Modelling of Thermal Sterilization of high-moisture snack foods: feasibility analysis and optimization

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Abstract

High-moisture snacks, such as steamed buns and rice cakes, are traditional and popular in Asian countries. However, their shelf life is short, primarily due to microbial spoilage. Current manufacturing methods address this shortcoming through the use of chemical preservatives. To satisfy consumers' demand for preservative-free food, thermal sterilization of a model high-moisture snack (steamed rice cakes) is investigated in this work. *Bacillus cereus* spores are heat-resistant pathogens typically found in rice products; hence, they constitute a suitable candidate to assess the effectiveness of thermal sterilization. A validated combination of predicted temperature profile of rice cakes based on thermal properties extracted experimentally with thermal inactivation kinetics of *B. cereus* spores allows us to assess the sensitivity of processing conditions to sterilization efficiency. Using both experimentation and modelling, it is shown that enhancement of heat transfer by improving convection from the heating medium (either water or steam) has a limited effect on inactivation due to the intrinsic kinetics of spore inactivation.

Keywords: heat transfer model; rice cakes; spore thermal inactivation; Inactivation kinetics; high-moisture snacks

1 Introduction

High-moisture soft solid snack foods such as rice cakes, glutinous rice dumplings and other desserts have become increasingly popular. Most of these snacks are freshly made and sold in the market, while industrially-produced versions use chemical preservatives to inhibit microbial growth and improve shelf life. With the increasing demand for preservative-free foods from consumers, this work explores the limits of thermal treatments for preventing bacterial spoilage without chemical preservatives. Steamed rice cakes, which are traditional high-moisture snacks in many Asian countries, are used as a case study.

Steamed rice cakes have a moisture content of 35%-50% depending on the formulation. They spoil after only 2-3 days due to the growth of microorganisms such as *Bacillus* spp, *Staphylococcus aureus* and *Staphylococcus epidermidis* (FDA 2012; Ji et al. 2007). Heat resistant spore-forming microorganisms can survive the steam cooking process, which includes *Bacillus cereus*, a common toxigenic food poisoning bacterium (Okahisa et al. 2008). *B. cereus* spores in food products may survive when cooked at or below 100 °C and following cooling may germinate and grow to dangerous levels in the absence of competing bacteria (Rajkovic 2014; Van Asselt and Zwietering 2006). Hence, *B. cereus* spores constitute a suitable candidate to investigate sterilization efficiency.

Thermal processing has been suggested as an effective method to achieve microbial stability for low moisture foods (moisture content of 0%-20% (Heldman 2005) or water activity lower than 0.85 (FAO 2015)) such as dry corn flour (VanCauwenberge et al. 1981), almonds (Jeong et al. 2011) or dry mashed potatoes (Ovrutskaia et al. 1980). While these studies provide the inactivation kinetics of the bacteria, process design requires coupling this information with a suitable thermal model. A more comprehensive analysis of the link between processing and inactivation kinetics has been performed for liquid pasteurization in bottles (Augusto et al. 2010; Moraga et al. 2011) and pipelines (Grijspeerdt et al. 2003; Chen and Hoover 2003; Nazarowec-White et al. 1999), as well as for vegetables in cans (Mafart et al. 2002; Marra and Romano 2003; Abdul Ghani et al. 1999; Kızıltaş et al. 2010) and retort pouches (Abdul Ghani et al. 2001; Abdul Ghani et al. 2002; Bhowmik and Tandon 1987), and for biological powders and solid dry materials by instant controlled pressure drop technology (Mounir et al. 2014). Pasteurization of milk and beer are examples of established technologies, where thermal modelling is mostly aimed at optimizing temperature profiles in existing devices. Most reports on thermal modelling in cans and pouches have focused on determining the temperature profile and thermal properties such as thermal conductivity and heat transfer coefficient from Computational Fluid Dynamics (CFD) simulations. While these temperature profiles are sometimes used to predict microbiological inactivation or the evolution of quality variables (Al-Baali and Farid 2007), the lethality of the process is usually not validated by independent microbial methods which will give more precise information about sterilization than the F-value calculated based on temperature measurement.

In this study, thermal processing of steamed rice cakes to achieve preservative-free microbial safety will be assessed by combining experimentation and modelling, which has not been previously reported for these high-moisture soft-solid foods. Firstly, the thermal properties of steamed rice cakes will be extracted from fitting temperature profiles of samples in both a water bath and an autoclave, the latter system covering a wider range of temperatures. Then the thermal inactivation kinetic parameters of *B. cereus* spores in steamed rice cakes will be measured in an oil bath set at various temperatures. Finally, the combination of thermal inactivation kinetics and modelling based on the thermal properties will be used to assess the effect of processing conditions and product dimensions on rice cake sterilization.

2 Materials and Methods

2.1 Preparation of steamed rice cake

Sticky rice flour, rice flour (Erawan Marketing Co., Bangkok, Thailand), sugar (Sugar Australia Pty Limited, Australia), and tap water were mixed in a ratio of 3:2:1:3.6 in a bench mixer (GS-6118, Homemaker, Kmart Australia) at low speed for 1 min to produce a dough which was formed into small rice balls weighing 50 g. The rice balls were cooked in a steamer (RC-4700-A, Homemaker, Kmart Australia) for 15 min. Subsequently, they were removed from the steamer and cooled down to room temperature before being packaged in single-layer food wrap (Cling Wrap, Berry Plastics Pty Ltd, Australia) close to the surface of the rice cakes. The volume of the rice cakes were measured by the seed displacement method and the density was calculated as weight/volume.

2.2 Measurements of time-temperature profiles of rice cakes in boiling water and autoclave for calculation of thermal conductivity and heat transfer coefficient

2.2.1 In boiling water

K-type thermocouples with a diameter of 1 mm (RS Components Pty Ltd, Smithfield, Australia) were inserted into the centre of the cakes, which were submerged in a boiling water bath as shown schematically in Figure 1(a). The evolution of temperature with time during thermal processing was recorded until the samples reached an equilibrium temperature. The size of the samples and the temperature and stirring conditions in the water bath were varied as shown in Table 2.

2.2.2 In autoclave

Wireless thermocouples (Hitemp 140-FP-36, MadgeTech Inc., Warner, NH, USA) with a diameter of 2.5 mm were inserted into the centre of the rice cakes. The samples were subsequently placed in an autoclave for 40 minutes and the time-temperature profiles were measured, a schematic of the experiment is shown in Figure 1(b). The sterilization temperature was set at either 105, 110, 115, 121, or 125 °C and the size of the rice cakes was varied. Table 1 contains all the experimental conditions used.

