HIGH PRESSURE
PROCESSING (HPP)

CONSIDERATIONS IN USING HPP TECHNOLOGY

Contents

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Introduction

This guidance document has been developed as an introductory guideline for new entrants looking to introduce High Pressure Processing (HPP) into their operations. This document is not exhaustive, only a starting point.

High pressure processing (HPP) is an excellent addition to a food technologist's tool kit. For some foods HPP provides a good technology solution to processing challenges. For other products the use of pressure alone has limitations and processes that combine pressure and temperature might be required to achieve the sought-after product characteristics, while also ensuring food safety. The desired outcome and product characteristics must be taken into account during validation studies and when assessing shelf life.

Reviews by Balasubramaniam, Martínez-Monteagudo, & Gupta, 2015 and Horn et al., 2019 provide detailed information on HPP and combined processing technologies. The reviews note that pressure alone, at or near ambient temperature, has very limited or no effect on spore destruction. This has clear implications for the HPP treatment of low-acid foods. HPP low-acid foods stored at ambient temperature will be at risk of growth and toxin production by *Clostridium botulinum* and rapid spoilage. Extended refrigerated storage of HPP low-acid foods will be at risk of growth and toxin production by cold-tolerant (psychrotrophic) *Clostridium botulinum*. Over time, spoilage is also likely to be a problem.

These risks are no different to those faced when preparing pasteurised products and foods cooked at conventional temperatures. HPP needs to be used wisely and with an appreciation of the limitations of the technology. A review by Rendueles et al. (2011) states that in general, HPP applied at ambient temperature destroys vegetative cells and inactivates certain enzymes with minimal change in the organoleptic properties of the treated product. Large differences in sensitivity to HPP among pathogenic microorganisms (*Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Salmonella* Typhimurium) have been reported, with reductions in the range 0.5-8.5 log units (Rendueles et al., 2011).

This document aims to assist those new to HPP navigate their way through some basic issues including product design, process validation and estimation of shelf life. The NSW Food Authority recommends that food manufacturers interested in validating pressure-pasteurisation treatments work with competent pressure-processing experts in designing and conducting validation trials.

Part 1 – An overview of HPP science and technology

HPP Technology

HPP subjects liquid or solid foods, with or without packaging, to pressures between 100 and 800 MPa (14,500 to 116,000 PSI). Process temperatures during pressure treatment can be specified to below 0°C and to above 100°C. Vessels are uniquely designed to withstand these pressures over many cycles. Commercial exposure times can vary from a millisecond pulse to over 20 minutes. Foods subject to HPP treatment at or near room temperature will not undergo significant chemical transformations due to the pressure treatment itself.

HPP acts instantaneously and uniformly through a mass of food independent of size, shape and food composition. Thus package size, shape and composition are not factors when determining the process. The work of compression will increase the temperature of the foods by approximately 3°C per 100 MPa, depending on the composition of the food. If a food contains a significant amount of fat, the temperature rise can be larger. Foods cool down to their original temperature on decompression if no heat is lost or gained from the vessel walls of the chamber during the hold time at pressure. A uniform initial temperature is required to achieve a uniform temperature increase in a homogenous system during compression. As the process leads to a very limited increase in temperature, HPP is considered a non-thermal process.

While the temperature of a homogenous food will increase uniformly due to compression, the temperature distribution in the mass of food during the holding period at pressure can change due to heat transfer to or from the walls of the pressure vessel. The pressure vessel must be held at a temperature equal to the final food temperature increase from compression for truly isothermal conditions. Temperature distribution must be determined in the food and reproduced each treatment cycle if temperature is an integral part of the HPP microbial inactivation process specification.

[Reference: IFT & FDA, 2000]

Factors affecting the effectiveness of HPP

The effectiveness of HPP in eliminating or reducing foodborne microorganisms depends on a number of factors related to the specific organisms of concern (e.g. type of organisms and growth phase) and the food (e.g. pH, water activity, temperature and composition).

Table 1. Factors influencing microbial sensitivity to HPP (adapted from Horn et al., 2019)

