



# Modeling the inactivation of *Neosartorya fischeri* ascospores in apple juice by high pressure, power ultrasound and thermal processing



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## ABSTRACT

*Neosartorya fischeri* is a mould that spoils acid foods and can produce mycotoxins. In this work, the efficacy of high pressure processing (HPP, 600 MPa) and power ultrasound (24 kHz, 0.33 W/mL) in combination with 75 °C for the inactivation of four week old *N. fischeri* ascospores in apple juice was investigated and compared with 75 °C thermal processing alone. The HPP-75 °C process was the most effective technique for inactivating *N. fischeri* spores, resulting in 3.3 log reductions after 10 min vs. no inactivation for thermosonication (TS) and thermal processing. Unexpectedly, activation shoulders were observed during the TS process. Then, the effect of different temperatures on the ascospore inactivation in apple juice by HPP-thermal, TS and thermal processing was investigated, and the log survivors vs. time were modeled. Faster inactivation was achieved at higher temperatures for all the technologies tested, indicating the significant role of temperature for the spore inactivation, alone or combined with other processes. The Weibull model described the spore inactivation better by 600 MPa HPP-thermal (50, 60, 75 °C) and thermal (85, 90 °C), whereas Lorentzian was more appropriate for the TS treatment (65, 70, 75 °C). In conclusion, HPP is the best food preservation technology due to higher spore inactivation in apple juice at the same temperature.

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## 1. Introduction

Extremely heat resistant ascospores from moulds *Byssoschlamys*, *Neosartorya*, and *Talaromyces* have been found (Hocking & Pitt, 1984; Silva & Gibbs, 2004, 2009; Silva, Gibbs, Nunez, Almonacid, & Simpson, 2014). These are often associated with the spoilage of pasteurized fruit products such as juices, purees, jellies, jams, and canned fruits (Beuchat, 1998; Pitt & Hocking, 1997; Silva et al., 2014). *Neosartorya fischeri* (anamorph *Aspergillus fischerianus*) is also a public health concern because of its capacity to produce mycotoxins terrein, fumitremorgins A and B, and verruculogen (Frisvad & Samson, 1991; Misawa, Nara, Nakayama, & Kinoshita, 1962; Nielsen, Beuchat, & Frisvad, 1989; Tournas, 1994). This species is widely distributed in soil (Pitt & Hocking, 1997) and was first isolated from canned strawberries in 1963 (Kavanagh, Larchet, & Stuart, 1963). *N. fischeri* can grow at temperatures between 10 and 52 °C (the optimal temperature is around 26–45 °C), in oxygen

levels as low as 0.1% at 25 °C (Nielsen et al., 1989), and a broad range of pH (3–8) as most fungi (Pitt & Hocking, 1997). The extremely heat resistant ascospores formed by the teleomorphs or sexual reproductive stage survive 85 °C for 10 min (Houbraken, Dijksterhuis, & Samson, 2012) and drought (<0.5% relative humidity) (Wyatt, 2014). Pitt and Hocking (1997) reported that the degree of heat resistance of ascospores of *N. fischeri* is comparable with that of many bacterial spores, and is higher than that of *Byssoschlamys fulva* ascospores, the most heat resistant mould ascospores known. The heat resistance of *N. fischeri* also increased with the ascospores age (Slongo, Miorelli, & Aragão, 2009; Tournas & Traxler, 1994), with 25 day old ascospores exhibiting changes in their ultrastructure and chemical composition when compared with 11 day old ascospores (Conner, Beuchat, & Chang, 1987). Based on the ascospore ornamentation, three varieties of *N. fischeri* (*var. fischeri*, *var. glabra*, and *var. spinosa*) have been identified (Samson, Nielsen, & Frisvad, 1990).

