Hurdle approach to increase the microbial inactivation by high pressure processing: Effect of essential oils

Elisa Gayán[1,2], J. Antonio Torres[1,*], and Daniel Paredes-Sabja[3,4]

[1]Food Process Engineering Group, Deptartment of Food Science and Technology, College of Agricultural Sciences, Oregon State University, Corvallis, OR, U.S.A.; [2]Tecnología de Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, C/Miguel Servet 177, CP 50013, Zaragoza, Spain; [3]Laboratorio de Mecanismos de Patogénesis Bacteriana, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile; [4]Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR, USA

* Correspondence author's email: J_Antonio.Torrres@OregonState.edu

Abstract

Consumer demand for improved-quality and fresh-like food products have led to the development of new non-thermal preservation methods. High pressure processing (HPP) is currently the novel non-thermal technology best established in the food processing industry. However, many potential HPP applications would require long treatment times to ensure an adequate inactivation level of pathogens and spoilage microorganisms. High hydrostatic pressure and the addition of essential oils (EOs) have similar effects on microbial structures and thus they may act synergistically on the inactivation of microorganisms. Therefore, the combination of high hydrostatic pressure with EOs is a promising alternative to expand the HPP food industry. In this work, findings on this scarcely investigated hurdle option have been reviewed with a focus on the mechanisms involved. The main mechanisms involved are: i) membrane permability induced by HPP and EOs facilitating the uptake of EOs by bacterial cells; ii) generation of reactive oxygen species via the Fenton reaction; iii) impairment of the proton motive force and electron flow; and iv) disruption of the protein-lipid interaction at the cell membrane altering numerous cellular functions. The effectiveness of a specific EO in enhancing the microbial inactivation level achieved by HPP treatments depends on the microbial ecology of the food product, the molecular mechanisms of the microbial inactivation by HPP, and the mode of action of the EO being used.

KEYWORDS: Hurdle technology; essential oil; microbial inactivation; high pressure processing; bacterial inactivation mechanism

1. Introduction

Consumer demand for fresh-like food products have led to the development of non-thermal preservation methods capable of extending food shelf-life and inactivating microbial pathogens without major changes in sensory and nutritional properties. High hydrostatic pressure processing (HPP), nowadays the most promising and widely utilized novel food preservation technology, consists of subjecting food products to hydrostatic pressure in the 100 to 700 MPa range to inactivate foodborne pathogens, spoilage microorganisms, and deteriorative enzymes with minimum effects on food quality (Mañas and Pagán 2005; Mújica-Paz et al. 2011; Torres et al. 2009; Torres and Velazquez 2005). Current HPP applications include processing of products such as fruit juices, smoothies, guacamole, seafood, processed meats, snacks and prepared meals (Rastogi et al. 2007).

Similarly to other food processing methods such as thermal treatments, bacterial pathogens have intrinsic mechanisms of pressure resistance, with gram-positive bacteria having higher resistance than gram-negative pathogens. Most striking, is the extreme pressure resistance of bacterial spores when compared to their vegetative counterparts (Patterson et al. 1995). Indeed, several studies have shown that while HPP treatments can effectively reduce the viable numbers of vegetative foodborne pathogens, they do not inactivate bacterial spores at ambient temperatures (San Martin et al. 2002), limiting HPP applications to be used only as pasteurization processes.

Numerous studies have shown that the kinetics of bacterial inactivation by HPP exhibit-first order kinetics plots with pronounced tails and/or shoulders (Koseki and Yamamoto 2007; Klotz et al. 2007; Saucedo-Reyes et al. 2009). The presence of a tail means that a small fraction of the population remains viable even after prolonged processing (Vurma et al. 2006). Tails could be due to in part to the large variability in

pressure resistance within the same bacterial species (e.g., *Escherichia coli* and *Listeria monocytogenes*), requiring the application of HPP for prolonged treatment times (Tay et al. 2003). Prolonged HPP treatments have high costs and can adversely affect the organoleptic attributes and ultimately reduce consumer acceptability (Cheftel 1995b, a), and thus limiting commercial and economical application of HPP technology (Lado and Yousef 2002). More troubling is the possibility that bacteria, particularly bacterial pathogens, might develop gigapascal-high pressure resistance (Vanlint et al. 2011). For example, exposure to multiple pressure cycles yielded *E. coli* O157:H7 strains with abnormal resistance to pressure (Hauben et al. 1997).

The limitations and challenges to achieve an effective HPP treatment could be overcome by hurdle technology approaches reducing processing time (Raso et al. 1998; Leistner 1992). Hurdle technology relies on the synergistic combination of moderate doses of bactericidal and/or bacteriostatic compounds in combination with conventional and novel food processing options to achieve an acceptable pathogen inactivation level (Rastogi et al. 2007). These combinations minimize the HPP treatment time and/or intensity resulting in an increased commercial feasibility while also improving the sensorial and nutritional quality of foods. However, the potential success of a specific hurdle strategy depends on an in-depth understanding of the microbial ecology of the particular product, and of the molecular mechanisms of microbial inactivation and microbial resistance of the particular compounds being employed to reduce the process intensity required. Consequently, a detailed knowledge of the key cellular pressure targets for each hurdle would help the development of combined hurdle/HPP preservation process by establishing the most effective treatment conditions. Therefore, the aim of this review is to provide an in-depth view of the mechanisms of inactivation

of bacterial cells of HPP technology and antimicrobial compounds and the effects of their combined application as a hurdle strategy.

