# 6
# High Pressure Processing

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## 6.1
## Introduction

In recent years there has been a consumer-driven trend towards better tasting and additive-free foods with a longer shelf life. Some of the scientific solutions, such as genetic modification and gamma irradiation, have met with consumer resistance. However, high pressure processing (HPP) is one technology that has the potential to fulfil both consumer and scientific requirements.

The use of HPP has, for over 50 years, found applications in diverse, nonfood industries. For example, HPP has made a significant contribution to aircraft safety and reliability where it is currently used to treat turbine blades to eliminate minute flaws in their structure. These microscopic imperfections can lead to cracks and catastrophic failure in highly stressed aero engines. HPP now gives a several-fold increase in component reliability, leading to a longer engine life and, ultimately, lower flight cost to the public.

Although the effect of high pressure on food has been known for about 100 years [1], the technology has remained within the R&D environment until recent times (see Table 6.1).

Early R&D pressure vessels were generally regarded as unreliable, costly and had a very small usable vessel volume. Hence the prospect of 'scaling up' the R&D design to a full production system was commercially difficult.

Equally the food industry, particularly in Europe, has mainly focussed upon cost reductions, restructuring and other programmes, often to the neglect of emerging technologies.

A situation had to develop where a need was created that could not be fully satisfied by current technology. This occurred on a small scale in Japan with the desire to produce delicate, fresh, quality, long shelf life fruit-based products for a niche market. The HPP products were, and are today, produced on small machines at a premium price to satisfy that particular market need.

*Food Processing Handbook*. Edited by James G. Brennan

ISBN: 3-527-30719-2

**Table 6.1** The history of HPP for food products.

| Year | Event(s) |
|---|---|
| 1895 | Royer (France) used high pressure to kill bacteria experimentally |
| 1899 | Hite (USA) used high pressure for food preservation |
| 1980s | Japan started producing high-pressure jams and fruit products |
| 1990s | Avomex (USA) began to produce high-pressure guacamole from avocados with a fresh taste and extended shelf-life |
| 2000 | Mainland Europe began producing and marketing fresh fruit juices (mainly citrus) and delicatessen-style cooked meats. High-pressure self-shucking oysters, poultry products, fruit juices and other products were marketed in the USA |
| 2001 | HPP fruit pieces given approval for sale in the UK. Launch of the first HPP fruit juices in the UK |

In the USA, issues relating to food poisoning outbreaks, notably with unprocessed foods such as fruit juices and oysters led to action by the Food and Drug Administration. They attempted to regulate the situation by requiring a significant reduction in the natural microbial levels of a fresh food. Those foods which did not achieve this reduction were required to be labelled with a warning notice. This meant that producers of freshly squeezed orange juice, for example, marketing their product as wholesome and healthy were faced with a dilemma. If they reverted to the established method of reducing microbial counts, i.e. by heating the juice, then the product became of inferior quality in all respects. If they continued to sell the juice 'untreated', they faced market challenges with a product essentially labelled to say that it could be harmful to the consumer.

Another example involved the producers of avocado-based products who knew that there was a huge demand for fresh quality product. However, the very short shelf life of the product coupled with the opportunity for harmful microbes to flourish with time effectively restricted this market opportunity.

Oysters are another case in point. They have traditionally been a niche market with romantic connotations but food poisoning outbreaks associated with *Vibrio* spp in the oyster population caused serious illness and even death, which effectively devastated the market.

In all these cases, the producers needed to look for a process that reduced the numbers of spoilage or harmful microorganisms but left the food in its natural, fresh state. HPP has been shown to be successful in achieving this aim. HPP guacamole is now produced in Mexico and sells very successfully in the US, while HPP oysters are being produced in Louisiana.

High pressure processing of food is the application of high pressure to a food product in an isostatic manner. This implies that all atoms and molecules in the food are subjected to the same pressure at exactly the same time, unlike heat processing where temperature gradients are established.

The second key feature of high pressure processing, arising from Le Chatelier's principle, indicates that any phenomenon that results in a volume decrease is enhanced by an increase in pressure. Thus, hydrogen bond formation is favoured by the application of pressure while some of the other weak linkages found in proteins are destabilised. However, covalent bonds are unaffected.

For food applications, 'high pressure' can be generally considered to be up to 600 MPa for most food products (600 MPa = 6000 bar = 6000 atmospheres = 87 000 psi). With increasing pressure, the food reduces in overall size in proportion to the pressure applied but retains its original shape. Hence, a delicate food such as a grape can be subjected to 600 MPa of isostatic pressure and emerge apparently unchanged although the different rates and extents of compressibility of the gaseous (air), liquid and solid phases may lead to some physical damage. Pressure kills microorganisms, including pathogens and spoilage organisms, leading to a high quality food with a significantly longer and safer chilled shelf life. The conventional way to do this is to process the food by heat, but this may also damage the organoleptic and visual quality of the food, whereas HPP does not.

In summary, the advantages of HPP are:

- the retention of fresh taste and texture in products, such as fruit juices, shellfish, cooked meats, dips, sauces and guacamole;
- the increase in microbiological safety and shelf-life by inactivation of pathogens and spoilage organisms and also some enzymes;
- the production of novel foods, for example gelled products and modification of the properties of existing foods, e.g. milk with improved foaming properties;
- the savings in labour which some HPP processes bring about, compared to more traditional techniques, e.g. self-shucking oysters;
- low energy consumption;
- minimal heat input, thus retaining fresh-like quality in many foods;
- minimal effluent;
- uniform isostatic pressure and adiabatic temperature distribution throughout the product, unlike thermal processing.

The current disadvantages of HPP are:

- Initial outlay on equipment, which remains high (in the region of $1.8 million for a typical production system). However, numerous companies have justified this cost by offsetting it against new product opportunities, supported by the relatively low running cost of the HPP equipment.
- Uncertainty introduced by the European 'novel foods' directive (May 1997) about the ease of gaining approval for new products has made some companies reluctant to apply. Better understanding of the regulations is making this easier and the fact that approval has now been granted for some HPP products has renewed confidence in the marketplace. Furthermore, many HPP products are in fact exempt from the regulations as they are 'substantially equivalent' to nonHPP products on the market.

High pressure has many effects on the properties of the food ingredients themselves as well as on the spoilage organisms, food poisoning organisms and enzymes. In addition to preserving a fresher taste than most other processing technologies, HPP can affect the texture of foods such as cheese and the foaming properties of milk. This chapter looks at how these effects can make HPP foods more marketable, less labour-intensive to produce and generally more attractive to the producer, retailer and consumer alike.

## 6.2
## Effect of High Pressure on Microorganisms

The lethal effect of high pressure on bacteria is due to a number of different processes taking place simultaneously. In particular, damage to the cell membrane and inactivation of key enzymes, including those involved in DNA replication and transcription are thought to play a key role in inactivation [2]. The cell membranes are generally regarded as a primary target for damage by pressure [3]. The membranes consist of a bilayer of phospholipids with a hydrophilic outer surface (composed of fatty acids) and an inner hydrophobic surface (composed of glycerol). Pressure causes a reduction in the volume of the membrane bilayers and the cross-sectional area per phospholipid molecule [4]. This change affects the permeability of the membrane, which can result in cell damage or death. The extent of the pressure inactivation achieved depends on a number of interacting factors as discussed below. These factors have to be considered when designing process conditions to ensure the microbiological safety and quality of HPP foods.

