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Trends in Food Science & Technology 19 (2008) 289–299

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

High pressure in combination with elevated temperature as a method for the sterilisation of food

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Application of high-pressure processing to foods can effect a decrease in the number of vegetative bacterial cells, and hence can result in pasteurisation. Inactivation of bacterial spores, however, is required for the sterilisation of foods. This article reviews the current status of the application of high-pressure treatments for the inactivation of bacterial spores, and particularly examines the requirement for a combination of high pressure and high temperature processing to achieve the sterilisation of foods.

Introduction

Consumer demand is increasing for products that are fresh tasting, additive-free and microbiologically safe yet are convenient to use, having an extended shelf-life and requiring minimal preparation time. The characteristics of an ideal processing method have been identified (Raso & Barbosa-Cánovas, 2003) as:

- able to inactivate spoilage and pathogenic microorganisms,
- not degrading organoleptic and nutritional values of products,
- not leaving residues,
- cheap and convenient to apply and
- acceptable to consumers and regulatory agencies.

Many of the processing methods used today possess some of the characteristics described but do not meet all the criteria. For example, chilling and freezing can maintain, to a certain degree, freshness of the food but do not kill microorganisms, only delay or inhibit their growth. A significant break in the chill/freeze chain, caused for example by equipment failure, can lead to growth of undesirable microorganisms. Thermal processing, on the other hand, can inactivate microorganisms and enzymes resulting in safer and more stable products but heat treatment can adversely affect the organoleptic qualities of the final product such as appearance, taste and flavour as well as its nutritional value.

The potential for combination treatments

In an attempt to find ideal processing characteristics two or more processing methods are commonly applied simultaneously. Combinations of treatment are often more effective at preventing microbial growth than those same conditions used in isolation, which means combining preservative factors can significantly improve the quality of foods whilst delivering the same level of microbial inactivation as conventional methods. Such a combination using high temperature applied with high hydrostatic pressure can successfully inactivate microorganisms, and the potential to use this combination for the sterilisation of foods is the focus of this review.

HP processing for inactivation of vegetative bacteria

The ability of high-pressure treatment to preserve foods has been known since 1899, when Hite (1899) conducted a series of experiments with the effect of pressure on different food systems. He observed that milk 'kept sweet longer' after treatment of *circa* 600 MPa for 1 h at room temperature. He also reported (Hite *et al.*, 1914) that, while pressure could be used to extend the freshness of fruits and fruit juices, this was not the case with vegetables. The difference was caused by the pressure treatment destroying vegetative microorganisms capable of growth in the

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0924-2244/$ - see front matter
doi:10.1016/j.tifs.2008.01.005

high-acid fruits, but failing to destroy the greater range of microorganisms, including bacterial spores that could subsequently grow in the low-acid vegetables. This illustrates a problem that remains today: pressure treatments of around 600 MPa at ambient temperature are very effective against vegetative bacteria, but a pressure treatment of up to 1500 MPa at ambient temperature can fail to inactivate bacterial spores. High-pressure treated products that are currently commercially available are typically high-acid foods such as fruit preparations, fruit juices and sauces such as acidified guacamole (Tewari, Jayas, & Holley, 1999) which do not support the growth of spore forming microorganisms but are prone to spoilage by microorganisms such as yeasts, moulds and lactic bacteria, which are relatively pressure sensitive.

Potential of HPT to inactivate bacterial spores

To achieve an acceptable decrease in the number of microorganisms in low-acid foods (e.g. poultry, red meat, cheese, milk, liquid whole egg and vegetable products), high pressure must be combined with a second inactivating process. This second factor is often high temperature.

This article focuses on research using high-pressure treatments applied in combination with temperature in excess of ambient (elevated) temperature, which we refer to as high-pressure thermal (HPT) processing and the potential for such treatments to achieve the sterilisation of foods. Accordingly, it reviews the effects of HPT processes on bacterial spores in various conditions, and discusses some problems that need to be resolved in order to successfully apply the method on an industrial scale.