2. HPP bacterial inactivation mechanisms

The antimicrobial HPP effect was demonstrated at the end of the 19th century in experiments performed by Hite (1899). HPP treatments cause several changes in the cell. Pressure in the range of 20 to 80 MPa inhibits cell division more than cell growth (Zobell and Cobert 1963), affects motility (Kitching 1957), and stops synthesis of DNA, RNA and proteins (Yayanos and Pollard 1969). Relatively moderate pressure (300-500 MPa) affects a variety of cellular processes and result mostly in sublethal bacteria injury, whereas at higher pressure the cellular membrane appears to be the primary site of damage resulting in a rapid increase in microbial inactivation rate (Ulmer et al. 2000; Casadei et al. 2002). Membrane lipids, typically in liquid state at room temperature, crystallize during pressure build up, altering the permeability of the cell membrane and the function of membrane proteins involved in the transport of solutes and ions (Cheftel 1995a, b) reducing the cells ability to maintain a pH gradient across the membrane (Wouters et al. 1998).

The pressure resistance of bacterial membranes depends on their fatty acid composition with unsaturated fatty acids resulting in more fluid membranes under high pressure and thus increasing their pressure resistance. HPP also causes partial or permanent membrane disruption of bacterial cell membranes. Some bacterial species, such as some *E. coli* strains undergo permanent membrane disruption and are unable to reseal their membranes after decompression (Benito et al. 1999; Pagán and Mackey 2000). However, some barotolerant *E. coli* strains are able to reseal their membranes after decompression and recover cell viability (Pagán and Mackey 2000). These

transient changes that affect membrane permeability can be exploited to introduce bactericidal and/or bacteriostatic compounds into bacterial cells to enhance the lethality of HPP treatments (Karatzas et al. 2001).

Another key target of HPP treatments is protein denaturalization, which may include conformational changes of ribosomes, and enzyme inactivation (Mañas and Mackey 2004). Interestingly, heat shock proteins including chaperones (DnaK, GrpE, GroES, and GroEL) and proteases involved in the degradation of denatured proteins (ClpB, ClpP and Lon), are synthesized in cells during exposure to sublethal pressures and in cells recovering from a pressure treatment (Welch et al. 1993; Aertsen et al. 2005; Aertsen et al. 2004). These proteins may refold or degrade damaged proteins enhancing the HPP resistance of bacterial cells.

In addition to the aforementioned target sites for the lethal effects of HPP treatments, other types of damage can also cause cell death. In this context, oxidative stress during HPP treatments appears to play an important role (Klotz et al. 2010), an hypothesis supported by the enhanced survival observed in the recovery of cells pressure-treated under anaerobic conditions (Cebrián et al. 2010), the increased pressure-sensitivity observed in *E. coli* strains with mutations in genes encoding for oxidative stress regulatory elements such as *oxyR* and *soxS*, as well as in genes encoding for HPII hydroperoxidase (*katE*) and superoxide dismutase (*sodAB*) (Aertsen et al. 2005), and the HPP-triggered release of iron from Fe-S clusters which generate free hydroxyl radicals via the Fenton reaction (Malone et al. 2006). These observations suggest that cell death caused by HPP treatments could be due in part to oxidative stress. In summary, HPP treatments are not an all-or-nothing events but a multi-target technology that may not inactivate all microorganisms and cause only injury to part of the cell population (Patterson 2005).

3. Hurdle technology based on HPP

An alternative to enhance the lethal effects of HPP on foodborne bacterial pathogens is to design a hurdle technology combining high pressure with mild heat treatment or nonthermal technologies (Kalchayanand et al. 2003). In addition to combining HPP with mild heat to enhance the inactivation of bacteria, HPP can be combined with bacteriocins (Lee and Kaletunç 2010), potassium sorbate (Mackey et al. 1995), or lysozyme (Tribst et al. 2008), all with effectiveness successfully demonstrated. The synergistic effects observed between HPP and these antimicrobial compounds are in part due to the HPP-induced damage in the bacterial cell membrane, facilitating the uptake of the antimicrobial agents into the cells (Wouters et al. 1998).

Essential Oils (EOs). A novel family of compounds successfully being employed in combination with HPP treatments is essential oils (EOs). These natural, volatile and complex compounds, produced as secondary metabolites by herbs, spices and aromatic plants, have antimicrobial effects and medical properties in addition to their unique aroma (Bakkali et al. 2008). In the food industry, they have been widely used as flavoring agents in food and beverages (Bakkali et al. 2008) and as antioxidants of lipids (Shahidi and Zhong 2010). Although the antimicrobial properties of EOs has been recognized since 1950s (Boyle 1955), their use as antimicrobial agents is a recent and growing trend reflecting the interest of producers and consumers to reduce the use of synthetic preservatives in foods, particularly to inhibit foodborne pathogenic, control spoilage bacteria and extend shelf life (Burt 2004). The biological properties of EOs are determined by its components, which are typically low molecular weight terpenes and terpenoids or aromatic and aliphatic molecules (Bakkali et al. 2008).