### 6.2.1
### Bacterial Spores

Bacterial spores can be extremely resistant to high pressure, just as they are resistant to other physical treatments, such as heat and irradiation. However, low/moderate pressures are more effective than higher pressures. It was concluded that inactivation of spores is a two-step process involving pressure-induced germination [5]. This has led to the suggestion that spores could be killed by applying pressure in two stages. The first pressure treatment would germinate or activate the spores while the second treatment would kill the germinated spores [6].

Temperature has a profound effect on pressure-induced germination. In general, the initiation of germination increases with increasing temperature over a specified temperature range [7, 8]. Applying a heat treatment before or after pressurisation can also enhance spore kill [9]. In recent years the application of high temperatures along with high pressures has shown promise as a way of producing shelf-stable foods [10]. It is claimed these products have superior sensory and nutritional quality compared to those produced by conventional thermal processing [11].

### 6.2.2 Vegetative Bacteria

Gram positive bacteria, especially cocci such as *Staphylococccus aureus*, tend to be more pressure-resistant than Gram negative rods, such as *Salmonella* spp. However, there are exceptions to this general rule. For example, certain strains of *Escherichia coli* O157:H7 are relatively resistant to pressure [12].

One proposed explanation for the difference in pressure response between Gram positive and Gram negative bacteria is that the more complex cell membrane structure of Gram negative bacteria makes them more susceptible to environmental changes brought about by pressure [13]. The cell walls of Gram negative bacteria consist of an inner and outer membrane with a thin layer of peptidoglycan sandwiched between. The cell walls of Gram positive bacteria are less complex, with only an inner plasma membrane and a thick peptidoglycan outer layer, which can constitute up to 90% of the cell wall.

### 6.2.3 Yeasts and Moulds

Yeasts are generally not associated with foodborne disease. They are, however, important in food spoilage due to their ability to grow in low water activity ($a_w$) products and to tolerate relatively high concentrations of organic acid preservatives. Yeasts are thought to be relatively sensitive to pressure, although ascospores are more resistant than vegetative bacteria. Unlike yeasts, certain moulds are toxigenic and may present a safety problem in foods. There is relatively little information on the pressure sensitivity of these moulds. In one study, a range of moulds including *Byssochlamys nivea*, *B. fulva*, *Eupenicillium* sp. and *Paecilomyces* sp. were exposed to a range of pressures (300–800 MPa) in combination with a range of temperatures (10–70 °C) [14]. The vegetative forms were inactivated within a few minutes using 300 MPa at 25 °C. However, ascospores were more resistant. A treatment of 800 MPa at 70 °C for 10 min was required to reduce a starting inoculum of $<10^6$ ascospores ml$^{-1}$ of *B. nivea* to undetectable levels. A treatment of 600 MPa at 10 °C for 10 min was sufficient to reduce a starting inoculum of $10^7$ ascospores ml$^{-1}$ of *Eupenicillium* to undetectable levels.

Information on the effect of pressure on preformed mycotoxins is limited. Brâna [15] reported that patulin, a mycotoxin produced by several species of *Aspergillus*, *Penicillium* and *Byssochlamys*, could be degraded by pressure. The patulin content in apple juice decreased by 42%, 53% and 62% after 1 h treatment at 300, 500, and 800 MPa, respectively, at 20 °C.

### 6.2.4
### Viruses

There is relatively little information on high-pressure inactivation of viruses, compared to the information available on vegetative bacteria. However, some reports suggest that Polio virus suspended in tissue culture medium is relatively resistant to pressure, with 450 MPa for 5 minutes at 21 °C giving no reduction in plaque-forming units [16]. However, Feline calicivirus, a Norwalk-virus surrogate, hepatitis A [16] and human rotavirus [17] were more pressure sensitive. These results would suggest that the pressure treatments necessary to kill vegetative bacterial pathogens would also be sufficient to cause significant inactivation of human virus particles.

There is also some evidence emerging that pressure combined with heat may have some effect on prions, but further work still needs to be completed in this area [18].

### 6.2.5
### Strain Variation Within a Species

Pressure resistance varies not only between species but also within a species. For example, Linton et al. [19] reported a 4 log difference in resistance of pathogenic *E. coli* strains to treatment at 600 MPa for 15 min at 20 °C. Alpas et al. [20] also demonstrated variability in pressure resistance within strains of *Listeria monocytogenes*, *Salmonella* spp, *S. aureus* and *E. coli* O157:H7. However, they found that the range of pressure differences within a species decreased when the temperature during the pressure treatment was increased from 25 °C to 50 °C. This finding would be helpful in a commercial situation, where the combination of pressure and mild heat could be used to enhance the lethal effect of the treatment.

### 6.2.6
### Stage of Growth of Microorganisms

Vegetative bacteria tend to be most sensitive to pressure when treated in the exponential phase of growth and most resistant in the stationary phase of growth [21]. When bacteria enter the stationary phase they can synthesise new proteins that protect the cells against a variety of adverse conditions, such as high temperature, high salt concentrations and oxidative stress. It is not known if these proteins can also protect bacteria against high pressure but this may explain the increase in resistance in the stationary phase.

### 6.2.7
### Magnitude and Duration of the Pressure Treatment

Pressure is similar to heat, in that there is a threshold below which no inactivation occurs. This threshold varies depending on the microorganism. Above the threshold, the lethal effect of the process tends to increase as the pressure increases but not necessarily as the time increases. This can lead to inactivation curves with very definite 'tails'. These nonlinear inactivation curves have been reported by many workers [12, 13, 22]. Metrick et al. [23] reported tailing effects for S. Typhimurium and S. Senftenberg. When the resistant tail populations were isolated, grown and again exposed to pressure, there was no significant difference in the pressure resistance between them and the original cultures.

Several theories to explain the tailing effect have been proposed. The phenomenon may be independent of the mechanisms of inactivation but be due to population heterogeneity such as clumping or genetic variation. Alternatively, tailing may be a normal feature of the mechanism of resistance (adaptation and recovery) [24]. In practice, the nonlogarithmic inactivation curves make it difficult to calculate accurate $D$ and $z$ values.

### 6.2.8
### Effect of Temperature on Pressure Resistance

The temperature during pressure treatment can have a significant effect on microbial resistance. As a general rule, pressure treatments carried out below 20 °C or above the growth range for the microorganism result in greater inactivation. Takahaski et al. [25] reported that the pressure inactivation of S. Bareilly, *V. parahaemolyticus* and *S. aureus* in 2 mM sodium phosphate buffer, pH 7.0, was greater at –20 °C than at +20 °C. The simultaneous application of pressure (up to 700 MPa) with mild heating (up to 60 °C) was more lethal than either treatment alone in inactivating pathogens such as *E. coli* O157:H7 and *S. aureus* in milk and poultry meat [26].

