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Inactivation of microorganisms by high isostatic pressure processing in complex matrices: A review

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ABSTRACT

The benefits of high pressure processing (HPP) for microbial inactivation in food production include reduced thermal treatment and minimized effects on sensory and nutritional profiles. These benefits have resulted in increasing commercial production of high pressure pasteurized foods. In this review, the current state of the art in terms of vegetative cell and bacterial spore inactivation by HPP in complex food matrices is assessed with an emphasis on mechanisms of inactivation and treatment of products that have low or non-uniform water activity (a_w) profiles. Low a_w can be the result of a high concentration in solutes, the presence of oils/fats, or the physical removal of water through dehydration. Microbial inactivation in low a_w environments remains a particular challenge for HPP and studies on microbial inactivation observed in the different types of low a_w food matrices are reviewed in detail.

Industrial relevance: HPP-treated food products with low a_w have been on the market since the nineties, but the mechanisms of microbial inactivation at low a_w are still not well understood, which hinders the development of new applications in low or inhomogeneous a_w food. This review summarizes the state of the art in terms of HPP microbial inactivation mechanisms in model systems and various low a_w food environments. Thereby, it identifies existing and potential new applications as well as the current gaps and future research needs.

Contents

1. Introduction

High pressure processing (HPP) is a non-thermal processing technology of food which was first investigated in the late nineteenth

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century by Hite (1899). Following this, early work focused on understanding the biological effects of high pressure on food microorganisms and how this could open opportunities for food preservation (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989). Since then, a significant research effort has enabled the development of this tool and its transfer to the industrial-scale. To date, it has been mainly applied in the food industry as a post-packaging pasteurization step allowing for vegetative microorganisms inactivation. HPP provides a gentle pasteurization method in comparison to conventional thermal processing with minimal effects on sensory and nutritional profiles (Heinz & Buckow, 2010). The inactivation of bacterial spores by HPP has also been investigated and high pressure high temperature sterilization could produce uniform, minimally processed foods of higher quality (Mathys, 2008) than heat treatment alone (Georget et al., 2013). It has, however, not yet been successfully introduced into the food industry, possibly due to limited knowledge regarding the inactivation mechanisms of high resistant bacterial spores as well as technical limitations (Reineke, 2012).

A common element to all decontamination (pasteurization or sterilization) strategies by HPP is the need to account for the food matrices hosting these microorganisms. Food matrices are complex environments which may offer shelter to microorganisms, even under harsh treatment conditions. Specifically, low water activity (a_w) matrices have been shown to be particularly challenging to achieve microbial decontamination by any kind of decontamination strategies, including HPP (Doona & Feeherry, 2007).

However, low a_w products do not constitute a homogeneous category and the low a_w can result from different compositions or properties. The a_w in a food matrix can be influenced, locally or overall, by a high concentration in solutes, the presence of oils/fats, or the physical removal of water through dehydration. As a result, inactivation challenges in low or inhomogeneous a_w environments might be associated with different, and possibly complementary, mechanisms.

In this work, the current state of the art in terms of microorganism inactivation by HPP in complex matrices is reviewed. First, the application and limitations of high pressure processing for food preservation are presented. Then, high pressure inactivation of microorganisms in food matrices with low or variable a_w is introduced through 1) the impact of different solutes and resulting microorganism protection against HPP inactivation, 2) the impact of dehydration of food systems on the a_w and resulting microorganism protection against HPP inactivation, and finally 3) the impact of fats and oils on local a_w in food matrices and resulting effect on microbial inactivation by HPP.

2. Application of high pressure processing for preservation and current limitations

2.1. Vegetative microorganisms' inactivation by HPP

The use of HPP to inactivate pathogenic or spoilage vegetative microorganisms has been largely investigated for the pasteurization of commercial products for decades (Heinz & Buckow, 2010). Hite was the first to conduct experiments with high pressure in combinations with foods to extend shelf life in 1899, and reported that milk stayed sweet longer after the treatment with high pressure (Hite, 1899). Since then, significant research effort has focused on understanding the underlying mechanisms of the inactivation of microorganisms under high pressure conditions. HPP offers a lower thermal input into the product by comparison with conventional thermal treatment and therefore increases the quality of the food while maintaining food safety (Balasubramaniam, Farkas, & Turek, 2008; Barba, Esteve, & Frígola, 2012; Bermúdez-Aguirre & Barbosa-Cánovas, 2011; Bolumar, Georget, & Mathys, 2014; Hogan, Kelly, & Sun, 2005; Smelt, 1998). Despite the steadily increasing commercial production of high pressure pasteurized food with more than 500,000 t/y (Tonello Samson, C. 2014, Hiperbaric, Spain, personal communication), some important scientific and technological questions are still unresolved.

