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

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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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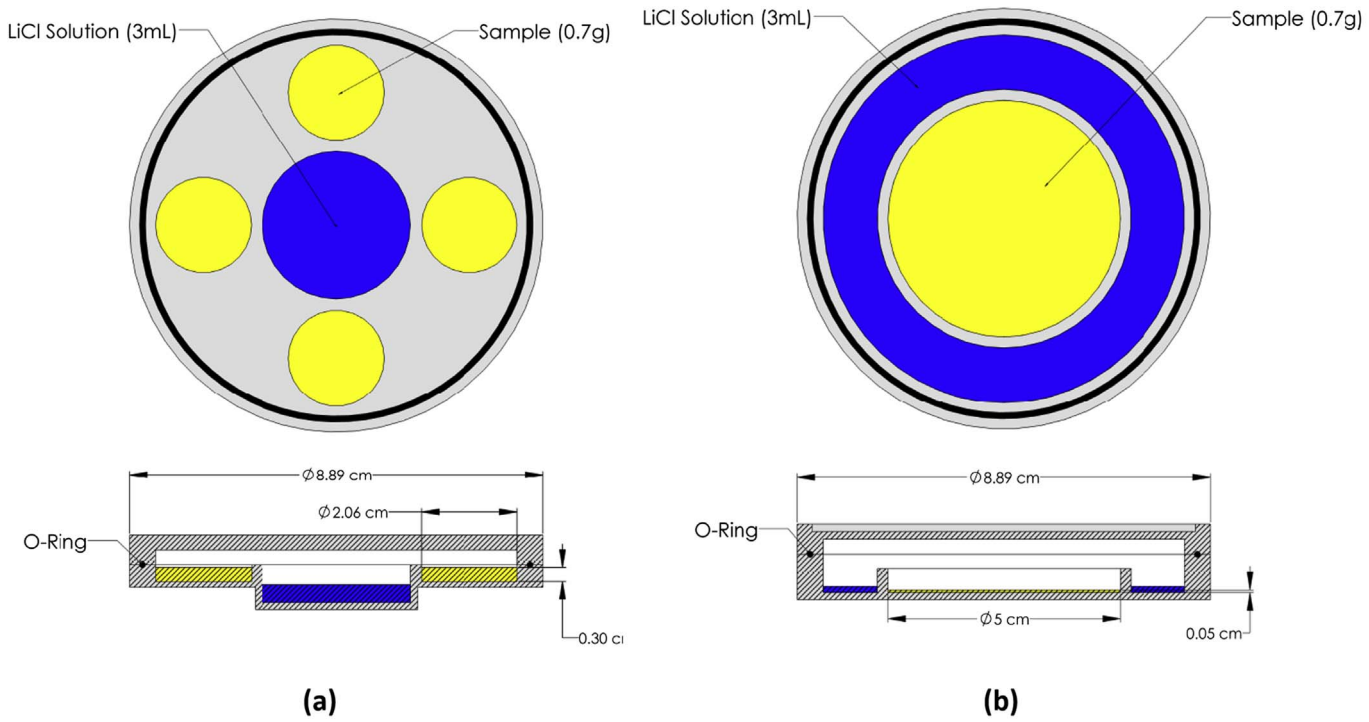

Fig. 1. Schematic representation of both test cells used in this study: TAC (a) and TAC II (b).

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

The original design of TAC cell as reported by our group (Tadapaneni, Syamaladevi, et al., 2017) was modified into an improved version, referred to as TAC II. TAC II was also fabricated using aluminum alloy 6061 for good heat transfer properties (Tadapaneni, Syamaladevi, et al., 2017). Fig. 1 shows the internal design changes in a TAC II, where the sample well diameter was expanded to hold the same amount of food sample with reduced depth (approximately 0.05 cm), and the outer channel would hold an unsaturated LiCl solution. With the increased aspect ratio (diameter/thickness) of a test food sample in a TAC II, the time required for a food sample to reach the set temperature and controlled relative humidity condition would be reduced. The food sample in the TAC II was estimated to have approximately 490% more exposed surface area than the original design with same sample size. Thus, it was anticipated that a food sample in a TAC II would adjust its moisture content relatively faster in response to the relative humidity of headspace at the treatment temperature.

In a closed system, LiCl solution with a specific molality helps to maintain the relative humidity in the headspace. Using osmotic coefficients of LiCl, the corresponding a_w values as a function of temperature were reported as a user-friendly chart (Tadapaneni, Syamaladevi, et al., 2017). For example, 9.37 mol kg^{-1} of LiCl solution in a closed cell would offer a_w of 0.45 at 20 °C. When the temperature increased to 80 °C, a_w of the same LiCl solution in the cell would increase to 0.50. The theoretical estimation of a_w values of LiCl solutions was experimentally verified in the TAC II at different temperatures by using a lid with temperature and RH sensor (HX15-W, Omega Engineering Inc., Stanford, CT).

