MINIREVIEW

High-pressure processing – effects on microbial food safety and food quality

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Received 30 October 2007; accepted 23 December 2007.
First published online 16 February 2008.

DOI:10.1111/j.1574-6968.2008.01084.x

Editor: Rustam Aminov

Keywords
high-pressure processing; nonthermal; minimally processed foods; food safety.

Abstract

High-pressure processing (HPP) is a nonthermal process capable of inactivating and eliminating pathogenic and food spoilage microorganisms. This novel technology has enormous potential in the food industry, controlling food spoilage, improving food safety and extending product shelf life while retaining the characteristics of fresh, preservative-free, minimally processed foods. As with other food processing methods, such as thermal processing, HPP has somewhat limited applications as it cannot be universally applied to all food types, such as some dairy and animal products and shelf-stable low-acid foods. Herein, we discuss the effects of high-pressure processing on microbial food safety and, to a lesser degree, food quality.

Introduction

The quality and safety of food products are among the most important factors influencing consumer choices in modern times, as well as being the most important considerations of food manufacturers and distributors (Ohlsson, 1994; Cardello *et al.*, 2007). It is therefore of utmost importance for the food industry to continue to seek out more effective methods to reduce undesirable changes in foods associated with food processing, such as loss of colour, flavour, texture, smell and, most importantly, nutritional value. High-pressure processing (HPP), also known as high hydrostatic pressure (HHP), is a relatively new, nonthermal food processing method that subjects foods (liquid or solid) to pressures between 50 and 1000 MPa (Fig. 1).

HPP treatment of food dates back over a century to the research of Hite (1899) in West Virginia, who performed experiments with milk, fruit and a variety of other foods at pressures far in excess of atmospheric pressure. In later years, Hite's studies extended to the pressure inactivation of viruses (Giddings *et al.*, 1929). Despite the pioneering efforts of Hite *et al.*, research in HPP was sporadic at best until the mid-1980s, which marked a resurgence of interest in commercial HPP treatment as an alternative to thermal processing of foods (Patterson, 2005). Over the last 20 years, significant advances in HPP technology have been made, in the form of semi-continuous systems to the scaling up of pilot units to successful commercially viable processes (Moreau, 1995). Current industrial HPP treatment of food is carried out using a batch or semi-continuous process; solid food can only be treated in a batch mode whereas liquid products can also be treated using a continuous or semi-continuous process (Hogan *et al.*, 2005). A prime example of the commercial importance of HPP food equipment at an industrial level is Avomex Inc., which began HPP-treating avocado using a 25 L batch processing unit in 1996 before investing in another 25 L as well as a 50 L vessel as product demand expanded. By 2000 the company had expanded further, investing in a semi-continuous unit and a larger 215 L batch processing vessel (Torres & Velazquez, 2005). It was in the early 1990s that the first commercial food applications of HP technology were seen, with the launch of the first HPP food, a high-acid jam marketed by the Japanese company Medi-Ya (Mertens, 1995). Due to the commercial success of jams, other products have since been marketed, such as HPP-treated jellies and shellfish in Japan, oysters and guacamole in the United States, and fruit juices in France, Mexico and the United Kingdom (Smelt, 1998; Torres & Velazquez, 2005). More recently, HPP has extended to include food products such as salsa, rice products, fish,

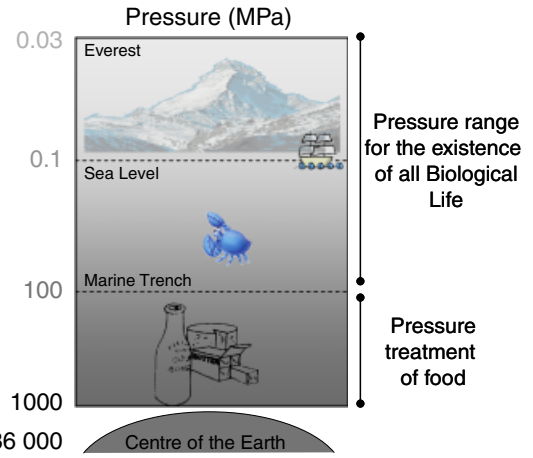

Fig. 1. Schematic representation of the pressures used in food processing.

meal kits [containing high pressure (HP)-treated cooked meats and vegetables], poultry products and sliced ready-to-eat meats (Murchie *et al.*, 2005; Goh *et al.*, 2007). HPP treatment of such foods has enabled the consumer to access foods with distinct advantages over thermally processed foods, such as minimally processed, fresh-tasting, high-quality convenient products with an extended shelf life.