2.3 Thermal inactivation of *B. cereus* spores in rice cakes in oil bath

2.3.1 Bacterial cultures

B. cereus ATCC 11778 and *B. cereus* ATCC 14579 were supplied by Thermo Fisher Scientific Inc. (Waltham, MA, USA). The culti-loops were used to streak on Heart Infusion (HI) agar, and the agar plate was incubated at 37 °C for 24 h. A single colony was inoculated in HI broth, then incubated at 37 °C for 24 h. The cultures were individually maintained at 4 °C and a glycerol stock of *B. cereus* strains was stored at -80 °C. The cultures were propagated in HI broth overnight at 37 °C for the next step.

2.3.2 Preparation of spore suspension

Spore suspensions of the two *B. cereus* strains were prepared using the procedure of Novak et al. (2005) with a few modifications. The cultures of each *B. cereus* strain were inoculated into 10 ml of HI broth and incubated at 37 °C overnight. The culture broths were surface spread on pre-poured Nutrient Agar (Oxoid, Basingstoke, UK) plates. The plates were incubated for one week at 30 °C (ATCC 11778) and 3 days at 37 °C (ATCC 14579). Dark phase microscopy was used to check for sporulation. Spores were harvested from multiple plates by flooding the agar surface with 2 ml 0.85% saline and gentle scraping with sterile L-shaped spreaders, washing with 0.85% saline 4 times and resuspending in 10 ml of saline. The spore suspension was heat treated at 80 °C for 10 min to inactivate vegetative cells. The concentration of spores was calculated by the plate count method where the spore suspension was serially diluted and plated on nutrient agar plates. After incubation at 30 °C for 18 h, the colonies were counted and converted into CFU/ml. Each spore suspension of 10^8 CFU/ml was stored at 4 °C in sterile screw-capped tubes and the spore suspensions of *B. cereus* ATCC 11778 and *B. cereus* ATCC 14579 were mixed 1:1 before inoculation into rice cakes. The application of mixed spores aims at ensuring the stability of the spores in food as one strain might be more sensitive to the food environment and result in inaccurate calculation of thermal inactivation.

2.3.3 Thermal inactivation of *B. cereus* spores in rice cake

Steamed rice cakes were pre-heated at selected temperatures of 90, 95, 100 and 105 °C, in a thermostatically-controlled oil bath. Thermocouples were used to record the temperature in the centre of rice cakes to make sure the inactivation experiment was performed at constant temperature. The small diameter of the thermocouples guarantees accurate spatial and temporal measurements at the centre of the sample; the measurement noise was insignificant. After reaching the target temperature, the rice cakes were immediately removed from the bath and inoculated with spores (1.6×10^5 CFU/g) at the centre, which constitutes the worst-case scenario as the centre is the coldest point requiring the longest time to achieve spore inactivation. After inoculation, the rice cakes were kneaded to keep them intact and prevent leakage of spores. The inoculated rice cakes were then placed into an oil bath at 90,

95, 100 or 105 °C for specific durations of 0, 3, 6, 9, 12, 15 and 18 min respectively. The rice cakes that were not inoculated were used as a negative control. After heating the samples were collected, transferred to ice chilled peptone water (0.1% bacteriological peptone, Oxoid) and immediately cooled in an ice bath to prevent further inactivation from residual heat. The experimental samples were homogenised for 1 minute in 200 ml of peptone water using a stomacher (BagMixer 400P, Interscience Co., St Nom, France) and serially diluted using 0.85% saline, surface plated on nutrient agar plates, then incubated aerobically for 24 h at 37 °C. Colonies formed on the nutrient agar plates were calculated and recorded as CFU/g.

3 Mathematical model of sterilization

3.1 Governing equations

The steamed rice cake is modelled as a sphere of radius *r*. In this study, two main assumptions are made:

1. Only conductive and convective heat transfer are taken into consideration.
2. Any rice cake swelling is neglected with a constant volume assumed throughout the sterilization process.
3. The wrapping thickness is insignificant and its conductive resistance negligible.

The basic heat transfer equation for conduction in spherical coordinates (Incropera and DeWitt 1985) is:

$$\frac{1}{r^2}\frac{\partial}{\partial r}\left(kr^2\frac{\partial T}{\partial r}\right)=\rho C_p\frac{\partial T}{\partial t}=\frac{1}{\alpha}\frac{\partial T}{\partial t} \tag{1}$$

where r is the radial coordinate, ρ is the density (kg/m^3), C_p is the specific heat capacity (J/kg·K) k is the thermal conductivity (W/m·K), and α is the thermal diffusivity (m^2/s).

The initial and boundary conditions are

$$T(r,0)=T_i, \tag{2}$$

$$\left.\frac{\partial T}{\partial r}\right|_{r=0}=0, \tag{3}$$

$$\left.-k\frac{\partial T}{\partial r}\right|_{r=r}=h\left[T(r,t)-T_\infty\right], \tag{4}$$

where T_i is the initial temperature of the sample, T_∞ is the bulk temperature of the surroundings, r is the radius of the steamed rice cakes (m), and h is the convective heat transfer coefficient (W/m^2·K).

In the calculation of effective thermal conductivity of steamed rice cakes from data extracted from cooked rice (Ramesh 2000), the sample (cooked rice) was a mixture of a solid and a fluid phase (assumed to be air). In that case, knowing the experimental effective thermal conductivity of cooked rice and that of air, the thermal conductivity of the solid phase can be estimated. The effective medium theory (EMT) formulation incorporates simultaneous parallel and series conduction models in heterogeneous chaotic media (Landauer 1952), which is used to calculate the effective thermal conductivity in a medium containing two randomly distributed components. Rice cake is a porous material with various sizes of random distributed air bubbles, as observed by microscopy and magnetic resonance imaging (unpublished data), which suggests the EMT is reasonable for calculating a thermal conductivity. Other well-known models do not have the same structural basis (Pietrak and Wisniewski 2015), including the Maxwell-Eucken model that calculates thermal conductivity of porous materials by assuming the disperse phase comprises individual spheres that do not come into contact with each other within a continuous matrix phase. Furthermore, EMT has been successful in predicting the thermal conductivity of fruits, starchy foods and fibrous porous materials (Mattea et al. 1986; Gong et al. 2014). Following EMT,

$$\phi\frac{k_f-k}{k_f+2k}+(1-\phi)\frac{k_s-k}{k_s+2k}=0, \tag{5}$$

where k_s, k_f and k are the solid, fluid and sample thermal conductivities, respectively, and ϕ is the volume fraction of the fluid phase. ϕ can be extracted from the sample density, ρ, and the fluid mass fraction, ω_f, as $\phi=\omega_f\rho/\rho_f$, where ρ_f is the fluid density.