Terpenes and Terpenoids. These are hydrocarbons containing 5-carbon (C5) base units called isoprenes. Terpenes have the molecular formula $(C_5H_8)_n$, or $CH_2=C(CH_3)-CH=CH_2$ (for n = 1), and are classified according to the number of isoprene units as monoterpenes (2 isoprene units), sesquiterpenes (3 isoprene units), diterpenes (4 isoprene units), triterpenes (6 isoprene units), and tetraterpenes (8 isoprene units). The most common are monoterpenes (C10) and sesquiterpenes (C15). Terpenes are the most representative molecules constituting 90% of the essential oils and allow a great variety of structures with several functions including carbides, alcohols, aldehydes, ketones, esters, ethers, peroxides and phenols. Monoterpenes and sesquiterpenes are formed from the coupling of two isoprene units (C10) and three isoprene units (C15), respectively (Figure 1). The extension of the chain increases the number of cyclizations allowing a great variety of structures. Terpenoids are terpenes with oxygen-containing functions such as alcohols, aldehydes or ketones, and their building block is the hydrocarbon isoprene. Examples of plants containing these compounds are angelica, bergamot, caraway, celery, citronella, coriander, eucalyptus, geranium, juniper, lavandin, lavander, lemon, lemongrass, mandarin, mint, orange, peppermint, petitgrain, pine, rosemary, sage, and thyme (Bakkali et al. 2008).

Aromatic compounds. These phenylpropane derivatives are less abundant than terpenes and comprise aldehydes, alcohols, phenols, methylene, and methoxy, nitrogenous or sulfured compounds. Typical plant sources for these compounds are anise, cinnamon, clove, fennel, nutmeg, parsley, sassafras, star anise, tarragon, and other members from the Apiaceae, Lamiaceae, Myrtaceae, and Rutaceae botanical families. Nitrogenous or sulfured components such as glucosinolates or isothiocyanate

derivatives (garlic and mustard oils) are also characteristic secondary metabolites of diverse plants, and of grilled and roasted products (Bakkali et al. 2008).

Antimicrobial activity of EOs. The antimicrobial effects of EOs have been extensively studied *in vitro*, where essential oils are brought into direct contact with the selected microorganisms, and their inhibition is monitored by means of direct inspection or by measuring a physical property that is directly related with microbial growth, such as optical density, impedance, or conductance (Burt 2004). Although the precise antimicrobial mechanisms of EOs are not yet fully understood, primarily because of the great number of compounds involved, several cellular targets have been identified (Carson et al. 2002). The primary target is the microbial cell cytoplasmic membrane because the lipophilic nature of EOs allows them to translocate through the cell wall and cytoplasmatic membrane, and consequently increasing their permeability by disrupting the phospholipid bilayer (Sikkema et al. 1994). Fluorescent probes with propidium iodide, ethidium bromide and rhodamine B have shown how EOs induces permeability alteration in the cytoplasmatic membrane and leakage of ions and cytoplasmic contents (Lambert et al. 2001; Ultee et al. 1999). This permeabilization induced by EOs has been associated with the leakage of ions and other cell contents (Lambert et al. 2001; Cox et al. 2000). The ability of EOs to disrupt the membrane integrity also results in the disruption and coagulation of protein complexes embedded in the cell membrane as observed by transmission electron microscopy (Gustafson et al. 1998). These two subsequent events can impair the proton motive force and electron flow altering the pH gradient and the electrical potential by changes in ion transport or by depolarization through structural changes in the membrane and membrane embedded protein complexes. Leakage of ATP and disruption of ATPases localized in the

cytoplasmic membrane disturb the energy generation system (Helander et al. 1998; Lambert et al. 2001). Two alternative and complementary mechanisms have been suggested whereby cyclic hydrocarbon EOs might be inducing the aforementioned cellular damage. Lipophilic hydrocarbon molecules could accumulate in the lipid bilayer and distort the lipid-protein interaction. Alternatively, lipophilic compounds could have direct interaction with the hydrophobic regions of membrane proteins and protein complexes (Sikkema et al. 1995). On the other hand, phenolic EOs may interfere with cellular metabolic routes including forming complexes with substrates, disrupting membranes, inactivating enzymes, and chelating metals (Cowan 1999). Bacterial cells can tolerate some leakage and impairment of membrane function without loss of viability; however, extensive loss of cell contents and membrane function will lead to cell death (Denyer and Hugo 1991).

In general, EOs are more active against gram-positive than gram-negative bacteria (Delaquis et al. 2002; Lambert et al. 2001) primarily due to the restricted diffusion of hydrophobic compounds through the external lipopolysaccharide wall that surrounds the peptidoglycan in gram-negative bacteria (Vaara 1992). There are also nonphenolic constituents such as allyl isothiocyanate (AIT) in garlic oil which are more effective against Gram-negative bacteria (Yin and Cheng 2003; Ogawa et al. 1998). However, some EOs (e.g., oregano, clove, cinnamon and citral) are effective against both groups (Skandamis and Nychas 2000). In addition, EOs can prevent the growth and germination of bacterial spores (Chaibi et al. 1997). Indeed, a concentration of 0.5 g/L of cinnamaldehyde completely inhibited germination of *Alicyclobacillus acidoterrestris* spores for up to 13 d (Chaibi et al. 1997); however, further research is required to understand their effect on the mechanism of spore germination. The factors affecting bacterial resistance to EOs include the physiological stage of bacterial cells

with logarithmically growing cells being more susceptible than stationary phase cells to EOs (Phillips and Duggan 2002). Another factor is the microbial growth temperature with lower values decreasing EOs resistance as compared to near optimum growth temperature (Karatzas et al. 2000).

