



# Influence of low water activity on the thermal resistance of *Salmonella* Enteritidis PT30 and *Enterococcus faecium* as its surrogate in egg powders

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## Abstract

Egg powders are increasingly popular ingredients, due to their functionality and compactness, in industrial food production and preparation at homes. However, there is a lack of studies that evaluate the thermal resistance of *Salmonella* Enteritidis PT30 and its potential surrogate *Enterococcus faecium* NRRL B-2354 in egg powders. This study examined the log-linear relationship between the thermal resistance of *Salmonella* Enteritidis (D-value) and the water activity ( $a_w$ ) of egg powders. The changes of  $a_w$  in the egg powders with temperature were measured using a Vapor Sorption Analyzer and a high-temperature cell. The  $D_{80^\circ\text{C}}$ -value of *S.* Enteritidis PT30 and *E. faecium* inoculated in the egg powders preconditioned to three  $a_w$  levels (0.3, 0.45, and 0.6) at 20 °C were determined using aluminum thermal death test cells. The  $a_w$  values increased ( $P < 0.05$ ) in all three egg powders when the temperature of the samples was raised from room temperature to 80 °C. The  $D_{80^\circ\text{C}}$ -values ranged from  $5.3 \pm 0.1$  to  $25.9 \pm 0.2$  min for *S.* Enteritidis while  $10.4 \pm 0.4$  to  $43.8 \pm 0.4$  for *E. faecium* in samples of the three different  $a_w$  levels. *S.* Enteritidis PT30 showed a log-linear relationship between  $D_{80^\circ\text{C}}$ -values and  $a_{w80^\circ\text{C}}$  for the egg powders. This study contributes to our understanding of the impact of  $a_w$  on the development of thermal treatments for low-moisture foods.

## Keywords

*Salmonella*, *E. faecium*, egg powders, thermal resistance, D-value, water activity

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## INTRODUCTION

Numerous *Salmonella* outbreaks have been associated with low-moisture foods such as raw almonds, chocolate, peanut butter, infants food, spices, wheat flour, and bakery products (Enache et al., 2017; Keller et al., 2013; Lathrop et al., 2014; Nascimento et al., 2012; Nummer et al., 2012; Villa-Rojas et al., 2013; Yang et al., 2014; Zweifel and Stephan, 2012). These outbreaks are attributed to *Salmonella*'s ability to survive in dry conditions for long storage periods (Enache et al., 2017). *Salmonella* in low-moisture foods has a high tolerance to thermal treatments, complicating the

processing of low-moisture food (Santillana-Farakos et al., 2013).

Low-moisture foods are defined as foods with water activity ( $a_w$ ) levels lower than 0.60 (Santillana-Farakos et al., 2013). Water activity measures the water that is not bound to food molecules, which can support the growth of microorganisms. Because most foodborne

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pathogens are not able to grow below an  $a_w$  value of 0.85, drying or decreasing the  $a_w$  is a long used technique to prevent food spoilage (Enache et al., 2017). Nonetheless, *Salmonella* is able to survive in low-moisture food where  $a_w$  is less than 0.6 for a long time (Santillana-Farakos et al., 2013). Thermal treatments are used to eliminate potential contaminated *Salmonella* in high-moisture foods. But, this has been a difficult task for low-moisture foods because low  $a_w$  environments enhance the thermal resistance of *Salmonella* (Xu et al., 2019). Also, the  $a_w$  of food changes with an increase in temperature, depending on whether food ingredients release or absorb water (Syamaladevi et al., 2016a). Most published studies have attributed the increased thermal resistance of *Salmonella* to low  $a_w$  (Liu et al., 2018b; Syamaladevi et al., 2016b; Xu et al., 2019).

In compliance with Food Safety Modernization Act, the food industry is looking into effective means to validate thermal treatments for low-moisture foods. However, to introduce *Salmonella* into an industrial facility is a microbial safety hazard. Non-pathogenic *Enterococcus faecium* NRRL B-2354 (*E. faecium*) has been selected as a surrogate for *S. Enteritidis* PT30 (Liu et al., 2018a). It has demonstrated good performance as a surrogate for *Salmonella* in the validation of a number of thermal treatments such as air drying, roasting, and infrared heating (Liu et al., 2018b). Since *S. Enteritidis* PT30 is the most common transovarial infection in eggs and its ability to survive for prolonged periods of time in low-moisture foods, it would be hazardous to validate thermal treatments using a *S. Enteritidis* strain inside facilities processing egg powder products (Whiley and Ross, 2015). To overcome this limitation, *E. faecium* could be evaluated as a surrogate for the validation of thermal treatments to avoid possible contaminations (Froehlich et al., 2015).

