



Original research

Modeling thermal destruction characteristics of *Bacillus cereus* in sorghum (*Sorghum bicolor* L. Moench) starch

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A B S T R A C T

This study evaluated the thermal destruction characteristics of *Bacillus cereus* in sorghum starch as to enhance its microbiological safety. Mature sorghum grains were sourced and processed into starch under hygienic conditions. The extracted starch was dried, inoculated with *Bacillus cereus* and subjected to thermal treatment. Design Expert 13 for Windows was used for experimental lay-out, comprising temperatures (80 - 90 °C) and a_w (0.4 - 0.8) and conducted in triplicate. Thermal destruction characteristics of *Bacillus cereus* in sorghum was obtained by plotting number of survivor (CFU/ml) against time and corresponding thermobacteriological parameters were obtained. Other thermal destruction characteristics parameters: z-value, F-value and enthalpy were obtained using appropriate equations. The thermal destruction characteristics data obtained was fitted into model using Design Expert 13 for Windows. The data obtained showed that the destruction characteristics of *Bacillus cereus* in sorghum starch had quadratic interactive effects between as temperature and a_w were varied. As temperature and a_w increased from 80 - 90 °C and 0.4 - 0.6, respectively. Thermal parameters such as D-value, k-value, F-value, z-value and activation energy (E_a) values ranged from 60 - 108 min; 156 - 205 min; 48.32 - 62.11 °C and 39.457 - 66.765 kJ/mol, respectively. The results indicated that *Bacillus cereus* in sorghum starch showed significant inactivation at an elevated temperature, 90 °C and 0.4 a_w . This study provided valuable thermal destruction characteristics data that could be employed as a guide to potential food processors, scientists and engineers in order to improve consumption safety of the product.

Keywords: *Bacillus cereus*; Thermal destruction; Sorghum; Starch; Modeling.

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1. Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a staple cereal crop mainly cultivated in tropical and subtropical regions in which it plays a crucial role in diets of millions in developing countries particularly Africa and Asia. It is the fifth or sixth most cultivated cereal crop globally, after wheat, rice, maize, barley and soybean recently, due to its adaptability to harsh environmental conditions (Awika, 2017; USDA, 2025). The major producing countries of sorghum are US, Nigeria, Brazil and India with an annual output of 8.7, 7.0, 6.0 and 5.5 MMT, respectively. African countries contributed approximately 46% of sorghum global output (Awika, 2017; USDA, 2025). Its grains are usually small, round, and typically red, yellow, or white,

depending on the variety (Silva et al., 2022). It is a good source of carbohydrates, dietary fiber, and minerals (Fe, P and K). It is also rich in bioactive compounds such as phenolic acids, flavonoids, and tannins, which have been linked to antioxidant and anti-inflammatory properties (Chen et al., 2021; Crozier et al., 2022). It has also been reported that sorghum grains contain low protein digestibility and high resistance starch which offer potential benefits for managing diabetes and obesity (Ducksbury et al., 2022; Taylor and Duodu, 2023). The versatility of sorghum starch for its nutritional purposes has led to its widespread application in the formulation of diverse foods like porridge, flatbread, and fermented beverages. Beyond its roles in food industry, sorghum starch is also used in non-food sectors to manufacture industrial starch, thickening