	Factor	Effect
Organisms	Growth phase	Microorganisms in the exponential phase of growth (a period of time where the cell numbers are doubling and growth is at its fastest) are more sensitive to pressure than in the stationary phase of growth (a period of time following the exponential phase in which the growth rate and death rate are equal).
	Spore-formers	Spores are highly resistant to pressure. A combination of pressure (>800 MPa) and heat (>80°C) or high pressure in combination with other antimicrobial treatments, is required to achieve a significant level of inactivation of spores in foods. Spores have been observed to germinate during pressure treatments of up to 400 MPa.
	Sub-lethally injured cells	Sub-lethal inactivation by HPP can lead to stressed or injured cells that can recover under certain conditions and present a risk of re-growth of the microorganism during the shelf life of a food. If pathogens are not permanently inactivated by HPP, inhibitory food formulations or storage conditions are required to inhibit the growth of injured cells after processing.
	Cell temperature prior to HPP	The temperature at which microorganisms are held prior to HPP treatment may affect their sensitivity to damage by pressure. This is because temperature is an important factor for bacterial growth.
Food	Food composition	Food constituents such as proteins, fats, sugars, salts and minerals can provide a protective effect and increase the microbial resistance to pressure.
	pH	As pH is lowered, most microorganisms become more susceptible to HPP inactivation and sub-lethally injured cells fail to repair.
	Water activity (a_w)	Reducing water activity tends to protect microorganisms against inactivation by HPP. Recovery of injured cells can also be inhibited by low water activity. HPP may not work with dry solids or powders. The pressure treatment may compact products (form a cake) that do not have sufficient moisture content.
	Antimicrobials	The combination of HPP and antimicrobial compounds can promote the elimination of pressure-resistant microorganisms, decrease the temperature needed to inactivate microorganisms and help prevent the repair of sub-lethally injured cells during storage.
Other	Pressurised temperature	The pressurised temperature affects the sensitivity of bacterial cells to pressure. Temperatures above 50°C rapidly decrease the pressure resistance with increasing temperature.

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Microbiological factors

HPP does not disrupt just one cellular structure or function. Cell death is due to a multiplicity of damage accumulated in different parts of the cell. When the accumulated damage exceeds the cell's ability to repair, cell death occurs. In some cases, the cell is damaged but repair can occur if post-treatment conditions are favourable. The cell membrane is the main bacterial structure damaged by HPP treatment, resulting in detrimental changes to cell permeability and function.

HPP usually has a higher destructive effect in organisms with a greater degree of organisation and structural complexity. The destruction of foodborne parasites, such as *Toxoplasma gondii, Cryptosporidium parvum, Anisakis simplex, Trichinella spiralis* and *Ascaris*, can be achieved in low pressure ranges (100 – 400 MPa).

Moulds and yeast have intermediate resistance, with mould mycelia being particularly susceptible. However, there have been reports of mould spores that are quite resistant to HPP. Yeasts including *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* have high susceptibility to HPP, but environmental factors such as pH can have an impact.

Organisms with simple structures, such as bacteria, are usually more resistant to HPP inactivation. Studies with foodborne vegetative pathogens show large variations in sensitivity when low pressures (300 MPa) are combined with intermediate temperatures. Differences are seen between species of pathogenic microorganisms and differences have also been reported between strains belonging to the same genus or species. Gram positive bacteria are generally more resistant than gram negative bacteria. Bacteria are more sensitive during exponential growth compared to the stationary phase.

Spores show great resistance to inactivation by high pressure. This is especially relevant for the spore forming foodborne pathogens *Clostridium botulinum, Clostridium perfringens* and *Bacillus cereus*. The production of shelf stable, low acid foods requires inactivation of spores by combining HPP treatment at pressures exceeding 800MPa and at temperatures above 80°C, or at increased pressure in combination with other antibacterial treatment. At moderate pressures (up to 400MPa), it is possible for spores to germinate into vegetative cells which may then multiply during product storage.

Viruses vary in pressure resistance depending on their structural complexity. Enveloped viruses are usually more sensitive than non-enveloped viruses.

Staphylococcal enterotoxins produced by *S. aureus* are very pressure tolerant. A combination of high pressure and temperature is required to denature the toxin to an inactive and safe state.

[References: Babtista et al., 2016; Balasubramaniam et al., 2015; Demazeau & Rivalain, 2011; Horn et al., 2019; Rendueles et al, 2011]

Food characteristics

Food composition

Some food components, such as proteins, fats, carbohydrates, amino acids, vitamins and minerals, can have a protective effect on microbiological cells. Microorganisms generally show a higher resistance to pressure in food systems than in buffers or broths.

During pressure treatment, the temperature of different food materials increases quickly due to physical compression and returns to its initial value upon decompression. The heat of compression of most of the high-moisture food materials is very similar to that of water, 3°C per 100 MPa at 25°C. However, fatty foods have higher compression heating due to their higher compressibility with long-chain unsaturated fatty acids and lower specific heat.