Egg powders have been in increasing demand in the food market, because of their functional properties, greatest stability, and less required storage space in comparison to egg products in the liquid form (Koç et al., 2012). Egg powders are used mainly in bakery foods, bakery mixes, mayonnaise, salad dressings, pastries, ice cream, and pasta (Rao and Labuza, 2012). Typical egg powder processing consists of several unit operations where contamination by *Salmonella* may occur. Egg whole and yolk are pasteurized before spray drying. Egg white powder production, on the other hand, includes a pasteurization step after spray drying, called dry-heating, that consists of heating the packaged powder in a room at 58 °C to 60 °C for 10 to 14 days (Boreddy et al., 2014; Lechevalier et al., 2013). In spite of these treatments, the survival of *Salmonella* in spray-dried egg powders has been reported in the United States, Great Britain, and Canada (Froehlich

et al., 2015). There have been multiple outbreaks of *Salmonella* in shell egg products in the United States from 1970 to the present (Wright et al., 2016). Eventually, these contaminated raw eggs could be processed to become egg powders. Because *Salmonella* is able to survive spray drying processing, an outbreak would be possible (Lian et al., 2015). Another scenario of *Salmonella*'s outbreak is the possibility that egg powders get contaminated after spray drying (Carrasco et al., 2012). Also the dry-heating process for egg white powder is a lengthy treatment that can be reduced in order to save energy (Boreddy et al., 2014). Therefore, it is necessary to study the thermal resistance of *S. Enteritidis* PT30 in egg powders to allow better designs of thermal treatments post-spray-drying that assure microbial safety.

In the present study, we evaluated the influence of  $a_w$  on  $D_{80^\circ\text{C}}$  [the necessary time (min) to reduce the microbial population by 90% at 80 °C] for *S. Enteritidis* (the most common *Salmonella* serotype linked to egg outbreaks; Whiley and Ross, 2015) and *E. faecium* in egg white, egg yolk, and whole egg powders. The outcomes of this research will facilitate the understanding of the relationship between low  $a_w$  and the thermal resistance of *S. Enteritidis* PT30, as well as how to improve thermal treatments for egg products in their powder form.

## MATERIALS AND METHODS

### Materials

Three types of egg powders were used in this study, namely, egg white powder (Hoosier Hill Farm LLC, USA), egg yolk powder (Magic Flavors, USA), and whole egg powder (Hoosier Hill Farm LLC, USA). These products were selected to analyze the impact of egg composition on the thermal resistance of *S. Enteritidis* PT30 and *E. faecium*. The egg powders chemical composition was determined by Siliker Inc., Northern California Laboratory (Salida, CA) utilizing standard analytical methods (AOAC, 2012).

Tryptic soy broth (TSB), tryptic soy agar (TSA), yeast extract (YE), and buffer peptone water were purchased from BD Diagnostics (Sparks, MD); ammonium iron (III) citrate was purchased from Sigma-Aldrich Corporation (St. Louis, MO); and sodium thiosulfate 5-hydrate was obtained from J.T. Baker (Center Valley, PA).

The stock cultures of *S. Enteritidis* PT30 and *E. faecium* were provided by Dr. Linda Harris's laboratory at the University of California, Davis. The cultures were stored at -80 °C in TSB supplemented with 20% (vol/vol) glycerol. Working cultures were elaborated by streaking to isolate each microorganism on TSA plates supplemented with 0.6% (wt/vol) yeast extract (TSAYE) where the plates were incubated 24 h at 37 °C.

### Moisture sorption isotherms at room and treatment temperature

The moisture sorption isotherms (MSIs) of the egg powders were determined at 20 °C using a Vapor Sorption Analyzer (VSA, Decagon Devices Inc, Pullman, WA) following the dynamic vapor sorption (DVS) method (Tadapaneni et al., 2017). Prior to the tests, the moisture content of the egg powder samples was determined using a vacuum oven at 10 kPa and 80 °C for 10 h (AOAC 927.05). Each egg powder sample (~5 g) was placed inside the VSA; the initial moisture content from the oven method was entered to the VSA's control software. The sample was exposed to a pre-set relative humidity. Once the sample moisture content reached the equilibrium,  $a_w$  and its respective equilibrium moisture content were recorded. After that, the VSA increased the relative humidity to obtain a different  $a_w$ . An increase of 10% in relative humidity was selected to get  $a_w$  intervals of 0.1 in the MSIs. The moisture content at each  $a_w$  was calculated from the weight changes (Syamaladevi et al., 2016a).

Because the VSA's maximum temperature operation is 60 °C, a recent method developed in our group was used to attain the MSIs of egg powders at 80 °C. In this method, we used a high-temperature cell (HTC) that housed a relative humidity and temperature sensor (Honeywell HumidIcon TM, Morristown, NJ) mounted in the lid (Tadapaneni et al., 2017). Before the tests, the egg powders were vacuum dried in an oven ADP-31 (Yamato Scientific America Inc., CA, USA) at 10 kPa and 50 °C for 24 h. Afterward, the samples were conditioned at different relative humidities using supersaturated salt solutions as follows: LiCl (11.3%), CH<sub>3</sub>COOK (22.5%), MgCl<sub>2</sub> (32.8%), K<sub>2</sub>CO<sub>3</sub> (43.2%), MgNO<sub>3</sub> (52.9%), NaNO<sub>2</sub> (65.8%), NaCl (75.3%), or KCl (84.3%) (Fisher Scientific, Houston, TX) in airtight containers (Greenspan, 1977). About 2–3 g of the pre-conditioned samples were placed inside the HTC and heated in an oil bath set at 80 °C. When the temperature and relative humidity of the HTC sensor reading became stable for 30 min,  $a_w$  was measured and recorded (Xu et al., 2019). The HTC was then removed from the oil bath and cooled to room temperature. Subsequently, the sample moisture content was determined from the oven method as described above. All data points are the average of two independent samples.