Interactions between high pressure and high temperature

High temperatures can result in relatively slow rates of heating and cooling, which can decrease product quality. One benefit of generating high pressure is that it causes temperature increase in the sample and pressure transmitting fluid (compression heating). For example, for water at an initial temperature of 20 °C an increase of 2–3 °C per 100 MPa (Cheftel, 1995) is to be expected. For an initial temperature of 90 °C, the increase in temperature due to compression heating is 5.3 °C per 100 MPa increase in pressure (Balasubramaniam, Ting, Stewart, & Robbins, 2004). Even higher values have been reported for other food products (Rasanayagam *et al*., 2003). At initial temperature of 25 °C, compression heating of olive oil resulted in a temperature rise of between 6.9 and 8.7 °C, of mayonnaise between 5.0 and 7.2 °C and of tomato salsa in the range of 2.6–3.0 °C. The successful use of compression heating can result in reduction of processing time and, as a consequence, higher product quality and lower energy consumption. Use of compression heating could also be made to increase inactivation of microorganisms in foods where an initial preheating to high temperatures was already achieved.

Measured effects of HPT processing on inactivation of vegetative bacteria

This article is focused on the greatest challenge in the use of HPT processing which is inactivation of bacterial spores. However, there is wide range of published research on the combined effect of high hydrostatic pressure and elevated temperature on vegetative forms of bacteria, which is briefly summarised here. Pressure acts on vegetative microbial cells *via* inhibition of protein synthesis, enzyme denaturation and decrease of lipid membrane fluidity (Bartlett, 2002; Simpson & Gilmour, 1997). The response of food-borne pathogenic bacteria to HPT processing is variable. It has been observed that bacteria exhibit the biggest pressure resistance at temperatures between 20 and 30 °C. For example, inactivation of *Escherichia coli* O157:H7 in poultry meat, treated with 400 MPa for 15 min at 20 °C, was less than 1 log, the same result as for a 50 °C only heat treatment (Patterson & Kilpatrick, 1998). When pressure of 400 MPa was combined with a temperature of 50 °C, however, a reduction by 6 log was observed. Similar situations have been reported in milk (Patterson & Kilpatrick, 1998). Pressure inactivation of *E. coli* O157:H7 was also studied in tomato juice (Bari, Ukuku, Mori, Kawamoto, & Yamamoto, 2007); at a temperature of 25 °C, moderate pressure of 300, 350 and 400 MPa caused a reduction in bacterial population by 3.0, 3.0 and 5.0 logs, respectively.

Recent studies on *Listeria monocytogenes* in model washed-curd cheeses (Lopez-Pedemonte, Roig-Sagues, De Lamo, Hernandez-Herrero, & Guamis, 2007) showed that by applying 500 MPa of pressure for 10 min, even at low temperature (5 °C) it is possible to achieve 4.5–5.5 log cfu/g reduction of counts (depending on the bacterial strain).

Another study compared the pressure resistance of *L. monocytogenes* with that of *Salmonella typhimurium* (Ritz, Pilet, Jugiau, Rama, & Federighi, 2006). Treatments were performed at room temperature using pressure of 600 MPa for 10 min. The initial bacterial concentration was 8.2 log cfu/ml suspended in pH = 7.0 buffer. Plate counting indicated complete inactivation. However, direct counting of viable cells using an epifluorescence microscope demonstrated that around 4 log/ml of viable cells had survived the treatment. Indeed, the experiments reported (Kalchayanand, Sikes, Dunne, & Ray, 1998; Patterson & Kilpatrick, 1998) so far allow for the conclusion to be drawn that the combination of pressure in the range of 200–500 MPa with a temperature of greater than 50 °C can drastically reduce the number of vegetative bacteria in a wide range of food products. However, the greatest challenge in the use of high-pressure processing for food sterilisation is the inactivation of bacterial spores.

Measured effects of HPT processing on inactivation of bacterial spores

Compared to vegetative cells, bacterial spores have increased resistance to environmental stresses including high temperatures and pressures. Many authors have

studied the use of pressure for the inactivation of bacterial spores. The range of pressures and temperatures tested and the inactivation measured is summarised in Tables 1 and 2. Differences in response to pressure between different species, and between strains of the same species are frequent. For example, spores of *Clostridium sporogenes* in fresh chicken breast have been shown to require a pressure of at least 680 MPa for 1 h at ambient temperature to achieve 5 logs inactivation (Crawford, Murano, Olson, & Shenoy, 1996). However, other workers have found that for a similar reduction in numbers, pressure in excess of 1000 MPa was required and even processing at 1500 MPa resulted in a reduction of only 1.5 log cycles (Maggi, Rovere, Miglioli, Dall'Aglio, & Loenneborg, 1996).

Effect of HPT processing on the inactivation of spores of *Clostridium botulinum*

C. botulinum is a heterogeneous species comprising four physiologically distinct groups. Groups I and II proteolytic and non-proteolytic *C. botulinum* are those most frequently associated with food poisoning.

