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Design of a novel test cell to study the influence of water activity on the thermal resistance of *Salmonella* in low-moisture foods

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ABSTRACT

A novel test cell was developed to study the influence of water activity (a_w) on thermal inactivation of food pathogens in low-moisture foods (LMF). The cell consisted of multiple wells with a shared headspace; a_w of the inoculated food sample was controlled by lithium chloride (LiCl) solution of selected molality during heating. The performance of the test cell was evaluated by studying sample heating uniformity using finite element simulation and by comparing measured headspace relative humidities (RH) with predicted RH provided by LiCl solutions. The new cells were used to determine thermal resistance (D-value) of *Salmonella* at 80 °C in organic wheat flour (OWF) maintained at a_w of 0.45. The D-value of *Salmonella* was also determined in conventional thermal death time test cells in which a_w of OWF samples increased from 0.45 at room temperature to 0.73 at 80 °C according to the measured isotherm. The results demonstrated that a_w significantly influenced the D-values of *Salmonella,* and the new test cells can be used to directly relate thermal resistance of food pathogens to water activities of LMF at processing temperatures.

1. Introduction

Foods at low water activities (a_w) are generally considered as microbiologically safe. However, a number of recent food-borne outbreaks reported across the globe have been connected to low moisture foods. These foods include peanut butter, cereals and cereal products, spices, dry fruits, cocoa based products, and many other ready-to-eat products (Chen et al., 2009; Keller et al., 2013; Komitopoulou and Penaloza, 2009; Lathrop et al., 2014; Scott et al., 2009). These outbreaks were mostly associated with the occurrence of *Salmonella* and *Escherichia coli* (Podolak et al., 2010). Several studies have reported the prevalence of *Salmonella* in foods and raw ingredients under desiccated conditions (Doyle and Mazzotta, 2000; Hiramatsu et al., 2005; Podolak et al., 2010; Van Doren et al., 2013). Factors influencing the ability of this bacteria to survive in low moisture environments and thermal treatments are complex. They include water activity (a_w), food composition, and storage conditions (Wesche et al., 2009).

Water activity, a_w, of a food system is a thermodynamic property. It is defined as the ratio of water vapor pressure of food to the saturated water vapor pressure at a given temperature. The a_w is considered to be a good quality indicator for the safety and stability of foods with respect to microbial growth and biochemical reactions (Roos, 2007, 2010). But a_w in foods changes with temperature; the degree of such change depends on the composition of foods, i.e., amounts of salts, lipids, proteins and other components (Chen and Grant, 1998; Labuza, 1968).

Several studies have attempted to investigate the influence of a_w on the thermal resistance of *Salmonella* in low-moisture foods such as peanut butter (He et al., 2011) and almonds (Villa-Rojas et al., 2013). But those studies only reported a_w at room temperature. It is well-known that in sealed containers the a_w of different food products behaves differently at elevated temperatures following unique sorption isotherms of specific foods (Syamaladevi et al., 2016a,b). It has also been shown that inactivation kinetics of bacteria, including *Salmonella,* are greatly influenced by slight changes in the a_w of food matrices. For example, with decreasing a_w of food, the time needed to achieve acceptable log-reductions of *Salmonella*

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Nomenclature

A	Surface area (m^2)
a_w	Water activity
C	Thermodynamic constant
C_p	Heat capacity at constant pressure (J kg^{-1} K^{-1})
CUT	Come-up Time (s)
D-value	Decimal reduction time (min)
DVS	Dynamic vapor sorption
FEM	Finite element method
GAB	Guggenheim, Anderson and de Boer
h	Heat transfer coefficient (W m^{-2} K^{-1})
K	Thermodynamic constant
k	Thermal conductivity (W m^{-1} K^{-1})
LiCl	Lithium chloride
M_w	Molar mass of water (kg/mol)
m_s	Molality of salt (mol/kg)
n	number of replicates
n_w	Amount of water (mol)
n_s	Amount of salt (mol)
N_0	Initial microbial population (CFU/g)
N	Microbial population at time t (CFU/g)
OWF	Organic Wheat Flour
RH	Relative Humidity
T_0	Initial temperature (°C or K)
T	Temperature (°C or K)
T_{ext}	Temperature of the heating fluid (°C or K)
TAC	Thermal Water-Activity Cell
TDT	Thermal death time
t	Time (in s or min)
V	Volume (m^3)
ν	Number of ions formed when one mol of salt is dissolved in water
X	Moisture content (dry basis) of the material
X_m	Monolayer moisture content (dry basis)

Greek symbols

∇	Delta operator
Φ	Osmotic coefficient
p	Density (kg m^{-3})

population increases (Baird-Parker et al., 1970; Chang et al., 2010; D'Aoust, 1989; Farakos et al., 2013). But it is difficult to directly measure a_w of food samples at temperatures applicable to thermal pasteurization of low moisture foods (Syamaladevi et al., 2016b). As a result, there have been no studies to date that quantitatively relate thermal resistance of *Salmonella* to a_w at the treatment temperatures, and isolate the influence of a_w at those temperatures on bacterial thermal resistance from other factors.

The objectives of this study were to 1) design a novel thermal test cell with ability to control a_w of the low-moisture food sample at thermal pasteurization temperatures; 2) identify a_w controlling agent that could provide relatively stable a_w over a wide range of temperatures; and 3) evaluate the thermal resistance of *Salmonella* in low-moisture foods at a high temperature using the new test cell against data from conventional thermal death time test cells.

2. Material and methods

2.1. Design of the thermal-water activity cell (TAC)

The hypothesis of this study was that by using a relative humidity (RH) controlling medium in an enclosed cell, a_w of food samples inoculated with target bacteria can be controlled at elevated temperatures. Decimal reduction times (D-value: at a given temperature, time required to reduce 90% of a microbial population) of the target bacteria could be determined, directly related to a_w at the test temperatures.