2.2. Equilibration time for OWF in TAC II and TAC

Organic wheat flour (OWF) (Eden Foods, Clinton, MI) represents a LMF category of cereal flour and flour products with high carbohydrate-low fat contents. Moreover, it was reported that wheat flours and flour products were involved in the outbreaks or recalls due to the detection of pathogens of concern such as *Salmonella* Typhimurium and *E. coli* (Harris & Yada, 2017). Thus, in this study, OWF with an initial a_w of 0.33 ± 0.3 at room temperature was selected as a test food matrix to evaluate the equilibrating proficiency of TAC II in comparison with that of TAC. The OWF samples were conditioned to a_w of 0.45 ± 0.02 at room temperature for 4–5 days using a custom-built humidity chamber as reported previously by Hildebrandt et al. (2016).

To understand the time needed for OWF sample to reach the equilibrium level with the controlled RH condition by LiCl solutions in the TAC II and TAC, multiple units of TAC II and TAC with 3 mL of 9.37 mol kg^{-1} LiCl solution (corresponding a_w at room temperature a_w = 0.45 ± 0.01) were preheated to 80 °C in a hot air oven. The pre-conditioned OWF sample (to 45% RH) of approximately 0.7 g was placed in an aluminum foil cup and spread as a thin layer in TAC II. The cup was placed in the center well of a TAC II with LiCl solution in the outer channel. Similarly, in TAC, OWF of same sample size (~0.7 g) was placed in the foil cups, and later those cups were placed in sample wells with LiCl solution in the center well. Both test cells were sealed and heated at 80 °C. Every 2 min, the moisture content of OWF samples in both test cells were gravimetrically tracked until no weight change in the samples was observed.

2.3. Time-temperature profile of OWF in TAC II

To evaluate the temperature come-up time of an OWF sample in a TAC II, a pre-conditioned OWF sample of approximately 0.7 ± 0.05 g was placed in the center well of a TAC II and spread out to a thin layer. A calibrated thermocouple probe (Type-T, THQSS-020U-6, Dia 0.5 mm, Omega Engineering Inc., Stanford, CT) was inserted through the lid and extended to the geometric center of OWF sample, which is typically considered to be a cold spot.

The TAC II with OWF sample and 3 mL of LiCl solution (9.37 mol kg^{-1}) was sealed and immersed in a pre-heated ethylene glycol-oil bath (HAAKE DC30/DL 30, Thermo Electron, Germany) with the set temperature at 80 °C. When the OWF sample in TAC II reached the set temperature of the oil bath, TAC II was removed and immediately put in stirring ice-water bath. The heating and cooling temperature profiles of OWF sample in TAC II were recorded using a thermocouple thermometer (Digisense DuaLogR 99100-50, Cole-Parmer Instruments Co., Vernon Hills, IL).

2.4. Thermal resistance of Salmonella Enteritidis PT30 in OWF using TAC II

Salmonella Enteritidis PT30 was selected in this study due to its association with foodborne outbreaks in LMF (Isaacs et al., 2005). The strain was obtained from Dr. Linda Harris at University of California, Davis, and was stored in tryptic soy broth (TSB) (Difco, Becton Dickinson, Sparks, MD) at −80 °C with added supplements which included 0.6% (w/v) yeast extract (YE) (Difco, Becton Dickinson, Sparks, MD) and 20% glycerol (Sigma Aldrich, St. Louis, MO).

The inoculum of *Salmonella* Enteritidis PT30 was prepared according to lawn-based pellet method described by Hildebrandt et al. (2016). A loop (10 μL) from the stored strain of *Salmonella* Enteritidis PT30 was inoculated to 9 mL of TSB + YE and incubated at 37 °C for 24 h. Further, 100 μL of the previous culture was transferred to another 9 mL of TSB + YE and incubated at 37 °C for 24 h. After two transfers, 1 mL of bacteria culture from TSB + YE was spread plated on tryptic soy agar (TSA, Difco, Becton Dickinson, Sparks, MD) supplemented with YE and incubated at 37 °C for 24 h. After incubation, the bacterial lawn was harvested by maximum recovery diluent (MRD) (Fisher Scientific, Pittsburgh, PA) and centrifuged (6000 g × for 15 min at 4 °C) to obtain pellets. The pelletized culture was added with 3 mL of MRD for re-suspension. One mL of re-suspended bacterial culture was added to 10 g of OWF sample. The whole sample was hand massaged in stomacher bags for approximately 3 min to break down any lumps. The mixture was further mixed with 90 g of OWF in stomacher bags and massaged with a stomacher (400 Seward, West Sussex, UK). After the inoculation, the OWF samples were placed into sterilized trays and equilibrated to 45% relative humidity for 4–5 days at room temperature in the humidity chamber as described in Section 2.2. After equilibration of the inoculated OWF samples, the population levels of *Salmonella* Enteritidis PT30 in OWF samples were verified. The OWF samples with bacterial colony counts of at least 10^7 to 10^8 log CFU/g were selected for the thermal resistance tests.