Proteolytic *C. botulinum* is one of the most heat resistant food poisoning organisms and is thus an important target of thermal processing of low-acid foods. Spores of *C. botulinum* are also pressure resistant. *C. sporogenes* is considered to be a non-toxinogenic equivalent of proteolytic *C. botulinum* and is often used where health and safety reasons do not permit work with *C. botulinum*. Although studies on *C. botulinum* are reviewed below, data also relating to *C. sporogenes* are shown in Table 1.

Studies on spores of *C. botulinum* strain TWM 2.357 in mashed carrot matrix (Margosch, Ehrmann, Ganzle, & Vogel, 2004) were performed at temperatures starting from 80 °C and reaching 116 °C in a single experiment with compression rates of 2 MPa/s and 6 MPa/s (Table 1). The processing time needed to achieve a reduction of 5.5 log decreased from over 70 min (at 2 MPa/s) to 10 min (at 6 MPa/s). In that series of experiments, a rapid decrease of temperature just after reaching maximum pressure (caused by conduction to the pressure vessel) was observed. This transient difference strongly accelerated spore inactivation. Reddy, Solomon, Tetzloff, and Rhodehamel (2003) reported that partial inactivation of spores of *C. botulinum* strains 62-A and BS-A was achieved in both synthetic buffer and model food systems. At a maximum temperature of 75 °C, in phosphate buffer (pH 7.0), log reductions of both strains increased from less than 2.0 for lower pressures (<689 MPa) to 3.0 log units for 827 MPa with a processing time of 20 min. Similar results were observed in crabmeat where the maximum inactivation was 3.2 log units for BS-A and 2.7 log units for 62-A using 827 MPa at 75 °C. These decreases resulted from treatments of 15 min and are greater than corresponding results for phosphate buffer (1.7 log for 62-A). Curiously, an increase in processing time of the crabmeat from 15 min to 20 min caused a decrease in inactivation level (2.5 log for BS-A, 2.3 log for 62-A). Spores of proteolytic *C. botulinum* type A required over 800 MPa at up to 88 °C for 9 min to effect a decrease of 3 log cycles (Table 1).

Spores of proteolytic strains of *C. botulinum* do seem to be substantially more pressure resistant than non-proteolytic strains (Margosch *et al.*, 2004). Spores of non-proteolytic *C. botulinum* are of moderate heat resistance and 10 min at 90 °C, or equivalent, is usually considered to be sufficient to ensure a 6-log reduction in viability (ECFF, 2006). However, this organism can grow at temperatures as low as 3 °C, so is a concern in refrigerated and pasteurized foods. Combining moderate heat treatment with high pressure could be an effective way of increasing the safety of long-life chilled foods.

Examples of inactivation of spores of non-proteolytic *C. botulinum* follow. Inactivation of spores of non-proteolytic type E, strains Alaska and Beluga, at 827 MPa for 5 min increased as the temperature of the process increased from 35 to 55 °C. At 35 °C, *C. botulinum* Alaska exhibited inactivation of less than 1.5 log, while at 50 °C a reduction of 5 log was achieved. For *C. botulinum* Beluga, the temperature needed to achieve the same levels of inactivation were, respectively, 40 and 60 °C. Processing temperatures below 35 °C showed little or no spore inactivation in 5 min. Also, lowering the high pressure at a temperature of 50 °C decreased the number of inactivated spores (at 689 MPa for 5 min, the reduction was less than 0.3 log). However, at 827 MPa, lowering the temperature to 40 °C, with exposure time increased from 5 min to 10 min, caused a 5-log reduction in spores of *C. botulinum* Alaska (Reddy *et al.*, 1999).

The mechanism of pressure resistance in *Clostridium* spores is not yet fully understood. However, a mechanism of inactivation of *Bacillus* spores has been proposed and is described below.

Effect of HPT processing on the inactivation of spores of *Bacillus* species

Mechanisms of spore inactivation

The mechanism of spore inactivation by high pressure has been most widely studied in *Bacillus subtilis*. Two distinct mechanisms have been proposed, depending on the severity of treatment (Paidhungat *et al.*, 2002; Wuytack, Boven, & Michiels, 1998).