The pressures applied to foods being processed is transmitted isostatically and instantaneously; thus the process is not dependent on the shape or size of the food (Smelt, 1998). The major advantage of this is that the food is treated evenly throughout, which can often be problematic in thermal processing of large or bulky food products. Pressurization of liquid or solid foods at room temperature is usually accompanied by a moderate temperature increase (*c.* 5–15 °C), termed adiabatic heating, depending on the food composition (Balasubramanian & Balasubramaniam, 2003). Foods cool down to their original temperature on decompression provided no heat is lost or gained during the pressure hold time (Hogan *et al.*, 2005).

Routinely used food preservation techniques are primarily evaluated based on their ability to eradicate pathogenic microorganisms, thereby improving food safety and extending product shelf life through the inactivation of spoilage microorganisms. HPP has a distinct advantage in this respect producing foods of superior quality and nutritional value than thermally processed products (Smelt, 1998). This review focuses on HPP treatment and its effects on the microbiology of food.

Effect of HPP on microbial food safety

Recent years have seen significant research on the inactivation of microorganisms in foods by HP (Dogan & Erkmen, 2004; Smiddy *et al.*, 2004, 2005; Donaghy *et al.*, 2007). Different microorganisms react with different degrees of resistance to HPP treatment, and indeed there can be vast HPP sensitivity among bacterial species and even strains (Alpas *et al.*, 1999; Benito *et al.*, 1999; Pagán & Mackey, 2000). Alpas *et al.* (1999) reported viability losses of between 0.5 and 8.5 logs among pathogenic bacterial strains. Prokaryotic cells tend to be more pressure resistant than eukaryotes (Patterson, 1999). Endospores tend to be extremely HPP resistant compared with vegetative cells, withstanding treatments of more than 1000 MPa (Smelt, 1998). HPP can induce germination of bacterial spores, the extent of which varies according to the growth medium and test organism (Smelt, 1998; Black *et al.*, 2005b). Spores of *Clostridium* spp. tend to be more pressure resistant than those of *Bacillus* spp. (Patterson, 1999). Combination treatments of heat and pressure applied simultaneously or sequentially, as well as pressure cycling treatments, have been studied, and although these methods achieve spore inactivation to some degree, complete efficacy depends on factors such as bacterial species, number of treatment cycles, pH, pressure, processing time and temperature (Mills *et al.*, 1998; Wuytack *et al.*, 1998; Farkas & Hoover, 2000; Torres & Velazquez, 2005). Wuytack *et al.* (1998) reported that germination of spores could be achieved using both low- and high-pressure treatments; however, spores germinated at lower pressures were in turn more sensitive to subsequent pressure treatments. Generally, gram-positive bacteria are more resistant to heat and pressure than gram-negative bacteria, and cocci are more resistant than rod-shaped bacteria (Smelt, 1998). Furthermore, it has been suggested that the complexity of the gram-negative cell membrane could be attributable to its HPP susceptibility (Murchie *et al.*, 2005). In comparison, yeasts and moulds are relatively HPP sensitive; however, ascospores of heat-resistant moulds such as *Byssochlamys*, *Neosartorya* and *Talaromyces* are generally considered to be extremely HPP resistant (Smelt, 1998; Chapman *et al.*, 2007). Pressure resistance of viruses varies considerably; HPP can cause damage to the virus envelope preventing the virus particles binding to cells or even complete dissociation of virus particles, which may be either fully reversible or irreversible (Hogan *et al.*, 2005). Prions, associated with neurological disorders, are generally even more difficult to destroy than bacterial spores, with some withstanding autoclave temperatures of 134 °C (Taylor, 1999); however, recent evidence suggests that some prions are affected by pressure in conjunction with a simultaneous treatment temperature of 60 °C (García *et al.*, 2004). García *et al.* (2004) suggested that the irreversible effects of HPP-thermal treatments of prions observed by another group (Brown *et al.*, 2003) were most likely due to, because HPP does not disrupt covalent bonds, changes in weak inter- and intramolecular interactions that affect the stability of the cross-β structure of amyloids thereby increasing digestion efficiencies with proteinase K. Prions are composed only of protein whose

response to HPP is driven by a protein volume change associated with conformational changes, aggregation and protein folding–unfolding (Garcia *et al*., 2005).

