



ORIGINAL RESEARCH ARTICLE

MODELING THERMAL DEATH TIME (D – VALUE) OF *BACILLUS CEREUS* IN ACHA (*Digitaria exilis*) STARCH FLOUR

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ABSTRACT

This study investigated thermal death time (D – value) of *Bacillus cereus* in acha starch flour, with the aim of providing thermo-bacteriological data that would enhance the safety of acha starch flour. The data would therefore serve as a guide to potential food processors, engineers and scientists thereby promoting the starch usage in food development and formulation beyond its present status. Acha starch was prepared, stored under hygienic conditions and sterilized in an autoclave prior to its thermal treatments. The sample water activity (a_w) was then adjusted, and the value confirmed via the a_w meter. Design Expert 13 for window was used for the experimental lay-out, comprising three inactivation temperatures and a_w values with all experiments conducted in triplicate. The *Bacillus cereus* thermal destruction characteristics were obtained by plotting number of survivor (CFU/g) against time and corresponding D-value was determined. The D-values obtained were analyzed descriptively and inferentially using Turkey's posthoc test (Design-Expert 7.00) for Window and fitted into a linear equation representing the dependent and independent variables. The D-value ranged from 20.4 to 12 min. as water activity and destruction temperature changed from 0.55 and 92.1 °C to 0.65 and 80 °C, respectively. The interactive effects of water activity and destruction temperature on natural logarithm of D-value of *Bacillus cereus* in acha starch flour was linear. The maximum D-value was observed at 80 °C temperature when the water activity was 0.55. This study, therefore, provides valuable thermo-bacteriological data that could be employed as a guide to potential food processors, scientists and engineers in order to improve consumption safety of the product.

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1.0 Introduction

Starch is an important food product and raw ingredient for both the food and non–food industries. It is used in the food industry to thicken, preserve, and improve the quality of baked dishes, confectioneries, pastas, soups and sauces, and mayonnaises (Egbarevba, 2019). In addition to its utilization in the food and beverage industries, cereal starch has several applications in health and medical, agriculture, textiles, pulp and paper, chemicals and pharmaceuticals, biofuel production, mining, and construction (Przetacsek–Rożnowska, 2017). Among cereals, starch is mainly processed from corn which accounts for about 80% of world output while other main sources are rice, barley and millet (Egbarevba, 2019). In 2015, 8.5 mt of starch was produced globally with approximately 11, 36, and 53% used as modified starch, native starch and sweetener, respectively (Egbarevba, 2019). It also served as a primary source of energy in the human diet and so has significant economic value (Laity et al., 2010; FAO, 2019). Therefore, the ever–increasing demands for starch in modern food and non–food industries have resulted into a dire need to source for starch from other readily available non–conventional sources. Acha (*Digitaria exilis*) is an underutilized and non–conventional grain cultivated mainly in West African sub-region. It belongs to the family of *graminaea* with its unique small kernel size of 0.4 – 0.5 mm making its processing tedious and laborious (Adoukonou–Sagbadja et al., 2007). It is also called hungry millet, hungry rice, fundi grain, white acha and acha millet. It thrives well on the marginal soils of the semi-arid regions where most cereals could not survive. Major acha producing countries in West Africa include

Cape Verde, Chad, Nigeria, and even stretches south to the boundaries of tropical rain forest zones (Glew et al., 2013). It serves as a staple grain to populations living in arid savannah which in terms of protein, crude fat, carbs, and vital minerals, compares favorably to rice, sorghum, maize, and millet (Cruz, 2004; Agu et al. 2020). Also, abundance of sulphur-based amino acids like methionine and cysteine in *acha* grains surpasses popular legumes in content (Enyiukwu and Bassey, 2020). It is combined with other flours to make bread and is utilized to make couscous, *acha* jollof, and porridges (Satimehin and Philip, 2012). *Acha* starch has a rich nutritional profile, offering a diverse range of essential nutrients, making it a valuable ingredient in local and international diets. Recently, *Bacillus cereus* has been frequently isolated from low moisture food products such as dried milk powder, powdered infant formulas, dried vegetables, herbs, spices and cereals despite their production involved heating or drying processes (Satimehin and Philip, 2012). Furthermore, several works have documented that heat resistance of microbial populations tends to increase as water activity (a_w) decreases (D'Aoust, 1997; Daryaei et al., 2018). The presence of pathogenic microorganisms such as *Bacillus cereus* in low moisture foods is a significant concern for food safety. These resilient microorganisms can withstand heat treatment, dry conditions and may pose health risks if consumed (Daryaei et al., 2018). This study then focused on modeling the D-value of *Bacillus cereus* at different destruction temperatures and a_w levels in *acha* starch flour with the view of providing thermobacteriological information as a guide to potential food processors, scientists and engineers in order to improve consumption safety of the product.

