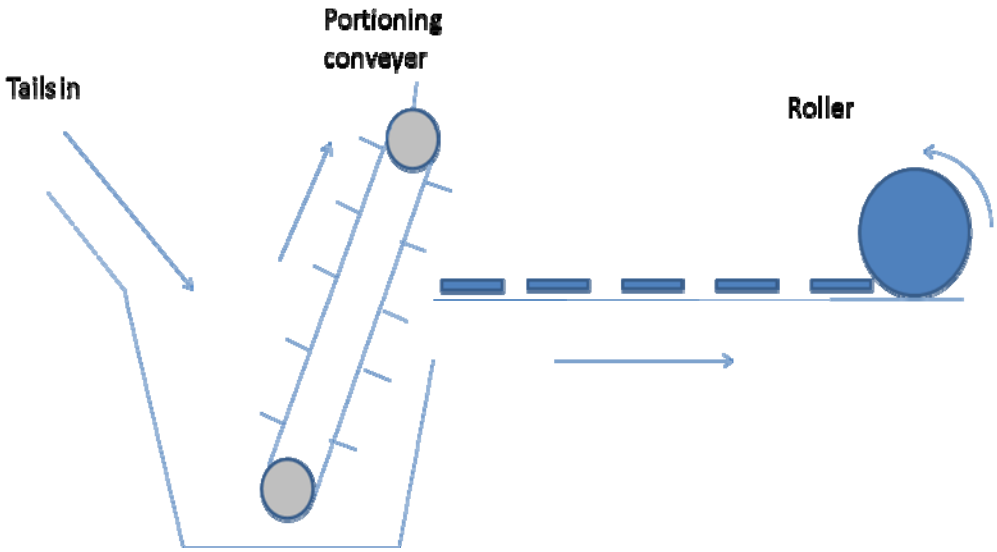

Figure 3: Automatic peeling process

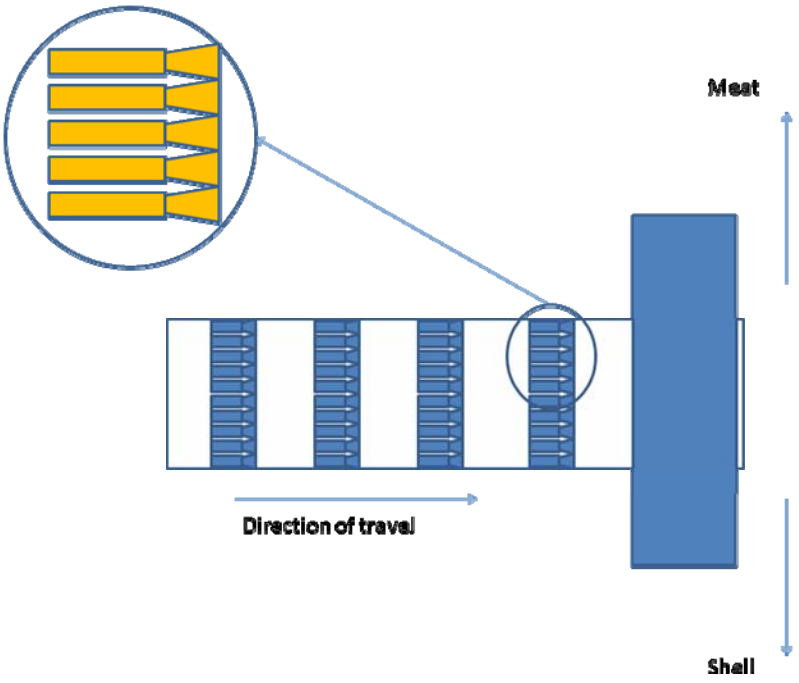

Figure 4: Plan view of horizontal belt

Before peeling, tails were weighed and placed in cold water for around 5 minutes to equilibrate their temperature. They were then drained and loaded into the peeler. The tails were placed into a hopper, deposited onto a vertical belt and tipped onto two horizontal belts travelling in parallel. Factory operators sorted the tail portions into groups of 5-6 and aligned them parallel to the direction of movement.

The tails were conveyed to a metal roller. Passing under the roller, the meat was squeezed from the shell, scraped from the roller and conveyed to a collection vessel. The shell was conveyed to a separate collection vessel. The weights of both meat and shell were measured and recorded and the associated meat yield calculated.

After peeling, tail meat was hand sorted into pairs on a horizontal belt before passing into a continuous belt blast freezer. Frozen scampi pieces were weighed after collection.

3.2 Brown crab

Live brown crabs were supplied by an industrial partner and were kept chilled until required. Crabs were sequentially numbered and the weight of each was recorded. Crabs were filled into baskets as previously described for *Nephrops norvegicus*. The weight of each batch was recorded before and after pressure treatment. The crabs were processed in two batches at 270 MPa for 2.5 minutes. After treatment the crabs were packed into insulated containers: 25 crabs were allocated for picking after HPP i.e. essentially 'raw', 25 were allocated for picking after HPP and cooking, and 25 untreated live control crabs were packed for cooking and picking using standard operating procedures at the industrial partner's site. The samples were packed into the insulated containers with ice-packs and transported to the factory where they were chilled overnight before picking. Whilst overnight storage was a logistical necessity for the trials, it is not known whether this storage period would have any effect on picking compared with picking immediately after treatment.

The 25 control crabs were soaked in fresh water for 1.5 hours according to the normal factory procedure prior to cooking and picking. The set of 25 crabs that were HPP treated cooked and then picked were loaded into trays and subjected to the factory's standard cooking procedure. As the crabs were a mixture of sizes the cooking procedure for large crabs was used. For the 25 crabs that were HPP treated, picked and then cooked, the crabs were picked in the low care area of the factory by an experienced crab picker. After picking, the weight of the brown meat, white meat and shell was recorded for each crab. The brown meat was drained because it was very watery following pressure treatment. The shell was discarded and the two types of meat were packed into vacuum bags and cooked following the normal factory procedure.

The 'control' 25 crabs and the 'HPP treated-cooked-and-picked' set of 25 were picked in the high care area of the factory by an experienced crab picker. The weight of the brown meat, white meat and shell was recorded. The two types of meat were packed into vacuum bags and all the samples were transported back to CCFRA in cool boxes.

On arrival at CCFRA, the white and brown meat was separated into small vacuum bags in a laminar flow cabinet, and stored for 6 days at a temperature between 0 and 2°C. Sensory evaluation was carried out on the samples on days 2, 5 and 6 after processing (see 3.8 for sensory methods). At each sampling interval the crab meat was tested for microbiological quality (see section 3.7 for methods) and high quality digital images were taken using a digital colour measurement system (DigiEye UK – see section 3.10).

3.3 Warm water prawns

An industrial partner provided frozen, raw, split-back, head-on, shell-on, black tiger prawns with a count of 16-20 prawns per kilogram. The prawns were tempered at the partner's factory and were between 0 and -2°C on arrival at the HPP facility. Around half of the prawns were de-boxed and filled into baskets (Figure 5) for pressure treatment as previously described for brown crab and *Nephrops norvegicus*.

Figure 5. Black tiger prawns loaded into baskets for HPP treatment

Prawns were pressure treated at 237 MPa for 2.5 minutes in three batches, but one of the batches was discarded because of equipment problems (see discussion for further details). The weight and temperature of the prawns was recorded before and after processing as was the temperature of the water used to pressurise the samples. After pressure treatment the samples were packed into insulated containers along with ice packs and were transported back to CCFRA. The samples arrived at CCFRA on the day of processing and were stored at between 0 and 2°C until required for peeling.

The samples were peeled on the second day after processing; this was because 24 hours after pressure treatment, the control samples (which had not been de-boxed from their original packaging) were still much colder than the HPP treated samples and there were concerns that this would unduly influence the results of the peeling trials.

The prawns were hand peeled by 3 CCFRA staff in 12 randomised batches in order to account for operator and time effects (see section 3.6 for details on experimental designs). Yields and speed of peeling were assessed. Prawns were tested for sensory quality 2 days after pressure treatment (see section 3.8 for method details). Prawns were assessed for texture changes 2 days after processing (see section 3.10).

3.4 Cod

For microbiological evaluations, skinless cod loins (*Gadus* spp) were purchased from a local supplier. The cod was cut into chunks and macerated in a food processor for 1 minute. Samples (50g) of the blended cod were filled into small stomacher bags and labelled. Two bags were processed for each treatment combination (designated Runs A & B). All of the bags were vacuum sealed (Multivac).

The samples were processed at either 200 or 600 MPa for 5 minutes using a laboratory scale HPP vessel (EPSI, Beligium). The vessel volume was 700-ml and the pressure fluid was a 3% w/w MKU solution (an oil based corrosion inhibitor supplied by EPSI). 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). After HPP, all of the samples were kept at 0-2°C until the day of microbiological evaluation. Samples were enumerated for Aerobic plate counts (APC), Coliforms and *Pseudomonas* on days 0, 5, 8 and 11 after processing. Details of the microbiological methods used can be found in section 3.7.

Samples were macerated rather than using whole fish in order to remove raw material variability. This approach was considered valid because HPP is generally considered to act independently of sample geometry and tissue structure unlike, for example, heat

processing, where maceration of the sample could have changed the rate of heating and the results would therefore not be transferable to whole fish.

High quality digital images were taken using a digital colour measurement system (DigiEye UK) and colour measurements were taken (see section 3.10 for details). Texture measurements were taken using a Stable Microsystems texture analyser (see section 3.10). Protein changes induced by HPP were explored using Gel Electrophoresis (see section 3.9 for method details).

Sections from whole cod were used for sensory analysis, colour and texture analysis. Whole gutted cod was supplied by an industrial partner; the cod was weighed, filleted and skinned. The fillets were portioned up into small vacuum bags and vacuum packed (Multivac). For sensory analysis, samples were treated at two pressures (200 and 600 MPa) and one time interval (5 minutes) using a laboratory scale HPP system (EPSI, Belgium). Treated samples and controls were stored at 0-2°C. Sensory analysis was carried out on days 0, 2, 6 and 9 after treatment. Texture analysis was carried out on five replicate pieces for each condition on day 0.

3.5 Salmon

Farmed salmon (*Salmo salar*) was supplied by an industrial partner. The samples arrived at CCFRA, packed on ice, two days after slaughter and gutting. All fish used in the studies were measured and weighed. Eight fish were filleted and de-skinned and around 6 circular samples were taken from the loin area using a cork borer 31mm in diameter. The cylindrical samples were trimmed to a height of 20 mm by cutting from the skin side. Subsequently the samples were grouped by fish and placed in vacuum bags on a fine mesh to allow access of gas on all sides. The vacuum bags were flushed with CO_2 before sealing, and it was ensured that the total gas volume was > 3 times the sample volume. The CO_2 concentration in the bags was measured using a gas analyser (Systec UK) at the start and end of packing and was > 95%. The sealed bags were placed in a chill store

operating at between 2 and 4°C for 24 hours. The following day, 10 fish were filleted and prepared as described previously but were packed in air before chilled storage.

One day later, air packed samples were trimmed to a height of 20 mm (from the skin side) and were vacuum packaged in pouches with 6 pieces in each pouch, each piece from a different fish. These samples were intended for colour and texture measurements (see section 3.10). Samples for microbiological analyses were prepared in the same way, with two fish pieces in each pouch (see section 3.7 for methods).

Fish samples stored in CO_2 flushed packs were opened and quickly re-packed into vacuum bags with 4 pieces in each pouch (again with each piece coming from a different fish). These samples were intended for colour and texture measurement. Samples for microbiological analyses were prepared in the same way, with two pieces in each pouch. The samples for microbiology were prepared first. The whole sampling and packaging process for the gas treated samples took approximately 20 minutes.

Salmon pieces were treated at different pressures, temperatures and times, with and without the presence of CO_2 as detailed in section 3.6. Microbiological enumeration, colour measurement, texture measurement and gel electrophoresis were carried out to assess the effects of HPP on the salmon samples.

3.6 Experimental design and statistical analysis

3.6.1 Nephrops, crab and warm water prawns

All pressure treatments for Nephrops were carried out at 300 MPa for 5 minutes. All pressure treatments for crab were again at a single condition (270 MPa for 2.5 minutes). Warm water prawns were pressure treated at 237 MPa for 2.5 minutes. Temperature was recorded but not controlled in all three cases. Conditions were selected based on experiences gained in the first phase of the project.

For the peeling trials with warm water prawn, the untreated and HPP treated samples were split into 12 batches approximately equal in weight. Three operatives were randomly assigned batches, each operative peeling 4 batches each. The weight of each batch was recorded, the weight of meat and shell after peeling was recorded and the time at the start and end of peeling was recorded. Yields and speed of peeling could therefore be calculated.

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3.6.2 Salmon

Trials on salmon utilised a full factorial design with 4 factors each at two levels with replication as shown in Table 1.

Pressure (MPa)	Time (min)	'Temperature'	CO_2 level
600	5	On ice	None
200	1	No ice	100%
600	5	No ice	100%
600	1	No ice	100%
200	5	No ice	None
200	5	On ice	100%
600	1	No ice	None
600	1	On ice	100%
200	1	On ice	100%
200	1	No ice	None
200	5	No ice	100%
600	5	On ice	100%
600	1	On ice	None
600	5	No ice	None
200	1	On ice	None
200	5	On ice	None
600	5	On ice	None

Pressure (MPa)	Time (min)	'Temperature'	CO_2 level
600	1	No ice	None
200	1	No ice	None
200	1	On ice	100%
200	5	No ice	None
200	5	On ice	100%
200	1	On ice	None
600	5	No ice	None
600	1	On ice	None
200	5	No ice	100%
200	5	On ice	None
600	1	On ice	100%
600	1	No ice	100%
200	1	No ice	100%
600	5	On ice	100%
600	5	No ice	100%

Table 1. Design employed for salmon

Samples were HPP treated either packed in ice or at ambient temperature and were designated 'on ice' or 'no ice' as appropriate. Some samples were flushed with CO_2 and stored for 2 days prior to vacuum packing and pressure treatment (designated '100% CO_2' or were packed in air followed by vacuum packing (designated 'None'). See section 3.5 for details.

3.6.3 Cod

Trials on cod were conducted at 200 or 600 MPa for 5 minutes in triplicate. Temperature of pressurisation was not controlled but the temperature of the pressure fluid was recorded before and after treatment.

3.7 Microbiological methods

Methods used for microbiological enumeration (where conducted) 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.

3.8 Sensory evaluation methods

Samples were subjected to sensory evaluation after processing. All samples were presented under three-digit code to a panel of three 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 (Table 2). Samples were 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.

9	Excellent Quality
8	Very Good Quality
7	Good Quality
6	Fairly Good Quality
5	Satisfactory Quality
4	Just Acceptable Quality
3	Poor Quality
2	Very Poor Quality
1	Bad Quality

Table 2. Nine point scale used for quality grading

In the case of *Nephrops norvegicus*, each sample was received raw, frozen and was first evaluated uncooked. The frozen sample was placed in a Pyrex bowl, covered and placed in a steamer, over 700 ml of boiling water in the base and steamed for 12 minutes. For each evaluation 10 pieces were assessed.

3.9 Gel electrophoresis

Where gel electrophoresis was used, approximately 0.2g of each sample was weighed and added to 10 ml of water and homogenised for 20 seconds using an Ultra-Turrex homogeniser. An aliquot of the homogenate (10-18 µl) was taken based on the weight of tissue used and made up to a final volume of 100 µl in Laemmli gel sample buffer (Laemmli 1970). This ensured that an equivalent amount of total available protein was subject to extraction in the Laemmli gel sample buffer.

The dispersions were boiled for 4 minutes and spun in a micro-centrifuge for 10 minutes. This procedure results in the solubilisation of the extractable protein fraction within the sample. The Laemmli gel sample buffer contained 0.1M DTT (dithiothreitol). DTT severs disulphide bonds, which occur within and between proteins. An aliquot of 10 µl of each sample extract was loaded onto all gels. A 7.5-25% gradient SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) gel was run using the BioRad Mini-Protean II system. Standard CCFRA protocols were used for running the gel, staining with colloidal Coomassie Blue stain (CCFRA TES-CM-0033) and performing densitometric analysis using the Totallab TL120 software (Nonlinear Dynamics Ltd, UK) (CCFRA TES-CM-0032). This gel electrophoresis technique resolves polypeptide subunits based on the molecular size and provides information about protein composition, as well as indication of irreversible aggregation or degradation of sununits.

3.10 Colour and texture measurements

Colour measurements were made on some products (as indicated in the results section) using a 'DigiEye' digital imaging system as shown in Figure 6 (DigiEye plc, Leicester, UK).