Moderately high pressures (50–300 MPa) activate nutrient–germinant receptors normally activated by alanine or AGFK (a mixture of asparagine, glucose, fructose and potassium ions), which then facilitates the release of Ca-DPA (chelate of Ca^{2+} and pyridine-2,6-dipicolinic acid). Ca-DPA triggers a cascade of later germination events starting with hydrolysis of spore cortex by cortex-lytic enzymes (CLE), degradation of small acid-soluble proteins (SASPs) and generation of ATP in a similar way to a nutrient triggered germination mechanism. The resultant spores are as sensitive to subsequent heat and pressure treatments as vegetative cells (Black *et al.*, 2005). It is yet unknown if

Table 1. Effect of high pressure and temperature on the inactivation of spores of *Bacillus* and *Clostridium* in buffer

Organism	Suspending medium/ foodmatrix	Pressure transmission fluid	Pressure (MPa)	Processing temperature (°C)	Initial pressure transmission fluid temperature (°C)	Maximum pressure transmission fluid temperature (°C)	Final pressure transmission fluid temperature (°C)	Pressure holding time (min)	Log reduction	Ref.
Clostridium botulinum Type A Proteolytic	Phosphate buffer pH 7.0	50% glycol water fluid	<689	75	N/A	N/A	N/A	20	<2.0	Reddy *et al.*, 2003
C. botulinum Type A, proteolytic	Phosphate buffer pH 7.0	50% glycol water fluid	827	75	60.3 ± 1.1	92.4 ± 2.3	74.3 ± 0.5	20	3.0	
C. botulinum Type E Alaska, Non-proteolytic	Phosphate buffer pH 7.0	2% sodium benzoate solution	827	<35	12.9 ± 0.4	43.0 ± 0.2	36.2 ± 0.4	5	<1.5	Reddy *et al.*, 1999
C. botulinum Type E Alaska, Non-proteolytic	Phosphate buffer pH 7.0	2% sodium benzoate solution	827	40	16.7 ± 0.8	46.3 ± 0.8	39.7 ± 0.9	10	5	
C. botulinum Type E Alaska, Non-proteolytic	Phosphate buffer pH 7.0	2% sodium benzoate solution	827	55	35.5	62.9	54.0	5	5	
C. botulinum Type E Alaska, Non-proteolytic	Phosphate buffer pH 7.0	2% sodium benzoate solution	689	50	N/A	N/A	N/A	5	<1.5	
C. botulinum Type E Alaska, Non-proteolytic	Phosphate buffer pH 7.0	2% sodium benzoate solution	827	35	12.9 ± 0.4	43.0 ± 0.8	39.7 ± 0.9	5	<1.5	
C. botulinum Type E Alaska, Non-Proteolytic	Phosphate buffer pH 7.0	2% sodium benzoate solution	827	50	28.9 ± 0.4	57.0 ± 1.1	49.1 ± 1.1	5	5	
C. botulinum Type E Beluga, Non-Proteolytic	Phosphate buffer pH 7.0	2% sodium benzoate solution	827	40	16.7 ± 0.8	46.3 ± 0.8	39.7 ± 0.9	5	<1.5	
C. botulinum Type E Beluga, Non-Proteolytic	Phosphate buffer pH 7.0	2% sodium benzoate solution	827	60	36.8 ± 0.5	67.2 ± 0.2	59.0 ± 0.4	5	5	

Clostridium sporogenes NCIMB 8053	Distilled water	10% Dromus oil in water emulsion	400	60	N/A	N/A	N/A	30	<1	Mills, Earnshaw, & Patterson, 1998
C. sporogenes NCIMB 8053	Distilled water	10% Dromus oil in water emulsion	Sequential 60 and 400 MPa cycle	60	N/A	N/A	N/A	90	<1	
C. sporogenes PA 3679	McIlvaine citrate phosphate buffer pH 4.0	2% hydraulic fluid solution (Hydrolubric 142)	404	45	N/A	N/A	N/A	15	2	Stewart, Dunne, Sikes, & Hoover, 2000
C. sporogenes PA 3679	McIlvaine citrate phosphate buffer pH 5.0	2% hydraulic fluid solution (Hydrolubric 142)	404	45	N/A	N/A	N/A	15	1.0	
C. sporogenes PA 3679	McIlvaine citrate phosphate buffer pH 4.0	2% hydraulic fluid solution (Hydrolubric 142)	404	70	N/A	N/A	N/A	15	6	
C. sporogenes PA 3679	McIlvaine citrate phosphate buffer pH 4.0	2% hydraulic fluid solution (Hydrolubric 142)	404	70	N/A	N/A	N/A	15	4	
C. sporogenes PA 3679	McIlvaine citrate phosphate buffer pH 7.0	2% hydraulic fluid solution (Hydrolubric 142)	404	70	N/A	N/A	N/A	15	<0.5	
C. sporogenes ATCC 7955	Chicken breast meat	Mineral Oil	689	80	N/A	100	N/A	5	5	Crawford *et al.*, 1996
C. sporogenes ATCC 7955	Citric acid buffer pH 6.5	5% Hydrolubric 120-B in water	650	55	N/A	N/A	N/A	15	0.4 ± 0.0	Paredes-Sabja, Gonzalez, Sarker, & Torres, 2007
C. sporogenes ATCC 7955	Citric acid buffer pH 6.5	5% Hydrolubric 120-B in water	650	75	N/A	N/A	N/A	15	1.8 ± 0.7	
C. sporogenes ATCC 3584	Citric acid buffer pH 6.5	5% Hydrolubric 120-B in water	650	55	N/A	N/A	N/A	15	0.6 ± 0.2	
C. sporogenes ATCC 3584	Citric acid buffer pH 6.5	5% Hydrolubric 120-B in water	650	75	N/A	N/A	N/A	15	2.5 ± 1.0	

