

Original Research Article

Effect of concentration *Saccharomyces cerevisiae* used as starter on the aroma profiling of fermented and dried cocoa beans and organoleptic of derived chocolate

Abstract

Fermentation is an essential step on the raw cocoa production. During the process, yeasts play an important role for the development of the cocoa volatile profile. Selected strain of *Saccharomyces cerevisiae* recognized as major aroma compounds producers, isolated from wine, was inoculated at different rate to fresh cocoa beans for fermentation. Fermented cocoa beans samples were then sun dried until to 7-8 % moisture. Identification and quantification of aroma compounds were analyzed by SPME-GC/MS. Inoculation of *S. cerevisiae* at 0.5g of yeast per kg of fresh beans (0.5 g.kg⁻¹) led to the production of higher contents of desirables aroma compounds than when it was used at 1 g of yeast per kg of fresh beans (1.0 g.kg⁻¹). Sensory profiling concluded that whatever the strain of selected *S. cerevisiae*, chocolate made from cocoa beans inoculated at 0.5 g.kg⁻¹ contained fresh fruits, flowery notes and high scores for general quality than those produced from cocoa beans inoculated at 1.0 g.kg⁻¹ characterized by undesirable flavor attributes. Inoculation of selected *S. cerevisiae* at 0.5 g.kg⁻¹ appeared to be a real potential producer of great volatile profile of raw cocoa beans.

Keywords: Cocoa, Fermentation, *Saccharomyces cerevisiae*, volatile fraction, chocolate, sensory quality

1-Introduction

Raw cocoa beans are one of the most important agricultural commodity products in the world [1]. Cocoa (*Theobroma cacao* L.) belongs to the family *Malvaceae* growing mainly in West Africa, Central and South America and South-East Asia. The four largest producing countries include Côte d'Ivoire, Ghana, Indonesia, and Ecuador [2]. Cocoa is one of many crops holding vital and economic importance for Côte d'Ivoire [3]. Cocoa beans originate as seeds in fruit pods of the tree *Theobroma cacao* and constitute the principal raw material of chocolate [4]. Cocoa is classified as

foodstuffs that rely on a microbial curing process because crude beans are reported to have an astringent and unpleasant taste [2]. Therefore, fresh cocoa beans may be fermented before manufacturing into chocolate. Fermentation is currently considered as one of the main post-harvest treatments impacted the global quality of raw cocoa material [5, 6]. So, several previous researches have shown that properly conducted fermentation process is a prerequisite for the production of high quality chocolates [5, 7]. The main reason for cocoa fermentation is to generate many biochemical transformations within the beans that induce several changes regarding color, taste and particularly aroma and flavour precursors of chocolate [7]. Since a long time, cocoa bean fermentation is reported to be performed spontaneously by a consortium of naturally occurred various microorganisms [8]. The microbial groups succession during this post-harvest treatment has been clearly established [9] coming from the environment, agricultural material and the hands of the farmers [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. The great roles of yeasts are principally to break down the citric acid in the pulp, to increase the pH, to transform fermentable sugars into ethanol and organic acids [12]. Therefore, several studies dealt with the elucidation of the role of yeasts population in the formation of cocoa aroma and flavour compounds were conducted [13, 14]. The development of aroma and flavour precursors requires yeast and acetic bacteria enzymatic action on carbohydrates, proteins and polyphenols present in the cocoa beans [4]. Most research reported that yeasts contribute greatly to the development of aroma and flavour compounds of raw cocoa [11, 15, 16]. *P. kudriavzevii* and *S. cerevisiae* are associated commonly with all performed fermentation methods, they appeared as the major yeasts producing most great cocoa aroma compounds [14]. Besides *S. cerevisiae* is reported to accelerate the cocoa fermentation [15], to be the principal isolated yeasts species associated with cocoa fermentation [17] and to 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 the genotype of the cocoa tree that has produced the beans, the producing origin, and how the beans have been fermented [19]. Combined actions of yeasts and acetic bacteria induce the complex biochemical changes inside the beans leading to the development of aroma volatile compounds that contribute as flavour precursors of chocolate [20]. Nowadays, spontaneous fermentations are more and more abandoned for the benefit of controlled fermentations. That

