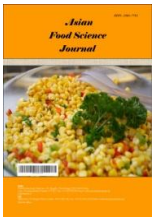

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Influence of Hurdle Applications on the Storage Stability of *Dambu-nama*

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Authors' contributions

This research work was carried out in collaboration among all authors. Author MOE designed the study, performed the statistical analysis. Author CCA wrote the protocol, wrote the first draft of the Manuscript and managed the analyses of the study. Author JOA managed the literature searches. All Authors read and approved the final manuscript.

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ABSTRACT

The microbiological, physico-chemical, sensory and storage characteristics of *dambu-nama* (DN) as influenced by addition of citric acid, salt and sugar as hurdles were investigated. Preliminary sensory evaluation suggested that at citric acid level above 0.3%, sugar and salt levels above 2% each, the products were either too harsh or sweet for acceptance. Four products comprising DN with 0.1% citric acid + 2% salt and 2% sugar ($DNC_{0.1}$), DN+ 0.2% citric + 2% salt + 2% sugar ($DNC_{0.2}$), DN + 0.3% citric acid + 2% salt + 2% sugar ($DNC_{0.3}$) and a control DN without citric acid , salt or sugar (DNC_0) were produced and subjected to microbiological, sensory and storage analyses. Treatment of *dambu-nama* with the hurdles resulted in significant ($p< 0.05$) decrease in total plate and mould counts. Thiobarbituric acid, tyrosine and total plate counts varied very little with no definite trend, there by ruling out their use as possible indices of quality. The pH of *dambu-nama* exhibited a slow but definite decline with storage. The decrease in pH was best described by first order reaction kinetics ($r^2 \geq 0.987$) and adequately by the Arrhenius activated energy complex theory ($r^2 \geq 0.991$) with activation energies (kJ/mol) of 32.6 (DNC_0), 27.6 ($DNC_{0.1}$), 23.1 ($DNC_{0.2}$) and 54.5 ($DNC_{0.3}$), respectively. Treatment with the hurdles increased shelf-life of *dambu-nama* by a factor of 2.3 to 8.4 with shelf-life (wks) of 7.7 to 4.3 (DNC_0), 17.8 to 10.3 ($DNC_{0.1}$), 33.3 to 21.0

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($DNC_{0.2}$) and 64.9 to 27.9 ($DNC_{0.3}$), respectively for storage at 20 to 35^{o}C. The objective of this study thus was to investigate quality changes in *dambu-nama* during normal and accelerated storage as influenced by the hurdles with the purpose of shelf-lives predictions.

Keywords: Influence; hurdles; physico-chemical; storage stability and dambu-nama.

1. INTRODUCTION

Dambu-nama is a Nigerian traditionally dried meat product. It is commonly obtained from beef, goat meat, mutton or camel meat and is popularly consumed in the Northern parts of Nigeria. Proximate analysis by [1] indicated that the product contains 46.51% protein, 15.65% fat and 5.76% ash. The common ingredients used for the production of *dambu-nama* are spices and seasoning such as garlic, ginger, onions, chillies, salt and neutralized hydrolysed vegetable proteins. According to [2] and [3], *dambu-nama* has good nutritive values but poor keeping quality at room temperature. It can serve as a snack or combined with other foods as part of daily diet for the consumers. It also provides a convenient dry pack for travellers and campers because of its light weight, easy- to pack, tender and ready- to- eat qualities [1].

Hurdle technology is the application of combinations of microbial and enzyme inhibitory agents (such as organic acids, salt and sugar) in food preservation. The technology is based on synergistic effects of the hurdles which are employed at very low concentrations. This trend emerged as a result of consumer demand for minimally processed and yet acceptable and safe food products. The major deteriorative agents are microorganisms which render the meat unacceptable and unfit for human consumption [4]. Chemical preservatives are used to create hurdles to these agents thereby assuring safety, stability and enhanced sensory and nutritional qualities of food products [5]. According to [6], the most important hurdles used in food preservation are temperature (high or low), water activity (a_w), acidity (pH), redox potential (Eh), preservatives (e.g. sugar, salt, citric acid nitrites, sorbate, sulphite) and competitive microorganisms (e.g. Lactic acid bacteria). In developing countries the application of hurdle technology is important for stability, safety and acceptability of meat products that do not require refrigeration. This is because of poor handling and processing methods which tend to lower safety and storage stability of traditional meat based products.

According to [7] and [8], citric acid is used in meat marinates as a chelator of pro-oxidants and to improve the water holding capacity and tenderness of beef muscle. [9] reported that the use of hurdles such as organic acids and salt solutions promoted shelf-life extension and inhibitthe growth of microorganisms. [10] also reported that the use of sodium lactate and lactic acid enhanced the chemical, microbiological and sensory characteristics of marinated chicken. The application of hurdles such as citric acid, salt, sugar and spices enhanced the storage stability, sensory, nutritional and microbiological stability of *dambu-nama* [11]. Therefore, there is the need for the application of hurdle technology to the processing and production of *dambu-nama*. Information about the influence of hurdles on the physico-chemical, microbiological, sensory and storage characteristics of *dambu-nama* are lacking.

2. MATERIALS AND METHODS

A 16 kg of pre-rigor hind quarters beef were purchased from the abattoir of Makurdi Modern Market within 2h of slaughter of cow. The sample was placed in plastic buckets with ice blocks and transported promptly to the laboratory where they were trimmed of fats, washed with chilled deionised water and packed in black polyethylene bags. The packs were kept in a household deep freezer (Model: HR 98; manufacturers: Thermacool) and used for *dambu-nama* production within 24hours. Food grade common salt (iodized NaCL), sugar, citric acid (BDH), vegetable oil, Spices (powdered mixture of dehydrated onions, ginger, garlic and chillies) and neutralized hydrolysed vegetable protein ("maggi cubes", Food Specialities Limited, Nigeria) were purchased from a supermarket in Makurdi. The spices and ground maggi cubes were mixed in a ratio 1:1 (w/w) to obtain the spices used for this study.

A 4×4×4×4 randomized experimental design comprising 4 levels each of citric acid (0.2, 0.4, 0.6, 0.8 %), salt (1, 2, 3, 4%), sugar (1, 2, 3, 4%) and spices (2, 3, 4, 6%) was employed to obtain a possible 256 combinations. Because of this large sample size, sensory evaluation based on

evolutionary operations (EVOP) using table of random numbers was employed for eliminations. The preliminary sensory result (data not included) suggested that citric acid was the most critical hurdle affecting acceptability of the *dambu-nama*. At concentrations of 0.4% and above (citric acid), > 2% for salt and sugar, the products were either too harsh or sweet for acceptance. With sugar and salt at 2% level each, a 4 × 4 design (0.1, 0.2, 0.3, 0.4% citrate; 1, 2, 3, 4, 5% spices) that gave 16 possible combinations was then used for further eliminations to obtain the three (3) most preferred product samples.