The test cell, referred to as the Thermal Water Activity Cell (TAC), was made of a metal alloy (aluminum alloy 6061). The TAC cells were fabricated as two variants: one version primarily intended for measuring the time-temperature profile of the food sample and RH of the headspace in the cell, and the other for microbiological studies. The first version included a lid with an embedded temperature sensor and a RH sensor (HX15-W, Omega Engineering Inc., Stanford, CT) and a base. The sensor was connected to a computer via a data logger for real time data recording of the relative humidity in the headspace above the sample, and another thermocouple probe (Type-T, THQSS-020U-6, Dia 0.5 mm, Omega Engineering Inc., Stanford, CT) measured the sample center's temperature. The base had four sample holding wells (1 mL volume each) and one well (3 mL volume) in the center to hold a salt solution. An O-ring between the lid and the base provided for a tight seal during heat treatments (Fig. 1). The second version of the TAC

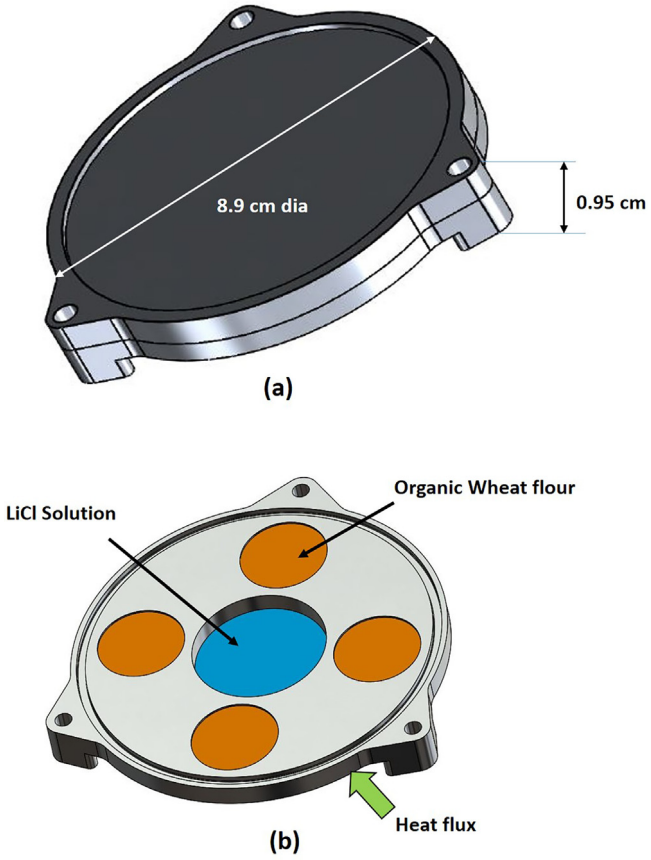

Fig. 1. 3D rendering of TAC used for heat transfer simulation (a) TAC complete assembly with lid and base (b) Arrangement of LiCl (indicated by blue color in center) and organic wheat flour (OWF) in sample wells (indicated by brown color in 4 wells). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

cell had the same dimensions as the first version but did not have the inserted sensors through the lid, as shown in Fig. 1.

During heating, the inoculated samples, preferably in a porous form, were fully exposed to the headspace RH controlled by the salt solution. At high temperatures, fast mass transfer through the porous samples to and from the headspace allowed rapid adjustment of sample a_w to reach an equilibrium with the head-space environment.

2.2. Lithium chloride (LiCl) - a_w controlling agent

Lithium chloride (LiCl) salt solution was selected to control the relative humidity of the headspace inside the TAC cell. In a closed system, LiCl solution generates specific RH values depending on its molality. At equilibrium, the a_w of the food sample can be estimated from the RH (Eq (1)):

$$a_w \approx \frac{RH}{100} \tag{1}$$

The osmotic coefficients (Φ) of LiCl with selected molalities (from 1 to 18.5 mol/kg) at various temperatures (20–100 °C) were determined by Gibbard and Scatchard (1973). These values were used in Eq. (2) to calculate the RH a_w values generated by LiCl at the respective temperatures (Gibbard and Scatchard, 1973; Gibbard et al., 1974):

$$\frac{RH}{100} = \exp(-\Phi M_w \nu m_s) = \exp\left(\frac{-\Phi \nu n_s}{n_w}\right) \tag{2}$$

where, M_w is the molar mass of water (kg/mol), ν is the number of ions formed when one mol of salt is dissolved in water, m_s is the molality of salt (mol/kg H_2O), n_w and n_s are the amount of water and salt, respectively (in mol).

2.3. Validation of predicted a_w values at different temperatures

To validate the predicted RH values provided by Eq. (2), the first version of TAC cells with the commercial RH sensor probe (RH1) as explained in Section 2.1 was tested experimentally with different molal solutions of LiCl. In verifying the precision of RH in TAC, another RH sensor (RH2) from the same manufacturer (HX15-W, Omega Engineering Inc., Stanford, CT) was used in place of RH1. Both sensors independently measured the headspace RH due to the LiCl solution in the TAC at 20 and 80 °C. Calibration standard LiCl solutions with a_w of 0.25 and 0.5 measured at 20 °C (Decagon Devices Inc. Pullman, WA) were used to assess the accuracy of the estimated values.

