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Application of linear and nonlinear models for the temperature and pH behavior of a solid state fermented cocoa shell (*Theobroma cacao* L.)

Willan Caicedo (orlando.caicedo@yahoo.es)[a,c], Derwin Viáfara (dviafara@uea.edu.ec)[b], Manuel Pérez (mperez@uea.edu.ec)[a], Dunia Chávez (dchavez@uea.edu.ec)[a], Andrea Guamán (agr20140094@uea.edu.ec)[c], Carolina Sócola (agr20140104@uea.edu.ec)[c], Felipe Norberto Alves Ferreira (felipe.alves@agroceres.com)[d]

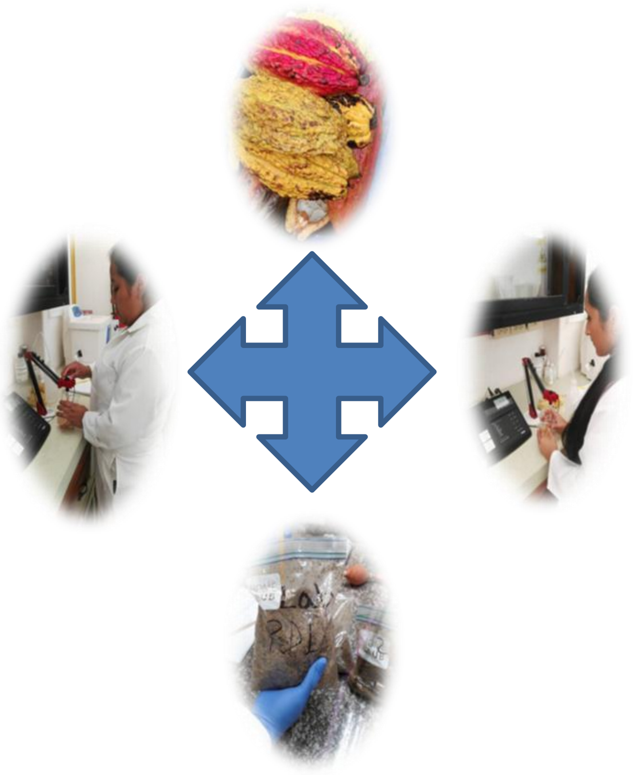
[a]*Departamento de Ciencias de la Tierra, Universidad Estatal Amazónica, Puyo, Pastaza, Ecuador*

[b]*Laboratorio de Bromatología, Universidad Estatal Amazónica, Puyo, Pastaza, Ecuador*

[c]*Granja Agropecuaria Caicedo, Puyo, Pastaza, Ecuador*

[d] *Department of Swine Nutrition, Agroceres Multimix, Rio Claro, São Paulo, Brazil*

Graphical Abstract



Abstract

Linear, quadratic, cubic, compound, exponential and logistic regression models were used to evaluate the temperature and pH dynamics of a solid-state fermented (SSF) cocoa shell (*Theobroma cacao* L.) treated with natural yogurt for use in animals. For this purpose, temperature and pH were checked in silage samples of 0, 1, 4, 8, 15 and 30 days. For the processing of the data, the statistical package SPSS version 22 was used. The behavior of the two variables were adjusted to a cubic model: temperature (R^2 = 0.88; Y = 23.815 + 0.181*X - 0.022*X^2 + 0.001*X^3) and pH (R^2 = 0.95; Y = 5.543 - 0.362*X + 0.027*X^2 - 0.001*X^3). In conclusion, the temperature and pH behavior were adjusted to a non-linear cubic model, and were kept within the optimal ranges for the production of SSF of optimum quality for use in animals.

Key words: *animal feed, SSF, nonlinear regression, cocoa byproduct*

Introduction

Agricultural and agroindustrial by-products contribute to environmental pollution. In the Ecuadorian Amazon Region a high amount of agricultural by-products is generated, among them, the cocoa shell that is discarded into the field without prior treatment. These resources can be a good source of nutrients and polyphenols that processed by solid state fermentation (SSF) generate foods of high nutritional value for use in animal feed (Borrás-Sandoval et al 2017).