Prior to thermal tests, the a_w of inoculated OWF samples was analyzed using AquaLab series 3 TE (METER Group Inc., Pullman, WA). The inoculated OWF samples with a_w of 0.45 ± 0.02 (at ~22 °C) were considered for the thermal treatments with TAC II. The thermal treatment procedures of inoculated OWF samples were followed according to the method reported by Tadapaneni, Syamaladevi, et al. (2017). Approximately 0.70 ± 0.05 g of inoculated OWF sample was loaded into the central well of the TAC II and spread into a thin layer. Also, 3 mL of LiCl with a molality of 9.37 mol kg^{-1} was added to the outer channel of the TAC II. The TAC II units were sealed and equilibrated for 8 h at room temperature to headspace relative humidity provided by LiCl solution. All the sample loading process was operated in the humidity chamber with a controlled environment (45% relative humidity) at room temperature.

The conditioned TAC II cells were then immersed in a well-stirred ethylene glycol-oil bath (Isotemp 5150 H11, Fisher 180 Scientific, Inc. PA) set at 80 °C. After the OWF samples reached the target temperature (based on the come-up time of OWF sample in TAC II), the TAC II cells were removed from the heated bath at nine different time intervals, and immediately placed in an ice-water bath for approximately 2 min.

The survivor population of *Salmonella* Enteritidis PT30 was determined by adding treated samples to sterile bags (VWR Sterile Sample Bags, Radnor, PA) with 6.3 mL of MRD and mixed for 3 min in a

stomacher. The mixed samples were 10-fold diluted in MRD, and further plated in duplicate onto TSA + YE with supplementation of 0.05% (w/v) ferric ammonium citrate (Sigma-Aldrich, St Louis, MO) and 0.03% (w/v) sodium thiosulfate (Sigma Aldrich, St. Louis, MO). The plates were incubated at 37 °C for 48 h for counting the bacterial colonies.

The thermal resistance data, i.e., D-values at 80°C was estimated using the linear regression of the first-order kinetic model which fitted the survivor curves of *Salmonella* Enteritidis PT30 (Peleg, 2006):

$$D_{80°C} = \frac{t}{(\log N_0 - \log N_t)} \quad (1)$$

where $D_{80°C}$ is the time required to reduce 90% of the microbial population at the temperature of 80°C, t is the time of isothermal treatment (min), N_0 is the initial population count of *Salmonella* Enteritidis PT30 (CFU/g), and N_t is the population of *Salmonella* Enteritidis PT30 at time t (CFU/g).

2.5. Inactivation kinetics of Salmonella Enteritidis PT30 in TAC II during cooling

The cooling of OWF sample thermally treated in a TAC II involved immersing the cell in an ice-water bath. During that process, there is a probability that water vapor present in the headspace of a TAC II might condense to the surfaces of TAC II due to cooling, especially for tests at higher head space relative humidity (> 50%). The water condensation would sharply increase the relative humidity of the headspace, making the inoculum more vulnerable to temperature and cause additional kill of *Salmonella* Enteritidis PT30 during cooling. Thus, it is important to estimate the log-reduction during the cooling of OWF sample in a TAC II with the assumption of conservative conditions which is at the dew point temperature at the specific relative humidity and treatment temperature.

For conservative conditions in the TAC II, we considered the D-values for *Salmonella* Enteritidis PT30 in a medium-high a_w (> 0.95). Based on the study reported by Álvarez, Mañas, Sala, and Condón (2003), the D and z-values of *Salmonella* Enteritidis PT30 in high a_w media (McIlvaine citrate phosphate buffer + sucrose; a_w at room temperature = 0.96) were used as reference data points for the high a_w environment in this study.

The psychrometric chart (Marcks, 2006) was used to estimate the dew point temperatures for a wide range of relative humidities (25, 45, 65 and 85% RH) and treatment temperatures (60–95 °C). From the cooling curve of the treated OWF sample in a TAC II, we considered two different time points to determine the inactivation of *Salmonella* Enteritidis PT30. The log reduction of *Salmonella* Enteritidis PT30 between those time points of the cooling process was calculated by the following relation:

$$\log\frac{N_2}{N_1} = -\int_{t_1}^{t_2}\frac{dt}{D} \quad (2)$$

where, N_1 is the initial microbial population (CFU/g) at time t_1 (s), and N_2 is the microbial population (CFU/g) at time t_2 (s). D is the decimal reduction time (s).

The equation for D in terms of reference temperature (T_{ref} in °C) is D_{Tref}:

$$D = D_{Tref}\, 10^{\frac{\left(T_{ref} - T(t)\right)}{z}} \quad (3)$$

where $T(t)$ is the temperature (°C) at time t (s). The temperature data was plotted against corresponding time points (t_1 and t_2) of cooling process and a significant linear relationship was found:

$$T(t) = -m \cdot t + c \quad (4)$$

where m is the slope and c is the intercept of the curve. From Eqs. (2)–(4):

$$\log\frac{N_2}{N_1} = -\int_{t_1}^{t_2}\frac{dt}{D_{Tref}\, 10^{\frac{\left(T_{ref} - (-m \cdot t + c)\right)}{z}}} \quad (5)$$

Using definite integration of Eq. (5), the log reduction of *Salmonella* Enteritidis PT30 in OWF sample during the cooling process for time points t_1 and t_2 was estimated.