### 6.2.9
### Substrate

The composition of the substrate can significantly affect the response of microorganisms to pressure and there can be significant differences in the levels of kill achieved with the same organism on different substrates. For example, *E. coli* O157:H7 treated under the same conditions of 700 MPa for 30 min at 20 °C resulted in a 6 log reduction in numbers in phosphate-buffered saline, a 4 log reduction in poultry meat and a <2 log reduction in UHT milk [12]. The reasons for these effects are not clear but it may be that certain food constituents like proteins and carbohydrates can have a protective effect on the bacteria and may even allow damaged cells to recover more readily.

There is evidence that the $a_w$ and pH of foods can significantly affect the inactivation of microorganisms by pressure. A reduction in $a_w$ appears to protect microorganisms from pressure inactivation. Oxen et al. [27] reported that in sucrose ($a_w \sim 0.98$), the pressure inactivation (at 200–400 MPa) of *Rhodotorula rubra* was independent of pH at pH 3.0–8.0. However, at $a_w$ values below 0.94 there was a protective effect, irrespective of solute (glucose, sucrose, fructose or sodium chloride). Most microorganisms are more susceptible to pressure at lower pH values and the survival of pressure-damaged cells is less in acidic environments. This can be of commercial value, such as in the pressure treatment of fruit juices where, in the high acid conditions, pathogens, such as *E. coli* O157:H7, which may survive the initial pressure treatment will die within a relatively short time during cold storage [28].

### 6.2.10
### Combination Treatments Involving Pressure

High pressure processing can be successfully used in combination with other techniques to enhance its preservative action and/or reduce the severity of one or all of the treatments. It is possible that this hurdle approach will be used in many of the commercial applications of HPP technology. Beneficial combination treatments include the use of pressure (usually <15 MPa) with carbon dioxide to improve the microbial quality of chicken, egg yolk, shrimp, orange juice [29] and fermented vegetables [30]. Pressure combined with irradiation has been proposed to improve the microbial quality of lamb meat [31] and poultry meat [32]. Pressure combined with heat and ultrasound has been successful in inactivating *B. subtilis* spores [33]. Various antimicrobial compounds have been used in combination with pressure. Nisin and pressure caused a significant reduction in numbers of *B. coagulans* spores [7]. Other antimicrobials such as pediocin AcH [34] and the monoterpenes [35] have also been combined with pressure treatment to enhance microbial inactivation with mixed success. The commercial value of these combinations still has to be assessed, given that one of the advantages of HPP is that it can be regarded as a 'natural' minimal processing technology.

### 6.2.11
### Effect of High Pressure on the Microbiological Quality of Foods

High pressure processing is already used commercially to enhance the microbiological quality of certain food products. A number of potential applications have also been reported in the scientific literature and there is a significant amount of ongoing research in this area. Fruit products have been most extensively studied. These products are acidic so, in terms of their microbiology, pathogens are generally not so important but spoilage microorganisms, particularly yeasts and moulds are of concern. The limiting factor for shelf life of such products is often the action of enzymes, particularly those which can cause browning, although the problem may be at least partially overcome by blanching or adding an oxygen scavenger such as ascorbic acid. Most fruit products are given a treatment of around 400 MPa for up to a few minutes. This can give a shelf life of up to several months provided the products are stored at 4 °C [36]. Pressure treatment of vegetables tends to be less successful due to their higher pH and the potential presence of spores, which can be very pressure-resistant. In addition, the quality of some vegetables deteriorates as a result of pressure processing, which can also be a limiting factor [37]. However, it should be noted that one of the most successful commercial products available to date is HPP guacamole. Research has shown that clostridial spores cannot outgrow in this product and it can have a shelf life of around 1 month at 4 °C without modification of colour, texture or taste.

The use of HPP to improve the microbiological quality of meat, fish and dairy products has been investigated by a number of workers. These products tend to have a more neutral pH and provide a rich growth medium for most microorganisms, with pathogens being of particular concern. The need to ensure microbiological safety is one of the reasons why, to date, there are relatively few commercial applications of HPP meat and dairy products available. However, research has shown that pressure processing can be successful, in terms of improving microbiological safety and quality, for the treatment of pork [38], minced beef [39], duck Foie Gras (liver pate) [40], fish [41], ovine milk [42] and liquid whole egg [43]. In many cases, the authors also comment on the ability of the pressure treatment to maintain or enhance sensory, nutritional or functional quality compared to conventional processing methods. In all cases, the optimum treatment conditions need to be carefully defined and thoroughly tested to ensure food safety is not compromised. For example, this may include extensive inoculation studies, under standardised conditions and using the most resistant strains of pathogens to ensure that the product will be microbiologically safe during its shelf life.

## 6.3 Ingredient Functionality

It is now well established [44, 45] that changes in protein structure and functionality occur during high pressure treatment. Studies carried out on volume changes in proteins, have shown that the main targets of pressure are hydrophobic and electrostatic interactions [46, 47]. Hydrogen bonding, which stabilizes the $\alpha$ helical and $\beta$ pleated sheet forms of proteins, is almost pressure-insensitive. At the pressures used in food processing, covalent bonds are unaffected [48] but at pressures of about 300 MPa sulfhydril groups may oxidise to S-S bonds in the presence of oxygen.

It is readily apparent from the above that pressure and temperature do not normally work synergistically with respect to protein unfolding, since the weak linkages that are most labile to heat, i.e. hydrogen bonds, are stabilised or only marginally affected by pressure whilst the bonds most labile to pressure (electrostatic and hydrophobic interactions) are far less temperature-sensitive. How-