On the other hand, in the production of SSF it is necessary to monitor the behavior of temperature and pH, since these parameters directly influence the conservation of the silage (Caicedo et al 2019a). It is considered an optimum temperature range between 15 and 25 ºC (Wang et al 2017), while an ideal pH is found with values below 5 (Kung and Shaver, 2001). The objective of this investigation was to evaluate the temperature and pH dynamics of a solid state fermented (SSF) cocoa shell (*Theobroma cacao* L.) treated with natural yogurt for use in animals.

Materials and Methods

Location

The study was carried out in the Bromatology laboratory of the Amazon State University, located in the Pastaza canton, Pastaza province, Ecuador, this area has a humid subtropical climate, with rainfall varying between 4000 and 4500 mm per year, altitude of 900 meters above sea level, average relative humidity of 87% and temperatures ranging between 20 and 28 ºC (INAMHI 2014).

Preparation of the SSF of cocoa shell

For the preparation of the silage, the cocoa shell was collected, washed, and ground in a knife mill with 1 cm sieve. Afterwards, a clean plastic was placed on a concrete floor and all the materials (chopped cocoa husk, molasses, calcium carbonate, vitaminized pecutrin, wheat powder and natural yogurt) were mixed homogeneously at room temperature of 22 °C for 5 minutes, after this time it was introduced in Ziploc plastic bags with a capacity for 1 kg sealed for: 1, 4, 8, 15 and 30 days under shade at room temperature. The silage formulation is observed in (Table 1).

Table 1. SSF formulation of cocoa shell

Raw materials	**% Inclusion**
Chopped cocoa shell	90.0
Wheat dust	6.0
Molasses	2.0
Vitaminized Pecutrin	0.5
Calcium carbonate	0.5
Natural yogurt	1.0

SSF temperature and pH check

The temperature and pH of the fermented material was measured in a total of 15 microsyls on days 1, 4, 8, 15 and 30 of the course of fermentation, three microsyls on each day of fermentation. A Martini 2012 digital thermometer (Caicedo 2013) was used to check the temperature in the microsyls. For the pH measurement, an aqueous extract consisting of a fraction of 25 g of silage and 250 ml of distilled water was used (Cherney and Cherney 2003).

Statistical analysis

For the processing of temperature and pH data, linear, quadratic, cubic, composite, exponential and logistic regression models were used, using the statistical software SPSS version 22.

Results and Discussion

The cubic model ($Y = a + bx + cx^2 + dx^3$) was the one that presented the best fit for the evaluation of the temperature behavior (Table 2, Figure 1) and pH (Table 3, Figure 2) of the cocoa shell SSF.

Table 2. Coefficients of the cubic regression model for the temperature of the cocoa shell SSF

	Non-standardized coefficients		Standardized Coefficients		
	B	Standard error	Beta	t	Sig.
Fermentation days	,181	,123	5,563	1,473	,027
Fermentation days **2	-,022	,012	-21,341	-1,832	,020
Fermentation days **3	,001	,000	15,852	1,920	,019
(Constant)	23,815	,254		93,870	,000

