Colegio de Postgraduados

Effect of spontaneous micro-fermentation on the physicochemical characteristics of *Theobroma cacao* L. beans

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ABSTRACT

Objective: To evaluate the physicochemical characteristics of cacao beans during the spontaneous fermentation process in tepemixtle wooden boxes of three different sizes.

Design/Methodology/Approach: Trinitarian-type cacao (*Theobroma cacao* L.) fruits were collected in a commercial orchard. The beans were fermented in boxes with the following operating conditions: volume of cacao with pulp, box size, a six-day period, and uncontrolled temperature and pH. The experiments were carried out in triplicate and an analysis of variance and a multiple comparison of means were performed with the Tukey test ($p<0.05$) using the R program.

Results: The parameters evaluated had the same behavior in the 4 kg box than in the 25 kg box and the 60 kg box, which indicates that the physicochemical and quality characteristics of cacao beans are not impacted by the decrease in the box size. Therefore, smaller boxes are viable for small-scale fermentations.

Study Limitations/Implications: Only cacao beans from the Trinitarian genetic group were evaluated.

Findings/Conclusions: Small-scale fermentations can be an option for the evaluation of varieties when the beans must be fermented for the sensory analysis of their qualitative attributes. This micro-fermentation methodology can also be incorporated into genetic improvement programs or the evaluation of the processes aimed at their optimization.

Keywords: color, quality, small scale.

Citation: Martinez-Valencia, B. B., Carrizales-Mérida, J. E., Caballero-Pérez, J. F., & Solís-Bonilla, J. L. (2024). Effect of spontaneous micro-fermentation on the physicochemical characteristics of *Theobroma cacao* L. beans. *Agro Productividad*. https://doi.org/ 10.32854/agrop.v17i5.2794

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 15, 2024.
Accepted: April 28, 2024.
Published on-line: May XX, 2024.

Agro Productividad, 17(5). May. 2024. pp: 57-65.

INTRODUCTION

Chocolate, cocoa, nibs, and other products are result of the fermentation of cacao (*Theobroma cacao* L.) beans. The flavor of cacao depends on multiple intrinsic and extrinsic factors that are involved before and after its harvest (Engeseth and Ac Pangan, 2018). Cacao beans are composed of an embryo and two cotyledons, they are surrounded by a mucilaginous pulp that can vary depending on their origin, and they have a bitter and astringent taste after being harvested (Dominguez-Perez *et al.*, 2020; Alexander J. Taylor, 2022; Rawel *et al.*, 2019; Santander *et al.*, 2019). Cacao pulp is made up of sugars (glucose, fructose, and sucrose), citric acid, and pectin, which serve as substrates for the microorganisms involved in fermentation. The high polyphenol content of cacao beans causes an astringent, bitter, and unpleasant flavor, which decreases to 5% during the fermentation process (Afoakwa *et al.*, 2008; Dominguez-Perez *et al.*, 2020). Therefore, cacao beans must undergo a post-harvest processing that comprises the fermentation, drying, and roasting stage to develop precursors that provide desirable aroma and flavor profiles (Rawel *et al.*, 2019; Dominguez-Perez *et al.*, 2020).

Spontaneous fermentation accomplishes three main tasks: (i) decomposition and reduction of the pulp surrounding the beans; (ii) formation of volatile organic compounds, which reduce bitterness and astringency; and (iii) hydrolytic reactions within the cotyledons (Amoa-Awua, 2015).

Traditionally, the fermentation method implemented by producers involves two transformation phases: (1) anaerobic and (2) aerobic. The length of these phases and the frequency of aeration of the mass in the aerobic phase varies depending on the producer (Escobar *et al.*, 2019). All the biochemical reactions that can take place inside the beans in both phases are determined by the action of microorganisms, whose biocatalytic activity generates physical and chemical transformations in the cacao beans. These microorganisms are significantly influenced by the transfer of oxygen through the aeration of the cacao bean mass, the transfer of the mass from the acids and ethanol (metabolites produced by microorganisms) to the bean, and the heat transfer caused by the exothermic reactions; together, these phenomena modulate the formation of flavor precursors (Santander Muñoz *et al.*, 2020). This process is conventionally called spontaneous fermentation and is carried out by cacao producers.

