Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/issn/15375110

Research Paper

Radiofrequency inactivation of *Salmonella* Enteritidis PT 30 and *Enterococcus faecium* in wheat flour at different water activities

Rossana Villa-Rojas [a], Mei-Jun Zhu [b], Bradley P. Marks [c], Juming Tang [a,*]

[a] Department of Biological Systems Engineering, Pullman, WA, USA
[b] School of Food Science, Washington State University, Pullman, WA, USA
[c] Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI, USA

ARTICLE INFO

Article history:
Received 24 November 2015
Received in revised form
12 December 2016
Accepted 3 January 2017
Published online 4 February 2017

Keywords:
Salmonella
Low-moisture
Radiofrequency heating
Inactivation
Enterococcus faecium
Surrogate

Salmonella persistence in low-moisture foods creates a significant need for effective pasteurisation processes, but conventional thermal treatments for low-moisture products are challenged by long treatment times and insufficient information on inactivation kinetics. Radiofrequency (RF) heating can reduce heating time and inactivate *Salmonella* without inducing significant quality damage. The objectives were to study RF heating of organic wheat flour, and evaluate *Enterococcus faecium* as a surrogate for RF inactivation of *Salmonella*. Temperature profiles and uniformity of the top and cross-section surface of RF heated flour were obtained with an infrared camera, using different electrode gaps, platforms, and different materials that surrounded the sample to make the electromagnetic field uniform. The flour was inoculated with *S.* Enteritidis PT 30 or *E. faecium*, equilibrated to a specific a_w, and then RF heated for 8.5 (0.25 a_w) or 9 min (0.45 and 0.65 a_w) to reach ≈75 °C minimum temperature (no holding time); survivors were then enumerated. The best temperature uniformity was obtained using a 90 mm electrode gap, placing small polystyrene cylinders above and underneath the sample container, and using a platform of polystyrene Petri dishes. *Salmonella* reduction of 7 log was achieved at 0.45 and 0.65 a_w at room temperature, while 5 and 3 log reductions were reached for *Salmonella* and *E. faecium*, respectively, at 0.25 a_w. These data suggest that RF heating has potential as an inactivation treatment for *Salmonella*, and that *E. faecium* is a feasible surrogate to validate the efficacy of RF treatments.

1. Introduction

The new FDA Food Safety Modernization Act (FSMA) addresses food safety with a preventive focus. All manufacturers who provide food products or ingredients to the US market will need comply with pending rules (Food and Drug Administration, 2013). One of the requirements in section 204(d)(2) is for the FDA to designate high risk foods that require additional record keeping to protect the public's health. Due to the association with several *Salmonella* outbreaks, some low-moisture foods, like nuts and nut products,

* Corresponding author. Biological Systems Engineering, Washington State University, P.O Box 646120, Pullman WA 99164-6120, USA. Fax: +1 509 335 2722.
E-mail address: jtang@wsu.edu (J. Tang).
http://dx.doi.org/10.1016/j.biosystemseng.2017.01.001

are included in the high risk food list (Food and Drug Administration, 2014; Food and Drug Administration, United States Department of Agriculture, & Homeland Security, 2011). This has forced the food industry and research community to study possible treatments to inactivate *Salmonella* in low-moisture foods. However, the efficacy of such treatments is challenging, because *Salmonella* becomes highly resistant to heat at low water activities (Archer, Jervis, Bird, & Gaze, 1998; Bari et al., 2009; Chen et al., 2009; Du, Abd, McCarthy, & Harris, 2010).

Heat treatments have been successfully implemented for both pasteurisation and sterilisation of high moisture products, and they show promise for low-moisture products. Jeong, Marks, and Orta-Ramirez (2009) observed that 1 log reduction of *Salmonella* on the surface of almonds could be achieved in 16 s if the air humidity was 70–90% (volume fraction) at 82.2 °C, and in 957 s if the air humidity was 5%. Radiofrequency (RF) heating is another promising technology to heat bulk low-moisture foods in short times and thereby inactivate pathogens in those products. Wang, Tiwari, Jiao, Johnson, and Tang (2010) compared hot air vs. RF-assisted heating for legumes (chickpeas, green peas, and lentils) as potential treatments for disinfestation. They found RF-assisted heating required less time (5–7.5 min) than did hot air (275–660 min) to reach the target temperature (60 °C) at the centre of the legume bed. Although changes in colour were not significantly different for either method compared to the untreated samples, hot air treated samples lost significant weight and moisture when compared to RF-treated or untreated samples. Kim, Sagong, Choi, Ryu, and Kang (2012) used RF heating to inactivate S. Typhimurium and *Escherichia coli* in spices, and found that 50 and 40 s treatments resulted in 2.8 and 4.3 log reductions in black pepper, and 3.4 and >5 log reduction in red pepper for S. Typhimurium and *E. coli*, respectively, without significant colour changes.

