Original Research Article

Impact of *Saccharomyces cerevisiae* Concentration on Aroma Profiles of Fermented Cocoa Beans and Sensory Qualities of Chocolate

Abstract

Ivorian cocoa is subject to various quality defects including the absence of the fine flavors desired by chocolate consumers. Fermentation of cocoa beans is a key post-harvest treatment involving yeast. Yeasts play a key role in the formation of cocoa flavor precursor compounds. This study aims to contribute to the improvement Ivorian cocoa aromatic quality, through controlled fermentation. Starter monoculture based on *Saccharomyces cerevisiae* strain TIA2019A isolated from wine fermentation and known to be aromatic were prepared at varying concentrations of 0.5 and 1.0 g yeast/kg fresh beans. These starter cultures were used for fermentation of cocoa beans during six (06) days with stirring (48 and 96 hours) according to the micro-fermentation technique in plastic boxes (50 x 30 x 30 cm^3). Fermented cocoa beans was sun-dried to a moisture content about 8%. Analysis of volatile organic compounds (VOCs) in cocoa beans was performed by SPME-GC/MS method. The results showed that the concentration 0.5 g of yeast/kg of *S. cerevisiae* strain allowed formation of high levels desirable VOCs between 100 and 600 µg/g; while lower levels of VOCs from 20 to 50 µg/g, were obtained at the concentration of 1.0 g of yeast/kg . Chocolates made from cocoa beans inoculated at the concentration 0.5 g/kg with *S. cerevisiae* selected strain, presented better score in fresh fruit flavors and overall quality than those produced from cocoa beans inoculated at the concentration 1.0 g/kg. Use of starter based on *S. cerevisiae* at low concentration allowed to optimize the organoleptic qualities of cocoa and derived chocolate. Selected yeast strains with VOCs producing performance can thus be used to improve the aromatic quality of Ivorian cocoa.

Keywords: Cocoa, Fermentation, *Saccharomyces cerevisiae*, VOCs, organoleptic qualities, chocolate,

1-Introduction

Raw cocoa beans are considered as luxury products in many countries **[1]**. The main producing countries include Côte d'Ivoire, Ghana, and Ecuador **[2]**. Cocoa is one of many crops holding vital and economic importance for Côte d'Ivoire **[3]** and constitute chocolate main raw material **[4]**. Unfermented cocoa beans have a bitter and astringent taste **[2]**. Therefore, fresh beans may be fermented before manufacturing into chocolate. Cocoa beans fermentation is the main post-harvest treatments influencing the generation of chocolate flavour precursors **[5, 6].** So, previouly studies reported that properly conducted fermentation contribute to the production of luxury chocolate **[5, 7].** Since a long time, cocoa beans fermentation was performed by a consortium of various indigenous microorganisms **[8]**, from agricultural material, environment and the farmers hands **[9, 10].** Composition of these microbial communities is very heterogeneous and vary according to cocoa producing country, region and season **[11]**. Cocoa fermentation process is initiated by yeasts populations which transform fermentable sugars into ethanol and organic acids **[12]**. In addition, several studies reported that the development of aroma and flavour precursors in cocoa beans requires yeast enzymatic action **[13, 14, 15, 16]**. *P. kudriavzevii* and *S. cerevisiae* are associated commonly with all performed fermentation methods and appeared as the greatest cocoa aroma flavour producers **[14]**. Besides *S. cerevisiae* accelerates cocoa beans fermentation **[15]**, is the main isolated yeast specie associated with cocoa fermentation **[17]** and produce most great cocoa aroma and flavour compounds **[16]**. Aroma and flavour compounds quality is one of the most important qualitative characteristics of cocoa beans **[18]**. They formation is reported to be depended on fermentation, geographical origin and genotype of the cocoa tree that has produced the beans **[19]**. Combined actions of yeasts and acetic bacteria induce the complex biochemical changes inside the beans leading to development of volatile organic compounds (VOCs) that contribute as chocolate flavor precursors **[20]**. Today, selected yeast strains are increasingly used as starters for cocoa beans fermentation to remedy quality variation from spontaneous process. Additional aims are the best control of fermentation and the production of optimal, standardized and reproducible aromatic quality. Thus an increasing amount of study was done on how to improve or change the flavor profiles of raw cocoa beans in relation to activities of selected *Saccharomyces cerevisiae* strains **[13, 21, 22**]. Ivorian cocoa sector is characterized by various cocoa producing regions, climatic and environmental factors, and methods fermentation **[23]** leading to the variable and fluctuating quality. Consequently, the aroma quality of cocoa sourced from country is reported to be standard.

This study aims to contribute to the improvement Ivorian cocoa aromatic quality, through controlled fermentation using starter monoculture based on *Saccharomyces cerevisiae* strain TIA2019A isolated from wine fermentation and known to be aromatic at varying concentrations.

2. Material and methods

2.1. Material

2.1.1. Vegetal material

Vegetal material used in this research work consisted of fresh cocoa beans from Forastero cocoa pods. 1500 cocoa pods were harvested during the major cocoa harvesting season in October 2015, from traditional farm, located in N'douci in the south of Côte d'Ivoire, 110 Km from Abidjan, between 5.51° North latitude and 4.45° West longitude.

2.1.2. Yeast strain inoculated as starter culture

Selected *Saccharomyces cerevisiae* strain TIA2019A isolated from wine fermentation was used in this study for cocoa beans fermentation. This strain supplied by Lallemand (Blagnac, France), recognized as producers of desirable volatile organic compounds (VOCs).