$DNC_{0.1}$ = *Dambu-nama* + 0.1% citric acid + 2% salt + 2% sugar + 4% spices
$DNC_{0.2}$ = *Dambu-nama* + 0.2% citric acid + 2% salt + 2% sugar + 4% spices
$DNC_{0.3}$ = *Dambu-nama* + 0.3% citric acid + 2% salt + 2% sugar + 4% spices

These three products, together with a control sample (DNC_0) containing only 4% spices (as in traditional products) which served as a control, were used for physico-chemical, microbiological and storage tests.

Essentially for each product, 4kg of beef were manually cut into thin slices (approximately 2 × 2 × 2mm, length × width × thickness) using sharp stainless steel knives. The meat slices were mixed with appropriate ratios of citric acid, salt, sugar and spices followed by simmering in stainless steel pressure cookers for about 10mins. The cooked meats together with resultant stock were pounded using previously washed mortar and pestle into a mash. After separation of the meat strands, they were dried in an air draft electric oven (Model: TT-9053, Techmel and Techmel USA) at 60°C for 30min. This enhanced products handling and moisture reduction. The strands were then deep fried in vegetable oil over an electric heater for 5min. After frying, the strands were drained in a plastic basket, dried in the oven at 60°C for 3h followed by cooling, packaging and storage in plastic containers with tight lids.

The total plate counts, *Enterobactericeae*, yeast and mould counts and storage tests were at ambient (30±2°C) for 6 months and accelerated temperatures (40, 50, 60 and 70°C) for 4 weeks respectively. The total plate count, pH, tyrosine value, thiobarbituric acid value (TBA) and sensory attributes were evaluated at the different storage conditions.

2.1 Chemical Quality Indices

2.1.1 Ph

The pH was determined by the use of pH meter as recommended by AOAC [12]. A 2.0 g of the sample was homogenised in 20 ml of de-ionized water in a beaker. The pH meter (Lab tech digital 152 R) was standardized using buffer solution of pH 4.01 and 9.20. The electrode was rinsed with de-ionized water and dipped into the homogenate allowing sufficient time for stabilization before taking the reading.

2.1.2 Thiobarbituric acid (TBA)

The method described by Kirk and Sawyer [13] was used. A 0.2g of sample was weighed into 25 ml volumetric flask and dissolved with small quantity of 1-butanol and made up volume and mixed. Then 5ml each was pipetted into dry stopper test tube and 5ml of TBA reagent added followed by filtration and heating in a water bath at 95°C for 120 min. After cooling, the absorbance (As) was read at 530nm in 10mm cell against a blank.

$$\text{TBA value} = 50 \times \frac{A_S A_b}{M} \quad (1)$$

where,

A_s = Absorbance reading
A_b = Blank reading
M = Sample weight

2.1.3 Tyrosine value

The tyrosine value was determined according to the method described for amino acid (Kure *et al.*, 2009).

$$C = \frac{Dillution \times 16}{Sample\ Wt \times N\% \times 10 \times Vol\ Loaded \times NH \times w\ (nleu)}$$

Where,

NH= Net height
w = width at half-height
Nleu = Norleucine

2.2 Microbiology

Duplicate sample (each 10g) were weighed aseptically from each *dambu-nama* product and

masticated in a sterile warring blender with 90ml chilled sterile peptone water. Serial dilutions were prepared as described by [14]. Total plate counts were evaluated on pour plates containing dextrose tryptone agar (DTA) with brom-cresol purple indicator. All plates were incubated for 48hrs at 30 ± 1^{o}C prior to enumeration.

Presumptive *Enterbacteriacae* and *Staphylococcus* counts were determined on violet red bile glucose agar (VRBGA) and mannitol salt agar (MSA) respectively. Yeast and mould counts were made on oxytetracycline + chloramphenicol potato dextrose agar(OCPDA). Yeast counts were made on OCPDA containing 0.25% sodium proprionate.Mould counts were obtained by difference [14].

2.3 Sensory Evaluation

Sensory evaluation of the products was by a consistent panel of twenty members (students and staff of Adamawa State University, Mubi) who are regular consumers of *dambu- nama*. Sensory testing was in an illuminated room. Samples were coded using random three digit set of random numbers and presented to the panellists in transparent plastic plates. Potable water was provided for rinsing of mouth between taste testings. The panel evaluated each product for appearance, flavour, mouth feel and general acceptability on a 7 point Hedonic scale (1= dislike extremely and 7= like extremely). Data obtained were subjected to multiple range (f-test) tests for significant ($p< 0.05$) differences. Means were separated using Tukey's LSD test as described by [15].

2.4 Statistical Analysis

Significant differences ($p < 0.05$) in chemical, microbiological and sensory attribute data were detected by analysis of variance. Tukey's least significant difference test was used for separating the means as described by [14].

Correlation and regression analyses were performed by the method of Gupta (1987). Regression analyses were carried out at varying storage times under ambient and accelerated conditions respectively as the independent variable and with sensory, microbiological or chemical parameters as the dependent variables.

2.5 Kinetic Calculations

Simplifying assumptions were made for calculation of kinetic parameters of changes in the quality indices during ambient and accelerated storage. Irreversible non cyclic, unimolecular reaction mechanism (A--B) was assumed for all physico-chemical and plate count changes.

Reaction order for change in the quality indices of *dambu-nama* at constant temperature was calculated (Villota and Hawkes,1992) from Eq.(2):

$$^{\mathrm{dC}}/_{\mathrm{dt}} = \pm KC^{0} \quad (2)$$

where C = values of quality index at time t (wk), K= reaction rate constant (wt^{-1}), n = reaction order.

Reaction rate constants were calculated using Eq. (3)

$$C\text{-}C_o = kt \quad (3)$$

for zero order reactions, and Eq. (4):

$$\ln\left(^{C_0}/_{\mathrm{C}}\right) = Kt \quad (4)$$

for first order reactions. C_o = initial value of quality index.

Temperature dependency of the rate constants was expressed by Arrhenius equation:

$$k = k_o \exp.\left(^{-E_a}/_{\mathrm{RT}}\right) \quad (5)$$

where k_o = frequency or collision factor (independent of temperature), E_a = activation energy (kJ/mol), R= universal gas constant (0.008314 kJ/mol ^{o}K), T = absolute temperature (^{o}K).