RF heating has potential as a heat treatment to control pests and pathogens (Alfaifi et al., 2014; Wang & Tang, 2004; Wang, Tang, Johnson, Mitcham, & Hansen, 2002); however, temperature uniformity is still a major challenge for this technology (Jiao, Tang, & Wang, 2014). Some parts of the food are being over treated, while others may be undertreated. Prolonging processing times to bring the cold spots to the minimum temperature required for pasteurisation increases the chances of quality damage due to extreme overtreatment of the hot spots.

Several studies have tried different approaches to improve heating uniformity of RF heating. Wang et al. (2010) studied the effect of forced hot air, shaking the container with a conveyor belt, and mixing food in the container during RF heating of legumes, as well as different combinations of all these. They found that using a combination of forced hot air and shaking the container reduced the standard deviation in temperature from 4.2 to 3.2 °C.

Previous studies reported that surrounding a peanut butter container with a plastic that has a similar dielectric constant to peanut butter reduced the temperature difference from 13 to 7 °C on the top surface and from 28 to 18 °C in the cross-section surface, with a similar effect reported for wheat flour (Jiao et al., 2014). However, there is no universal solution, and different products may require different approaches.

Once any process or technology is proposed for commercial application, validation of that process is essential, either via the use of microbial inactivation models (and appropriate dynamic product and process data) or via the use of a non-pathogenic surrogate inoculated onto the product and subjected to the actual process (Awuah, Ramaswamy, & Economides, 2007; Chen et al., 2009). However, there are very few established procedures to validate a process in low-moisture products. The Almond Board of California had documented procedures for the use of *Enterococcus faecium* NRRL B-2354 as a surrogate to validate thermal inactivation of *Salmonella* in almonds (Almond Board of California, 2007). *E. faecium* has also been studied to validate processes such as extrusion of carbohydrate—protein meal (Bianchini et al., 2014). However, the validity of *E. faecium* as an appropriate surrogate has been demonstrated for very few other products or processes.

The objectives of this research were to assess RF as an inactivation treatment for S. Enteritidis PT 30 in organic wheat flour and to evaluate the use of *E. faecium* NRRL B-2354 as a non-pathogenic surrogate for treatment validation.

2. Material and methods

2.1. Bacteria strains and wheat flour

S. Enteritidis PT 30 and *E. faecium* NRL B-2354 were acquired from Dr. Linda Harris at UC-Davis. They were kept in a stock solution of trypticase soy broth (TSB) supplemented with 0.6% (w/v) yeast extract (YE) and 20% glycerol at −80 °C until used. S. Enteritidis PT 30 was chosen because of its relation to a low moisture food outbreak (Isaacs et al., 2005), high resistance to thermal inactivation (Anderson, Keller, Gradl, Pickens, & Li, 2013) and various studies publish on its survival, persistence and inactivation kinetics in different matrixes with different technologies (Danyluk, Uesugi, & Harris, 2005; Harris, Uesugi, Abd, & McCarthy, 2012; Jeong et al., 2009; Jeong, Marks, Ryser, & Harte, 2012; Komitopoulou & Pen, 2009; Smith & Marks, 2015; Villa-Rojas et al., 2013). The studied food matrix was soft white wheat organic pastry flour (Eden Foods, Clinton, MI).

2.2. Radiofrequency-assisted heat treatment

2.2.1. Temperature uniformity and profile of RF treatments

A bench-top, 0.5 kW, 27 MHz RF heating unit (Thermail E0-1, W.T. LaRose & Assoc. Inc., Troy, NY) was used to heat treat the samples (Fig. 1 A and B). An infrared camera (Thermal CAMTM SC-3000, FLIR Systems, Inc., North Billerica, MA) was used to obtain temperature profiles of the top surface (Fig. 1C) and/or a cross-sectional (Fig. 1D) for all samples. The heating pattern of the RF equipment was evaluated by heating a foam slab made out of polyurethane, with the same dimension of the upper electrode (254 × 75 mm). The foam would show the location of the hot and cold spots within the equipment.

The effects of the electrode gap, platform, and surrounding material on the heating uniformity of the sample were also evaluated. The temperature uniformity was calculated with the uniformity index (UI) as explained by Alfaifi et al. (2014),