2.2. Methods

2.2.1. Postharvest treatments

Pod opening was carried out within Two days of harvesting using wooden clubs. The extracted cocoa beans were immediately transferred to plastic boxes. Four (04) samples (duplicated) were inoculated respectively with S. cerevisiae strain TIA2019A (inoculation concentrations : 0.5 and 1.0 g yeast/kg fresh beans). For 0.5 g yeast/kg fresh beans concentration, 12.5 g dehydrated yeast strain were rehydrated with 75 mL sterile distilled water. About concentration 1.0 g yeast/kg fresh beans, 25 g dehydrated yeast strain were used in the same volume of sterile distilled water. In all cases two inocula were obtained, and the inoculum obtained was used to inoculate 25 Kg of fresh cocoa beans **[3, 15].** Un-inoculated sample constituted the control. Cocoa beans fermentation was performed in perforated plastic boxes (50x 30x30 cm^3) during six (06) days with stirring (48 and 96 hours) **[13]**. Fermented cocoa beans were sun dried until 7-8% moisture on plastic tarpaulin.

2.2.2. Extraction, identification and quantification of volatile organic compounds (VOCs)

The volatile compounds were extracted by SPME fiber (DVB/Carboxen/PDMS) on 2.5 g of cocoa powder **[24]**. Analysis and identification of cocoa flavour compounds were carried out by GC-MS (Agilent 6890 and 5973 N). Quantification of VOCs was performed using the method previously described by **Assi-Clair *et al*. [13]**.

2.2.3. Sensorial analyses of chocolate made from raw cocoa beans

Evaluation of sensory properties of resulted chocolate, prepared in the CIRAD chocolate factory laboratory, from raw cocoa samples was carried out according to **ISO 13.299.** A descriptive analysis was performed using a panel of twelve (12) judges who are familiar with sensory evaluation of chocolate. Ten (10) descriptors such as, odor intensity, sweety, bitter, sour, astringency, cocoa aroma, fresh fruit aroma, dried fruit aroma floral aroma and general quality (general appreciation of chocolate quality) were used to describe the odor, taste, flavor and mouthfeel of the chocolates. A hedonic scale from 0 to 10 was used to score each descriptor. Four tasting session were carried out to enable panelists to taste chocolates in duplicate. The results of tasting were analyzed using microsoft Excel version 2013 software.

2.2.4. Statistical analysis

Statistical analyses of results were performed with XLSTAT software version 19.02.2017. Ki-deux concordance test (α=0.001) was used to show significant differences between the volatile compound contents in cocoa samples. Sensorial analyses results were analyzed using the Fisher's test (α=0.05).

3-Results

A total of 36 VOCs in six families, including 06 alcohols, 05 aldehydes, 05 ketones, 08 esters, 04 acids, 03 pyrazines and 05 others aroma compounds were identified in all cocoa samples analyzed **(Table 1**). Some of these volatile ccompounds promote specific desirable aromas in cocoa beans, while others confer undesirable sensory attributes.

Table 1. Volatile organic compounds (VOCs) identified in inoculated cocoa beans and control

Chemical families	Retention Time (mn)	VOCs	Kovats index[a]	Kovats index calculated	Odor attributes[b]	References
Alcohols	2.25	Ethanol	929	925		
	5.80	2-Methyl-1-propanol	1101	1118	Wine	**[25]**
	6.87	2-Pentanol	1122	1139	Green, mild green	**[7]**
	10.54	3-Methyl-1-butanol	1214	1203	Malty, bitter, chocolate	**[14]**
	16.85	2-Heptanol	1326	1315	Soft, citrus	**[25]**
	47.93	2-Phenylethanol	1891	1865	Honey, flowery	**[26]**
Aldehydes	1.45	Propanal 2-methyl	817	804	Malty, chocolate	**[13]**
	2.02	Butanal 2-methyl	910	906	Malty, chocolate, cocoa	**[16]**
	2.06	Butanal 3-methyl	912	912	Malty, chocolate, cocoa	**[14]**
	26.40	Benzaldehyde	1516	1508	Bitter,almond, grass	**[27]**
	33.14	Phenylethanal	1634	1604	Honey, green, flowery	**[28]**
Ketones	2.70	2-Pentanone	983	964	Fruity	**[24**
	8.82	2-Heptanone	1181	1172	Fruity, green	**[25]**
	13.36	Acetoïn	1250	1255	Buttery, cream	**[28]**
	20.05	2-Nonanone	1389	1369	Flowery, fatty	**[28]**
	33.35	Acetophenone	1642	1612	Flowery, sweet	**[29]**
Acids	23.11	Aceticacid	1452	1430	Sour, viniegra	**[30]**
	28.43	Propanoïc acid	1523	1514	Pungent, rancid	**[31]**
	30.31	2-Methyl propanoïc acid	1568	1544	Rancid	**[30**
	35.91	3-Methylbutanoïc acid	1676	1643	Sweaty	**[30]**

Table 1 (Continued).