Information relating to the effect of HPP on the possibility of toxicity of HPP foods or associated allergens is rare, but warrants research; while the same observation can be made for research on bacterial and algal toxins (Torres & Velazquez, 2005; Rastogi *et al*., 2007), recent efforts have been made to address this, such as a study on the induction of Shiga toxin caused by HPP (Aertsen *et al*., 2005), and the apparent complete inactivation of Staphylococcal enterotoxins in cheese using ultrahigh pressure homogenization alone or in combination with HHP (López-Pedemonte *et al*., 2006). Among the bacterial species, the food poisoning bacteria *Staphylococcus aureus*, *Listeria monocytogenes*, *Vibrio* spp., *Salmonella* spp. and *Escherichia coli* O157:H7 are among the most extensively studied in terms of HPP (Styles *et al*., 1991; Mackey *et al*., 1994; Patterson & Kilpatrick, 1998; Berlin *et al*., 1999; Calik *et al*., 2002; Smiddy *et al*., 2005; Jofré *et al*., 2008).

The growth phase of bacteria also plays a role in determining their pressure sensitivity/resistance. Cells in the stationary phase of growth are generally more pressure resistant than those in the exponential phase (McClements *et al*., 2001; Mãnas & Mackey, 2004; Hayman *et al*., 2007); this is likely due to the synthesis of proteins that protect against a range of adverse conditions, such as high salt concentrations, elevated temperatures and oxidative stress (Hill *et al*., 2002). It has also been shown that the higher resistance of stationary phase cells to HPP is partly due to the presence of the RpoS protein in *E. coli* and SigB in *Listeria monocytogenes* (Robey *et al*., 2001; Wemekamp-Kamphuis *et al*., 2004), resulting in a mounted bacterial stress response.

The type of substrate and composition of the food can have a dramatic effect on the response of microorganisms during pressure treatment. Carbohydrates, proteins, lipids and other food constituents can confer a protective effect (Simpson & Gilmour, 1997; Garcia-Graells *et al*., 1999). This is probably due to the fact that, in contrast to heat, HPP does not denature covalent bonds, which in turn leaves primary protein structure largely unaffected (Murchie *et al*., 2005). Some foods appear to give more protection than others, and this may be due to the ability of rich media to provide essential vitamins and amino acids to stressed cells (Black *et al*., 2007). Complex media and some foods containing numerous ingredients have recently been shown to exert a baroprotective effect on microorganisms. Hauben *et al*. (1998) showed that Ca^{2+} and other cations protected *E. coli* from the effects of HPP while van Opstal *et al*. (2003) demonstrated the protective effect of high sucrose concentrations on *E. coli* exposed to high pressure. More recently, Black *et al*. (2007) reported the increased survival of *Listeria innocua* in simulated milk ultra filtrate with added calcium, magnesium, citrate and phosphate. Magnesium is known to have a stabilizing effect on ribosome structure; however, loss of essential ions like magnesium could trigger ribosome destabilization (Niven *et al*., 1999). Calcium stabilizes the outer membrane of cells as its concentration increases; therefore HPP would then have to inactivate bacterial cells via less sensitive targets (Hauben *et al*., 1998). Sucrose protects bacterial cells from the damaging effects of HPP inactivation by stabilizing membrane protein functionality (Mañas & Pagán, 2005). Furthermore, recent evidence from our laboratory has revealed that compatible solutes (such as betaine and carnitine) can also act as baroprotective compounds (Smiddy *et al*., 2004; Sheehan *et al*., 2006); however, because of this phenomenon, the use of laboratory media and buffers in place of real food situations is of little use, as a more severe treatment may be needed in foods to achieve the same levels of inactivation, as was observed by Patterson *et al*. (1995), Dogan & Erkmen (2004) and Smiddy *et al*.(2005). Compatible solutes play a role as stabilizers of enzyme function and as osmotic balancers (Hill *et al*., 2002).