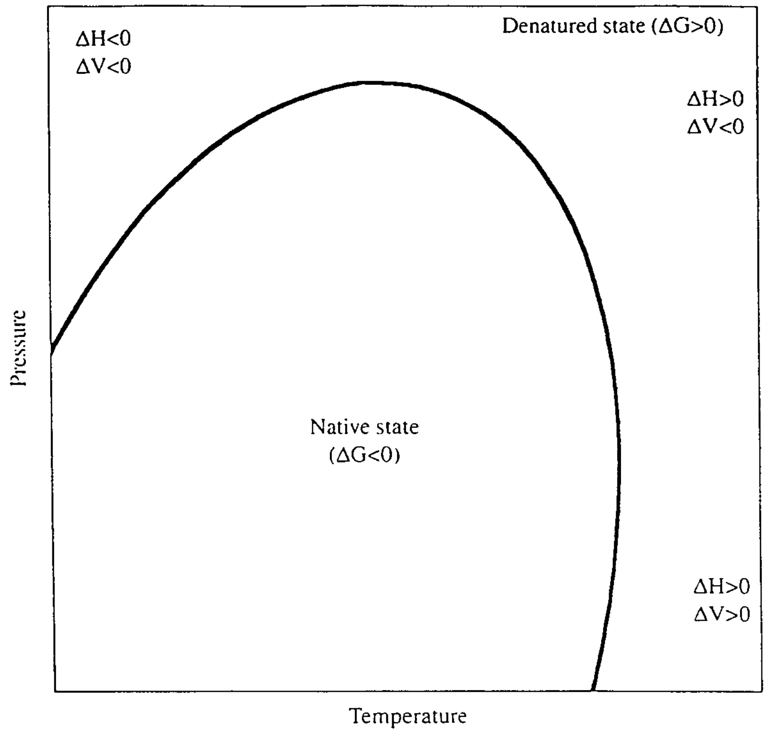


**Fig. 6.1** Phase diagram for native/denatured proteins. Most proteins denature in the range 40–80 °C.

ever, in the presence of oxygen, both increasing temperature and pressure encourage disulphide bond formation and/or interchange. Such considerations help to explain the effects of pressure and temperature on the phase diagram for most, if not all, native/denatured protein systems (see Fig. 6.1) where for example it is seen that, up to a certain temperature, pressure stabilises the protein against heat denaturation. On removal from the denaturing environments, the proteins will, if free to do so, refold to a native-like structure. However, because the pressure/temperature dependence of many weak linkages differ so markedly, on pressure release the 'reformed' structures often differ from that of the native structure, i.e. conformational drift occurs. Thus the amount of $\alpha$ helix, $\beta$ sheet and random coil present will vary, as also will such properties as surface hydrophobicity and charge. As these 'renatured' pressure-treated proteins yield such different structures, it is not surprising that they exhibit marked differences in behaviour to the native protein or its heat-denatured product. Thus their functional properties vary.

## 6.4 Enzyme Activity

Enzymes, being proteins, will at sufficiently high pressure undergo conformational changes and thus lose activity. Most enzymes of importance in food deterioration are relatively resistant to pressure and complete inactivation is difficult to achieve. Thus polyphenoloxidases, the enzymes responsible for browning in fruits and vegetables, require pressures of 800 MPa, at room temperature, or more to bring about complete inactivation. The degree of inactivation is usually dependent on pressure, temperature and time and, not unnaturally, pH is a further very important factor [49].

A recent review by Ludikhuyze et al. [50] has summarised present knowledge regarding the combined effects of pressure and temperature on those enzymes that are related to the quality of fruits and vegetables. The enzymes of importance include the polyphenoloxidases, pectin methylesterases, which induce cloud loss and consistency changes, and lipoxygenases which are responsible for the development of off flavours, loss of essential fatty acids and colour changes. These authors discuss how complete characterisation of the pressure/temperature phase diagram for inactivation of these enzymes has been achieved and also suggest how this information could be useful in generating integrated kinetic information with regard to process engineering associated with HPP treatment. Interestingly, these authors found that, for most of the enzymes so far studied, the reaction kinetics were first order, the only exception being pectin methylesterase which only gave fractional conversion and thus the kinetics were difficult to resolve. As discussed in Section 6.3, these studies showed that most enzymes (proteins) have maximum stability to pressure at temperatures around 25–40 °C.

In these model systems, the loss of activity is invariably due to a change in the conformation of the protein, i.e. unfolding/denaturation, which tends to be irreversible. However, although the loss of enzyme activity in the majority of cases is associated with major structural changes, this is not always the case. For example, the secondary and tertiary structure of papain is little affected by pressures up to 800 MPa [51], but a significant loss of activity is observed on treatment at these pressures. This loss in activity can be largely inhibited by applying pressure in the absence of oxygen, since treatment at 800 MPa in air causes a loss in activity of about 41%, but only 23% of the initial activity is lost on pressure treatment after flushing with nitrogen; and, after flushing with oxygen, the loss in activity is 78% at 800 MPa. Gomes et al. [51] suggested that, in this case, the loss in activity is related to specific thiol oxidation at the active site. The active site in papain contains both a cysteine group and a histidine group and, at pH 7, they exist as a relatively stable $S^{-}$-$N^{+}$ ion pair. In an aqueous environment, pressure causes this linkage to rupture due to the associated decrease in volume due to electrostriction of the separated charges. However, steric considerations mean the $S^{-}$ ion can not form a disulphide linkage and thus, in the presence of oxygen, the ion oxidises to the stable $SO_3^{-}$ [52].

Since many enzymes are relatively difficult to denature, it is not surprising that, when whole foods are subjected to pressure, the effects on enzyme activity are difficult to predict. Thus many fruits and vegetables, when subjected to pressure, undergo considerable browning since at pressures below that necessary to inactivate the enzyme some change occurs which makes the substrate more available [53]. For this reason, combined with the cost of the process, high pressure is an effective means of preventing enzymic browning only if applied to the food with appropriate control. Although the application of high pressure may well accelerate the activity of polyphenoloxidase (PPO) in some fruits and vegetables, it can be controlled and thus in the commercial manufacture of avocado paste (guacamole) treatment at 500 MPa for a few minutes is adequate to extend the colour shelf life of the product so that it has a shelf life at chill temperatures of 8 weeks compared to a few hours under the same conditions if not subjected to pressure. From the previous discussion, it is apparent that 500 MPa does not fully inactivate PPO but it brings about sufficient decrease in activity to permit the extended shelf life. However, where modification of enzymic activity is required, pressure treatment may be of benefit as in optimising protease activity in meat and fish products [54] or in modifying the systems that affect meat colour stability. For example, Cheah and Ledward [55, 56] have shown that subjecting fresh beef to pressures of only 70–100 MPa leads to a significant increase in colour stability due to some, as yet unidentified, modification of an enzyme-based system that causes rapid oxidisation of the bright red oxymyoglobin to the brown oxidised metmyoglobin.

Enzymes, as being responsible for many colour changes in fruit, vegetables and meat systems, are also intimately related to flavour development in fruits and vegetables. Thus, lipoxygenase plays an important role in the genesis of volatiles [57], as this enzyme degrades linoleic and linolenic acids to volatiles such as hexanal and *cis*-3-hexenal. The latter compound transforms to *trans*-2-hexenal which is more stable. These compounds are thought to be the major volatile compounds contributing to the fresh flavour of blended tomatoes [58]. Tangwongchai et al. [59] reported that pressures of 600 MPa led to a complete and irreversible loss of lipoxygenase activity in cherry tomatoes when treated at ambient temperature. This loss of enzyme activity resulted in flavour differences between the pressure processed tomato and the fresh product. Compared to unpressurised tomatoes, treatment at 600 MPa gave significantly reduced levels of hexanal, *cis*-3-hexenal and *trans*-2-hexenal, all of which are important contributors to fresh tomato flavours. It is well established that high pressure can very satisfactorily maintain the flavour quality of fruit juices, as well as their colour quality, but obviously with regard to the fresh fruit differences become apparent and it is likely therefore that the technology will not be of benefit for some whole fruits, although its use for fruit juices cannot be disputed.