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agents and biofuels, adhesive, biodegradable packaging materials (Weiss et al., 2022; McGinna, 2024). This gluten-free starch has gained prominence in recent years due to the increasing demand for functional diets (Awika, 2017). The widespread applications of sorghum starch in both food and non-food industries pose a significant role in global food safety particularly with the increasing prevalence of foodborne diseases caused by pathogenic microbial contamination. Recently, numerous outbreaks have been reported involving low-moisture foods traditionally considered microbiologically safe because it was understood that bacterial growth is inhibited under such conditions (Syamaladevi et al., 2016; Tadapaneni et al., 2017; Jiao et al., 2019). These outbreaks affected a wide range of low moisture foods – food powders such as flour, cake mix, and chia powder; different kinds of nuts, including pistachios, almonds, and peanuts; spices; and more complex foods such as peanut butter and German chocolate (Jiao et al., 2019; Perez-Reyes et al., 2025). Among the pathogenic bacteria, *Bacillus cereus*, a gram-positive, spore-forming bacterium, is widely recognized for its ability to cause foodborne illnesses (Satimehin and Philip, 2012; Jiao et al., 2019; Perez-Reyes et al., 2025). Therefore, presence of *B. cereus* in low moisture food such as sorghum starch is a significant concern for food safety. This resilient bacterium can withstand heat treatment, dry conditions and pose health risks if consumed (Pereira et al., 2019; Perez-Reyes et al., 2025). Therefore, this study focused on thermal destruction characteristics of *Bacillus cereus* in sorghum starch at different processing conditions that would help to establish standardized protocols for producing microbiologically safe sorghum starch flour without compromising its functional properties.

2. Materials and Methods

2.1. Source of materials

Dried mature sorghum grains were purchased from Olorunshogo market, Ile-Ife, Osun State, Nigeria. The *Bacillus cereus* strain was obtained from the Food Microbiology laboratory of the Food Science and Technology Department, Obafemi Awolowo University, Ile-Ife. All other chemicals used for this research work were of analytical grades.

2.2. Starch preparation

Sorghum grains (5 kg) were washed and destoned with portable water. The cleaned sorghum grains were steeped in water for 24 h at 28 °C. Thereafter, the steep solution was discarded and the grains were re-washed with potable water. The steeped sorghum grains were wet-milled using a laboratory milling machine (Falling Number 3100, Huddinge, Sweden). The slurry obtained was diluted with 5 L of distilled water and subsequently screened using a muslin cloth. The filtrate was subsequently allowed to settle for 3 h in a stainless-steel decanting bowl, after which the supernatant was then discarded. The starch slurry was air dried. The starch flakes obtained was milled using laboratory milling machine (Retsch GM300, Germany) and sieved (sieve size: 500 µm) to obtain a smooth fine sorghum starch flour. Then, the starch flour obtained was stored in a zip-lock bag at room temperature till the time of usage.

