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Effect of *Saccharomyces cerevisiae* Strain TIA2019A Concentration as Starter Culture on Volatile Aroma Fingerprint of Cocoa Beans and Sensory Perception of End-chocolate

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cocoa from Côte d'Ivoire is currently characterized by low technological quality including the absence of desired fine flavors. Various yeasts are essential for cocoa fermentation and play a key role in the formation of cocoa volatile aroma precursors. This study investigated the improvement of aromatic quality of dry fermented cocoa beans through inoculation of yeats reported to be high potential aroma compounds producer to the cocoa fermentation. Starter culture based on *Saccharomyces cerevisiae* strain TIA2019A from wine fermentation were prepared at 2 concentrations about 0.5 and 1.0 g yeast per kg of fresh cocoa beans. Cocoa 6 days-fermentation with about 25 kg of fresh beans were carried out in plastic boxes (50×30×30 cm^3) for 06 days with 2 turnings at 48 and 96 hours. Fermented cocoa beans was then sun-dried until a moisture content about 8%. Volatile organic compounds (VOCs) fingerprint of dry fermented cocoa beans were determined using SPME-GC/MS method. Main results showed that dry fermented cocoa beans fermented from inoculated 0.5 g of *S. cerevisiae* per kg of fresh cocoa recorded higher contents of relevant VOCs comprised between 100 and 600 $\mu g.g^{-1}$ than cocoa beans inoculated at 1.0 g of yeast per kg. Chocolate made from cocoa beans inoculated at 0.5 g of *S. cerevisiae* per kg of cocoa recorded better score for fresh fruit flavors and global organoleptic quality than those produced from cocoa beans inoculated at 1.0 g of *S. cerevisiae* per kg of cocoa. Utilization of starter based on *S. cerevisiae* at low concentration promoted the organoleptic qualities of cocoa and derived chocolate. Selected yeast strains with VOCs producing performance can thus be used to improve aroma quality of dry cocoa beans and the organoleptic quality of chocolate produced thereof.

Keywords: Cocoa; fermentation; Saccharomyces cerevisiae; aroma compounds; quality; chocolate.

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) is an important food source for many countries due to the frequent consumption of its various end-products such as biscuits, chocolate, chocolate drinks and sweets [1]. Côte d'Ivoire, Ghana, and Ecuador are reported to be the main cocoa producing countries [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 cocoa 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, many previous studies reported that properly conducted fermentation promote the production of high quality chocolate [5,7]. Since a long time, cocoa beans fermentation was spontaneously performed by a consortium of various indigenous microorganisms' communities [8] from agricultural material, environment and the farmers hands [9,10]. The diversity of these microbial communities is very large and heterogeneous depending on cocoa producing country, region and season [11]. Cocoa fermentation process is initiated by various yeasts strains which convert fermentable sugars into ethanol and various organic acids [12]. Additionaly many studies reported that the development of aroma and flavour precursors in cocoa beans requires yeast enzymatic activities [13-16]. *P. kudriavzevii* and *S. cerevisiae* are commonly associated with any fermentation technic and appeared as the greatest aroma flavour producers [14]. *S. cerevisiae* is also reported to contribute to accelerate cocoa beans fermentation [15] but is the main yeast specie frequently involved in cocoa fermentation [17]. The presence of aroma compounds is one of the most important indicators of cocoa beans quality [18]. The generation of aroma compounds depended on the genotype of the cocoa tree, the fermentation time and the quality of soils [19]. Resulting biological activities from yeasts and acetic bacteria inducing various changes of biochemical composition inside the beans leading to development of VOCs that probably chocolate sensory quality [20]. Today, many papers reported that specific yeast strains are increasingly used as starters for cocoa beans fermentation to mitigate quality variation [11,16]. Nowadays, many chocolate manufacturers are very interested in the improvement of fermentation processing leading to optimized, standardized and reproducible aromatic quality of final dry fermented cocoa beans. Since few time, an increasing studies dealing with the improvement or the modulation of aroma quality notably fingerprint of VOCs of dry fermented cocoa beans using selected *Saccharomyces cerevisiae* strains [13,21,22]. The cocoa sector of Côte d'Ivoire include various cocoa producing regions with different climatic and environmental factors and primary postharvest processing [23] leading to the fluctuating and low aroma quality. This study aims to contribute to the improvement of aromatic quality of cocoa sourced from Côte d'Ivoire using *Saccharomyces cerevisiae* strain TIA2019A.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Cocoa beans sampling

