LWT - Food Science and Technology 92 (2018) 371–379

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Improving design of thermal water activity cell to study thermal resistance of *Salmonella* in low-moisture foods

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ARTICLE INFO

Keywords:
Food safety
Relative humidity
Thermal inactivation
Pathogens

ABSTRACT

Water activity (a_w) influences the thermal resistance (D-value) of pathogens in low-moisture foods (LMF). However, the influence of food matrices on a_w at elevated temperatures is complicated. A recent study reported that a novel thermal water activity cell (TAC) could be used to control a_w of LMF using LiCl solution (select molality) during the isothermal determination of D-values of *Salmonella*. This research proposed a new version of TAC (TAC II) that could significantly reduce the time to establish the moisture-equilibrium between LMF and LiCl solutions during D-value measurements. Wheat flour (WF) samples inoculated with *Salmonella* (a_w at room temperature = 0.45) were treated in TAC II at 80 °C under controlled relative humidity (RH) (50%) provided by LiCl solution (9.37 mol kg^{-1}). Results showed that the moisture-equilibrium was established to the controlled RH condition at 80 °C in < 4 min for tests with TAC II, as opposed to > 14 min for TAC. The D-value of *Salmonella* PT30 in WF (80 °C, RH 50%) tested with TAC II (20.7 ± 1.1 min) was significantly higher ($p < 0.05$) than the reported result obtained from TAC. TAC II provides more accurate estimation of D value for giving relative humidity at high temeprature, and it offers to improve our understanding of the isolated effect of a_w (corresponding RH) at high temperatures on the D-values of *Salmonella* in LMF.

1. Introduction

Low-moisture foods (LMF) were traditionally considered to be associated with foodborne illnesses caused by microbiological contamination (bacterial or fungal). That notion has changed due to recent foodborne outbreaks or product recalls of LMF (CDC, 2017; FDA, 2017; Harris, Beuchat, Danyluk, & Palumbo, 2017). These incidents have often been caused by pathogens such as *Salmonella* spp. in different food products or raw ingredients under desiccated conditions. These products include chocolate, powdered dairy powder, spices and seasonings, cereal and nut products (Chen et al., 2009; Podolak, Enache, Stone, Black, & Elliott, 2010). Consequently, U.S. Food and Drug Administration is considering to designate the LMF category in the high-risk foods list under section 204(d)(2) of Food Safety Modernization Act (FSMA) (FDA, 2014). With the implementation of FSMA, there is an urgent need for the food processors to develop the preventive controls and assess the efficacy of thermal processing operations to mitigate food safety hazards in LMF (Brackett, Ocasio, Waters, Barach, & Wan, 2014; Grover, Chopra, & Mosher, 2016; Taylor, 2011). This requires additional research to identify key factors contributing to the enhanced thermal resistance of *Salmonella* spp. in LMF.

Factors that make *Salmonella* spp. in LMF resistant to the elevated temperatures are quite complex and may include several intrinsic characteristics such as water activity (a_w) and mobility, moisture content, food composition, and other factors (Wesche, Gurtler, Marks, & Ryser, 2009) and extrinsic parameters such as time-temperature regime, relative humidity (RH), heating mode. Out of these factors, a_w is a measure of thermodynamically available moisture in a food system, and is considered as a more useful indicator than the moisture content for predicting the stability and safety of foods (Y. H. Roos, 2007; Yrjö H. Roos, 2010). Many studies have shown that reduced a_w of LMF products could significantly enhance the thermal resistance of *Salmonella* spp. (Archer, Jervis, Bird, & Gaze, 1998; He, Guo, Yang, Tortorello, & Zhang, 2011; Lang et al., 2017; Villa-Rojas et al., 2013). In those published studies, the a_w of food measured at room temperature was used as an important factor to define the thermal resistance data (D-value – the time required to inactivate 90% of the microbial population at a target temperature) of bacteria at the treatment temperatures. However, the a_w of food of a fixed moisture content in a closed system often changes with temperature (Tadapaneni, Yang, Carter, & Tang, 2017), and the degree of such change can be estimated by the temperature specific moisture sorption isotherms (Syamaladevi, Tadapaneni, et al., 2016; Syamaladevi, Tang, Villa-Rojas et al., 2016; Tadapaneni, Syamaladevi, Villa-Rojas, & Tang, 2017). The change in a_w may significantly affect

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https://doi.org/10.1016/j.lwt.2018.02.046
Received 9 December 2017; Received in revised form 15 February 2018; Accepted 16 February 2018
Available online 19 February 2018
0023-6438/