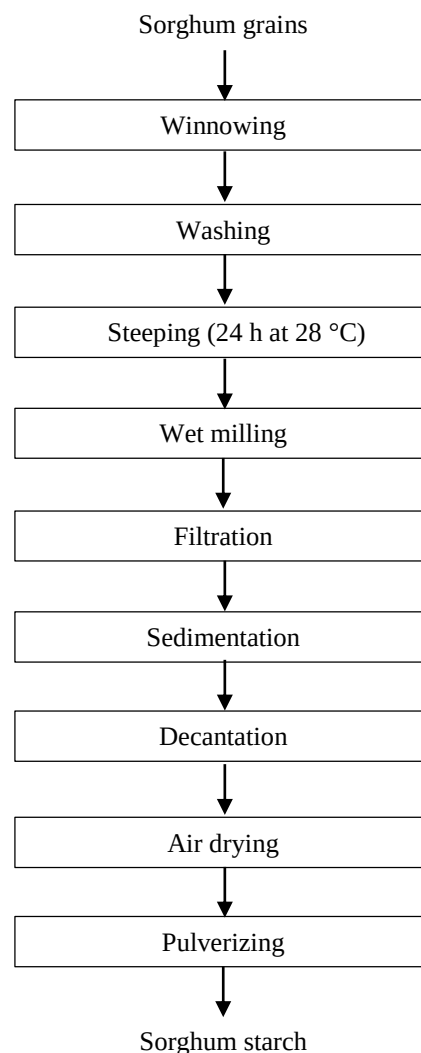


Fig. 1. A flow chart of the sorghum starch production process

2.3. Bacteria strain, inoculation and thermal treatment

A properly stored sorghum starch flour (5 g) was weighed into 25 ml conical flask and sterilized at 121 °C for 15 min. in an autoclave. The sample was then transferred to a water activity (a_w) conditioning system to adjust the sample to a targeted a_w value. Prior to thermal treatment, the sample was conditioned for 6 days at the targeted a_w level to allow the entire sample to equilibrate to the targeted a_w , which was subsequently confirmed via a a_w meter (Aqualab, Model Series 3 TE, Serial 09048826B). The experiment was conducted at various inactivation temperatures (80, 85, and 90 °C) and a_w levels (0.4 – 0.8). For interactive effect of inactivation temperature and a_w on D-value, Design Expert 13 for Window was used for the experimental lay-out (Table 1) and all experiments were conducted in triplicate. The sample in the conical flask was inoculated with 1 ml of 18 h old *B. cereus* ATCC 9818 strain suspension. Slant containing *B. cereus* was scrapped and washed with sterile distilled water. The optical density of the properly mixed

inoculum was adjusted until 0.2 was obtained with a spectrophotometer using wavelength 600 nm. The count of 1 ml was then determined with pour plate method (Adeniran et al., 2020). A digital hot air oven (Memmert GmbH +Co KG, Germany) was set at predetermined inactivation temperature and the conical flasks were placed in the hot air oven and timed. The sample come-up time was then verified using a non-inoculated sample in a conical flask with a k-type thermocouple (Nabertherm GmbH, Germany, Model No: 540300066) located at the center of the flask. The come-up time for the sample core to reach within 0.5 °C of the targeted inactivation temperature was used as time 0 for the thermal inactivation. Once a particular flask has reached its predetermined time, it was removed from the hot water bath. The samples were removed at an interval of 30 min starting with the time 0 samples. The flasks removed was left to cool and 1 g of the sample was introduced into tubes of serial dilution. 1 ml was transferred to dilution test tubes to serially dilute the sample. 1 ml from the dilution tubes was plated in duplicate on nutrient agar plates prepared based on Manufacturer's specification of 28 g to 1000 ml of distilled water and plates was incubated for 24 h at 37 °C. This procedure was repeated for the other test tubes at their individual times. After incubation, the number of colonies present on the plates was counted to ascertain the level of survival of the organism.

Table1. Experimental layout of combined effect of inactivation temperature and water activity of *Bacillus cereus* ATCC 9818 in sorghum starch flour.

Run	Water activity	Temperature, °C
1	0.6	77.9
2	0.8	80
3	0.6	85
4	0.6	85
5	0.6	85
6	0.3171	85
7	0.8828	85
8	0.6	85
9	0.4	90
10	0.8	92.1
11	0.6	90
12	0.6	85
13	0.4	80

2.3.1. Conversion of number of microbial survivor to CFU/g

Number of *Bacillus cereus* survival to colony-forming unit per g (CFU/g) was calculated using Eq. (1):

$$\text{CFU/g} = \frac{N \times df}{g} \quad (1)$$

Where; log CFU/g = No of survivors; N = No. of microbial survival; df = dilution factor; g = mass of sample used. Then, CFU/g was plot against microbial destruction time and D-value was obtained as documented by Adebayo et al. (2025).

2.3.2. Determination of rate constant (k-value)

The k-value was obtained from the slope of graph of natural logarithm of number of survival (CFU/g) versus time (Eq. (2)):

$$\ln \frac{N}{N_0} = -kt = \ln N - \ln N_0 = kt \quad (2)$$

2.3.3. Determination of F-value

F-values was calculated using the Eq. (3):

$$F = D \log \left(\frac{N_0}{N} \right) \quad (3)$$

Where; D = D-value, min; N_0 = Initial population of microorganism, N = Number of final population of microorganism