Generally, low water activity (a_w) protects cells against HPP, but microorganisms that are injured by HPP are usually more sensitive to a_w (Smelt, 1998). The nature of the solute (i.e. sugar or salt) can have a significant effect on cell survival after pressure treatment, and in particular on the pressure resistance of spores. Patterson (1999) showed that ionic solutes such as NaCl and $CaCl_2$ conferred more protection on *Bacillus coagulans* than nonionic solutes such as sucrose and glycerol, and concluded that the spores of *B. coagulans* were better protected by high ion concentration rather than low a_w. Cheftel (1995) also observed that sucrose and salt content seem to exert a considerable baroprotective effect against HPP inactivation of microorganisms.

HPP only affects noncovalent bonds (i.e. ionic, hydrophobic and hydrogen bonds), which means that primary protein structures remain intact while alterations may occur in secondary, tertiary and quaternary structures in the form of protein unfolding for instance (Ross *et al*., 2003; Rastogi *et al*., 2007). Hoover *et al*. (1989) noted that the pH range for the growth of microorganisms narrows as a result of HPP causing an inhibitory effect on membrane ATPase, a vital enzyme in the acid–base physiology of cells. The environment around the microorganism can significantly influence HPP inactivation, e.g. low pH in a suspending medium can render pathogens more sensitive to the effects of HPP (Alpas *et al*., 2000). Studies carried out comparing buffers and food systems have indicated that pressurization of buffers generates an extensive shift in pH, which would render such data unrealistic in food situations (Patterson *et al*., 1995; Smelt, 1998). It is known that the pH of acidic solutions decreases as pressure increases (Patterson, 2005). When the treatment pressure is released, reversion to the

original pH value occurs. It is unknown whether the sudden pH change has an effect on microbial survival as well as the effect of pressure alone. Vegetative bacteria are relatively sensitive to pressure, heat and low pH (Smelt, 1998).

Temperature during HPP treatment can exert a significant impact on microbial survival and subsequent growth (Hogan *et al.*, 2005). Increased inactivation is usually observed at temperatures above or below ambient temperature. Refrigeration temperatures can enhance inactivation; for example, ewes' milk pressurized at 2 or 10 °C results in lower microbial numbers than in milk treated at 25 °C (Gervilla *et al.*, 1997). Increases in temperatures can trigger bacterial spore germination, which in turn renders the overall population more susceptible to high pressure (Black *et al.*, 2005b). Patterson & Kilpatrick (1998) reported inactivation of a pressure-resistant *E. coli* using a treatment of 400 MPa at 50 °C for 15 min. Neither 50 °C nor 400 MPa alone could achieve the level of inactivation reported (5–6 log reduction). Temperature and HPP can cause considerable microbial inactivation when applied alone, but it has been observed that these two treatments combined can confer dramatically improved inactivation levels, particularly with regard to bacterial spores. Combinations of temperatures used before, during and after HPP treatments have been conducted and their effects studied. Patterson (1999) noted that heating before HPP treatment, in contrast to heating during HPP, was more effective at inactivating spores, referencing a study carried out on *Clostridium sporogenes* spores. The use of mild–high temperatures during HPP treatment has also been studied with respect to *Bacillus cereus* spore producers (van Opstal *et al.*, 2004). This study found that inactivation levels of ≥ 5 log units was achieved for the *B. cereus* strains using a treatment of 500 MPa in combination with a temperature of 60 °C. Interestingly, it has also been observed that bacterial spores can be successfully inactivated by first inducing spore germination using relatively low HPP parameters, followed by complete inactivation and death of the spores using relatively mild heat treatments (Smelt, 1998).

Pressure treatment of microbial cells induces many changes in the bacterial cell, including inhibition of key enzymes, inhibition of protein synthesis, alterations in cell morphology and the cell membrane, as well as affecting the genetic mechanisms of the microorganism such as disruption of transcription and translation and cellular functions responsible for survival and reproduction (Patterson, 1999; Murchie *et al.*, 2005; Torres & Velazquez, 2005; Vogel *et al.*, 2005; Huppertz *et al.*, 2006; Abe, 2007). Bacterial membrane damage has multiple effects, causing leakage of cellular material through the inner and outer membranes as well as nutrient uptake and disposal of cell waste, as seen in studies reporting increased sensitivity to sodium chloride (Chilton *et al.*, 1997) and uptake of ethidium bromide and propidium iodide (Mackey *et al.*, 1995; Ritz *et al.*, 2001). Membrane permeabilization has been attributed to the compression and reduction of the phospholipid bilayer (Hoover *et al.*, 1989). Abe (2007) stated that ribosome dissociation has also been shown to limit cell viability at high pressures. The various morphological changes include separation of the cell membrane from the cell wall, contraction of the cell membrane, compression of gas vacuoles, cell lengthening and release of intracellular material (Patterson, 2005).