### Inoculation of egg powders

The absence of *S. Enteritidis* PT30 and *E. faecium* in egg powders samples was corroborated by diluting 10 random 1 g samples in 9 ml 0.1% (w/v) buffer peptone water, and then plating on modified TSAYE [Tryptic soy agar supplemented with 0.6% (w/v) yeast extract

(TSAYE) plus 0.05% ammonium iron (III) citrate, and 0.03% sodium thiosulfate pentahydrate] and e-TSAYE (TSAYE plus 0.05% ammonium iron (III) citrate, and 0.03% esculin thiosulfate) for *E. faecium* (Liu et al., 2019; McLaughlin and Balaa, 2006).

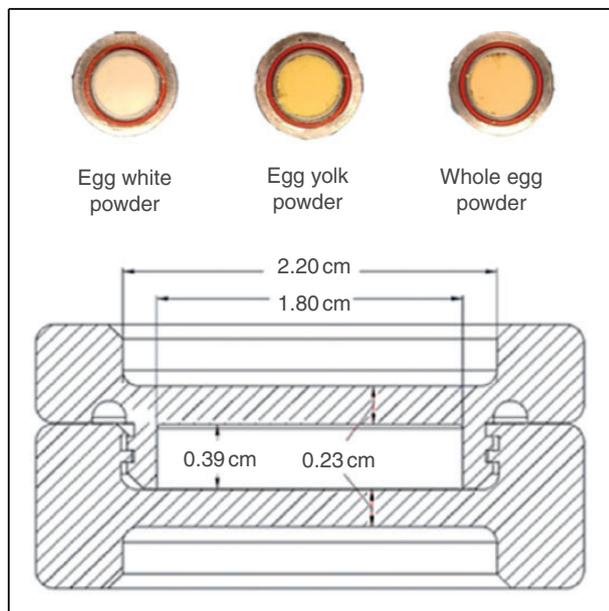
The inoculum was prepared following a modified lawn-harvest method designed for *S. Enteritidis* PT30 and *E. faecium*. A single colony of each microorganism was subjected to two successive transfers in 9 ml of TSBYE (Tryptic soy broth supplemented with 0.6% (w/v) yeast extract) and to incubation periods of 24 h at 37 °C. Then 1 ml of TSBYE culture was plated onto TSAYE plates (150 × 15 mm) (Hildebrandt et al., 2016). Afterward, the bacterial lawns were harvested using 20 ml of sterile 0.1% (w/v) buffer peptone water and centrifuged at 6000g at 4 °C for 15 min. The supernatants were removed, and the pellet was resuspended in 3 ml of 0.1% (w/v) buffer peptone water to get a ~10<sup>10</sup> Colony Forming Units per milliliter (CFU/ml) suspension of *S. Enteritidis* PT30 or *E. faecium*. Three batches of each egg powder (100 g) were inoculated as follows: 10 g were inoculated with 1 ml of *S. Enteritidis* PT30 or *E. faecium*, and the samples were hand-mixed until the pellets were wholly integrated to the sample and no clumps were visible. The 10 g were then further combined with 90 g of egg powder samples. The uniform distribution of the inoculum was confirmed by sampling ten 1 g from each egg powder randomly and enumerated on TSA plates as described below (Xu et al., 2019).

The inoculated samples of the egg powders were placed onto Petri dishes (150 × 15 mm) without lids, and immediately conditioned at selected  $a_w$  (0.30 ± 0.02, 0.45 ± 0.02, and 0.60 ± 0.02) at room temperature for four to five days in equilibration chambers (EW-34788-00, Cole Parmer, IL, USA) designed at Michigan State University (Smith and Marks, 2015). The population of *S. Enteritidis* PT30 and *E. faecium* for inoculated egg powders samples was 8.7–8.8 Log<sub>10</sub> CFU/g after equilibration.

### Isothermal treatments and survival bacteria enumeration

The inoculated egg powders samples (~0.5 g) were loaded into aluminum thermal death time test (TDT) cells with 18 mm inner diameter and 4 mm height (Figure 1) (Chung et al., 2008). The TDT cells were firmly sealed and were immersed in an oil bath (Neslab GP-400, Newington, NH) preheated to 80 °C. When the TDT cells reached 80 °C, they were removed at predetermined time intervals, and immediately cooled in ice water to stop the thermal inactivation.

After the isothermal treatments, *S. Enteritidis* PT30 and *E. faecium* survivors were enumerated. Briefly, the



**Figure 1.** Representative images of thermal death time cells (Xu et al., 2019).

heat-treated samples were transferred from the TDT cells to sterile stomacher bags and diluted with 0.1% (w/v) buffer peptone water to get a 10-fold dilution. The samples were then homogenized for 2 min at 260 r/min with a Seward Stomacher (Seward, London, UK) (Xu et al., 2019). Egg powder samples were appropriately tenfold serial diluted in 0.1% (w/v) buffer peptone water and then spread-plated in triplicate onto modified TSAYE for *S. Enteritidis* PT30 and e-TSAYE for *E. faecium* (Liu et al., 2019; McLaughlin and Balaa, 2006). The plates were incubated at 37 °C for 48 h; the colonies were counted and converted in log CFU/g. Three technical replicates (samples from the same batch) were evaluated in each inactivation curve. The experiments were repeated three times independently (three batches inoculated with independently grown inoculum).