A challenging aspect of interpreting published research is the often large differences in the antimicrobial activity reported for the same plant EOs. This can be explained by the variability in the chemical composition and in the relative concentration of each EO constituent due to differences in harvesting season, plant genotype, climate effects, geographical source, plant drying procedures, and subsequent extraction typically via steam distillation (Monzote et al. 2006; Angioni et al. 2006). The chemical profile of EOs depends also on the extraction technique which varies with the intended application of the extract (Bakkali et al. 2008). For example, extracts containing oxygenated compounds have significantly more antimicrobial activity than hydrocarbon monoterpenes (Ait-Ouazzou et al. 2011).

Inactivation of foodborne bacterial pathogens with EOs. EOs are active against important bacterial pathogens such as *Salmonella, Staphylococcus aureus, Escherichia coli, Listeria monocytogenes* and *Yersinia enterocolitica*. However, the precise inactivation conditions of foodborne pathogens depend on the microbial ecology and complexity of the food matrix. The composition of the food matrix, pH and water activity, as well as the food storage temperature and headspace composition will directly affect the antimicrobial activity of EOs (Burt 2004). Bacterial pathogens are protected from EOs in foods with high levels of starch and fat, primarily because these food constituents trap EOs reducing their concentration in contact with bacterial cells (Mejlholm and Dalgaard 2002). Low pH enhances the antimicrobial activity of EOs by

increasing their association with bacterial membrane lipids (Juven et al. 1994) while high moisture and salt content facilitates their action (Wendakoon and Sakaguchi 1993; Tassou et al. 1995; Skandamis and Nychas 2000). Low oxygen, modified atmosphere and vacuum packaging conditions enhance synergistically the antimicrobial activity of EOs (Tsigarida et al. 2000; Skandamis and Nychas 2001). Because *Listeria monocytogenes* can tolerate anaerobic environments, grow in refrigerated products to high numbers (Vasseur et al. 1999), and cause listeriosis with most severe consequences in unborn children, neonates, pregnant women and the elderly even from low infective doses, its viability and growth rate reduction by EOs has driven considerable attention, particularly in refrigerated ready-to-eat foods (Rasooli et al. 2006; Lisbalchin and Deans 1997). Strong antilisterial activity is often correlated with EOs containing a high percentage of monoterpenes, eugenol, cinnamaldehyde, thymol, citronellol, limonene and geraniol (Somolinos et al. 2008).

4. Combined Application of HPP and EOs

The precise mechanism of inactivation for combined HPP/EOs treatments is unclear; however, the available evidence suggests a synergistic effect (Karatzas et al. 2001) (Vurma et al. 2006). For example, HPP treatments can injure bacterial cells by temporally disrupting membranes, and depending on their nature (see above), EOs will preferentially disrupt cytoplasmic membranes causing an increased permeability, decreasing the pH gradient in the cell, or alter osmoregulatory functions associated with the cytoplasmic membrane. These effects have been observed when combining HPP with the monoterpenoid phenol carvacrol ($C_6H_3CH_3(OH)(C_3H_7)$) inactivating *L. monocytogenes* (Karatzas et al. 2001). Cells treated with HPP and carvacrol did recover on non-selective media (Kalchayanand et al. 1998) but not in media with high NaCl

levels (O'Byrne and Booth 2002) or with carvacrol concentrations that when applied alone which caused only sublethal injury (Karatzas et al. 2001). These observations support the notion that HPP treatments alone causes only sublethal injuries, and thus full inactivation of bacterial pathogens requires the inhibition of cell repair, or the injury needs to be made lethal by combining HPP treatments and EOs. The effects of the latter strategy are described next and summarized in Table 1. The synergistic effect has been demonstrated in the fungus *Colletotrichum gloeosporioides* causing Anthracnose, the main papaya post-harvest disease. Spores of *C. gloeosporioides* in a saline solution were inhibited by a 350 MPa pressure treatment for 30 min. In combination with 0.75 mg/mL of citral or lemongrass oil, the pressure needed to achieve the same spore inhibition was 150 MPa (Palhano et al. 2004). However, effects observed in model solutions need to be interpreted with caution since food matrix components can affect the antimicrobial effect of EOs. For example, Karatzas et al. (2001) showed that although the synergistic effect of carvacrol and HPP observed in a buffer system also occurred in milk, the effect was at least two orders of magnitude lower in milk (3.2 log reduction) when compared to those observed in buffer (> 6 log reduction). These results are in agreement with reports that the effectiveness of EOs decrease in foods due to the presence of components, such as proteins and fats, which immobilize and inactivate components in EOs (Smid and Gorris 1999). The addition of mint EO to yogurt drink enhanced HPP inactivation of *L. innocua* and *L. monocytogenes* by more than one decimal reduction. Furthermore, this combination reduced the pressure treatment from 600 MPa/300 s to 100-300 MPa/210 s while ensuring more than 5 decimal reductions of *L. innocua*. HPP treatments alone, or combined with mint EOs, did not cause serum protein separation nor change the drink pH, water activity and color (Evrendilek and Balasubramaniam 2011). Commercially sterile sausages contaminated with