As well as being involved in flavour development in fruits and vegetables, enzymes are intimately involved in the textural changes that take place during growth and ripening. The enzymes primarily responsible are believed to be polygalacturonase and pectin methylesterase. Tangwongchai et al. [60] showed that, in whole cherry tomatoes, these two enzymes are affected very differently by pressure. Although a sample of purified commercial pectin methylesterase was partially inactivated at all pressures above 200 MPa, irrespective of pH, in whole cherry tomatoes no significant inactivation was seen even after treatment at 600 MPa, presumably because other components in the tomato offered protection, or the isoenzymes were different. Polygalacturonase was more susceptible to pressure, being almost totally inactivated after treatment at 500 MPa. It is interesting to note that these authors observed, both visually and by microscopy, that whole cherry tomatoes showed increasing textural damage with increasing pressures up to about 400 MPa. However at higher pressures (500–600 MPa) there was less apparent damage than that caused by treatment at the lower pressures, the tomatoes appearing more like the untreated samples. These authors concluded that the textural changes in tomato induced by pressure involve at least two related phenomena. Initially, damage is caused by the greater compressibility of the gaseous phase (air) compared to the liquid and solid components, giving rise to a compact structure which on pressure release is damaged as the air rapidly expands, leading to increases in membrane permeability. This permits egress of water and the damage also enables enzymic action to increase, causing further cell damage and softening. The major enzyme involved in the further softening is polygalacturonase (which is inactivated above 500 MPa) and not pectin methylesterase (which in the whole fruit is barotolerant). Thus, at pressures above 500 MPa, less damage to the texture is seen.

From the brief overview above it is apparent that high pressure has very significant effects on the quality of many foods, especially fruits and vegetables, if enzymes are in any way involved in the development of colour, flavour or texture.

Although to date pressure has largely been concerned with the preservation of quality, either by inhibiting bacteria or inactivating enzymes, it does offer potential as a processing aid in assisting reactions which are pressure-sensitive. An example that may have commercial application is that moderate pressures (300–600 MPa) cause significant increases in the activity of the amylases in wheat and barley flour, because the pressure-induced gelatinisation of the starch makes it, the starch, more susceptible to enzymic attack [61]. Higher pressures lead to significant decreases in activity due to unfolding and aggregation of the enzymes. This technology thus has potential in producing glucose syrups from starch by a more energy-efficient process than heat.

The effects of pressure on enzyme activity suggest that it may well be a very effective processing tool for some industrial applications in the future.

## 6.5 Foaming and Emulsification

The structural changes undergone by a protein will affect its functionality and, for example, pressure-treated $\beta$-lactoglobin, at 0.01% concentration has significantly improved foaming ability compared to its native counterpart (see Fig. 6.2). How-

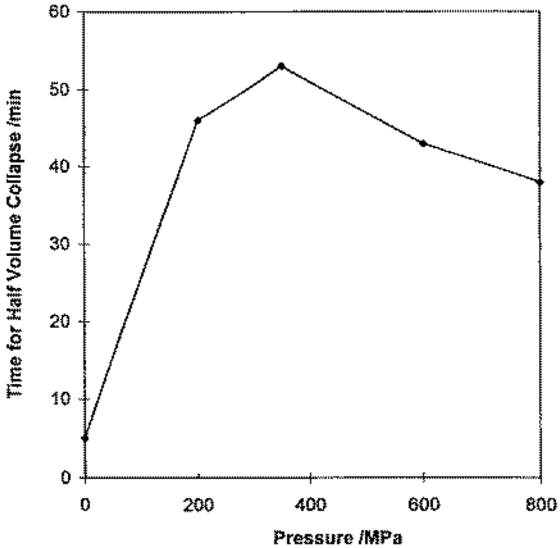


**Fig. 6.2** Effects of high HPP treatment on the foam stability of $\beta$-lactoglobulin at pH 7.0. The time for half volume collapse is plotted as a function of pressure applied for 20 min; from Galazka et al. [62].

ever, if pressure is applied to a relatively concentrated solution of $\beta$-lactoglobin (0.4%), disulphide bond formation may lead to significant aggregation so that it is less useful as a functional ingredient. If disulphide bond-induced aggregation does occur on pressure treatment, then only dilute solutions should be treated, to avoid loss of functionality due to increased size. In addition, other factors that aid aggregation or disulphide bond formation, such as pressures above that necessary to cause unfolding, extended treatment times, alkaline pH and the presence of oxygen, should be avoided so as to optimise functionality.

In addition to $\beta$-lactoglobin, many studies have been reported on the effect of high pressure on the emulsifying and foaming properties of other water-soluble proteins, including ovalbumin, vegetable proteins such as soy and pea and casein. One advantage of pressure treatment on proteins to improve or modify their functional properties is that the process is invariably easier to control than thermal processing, where the effects are rather drastic and less easy to control. For example, model emulsions prepared with high pressure treated (<600 MPa) protein after homogenisation show that pressurisation induced extensive droplet flocculation which increased with protein concentration and severity of treatment [63]. However, it was also noted that moderate thermal processing (80 °C for 5 min) had a far greater effect than pressure treatment at 800 MPa for 40 min on the state of flocculation of ovalbumin-coated emulsion droplets. The increase in emulsion viscosity is due to the formation of a network from the aggregated dispersed oil droplets and denatured polymers in aqueous solution.

The level of pressure-induced modification can be controlled more efficiently by altering the intensity of high pressure treatment rather than by controlling the temperature in thermal processing. Thus, HPP can be viewed as a novel way of manipulating the microstructure of proteins such as ovalbumin while maintaining the nutritional value and natural flavour of such compounds.

As with ovalbumin, emulsions made with pressure treated 11S soy protein were found to have poorer emulsifying and stabilising ability with respect to initial droplet size and creaming behaviour than the native protein. This is probably due to the enhanced association of subunits and/or aggregation induced by the formation of intermolecular disulphide bridges via a SH/-S-S interchange. As with ovalbumin, moderate heat treatment (80 °C for 2 min) had a far greater effect than high pressure treatment on the changes in emulsion stability and droplet size distribution.

Though only in its infancy, the ability of HPP to modify the structure and surface hydrophobicity of a protein does suggest that, as well as a preservation technique, HPP may well have commercial/industrial application for modifying the functional properties of potential emulsifiers and foaming agents.

## 6.6 Gelation

At sufficiently high concentrations and at the appropriate pH many proteins, especially if they have potential disulphide bond forming abilities, will gel or precipitate, but the texture of the gels formed will be markedly different to their heat-set counterparts. Such pressure-set gels will normally contain a relatively high concentration of hydrogen-bonded structure(s) and thus will melt or partially melt on heating. In addition, they will be less able to hold water, i.e. they will synerese and be much softer in texture and 'glossier' in appearance. For example, myoglobin (a protein with no amino acids containing sulphur) will unfold at sufficiently high pressure on pressure release and then normally revert back to a soluble monomer or dimer; but at its isoelectric point (pH 6.9) it will precipitate. However, unlike the precipitate formed on heat denaturation, it will be relatively unstable and, because it is primarily stabilised by hydrogen bonds, will dissolve or melt on gently raising the temperature [64].

If, as suggested, pressure-set gels are stabilised, at least to some extent by hydrogen bonds, then it would be expected that heat treatment of such a system will destroy this network and enable a 'heat' set gel to form. Such effects are apparent in both whey protein concentrate gels at pH 7 and in the toughness or hardness of fish and meat flesh, i.e. myosin gels. For example, calorimetric studies on fish and meat myofibrillar proteins and whole muscle have clearly demonstrated the presence of a hydrogen-bonded network in pressure-treated muscle, myofibrillar protein and myosin [65, 66], that is destroyed on heat treatment. Just as the thermal stability of different myosins reflects the body temperature of the species, that from cod being less stable than that from beef or pork, so their relative pressure sensitivities also vary, with cod myosin denaturing at about 100–200 MPa at 20 °C, whilst that from turkey and pork only unfolds at pressures above 200 MPa.