necessitate using of microorganisms characterized by strong resistances to various stresses and real potentials for production of desired metabolites in a mixed starter culture [21]. Additional aims are the best control of the process and the production of optimal, standardized and reproducible aromatic quality. In this way, most research related to the improvement or altering the aroma and flavour profiles of raw cocoa linked to the activities of *S. cerevisiae* strains were more and more conducted [13, 22]. Although Côte d'Ivoire is the world leader of raw cocoa, Ivorian cocoa sector is characterized by various cocoa producing regions, climatic and environmental factors, and methods of fermentation [23] leading to the variable and fluctuating quality. Consequently, the aroma quality of cocoa sourced from this country is reported to be standard [11]. Additionally, up to today, few studies dealing with the aroma and flavour compounds of cocoa have been carried out at Côte d'Ivoire. This research aims to contribute to the improvement of raw cocoa material aroma quality and the sensory quality of resulted chocolate by the inoculation at two concentrations of aroma-potential *S. cerevisiae* strain for the fermentation of the cocoa beans in Côte d'Ivoire.

2. Material and methods

2.1. Materials

Fresh cocoa seeds used in this work were originated from the cultivar Ivorian first generation of hybrids (Amelonado × West African Trinitario) and open-pollinated progenies. A total of 1500 cocoa pods were harvested in targeted farms located at N'douci, an Ivorian cocoa-producing region located at the South of Côte d'Ivoire, 110 km from Abidjan, during the main harvest season (October 2015). Hydrochloric solution (0.5%)-disinfected cocoa pods were manually opened delayed by 2 days after harvesting with a piece of wood billet as bludgeon for the extraction of fresh cocoa beans.

2.2. Methods

2.2.1. Field experiment and sampling of raw cocoa beans

After pod opening, the beans were immediately transferred in 0.045-m³ plastic boxes. Inoculated fermentations were performed after inoculation of a pure yeast starter culture containing selected *Saccharomyces cerevisiae* strain. The *S. cerevisiae* strain was obtained from the yeast culture collection of LALLEMAND (Blagnac, France). The quantities (12.5 g and 25g) of lyophilized

yeast of *S. cerevisiae* strain were separately weighed to reach a concentration of approximately 0.5 g of yeast per kg of cocoa beans and 1.0 g of yeast per kg of cocoa beans. Each quantity was dissolved in 75 ml of sterile and distilled water. Each micro-fermentation batch included 30 kg of cocoa beans [15] and 2 batches were constituted. One fresh cocoa bean batch was inoculated with 12.5 g of lyophilized strain dissolved in 75 ml and the other was inoculated with 25 g. Spontaneous fermentation of fresh cocoa bean batch was performed as the control. All workers wearing gloves helped with the removal of the cocoa beans from the pods and for the inoculation of cocoa beans [3]. All fermentations were monitored over 6 days, stirring after 48 and 96 h [13]. Each fermented cocoa bean sample was placed on tarpaulin plastic in 5-cm-thick layers and then sun-dried for 6 days before chemical analyses. All fermentations were performed in duplicate.

2.2.2. Volatile compound extraction

The volatile compounds of cocoa samples (2.5 g) were extracted using the technique of solid-phase microextraction in the headspace (SPME-HS) [24], using a 50/30- μm divinylbenzene/carboxene/polydimethylsiloxane (DVB/ CAR/PDMS) fiber provided by Supelco to extract volatiles. The fiber was previously conditioned in the chromatograph injector at 250 °C for 3 min and then exposed to the sample headspace at 50 °C for 45 min. Extracted aroma volatile compounds were analyzed using an Agilent 6890 N gas chromatography–mass spectrometer (GC–MS) equipped with a Hewlett Packard capillary column DBWAX, 30 m length \times 0.25 mm internal diameter \times 0.25 μm film thickness (Palo Alto, CA, USA). The GC oven temperature was initially set at 40 °C for 5 min, increased to 140 °C at a rate of 2°C/min and then increased at a rate of 10°C/min to 250 °C for 66 min. The carrier gas was high-purity helium at 1 ml min⁻¹. Injection mode was splitless at 250 °C for 2 min. The selective mass detector was a quadrupole (Hewlett Packard, Model 5973), with an electronic impact ionization system at 70 eV and at 230 °C.

