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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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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