Temperatures between 85 and 95 °C are commonly used to prolong the shelf life of fruit juices (Sant'Ana, Rosenthal, & Massaguier, 2010). However, it has been recognized that the thermal process may activate the dormant ascospores of moulds which

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subsequently cause deterioration, hence resulting in economic loss (Katan, 1985; Slongo & Aragão, 2006; Splittstoesser, Nielsen, & Churey, 1993). Increasing the intensity (temperature or processing time) of the heat treatment is not desirable, due to quality reasons and consumer demands for 'fresh-like' fruits. Food preservation by non-thermal methods such as high pressure processing (HPP) and power ultrasound in combination with mild heat have been investigated due to reduced treatment temperatures and processing times (Evelyn & Silva, 2015a, 2015b, 2015c). HPP is an established commercial food processing technology and can be combined with temperature for the inactivation of resistant microbial spores (Evelyn & Silva, 2015c; Sarker, Akhtar, Torres, & Paredes-Sabja, 2015; Wilson, Dabrowski, Stringer, Moezelaar, & Brocklehurst, 2008) and enzymes (Sulaiman, Soo, Yoon, Farid, & Silva, 2015). With respect to the heat resistant mould ascospores such as *Byssochlamys fulva*, *Byssochlamys nivea*, *N. fischeri*, *Neosartorya spinosa*, *Talaromyces avellaneus*, *Talaromyces macrosporus*, the efficacy of 600–900 MPa of HPP pressure (cycle, oscillatory or continuous) in conjunction with heat (25–90 °C) using 3–15 week old spores was up to 5.7 log reductions (Butz, Funtenberger, Haberditzl, & Tauscher, 1996; Chapman et al., 2007; Ferreira, Rosenthal, Calado, Saraiva, & Mendo, 2009; Hocking, Begum, & Stewart, 2004; Maggi, Gola, Spotti, Rovere, & Mutti, 1994; Palou et al., 1998; Reyns, Veraverbeke, & Michiels, 2003; Voldřich, Dobiáš, Tichá, Čerovský, & Krátká, 2004). Among the research studies, only Voldřich et al. (2004) modeled the inactivation kinetic for *Talaromyces* spores, reporting a first order kinetics and a decrease in the decimal reduction time (*D-value*) at 600 MPa as the temperature increased from 17 to 60 °C.

Power ultrasound (frequency ranging from 20 to 100 kHz) is a promising non-thermal technology for food preservation. This technology relies on the application of pressure waves called cavitation to the food/beverage, causing microbial cell death (Feng & Yang, 2011; Piyasena, Mohareb, & McKellar, 2004). Power ultrasound has been combined with mild heat (thermosonation, TS) to inactivate bacterial and fungal vegetative cells and spores, and a synergistic effect was observed (Earnshaw, Appleyard, & Hurst, 1995; Evelyn & Silva, 2015a; Garcia, Burgos, Sanz, & Ordonez, 1989; López-Malo, Palou, Jiménez-Fernández, Alzamora, & Guerrero, 2005; Ordoñez, Aguilera, García, & Sanz, 1987; Zenker, Heinz, & Knorr, 2003). With respect to mould ascospores, Jimenez-Munguia, Arce-Garcia, Argai, Palou, and López-Malo (2001) reported that the inactivation of *Penicillium digitatum* and *Aspergillus flavus* ascospores by TS (20 kHz, 40–45 °C) in sabouraud broth increased with the treatment time and amplitude. The addition of boiling chips and air bubbles to the broth medium reduced the *D-values*. López-Malo et al. (2005) found lower *D-values* for TS (20 kHz, 40–60 °C) inactivation of *P. digitatum* and *A. flavus* ascospores in sabouraud broth compared to the thermal treatment alone. The authors also concluded an increase in the ultrasound amplitude and decrease in pH resulted in lower *D-values*. Coronel, Jiménez, López-Malo, and Palou (2011) proposed the Weibull model for the inactivation of *A. flavus* ascospores in broth by TS combined with vanillin. No studies have been carried out on the TS inactivation and kinetics modeling of heat resistant mould ascospores relevant to the fruit industry, such as *N. fischeri*. In particular, no work using fruit products has been reported, being broth inoculated with microorganisms the medium processed.

