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OPTIMIZATION OF THE COCOA BEANS (*Theobroma cacao* L.) FERMENTATION – THE IMPACT OF A PRE-FERMENTATION BEANS EXPOSURE ON FERMENTATION TIME AND FINAL FLAVOR

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ABSTRACT

Cocoa beans (*Theobroma Cacao* L.) are the main ingredient for chocolate making. The most important processing step of the bean is the fermentation. Indeed, the aroma precursors necessary for the flavor development are formed at that time. Its quality is then fundamental for a good quality chocolate. The purpose of this study was to improve fermentation quality of cocoa beans via an adjustment of the pulp quantity and the inoculation by fruit flies (*Drosophila Melanogaster*) prior to fermentation. The impact on the physical-chemical quality of the fermentation (duration of the process and homogenization) and/or the sensory quality of the beans was then assessed. Two distinct Trinitario varieties of beans were tested, older Imperial College Selections (ICS) and newer Trinidad Selected Hybrids (TSH). The beans were exposed in both the sun and shade (50% light) for different times (5h and 24h), to be subject to a slight drying in order to decrease the pulp quantity by on one hand, and an inoculation by *D. Melanogaster* on the other hand and then each exposure level was fermented for 6 days. For each of the three sets of experiments, fermentation temperature (°C) and acidity (pH) of testa and cotyledon monitoring was measured during every day of the fermentation, as well, as a cut-test and cocoa liquor tasting after drying, from days 3, 5 and 6 of the fermentation.
All the results show that beans exposed to the sun did not ferment properly. However, beans exposed to the shade were better fermented than the others. No significant difference was observed between the two varieties used and the natural inoculation of the beans by *D. Melanogaster* in the shade improves the quality of the fermentation. Therefore for these varieties, increasing the quantity of pulp had a positive effect on the bean quality, which fermented better and had a more interesting sensory profile.

Keywords: cocoa, fermentation, exposure, fruit flies, pulp reduction

INTRODUCTION

Cocoa beans (*Theobroma cacao* L.) are the principal ingredient for chocolate production (Beckett, 2008). Three main varieties of cocoa are cultivated and possess different characteristics: the Forastero, referred as "bulk cocoa" due to its strong and neutral cocoa flavor; the Criollo, having a rich balanced fruity/floral flavor but delicate to grow as the tree is sensitive to diseases; and the Trinitario, which is a natural hybrid between the two previous one. It has the characteristic to be more resistant to diseases, but possesses aromatic flavor (Ferrão J.E.M., 2002). Recent research, based on genetic, highlighted a more precise classification of 10 different varieties: Marañon, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Purús, Nacional and Guiana (Motamayor J.C. and al., 2008)
When the beans are collected, each stage of the transformation process has an impact on their properties and quality. Among these steps, 3 are essential for the flavor development: the fermentation and drying, the roasting and the conching (Afoakwa, 2008; Owusu M., 2011). A good fermentation is crucial to obtain a good quality chocolate, as a large number of chemical reactions occur, allowing the development of essential compounds to produce the aromas (De Vuyst & al, 2010) during the further steps.

Beans have to be inoculated by microorganisms in order to obtain a proper fermentation and a development of chocolate flavor (Ho V.T.T. and al., 2014). As long as the pods are not damaged, the internal part is considered sterile (Ostovar & Keeney, 1973; Watson R. & al, 2013). After the pod cracking, beans are naturally inoculated by various microorganisms that come from the pod surface, the hands of the workers, the knives used, and other materials being in contact with them at one point before the fermentation (Schwan F. & Fleet H., 2014). The quantity and diversity of microorganisms contributing to the fermentation process influences good processing and quality (Ostovar & Keeney, 1973). Indeed, a succession of different microbial activities occurs: mainly yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) (De Vuyst & al, 2016). The process starts during the first 24 to 48h after opening the pods (Ho V.T.T. and al., 2014). The low pH from 3 to 3.5 and the