2.3.4. Determination of z-value

z-value was obtained using Eq. (4):

$$\log \frac{D_1}{D_2} = \frac{T_1 - T_2}{z} \quad (4)$$

Where: D_1 = D-value at initial temperature, min; D_2 = D-value at final temperature; T_1 = Initial temperature, °C; T_2 = Final temperature, °C; z = z-value, °C

2.3.5. Determination of Activation Energy (Ea)

Activation energy (E_a) was calculated using Eqs. (5) and (6):

$$\log \frac{k_1}{k_2} = \frac{-E_a}{2.3R} \left(\frac{T_2 - T_1}{T_1 T_2} \right) \quad (5)$$

$$E_a = \frac{2.3RT_1 T_2}{z} \quad (6)$$

R = 8.3144; z = z-value, °C; E_a = Activation energy, kJ/mol

2.4. Statistical Analysis

Data obtained was analyzed descriptively and inferentially using Tukey's posthoc test (Design-Expert 7.00) for Window.

3. Results and Discussion

3.1. Effect of inactivation temperature and water activity (a_w)

The effect of inactivation temperature and water activity (a_w) on D-value of *B. cereus* ATCC 9818 in sorghum starch was presented in Table 2. At a_w 0.40, D-value of *B. cereus* in sorghum starch decreased from 98 to 69 min. as inactivation temperature increased from 80 to 90 °C, respectively. While at a_w 0.60, D-value decreased from 112 to 76.4 min., and at a_w 0.80, D-value decreased from 124 to 81.2 min. The result showed that as a_w increased (0.40 to 0.80), there is a corresponding increase in D-value of *B. cereus* in sorghum starch flour. The high D-value obtained at 0.80 a_w showed that *B. cereus* demonstrated more thermal resistance at high a_w (0.8). Adebayo et al. (2025) documented similar trend for *B. cereus* in starch wherein D-value of *B. cereus* in *acha* starch increased from 20.4 to 12 min. as a_w increased from 0.65 to 0.55, respectively as the starch samples were treated at temperature 80 to 90 °C. Although, Wei et al. (2020) reported that D-value of *Salmonella* spp. in non-fat

Table 2. Thermal destruction parameters of *Bacillus cereus* ATCC 9818 in sorghum starch flour as influenced by water activity and inactivation temperature.

S/N	a _w	Temperature (°C)	D-value (min)	F-value (min)	z-value (°C)	Activation Energy, E _a (kJ/mol)
1	0.40	80.00	98.00 ± 7.45 ^c	182.00±8.14 ^c	36.70±2.13 ^a	66.765±4.39 ^c
2		85.00	74.00 ± 4.11 ^b	142.00±6.28 ^b		
3		90.00	69.00 ± 3.21 ^a	120.00±5.03 ^a		
4	0.60	80.00	112.00 ± 8.05 ^c	208.00±9.32 ^c	48.30±2.22 ^b	50.730±2.46 ^b
5		85.00	86.00 ± 5.71 ^b	167.00±7.11 ^b		
6		90.00	76.40 ± 3.52 ^a	156.00±6.72 ^a		
7	0.80	80.00	124.00 ± 7.23 ^c	223.00±9.45 ^c	62.10±3.61 ^c	39.457±2.19 ^a
8		85.00	92.00 ± 6.11 ^b	196.00±9.22 ^b		
9		90.00	81.00 ± 4.71 ^a	164.00±6.82 ^a		

dry milk powder increased from 9.82 to 10.46 min as a_w decreased from 0.30 to 0.2, respectively. This implied that *Salmonella* spp. demonstrated increasing thermal resistance as a_w decreased (0.3 to 0.2) in non-fat dry milk powder when was treated at temperature 80 °C. The differences in D-value trends observed may be associated with high-protein matrix food, types of microorganism or a combination of these factors (Santillana-Farakos et al., 2014; Jin et al., 2018). The D-value obtained compared favorably with the D-value of 112.87 to 32.36 min. reported for *Salmonella* in wheat flour at 80 to 90 °C of a_w 0.11 to 0.33, respectively (Dodier, 2015).

