Sensing Cocoa (Theobroma cacao L.) Beans Fermentation by Electronic Nose System

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Abstract

Fermentation is a very important postharvest process where many processing properties and sensory attributes are developed. However, cocoa fermentation still remains empirical due to its complex mechanisms that evolved many microbiological changes. Some equipment such as HPLC, GC-MS, and near infrared spectroscopy may be useful to study cocoa fermentation, however they are relatively expensive, timing consuming and inaccessible to cocoa farmers. In this study, a machine learning based electronic nose system was developed to determine the fermentation time of cocoa beans. The system achieved a misclassification rate as low as 14.2 % with relatively show time and low cost.

Keyword: cocoa; fermentation; electronic nose; machine learning

1.Introduction

Chocolate is one of the most profitable merchandise of the global confectionary industry. The chocolate market worth 98.3 billion dollars in 2016 and the retail sale of chocolate in US alone is estimated to be 22.4 billion for 2017 (Duncan 2017). Cocoa bean (Theobroma cacao L.) is the major raw material in chocolate products. Globally, the production of cocoa bean was 4.031 million tons in 2016. Consumers are willing to pay more money for better quality chocolate, which creates price gap between mediocre chocolate and fine making chocolate. In most cases, the quality of cocoa bean is pivotal to the value of the final the chocolate products (Afoakwa et al., 2008).

The quality of cocoa bean is influenced by its variety, soil, climate, crop management and mainly by post-harvest processing (De Brito et al., 2001). Fermentation is a prerequisite for the development of cocoa flavor precursors and better processing properties (Hue et al., 2016). During there are many microbial, physiochemical and enzymatic effects that greatly change the properties of cocoa. Some researchers (Biehl et al., 1982; Biehl et al., 1985) have reported that the PH of cocoa beans can influence the formation of flavor precursors by either inhibiting or stimulating the activities of proteolytic enzymes such as endoprotease (Biehl et al., 1982; Biehl et al., 1985). Those proteolytic enzymes transform seed proteins into precursors for Maillard reaction triggered at roasting process (Biehl et al., 1993).

Cocoa fermentation still remains empirical even it has been studied for more than one hundred years. Fermentation conditions and fresh bean qualities are very difficult to control which give rise to beans of inconsistent fermentation quality, which obliges processors continuously to make changes of their formulations (Zhao et al., 2015). The formation of flavor compounds during fermentation involves a successional growth of various species of yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and, possibly, species of Bacillus, other bacteria and filamentous fungi (De Vuyst et al., 2010). In the beginning of fermentation, yeasts transform carbohydrates in cocoa pulp into ethanol and carbon dioxide. In the meantime, LAB converts citric acid and other remaining carbohydrates in the pulp to lactic acid, slightly increasing the pH of cocoa beans (Lefeber et al., 2012). In the following stage, AAB oxidizes the produced ethanol into acetic acid (Camu et al., 2007; Sandhya et al., 2016). The microbial oxidation of ethanol into acetic acid increases the temperature, which kill the seed embryo and diffusing acetic acid inside the beans. The diffused acetic acid disintegrates the cellular membranes inside cocoa beans and triggers enzymatic conversions of substrates in the cotyledon to develop characteristic flavor precursors and color of fully fermented cocoa beans (Thompson et al., 2013). In the last stage, various species of Bacillus grow when the pH of the cocoa bean becomes less acidic and the temperature increases to 40– 50 °C due to the oxidative metabolism of ethanol.

Currently, the standard methods for determining the fermentation degree of cocoa bean is cut test. This method consists in longitudinally cutting and counting the proportion of purple and brown beans on a representative dried sample of 300 beans (Wood and Lass 2008). However, cut test is relatively time consuming and the determination is based on human observations which are inevitable inconsistent and bias. Sensory tests are alternative methods for cut test, however, it is also time consuming and required a well-trained sensory panel. Some chocolate manufacturers and researchers have applied techniques such as gas chromatography-Mass spectrometry (GC-MS) (Grün et al., 2008; Caligiani et al., 2007), High-performance liquid chromatography (HPLC) (Pätzold et al., 2006; Tomlins et al., 1990; Sandhya et al., 2016) and near infrared spectroscopy (Hue et al., 2014) to determining cocoa fermentation degree by mapping the profiles of compounds such as ammonia nitrogen, free amino acids, and volatile compounds. Those methods were reported to be useful, however, those technologies are expensive and difficult to conduct.

