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Modeling of the microbial inactivation by high hydrostatic pressure freezing

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This work aimed at investigating the effect of HPF processes on the inactivation kinetics of selected microbial strains, *Escherichia coli O157:H7* ATCC 26 and *Lactococcus lactis* ssp. *Cremoris* suspended in McIlvine buffer. The effects of different processing conditions, namely pressure level (200–400 MPa), temperature (0, −10, −20 °C), treatment time (0, 2, 5, 7, 10 min) and number of phase transitions (single or multiple transitions) were investigated in the experimental trials. The mechanism of cell inactivation in complex processes, in which pressure and temperature are simultaneously changed, was also studied and the synergistic effect of the two processing parameters, if any, was identified. Experimental data were analyzed and fitted with mathematical models representing the kinetics of microbial inactivation in HPF processes.

The results obtained so far demonstrated that the factor controlling the process efficiency is the pressure level at constant operating temperature. The level of inactivation obtained increased with decreasing the processing temperature at constant pressure or increasing the number of phase transitions, and with decreasing the size of ice crystals formed in the product. Finally, the kinetics of microbial death was described by a modified Weibull model, whose parameters are dependent on processing conditions.

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1. Introduction

It is well known that pressure influences the liquid/solid phase transition of water by affecting the kinetic of ice crystal formation as well as reducing the latent heat of fusion (Bridgman, 1912; Kalichevsky, Knorr, & Lillford, 1995; Le Bail, Chevalier, Mussa, & Ghoul, 2002; Li and Sun, 2002).

The phase diagram of water is characterized by many triple points and one or two critical points. Moreover, sixteen crystalline phases, where the oxygen atoms are in fixed positions relative to each other but the hydrogen atoms may or may not be disordered but obeying the ‘ice rules’, are detected and three amorphous (non-crystalline) phases (Zheligovskaya and Malenkov, 2006).

The different pathways, which may be followed to induce the phase transition of water from liquid to solid state, have been considered by Knorr, Schlueter, and Heinz (1998), who classified the freezing methods under pressure (HPF, High Pressure Freezing) in terms of the way in which the phase transition occurs. After the expansion step of the freezing cycle, crystal growth proceeds at atmospheric pressure, increasing the amount of water frozen (Otero and Sanz, 2006).

Concerning the effect of HPF processes on the size and distribution of ice crystals, it has been observed that they do not show any specific orientation and are uniformly distributed in the frozen food (Sanz, Otero, de Elvira, & Carrasco, 1997; Martino, Otero, Sanz, & Zaritzky, 1998; Otero, Martino, Zaritzky, Solas, & Sanz, 2000; Li and Sun, 2002). Therefore, several scientific studies have highlighted the possibility of applying HPF technology as an alternative to traditional methods for the freezing of different foods, namely carrot (Fuchigami, Kato, & Teramoto, 1997; Fuchigami, Miyazaki, Kato, & Teramoto, 1997), potato (Koch, Seyderhelm, Wille, Kalichevsky, & Knorr, 1996; Luscher, Schluter, & Knorr, 2005; Urrutia-Benet et al., 2006, Urrutia-Benet, Arabas, et al. (2007) and Urrutia-Benet, Balogh, et al. (2007)), Chinese cabbage and tofu (Fuchigami & Teramoto, 1997; Fuchigami, Kato, & Teramoto, 1998; Kanda, Aoki, & Kosugi, 1992), peach and mango (Otero et al., 2000), strawberry (Van Buggenhout, Grauwet, Van Loey, &

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Hendrickx, 2007), eggplant (Otero et al., 1997), as well as fish (redfish, salmon, cod and whiting fillet (Schubring, Meyera, Schluter, Boguslawski, & Knorr, 2003), Atlantic salmon (Zhu, Ramaswamy, & Le Bail, 2006)) and meat products (Martino et al., 1998; Molina-Garcıa et al., 2004). Most of these studies discussed the advantageous effects of carrying out the freezing process under pressure, with respect to traditional freezing, on quality, texture and structure of food products, because of the formation of smaller ice crystals, which allow preventing the breakdown of the cellular walls and modifications of the texture of food tissues.

While the advantages of HPF on preserving the quality of frozen products have been assessed, few scientific contributions dealing with the impact of this novel freezing technique on microorganisms are available.

It is well known that traditional freezing has no appreciable effects on the microbial load of fresh foods. The low temperatures used during freezing as well as during storage of frozen products are able to slow down the metabolic functions of microorganisms and, in some cases, even inhibit the cell growth. During thawing, instead, microorganisms, reported under optimal growing conditions, recover their activity, which, in turn, can cause significant changes on product quality and safety.

Among the results available in the literature, Hashizume, Kimura, and Hayashi (1995) investigated the effects of HP treatments, performed at initial temperatures in the range between −20 °C and 50 °C, on *Saccharomyces cerevisiae* cells suspended in a solution at 0.85% NaCl. They observed that the cells were more efficiently inactivated when the suspension was in the frozen state (Hashizume et al., 1995). Similarly, Urrutia-Benet, Arabas, et al. (2007) and Urrutia-Benet, Balogh, et al. (2007), studied the inactivation of *Bacillus subtilis strain* PS832, suspended in ACES buffer, processed with HP at different initial temperatures (15 °C, −25 °C, −45 °C). The authors concluded that higher inactivation level was obtained when microbial cells were treated in the frozen state. On the contrary, Ponce, Pla, Capellas, Guamis, and Mor-Mur (1998) studied the resistance of *Escherichia coli* in liquid whole egg at several pressures (300, 350, 400 and 450 MPa) and different initial temperatures (50, 20, 2 and −15 °C). The authors observed that *E. coli* cells were more resistant to pressure at ambient temperature (20 °C) and in the frozen state (− 15 °C) than at 50 °C and 2 °C.