The D-value obtained was higher than 14.53 to 0.49 min reported for *Salmonella* in wheat flour as a_w increased from 0.3 to 0.7 and inactivation temperature increased from 75 to 80 °C, respectively (Smith et al. 2016). These differences may be associated with particle size, food composition and structure, high surface area, moisture content variability, microbial load and strain variability and type of microorganism (Hildebrandt et al., 2016). Impact of inactivation temperature on D-value of *B. cereus* on sorghum starch sample was also presented in Table 2. At inactivation temperature, 80 °C, D-value increased from 98 to 124 min. as a_w increased from 0.4 to 0.8. While at inactivation temperature, 85 °C, D-value increased from 74 to 92 min; and at inactivation temperature 90 °C, D-value increased from 69 to 81 min. The highest D-value 124 min was observed at inactivation temperature, 80 °C and a_w 0.8. Also, the least D-value (69 min) occurred at inactivation temperature 90 °C

and a_w 0.4. The result showed that at elevated inactivation temperature; there is corresponding decrease in D-value. Similar trends were documented for D-value of *B. cereus* in rice and wheat flour wherein D-value ranged from 2.5 to 0.22 min. as temperature increased from 100 to 140 °C and from 40.19 to 30.84 min as temperature increase from 90 to 100 °C, respectively (Jin et al., 2018). These differences may be associated with particle size, food composition and structure, high surface area, moisture content variability, microbial load and strain variability and thermal conductivity (Hildebrandt et al., 2016). D-values are very important in thermal destruction because they ensure the effectiveness of sterilization by providing the basis for calculating other thermobacteriological parameters, like the F-value, which ensures the desired level of microbial reduction. Thus, it is a key parameter in ensuring the safety and quality of food products by effectively managing the thermal destruction of pathogens.

Interactive effect of inactivation temperature and a_w on the D-value of *Bacillus cereus* in sorghum starch was presented in Fig. 2. In order to select a model that best fits the experimental results, ANOVA was performed on the transformed data. A quadratic model was selected after a natural logarithm transformation was applied to D-value response to ensure normality. The model for the D-value was highly significant ($p < 0.0002$) with a coefficient of determination (R^2) of 0.8768. Inactivation temperature ($p < 0.0001$) was highly significant independent factor for D-value model while

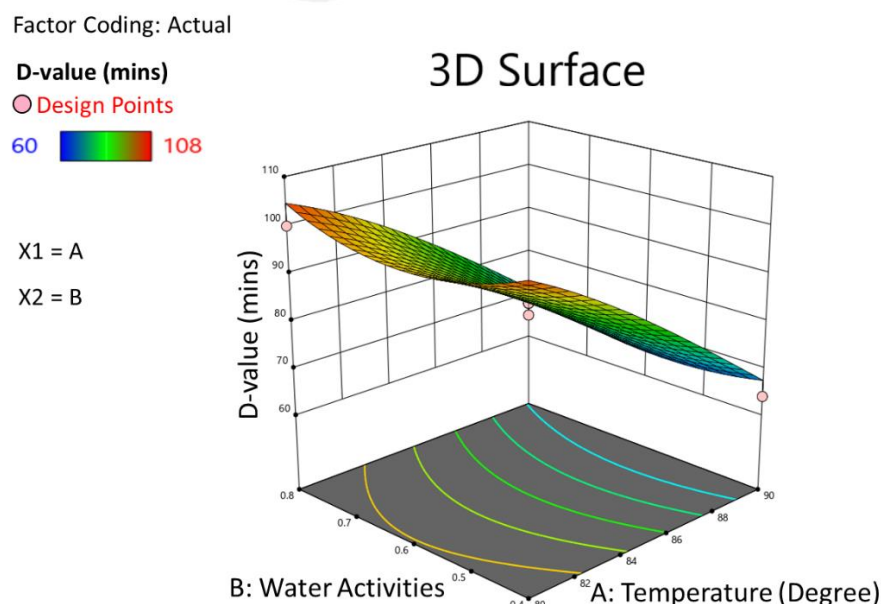
Fig. 2. Interactive effect of inactivation temperature and water activity on the D-value of *Bacillus cereus* ATCC 9818 in sorghum starch flour.