Nowadays, most cacao is fermented spontaneously by producer cooperatives as an uncontrolled and non-industrialized bioprocess (John *et al.*, 2020, Rottiers *et al.*, 2019). A prolonged process can produce unpleasant flavors, as a result of the increase in bacilli and filamentous fungi (Moreno-Zambrano *et al.*, 2018; Mota-Gutiérrez *et al.*, 2018). Fermentation takes place for 5 to 7 days, depending on the variety and the climate of the place (Schwan and Fleet, 2014; Schwan and Wheals, 2004). Overall, spontaneous cacao fermentations take place in approximately 100-kg wooden boxes, in containers made from some unusual materials (such as buckets or plastic trays), or in piles on the ground. Each producer cooperative has its own practices and preferred styles, which differ by region, culture, and fermentation, drying, and roasting times (Levai *et al.*, 2015). Nevertheless, cacao fermentation remains a successful strategy for the improvement of product quality (J. Taylor *et al.*, 2022). However, most small-scale producers in southern Chiapas, whose main product is chocolate, do not ferment the cacao beans; consequently, they must add three times more sugar to reduce the bitter and astringent taste of the washed cacao bean. This problem is caused by the producers' lack of knowledge about the fermentation processes and the small size of their plots. Some cooperatives work with 100 kg of cacao, which could hardly be fermented in such plots.

Meanwhile, breeding programs that use individual plants of the varieties under study must ferment the grains for the sensory analysis of their qualitative attributes (Clapperton *et al.*, 1994). Often, only small quantities of cacao beans are available in evaluation, validation, or conservation plots (approximately 3 to 4 kg per plant). Micro-fermentation methods are particularly useful to research fermentation process variables (*e.g.*, temperature, pH, moisture), as well as to identify microorganisms with biotechnological potential. These types of processes facilitate the evaluation work, because they do not require large quantities of cacao. Therefore, the objective of this research was to evaluate the physicochemical characteristics of cacao beans during the spontaneous fermentation process in *tepemixtle* (*Ocotea veraguensis* (Meisn) Mez) wooden boxes of three different sizes.

MATERIALS AND METHODS

Raw material

Cacao fruits from the Trinitarian genetic group were harvested ripe, without mechanical or biological damage, in the plots of producers from Tuzantán, Chiapas, Mexico. They were subsequently transferred to the post-harvest laboratory facilities, where they were washed with water and sprayed with 70% alcohol, before they were opened and the cacao beans with pulp were removed.

Fermentation and drying

The beans with pulp were fermented under a completely randomized design, using *tepemixtle* wooden boxes of three sizes. Box 1 measured 15×15×15 cm and had a capacity of 4 kg; box 2 measured 30×30×30 cm and had a capacity of 25 kg; and box 3 measured 45×45×45 cm and had a capacity of 60 kg. The boxes were covered with a black polyethylene sheet and tied with rope to avoid temperature losses. The operating conditions over the course of 6 days were uncontrolled temperature and pH. Sampling was always carried out at the same time (every 24 h) for each fermentation time (24, 48, 72, 96, 120 and 144 h), the mass was turned over and a 50 g sample was extracted to carry out the physicochemical analyses. The evaluations were carried out in triplicate.

After fermentation, the cacao beans were spread on wooden trays for drying, moved approximately every 4 h each day to facilitate evaporation, until they reached 7% water content. Samples were stored in hermetically sealed containers.

Fermentation monitoring

During fermentation, the temperature was measured every 24 h, placing the thermometer at half the height of the box and taking data at five points. The following data were likewise recorded for the cacao beans with pulp: change in pH (AOAC 981.12), total titratable acidity (AOAC 939.05), moisture, and ash (AOAC 972.15).