(*continued on next page*)

Table 1 (*continued*)

Organism	Suspending medium/ foodmatrix	Pressure transmission fluid	Pressure (MPa)	Processing temperature (°C)	Initial pressure transmission fluid temperature (°C)	Maximum pressure transmission fluid temperature (°C)	Final pressure transmission fluid temperature (°C)	Pressure holding time (min)	Log reduction	Ref.
C. sporogenes ATCC 7955	Citric acid buffer pH 4.75	5% Hydrolubric 120-B in water	650	55	N/A	N/A	N/A	15	0.8 ± 0.1	
C. sporogenes ATCC 7955	Citric acid buffer pH 4.75	5% Hydrolubric 120-B in water	650	75	N/A	N/A	N/A	15	5.7 ± 0.2	
C. sporogenes ATCC 3584	Citric acid buffer pH 4.75	5% Hydrolubric 120-B in water	650	55	N/A	N/A	N/A	15	0.8 ± 0.1	
C. sporogenes ATCC 3584	Citric acid buffer pH 4.75	5% Hydrolubric 120-B in water	650	75	N/A	N/A	N/A	15	6.0 ± 0.0	
Bacillus cereus ATCC 14579	McIlvane Citrate phosphate buffer pH 7.0	5% mobile Hydrasol 78 in water	690	40	N/A	N/A	N/A	2	8	Raso, Gongora-Nieto, Barbosa-Canovas, & Swanson, 1998
Bacillus subtilis As 1.1731	Milk buffer pH 7.0	bis (2-ethylhexyl) sebacate	479	46	87	N/A	N/A	14	6	Gao & Jiang, 2005

N/A = data not available in the reference cited.

Table 2. Effect of high pressure and temperature on the inactivation of spores of *Bacillus* and *Clostridium* in food matrices

Organism	Suspending medium/food matrix	Pressure transmission fluid	Pressure (MPa)	Processing temperature (°C)	Initial pressure transmission fluid temperature (°C)	Maximum pressure transmission fluid temperature (°C)	Final pressure transmission fluid temperature (°C)	Pressure holding time (min)	Log reduction	Ref.
Clostridium botulinum Type B, proteolytic	Mashed carrot	80% ethanol 20% rhizinus oil mixture	600 MPa Ramp at 2 MPa/s	80	80	100	80	70	5	Margosch *et al.*, 2004
C. botulinum Type A, proteolytic	Mashed carrot	80% ethanol 20% rhizinus oil mixture	600 MPa ramp at 6 MPa/s	80	80	116	80	6	5	
C. botulinum Type B, proteolytic	Mashed carrot	80% ethanol 20% rhizinus oil mixture	800	80	80	116	80	4	2.3	
C. botulinum Type A, proteolytic	Crabmeat	50% glycol water fluid	827	75	62.0 ± 3.2	95.6 ± 4.4	76.1 ± 1.0	15	3.2	Reddy *et al.*, 2003
C. botulinum Type A, proteolytic	Crabmeat	50% glycol water fluid	827	75	60.3 ± 1.1	92.4 ± 2.3	74.3 ± 0.5	20	2.3	
Bacillus amyloliquefaciens	Mashed carrot	80% ethanol 20% rhizinus oil mixture	800	70	N/A	N/A	N/A	64	2.1	Margosch *et al.*, 2004
B. amyloliquefaciens	Mashed carrot	80% ethanol 20% rhizinus oil mixture	800	80	N/A	N/A	N/A	4	1.15	
B. amyloliquefaciens	Egg patty mince	Propylene glycol	700	105	67	N/A	N/A	3	5	Rajan *et al.*, 2006
B. amyloliquefaciens	Egg patty mince	Propylene glycol	700	110	73	N/A	N/A	3	7	
B. amyloliquefaciens	Egg patty mince	Propylene glycol	700	121	84	N/A	N/A	3	ND	

N/A = data not available in the reference cited.

pressure acts on germinant receptors by directly changing their conformation or by a decrease of spore inner membrane fluidity in which they are sited.