2.4. Heat transfer performance of TAC

2.4.1. Modeling and simulation of TAC

The physical model of the TAC cell is shown in Fig. 1. The performance of the TAC cell at an elevated temperature was analyzed by modeling heat transfer governing equation. Heat transfer in solid media, i.e., aluminum alloy cell and food sample, is mainly governed by conduction, which is mathematically expressed as:

$$\rho Cp \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) \tag{3}$$

where ρ is the density (kg m^{-3}); Cp is the heat capacity at constant pressure (J kg^{-1} K^{-1}); t is the time (s); ∇ is the delta operator; k is the thermal conductivity (W m^{-1} K^{-1}) and T is temperature (°C). For TAC immersed in a well-stirred oil bath, convective heat transfer occurs at the interface of the TAC surface and heating medium (oil), given by:

$$q_o = h\,(T_{ext} - T) \tag{4}$$

where q_o is the inward heat flux; h is the heat transfer coefficient (W m^{-2} K^{-1}) at the heating oil and TAC interface; and T_{ext} is the temperature of the heating fluid (°C). The thermal properties of aluminum alloy used in heat transfer modeling are listed in Table 1. The estimation of the heat transfer coefficient (h) in this model was based on the thermo-physical properties of heating fluid and configuration of the TAC (Miller et al., 1994).

A disc of aluminum alloy with identical thermal properties to that of the TAC cell was used to estimate the surface temperature of metal alloy used in the fabrication of TAC cells. The value of h was determined by the lumped system analysis (Eq. (5)) (Holman, 1989):

$$h = -\frac{V\rho C_p}{A}\left(ln\frac{T - T_{ext}}{T_0 - T_{ext}}\right) \tag{5}$$

where T_0, A, and V are the initial temperature (°C), surface area (m^2), and volume (m^3), respectively, of the aluminum alloy disc. By analyzing the experimental thermal data of the disc, the proximal h value was determined to be 645 W m^{-2} K^{-1} and this value was applied to the simulation of heat transfer in the TAC cell.

Finite element method (FEM) based software (4.2a COMSOL Multiphysics, Burlington, MA, USA) was used to solve the governing heat transfer equation (Eq. (3)) for this model. The system configuration to run this model was a Dell workstation with Intel® Core™ i7-2600, 16 GB RAM, and Windows 7 Professional 64-bit operating system. The run time for the simulation model was approximately 32 min.

2.4.2. Validation of the finite element model

The simulation model was evaluated for its validity by performing the experiments with a prototype TAC cell. The food matrix used in this study was soft white wheat organic flour (OWF) (Eden Foods, Clinton, MI). For the proximate analysis of OWF, duplicated samples were sent to the Northern California Laboratory of Silliker Inc. (Salida, CA). The proximate compositions of the OWF were – moisture: 8.34 ± 0.17% (w/w); ash: 1.55 ± 0.04% (w/w); fat: 3.28 ± 0.12% (w/w); protein: 7.92 ± 0.48% (w/w) and carbohydrate (by difference): 78.91 ± 0.16% (w/w). The thermal and physical properties of OWF are listed in Table 1. Samples of OWF (0.70 ± 0.05 g) pre-conditioned to 0.45 ± 0.02 a_w at room temperature was loosely placed in the sample wells and 3 mL of LiCl solution was added to the central well. The OWF sample-filled TAC cell was sealed and equilibrated at ambient temperature for 1 h. Following the equilibration time, the TAC was immersed in a heated ethylene glycol bath (HAAKE DC 30/DL30, Thermo Electron, Germany) with the temperature set at 80 °C. The temperature profile of OWF was measured by a pre-calibrated thermocouple probe attached to the data logger (DL2e, Delta-T Devices Ltd., Cambridge,

Table 1
Thermal and physical properties of aluminum and organic wheat flour (OWF) included in the simulation of the TAC model.

Properties	Aluminum (Alloy 6061)[a]	OWF[b]
Density, ρ (kg m^{-3})	2702	901
Specific Heat, Cp (J kg^{-1} K^{-1})	903	1025
Thermal conductivity, k (W m^{-1} k^{-1})	180	0.078

[a] (Adams and Aliya, 1990).
[b] (Liu et al., 2015).

UK). The temperature was recorded at 5 s intervals. The experimental come-up time for the OWF was evaluated against come up time for OWF generated from the simulated model.

2.5. Sorption isotherms of OWF

The adsorption and desorption isotherms of OWF at 20 °C were generated using an AquaLab Vapor Sorption Analyzer (VSA) (Decagon Devices, Inc. Pullman, WA) following the dynamic vapor sorption (DVS) method (Yu et al., 2008). The a_w values of OWF at 80 °C were determined with a sealed thermal cell containing a commercial relative humidity sensor (HX15-W, Omega Engineering, Inc.) developed in our laboratory (Syamaladevi et al., 2016a). More details on the experimental procedure to develop sorption isotherms at elevated temperatures are reported in Syamaladevi et al. (2016a). The Guggenheim, Anderson and de Boer (GAB) relation based on the monolayer and multilayer adsorption was considered as the appropriate model to estimate the effect of temperature on isotherms of foods (Syamaladevi et al., 2016a). Therefore, the GAB equation (Eq. (6)) was used to fit the water sorption isotherm data for OWF samples at 20 and 80 °C:

$$\frac{X}{X_m} = \frac{CKa_w}{(1 - Ka_w)(1 - Ka_w + CKa_w)} \quad (6)$$

where X is the moisture content (dry basis) of the material, X_m is the monolayer moisture content (dry basis), and C and K are thermodynamic constants based on multi-layer adsorption of moisture.