Moisture determination

Three g of bean with pulp were weighed and placed in a Yamato® convection drying oven for 24 h at 60 °C. Subsequently, they were weighed again and the moisture content of the grain and pulp was determined by gravimetric analysis.

Determination of oil content

To determine the oil content, 3 g of dried and ground cacao beans were weighed and placed in a cartridge to start the extraction process. The Soxleth method was followed

for this purpose, using hexane for 4 h at 70 °C. Subsequently, the excess hexane was removed with a Heidolp® rotary evaporator and the oil sample was poured in a flask and subsequently placed in an oven at 60 °C for 24 h to eliminate hexane traces. The oil content of the sample was determined by gravimetry. The experiments were carried out in triplicate. The analysis of variance and multiple comparison of means were carried out with the Tukey test ($p \leq 0.05$), using the R program (Enio *et al.*, 2014; R Core Team, 2018).
2.6 Colorimetric determination

The color of the cacao beans was observed throughout the process. Color measurements were taken from three experimental units, from which three cacao beans were extracted at random. A longitudinal cut was made to examine and grade their internal state (Chire *et al.*, 2016). The L*, a*, and b* readings were recorded using a colorimeter. The ΔE* color changes were calculated by the equation:

$$\Delta E^* = \left[(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2\right]^{1/2}$$

In each experimental unit, the average color (L*, a*, and b*) was measured every 24 h throughout the fermentation process.

Statistical analysis

All analyses were performed in triplicate. An analysis of variance and a Tukey's mean comparison test were applied to the results with the R statistical program (Enio *et al.*, 2014; R Core Team, 2018).

RESULTS AND DISCUSSION

Physicochemical characterization of cacao beans during the spontaneous fermentation process

Figure 1 shows the behavior of temperature and pH of the three box sizes throughout time. The initial average temperature of spontaneous fermentation was 32 °C and gradually increased until it reached its maximum level at 72 h: 40.3 in box 1, 42.6 in box 2,

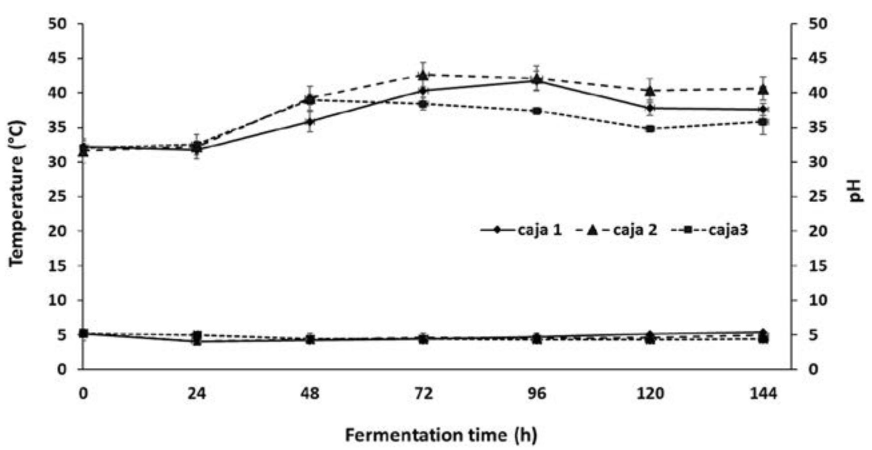

Figure 1. Behavior of temperature (°C) and pH of grains with pulp, during 6 days of spontaneous fermentation, in three wooden boxes of different sizes.

and 38.4 °C in box 3. Although the three boxes behaved similarly during the spontaneous fermentation process after 72 hours, box 1, despite having the smallest dimensions, reached higher temperatures than box 3. The constant increase in temperature may be associated with the release of heat throughout the whole process. This release may be caused by the conversion of the available fermentable substrate (sugars) into the desired metabolite (Melo *et al.*, 2020). With an adequate fermentation, the temperature of the bean mass should reach 45 to 48 °C in approximately 72 h (Melo *et al.*, 2020). The temperatures throughout the process favor the production of ethanol and the death of the bean embryo (Crafack *et al.*, 2014). The production of ethanol allows the succession of microorganisms (such as acetic acid bacteria), responsible for oxidizing ethanol into acetic acid. According to García *et al.* (2019), low temperatures during fermentation can be caused by the type of bioreactor material and the amount of grain with pulp. These authors recommend filling the fermentation boxes to the brim to avoid heat losses.