anaerobic environment favor the activity of yeasts and LAB (Watson R. & al, 2013). They mainly transform the sugar of the pulp (about 9 to 13%) (Lima L. and al., 2011) to ethanol. This action allows a modification of the micro-environment: the pulp is degraded and flows, the environment becomes aerobic and the pH increases to 4.5 (Carr J.C., 1982). After 48 to 96h of fermentation, AAB are then in proper condition to grow and degrade the ethanol into acetic acid, which enters the cotyledons. The resulting consequences are the decrease of the bean's pH, the death of the germ, and more important the start of important biochemical reactions forming aromas' precursors (namely free amino acids and peptides from enzymatic degradation of the storage proteins, and reducing sugars from enzymatic degradation of sucrose), necessary for the chocolate flavor development in the further steps of the transformation process and from which typical aromas can be formed (Afoakwa and al., 2013; Lagunes-Gálvez S. and al., 2007; Watson R. & al, 2013). More than 600 flavor compounds have been identified in cocoa products (Crafack and al., 2014)

Cocoa bean fermentation is a natural and spontaneous process (De Vuyst & al, 2016) which is difficult to standardize and homogenize, but is crucial for the development of good quality beans. In order to improve the quality of the fermentation and the final flavor of the chocolate, this study investigated a pre-conditioning of the beans through an exposure to the fresh air prior to fermentation whilst varying the following parameters: the variety of beans (old variety (Imperial College Selections - ICS and new variety Trinidad Selected Hybrids - TSH supposed to have more pulp), the exposure place (shade and sun) and the exposure time (5 and 24h).

Impacts on fermentation quality and final flavor was tested by investigating exposure to the shade to determine the influence of the inoculation of the fresh beans by fruit flies (*D. Melanogaster*); whilst exposure to the sun was done to determine the impact of the pulp reduction through a pre-drying by the heat of the sun.

MATERIALS & METHODS

Three experiments were carried out following the same experimental protocol, but with some variations, like the climatic conditions, as detailed in the Table 1 below.

	Shade 5h	Sun 5h	Shade 24h	Sun 24h
Exp.1			Missing data : day 5 and 6	Missing data : day 6
Exp.2		Rain: no data		Rain: less exposure
Exp.3	Watering	Strong sun	Watering	Strong sun

Table 1: Variations of parameters during sun and shade exposure between the 3 experiments.

1. Manipulations

Exposure
Beans were collected in two different estates: San Pedro Estate, Gran Couva, Trinidad for the ICS variety, and Monserrat Cocoa Farmer's Cooperative Society Limited, Gran Couva, Trinidad for the TSH variety. They are weighed in batches of 11 kg and then spread on tarpaulins for exposure to the sun and on the shade (in a green-house).

Fermentation & drying
The beans were fermented for 6 days in the Cocoa Research Centre's mini fermentation facility after their respective exposure times in Styrofoam coolers covered with banana leaves and jute bags. Turning was done on days 3 and 5 of the fermentation.

Drying
This was carried out in a tunnel dryer fashioned from a modified greenhouse dedicated to this activity on the rooftop of the Cocoa Research Centre. Beans were placed in wood trays and turned regularly to avoid mold development until a moisture content of 6 - 7 % (occurring between one and two weeks of drying). Final moisture contents were assessed by a digital moisture meter (Burrows, model DMC 700, series 8211/01382/122) calibrated for cocoa. Dried, beans were placed in snap seal bags and stored at a room temperature of approximately 22°C.

Cocoa liquor production
Cocoa liquor production followed a slightly modified method of Sukha et al (2008). As a summary, 165 g batches of sorted beans from the different experiments were roasted in a forced-air oven (Shel Lab 1350 FX, Sheldon manufacturing, USA) for 30 min at 140°C. After cooling they were broken and winnowed via a John Gordon cocoa breaker and winnower (Crompton controls series 2000, Commodity Processing System, UK) and fine cleaned by hand to remove any residual germ and small bits of shell. Pre grinding was done in a Magic Bullet (MB1001B, US) and added gradually to a stone melangeur (Cocoatown ECGC12SLTA, USA). The grinding was done for 90 minand the resulting liquors stored in sterile specimen containers at -18°C for at least 2 weeks prior to sensory evaluation.