2.6. Thermal resistance of Salmonella

2.6.1. Salmonella – inoculum preparation

Salmonella enterica Enteritidis PT30 was acquired from Dr. Linda Harris at University of California-Davis and kept in a stock solution of tryptic soy broth (TSB, Difco, Becton Dickinson, Sparks, Md) supplemented with 0.6% (w/v) yeast extract (YE, Difco, Becton Dickinson, Sparks, Md), and 20% glycerol (Sigma Chemical Co., Saint Louis, Mo) and stored at −80 °C until use. The lawn-based pelletized method for inoculum preparation was followed as reported by Hildebrandt et al. (2016). A loop (ca. 10 μL) of the *Salmonella* strain stored in 20% glycerol at −80 °C was transferred to 9 mL of TSB-YE and incubated for 24 h at 37 °C. Subsequently, 100 μL were transferred to 9 mL of TSB-YE and incubated for 24 h at 37 °C. The lawn was harvested with Maximum Recovery Diluent (MRD, Fisher Scientific, Pittsburgh, PA), followed by pelletizing the culture by centrifugation (3000 g for 15 min at 4 °C). The pellets were re-suspended in 3 mL of MRD, and 1 mL of the suspension was inoculated into 10 g of OWF (initial $a_w = 0.34 \pm 0.03$). The mixture was hand massaged in a sealed bag for 3 min until lumps were dissolved, then added to 90 g of OWF in stomacher bags and stomached for 3 min (400, Seward, West Sussex, UK). Inoculated OWF samples were equilibrated to the humidity level of 45% in a controlled RH chamber (designed and built by Michigan State University) for about 4–5 days at room temperature (~22 °C). The initial microbial count in inoculated OWF samples was 10^8 CFU/g before equilibration; the population was enumerated before each heat treatment to account for losses during equilibration. Prior to thermal treatments, a_w values of inoculated OWF samples at room temperature were measured with an AquaLab series 3 TE (Decagon Devices Inc., Pullman, WA, USA); samples with a_w of 0.45 ± 0.02 were used. At least three biological replicates were used for each test cell.

2.6.2. Thermal treatments of inoculated OWF samples

Prior to thermal treatment, each sample well of a TAC cell was filled with 0.70 ± 0.05 g of inoculated OWF sample, the central well was filled with 3 mL of LiCl solution with concentration of 9 mol/kg (corresponding to an equilibrium relative humidity of 45% at room temperature). The OWF samples were conditioned in the sealed TAC cells at room temperature for 1 h to equilibrate with the relative humidity in the enclosed headspace. Changes in *Salmonella* population as affected by the in-cell conditioning were assessed in a preliminary study in which samples in three replicates prior to and after the equilibration were enumerated. The average losses in population after equilibration to a_w of 0.45 were less than 0.5-Log CFU/g. After conditioning, the TAC cells were immersed in a well-stirred oil bath (Isotemp 5150 H11, Fisher 180 Scientific, Inc., PA) at 80 °C. After reaching the set temperature, TAC cells were removed from the oil bath at 5 different time intervals (n (replicates) = 3) and cooled in an ice-water bath for 2 min.

The reproducibility of the data by TAC cell was evaluated by a two-time independent inactivation kinetic study involving three biologically independent replicates of OWF sample in each TAC cell.

To compare results from the TAC cell with conventional heating methods used by previous researchers, inoculated OWF samples were treated in Thermal Death Time (TDT) cells at 80 °C. TDT cells were designed and fabricated at Washington State University (Chung et al., 2008) and were used in several other studies (Syamaladevi et al., 2016a). A TDT cell had a 1 mL sample holding cavity with dimensions of 18 mm diameter and 4 mm height. The come-up-time (CUT, defined as the time needed to reach within 0.5 °C of the set temperature) for OWF sample's cold spot (geometric center) in TDT cells was measured by using a pre-calibrated T-type thermocouple attached to the thermometer (Digi-Sense DuaLogR EW99100-50, Cole-Parmer Instruments Co., Vernon Hills, IL). Each TDT cell was filled with 0.70 ± 0.05 g of the inoculated OWF sample equilibrated to 0.45 a_w (measured at room temperature before treatment) and treated at 80 °C similar to the isothermal treatment of TAC cells. TDT cells were removed from the oil bath at different time intervals (n (replicates) = 6) and were cooled in an ice-water bath for 2 min.

To obtain survivor curves for *Salmonella*, treated samples were recovered from TAC and TDT cells, transferred to sterile bags (VWR® Sterile Sample Bags, Radnor, PA) along with 6.3 mL of MRD and stomached for 3 min at 230 rpm (Stomacher® 400 Circulator, Seward Laboratory Systems Inc, Bohemia, NY). Post-stomaching, the samples were tenfold serially diluted in MRD, duplicate-plated (three consecutive serial dilutions) onto Tryptic Soy Aagar (TSA, Difco, Becton Dickinson, Sparks, Md) supplemented with 0.6% (w/v) yeast extract, 0.05% (w/v) ferric ammonium citrate (Sigma-Aldrich, St Louis, MO), and 0.03% (w/v) sodium thiosulfate (Sigma-Aldrich, St Louis, MO) (to identify *Salmonella* colonies as those having a dark center) and incubated for 48 h at 37 °C before counting colonies.

Thermal resistance, as reflected by D-values in min, was calculated by fitting the first order kinetics model to the survivor curves by (Peleg, 2006):

$$\log N = \log N_0 - \frac{t}{D} \quad (7)$$

where t is the time of isothermal treatment (min); N is the population at time t (CFU/g); N_0 is the initial population (CFU/g) and D is the time (in min) required to reduce the microbial population by 90% at a determined temperature (°C).

2.7. Statistical analysis

Statistical analyses were performed using Minitab 14 (Minitab Inc., State College, PA) with a standard test criterion of $\alpha = 0.05$. Differences between microbial populations pre-and post-

equilibration in TAC, between two-time independent sets of data (D-values) from TAC (to ascertain repeatability of the TAC results) and between D-values of TAC and TDT tests were all evaluated using the student's t-test.