### Modeling the inactivation kinetics

The first-order kinetic model was used to describe the bacteria inactivation in the egg powders as follows

$$\log\left(\frac{N}{N_0}\right) = -t/D \quad (1)$$

where  $N$  and  $N_0$  (CFU/g) are the populations at times  $t$  and 0, respectively,  $t$  is the time of the isothermal treatment (min), and  $D$  is the necessary time (min) to reduce the microbial population by 90% at a selected temperature (Villa-Rojas et al., 2013).

The data were fitted to the model to obtain the thermal inactivation curves, using the Excel software program (Microsoft Corporation, Redmond, WA, USA). The goodness of fit was evaluated using the regression coefficient ( $R^2$ ) and root mean square error (RMSE).

$$R^2 = 1 - \frac{\left( \sum_{i=1}^n \left( \log\left(\frac{N}{N_0}\right)_{i,obs} - \log\left(\frac{N}{N_0}\right)_{i,model} \right)^2 \right)}{\sum_{i=1}^n \left( \log\left(\frac{N}{N_0}\right)_{i,obs} - \log\left(\frac{N}{N_0}\right) \right)^2} \quad (2)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^n \left( \log\left(\frac{N}{N_0}\right)_{i,model} - \log\left(\frac{N}{N_0}\right)_{i,obs} \right)^2}{n - p}} \quad (3)$$

where  $\log\left(\frac{N}{N_0}\right)_{i,obs}$  is the measured log reduction,  $\log\left(\frac{N}{N_0}\right)_{i,model}$  is the predicted log reduction from the model,  $\log\left(\frac{N}{N_0}\right)$  is the mean of the log reduction,  $n$  is the total number of observations, and  $p$  is the number of model parameters.

The inactivation curves were drawn by plotting the logarithm of the surviving microorganisms versus heating times. The  $D_{80^\circ C}$ -values of both bacteria were calculated from the slope of their corresponding inactivation curves. According to equation (1), the inactivation curve's slope is  $-\frac{1}{D_{80^\circ C}}$ , where  $D_{80^\circ C}$  is the necessary time (min) to reduce the microbial population by 90% at 80 °C. The mean  $D_{80^\circ C}$ -values and Standard deviation for each product were based on three independent replicates.

The relationship between  $a_w$  and thermal resistance is given by the following equation where  $Z_{aw}$  is the  $a_w$  change necessary to alter the  $D_{80^\circ C}$ -value by 1-log cycle and  $A$  is a constant (Xu et al., 2019)

$$\log D_{80^\circ C} = \frac{1}{Z_{aw}}(a_{w,80^\circ C}) + A \quad (4)$$

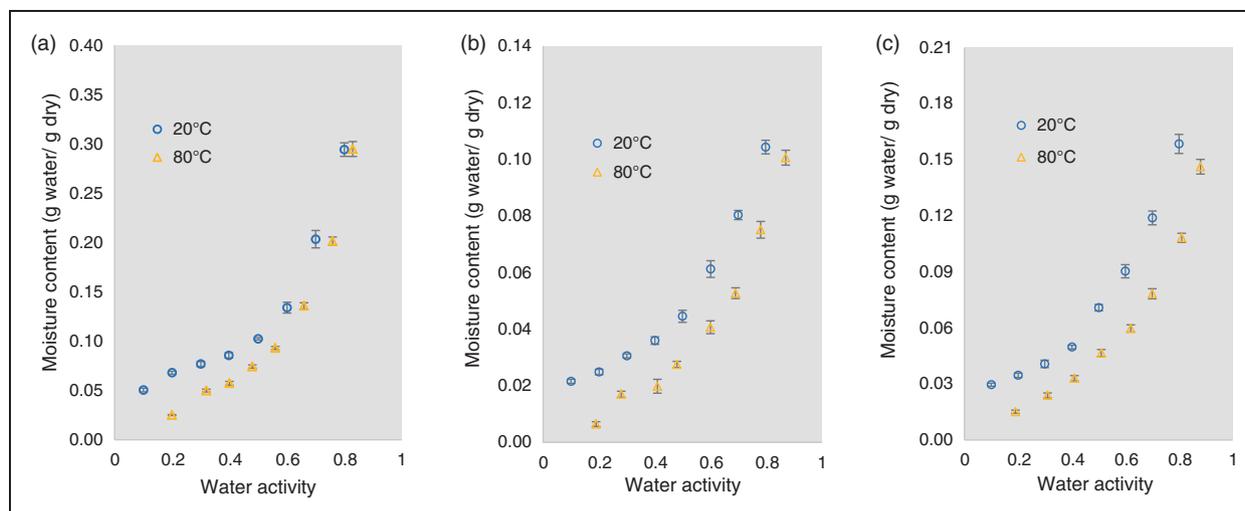
### Statistical analysis

The  $a_w$  and thermal resistance data of both microorganisms were analyzed for statistical significance ( $P < 0.05$ ) using ANOVA in Minitab 17 (Minitab Inc, State College, PA).

