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# Phase transitions during high pressure treatment of frozen carrot juice and influence on *Escherichia coli* inactivation

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ABSTRACT

Influence of high pressure (HP) treatment (200–400 MPa; 0–10 min) on phase transition behavior of frozen carrot juice and the resulting influence on the inactivation kinetics of *Escherichia coli* ATCC 25922 were evaluated. Experiments were carried out in a specially designed container to prevent heat exchange from the environment, except for the compression heating and decompression cooling. Solid to solid and solid to liquid transitions were recognized during HP treatment. Transition to Ice-III was observed from the temperature-pressure profiles when the application pressure was >350 MPa. Inactivation of *E. coli* in frozen carrot juice followed the first order kinetics with *D* values between 2.62 and 2.12 min at 300–400 MPa pressure levels, much shorter than those observed in unfrozen carrot juice. The combination of frozen state, phase transition status and pressure level likely contributed to the better inactivation of *E. coli* in frozen carrot juice.



## 1. Introduction

High pressure (HP) processing is a promising alternative to thermal processing for extending the shelf-life and improving the safety of food products (Bari, Ukuku, Mori, Kawamoto, & Yamamoto, 2007; Picouet, Sárraga, Cofán, Belletti, & Dolors, 2015; Ramaswamy, 2011; Vervoort et al., 2012). Studies have confirmed that HP processing can effectively inactivate vegetative cells of pathogenic microorganisms (Ramaswamy, Zaman, & Smith, 2008) without changing the sensory and nutritional properties of the food (Dede, Alpas, & Bayindirli, 2007; Oey, Lille, Loey, & Hendrickx, 2008; Picouet et al., 2015). HP processing does not affect the low molecular and covalently bound food compounds, such as vitamins, flavoring agents, etc., and hence HP processing can provide "fresh-like" carrot juice with better sensory properties for long refrigerated storage (Picouet et al., 2015).

HP effects on microorganisms depend on several factors, but the effects of pressure level and time have been studied most often. A number of studies have demonstrated that the combination of pressure and elevated temperatures have synergist acceleration of microbial destruction and enzyme inactivation kinetics (Ramaswamy, Riahi, & Idziak, 2003). Temperature effect on pressure destruction kinetics of microbial spores has also been shown to be significant when the temperatures are elevated and approached the lethal levels (Shao, Ramaswamy, & Zhu, 2007). HP effects on destruction kinetics have also been recognized to depend on product properties such as pH, soluble solid concentrations, composition (fat, protein and carbohydrate content) (Ramaswamy, Jin, & Zhu, 2009). In some cases, protection effect of pressure on thermal destruction kinetics at elevated temperature levels in the lethal range has been reported (Shao et al., 2007). This has been reported to be due to the increase in temperature and pressure causing opposite effects on volume expansion; with temperature contributing to volume increase while pressure reversing that effect. For similar reasons, some studies have shown that at sub-lethal levels, the pressure destruction effects are elevated at lower temperatures (Su et al., 2014).

There are far fewer studies in HP processing research that use the combination of pressure and refrigerated conditions. Research related to the use of subzero (frozen) temperatures on inactivation kinetics are even more scarce (Luscher, Balasa, Fröhling, Ananta, & Knorr, 2004; Picart, Dumay, Guiraud, & Cheftel, 2005). Su et al.

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(2014) reported a combination or interaction effect of pressure and subzero temperature for the inactivation of microorganisms in food. Phase transitions of Ice-I/Ice-III by pressurizing frozen systems above 200 MPa was shown to be responsible for bacterial destruction (Fernández et al., 2007). However, there is little information on the evaluation of temperature profiles and phase transition in frozen samples under HP processing conditions, especially using a real food matrix.

Fruit and vegetables are important components of a healthy diet. Their daily consumption in adequate quantities could help prevent several major diseases (Picouet et al., 2016), such as heart problem, cancer, diabetes and obesity, as well as the prevention and alleviation of several micronutrient deficiencies. Among common fruits and vegetables, carrots are high in fibers, carotenoids, vitamins C and E, and phenolics such as *p*-coumaric, chlorogenic, and caffeic acids (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001). However, the low-acid condition in carrot juice is conducive to the growth of pathogenic microorganisms (Patterson, McKay, Connolly, & Linton, 2012). So storage of raw, unprocessed carrot juice may also lead to microbiological safety problems and shortened shelf-life. Therefore, extending the shelf-life using mild processing technologies that minimally affect the sensory and nutritional properties will be of interest for the food industry. Van Opstal, Vanmuysen, Wuytack, Masschalck, and Michiels (2005) reported that pressure inactivation of *Escherichia coli* MG1655 was significantly lower in carrot juice than in buffer (150–600 MPa, 5–45 °C). Pilavtepe-Çelik, Buzrul, Alpas, and Bozoğlu (2009) reported that carrot juice even had a protective effect on *E. coli*.

Therefore, the objective of this study was to evaluate the phase transition behavior of frozen carrot juice during HP treatment and to extend the work of Su et al. (2014) on *E. coli* inactivation kinetics in frozen carrot juice. A specially designed container was used to isolate the sample and to achieve the ice phase transition under HP at room temperatures. This helped to overcome a serious practical problem of maintaining the entire pressure chamber at subzero temperatures. Maintaining subzero temperatures in commercial HP vessels is not only impractical but also be very expensive and energy intensive. Inactivation kinetic of *E. coli* was used for comparing the HP treatment effectiveness in frozen vs unfrozen carrot juice.