3. RESULTS AND DISCUSSION

3.1 Ambient Storage Changes; Chemical Indices of Quality

Variation in pH, TBA and tyrosine value during ambient storage 30 ± 1^{o}C of the *dambu-nama* as influenced by treatment with a combination of citric acid, salt and sugar are presented in Table 1. The pH showed a gradual but consistent decrease within 6 months of storage ranging from 5.92 to 5.60 (DNC_0), 5.55 to 5.00 ($DNC_{0.1}$), 5.50 to 4.84 ($DNC_{0.2}$) and 5.20 to 4.40 ($DNC_{0.3}$). TBA values fluctuated during storage but anchored with high value at end of six months. Thioburtituric acid (TBA) values (mg maloaldehyle/kg sample) range from 0.019 to 0.051 (DNC_0), 0.016 to 0.045 ($DNC_{0.1}$), 0.018 to

0.022 ($DNC_{0.2}$) and 0.017 to 0.045 ($DNC_{0.3}$) within six months of ambient storage. Tyrosine values did not vary ($p > 0.05$) during storage. Mean tyrosine values (mg tyrosine/ 100g were 5.50 ± 0.06 (DNC_0), 5.49 ± 0.05 ($DNC_{0.1}$), 5.03 ± 0.17 ($DNC_{0.2}$) and 4.92 ± 0.13 ($DNC_{0.3}$), respectively. The influence of hurdles on the microbial load of the *dambu-nama* during ambient storage is as presented in Table 1. It can be observed that total plate counts did not vary significantly ($p > 0.05$) during ambient storage. The values (cfu/g) ranged from 150 to 1.2×10^3 (DNC_0), < 30 to 280 ($DNC_{0.1}$), < 30 to 250 ($DNC_{0.2}$) and < 30 to 250 ($DNC_{0.3}$). The total plate counts did not show a definite trend within the first 4 months of ambient storage.

Changes in physico-chemical indices of quality of the *dambu-nama* products during ambient storage were carried out with the purpose of selecting indices suitable for shelf-life prediction studies. Of all the physico-chemical parameters examined, pH proved to be the most reliable index for monitoring quality of *dambu-nama* products during storage. TBA, tyrosine value and also total plate counts did not show definite trends during storage of the products. The pH showed a consistent and gradual decrease during storage with a high positive correlation with organoleptic changes. The pH dropped from 1.8 to 2.8%, 3.0 to 5.4%, 5.0 to 5.8% and 5.2 to 6.0% after 24 weeks storage of *dambu-nama* at 30, 40, 50, 60 and 70°C respectively. The pH drop was not a consequence of microbial activity since significant microbial growths were not detected. The variations in pH can be attributed to protein-protein reactions during storage leading to release of free H^+, TBA value (mg malonaldehyde/kg sample) has been used to evaluate the degree of rancidity of lipids in foods. The tyrosine value has been used to assess the degree of autolytic and bacterial proteolysis in meat products by Pearson (1981). In addition to tyrosine, the reaction measures tryptophan, phenolic groups, sulphydryl compounds, hydrogen sulphide etc. (Pearson, 1981). Low tyrosine values recorded in the products studied support the results of low microbial activities in the *dambu-nama* during storage.

3.2 Organoleptic Changes

The mean sensory scores on of *dambu-nama* samples during ambient (30± 1°C) storage are as provided in Table 2. All the products had excellent to good sensory attributes within 24 weeks of storage. However, the products treated with the hurdles (citric acid, salt and sugar) had higher sensory scores. The appearance and mouth feel of the products were stable within the first 12 weeks before gradual decline in quality. The flavor attribute was stable in all products in the first 8 weeks before the unset of gradual decline. The overall acceptability exhibited a gradual but steady decline thereby making it a possible organoleptic index for shelf-life predication purposes.

3.3 Accelerated Storage Changes

Changes in pH and overall acceptability of *dambu-nama* samples during accelerated testing at elevated temperatures (40 – 70°C) are shown in Tables 3 and 4, respectively. The pH and overall acceptability decreased with storage time, at faster rates than normal ambient storage with the changes being more for *dambu-nama* samples without hurdles. From the results, it can be observed that, as expected, the rates of degradation increased with temperature. For DNC_0, the pH and overall acceptability values varied from 5.92 to 4.85 and 6.6 to 4.4 (40°C), 5.92 to 4.18 and 6.6 to 4.0 (50°C), 5.92 to 3.96 and 6.6 to 3.8 (60°C), 5.92 to 3.44 and 6.6 to 3.6 (70°C) within 12 days. For $DNC_{0.1}$, the values ranged from 5.55 to 4.13 and 6.8 to 5.2 (40°C), 5.55 to 4.02 and 6.8 to 5.0 (50°C), 5.55 to 3.85 and 6.8 to 4.8 (60°C), 5.55 to 3.40 and 6.8 to 4.0 (70°C). That of $DNC_{0.2}$ were 5.55 to 4.50 and 6.9 to 5.4 (40°C), 5.55 to 4.10 and 6.9 to 5.0 (50°C), 5.55 to 3.88 and 6.9 to 4.8 (60°C), 5.55 to 3.88 and 6.9 to 4.2 (70°C) while that of $DNC_{0.3}$ varied from 5.52 to 3.83 and 6.8 to 5.4 (40°C), 5.52 to 3.68 and 6.8 to 4.6 (50°C), 5.52 to 3.50 and 6.8 to 4.2 (60°C), 5.52 to 3.24 and 6.8 to 3.8 (70°C) within 12 days of accelerated testing.

3.4 Kinetics of Ph and Overall Acceptability Changes

The data obtained for changes in pH (Table 3) and overall acceptability (Table 4) during accelerated storage of the *dambu-nama* products were fitted respectively with zero (Equation 3) and first (Equation 4) order reaction kinetics models. Based on linearity of the plots (Fig. 1 and 2 respectively) and the coefficients of regression ($r^2 \geq 0{,}978$), variations in pH were best described by first order kinetics while overall acceptability scores were adequately described by zero order kinetics. The regression parameters generated via linear regressional analysis are provided respectively in Tables 5 and 6 for pH and overall acceptability changes.