2. Materials and Methods

2.1 Source of Materials

Acha (*Digitaria exilis*) grains devoid of defects was purchased from central market in Ilorin, Kwara State, Nigeria while all chemicals needed for this work were of analytical grades.

2.2 Sample Preparation

Acha starch was prepared according to Adebayo et al. (2024). *Acha* grains (5 kg) was washed and destoned with portable water. The cleaned *acha* grains was steeped in water for 24 h at 28 °C. Thereafter, the steep solution was discarded and the grains was re-washed with potable water. The steeped *acha* grains was 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 discarded. The starch slurry was then air dried. The starch flakes obtained were milled in a laboratory milling machine (Retsch GM300, Germany) and sieved (sieve size: 500 µm) to obtain the starch flour.

2.3 Bacteria Strain, Inoculation and Thermal Treatment

A properly stored *acha* starch flour (5 g) was weighed into 25 ml conical flask and was sterilize in an autoclave. The sample was transferred to water activity (a_w) conditioning system to adjust the sample to a targeted a_w value. Prior to thermal treatment, sample was conditioned for 4 to 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 the a_w meter (Aqualab, Model Series 3 TE, Serial #09048826B). Design Expert 13 for window was used for the experimental lay-out, comprising three inactivation temperatures (80, 85, and 90 °C) and three a_w values and all experiments were conducted in triplicate (Table I). The sample was inoculated with 1 ml of *Bacillus* strain suspension. The hot water bath (Neslab GP-400, Newington, NH) was set at already predetermined temperatures (80, 85, and 90 °C). Then, the flask was placed in the hot water bath and timed. Come-up time was verified using a non-inoculated sample in a conical flask with a K-type thermocouple located at the center of the flask. The come-up time for the sample core to reach within 0.5 °C of the targeted 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 10 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 were then incubated for 24 h at 37 °C. The same procedure was applied to the other test tubes at their individual times. After incubation, the number of colonies present on the plates were then counted to ascertain the level of survival of the organism.

Table I: Interactive effect of water activity and temperature on D-value of *Bacillus cereus* in *acha* starch flour

	Block I	Temperature, °C	Water activity	D-value, min
1	Block I	90	0.45	
2	Block I	85	0.55	
3	Block I	80	0.45	
4	Block I	85	0.55	
5	Block I	85	0.55	
6	Block I	90	0.65	
7	Block I	80	0.65	
8	Block I	85	0.69	
9	Block I	85	0.40	
10	Block I	85	0.55	
11	Block I	77.93	0.55	
12	Block I	85	0.55	
13	Block I	92.07	0.55	

2.4 Conversion of Number of microbial Survivor to CFU/g

The number of colonies counted was converted to colony-forming unit per g (CFU/g) using equation 1:

$$\text{CFU/g} = \frac{N \times df}{m} \quad |$$

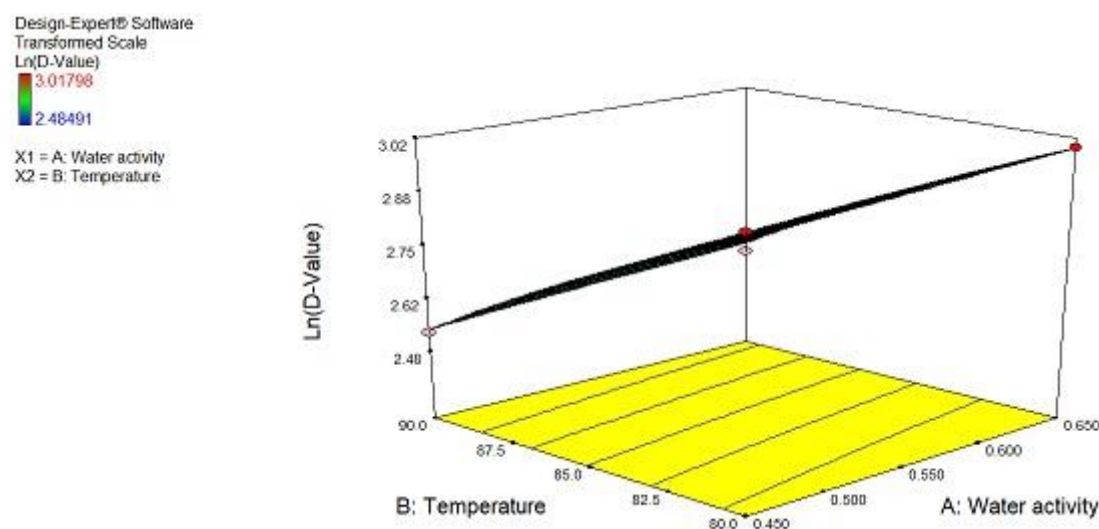
Where; log CFU/g = No of survivors; N = No. of microbial survivors; Df = dilution factor; M = mass of sample. Then, CFU/g was plot against microbial destruction time and D-value was obtained as documented by Toledo (2007).