3.2 Thermal inactivation model of *B. cereus* spores

The experimental data of thermal death curves, which show log numbers of spore survival as a function of time, are fitted using first order kinetics:

$$\log N(t) = \log N_0 - t / D_T \tag{6}$$

N and N_0 are the remaining and initial number of microorganisms, respectively, t is time and D_T is the decimal reduction time at temperature T. The D-value is defined as the time required to achieve a 1-log reduction in bacterial numbers. It varies with T as follows:

$$\log D_T = \log D_{ref} - \left(T - T_{ref}\right) / z \tag{7}$$

Where z is the thermal death constant and D_{ref} is the D-value at the reference temperature T_{ref}, 90 °C in this study.

4 Results

4.1 Modelling of heat transfer during thermal treatment of steamed rice cake

Two sterilization processes, submersion in boiling water and autoclave with steaming, were investigated to characterise the thermal properties of rice cakes in different heating media. This allowed the development of a mathematical model to describe the temperature distribution of rice cakes during thermal processing with various parameters such as processing temperature, heat transfer coefficient, and thermal conductivity of samples.

The conductive heat transfer Eqs. (1)-(4) were used to predict the experimentally measured temperature in the centre of the rice cake during heating. The thermal conductivity is estimated using the EMT model (Eq. 5) on the basis that it is not expected to vary to the same extent as the heat transfer coefficeint upon variation in processing conditions. By calculating the conductivity separately, the temperature profiles can be predicted by fitting only one variable, the heat transfer coefficeint.

The thermal conductivity of the steamed rice cakes was estimated using bulk density, porosity, and experimental thermal conductivity of cooked rice at 70% moisture content and 70 °C from Ramesh (2000) using EMT (Eq. (5)). This assumes the fluid in the void area of cooked rice is air. The calculated thermal conductivity of the solid (k_{solid}) is shown in Table 2. There is a small variation in the thermal conductivity of the solids in cooked rice with different bulk densities and porosities. This variation might be related to the assumption that dry air constitutes the void area of cooked rice instead of moist air with different levels of humidity. The average solid thermal conductivity was k_{solid} =0.8±0.06 W/m K.

The thermal conductivity of steamed rice cakes depends on the material properties including the formulation and porosity, and temperature (Sweat 1995). A steamed rice cake is a more condensed system than cooked rice, with a bulk density of 1240 kg/m^3, which is higher than that of 720 - 850 kg/m^3 for cooked rice and 1157 kg/m^3 for the solids in cooked rice calculated by $\phi = \frac{\rho_t - \rho_b}{\rho_t}$ (ϕ is porosity, ρ_t is true density of the solids in cooked rice and ρ_b is the bulk density of cooked rice). Therefore, it is reasonable to assume that the low porosity of steamed rice cakes does not influence the effective thermal conductivity of samples and the thermal conductivity of the solids (0.8 W/mK) is equal to that of rice cakes. A range of thermal conductivity values (0.1-0.6 W/mK) for raw rice and cooked rice has been reported in the literature (Muramatsu et al. 2007; Ramesh 2000; Mohsenin 1980). In addition to variations arising from differences in temperature, water content and porosity of the samples, there are also challenges and limitations in the experimental procedures used to obtain thermal conductivity. This includes moisture migration through samples with high moisture content in the guarded hot plate method that influences the steady state assumption (Reidy and Rippen 1971), and the location of the

thermal probe in the probe method that cause deviation particularly in multi-phase materials. Hence, future work is required to improve experimental measurement procedures to obtain more accurate determination for the thermal conductivity of rice cakes and cooked rice. In this study, we assume that thermal conductivity is independent of temperature to simplify the model and calculate the heat transfer coefficient in both systems for sterilization efficiency investigation in section 4.2. The effect of thermal conductivity on inactivation efficiency of *B. cereus* spores will be discussed later (section 4.4) as part of a sensitivity analysis. Therefore, the sterilization of rice cakes with fillings or different formulations, and other high-moisture food products that have a different thermal conductivity can also be evaluated using this model.

The measured time-temperature profiles of steamed rice cakes in boiling water and autoclave are shown in Figure 2(a) and (b). Taking an average effective thermal conductivity (0.8 W/m·K) of steamed rice cakes as a constant, the experimental results were fitted using the Levenberg–Marquardt algorithm built into Matlab with Eqs. (1)-(4). The heat transfer coefficient, *h*, was estimated from the fitting and is reported in Table 2.

Heat is transferred rapidly from the heating medium to the rice cakes before slowing down as it approaches equilibrium as the driving temperature difference between the heating medium and rice cakes decreases (Figure 2(a) and (b)). Since the *Biot* number ($Bi = hr/k$) in this study is 2.2-5.0, which falls between 1 and 100, the heat transfer rate depends significantly on both the heat transfer coefficient in the sterilization system and the thermal properties of the rice cakes.

In the water bath, the heat transfer coefficient increased with the water bath temperature, water turbulence (stirring), and showed no specific trend with the rice cake radius. The heat transfer coefficient is a complex function of Reynolds number, *Re*, bulk fluid (heating medium) viscosity, interfacial viscosity and the thermal conductivity of the rice cake, the density of the fluid and its specific heat capacity, which are all temperature dependant. For flow around a sphere, the Nusselt number, *Nu,* is usually correlated (Incropera and DeWitt 1985) as:

$$Nu = hD/k = 2 + (0.4\mathrm{Re}^{1/2} + 0.4 + \mathrm{Re}^{2/3})\mathrm{Pr}^{2/5} \quad (8)$$

where *Pr* is the Prandtl number, the ratio of momentum diffusivity to thermal diffusivity. The *h* tends to increase with *T* between 80 °C and 100 °C because the reduction in viscosity with *T* is more pronounced than for the other variables. According to Eq. (8), *h* tends to decrease with the diameter of the sphere. However, this correlation is only valid for smooth surfaces. Table 2 shows an unexpected variation in *h* with cake diameter, which could be related to the inherent uncertainty on the true surface area, which in this case is evidently not smooth. The presence of stirring using a stirrer bar at the bottom of the water bath increased the value of *h*, but not dramatically. However, inducing forced convection using more intense mixing devices is anticipated to increase *h* significantly.

The autoclave system consisted of a cylindrical, 80-cm diameter vertical pipe with a water pool at the bottom (Figure 1(b)). The sample was located at the centre of the pipe over a grid that allowed free steam circulation. The water pool was heated using electrical resistance until boiling. Initially, a valve at the top of the autoclave allowed the air (pushed by the ascending steam) to leave the system. Subsequently, this valve was closed and the pressure was allowed to increase up to 2 bars (corresponding to a saturation temperature of 121 °C). The temperature versus time curve inside the autoclave (Figure 2(b)) includes a heating phase (from 25 °C to 100 °C) and a latent and heating phase (from 100 °C to 121 °C). The steam velocity was distributed unevenly, particularly in the heating phase, which likely encompasses considerable variations in h. Hence, the values in Table 2 should be considered as an average. Moreover, since the steam we used was not overheated, the main component of heating was assumed to be performed by vapour condensation.

