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Djina Yves
Biocatalysis and Bioprocesses Laboratory, Faculty of Food Science and Technology, NANGUI ABROGOUA University, Abidjan, Côte d'Ivoire

Disseka Kwithony William
Biocatalysis and Bioprocesses Laboratory, Faculty of Food Science and Technology, NANGUI ABROGOUA University, Abidjan, Côte d'Ivoire

N'zebo N'Zebo Jean-Michel
Biocatalysis and Bioprocesses Laboratory, Faculty of Food Science and Technology, NANGUI ABROGOUA University, Abidjan, Côte d'Ivoire

Faulet Meuwiah Betty
Biocatalysis and Bioprocesses Laboratory, Faculty of Food Science and Technology, NANGUI ABROGOUA University, Abidjan, Côte d'Ivoire

Gonnety Tia Jean
Biocatalysis and Bioprocesses Laboratory, Faculty of Food Science and Technology, NANGUI ABROGOUA University, Abidjan, Côte d'Ivoire

Corresponding Author:
Djina Yves
Biocatalysis and Bioprocesses Laboratory, Faculty of Food Science and Technology, NANGUI ABROGOUA University, Abidjan, Côte d'Ivoire

Optimization of the Preservation of Attiéké, a Local Ivorian Product, Through Predictive Microbiological Modeling

Djina Yves, Disseka Kwithony William, N'zebo N'Zebo Jean-Michel, Faulet Meuwiah Betty and Gonnety Tia Jean

Abstract
The objective of this study is to propose methods for predicting the use-by date (DLC) of Attiéké, providing reliable results in a shorter time than aging tests, which can be tedious and costly for manufacturers. The methods used include the Peck model and a centered composite experimental design. The modeling of the influence of attiéké's moisture content and storage temperature on bacterial growth parameters (growth rate μmax and lag time T-lag) allowed for the prediction of the DLC. The DLC predicted by the acceleration law was identical to that obtained from aging tests, while the DLC predicted by the experimental design was close to the aging test values, with some discrepancies at certain temperatures.

Keywords: Attiéké, use-by date, modeling, experimental design, temperature

Introduction
Attiéké is a traditional Ivorian dish made from cassava (*Manihot esculenta* Crantz), a root crop widely cultivated in tropical regions (Adayé, 2020) [1]. This food product, presented as a steamed semolina, is highly appreciated not only in Côte d'Ivoire but also in several West African countries (Kakou, 2000; Djéni *et al.,* 2014) [2, 3]. It constitutes an essential food source, particularly in urban areas where it is consumed daily. Moreover, *attiéké* is increasingly exported to countries in the Sahel, as well as to Europe and the United States, reflecting its growing international recognition. In 2015, approximately 5,818 and 20,000 tons of *attiéké* were exported to Mali and Burkina Faso, respectively (FIRCA, 2018) [4]. In recent years, export volumes have increased significantly, largely due to the development of dried and dehydrated forms, which have expanded preservation and transport possibilities (Adayé, 2020) [1].

However, in response to this rising demand, it is crucial to optimize *attiéké* production while ensuring both quality and food safety. This calls for rapid, reliable, and cost-effective methods to assess the product's shelf life. Indeed, the traditional method of aging tests—based on a series of lengthy and expensive trials-no longer meets the needs of the modern agri-food industry. Furthermore, this approach has ecological limitations due to the substantial resources required to conduct such tests. Consequently, alternatives such as accelerated aging tests and experimental design methodologies are increasingly favored. These methods are less expensive and more environmentally friendly, allowing for a reduction in the number of tests and the duration of studies, while still yielding reliable results (Andrianina, 2018) [5].

Modeling, in particular, is a valuable tool for predicting the use-by date (DLC) of food products by considering their initial physicochemical and microbiological characteristics. This approach is especially relevant for highly perishable foods such as *attiéké*, which require strict shelf-life management. Modeling allows for the simulation of storage conditions and a more accurate estimation of the DLC without relying on time-consuming experimental trials.