Table 3. Regression analysis of inactivation temperature and a_w on D-value of *Bacillus cereus* ATCC 9818 in sorghum starch flour.

Source	Sum of square	Df	Mean square	F-value	p-value	Decision
Model	0.3774	5	0.00755	27.78	0.0002	significant
A-Temperature	0.3315	1	0.3315	121.56	<0.0001	
B-Water activity	0.0019	1	0.0019	0.7049	0.4289	
AB	0.0008	1	0.0008	0.2776	0.6146	
A ²	0.0142	1	0.0142	5.23	0.0561	
B ²	0.0235	1	0.0235	8.63	0.0218	
Residual	0.0191	7	0.0027			
Lack of fit	0.0191	3	0.0064			
Pure error	0.0000	4	0.0000			
Corrected Total	0.3965	12				

Table 4. Processing conditions for desirability level (D-value) of D-value of *Bacillus cereus* ATCC 9818 in sorghum starch flour.

Number	Inactivation temperature (°C)	Water activity	D-value (min)	Desirability	Decision
1	90.00	0.503	65.549	0.879	significant
2	90.00	0.502	65.563	0.879	
3	90.00	0.505	65.537	0.879	
4	90.00	0.507	65.523	0.879	
5	90.00	0.493	65.656	0.879	
6	90.00	0.518	65.440	0.879	

a_w ($p < 0.4289$) was not. The numerical model describing the effect of temperature and a_w on the natural log of the D-value was presented in Eq. (7):

$$\ln(D) = -4.00978 + 0.256977T - 1.38249a_w + 0.013755Ta_w \quad (7)$$

Where; T = Temperature, °C and a_w = water activity.

The model F-value of 27.78 for the D-value, imply that the model was significant and there was only a 0.02% chance that this F-value could occur due to noise. The p-value less than 0.05 indicates model terms were significant (Table 3). The adequacy and goodness of fit for the model were evaluated using R^2 and adjusted R^2 . The high adjusted R^2 values indicate that temperature is a critical parameter in determining the microbial destruction kinetics. The R^2 values of 0.8768 observed for the D-value models, along with low p-values 0.0002 for D-value, depict the statistical significance of the models. The coefficient of variation (C.V. %) values of 1.35% observed for the D-value model indicates a high level of precision and reliability in the experimental data. The model's robustness is further confirmed by the adequate precision values of 17.34, which indicate an adequate signal-to-noise ratio. This implies that the model can be used to navigate the design space.

Predicted optimal values for achieving maximum microbial destruction (minimum D-value) suggest using a higher temperature (90 °C) and (a_w 0.5) (Table 4). The summary statistics for the fitted model are presented in Table 5. The model demonstrated a strong fit, with an R^2 value of 0.8768 for D-value of thermal destruction of *Bacillus cereus* in sorghum starch. The lack of fit test results for various models were shown in Table 6. This test did not produce an F-value and p-value. This could be attributed to the model's complexity, insufficient degrees of freedom, or lack of sufficient

replicate measurements in the experimental design. These factors may have led to the inability to perform the test.

Table 5. Model summary statistics (D-value) of *Bacillus cereus* ATCC 9818 in sorghum starch flour.

Model summary	D-value (min)
Standard deviation	0.0819
Mean	-6.05
C.V%	1.35
R ²	0.8768
Adjusted R ²	0.8522
Predicted R ²	0.7357
Adequate precision	17.3419

Table 6. Analysis of variance of lack of fit test (D-value) of D-value of *Bacillus cereus* ATCC 9818 in sorghum starch flour.