2.2.3. Separation and quantification of volatile aroma compounds

Volatile compounds were identified using three criteria: by comparison of the retention index with CIRAD aromatic database, by comparison of the mass spectra with the Wiley 275L library of mass spectra, and whenever possible, the identification was confirmed using pure standards of the components. For each cocoa sample, three replicates were carried out, considering that from one sample to the other the same compound behaves similarly. The dispersion coefficients in the headspace and absorption on the fiber were neglected. Moisture content of the cocoa beans was

determined in cocoa nibs using the oven-drying method according to the International Organization for Standards (ISO 1666:1996) method. The content of each volatile compound was calculated according to the following formula:

$$qi(\mu\text{g}\cdot\text{g}^{-1}) = \frac{25 \times A_i}{A_{but} \times m_e \times W}$$

where q_i is the amount of compound i , A_i is the area of compound i , A_{but} is the area of butanol (standard), 25 is the content of butanol in $\text{mg}\cdot\text{L}^{-1}$, m_e is the mass of sample introduced into the vial in g and W is water content of the sample

2.2.4. Sensory analysis of chocolates

Chocolates were produced from 6-day-fermented cocoa beans after inoculation with selected *S. cerevisiae* strain and the control according to the process previously described by Assi-Clair *et al.* [13] The recipe of chocolates was 100% of cocoa solids. Only 5% of additional cocoa butter was added and lecithin was added. The conching process was done for 2 h and the chocolate tasting tests were done after 2-day storage at 4 °C in a refrigerator. Determination of sensorial profile of resulted chocolate was performed with a descriptive analysis performed according to ISO 13.299 in the CIRAD chocolate factory laboratory. For this purpose, twelve judges among 5 women and 7 men who had been previously selected and trained performed sensorial analyses of the chocolates. The global quality and the intensity of each attribute were evaluated simultaneously using a scale varying from 0 to 10, with 0.25 increments, and a total score for each sample was assigned

2.2.5. Statistical analysis

The statistical analyses were carried out with the STATISTICA software version 7.1. The Kideux test ($\alpha = 0.001$) was performed to indicate the significant differences between volatile compounds contents in cocoa samples. The sensorial analyses results were analyzed using Microsoft Excel Program, 2013 (Microsoft Corporation, Redmond, Washington, USA). The means were separated by the Fisher's test ($\alpha = 0.05$).

3-Results

In this research, a total of 36 volatile compounds grouped into seven main groups including alcohols (06), aldehydes (05), ketones (05), esters (08), acids (04), pyrazines (03) and others compounds (05) were identified in fermented and dried cocoa beans (Table 1). The numbers of individual esters and alcohol compounds were higher than others compounds. In this study, less volatile compounds classified into similar groups were detected than in the previous investigations [25]. These observations could be explained by the analytical approach of the volatiles, the environmental conditions and genetic impact.[7].

Table 1. Odor description and Kovats retention index of volatile compounds identified in cocoa beans inoculated with *S. cerevisiae* strain

Chemical groups	Retention Time (mn)	Volatile compounds	Kovats retention index ^a	Kovats retention index calculated	Odor description ^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]
	2.70	2-Pentanone	983	964	Fruity	[24]
Ketones	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]
	23.11	Acetic acid	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 groups	Retention Time (mn)	Volatile compounds	Kovats retention index ^a	Kovats retention index calculated	Odor description ^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	Pyrazine 2,3, 5-trimethyl	1408	1383	Cocoa, roasted	[7]
	24.87	Pyrazine 2,3, 5,6-Tetramethyl	1489	1452	Roasted, chocolate	[7]
Others compound						
Lactones	31.71	Butyrolactone	1618	1583	ND	
Sulfur compounds	1.26	Dimethylsulfur	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]