Electronic nose is an array of many gas sensor, mimicking the discrimination of the mammalian olfactory system for smells (Persaud and Dodd 1982). Each gas sensor gives a fingerprint response to given odors, and the response pattern of gas sensor can be recognized by certain algorithms and then performs odor identification and discrimination (Arshak et al., 2004). E-nose has been applied to access the qualities of some food materials include sausages (Eklöv et al., 1998), vegetable oils (Hai and Wang 2006), milk (Capone et al., 2001), meats (Rajamäki et al., 2006) and fruits (Saevels et al., 2004). In addition, the applications of e-nose in food quality evaluation, discrimination, and control are also very broad. However, the applications of e-nose in cocoa quality and processing controls were barely reported. Therefore, it is potentially useful to develop a universal, affordable, and fast measuring methods for cocoa bean quality determination.

Artificial neural network (ANN) is computational model used in machine learning, mimicking the cognitive processes of human. Like the human cerebral cortex, a ANN consist of layers of artificial nodes. In the basic model of the ANN, nodes are separated into different layers and connections are built between nodes that are in adjacent layers. The weight is assigned to connection between two nodes. each node calculates all the weighted inputs from connected nodes in the previous layers and processed them by transfer function. The results from the function are transferred to the connected nodes in the next layer. The effects of the synapses are represented by connection weights that modulate the effect of the associated input signals, and the nonlinear characteristic exhibited by neurons is represented by a transfer function. The learning capability of an artificial neuron is achieved by adjusting the weights in accordance to the chosen learning algorithm (Abraham 2005).

In this study, the fermentation of cocoa (Theobroma cacao L.) beans was monitor by self-built electronic nose system. The responses of the e-nose were processed by artificial neural network. The temperature and PH of cocoa beans during fermentation were recorded and cut tests were conducted as reference.

2. Materials & methods

2.1 Cocoa fermentation

75 kg fresh cocoa beans (Theobroma cacao L.) were evenly distributed to 3 Styrofoam coolers (60 × 30 × 30 cm). The three coolers were placed adjacent to each other in a fermentation room with ambient temperatures varied from 20-30 °C. The cocoa beans were turned and mixed every two days.

2.2 PH, temperature measurements

Temperature, PH measurements and cut tests were taken every day (Days 0-7) after the first electronic nose reading was obtained. A thermometer (model EW-94469-40, Cole-Parmer, Vernon Hills, IL) was inserted at three different depths (top, middle and bottom) in each of the three Styrofoam coolers in order to obtain three replicates of readings for each treatment. PH measurements were carried out using an Oakton Acorn series PH meter (model WD-35613-70, Oakton, IL). the testa was separated from the cotyledons and placed in separate ceramic mortars. 10mL of distilled water was added to each and then the mixture was grounded using a ceramic pestle.

2.3 E-nose system

The design of the e-nose is based on Tan and Kerr (2018)'s work with some upgrade. The diagram of the e-nose system is shown in Fig. 1. The system consisted of five major components, including a micro pump (NMP830, KNF, Trenton, NJ), a 3-way solenoid valve (225T031, NR, Caldwell, NJ), an Arduino board microcontroller (Uno, Arduino), e-nose (gas sensors and chamber), and data acquisition system. The e-nose chamber was built from a 10cm χ 10cm χ 5cm nylon box with a 1.5cm thick Teflon top. Sensors alone with their socket were inserted into the top with sensor head inside the chamber. The e-nose had nine gas sensors from Figaro USA, INC (Arlington Heights, IL). The specification of each sensor was summarized in Table 1. The pump is always open during sampling (30s) and cleaning (100s) and closed when e-nose is reacting with gas. The valve alternated its direction to switch the e-nose from sampling model to cleaning model.

The signals (output voltage as a function of time) were collected by three data acquisition boards (Model NI9219, National Instruments, Austin, TX). A program was developed using LabView software (Version 2015, National Instruments, Austin, TX) to collect data from the DAQ. Three characters (relative peak, relaxation time, and rising time) of the responses of each gas sensor were extracted. The 'relative peak' was defined as the output peak value minus the baseline values of each sensor. The 'relaxation time' was defined as the time that the output voltage decreased from the peak value to 80% of its relative peak value. The 'rising time' was defined as the time needed before the responses of each sensor reached its relative peak.