Several studies have focused on *attiéké*, including the production of cassava starter cultures for its manufacture (Assanvo *et al*., 2002) [6], cold storage preservation (Sahore and Nemlin, 2012), the composition of dehydrated *attiéké* (Yao *et al.,* 2006) [7], and the quality of *attiéké* produced by mechanical granulation (Dédédji *et al.,* 2008) [8]. While these studies have contributed to improving knowledge about the production and preservation of *attiéké*, the modeling of bacterial flora growth and the prediction of its use-by date have not yet been

thoroughly investigated. This study therefore aims to address this gap by proposing a model for bacterial growth in fresh *attiéké*, in order to reliably predict its use-by date.

Materials And Methods

Biological material

The biological material used in this study consisted of attiéké produced following the traditional Ebrié cooking method (n'tonié type) (Figure 5). The attiéké was collected immediately after cooking from a local producer based in the Abobo municipality (Abidjan, Côte d'Ivoire).

Sampling

Samples were collected under strict aseptic conditions (Figure 6). Using sterile gloves, the samples were directly transferred—while still hot—into sterile stomacher bags. These bags were then sealed using a stapler and transported in an insulated cooler equipped with a digital thermometer. The temperature was maintained at 5 ± 3 °C using dry ice until arrival at the laboratory.

Sample preparation

To perform the shelf-life modeling, three attiéké samples with different moisture contents were prepared: 50%, 65%, and 80%. The initial sample had a moisture content of 50%, measured using a halogen moisture analyzer (KERN, Germany).

- To obtain 65% moisture, 30 g of sterile ultrapure water were added to 70 g of attiéké.
- To reach 80%, 60 g of sterile ultrapure water were added to 40 g of attiéké. All mixtures were prepared under fully aseptic conditions.

Physicochemical analyses

Analyses were carried out according to AOAC standardized methods:

- **Moisture content**: by mass loss (AOAC, 2020) [9]
- **Ash content**: by incineration (AOAC, 2000) [9]
- **Lipids**: by Soxhlet extraction **(**AOAC, 2000) [9]
- **Proteins**: by the Kjeldahl method (AOAC, 2000) [9]
- **Fibers**: by the Van Soest method (1963) [10]
- **Metabolizable carbohydrates**: by difference (AOAC, 2000) [9]
- **pH**: using a pH meter (AOAC, 2000) [9]
- **Energy value**: calculated according to the Atwater and Benedict method (1902) [11]

Determination of the Use-by Date (UBD)

Accelerated shelf-life testing using primary method

This test involved daily monitoring of bacterial flora evolution (aerobic mesophilic bacteria – AMB, sulfite-reducing anaerobes – SRA, *Escherichia coli*, total coliforms, *Staphylococcus aureus*, yeasts and molds) in attiéké samples stored at 15 °C, 25 °C, and 30 °C. Monitoring continued until microbial loads exceeded the specifications set by the NI 4684v2018 standard for attiéké.

Accelerated shelf-life testing using secondary method

Based on the Peck model, this test was performed at 30 °C with a moisture content of 65%, conditions favorable to aerobic flora growth. The UBD obtained under accelerated conditions was compared with that from natural testing, enabling the development of a mathematical model for microbial growth rate prediction.

Estimating the use-by date using the accelerated growth method (PECK'S LAW)

Peck a proposé un modèle mathématique corrélant le taux de croissance de la flore bactérienne d'un produit avec la température de stockage et la teneur en humidité de l'aliment (Bennaceur, 2013) [12]. This mathematical model can be summarised as follows:

Model equations

Equation 1

$$FA = \frac{\mu N}{\mu A} = \left(\frac{HA}{HN}\right)^{n} \times e^{\left[\left(\frac{Ea}{R}\right)\left(\frac{1}{TN}-\frac{1}{TA}\right)\right]}$$