Freezing processes under pressure, instead, in which the effects of high pressure and low temperature are coupled, may affect the microbial cells, representing a valid alternative to the traditional freezing process to be applied to produce shelf-stable and safe products. Data reported in the literature mainly refer to the comparison of the lethality caused by the traditional freezing with respect to that induced by freezing cycles under pressure. Volkert, Ananta, Luscher, and Knorr (2008) studied the effects of different freezing cycles (static air freezing (T = −30 °C, t = 3 h), HPF (P = 210 MPa, T = − 21 °C) and spray freezing, SF (T −30 °C) on the inactivation of *Lactobacillus rhamnosus* GG suspended in phosphate buffer saline (PBS) or in skim milk (20% w/v). The cell viability was determined by flow cytometer. The authors observed similar viability of the microbial cells suspended in PBS after freezing with HPF process or with a static freezer. A higher lethality of HPF process was detected when the microorganisms were suspended in skim milk. However, the authors did not neither determined the level of inactivation of the microbial cells after freezing nor discussed the inactivation mechanisms. Moreover, Picart, Dumay, Guiraud, and Cheftel (2004) observed that freezing cycles carried out at 207 MPa and −21 °C were able to inactivate different microbial strains (*Listeria innocua and Micrococcus luteus* (2–2.5 log cycles); *Pseudomonas fluorescens* (4.6 log cycles)) inoculated in salmon mince.

The aim of this work was to investigate the effects of different freezing processes under pressure on vegetative microbial cells. An extensive experimental design was performed to determine the lethality of HPF process on two different strains, *Escherichia coli O157:H7* ATCC 26 and *Lactococcus lactis* ssp. *Cremoris*, grown in the stationary phase. HPF cycles were carried out at different operating conditions (pressure, temperature and holding times). Experimental data were collected to determine the role of each processing parameter and to model the kinetics of microbial inactivation in a complex process, in which pressure and temperature variations and phase transitions occur.

2. Materials and methods

2.1. Sample preparation

Escherichia coli (O157:H7, ATCC 26) and *Lactococcus lactis* ssp. *cremoris* (ATCC 19257) were used in the experimental campaign. *E. coli* is a straight, rod-shaped, Gram-negative bacterium, facultative anaerobic, non-sporulating bacterium (Doyle & Schoeni, 1984). *L. lactis* ssp. *cremoris* is Gram-positive coccus, non-motile and not able to form spores (Roissart & Luquet, 1994).

E. coli and *L. lactis* were inoculated into Tryptic Soy Broth (TSB, Liofilchem, Italy) and Brain-Heart Infusion Broth (BHI, Oxoid, Basingstoke, England) at their early stationary phases and incubated for 24 h at 37 °C and for 30 h at 26 °C, respectively (Alpas et al., 2000; Schleifer et al., 1985). Samples of microbial cells, centrifuged at 5000 rpm and 4 °C for 10 min, were suspended in a McIlvine buffer at the same pH of the growth medium, reaching a final microbial concentration of 10^8 cfu/mL. Samples were stored under refrigerated conditions (4 °C) before being processed with HPF.

2.2. Experimental apparatus

2.2.1. The multi-vessel system U111

The experimental trial was carried out in a high pressure multi-vessel system U111 (UNIPRESS-Polish Academy of Sciences, Warsaw, Poland), which can be operated at pressures up to 700 MPa and temperatures in the range between − 40 °C and 100 °C. The system includes five high pressure reactors, made from Cu–Be alloy and working in parallel, immersed in a thermostatic bath. Each reactor is equipped with a manual cut-off valve and a K-type thermocouple, placed at the bottom of each reactor and able to measure the temperature of the liquid in contact with the samples. The pressurization system consists of a hydraulic low-pressure pump connected to a high pressure intensifier, while a high pressure manual valve is used to release the pressure. The compression rate can be changed in the range between 2.5 and 25 MPa/s. During the experimental design, the compression rate was always set at 10.5 MPa/s. A silicon oil was used as pressurization and heating fluid of the thermostatic bath. Due to the technical features of the high pressure system, the pressurization causes an average temperature increase of 4 °C/100 MPa, while during the holding time under pressure, the temperature quickly reduces to the initial setup value. The multi-vessel system is also equipped with a control unit, which allows setting up the pressure level, set up, and control the pressurization ramp, start the filling of the intensifier and reactors, as well as measure and control the pressure and temperature levels in the reactors. A data acquisition system, U111-DAS, records the pressure as well as the temperature measured in the reactors and that of the thermostatic bath.

2.2.2. The optical HP-LT microscopic cell

A miniaturized High Pressure - Low Temperature (HP-LT) microscopic cell (UNIPRESS-Polish Academy of Sciences, Warsaw,

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