**Table 1.** Proximate composition of egg powders samples

	Egg white powder	Egg yolk powder	Whole egg powder
Moisture (% w/w)	6.9 ± 0.3	3.7 ± 0.3	4.3 ± 0.1
Ash (%w/w)	5.3 ± 0.1	2.0 ± 0.2	5.2 ± 0.1
Fat (%w/w)	0.6 ± 0.0	55.1 ± 0.3	43.2 ± 0.1
Protein (% w/w)	84.3 ± 0.7	34.7 ± 0.7	46.4 ± 0.1
Carbohydrate (by difference – %w/w)	2.9 ± 0.8	4.5 ± 0.5	0.9 ± 0.2

Values are represented as mean ± SD; n = 3.



**Figure 2.** Adsorption isotherms of egg powders at 20 °C and 80 °C: (a) egg white, (b) egg yolk, and (c) whole egg. Water content data points are average of at least two independent samples (mean ± SD).

## RESULTS AND DISCUSSION

### Egg powders

The chemical composition of the egg powders is shown in Table 1. According to the results, these powders have either a high-fat content (egg yolk powder) or high content of protein (egg white powder). A third one had a composition close to 50–50% of protein and fat (whole egg powder). The presence of *S. Enteritidis* PT30 and *E. faecium* in the egg powders were not detected before inoculation.

### Changes in egg powder's water activity with temperature

The adsorption MSIs of egg powders at 20 and 80 °C are presented in Figure 2. Type II isotherm describes the behavior of the egg powder curves at 20 °C, as it had a sigmoidal shape with an upward concavity (Andrade-P. et al., 2011). When the moisture content was high, most of the water in food had a vapor pressure close to the pure water of 1.0. However, when the moisture content

of food was low, the vapor pressure was reduced accordingly, following the sigmoid shape observed in egg powders MSIs at 20 °C (Al-Muhtaseb et al., 2002). This type of isotherm is the most common for foods rich in proteins as egg powders (Table 1), due to their plasticized nature, which results in an increase of availability of polar groups (Labuza and Altunakar, 2007).

The observed shape in the adsorption MSIs of the egg powders at 80 °C corresponds to Type III, where the lateral interactions between adsorbed molecules are stronger than the interactions between the adsorbent surface and adsorbate (Kruk and Jaroniec, 2001). This phenomenon is favored at temperatures close to 80 °C, as the denaturation of ovalbumin, ovotransferrin, and lipoproteins, which are the egg powders main proteins, is between 61 °C and 84 °C (Denmat et al., 1999; Rao and Labuza, 2012). The elevated temperatures above that for denaturation affect the stability of noncovalent interactions in egg powder proteins, making more of the side groups exposed to the surrounding solvent (Ustunol, 2014). Gelation of the egg proteins is another process that occurs at elevated temperatures (55–70 °C). In this process, the heat induces the

**Table 2.** D<sub>80°C</sub>-Values of *S. Enteritidis* PT30 and *E. faecium* in egg powders samples

Egg Powder	a <sub>w,20°C</sub> ± 0.02	a <sub>w,80°C</sub>	<i>S. Enteritidis</i> PT30			<i>E. faecium</i>		
			D <sub>80°C</sub> (min)	R <sup>2</sup>	RMSE	D <sub>80°C</sub> (min)	R <sup>2</sup>	RMSE
Egg White	0.30	0.48	25.8 ± 0.3	0.97	0.22	43.8 ± 0.4	0.96	0.24
	0.45	0.56	13.3 ± 0.3	0.94	0.44	21.5 ± 0.1	0.97	0.24
	0.60	0.66	6.1 ± 0.1	0.97	0.28	13.2 ± 0.1	0.97	0.35
Egg Yolk	0.30	0.53	25.9 ± 0.2	0.99	0.16	42.4 ± 0.4	0.99	0.11
	0.45	0.60	13.6 ± 0.2	0.97	0.44	21.6 ± 0.2	0.96	0.33
	0.60	0.72	6.1 ± 0.0	0.96	0.31	12.5 ± 0.3	0.97	0.30
Whole Egg	0.30	0.45	20.5 ± 0.2	0.97	0.34	32.2 ± 0.5	0.99	0.23
	0.45	0.62	7.0 ± 0.1	0.98	0.28	15.5 ± 0.3	0.96	0.39
	0.60	0.74	5.3 ± 0.1	0.99	0.26	10.4 ± 0.4	0.98	0.30

Mean ± SD, n = 3. R<sup>2</sup>: coefficient of determination; RMSE: root mean square error.

aggregation of the proteins into filaments or densely branched clusters which interact as “sticky” molecules (Hsien and Regenstein, 1992). Moreover, between 44 °C and 65 °C, there is a phase change from solid to liquid of the fats (melting) present in the egg yolk powder (Marques et al., 2015).