2.5. Statistical Analysis

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

3. Results and Discussion

Interactive effect of water activity and destruction temperature on D-value of *Bacillus cereus* in *acha* starch flour is presented in Figure 1.

**Figure 1:** Effects of water activity and temperature on D-value of *Bacillus cereus* in *acha* starch flour

It was observed that the D-value ranged from 20.4 to 12 min. as water activity and destruction temperature changed from 0.65 and 80 °C to 0.55 and 92.1 °C, respectively. It showed that lower D-values were obtained at lower water activities and higher destruction temperatures and vice versa. The D-value obtained was higher than 5 to 3.4 min reported for ginger but lower than 92.2 - 17.2 min and 148.5 - 23.3 min for cocoa nibs and cocoa beans, respectively (Pereira et al., 2019; Yehia, 2022). 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 ((Daryaei et al., 2018). Knowledge of the D-values is very important in the application of food processing as it allows producers to determine the necessary time and temperature combination to ensure safety and preservation of food products. Additionally, the results revealed that as water activity decreased and destruction temperature increased, there is corresponding decrease in D-value of *Bacillus cereus* in *acha* starch flour.

The interactive effects of water activity and destruction temperature on natural logarithm of D-value of *Bacillus cereus* in *acha* starch flour was linear. The maximum D-value was observed at 80 °C temperature when the water activity was 0.55. However, in order to select a model that best fits the experimental results, F-value and p-value were used for evaluation. Both quadratic versus 2FI and linear versus mean models were selected for data fitting with F-value and p-value of 370 and <0.001; and 4.83 and 0.0483, respectively. The numerical model describing the effect of water activity and destruction temperature (°C) on D-value of *Bacillus cereus* in *acha* starch flour was shown in equation 2:

$$\ln D = 2.79 + 0.0264a_w - 0.201T - 0.000869T * a_w - 0.00214a_w^2 - 0.0193T^2 \quad 2$$

Where: a_w is water activity, T is temperature, °C and D is D-value, min.

The experimental data of the dependent variable was fitted into models to illustrate the relationship between the dependent variable and independent variables. Multiple regression analysis carried out on the experimental data and the generated models were shown in Table 2. The result showed that D-value was significantly influenced ($p < 0.05$) by both water activity and destruction temperature (Table 2).

Table 2: Regression analysis of water activity, temperature and D-value of *Bacillus cereus* in *acha*

starch flour		
Model Terms	Model F-value and probability levels	
	F value	P value
Models	248	< 0.0001
A: Water activity	20.8	0.00260
B: Temperature	0.00121	< 0.0001
AB	0.0113	0.918
A ²	0.118	0.741
B ²	9.63	0.0172
Lack of fit	370	<0.0001
R ²	0.994	-
Adjusted R ²	0.990	-
Predicted	0.960	-
SD	0.0164	-
Mean	2.77	-
CV (%)	0.591	-

Analysis of variance (ANOVA) was used to access the extent to which the selected quadratic vs 2FI and linear vs mean models adequately represented the data obtained for the D-value of *Bacillus cereus* in *acha* starch flour (Liyama-Pathirana and Shahidi, 2005). The result of ANOVA for the respective D-values (responses) with their corresponding regression coefficients of determinations (R²) for the D-value of *Bacillus cereus* in *acha* starch flour were generated by the software (Table 2). The quadratic vs 2FI model F-value of 248 implies the model is significant and there is only a 0.01% chance that the model F-value this large could occur due to noise. Therefore, p-values <0.0001 indicate model terms are significant. In this case A, B, AB, A² and B² are significant model terms. The adequacy and goodness-of-fit were evaluated using the regression coefficient (R²), predicted R² and adjusted R² (Table 2). R² is the percent of variance in the dependent variable (D-value) that is explained collectively by all the independent variables (water activity and temperature). Inability of the model to give an (Predicted R² is not too close to the Adjusted R² as expected, the difference < 0.03) explanation of the variance in the response suggest that it might be necessary to give consideration to other factors that are relevant to the D-value of *Bacillus cereus* in *acha* starch flour besides the two independent variables (water activity and temperature) used for the experiment. These factors may include: variation in agronomic characteristics within and among cultivar, pH of the starch sample, particle size, thermal conductivity, food composition, moisture content variability, microbial load and strain variability (Daryaei et al., 2018).