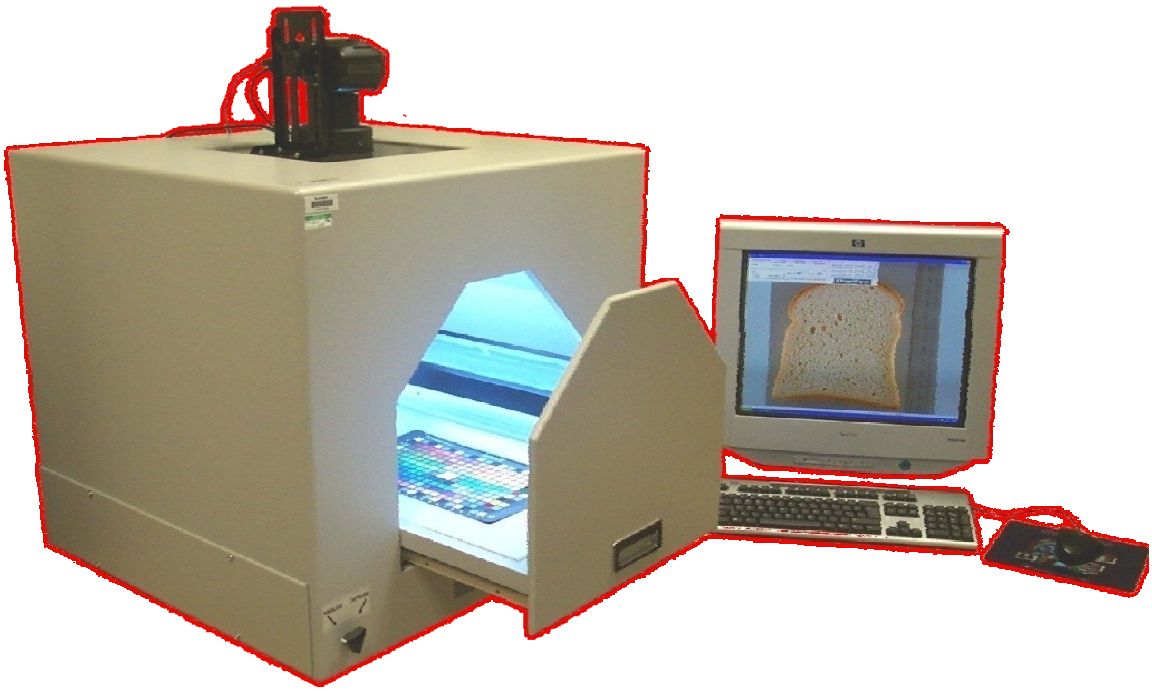


Figure 6. DigiEye imaging system used for all photography and colour measurement

This enabled a calibrated colour image of samples to be taken which could subsequently be measured using the CIE L^* a^* b^* uniform colour space system (ASTM 2000;CIE 1986). A diffuse lighting source closely approximating to the D_{65} illuminant (a commonly used daylight standard) was used to illuminate each sample as a digital image was taken. Mean measurements were reported for L^* , a^*, and b^*. A detailed discussion of the CIE L^* a^* b^* scale and its interpretation has previously been published by CCFRA (Whitworth 2006). For simple reference, the basic concept of the CIE L^* a^* b^* colour space is summarised in Figure 7.

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

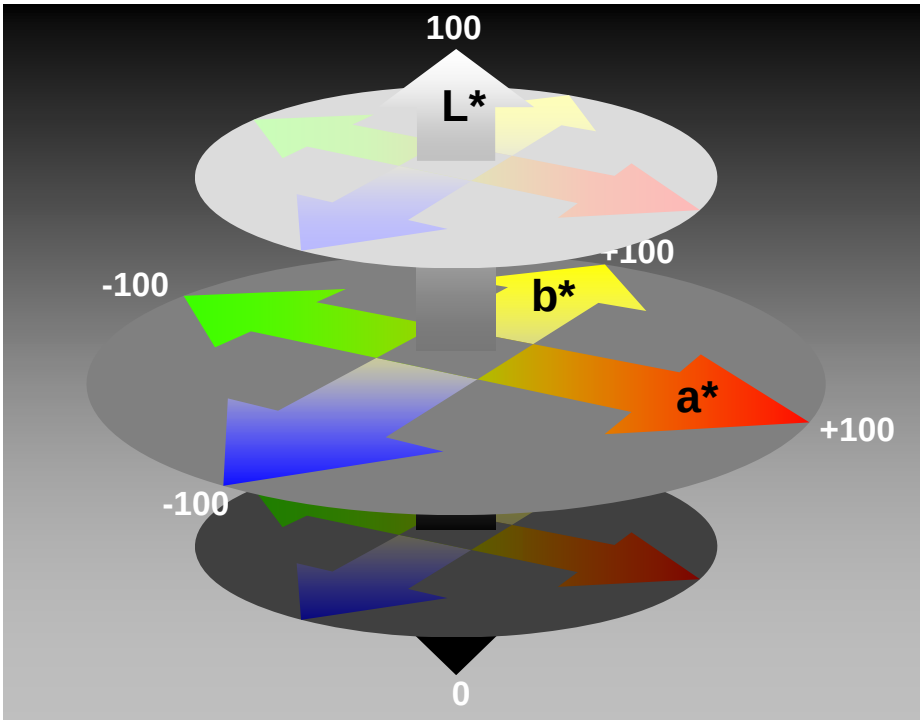


Figure 7. Basic concept of the CIE L a* b* colour space*

Texture measurements were made on all products apart from crab using a Stable Micro Systems texture analyser ((Godalming, Surrey, UK). For cod and salmon, samples were kept on ice until prior to testing. A compression test was used with a pre-test speed of 2 $mm.s^{-1}$ and a test speed of 0.8 $mm.s^{-1}$. The post test speed was 10 $mm.s^{-1}$, strain was 60% and the trigger force was 4g. Samples were tested by placing on the base of the instrument.

For prawns and Nephrops, a cutting force test was used rather than a compression test; a Stable Micro Systems texture analyser fitted with a blade was used and the force required to cut through the sample was measured. The stage had a slot through which the blade could pass in order to ensure that the test piece was cut completely. The pre test speed of the instrument was 2 $mm.s^{-1}$, the test speed was 2 $mm.s^{-1}$ and the post test speed was 10 $mm.s^{-1}$. For warm water prawns, the peak cutting force was considered the best measure by which to compare samples. For Nephrops, the area under the force/distance curve was considered a better measure for comparison purposes.

4 Results

4.1 Nephrops norvegicus

4.1.1 Temperature and weight changes after HPP

Pressure treatment on Nephrops was carried out at 300 MPa for 5 minutes. The water temperature during the 3 runs was on average 16.2°C (Table 3, n=3, s.d.=0.29°C). The temperatures of the raw material was between 0 and 1°C on arrival but after HPP temperature ranged between 12 and 15°C. There was some weight reduction after pressure treatment (Table 3); some of this weight loss was simply due to physical losses in the vessel. The baskets were not designed specifically for this application and so some product simply came out of the basket during the process. It is also possible, however, that some of the weight-loss was due to protein 'cook-out' during pressurisation. Upon pressure release, surface foam was noted on the water used for pressurisation (much the same in appearance as seen during the cooking of shellfish); this effect caused equipment problems part way through the trials on warm water prawns (see section 4.3), because a level sensor was 'blinded' by foam accumulating on its surface. This problem has been resolved in commercial seafood HPP vessels because electronic level sensors have been replaced with Inox steel buoys (essentially 'ball-cock' type floats) that are less susceptible to 'blinding' by any foam generated during processing (personal communication with NC Hyperbaric).

	Run 1	Run 2	Run 3
Water temperature before HPP (°C)	16.0	16.5	16
Weight before HPP (kg)	18.4	18.4	*
Weight after HPP (kg)	17.5	16.5	9.0
Sample temperature after HPP (°C)	14.0	12.0	15.0

*Table 3. Nephrops sample weights and temperatures. * indicates not recorded*

4.1.2 Sensory evaluation results

A summary of the quality grades awarded are detailed in Table 4; individual comments from assessors for each sample are presented in Tables 5-8. Uncooked HPP samples appeared to be of a similar quality to untreated samples. HPP treated samples scored slightly lower on cooked assessment, having slightly reduced sweetness and some bitter notes. Texturally, HPP treated samples had a slightly softer bite and were perceived as a little more fibrous in texture. It seems likely (though not formally tested) that HPP treated samples would be perceived as different in, for example, a triangle test (informal testing with a small number of panellists supports this hypothesis). If however, the Nephrops were subsequently processed, e.g. added to a recipe dish or breaded etc, it is debatable whether or not the difference between treated and untreated products would be detectable.

Sample Name	***Treatment Details***	***Uncooked Quality Grade***	***Cooked Quality Grade***
Nephrops Control (Untreated)	Untreated	7	6
Nephrops Treated	300 MPa for 5 minutes	7	5

Table 4. Quality grades awarded for Nephrops

	Raw Assessment Control – Frozen
Appearance	• Slightly dull • Pale pink in colour with slight grey tints • Slight pink veining • Clean
Odour	• Weak shellfish odour
Overall Quality	**7 (Good)**

Table 5. Individual comments for raw control

	Raw Assessment Treated – Frozen
Appearance	• Slightly dull • Pale pink in colour with slight grey tints • Slight pink veining • Clean
Odour	• Weak shellfish odour
Overall Quality	**7 (Good)**

Table 6. Individual comments for treated raw assessment

	Cooked Assessment – Control
Appearance	• Slightly dull • Pale pink in colour with slight grey and yellow tints • Slightly shrivelled • Moist
Odour	• Freshly cooked shellfish odour
Flavour	• Moderate shellfish flavour • Slightly sweet and slightly salty • Slight seaweed notes
Texture/Mouthfeel	• Very slightly firm bite • Very slightly chewy and spongy on breakdown • Slightly moist • Slightly fibrous and gritty
Overall Quality	**6 (Fairly Good)**

Table 7. Individual comments for cooked control

	Cooked Assessment – Treated
Appearance	• Slightly dull • Pale pink/orange in colour with slight grey and yellow tints • Moderately shrivelled • Moist
Odour	• Freshly cooked shellfish odour
Flavour	• Moderate shellfish flavour • Very slightly sweet and slightly salty/savoury • Slight seaweed and bitter notes
Texture/Mouthfeel	• Softer to bite • Slightly chewy and spongy on breakdown • Slightly moist • Moderately fibrous and gritty
Overall Quality	**5 (Satisfactory)**

Table 8. Individual comments for treated cooked assessment

4.1.3 Instrumental texture analysis

For the Nephrops there was not an easily identifiable peak force that could be used as a characteristic indicator of texture, therefore the force under a force/distance curve was used to compare samples. Samples showed considerable variation from one piece to another. The mean area under the force distance curve for untreated samples was 972 g.mm compared with 1103 g.mm in the HPP samples, suggesting that HPP samples were more tough. However, the differences measured were not statistically significant ($p>0.05$). This is likely to be due to the within-sample variation as previously discussed, exacerbated by the relatively small number of texture measurements made (8-9 measurements). The results of the texture analysis are reported in Table 9. Although a statistically significant difference was not detected between the samples, the apparent increased toughness was supported by the sensory evaluation data.

Sample No.	**Untreated (g.mm)**	**HPP (g.mm)**
1	1393.0	906.2
2	900.4	1188.0
3	982.4	1385.0
4	431.5	972.0
5	873.2	1364.0
6	721.6	991.5
7	934.4	894.1
8	1642	1123.0
9	867.7	
Mean	**972**	**1103**
s.d.	**355**	**195**

Table 9. Instrumental texture measurements of Nephrops

4.1.4 Peeling yield results

Peeling yield from HPP treated Nephrops was 0.9% higher than in untreated samples. In addition, although a somewhat subjective point, HPP treated samples appeared to be visually more intact after mechanical peeling compared with untreated controls. This could be due to the slight firming of the samples that was reported in sensory and instrumental analysis. This observation could be of commercial significance because mechanically peeled tails are typically much more physically damaged compared with hand peeled tails. Yields from hand peeled tails are also typically better than those from mechanical peeling; this along with improvements in visual appearance has led to Nephrops being shipped to the Far East for manual peeling. Although the physical appearance of the samples seemed to be improved, the yield improvement of 0.9% was probably not high enough to be of commercial significance. However, yield improvements in phase 1 of the project were as high as 3%, which would certainly be of commercial significance. There were no major, obvious differences between HPP and untreated samples after processing (Figure 8).

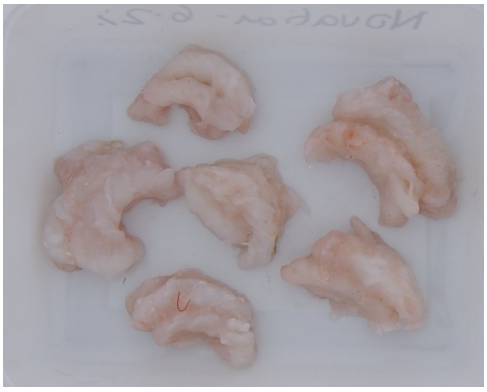
Untreated, frozen-thawed Nephrops	HPP treated, frozen-thawed Nephrops
	

Figure 8. Untreated and HPP treated Nephrops

4.1.5 General observations for Nephrops

The observed improvements in peeling yields using HPP were probably not sufficiently large to be of commercial interest. However, it is important to note that the conditions used for this experiment may not have been the optimum for maximum peeling yield as there are many variables to consider, including raw material temperature and initial state (i.e. frozen, thawed or fresh-never-frozen), seasonal influences on the raw material, HPP vessel pressure, temperature and time, and time from pressure treatment to peeling. This latter factor was kept as short as possible in these trials but given that the peeling equipment was several hours' drive from the HPP unit, the samples could be not peeled immediately after pressure treatment. Reversible protein modifications are known to occur at lower HPP conditions, so it is not inconceivable that the time from HPP treatment to peeling could have an influence on the subsequent yield.

It is also true to say that results observed on small scale HPP units are not always directly transferrable to large scale units; as a result optimum conditions derived at laboratory scale may not be optimum on a commercial system. The presents the industry with a difficult situation. Capital costs for HPP equipment are relatively high, but without having access to a large scale machine for detailed trials, development trials, for example on a contract consultancy basis, are likely to be excessively high for many small businesses. An approach to overcome equipment access problems is discussed in section 6.

4.2 Brown crab

4.2.1 Temperature and weight changes after HPP

The weight of each batch before and after processing and the initial temperature of the water used for pressurisation are recorded in Table 10. Both batches were around 0.5 kg heavier after HPP; it is not known how much of this weight gain was simply residual water in the baskets after treatment and how much was potentially water uptake in the

crab. As will be described shortly, at least some water uptake is likely to have occurred in the actual crabs.

	Run 1	Run 2
Water temperature before HPP (°C)	11.0	11.5
Weight before HPP (kg)	16.1	13.9
Weight after HPP (kg)	16.5	14.4

Table 10. Water temperature and batch weights

4.2.2 Crab yield changes

HPP treated, picked and then cooked meat gave significantly higher yields compared with untreated-cooked samples and HPP-cooked-picked samples ($p<0.05$). Brown meat yield was 23% in the HPP sample compared with 18% in the control. Similarly, white meat yield was 12.9% compared with 8.3% in the control. Yield from HPP treated-cooked-and-picked was not significantly different from the control ($p>0.05$) so this approach did not appear to offer any benefits over conventional processing. Although HPP gave significant improvements in yield, the appearance of the product and the sensory quality was very poor in this particular set of experiments. It is difficult to say with certainty what yield improvements were due to extra meat removal and what was due to water ingress (Figure 9).

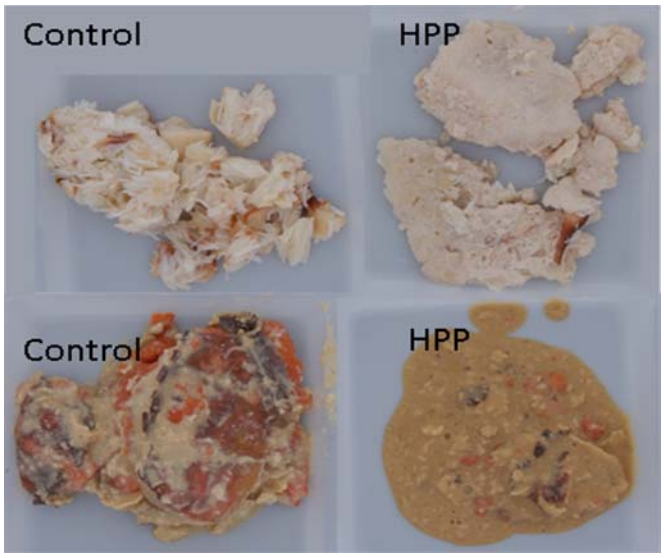


Figure 9. Cooked crab meat appearance in HPP and control samples

The brown meat in particular had a very watery appearance and the white meat had a proteinaceous like coating over its surface (having a 'scrambled-egg' type appearance). This effect on white meat was also observed in the first phase of the work at some processing conditions (Figure 10).