Chemical families	Retention Time (mn)	VOCs	Kovats index[a]	Kovats index calculated	Odor attributes[b]	References
Esters	1.50	Methylacetate	813	822	Fruity	**[14]**
	1.82	Ethylacetate	872	875	Nail polish, fruity	**[13]**
	3.28	Isobutylacetate	1008	1008	Fruity, banana	**[16]**
	4.68	2-Pentyl acetate	1080	1073	Fruity	**[25]**
	6.28	Isoamylacetate	1118	1137	Banana	**[32]**
	6.71	Amylacetate	ND		Banana	**[16]**
	42.99	2-Phenylethyl acetate	1810	1793	Honey, flowery	**[30]**
Pyrazines	20.61	2,3, 5-trimethyl Pyrazine	1408	1383	Cocoa, roasted	**[7]**
	24.87	2,3, 5,6-Tetramethyl Pyrazine	1489	1452	Roasted, chocolate	**[7]**
			Others compound			
Lactones	31.71	Butyrolactone	1618	1583	ND	
Sulfur compounds	1.26	Dimetylsulfur	716	726	ND	
Hydrocarbons	3.70	Toluen	1042	1028	ND	
	5.93	Ethylbenzen	1125	1125	ND	
	11.87	Styren	1250	1227	ND	
Terpene alcohols	24.79	Linalool oxid	1423	1451	Sweet, flowery	**[27]**
	29.96	Linalool	1537	1540	Rose, flowery	**[33]**

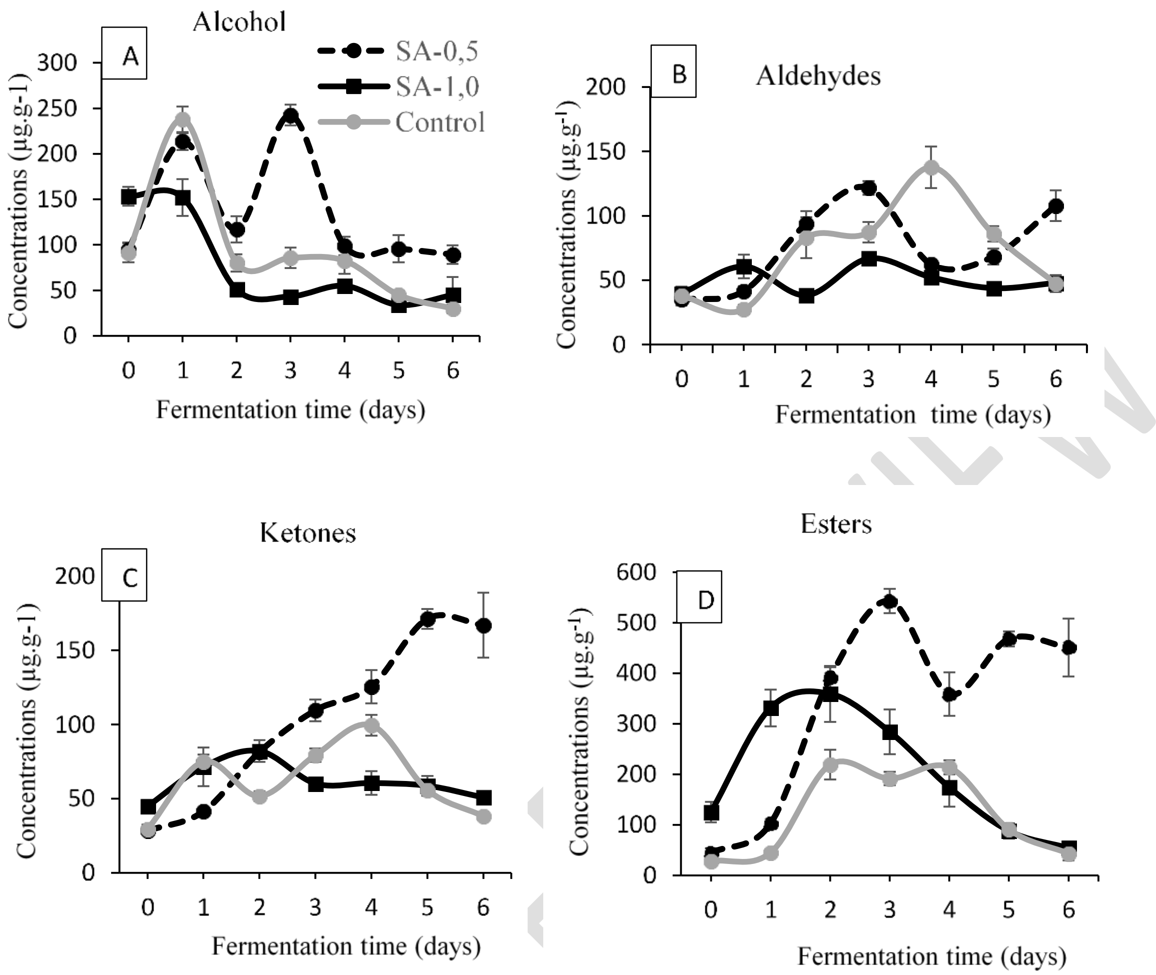
3.1. Effect of *S. cerevisiae* inoculum concentrations on the production of different chemical volatile organic compounds groups.

Fig. 1A show changes in total alcohol contents produced in cocoa beans inoculated at two concentrations (0.5 and 1.0 g yeast/kg fresh beans) with selected *S. cerevisiae* strain. Total alcohol contents in inoculated cocoa beans at concentration 0.5 g/kg were higher ($p < 0.001$) than those detected in inoculated cocoa beans with concentration 1.0 g/kg and control, during fermentation. The highest contents were obtained after one day and were 152.06 and 238 µg/g respectively for cocoa beans inoculated at 1.0 g/kg and the control; while for cocoa beans inoculated at 0.5g/kg, alcohol concentration change to maximum of 242.56 µg/g after three fermentation days.