barotolerant *L. monocytogenes* were treated with selected combinations of TBHQ (100 to 300 ppm), nisin (100 and 200 ppm), and HPP (600 MPa, 28 ^{o}C, 5 min). HPP alone resulted in a modest decrease in the number of positive samples; however, *L. monocytogenes* was not detected in any of the inoculated commercial sausage samples after treatment with HPP-TBHQ or HPP-TBHQ-nisin combinations. These results suggest that addition of TBHQ or TBHQ plus nisin to sausage followed by in-package pressurization is a promising method for producing *Listeria*-free ready-to-eat products (Chung et al. 2005). Somolinos et al. (2008) reported a synergistic effect of HPP and citral on the inactivation of *L. monocytogenes* and *E. coli*. Citral is used as a flavoring in a variety of foods and is particularly recommended as an antimicrobial additive for soft drinks, orange juice, and apple juice where its odor is likely to be acceptable. The combination of citral and HPP achieved a higher degree of inactivation or a higher proportion of sublethally injured cells. They demonstrated that the extent of sublethal injury after HPP-citral treatments depend on the type of microorganism, and the pH and composition of the treatment medium. Chung (2008) studied the synergistic effect of butyl hydroquinone (BHQ) and HPP treatments on the inactivation of barotolerant strains of *E. coli* O157:H7 and *L. monocytogenes*. The pressure lethality threshold for the combination treatment was much lower for *E. coli* O157:H7 (200 MPa) than for *L. monocytogenes* (> 300 MPa). Differences in sensitivity to the treatment between these two pathogens may be attributed to differences in cell envelope composition and structure previously described. Malone et al. (2008) studied the molecular mechanism of the synergistic effect of tert-butyl hydroquinone (TBHQ) combined with HPP using selected *E. coli* mutants in genes maintaining redox homeostasis and anaerobic metabolism chosen because phenolic compounds have an antioxidant action owing their ability to scavenge oxidative-free radicals (Bors 1987). However, this work showed that

TBHQ contribute to the oxidative damage of HPP. It has been suggested that pressure treatment results in the release of iron from Fe-S clusters leading to the generation of hydroxyl free radical via the Fenton reaction (Malone et al. 2006). The release of iron ions in the cytosol from the pressure damaged [Fe-S] proteins results in the activation of TBHQ forming TBQ, semiquinone anion radical, and reactive oxygen species (ROS) leading to oxidative damage of DNA and cell membranes, or the generation of substrates for the Fenton reaction (Green and Paget 2004; Malone et al. 2008).

5. Conclusions and future directions

In conclusion, the combination of HPP with most EOs can be effectively employed to enhance food safety. This combination could be useful when inactivating pressure-resistant *L. monocytogenes* in food, one of the most important pathogens in ready-to-eat products, as well as other foodborne bacterial pathogens. Most promising is the ability of EOs to act synergistically with HPP, which allows a reduction on the concentrations of EOs incorporated during the formulation, and also of the HPP treatment intensity, leading to food products with higher sensorial properties and reductions in processing costs. An unexploited field that needs further attention to enhance HPP's preservation effect is the synergistic effect of EOs and HPP on bacterial spores, which is currently the main barrier for HPP not being considered a sterilization technology.

Table 1. Evaluation of the microbial inactivation by hurdle technology approaches based on high pressure processing

Compound tested	HPP Treatment	Microorganism	Media	Microbial reduction	Reference
Allyl isothiocyanate (80 µg/mL)	200 MPa/10 min (4 and 40 ºC)	*E. coli*	Low salt cucumbers	> 5 Log cycles	Ogawa et al. (1998)
Butylated hydroxyanisole (1.55 mM)	300 MPa/10 min	*L. monocytogenes*	Laboratory media	4 Log cycles	Mackey et al. (1995)
α-terpinene (150 µg/mL)	177 MPa/60 min	*S. cerevisae*	Laboratory media	2.8 Log cycles	Adegoke et al. (1997)
(R)-(+)-limonene (200 µg/mL)	177 MPa/60 min	*S. cerevisae*	Laboratory media	> 6 Log cycles	Adegoke et al. (1997)
Carvacrol (2.5-3 mM)	250-300 MPa/20 min	*L. monocytogenes*	Laboratory media	> 6 Log cycles	Karatzas et al. (2001)
Carvacrol (3 mM)	300 MPa/20 min	*L. monocytogenes*	Milk	3.2 Log cycles	Karatzas et al. (2001)
Citral (0.75 mg/mL)	150 MPa/30 min	*Colletotrichum gloeosporioides spores*	Laboratory media	> 7 Log cycles	Palhano et al. (2004)
Mint essential oil (0.5 and 1 µL/mL)	100-300 MPa/3.5 min	*L. innocua and L. monocytogenes*	Yogurt	> 5-6 Log cycles	Evrendilek and Balasubramaniam (2011)
TBHQ (100 ppm)	400 MPa/5min	*L. monocytogenes*	Laboratory media	4.2 Log cycles	Chung et al. (2005)
TBHQ (300 ppm)	600 MPa/5min	*L. monocytogenes*	Sausages	> 9 Log cycles	Chung et al. (2005)
TBHQ (100 ppm)	400 MPa/10 min	*L. monocytogenes*	Laboratory media (pH 7)	1.15 Log cycles	Somolinos et al. (2008)
Citral (1000 ppm)	300 MPa/10 min	*E. coli*	Laboratory media (pH 7)	0.3 Log cycles	Somolinos et al. (2008)
TBHQ (50 ppm)	500 MPa/1 min	*L. monocytogenes*	Laboratory media	6 Log cycles	Chung and Yousef (2008)
TBHQ (50 ppm)	200MPa/1 min	*E. coli*	Laboratory media	> 8 Log cycles	Chung and Yousef (2008)