In an autoclave, h tends to increase as temperature increases from 105 to 121 °C. Since the viscosity of water vapour in the autoclave is relatively constant over this temperature range (0.000013 kg/m·s), the increase in h is likely to be associated with the velocity of water vapour, as the evaporation rate increases with the temperature of the boiling resistance. In this study, only one temperature-independent value of h was extracted from the measurements, corresponding to an average over the entire profile. Table 2 shows that on average, h was greater in the autoclave than in boiling water. In agreement with Eq. (8), the extracted h decreased with the diameter of the rice cakes.

Figure 2 (c) and (d) depict an example of the temperature distribution in rice cakes at different processing times in stirred boiling water using k = 0.8 W/m·K and h = 128 W/m^2·K from Figure 2(a). Since Bi was very high (Bi>2), the temperature profile within the material should have been non-homogeneous. Because of this absence of temperature homogeneity, higher microbial concentration was to be expected in the center which had to be the coldest point. Hence, in the sensitivity analysis (section 4.4), the microbial inactivation in the centre of the rice cakes was taken as an indication of the processing time required for industrial application.

The computational model seems to provide reasonable predictions of the experimentally-measured temperature inside the rice cake by fitting h, varying from ca. 100 to 300 W/m^2·K, across the range of conditions evaluated in both systems. Section 4.4 investigates how sensitive the model is to variations in system variables (i.e. a sensitivity analysis) and is used to assess routes for potentially improving the sterilization efficiency. The goal of the present study was to determine the balance between the final processing temperature and heat transfer coefficient in the sterilization system for optimising heat transfer kinetics during sterilization of the food product.

4.2 Inactivation kinetics of *B. cereus* spores

The log numbers of spore survivors were measured as a function of time at each measured temperature, with the results shown in Figure 3(a). Two parameters, *D*-value and *z*-value, in thermal inactivation model (Eqs. (6) - (7)) for *B. cereus* spores were calculated and are shown in Figure 3(b) and Table 1.

The number of spores remained relatively constant over the first 3-6 minutes (Figure 3(a)). Peleg (2000) has suggested that such a lag phase is possibly due to cumulative damage instead of an instantaneous lethal effect as there are multiple target sites for thermal inactivation (Xiong et al. 1999), which becomes irreparable and lethal only beyond a certain critical level. Survival curves can have a variety of shapes such as linear, sigmoid, concave, and convex. The shape depends on the category and physiological conditions of the microorganisms, the sterilization methods, and the experimental conditions (Casolari 1988). In the first-order kinetics model, which is well developed and widely used to calculate thermal inactivation of microbes, the linear part is usually used to calculate the *D*-value and *z*-value.

In this study, time periods from 6 min and above were used to calculate *D*-value which had R^2 values of 0.921 or more (Figure 3, Table 3). The D_{100}, D_{90} and *z*-value obtained in this study were 4 min, 10.4 min, and 25.6 °C, respectively. This corresponded well to the values reported in the literature. For example, D_{100} values for *B. cereus* ATCC 4342, 7004, 9818 spores were between 0.062 and 10.9 min depending on the sporulation temperature (González et al. 1999) and between 0.39 and 21.40 min depending on the solutes and water activity (Mazas et al. 1999); D_{90} for a mixture of *B. cereus* DSM 4313 and DSM 626 spores in pork luncheon roll was reported as 10.1 min (Byrne et al. 2006) while the *z*-value of 25.6 °C in the present study was higher than the reported values of 7.32-12.1 °C in spore suspensions. The difference was likely due to differences in the strains of *B. cereus* used and different heating media (spore suspensions in McIlvaine buffer, distilled water or pork luncheon roll in the literature and steamed rice cakes in this study), and different experimental conditions such as sample size. However, high z-values of 30.3 °C have also been reported for *Salmonella weltevreden* in plain wheat flour with a water activity of 0.56-0.60 (Archer et al. 1998), indicating that the results of the present study were reasonable.

4.3 Validation of the mathematical model combining heat transfer and thermal inactivation of *B. cereus* spores

With the prediction of temperature distribution in rice cakes using *k* and *h*, the inactivation model of *B. cereus* spores which is a function of *D*-value, *z*-value and temperature was used to predict the spore survivor distribution in rice cakes. With the parameters obtained in sections 4.1 and 4.2, the spatial distribution of *B. cereus* spore survivors is expressed as a function of *k*, *h*, *D*-value, *z*, and temperature as shown in Eqs (1) (4) (6) and (7), during the thermal processing of the steamed rice cakes. This combined model could predict the processing time required for a 6 log reduction (6D) of the microbial

population at the critical point (in this study it was the centre of rice cakes that required the longest time to sterilize).

The validation of the combined model is shown in Figure 4. The trends of the experimental and predicted curves were similar and the experimental curve only deviated significantly from the predicted curve at 12 min ($p < 0.05$). The predicted inactivation of spores largely follows a convex curve similar to that described previously for *Geobacillus stearothermophilus* (Iciek et al. 2006), the experimental results show a small deviation at 9-12min. This deviation may be due to heat-activated spores producing vegetative cells whicha re more heat-sensitive and can be expected to be killed more rapidly than predicted and might be responsible for the underestimation of killing at 9-12 min.

However, in this model, the differences between all the data points were less than 10% which is acceptable and 99.4% of the variance in the experiment can be explained by the model (Figure 5 (b), $R^2 = 0.994$). This suggested that the model can be extrapolated and used to predict the industrially relevant 6D of *B. cereus* spores in rice cakes under different sterilization conditions.

4.4 Prediction of inactivation using combined model of heat transfer and thermal inactivation kinetics of *B. cereus* spores

In terms of heat transfer rate, the most important factors that can be modified through changing product and processing conditions are the heat transfer coefficient, processing temperature, size and shape of rice cakes. The thermal inactivation kinetics of *B. cereus* spores obtained previously was used to predict the distribution of microorganisms in steamed rice cakes under these different processing parameters. Two additional geometries with the same volume as that of the original spherical cakes (diameter of 4 cm) were investigated: a disk with a diameter (D = 7.52 cm) 10 times its thickness (L = 0.75 cm) and a cylinder of length (L = 16.2 cm) 10 times its diameter (D = 1.62 cm).