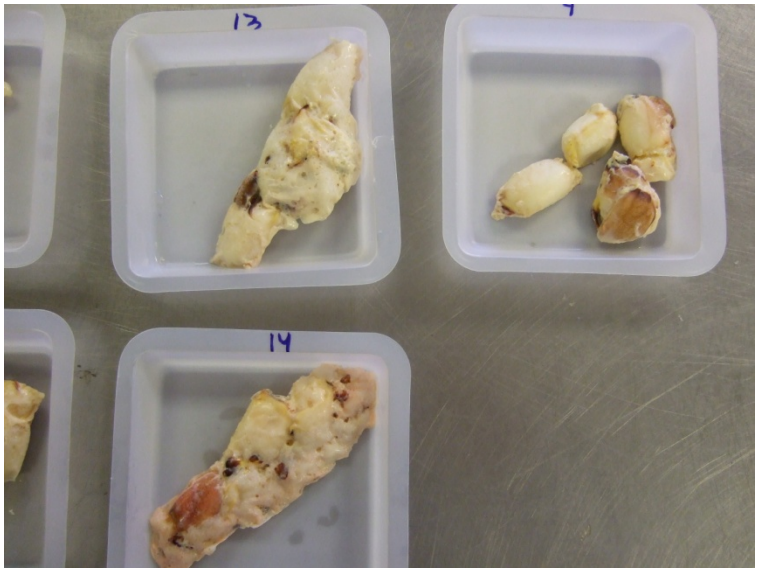


Figure 10. Cooked HPP crab meat showing proteinaceous material on the meat surface

It should also be noted that, in the first phase of the project, brown meat could be readily extracted at some conditions without obvious signs of excessive water ingress (Figure 11).

Figure 11. Brown meat extraction

4.2.3 Sensory results

Quality assessment was carried out on untreated-cooked-picked crab meat (the control), HPP-picked-cooked meat ('Run 1') and HPP-cooked-picked meat ('Run 2'). Samples were assessed at days 2, 5 and 6 after manufacture. The white and dark meats were assessed separately. A summary of the quality grades awarded are detailed in Figure 12 and Figure 13.

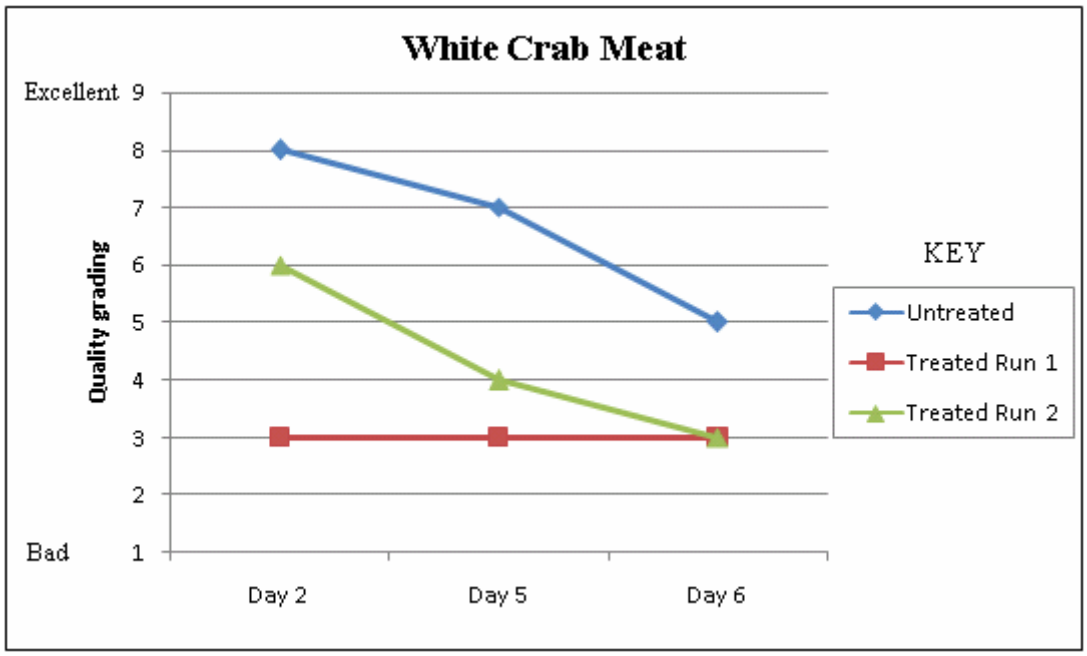


Figure 12. White meat quality grades over storage

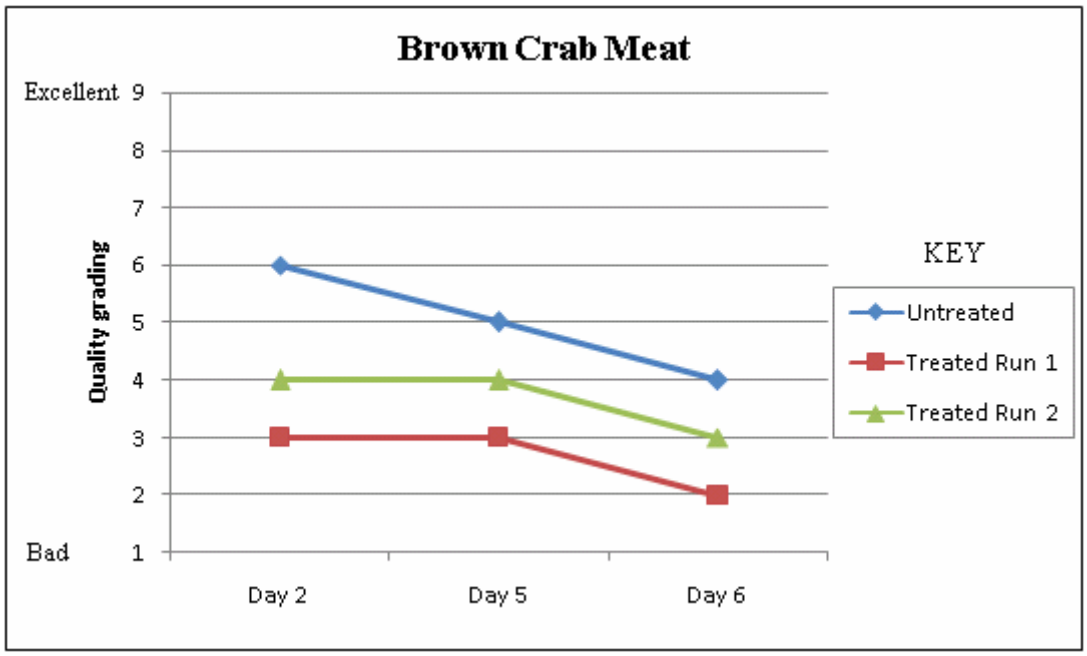


Figure 13. Brown meat quality grades over storage

Throughout the trial, the control (untreated) sample was graded higher than the two HPP treated samples for both the white and dark meat. The HPP-picked-cooked sample was graded the lowest.

At Day 2, compared to the two treated samples, the white meat of the control (untreated) sample was brighter and cleaner, with no processed notes found in the flavour, and it had a firmer texture. The brown meat was less broken down, had a stronger crab flavour and was not slimy in the mouth. The HPP-picked-cooked sample was graded the lowest for both the white and dark meat mainly due to the fact that it did not resemble crab meat in appearance.

At Day 5, the control (untreated) sample was graded very slightly lower than at Day 2 with a loss of brightness in the white meat and slight loss of freshness in the brown meat. The HPP-picked-cooked sample was graded the same as at Day 2; it had become slightly stronger in flavour with a sour note. The HPP-cooked-picked sample white meat had become slightly duller and clumped in appearance, with a slight loss of freshness and a softer texture. The brown meat had remained as Day 2.

At Day 6, the control (untreated) sample had become less bright with bitter, seaweed notes in the white meat. The brown meat had developed a fruity, acidic odour and acidic flavour. The HPP-picked-cooked sample had developed metallic notes in the white meat. The brown meat appeared to have black, 'bloody' and yellow areas. The HPP-cooked-picked sample had developed a green/grey tint and a 'sweetcorn', less-fresh odour. Neither of the treated brown meat samples was assessed for flavour and texture.

4.2.4 Crab microbiological results

Microbial counts in brown and white crab meat over a six day period are shown in Figures 14-17. Coliforms were not detected in any samples over the entire storage period. Generally speaking the HPP process had a positive effect in terms of reducing microbial counts in the picked crab meat. The pressures used for the picking experiment were quite low so it would not necessarily be expected that HPP would have a dramatic effect on microbial load. Pseudomonads were generally found to be pressure sensitive, as was reported in the first phase of the work, but there was some variability in the data.

This could have been due to the practical need to sub-sample processed meat to evaluate over the product shelf-life, i.e. some cross contamination could have occurred.