For aldehydes, the results showed that, the total contents increased up to three fermentation days with maximum averages of 121.71 and 66.7 µg/g respectively in inoculated cocoa beans at 0.5 and 1.0 g yeast/kg fresh beans, after that a decrease was observed. For the control, total contents of aldehydes increased up to four day with a maximum average of 137. 7 µg/g and decreased after. However, at six fermentation days, total aldehyde contents in inoculated cocoa beans at 0.5 g/kg were significantly higher ($p < 0.001$) than contents obtained in inoculated cocoa beans at 1 g/kg and control (**Fig. 1B)**.

The results for ketones showed that total contents in inoculated cocoa beans at 0.5 g/kg, increased up to six fermentation days with maximum averages of 166.97 µg/g. Whereas, for cocoa beans inoculated at 1.0 g/kg, total ketone contents increased up to two fermentation days with maximum average of 82.08 µg/g and decreased after. For spontaneous fermented cocoa beans (control), maximum average was 99.44 µg/g after four days. In general, total ketone contents in inoculated cocoa beans at 0.5 g/kg were (<0.001) higher than contents detected in inoculated cocoa beans with 1.0 g/ kg and control, during fermentation process **(Fig. 1C)**.

Fig. 1D show changes in total esters contents obtained in inoculated cocoa beans and control. On 2nd and 3rd days of fermentation, total esters contents increased up to maximum averages of 359.29 and 542.82 µg/g respectively in inoculated cocoa beans at 1.0 g/kg and with 0.5 g/kg, a decreased was observed after that. For control, the maximum contents were 218.91 µg/g (after fourth days of fermentation). Our results showed that, contents of total esters were significantly higher ($p < 0.001$) in raw cocoa beans inoculated with 0.5 g/kg than other fermented cocoa beans.



SA-0,5 : inoculated cocoa beans at concentration 0.5 g/kg
SA-1,0 : inoculated cocoa beans at coccentration 1.0 g/kg
Control : spontaneous fermented cocoa beans

Fig. 1. Changes in total contents of : **A**) alcohol family ; **B**) aldehydes family **C**)ketones family ; **D)** esters family , obtained in inoculated cocoa beans and control according to the fermentation days.

3.2. Effect of *S. cerevisiae* inoculum concentrations on the production of specific aroma compounds

Fig. 2 show changes in contents of desirable aroma compounds found in inoculated cocoa beans and spontaneous fermented cocoa beans (control). Two high alcohols contents, such as 3-MethylButanol, and 2-Phenylethanol were significantly ($p < 0.001$) important in inoculated cocoa beans at 0.5 g/kg (**Fig. 2A, 2B)**. Two main aldehyde compounds were identified as 3-Methyl butanal, and Benzaldehyde in this research work. Contents of 3-Methylbutanal, and Benzaldehyde were significantly ($p < 0.001$) higher in inoculated cocoa beans with 0.5 g/kg, after six fermentation days compared to the control and inoculated cocoa beans at 1.0 g/kg (**Fig. 2C, 2D)**.

For specific ketones, contents of 2-Pentanone and Acetoin were significantly ($p < 0.001$) higher in inoculated cocoa beans at 0.5 g/kg after two fermentation days. However, at six fermentation days, concentration of 2-Pentanone was significantly ($p < 0.001$) higher in raw cocoa beans inoculated at 1.0 g/kg compared to the other fermented raw cocoa beans **(Fig. 2E, 2F).** Main esters contents, such as methyl Acetate and ethyl Acetate were significantly ($p < 0.001$) higher in inoculated cocoa beans with 0.5 g/kg than control and those inoculated at 1.0 g/kg **(Fig. 2G, 2H)**.