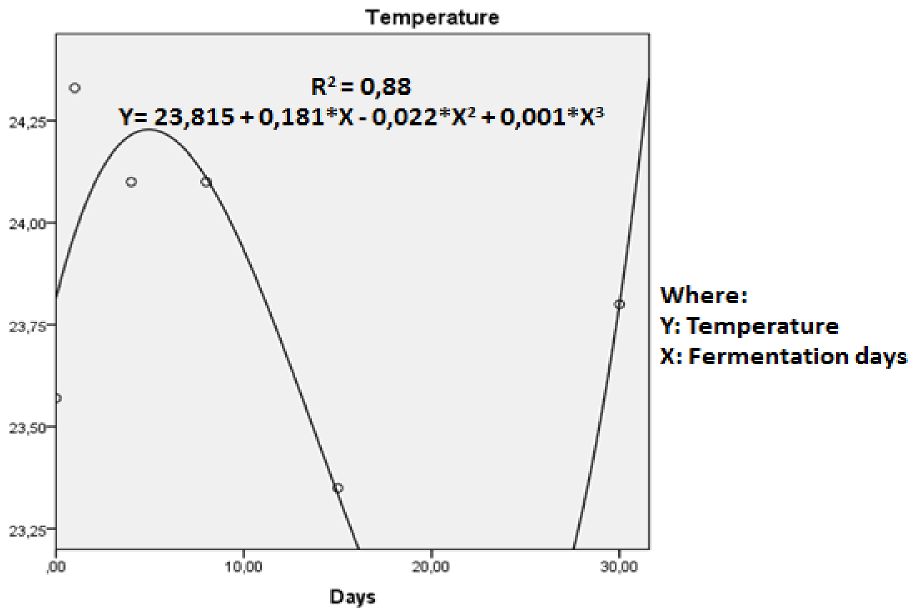


Figure 1. Cubic regression curve for temperature

The temperature fluctuation in microsyls is due to cellular respiration, microbial activity and climatic conditions where silage is performed (Muck 2010; Da Silva et al 2014; Fabiszewska et al 2019). However, Zhou et al (2019) mention that temperatures between 20 and 25 °C, guarantees the growth of lactic acid bacteria colonies such as *Lactobacillus buchneri*, these favors a rapid increase in the concentration of lactic acid, and the rapid decrease in pH to favor the silage process (Muck 2013; Wang et al 2017; Caicedo et al 2019b), in this study the cocoa shell SSF remained within the optimum temperature ranges for the production of good quality silage. On the other hand, temperatures below 10 °C or above 37 °C affect the fermentation process, leading to poor quality silage and low aerobic stability (Zhou et al 2019; Bernardes et al 2018).

Table 3. Coefficients of the cubic regression model for the pH of the cocoa shell SSF

	Non-standardized coefficients		Standardized Coefficients		
	B	Standard error	Beta	t	Sig.
Fermentation days	-,362	,083	-6,880	-4,381	,048
Fermentation days **2	,027	,008	15,856	3,273	,032
Fermentation days **3	-,001	,000	-9,745	-2,839	,045
(Constant)	5,543	,171		32,466	,001

On the other hand, immediately after the fermentation of the raw material, a variation in pH occurs, which reaches values between 5.5 and 6 due to the low concentration of lactic, acetic and propionic acid in the medium (Kung et al 2018). After 96 hours of fermentation in the silage there is a good production of lactic acid product of the action of lactic bacteria (Muck 2013), reducing the pH to values lower than 4.5, a pH lower than these values allows to obtain an optimal silage product nutritional, microbiological and organoleptic quality, without danger of transmitting diseases for animals (Wang et al 2016; Hartinger et al 2019; Tyrolová et al 2017).

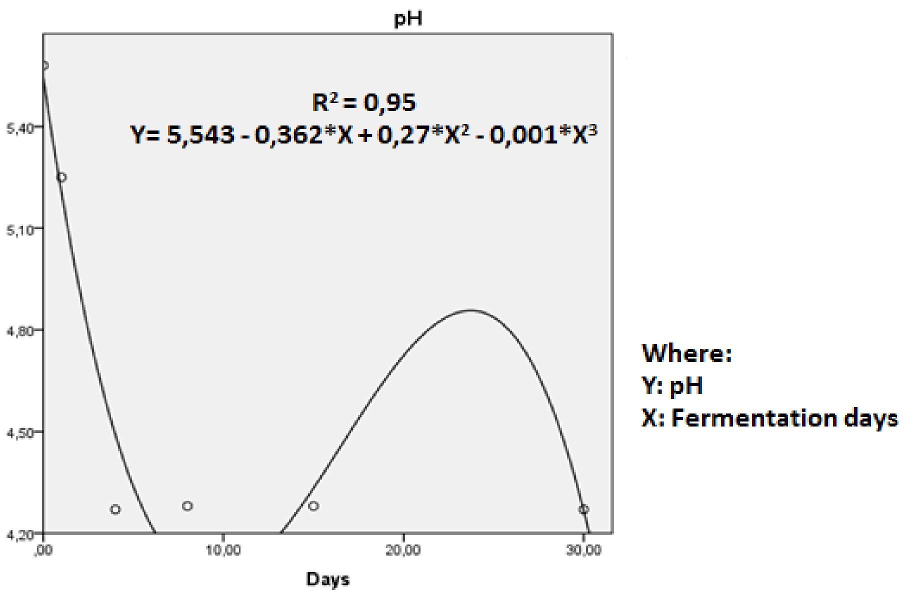


Figure 2. Cubic regression curve for pH

Conclusions

The temperature and pH behavior were adjusted to a non-linear cubic model, and were kept within the optimal ranges for the production of SSF of optimum quality for use in animals.

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