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Favourable effects of soluble gas stabilisation and modified atmosphere for supressing regrowth of high pressure treated *Listeria innocua*

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ABSTRACT

Bacteria can be injured or inactivated by high pressure processing (HPP), depending on the pressure level, holding time and bacterial strains and species. Fish soup was in this study treated with high pressure (400 and 600 MPa, 2 min) in combination with different packaging regimes; vacuum, modified atmosphere packaging (MAP), soluble gas stabilisation (SGS), and a combination of the two latter ones (SGS-MAP). The fish soup was before treatment inoculated with *Listeria innocua*, and stored at 5 °C and further analysed for a period up till 49 days. HPP gave a reduction of *L. innocua* of 3.5 and 7.3 log cfu/g immediately after exposure to 400 and 600 MPa, respectively. Both SGS and MAP showed a significant ($P < 0.01$) interaction with pressure. SGS in combination with pressure significantly ($P < 0.001$) inhibited the growth of *L. innocua* during storage. The same was observed when packaging in MAP ($P < 0.001$). Bacterial plating on non-selective and selective agar revealed that over 99.9% of the surviving cells after HPP treatment in combination with SGS or SGS-MAP were sublethally injured.

1. Introduction

High pressure processing (HPP) is a processing technique used at low or moderate temperatures to enhance the shelf life of food and inactivate microorganisms (Alpas et al., 1999; Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008; Rendueles et al., 2011; San Martin, Barbosa-Canovas, & Swanson, 2002). HPP has been used for the preservation of various food products, and there are several commercial products available especially in the juice and cooked meat segment.

Listeria is widespread in nature, and can occasionally be detected in food. *Listeria monocytogenes* can cause foodborne disease, and is often the cause of food recalls (Teratanavat & Hooker, 2004). Compared with other non-spore forming bacteria *L. monocytogenes* has the capacity to survive many food hurdles, and the ability to grow at very low temperature. A growth temperature range from −1.5 to 50 °C permits growth of *Listeria* in refrigerated foods (Maukonen et al., 2003). The use of *L. monocytogenes* for microbial studies in a production facility, and equipment used for food production, is challenging. Several strains of *Listeria innocua* have been found to be more heat resistant than *L. monocytogenes*, and accepted as suitable surrogates when studying thermal inactivation (Ahn & Balasubramaniam, 2007; Lorentzen, Ytterstad, Olsen, & Skjerdal, 2010), as *L. innocua* has high phenotypic similarity to *L. monocytogenes*. *L. innocua* has previously been used as a surrogate in HPP studies (Pathanibul, Taylor, Davidson, & Harte, 2009) and the growth kinetics under HPP has been investigated (Buzrul & Alpas, 2004; Saucedo-Reyes, Marco-Celdran, Pina-Perez, Rodrigo, & Martinez-Lopez, 2009).

The effectiveness of HPP for inactivation of microorganisms in foods depend on several factors; e.g. type of microorganism, pressure level, holding time, type of food and food additives. Studies have shown that HPP can give high microbial inactivation immediately after processing. However, if not all bacteria are killed and only injured, bacteria like *Listeria* can have the ability to recover during storage, and start to grow after a shorter or longer recovery period. Several studies have reported recovery of HPP injured bacteria (Bozoglu, Alpas, & Kaletunç, 2004; Bull, Hayman, Stewart, Szabo, & Knabel, 2005; Munoz-Cuevas et al., 2013; Picouet, Cofan-Carbo, Vilaseca, Ballbè, & Castells, 2010). To minimise the outgrowth of HPP resistant cells, it can be effective to combine HPP with one or several other treatments. Examples can be heat,

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packaging and antimicrobial agents (Bolumar, Andersen, & Orlien, 2011; Marcos, Aymerich, Garriga, & Arnau, 2013; Patterson & Kilpatrick, 1998).

The use of modified atmosphere (MA) packaging (MAP) is a well-known method for extending the shelf life and maintaining high quality of different food products (Lambert, Smith, & Dodds, 1991; Sivertsvik, Jeksrud, & Rosnes, 2002). A way to increase the effect of MAP, is to dissolve CO_2 into the product prior to packaging. This method is called soluble gas stabilisation (SGS) (Sivertsvik, 2000), and have the potential to prevent packaging collapse even at high filling degree, without compromising with the quality and shelf life of the packaged product (Rotabakk, Birkeland, Jeksrud, & Sivertsvik, 2006; Rotabakk, Birkeland, Lekang, & Sivertsvik, 2008; Sivertsvik & Birkeland, 2006; Sivertsvik, Rosnes, & Jeksrud, 2004).

L. monocytogenes demonstrates the ability to grow in CO_2 rich atmosphere, but increased levels of CO_2 reduces the grow rate (Farber, 1991; Provincial et al., 2013). Combination of different hurdle effects to decrease the growth of *L. monocytogenes* in CO_2 systems is therefore necessary to investigate (Van Houteghem et al., 2008).