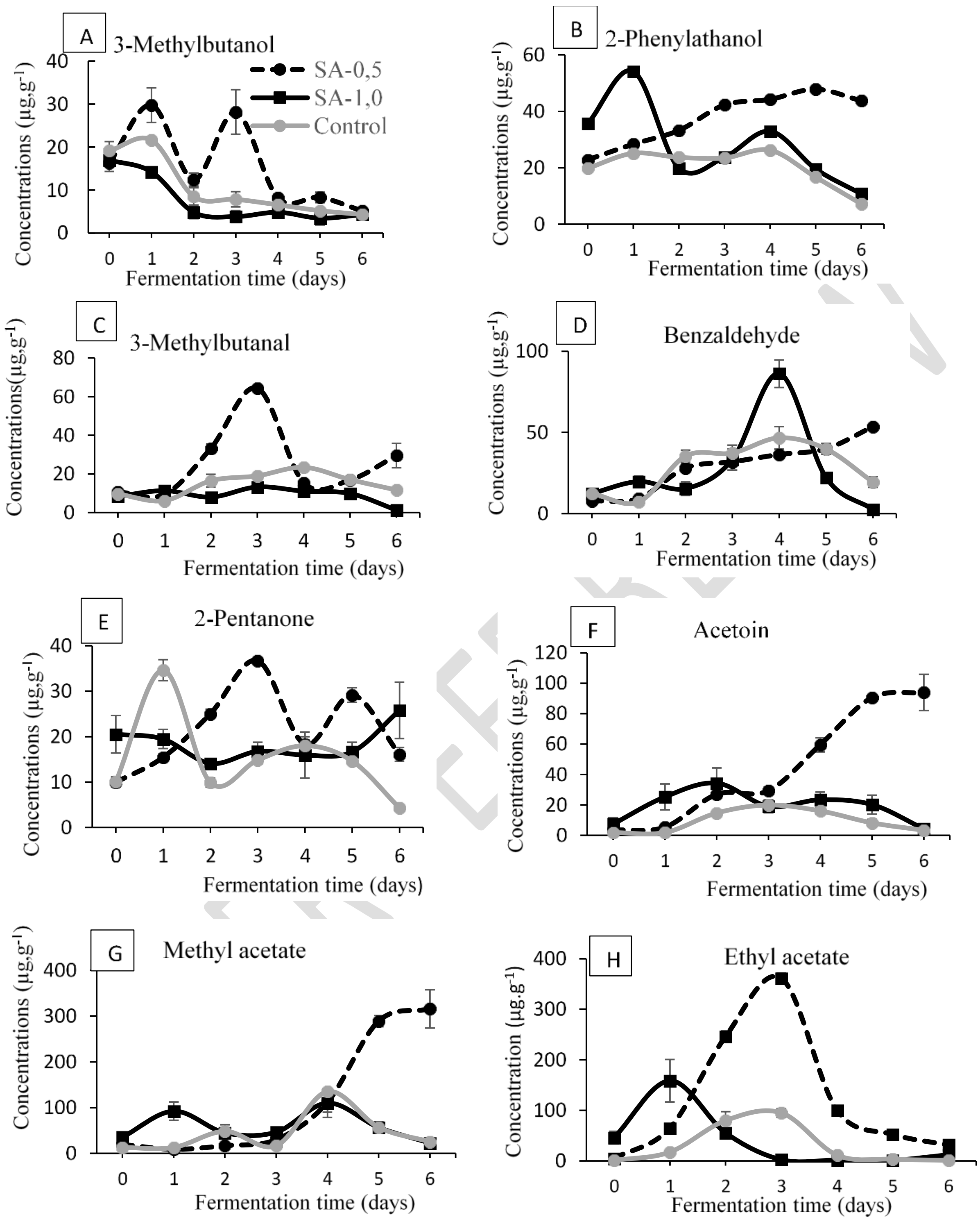


Fig. 2. Changes in contents of some desirable aroma compounds such as : **A**) 3-methylbutanol, **B**) 2-phenylethanol ; **C**) 3-methylbutanal, **D**) benzaldehyde ; **E**) 2-pentanone, **F**) ; acetoin ; **G)** methyl acetate, **H**) ethyl acetate, found in inoculated cocoa beans according to the fermentation days.

3.3. Sensory profile of chocolates produced

Sensory analysis were performed on the chocolates formulated from different inoculated cocoa beans and control (spontaneously fermented cocoa beans). Ten (10) attributes were analyzed from tasting. Chocolates prepared from inoculated cocoa beans at concentration 0.5 g/kg was characterized by highest score for fresh fruit (6.33) and the best general quality (6.58) compared to control and chocolate made from inoculated cocoa beans at 1.0 g/kg which was bitter (**Fig. 3).**

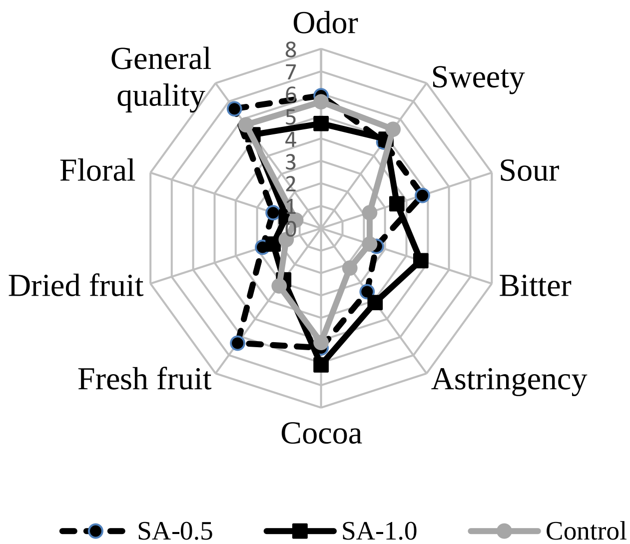


Fig. 3. Sensory profiles of chocolates produced from inoculated fermented cocoa beans and control (spontaneous fermented cocoa beans).

4. Discussion

Several previous studies reported that yeasts action is essential for cocoa bean fermentation and training of desirable chocolate flavor **[16, 20, 32, 34]**. Therefore, in this work, selected *S. cerevisiae* strain was used as starter culture at different inoculum levels in order to observe their influence on the production of VOCs (Volatile Organic Compounds) during cocoa beans fermentation and on

sensorial properties of resulted chocolate. Thirty six (36) VOCs classified in seven families such as alcohols, aldehydes, ketones, esters, acids, pyrazines and other compounds were identified in fermented and dried cocoa beans samples in this research work. However, studies related to the use of other *Saccharomyces cerevisiae* strains as starter have identified more volatile organic compounds (VOCs) in the cocoa beans analyzed than the present study **[13, 16]** , while few flavor compounds have been counted by others **[14, 23].**
Our results showed that before fermentation, all cocoa beans had initial contents of alcohols, aldehydes and ketones. Initial contents of total alcohols observed in all cocoa beans could be explained by beginning of fermentation process before inoculation. This is probably due to the contamination of fresh beans by wild yeasts during manual opening of cocoa pods. Initial contents of total aldehydes and ketones could be explained by the fact that some of these compounds are intrinsic to the bean **[32].** In this study, the highest concentrations of all chemical groups of volatile compounds were found at three and four days of fermentation, a decrease was observed after when fermentation time increased. This decrease could be explained by their conversion into other organic compounds namely lactic and acetic acids due to the growth and activity of both lactic and acetic bacteria. The results of this study are opposed to those obtained by **Rodriguez-Campos *et al.*[25]** who reported an increase in contents of total aldehydes, ketones, alcohols, pyrazines and acids during fermentation process. According to these authors, the high contents of these compounds were detected after six or eight fermentation days. The highest contents of all volatile compounds after three and four fermentation days observed in this research work confirm that is not necessary to extend cocoa beans fermentation time to seven and eight days.