2.6. Data analysis

The obtained data for the tests with TAC II was analyzed against the reported data from tests of TAC and TDT cells using student's t-test in Minitab 17 (Minitab Inc., State College, PA) with the level of significance set at 0.05. In this study, all the tests were performed as at least in three independent replicates.

3. Results and discussion

3.1. Rate of moisture change of OWF in TAC II

The molality of LiCl solution (a_w 0.45) can be estimated as 9.37 molkg^{-1}, the a_w of this solution during the thermal treatment (at 80 °C) in TAC II was theoretically estimated as 0.50.

Fig. 2 shows the change in the mass of an OWF sample for every 2 min in the TAC II and TAC cells under a controlled relative humidity (50%) by a LiCl solution at 80 °C. For the initial 2 min, there was a loss of approximately 3.6% in the weight of the OWF sample in TAC II. On further heating of the samples in the TAC II with above-mentioned RH condition, the weight continued to decrease till 6 min (by 0.6%). With more heating, the moisture content of the samples remained stable at approximately 7.5% db (dry basis). The overall weight loss of OWF sample at the end of the test (20 min) with the TAC II was approximately 4.4%.

In contrast, for the initial 2 min of heating, the OWF samples in the TAC cell experienced a moisture loss of approximately 2%. As the OWF samples were further heated in the TAC cell, additional moisture loss continued till 14 min of heating time after which the moisture content of OWF sample remained constant at approximately 8% db.

The observed weight change in the OWF samples during heating in TAC II and TAC resulted from the difference in a_w between OWF and LiCl at the elevated temperature. It was reported that in a closed system in which the sample moisture content did not change, the water activity of OWF with initial level of 0.45 at 20 °C would increase to 0.73 at 80 °C as indicated by the reported moisture sorption isotherms of OWF (Tadapaneni, Syamaladevi, et al., 2017). But, the a_w of LiCl solution in TAC II remained relatively stable (0.50 ± 0.01) at 80 °C, similar to that in TAC (Tadapaneni, Syamaladevi, et al., 2017). The difference between the vapor pressures of OWF sample and LiCl solution would induce moisture migration from the OWF sample to reach the equilibrium with the headspace in TAC II and TAC. From the reported moisture sorption isotherms of OWF at 20 and 80 °C (Tadapaneni, Syamaladevi, et al., 2017), it can be determined that, in order for this OWF sample to reach a a_w of 0.50 at 80 °C, the sample moisture content would be reduced from approximately 12% db to 7.5% db. This estimation is consistent with our observations in Fig. 2.

The results in Fig. 2 suggest that OWF samples, when heated in TAC II, experienced faster equilibration with the headspace relative humidity (< 4 min) because of the larger exposure area and thinner sample layer, while samples in the TAC cell had longer equilibration time (> 14 min) due to smaller exposure area and thicker sample layer. The data from this test indicate that the TAC II should be considered a more efficient method to control a_w of LMF under controlled relative humidity at elevated temperatures.

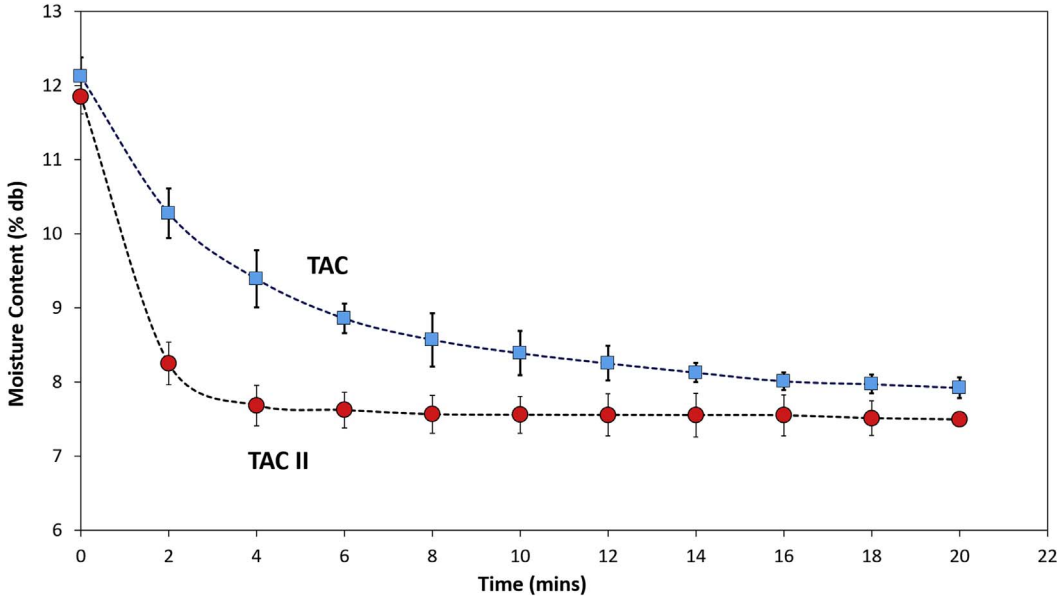

Fig. 2. Moisture balance during the heating of OWF in the TAC II and TAC cells at 80 °C with LiCl solution of 9.37 mol kg^{-1} (providing RH of 50%).