Due to the importance of *N. fischeri* spores in high acid fruit products, more research is needed to provide predictive models for the HPP-thermal and TS inactivation and design appropriate processes. Therefore, in this research the inactivation of *N. fischeri* ascospores in apple juice by HPP-thermal and TS processes were carried out, and the main objectives were as follows: (i) to compare the HPP-thermal, TS and thermal inactivation of ascospores at

75 °C; (ii) to model the 600 MPa HPP-thermal inactivation of ascospores; (ii) to model the TS inactivation of ascospores; and (iv) to model the thermal inactivation of ascospores.

## 2. Material and methods

### 2.1. Microbiology

#### 2.1.1. Mould

*N. fischeri* var *fischeri* JCM 1740 was obtained from the Japan Collection of Microorganism (= ATCC 1020, DSM 3700, CBS 101.12, IAM 13864). This strain was isolated from canned apples in the USA.

#### 2.1.2. Ascospore production

Ascospores of *N. fischeri* were obtained after growth for four weeks at 30 °C on malt extract agar (MEA). The spores were collected by flooding the surface of the culture plates with 5 mL sterile distilled water (SDW), and gently rubbing the agar surface with a sterile bent glass rod. The spore suspension was subsequently filtered through layers of gauze to remove any remaining hyphal fragments. Spore pellets were obtained after centrifugation in sterile SDW at 4000 × *g*, 15 min, 4 °C and the procedure was repeated three times. The final spore suspension was then stored at 2 °C in SDW containing glass beads until use.

#### 2.1.3. Apple juice inoculation and preparation

Apple juice (pH 3.7, 10.6 ± 0.1°Brix) was obtained from a local supermarket and used as the treatment medium to suspend *N. fischeri* ascospores. For HPP-thermal and thermal experiments, aliquots (ca. 0.5 mL) of *N. fischeri* spore solution were inoculated into 3.0 mL of apple juice to yield an initial juice spore concentration of approximately 10<sup>6</sup> cfu/mL of juice. The inoculated juice was packed in 8 × 8 cm food grade retort pouches (Cas-Pak, New Zealand) composed of polyester coated with silicon oxide, and laminated to nylon and cast polypropylene (PET-SIOX(12)//ON(15)//RCP(70)). The pouches can withstand temperatures of up to 130 °C which are suitable for thermal processing and high pressure applications. Regarding the TS experiments, *N. fischeri* spore solution was inoculated aseptically by adding a small volume of inoculum to the apple juice contained in a round-bottom flask (5 mL of spore solution into 95 mL of apple juice) before the TS thermal pretreatment. The initial spore concentration after the thermal pretreatment and before TS was approximately 10<sup>5</sup> cfu/mL of juice.

#### 2.1.4. Spore enumeration

The mould ascospore concentration in apple juice before and after processing (thermal, HPP and TS) was determined by spread plating onto MEA. A heat shock (75 °C, 5 min) of raw unprocessed apple juice was required to obtain the initial ascospore count (1.3 × 10<sup>7</sup> cfu/mL) in the untreated juice for HPP and thermal processes (Katan, 1985; Splittstoesser et al., 1993). *N*<sub>0</sub> for TS process was thermally pretreated apple juice at 80 °C for 30 min. Prior to plating, spore samples were decimal diluted using 9 mL 0.1% (w/v) sterile buffered peptone water (BPW; Difco, Becton Dickinson, USA). Each tube dilution was mixed repeatedly using a high speed vortex mixer to yield a uniform spore suspension, and plated twice. The plates were then incubated at 30 °C for 3–5 days until visible colonies were formed. Plates with 20–100 colonies were used for enumeration. Ascospore concentration was expressed in cfu per milliliter (cfu/mL) of juice sample.

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