The impact of selected yeast concentration on raw cocoa beans aroma profiles indicated that *S. cerevisiae* strain used at 0.5g/kg contributed globally to the production of high concentrations of almost all volatile compounds such as alcohols, ketones, esters and acids than when it was used at 1 $g.kg^{-1}$. This could be explained by compétition between yeasts in the use of the substrate **[35]**. Besides, *S. cerevisiae* strain inoculated at 0.5 g/kg promoted more high contents of desirable volatile compounds such as, 2-Phenylethanol, 3-Methylbutanol, 3-Methylbutanal, 2-Pentanone, Acetoin and Methyl acetate than the concentration 1.0 g/kg. These volatils compounds produce respectively malty, flowery, buttery and fruity flavor notes in unroasted and roasted cocoa beans **[7, 14, 36, 37].** The results of this research work were similar to those obtained by **Sandhya *et al.***

[22]. According to these authors low inoculum of starter regulate microbial succession, leads to consistent fermentation and to the development of high qualitative characteristics of the raw cocoa.

The results about the impact of selected *S.cerevisiae* concentration on sensory profiles of chocolate indicated that chocolates made from cocoa beans inoculated at 0.5g/kg were characterized by desirable flavors such as fresh fruit notes and high score for general quality; whereas those produced from cocoa beans inoculated with 1.0 g/kg were influenced by bitter attribute. The fresh fruit notes on chocolate could be due to the high concentrations of Methyl acetate, 2-Pentanone correlate to fruity flavor in cocoa **product [13, 25, 37, 39]**. However bitter attribute could be explained by high concentrations of acids mainly lactic acid **[14, 16, 40]**. The concentration 0.5g/kg contributed better to sensorial quality of cocoa beans than inoculum concentration 1.0 g/kg. The same results were almost obtained by **Sandhya *et al*. [22]** who reported that chocolate prepared from cocoa beans inoculated with 10 % inoculum of starter consortia had an acceptable sensorial properties than those made from cocoa beans inoculated with high density cell culture (30-60 %). This research also revealed that chocolate obtained from inoculated cocoa beans at 0.5 g/kg was better compared to control (chocolate made from spontaneous fermented cocoa beans). These results were similar to those obtained by a large number of researchers **[13, 16, 20, 42],** but opposed to the results of **Moreira *et al.* [43].** study which showed that chocolate prepared from cocoa beans inoculated with yeast cocktail (*S. cerevisiae + Pichia Kluyveri*) had no specific flavors and was bitter than chocolate produced by spontaneous fermented cocoa beans. All these results confirm that yeasts have a greater impact on the sensorial quality of cocoa beans and final chocolates **[11, 16, 20, 27, 34]**. Although the profile of cocoa beans is correlated to the metabolic activities of yeasts, it is fully developed on subsequent bean roasting and conching during the chocolate making process **[11, 13, 32, 41].**

5. Conclusion

At the end of this study let us note that *Saccharomyces cerevisiae* strain used with concentration 0. 5 yeast/kg fresh cocoa beans (0.5g/kg) produced desirable chocolate key volatile organic compounds (VOCs) such as esters (methyl acetate) and alcohols (3-methylbutanol, 2-phenylethanol) than when it was used at 1.0g/kg. Sensory profiling concluded that the concentration 0.5 g/kg led to the chocolate with better fruity notes and higher scores of general quality than chocolate produced from 1.0 g/kg inoculated cocoa beans. Use of starters based on *S.*

cerevisiae at low concentration allowed to optimize the organoleptic qualities of cocoa and derived chocolate. Selected yeast strains with VOCs producing performance can thus be used to improve the aromatic quality of Ivorian cocoa

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

References

1 Kresnowati MP, Suryani L, Affifah M. Improvement of Cocoa beans fermentation by LAB starter addition. *Journal of Medical and Bioengineering*, 2013; 2(4): 274-278.

2 Afoakwa EO, Quao J, Takrama J, Budu AS, Saalia FK. Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. *Journal of Food Science and Technology*, 2013; 50(6): 1097-1105.

3 Guehi TS, Zahouli IB, Ban-Koffi L, Fae MA, Nemlin JG. Performance of different drying methods and their effects on the chemical quality attributes of raw cocoa material. *International journal of food science & technology*. 2010; *45*(8): 1564-1571.

4 Afoakwa EO, Budu AS, Brown HM, Takrama JF, Ansah EO. Effect of roasting conditions on the browning index an appearance properties of pulp pre-conditioned and fermented cocoa (T*heobroma cacao*) beans. *Journal of Nutritional Health & Food Science*. 2014; 2(1): 1-5.

5 Pacheco-Montealegre ME; Dávila-Mora, LL, Botero-Rute LM, Reyes A, Caro-Quintero A. Fine resolution analysis of microbial communities provides insights into the variability of cocoa bean fermentation. *Frontiers in Microbiology*. 2020; 11, p650.

6 Ordoñez-Araque RH, Landines-Vera EF, Urresto-Villegas JC, Caicedo-Jaramillo CF. Microorganisms during cocoa fermentation: systematic. review. *Foods and Raw Materials.* 2020 ; 1(8): 155-162. DOI: http://doi.org/10.21603/2308-4057-2020-1-155-162.