Table 1. Physico-chemical and microbiological changes during ambient storage of *dambu-nama* treated with some hurdles

Parameter	Sample	Ambient (30±1°C) Storage Time (months)			
		0	2	4	6
pH	DNC_0	5.92	5.90	5.80	5.60
	$DNC_{0.1}$	5.55	5.40	5.20	5.00
	$DNC_{0.2}$	5.50	5.35	4.90	4.84
	$DNC_{0.3}$	5.20	5.00	4.84	4.40
TBA (mg maloaldehyde/kg)	DNC_0	0.019	0.028	0.049	0.051
	$DNC_{0.1}$	0.016	0.024	0.018	0.045
	$DNC_{0.2}$	0.018	0.026	0.023	0.022
	$DNC_{0.3}$	0.017	0.024	0.046	0.045
Tyrosine Value (mg/100g)	DNC_0	5.41	5.50	5.54	5.54
	$DNC_{0.1}$	5.42	5.50	5.50	5.53
	$DNC_{0.2}$	4.80	5.05	5.05	5.20
	D $NC_{0.3}$	4.76	5.02	5.03	4.88
Total Plate Count (cfu/g)	DNC_0	150	1.2×10^2	1.7×10^2	1.2×10^3
	$DNC_{0.1}$	<30	160	150	280
	$DNC_{0.2}$	<30	<30	200	250
	$DNC_{0.3}$	<30	<30	250	200

DNC_0 = Dambu-nama + 4% spices (control), $DNC_{0.1}$ =Dambu-nama + citric acid (0.1%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.2}$ = Dambu-nama + citric acid (0.2%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.3}$ = Dambu-nama + citric acid (0.3%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), TBA = thiobarbituric acid, cfu/g = colony forming unit per gram.

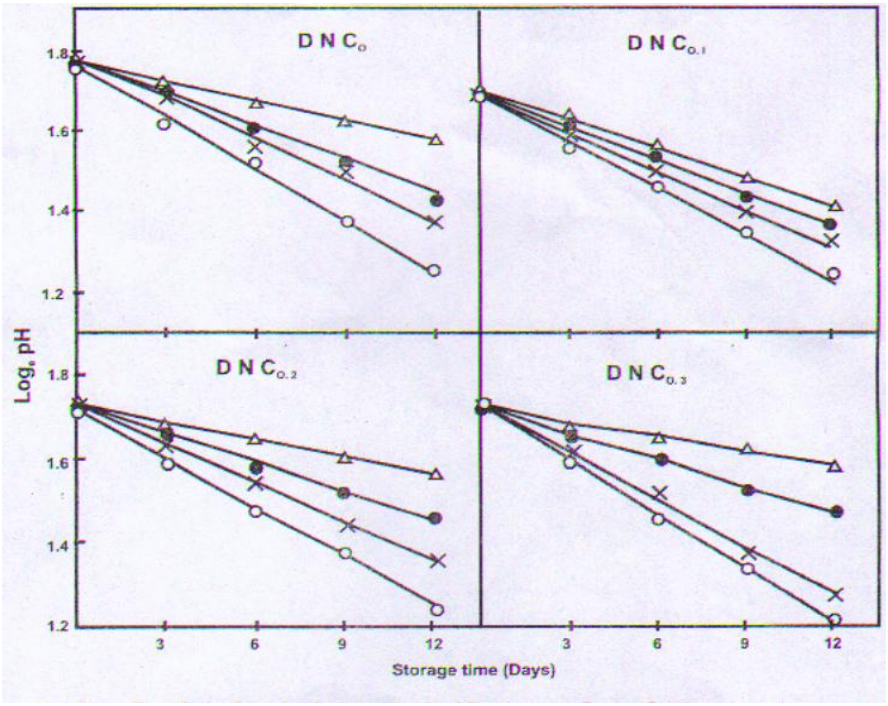

Fig. 1. First Order Plots for Changes in pH of *Dambu-nama* During Storage at 40(△), 50(●), 60(×) and 70(○) °C respectively.
DNC_0 = Dambu-nama, $DNC_{0.1}$ = Dambu-nama + 0.1% citric acid + 2% salt + 2% sugar, $DNC_{0.2}$ = Dambu-nama + 0.2% citric acid + 2% salt + 2% sugar; $DNC_{0.3}$ = Dambu-nama + 0.3% citric acid + 2% salt + 2% sugar score;

Table 2. Mean Sensory Scores of *Dambu-nama* during ambient storage (30±1°C)

Attributes	Samples	Ambient storage time (Weeks)						
		0	4	8	12	16	20	24
Appearance	DNC_0	6.2	6.0	6.0	5.8	5.6	5.4	5.0
	$DNC_{0.1}$	6.8	6.6	6.4	6.4	6.2	6.0	5.8
	$DNC_{0.2}$	6.8	6.8	6.8	6.6	6.4	6.2	6.0
	$DNC_{0.3}$	6.8	6.8	6.8	6.6	6.6	6.4	6.2
Flavour	DNC_0	6.2	6.0	6.0	5.4	5.2	5.0	4.8
	$DNC_{0.1}$	6.8	6.8	6.8	5.6	5.4	5.2	5.0
	$DNC_{0.2}$	7.0	6.8	6.8	6.6	5.8	5.6	5.0
	$DNC_{0.3}$	6.6	6.6	6.6	6.4	6.2	6.0	5.6
Mouthfeel	DNC_0	6.6	6.6	6.4	6.2	6.4	6.2	6.4
	$DNC_{0.1}$	6.8	6.8	6.8	6.6	6.6	6.4	6.4
	$DNC_{0.2}$	6.8	6.8	6.8	6.8	6.4	6.4	6.4
	$DNC_{0.3}$	6.6	6.6	6.6	6.2	6.4	6.4	6.4
Overall acceptability	DNC_0	6.0	6.5	6.3	6.0	5.8	5.4	5.2
	$DNC_{0.1}$	6.8	6.6	6.4	6.2	6.0	5.6	5.4
	$DNC_{0.2}$	6.9	6.9	6.7	6.5	6.4	6.2	6.0
	$DNC_{0.3}$	6.8	6.6	6.4	6.2	6.0	5.8	5.6

DNC_0 = Dambu-nama + 4% spices (control), $DNC_{0.1}$ =Dambu-nama + citric acid (0.1%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.2}$ = Dambu-nama + citric acid (0.2%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.3}$ = Dambu-nama + citric acid (0.3%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%)