In recent times, research has focused on how HPP causes bacterial, viral and fungal inactivation. As a result, there has been more interest at the genetic and proteomic level to examine the stress response mounted by the microorganisms of interest. Microarray and proteome analysis has yielded valuable insight into possible genes or proteins that seem to be involved in conferring HPP resistance or survival. Such work has mainly concentrated on food pathogens such as *E. coli* (Ishii *et al.*, 2005; Malone *et al.*, 2006), *Listeria monocytogenes* (Bowman *et al.*, 2006) and lactic acid bacteria such as *Lactobacillus sanfranciscensis* (Vogel *et al.*, 2005). These studies and others (Aertsen *et al.*, 2004a, b, 2005) have revealed that HPP treatment induces, among others, oxidative stress, heat- and cold-stress responses, an SOS response, up-regulation of genes for chemotaxis, phosphotransferase systems, flagellar systems and genes involved in cell elongation and septum development. Further research is necessary to fully understand the implications that HPP has on bacterial survival, particularly in regard to food systems and *in vivo,* following ingestion. Based on this understanding, it may be possible to examine the potential of applying such information to the growing area of patho-biotechnology (i.e. describes the exploitation of pathogenic stress-survival strategies for beneficial food and medical applications and design of more versatile probiotic cultures; for reviews see Sleator & Hill, 2006, 2007, 2008) and determining whether transfer of HPP-resistance genes from one bacterium to another would confer nutritional or therapeutic benefit. Transfer of the betaine uptake system BetL (which confers high pressure resistance in *Listeria monocytogenes*), for example, into the probiotic strains *Lactobacillus salivarius* UCC118 and *Bifidobacterium breve* UCC2003 resulted in improved probiotic function both *in vitro* and *in vivo* (Sheehan *et al.*, 2006, 2007). The presence of *betL* enhanced the viability and facilitated the growth of both *Lactobacillus salivarius* UCC118 and *Bifidobacterium breve* UCC2003 under a range of stresses including acid, osmoprotectants, reduced temperature and extremes of pressure, which indicated a significant improvement of robustness of the probiotic strain. The clearest evidence of improved probiotic function was the observed protection against listerial infection in the spleen of mice (Sheehan *et al.*, 2007). BetL encodes a betaine transporter

from *Listeria monocytogenes,* which is highly specific for betaine and does not transport other compatible solutes such as carnitine and proline (Sleator *et al*., 1999). Abee & Wouters (1999) have a comprehensive section on betaine and its transport system for further reference.

Effect of HPP on food quality

HPP has the potential to produce high-quality foods that display characteristics of fresh products, are microbiologically safe and have an extended shelf life (Hogan *et al*., 2005; Patterson, 2005). HPP foods are currently considered novel foods as they fulfil two criteria: a new manufacturing process has been employed in their production, and their history of human consumption, to date, has been minimal (Hogan *et al*., 2005). Consumer perception of food quality depends not only on microbial quality but also on other food factors such as biochemical and enzymatic reactions and structural changes (Cheftel, 1995; Patterson, 2005). HPP can have an effect on food yield and on sensory qualities such as food colour and texture (Hogan *et al*., 2005).

The appearance and colour of food has been shown to significantly influence consumer sales. While some degree of protein denaturation can take place during HPP treatment of certain high-protein foods, the resulting changes in physical functionality and/or changes in raw product colour are significantly less than those experienced using conventional thermal processing techniques (Hogan *et al*., 2005). Indeed, HPP-induced protein denaturation can be reversible depending on treatment conditions (temperature, time and pressure) and also the type of protein (Rastogi *et al*., 2007). Using HPP conditions of 100–300 MPa, proteins usually, though not always, denature, dissolve or precipitate reversibly (Thakur & Nelson, 1998). In fresh meat and poultry, pressure-induced colour changes are due to changes in myoglobin, heme displacement/release or ferrous atom oxidation, which can result in a cooked-like appearance (Hugas *et al*., 2002). This means that the meat cannot then be sold as fresh meat. HPP of white or cured meats on the other hand rarely causes major colour changes (Cheftel & Culioli, 1997).