3. Results and discussion

3.1. Performance of TAC

Fig. 2 compares simulated and experimental temperatures at the geometric center of an OWF sample in a TAC cell. The simulated result followed very closely the experimental data. After 30 s heat treatment, the temperature difference between the two sets of data was within 0.5 °C. Thus, it is appropriate to use the simulation to evaluate heating uniformity within the TAC cell.

The temperature distribution in different parts of the simulated TAC model after 85 s heating at 80 °C is shown in Fig. 3. The cold spot in the OWF samples and LiCl solution was at the geometric center of each well. The cold spot of the OWF samples was approximately 0.35 °C lower than the hot spot of those samples, while the cold spot in LiCl solution was approximately 0.42 °C lower than the hot spot. The simulation suggests that the TAC cell was able to provide good temperature uniformity after more than 85 s of heating.

3.2. Relative humidity of LiCl at different temperatures– theoretical and experimental estimation

Using Eq. (2), the graphical representation of the relationship between relative humidity (RH) in a closed headspace (and, thus, a_w of the solution) and temperature for different mole LiCl solutions was established. The data theoretically predicts the behavior of LiCl solutions at different temperatures (Fig. 4). Theoretically, the a_w of a LiCl solution with a given molality increases slightly with temperature (Fig. 4). For example, LiCl of 13.41, 9.00 and 6.63 mol/kg have a_w of 0.24, 0.47 and 0.65, respectively, at 20 °C. When the temperature is increased to 80 °C, the a_w of LiCl is predicted to be 0.31, 0.52 and 0.68, respectively. These values were verified with experimental measurements. At 20 °C, the measured a_w values of LiCl solutions of 13.41, 9.00 and 6.63 mol/kg were 0.25 ± 0.01, 0.46 ± 0.01 and 0.63 ± 0.03, respectively. These values did not significantly deviate from the predicted values at 20 °C. At 80 °C the measured a_w values of LiCl solutions of 13.41, 9.00 and 6.63 mol/kg were 0.30 ± 0.01, 0.48 ± 0.02 and 0.65 ± 0.01, respectively. The measured a_w values were slightly lower than the corresponding theoretical values at 80 °C. A possible reason for this deviation is that Eq. (2) predicts RH at ambient pressure. The increased overall internal pressure in a TAC cell at 80 °C could slightly suppressed water vapor pressure and thus the measured RH values. Nevertheless, the deviations were less than 3–6% of experimental a_w values, well within the accuracy of RH sensors at high temperatures. Both theoretical and experimental data indicate that the presence of LiCl solutions inside TAC provided stableRH inside the TAC in the tested temperature range.

3.3. Sorption isotherms of organic wheat flour (OWF)

The experimental data for moisture sorption isotherms of OWF samples are shown as scattered points in Fig. 5. In general, a_w values of OWF samples increased with moisture content, as expected. At 20 °C, the absorption curve was below the desorption curve between a_w of 0.2 and 0.7. This phenomenon is commonly referred to as hysteresis (Labuza and Rahman, 2007). Hysteresis is generally observed in hygroscopic products due to the colligative, capillary effects and surface interactions occurring between moisture and the polymeric structures (Labuza and Altunakar, 2008). But hysteresis was not observed in OWF samples at 80 °C, consistent with our measurement results for other food materials (e.g., dates, low fat skim milk, almond flours) at temperatures above 60 °C.

The different hysteresis behaviors between the sorption isotherms at 20 °C and 80 °C may be attributed to the moisture diffusion as a function of temperature and the changes in the physical state of the molecules of the major food composition. During the adsorption process of the OWF sample at 20 °C, the migration of water molecules from ambient environment to the

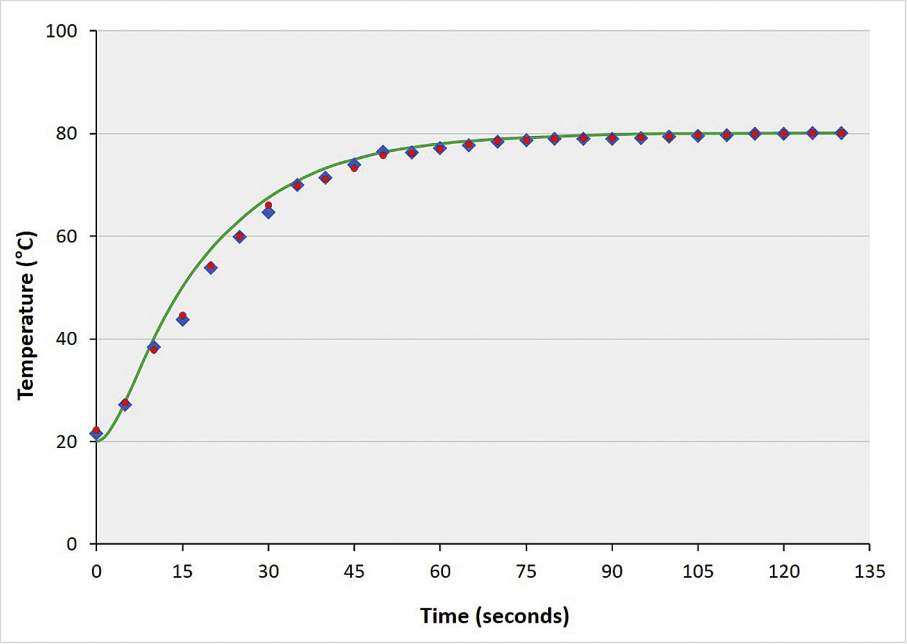

Fig. 2. Simulated (——) vs experimental (♦, ●) temperatures in the center of an OWF sample when heated in a water bath at 80 °C.