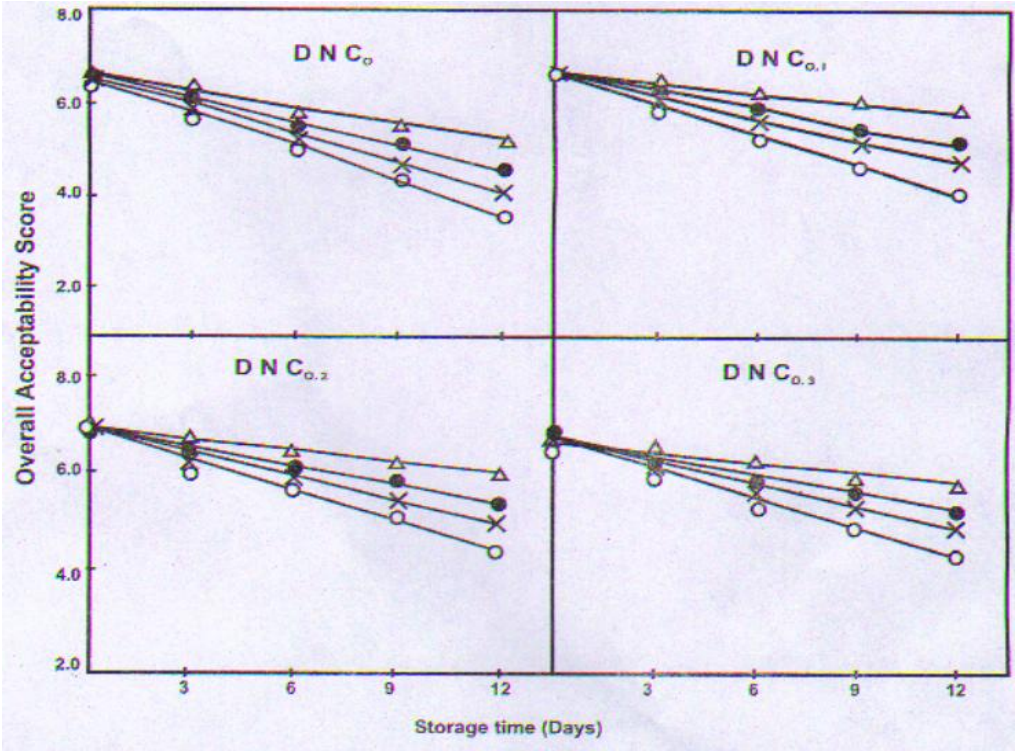

Fig. 2. **Zero Order Plots for Overall Acceptability of *Dambu-nama* during storage at 40(△), 50(●), 60(×) and 70(○) °C respectively.**
DNC_0 = Dambu-nama, $DNC_{0.1}$ = Dambu-nama + 0.1% citric acid + 2% salt + 2% sugar, $DNC_{0.2}$ = Dambu-nama + 0.2% citric acid + 2% salt + 2% sugar; $DNC_{0.3}$ = Dambu-nama+ 0.3% citric acid + 2% salt + 2% sugar score ; 7 = like extremely, 4 = neither like nor dislike, 1 = dislike extremely.

Table 3. Influence of hurdles on ph changes in *dambu-nama* during accelerated storage elevated temperatures

Sample	Storage time (days)	pH Temperature (°C)			
		40	50	60	70
DNC_0	0	5.92	5.92	5.92	5.92
	3	5.60	5.47	5.37	5.13
	6	5.30	5.00	4.76	4.62
	9	5.10	4.62	4.54	3.65
	12	4.85	4.18	3.96	3.44
$DNC_{0.1}$	0	5.55	5.55	5.55	5.55
	3	5.23	5.02	4.95	4.85
	6	4.76	4.81	4.56	4.41
	9	4.44	4.65	4.13	3.97
	12	4.13	4.02	3.85	3.40
$DNC_{0.2}$	0	5.55	5.55	5.55	5.55
	3	5.37	5.26	5.16	4.85
	6	5.14	4.88	4.71	4.21
	9	4.86	4.55	4.22	3.97
	12	4.50	4.10	3.88	3.76
$DNC_{0.3}$	0	5.52	5.52	5.52	5.52
	3	5.42	5.26	5.05	4.96
	6	5.23	4.95	4.52	4.31
	9	5.13	4.60	4.05	3.82
	12	4.86	4.39	3.60	3.39

DNC_0 = Dambu-nama + 4% spices (control), $DNC_{0.1}$ =Dambu-nama + citric acid (0.1%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.2}$ = Dambu-nama + citric acid (0.2%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.3}$ = Dambu-nama + citric acid (0.3%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%).

The reaction rate constants generated for pH (K_{pH}) via first order kinetics and reaction rate constants for overall acceptability (K_A) derived from zero order kinetics were related to absolute temperature using Arrhenius kinetic model (Eqn.5). The Arrhenius kinetics plots are shown in Fig. 3 and 4 respectively for variations in pH and overall acceptability of the *dambu-nama* products. The plots were linear and crossed each other indicating strong interactions of the effect of the hurdles applied. For pH changes, the plots cross at about 53.8°C. The Arrhenius regression parameters are given in Table 3 and 4 for pH and overall acceptability respectively. The regression coefficients ranged from 0.973 to 0.999 for pH changes and 0.962 to 0.990 for overall acceptability variations for *dambu-nama*. The activation energies varied from 23.1 kJ/mol ($DNC_{0.2}$) to 54.5 kJ/mol ($DNC_{0.3}$).

3.5 Shelf-life Prediction

The shelf-lives of the *dambu-nama* products were estimated using the assumption of an average out going quality level (AOGQ) of 5 (good quality) on a 7- point scale with 7 as excellent quality. The data obtained for changes in pH (Table 1) and overall acceptability (Table 2) during ambient storage of the various *dambu-nama* products were related using linear regression analysis. The relationships developed are provided in equations 6 to 7 as follows:

$$pH=4.0642+0.2988A \quad (6)$$

for DNC_0 (r^2 =0.948) with A = overall acceptability

$$pH=2.8304+0.3995A \quad (7)$$

for $DNC_{0.1}$ (r^2 = 0.994),

$$pH=0.0605+0.7826A \quad (8)$$

for $DNC_{0.2}$ (r^2 =0.936) and

$$pH=0.892+0.64A \quad (9)$$

for $DNC_{0.3}$ (r^2 = 0.971)

From Equations.6 to 7, the critical pH values (pH_c) equivalent to overall acceptability score of 5 (good) developed earlier (Table 2) were evaluated. The pH_c values were 5.56 (DNC_0), 4.83 ($DNC_{0.1}$), 3.97 ($DNC_{0.2}$) and 4.09 ($DNC_{0.3}$) respectively.