Fresh cocoa beans extracted from 1500 cocoa pods of the cultivar Ivorian first generation of hybrids (Amelonado×West African Trinitario) and open-pollinated progenies were harvested from target peasant cocoa planation in October 2015 during the main cocoa harvesting season. This cocoa farm is located in N'douci in the south of Côte d'Ivoire, between 5.51° North latitude and 4.45° West longitude.

2.1.2 Yeast strain inoculated as starter culture

Specific relevant aroma compounds producing *Saccharomyces cerevisiae* strain TIA2019A from wine fermentation was used for cocoa beans fermentation. This *Saccharomyces cerevisiae* strain recognized as high producer of desirable VOCs.was provided by Lallemand (Blagnac, France).

2.2 Methods

2.2.1 Primary postharvest processing

Harvested cocoa pods was stored for 2 days at the field before opening with wooden clubs. The extracted fresh cocoa beans were immediately transferred to plastic boxes. Four cocoa beans samples (duplicated) were inoculated respectively with *S. cerevisiae* strain TIA2019A at concentrations of 0.5 and 1.0 g yeast per kg of fresh beans. For 0.5 g of *S. cerevisiae* per kg of fresh cocoa beans, 12.5 g dehydrated yeast strain were rehydrated in 75 mL sterile distilled water. About concentration 1.0 g of *S. cerevisiae* per kg of fresh beans was prepared by rehydrating 25 g of dry yeast were used in in 75 mL of sterile distilled water. In any case, two inocula preparation were obtained in order to inoculate 25 kg of fresh cocoa beans [3,15]. A control cocoa beans sample was constituted by uninoculated fresh cocoa beans. Each cocoa beans fermentation was performed in perforated plastic boxes (50x 30x30 cm^3) during six (06) days with 2 turings at 48 and 96 hours [13]. Fermented cocoa beans were sun dried until 7-8% moisture on plastic tarpaulin for 8 hours.

2.2.2 Extraction, identification and quantification of VOCs

The VOCs were extracted by SPME fiber (DVB/Carboxen/PDMS) on 2.5 g of cocoa powder [24]. 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 Sensory perception of chocolate produced from inoculated dry fermented cocoa beans

Evaluation of sensory attributes of end-chocolate produced from each separate dry fermented cocoa beans sample was carried out according to ISO 13.299. A descriptive analysis was performed using a panel of twelve (12) judges expert in chocolate sensory quality evaluation. Ten (10) descriptors including odor intensity, sweety, bitter, sour, astringency, cocoa aroma, fresh fruit aroma, dried fruit aroma floral aroma and global quality were used to describe the odor, taste, flavor and mouthfeel attributes of each end-chocolate. 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.

2.3 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 grouped in 6 chemical families, including alcohols (06), aldehydes (05), ketones (05), esters (08), acids (04), pyrazines (03) and others aroma compounds (05) were identified in all cocoa samples analyzed (Table 1). Family of esters recorded most VOCs while family of pyrazines presented least VOCs.

Table 1. Global volatile organic compounds (VOCs) found in dry fermented inoculated cocoa beans and control

Chemical families	Retention Time (mn)	VOCs	Kovats index[a]	Kovats index calculated	Odor attributes[b]	References
	2.25	Ethanol	929	925		
	5.80	2-Methyl-1-propanol	1101	1118	Wine	[25]
Alcohols	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]
	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]
Aldehydes	26.40	Benzaldehyde	1516	1508	Bitter,almond, grass	[27]
	33.14	Phenylethanal	1634	1604	Honey, green, flowery	[28]
	2.70	2-Pentanone	983	964	Fruity	[24
	8.82	2-Heptanone	1181	1172	Fruity, green	[25]
Ketones	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]
	23.11	Aceticacid	1452	1430	Sour, viniegra	[30]
	28.43	Propanoïc acid	1523	1514	Pungent, rancid	[31]
Acids	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
	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]
Esters	6.28	Isoamylacetate	1118	1137	Banana	[32]
	6.71	Amylacetate	ND		Banana	[16]
	42.99	2-Phenylethyl acetate	1810	1793	Honey, flowery	[30]