4.4.1 Inactivation of *B. cereus* spores in spherical rice cakes

Figure 5 shows that predicted inactivation increased significantly with (i) heat transfer coefficient (h) when h is between 0 and 500 W/m^2·K, (ii) thermal conductivity (k), and (iii) processing temperature, and decreases with the diameter of the rice cakes. However, no significant difference was observed when h was over 500 W/m^2·K. The h values calculated by fitting the experimental data in this study were between 100 and 300 W/m^2·K, which shows that increasing h in the sterilization system can significantly increase the inactivation of *B. cereus* spore in the rice cakes and reduce the time required for sterilization. However, when h is large enough (> 500 W/m^2·K), heat transfers quickly to the centre of the rice cakes (160 s and 1200 s for the centre of disk-shaped and spherical rice cakes, respectively, to reach 120 °C when h is 500 W/m^2·K). Therefore, inactivation would depend mostly on the innate heat resistance of the bacteria. This imposes a restriction on how much inactivation times can be reduced by simply varying the fluid circulation profile around the sample. At a sufficiently high value of h, the

355 temperature on the surface of the rice cake is essentially constant and inactivation depends solely on
356 the conductivity of the rice cake and the intrinsic kinetics of the bacterial inactivation process. Even
357 after the rice cake reaches thermal equilibrium with the surrounding fluid, the time dependence of the
358 inactivation kinetics limits how much the sterilization time can be reduced.

359 Smaller *D*-values require less time to reach the target *B. cereus* spore inactivation and increasing heat
360 flow, by increasing *k* may decrease the *D*-value. In this case, *D*-value influences the inactivation more
361 than *k* (as shown in Figure 5(b) and (c)), the range of thermal conductivities in food products is small,
362 k = 0.01-5W/m·K, resulting in slow heat transfer that may increase the *D*-value of spores. Our results
363 show that other factors that have a stronger effect on the *D*-value than *k*, such as water activity or pH,
364 are more promising to increase inactivation. This suggests that various formulations and the filling can
365 be applied to modify the organoleptic properties of steamed rice cakes without significantly influencing
366 the sterilization efficiency if the inactivation kinetics of the bacteria do not change significantly. In this
367 case, the inertia of the intrinsic kinetics of inactivation sets a limit on the additional efficiency that can
368 potentially be gained by increasing *h*. The heat resistance of spores is influenced by various factors,
369 such as the species of the microorganism, the composition of the sporulation medium (pH, divalent
370 metallic cations, phosphate concentration and other additives), the temperature of spore cultivation, the
371 water activity and the suspending medium (Brown and Melling 2012). The important parameters in
372 microorganism inactivation kinetics such as the *D*-value and *z*-value greatly depend on these factors.
373 Steamed rice cake has a water activity of approximately 0.92 (Ji et al. 2007) and a pH of 5.73 ± 0.05
374 (measured experimentally in this study). Casadei et al. (2001) found that a low pH of the suspending
375 medium increased the thermal resistance of *B. cereus* spores, i.e., the *D*-value of the spores decreased
376 when the pH of the matrix increased in the range of 3 to 7. Increasing the pH of rice cakes by adding
377 fillings could potentially be one way to get a lower *D*-value, thus improving sterilization efficiency.
378 The water activity of the food material also significantly influences the heat resistance of spores.
379 Gaillard et al. (1998) reported that a higher water activity resulted in a lower thermal resistance.
380 However, variations were also observed at higher levels of water activity (Mazas et al. 1999). Further
381 research is required to understand how the pH, water activity and other physicochemical properties of
382 food products influence the thermal resistance of *B. cereus* spores. Methods to change the water activity
383 and pH of rice cakes with acceptable sensory properties might be considered to improve the sterilization
384 efficiency.

385 Trends are also predicted for changes in processing temperature and in the sizes of rice cakes (data not
386 shown for brevity). Inactivation changes significantly in rice cakes of different diameter. The results
387 from the model prediction shows, as intuitively expected, that rice cakes with a smaller size under
388 thermal processing at higher temperature have a higher sterilization efficiency. The 6D of *B. cereus*
389 spores in rice cakes with a diameter of 1 cm at a processing temperature of 120 °C and an *h* value of
390 500 W/m^2·K is 2.8 min, while that of rice cakes with a diameter of 4 cm is 8.9 min.

4.4.2 Inactivation of *B. cereus* spores in disk-shaped rice cakes

The sterilization of disk-shaped rice cakes is predicted to be much more efficient (less time required) than for spheres of the same volume (Figure 6(b)). The trends in inactivation were the same as those for spherical rice cakes with changing *h* values and processing temperatures (Figure 6(a)). However, *h* did not have a large effect on inactivation between 25 and 500 W/m^2·K. Thus, compared to spherical rice cakes, the inactivation of disk-shaped rice cakes is predicted to be much less dependent on *h*. The 6D of *B. cereus* spores in disk-shaped rice cakes (radius of 3.76 cm and thickness of 0.75 cm) at a processing temperature of 120 °C and *h* value of 500 W/m^2·K is 2.8 min.

4.4.3 Inactivation of *B. cereus* spores in cylindrical rice cakes

The sterilization of rice cakes with a cylindrical shape is also much more efficient (less time required) than for a spherical shape (Figure 6(b)). The inactivation increased with increasing *h* value (data not shown). However, the *h* value did not have a large effect on inactivation between 25 and 500 W/m^2·K. The trend in inactivation was the same as that for spherical rice cakes with changes in processing temperatures. The 6D of *B. cereus* spores in rice cakes of cylindrical shape (radius of 0.81 cm and length of 16.22 cm) with the same volume as spherical rice cakes with a 4 cm diameter at a processing temperature of 121 °C and an *h* value of 500 W/m^2·K is predicted to be 3.4 min.

The size and shape of rice cakes play an important role in sterilization efficiency. Less time is required for smaller rice cakes to reach the target processing temperature. The sterilization efficiency of disk- and cylindrically-shaped rice cakes is much higher than that of spherically-shaped rice cakes, mainly due to the heat transfer rate differences. Heat transfers rapidly from the surface to the centre of disk- and cylindrically-shaped rice cakes, which results in small differences in spore inactivation when *h* varies between 25 and 5000 W/m^2·K. The effects of both size and shape on the heat transfer rate are related to the differences in the linear distance between the surface and the centre of the rice cake. Smaller spherical rice cakes have less distance between the surface and the centre. For the same volume, disk-shaped rice cakes have the smallest linear distance between the surface and the centre, followed by cylindrical and spherical rice cakes.

A sensitivity analysis of inactivation indicated that for spherical rice cakes, sterilization efficiency can be significantly improved by increasing *h* values, for example, by placing them in a flow condition where the experimental *h* varies from 50 to 250 W/m^2·K. Using a higher temperature (such as 121 °C, widely used as a retorting temperature) and a smaller size of samples can also be predicted to contribute to sterilization efficiency. In addition, steamed rice cakes could be made into disk or cylindrical shapes to achieve a more efficient sterilization.