The first order reaction kinetics for pH changes from Equation 4 at a specified temperature gives:

$$\ln pH = \ln pH_0 - K_{pH}\, t \quad (10)$$

Where pH = pH at time t(wk), K_{pH}= reaction rate constant (wk^{-1}) and pH_0 = initial pH of the *dambu – nama.*

Based on the assumption of AOGQ level of 5 (good) on a 7-point scale, good manufacturing practices and also effective packaging in opaque and moisture proof materials, equation 8 was transformed to give:

$$\ln pH_c = \ln pH_0 - k_{pH}\, t_s \quad (11)$$

where t_s = shelf life (wk), pH_c = critical pH of the product at expiration, pH_0= initial pH of *dambu-nama*, K_{pH}= reaction rate constant at specified temperature (^{0}C).

From the Arrhenius plots (Fig. 3), K_{pH} values where predicted at various ambient temperature ranging from 20 – 30^{0}C using equation 10 as follows:

$$\ln K_{pH} = \ln K_{0pH} - {}^{E_a}/_{R}\left({}^{1}/_{T}\right) \quad (12)$$

where K_{pH} = reaction rate constants at absolute temperature T, K_{0pH} = frequency factor (provided in Table 3), E_a = activation energy (kJ/mol), R = universal gas constant (0.008314 kJ/mol. K). E_a/R which is the slope index of the Arrhenius plots are also provided in Table 3. The predicted K_{pH} at selected ambient temperatures are provided in Table 9. Using the K_{pH} values at the selected storage temperatures and appropriate critical pH values (pH_c), the shelf-lives of the *dambu-nama* products were estimated using equation 9. Plots of predicted shelf-lives of the products are provided in Fig. 5. The shelf-lives at 20–30^{o}C storage ranged from 7.7 to 4.3 wks (DNC_0), 17.8 to 10.3 wks ($DNC_{0.1}$), 33.3 to 21.0 wks ($DNC_{0.2}$) and about 65 to 28 wks ($DNC_{0.3}$) respectively.

Table 4. Influence of hurdles on overall acceptability of *dambu-nama* during accelerated storage at elevated temperatures

Sample	Storage time (days)	Overall acceptability score			
		Temperature (^{o}C)			
		40	50	60	70
DNC_0	0	6.6	6.6	6.6	6.6
	3	6.4	6.2	6.0	5.8
	6	5.8	5.6	5.4	5.0
	9	5.6	5.2	4.8	4.4
	12	5.4	4.6	4.2	3.6
$DNC_{0.1}$	0	6.8	6.8	6.8	6.8
	3	6.6	6.4	6.2	6.0
	6	6.4	6.0	5.8	5.4
	9	6.2	5.6	5.4	4.8
	12	6.0	5.4	4.6	4.2
$DNC_{0.2}$	0	6.9	6.9	6.9	6.9
	3	6.6	6.4	6.2	6.0
	6	6.4	6.2	6.0	5.6
	9	6.2	5.9	5.4	5.2
	12	6.0	5.4	4.9	4.4
$DNC_{0.3}$	0	6.8	6.8	6.8	6.8
	3	6.6	6.4	6.4	6.0
	6	6.4	6.0	6.0	5.4
	9	6.0	5.8	5.4	5.0
	12	5.9	5.4	5.0	4.8

Values are means of 20 panelists response on a scale with 7 = Like Extremely, 6 = Like very much, 1 = Dislike Extremely DNC_0 = Dambu-nama + 4% spices (control), $DNC_{0.1}$ =Dambu-nama + citric acid (0.1%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.2}$ = Dambu-nama + citric acid (0.2%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.3}$ = Dambu-nama + citric acid (0.3%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%).

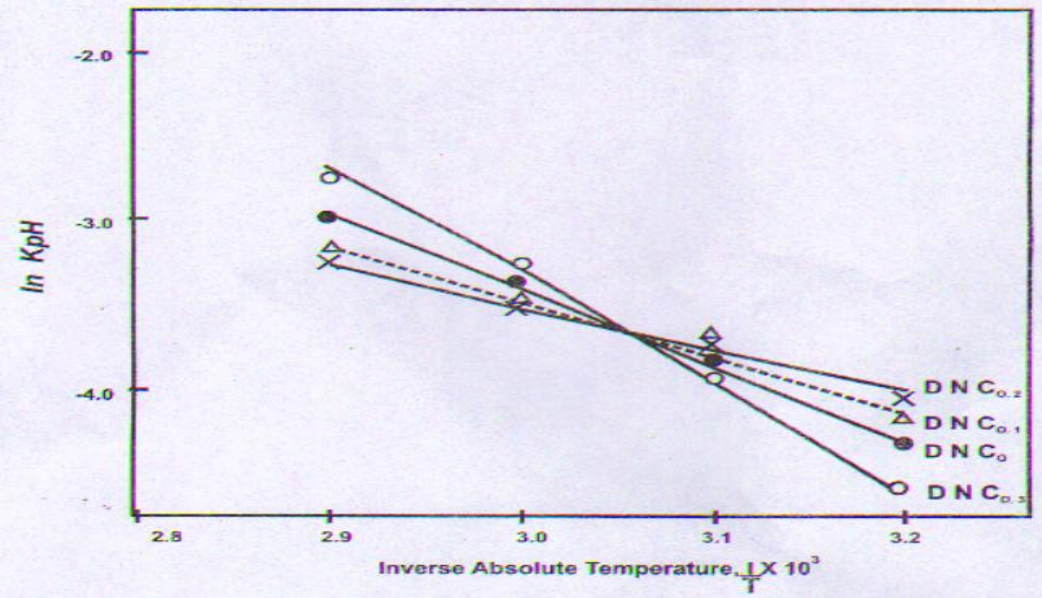

Fig. 3. **Arrhenus Relationship for pH Changes During Accelerated Storage of** *Dambu-nama*
DNC_o = Dambu-nama, $DNC_{o.1}$ = Dambu-nama + 0.1% citric acid + 2% salt + 2% sugar,
$DNC_{o.2}$ = Dambu-nama + 0.2% citric acid + 2% salt + 2% sugar;
$DNC_{o.3}$ = Dambu-nama+ 0.3% citric acid + 2% salt + 2% sugar score ;

Table 5. First order reaction regression parameters for changes in ph of *dambu-nama* during accelerated storage