Very high pressures (>500 MPa) induce rapid germination in a different manner which does not facilitate germinant receptors. Instead, Ca-DPA is released directly in the process that is not physicochemical but physiological in nature. Later, germination events follow the same mechanism as for moderate pressures, including spore cortex degradation by CLE enzymes, which is necessary for completion of the germination process (Wuytack, Soons, Poschet, & Michiels, 2000).

Two possible mechanisms were recently proposed to explain the Ca-DPA release (Black *et al*., 2007). One hypothesis suggests that pressure activates protein channels that normally allow for DPA movement across the spore's inner membrane. However, again the precise target of that action is unknown, it might be the membrane itself or membrane proteins. A second possible explanation is that pressure creates new pores in the inner membrane that are independent from the membrane proteins.

Inactivation of spores

Bacillus spores are thought to be susceptible to pressure-induced germination by use of relatively moderate pressures of 100–600 MPa. Pressure-induced germination may enable an inactivation of spores by mild heat and/or pressure treatment (Wuytack *et al*., 1998). However, this concept cannot reliably be adopted commercially due to the distribution in the variability of the effects of high pressure on spore germination.

Gao and Jiang (2005) used a response surface plot to predict optimal treatment conditions that will achieve a 6-log reduction in viable counts of *B. subtilis*. The optimum treatment was found to be 479 MPa at a temperature of 46 °C for 14 min. Spores were suspended in milk buffer of pH = 7.0.

Bacillus amyloliquefaciens form highly pressure resistant spores and it has been suggested that it should be adopted as the target organism for the development of high-pressure processes (Rajan, Ahn, Balasubramaniam, & Yousef, 2006).

Studies on 18 strains of both *B. subtilis* and *B. amyloliquefaciens* (Margosch, Ganzle, Ehrmann, & Vogel, 2004) showed that there is a substantial difference in pressure resistance between the strains. Ten strains of *B. subtilis* were tested in mashed carrots, with 800 MPa pressure applied at 70 °C. The number of spores of three laboratory-derived strains was reduced by 6 log within the first minute of treatment. Four food isolates and one laboratory strain were more pressure resistant, being reduced by more than 4 log only after 16 min of treatment. Two isolates from ropy bread formed highly resistant spores. These were only inactivated by 2 logs after 16 min of pressure treatment. All of the *B. amyloliquefaciens* strains (isolated from ropy bread) exhibited essentially the same resistance to pressure and heat. A similar 16 min treatment as for *B. subtilis* resulted in 90% of spores still being viable. *B. amyloliquefaciens* strain Fad 11/2 formed the most resistant spores, and 800 MPa at 70 °C had no effect on spore numbers. The recovery media used was ST1. In the same study, the effect of sporulation conditions was assessed. One of the strains of *B. subtilis* (ATCC 19659) had been grown at 30, 44 and 48 °C. After 1 s of pressure holding time (the conditions were the same as above), the respective decrease in number of survivors was 2.5, 5 and 6 log. In addition to the role of temperature, the role of minerals in spore resistance was also investigated. This was done by addition of 5 mM $CaSO_4 \cdot 2H_2O$, $MnSO_4 \cdot H_2O$, or $ZnSO_4 \cdot 7\ H_2O$ to ST1 agar plates. These were incubated at 30 °C. After 1 s pressure holding time inactivation of four orders of magnitude was reported. In conclusion, increased sporulation temperature and presence of minerals have a negative effect on spore resistance to HPT processing. This is in line with the work of Igura, Kamimura, Islam, Shimoda, and Hayakawa (2003), which indicates that deficiency of the certain ions (Ca^{2+} or Mg^{2+}) prevented activation of cortex-lytic enzymes, and as a consequence, inhibited germination, leading to higher resistance.