Thus, the simultaneous or sequential treatment of proteins with heat and pressure does raise the possibility of generating gels with interesting and novel textures. The likely mechanisms are discussed in more detail by Ledward [63].

As well as being able to modify the functional properties of water soluble proteins such as $\beta$-lactoglobulin and myoglobin and generate heat-sensitive gels in proteinaceous foods such as meat and fish, high pressures can also be used to texturise many insoluble plant proteins such as gluten and soya. An extensive

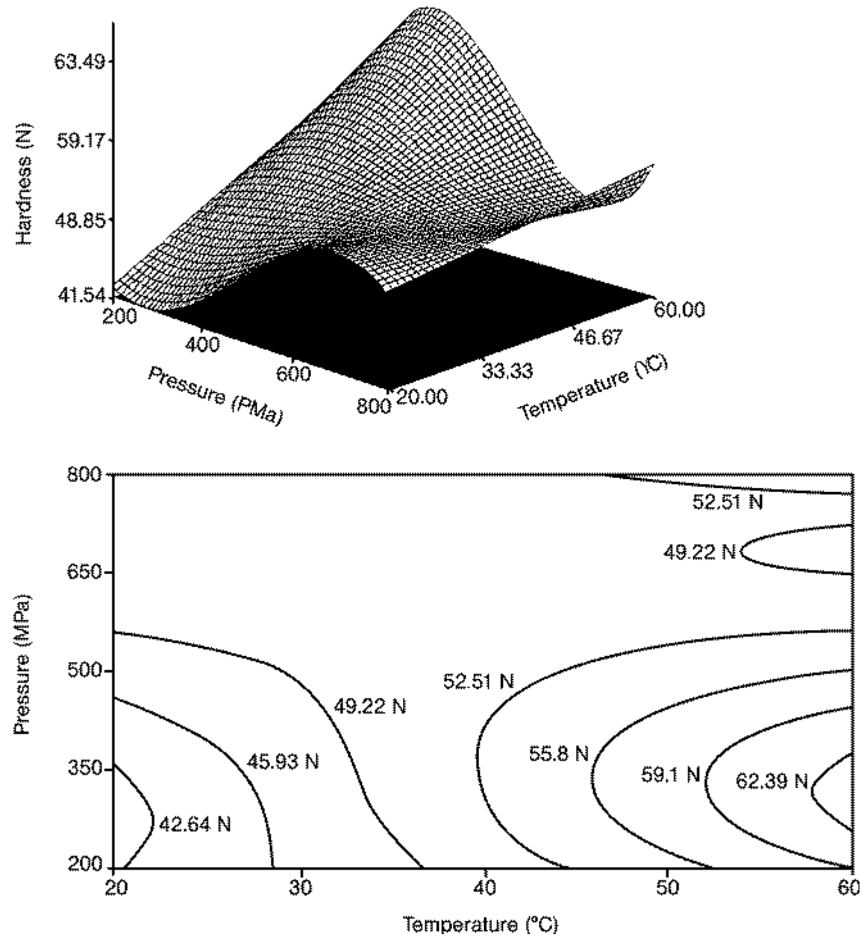


**Fig. 6.3** Response surface and contour plots of the hardness of soy protein gels prepared from a commercial soy concentrate mixed with 3.75 times its weight of water after treatment at temperatures of 20–60 °C and pressures up to 800 MPa for 50 min; from Apichartsrangkoon [69].

study has been carried out by Apichartsrangkoon et al. [67, 68] on the use of various pressure/temperature treatments to texturise wheat gluten and soy protein and it can be seen from Fig. 6.3 how pressure/temperature and time can be used to generate a range of soya gels of different rheological properties.

The above are but a few examples of the rapidly expanding literature on the use of pressure and/or temperature to generate gels with very different rheological properties. The potential of such technology in the development of new proteinaceous foods is very exciting.

From the above brief review it is readily apparent that, although our knowledge of the effects of pressure on proteins has advanced in recent years, a great deal more research is needed before our understanding approaches that of the effect of other parameters on proteins, such as temperature and pH.

## 6.7 Organoleptic Considerations

Since covalent bonds are unaffected by pressure, many of the small molecules that contribute to the colour, flavour or nutritional quality of a food are unchanged by pressure. This is a major advantage of the process and has led to its successful application to such products as guacamole, fruit juices and many other fruit-based jams and desserts.

However, if the organoleptic quality of a food depends upon the structural or functional macromolecules, especially proteins, pressure may affect the quality. The most obvious case of this is with meat and fish products where, at pressures above $\sim$100 MPa for coldwater fish and 300–400 MPa for meats and poultry, the myosin will unfold/denature and the meat or fish will take on a cooked appearance as these proteins gel. In addition, in red meats such as beef and lamb, myoglobin, the major haem protein responsible for the red colour of the fresh product, will denature at pressures around 400 MPa and give rise to the brown haemichrome, further contributing to the cooked meat appearance. However, since the smaller molecules are not affected, the flavour of the fish or meat will be that of the uncooked product. On subsequent heat treatment, these foods will develop a typical cooked flavour.

Although in many circumstances the flavour of a food will remain unchanged after pressure treatment, Cheah and Ledward [55] and Angsupanich and Ledward [65] have shown that, under pressure, inorganic transition metals (especially iron) can be released from the transition metal compounds in both meat and fish. These may catalyse lipid oxidation and thus limit shelf life and may also contribute to the flavour when the product is subsequently cooked. This release of inorganic iron takes place at pressures above 400 MPa and does not appear to be a problem with cured meats or shellfish. This phenomenon may though limit the usefulness of HPP for many uncured fish and meat products.

Since enzyme activity is affected by pressure, many foods whose flavour, texture or colour is dependant on enzymic reactions, may have their sensory properties modified on pressure treatment. Also, in multiphase foods, such as fruits and vegetables, pressure can give rise to significant textural changes due both to modification of the enzyme activity and because of the different rates and extents of compression and decompression of the aqueous, solid and gaseous phases. This may lead to physical damage, as described above for cherry tomatoes.

## 6.8 Equipment for HPP

The key features of any HPP system used for food processing include: a pressure vessel, the pressure transmission medium and a means of generating the pressure. The emergence of production-sized HPP equipment for food occurred more or less simultaneously in the USA and Europe in the mid-1990s.

For convenience, potable water is used as the pressurising medium. The water is usually separated from the food by means of a flexible barrier or package. Products such as shellfish, however, are usually immersed directly into the water as beneficial effects upon yield can be achieved. Up to the minute details of HPP technology and applications can be found on www.avure.com and www.freshunderpressure.com.

### 6.8.1 'Continuous' System

'Continuous' output of pumpable foods such as fruit juice is achieved by arranging three or more small pressure vessels (isolators) in parallel. Each vessel is automatically operated 'out of phase' with the others so as to give an effective 'continuous' output of treated food. Each 'isolator' typically comprises a 25-l pressure vessel incorporating a floating piston and valve block.