Table 2. Heat of compression values of selected foods

Substance at 25°C	Temperature change per 100 MPa
Water, juice, tomato salsa, 2% milk, and other water-like substances	3.0
Mashed potato	3.0
Salmon	3.2
Chicken fat	4.5
Beef fat	6.3
Olive oil	8.7 to 6.3[a]
Soy oil	9.1 to 6.2[a]

[a] Substances exhibited decreased temperature rise as pressure increased.

pH

As pH is lowered, most microorganisms become susceptible to HPP inactivation and sub-lethally injured cells fail to repair. Experience with acid foods suggests that foods with a water activity close to one, and pH values less than 4.0, can be made commercially sterile using a pressure of 580 MPa and a process hold time of 3 min. This treatment has been shown to inactivate 10^6 cfu/g of *E. coli* O157:H7, *Listeria* spp., *Salmonella* spp., or *Staphylococcus* spp. in salsa and apple juice.

Acid foods with pH values between 4.0 and 4.5 can be made commercially sterile using a pressure of 580 MPa and a hold time of 15 min, with an initial temperature of approximately 22°C.

Water activity

Reducing the water activity (a_w) of a food appears to protect microorganisms against inactivation by HPP. However, it is expected that microorganisms may be sub-lethally injured by pressure and recovery of sub-lethally injured cells can be inhibited by low a_w. Consequently, the net effect of a_w may be difficult to predict.

A technical paper by Georget et al. (2015), concluded that HPP inactivation of microorganisms at low or variable a_w is reduced, but that the impact appears in part to be related to a_w and in part to the impact of the solute.

Temperature

An increase in food temperature above room temperature and to a lesser extent a decrease below room temperature, increases the inactivation rate of microorganisms during HPP treatment. Temperatures in the range of 45 to 50°C appear to increase the rate of inactivation of foodborne pathogens and spoilage microorganisms and thus warrant the development of processes which incorporate a uniform initial food temperature in this range. Process temperatures in the range of 90 to 110°C in conjunction with pressures of 500-700 MPa have been used to inactivate spore forming bacteria such as *Clostridium botulinum*. The use of elevated temperatures as part of a specified HPP process will require monitoring the food temperature during the process to ensure every element of the food reaches the specified value.

Rendueles et al. (2011) explored some reasons why temperature might affect inactivation. They report that the decrease in resistance to pressure at low temperatures, may be due to changes in membrane structure and fluidity through weakening of hydrophobic interactions and crystallisation of phospholipids. Moderate heating (40–60°C) can also enhance microbial inactivation by pressure, which in some cases makes application of lower pressure an option.

Additives

The inclusion of antimicrobial hurdles can improve the ability of HPP to inactivate microorganisms during processing or inhibit their growth during storage. Increases in pressure can result in increased membrane permeability. This may explain why bactericidal compounds, such as nisin and lysozyme, are more effective in combination with HPP treatment.

[References: Balasubramaniam et al., 2015; Babtista, 2016; IFT & US FDA, 2000; Horn et al., 2019; Patterson et al, 1995; Rendueles et al., 2011]

Part 2 – Considerations for manufacturers

Sub-lethal inactivation and recovery

Following an HPP treatment, vegetative bacterial cells may either be:

- Unaffected, healthy and able to grow post treatment,
- Stressed or injured, and if given favourable environmental conditions and time can recover and able to grow again, or
- Inactivated without the ability to recover.

Immediately after HPP treatment, the population of bacterial cells in/on the food is likely to be a combination of the three states. Healthy cells will be able to grow and can be cultured and enumerated on selective and non-selective media. However, stressed cells will only grow on non-selective media.

Both stressed and injured cells can continue with normal growth after a period of time if given favourable conditions. **Sub-lethal inactivation of bacterial cells should be taken into account when validating an HPP treatment for a particular product. It is important that injured or stressed bacterial cells are not able to recover and grow during the shelf life of the product. It is also important to include a culture isolation procedure that will detect stressed or injured bacterial cells throughout the shelf life of the product during the validation process.**