One of these issues is the impact of different intrinsic and extrinsic factors on the inactivation mechanisms of vegetative bacteria and bacterial spores under pressure. To unravel the impact of the different pressure and temperature combinations on a possible cell death or recovery, detailed analyses about the physiological state of the cells and how they are influenced by different food constitutes are needed. According to Le Chatelier's principle in a system facing a shift of equilibrium, all cellular components are affected by high pressure, including the cell membrane and its membrane proteins, enzymes and ribosomes as well as all the cell metabolism (Heremans, 2002; Smelt, Hellemons, & Patterson, 2001; Winter & Jeworrek, 2009). In general, prokaryotic cells show a higher resistance towards pressure than eukaryotic cells. Yeast and molds are in general more pressure sensitive although ascospores of some molds such as *Byssochlamys* and *Talaromyces* can be very pressure-resistant (Chapman et al., 2007; Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008; Smelt, 1998). Within prokaryotes, gram positive microorganisms such as *Bacillus*, *Listeria*, *Staphylococcus* and *Clostridium* have a thicker peptidoglycan layer and are therefore generally more pressure resistant than gram-negative microorganisms (Considine et al., 2008; Dumay, Chevalier-Lucia, & Lopez-Pedemonte, 2010; Smelt, 1998).

The mechanisms leading to cell death have been investigated in several bacterial species (Huang, Lung, Yang, & Wang, 2014). However, the particular events leading to inactivation are not well understood (Buckow & Heinz, 2008; Cheftel, 1995; Klotz, Manas, & Mackey, 2010). High pressure between 300 and 800 MPa at ambient temperatures can lead to the unfolding and denaturation of important cell enzymes and proteins in vegetative microorganisms (Knorr, Reineke, Mathys, Heinz, & Buckow, 2011; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007), but the specific pressure effects on microorganism are more complex and several different mechanisms leading to cell death can occur simultaneously when high pressures are applied. Primarily, pressure at a sufficiently high level, can induce enzyme inactivation, membrane protein denaturation and cell membrane rupture caused by a phase transition of the membrane and change in its fluidity (Abe, 2013; Ananta, Heinz, & Knorr, 2005; Winter & Jeworrek, 2009). The pressure level needed to achieve a 5 $\log_{10}$ reduction of pathogenic microorganism in different food-products ranges from 300 to 800 MPa (Hendrickx & Knorr, 2002) and often synergism between pressure and temperature is observed (Buckow & Heinz, 2008). By increasing the process pressure, it is possible to decrease the temperature needed to achieve the same inactivation. According to Smelt et al. (2001) the pressure induced effects leading to cell death of vegetative microorganisms can be attributed to four factors:

(I) Protein and enzyme unfolding, including partial or complete denaturation;
(II) Cell membranes undergoing a phase transition and change of fluidity;
(III) Disintegration of ribosomes in their subunits; and
(IV) Intracellular pH changes related to the inactivation of enzymes and membrane damage (Knorr et al., 2011; Molina-Gutierrez, Stippl, Delgado, Gänzle, & Vogel, 2002).

2.2. Sporulated microorganisms' inactivation by HPP

While high pressure pasteurization is already established, high pressure sterilization is not yet implemented within the food industry, even though the process of Pressure Assisted Thermal Sterilization for mashed potatoes filed at the FDA was accepted in 2009 (Illinois Institute of Technology, 2009). This lies mostly in the current limitation in achieving full spore inactivation and a gap of knowledge in the mechanistic impact of high pressure in combination with high temperature on spores. Moreover, the lack of alignment in the choice of a (product-specific) bacterial strain(s) for validation and the absence of industrial-scale systems with adequate temperature homogeneity at