2. Evaluations

Temperature
The temperature was measured daily with a thermometer (model EW-94469-40 Digi-sense, Cole-Parmer, US) every day at the same hour, at three points in the fermentation mass.

pH
Nine beans were sampled daily from the same three points where temperature was measured and the beans were cut in half with secateurs. The testa and the cotyledons of these beans were separated (in sets of three to give three repetitions of measurement) and placed in ceramic mortar bowls. Distilled water (10mL) was added to both the testa and cotyledons and macerated with a pestle. The pH was measured with a pH-meter (model WD-35613-70, Oakton, US).

Cut-test
A sample of 30 randomly selected dried beans were cut in half along their longitudinal axis and assessed for fermentation degree. Depending on their color, appearance (visible fissuring) and smell, using a cut test chart (Sukha and Rohsius, 2004) they were categorized into: over fermented, slightly over fermented, well-fermented, partly purple partly brown, purple turning brown, purple, slaty, moldy, moldy and infested. The percentage of fermented beans is made by grouping 3 categories: "well-fermented", "partly purple partly brown" and purple turning brown". Cut-tests were done on beans fermented for 3, 5 and 6 days.

Sensory analysis
Descriptive analysis of cocoa liquor was based on a cocoa liquor flavor wheel (37 descriptors) according to CAOBISCO/ECA/FCC, (2015) with two other parameters evaluated: Global quality of the liquor and Uniqueness.
A trained sensory panel of 5 persons from the Cocoa Research Center carried out the flavor evaluations with 3-digit coded samples that were randomized in a factorial statistical design that incorporated hidden reference liquors to check the consistency and the uniformity of the panel.

The evaluation was standardized with samples being heated for 15 min and analyzed during 5 min: about 3 min to taste and rank the product, and 2 min to clean the palate with hot water and neutral crackers (Carrs Table Water Crackers, UK).

3. Results expression

The statistics carried out are variance analysis (ANOVA), with the statistic software NCSS. A LSD (Least Significant Difference) is used to separate and compare the means. A significant difference is accepted with a margin of error of 5% (p-Value < 0,05). For the sensory analysis, a principal component analysis (PCA) is realized via the R software (version 3.3.3).

RESULTS & DISCUSSION

1. Physical-chemical analysis

For the 3 experiments, both ICS and TSH varieties show the same evolution of the fermentation temperature indicating that the variety has no effect on the fermentation quality. Beans subjected to pulp reduction (exposed to a strong sun for 24h) do not ferment and the bean mass temperature does not exceed 33.5°C. This is also confirmed with the cut-test, where the fermentation doesn't exceed 20% (figure 1).

However when the temperature raises more than 44°C, beans ferment properly and is also reflected by the evolution of the pH trends which decreases for the cotyledon and increases for the testa until they cross at one point usually coinciding with the end point of fermentation. The pH of the beans exposed 5h to the shade (experiment 3) increases from 3.92 (Day 0) to 5.59 (Day 6) for the testa and decreases from 5.48 to 4.97 for the cotyledon. The two pH values cross at Day 5, day where the cut-test result showed a good fermentation of 96.7%.
It was also noticed that the faster the temperature increased, the faster the beans fermented.

Beans exposed for 5h ferment faster and better than those exposed for 24h in both exposure regimes (shade and sun). This corresponds to beans whose pulp quantity decreased only slightly during the exposure, depending on the climatic conditions of the different experiments. Those results are in agreement with the literature (Schwan F. et Wheals A.E., 2010). Indeed, it is known that a decrease of the pulp quantity from 10 to 20% allows a faster fermentation, without impacting the sensorial quality of the final chocolate. An important pulp reduction inhibits the fermentation and a pulp augmentation slower a little bit the fermentation.

