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Prediction of fermentation index of **cocoa** beans (*Theobroma cacao* L.) based on color measurement and artificial neural networks

Noemí León-Roque [a], Mohamed Abderrahim [b], Luis Nuñez-Alejos [c], Silvia M. Arribas [d], Luis Condezo-Hoyos [b,*]

[a] Universidad Nacional Pedro Ruiz Gallo, Facultad de Ingeniería Química e Industrias Alimentarias, Departamento de Ingeniería en Industrias Alimentarias, Lambayeque, Perú
[b] Universidad Carlos III de Madrid, Departamento de Ingeniería de Sistemas y Automática, Leganés, Madrid, Spain
[c] Programa Nacional de Alimentación Escolar Qali Warma, Cajamarca, Jaén, Perú
[d] Universidad Autónoma de Madrid, Facultad de Medicina, Departamento de Fisiología, Madrid, Spain

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ABSTRACT

Several procedures are currently used to assess fermentation index (FI) of cocoa beans (*Theobroma cacao* L.) for quality control. However, all of them present several drawbacks. The aim of the present work was to develop and validate a simple image based quantitative procedure, using color measurement and artificial neural network (ANNs). ANN models based on color measurements were tested to predict fermentation index (FI) of fermented cocoa beans. The RGB values were measured from surface and center region of fermented beans in images obtained by camera and desktop scanner. The FI was defined as the ratio of total free amino acids in fermented *versus* non-fermented samples. The ANN model that included RGB color measurement of fermented cocoa surface and R/G ratio in cocoa bean of alkaline extracts was able to predict FI with no statistical difference compared with the experimental values. Performance of the ANN model was evaluated by the coefficient of determination, Bland-Altman plot and Passing-Bablok regression analyses. Moreover, in fermented beans, total sugar content and titratable acidity showed a similar pattern to the total free amino acid predicted through the color based ANN model. The results of the present work demonstrate that the proposed ANN model can be adopted as a low-cost and *in situ* procedure to predict FI in fermented cocoa beans through *apps* developed for mobile device.

1. Introduction

There are several procedures currently used to assess fermentation index (FI) of cocoa beans for quality control [1–3]. Cut-test is a simple, often used procedure, based on color changes registered during fermentation [1]. Cut-test consists of cutting beans lengthwise in halves and examining their internal color using a score based on purple and brown beans [2,3]. However, this method is not fully quantitative and the color evaluation is very subjective. The measurement the absorbance ratio A460 nm/A530 nm of methanolic acid extracts could potentially be used [1,3]. This absorbance ratio has shown a good non-linear relationship with sensorial color category (cut-test) and Hunter L, a, b color indicators during the fermentation [1], However, it has not been used as FI in fully fermented cocoabeans, where minimal color differences between samples could occur.

Fermentation degree of cocoa beans is traditionally measured by ammonia (NH_3) content [4]. A positive correlation between ammonia content and fermentation level has been observed [4]. However, this is assessed by the Conway technique [5], which is time consuming and, therefore, not appropriate for routine analysis. Changes in the amount and composition of free amino acids have been reported during fermentation and, therefore, could also provide information on FI. Thus, fermented beans are characterized by high amounts of total free amino acids and increased ratio of hydrophobic free to acidic free amino acids, which provide information on the degree of fermentation [6]. A positive correlation ($r=0.7$; $p<0.01$) between the total amount of free amino acids and the ratio of hydrophobic to acidic free amino acids has been observed in raw cocoa from different origins [6]. Individual and total free amino acids in cocoa samples can be quantified by liquid chromatography. However, this technique is not always available in all laboratories and it requires a long time of analysis -approximately 1 h per sample- [6].

Computer vision-based analytical chemistry (CV-AC), which focuses on chemical analysis based on color changes, is gaining

* Corresponding author.
E-mail address: lcondezotm@hotmail.com (L. Condezo-Hoyos).

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increasing interest [7]. CV-AC has several significant advantages, such as simplicity of use, and the fact that it is easily combinable with portable and widely distributed imaging devices, resulting in friendly analytical procedures in many areas that demand out-of-lab applications for *in situ* and real-time monitoring [7]. A limited number of studies using linear and non-linear tools to modeling have been reported on CV-AC in food science. Thus, multiple linear regression have been developed to predict antioxidant activity ($R^2=0.97$, $p<0.001$) and total phenolic compounds ($R^2=0.94$, $p<0.001$) in colored carrot, based on CIELab data obtained by a computer vision system [8]. Similarly, level of ammonia in iceberg lettuces has been predicted from color parameter ($R^2=0.73$, $p<0.001$) by multiple linear regression [9]. Least-squares support vector machine, a non-linear procedure, and fractal color has been used to predict acrylamide content (root mean square error (RMSE)=15.70 ng/g, coefficient of determination (R^2)=0.99) [10]. Artificial neural networks (ANNs) are very powerful tools to model non-linear trends within the data where there are highly complex relationships to be modeled [11]. Cocoa bean fermentation is a complex biochemical process during which several changes occur in macromolecules such as proteins and carbohydrates as well as in organic acids and sugars [2]. ANNs is a heuristic tool useful when theoretical relationship between input and output variables is lacking [12] as fermentation index and color of cocoa beans. The multiple-layer perceptron is the most used architecture ANNs to approximate any continuous functions and for high sample/variable ratio to develop a predictive model [13].