References

Adegoke GO, Iwahashi H, Komatsu Y (1997) Inhibition of *Saccharomyces cerevisiae* by combination of hydrostatic pressure and monoterpenes. J Food Sci 62 (2):404-405

Aertsen A, De Spiegeleer P, Vanoirbeek K, Lavilla M, Michiels CW (2005) Induction of oxidative stress by high hydrostatic pressure in Escherichia coli. Appl Environ Microbiol 71:2226-2231

Aertsen A, Vanoirbeek K, De Spiegeleer P, Sermon J, Hauben K, Farewell A, Nystrom T, Mivhiels CW (2004) Heat shock protein-mediated resistance to high hydrostatic pressure in *Escherichia coli*. Appl Environ Microbiol 70:2660-2666

Ait-Ouazzou A, Cherrat L, Espina L, Lorán S, Rota C, Pagán R (2011) The antimicrobial activity of hydrophobic essential oil constituents acting alone or in combined processes of food preservation. Innov Food Sci Emerg Technol 12:320-329

Angioni A, Barra A, Coroneo V, Dessi S, Cabras P (2006) Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp *stoechas* essential oils from stem/leaves and flowers. J Agric Food Chem 54:4364-4370

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils – A review. Food Chem Toxicol 46:446-475

Benito A, Ventoura G, Casadei M, Robinson T, Mackey B (1999) Variation in resistance of natural isolates of *Escherichia coli* O157 to high hydrostatic pressure, mild heat, and other stresses. Appl Environ Microbiol 65:1564-1569

Bors WASM (1987) Radical scavenging by flavonoids antioxidants. Free Radic Res Commun 2:289-294

Boyle W (1955) Spices and essential oils as preservatives. The American perfumer and essential oil review 66:25-28

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods - a review. Int J Food Microbiol 94:223-253

Carson CF, Mee BJ, Riley TV (2002) Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob Agents Chemother 46:1914-1920

Casadei MA, Manas P, Niven G, Needs E, Mackey BM (2002) Role of membrane fluidity in pressure resistance of *Escherichia coli* NCTC 8164. Appl Environ Microbiol 68:5965-5972

Cebrián G, Michiels CW, Mañas P, Condón S (2010) Biological approach to modeling of *Staphylococcus aureus* high-hydrostatic-pressure inactivation kinetics. Appl Environ Microbiol 76:6982-6990

Chaibi A, Ababouch LH, Belasri K, Boucetta S, Busta FF (1997) Inhibition of germination and vegetative growth of *Bacillus cereus* T and *Clostridium botulinum* 62A spores by essential oils. Food Microbiol 14:161-174

Cheftel JC (1995a) High pressure, microbial inactivation and food preservation. Comptes Rendus de l'Academie d'Agriculture de France 81 (1):13-38

Cheftel JC (1995b) Review: High-pressure, microbial inactivation and food preservation. Food Sci Technol Int 1:75-90

Chung YK, Vurma M, Turek EJ, Chism GW, Yousef AE (2005) Inactivation of barotolerant *Listeria monocytogenes* in sausage by combination of high-pressure processing and food-grade additives. J Food Prot 68:744-750

Chung YK, Yousef AE (2008) Inactivation of barotolerant strains of *Listeria monocytogenes* and *Escherichia coli* O157:H7 by ultra high pressure and tert-butylhydroquinone combination. Journal of Microbiology 46:289-294

Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12:564–582

Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG (2000) The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J Appl Microbiol 88:170-175

Delaquis PJ, Stanich K, Girard B, Mazza G (2002) Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int J Food Microbiol 74:101-109

Denyer SP, Hugo WB (1991) Biocide-induced damage to the bacterial cytoplasmic membrane. The Society for Applied Bacteriology, Technical Series, Oxford Blackwell Scientific Publication, Oxford, UK

Evrendilek GA, Balasubramaniam VM (2011) Inactivation of *Listeria monocytogenes* and *Listeria innocua* in yogurt drink applying combination of high pressure processing and mint essential oils. Food Control 22:1435-1441

Green J, Paget MS (2004) Bacterial redox sensors. Nature Reviews Microbiology 2:954-966

JE, Liew YC, Chew S, Markham J, Bell HC, Wyllie SG, Warmington JR (1998) Effects of tea tree oil on *Escherichia coli*. Lett Appl Microbiol 26:194-198

Hauben KJ, Bartlett DH, Soontjens CC, Cornelis K, Wuytack EY, Michiels CW (1997) *Escherichia coli* mutants resistant to inactivation by high hydrostatic pressure. Appl Environ Microbiol 63:945-950

Helander IM, Alakomi HL, Latva-Kala K, Matilla-Sandholm T, Pol I, Smid EJ, Gorris LGM, von Wright A (1998) Characterization of the action of selected essential oil components on gram-negative bacteria. J Agric Food Chem 46:3590-3595

Hite BH (1899) The effects of pressure in the preservation of milk. Bulletin West Virginia University Agricultural Experiment Station, Morgantown 58:15-35

Juven BJ, Kanner J, Schved F, Weisslowicz H (1994) Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J Appl Bacteriol 76:626-631

Kalchayanand N, Dunne CP, Sikes A, Ray B (2003) Inactivation of bacterial spores by combined action of hydrostatic pressure and bacteriocins in roast beef. J Food Saf 23:219-231