## 2. Materials and methods

### 2.1. Escherichia coli strain and culture preparation

The culture of *E. coli* ATCC 25922 (CGMCC 1.2385) was obtained from China General Microbiological Culture Collection Center (CGMCC, Beijing, China). A fresh culture was prepared every 2 weeks to ensure their viability. To prepare the inoculation stock, several loops of isolated colonies of stock culture were transferred to 50 mL sterile nutrient broth (Sinopharm Chemical Reagent Co., Ltd. Shanghai, China) in 100 mL Erlenmeyer flasks and incubated at 37 °C for 24 h with agitation (150 rpm). Several loops of the incubated broth were then transferred to another 50 mL sterile nutrient broth and incubated at 37 °C for 24 h incubation. Following this, 30 mL incubated broth was aseptically transferred into a 50 mL sterilized centrifuge tube and centrifuged at 3200*g* for 5 min at 20 °C (5810R, Eppendorf AG, Germany). The cell pellet obtained was re-suspended in 20 mL nutrient broth and enumerated using the pouring plate method on a brain heart infusion agar plate (BHIA) (Hangzhou Tianhe Microorganism Reagent Co., Ltd., Hangzhou, China), and incubated at 37 °C for 48 h and counted. The *E coli* selective media was not used in this study since a pure culture of *E. coli* was used and in order to obtain counts of both surviving and injured cells (Ramaswamy et al., 2003). The initial population of *E. coli* in the inoculated culture stock was approximately $10^8$ colony forming units (CFU)/mL.

### 2.2. Sample preparation

Fresh carrots were purchased from Wal-Mart store near Zhejiang University (Hangzhou, China). The carrots were peeled and squeezed with a juice extractor (JYZ-B550, Joyoung Co., Ltd., Hangzhou, China), and the juice was then centrifuged at 3200*g* for 10 min to remove the suspended solids. The supernatant was filtered through a 0.23-mm-pore-diameter filter. The clear carrot juice had a pH of 6.5 ± 0.3 (FE 20, Mettler-Toledo, Shanghai, China). The juice was subjected to a thermal treatment, in a thermostatically controlled water bath (DKS-224, Zhongxin Medical Instrument Co., Ltd., Jiaxing, China) at 65 °C for 30 min. Microbial enumeration on BHI agar in heat treated samples returned negative counts.

The prepared carrot juice was transferred aseptically into sterile bags (110 mm × 185 mm, BagLight PolySilk, Interscience, Paris, France) (about 150 mL for each bag) and sealed using a heat sealer (FS-300, Yongkang Teli Packing Machinery Co., Ltd., China). The bags were frozen stored in a freezer (BC/BD-103HA, Haier, China) at −20 °C for subsequent handling.

Carrot juice was fully thawed at 4 °C for ~4 h prior to inoculation of *E. coli* culture. After thawing, carrot juice was aseptically transferred to a sterile beaker. The previously prepared *E. coli* pellet was re-suspended in 20 mL carrot juice and then added to the rest of carrot juice in a beaker. After stirring at 500 rpm (RCT BS25, IKA, Staufen, Germany) for 1 min, the inoculated carrot juice was aseptically transferred to a sterile 2.0 mL cryogenic vial (430 659, Corning Inc., USA), up to the brim and closed with sterile screw cap leaving no headspace to avoid possible cracking during HP treatment.

### 2.3. Insulated container

The cryogenic vials were individually vacuum packed in two layers of polyethylene bags and divided into two batches. One batch of the cryogenic vials were then loaded into a specially designed plastic container consisting of a compressed sponge as previous study used (Su et al., 2014) and slightly modified, as shown in Fig. 1. A K-type thermocouple (OMEGA Engineering, Stamford, CT, USA) was installed into the container for temperature recording using a data logger (34970A, Agilent Technologies GMBH, Germany). Four cryogenic vials were placed in one container with one of them used for recording sample temperature during HP treatment. The plastic container was filled water to completely soak the sponge and frozen at −20 °C for 24 h, and were secured by vacuum-packing in a flexible thermo-stable PA/PE pouches (30 cm × 42 cm) prior to HP treatment (details shown in Fig. 1). Samples of *E. coli* inoculated carrot juice frozen stored at −20 °C, prepared in a similar manner but without HP treatment, were used as control. The second batch was not frozen but stored at 4 °C and treated likewise for providing HP inactivation data under unfrozen conditions.

### 2.4. High pressure equipment

HP treatments were carried out in a laboratory-scale HP equipment (UHPF-750, Baotou Kefa High Pressure Technology Co., Ltd., China) with a maximum chamber capacity of 5 L. The high pressure unit was connected to a data logger for temperature and pressure (current signal) recoding during HP treatment. Water was used as the pressure-transmitting medium. The pressure vessel was maintained at room temperature (~20 °C) and with some added ice if the temperature was above 20 °C before pressure treatment. The near room temperature setup was used so that the