[Reference: Horn et al., 2019]
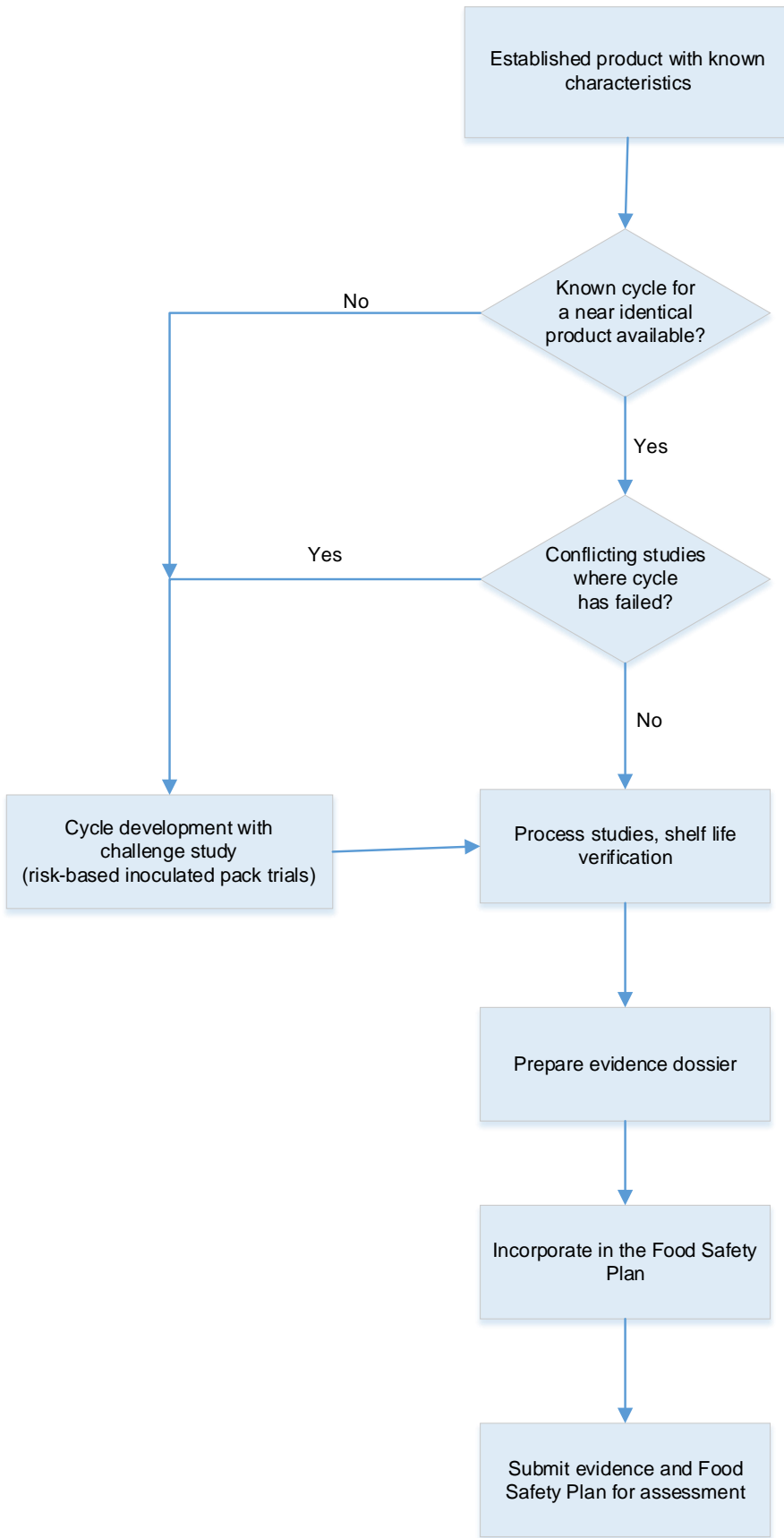
Processing conditions

When product development is well advanced[1] and an HPP packaging system has been identified, processing cycle must be resolved. When developing processing conditions for a specific product, it is important to consider both the effects of food composition on microbiological inactivation and the effect of HPP on the properties of the food.

A Hazard Analysis and Critical Control Point (HACCP) plan is essential to ensure that ingredients entering the process have low counts of pathogens and spoilage microorganisms. Shorter hold times (the length of time where the food is held at the desired pressure) are possible if the product is to be refrigerated. Actual hold-time values must be determined from challenge packs and storage studies for at least twice the length of the intended shelf-life of the product.

Small manufacturers that contract an HPP processor will generally look for a published cycle for a food that closely matches their product. The cycle should originate from a scientific journal, an expert research organisation or the equipment manufacturer. **HPP process conditions must be validated in the product of interest rather than extrapolating data from other food matrices or broth**. **Figure 1** describes an approach for the development of an HPP treatment cycle.

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[1] For further reference on product development and shelf life refer to the '*Shelf Life Testing*' document on the Food Authority's website.

Figure 1. HPP cycle development

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                    ┌─────────────────────────────┐
                    │  Established product with    │
                    │    known characteristics     │
                    └─────────────────────────────┘
                                  │
                                  ▼
                            ◇ Known cycle for ◇
         No ─────────────────◇ a near identical ◇
         │                   ◇ product available? ◇
         │                            │
         │                           Yes
         │                            ▼
         │                    ◇ Conflicting studies ◇
         │          Yes ──────◇   where cycle        ◇
         │          │         ◇   has failed?        ◇
         │          │                  │
         │          │                  No
         ▼          ▼                  ▼
   ┌──────────────────────┐   ┌──────────────────────┐
   │ Cycle development    │   │ Process studies,     │
   │ with challenge study │──▶│ shelf life           │
   │ (risk-based          │   │ verification         │
   │ inoculated pack      │   └──────────────────────┘
   │ trials)              │             │
   └──────────────────────┘             ▼
                            ┌──────────────────────┐
                            │ Prepare evidence     │
                            │ dossier              │
                            └──────────────────────┘
                                       │
                                       ▼
                            ┌──────────────────────┐
                            │ Incorporate in the   │
                            │ Food Safety Plan     │
                            └──────────────────────┘
                                       │
                                       ▼
                            ┌──────────────────────┐
                            │ Submit evidence and  │
                            │ Food Safety Plan     │
                            │ for assessment       │
                            └──────────────────────┘
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Challenge study