5 Conclusions

A model has been established for optimising processing conditions for the conventional sterilisation of high-moisture snack foods. A key innovation has been to combine thermal inactivation kinetics of *B. cereus* spores with a multiphysics model that predicts the temperature profile within rice cakes whilst they undergo sterilisation using a boiling water or autoclave system. This combination enables the prediction of the inactivation of spores under various industrially-relevant processing scenarios, which cannot be achieved by focusing on temperature alone. Through validation against experiments and a sensitivity analysis, it was found that increasing the heat transfer coefficient (via flow dynamics) and processing temperature can be used to improve inactivation, but a major limiting factor is conduction within the rice cake that is governed by its low thermal conductivity. Thus, decreasing the diameter of the rice cakes also increases the rate of inactivation, and this effect can be mirrored by altering their shape into disks and cylinders to decrease the distance between the surface and the centre. Future work will focus on adapting the model to investigate the influence of different fillings within rice cakes, where it is anticipated that faster inactivation would be achieved in systems where the filling has a higher thermal conductivity than the rice cake. The model and approach can also be expected to be useful for optimising sterilization processes and devices for other high-moisture food systems.

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556 **Figure 1** Schematic diagram for measurement of time-temperature profiles of rice cakes in (a) a boiling
557 water system and (b).an autoclave

558 **Figure 2** Time-temperature profiles of 4-cm diameter steamed rice cakes during thermal treatment in
559 (a) stirred boiling water; (b) autoclave; Prediction of temperature distribution of steamed rice cake
560 during heat treatment in boiling water, time intervals: (c) 300 s; (d) 720 s.

561 **Figure 3** Thermal inactivation kinetics of *B. cereus* spores in rice cakes in an oil bath: (a) thermal death
562 curves, D-values at each measured temperature calculated as the negative slopes of the linear part; (b)
563 determination of the z-value from log D versus temperature.

564 **Figure 4** Validation of combined model: (a) inactivation data of experiment and model prediction; (b)
565 comparison of experiment and prediction.

566 **Figure 5** Inactivation prediction of *B. cereus* in spherical rice cakes in sterilization systems of (a)
567 different heat transfer coefficients; (b) thermal conductivities and (c) *D*-values.

568 **Figure 6** Inactivation prediction of (a) *B. cereus* in disk-shaped rice cakes in sterilization systems of
569 different heat transfer coefficients; (b) *B. cereus* in cylindrical, disk-shaped and spherical rice cakes.
570 Volume of rice cakes: 33.5 cm^3; Temperature: 121 °C; Heat transfer coefficient: 500 $W/m^2 \cdot K$.

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572 **List of Tables**

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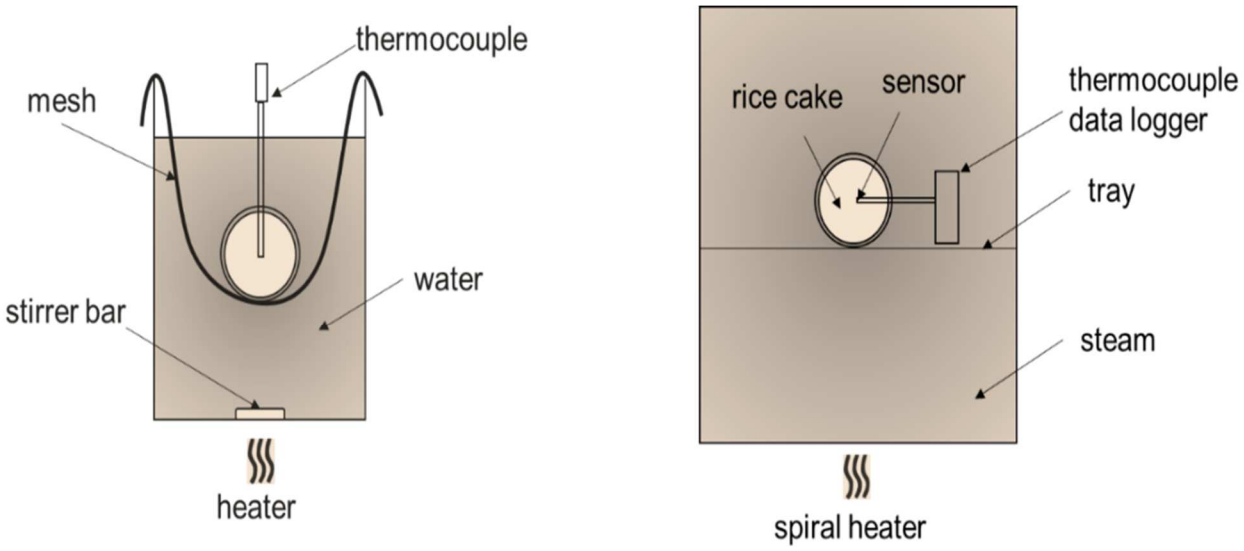

Figure 1

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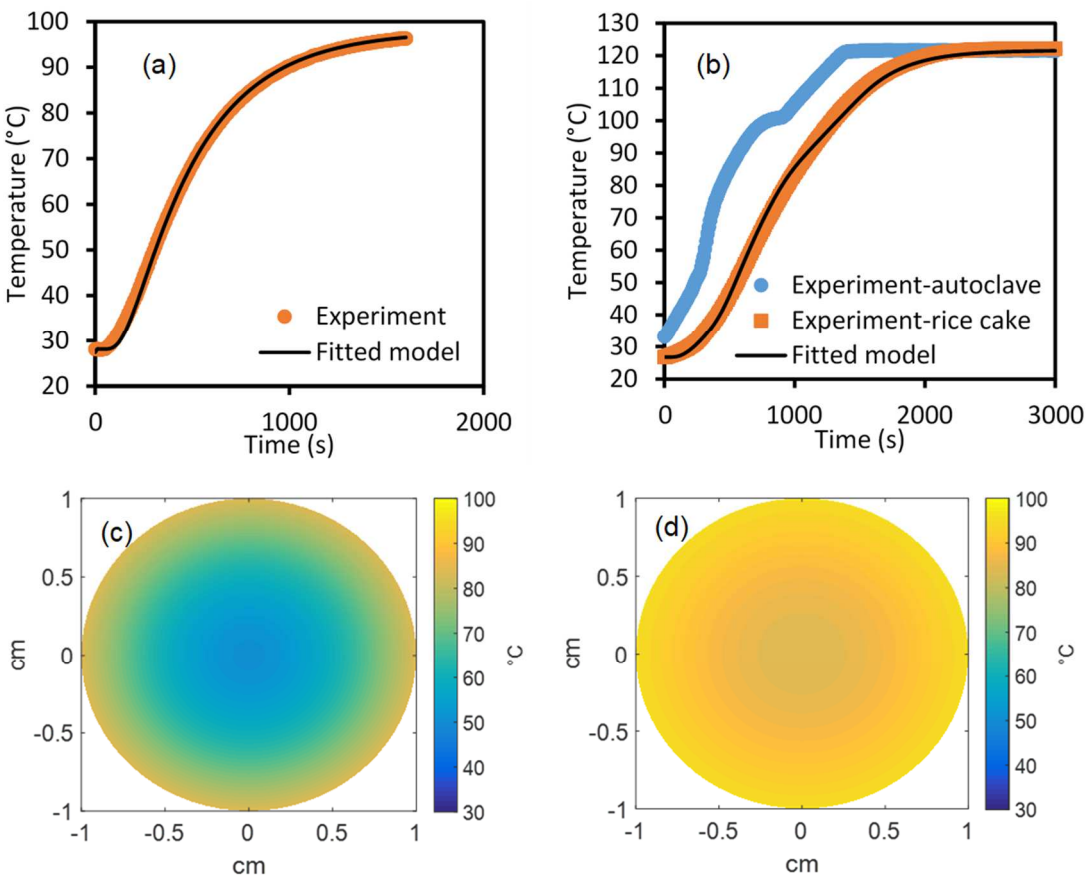