In the three box sizes evaluated, a stable pH was observed, with similar behavior during the sampling days. The initial pH was 5.17±0.057 for box 1, 5.2±0.01 for box 2, and 5.27±0.057 for box 3. The pH decreased as the days of fermentation increased, although box 1 recorded a slight increase towards the end of the fermentation (5.42±0.01). This phenomenon can be correlated with the production of organic acids (lactic acid, acetic acid, citric acid, among others) by fermenting microorganisms (Balogu *et al.*, 2014). These organic acids decreased the pH of treatment 2 (box 2) and treatment 3 (box 3), which could potentially vary the microbial profile and the population dynamics of the fermentation system and possibly prevent the microorganisms from generating the appropriate acids and conditions that enable them to enter the grain (Balogu *et al.*, 2014). Santos *et al.* (2020), M. D. P. López *et al.* (2019), and Balogu *et al.* (2014) recorded a dynamic pH range (6.64-3.52), which was similar to the ranges reported in this study. The variations observed are perhaps due to the different treatments, geographical areas, and varieties of cacao beans.

Figure 2 shows the dynamics of the oil content and ash content throughout time. The oil content started at 36.45±0.165 in box 1, 40±0.09 in box 2, and 39.22±1.15 in

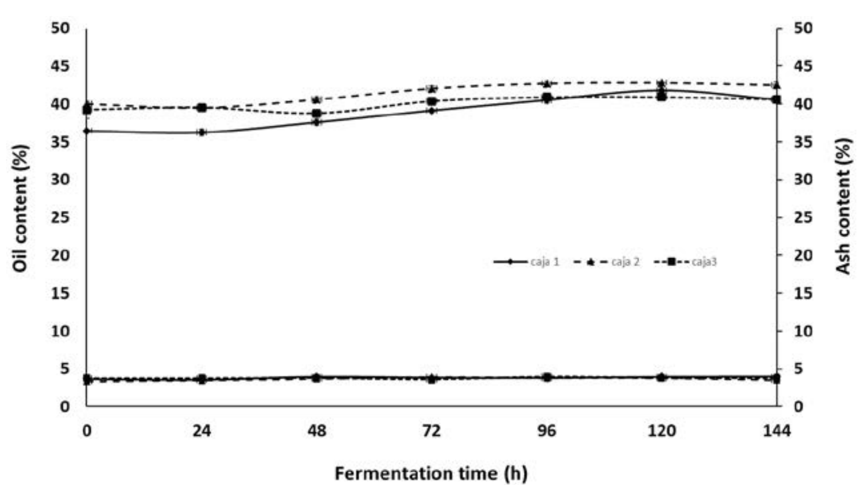

Figure 2. Behavior of oil (%) and ash (%) content, during 6 days of spontaneous fermentation, in three sizes of wooden boxes.

box 3. Throughout the days of fermentation, the three treatments had a similar behavior and had a slight increase in their oil content, resulting in 40.57±0.2362 in box 1, 42.49±0.358 in box 2, and 40.62±0.158 in box 3 at the end point of fermentation (144 h). In relation to this variable, Guehi *et al.* (2007) mention that the fat content of cacao is often quantified and assumed to be stable based on two facts: 1) that, with the exception of lipase activity, fermentation only affects hydrophilic compounds or bonds; and 2) the low risk of oxidation of cacao butter, as a consequence of the low content of unsaturated free fatty acids. This oxidation stability has been confirmed during the shelf life of cacao butter (Hu and Jacobsen, 2016). However, fermentation leads to modifications such as temperature increase, alcohol formation, grain acidification, or modification of enzymatic activities (that can lead to lipid degradation), enzyme breakdown, hydrolysis, or oxidation. Regarding modifications to fats during fermentation, Ndife *et al.* (2013) described a decrease in fat content, while Krähmer *et al.* (2015), Servent *et al.* (2018), and K.T. Dewandari *et al.* (2021) reported an increase in fat content. In terms of plant tissue, lipases are also known to degrade triglycerides, while the optimal pH of cacao lipase activity is reached during cacao fermentation, due to the production and spreading of acetic acid (Servent *et al.*, 2018).