Juice is pumped into the vessel under low pressure, forcing the piston down. With valves closed, a high-pressure pump is used to pump water beneath the piston, so forcing it up and thereby pressurising the juice. After the set pressure and hold time conditions have been satisfied, the high-pressure water is vented and the treated juice is pumped out to the production line, by means of low-pressure water pushing the piston to the top of the vessel.

An illustration of a 'continuous' system is shown in Fig. 6.4.

The 'continuous' system has the advantage of treating juices in their unpackaged condition as an integral part of the production process. If juices were previously thermally treated, the HPP system simply replaces the thermal system, with few changes to the remainder of the production process.

The HPP juice may be packed into any suitable package. If a very long chilled shelf life is required however, then the producer should consider the use of aseptic packaging conditions (see Chapter 9). Packaging materials or methods to inhibit the oxidation of the juice by ingress of oxygen through the packaging material over time may also need to be considered.

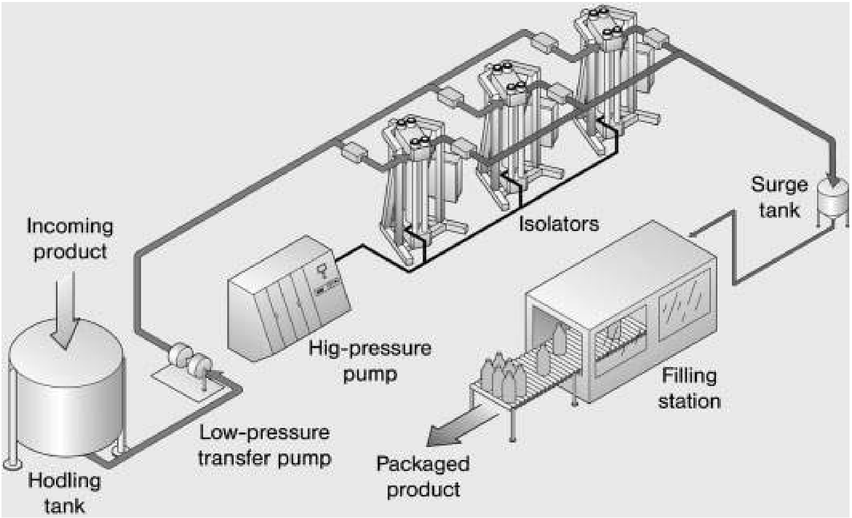


**Fig. 6.4** Illustration of a 'continuous' HPP system; courtesy Avure Technologies AB.

The HPP production line can effectively be run for extended periods without stopping, as the juice now has a long shelf life and can be directed to chilled storage prior to distribution. The cost savings by adopting this strategy can be very considerable compared to the 'normal' production strategy of producing small quantities and shipping immediately.

As the HPP system incorporates its own 'clean in place' (CIP) system, the whole production system can be cleaned and sanitised together. Risk assessment under HACCP (see Chapter 11) includes the HPP system in conjunction with the remainder of the production equipment, methods and materials.

The 'continuous' system is suitable for most 'pumpable' products, such as fruit juices, purees, smooth sauces and soups, vegetable juices and smoothies.

### 6.8.2
### 'Batch' System

These vessels, typically 35–680 l in capacity, use water as the pressurising medium and accept prepackaged food products (see Fig. 6.5).

When a high water content food is subjected to isostatic pressure up to 600 MPa, it compresses in volume by 10–15%, depending on the food type and structure. As the pressurising medium is water, the food packaging must be capable of preventing water ingress and accepting the volume reduction.

The packaging has to withstand the pressure applied but it should be noted that standard plastic bottles, vacuum packs and plastic pouches are usually suitable. These flexible materials have essentially no resistance to volume under the

**Fig. 6.5** Illustration of a 'batch' HPP system; courtesy Avure Technologies AB.

effects of high pressure. Hence, a plastic bottle of fruit juice, having a small (standard) headspace, will compress by the volume of the headspace plus the compressibility of the juice. Provided that the pack is resilient enough to withstand the volume reduction imposed, then the pressurising water will be kept out of the bottle. Once depressurised, the bottle and contents will revert to their original state, minus the spoilage organisms.

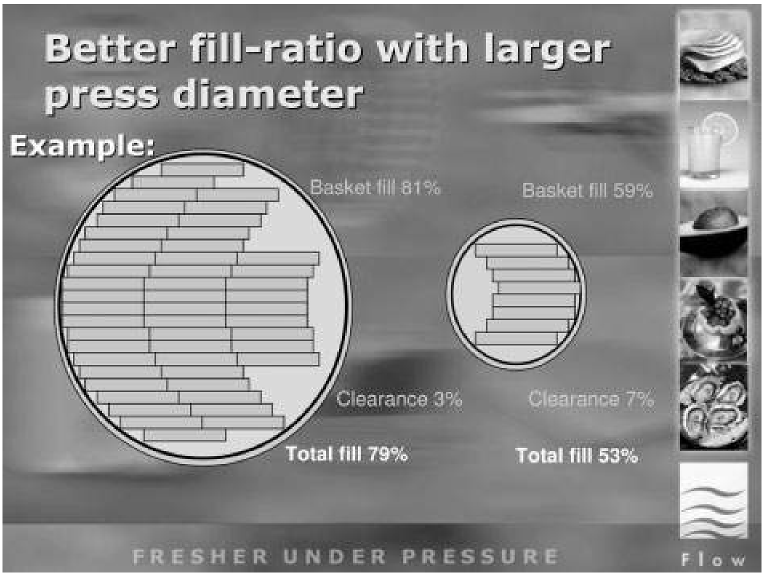


**Fig. 6.6** Schematic representation showing how a better fill ratio is attainable with a larger pressure vessel; courtesy Avure Technologies AB.

A large vessel size gives better utilisation of the available space; and therefore the greatest production output per batch cycle. A 320-l vessel could be filled with a 320-l plastic bag of food, therefore obtaining the maximum use of available volume. Normally however, the batch vessel will be used for a variety of prepacked consumer food products of different size and styles. Fruit juice bottles, for example, will not normally pack together without gaps and therefore the effective output of the vessel will reduce accordingly (see Fig. 6.6).

Batch systems are ideal where wide ranges of products are required and the products comprise solids and liquids combined. Cooked meats, stews, guacamole, fruits in juice, shellfish and ready meals are typical batch HPP examples. Batch systems filled with consumer type packs of food benefit from both the packaging and the food being HPP-treated. This considerably aids quality assurance through distribution and shelf life and may reduce or simplify some production processes.

## 6.9 Pressure Vessel Considerations

In a commercial environment, a HPP system is likely to be run at least 8 h day$^{-1}$, possibly continuously, and so the design criteria is critical.

'Standard' pressure vessels use a thick steel wall construction, where the strength of the steel parts alone is used to contain the pressure within. This is fine for a vessel expected to perform a low number of cycles. However, every time the pressure is applied, the steel vessel expands slightly as it takes up the strain. This imposes expansion stresses within the steel structure and, with time, can lead to the creation of microscopic cracks in the steel itself. If undetected, it is possible for the vessel to fail and rapidly depressurise, causing a safety hazard and destroying the vessel.