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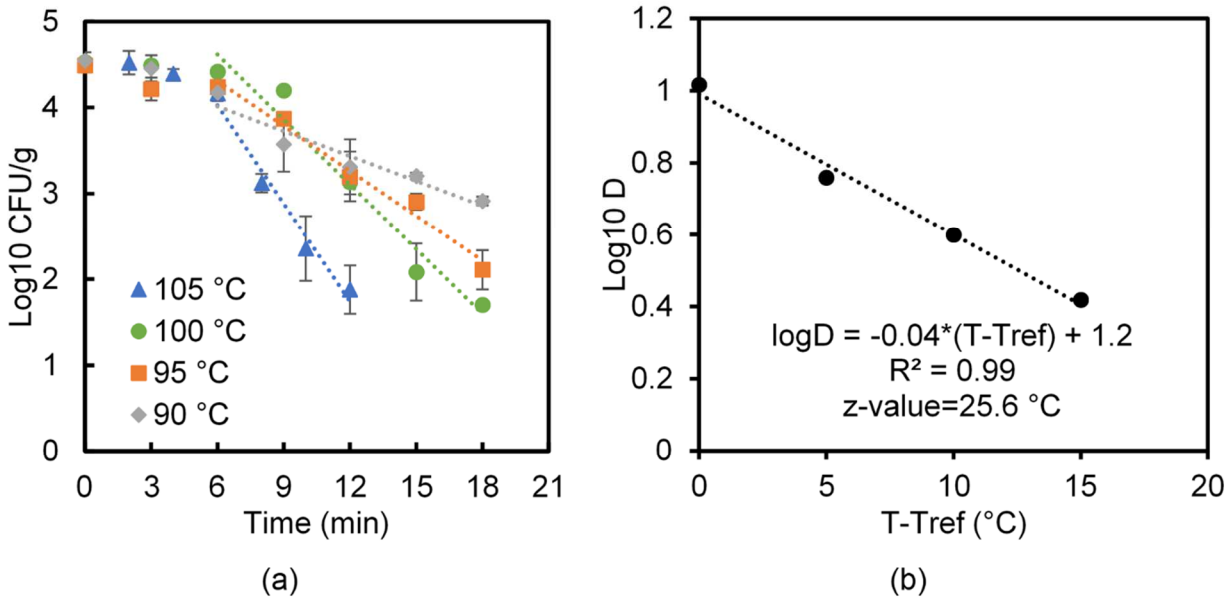

Figure 3

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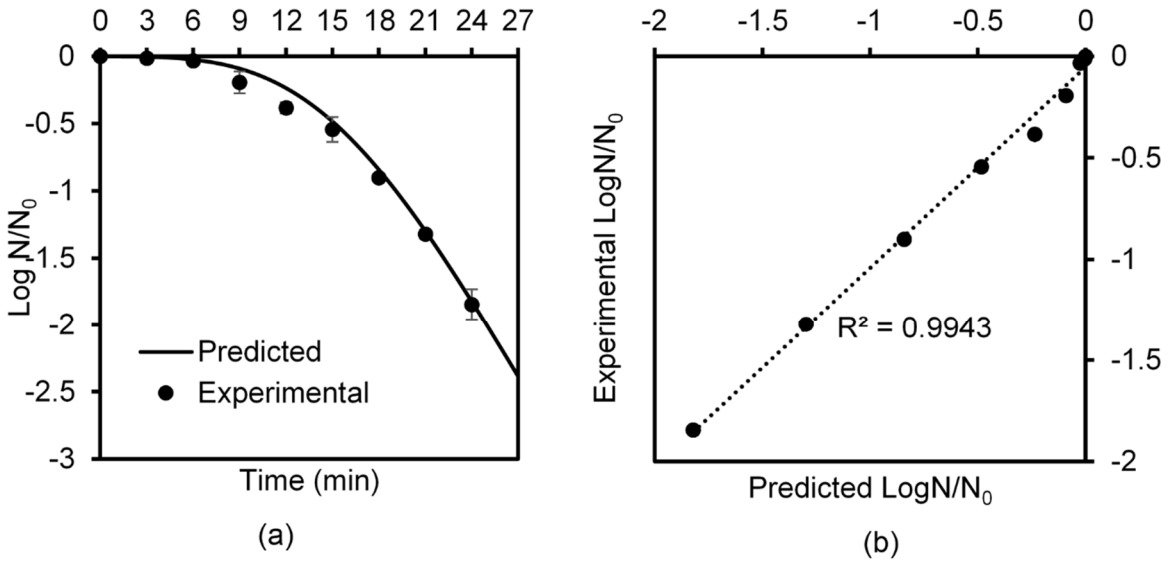
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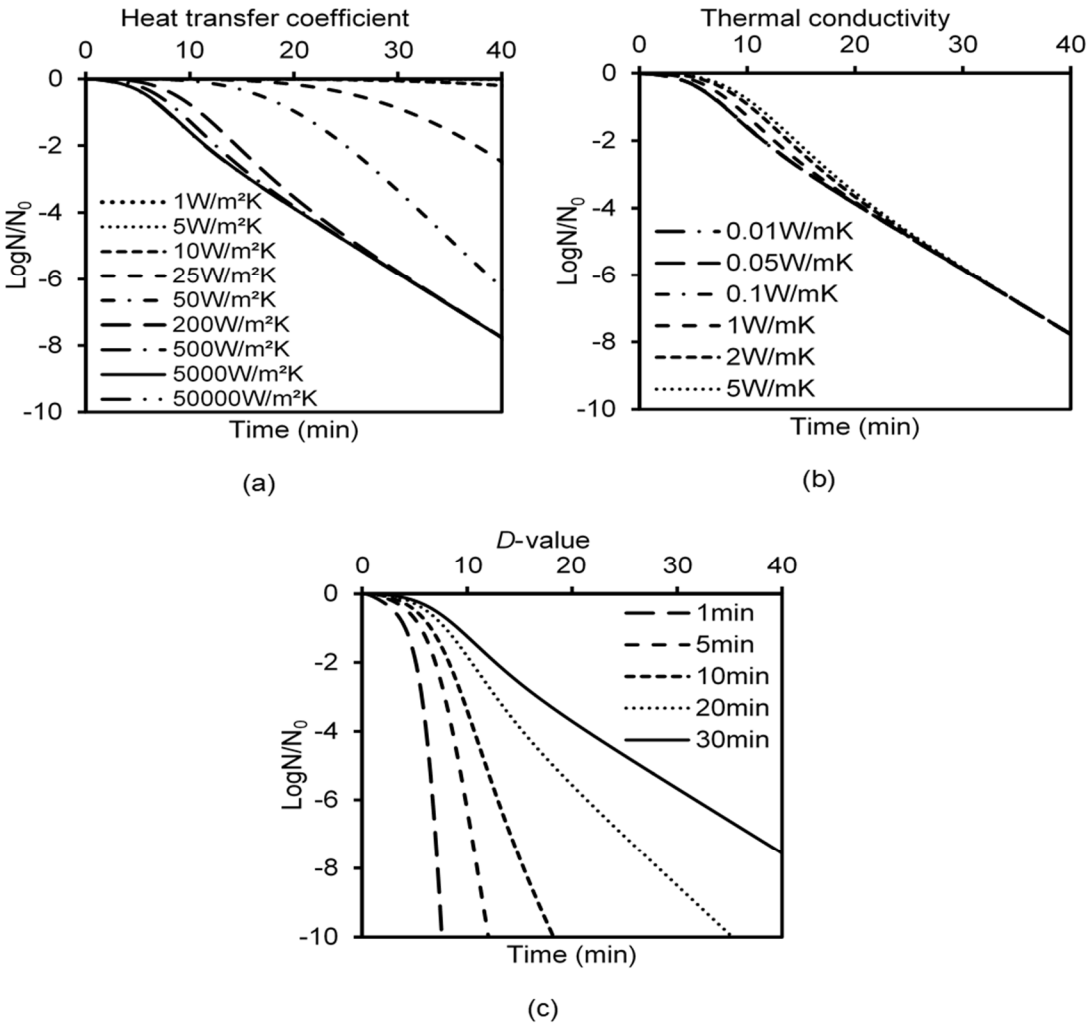
-0.5
-1
-1.5
-2
-2.5
-3
Log N/N0
Predicted
Experimental
Time (min)
(a)
-2 -1.5 -1 -0.5 0
0
-0.5
-1
-1.5
-2
Experimental LogN/N0
R² = 0.9943
Predicted LogN/N0
(b)