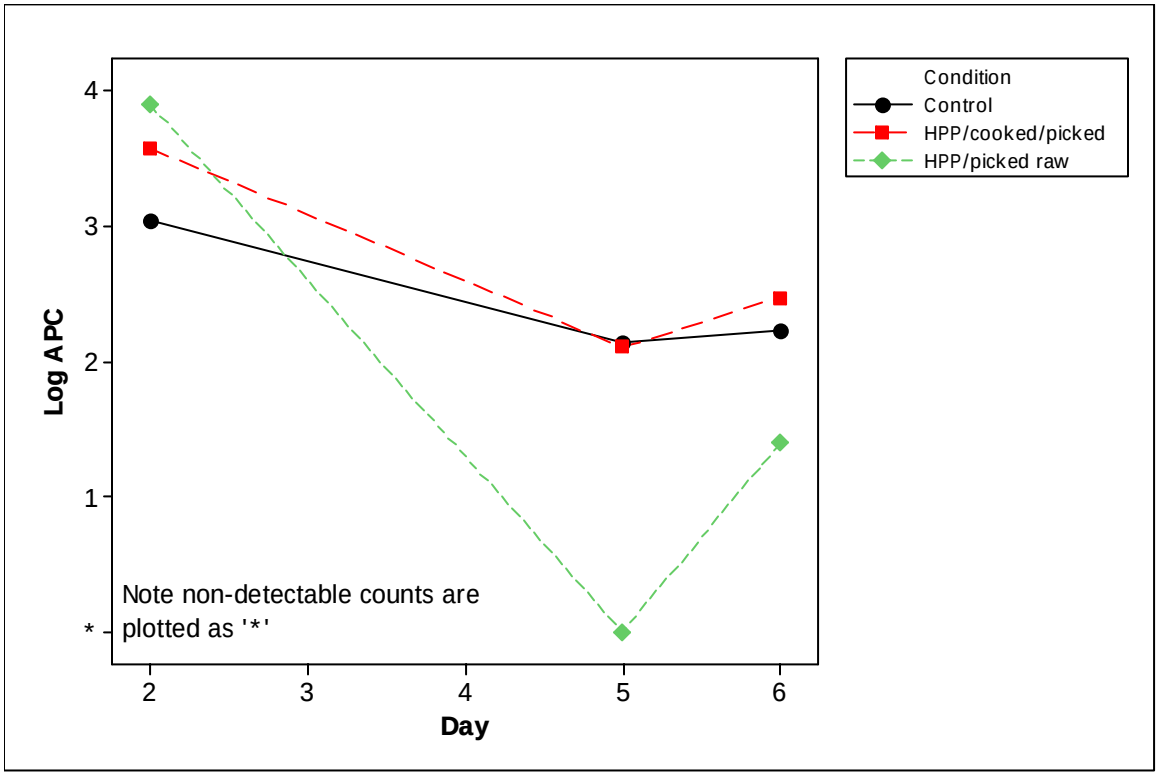


Figure 14. Changes in APC in brown meat over storage

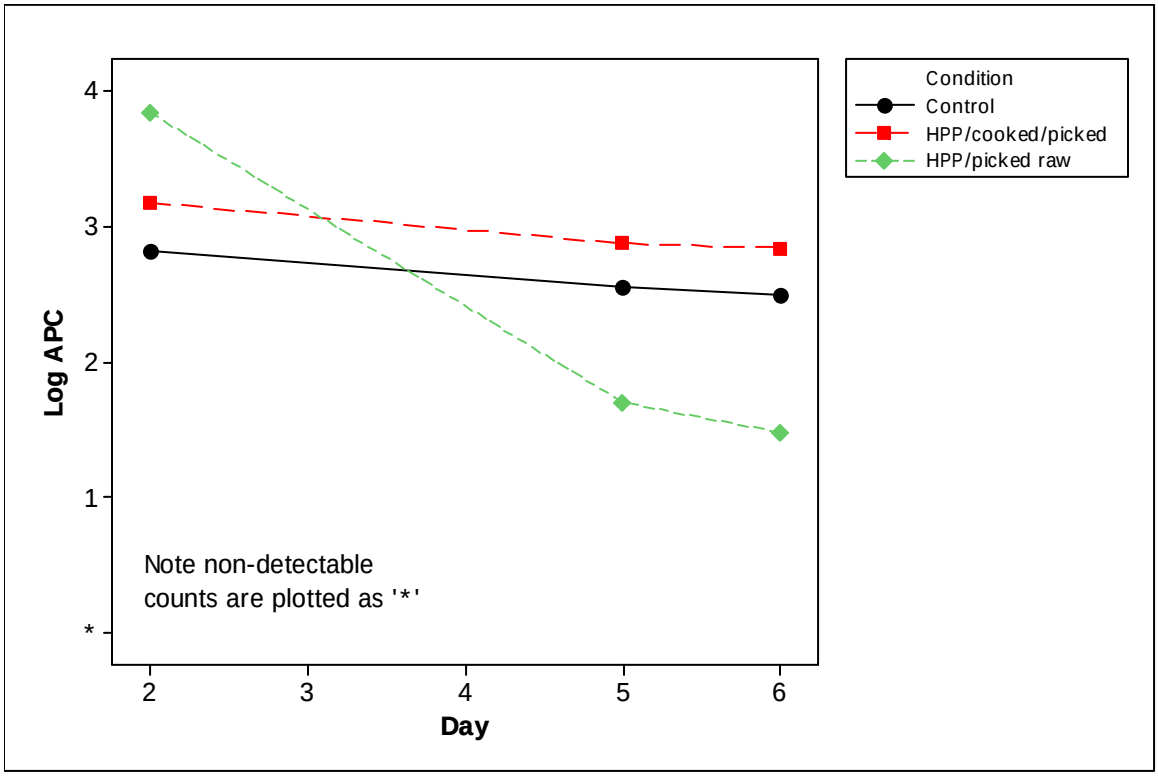


Figure 15. Changes in APC in white meat over storage

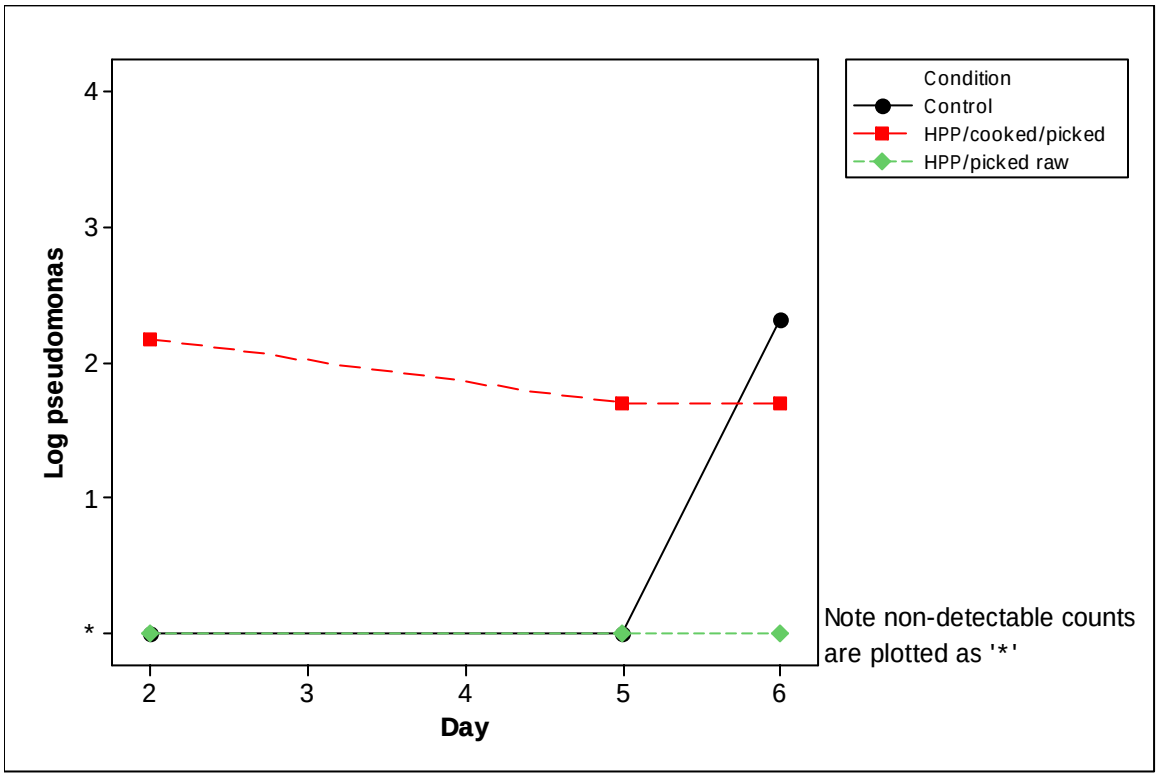


Figure 16. Changes in pseudomonad levels in brown meat over storage

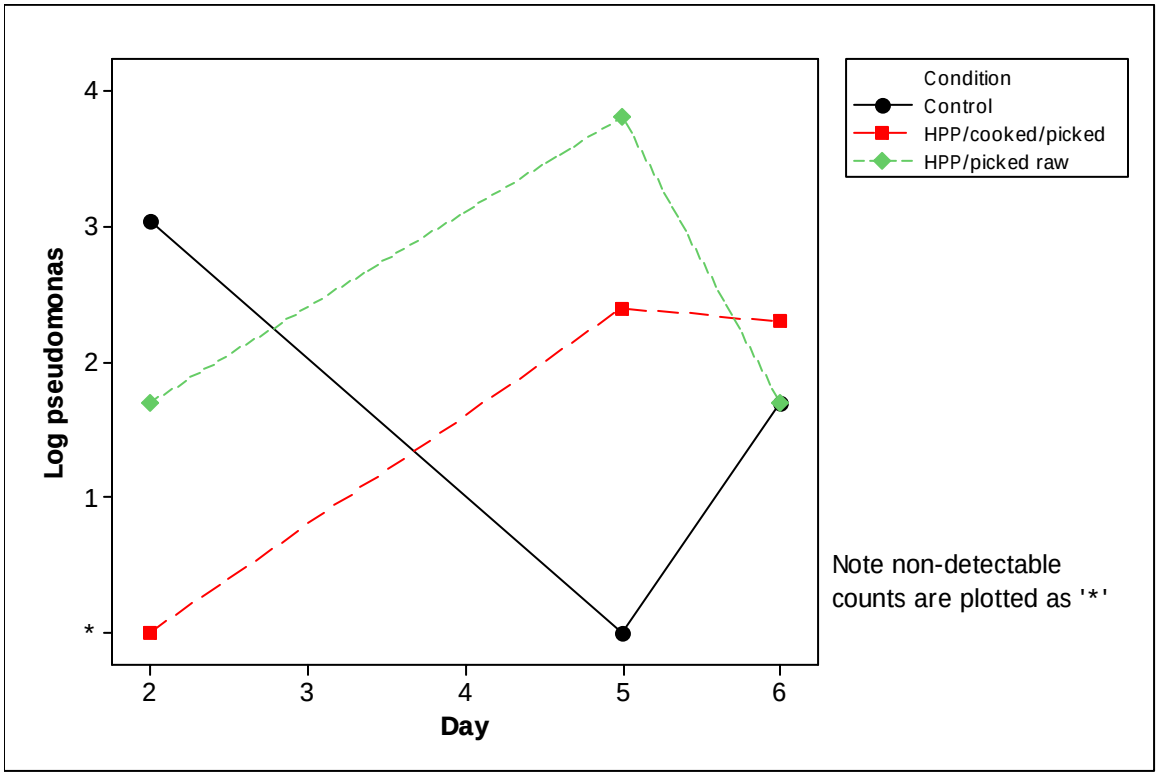


Figure 17. Changes in pseudomonad levels in white meat over storage

4.2.5 General observations for crab

Yield increases were significant but the quality of the crab from these trials was poor. It is important to stress, however, that these results DO NOT indicate that HPP does not have potential for crab meat extraction; in fact the opposite is true. It does, however highlight that more development work is required to optimise the time, pressure and temperature conditions to ensure optimum meat extraction with minimum effects on product quality. As was discussed for Nephrops, a great many factors could come in to play that influence the success (or otherwise) of the HPP process. Aside from the process factors, factors such as species, season and time from HPP to picking could all influence the efficacy of the process. Again as has been mentioned, scale-up of results from laboratory scale equipment to industrial equipment can be problematic and there is a need for the industry to have ready, affordable access to industrial scale HPP machines. At the moment, the author is only aware of one industrial machine in the UK that is available for

contract trials. This vessel is a 35 litre Avure system at the Agri-Food and Biosciences Institute in Belfast.

4.3 Warm water prawns

4.3.1 Temperature and weight changes after HPP

Water temperatures and product weights before and after HPP processing are reported in Table 11.

	Run 1	Run 2
Water temperature before HPP (°C)	15.0	11.5
Weight before HPP (kg)	12.5	6.2
Weight after HPP (kg)	12.3	6.4

Table 11. Water temperature and batch weights

All samples were pressure treated at 237 MPa for 2.5 minutes. One batch of prawns (data not presented) was damaged and so was not analysed because of an error in the pressure cycle. This was caused by a level switch failing as a result of foam coming from the product (see section 4.1 for details on how this issue has been resolved in commercial systems). In one of the runs there was a product weight reduction of 0.2 kg after pressure treatment; in the second run there was a weight gain of around 0.2 kg. Both of these results could easily be explained by either residual water in the baskets or some amounts of product losses in the high pressure vessel. Results from the first phase of the project would suggest that, in general, HPP treatment of warm water prawns results in a weight gain. After pressure treatment the samples were packed into insulated containers and transported back to CCFRA for hand peeling trials and sensory evaluation.

4.3.2 Sensory results

Each sample was received raw by the Sensory panel and was first evaluated uncooked for odour and appearance. The samples were placed in a steamer, over half a litre of boiling water in the base and steamed for 2.25 minutes before being assessed for cooked quality.

A summary of the quality grades awarded are detailed in Table 12.

Sample Name	**Treatment Details**	**Uncooked Quality Grade**	**Cooked Quality Grade**
Warm Water Prawns Control (Untreated)	Untreated	7	7
Warm Water Prawns Treated	237 MPa for 2.5 minutes	7	6

Table 12. Quality grades awarded for warm water prawns

When assessed raw for odour and visual appearance, the treated and untreated samples were essentially indistinguishable from one another. On assessing the samples cooked, HPP treated samples scored slightly lower but were still considered to be of fairly good quality. Individual comments are recorded in Tables 13-16.

	Raw Assessment **Control**
Appearance	Slightly dull Slightly moist Pale grey/green in colour with slight blue tints Slight pink veining Clean and plump
Odour	Weak prawn odour Slight seaweed note
Overall Quality	**7 (Good)**

Table 13. Summary comments for raw controls

	Raw Assessment **Treated**
Appearance	Very slightly dull Slightly moist Pale grey/blue in colour with slight green tints Slight pink veining Clean and slightly plump
Odour	Weak prawn odour Slight seaweed note
Overall Quality	**7 (Good)**

Table 14 .Summary comments for raw HPP samples

	Cooked Assessment **Control**
Appearance	Moderately bright Externally - moderate pink/orange in colour with slight grey/brown tints Plump, slightly ragged Internally – creamy/white coloured flesh, dense and moist
Odour	Freshly cooked prawn odour with a slight seaweed note
Flavour	Moderate prawn flavour Moderately sweet and slightly salty Slight seaweed and eggy notes
Texture/Mouthfeel	Moderately firm meaty bite Slightly chewy and rubbery on breakdown Slightly moist Slightly fibrous
Overall Quality	**7 (Good)**

Table 15. Summary comments for cooked controls

	Cooked Assessment Treated
Appearance	Slightly bright Externally - slightly pink/orange in colour with moderate grey/brown tints Plump, slightly ragged and curling to cut edge Internally – creamy coloured flesh, dense and moist
Odour	Freshly cooked prawn odour with a slight seaweed note
Flavour	Slight prawn flavour Slightly sweet and salty Slight seaweed , eggy and bitter notes
Texture/Mouthfeel	Slightly firm bite Slightly soft and spongy on breakdown Slightly moist Very slightly fibrous
Overall Quality	**6 (Fairly Good))**

Table 16. Summary comments for cooked HPP samples

In terms of odour and appearance, cooked samples seemed very similar to one another. The flavour of HPP treated prawns seemed to be a little less intense and the texture was described as 'slightly soft and spongy' compared with 'Slightly chewy and rubbery on breakdown' in the control samples. As was the case with Nephrops, the HPP treated prawns were probably sufficiently different to be detectable when comparing against the control but it is debatable whether the difference would be noted if the samples were tested in isolation rather than alongside the controls.

4.3.3 Instrumental texture analysis

For warm water prawns the peak force required to cut through the prawn was considered to be a reasonable characteristic measure of texture. As was found with Nephrops, prawn samples showed considerable variation from one piece to another. The mean peak force

for untreated samples was 2659g (n = 6, s.d.508g) compared with 2735g (n=5, s.d. = 392g) in the HPP samples, again suggesting that HPP samples were more tough. However, the differences measured were not statistically significant ($p>0.05$). This is likely to be due to the within-sample variation as previously discussed, exacerbated by the relatively small number of texture measurements made (6 measurements per sample). The results of the texture analysis are reported in Table 17. The apparent increased toughness was a little more difficult to reconcile with the sensory data as summary descriptions from the sensory panel suggested that the HPP treated samples were more soft and spongy on breakdown. The instrumental texture measurement was essentially measuring something akin to the 'initial bite' into the sample, so it is possible that the sensory panel were picking up differences experienced during chewing of the sample that would not be picked up by the instrumental test used.

Sample No.	**Untreated peak force (g)**	**HPP treated peak force (g)**
1	2441.5	2259
2	2684.6	2660.3
3	3628.2	2357
4	2259.3	3297.5
5	2282.7	2981.2
6	2657.5	2857.8
Mean	**2659**	**2735**
s.d.	**508**	**392**

Table 17. Peak force to cut the prawns

4.3.4 Peeling yield results

Yield improvements in HPP treated samples were substantial; peeling yield in the controls was 49.2% and this increased to 52.9% in the HPP samples. This effect was statistically significant ($p<0.05$) and was certainly of practical significance. The result

was especially positive, considering that the quality of the peeled product was almost as good as the control and could no doubt be further optimised. The results were also in good agreement with the small scale trials carried out in the first phase of the work, although here yield increases as high as 8% were observed.

There were no significant differences in peeling yields produced by the different operators involved in the experiments ($p>0.05$) and HPP or control samples were randomly assigned so these influences can be ruled out as a possible reason for the improved yield.

There was no significant difference in the peeling time required to peel HPP and untreated samples ($p>0.05$) so it would not appear that there are productivity improvements that could be achieved by pressure treatment. Yield increases alone could make it commercially viable for seafood processors to explore HPP for warm water prawns and other peeling processes.

4.3.5 General observations for warm water prawns

Yield improvements were substantial and product quality did not appear to suffer greatly as a result of pressure treatment. It also seems likely that product quality could be further improved by process optimisation using commercial scale equipment. The only draw back for the use of HPP on warm water prawns is perhaps one of practicality – since all of this material is imported from overseas it would require HPP vessels to be installed at the peeling site and this may be commercially difficult to justify given the low costs of labour in these regions. Nevertheless, these results should be taken as indicative of the sorts of yield benefits that might be achievable for any seafood product requiring peeling, once the process conditions are optimised.

4.4 Cod

4.4.1 Microbiological results

The microbiological results for the storage trial on cod are reported in Figures 18-20. All results plotted are the average of microbial counts from two separate fish samples.

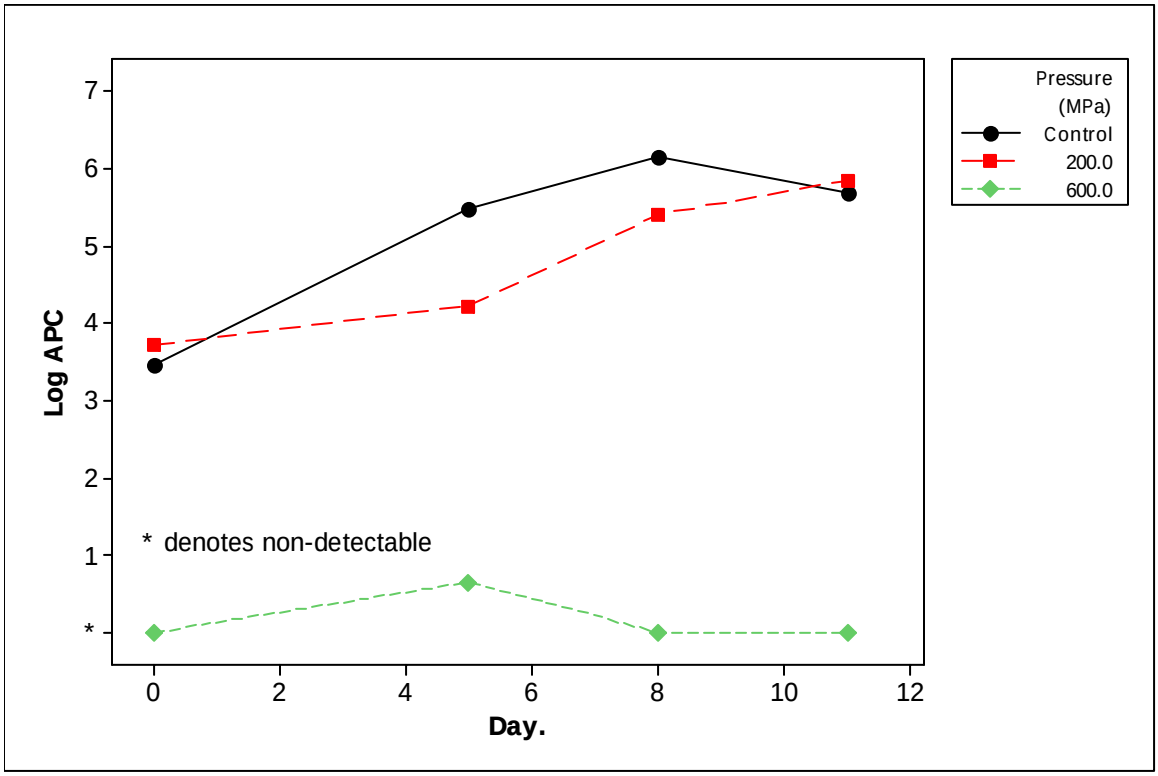


Figure 18. APC counts in cod over 11 day storage

Aerobic plate counts, coliforms and pseudomonads were reduced to non detectable levels using a process of 600 MPa for 5 minutes even after 11 days at chilled storage. The lower pressure of 200 MPa did not suppress APC counts by any significant degree but coliforms were at non detectable levels for 5 days of chilled storage, and pseudomonads gradually declined over the first 8 days of storage.

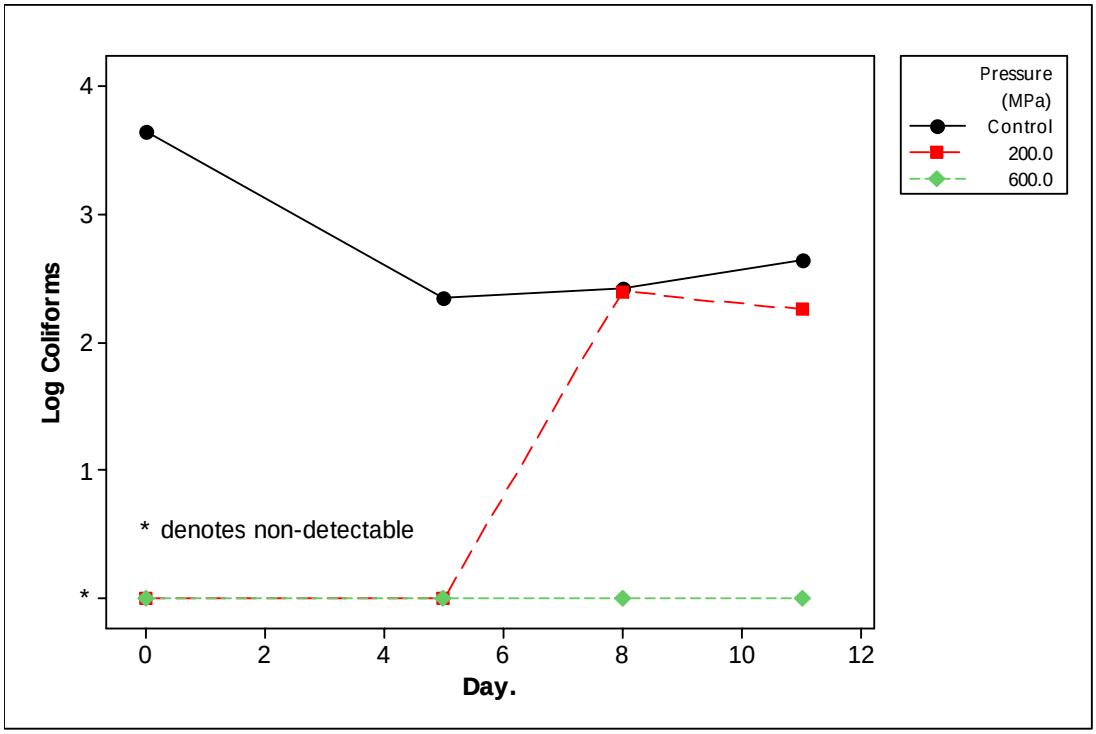


Figure 19. Coliform counts in cod over 11 day storage

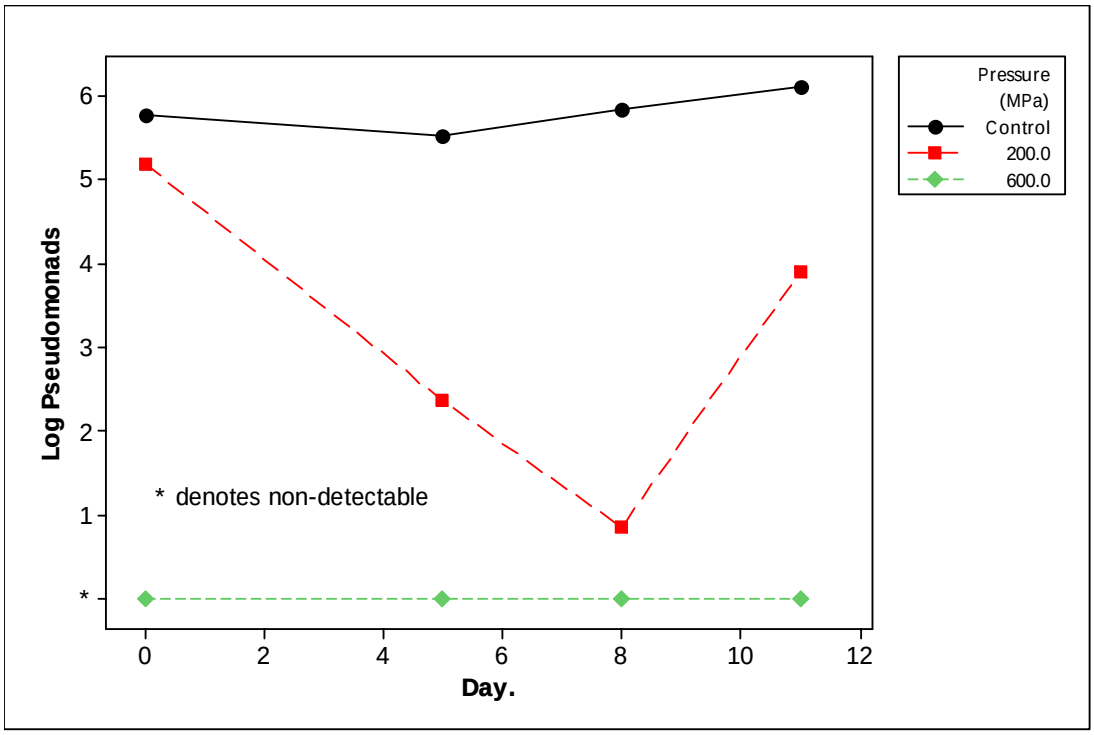


Figure 20. Pseudomonad counts in cod over 11 day storage

Given the right time, pressure and temperature combination, HPP is clearly an excellent means of controlling microbial growth in seafood products. Lower range pressure treatments, e.g. for shucking/peeling applications, may give some useful levels of reduction for key seafood spoilage organisms such as pseudomonads, but are highly unlikely to yield what might be considered a 'pasteurised' product.