Chemical families	Retention Time (mn)	VOCs	Kovats index[a]	Kovats index calculated	Odor attributes[b]	References
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	[32]

3.1 Effect of *S. cerevisiae* Concentrations as Starter Culture on VOCs Families of dry Fermented Cocoa Beans

Fig. 1A show changes in total alcohols contents produced in *S. cerevisiae* inoculated cocoa beans with 2 concentrations namely 0.5 and 1.0 g of yeast per kg fresh beans. Results showed that alcohols content of cocoa beans inoculated at concentration 0.5 g of yeast per kg was higher ($p < 0.001$) than those quantified both in cocoa beans inoculated at 1.0 g of yeast per kg and control. Actually, the highest alcohols contents were found in 1 day-fermented cocoa beans nearby 152.06 and 238 $\mu g.g^{-1}$ in cocoa beans inoculated at 1.0 g of yeast per kg and the control samples respectively. Yet, 3 days-fermented cocoa beans inoculated with 0.5 of yeast per kg recorded 242.56 $\mu g.g^{-1}$ of alcohols.

The results about of aldehydes, increasing contents nearby 121.71 and 66.7 $\mu g.g^{-1}$ were mesured in up to 3 days-fermented inoculated cocoa beans at 0.5 and 1.0 g of yeast per kg respectively. In parallel, average of 137.7 $\mu g.g^{-1}$ was quantified in control samples. However, in 6 days-fermented cocoa beans samples, aldehydes content found in inoculated cocoa beans at 0.5 g of yeast per kg were significantly higher ($p < 0.001$) than those quantified in cocoa beans inoculated with higher *S. cerevisiae* concentration and control (Fig. 1B).

The results about ketones showed that inoculated cocoa beans at 0.5 g of yeast per kg recorded maximum content after 6 days of fermentation (166.97 $\mu g.g^{-1}$) while 2 days-fermented cocoa beans inoculated at 1.0 g of yeast per kg contained 82.08 $\mu g.g^{-1}$. The control recorded maximum average content nearby 99.44 $\mu g.g^{-1}$ after four days of fermentation. Basically, total ketone contents quantified in inoculated cocoa beans at 0.5 g of yeast per kg were higher ($p<0.001$) than those found in both cocoa beans inoculated with double *S. cerevisae* concentration and control samples (Fig. 1C).