Meanwhile, the ash content showed a stable behavior during the fermentation of the three boxes, with a slight increase. The starting values were 3.67±0.40 in box 1, 3.34±0.11 in box 2, and 3.76±0.31 in box 3. Finally, after 144 hours of fermentation, the final values were 3.93±0.163 in box 1, 3.54±0.38 in box 2, and 3.78±0.03 in box 3. This change is perhaps due to the formation of bioactive compounds that are triggered during the spontaneous fermentation process (K.T. Dewandari *et al.*, 2021).

The moisture percentage of fresh beans with pulp does not have statistically significant differences ($p \leq 0.05$) between treatments, during the 48 h of fermentation. Box 3 and boxes 1 and 2 have differences at 72, 96, and 120 h (Figure 3). Furthermore, at the end of fermentation, a 16.83%, 23.68%, and 16.60% moisture loss can be observed in box 1, box 2, and box 3, respectively (Figure 3). Similar results were obtained by García *et al.*,

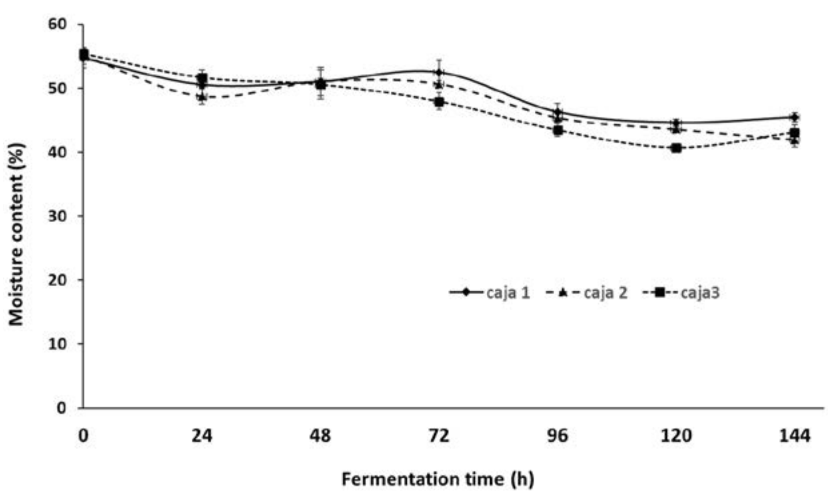

Figure 3. Behavior of moisture content (%), during 6 days of spontaneous fermentation, in three sizes of wooden boxes.

(2019), who reported a 59.5 to 53.8% moisture decrease —*i.e.*, 10% at the end of the 96 h fermentation in wooden boxes.

Color analysis

The relationship of color change (ΔE) of cacao beans in the three treatments during the spontaneous fermentation process was studied. Table 1 shows no significant difference ($p>0.05$) between the treatments; however, a progressive increase in color change (Table 1) and a decrease in luminosity were recorded, from non-fermented samples to completely fermented samples. The cacao beans turned darker as fermentation increased. According to Afoakwa *et al.* (2011), these changes may be caused by the degradation of anthocyanins resulting from enzymatic hydrolysis, which is accompanied by the bleaching and subsequent darkening of the cacao beans. The color change in the cotyledons of cacao beans during fermentation is a response variable that indicates the degree of fermentation (Marciano *et al.*, 2021). The cut test is the simplest and most widely used method to visually evaluate the quality of a random sample of the beans from a plot (Kongor *et al.*, 2013). However, even if it is carried out by trained personnel, it is considered a subjective method and, despite its ease of use, it is difficult to standardize (Kongor *et al.*, 2013). An accurate measurement technique is the CIELAB L*, a*, and b* system. Chire *et al.* (2016) reported color changes of 15.6 after three days of fermentation; those results are similar to those identified in this research.