Where a cycle has to be developed from scratch, a food manufacturer would usually require the services of an HPP expert and a microbiological laboratory with experience in conducting challenge studies. A microbiological challenge study is a useful tool for determining the ability of food to support the growth of spoilage organisms or pathogens. It also plays an important role in the validation of processes that are intended to deliver some degree of lethality against a target organism or a group of target organisms.

The design, implementation and assessment of microbiological challenge studies is complex and it relates to how the product is formulated, manufactured, packaged, distributed, prepared and consumed. Failure to account for specific product and environmental factors in the design of the study could result in inaccurate conclusions.

In a challenge study, pathogens of interest are added to the food product prior to packaging and HPP treatment. The product is then stored and tested for these organisms during its shelf life. The aim of the study is to simulate what can happen to pathogens in a product during processing, distribution and subsequent handling **(Figure 2).**

A more detailed overview of factors to be considered when designing a challenge study can be found in **Table 3**.

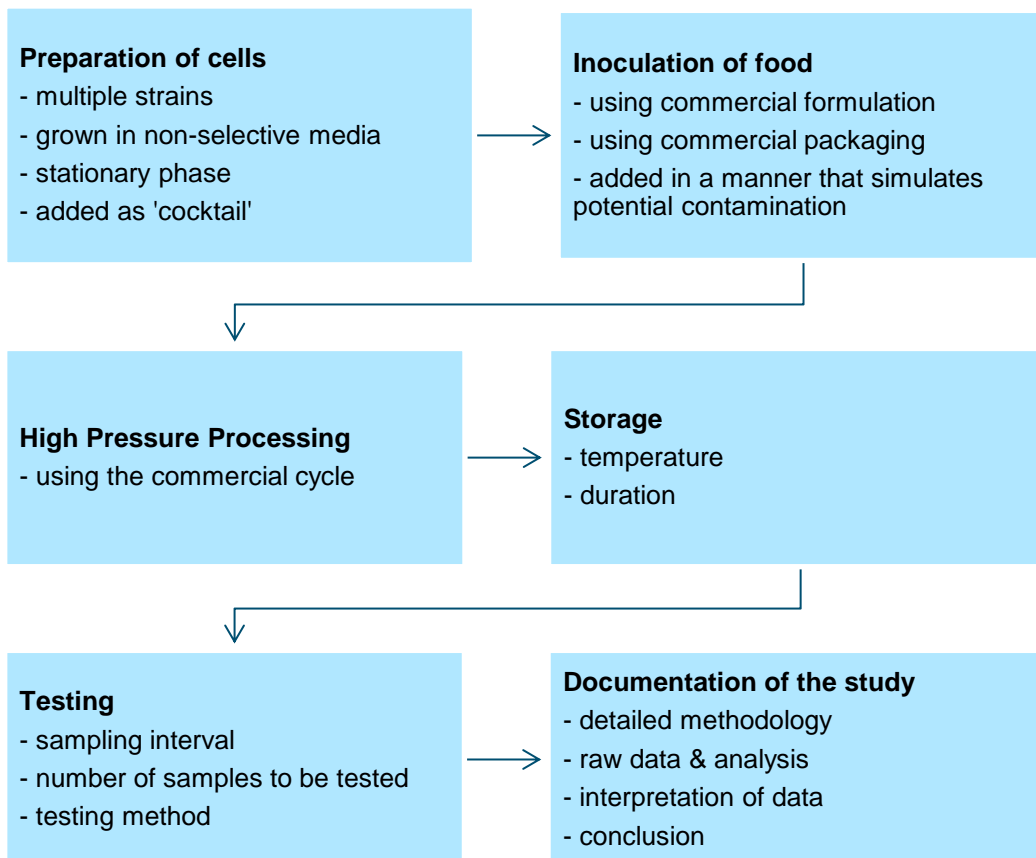**Figure 2. The steps of a challenge study**

Preparation of cells
- multiple strains
- grown in non-selective media
- stationary phase
- added as 'cocktail'

Inoculation of food
- using commercial formulation
- using commercial packaging
- added in a manner that simulates potential contamination