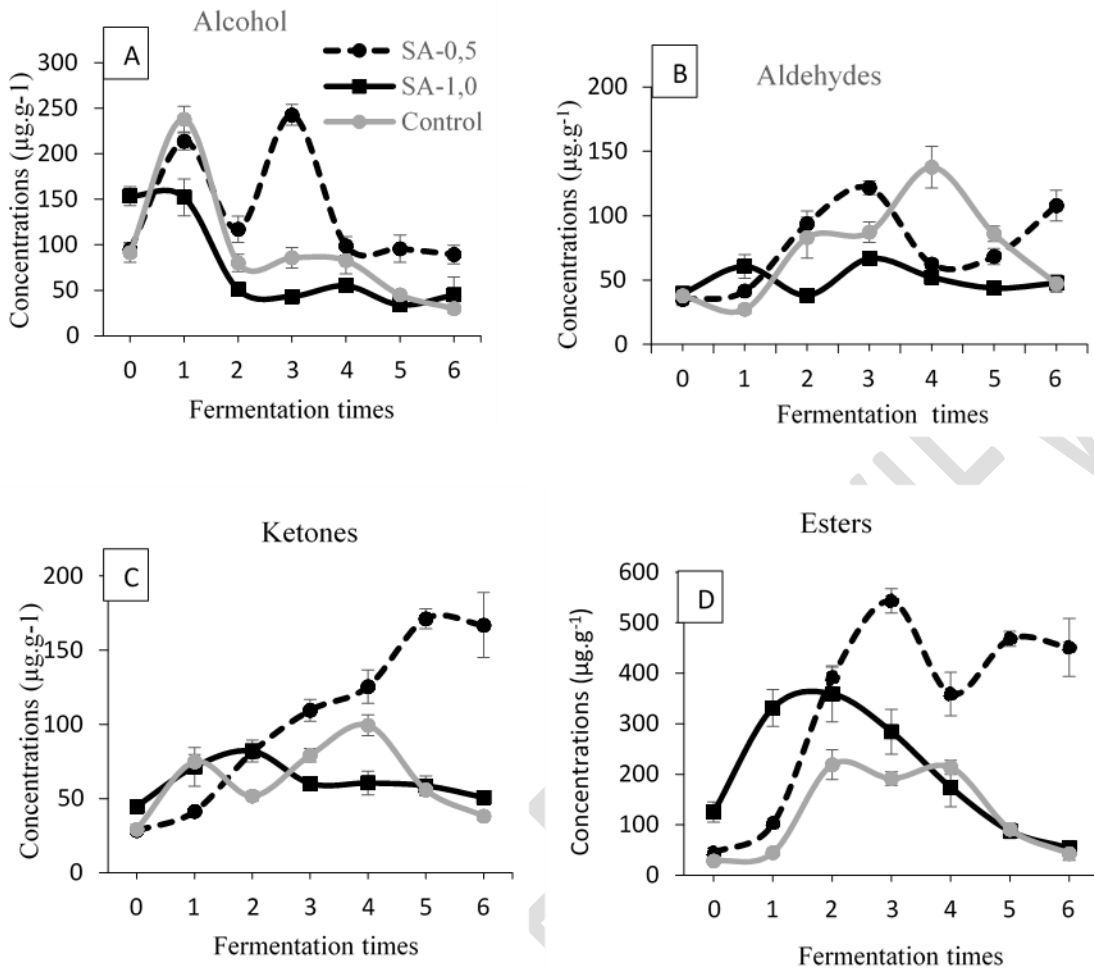
3.1. Effect of *S. cerevisiae* inoculum concentrations on the production of different chemical volatile compounds groups.

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

For aldehydes, the results showed that, the total concentrations increased up to three fermentation days with maximum averages of 121.71 and 66.7 µg.g⁻¹ respectively in raw cocoa beans inoculated at 0.5 and 1 g of dehydrated yeast per kg of fresh cocoa beans, after that we observed a decrease. For the control, total concentration of aldehydes increased up to four fermentation day with a maximum average of 137.7 µg.g⁻¹ and decreased after. However, at six fermentation days, concentrations of total aldehydes in cocoa beans inoculated at 0.5 g.kg⁻¹ were significantly higher ($p < 0.001$) than those obtained in cocoa beans inoculated at 1 g.kg⁻¹ and control (**Fig. 1B**).

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

Fig. 1D show changes in total concentrations of esters found in cocoa beans inoculated with starter culture at different concentrations. Total concentration of esters increased up to maximum averages of 359.29 and 542.82 µg.g⁻¹ respectively in cocoa beans inoculated at 1 g.kg⁻¹ and with 0.5 g.kg⁻¹ at two and three fermentation days, after that a decreased was observed. For the control, the maximum contents were 218.91 µg.g⁻¹ (after four fermentation days). Our results showed that, contents of total esters were significantly higher ($p < 0.001$) in raw cocoa beans inoculated with 0.5 g.kg⁻¹ than other fermented cocoa beans.