Table 1. Color change (ΔE) every 24 hours, during 6 days of fermentation.

Treatments	Hours					
	0	24	72	96	120	144
Box 1	7.945±1.155ab	13.644±2.754a	12.407±1.819a	14.570±1.211b	17.129±0.783a	17.269±2.248a
Box 2	6.057±0.855b	15.758±1.743a	14.014±3.236a	18.992±1.556ab	15.138±1.889a	14.089±3.040a
Box 3	10.933±2.450a	14.780±1.224a	16.332±3.061a	16.961±1.089a	17.098±0.956a	17.969±1.455a

CONCLUSIONS

The use of small-scale spontaneous fermentations can be an option for varieties whose beans must be fermented for the sensory analysis of their qualitative attributes. Additionally, this micro-fermentation methodology can be incorporated into genetic improvement programs or in the evaluation of the processes aimed at its optimization.

ACKNOWLEDGEMENTS

The authors would like to thank the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) for financing the project "Caracterización bioquímica y desarrollo de tecnología en los procesos de agregación de valor de cacao."

REFERENCES

Adrien Servent, Renaud Boulanger, Fabrice Davrieux, Marie-Neige Pinot, Eric Tardan, Nelly Forestier-Chiron, Clotilde Hue. 2018. Assessment of cocoa (*Theobroma cacao* L.) butter content and composition throughout fermentations. *Food Research International*,107, 675-682. Doi: https://doi.org/10.1016/j.foodres.2018.02.070.

Afoakwa, E. O., Quao, J., Takrama, J., Budu, A. S., Saalia, F. K. 2013. Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. *Journal of food science and technology, 50*(6), 1097-1105. Doi: https://doi.org/10.1007/s13197-011-0446-5

Afoakwa, E. O., Paterson, A., Fowler, M., Ryan, A. 2008. Flavor formation and character in cocoa and chocolate: A critical review. *Food Science and Nutrition, 48*(9), 840-857. Doi: https://doi.org/10.1080/10408390701719272.

Alexander J. Taylor, Eduardo Cárdenas-Torres, Michael J. Miller, Sihai Dave Zhao, Nicki J. Engeseth. 2022. Microbes associated with spontaneous cacao fermentations - A systematic review and meta analysis. *Current Research in Food Science*, 5, 1452-1464. Doi: https://doi.org/10.1016/j.crfs.2022.08.008.

Amoa-Awua, W. K. Methods of cocoa fermentation and drying. En Cocoa and coffee fermentations, 1ª ed.; Editor Rosane F. Schwan, Graham H. Fleet. CRC Press, 2015. 1, pag. 71-128. Doi: https://doi.org/10.1201/b17536

AOAC. 2005. Association of Offical Analytical Chemistry. 18th Ed. Gaithersburg, MD (Estados Unidos): AOAC International.

Balogu, T. V., Onyeagba, R. A., Onwuchekwa, E., & Mohammed, S. S. D. 2014. Microbial and physicochemical dynamics of on-farm cocoa fermentation: A function of bioreactors. *Jewel J Sci Res, 2*(2), 85-92.

Chire, Gabriela C., Piero A. Verona, Jorge H. Guzmán. 2016. Cambios en el color durante el beneficio del grano de cacao (*Theobroma cacao* L.) peruano procedente de Piura. *Ciencia e Investigación, 19*(1): 29-34.

Clapperton, Y., J. Chan, D. Lim, R. Lockwood, L. Romanczyk y J. Hammerstone. 1994. The contribution of genotype to cocoa (*Theobroma cacao* L.) flavour. *Tropical Agriculture, 71*(4),303-308.