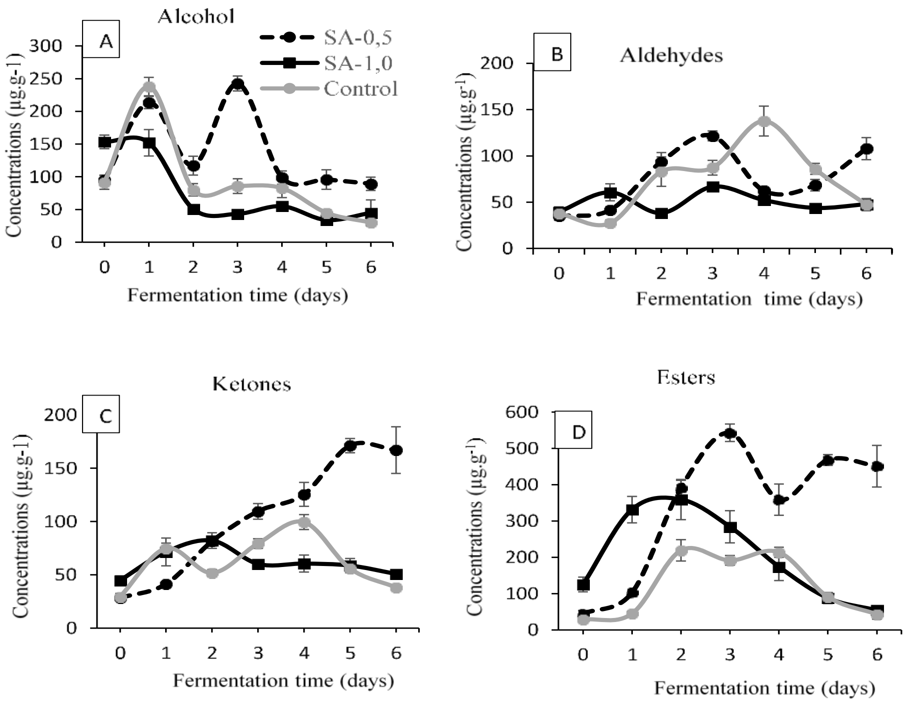

Fig. 1. Changes in total contents of main volatile organic compounds (VOCs) families: A) alcohols ; B) aldehydes ; C) ketones ; D) esters ; found in dry fermented cocoa beans inoculated with aromatic *S. serevisiae* strain and control during the fermentation

SA-0,5 : fresh cocoa beans inoculated with concentration 0.5 g of S. cerevisiae per kg
SA-1,0 : fresh cocoa beans inoculated with concentration 1.0 g of S. cerevisiae per kg
Control : cocoa beans spontaneously fermented without inoculation of S. cerevisiae