Studies combining pressure and gases have shown to be effective for inactivation of bacteria *in vitro* (Debs-Louka, Louka, Abraham, Chabot, & Allaf, 1999; Thom & Marquis, 1984), but little is reported in real food systems. Amanatidou et al. (2000) showed that a combination of HPP (150 MPa, 10 min, 5 °C) and MAP (50% oxygen and 50% carbon dioxide) extended the shelf life of salmon, and had a positive effect on reducing *L. monocytogenes*. In a recent study of Al-Nehlawi, Guri, Guamis, & Saldo (2014) combining HPP (350 MPa, 10 min) and CO_2 (99%) reduced the growth of *L. innocua*, *Leuconostoc carnosum*, *Brochothrix thermosphacta*, and *Campylobacter jejuni* in poultry sausage during storage. Dissolving carbon dioxide in milk prior to heat treatment has shown to increase the thermal inactivation of microorganisms (Loss & Hotchkiss, 2002). This approach has to our knowledge, not been used on products subjected to HPP.

The aim of this study was to assess the effects of combining high pressure processing and different packaging regimes for inactivation of *L. innocua* in fish soup and preventing its regrowth. *L. innocua* was used as a non-pathogenic surrogate for *L. monocytogenes* in this study, and it is expected to have similar sensitivity to pressure (Basaran-Akgul et al., 2010).

2. Materials and methods

2.1. Design

Different HPP treatments (0.1, 400 and 600 MPa) were combined with four different packaging regimes (vacuum; MAP; SGS; SGS and MAP). *L. innocua* was added to the soup prior to the treatments. The effect of how these 12 different treatment variants would affect the shelf life and reduction of *L. innocua* in a milk-based fish soup was studied. After 0, 12, 26, 39 and 48 days of storage, fish soup samples were assessed for cultivable *L. innocua*, head space gas composition and pH analyses. All analyses were carried out with three technical replicates for each treatment, if nothing else is stated. The experiment was performed twice, on different days.

2.2. Samples

Fish soup was diary based, and made using the recipe of a commercial product. Ingredients consisted of approx. 2% fish bouillon, 8–8.5% fat (from butter, milk and cream), 0.66% salt and the rest being mainly water. The soup was cooked, packed and treated with a sterilisation program $F_{121^{\circ}C}^{10^{\circ}C} \geq 3$ min based on core temperature. The soup was stored at 1 °C prior to use.

2.3. Bacteria and growth conditions

L. innocua ATCC 33090 obtained from Oxoid (Hampshire, UK) was used in this study. The stock culture was maintained in Microbank (Pro-Lab Diagnostics, Richmond Hill, Canada) at −80 °C. Prior to use, *L. innocua* was grown overnight in tryptic soy broth added 0.6% yeast extract (both Oxoid) at 37 °C. Then it was re-cultured and grown at 30 °C at 150 rpm for 20 h to a cell density of approx. 10^8 cells ml^{-1}. The bacteria were up-concentrated to 10^{10} by centrifugation (3500 × *g* for 10 min at 20 °C). To be able to detect possible differences between the different packaging regimes a high initial concentration of *L. innocua* was chosen.

2.4. Soluble gas stabilization (SGS)

SGS treatment was carried out before bacterial inoculation, in batches with 4 L of soup placed inside a heat-sealed 20 μm PA/70 μm PE bag (700 × 500 mm, Star-pack produktie B.V., The Netherlands) where the atmosphere was evacuated twice (5000 Pa vacuum, CVP Fresh Vac Model A-600, Downers Grove, Il., USA) and flushed with 100% food grade CO_2. The initial atmosphere inside the bags immediately after packaging was measured to be 97.7 ± 0.5% CO_2. The SGS-bags were large enough to ensure excess availability of CO_2. After packaging, the packaged soup was repeatedly agitated for 30 s to increase the mass transfer speed while stored at 1 °C for 24 h to reach equilibrium.

2.5. Bacterial inoculation and vacuum packaging

L. innocua was added to the fish soup, diluted 1:100, giving an initial concentration of 10^8 bacteria per ml soup. All samples, both SGS and non-SGS treated, were vacuum packed (Webomatic vacuum chamber, Werner Bonk, Bochum, Germany) in heat-sealed 20 μm PA/70 μm PE bag (180 × 140 mm, Lietpak, Vilnius, Lithuania). The oxygen transmission rate for the bag was 0.9 $cm^3 d^{-1} atm^{-1}$, measured at 296 K, 75% RH. Samples to be repacked in MAP, after the HPP treatment had a volume of 130 ml while, the remaining samples contained 50 ml fish soup. A vacuum of 1000 Pa was used on the non-SGS treated soup, while the SGS treated soup had a release of CO_2 during vacuum packaging and was subjected to a vacuum of 20,000 Pa.

2.6. Pressure treatment

Two third of the samples were pressurised at 400 and 600 MPa for 2 min at 20 °C in a high hydrostatic pressure machine QFP 2L-700 (Avure Technologies Inc., Columbus, USA). Come up time was approx. 65 and 100 s for 400 and 600 MPa, respectively, whereas the pressure release was immediate. The duration of treatment did not include the come up time. The rest of the samples were non-pressurised control samples (0.1 MPa). Prior and after treatment, samples were kept on ice.

2.7. Modified atmosphere packaging (MAP)

In MAP, the soup was repackaged after HPP on an automatic packaging machine (Mondini CV/VG-S, G. Mondini SpA, Cologne, Italy) in PP-EVOH_PP trays (Tray E1540, 380 ml, EDV packaging solutions, Barcelona, Spain). The opening was performed in a class II cabinet and repackaging was performed in a clean room to ensure as sterile conditions as possible. The head space was evacuated and subsequently flushed with a gas mix (71.3 ± 0.7% CO_2 and