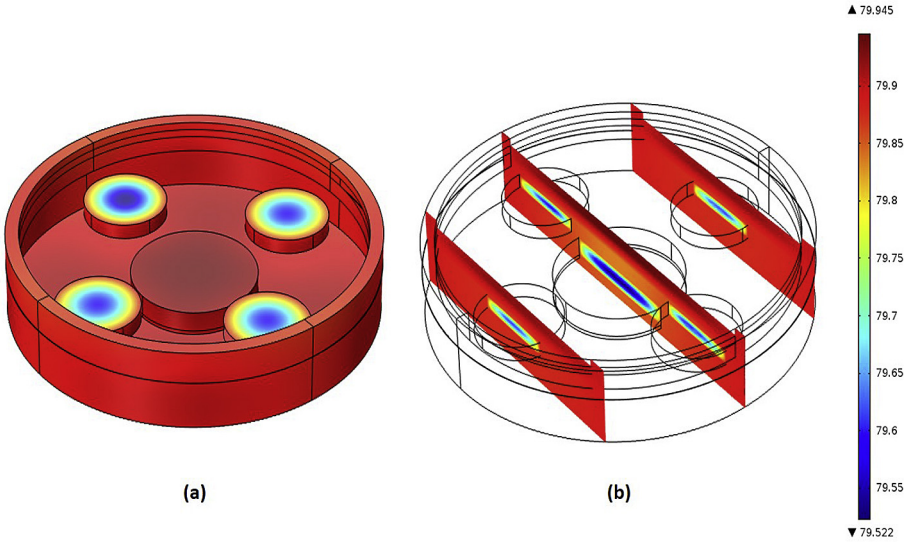

Fig. 3. Predicted temperature profile (in °C) on the top surface (a) and cross-sectional area (b) of the LiCl and OWF samples inside TAC at after heating for 85 s in a heated water bath (at 80 °C).

food sample was low for the a_w less than 0.2 because of the small difference in water vapor pressure between ambient environment and the sample. With an increase in a_w (>0.2), the difference in the water vapor pressure between the OWF sample and ambient air was increased, which resulted in the absorption of moisture by the OWF samples and the swelling of the amorphous region. In the desorption process at 20 °C (between a_w of 0.2 and 0.7), the migration of the moisture from the OWF sample to the air was affected by the immobility of water molecules within the sample due to the glassy state of starch as the room temperature was below the glass transition temperature. It has been reported that when the water content of the wheat starch is reduced from 20% to 7%, the glass transition temperature increases from 30 to 125 °C (Zeleznak and Hoseney, 1987).

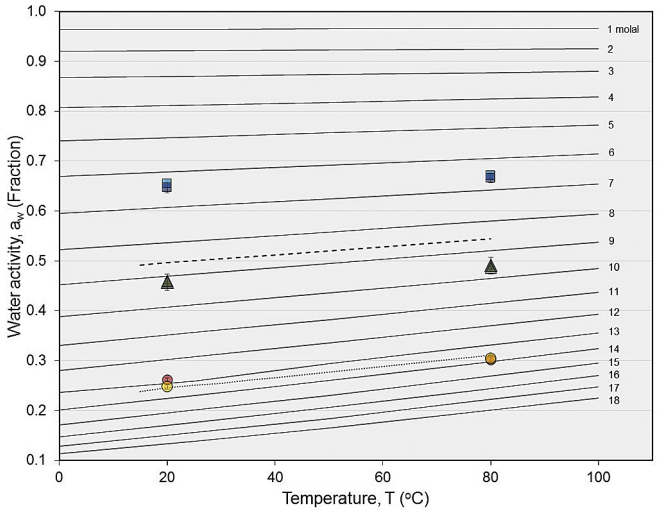

Fig. 4. Prediction of water activities (or relative humidity - RH) of LiCl of different molalities (1–18 mol/kg) at different temperatures (0–100°C). Experimentally determined RH values for different molal solutions of LiCl inside the TAC at 20 and 80°C using two commercial RH sensors to validate the TAC are represented by ■ ■ (7.35 mol/kg, ▲ ▲(9 mol/kg) and ● ● (13.41 mol/kg) and calibration LiCl standards (▪ ▪ ▪ 8.57 mol/kg and ▪▪▪ 13.41 mol/kg) from Decagon Devices Inc.

At 80 °C, on the other hand, the mobility of water in OWF samples was greatly increased. The desorption curve for OWF samples overlapped with the adsorption curve. Similar results were reported by Chuma et al. (2012).

The isotherm data of OWF samples were fitted with the GAB equation (Eq. (6)), as shown in Fig. 6. The GAB model was based on a multi-layer adsorption process and predicts the experimental data well. Adsorption and desorption isotherms of OWF samples at 20 and 80 °C indicate a Type II sigmoidal trend, classified by the Brunauer–Emmett–Teller (BET) theory for starch-rich foods (Brunauer et al., 1940). According to the GAB model, the sigmoid curve of OWF isotherms can be described as having two stages – for lower a_w range (0.2–0.4) multilayers were formed and the micro-pores were filled up and at higher a_w range (0.6–0.8) large pores expanded with the dissolution of solutes (Labuza and Altunakar, 2008).