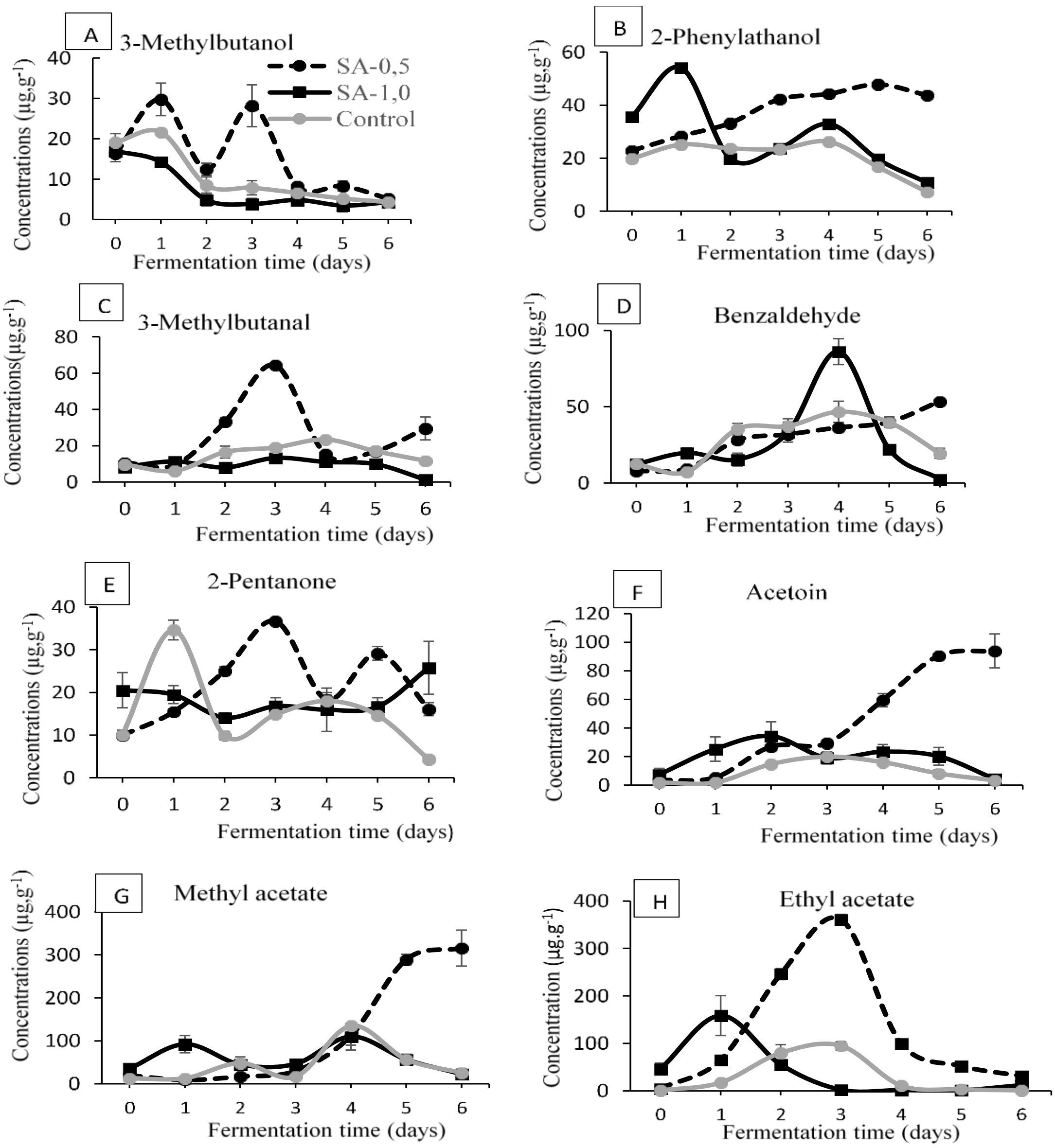

Fig. 2. Changes in contents of some desirable aroma compounds such as : A) 3-methylbutanol, B) 2-phenylethanol ; C) 3-methylbutanal, D) benzaldehyde ; E) 2-pentanone, F) ; acetoin ; G) methyl acetate, H) ethyl acetate, found in dry fermented cocoa beans inoculated with aromatic *S. serevisiae* strain and control during the fermentation

The changes in total esters contents in *S. cerevisiae* inoculated cocoa beans were indicated at Fig. 1D. At 2 and 3 days of fermentation, the contents increased up to maximum averages of 359.29 and 542.82 $\mu g.g^{-1}$ in inoculated cocoa beans at 1.0 g of yeast per kg and at 0.5 g of yeast per kg respectively and then decreased for the following days. The maximum esters contents around 218.91 µg/g were quantified in control after 4 days of fermentation. Our results showed that, total contents of esters were significantly higher ($p < 0.001$) in dry-fermented cocoa beans inoculated with 0.5 g of yeast per kg than other tested cocoa beans samples.

3.2 Effect of *S. cerevisiae* Concentrations as Starter Culture on the Specific Main VOCs of Dry Fermented Cocoa Beans

Fig. 2 shows changes in contents of cocoa desirable aroma compounds found in inoculated samples compared to those in the control. Two

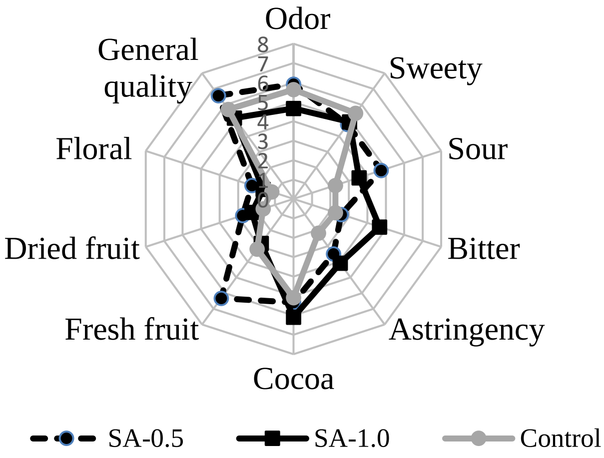

Fig. 3. Sensory attributes of chocolates produced from aromatic *S. cerevisiae* inoculated cocoa beans fermented for 6 days

main alcohols contents, such as 3-MethylButanol and 2-Phenylethanol were significantly ($p < 0.001$) high in cocoa beans inoculated at 0.5 g of yeast per kg (Fig. 2A,B). 3-Methyl butanal and Benzaldehyde were found in 6 days fermented cocoa beans inoculated at 0.5 g of yeast per kg as the main compounds of the aldehyde family with contents of 29.5 and 53.5 $\mu g.g^{-1}$ respectively , significantly ($p < 0.001$) higher than those measured in cocoa beans inoculated at 1.0 g of yeast per kg and control (Fig. 2C, D).