3.2. Heating proficiency of TAC II

Fig. 3 shows the time-temperature profile at the geometric center of an OWF sample layer in a TAC II which is typically the cold spot during the heating and hot spot during the cooling process. The come-up time of OWF sample to reach 79.5 °C was 135 s. During the cooling of the heated TAC II the temperature of the sample dropped from 80 to 63 °C in 5 s after which additional inactivation of *Salmonella* Enteritidis PT30 was ceased.

The wider sample well with thinner sample layer would offer more uniform heating to the OWF samples during the thermal treatments in the TAC II for both heating and cooling process.

3.3. Thermal inactivation of Salmonella Enteritidis PT30 in TAC II

Fig. 4 shows the normalized survival data of *Salmonella* Enteritidis PT30 population in OWF samples treated in the TAC II at 80 °C, in comparison with the reported survivor curves for *Salmonella* Enteritidis PT30 in OWF when treated with TAC and thermal death time (TDT) cell at 80 °C (Tadapaneni, Syamaladevi, et al., 2017). A log-linear trend was observed for the surviving colonies of *Salmonella* Enteritidis PT30 in OWF sample when treated with the TAC II which was similar to the trend observed in the reported survival curve data from TAC and TDT cells (Tadapaneni, Syamaladevi, et al., 2017).

Approximately 4-log reductions of *Salmonella* were observed in the course of thermal treatment (~80 min). At the end of the treatment times of 15 min in TAC and TDT cells, there were approximately 2.5

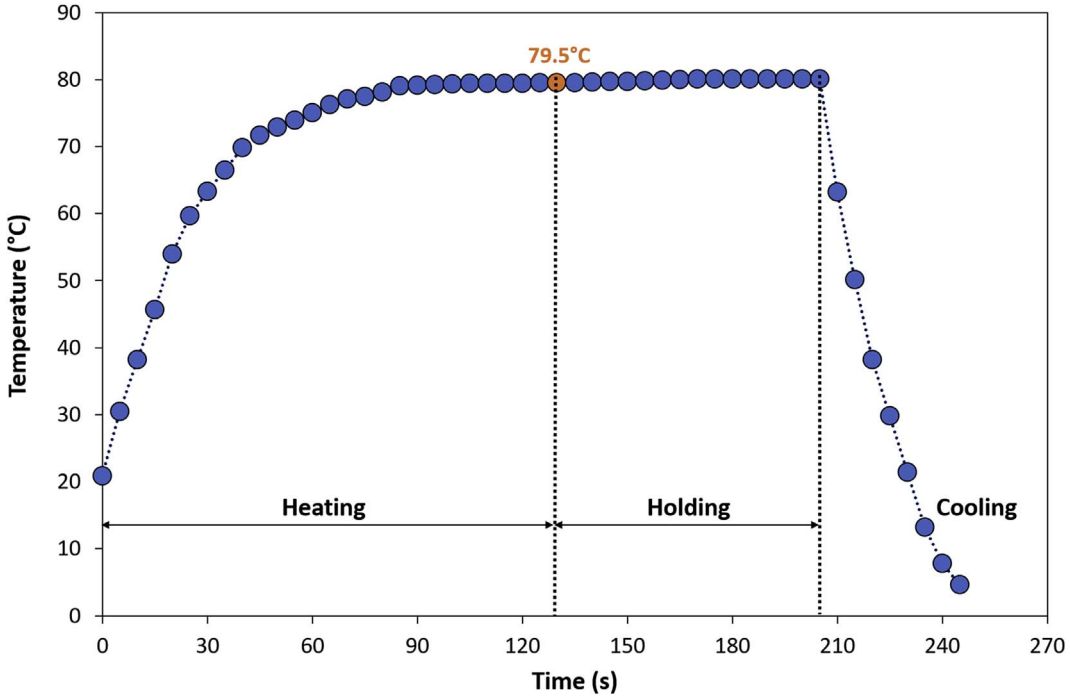

Fig. 3. Time-temperature profile of OWF in a TAC II when heated to 80 °C in an oil bath and cooled in an ice-water bath.

