



## Comparison of bacterial inactivation with novel agitating retort and static retort after mild heat treatments



Mehmet Baris Ates<sup>a,b,\*</sup>, Dagbjørn Skipnes<sup>a</sup>, Tone Mari Rode<sup>a</sup>, Odd-Ivar Lekang<sup>b</sup>

<sup>a</sup> Nofima AS, Richard Johnsens Gate 4, P.O. Box 8034, N-4068 Stavanger, Norway

<sup>b</sup> The Norwegian University of Life Sciences, Department of Mathematical Science and Technology, P. O. Box 5003, N-1432 Ås, Norway

### ARTICLE INFO

#### Article history:

Received 17 September 2013

Received in revised form

21 January 2014

Accepted 8 March 2014

Available online 20 March 2014

#### Keywords:

Agitating retort

Thermal inactivation

*Listeria innocua*

### ABSTRACT

Lower thermal load on foods is desirable for food producers and consumers as the food gets higher quality. With reduced thermal load, the investigation of food safety is of importance. In this study, microbial inactivation efficacy of a new retort process with high frequency longitudinal agitation was compared to static retort process which was used as a benchmark. As a model, fish soup samples, inoculated with approximately  $10^8$  cells/ml *Listeria innocua*, was exposed to mild heat treatments at 62, 65 and 68 °C. Results clearly demonstrated that agitating mode can provide equivalent lethality to the model organism *L. innocua* within significantly shorter heating times compared to static mode. Bacteria were not detected on TSA-YE plates after 11.5, 6.8 and 5.5 min processing in agitating mode; 77, 67 and 52 min processing in static mode at 62, 65 and 68 °C respectively. Bacterial inactivation in agitating mode was generally correlated with estimated inactivation based on product core temperature, D- and z-values for *L. innocua*. This may indicate that distribution of the heat load over the soup was enhanced through agitation. Results showed that utilization of high frequency longitudinal agitation mechanism in retorts is promising for reducing the thermal load on food products without compromising on food safety related to non-spore forming pathogens.

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### 1. Introduction

Thermal processing in hermetically sealed containers is a common method to produce foods with extended shelf-life utilized historically since the Napoleonic era. Static retorts have been widely used for industrial applications of thermal processing. Early versions of these retorts used steam as the heating medium but later on water, steam/air, raining and spray water systems were also developed as heating media. In 1920s, agitating retorts were introduced for the first time where agitation mechanism was based on axial rotation (rolling of cans) (Eisner, 1988). Rotary retort was later developed with end-over-end rotation principle (Clifcorn, Peterson, Boyd, & O'Neil, 1950).

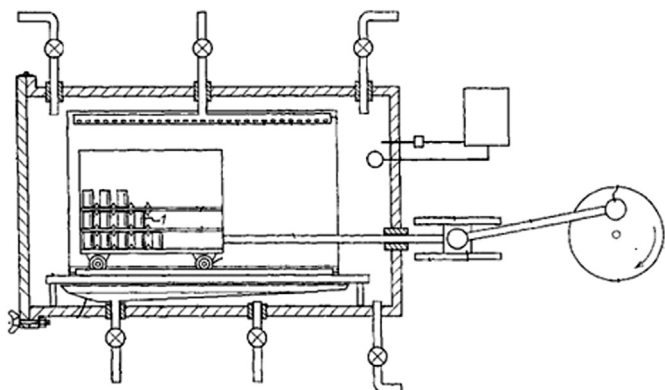
Development of agitating retorts was the outcome of a need to overcome some weaknesses of conventional static retorts. The weaknesses included differing temperature zones within heated product, over-cooking, lack of consistency in texture and flavor in processed products and slow heat penetration (Eisner, 1988). On

the other hand, agitation of packed foods has enabled more uniform distribution of heat and process time reduction. These benefits often give higher quality food products while being only applicable for liquid and semi-liquid foods (Rosnes, Skara, & Skipnes, 2011). Furthermore, product consistency, headspace, fill-in weight and rotation speed has to be strictly controlled to prevent under-processing as these parameters influence the heat transfer effectiveness of agitating process (Auwah, Ramaswamy, & Economides, 2007). Recently, an agitating retort with high frequency longitudinal agitation mechanism was developed in 2006 (Fig. 1). The new process allows rotation speeds beyond the 20–40 rpm range with the help of reciprocating agitation mechanism (Rosnes et al., 2011). Although critical factors for the new retort process are believed to be same as rotary retorts, investigation of food safety through microbial inactivation studies is of importance with such novel processes (Walden, 2008).

Microbial inactivation studies with artificially inoculated foods are typically used for food safety studies with novel processes. Lower number of survivors than the hazardous level in the food product over a determined shelf-life period after thermal processing is desirable (NACMF, 2010). Traditionally, process lethality calculations have been based on thermal resistance data for

\* Corresponding author. Nofima AS, Richard Johnsens Gate 4, P.O. Box 8034, N-4068 Stavanger, Norway Tel.: +47 96 95 27 94.

E-mail addresses: [baris.ates@nofima.no](mailto:baris.ates@nofima.no), [brsates@gmail.com](mailto:brsates@gmail.com) (M.B. Ates).



**Fig. 1.** Principle for the new retort system: Products (1) within the basket are agitated in longitudinal direction. The corresponding patent can be referred to for descriptions on each number shown in the figure modified from (Walden & Ferguson, 2007).

bacteria and spores. Thermal resistance has been mathematically expressed by decimal reduction time ( $D$ -value) and  $z$ -value.  $D$ - and  $z$ -values are based on the assumption that thermal inactivation of bacteria follows log-linear kinetics.  $D$  value is the duration of heat treatment at a specific temperature necessary to kill 90% of the microbial population and  $z$  value is the temperature change required to shift  $D$  value by 1 log unit (Stumbo, 1973). Other available thermal inactivation models for microorganisms have been reviewed by (Smelt & Brul, 2014) for interested readers.