All these processes involve a series of physical and chemical changes that affect the a<sub>w</sub> values of the egg powders. We observed an increase (P < 0.05) of a<sub>w</sub> values at 80 °C (a<sub>w,80°C</sub>) at each moisture content for all the egg powders. The increase of a<sub>w</sub> in samples of different egg powders (a<sub>w,20°C</sub>) at 80 °C are presented in Table 2. As an example, the a<sub>w</sub> conditioned to 0.30, 0.45, and 0.60 at 20 °C increased to 0.48, 0.56, and 0.66 at 80 °C, respectively, in egg white powder samples. These changes in a<sub>w</sub> are the results of the increasing mobility of water molecules. Egg powders held less water at higher temperatures than at lower temperatures (Labuza and Altunakar, 2007). Most of the foods are subjected to thermal treatments where the a<sub>w</sub> may change. These changes in a<sub>w</sub> could affect the thermal inactivation of pathogens like *Salmonella* as shown in Table 2.

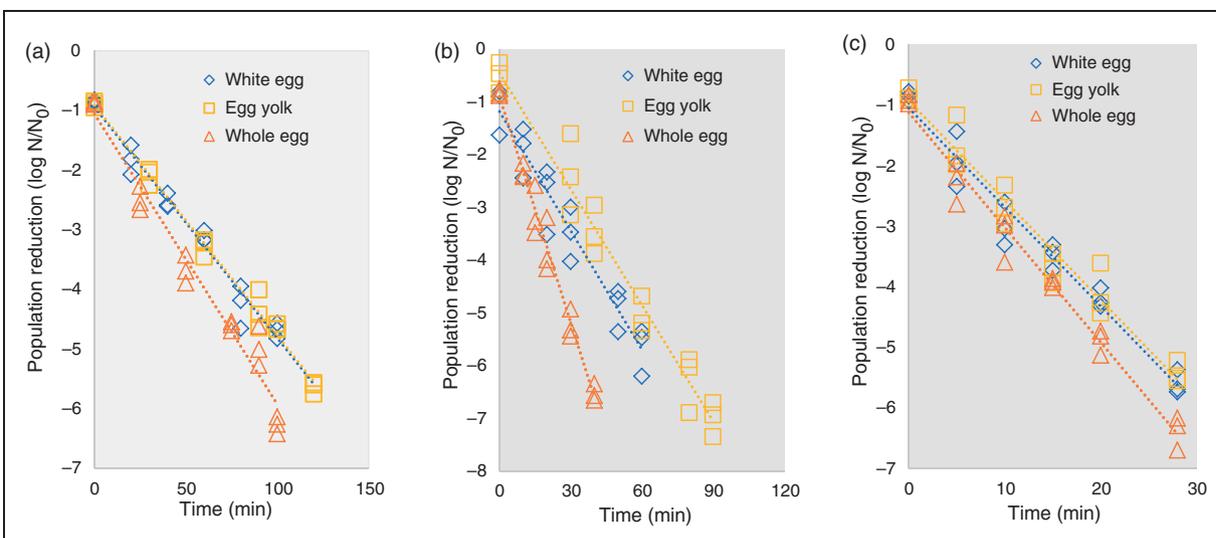
### D<sub>80°C</sub>-values of *S. Enteritidis* PT30 and *E. faecium* in egg powders

The come-up time for the egg powders samples to reach the target temperature (80 °C) inside the TDT cell was ~150 s. The end of the come-up time was taken as time zero of the survival curves, so it did not interfere with the calculation of the slopes (Xu et al., 2019). Thermal inactivation kinetics of both strains fitted well the first-order model as reflected by the high R<sup>2</sup> values and low RMSE values (Table 2). The inactivation curves of *S. Enteritidis* PT30 for the egg powders are presented in Figure 3.

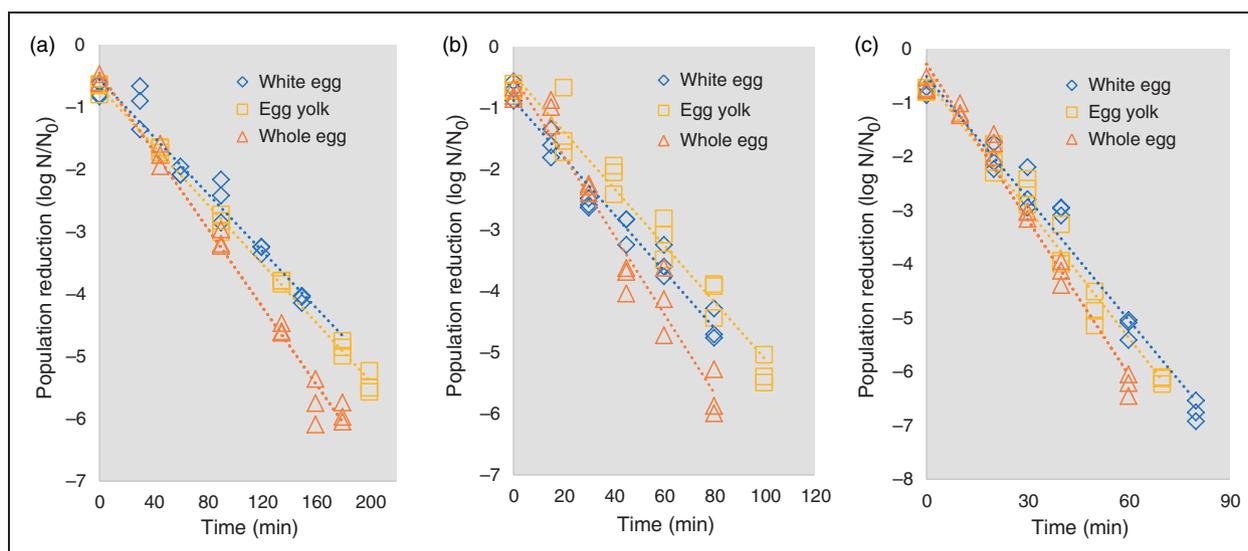
The D<sub>80°C</sub>-values obtained for *S. Enteritidis* PT30 from the egg powder samples at the selected a<sub>w,20°C</sub>