SA-0,5 : cocoa beans inoculated with *S. cerevisiae* strain at the rate 0.5 g.kg⁻¹
 SA-1,0 : cocoa beans inoculated with *S. cerevisiae* strain at the rate 1.0 g.kg⁻¹
 Control : spontaneous fermented cocoa beans

Fig. 1. Changes in total concentrations of : **A)** alcohol family ; **B)** aldehydes family **C)**ketones family ; **D)** esters family , found in cocoa beans inoculated by selected aroma *S. cerevisiae* strain according to the fermentation times. Bars are ± standard deviation. N=4

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

Fig. 2 show changes in concentrations of specific aroma compounds found in cocoa beans inoculated with selected *S. cerevisiae* at two concentrations. The results showed that the two high alcohols concentrations, such as 3-Methyl-1-butanol and 2-Phenylethanol were significantly ($p < 0.001$) important in raw cocoa beans inoculated at 0.5 g.kg^{-1} (**Fig. 2A, 2B**).

The results of identification of aldehydes have shown two main compounds which were namely identified as 3-methylbutanal and benzaldehyde. Contents of 3-methylbutanal and Benzaldehyde were significantly ($p < 0.001$) higher in raw cocoa beans inoculated with 0.5 g.kg^{-1} , after six fermentation days compared to the control and inoculated cocoa beans at 1 g.kg^{-1} (**Fig. 2C, 2D**).

For specific ketones, contents of 2-Pentanone and Acetoin were significantly ($p < 0.001$) higher in raw cocoa beans inoculated at 0.5 g.kg^{-1} 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 g.kg^{-1} compared with the other fermented raw cocoa beans (**Fig. 2E, 2F**).

concentrations of main esters, such as Methyl acetate and Ethyl acetate were significantly ($p < 0.001$) higher in raw cocoa beans inoculated with 0.5 g.kg^{-1} compared with those inoculated at 1 g.kg^{-1} (**Fig. 2G, 2H**).