Milder heat treatment is generally applied on foods designed to have short shelf-life under refrigeration temperatures. For such foods, *Listeria monocytogenes* contamination is a large problem as the bacterium can cause lethal diseases. Compared to other non-spore forming food-borne pathogens, *Listeria* is generally reported to have higher heat resistance as well as being able to grow at temperatures from  $-1.5$  °C up to 44 °C (Hudson & Mott, 1993). Furthermore, *L. monocytogenes* has been reported to grow in foods from  $a_w$  of 0.91–0.93 and pH value of 4.2 (FAO/WHO, 2004). It is generally agreed that sufficient pasteurization can eliminate *L. monocytogenes*. Mild heated products are required to be heated for at least 2 min at 70 °C (at the coldest point) in order to achieve 6-log kill effect on *L. monocytogenes* (FAO, 1999, p. 34; Rosnes et al., 2011).

In research studies, *Listeria innocua* is proposed as a potential surrogate microorganism for *L. monocytogenes*. This is because *L. innocua* is safer to work with as well as having major phenotypic similarity with *L. monocytogenes* (Kamat & Nair, 1996; Lorentzen, Ytterstad, Olsen, & Skjerdal, 2010; Miller, Gil, Brandão, Teixeira, & Silva, 2009). In a recent review, Milillo et al. (2012) recommended more precise selection of surrogate microorganisms for *L. monocytogenes* based on experimental conditions. Although several strains of *L. innocua* have been found to be more heat tolerant than *L. monocytogenes* (Friedly et al., 2008; O'Bryan, Crandall, Martin, Griffis, & Johnson, 2006; Sorqvist, 2003), these results may sometimes vary with process conditions and product matrix (Murphy, Duncan, Beard, & Driscoll, 2003). Lorentzen et al. (2010) compared the survival of *L. innocua* ATCC 33090, *L. monocytogenes* NCTC 11994 and No. 4006 and found ATCC 33090 to be the most heat tolerant one. *L. innocua* is apparently a useful model organism for inactivation studies but should not be directly used for thermal validation purposes.

To the best of our knowledge, there are no studies published regarding microbial inactivation efficacy of the retort process with high frequency longitudinal agitation mechanism. Therefore, objective of this study was to investigate bacterial inactivation

over the whole food product with agitating retort process in comparison to static process which was used as a benchmark. This was done by conducting bacterial inactivation experiments through a large set of heat treatments with fish soup samples inoculated with *L. innocua* model organism. The product was intended to have limited shelf life at refrigerated temperatures lower than 3.3 °C.

## 2. Materials and methods

### 2.1. Fish soup preparation

A common recipe for making fish soup was used. Ingredients consisted of approximately 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 sterilization program of  $F_{121^{\circ}\text{C}}^{10^6} \geq 3$  min based on core temperature. As a next step, the soup was packed in polypropylene plastic trays (Promens, Kristiansand, Norway) with dimensions  $9 \times 4 \times 13.2$  cm and sealed with a plastic film. Samples were then stored at 1 °C until the day of experiments.

### 2.2. Culture preparation and inoculation into fish soup samples

*L. innocua* ATCC 33090 (Oxoid, Hampshire, U.K) was stored in Microbank (Pro-Lab Diagnostics, Canada) at  $-80$  °C. *L. innocua* was initially grown in Tryptic Soy Broth (Oxoid) supplemented with 0.6% w/w Yeast Extract (Merck, Darmstadt, Germany) (TSB-YE) at 37 °C for 20 h at 150 rpm. The overnight culture was then sub-cultured in TSB-YE with 20 h incubation at 30 °C, 150 rpm. Resulting cell density was  $10^8$ – $10^9$  cells/ml. In order to concentrate the cells further, bacteria were centrifuged at  $3500 \times g$  for 4 min and cell pellets were collected and re-suspended in peptone water (Merck) to obtain  $10^{10}$ – $10^{11}$  cells/ml. Bacteria were then added to fish soup samples to obtain an initial concentration of approximately  $10^8$  cells per ml fish soup. Finally, inoculated fish soup samples, each having 350 g weight, were separately packed and shaken thoroughly to distribute the bacteria evenly before subsequent heat treatments.

### 2.3. Heat treatments

A batch retort (Steriflow, Roanne, France) was used for all heat treatments since it was possible to run the retort both in agitating and static heating modes. The retort was previously calibrated and checked for even heat distribution and the heat transfer medium was steam and raining water. The range used in agitating mode was 80–100 strokes per minute (spm). There were two process batches where three replicates of inoculated fish soup were heated in each ( $n = 6$ ). Core temperature histories of three additional soup samples were recorded during treatments. A negative control (without bacteria) was also processed and analyzed in each process batch.

For a detailed assessment of bacterial inactivation pattern with longitudinal agitating heating mode in comparison to static mode, three different mild temperatures (62, 65 and 68 °C) were selected for investigation. Retort program phases were (1) come-up period for retort water temperature; (2) heating; (3–4) cooling (Fig. 2). Phases 1, 3 and 4 were always kept constant for each thermal treatment. Only the effect of selected time–temperature combinations for heating phase (2) on bacterial inactivation was studied. For this purpose, time intervals analyzed in agitating mode were 4–9.5 min (at 62 °C), 3–4.8 min (at 65 °C) and 1.5–3.5 min (at 68 °C). In static mode, time intervals 40–70 min (at 62 °C), and 30–65 min (at 65 °C) and 25–50 min (at 68 °C) were investigated. Additionally,

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