values (0.30, 0.45, and 0.6) and a<sub>w,80°C</sub> are summarized in Table 2. These values are significantly higher compared to the D-values of *Salmonella* in high-moisture food (a<sub>w</sub> > 0.96), which are usually between 0.06 and 0.25 min at 70 °C (Silva and Gibbs, 2012). As an example, the D<sub>80°C</sub>-values for egg white powder samples was 25.8 ± 0.3 min, 13.3 ± 0.3 min, and 6.1 ± 0.1 min at a<sub>w,20°C</sub> values of 0.30 ± 0.02, 0.45 ± 0.02, and 0.60 ± 0.02, respectively. This increase in the thermal resistance of *Salmonella* with reducing a<sub>w</sub> has also been observed in wheat flour, almond flour, whey protein, milk powder, and cocoa powder conditioned at similar a<sub>w</sub> values at room temperature before thermal treatments (Liu et al., 2019; Tsai et al., 2019; Xu et al., 2019). Because bacteria cells adapt quickly to a low a<sub>w</sub> environment by losing water, their proteins and ribosomes become more stable, and water loses mobility inside the bacteria cell (Leuenberger et al., 2017). This mechanism might hinder the thermal denaturation. Other mechanisms related to *Salmonella* survival in low-moisture environments might be due to osmoprotectant effect or biofilm formation that might contribute to enhance the thermal resistance of *Salmonella* (Finn et al., 2013; Villa-Rojas et al., 2017).

There are no differences (P > 0.05) between the D<sub>80°C</sub>-values of *S. Enteritidis* PT30 of egg white and that of yolk powder samples, at the selected a<sub>w</sub> values where their inactivation curves nearly overlap. The D<sub>80°C</sub>-values obtained of whole egg powder are smaller compared with the obtained ones for yolk and white egg. We can infer that D<sub>80°C</sub>-values of egg white and yolk are similar since the yolk is described as fat particles suspended in a protein solution, so bacteria are attached mainly to proteins in both powders (Li-Chan and Kim, 2008). However, the mixing of the whole egg before spray drying operation generates chemical and physical interactions between its components, which might affect the attachment of the bacteria to the



**Figure 3.** Thermal inactivation kinetics curves of *S. Enteritidis* PT30 in egg powders with different  $a_w$  values at 80 °C: (a)  $0.30 \pm 0.02$ , (b)  $0.45 \pm 0.02$ , and (c)  $0.60 \pm 0.02$ . The tests were conducted in independent replicates.

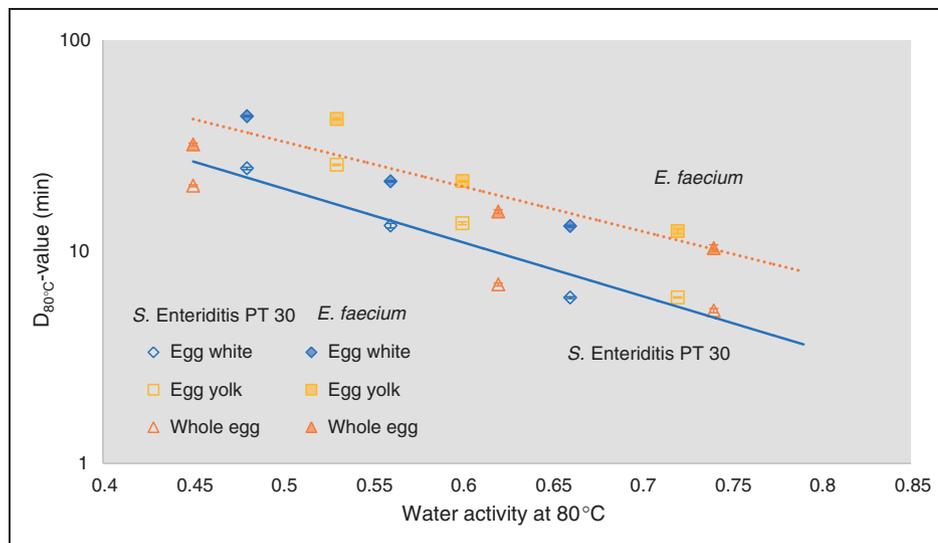


**Figure 4.** Thermal inactivation kinetics curves of *E. faecium* in egg powders with different  $a_w$  values at 80 °C: (a)  $0.30 \pm 0.02$ , (b)  $0.45 \pm 0.02$ , and (c)  $0.60 \pm 0.02$ . The tests were conducted in independent replicates.

powder (Lechevalier et al., 2013). We corroborate this hypothesis by evaluating a mix of 50% egg white powder and 50% egg yolk powder, which was also conditioned to  $a_w$  levels of  $0.30 \pm 0.02$ ,  $0.45 \pm 0.02$ , and  $0.60 \pm 0.02$ . The obtained  $D_{80^\circ\text{C}}$ -values for this mixture at each  $a_w$  level selected were  $25.43 \pm 0.15$ ,  $12.45 \pm 0.33$ , and  $6.05 \pm 0.10$  min, respectively, which are similar to the  $D_{80^\circ\text{C}}$ -values obtained for yolk and white egg powders. Similar thermal resistances of *Salmonella* have been observed in foods products with a high-carbohydrate content (wheat flour) and products with a high-fat content (soybean oil) (Jin et al., 2018). Rachon et al.