4.4.2 Sensory evaluation results

Samples were evaluated for sensory quality at days 0, 2, 6 and 9 after processing. Samples were assessed for raw odour and appearance and were then cooked by placing in a steamer with 700 ml of boiling water in the base, for between 5 and 9 minutes (to a core temperature of 70°C). A summary of the quality grades awarded are detailed in Figures 21 and 22.

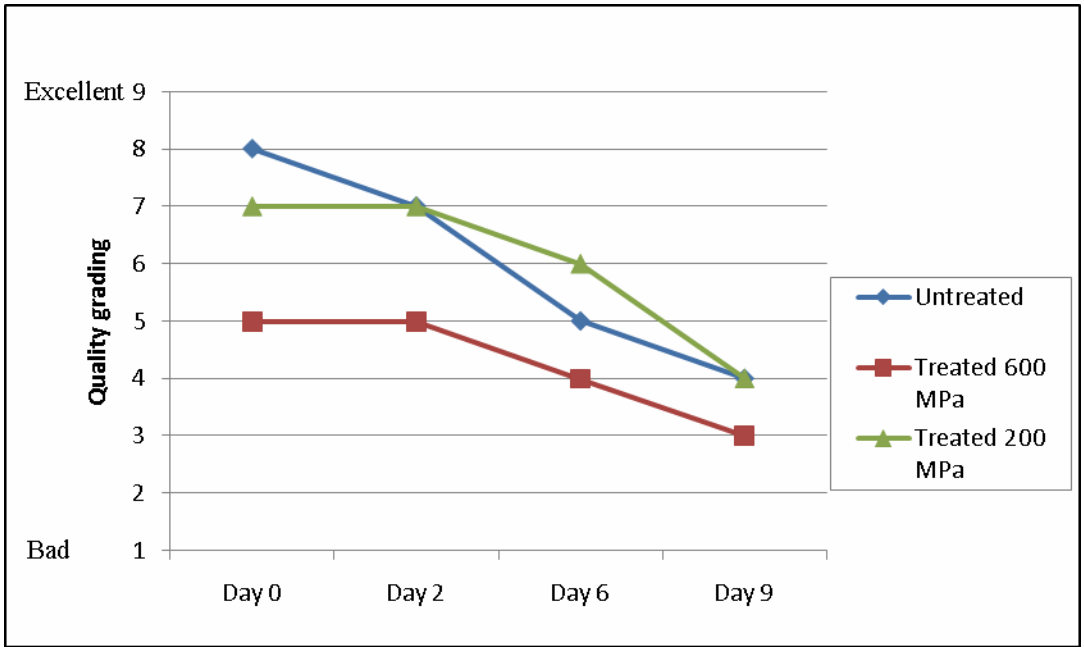


Figure 21. Raw cod sensory assessment

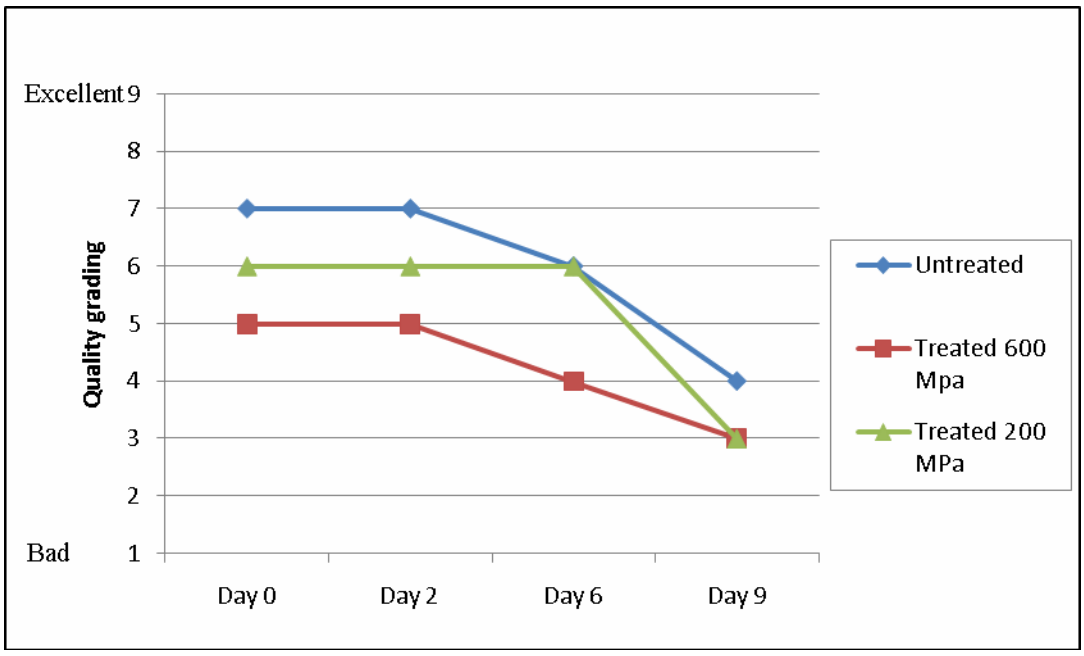


Figure 22. Cooked cod sensory assessment

Throughout the storage trial for both the uncooked and cooked assessments, the control (untreated) sample and the treated 200 MPa sample were graded similarly. The treated 600 MPa sample was graded the lowest.

For the raw assessment at Day 0, the control (untreated) sample was graded slightly higher than the treated 200 MPa sample as it had slightly better flake definition. The treated 600 MPa sample was graded the lowest as it was less bright and had a 'cooked', dense, compressed appearance. For the cooked assessment, the control sample was graded slightly higher as it was slightly brighter in appearance with a less chewy texture. The 600 MPa sample was graded the lowest as it had a dense, compact appearance.

For both the raw and cooked assessments at Day 2, all samples were similar to Day 0.

For the raw assessment at Day 6, the treated 200 MPa sample was grade the highest; the other samples had developed a seaweed note in the odour. For the cooked assessment, the control and treated 200 MPa sample were graded equally; the 600 MPa sample was downgraded as it had ammonia notes in the odour and a chewier texture.

For the raw assessment at Day 9, the 600 MPa sample was graded the lowest and considered 'Poor' due to a very moist, slimy appearance and a strong odour. For the cooked assessment, both of the treated samples were graded lower than the control sample and considered 'Poor', with sour notes in the flavour of the 600MPa sample and a slimy texture in the 200 MPa sample.

The compacted appearance of raw fish treated at 600 MPa is not a great surprise as this was reported in the first phase of the project and is a fairly well known phenomenon to those working with HPP. However, given that the microbiological inactivation results were so good the product deterioration over storage was a little surprising. It is likely that this degradation was due to non microbial spoilage mechanisms. Enzymes exhibit a variable response to HPP; in some cases they are readily inactivated, and in others they are very resistant or even stimulated by HPP. This could be one potential reason for the product deterioration in the pressure treated samples. Non microbial spoilage *can* be overcome in HPP products through interventions such as packaging selection (e.g. good oxygen barrier properties) and various mild pre-treatments. For example, mild blanching can be used to control enzyme degradation in HPP treated fruit. Solutions for non microbial degradation in seafoods were not developed within the scope of this project.

4.4.3 Instrumental texture analysis

Texture analysis results are shown in Figure 23. As described for Nephrops and warm water prawns there was a lot of sample-to-sample variation which made it impossible to derive any statistically significant effects as any differences between treatments were masked by variation within treatments. Samples processed at 600 MPa gave similar peak forces to the control samples; the mean force for samples treated at 200 MPa was slightly lower than for the control (9521 g compared with 11568 g) but this may be attributable to no more than random variation.

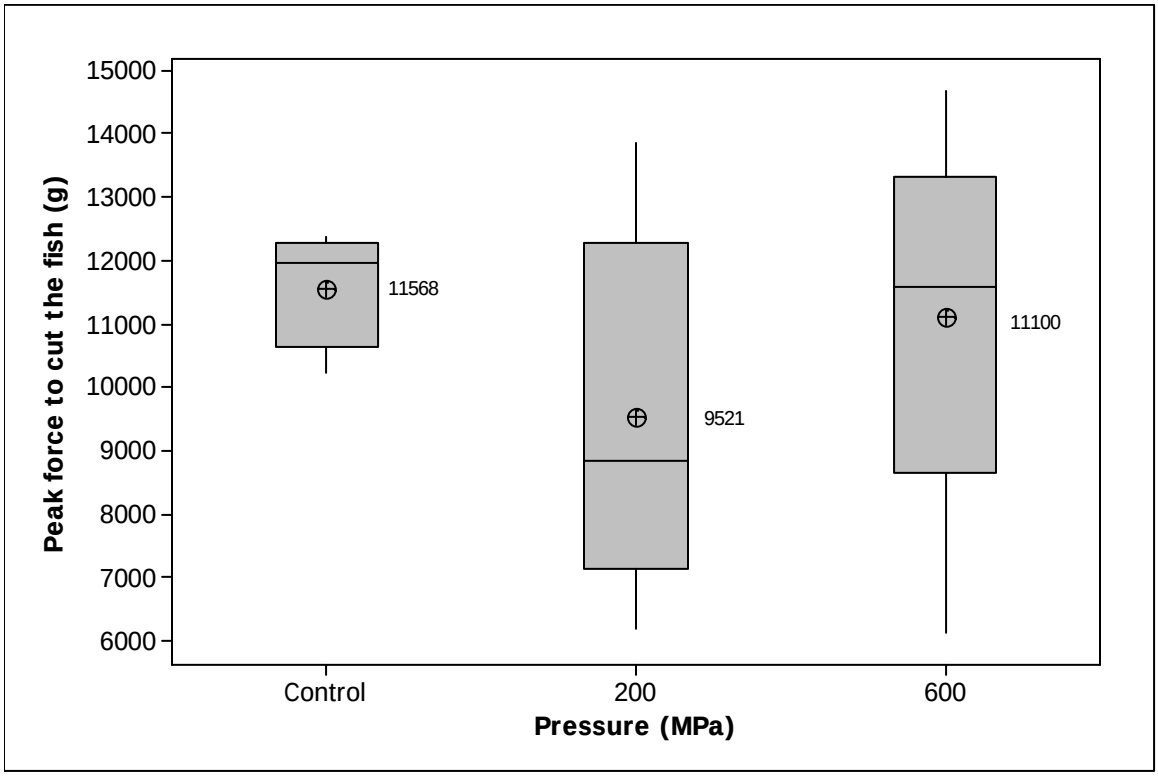


Figure 23. Peak force to cut cod

Quoted figure is the mean of 5 separate measurements

4.4.4 Gel electrophoresis results

The gel electrophoresis patterns of the protein extracts taken from the cod samples are shown in Figure 24 (Lane identification is shown in Table 18). An example of the densitometric analysis graph is shown in Figure 25. The assignment of fish muscle protein as resolved by gel electrophoresis according to (Thys, Blank, & Schachat 1998) is shown in Figure 26.

Sample	Treatment	Species	Laboratory No.	Gel Lane No.
A	Control No HPP No CO_2	Salmon	CMS/08/105	1
B	200MPa 0° C 1min No CO_2	Salmon	CMS/08/106	2
C	600MPa 0° C 1min No CO_2	Salmon	CMS/08/107	3
D	600MPa 0° C 5min No CO_2	Salmon	CMS/08/125	4
E	Control 100% CO_2	Salmon	CMS/08/108	5
F	200MPa 0° C 1min 100% CO_2	Salmon	CMS/08/109	6
G	600MPa 0° C 1min 100% CO_2	Salmon	CMS/08/110	7
H	600MPa 0° C 5min 100% CO2	Salmon	CMS/08/124	8
I	Control GE	Cod	CMS/08/111	10
J	200MPa 5min GE	Cod	CMS/08/112	11
K	600MPa 5min GE	Cod	CMS/08/113	12

Table 18 Lane identification.

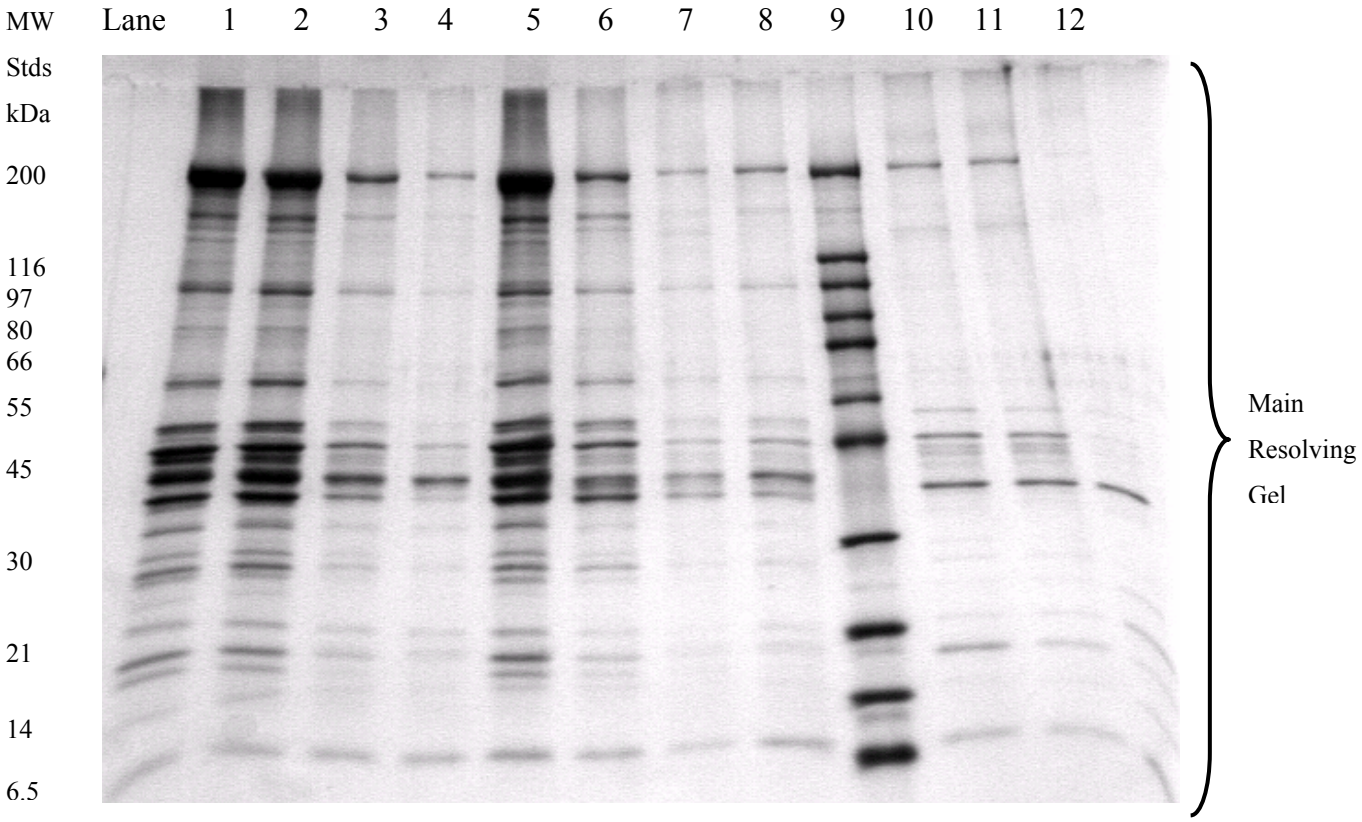


Figure 24. SDS-PAGE analysis of salmon and cod samples.