Figure 4

587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20
30
40
0
-2
-4
-6
-8
-10
LogN/N0
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5W/m²K
10W/m²K
25W/m²K
50W/m²K
200W/m²K
500W/m²K
5000W/m²K
50000W/m²K
Time (min)
(a)
Thermal conductivity
0.01W/mK
0.05W/mK
0.1W/mK
1W/mK
2W/mK
5W/mK
Time (min)
(b)
D-value
1min
5min
10min
20min
30min
Time (min)
(c)

Figure 5

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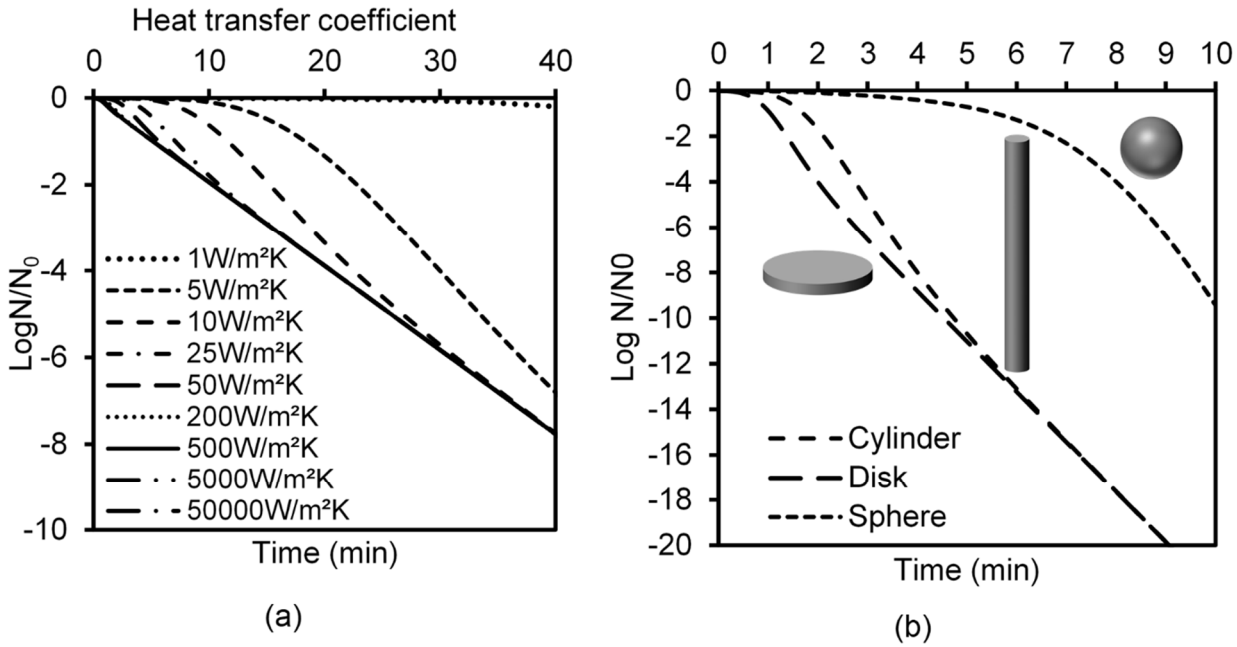

Figure 6

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590

591 **Table 1**

k_{air} (W/m K)	Bulk density (kg/m^3)	Porosity	$k_{cooked\ rice}$ (W/m K)	k_{solid} (W/m K)
0.0292	720	0.378	0.355	0.749
0.0292	800	0.309	0.460	0.803
0.0292	850	0.265	0.543	0.877

592

593

Table 2

Parameters in boiling water		h (W/m^2·K)	Parameters in autoclave		h (W/m^2·K)
Temperature (°C)	80	112.2	Temperature (°C)	105	105.9
	100	128.4		110	125.9
Diameter (cm)	3	114.4		115	141.1
	4	128.4		121	186.8
	5	106.6		125	181.4
Water	Stirred	128.4	Diameter (cm)	3	265.3
	Not stirred	98.3		4	186.8
				5	116.8

597 **Table 3**

Temperature (°C)	*D*-value (min)	R^2 for D-value calculation	*z*-value (°C)
90	10.4±1.85	0.921	25.6±4.97
95	5.70±0.63	0.980	
100	4.00±0.20	0.960	
105	2.60±0.33	0.973	

598