2. Sensory analysis

The most relevant sensory attributes for those samples, analyzed, included cocoa flavor, browned fruit, fresh fruit, floral, acidity,

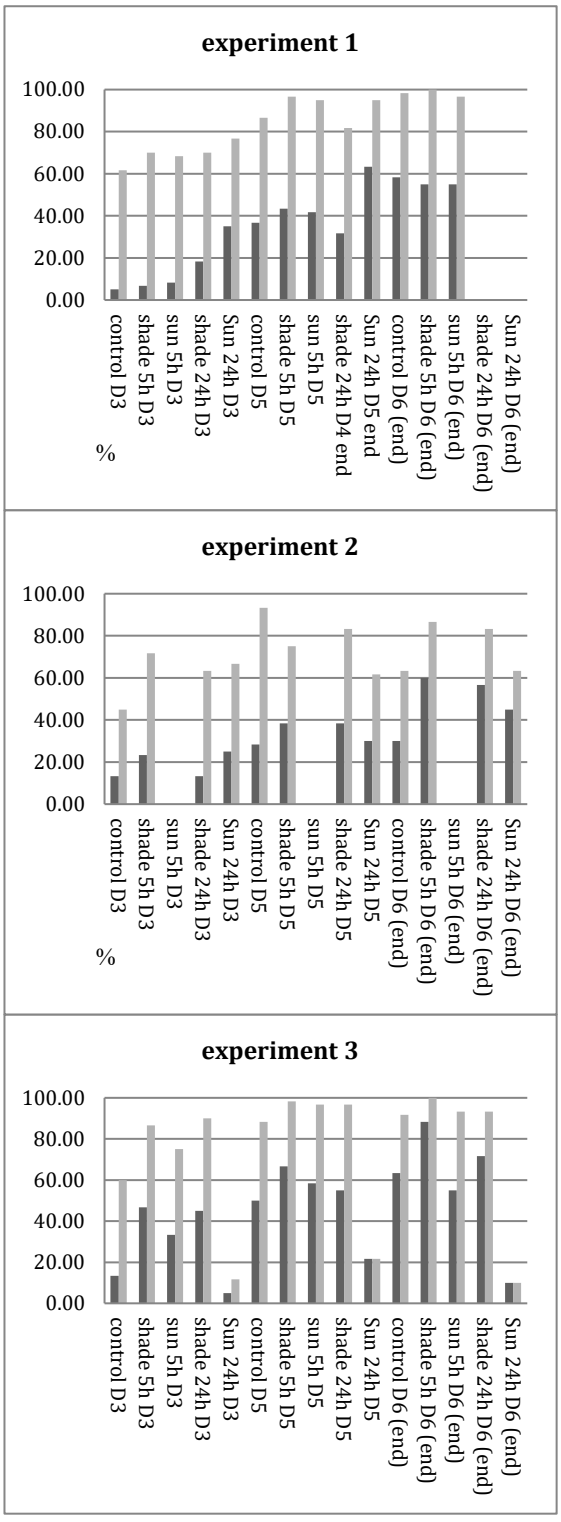

Figure 1 : Cut test results showing the evolution of the well-fermented beans ( ) and the fermented beans ( ) depending on the parameters and the days of fermentation

astringency, bitterness, global quality and uniqueness.

Variety

TSH variety beans are perceived more astringent. However, the ICS variety possesses stronger flavors (fresh fruits, floral) and so a better sensory quality.

%

Exposure place

Inoculation through fruit flies

An exposure to the shade increases the overall presence of fruity aromas and intensity in the cocoa liquor (Table 2), meaning that an inoculation of the beans prior to the fermentation by *D. Melanogaster* has a positive impact on the flavor quality. Indeed, for the experiments 1 and 2, the cocoa, fresh fruit and brown fruit aromas are perceived stronger.