Effects of HPT processing on food quality

Thermal processing not only inactivates microorganisms but also has a significant impact on the flavour, colour, texture and nutritive value of the treated food. The extent of the loss of organoleptic, textural or nutritive quality depends on the magnitude of the heat treatment. Much of the interest in new processing methods, such as high-pressure thermal processing, is that they have the potential to achieve a better quality product while maintaining or improving product safety. To date, most studies on the effect of high-pressure treatment on food quality have been carried out at moderate temperatures; data on high-pressure sterilised food (which is the topic of this review) are sparse (Matser, Krebbers, van den Berg, & Bartels, 2004).

Tests with basil subjected to high-pressure sterilisation treatment (Krebbers, Matser, Koets, Bartels, & van den Berg, 2002) gave promising results. Fresh basil samples were pre-heated to an initial temperature of HPT treatment, and pressurized twice at set pressure and temperature conditions for 30 s with an interval of 30 s at 0.1 MPa between the treatments. Pressure and temperature combinations tested were 860 MPa at 75 °C and 700 MPa at 85 °C. Because of adiabatic heating, maximum temperature during the first pulse reached 112 °C regardless of conditions used. Both set of conditions resulted in around 90% retention of flavour components, which was a much better result than freezing (at −30 °C), drying (20 s steam blanching followed by 2 h at 75 °C) and conventional sterilisation at conditions designed to mimic a temperature profile during HPT treatment (200 s at 85 °C followed by 210 s at 112 °C and 120 s cooling at 20 °C). All of the conventional preservation methods resulted in loss of 60–90% of essential oil

content. However, it is notable that although the aroma of HPT treated basil was more intense than that of conventionally processed basil, the texture and colour resembled heat-treated basil more than fresh.

Both high pressure and high temperature may inflict loss of tissue firmness by cell wall breakdown and loss of turgidity. However, Leadley, Tucker, and Fryer (2008) demonstrated that although considerable softening occurred in high-pressure sterilised green beans, it was not as severe as in thermal processed samples. In this experiment, the HPT samples were pre-heated to 86 °C then subjected to 2 × 2 min pressurization at 700 MPa, interrupted by 1 min hold at ambient pressure with a peak temperature of 117 °C. These pressure-treated samples were found to be approximately twice as firm as samples heated for the equivalent of 3 min at 121.1 °C under ambient pressure, not only just after processing but also after 7 months of storage.

Vitamin content of foods can also be significantly reduced by thermal processing and storage. Another study on green beans (Krebbers, Matser, Koets, & van den Berg, 2002) showed that retention of ascorbic acid after HPT treatment was much higher (76% of fresh material content) than after traditional blanching for 4 min at 90 °C (10%). The HPT process used was preheating at 75 °C for 2 min followed by two pulses of 1000 MPa for 80 s separated by 30 s at 0.1 MPa. The maximum temperature during treatment was 105 °C.

The effect of high pressure on colour at moderate temperatures is dependant on the food matrix. The colour degradation kinetics of tomato puree, treated at 300–700 MPa and 65 °C for 60 min, was shown to be similar to untreated material, independent of treatment pH (Rodrigo, van Loey, & Hendrickx, 2007). Similar results were obtained for strawberry juice at pH 2.5 in the same study. However, when strawberry juice was treated at pH 3.7 or pH 5, faster colour degradation was observed in the HP treated juice than the untreated equivalent.

The effects of HPT processing on flavour, texture, colour and nutritional value are important issues and knowledge gained at low and moderate temperatures needs to be extended to high temperatures as HPT sterilisation processes are developed.

Technical issues for experimental systems

There are many technical issues that must be addressed before attempting to carry out meaningful microbial inactivation studies relating to HPT processing. These fall into two general groups: problems that arise from dealing with biological systems (both food matrix and bacterial cells and spores) and issues centred on the equipment and its characteristics.

Equipment-centred issues

The combination of high-pressure and thermal processing of materials present problems of comparability of results and scale-up due to the individual characteristics of the equipment used (Balasubramaniam *et al*., 2004). Most of the problems arise from differing thermal characteristics. Many materials will exhibit the phenomenon of compression heating when subjected to the pressures involved in HPT processing. The temperature change is dependant not only on the pressure change but also on the material and the initial temperature. This not only applies to the sample, but also to materials used in the equipment, such as plastic sample holders and the pressure transmission fluid. For example, the rate of heat loss from the sample, the rate of pressure change and sample size can have effects. Although the pressure within a sample is uniform, the temperature may vary so producing inconsistent treatment within a sample. All of these characteristics have to be taken into consideration in creating a time, temperature and pressure profile, knowledge of which is vital to the production of reliable, comparable results.