Note molecular weights refer to marker lane 9

200 MPa pressure treatment over 5 minutes led to a 8% reduction in the amount of protein extracted (Lane 11,Table 19) compared to the control (Lane 10, Table 19). The proportions of the lower-molecular-weight proteins (9 and 18 kDa) were diminished by this treatment. Elevation of the pressure to 600 MPa resulted in a 76% reduction in the total protein, together with a gross change in protein composition (Lane 12, Table 19).

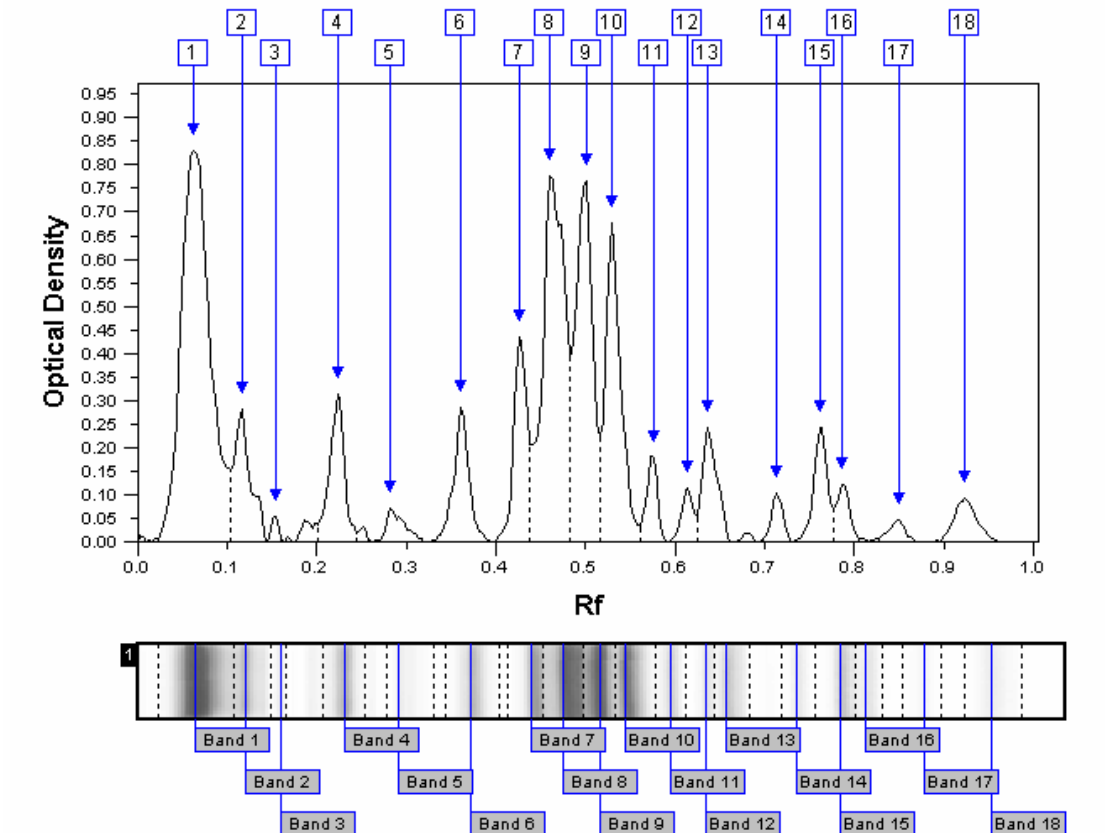


Figure 25. Example of a Lane Profile and Band Numbering

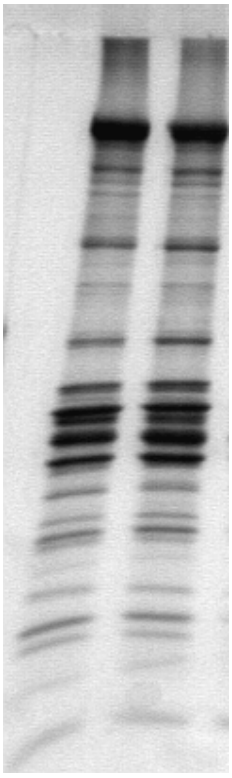


Figure 26. Assignment of Fish (Salmon) Muscle Proteins

COD								
Control GE			**200MPa 5min GE**			**600MPa 5min GE**		
Lane 10			**Lane 11**			**Lane 12**		
Band No	Band %	MW (kd)	Band No	Band %	MW (kd)	Band No	Band %	MW (kd)
1	14.7	205	1	12.2	205	1	62.9	36
2	5.0	137	2	6.8	141	2	18.4	21
3	3.9	52	3	11.0	61	3	18.7	8
4	13.0	45	4	2.8	52			
5	12.2	41	5	11.7	45			
6	29.8	36	6	19.7	42			
7	12.1	18	7	24.4	36			
8	9.4	9	8	4.9	18			
			9	6.5	9			
Total area			Total area			Total area		
183			168			47		

Table 19. Densitometric Analysis of Cod Samples, Lanes 10-12, GE

4.4.5 Light microscopy analysis of samples

Cod samples treated at 600 MPa showed dramatic structural changes in comparison to raw cod and cod treated at 200 MPa (Figure 27 and Figure 28). Essentially the structure of the flesh appeared cooked: multiple cross-breaks across the muscle fibres were observed at right angles to the axis of the fibres. The gel electrophoresis, microscopy and visual appearance results essentially confirmed that significant protein modifications occurred as a result of pressure treatment at 600 MPa, akin to that which would be expected by cooking of the product.

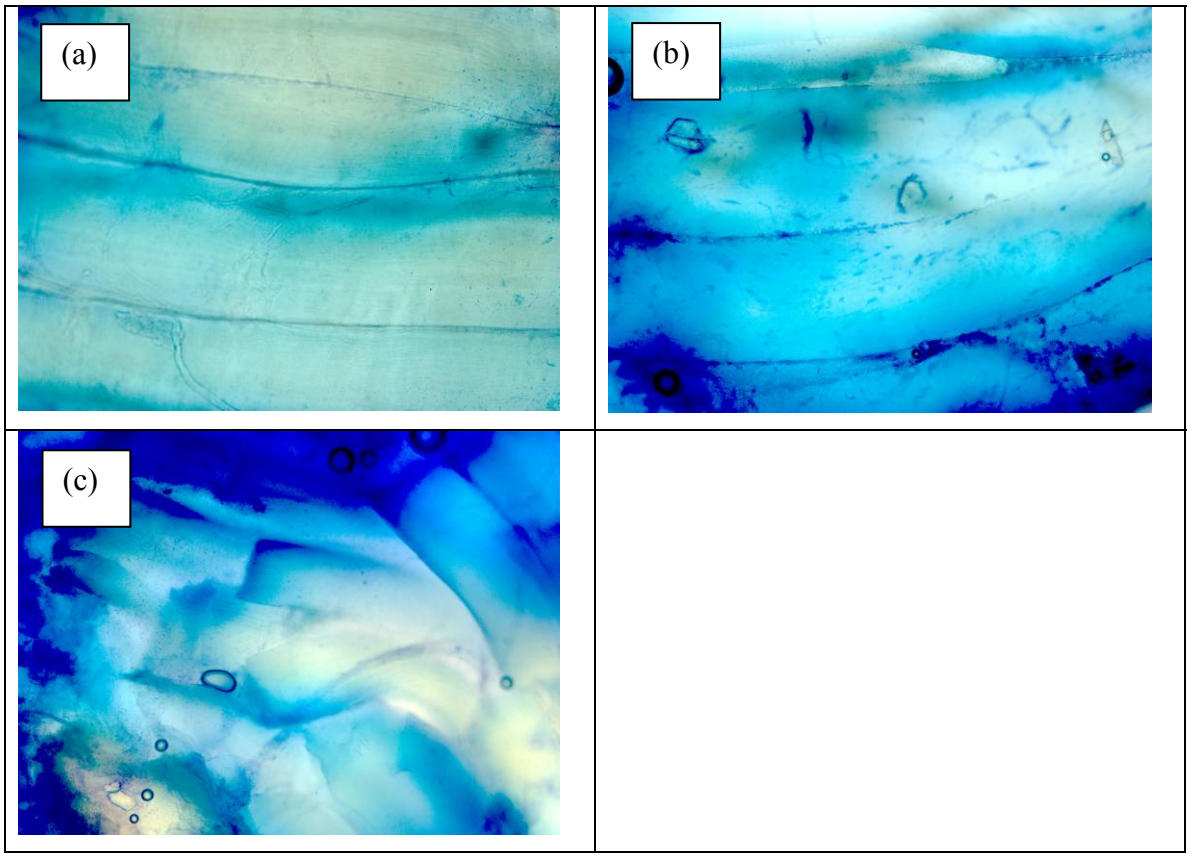


Figure 27. Images clockwise: (a) Untreated cod x 91 magnification, (b) 200 MPa 5 mins cod x 91 magnification, (c) 600 MPa 5 mins cod x 91 magnification

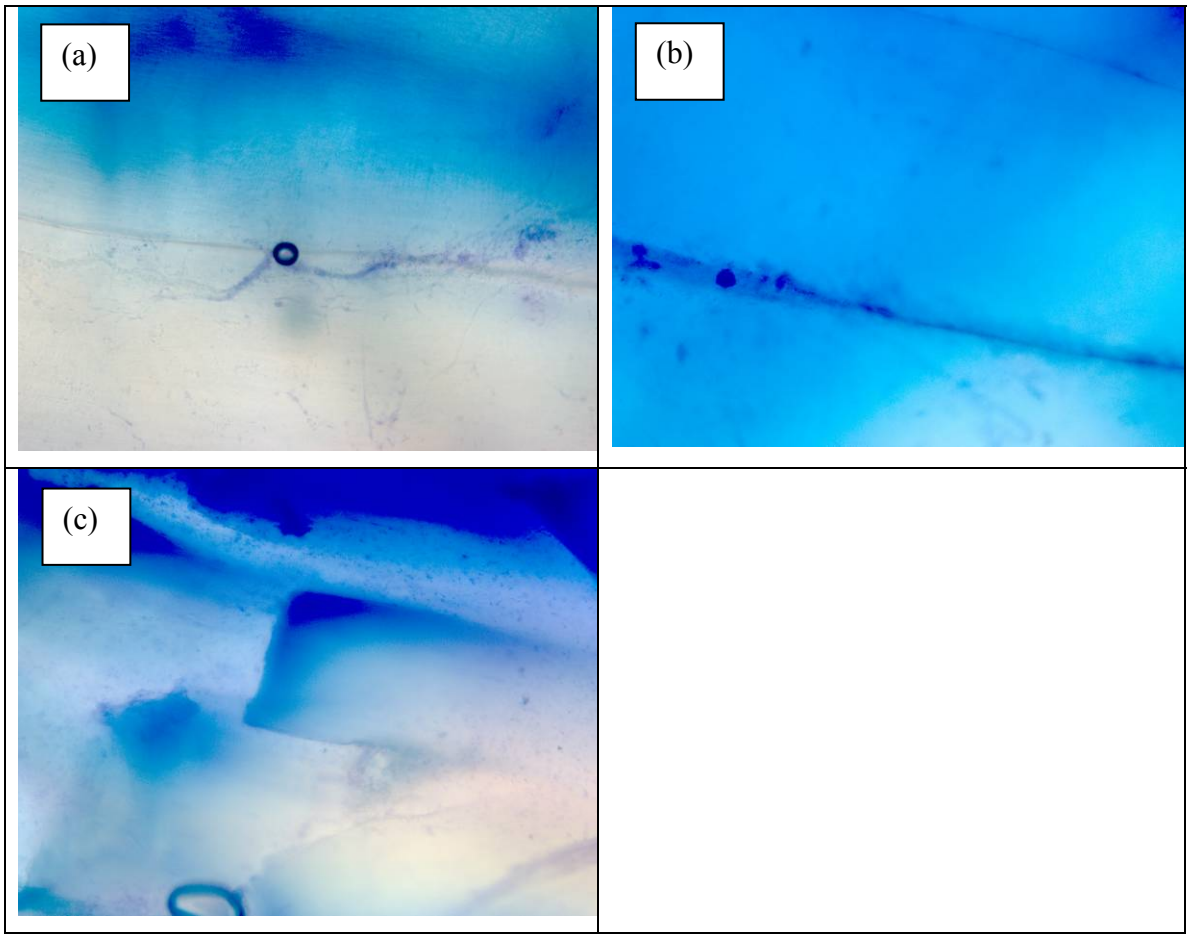


Figure 28. Images clockwise: (a) Untreated cod x 182 magnification, (b) 200 MPa 5 mins cod x 182 magnification, (c) 600 MPa 5 mins cod x 182 magnification

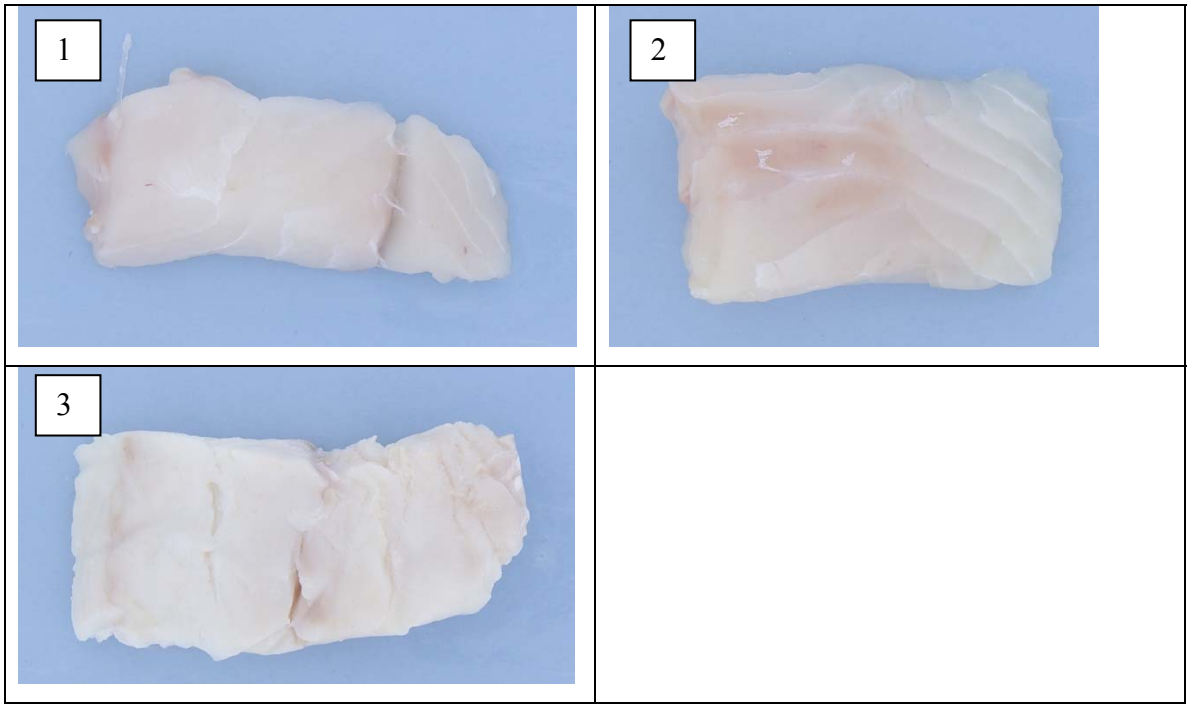


Figure 29. Cod samples at day 0. Samples (clockwise from top left) are: (1) Control,(2) 200 MPa for 5 minutes, (3) 600 MPa for 5 minutes

Cod samples treated at 200 MPa still look similar to untreated samples. Pressure treatments of 600 MPa give the fish a cooked appearance and the flesh takes on a compressed appearance and texture (Figure 29 and Figure 30).