Equation 2

$$\mu N = FA \times \mu A$$

Equation 3

$$DLC = \frac{\ln(3.10^{4}) - \ln(N0)}{\mu N}$$

Parameters

- **FA** : acceleration factor
- **μN** : growth rate under normal conditions (CFU/g)
- **μA** : growth rate under accelerated conditions (CFU/g)
- **n** : experimental constant (between 1.5 and 15)
- **Ea** : activation energy (J/mol)
- **R** : universal gas constant ($J \cdot mol^{-1} \cdot K^{-1}$)
- **TN, HN:** temperature (K) and humidity (%) under normal conditions
- **TA, HA:** temperature (K) and humidity (%) under accelerated conditions
- **Ln(N0)**: natural logarithm of the initial bacterial load
- **Ln(3·10⁴):** natural logarithm of the maximum load allowed by the NI 4684:2018 standard (for AMB)

Experimental design

A Central Composite Design (CCD) was generated using Design Expert 7 software to evaluate the interaction effects of two factors (temperature and moisture content) on:

- the maximum growth rate (μmax)
- the lag time (T-lag) of bacterial flora

Studied factors

Table 1: Experience matrix

Coded factor	Temperature (°C)	Moisture (%)
-1	15	50
0	25	60
+1	30	80

Table 2: Experimental domain

Trial No.	Temperature (K)	Moisture (%)
1	303.15	50
2	303.15	60
3	303.15	80
4	288.15	50
5	288.15	60
6	288.15	80
7	298.15	50
8	298.15	60
9	298.15	80
10	298.15	50
11	288.15	60
12	303.15	80
13	288.15	80

The mathematical model provided by the centred composite experimental design is a second-degree polynomial model defined as follows:

$$Y= b0+b1T+b2H+b3TH+b5T^2 +b6H^2+e$$

Y = optimal value of µmax or T-lag;
b0 = theoretical mean value of the response;
b1, b2, b3, b4, b5, b6, b7 = respectively the main effects of factors T, H, TH, T2, H2;
e = error term.

Microbiological analyses

Microbial counts were performed in accordance with good laboratory practices and the NI 4684:2018 standard. The following microbial groups were analyzed:

- Aerobic mesophilic bacteria (AMB)
- *Escherichia coli*
- *Staphylococcus aureus*
- Sulfite-reducing anaerobes (SRA)
- Yeasts and molds
- *Bacillus cereus*

Statistical analyses

The µmax and T-lag values obtained using MS Excel 2013 for the 13 trials were imported into Design Expert 7 software. This software was used to analyze interactions between temperature (K) and moisture (%) on bacterial growth, generating 2D graphical representations and predictive equations. A Python 3.7.9 script was developed to automate the prediction of attiéké's use-by date based on its water content and storage temperature.

Results

1. Evaluation of the Physicochemical Characteristics of Attiéké

The physicochemical characteristics of the attiéké sample are presented in Table 2 below. The results demonstrate compliance of the sample with the specifications of the NI 4684:2018 standard.

Table 3: Physicochemical Analysis Results of Attiéké

Parameter	Value	NI 4684 : 2018 Standard
Moisture (%)	50.84	45–55
Ash (%)	1.26	<1.4
Protein (%)	1.4	1–2%
Lipid (%)	1.03	1–3%
Fiber (%)	0.93	<1%
Carbohydrates (%)	89.80	80–90%
Energy value (Kcal/100g)	369.03	300–400
pH	4.0	4–5
Titratable acidity (meq/100g)	4.23	3.5–4.8
Aw	0.889	–

The conformity of the attiéké sample to the standard criteria is a prerequisite for predicting the maximum shelf life while maintaining compliance with existing quality standards.

2. Evaluation of the Microbiological Quality of Attiéké

The results of the microbiological quality evaluation of attiéké samples are illustrated in Figure 1 below, which shows the evolution of bacterial load in different types of attiéké depending on time and storage conditions.

