# Ultrasonic decontamination in smoked salmon experimentally contaminated with *Listeria monocytogenes*: Preliminary results

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## Abstract

The purpose of this work was to evaluate the effects of ultrasound (sonication) and their combination with temperature (thermosonication) on the inactivation of *Listeria monocytogenes* (LM) in smoked salmon. The trial was conducted on smoked salmon samples experimentally contaminated with a cocktail of 4 strains of *Listeria monocytogenes* (LM ATCC 19114, LM ATCC 15313, LM ATCC 19111 and LM ATCC 7644) at a final concentration of 8 log cfu/g and kept at 4°C until its use. Thermosonication treatments between 40°C and 50°C for 5, 10 and 15 minutes proved to be more effective without altering the sensory characteristics of the food.

## Introduction

The Genus *Listeria* is found naturally in agricultural environments such as soil, manure and water (Jeyaletchumi *et al.*, 2012). The scientific literature frequently discusses the ability of these microorganisms to survive in the environment and in the equipment used to produce food as well as in domestic refrigerators (Azizoglu *et al.*, 2014; Vergara *et al.*, 2014).

*Listeria monocytogenes* is a pathogenic bacterium that can cause a rare but dangerous infection called listeriosis. The severity of listeriosis can range from mild gastroenteritis to severe illness (septicaemia, encephalitis, meningitis, abortion, etc.) and can lead to a high mortality rate in immunodeficient subjects (Swaminathan and Gerner-Smidt, 2007). In fact, some people have a higher risk of developing listeriosis, in particular the elderly (> 65 years), newborns and infants (<5 years), and about 17% of cases of listeriosis affect pregnant women (Buzby, 2001; Okutani *et al.*, 2004; Swaminathan and Gerner-Smidt, 2007; Dilber *et al.*, 2009; Smith *et al.*, 2009; Gillespie *et al.*, 2010; CDC, 2015).

According to the Food and Drug Administration (FDA), around 2,500 people suffer from listeriosis in the United States each year (FDA, 2011). The mortality rate could be between 20% and 30% of those who contract listeriosis (Swaminathan and Gerner-Smidt, 2007). *Listeria monocytogenes* is responsible for 19% of total deaths due to the consumption of contaminated food in the United States (Scallan *et al.*, 2011).

According to a recent opinion of the European Food Safety Authority (EFSA), in Europe the cases of listeriosis have increased in particular between two groups of the population: individuals over 75 years of age and women between 25 and 44 years (likely due to pregnancy). The opinion covers the period 2008-2015 (EFSA, 2018).

The microorganism is commonly isolated from meat and meat products, fishery products, milk by-products and ready-to-eat foods (RTE), stored at refrigeration temperatures.

Conventional heat treatments (pasteurization or sterilization) are the techniques more frequently used for microbial decontamination of food products.

However, the demand for new technologies that are effective and have minimal impact on nutritional and sensory characteristics is constantly growing. Possibly, these technologies should be environmentally friendly, with a lower consumption of energy and therefore cheaper than conventional processes.

Among the alternative technologies for microbial inactivation, the scientific literature proposed pulsed electric fields, microfiltration, high hydrostatic pressures, and ultrasounds.

In recent years, ultrasounds have been shown to have a wide variety of applications, *e.g.* to increase meat tenderness or decontaminate foods. Sonication creates longitudinal waves that originate zones of alternate compression and expansion. (Sala *et al.*, 1995). These zones of pressure change cause the so-called "*cavitation*" that generates gas bubbles in the product. These bubbles have a larger surface area during the expansion cycle, which increases gas diffusion, causing the bubble to expand. A point is reached at which the supplied ultrasonic energy is not sufficient to maintain the vapour phase in the bubble, thus generating a rapid condensation. The condensed molecules collide violently and create shock waves. These shock waves form regions of very high temperature and pressure, reaching up to 5500°C and 50,000 kPa (Piyasena *et al.*, 2003). The pressure variations resulting from these implosions are the main bactericidal effect in ultrasounds. Hot spots can kill some bacteria, but they are very localized and do not affect a large enough area. The effectiveness of ultrasound depends on several factors, such as the type of bacterium treated, the product characteristics, the wave amplitude, the exposure/contact time and the treatment temperature. Several authors pointed out that the effectiveness can be greater if other decontamination technologies are associated with ultrasounds such as heat, low pH, chlorination, etc. In fact, in combination with heat, ultrasound could accelerate the rate of food decontamination, thus reducing the duration and intensity of the heat treatment and the consequent damage (Zencher *et al.,* 2001). In particular, the following treatment combinations have been studied: ultrasounds and high-pressure treatments (manosonication), heat treatments (thermosonication), and the combination of the three technologies (manothermosonication)

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(Baumann *et al*., 2005; Manas *et al*., 2000; Raso *et al.,* 1998; Sala *et al*., 1995).