Furthermore, at a specific moisture content with an increase in temperature of the OWF samples, there was an increase in the a_w of the OWF sample. For example, at 10% moisture content (dry basis) for the adsorption isotherm, the a_w of the OWF sample in a closed system at 20 °C was 0.4; and when heated to 80 °C the a_w of the OWF sample was increased to 0.65 (Fig. 5). Similarly, for desorption isotherms at 10% moisture content (dry basis), the a_w of the OWF sample increased from 0.3 to 0.67 as the temperature of the sample increased from 20 °C to 80 °C. This data agree with the reported sorption isotherms of carbohydrate-rich foods at 20 °C and 80 °C (Bandyopadhyay et al., 1980; Syamaladevi et al., 2016a,b). At high temperatures, for a given moisture content, the binding forces between the food macromolecules and water in the OWF sample are weakened, allowing more water molecules to escape from the samples as vapor into the headspace of the closed system and thus, causing the a_w of the OWF to rise (Palipane and Driscoll, 1993).

3.4. Thermal resistance of Salmonella in TAC and TDT

The thermal inactivation kinetics of *Salmonella* in OWF (initial a_w of 0.45 measured at room temperature) treated in TAC and TDT cells at 80 °C are shown in Fig. 6. The data points at t = 0 min reflected the changes in *Salmonella* populations during the come-up-time (CUT, time to reach 79.5 °C). These data show that during CUT,

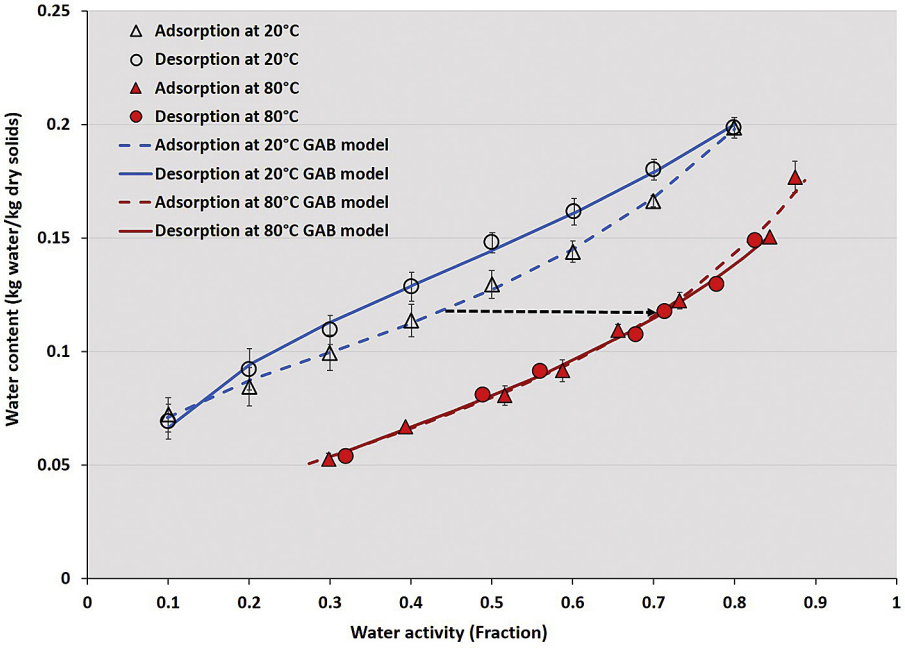

Fig. 5. Sorption isotherms (relationship between water content and a_w at constant temperature) of OWF at 20 and 80°C; ▬▬▶ indicates that for the adsorption process, at the same moisture content, a_w increases with rising temperature.

inoculated samples in the TAC cells had a 1-Log CFU/g reduction in *Salmonella* population while those in the TDT cells experienced a 2-Log CFU/g reduction. This difference in inactivation during the CUT of TAC and TDT cells is possibly related to variation in a_w of the OWF caused by the change of temperature during heating.

After the CUT (sample temperature reaching 79.5 °C), the relationships between survivors and treatment time followed a log-linear trend. Over the total treatment time of 15 min, inoculated OWF in the TAC cells underwent an approximately 3-Log CFU/g reduction, while samples treated in the TDT cells had an approximately 6-Log CFU/g reduction in *Salmonella* populations. The $D_{80°C}$-value for *Salmonella* in OWF samples treated in TAC cells was determined to be 7.3 ± 0.7 min, while that of OWF samples treated using TDT cells was 4.3 ± 0.2 min (Table 2). D-values calculated for the two-time independent TAC cell inactivation studies (each with 3 biologically independent replicas) were not significantly different ($p > 0.05$), indicating that the results from TAC cell were reproducible.

Salmonella in OWF samples treated in TAC cells had significantly higher heat resistance (D_{80} value) than samples treated in TDT cells