Concerning the ketones, the contents of 2-Pentanone (36.7 $\mu g.g^{-1}$) and Acetoin (29.3 $\mu g.g^{-1}$) were significantly ($p < 0.001$) higher in inoculated cocoa beans at 0.5 g of yeast per kg after 3 fermentation days. However, at 6 fermentation days, concentration of 2-Pentanone (25.8 $\mu g.g^{-1}$) was significantly ($p < 0.001$) higher in raw cocoa beans inoculated at 1.0 g of yeast per kg compared to the other fermented raw cocoa beans (Fig. 2E, F). Main esters contents, such as methyl Acetate and ethyl Acetate were significantly ($p < 0.001$) higher in inoculated cocoa beans with 0.5 g of yeast per kg than control and those inoculated at 1.0 g of yeast per kg. The level was 361.3 $\mu g.g^{-1}$ for ethyl Acetate at 3 fermentation days, while for methyl Acetate, the level was 315.8 $\mu g.g^{-1}$ at the end of fermentation (Fig. 2G, H).

3.3 Sensory Profile of Chocolate Produced from *S. cerevisiae* Inoculated Cocoa Beans

Sensory analysis were performed on the chocolates made from different *S. cerevisiae* inoculated cocoa beans and control samples. Chocolates produced from cocoa beans inoculated at concentration 0.5 g of yeast per kg was characterized by highest scores of 6.33 and 6.58 for fresh fruit and general quality respectively among all chocolate samples while chocolate made from inoculated cocoa beans at 1.0 of yeast per kg which was most bitter with score of 4.67 (Fig. 3).

4. DISCUSSION

Thirty six (36) VOCs classified in seven families such as alcohols, aldehydes, ketones, esters, acids, pyrazines and other compounds were identified in dry fermented cocoa beans samples in this research work. However, studies related to the use of other *S. cerevisiae* as starter culture have identified more 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 VOCs contents of alcohols, aldehydes and ketones. The presence of alcohols in raw cocoa beans could be explained by beginning of fermentation process due to the endogenous enzymes before yeasts inoculation. This fact could be probably ascribed to the contamination of underfermented beans by wild yeasts due the broken of cocoa pods and the crossed contamination by yeasts through the injuries [7]. The presence aldehydes and ketones could be explained by the fact that some of these compounds are intrinsic to the bean [33]. The highest concentrations of all chemical groups of VOCS were found at 3-4 and days-fermented cocoa samples. The decrease followed in the changes of VOCs contents could be explained by their conversion into other organic compounds namely lactic and acetic acids due to the growth and activity of both lactic, Bacillus and molds [34]. Our results are different from those obtained by Rodriguez-Campos et al. [25] who reported an increase in contents of total aldehydes, ketones, alcohols, pyrazines and acids during cocoa fermentation process. Actually, Rodriguez-Campos et al. [25] reported that contents of some VOCs families increased during cocoa fermentation. Otherwise, our results about any VOCs family of 3-4 days fermented cocoa beans samples suggest that it is not necessary to promote aroma quality of dry fermented cocoa beans by extending of fermentation time up to 6 or 7 days.

The effect of *S. cerevisiae* concentration on, the cocoa beans aroma compounds fingerprint indicated that inoculated at 0.5 g of yeast per kg globally promoted the generation of almost identified VOCs reaching high concentrations more than cocoa beans inoculated at 1.0 g of yeast per kg. This observation could be explained by the interactions between various yeast strains invloved in the fermentation process particularly by the competition for both space and substrates namely carbohydrates [35]. Besides, *S. cerevisiae* inoculated at 0.5 g of yeast per 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 cocoa beans inoculated at higher concentration of *S. cerevisiae* as previously reported 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. These desirable VOCs induced respectively malty, flowery, buttery and fruity flavor notes in unroasted and roasted cocoa beans according many researches **[**7,14,36,37].