Chemical changes in the sample

The extreme pressures involved in HPT treatments alter the pK_a of weak acids and bases within samples. This reversible change combined with temperature dependence of pH can result in a significant shift in pH in samples undergoing HPT treatment (Hamann, 1982; Mathys, Heinz, & Knorr, 2006). The importance of this to spore inactivation is not clear as the literature is contradictory. It has been reported that inactivation of *C. botulinum* was greater in mashed carrot than buffer and this was attributed to a differential shift in pH during high pressure (Margosch *et al*., 2004), whereas an apparent increase in inactivation was attributed to poorer outgrowth of spores in the pressure-treated food matrix (Raso & Barbosa-Cánovas, 2003).

Biological issues

A potential source of inconsistency between laboratories is the way that microbiological samples are prepared, processed and enumerated (Balasubramaniam *et al*., 2004). Variations in methods used in the production of vegetative cells and spores may result in unwanted variation that is difficult to separate from processing effects. For example, it has been shown that different sporulation media affect the resistance of spores of *C. botulinum* to HPT processing (Margosch *et al*., 2004) and there is considerable variation in the pressure and heat resistance between spores of different strains of *C. botulinum* (Margosch *et al*., 2004) (Tables 1 and 2). The enumeration of cells and spores following treatment is also a potential source of error. The enumeration techniques employed must take into account the resuscitation of sub-lethally injured organisms.

High pressure-mediated survival

A recent publication has shown that some intermediate combinations of high pressure and high temperature resulted in slower inactivation of *C. botulinum* and *B. amyloliquefaciens* endospores than thermal treatment alone (Margosch *et al*., 2006). There may be many reasons why this apparently antagonistic relationship had not previously been

widely reported. As mentioned above, it can be difficult to compare experiments, often because the thermal characteristics of apparatus vary considerably. However, the authors were careful to mimic the temperature profile of the HPT system in the thermal treatment at atmospheric pressure allowing comparison, and the number of spores undergoing treatment was higher than many other researchers use.

Kinetics/modelling

The kinetics of thermal inactivation of microorganisms is often described using a first order kinetics relationship despite many inactivation curves being non-linear. This is based on the assumption that all bacterial cells or spores in a population are equally resistant to some lethal factor and therefore a linear relationship between the decline in the logarithm of the number of survivors and time of treatment would be expected. However, curves obtained during high-pressure inactivation rarely obey first order linear kinetics. Frequently, in the first phase of high-pressure treatment the number of survivors decreases linearly, but the rate of inactivation gradually declines resulting in a ''tail'' of survivors (Cerf, 1977). Since there is a substantial number of experiments where tailing is reported, it seems unlikely that all of these are the result of experimental artifacts or mixed population. However, factors such as heterogeneous treatments, clumping between spores, adhesion to equipment, protective effects of dead spores and multiple inactivation mechanisms should not be ignored. Such non-linear kinetics require the application of appropriate mathematical functions for successful data analysis, although the fitting of data using primary models do not necessarily allow for prediction. Among models used to fit data are the log-logistic (Cole, Davies, Munro, Holyoak, & Kilsby, 1993), modified Gompertz (Bhaduri *et al*., 1991), Baranyi (Baranyi & Roberts, 1994) and the Weibull model, which has been used to describe thermal inactivation (van Boekel, 2002) and has been successfully applied to the thermal inactivation of spores of *Bacillus cereus* and *C. botulinum* (Mafart, Couvert, Gaillard, & Leguerinel, 2002). It fitted experimental data from HPT processing as well or better than log-logistic model (Chen, 2007; Chen & Hoover, 2003). Recently, Ratkowsky's square root model had also been successfully applied to predict *E. coli* inactivation kinetics by high-pressure processing (Koseki & Yamamoto, 2007). This model can be extended to include other factors influencing the process such as temperature, pH, water activity and nutrients (Ross, Ratkowsky, Mellefont, & McMeekin, 2003). As shown in Tables 1 and 2, the influence of temperature and pressure on inactivation is substantial. Therefore, such an extendable model is desirable in designing future experiments that will allow for better understanding of such complicated processes.

Summary

Interest in HPT processing technologies is predominantly in its potential for application to the sterilisation of foods. As such, the more resistant organisms need to be targeted, and these are the spores of pathogenic bacteria such as *C. botulinum*. It is apparent from the literature that the nature of the food matrix and its chemical environment are relevant to inactivation. Additionally, work carried out to date can be difficult to compare due to differences in equipment and experimental methodologies.

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