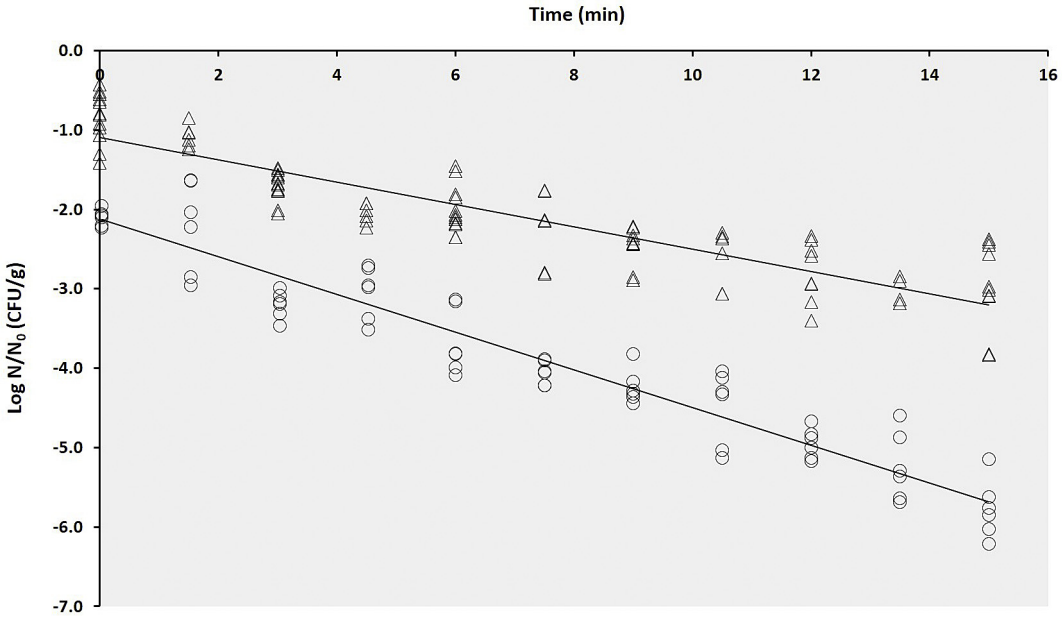

Fig. 6. Survival trend of *Salmonella* Enterica PT30 in OWF samples (with initial a_w of 0.45 ± 0.02 measured at room temperature) when treated with TDT cells (○, n (replicates) = 3) and TAC cells (Δ, n (replicates) = 6) at 80 °C; Straight lines indicate the fits of first order kinetic model given by Eq 7.

Table 2
D-values (min) at 80 °C for *Salmonella* Enteritidis in organic wheat flour (OWF) with a_w of 0.45 ± 0.02 (measured at room temperature), using TDT and TAC cells.

Food Sample (n = replicates)	D- value ± SE (min)	a_w at 80 °C
OWF in TDT (n = 3)	4.3 ± 0.2^A	0.73 ± 0.02
OWF in TAC (n = 6)	7.3 ± 0.7^B	0.46 ± 0.01

Different letters indicate significant difference when compared to each other ($p < 0.05$).

($p < 0.05$). In TAC cells, the bacterial population was less susceptible to inactivation at 80 °C due to the stable and relatively low a_w (~0.45) of OWF samples maintained with LiCl solution during the heat treatment. The relatively high thermal resistance of *Salmonella* in TAC cells may be related to the limited denaturation of key bacterial cell proteins due to the low availability of water molecules and stabilization of proteins with compatible solutes (Earnshaw et al., 1995; Syamaladevi et al., 2016b).

In a cross-laboratory comparison study, it was reported that the $D_{80°C}$-value for *Salmonella* in the identical food matrix (OWF) with initial a_w of 0.46 (measured at room temperature), as determined by TDT cell method, was 4.2–4.3 min (for lawn-based pelletized inoculation method) (Hildebrandt et al., 2016). These $D_{80°C}$-values are very consistent with the thermal inactivation data ($D_{80°C}$ = 4.3 ± 0.2 min) from TDT cells observed in the present study.

The a_w of OWF samples in TDT cells increased from 0.45 ± 0.02 at room temperature to 0.73 ± 0.02 when heated to 80 °C as shown by the adsorption isotherms in Fig. 5. It is well documented that increased a_w in foods generally reduce the thermal resistance of *Salmonella* (Syamaladevi et al., 2016a). Thus, in TDT cells *Salmonella* populations were more vulnerable to heat treatment (80 °C), reflected by the low $D_{80°C}$-value when compared to that of TAC cells.

During thermal treatment of OWF samples with TAC cells, LiCl solution helped to stabilize the relative humidity in the closed environment, which in turn controlled a_w of the small food samples. The above results demonstrated that a_w at elevated temperatures indeed played a critical role in influencing the thermal resistance of *Salmonella*. In future research, it will be possible to use different molar concentrations of LiCl solutions to control RH, as illustrated in Fig. 4, and thus a_w of inoculated food samples in TAC cells. Systematic tests can, then, be conducted to determine the influence of a wide range of a_w on D-values of *Salmonella* and other food pathogens or their surrogates in different food matrices at pasteurization temperatures. These studies could isolate the effect of a_w from other variable factors like food composition and the history of bacteria on thermal resistance of foodborne pathogens in low moisture foods. This is a critical step toward gaining an in-depth understanding of the dominant factors influencing the efficacy of thermal processes for control of foodborne pathogens in low moisture food systems.

In commercial manufacturing of low-moisture foods, different time-temperature profiles are used in different processes, in either open (e.g., baking/roasting) or closed (e.g., in-package pasteurization) systems. Based on the process dynamics, interactions between the components (sugar/fat/salts) in the food matrices and the available water molecules would occur. These interactions would affect the a_w of the food product and thus influence the thermal resistance of *Salmonella* in low-moisture foods. Obtaining quantitative relationships between a_w and the thermal resistance of the pathogens should help establish reliable mathematical tools to design reliable thermal processes and select appropriate processing conditions for pathogen control.

4. Conclusion

Simulated results based on the finite element method showed that the new test cell (TAC) developed in this study provided good heating uniformity in OWF samples when heated to high temperature (80 °C). The TAC cell was able to control a_w of OWF samples (0.45) at high temperatures by selecting an appropriate LiCl solution (9 mol/kg). Sorption isotherms of the OWF samples at 20 and 80 °C indicated that during thermal treatment of OWF samples in a closed system such as in TDT cells, the a_w of OWF samples increased (0.45 at 20 °C to 0.73 at 80 °C). The thermal resistance of *Salmonella* populations in OWF samples in TAC cells, where a_w of the OWF samples was controlled at the treatment temperature, was significantly higher ($p < 0.05$) than that obtained from TDT cells where a_w of OWF samples varied as indicated by the isotherms. TAC cells can serve as an effective tool to improve our understanding about the thermal resistance of *Salmonella* in low-moisture foods by isolating the influence of a_w from other variable factors at the treatment temperatures.

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