The results related to the effect of *S. cerevisiae* concentration on the sensory perception of chocolate made from dry fermented and inoculated cocoa beans indicated that chocolates produced from cocoa beans inoculated at 0.5 g of yeast per kg recorded high scores for many desirable sensory descriptors such as fresh fruit notes and global quality; whereas those made from cocoa beans inoculated at 1.0 g of yeast per kg were characterized 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 [13,25,37,38]. However bitter attribute could be explained by high concentrations of acids mainly lactic acid [14,16,39]. The inoculum at 0.5 g of yeast per kg great produced desirable sensory attributes in cocoa beans than inoculum concentration 1.0 g of yeast per kg. The similar results were previously obtained by Sandhya et al. [22] who reported that chocolate made from cocoa beans inoculated with low inoculum of starter consortia had an acceptable sensorial properties than those made from cocoa beans inoculated with high density cell culture. Our results also revealed that chocolate obtained from inoculated cocoa beans at 0.5 g of yeast per kg was better compared to the control. This observations is similar to those obtained by many papers [13,16,20,40], but opposed to the finding of Moreira et al. [41] about *S. cerevisiae* + *Pichia Kluyveri* cocktail as mix starter cultures inducing no specific flavor. Our results confirm that yeasts have a greater impact on the sensorial quality of cocoa beans and final chocolates as previously reported [11,16,20,27,34]. However, although the fingerprint of VOCs of dry fermented cocoa beans is favored by the metabolic activities of yeasts involved in the fermentation, chocolate's sensory perception is not necessarily the result of yeasts action, it derive from subsequently roasting and conching processing [11,13,32,42].

5. CONCLUSION

The results of this study let us note that aromatic *S. cerevisiae* strain used at concentration of 0. 5 g per kg of cocoa beans promoted more generation of desirable dry fermented cocoa beans key VOCs such as various esters namely methyl acetate and alcohols such as 3-methylbutanol and 2-phenylethanol than applied at 1.0 g per kg. In the same, sensory perception of chocolate produced thereof shows that chocolate made from cocoa beans inoculated at 0. 5 g per kg recorded better fruity notes and higher global quality than chocolate produced from cocoa beans inoculated 1. 0 g per kg. Utilization of aromatic yeasts strains as starter culture at low concentration could improve both the aroma quality of dry cocoa beans and the organoleptic quality of chocolate produced thereof although no great link exists between VOCs of cocoa beans and chocolate sensory properties.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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 this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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 (Theobroma 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;11:650.
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. 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 :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. Functional biodiversity of yeasts isolated from colombian fermented and dry cocoa beans. Microorganisms. 2020;8: 1086.
DOI:10.3390/microorganisms8071086.
13. Assi-Clair BJ, Koné MK, Kouame K. 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. Koné 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. Yeasts as producers of flavor precursors during cocoa bean fermentation and their relevance as starter cultures: A review. Fermentation. 2022;8(7):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;161.
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. 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. Available: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. Available: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. Food Research International. 2019;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. Castro-Alayo EM, Idrogo-Vásquez G, Siche R, Cardenas-Toro FP. Formation of aromatic compounds precursors during fermentation of Criollo and Forastero cocoa. Heliyon. 2019;5:e01157. Available:https://doi.org/10.1016/j.heliyon.2019.e01157
33. Ho VTT, Zhao J, Fleet G. Yeasts are essential for cocoa bean fermentation. International Journal of Food Microbiology. 2014;174:72–87.
34. Dulce VR, Anne G, Manuel K, Carlos AA, Jacobo RC, de Jesús CES, Eugenia LC. Cocoa bean turning as a method for redirecting the aroma compound profile in artisanal cocoa fermentation. Heliyon. 2021;7(8).
35. Sablayrolles JM, Salmon JM. Déroulement et contrôle de la fermentation. In INRA Montpellier, UMR 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. 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;33.
39. 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.
40. Mendoza Salazar MM, Martínez Álvarez OL, Ardila Castañeda MP, 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
41. 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.
42. 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.

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