We know that the diversity of the microorganisms is necessary to obtain a good fermentation and chocolate quality (De Vuyst L., Weckx S., 2016). The different yeasts, LAB and AAB secrete different components, which will constitute thereafter the chocolate aromas (Crafack M. et al, 2014). Based on several studies (Bainbridge & Davies, 1912; Ostovar & Keeney, 1973), the hypothesis has been made that insects, and more especially the fruit flies *D. Melanogaster* are responsible of the transfer of the microorganisms of the environment to the beans, and so are responsible for a good inoculation. Ostovar & Keeny (1973) showed that fruit flies carry different species of yeasts, LAB and AAB, all of them found thereafter in the beans in fermentation. It supports the hypothesis concerning the fruit flies' role, that contribute to the input of a microbial diversity and quantity during the beans inoculation time, improving the quality of the fermentation, a successful fermentation being dependent of a great microbial quality (quantity and diversity) (De Vuyst L. & Weckx S., 2016). This was confirmed with the results of this sensory evaluation, where beans exposed to fruit flies had their sensory quality significantly increase.

Pulp reduction

An exposure to the sun (slight pulp reduction) doesn't make any variation on the sensory profile of the beans. However, a strong pulp reduction (experiment 3) increases the bitterness and astringency of the beans, greatly decreasing the sensory quality.

An exposure to the sun also decreases acidity, regardless to a slight or strong pulp reduction. This result is consistent with a study conducted by Afoakwa et al. (2012), showing that removing portions of cocoa bean pulp contributes to less acid production during fermentation, leading to less acid beans (Afoakwa E.O. et al., 2012).

Surprisingly, increasing the pulp quantity (watering of the beans for the 3rd experiment) gives the best cocoa liquor sensory profile.

Exposure time

Beans exposed for 5h in general have a stronger presence of cacao, fresh fruit, browned fruit and floral aromas, increasing their quality. On the contrary, beans exposed for 24h develop astringency, bitterness and moldy flavors.

Based on these results, it is recommended to expose the beans for 5h.

Fermentation time
It would appear that for these varieties (ICS and TSH) in these experiments, the best fermentation time was 5 days. The ideal range of fermentation time varies depending on the cocoa variety. Trinitario and Nacional usually ferment between 3 and 5 days (Barel M., 2013).

	A: Variety			B : Shade.Sun			C : Hours			D: Days		
	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
Cacao					shade	shade			5h			5 days
Acidity						shade	5h	null	null		3 days	3 days
Bitterness						sun		24h	24h			
Astringency		ICS	TSH			sun			24h			
Fresh fruits			ICS	shade		shade	5h	5h	5h	5 days	3 days	3 days
Browned fruits					shade	shade			5h			
Floral			ICS				5h	5h	null		3 days	3 days
Moldy						shade	24h			6 days	6 days	6 days
Global quality			ICS			shade	5h		5h	3 days		3 days
typicité			ICS		shade	shade	5h	5h	5h	5 days		3 days

Table 2: Summary of the different statistical results (ANOVA) for each sensory attribute depending on the factors and experiments. The parameter that gives the significantly (p-Value < 0,05) highest result is written in the case.

CONCLUSION

No differences have been found between ICS and TSH varieties regarding the fermentation quality (and no differences on the pulp quantity has been found during the manipulations), but the ICS beans (old variety) have a better sensory profile.

During this study, it has been found that an exposure to fruit flies *D. Melanogaster* has a significant positive impact on the sensorial quality of the beans. A slight reduction of the pulp allows a faster fermentation, but a larger pulp reduction or pre-drying has a negative impact and inhibits entirely the fermentation.
This study also highlighted that increasing the quantity of pulp has a surprisingly positive effect on the beans quality, which ferment better (even if slower) and has a most interesting sensory profile. This is different to what is desirable for other varieties like CCN 51 in Ecuador where there is a significant pre drying stage to reduce pulp volume followed by fermentation and drying.
A five-hours exposure allows a better and faster fermentation, as well as an increase in the sensory quality, whereas 24h of exposure decreases the sensory profile of the beans.

The recommendation emphasized is to expose the beans to the shade for 5h, followed by fermentation of approximately 5 days.

It would be useful to repeat the experiment in order to confirm this action of fruit flies on the beans sensory quality, as well as the effect of increasing the pulp volume prior to fermentation.

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