For the purposes of this work, only sonication and thermosonication treatments have been considered. Room temperature sonication treatments on *Listeria monocytogenes* do not have a powerful effect; in fact, the decimal reduction time is 4.3 minutes (Pagan *et al.,* 1999). The combination of heat and high-power ultrasound was first explored by Ordonez *et al*. (1987). Thermosonication combines moderate heat (37°C to 75°C) with ultrasound treatment. According to some authors, this treatment, compared to sonication alone, has a real effectiveness if the temperature is above 50°C (Lopez-Malo *et al*., 1999; Piyasena *et al.,* 2003; Knorr *et al*., 2004; Dubrovic *et al*., 2011; Herceg *et al.,* 2012). Wrigley and Llorca (1992) reported that a treatment at 40°C for 30 minutes can reduce *Salmonella Typhimurium* by 3 log/cfu. Instead, Scouten and Beuchat (2002) reported that a treatment at 55°C for 5 minutes can reduce *Salmonella enterica* in alpha-alpha seeds by 3.62 log/cfu. Thermosonication was applied effectively to reduce the count of *L. monocytogenes* in cell suspension (Ugarte-Romero *et al*., 2007). Baumann *et al.* (2005) observed that after 5 minutes of treatment with thermosonication at 60°C in apple cider *Listeria monocytogenes* ATCC 10403S was inactivated during a 6-hour storage period at room temperature. The aim of the present work was to investigate the time-treatment parameters in smoked salmon samples experimentally contaminated with *L. monocytogenes* and immediately subjected to sonication and thermosonication at different time-temperature combinations.

## Materials and Methods

120 samples of salmon, in packs of 200 g., produced by the same manufacturer, were purchased at a local supermarket on the day of the trial and kept in refrigerated conditions (4°C) for 1 h until the time of treatment.

### Bacterial strains and preparation of the cocktail

The bacterial strains used were four; *Listeria monocytogenes* ATCC 19114, *Listeria monocytogenes* ATCC 15313, *Listeria monocytogenes* ATCC 19111 and *Listeria monocytogenes* ATCC 7644.

The individual microorganisms were rehydrated in 9 mL of Buffered Peptone Water (BPW, Oxoid), and after 20 minutes the cultures were streaked on Tryptic Soy Agar (TSA, Oxoid) and incubated at 35°C for 22 h. A colony was taken from each TSA plate, diluted in 10 mL of Tryptic Soy Broth (TSB, Oxoid) and incubated at 35°C for 22 h. Cells were collected by centrifugation at 4000 rpm for 20 minutes at 4°C and washed three times with BPW. The individual bacterial strains were resuspended in BPW to obtain a final concentration of about 108 cfu/mL. The final concentration of the inoculum was determined by a spectrophotometer at a wavelength of 550 nm. The bacterial cocktail was prepared by mixing equal volumes of each ATCC strain and was tested before use by the pour plate technique according to ISO 11290-2 standard method. The smoked salmon was weighed, maintaining sterile conditions, to give samples of 25 g. A spot inoculation method was then used to inoculate *L. monocytogenes* on smoked salmon samples (Mahmoud, 2010). Subsequently, the samples have been packaged under vacuum and subjected to sonication and thermosonication treatments within 10 min from inoculation.

### Sonication and thermosonication treatments

Waveco®, an ultrasonic bath with a capacity of 30 litres (Next Cooking Generation, Milano, Italy) was used for all ultrasound treatments. All samples were processed at 20 kHz with 100% amplitude with focused ultrasonic wave according to the patent (International Application No.: PCT/IB2017/053465) at 20°C, 25°C, 30°C, 40°C and 50°C for 5, 10 and 15 minutes. Before and after each ultrasound treatment, all equipment was sanitized. During each trial, samples were taken before (in triplicate) and after treatment (in quintuplicate) to assess the initial contamination and the effectiveness of the decontamination treatment.

### Bacterial enumeration

All samples were analysed according to the ISO 11290-2 standard method.