Samples	Parameter	Temperature (°C)			
		40	**50**	**60**	**70**
DNC_0	r^2	0.996	0.998	0.985	0.975
	S.E	0.07	0.08	0.03	0.05
	pH_o	5.89	5.94	5.91	5.93
	$K_{pH}(wk^{-1})$	0.0164	0.0202	0.0324	0.0475
$DNC_{0.1}$	r^2	0.996	0.938	0.994	0.993
	S.E	0.01	0.04	0.02	0.02
	pH_o	5.58	5.53	5.48	5.54
	$K_{pH}(wk^{-1})$	0.0152	0.0241	0.0304	0.0393
$DNC_{0.2}$	r^2	0.974	0.987	0.996	0.986
	S.E	0.05	0.03	0.01	0.03
	pH_o	5.62	5.62	5.60	5.47
	$K_{pH}(wk^{-1})$	0.0173	0.0250	0.0306	0.0382
$DNC_{0.3}$	r^2	0.965	0.995	0.998	0.998
	S.E	0.07	0.02	0.01	0.01
	pH_o	5.56	5.55	5.58	5.55
	$K_{pH}(wk^{-1})$	0.0103	0.0197	0.0369	0.0639

r^2 = Coefficient of regression, S.E. = standard error of estimate, pH_o = initial pH of dambu-nama, K_{pH} = reaction rate constant for pH changes, DNC_0 = Dambu-nama + 4% spices (control),
$DNC_{0.1}$ =Dambu-nama + citric acid (0.1%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%),
$DNC_{0.2}$ = Dambu-nama + citric acid (0.2%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%),
$DNC_{0.3}$ = Dambu-nama + citric acid (0.3%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%).

Table 6. Zero order reaction regression parameters for changes in overall acceptability of *dambu-nama* during accelerated storage

Samples	Parameters	Temperature (°C)			
		40	50	60	70
DNC_0	r^2	0.955	0.995	1.0	0.998
	S.E	0.15	0.08	<0.01	0.08
	A_o	6.6	6.64	6.6	6.65
	K_A	0.1067	0.1667	0.2000	0.2467
$DNC_{0.1}$	r^2	1.0	0.989	0.983	0.996
	S.E	<0.01	0.09	0.15	0.09
	A_o	6.8	6.76	6.8	6.72
	K_A	0.0667	0.12	0.1733	0.2133
$DNC_{0.2}$	r^2	0.991	0.978	0.981	0.976
	S.E	0.04	0.11	0.15	0.20
	A_o	6.86	6.86	6.84	6.78
	K_A	0.0733	0.1167	0.1600	0.1933
$DNC_{0.3}$	r^2	0.983	0.990	0.994	0.975
	S.E	0.08	0.08	0.07	0.19
	A_o	6.84	6.76	6.84	6.64
	K_A	0.0867	0.1133	0.1533	0.1800

r^2 = Coefficient of regression, S.E. = standard error of estimate, A_o = predicted initial Overall Acceptability, K_A = reaction rate constants for acceptability changes, DNC_0 = Dambu-nama + 4% spices (control), $DNC_{0.1}$ =Dambu-nama + citric acid (0.1%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.2}$ = Dambu-nama + citric acid (0.2%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.3}$ = Dambu-nama + citric acid (0.3%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%).

Shelf- life of a food product is the period before microbiological, physical, chemical or sensory quality factors deteriorate beyond the set limits. Upon storage of processed foods, physico-chemical and/or microbiological changes occur which may lead to deterioration in sensory properties and therefore become limiting factors for the shelf-life of the food. Because reaction rates are temperature dependent, most quality changes at normal ambient storage temperatures are slow making shelf-life determination tedious, expensive and time- consuming. Many researchers have suggested the use of accelerated testing and subsequent extrapolation to various anticipated ambient storage temperatures for prediction of commercial shelf-life. Changes in pH and overall acceptability of the *dambu-nama* products during storage at elevated temperatures were related via first order reaction kinetics (Equation 37) and Arrhenius activated complex theory (Equation 38). Based on initial value of *dambu-nama* pH, the models predicted acceptability scores and hence shelf-life at different storage temperatures. The criteria for shelf-life prediction were the application of GMP, effective packaging in moisture- barrier materials and that *dambu-nama* was acceptable (i.e. did not fail) if the overall acceptability, as predicted by the models were ≥ 5 which represents an average outgoing quality of good attributes on a 7-point scale. In all the products, the shelf-life decreased with increase in storage temperature. This was due to enhancement of deteriorative physico-chemical reactions at higher temperature. As can be observed from Fig. 5, the application of citric acid, salt and sugar as hurdles to *dambu-nama* significantly increased shelf-life of the product at all temperatures studied. The application of the hurdles increased shelf-life by factors of about 2.3 ($DNC_{0.1}$), 4.3 to 4.9 ($DNC_{0.2}$) and 6.5 to 8.4 ($DNC_{0.3}$), suggesting that citric acid, within the concentrations investigated, was the critical hurdle for shelf-life extension in *dambu-nama*.

Table 7. Arrhenius regression parameters for changes in ph of *dambu-nama* products

Regression parameters	Products			
	DNC_0	$DNC_{0.1}$	$DNC_{0.2}$	$DNC_{0.3}$
n	4	4	4	4
r^2	0.973	0.979	0.985	0.998
S.E.	0.14	0.08	0.06	0.02
Intercept	8.3384	6.4748	4.8501	11.9328
K_{opH}	4.2×10^3	0.69×10^3	0.13×10^3	0.13×10^5
Slope	-3918.56	-3320.49	-2776.41	-6555.06
E_a (KJ/mol)	32.6	27.6	23.1	54.5

n = number of points, r^2 = Coefficient of regression, S.E. = standard error of estimate, K_{opH} = frequency factor for pH changes, E_a = activation energy based on ln pH = ln K_{opH} - E_a/R(1/T), R = universal gas constant (0.008314KJ/mol^{0}C), T = absolute temperature, DNC_0 = Dambu-nama + 4% spices (control), $DNC_{0.1}$ =Dambu-nama + citric acid (0.1%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.2}$ = Dambu-nama + citric acid (0.2%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.3}$ = Dambu-nama + citric acid (0.3%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%).

Table 8. Arrhenius regression parameters for variations in overall acceptability of *dambu-nama* products

Regression parameters	Products			
	DNC_0	$DNC_{0.1}$	$DNC_{0.2}$	$DNC_{0.3}$
n	4	4	4	4
r^2	0.962	0.964	0.974	0.990
S.E.	0.10	0.13	0.096	0.04
Intercept	7.1198	10.6702	8.5415	6.1355
K_{oA}	1.24×10^3	4.3×10^4	5.12×10^3	0.46×10^3
Slope	-2909.05	-4161.28	-3473.16	2682.80
E_a (KJ/mol)	24.19	34.60	28.88	22.30

n = number of points, r^2 = Coefficient of regression, S.E. = standard error of estimate, ln K_A = ln K_{oA} - E_a/R(1/T), K_A = rate constants for changes in overall acceptability, K_{oA} = frequency factor E_a = activation energy, R = universal gas constant, T = absolute temperature, DNC_0 = Dambu-nama + 4% spices (control), $DNC_{0.1}$ =Dambu-nama + citric acid (0.1%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.2}$ = Dambu-nama + citric acid (0.2%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.3}$ = Dambu-nama + citric acid (0.3%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%).