Source	Sum of square	Df	Mean square
Linear	0.0631	6	0.0105
2FI	0.0624	5	0.0125
Quadratic	0.0191	3	0.0064
Cubic	0.0184	1	0.0184
Pure error	0.0000	4	0.0000

The effect of inactivation temperature and a_w on F-value of *Bacillus cereus* in sorghum starch is presented in Table 2. F-value ranged from 223 to 120 min. as inactivation temperature raised from 80 to 90 °C, respectively. While, at a_w 0.4, F-value ranged from 182 to 120 min; at a_w 0.6, F-value ranged from 208 to 156 min. and at a_w 0.8, F-value ranged from 223 to 164 min. as inactivation temperature raised from 80 to 90 °C. Hence, it could be deduced that as a_w increased, F-value of *Bacillus cereus* in sorghum starch also increased correspondingly. Table 2 also showed that at inactivation temperature 80 °C, F-value ranged from 223 to 182 min; at

inactivation temperature 85 °C, it ranged from 196 to 142 min; and at inactivation temperature 90 °C, it ranged from 164 to 120 min. The highest F-value (223 min) was observed at 80 °C and a_w 0.8. While, the lowest F-value (120 min) was observed at 90 °C and a_w 0.4. Hence, it could be deduced that as inactivation temperature increased, F-value of *B. cereus* in sorghum starch reduced for all a_w investigated. The F-value is crucial in assessing the thermal destruction of *B. cereus* in food safety. It measures the time required at a specific temperature to achieve a desired level of microbial reduction. It is specifically used to ensure that the thermal process effectively kills *B. cereus* and meets food standards and with its applications. In addition, application of the F-values help optimize heat treatments reinforces consumer confidence in the safety and reliability of the products they purchase.

The z-value of *B. cereus* in sorghum starch ranged from 36.7 to 62.1 °C as a_w increased from 0.4 to 0.8 when inactivation temperature ranged between 80 to 90 °C (Table 2). The results showed that as a_w increased, there is a corresponding increase in z-value of *Bacillus cereus* in sorghum starch. This showed that increase in a_w led to increase in the heat resistivity of *Bacillus cereus* in sorghum. The high z- value obtained could be due to starch gelatinization, viscosity storage modulus and the heated cells exhibiting varying degrees of lag periods at different temperatures. In food processing, z-value is an important parameter in thermal process because it measures heat resistance of bacteria. Specifically, z-value represents the temperature increase needed to reduce the time required for a given level of microbial destruction by one logarithmic cycle. Thus, z-value is a key parameter in ensuring the safety and quality of food products by effectively managing the thermal destruction of pathogenic bacteria.

Activation energy (E) of *B. cereus* in sorghum starch flour ranged from 39.457 to 66.765 kJ/mol as a_w reduced from 0.4 to 0.8. (Table 2). This data suggests that higher energy is required for *B. cereus* to undergo a significant reduction in sorghum starch as inactivation temperature raised from 80 – 90 °C. However, the result obtained was lower than 493 kJ/mol and 494 kJ/mol reported for the thermal resistance of *B. cereus* in egg whites and glucose, respectively (Coroller et al., 2001; Soni et al., 2018). These differences may be due to food matrixes like carbohydrate and protein which may protect the bacteria from heat, high moisture content, heat distribution and bacterial load. The information of activation energy can help food processors or manufacturers to select the suitable temperature and time combination to ensure sufficient microbial reduction, thereby reducing the risk of foodborne illness while preserving product quality.

4. Conclusion

This study focused on modeling thermal destruction characteristics of *B. cereus* ATCC 9818 in sorghum starch flour with the aim of providing the starch flour thermal inactivation characteristics on its safety. The key findings from this study would provide valuable thermo-bacteriological data that could be employed by potential sorghum starch processors to enhance its safety and broaden its acceptability among the potential users.

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Conflict of interest

The authors declare that there is no conflict of interest.

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