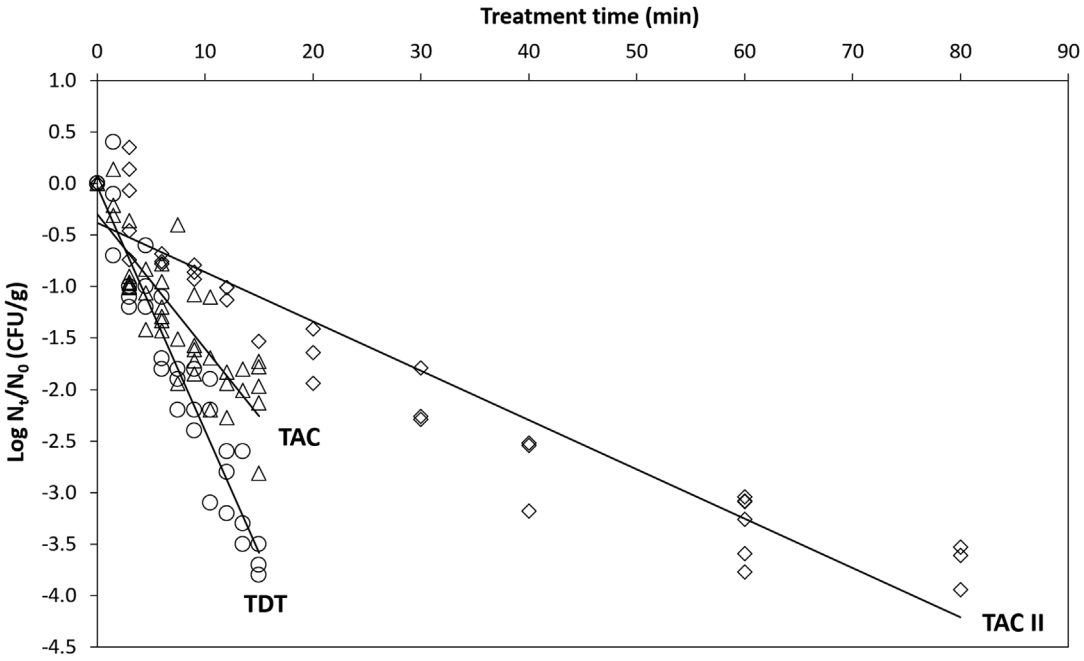

Fig. 4. Survival count of *Salmonella* Enteritidis PT30 in OWF when treated at 80 °C in the TAC II vs reported data from TAC and TDT cells.

and 4-log reductions in *Salmonella* Enteritidis PT30 colonies, respectively (Fig. 4).

Table 1 shows the $D_{80°C}$-values for *Salmonella* Enteritidis PT30 in OWF samples of initial a_w of 0.45 as determined with the TAC II, TAC and TDT cells with corresponding equilibrium a_w values at 80 °C. Based on the thermal inactivation data, the $D_{80°C}$-value for *Salmonella* Enteritidis PT30 in OWF sample determined in TAC II was 20.7 ± 1.1 min. The reported $D_{80°C}$-values determined from TAC and TDT cells were 7.5 ± 0.7 and 4.2 ± 0.2 min, respectively (Tadapaneni, Syamaladevi, et al., 2017).

In both TAC II and TAC cells, the a_w of OWF sample was stabilized at 0.50 at the treatment temperature (80 °C) due to the stable RH in the headspace maintained by the LiCl solution. However, a significantly higher $D_{80°C}$-value was determined with TAC II when compared to $D_{80°C}$-values measured with TAC and TDT cells for *Salmonella* Enteritidis PT30 in OWF ($p < 0.05$). The higher $D_{80°C}$-value for *Salmonella* Enteritidis PT30 in the TAC II was due to the shorter equilibration time so that most of the bacterial population was exposed to the 50% relative humidity (equivalent to $a_w = 0.50$) environment at 80 °C as controlled by the LiCl solution. The *Salmonella* Enteritidis PT30 population in the TAC cell had limited exposure to controlled RH condition in headspace due to a deeper sample well in the TAC cell. The thicker sample bed in the TAC cell reduced the rate of moisture exchange between the sample and the headspace and delayed the equilibration of OWF sample as shown in Fig. 2. Thus, the $D_{80°C}$-value of *Salmonella* Enteritidis PT30 obtained from TAC cell would not reflect the true thermal resistance data of the test culture for a stable water activity at the treatment temperature.

Table 1
$D_{80°C}$-values for *Salmonella* Enteritidis PT30 in organic wheat flour (OWF) (initial a_w at room temperature = 0.45) as treated with different test cells.

Test cell treatment of OWF (n = replicates)	$D_{80°C}$ ± SD (min)	a_w at 80 °C[a]
TAC II (n = 3)	20.7 ± 1.1[a]	0.50 ± 0.01
TAC (n = 6)	7.5 ± 0.7[*b]	0.50 ± 0.01
TDT (n = 3)	4.2 ± 0.2[*c]	0.73 ± 0.02

Different letters show significant difference when compared to each other (α = 0.05).
[a] (Tadapaneni, Syamaladevi, et al., 2017).

Conversely, the lower $D_{80°C}$-value of *Salmonella* Enteritidis PT30 in the OWF sample treated with TDT cells was due to the increase in the a_w of the OWF sample from 0.45 ± 0.02 (recorded at room temperature) to 0.73 ± 0.02 when TDT cells were heated at 80 °C (Tadapaneni, Syamaladevi, et al., 2017). The D-value of bacteria and the a_w of food samples are inversely related, i.e., with an increase in a_w of a food sample, the thermal resistance of bacteria is reduced (Syamaladevi, Tadapaneni, et al., 2016; Syamaladevi, Tang, Villa-Rojas et al., 2016). Further, it is well studied that the structural integrity of key cellular proteins in a bacteria is highly dependent on the moisture of its environment (Earnshaw, Appleyard, & Hurst, 1995; Syamaladevi, Tang, Villa-Rojas et al., 2016). The increase in availability of water molecules at elevated temperature would enhance the denaturation of those cellular proteins and consequently, damages the viability of the bacterial population.