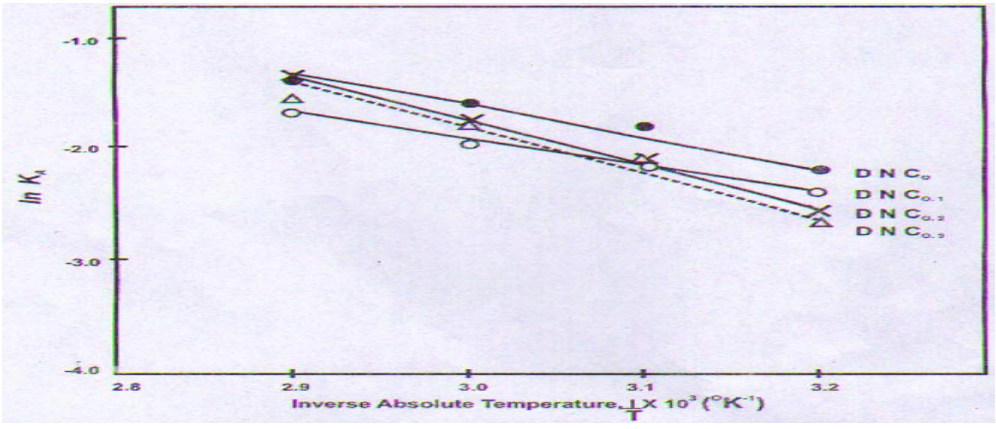

Fig. 4. Arrhenus Relationship between Degradation Rate Constant for Overall Acceptability (K_A) and Temperature During Accelerated Storage of *Dambu-nama*
DNC_0 = Dambu-nama, $DNC_{0.1}$ = Dambu-nama + 0.1% citric acid + 2% salt + 2% sugar, $DNC_{0.2}$ = Dambu-nama + 0.2% citric acid + 2% salt + 2% sugar; $DNC_{0.3}$ = Dambu-nama + 0.3% citric acid + 2% salt + 2% sugar score ;

Table 9. Predicted parameters for shelf life estimations of *dambu-nama*

Samples	Temperature (°C)	Predicted		
		pH_c	$K_{pH}(wk^{-1})$	Shelf-life (wks)
DNC_0	20		0.0081	7.7
	25		0.0099	6.3
	30	5.56	0.0121	5.2
	35		0.0146	4.3
$DNC_{0.1}$	20		0.0078	17.8
	25		0.0094	14.8
	30	4.83	0.0113	12.3
	35		0.0135	10.3
$DNC_{0.2}$	20		0.0098	33.3
	25		0.0115	28.3
	30	3.97	0.0134	24.3
	35		0.0155	21.0
$DNC_{0.3}$	20		0.0037	64.9
	25		0.0049	49.0
	30	4.09	0.0065	36.9
	35		0.0086	27.9

DNC_0 = Dambu-nama + 4% spices (control), $DNC_{0.1}$ =Dambu-nama + citric acid (0.1%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.2}$ = Dambu-nama + citric acid (0.2%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%), $DNC_{0.3}$ = Dambu-nama + citric acid (0.3%) + Salt (2.0%) + Sugar (2.0%) + Spices (4.0%).
**Based on average outgoing quality (AOGQ) of 5 (good) on a scale with 7 = excellent, 4 = fair and 1 = extremely poor overall acceptability. ln pH_c = ln pH_o - K_{pH} t_s; pH_c = critical pH, K_{pH} = reaction rate constant (wk^{-1}), t_s = shelf-life (wk)*

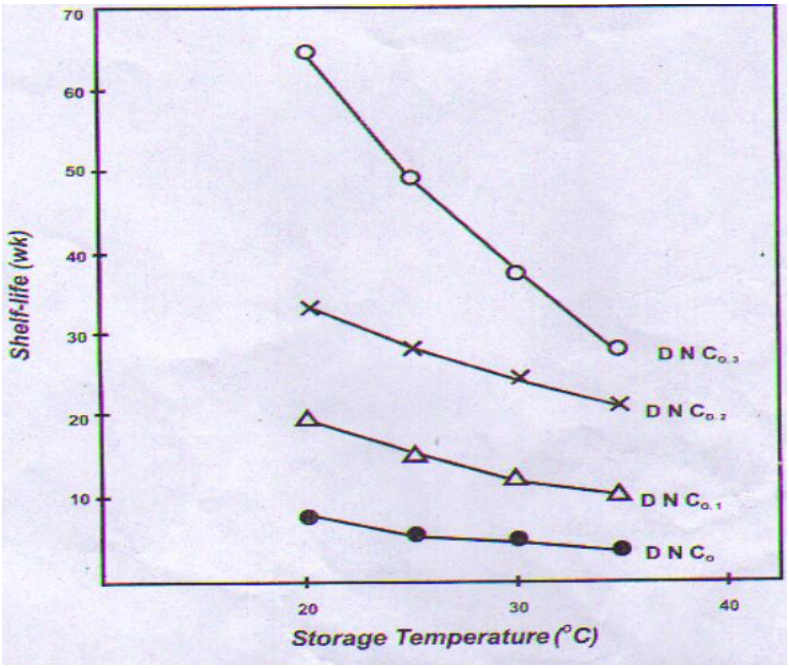

Fig. 5. Shelf-life Prediction Curves for *Dambu-nama* at Different Storage Temperatures
DNC_0 = Dambu-nama, $DNC_{0.1}$ = Dambu-nama + 0.1% citric acid + 2% salt + 2% sugar,
$DNC_{0.2}$ = Dambu-nama + 0.2% citric acid + 2% salt + 2% sugar;
$DNC_{0.3}$ = Dambu-nama+ 0.3% citric acid + 2% salt + 2% sugar score ;

4. CONCLUSION

The incorporation of citric acid (0.1 to 0.3%), 2% salt and 2% sugar as hurdles into *dambu-nama* resulted in the following findings:

Addition of the hurdles into *dambu-nama* inhibited growth of bacteria and moulds in the meat product. The pH of *dambu-nama* was shown to be a reliable quality index that demonstrated a high correlation with sensory attributes. Addition of the hurdles, extended the shelf-life of *dambu-nama* by factors of 2.3 to 8.4.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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