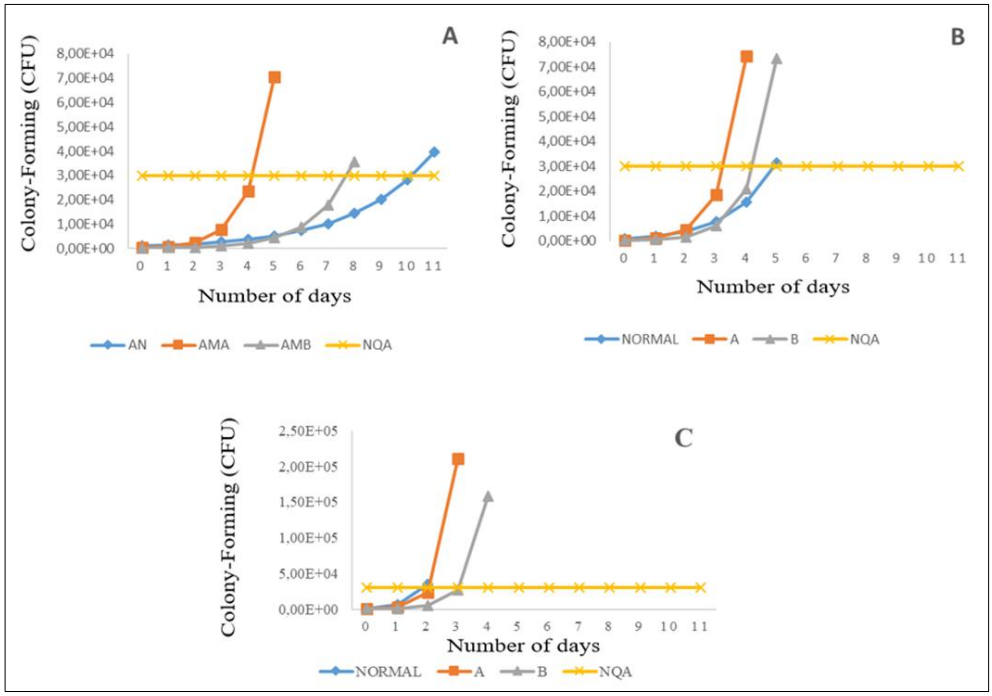

Fig 1: Monitoring Curve of Bacterial Load in Normal Attiéké (AN), Modified Attiéké A (AMA), and Modified Attiéké B (AMB

- **Graph A:** Storage at 15°C leads to exceeding the Acceptable Quality Level (AQL) after 11 days for normal attiéké, 8 days for attiéké with 65% moisture, and 5 days for attiéké with 80% moisture.
- **Graph B:** Storage at 25°C leads to exceeding the AQL after 6 days for normal attiéké, 3 days for attiéké with 65% moisture, and 4 days for attiéké with 80% moisture.
- **Graph C:** Storage at 30°C leads to exceeding the AQL after 2 days for normal attiéké, 2 days for attiéké with 65% moisture, and 3 days for attiéké with 80% moisture.

3. Assessment of the Use-by Date (UBD)

Figure 2 shows the influence of temperature and humidity on the growth parameters of bacterial flora. The results show that an increase in temperature and humidity leads to accelerated bacterial growth.