7 Rottiers H, Tzompa Sosa DA, De Winne A,· Ruales J, De Clippeleer J, · De Leersnyder I, · De Wever J, · Everaert H, · Messens K, Dewettinck K. Dynamics of volatile compounds and favor precursors during spontaneous fermentation of fne favor Trinitario cocoa beans. *European Food Research and Technology*. 2019; doi.org/10.1007/s00217-019-03307-y.

8 Crafack M, Mikkelsen MB, Saerens S, Knudsen M, Blennow A, Lowor S, Takrama J, Swiegers JH, Petersen GB, Heimdal H, Nielsen DS. Influencing cocoa flavor using *Pichia Kluyveri* and *Kluyveromyces marxianus* in a defined mixed starter culture for cocoa fermentation. *International Journal of Food and Microbiology*. 2013; 167: 103-116.

9 Okiyama DCG, Navarro SLB, Rodrigues CEC. Cocoa shell and its compounds: applications in the food industry. *Trends Food Sciences and Technology*. 2017 ; 63: 103–112. doi: 10.1016/j.tifs.2017.03.007.

10 Díaz-Muñoz C, Van de Voorde D, Comasio A, Verce M, Hernandez CE, Stefan Weckx S, De Vuyst L. Curring of cocoa beans : fine scale monitoring of the starters cultures applied and metabolomics of the fermentation and drying steps. *Frontiers in microbiology.* 2021; Doi : 10.3389/fmicb.2020.616875.

11 Koné KM, Guéhi TS, Durand N, Ban-Koffi L, Berthiot L, Fontana TA, Brou K, Boulanger R, Montet D. Contribution of predominant yeasts to the occurrence of aroma compounds during cocoa bean fermentation. *Food Research International*. 2016; 89: 910-917.

12 Delgado-Ospina J, Triboletti S, Alessandria V, Serio A, Sergi M, Paparella A, Kalliopi Rantsiou K, Chaves-López C. (2020). Functional Biodiversity of Yeasts Isolated from Colombian Fermented and Dry Cocoa Beans. *Microorganisms.* 2020; 8:1086; doi:10.3390/microorganisms8071086.

13 Assi-Clair BJ, Kone MK, Kouame K. et al. Effect of aroma potential of Saccharomyces cerevisiae fermentation on the volatile profile of raw cocoa and sensory attributes of chocolate produced thereof. European Food Research and Technology, 2019; 245: 1459–1471.

14 Kone KM, Assi-Clair BJ, Kouassi ADD, Yao AF, Ban-Koffi L, Durand N, Lebrun M, Maraval I, Bonlanger R, Guehi TS. Pod storage time and spontaneous fermentation

treatments and their impact on the generation of cocoa flavour precursor compounds. *International Journal of Food Science and Technology*. 2021 ; doi:10.1111/ijfs.14890.

15 Ramos CL, Dias DR, Miguel MGCP, Schwan RF. Impact of different cocoa hybrids (Theobroma cacao L.) and *S. cerevisiae* UFLA CA1 inoculation on microbial communities and volatile compounds of cocoa fermentation. *Food Research International*. 2014; 64: 908-918.

16 Batista NN, Ramos CL, Dias DR, Pinheiro ACM, Schwan RF. The impact of yeast starter cultures on the microbial communities and volatile compounds in cocoa fermentation and the resulting sensory attributes of chocolate. *Journal of Food Science and Technology*. 2016 ; 53(2) : 1101–1110.

17 Arana-Sánchez A, Segura-García LE, Kirchmayr M, Orozco-Ávila I, Lugo-Cervantes E, Gschaedler-Mathis A. Identification of predominant yeasts associated with artisan Mexican cocoa fermentations using culture-dependent and culture-independent approaches. *World Journal of Microbiology and Biotechnology*. 2015; 31(2): 359-369.

18 Munoz MS, Cortina JR, Vaillant FE, Parra, SE. 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*. 2020; 60: 1593–1613.

19 Papalexandratou Z, Kaasikc K, Villagra Kauffmanna L, Skorstengaardc A, Bouillon G, Espensena JL, Hansene LH, Jakobsenc RR, Blennowd A, Krychc L, Castro-Mejíac JL, Nielsen DS. Linking cocoa varietals and microbial diversity of Nicaraguan fine cocoa bean fermentations and their impact on final cocoa quality appreciation. *International Journal of Food Microbiology*. 2019 ; 304 (2019) : 106–118.

20 Gutiérrez-R, Hugo G, Suárez-Quiroz ML, Hernández-Estrada ZJ, et al. Yeasts as producers of flavor precursors during cocoa bean fermentation and their relevance as starter cultures: a review. Fermentation. 2022;. 8(7), p.331.

21 Rebeki-Bekki MA. Production de metabolites par les levures : caractérisation et identification des arômes et des alcools. Thèse de l'Université d'Oran d'Algérie. 2014 ; 161p.

22 Sandhya MVS, Yallappa BS, Varadaraj MC, Puranaik J, Rao JL, Janardhan P, Murthy PS. Inoculum of the starter consortia and interative metabolic process in enhancing quality of

cocoa bean (Theobroma cacao) fermentation. *Food science and technology*. 2016; 65: 731-738.

23 Kouassi ADD, Koné KM, Assi-Clair BJ, et al. Effect of spontaneous fermentation location on the fingerprint of volatile compound precursors of cocoa and the sensory perceptions of the end-chocolate. *Journal of Food Science and Technology*. 2022; 59 (11): 4466-4478.