25 g of untreated (control) and treated salmon at different times and temperatures were transferred to a sterile stomacher bag (Gosselin SM2B-01, Villeurbanne, France) containing 225 mL of Maximum Recovery Diluent (MRD, Oxoid), homogenized in a stomacher (BagMixer, Interscience) at room temperature for 1 min. 1 mL of the homogenized sample was then diluted 10 times in series in 9 mL of sterile MRD and the appropriate dilutions were streaked onto appropriate selective media.

### Statistical analysis

The effects of temperature and time were analysed through a one-way ANOVA. The level of significance was set at $P < 0.05$. The data was analysed using the software Excel 2010 (Microsoft Corporation).

## Results and Discussion

Survival and reduction of *L. monocytogenes* cocktail in smoked salmon at five temperatures are shown in Table 1 and in Figure 1.

**Table 1. Inactivation of *Listeria monocytogenes* cocktail after ultrasonic treatments in smoked salmon.**

| Time | 20°C | 25°C | 30°C | 40°C | 50°C |
|---|---|---|---|---|---|
| 5 | 0.58 | 0.72 | 1.24 | 1.91 | 2.44 |
| 10 | 0.88 | 1.00 | 1.39 | 2.05 | 1.90 |
| 15 | 0.97 | 1.08 | 2.02 | 2.14 | 2.12 |

*Average values expressed in log cfu/ml.

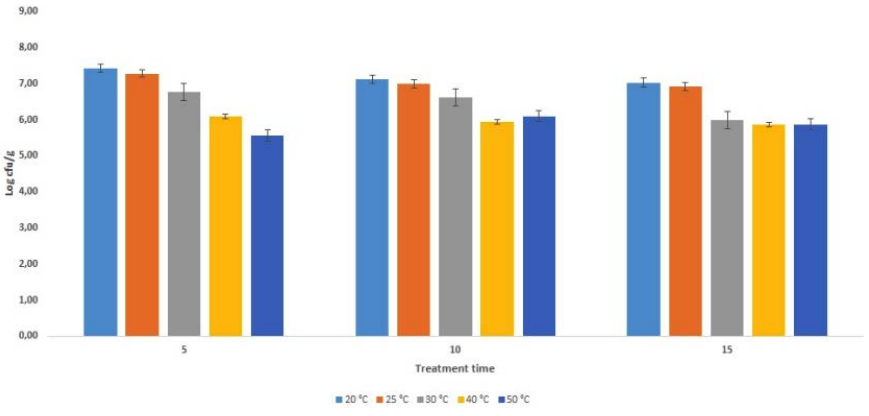


**Figure 1. Survival of *Listeria monocytogenes* cocktail after ultrasonic treatments in smoked salmon.**

Inactivation at low temperatures (20°C, 25°C) was much less evident, if not negligible, compared to the other temperatures (30°C, 40°C and 50°C).

The ultrasonic inactivation was 2.02, 2.12 and 2.44 log cfu/gr at 30°C for 15 minutes, at 40°C for 15 minutes, and at 50°C for 5 minutes, respectively.

However, no statistically significant difference was observed between the samples during the study.

The results obtained from this work are in accordance with other authors' results obtained in other foods. Baumann *et al.,* (2005) obtained a similar reduction in apple cider treated at 50°C, 55°C and 60°C. Earnshaw *et al.* (1995) reported a similar inactivation increase in whole milk, following the application of ultrasounds at high temperatures.

It is not clear why the treatment at 50°C for 10 minutes has given worse inactivation values than the other times and compared to the treatment at 40°C for the same time.

An explanation of this phenomenon has been described by Mason (1999), who hypothesized that, at certain temperatures, there might be an increase in the vapour pressure that suppresses, through a damping effect, the energy released when a cavitating bubble implodes thus lowering the rate of inactivation. Similar phenomena have already been observed and underlined by Pagan *et al.* (1999) during a 40°C manosonication treatments and Baumann *et al.* (2005) during thermosonication treatments at 60°C.

## Conclusions

The results of this study indicate that ultrasound treatments can improve the inactivation of *Listeria monocytogenes* at sublethal temperatures, maintaining the sensory characteristics of the treated samples. In fact, the smell has not undergone changes in any of the samples; instead, the colour has gradually decreased in intensity at higher temperatures, however, when the vacuum packs were opened, it immediately resumed tone. Therefore, this treatment regime could provide a solution for the smoked salmon industry, to achieve the food safety regulatory requirements. Our preliminary data can offer a basis to further investigate the effects of sonication or thermosonication in smoked salmon inoculated with *L. monocytogenes*.

The next steps will be the evaluation of shelf life and the effect on the pathogenic bacteria present in the samples, in particular the evolution of cell damage and repair phenomena.

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