Crafack, M., Keul, H., Eskildsen, C. E., Petersen, M. A., Saerens, S., Blennow, A., Skovmand-Larsen, M., Swiegers, J. H., Petersen, G. B., Heimdal, H., Nielsen, D. S. 2014. Impact of starter cultures and fermentation techniques on the volatile aroma and sensory profile of chocolate. *Food Res Int*., 63, 306–316. Doi: https://doi.org/10.1016/j.foodres.2014.04.032.

Enio G., Jelihovschi, J. C. Faria, Ivan Bezerra Allaman. 2014. Scott Knott: A Package for Performing the Scott-Knott Clustering Algorithm in R. *Trends in Applied and Computational Mathematics, 15*(1), 3-17. Doi: http://www.sbmac.org.br/tema/seer/index.php/tema/article/view/646/643.

García González, E., Serna Murillo, A. M., Córdoba Pantoja, D. A., Marín Aricapa, J. G., Montalvo Rodríguez, C., Ordoñez Narváez, G. A. 2019. Estudio de la fermentación espontánea de cacao (*Theobroma cacao* L.) y evaluación de la calidad de los granos en una unidad productiva a pequeña escala. *Revista Colombiana De Investigaciones Agroindustriales, 6*(1), 29-40. Doi: https://doi.org/10.23850/24220582.163.

Guehi, T. S., Dingkuhn, M., Cros, E., Fourny, G., Ratomahenina, R., Moulin, G., Clément, A. 2007. Identification and lipase-producing abilities of moulds isolated from ivorian raw cocoa beans. *Research Journal of Agriculture and Biological Sciences, 3*(6), 838-843.

H.M. Rawel, G. Huschek, S.T. Sagu, T. Homann. 2019. Cocoa bean proteins characterization, changes and modifications due to ripening and post-harvest processing Nutrients, 11 (2) (2019), p. 428. Doi: https://doi.org/10.3390/nu11020428.

H. Rottiers, D.A. Tzompa Sosa, A. De Winne, J. Ruales, J. De Clippeleer, I. De Leersnyder, J. De Wever, H. Everaert, K. Messens, K. Dewettinck. 2019. Dynamics of volatile compounds and flavor precursors during spontaneous fermentation of fine flavor Trinitario cocoa beans. *European Food Research and Technology, 245*(9), 1917-1937. Doi: https://doi.org/10.1007/s00217-019-03307-y

Hu, M., Jacobsen, C. 2016. Oxidative stability and shelf life of foods containing oils and fats: AOCS Press.

John, W. A., Böttcher, N. L., Abkamp, M., Bergounhou, A., Kumari, N., Ho, P.-W., & Ullrich, M. S. (2019). Forcing fermentation: profiling proteins, peptides and polyphenols in lab-scale cocoa bean fermentation. *Food Chemistry*, 278, 786-794. Doi: https://doi.org/10.1016/j.foodchem.2018.11.108.

Krähmer, A., Engel, A., Kadow, D., Ali, N., Umaharan, P., Kroh, L. W., & Schulz, H. 2015. Fast and neat – Determination of biochemical quality parameters in cocoa using near infrared spectroscopy. *Food Chemistry*, 181, 152-159. Doi: http://dx.doi.org/10.1016/j.foodchem.2015.02.084

K T Dewandari, R Rahmawati, S J Munarso. 2021. The effect of techniques and fermentation time on cocoa beans quality (*Theobroma cacao* L.). *IOP Conf. Ser.: Earth Environ. Sci*. 653. Doi: 10.1088/1755-1315/653/1/012046

Kongor, J. E., Takrama, J. F., Budu, A. S., Mensah-Brown, H., Afoakwa, E. O. 2013. Effects of fermentation and drying on the fermentation index and cut test of pulp pre-conditioned Ghanaian cocoa (*Theobroma cacao*) beans. *Journal of Food Science and Engineering*, 3, 625-634.