The moisture content of a bacteria cell can quickly change in response to (within seconds) to its changing external environment (Syamaladevi, Tang, & Zhong, 2016). Thus, the a_w of the carrier (food or non-food) for a bacteria greatly influences its survival in the low-moisture environment (Hildebrandt et al., 2017). For different carriers, bacteria such as *Salmonella* spp. may have different thermal resistance due to change in a_w at the treatment temperature as dictated by their respective moisture sorption isotherms. To evaluate the thermal resistance of *Salmonella* spp. independent of the carrier's chemical composition, a standardized calibration procedure is needed. The improved design in TAC II offers the advantage of studying the isolated influence of carrier composition on the thermal resistance of *Salmonella* spp. and other foodborne pathogens at constant temperature and a_w values.

3.4. Inactivation of Salmonella Enteritidis PT30 during cooling in TAC II

From the psychrometric chart, the dew point temperature and the amount of water in the headspace air of TAC II for different treatment temperatures with different controlled relative humidity (RH) conditions were determined as shown in Fig. 5 (a) and (b), respectively. The estimate data for dew point temperatures for different treatment temperatures indicate that at the controlled RH if the treatment temperature is increased, the dew point temperature also increases corresponding to the RH condition. Similarly, the amount of moisture in the headspace would also increase with the rise in treatment temperature at

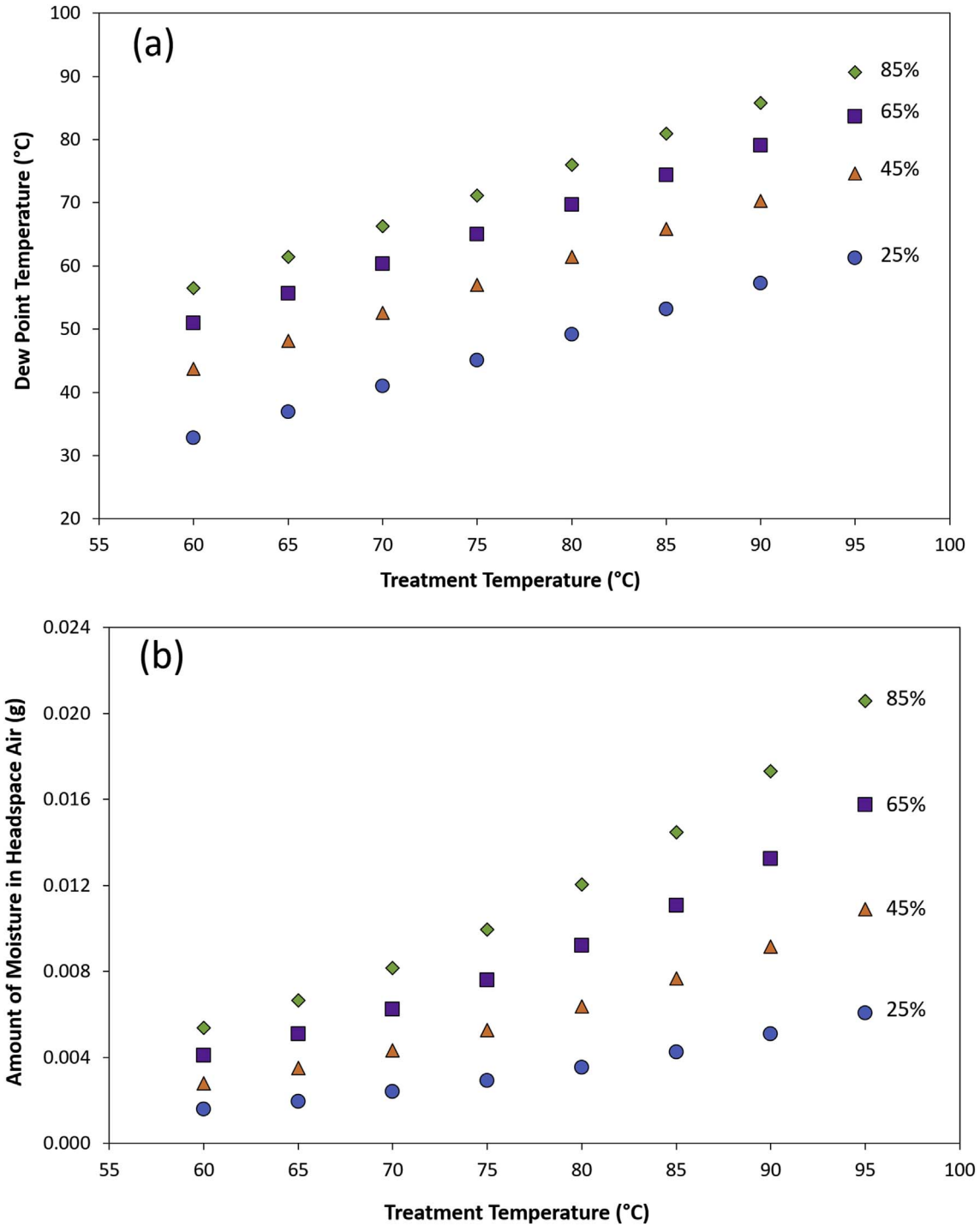

Fig. 5. Estimated dew point temperatures in °C (a) and the amount of moisture in g (b) for different controlled RH conditions (25, 45, 65 and 85%) in the headspace of a TAC II as determined from the psychrometric chart.

corresponding RH value.