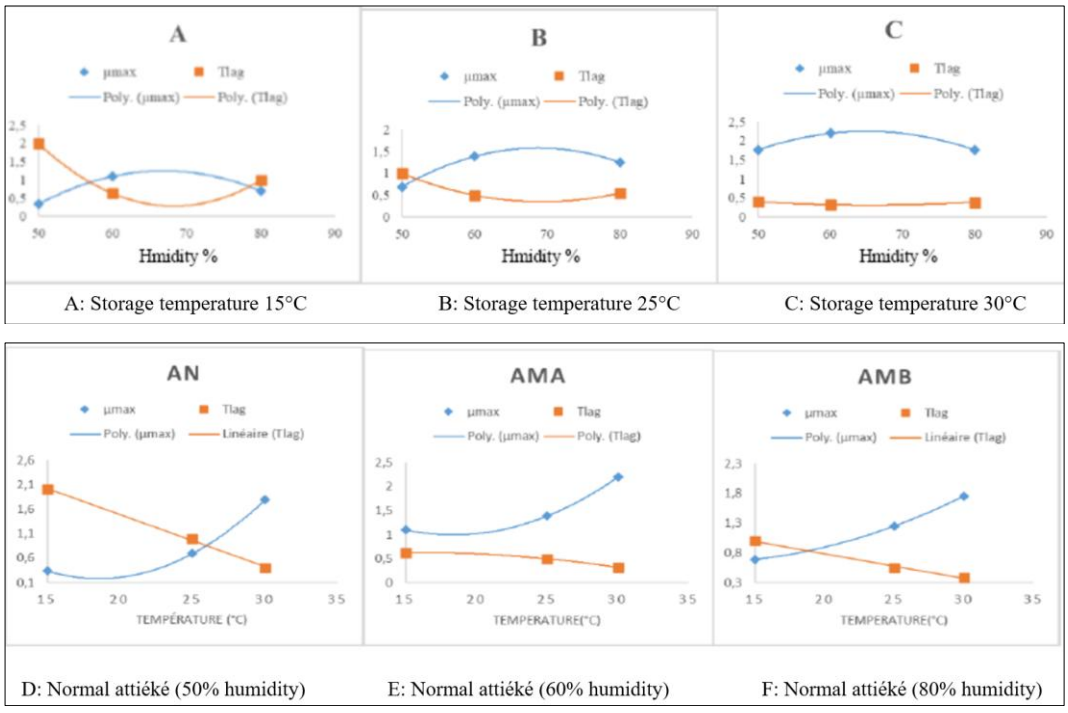

Fig 2: Graphical representation of the influence of temperature (A, B and C) and humidity (D, E and F) on µmax and T-lag

- **Graphs A, B and C:** The growth rate of bacterial flora increases with humidity, regardless of the attiéké sample (H=50%, H=65% and H=80%).
- **Graphs D, E and F:** The growth rate of bacterial flora is faster at higher temperatures for all attiéké samples.4. UBD Prediction Using the Peck Model.

The prediction of the UBD using the Peck model for normal attiéké at 25 °C is presented in Table 4. The predicted values are identical to the UBD obtained through shelf-life testing, indicating strong correlation.

Table 4: UBD Prediction of Attiéké According to the Peck Model

Acceleration Condition	AF	Estimated µmax (Normal)	Predicted UBD	Actual UBD
H = 60%, T = 30°C	0.3154	0.6930	5	5
H = 80%, T = 30°C	0.3948	0.6940	5	5

AF: Acceleration factor for the growth of bacterial flora in attiéké between 25°C and 30°C

5. Tertiary Model Results: Experimental Design

The response surface model was evaluated for reliability based on significant and non-significant coefficients. Table 5 shows that some model coefficients are significant ($p < 0.05$), while others are not ($p > 0.05$).

Table 5 : Relevance of Model Coefficients

Parameter µmax	p-value	Relevant	Parameter T-lag	p-value	Relevant
b0	<0.0001	Yes	β0	0.0023	Yes
b1	<0.0001	Yes	β1	0.0020	Yes
b2	0.0106	Yes	β2	0.0052	Yes
b3	0.2097	No	β3	0.0251	Yes
b4	0.0012	Yes	β4	0.6829	No
b5	0.0002	Yes	β5	0.0188	Yes

The model shows a correlation of $R^2 = 0.9774$ for µmax and $R^2 = 0.8987$ for T-lag, which allows for accurate prediction of the Attiéké UBD.

Table 6: Model Correlation Coefficients with Moisture and Temperature Factors

Parameter	µmax	T-lag
R^2	0.9774	0.8987

6. UBD Prediction Using the µmax Model

The results of UBD prediction using the µmax model are presented in **Table 6**. The predictions are close to those obtained by primary aging tests, with slight differences.

Table 7: Results of shelf life prediction according to the MSR model

Temperature (°C)	Temperature (K)	Moisture (%)	UBDN (Days)	UBDP (Days)
15	288.15	50	11	10
25	298.15	50	5	4
30	303.15	50	2	2

UBDN: Use-by date obtained through primary aging test (days)
UBDP: Use-by date predicted by the µmax model (days)

Discussion

Fresh attiéké is a highly perishable food product, with a shelf life at room temperature generally less than one week (Adayé, 2020) [1]. Cold storage, however, can extend this period to about two weeks (Sahore and Nemlin, 2012) [13].