The aim of this study was to predict FI in fully fermented cocoa beans using RGB color and absorbance, and ANNs. Color-based ANN models for the prediction of FI were validated using total free amino acid by coefficient of determination, Bland-Altman plot and Passing-Bablok correlation analysis.

2. Experimental

2.1. Reagents and solutions

All chemicals used were of analytical-reagent grade with no further purification. Anthrone, D-glucose, gallic acid, o-phthalaldehyde (OPA), sodium borate, L-arginine, 2-mercaptoethanol, phenol red indicator, polyvinylpyrrolidone (PVPP) and sodium hydroxide were purchased from Sigma-Aldrich (Madrid, Spain). Absolute ethanol, Folin-Ciocalteu reagent, hydrochloric acid and sulfuric acid were acquired from Merck (Barcelona, Spain). Milli Q ultrapure grade water (<18.2 mΩ) was used for the preparation of solutions.

Stock solution of L-arginine (10 mg mL^{-1}) was prepared in 0.4 mol L^{-1} of hydrochloric acid and freshly working solutions 0, 0.125, 0.25, 0.5, 1.0 were prepared from stock by serial dilution. Glucose stock solution 1 mg mL^{-1} was prepared in water and it was used to prepare working solutions 0, 0.0375, 0.075, 0.15, 0.3 mg L^{-1} by serial dilution.

2.2. Cocoa beans

2.2.1. Samples

Two raw white fine cocoa beans of criollo varieties from Piura (M_1) and Cajamarca-Perú (M_2) and one native criollo variety (M_3) from Tumbes-Peru were used. Raw samples were harvested in December 2015 and fermented as below.

2.2.2. Fermentation and drying

The cocoa fermentation was carried out in three different regions from Perú (Piura, Cajamarca and Tumbes) according to the producer usual practices. Raw cocoa beans were fermented by the box method using boxes of $1.2\times0.5\times0.5$ m provided of two compartments for progressive fermentation. At 48 h, when the fermentation temperature was 40 °C, the samples were stirred every 24 h for 6 days to avoid the excessive increase of the temperature (≤ 48 °C). The fermented cocoa beans were sun-dried to a final moisture content close to 6.5%, which was measured with a cocoa moisture meter (Aqua-Boy KMP, Leeds, UK). Fermented cocoa beans (100–200 g) were sampled from each bag using a specific probe and the quartering method. The samples were packed in labeled polyethylene bags and stored at 4 °C until analysis.

2.2.3. Image acquisition, processing and analysis

2.2.3.1. Acquisition. Forty fermented cocoa beans of each sample M_1, M_2 and M_3 were randomly chosen and manually peeled off. Firstly, whole bean image was acquired in a desktop scanner as shown below and secondly, each bean was cut manually in halves using a surgical knife, as for the traditional cut-test, and image was also acquire. Thereafter, fermented cocoa beans were individually ground in a coffee blender (Selecline, France) using five cycles of 10 s to avoid sample heating and stored at 4 °C until analysis. Fifty milligrams of ground fermented cocoa beans were mixed with 1 mL of NaOH (0.28 mol L^{-1}) and shaken at maximum speed in a "Movil Rod" (J.P. Selecta S.A, Barcelona, Spain) shaker for 5 min at room temperature. The fermented cocoa extracts were centrifuged at 5000 rpm for 5 min at room temperature and supernatant was stored at 4 °C until analysis. For fermented cocoa beans, the images were acquired with an HP PSC 1510 desktop scanner (Hewlett-Packard Development Company, USA) configured as follows: resolution=1200 dpi, color depth=32 bits per pixel and format file=TIFF compressed format. A specific white cover was designed and printed in a MakerBot 3D printer (NY, USA) to cover the beans to avoid external light interference and to improve the light reflection [14]. The images of fermented cocoa extracts were acquired using a mobile phone Motorola Moto G under constant conditions of light on 96-well plate.

2.2.3.2. Processing and analysis. The images of fermented cocoa beans for each sample were preprocessed before analysis using MATLAB Toolbox Image Processing. The images were corrected for the non-uniform background illumination and converted into a binary format for analysis. Images of each cocoa bean were segmented from total images (Supplementary Fig. 1). RGB values were measured using a plugins from ImageJ (http://imagej.nih.gov/ij/). For cocoa half beans, the mean RGB values of each two halves were calculated.

2.2.4. Chemical measurement

2.2.4.1. Absorption spectrum of extracts from fermented cocoa beans. Absorption spectra were acquired in a Synergy HT Multi-Mode Microplate Reader (Biotek, Rochester, VT, USA) at 400–500 nm.

2.2.4.2. Free amino acids and fermentation index. Free amino acids from cocoa beans were recovered using an adapted and modified extraction procedure previously described [6,15], which was optimized to remove phenolic compounds in fermented cocoa samples. Fifty milligrams of unshelled and ground cocoa beans were extracted with 1 mL of aqueous hydrochloric acid solution (0.4 mol L^{-1}), containing 5.6% w/v of PVPP to remove phenolic compounds, under constant shaking in a "Movil Rod" for 30 min at room temperature (20 °C). The samples were centrifuged at $2100\times g$ for 10 min at 4 °C and supernatant was stored at −20 °C until analysis. The efficiency in phenolic compounds removal by PVPP was checked through comparison with a sample without PVPP using a rapid microplate high-throughput methodology of Folin-Ciocalteu assay [16]. Free amino acids were determined by a