Kalchayanand N, Sikes A, Dunne CP, Ray B (1998) Interaction of hydrostatic pressure, time and temperature of pressurization and pediocin AcH on inactivation of foodborne bacteria. J Food Prot 61:425-431

Karatzas AK, Bennik MHJ, Smid EJ, Kets EPW (2000) Combined action of S-carvone and mild heat treatment on *Listeria monocytogenes* Scott A. J Appl Microbiol 89 (296-301)

Karatzas AK, Kets EPW, Smid EJ, Bennik MHJ (2001) The combined action of carvacrol and high hydrostatic pressure on *Listeria monocytogenes* Scott A. J Appl Microbiol 90 (463-469)

Kitching JA (1957) Effects of high hydrostatic pressures on the activity of flagellates and ciliates. J Exp Biol 34:494-510

Klotz B, Mañas P, Mackey BM (2010) The relationship between membrane damage, release of protein and loss of viability in *Escherichia coli* exposed to high hydrostatic pressure. Int J Food Microbiol 137 (214-220)

Klotz B, Pyle DL, Mackey BM (2007) New mathematical modeling approach for predicting microbial inactivation by high hydrostatic pressure. Appl Environ Microbiol 73 (8):2468-2478
Koseki S, Yamamoto K (2007) Modelling the bacterial survival/death interface induced by high pressure processing. Int J Food Microbiol 116 (1):136-143
Lado BH, Yousef AE (2002) Alternative food-preservation technologies: efficacy and mechanisms. Microb Infect 4:433-440
Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE (2001) A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 91:453-462
Lee J, Kaletunç G (2010) Inactivation of *Salmonella Enteritidis* strains by combination of high hydrostatic pressure and nisin. Int J Food Microbiol 140:49-56
Leistner L (1992) Food preservation by combined methods. Food Res Int 25:151-158
Lisbalchin M, Deans SG (1997) Bioactivity of selected plant essential oils against *Listeria monocytogenes*. J Appl Microbiol 82:759-762
Mackey BM, Forestiere K, Isaacs N (1995) Factors affecting the resistance of Listeria monocytogenes to high hydrostatic pressure. Food Biotechnol 9:1-11
Malone AS, Chung YK, Yousef AE (2006) Genes of *Escherichia coli* O157:H7 that are involved in high-pressure resistance. Appl Environ Microbiol 72:2661-2671
Malone AS, Chung YK, Yousef AE (2008) Proposed mechanism of inactivating *Escherichia coli* O157:H7 by ultra-high pressure in combination with tert-butylhydroquinone. J Appl Microbiol 105:2046-2057
Mañas P, Mackey BM (2004) Morphological and physiological changes induced by high hydrostatic pressure in exponential- and stationary-phase cells of *Escherichia coli*: Relationship with cell death. Appl Environ Microbiol 70:1545-1554
Mañas P, Pagán R (2005) Microbial inactivation by new technologies of food preservation. J Appl Microbiol 98:1387-1399
Mejlholm O, Dalgaard P (2002) Antimicrobial effect of essential oils on the seafood spoilage micro-organism *Photobacterium phosphoreum* in liquid media and fish products. Lett Appl Microbiol 34:27-31
Monzote L, Montalvo AM, Almanonni S, Scull R, Miranda M, Abreu J (2006) Activity of the essential oil from *Chenopodium ambrosioides* grown in cuba against *Leishmania amazonensis*. Chemotherapy 52:130-136
Mújica-Paz H, Valdez-Fragoso A, Tonello Samson C, Welti-Chanes J, Torres JA (2011) High-pressure processing technologies for the pasteurization and sterilization of foods. Food and Bioprocess Technology 4 (6):969-985. doi:doi: 10.1007/s11947-011-0543-5
O'Byrne CP, Booth IR (2002) Osmoregulation and its importance to food-borne microorganisms. Int J Food Microbiol 74:203-216
Ogawa T, Matsuzaki H, Isshiki K (1998) Bacterial control by hydrostatic pressure treatment with addition of allyl isothiocyanate. Nippon Shokuhin Kagaku Kogaku Kaishi 45 (6):349-356
Pagán R, Mackey B (2000) Relationship between membrane damage and cell death in pressure-treated *Escherichia coli* cells: Differences between exponential- and stationary-phase cells and variation among strains. Appl Environ Microbiol 66:2829-2834
Palhano FL, Vilches TTB, Santos RB, Orlando MTD, Ventura JA, Fernandes PMB (2004) Inactivation of *Colletotrichum gloeosporioides* spores by high