High Pressure Processing
- using the commercial cycle

Storage
- temperature
- duration

Testing
- sampling interval
- number of samples to be tested
- testing method

Documentation of the study
- detailed methodology
- raw data & analysis
- interpretation of data
- conclusion

Table 3. Factors to consider when designing a challenge study

Factors	Comments
Test product	• The product used in the challenge study must have the same formulation and undergo processing steps used during commercial operations (cooking temperature/time, cooling process, slicing, packaging, HP processing parameters etc). • Product packaging for the challenge study should be representative of typical commercial production e.g. packed under vacuum or using modified atmosphere.
Number of studies	• A challenge study must be conducted at least twice using different batches to ensure reproducibility of results.
Pathogens of concern	• Knowledge of the food formulation and history of the food (for example, association with known illness outbreaks and/or evidence of potential growth) is essential when selecting the appropriate challenge pathogens. The type of pathogens to be considered varies depending on the characteristics of the product. • Some pathogens may pose unacceptable risk and be unsuitable for handling in a challenge study. In this case, surrogates should be used. Surrogates are typically non-pathogenic proxies for the pathogen of concern that have similar or more robust survival capabilities under the HPP conditions being studied. Examples of surrogate/pathogen pairs include *Clostridium sporogenes/C. botulinum*; *Listeria innocua/L. monocytogenes;* and non-pathogenic *Escherichia coli/E. coli* O157:H7. The use of surrogates should be limited to only those cases where specific pathogens absolutely cannot be used for product or personnel safety reasons.
Type and number of strains	• A challenge study should include at least three to five strains for each pathogen because there is a wide variation in resistance to pressure between strains of the same bacteria. • It is recommended to use strains isolated from a similar food type, known foodborne outbreak, the food processing environment or from a clinical specimen. • Strains that are more resistant to HPP should be included.
Inoculum levels	• An inoculum composed of multiple strains (i.e. cocktail) is recommended. • High numbers of organisms are typically used e.g. 10^6 to 10^7 CFU/g to demonstrate the extent of reduction in challenge organisms. • Stationary phase cells grown on non-selective media under optimal growth conditions should be used, as they are more resistant to pressure. • It is important to verify the numbers of viable organisms in the inoculum used – both for the inoculum suspension itself and the inoculated food to obtain a zero-time count.

Factors	Comments
Method of inoculation	• The inoculum should be added to food in a manner that realistically simulates potential contamination that might occur during manufacture, preparation, transport or display of the product. • The method of inoculation should not change the critical parameters of the product formulation, for example adding too much liquid to a solid food might change the pH or water activity of the product. • For products packaged under vacuum or in modified atmosphere packaging, it is recommended to inoculate the food prior to packaging to avoid disturbing the gaseous environment.
High Pressure Processing parameters	• Equipment used for the challenge study should provide comparable pressure and temperature against time profile as the commercial equipment that will be used. • The holding time should be the same as for the commercial product.
Duration of the study	• The challenge study should be conducted for at least the intended shelf life of the product. • Ideally products should be held for some period beyond the end of the intended shelf-life to add an additional margin of safety. The additional time may be important for recovery of cells injured by the HPP. For example, 25% longer than the intended shelf-life for products with shelf-life 3-6 months to 50% for products with shelf-life of 7-10 days.
Storage conditions	• A challenge study should be done at the ideal storage temperature and at mildly elevated temperature to mimic the expected temperature range during commercial distribution and storage. • The temperature of the product should be recorded throughout the study. • Samples inoculated with pathogens must be segregated and clearly label to prevent inadvertent consumption.
Sampling intervals	• Samples should be analysed: initially after inoculation (before HPP), after HPP and then five to seven times over the duration of the study. • For long shelf-life products, it may be necessary to have more than seven sampling points. • Depending on the product characteristics, it may be appropriate to test on a more frequent basis early in the study (e.g. daily or multiple times per day) and then reduce the frequency of testing to longer intervals later in the study (e.g. weekly or fortnightly).
Number of samples to be tested	• At least two sample replicates must be tested at each sampling point to account for product variability. This is because the distribution of microorganisms in foods is typically not uniform.