(2016) observed that at 80 °C the inactivations rates for *Salmonella* in a high protein product (meat powder) are higher compared to the rates obtained for high-carbohydrate products (confectionary products), while at higher temperatures ( $<100^\circ\text{C}$ ) the inactivation rate was slightly higher for high-carbohydrate product, indicating a protective effect of sugars. These facts suggest that the inactivation rates heavily rely on product composition,  $a_w$ , and temperature.

The inactivation curves of *E. faecium* for the egg powders samples are presented in Figure 4. Under the same  $a_w$  conditions, the  $D_{80^\circ\text{C}}$ -Values obtained for *E.*



**Figure 5.** The log-linear relationship between  $D_{80^{\circ}\text{C}}$ -values of *S. Enteritidis* PT30 (open symbols) and *E. faecium* (close symbols) with  $a_{w,80^{\circ}\text{C}}$  of egg powders.

*faecium* from the inactivation curves are higher ( $P < 0.05$ ) compared to those obtained for *S. Enteritidis* PT30 in egg powders samples (Table 2). As an example, the  $D_{80^{\circ}\text{C}}$ -value obtained of *E. faecium* in egg white powder at  $a_{w,20^{\circ}\text{C}}$  of  $0.30 \pm 0.02$  was  $43.8 \pm 0.4$  min, while for *S. Enteritidis* PT30  $D_{80^{\circ}\text{C}}$ -value was  $25.8 \pm 0.3$  min at the same  $a_{w,20^{\circ}\text{C}}$ . Consistently, *E. faecium* showed a higher thermal resistance than *Salmonella* in different low-moisture foods, such as almond kernels, balanced carbohydrate meals, and wheat flour (Liu et al., 2018a; Xu et al., 2018). These results indicate that *E. faecium* is an appropriate surrogate for *S. Enteritidis* PT30 in egg powders.

**Influence of  $a_{w,80^{\circ}\text{C}}$  on thermal resistance of microorganisms.** Our results showed that there were changes in the selected  $a_w$  of the egg powders samples when heated from  $20^{\circ}\text{C}$  to  $80^{\circ}\text{C}$ . Figure 5 shows a semi-log linear relationship ( $R^2 = 0.85$ ) between the  $D_{80^{\circ}\text{C}}$ -values and the  $a_{w,80^{\circ}\text{C}}$  for both strains. With the increase of the  $a_{w,80^{\circ}\text{C}}$ , there was a sharp reduction of the  $D_{80^{\circ}\text{C}}$ -values, which is consistent with previous reports on thermal inactivation studies of *S. Enteritidis* PT30 in other low-moisture foods (Xu et al., 2019). This suggests that the thermal inactivation of bacteria relies on  $a_w$  value at treatments temperature and of the food matrix.  $Z_{aw,80^{\circ}\text{C}}$  (defined in equation (4), as the value of product water activity increase at  $80^{\circ}\text{C}$  required for one-log reduction in  $D_{80^{\circ}\text{C}}$ ) is a useful parameter since it indicates the sensitivity of the thermal resistance to the  $a_{w,80^{\circ}\text{C}}$  changes.  $Z_{aw,80^{\circ}\text{C}}$  for the three egg powders samples was calculated for each microorganism from the negative inverse of their corresponding slope in Figure 5.

The  $Z_{aw}$  values for *E. faecium* (0.47) was larger ( $P < 0.05$ ) than that of *S. Enteritidis* PT30 (0.39). The obtained values of  $Z_{aw}$  for *S. Enteritidis* PT30 in egg powders are similar to  $Z_{aw}$  value (0.32) obtained for whey proteins, silicon dioxide, and wheat and almond flours (Liu et al., 2018b; Xu et al., 2019).

## CONCLUSION

In this study, we observed that the  $a_w$  of egg powders increased ( $P < 0.05$ ) with rising temperatures. The  $D_{80^{\circ}\text{C}}$ -values for *S. Enteritidis* PT30 and *E. faecium* had a log-linear relationship with the  $a_w$  at  $80^{\circ}\text{C}$  and that the composition influenced the difference in the obtained D-values. The  $D_{80^{\circ}\text{C}}$ -values obtained for *E. faecium* was higher compared to  $D_{80^{\circ}\text{C}}$ -values of *S. Enteritidis* PT30, indicating that *E. faecium* is a suitable surrogate of *Salmonella* in egg powders for thermal process validation. This study suggests that to develop an efficient thermal treatment, the  $a_w$  of egg powders should be elevated to allow lower  $D_{80^{\circ}\text{C}}$ -values in order to reduce the treatment time. However, further research should be done in more complex food matrixes related to the inactivation of *Salmonella* by thermal treatments.

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