In this study, samples were taken immediately after cooking using sterile gloves and bags. This approach helped minimize the risks of post-cooking contamination related to ambient air, handling, and packaging. The initial analysis showed that the microbial load and physicochemical characteristics of the attiéké complied with the requirements of the NI 4684: 2018 standard. The bacterial flora present consisted solely of mesophilic aerobic germs (MAG), which justified limiting the aging tests to this group, with daily monitoring until the critical threshold of 3.10^4 CFU/g was exceeded, as recommended by the standard. The absence of *Staphylococcus aureus* indicates compliance with hygiene standards during production, packaging, and transport of the attiéké. Similarly, the absence of *Bacillus cereus* and sulfite-reducing anaerobes (SRA) suggests effective thermal processing, capable of eliminating bacterial spores (Yobouet *et al.,* 2016) [14]. Lastly, the absence of *Escherichia coli* and *Salmonella* points to the absence of fecal contamination (Roos *et al*., 1995; Watts *et al.,* 1991) [15] [16].

Water activity (aw) is a key factor in the microbiological stability of food. For carbohydrate-rich products such as attiéké, reducing aw through methods like drying or freezing can increase shelf life (Gnagne *et al.,* 2016) [17]. A food product is considered microbiologically stable when its aw is below 0.6. However, fresh attiéké has an aw value above 0.889, which promotes microbial growth (Slade and Levine, 1991) [18].

Moreover, temperature has a significant impact on food degradation kinetics. According to Arrhenius' law, a 10 °C increase doubles the degradation rate (Toledo *et al.,* 2007) [19]. Thus, higher temperatures and humidity levels than standard storage conditions accelerate the deterioration of attiéké. This is reflected in an increased microbial growth rate (µmax) and a shortened lag time (T-lag) as temperature and humidity rise.

The use of the acceleration law based on the Peck model enabled the determination of a microbial growth acceleration factor for attiéké under varying temperature and humidity conditions. This model allowed the prediction of attiéké's shelf life based on the µmax growth rate measured under accelerated conditions (30°C and relative humidity of 60% or 80%). The predicted values were very close to those obtained during standard shelf-life tests.

Additionally, the experimental approach based on the response surface methodology made it possible to develop a mathematical model describing the evolution of µmax and T-lag as a function of temperature and humidity. The resulting model is statistically valid and allows reliable shelf-life prediction, with an average deviation of one day compared to the results from tests conducted at 15°C and 25°C.

However, while the acceleration law allows for more precise predictions, the response surface method stands out for its robustness, as it covers a broader range of experimental conditions.

In conclusion, modeling microbial growth parameters provides a reliable tool for predicting the microbiological shelf life of attiéké. However, the results of this study apply only to samples containing MAG. It would be relevant to extend the investigation to attiéké containing other types of microorganisms such as *Staphylococcus aureus* or yeasts, to validate the applicability of the model to a broader range of microbial flora.

Conclusion

The study conducted on the microbiological shelf life of fresh attiéké demonstrated the effectiveness of a combined modeling approach for predicting the product's use-by date (UBD). The results revealed that the dominant microbial flora in attiéké consisted solely of mesophilic aerobic germs (MAG), indicating good hygiene control during production.

Variations in temperature and relative humidity showed a significant impact on the microbial growth rate, with an increase in µmax and a reduction in lag time (T-lag) under

accelerated conditions. The response surface model and the acceleration model (Peck) proved to be complementary, offering robust prediction and rapid estimation of the UBD under different storage conditions, respectively.

Therefore, this modeling approach provides a reliable and reproducible method for estimating the shelf life of attiéké based on storage conditions. It is a highly valuable tool for producers, processors, and distributors aiming to ensure the sanitary quality of the product while optimizing its distribution.

Further studies incorporating other microbial flora such as *Staphylococcus aureus* or yeasts would help broaden the applicability of the developed models.

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