2.4 Artificial Neural Network (ANN) setup

The three characters of each sensor were scaled to 0-1 before serving as training data. ANN training was conducted by neural Matlab network toolbox (R2017a, MathWorks, Natick, MA). There were 60 repetitions at each day of fermentation, of which 50 % repetitions were used for training the ANNs while the rest were used for validation. The scaled target data were 0, 0.13, 0.28, 0.42, 0.57, 0.71, 0.85 and 1, representing fermentation times of 0, 1,2,3,4,5,6 7 days respectively. At the beginning of training, initial weights between 0 to 1 were randomly assigned. Training was done using a backpropagation function, which updates weight and bias values according to the Levenberg-Marquardt optimization. Settings for the routine are shown in Table 2. Hyperbolic tangent sigmoid ("tansig") functions were used for hidden layers and output layers

2.8 Statistical methods

All results presented as the mean and superscript letters which indicated significant differences amongst treatments at the 95% level of confidence by Tukey's HSD. The results were compared by one-way ANOVA using JMP (Pro 13, SAS Institute Inc., Cary NC).

3. Results and discussion

3.1 Temperature and PH variation during cocoa fermentation

The trendlines in Fig. 1 and Fig. 2 shown the change of temperature and PH respectively during fermentation. Generally, in the fermentation process, the temperature varied between 28 to 50 °C, and the peak temperature was observed in the fourth day of fermentation when microbial action on producing ethanol and acids was about to over. The temperature of cocoa beans changes in the fermentation process was due to heat generated activities of microorganisms which transformed the substances in pulp into alcohol, carbon dioxide, organic acid and other volatiles.

The PH in testa increased from 3.6-4.5 during fermentation, however, the PH in cotyledon during drastically from 6.3 to 4.5. The observations were due to the organic acids including acetic, oxalic, phosphoric, succinic, and malic acids produced by several yeasts, penetrating the testa and gradually absorbed by the cotyledon.

3.2 Fermentation time determination by ANN

Table 3 shown the performance of the trained ANN. 14.2% overall misclassification rate was achieved. The ANN misclassified 33.3% of the verification samples from the first fermentation day. This was because cocoa fermentation didn't produce enough volatiles to reach the thresholding sensitivity of some gas sensor in the first day. In addition, we cocoa bean generated high content of water vapor in the headspace, camouflaging the volatiles. In addition, ANN may scarify the accuracy for samples from the first day in order to achieve high overall performance.

4. Conclusion

The ANN based e-nose system was proved to be successful in determining the fermentation degree of cocoa bean. Compared to traditional methods, the proposed method is much cheaper and fast. However, to make more powerful system that works for other cocoa beans, a massive data library need to be established to provide enough number of training data.

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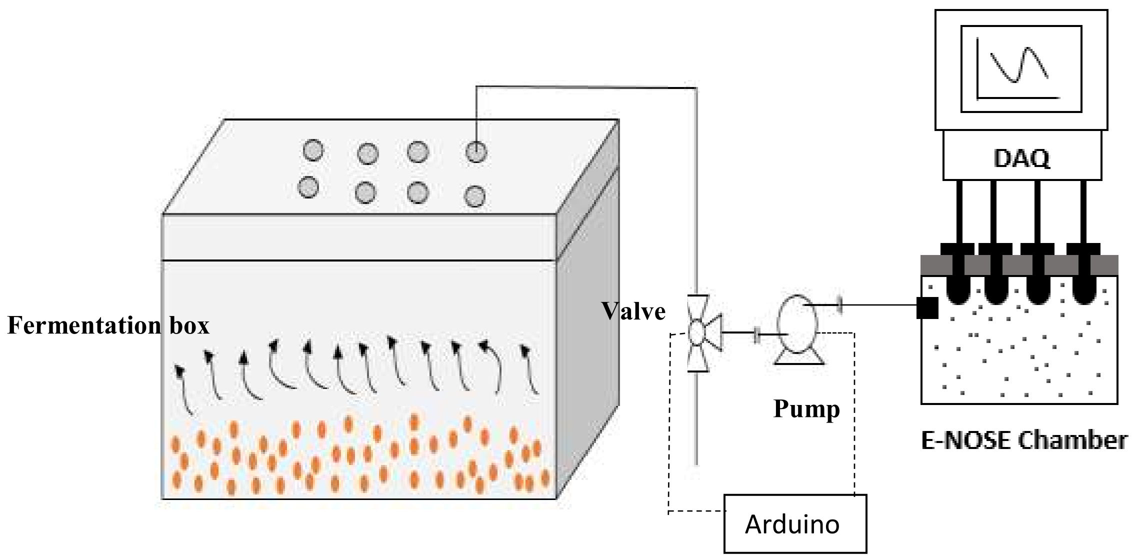