To verify if there is any additional inactivation during cooling of a test sample in a TAC II especially for high RH conditions (> 50%), a conservative case for the TAC II is discussed here. For example, if OWF is thermally treated in a TAC II with controlled RH of 65%. The dew point temperature of 65% RH headspace should be 69.7 °C according to Fig. 5a. During cooling of a thermally treated OWF sample in a TAC II, when the air temperature reaches the dew point, there would be the onset of water vapor condensation. This moisture condensate, when mixed with OWF sample, would increase the a_w of the sample and provide additional thermal inactivation of *Salmonella* Enteritidis PT30 above a lethal temperature. Thus, in this study, we considered the reported D-value of *Salmonella* Enteritidis in the high a_w system for the theoretical estimation of the log-reduction in *Salmonella* Enteritidis PT30 population during the cooling of OWF sample in a TAC II.

The reported $D_{71°C}$ and z-values for *Salmonella* Enteritidis in a buffer system with a_w of 0.96 (at room temperature) were 3 s and 6.38 °C, respectively (Álvarez et al., 2003). These values were considered to be equivalent reference data point for a high a_w system. From the cooling curve of treated OWF sample in a TAC II (Fig. 3), we considered the assumption that it takes approximately 2.5 s for the OWF sample to cool down from 80 to 71 °C, and another 2.5 s for the sample to cool down

from 71 to 63 °C. Therefore, for the cooling process from the dew point temperature (71 °C) to 63 °C, the additional log reduction in *Salmonella* Enteritidis PT30 in the OWF sample by thermal tests with the TAC II was 0.3. This is within experimental variations in the microbial enumeration.

During cooling of the OWF sample in the TAC II from 80 °C to 65 °C, the amount of moisture in the headspace of TAC II for 65% RH condition (for the extreme condition) was determined to be 0.003 g (Fig. 5b). If all the condensed moisture were to form one spherical drop of water, the size of that drop was estimated to be very small (dia ≈1.2 mm). In reality, water vapor in the headspace air of the TAC II would condense into much smaller droplets on to the flat surface of the lid in the TAC II. The flat surface of the lid would offer high surface adhesion to the condensed water droplets. Thus, there would be the limited possibility of those water droplets to fall onto the OWF sample in the TAC II. As a precautionary step for such thermal resistance tests of samples with very high RH conditions, it is also recommended to have a slightly concave surface towards the inner side of the lid in the TAC II. This concave surface of the lid would make water droplets move to the outer region and fall into LiCl solution and thus, preventing the condensed water droplets from contaminating the sample in the center well during the cooling process.

Based on the data estimated for the most conservative testing parameters for the TAC II, it would be safe to say that there would be no significant addition to the inactivation of *Salmonella* spp. during the cooling process of food samples in the TAC II even for high RH conditions.

Overall, the data showed and discussed in this study support the fact that the a_w of food system at high temperature plays a key role in the survival of *Salmonella* spp. under low-moisture conditions. During thermal treatments, the design of the TAC II offers a more appropriate method to evaluate the individual influence of a_w on the thermal resistance of *Salmonella* spp. and other potential pathogens with their identified surrogates in different food and non-food matrices (such as silicon dioxide). Further studies with the TAC II with different food systems would be needed to understand other critical information like z_T or z_{aw} (value to change the D-value by a factor of 10) in terms of temperature or a_w of LMF, respectively.

4. Conclusions

In this study, an improved design of thermal water activity cell (TAC II) was introduced where the reduced sample depth offered faster come-up time for the OWF sample to attain equilibrium with the controlled RH condition provided by LiCl solutions. The time-temperature profile of OWF sample in the TAC II indicated rapid heating and cooling of the sample. The thermal inactivation data of *Salmonella* Enteritidis PT30 in OWF samples determined with the TAC II at 80 °C shows that controlled RH condition at a high temperature increased the thermal resistance of bacteria when compared to the similar treatment of inoculated OWF using the traditional thermal death time cells. For conservative RH conditions (above 50% RH), it was estimated that there was no detectable additional inactivation of *Salmonella* Enteritidis PT30 during the cooling of treated OWF in the TAC II. The improved design of TAC II has provided more accurate estimation of D value of *Salmonella* spp. in real food matrix for giving relative humidity at high temperature. The data from this study offers a better approach in bridging the existing knowledge gaps associated with the influence of a_w on the thermal resistance of pathogens in the LMF and developing preventive controls as demanded by the FSMA.

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