24 Rodriguez-Campos J, Escalona-buendía HB, Orozco-Avila I, Lugo-Cervantes E, Jaramillo-Flores ME.. Dynamics of volatile and non-volatile compounds in cocoa (*Theobroma cocoa L*.) during fermentation and drying process using principal components analysis. *Food Research International*. 2011; 44: 250–258.

25 Rodriguez-Campos J, Escalona-Buendía HB, Contreras-Ramos SM, OrozcoAvila I, Jaramillo-Flores E, Lugo-Cervantes E. Effect of fermentation time and drying temperature on volatile compounds in cocoa. *Food Chemistry*. 2012; 132(1): 277-288.

26 Ducki S, Miralles-Garcia J, Zumbé A, Tornero A, Storey DM. Evaluation of solid-phase micro-extraction coupled to gas chromatography–mass spectrometry for the headspace analysis of volatile compounds in cocoa products. *Talanta*. 2008; 74(5): 1166-1174.

27 Owusu M, Petersen MA, Heimdal H. Effect of fermentation method, roasting and conching conditions on the aroma volatiles of dark chocolate. *Journal of Food Processing and Preservation*. 2011; 36: 1-11.

28 Moreira IMV, Vilela LF, Santos C, Lima N, Schwan RF. Volatile com pounds and protein profiles analysesof fermented cocoa beans and chocolates from different hybrids cultivated in Brazil. *Food Research International*. 2018; 109:196-203 .https://doi.org/10.1016/j.foodres.2018.04.012.

29 Marseglia A, Musci M, Rinaldi M, Palla G, Caligiani A. Volatile finger print of unroasted and roasted cocoa beans (*Theobroma cacao* L.) from different geographical origins. *Food Research International*. 2020 ; 132 :101-109; https://doi.org/10.1016/j.foodres.2020.109101.

30 Hinneh M, Van de Walle D, Tzompa-Sosa, DA, De Winne A, Termote S, Messens K., Dewettinck K.. Tuning the aroma profiles of FORASTERO cocoa liquors by varying pod storage and bean roasting temperature. 2019; *Food Research International*, 125, 108550.

31 Akoa SP, Boulanger R., Onomo PE, Lebrun M, Ondobo ML, Lahon MC, Djocgoue PF. Sugar profile and volatile aroma composition in fermented dried beans and roasted nibs from six controlled pollinated Cameroonian fine-flavor cocoa (*Theobroma cacao* L.) hybrids. *Food Bioscience*. 2023; 53; 102603.

32 Ho VTT, Zhao J, Fleet G. Yeasts are essential for cocoa bean fermentation. *International Journal of Food Microbiology*. 2014; 174: 72–87.

33 Castro-Alayo EM, Idrogo-Vásquez G, Siche R, Cardenas-Toro FP. Formation of aromatic compounds precursors during fermentation of Criollo and Forastero cocoa. 2019; Heliyon 5:e01157. https://doi.org/10.1016/j.heliyon.2019.e01157

34 Chagas Juniora GCA, Ferreiraa NR, Gloriac MBA, Da Silva Martins LH, Lopesa LS. Chemical implications and time reduction of on-farm cocoa fermentation by *Saccharomyces cerevisiae* and *Pichia kudriavzevii*. *Food Chemistry*. 2020 ; 127834.

35 Sablayrolles JM, Salmon JM. Déroulement et contrôle de la fermentation. In INRA Montpellier, U M R Sciences pour l'œnologie, France. 2010; 1-29.

36 Quelal OM, Hurtado, DP, Benavides AA, et al. Key aromatic volatile compounds from roasted cocoa beans, cocoa liquor, and chocolate. Fermentation. 2023; 9 (2): 166.

37 Crafack M, Keul H, Eskildsen CE, Petersen MA, Saerens S, Blennow A, *et al*. Impact of starter cultures and fermentation techniques on the volatile aroma and sensory profile of chocolate. *Food Research International*. 2014 ; 63(Part C) : 306-316.

38 Aprotosoaie AC, Luca SV, Miron A. Flavor chemistry of cocoa and cocoa products-an overview. Comprehensive Reviews in *Food Science and Food Safety*. 2016 ; 15: 73-91.

39 Deuscher Z, Gourrat K, Repoux M, Boulanger R, Hélène Labouré H, Le Quéré JL. Key Aroma Compounds of Dark Chocolates Differing in Organoleptic Properties: A GC-O Comparative Study. *Molecules*. 2020 ; 33p.

40 Bastos VS, Uekane TM, Bello NA, de Rezende CM, Flosi Paschoalin VM, Del Aguila EM. Dynamics of volatile compounds in TSH 565 cocoa clone fermentation and their role on chocolate flavor in Southeast Brazil. *Journal of Food Science and Technology*. 2019; 56: 2874–2887.

41 Lima LJR, Almeida MH, Nout MJ, Zwietering MH. *Theobroma cacao* L., "The Food of the Gods": Quality Determinants of Commercial Cocoa Beans, with Particular Reference to the Impact of Fermentation, *Critical Reviews in Food Science and Nutrition*. 2011; 51(8): 731-761.

42. Mendoza Salazar, M.M.; Martínez Álvarez, O.L.; Ardila Castañeda, M.P.; Lizarazo Medina, P.X. Bioprospecting of indigenous yeasts involved in cocoa fermentation using sensory and chemical strategies for selecting a starter inoculum. *Food Microbiology*. 2022; 101, 103896.

43. Moreira, I.; Costa, J.; Vilela, L.; Lima, N.; Santos, C.; Schwan, R. Influence of *S. cerevisiae* and *P. kluyveri* as starters on chocolate flavour. *Journal of the Science of Food and Agriculture*. 2021; 101, 4409–4419.