Factors	Comments
Testing method	• Ideally, the entire product sample should be homogenised and used in testing. • The procedure to enumerate the number of surviving cells should be appropriate to detect stressed or injured cells. Direct plating onto selective agar can overestimate the extent of death. Enrichment procedures should be considered to detect stressed or injured bacterial cells during the shelf-life of the product. Sub-lethally injured cells may be able to recover and grow during the shelf-life of the product.
Physical parameters	• It is important to measure relevant physical factors such as pH, water activity (a_w), moisture, salt content and preservative levels as part of the challenge study in parallel with the microbial analysis. • No sensory assessment, other than changes in appearance, should be performed on challenge test samples.
Documentation of the study	• The study should be documented, including a detailed description of the methodology, raw data and analysis, interpretation of data and conclusions. • Interpretation of results should be done by an experienced food technologist or microbiologist. • Once the challenge study is completed, the data should be analysed to see how the pathogens behaved over time. Trend analysis and appropriate graphical plotting of the data will show whether the challenge organisms died, remained stable, or increased in numbers over time. • Data analysis will show whether the proposed HP process can deliver the required level of lethality. Based on this information, adjustments can be made to the process, if necessary, to achieve the desired lethality.

Cultures of pathogenic bacteria should never be introduced into a processing facility. Challenge studies should only be done in an off-site laboratory or research facility.

Table 4 provides guidance on food safety risks that might be considered for products produced using Good Manufacturing Practices (GMP). The manufacturer should still complete a risk assessment for their product.

Table 4. Food safety risks associated with food types

Product	Shelf life	Risks	Equivalence target
Chilled acid food with pH below 4.2	Long	Survival of vegetative cells of pathogenic bacteria	Pasteurisation[2]
Chilled acid food with pH from 4.2 to <4.6	Long	Above risk plus potential growth of vegetative cells of some pathogenic bacteria	Pasteurisation
Low acid chilled foods	0 to 5 days	Above risks	Cook time as recommended for the type of food[3]
Low acid chilled foods	5 to 10 days	Above risks plus *Listeria monocytogenes*	Recommended *Listeria*-safe cooking process[4]
Low acid chilled food	10+ days	Above risks plus cold tolerant *Clostridium botulinum*	Recommended psychrotrophic *Clostridium botulinum* cooking process[4]
Low acid shelf stable foods	Long	Above risks plus conventional *Clostridium botulinum*	Suitable retorting process

[Reference: Hayman et al., 2004; Horn et al., 2019; IFT/FDA, 2001; NACMCF, 2009; Notermans et al., 1993; NZ MPI, 2016]

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[2] For juice, see https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=120.24

[3] For example, see https://www.foodauthority.nsw.gov.au/consumer/food-at-home/cooking-temperatures

[4] For example, see https://www.foodstandards.gov.au/publications/Documents/Safe%20Food%20Australia/Appendix%203%20-%20Limits%20for%20food%20processes.pdf

Shelf-life study

Shelf-life is the period of time which a food maintains its safety and/or quality under reasonably foreseeable conditions of distribution, storage and use. Validating product shelf-life is obtaining and documenting evidence that proves that the shelf-life of a food is accurate and the food will maintain its safety and/or quality until the end of that shelf-life.

In estimating and setting shelf-life, the primary objective should be food safety. All microorganisms have minimum/maximum and optimal requirements for survival and growth in foods. Considering the food characteristics (e.g. food formulation and composition, pH, water activity, antimicrobial substances, temperature of storage, packaging etc) will allow food manufacturers to determine what organisms may survive and grow in their food – particularly those that can cause illness i.e. pathogens – and control the safety of the product during its shelf-life by preventing or minimising survival and growth of specific pathogens.

A shelf-life study is a study in which an un-inoculated food product is stored under the expected conditions of storage and distribution for a period of time. The product is tested at certain intervals to determine the levels of background microflora surviving the processing steps or their changes during the proposed shelf-life.

A shelf-life study should be repeated if there are major changes made to the product formulation, processing or packaging to ensure that the shelf life has not been compromised by the changes.

Table 5. Factors to consider when designing a shelf-life study

Factors	Comments
Test product	• The product used in the shelf-life study must have the same formulation and undergo processing steps used during commercial operations (cooking temperature/time, cooling process, slicing, packaging, HP processing parameters etc). • Product packaging for the shelf-life study should be representative of typical commercial production e.g. packed under vacuum or using modified atmosphere.
Number of studies	• A shelf-life study should be repeated with several batches (at least three) to determine the variability between batches.
High Pressure Processing parameters	• Equipment used for the shelf-life study should provide comparable pressure and temperature against time profile as the commercial equipment that will be used. • Holding time should be the same as for the commercial product.