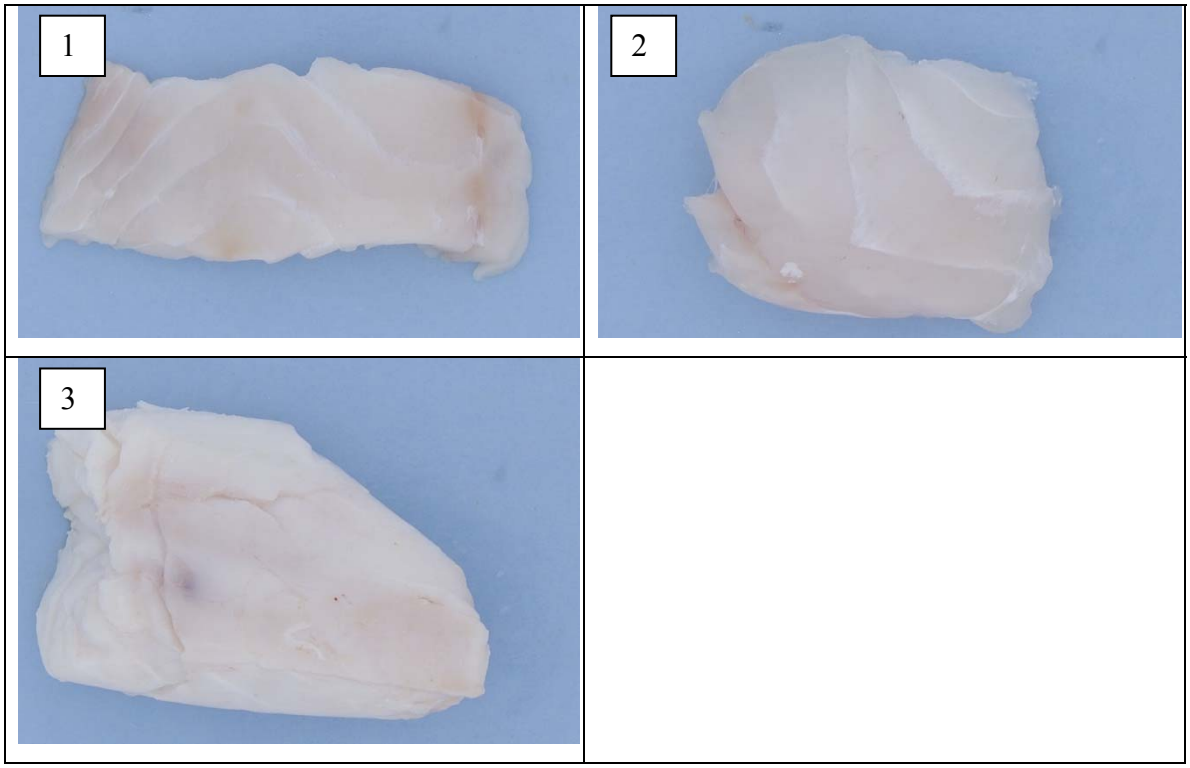


Figure 30. Cod samples at day 0. Samples (clockwise from top left) are: (1) Control,(2) 200 MPa for 5 minutes, (3) 600 MPa for 5 minutes

4.4.6 General observations for cod

Work on cod has demonstrated that HPP is extremely efficient as a means of controlling microbial loading on chilled seafood products. Key spoilage organisms such as pseudomonads can be reduced to non-detectable levels and the general microflora as described by aerobic plate counts can be reduced to very low levels for at least 11 days (the duration of the shelf life trial in these studies). However, raw fish suffers major changes in appearance when treated at a pressure of 600 MPa, primarily as a result of major protein changes as would be seen in cooking. There also appears to be non-microbial spoilage that is not controlled by HPP alone. It is likely that these non-microbial spoilage issues could be controlled by other techniques used in combination

with HPP (as has been seen in other commercial HPP products where similar issues arise) but such techniques have not been explored within this project.

4.5 Salmon

4.5.1 Microbiological results

The effects of pressure, temperature, time and the presence of CO_2 prior to treatment are shown in Figure 31. Initial log counts in the control salmon were, on average, 3.3 (n=3, s.d. = 0.7). Treatments of 600 MPa generally reduced total viable counts to non detectable or very low levels. Pressure level was a highly significant factor in the degree of lethality achieved ($p<0.05$). Treatment with carbon dioxide prior to HPP treatment did have a weak *statistically* significant effect on log TVC after processing ($p=0.0513$), with marginally fewer survivors in samples pre-treated with CO_2. However, in *practical terms*, pre-treatment with CO_2 had little impact on the overall lethality of the process (Figure 31), with the mean difference in log TVCs between samples pre-treated with CO_2 and those that were untreated being only 0.3 log units. Time at pressure and whether or not the sample was packed on ice or at room temperature did not appear to have an effect on the lethality of the process ($p>0.05$) within the conditions tested.

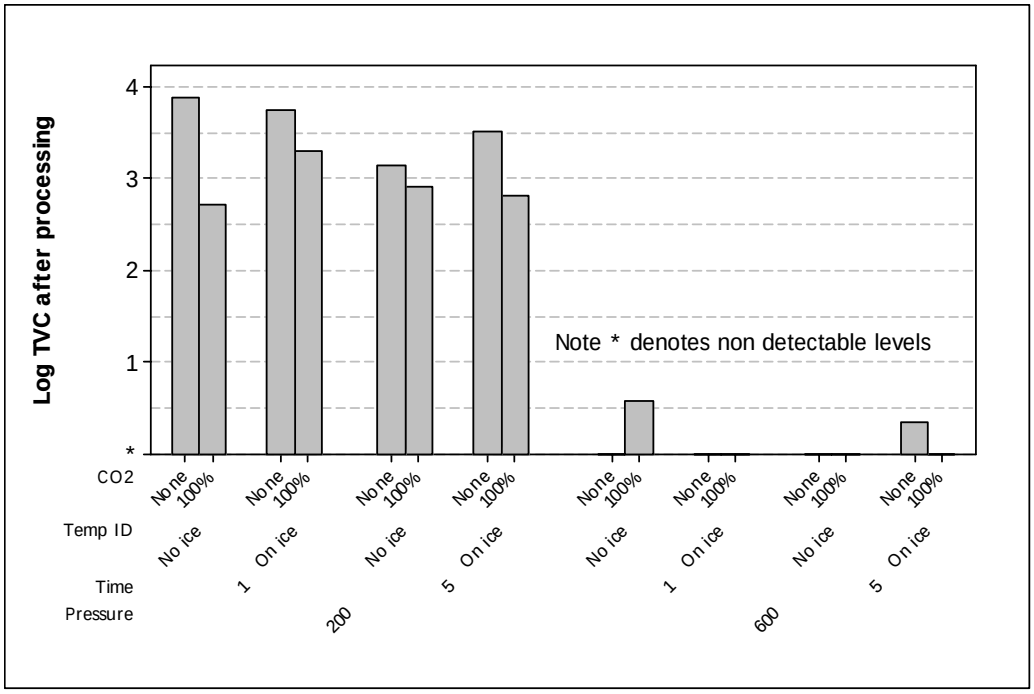


Figure 31. Total viable counts in salmon after a range of pressure treatments

4.5.2 Instrumental texture analysis

Pressure, time and pre-treatment with CO_2 all had a statistically significant effect ($p<0.05$) on the texture of the fish (as determined by compression force). The salmon became more firm when treated at higher pressures and when hold time increased. Samples pre-treated with CO_2 or packed on ice were softer in texture (Figure 32).

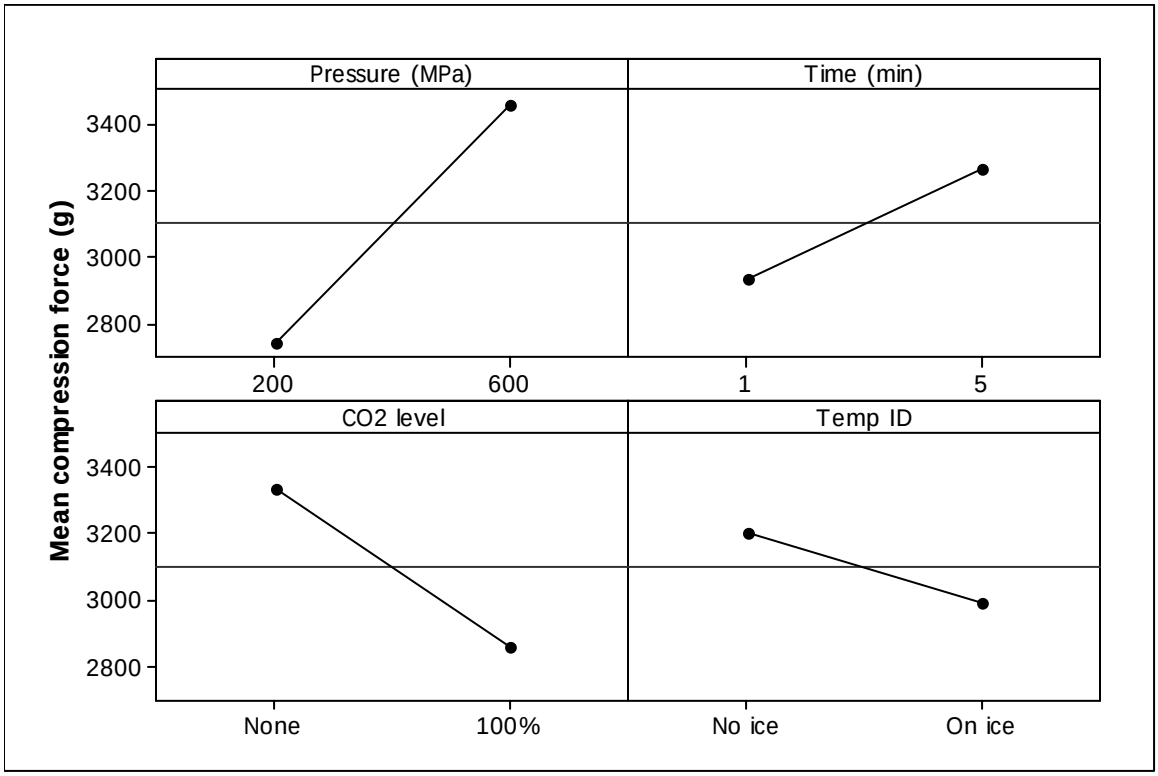


Figure 32. Main effect plot showing influence of pressure, time, CO_2 level and temperature on compression force in salmon

4.5.3 DigiEye colour measurement

Pressure and temperature both significantly influenced *L** ($p<0.05$, see Figure 7 for an explanation of colour terms). Samples became lighter as pressure increased and samples packed on ice were also lighter compared with samples packed at room temperature (Figure 33). Process time and pre-treatment with CO_2 did not influence L* values significantly ($p>0.05$).

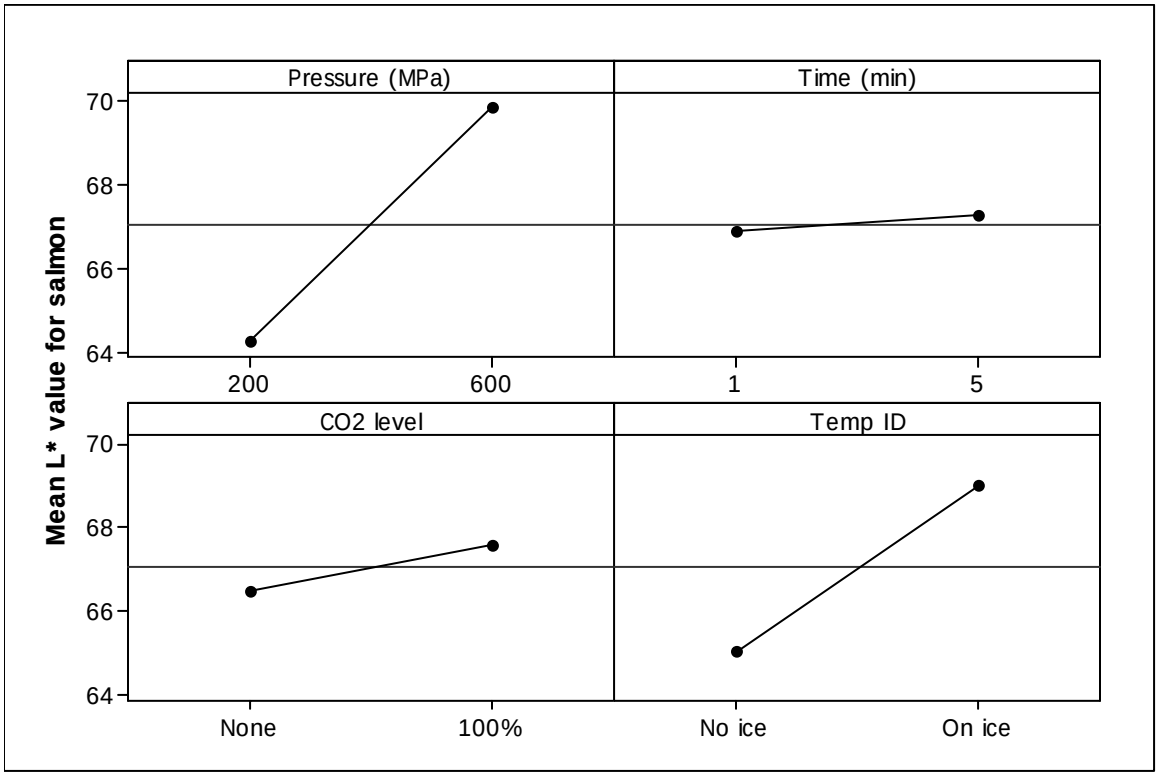


Figure 33. Main effect plot showing the influence of pressure, temperature, time and CO_2 levels on L values in salmon.*

Values of *a** were significantly influenced by pressure, time, CO_2 level and a pressure/time interaction ($p<0.05$). The influence of pressure, temperature, time and CO_2 levels on *a** are shown in Figure 34. Samples became less red as pressure increased (i.e. a downward shift in *a**). Longer hold times and pre-treatment with CO_2 also resulted in samples becoming less red. Values of *b** were significantly influenced by pressure, time, temperature, CO_2 pre-treatment and a pressure/time interaction ($p<0.05$). The influence of each factor on b* values is shown in Figure 35. Samples became less yellow as pressure and hold time increased and where a CO_2 pre-treatment was used. Packing on ice helped to reduce this downward *b** shift but the effect was small.

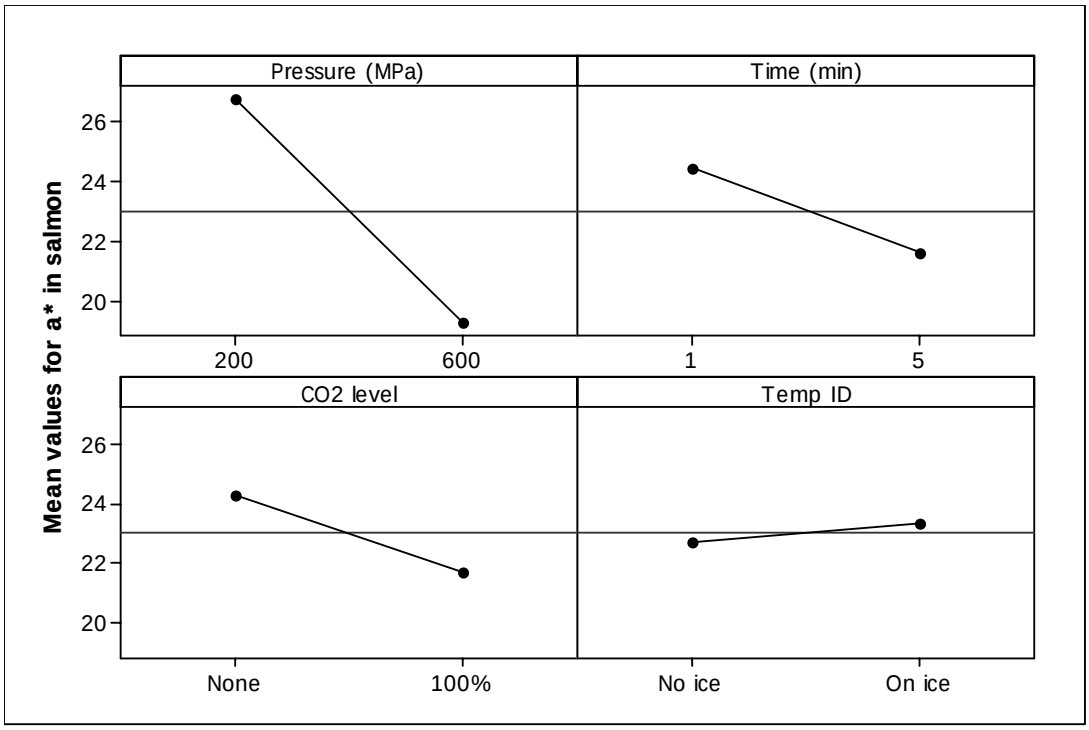


Figure 34. Main effect plot showing the influence of pressure, temperature, time and CO_2 levels on a values in salmon*

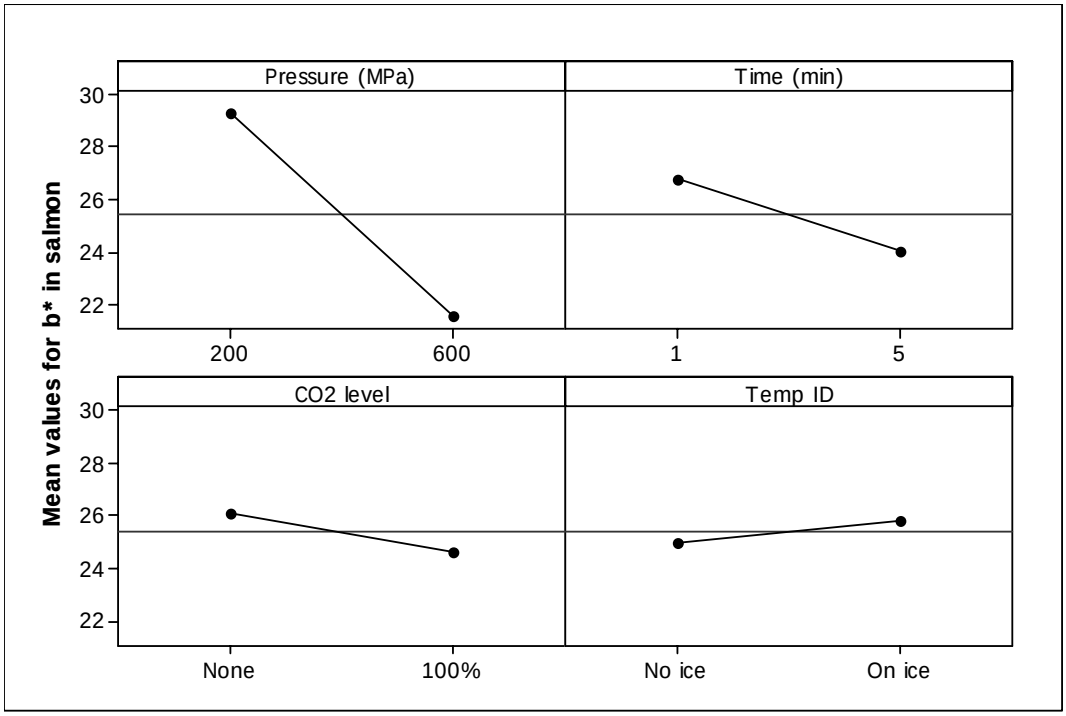


Figure 35. Main effect plot showing the influence of pressure, temperature, time and CO_2 levels on b values in salmon*

Figure 36 shows the practical implications of changing pressure, temperature and hold time and of pre-treating with CO_2. As can be seen in the images, increasing pressure has a dramatic effect on colour but also increasing hold time even at low pressures can have a detrimental effect on colour. The effect of CO_2 pre-treatment on colour as seen by the naked eye is marginal at 600 MPa but noticeable at 200 MPa, where the CO_2 treated samples take on a more pink cooked appearance.