hydrostatic pressure combined with citral or lemongrass essential oil. Int J Food Microbiol 95:61-66
Patterson MF (2005) A review: Microbiology of pressure-treated foods. J Appl Microbiol 98:1400-1409
Patterson MF, Quinn M, Simpson R, Gilmour A (1995) Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate buffered saline and foods. J Food Prot 58:524-529
Phillips CA, Duggan J (2002) The effect of temperature and citric acid, alone, and in combination with nisin, on the growth of Arcobacter butzleri in culture. Food Control 13:463-468
Raso J, Barbosa-Cánovas GV, Swanson BG (1998) Sporulation temperature affects initiation of germination and inactivation by high hydrostatic pressure of *Bacillus cereus*. J Appl Microbiol 85:17-24
Rasooli I, Rezaei MB, Allameh A (2006) Ultrastructural studies on antimicrobial efficacy of thyme essential oils on *Listeria monocytogenes*. Int J Infect Dis 10:236-241
Rastogi NK, Raghavarao KSMS, Balasubramaniam VM, Niranjan K, Knorr D (2007) Opportunities and challenges in high pressure processing of foods. Crit Rev Food Sci Nutr 47 (1):69-112
San Martin MF, Barbosa-Cánovas GV, Swanson BG (2002) Food processing by high hydrostatic pressure. Crit Rev Food Sci Nutr 42 (6):627-645
Saucedo-Reyes D, Marco-Celdrán A, Pina-Pérez MC, Rodrigo D, Martínez-López A (2009) Modeling survival of high hydrostatic pressure treated stationary- and exponential-phase *Listeria innocua* cells. Innovative Food Science & Emerging Technologies 10:135-141
Shahidi F, Zhong Y (2010) Novel antioxidants in food quality preservation and health promotion. Eur J Lipid Sci Technol 112:930-940
Sikkema J, Debont JAM, Poolman B (1994) Interactions of cyclic hydrocarbons with biological membranes. J Biol Chem 269:8022-8028
Sikkema J, Debont JAM, Poolman B (1995) Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 59:201-222
Skandamis PN, Nychas GJE (2000) Development and evaluation of a model predicting the survival of *Escherichia coli* O157:H7 NCTC 12900 in homemade eggplant salad at various temperatures, pHs, and oregano essential oil concentrations. Appl Environ Microbiol 66:1646-1653
Skandamis PN, Nychas GJE (2001) Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. J Appl Microbiol 91:1011-1022
Smid EJ, Gorris LGM (1999) Natural antimicrobials for food preservation. In: Rahman MS (ed) Handbook of Food Preservation. Marcel Dekker Inc., New York, NY, pp 285-308
Somolinos M, García D, Pagán R, Mackey B (2008) Relationship between sublethal injury and microbial inactivation by the combination of high hydrostatic pressure and citral or tert-butyl hydroquinone. Appl Environ Microbiol 74:7570-7577
Tassou CC, Drosinos EH, Nychas GJE (1995) Effects of essential oil from mint (Mentha-piperita) on *Salmonella Enteritidis* and *Listeria monocytogenes* in model food systems at 4 degrees and 10 degrees. J Appl Bacteriol 78:593-600

Tay A, Shellhammer TH, Yousef AE, Chism GW (2003) Pressure death and tailing behavior of *Listeria monocytogenes* strains having different barotolerances. J Food Prot 66:2057-2061

Torres JA, Sanz PD, Otero L, Pérez Lamela C, Saldaña MDA (2009) Engineering principles to improve food quality and safety by high pressure processing. In: Ortega-Rivas E (ed) Processing effects on safety and quality of foods. Contemporary Food Engineering. CRC Taylor & Francis, Inc., Boca Raton, FL, pp 379-414

Torres JA, Velazquez G (2005) Commercial opportunities and research challenges in the high pressure processing of foods. J Food Eng 67 (1-2):95-112

Tribst AAL, Franchi MA, Cristianini M (2008) Ultra-high pressure homogenization treatment combined with lysozyme for controlling *Lactobacillus brevis* contamination in model system. Innov Food Sci Emerg Technol 9:265-271

Tsigarida E, Skandamis P, Nychas GJE (2000) Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 degrees C. J Appl Microbiol 89:901-909

Ulmer HM, Gaenzle MG, Vogel RF (2000) Effects of high pressure on survival and metabolic activity of *Lactobacillus plantarum* TW1.460. Appl Environ Microbiol 66:3966-3973

Ultee A, Kets EPW, Smid EJ (1999) Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl Environ Microbiol 65:4606-4610

Vaara M (1992) Agents that increase the permeability of the outer membrane. Microbiol Rev 56:395-411

Vanlint D, Mitchell R, Bailey E, Meersman F, McMillan PF, Michiels CW, Aertsen A (2011) Rapid acquisition of gigapascal-high-pressure resistance by Escherichia coli. MBio 2:e00130-10. . mBio 2 (1):e00130-00110. doi:10.1128/mBio.00130-10

Vasseur C, Baverel L, Hebraud M, Labadie J (1999) Effect of osmotic, alkaline, acid or thermal stresses on the growth and inhibition of *Listeria monocytogenes*. J Appl Microbiol 86:469-476

Vurma M, Chung YK, Shellhammer TH, Turek EJ, Yousef AE (2006) Use of phenolic compounds for sensitizing *Listeria monocytogenes* to high-pressure processing. Int J Food Microbiol 106:263-269

Welch TJ, Farewell A, Neidhardt FC, Bartlett DH (1993) Stress response of *Escherichia coli* to elevated hydrostatic pressure. J Bacteriol 175:7170-7177

Wendakoon CN, Sakaguchi M (1993) Combined effect of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract. J Food Prot 56:410-413

Wouters PC, Glaasker E, Smelt JPPM (1998) Effects of high pressure on inactivation kinetics and events related to proton efflux in *Lactobacillus plantarum*. Appl Environ Microbiol 64:509-514

Yayanos AA, Pollard EC (1969) A study of the effects of hydrostatic pressure on macromolecular synthesis in *Escherichia coli*. Biophys J 9:1464-1482

Yin MC, Cheng WS (2003) Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. Meat Science 63:23-28

Zobell CE, Cobert AB (1963) Filament formation by *Escherichia coli* at increased hydrostatic pressures. J Bacteriol 87:710-719