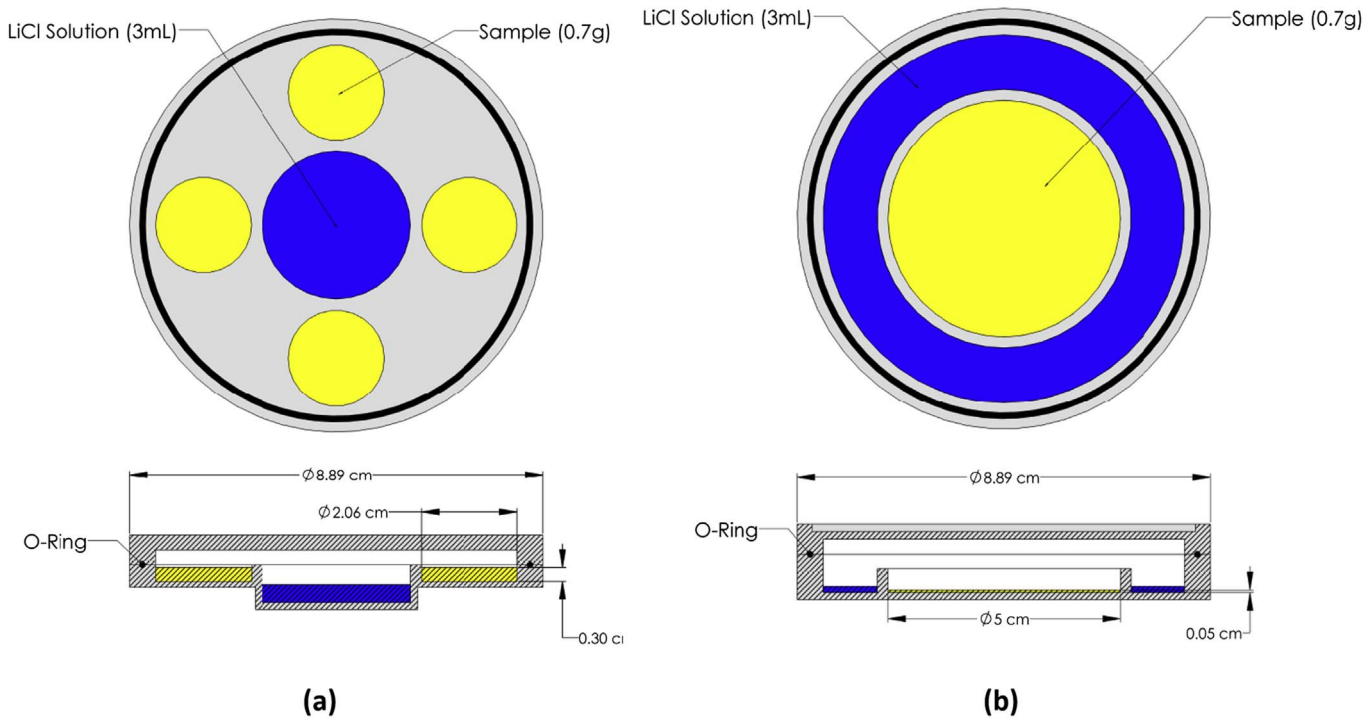

Fig. 1. Schematic representation of both test cells used in this study: TAC (a) and TAC II (b).

the thermal resistance of bacteria (Baird-Parker, Boothroyd, & Jones, 1970; Chang, Han, Reyes-De-Corcuera, Powers, & Kang, 2010; D'Aoust, 1989; Farakos, Frank, & Schaffner, 2013). Therefore, it is very important to determine the a_w of the foods at the treatment temperatures and connect such information with the thermal resistance of *Salmonella* spp. for development of effective thermal processes.

To isolate the effect of a_w at treatment temperatures on the thermal resistance of *Salmonella* spp. in LMF from other influencing factors, we developed a novel thermal water activity cell (TAC) which held a specific molality of lithium chloride (LiCl) solution to control the a_w of a food sample over a wide temperature range (Tadapaneni, Syamaladevi, et al., 2017). In that controlled RH environment, the thermal resistance of *Salmonella* Enteritidis PT30 in a LMF sample can be determined. In our previous design of TAC, approximately 0.7 g of food samples were added to each of four sample wells which had surface area of 3.33 cm^2, and sample thickness of approximately 0.3 cm, depending on the sample bulk density. This sample size is necessary to allow an adequate level of bacterial inoculation (10^7–10^8 CFU) in a relatively homogeneous mass.

When the food sample is heated in a TAC cell with LiCl solution of a specific molality, the vapor pressure of the sample changes according to its moisture sorption isotherms while LiCl solution generates a controlled RH relative humidity (RH) in the headspace. The difference between the vapor pressures of a food sample and that of the LiCl solution causes the moisture content of the food sample to change until their a_w reach an equilibrium at the treatment temperature. Thus, during the heating, two simultaneous physical processes take place: (1) the temperature of the food sample is increased to the set temperature, and (2) moisture migrates within the food sample and exchanges with the headspace environment. It is desirable that most of the thermal inactivation of the inoculated bacteria occurs after a relatively short temperature come-up time (e.g., the time required for the sample to reach 0.5 °C within the set temperature) and also a short sample moisture equilibrium time.

However, in our previous design of TAC (Tadapaneni, Syamaladevi, et al., 2017), the food samples in the four deep sample wells (~0.3 cm) of TAC might not be fully exposed to a stable controlled RH environment, while the moisture content of the food sample underwent a change to reach an equilibrium at the treatment temperature. Consequently, the calculated D-values from the measured reduction of bacteria population might not reflect the true thermal resistance of the bacteria in the food samples at the equilibrium stage.

Another concern is a possible condensation of moisture on the surface of samples during the cooling of TAC after each thermal treatment. If this happens, the inoculated bacteria would be exposed to a high moisture food matrix while the sample temperature is still above the lethal level, causing a significant reduction in the bacterial population during cooling and underestimation of thermal resistance data for the test microorganism.

Therefore, the objectives of the present study were: (1) to improve the design of TAC by increasing the exposure surface area of both food sample and LiCl solution, which would shorten the time for the food sample to reach equilibrium with the controlled RH condition in the headspace, and (2) to evaluate the thermal inactivation of *Salmonella* Enteritidis PT30 in a LMF sample (organic wheat flour) during cooling of the improved test cell as affected by the treatment temperature and relative humidity.

2. Material and methods

2.1. Design and development of TAC II

For meeting the objectives of this study as mentioned in the previous section, the improvements to TAC were based on the following design criteria:

- sample well in TAC to accommodate at least 0.7–1 g of test food sample for ease sample handling during preparation and post-treatment analysis
- increase aspect ratio (diameter/height) of the test food sample to accelerate the moisture equilibration process
- separation of the sample well and LiCl solution to prevent any potential cross-contamination