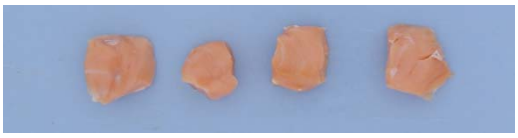
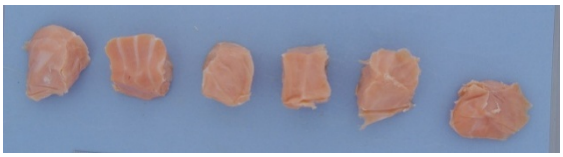
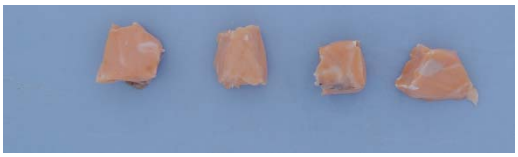
Control – no CO_2 pre-treatment	Control with CO_2 pre-treatment
	
200 MPa 1 min no CO_2 pre-treatment	200 MPa 1 min with CO_2 pre-treatment
	
200 MPa 5 mins no CO_2 pre-treatment	200 MPa 5 mins with CO_2 pre-treatment
	
600 MPa 1 mins no CO_2 pre-treatment	600 MPa 1 min with CO_2 pre-treatment
	
600 MPa 5 mins no CO_2 pre-treatment	600 MPa 5 mins no CO_2 pre-treatment
	

Figure 36. Salmon colour after a range of treatments

4.5.4 Gel electrophoresis results for salmon

The gel electrophoresis patterns of the protein extracts taken from the salmon samples are shown in Figure 24 (Lanes 1-8, see Table 18 to identify the lanes of each treatment). An example of the densitometric analysis graph is shown in Figure 25. The assignment of fish muscle protein as resolved by gel electrophoresis according to (Thys, Blank, & Schachat 1998) is shown in Figure 26.

The effect of 200 MPa pressure on the salmon sample in the absence of CO_2 appeared to be minor, as can be seen in Lanes 1 and 2 (Figure 24). However, the densitometric analysis showed that, overall, 33% more protein was extracted (Lane 2, Table 20) compared to the control sample (Lane 1, Table 20). This is consistent with reports that large increases in the solubilisation of myofibrillar proteins due to intense depolymerisation of such muscle proteins can occur following high pressure treatments (Cheftel & Culioli 1997); (Sequeira-Munoz et al. 2006). Furthermore, proportionally less of the Mysoin Heavy Chain (205 kDa) was extracted by this treatment compared to the rest of the proteins. Raising the pressure to 600 MPa over 1 minute caused a 70% reduction in the total extracted protein and increased the relative proportion of the Tropomysin (38 kDa, see Lane 3, Table 20). The proteins may have become more compacted and more covalently linked together, rendering them more resistant to extraction with SDS (Ashie & Simpson 1996). The 600 MPa treatment over 5 minutes caused a 90% reduction in the total extracted protein (Lane 4, Table 20).

SALMON											
Control No HPP No CO_2			**200MPa 0° C 1min No CO_2**			**600MPa 0° C 1min No CO_2**			**600MPa 0° C 5min No CO_2**		
Lane 1			**Lane 2**			**Lane 3**			**Lane 4**		
Band No.	Band %	MW (kd)	Band No.	Band %	MW (kd)	Band No.	Band %	MW (kd)	Band No.	Band %	MW (kd)
1	23.2	205	1	19.1	205	1	21.2	205	1	17.8	205
2	4.6	161	2	3.7	164	2	1.9	160	2	9.9	45
3	0.4	133	3	1.5	152	3	4.0	97	3	51.6	38
4	4.6	97	4	0.5	138	4	3.3	59	4	4.3	21
5	1.3	76	5	4.9	97	5	5.7	51	5	16.4	8
6	4.3	59	6	1.4	77	6	10.2	45			
7	5.3	50	7	4.9	59	7	25.9	38			
8	16.4	(45/43)	8	6.5	51	8	11.2	36			
9	13.7	38	9	8.9	45	9	1.6	32			
10	10.5	36	10	7.5	43	10	3.5	28			
11	2.1	32	11	14.4	38	11	1.5	21			
12	1.3	29	12	11.1	36	12	2.5	19			
13	3.7	28	13	2.3	32	13	7.5	8			
14	1.2	22	14	1.3	29						
15	3.1	19	15	4.0	28						
16	1.7	17	16	1.0	21						
17	0.8	13	17	2.8	19						
18	2.1	8	18	1.6	17						
			19	0.9	14						
			20	1.8	8						
Total area			Total area			Total area			Total area		
1105			1475			336			116		

Table 20. Densitometric Analysis of Salmon Samples, Lanes 1-4, No CO2

Pre-treatment with CO_2 caused little or no change to the total protein extracted and its composition (Lane 5, Table 21) compared to the control in the absence of CO_2 (Lane 1,

Table 20). However, the 200 MPa pressure treatment with CO_2 pre-treatment led to a 60% reduction in the amount of protein extracted (Lane 6, Table 21). Elevating the pressure to 600 MPa over 1 minute caused a 90% reduction in the amount of extracted protein, as well as affecting the composition. Surprisingly, the 600 MPa treatment over 5 minutes caused slightly less reduction (85%) in the overall amount of protein that was extracted (Lane 7, Table 21) compared to the 5 minute treatment at the same pressure. The protein composition was also different.

SALMON											
Control 100% CO_2			**200MPa 0° C 1min 100% CO_2**			**600MPa 0° C 1min 100% CO_2**			**600MPa 0° C 5min 100% CO_2**		
Lane 5			**Lane 6**			**Lane 7**			**Lane 8**		
Band No	Band %	MW (kd)	Band No	Band %	MW (kd)	Band No	Band %	MW (kd)	Band No	Band %	MW (kd)
1	19.7	205	1	17.9	205	1	11.8	205	1	18.0	205
2	4.6	161	2	4.7	161	2	5.7	97	2	6.4	97
3	1.4	136	3	2.4	148	3	8.2	58	3	3.8	58
4	4.7	97	4	3.6	97	4	6.1	50	4	6.8	51
5	2.2	77	5	4.1	59	5	9.9	45	5	9.1	45
6	3.9	59	6	7.6	51	6	31.1	39	6	31.3	38
7	5.7	51	7	11.0	45	7	19.5	36	7	12.8	36
8	18.0	45/43	8	6.4	43	8	7.7	8	8	11.7	9
9	13.9	38	9	16.6	38						
10	9.4	36	10	15.3	36						
11	2.2	32	11	1.4	32						
12	1.2	29	12	2.5	28						
13	3.6	28	13	2.7	19						
14	1.1	21	14	3.9	8						
15	3.4	19									
16	1.7	17									
17	0.7	16									
18	2.6	8									
Total area			Total area			Total area			Total area		
1166			462			116			183		

Table 21. Densitometric Analysis of Salmon Samples, Lanes 5-8, 100% CO_2

4.5.5 General observations for salmon

As was seen in cod, HPP was a very efficient method for controlling microbial loading in seafood products, but detrimental colour and texture changes were observed at higher pressures, thought to be due primarily to protein modifications. Crucially in salmon and in fact generally for seafood products processed using HPP, pressure, temperature and hold time can all influence the eventual sensory properties of the product. This means that there must always be a development process to determine the conditions that optimise product quality. Trials on laboratory HPP units cannot always be successfully scaled up to commercial vessels. Once again this highlights the need for a mainland large scale vessel to be available for detailed development trials.

Pre-treatment with carbon dioxide prior to pressure treatment did not appear to offer any significant benefits in terms of microbial reduction and exacerbated undesirable colour changes. It did appear to confer some benefits to texture in that it reduced the firming effect of high pressure. However, it seems unlikely that this benefit would justify the added complexity and processing on-costs that would result from using a CO_2 pre-treatment with HPP.

5 Conclusions from the project

5.1 Shucking, peeling/de-shelling

High Pressure Processing has significant potential for enhancing peeling and picking yields from seafood products but conditions must be carefully optimised to ensure minimal quality changes whilst maximising yield. Even where yield benefits have not appeared to be large enough to be of commercial significance (e.g. in Nephrops) it would be unwise to conclude from this study that better yields could not be achieved with more refinements to the processing conditions; higher yields were in fact achieved in the first phase of the project.

Whilst this project has provided platform knowledge for identifying suitable conditions for processing on a species-by-species basis, detailed development work was outside the scope and would need to be conducted by seafood processors having an in-depth understanding of their individual products and current process procedures. Many factors could influence the efficacy of the process that simply could not be considered within the practical and budgetary limitations of this study. Examples include:

- Raw material variation over the year
- Tempering temperatures of frozen raw materials
- The use of fresh rather than frozen materials
- Time and temperature from HPP treatment to peeling
- Post process handling effects on ease of picking/peeling
- Other variations of pressure/temperature/time not considered
- Variables relating to automated peeling systems
- Scaling issues from laboratory to commercial scale equipment

Commercial processes exist for the shucking of bi-valves and the results from phase 1 of this project highlighted just how effective HPP was for this application. Oysters and mussels were readily shucked, still appeared raw and significant yield benefits were achievable.

Commercial processes also exist for HPP assisted de-shelling of lobsters and excellent results have been reported for the de-shelling of a range of crab types including Alaskan King Crab and Dungeness crab (personal communication with Avure). Again it is therefore important to stress that the relatively poor results obtained for brown crab in these experiments should not be taken as 'proof' that HPP is unsuitable for de-shelling brown crab. Instead it indicates the need for detailed trials by processors on a species-by-species basis to identify optimum conditions.

Natural variability in raw materials has highlighted the need to carry out a reasonable numbers of trials in order to separate genuine trends arising from process conditions, from those of random variation due to the raw material. This process need not be excessively costly because large numbers of trials can be carried out quickly using HPP.

A final point of note with respect to the use of HPP for the processing of bi-valves and crustaceans is the fact that, whilst a number of HPP equipment suppliers exist, Avure Technologies Inc. holds a number of patents relating to the application of HPP for these products. Processors interested in pursuing HPP for shucking and de-shelling should be aware of these Intellectual Property issues when discussing applications with HPP equipment suppliers.

5.2 HPP for fish

HPP is an extremely effective method for inactivating microorganisms and a large body of data is now available to demonstrate this both for seafood and a wide range of other food products. Within the project, key seafood spoilage organisms such as

pseudomonads were generally found to be very pressure sensitive. Shelf-life trials on cod demonstrated that fish samples could be held for 11 days with non-detectable levels of aerobic plate counts, coliforms and pseudomonads. However, non microbial spoilage still occurred and more detailed studies are required to try to resolve this problem.

Pressures required for pasteurisation (i.e. in order to achieve a 6-log reduction of *Listeria monocytogenes* in the case of chilled products having a shelf life of less than 10 days) are likely to induce undesirable changes in the colour and texture of fresh fish. However, practical experience has shown that HPP treatment of *cooked* seafood products has only a minimal effect on product quality. This opens up opportunities for HPP to be used as a post-pack safety treatment for ready-to-eat seafood in much the same way as it is used as a post-pack *Listeria monocytogenes* inactivation step in cooked meats.

6 Future work and dissemination plans

As has been previously stated, future work now needs to be completed by seafood processors at an industrial scale in order to refine and optimise processing conditions for each individual species. HPP technology is now well established outside the UK and the benefits of using HPP are tangible. Critically, however, industrial product development relies on access to full scale equipment or, at the very least, small scale equipment that can be directly scaled to commercial conditions. Currently, seafood processors only have access to 1 large scale system in the UK where trials can be carried out on a contract basis. This situation must be addressed if HPP is to achieve significant commercial uptake in the UK seafood processing industry. Not only is ready access to equipment required but the cost for development trials must be low so that the technology is accessible to the many small businesses that make up the UK seafood processing industry. It seems likely that can only be achieved through some form of subsidy.

One possible route forward would be the development of a ‘pay-per-use’ HPP facility where small seafood processors could access equipment for commercial runs without having the risks associated with making a large capital expenditure. Again, the approach

is likely to require some form of grant to build a facility and to purchase a commercial scale HPP vessel. Such an approach would only be viable if there was a genuine industrial need for HPP technology. In order to gauge interest in a 'pay-per-use' facility, a 'HPP awareness day' is being organised and hosted at CCFRA in October 2008 with the help of Seafood Cornwall. This event will give seafood processors in the South West of England an opportunity to hear more about HPP from CCFRA and equipment suppliers. Uniquely, it will also give processors an opportunity to see HPP in action first hand. The event will be free of charge and is being funded as a dissemination exercise from the European Union project 'novelQ' (www.novelq.org) which is a project concerned with removing barriers to the commercialisation of a range of emerging preservation technologies. If there were sufficient interest, such events could be carried out for other regions of the UK but funding would be needed to cover the costs of these events.

Two reports have been produced from the project and both will be available free of charge on the Seafish Authority website. In addition, the results from the project have been disseminated via oral and poster presentations in the UK and at international conferences in the USA and Japan. At least one written peer reviewed publication is planned that will be jointly co-authored by CCFRA and Norconserv; this latter group having provided key technical support throughout the project. A series of 1 page 'research summary sheets' will be produced on a species-by-species basis so that seafood processors can get a concise summary of the benefits of HPP for particular products of interest.

7 References

Ashie, I. N. A. & Simpson, B. K. 1996, "Application of high hydrostatic pressure to control enzyme related fresh seafood texture deterioration", *Food Research International*, vol. 29, pp. 569-575.

ASTM 2000, *Standards on color and appearance measurement. American Society for Testing and Materials, Philadelphia*, 6th Edition edn, American Society for Testing and Materials, Philadelphia.

Cheftel, J. C. & Culioli, J. 1997, "Effects of high pressure on meat: a review", *Meat Science.1997*, vol. 46(3): pp. 211-236.

CIE 1986, *Colorimetry. CIE publication 15.2. Commision Internationale de l'Éclairage, Vienna*, 2nd Edition edn, Commision Internationale de l'Éclairage, Vienna.

Laemmli, U. K. 1970, "Cleavage of structural proteins during the assembly of the head of the bacteriophage T4", *Nature*, vol. 227, pp. 680-685.

Leadley, C. R&D report 226: high pressure sterilisation - temperature distribution studies in a laboratory scale high pressure vessel. 2006. Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire, GL55 6LD.
Ref Type: Generic

Sequeira-Munoz, A., Chevalier, D., Le Bail, A., Ramaswamy, H., & Simpson, B. K. Physicochemical changes induced in carp (Cyprinus carpio) fillets by high pressure processing at low temperatures. Innovative Food Science & Emerging Technologies 7, 13-18. 2006.
Ref Type: Generic

Thys, T. M., Blank, J. M., & Schachat, F. H. 1998, "Rostral-Caudal variation in Troponin T and Parvalbumin correlates with differences in relaxation rates of cod axial muscle", *The Journal of Experimental Biology*, vol. 201, pp. 2993-3001.

Whitworth, M. Objective measurement of food appearance. R&D Report 231. Campden & Chorleywood Food Reasearch Association, Chipping Campden, Gloucestershire, GL55 6LD, UK. 2006.
Ref Type: Generic