The technique used to create a long life and guaranteed 'leak before break' vessel is known as 'Quintus™ wire winding'.

In this case, a relatively thin wall pressure vessel (too thin to contain the working pressure of its own) is wrapped in steel wire. The wire wrapping, which can amount to several hundred kilometres of wire for a large vessel, is stretched and wrapped onto the vessel by a special machine and powered turntable.

The wire attempts to return to its unstretched state and in doing so exerts a compressive force upon the pressure vessel. The compressive force of the wire wrapping is engineered to slightly exceed the expansion force of the HPP water pressure. Hence, the thin-walled pressure vessel, even with 600 MPa water pressure within it, is actually still under compression from the wire windings. This means that the only forces within the steel of the pressure vessel are compressive and therefore cracks cannot propagate.

A wire-wound frame is made to hold in place 'floating' top and bottom plugs, so as to allow access to the vessel for loading and unloading. The frame con-

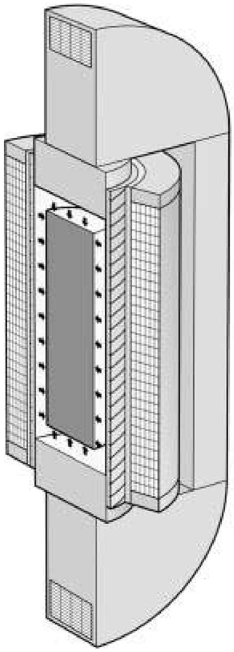

Fig. 6.7 A Quintus™ wire-wound pressure vessel; copyright Avure Technologies AB.

struction is similar to that described for the vessel (see Fig. 6.7). A wire-wound vessel has theoretically unlimited life expectancy.

High-grade forged pressure vessel steel is not the preferred material for contact with foods and so the wire-wound vessel incorporates a replaceable inner liner of stainless steel.

### 6.9.1 HP Pumps

Normal pumps cannot achieve the required pressures for HPP food applications. Therefore, a standard hydraulic pump is used to drive an intensifier pump, comprising a large piston driven back and forth by the hydraulic oil in a low-pressure pump cylinder. The large piston has two smaller pistons connected to it, one each side, running in high-pressure cylinders. The ratio of large and small piston areas and hydraulic pump pressure gives a multiplication of the pressure seen at the output of the high-pressure cylinders. The small, high-pressure pistons are pumping the potable water used as the pressurising medium in the HPP food process.

The pressure and volume output from the intensifier is dependent upon the overall sizes of the pistons and hydraulic pressure. However, it is often more convenient to use several small intensifiers working 'out of phase' to give a smooth pressure delivery and some degree of redundancy, rather than use a single, large intensifier.

### 6.9.2
### Control Systems

Both the batch and continuous HPP systems are mainly sequentially operated machines and so are controlled by standard PLCs. Verification of each sequence before proceeding to the next is essential in some cases, due to the very high pressures involved and the materials used for the sanitisation of food processes. Many critical sequences will include 'fail safe' instrumentation and logic.

The user interface forms an important part of any process and the demands today are for even the most complex machine or system to be operable by non-engineering operatives. The operator is therefore presented with a screen or monitor with the process parameters and operator requirements presented in a format similar to that of a home PC running the most popular software. System logic design and password protection means that any suitably trained person can carry out normal production and maintenance.

Fault conditions are automatically segregated into those that the operator can deal with and those that dictates either the automatic safety shutdown of the system (or part thereof) or a sequence halt. In every case, the fault and probable solution is available to the user.

## 6.10
## Current and Potential Applications of HPP for Foods

France was the first country in the European Community to have HPP food products commercially available. Since 1994, Ulti have been processing citrus juices at 400 MPa. Orange juice is the main product, although some lemon and grapefruit juice is also produced. Their motivation for moving into HPP was a desire to extend the shelf life of their fresh fruit juice, then only 6 days at chilled temperature. High pressure treatment allowed a shelf life of up to 16 days. This reduced logistical problems and transportation costs, without harming the sensory quality and vitamin content of the juice. HPP-processed fruit juice and fruit smoothies have also been available in the UK since 2002.

At present, a number of products are currently in development, including fruit, delicatessen and duck fat liver products. Before commercialisation is approved, convincing physicochemical, microbial and toxicity analyses must be carried out.

Elsewhere in Europe, Espuña of Spain process sliced ham and delicatessen meat products, in flexible pouches, using an industrial 'cold pasteuriser' unit. Throughputs of 600 kg $h^{-1}$ are achievable, using operating pressures of 400–500 MPa and hold times of a few minutes. The organoleptic properties of fresh ham are preserved; and an extended shelf life of 60 days under chilled storage has been reported.

As discussed above, HPP guacamole has been commercially available for some time in the USA. It is actually manufactured across the border in Mexico, to take full advantage of low raw material costs and avoid the import costs associated with the import of raw avocados to the USA. Its market share continues to grow and is reportedly based on the consumer preference for the 'fresher' taste of guacamole processed in this manner. The same company now produces HPP meal kits, consisting of pressure-treated cooked meat, salsa, guacamole, peppers and onion. Only the flour tortilla is not pressure-treated. The products have a chilled shelf life of at least 35 days.

'Gold Band' oysters from Motivatit Seafoods Inc. www.the perfectoyster.com are now achieving top national awards in America for HPP shucked oysters. They report up to 75% yield increase with HPP compared to manual shucking processes and now 'contract shuck' oysters for other famous oyster companies in the region. Shellfish shucking using HPP has also recently started in Australia and is expected in Europe within the near future.

High Pressure Research Inc. (Corvallis, Ore.) is another USA-based company to produce HPP products. Their range includes oysters, salmon, yoghurt, spreads, fruit and fruit juices. These products are currently appearing in major grocery chains and supermarkets; and a refrigerated shelf life of 60 days is possible. Meanwhile, pilot plant work is underway on the production of Spanish rice, oriental chicken, vegetarian pasta salad and seafood creole. Evaluation of these products after storage at room temperature for various times is to be carried out by the US Army at Natick Laboratories.

The Japanese market for HPP foods is better developed than in the West. A range of fruit products, jams and yoghurts has been available for a number of years, and the most recent innovations have been rice products. Rice cakes and convenience packs of boiled rice are now available commercially. A recent Japanese patent described a HPP technique to improve the perceived 'freshness' of fruit flavours. The process described involves adding a small amount of fruit juice to a fruit flavour and then processing at 100–400 MPa for 1–30 min. The processing temperature is maintained between freezing and room temperature.

Markets demanding better food quality, extended chilled shelf life and higher food safety can now be commercially accessed using high pressure. These new market opportunities and new products can offset the capital cost of the equipment, as has been seen in many commercial application successes. The textural changes possible using high pressure have started to lead to the development of new, or novel, foodstuffs. Food producers and retailers should carefully consider the currently accepted high costs associated with short production runs of fresh quality, limited shelf life foods. Compare those to the savings associated with longer production runs of longer shelf life and safer HPP foods, while still benefiting from the original organoleptic food quality. HPP offers foods with quality and convenience, in keeping with today's consumer trends.

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