The high values of adjusted R^2 (0.994) recorded in this study (Table 3) indicated that water activity and destruction temperature are all significant parameters for the determination of D-value of *Bacillus cereus* in *acha* starch flour. The nonsignificant lack of fit ($p < 0.0001$) further enhanced the reliability of the model.

Table 3: Fit Statistics

	<i>Acha</i> starch flour
Standard deviation	0.0164
Mean	2.77
C.V %	0.591
R^2	0.994
Adjusted R^2	0.990
Predicted R^2	0.960
Adequate precision	0.0134

The R^2 value of 0.994 and low p-value (< 0.0001) of the model depicts the statistical significance of the quadratic vs 2FI model. The results also showed that there were excellent correlations among the independent variables. Thus, the fitted model could sufficiently portray the independent variables (Adebayo *et al.*, 2021).

Coefficient of variation (CV) is a measure expressing standard deviation as a percentage of the mean (Chen *et al.*, 2003). The CV illustrates the ratio of the standard deviation of the estimate to the mean values of the observed dependent variables. It also represents the degree of reproducibility and repeatability of the models. It described the extent to which the data were dispersed (Table 3). The value of 0.591% recorded in this study signify that the models cannot be considered reproducible ($CV < 10\%$) (Chen *et al.*, 2003).

Table 4: Analysis of variance for lack of fit test

Source	Sum of square	df	Mean square	F-value	p-value
Mean vs Total	100	1	100		
Linear vs Mean	00259	2	0.00129	4.82	0.0483
2FI vs Linear	3.02E-006	1	3.02E-006	0.00609	0.939
Quadratic vs 2FI	0.330	5	0.165	370	< 0.0001
Cubic vs Quadratic	0.00177	5	0.000883	38.9	0.000895
Pure Error	0.000113	4	2.27E-005		

Table 5: Processing condition for desirability level

Number	Water activity	Temperature ($^{\circ}\text{C}$)	Desirability
1	0.530	86.9	1.00
2	0.525	81.50	1.00
3	0.510	89.10	1.00
4	0.630	80.90	1.00
5	0.579	81.4	1.00
6	0.649	85.4	1.00
7	0.609	83.5	1.00
8	0.490	81.0	1.00
9	0.578	81.0	1.00
10	0.559	89.8	1.00
11	0.490	81.0	1.00
12	0.63	85.2	1.00
13	0.569	86.0	1.00
14	0.610	83.7	1.00
15	0.482	87.4	1.00
16	0.501	81.5	1.00
17	0.463	82.8	1.00
18	0.636	80.6	1.00
19	0.471	81.1	1.00
20	0.623	83.4	1.00
21	0.452	82.3	1.00
22	0.612	88.9	1.00
23	0.469	80.8	1.00
24	0.645	81.3	1.00
25	0.533	80.6	1.00
26	0.645	83.6	1.00
27	0.513	80.3	1.00
28	0.630	85.3	1.00
29	0.570	89.7	1.00
30	0.482	89.6	1.00

Selected

ANOVA of the regression parameters of the predicted response surface models for D-value of *Bacillus cereus* in *acha* starch flour indicated the mean vs total, 2FI vs linear and cubic vs quadratic interaction did not produce a significant effect in each case ($p < 0.01$) (Table 4). Thus, none of the two effects of independent variables was primarily responsible for determining the term that may cause significant effects in the response. The relation between independent and dependent variables was viewed through a three-dimensional representation of the responses surface conformed by the models.

Predicted minimum value for D-value of *Bacillus cereus* in *acha* starch flour suggested the desirability of using water activity (0.482) and destruction temperature (89.6 °C) to achieve the minimum D-value of *Bacillus cereus* in *acha* starch flour (Table 5).

4. Conclusion

This study investigated modeling thermal death time (D-value) of *Bacillus cereus* in *acha* starch flour (a low – moisture food) with the aim of providing the product's thermal destruction characteristics on its safety. The key findings from this study showed minimum D-value was obtained at lower water activity and high destruction temperature while interactive effect of water activity and destruction temperature on D-value is linear. This information will provide valuable thermo-bacteriological data that could be employed to enhance the safety of *acha* starch flour utilization in food processing industries.

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