Factors	Comments
Duration of the study	- Initially, the study should run up to and beyond the target shelf-life. For example, 25% longer than the intended shelf-life for products with shelf-life 3-6 months to 50% for products with shelf-life of 7-10 days. - If, after this time, the microbiological safety criteria are still met, the testing may be continued until they are exceeded. - If a product fails to comply with the microbiological safety criteria before its target shelf-life, the testing should stop. The shelf-life of the product should be shortened or product development and further testing should be undertaken until the desired shelf-life is achieved. - For products with a long shelf-life, 'accelerated' studies may be performed in which the product is exposed to elevated temperatures to speed up the development of spoilage and deterioration and shorten the time that the study takes. This would only be useful if the spoilage patterns remain the same as for the normal storage conditions.
Storage conditions	- A shelf-life study should be done at the ideal storage temperature, at mildly elevated temperature to mimic the expected temperature range during commercial distribution and storage, and at a temperature representing the worst case scenario to mimic poor temperature control. - The temperature of the product should be recorded throughout the study.
Sampling intervals	- Samples should be analysed: before HPP, after HPP and then five to seven times over the duration of the study. - Depending on the product characteristics, it may be appropriate to test on a more frequent basis early in the study (e.g. daily or multiple times per day) and then reduce the frequency of testing to longer intervals later in the study (e.g. weekly or fortnightly).
Number of samples to be tested	At least two sample replicates must be tested at each sampling point to account for product variability. This is because the distribution of microorganisms in foods is typically not uniform.
Microorganisms to be tested	- The type of pathogens to be tested varies depending on the characteristics of the product. Knowledge of the food formulation and history of the food (for example, association with known illness outbreaks and/or evidence of potential growth) is essential when considering which pathogens are to be tested. - In addition to pathogens, general microbiological tests such as a standard plate count (SPC) and spoilage organisms typical for the product (e.g. lactic acid bacteria or yeast and mould) should be considered. The presence/absence of spoilage bacteria cannot be used as an indicator of safety, but it can help in determining the appropriate shelf life of the product.

Factors	Comments
Testing method	• Ideally, the entire product sample should be homogenised and used in testing. • The procedure to enumerate the number of surviving cells should be appropriate to detect stressed or injured cells. Direct plating onto selective agar can overestimate the extent of death. Enrichment procedures should be considered so stressed or injured bacterial cells can be detected during the shelf life of the product. Sub-lethally injured cells may be able to recover and grow during the shelf life of the product.
Physical parameters	• It may be important to measure physical factors such as pH, water activity, moisture, salt content and organoleptic acceptability as part of the shelf-life study in parallel with the microbial analysis.
Applying a margin of safety	• It is recommended that a margin of safety is applied to the shelf-life of the food. Applying a margin of safety will reduce the labelled shelf-life of the food to a shorter time interval. However, this allows the food manufacturer to take account of reasonably foreseeable conditions which may affect product safety. • The rationale behind an applied margin of safety should be documented.
Documentation of the study	• The study should be documented, including detailed overview of the methodology, raw data and analysis, interpretation of data and conclusions. • Interpretation of results should be done by an experienced food technologist or microbiologist. • The results of shelf-life studies reflect the natural contamination of food products. However, food manufacturers should be aware that 'absence' results are not proof that the food from which the samples came is not contaminated, due to the low probability of detecting pathogens in a contaminated batch, the low number of microbial cells initially present and their uneven distribution in the food. • If a product failed to comply with the microbiological safety criteria before its target shelf-life, the testing should stop. The manufacturer should shorten the shelf-life or continue with product development and further testing until the desired shelf-life is achieved.

[References: FSAI, 2019; NSW Food Authority, 2010; NZ MPI, 2016]

Shared responsibility for subcontract[5] HPP processing

The contract of manufacture must be correctly defined, agreed and controlled in order to avoid misunderstandings which could result in a product or work of unsatisfactory quality. There must be a written agreement between the manufacturer and the processor which clearly establishes the duties of each party.

The manufacturer is responsible for assessing the competence of the processor to successfully carry out the work required. The manufacturer should ensure that the processor is fully aware of any problems associated with the product which might pose a food safety hazard. The manufacturer should ensure that all processed products delivered to the processor have been released by an authorised person. A manufacturing record should log transport to the processor, storage at the processor, processing times and cycle numbers and the return transport.

The processor must have:

- adequate premises and equipment

- knowledge and experience; and,

- competent personnel to satisfactorily carry out the work ordered by the manufacturer.

- Records of the relevant cycles should:

    - be available to the manufacturer,

    - have a clear link between the manufacturers batch number and the processors cycle number(s); and

    - ensure that any records relevant to assessing the quality of a product in the event of complaints or a suspected defect are accessible and specified in the complaints handling or recall procedures of the manufacturer.

Technical aspects of the agreement should be drawn up by competent persons suitably knowledgeable in food manufacturing and GMP (Good Manufacturing Practice). All cycle parameters for processing must be included. The agreement should specify the way in which the authorised person releasing the batch for sale ensures that each batch has been processed and checked for compliance with the requirements for food safety.

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[5] Concepts derived from PIC/S Guide to GMP http://www.tga.gov.au/publication/manufacturing-principles-medicinal-products#partl

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