In addition to colour, food textural properties can have an enormous impact on product sales, e.g. soft/spongy foods could be perceived to be 'going off' or decaying. Therefore, a thorough understanding of the rheological properties of foods is essential for product development and quality control. The physical structure of most high-moisture food products remains unchanged after HPP exposure as the pressure exerted does not generate shear forces (Hogan *et al*., 2005); however, colour and texture may change in gas-containing products post-HPP treatment due to gas displacement and liquid infiltration into the collapsed gas pockets from the surrounding food structure. Shape distortion and physical shrinkage can occur due to the collapse of air pockets, generally causing irreversible compression of whole foods such as fruit (Hogan *et al*., 2005); however, fruit fragments are much less sensitive to such HPP treatment. Despite causing some undesirable textural changes, HPP can also be used to induce beneficial changes in product texture and structure such as melting of Mozzarella cheese during processing (O'Reilly *et al*., 2002).

One of the main benefits of HPP of food is the extension of shelf life while retaining the sensory characteristics of fresh food products (Patterson, 2005). Palou *et al*. (2002) reported that the delicate sensory attributes of avocado could be preserved using HPP while also conferring a reasonably safe and stable shelf life. In a sensory evaluation study that contained meat products treated with HPP and/or heat and untreated controls, panellists were unable to distinguish between them (Hugas *et al*., 2002). Product yield is of immense economic importance to food manufacturers and HPP treatment in general gives a higher food product yield compared with heat treatment, with effects depending on product type and treatment intensity (Hugas *et al*., 2002). Perhaps the best-documented example of a successful HPP effect on industry is the treatment of oysters. HPP denatures the adductor muscle, which enables easy opening of the oyster shell without causing knife damage to the product, thereby reducing the labour cost and risks associated with hand-shucking (Torres & Velazquez, 2005). HPP treatment increases the microbiological safety and shelf life of oysters by up to 3 weeks under refrigeration conditions and yield increases of up to 25% have been reported (Murchie *et al*., 2005; Torres & Velazquez, 2005).

Conclusion

HPP has significant potential and realized success in the food industry, as a new and novel technology that can achieve the same food safety standards as that of heat pasteurization, while at the same time meeting the demand for fresh-tasting minimally processed foods. HPP application can inactivate microorganisms and enzymes and modify structures, with little or no effect on the nutritional and sensory quality of foods. HPP is an industrially tested technology that offers a physical alternative to a wide range of food processing technologies. It prolongs shelf life while preserving organoleptic qualities by inactivating micro organisms and enzymes and leaving flavours and vitamins intact. Furthermore, combining HPP with other microbial agents such as lacticin 3147 (Ross *et al*., 2000), lactoperoxidase (Garcia-Graells *et al*., 2003) and nisin (Black *et al*., 2005a) has been shown to work synergistically to increase bacteriocidal effects. The combination of HPP with alternative nonthermal treatments for use as a hurdle technology

has immense possibilities, which are outside the scope of this article; however, comprehensive reviews discussing this have been published recently (Raso & Barbosa-Cánovas, 2003; Ross *et al.*, 2003). Nonetheless, food processors still face challenges in the form of extremely resistant bacterial spores and the capital cost of HPP equipment; however, with continued research and increased availability and accessibility of HPP (thereby reducing equipment costs), these problems will soon be overcome. For example, the large capital investment can be offset by operating HPP plants at full capacity and lowering processing costs by managing the pressures and processing times used (Rogers, 1999). HPP foods also have a distinct advantage over foods processed by other means, in that they have the potential to be marketed as value-added foods due to the retention of organoleptic and nutritional qualities similar to those of 'fresh' unprocessed products (Rastogi *et al.*, 2007). Information regarding the success of current food manufacturers and companies employing HPP technology to successfully process food in an efficient and safe manner for consumption as well as the scaling up from pilot units to commercially viable units (as in the case of Avomex Inc.) can only encourage other companies to realize the potential of HPP and the many benefits it can provide to both the consumer and industry, either alone or in combination with other processes as an alternative to thermal processing.

Acknowledgements

The authors acknowledge the financial assistance of the Department of Agriculture, Fisheries and Food through the Food Institutional Research Measure (FIRM) and The Alimentary Pharmabiotic Centre. Dr Roy Sleator is funded by the Health Research Board (Ireland).

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