Fig. 1: The diagram of the e-nose system for cocoa fermentation

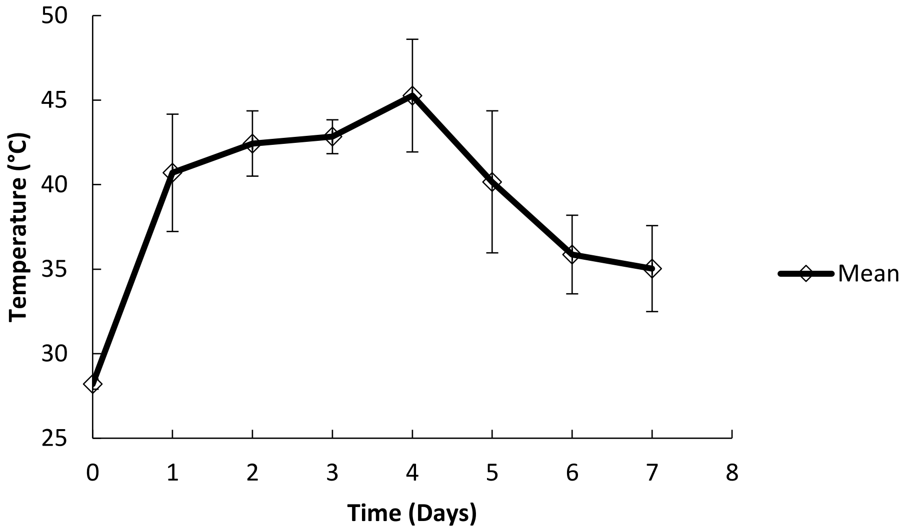

Fig. 2: The mean Temperature for the cocoa beans in the process of fermentation

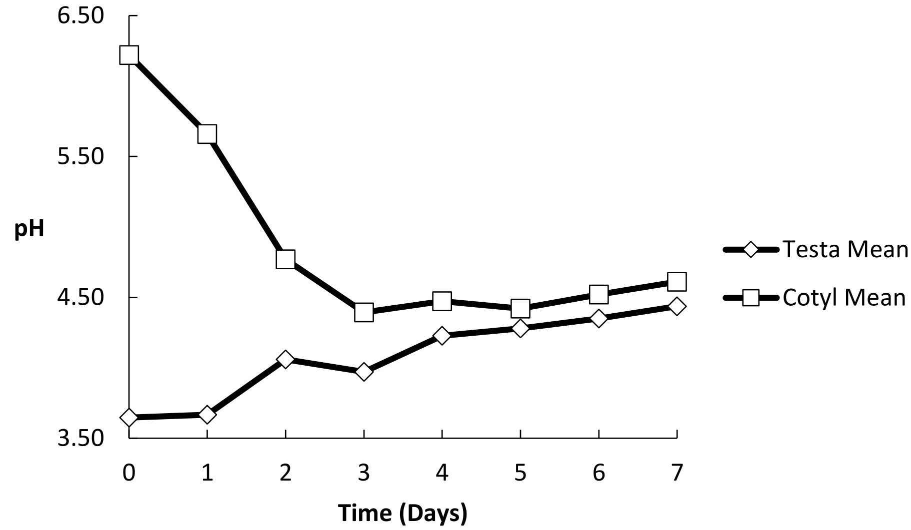

Fig. 3: The mean pH for the cocoa testa and cotyledon as a function of time in the fermentation

Table 1: features and specification of the gas sensors being used for e-nose system

Sensors	Features & specification
TGS821	Hydrogen
TGS 826	High sensitivity to ammonia and ethanol
TGS813	High sensitivity to methane, propane, and butane
TGS2602	High sensitivity to VOCs and odorous gases
TGS822	High sensitivity organic solvent vapors such as ethanol
TGS2610	High sensitivity to LP and its component gases (e.g. propane and butane)
TGS2620	High sensitivity to alcohol and organic solvent vapors
TGS830	R11, R113, other halocarbons
TGS823	High sensitivity to organic solvent vapors such as ethanol

Table 2: Initial settings for training artificial neural network (ANN)

Mu	Mu-dec	Mu-inc	Iterations	Validation check
0.001	0.1	0.1	1000	5000

Table 3: Performance of ANN for classify the fermentation time of cocoa

Fermentation time (day)	Misclassification rate (%)
0	33.3
1	16.7
2	6.7
3	13.3
4	13.3
5	16.7
6	6.7
7	6.7
Overall	14.2