Levai, L.D., Meriki, H.D., Adiobo, A. 2015. Postharvest practices and farmers' perception of cocoa bean quality in Cameroon. *Agric & Food Secur* 4, 28. Doi: https://doi.org/10.1186/s40066-015-0047-z

Leydy A. Domínguez-Pérez, Lilia M. Beltrán-Barrientos, Aarón F. González-Córdova, Adrián Hernández-Mendoza, Belinda Vallejo-Córdoba. 2020. Artisanal cocoa bean fermentation: From cocoa bean proteins to bioactive peptides with potential health benefits. *Journal of Functional Foods*, 73,1-10. Doi: https://doi.org/10.1016/j.jff.2020.104134.

López Hernández, M.D.P, Criollo N.J., Hernández, G.M.S., Lozano, T.M.D. 2019. Physicochemical and microbiological dynamics of the fermentation of the CCN51 cocoa material in three maturity stages. *Revista Brasileira de Fruricultura, 41*(3), 1-13. Doi: https://doi.org/10.1590/0100-29452019010

Marciano M. Oliveira, Breno V. Cerqueira, Sylvio Barbon, Douglas F. Barbin, 2021. Classification of fermented cocoa beans (cut test) using computer vision. *Journal of Food Composition and Analysis*, 97. Doi: https://doi.org/10.1016/j.jfca.2020.103771.

Melo, T.S., Pires, T.C., Engelmann, J.V.P. 2020. Evaluation of the content of bioactive compounds in cocoa beans during the fermentation process. *J Food Sci Technol* 58, 1947-1957. Doi: https://doi.org/10.1007/s13197-020-04706-w

M.M. Santander, C.J. Rodríguez, F.E. Vaillant, S.P. Escobar. 2019. An overview of the physical and biochemical transformation of cocoa seeds to beans and to chocolate: *Flavor formation Food Science and Nutrition, 21* (2019), pp. 1-21. Doi: 10.1080/10408398.2019.1581726

Moreno-Zambrano Mauricio, Grimbs Sergio, Ullrich Matthias S. and Hütt Marc Thorsten. 2018. A mathematical model of cocoa bean fermentation. *R. Soc. Open Sci. 5*(10), 1-18. Doi: http://doi.org/10.1098/rsos.180964

Mota-Gutiérrez, J., Botta, C., Ferrocino, I., Giordano, M., Bertolino, M., Dolci, P., Cocolin, L. 2018. Dynamics and biodiversity of bacterial and yeast communities during fermentation of cocoa beans. *Applied and Environmental Microbiology, 84*(19), 1-17. Doi: https://doi.org/10.1128/AEM.01164-18

Nicki J Engeseth, Marlon Fernando Ac Pangan, 2018. Current context on chocolate flavor developmenta review. *Current Opinion in Food Science*, 21, 84-91. Doi: https://doi.org/10.1016/j.cofs.2018.07.002

Ndife, J., Bolaji, P., Atoyebi, D., Umezuruike, C. 2013. Production and quality evaluation of cocoa products (plan cocoa powder and chocolate). *American journal of food and nutrition, 3*(1). Doi: https://doi.org/10.5251/ajfn.2013.3.1.31.38

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Recuperado de http://www.R-project.org/

Rawel, H. M., Huschek, G., Sagu, S. T., Homann, T. 2019. Cocoa bean proteins characterization, changes and modifications due to ripening and post-harvest processing. *Nutrients, 11*(2), 428. https://doi.org/10.3390/nu11020428.

Santander Muñoz, M., Rodríguez Cortina, J., Vaillant, F. E., & Escobar Parra, S. 2020. An overview of the physical and biochemical transformation of cocoa seeds to beans and to chocolate: Flavor formation. *Critical reviews in food science and nutrition, 60*(10), 1593-1613. Doi: https://doi.org/10.1080/10408398.2019.1581726

Schwan, R. F., & Fleet, G. H. (Eds.). (2014). Cocoa and coffee fermentations. CRC Press. Doi: https://doi.org/10.1080/10408690490464104

Schwan, R. F., & Wheals, A. E. (2004). The microbiology of cocoa fermentation and its role in chocolate quality. *Critical reviews in food science and nutrition, 44*(4), 205-221. Doi: https://doi.org/10.1201/b17536