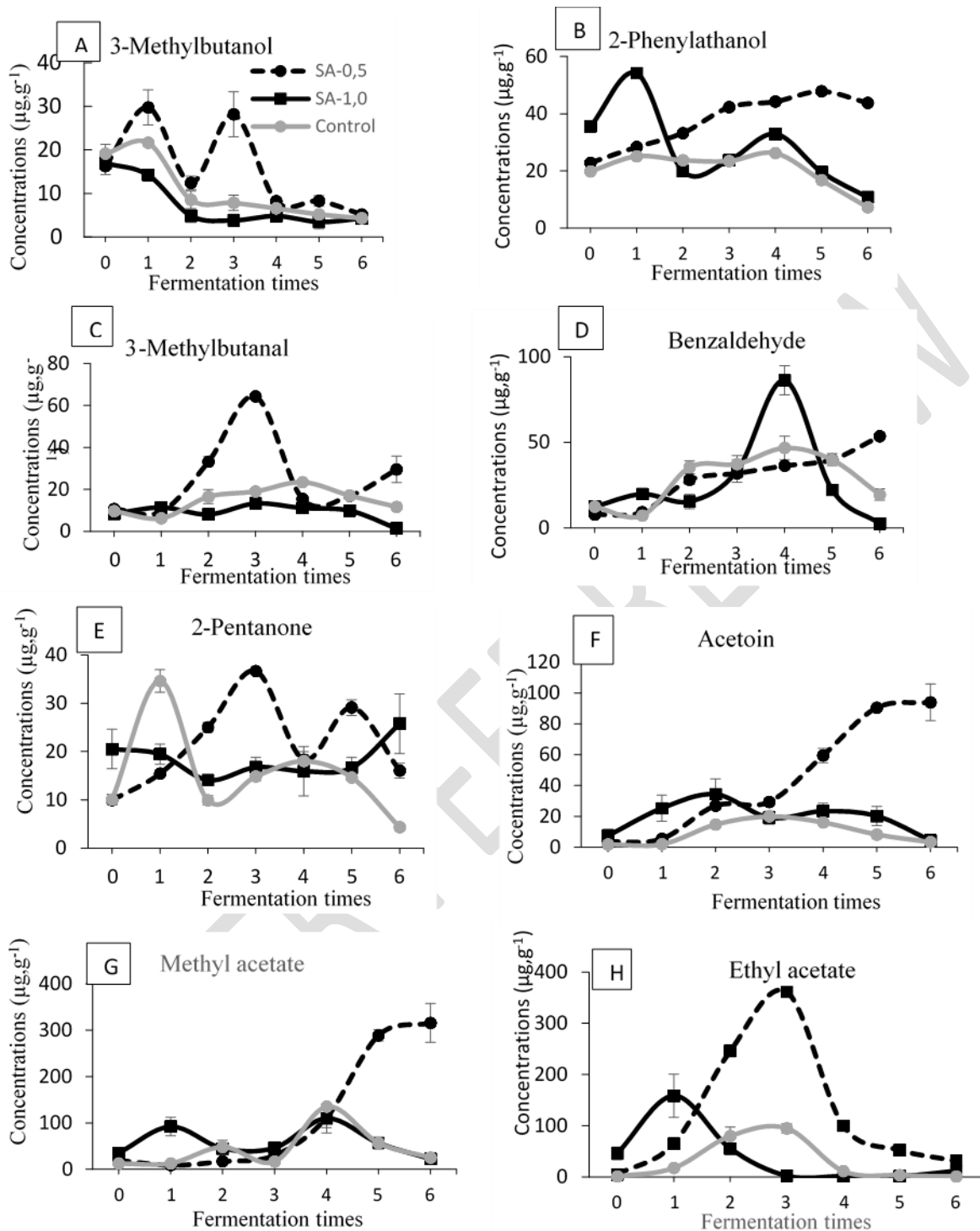


Fig. 2. Changes in concentrations of some specific aroma compounds such as : **A)** 3-methyl 1-butanol, **B)** 2-phenyl ethanol ; **C)** 3-methylbutanal, **D)** benzaldehyde ; **E)** 2-pentanone, **F)** acetoin ; **G)** methyl acetate, **H)** ethyl acetate, found in cocoa beans inoculated by selected aroma *S. cerevisiae* strain according to the fermentation times. Bars are \pm standard deviation. N=4

3.3. Effect of yeast inoculum concentration on the sensory quality of resulted chocolates

To correlate the results of aroma compounds analyzes with the sensory profile of chocolate, a sensory analysis were performed on the chocolates formulated from different inoculated cocoa beans. Acid, bitter, sweet, astringent, cocoa, flowery and fruity attributes were analyzed from tasting. **Fig.3.** shows the sensory profiles of chocolates issued from inoculated *S. cerevisiae* cocoa beans and spontaneously fermented cocoa beans (control). Chocolates prepared from inoculated *S. cerevisiae* cocoa beans at 0.5 g. kg⁻¹ is 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 g. kg⁻¹ which was bitter.

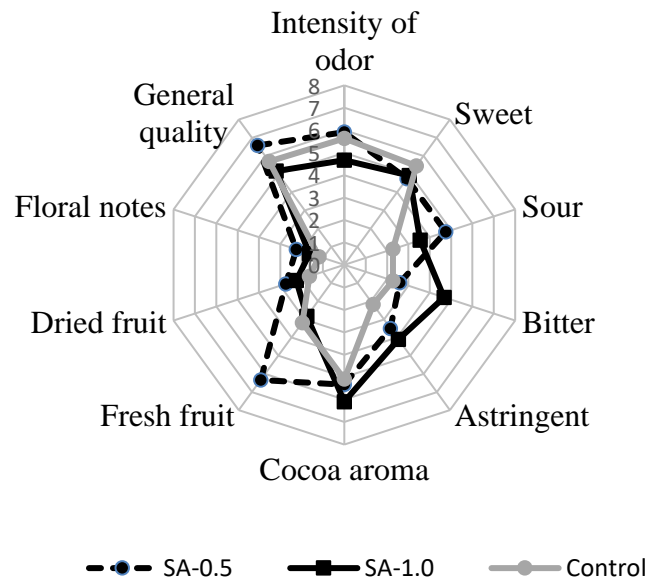


Fig. 3. Sensory differences in chocolates produced from fermented and sun-dried cocoa beans of the control (spontaneous process) and inoculated fermentations with selected *S. cerevisiae* strain. Comparisons were made by a panel of twelve selected and trained judges.

4. Discussion

Several previous studies reported that yeast growth and activity are essential for successful cocoa bean fermentation and for the development of desirable chocolate flavor [16, 20, 32, 34]. Therefore, in this work, selected *S. cerevisiae* strains were used as starter culture at different inoculum levels in order to observe their influence on the production of aroma compounds during the fermentation of cocoa beans and on sensorial properties of resulted chocolate. Our results on impact of *S. cerevisiae* strain on the volatile aroma profile 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. Our results were almost similar to those obtained by Sandhya *et al.* [22] who found alcohol average concentration of 15 mg.g⁻¹ in cocoa beans at the beginning of fermentation. Initial concentrations of total aldehydes and ketones could be explained by the fact that some of these components will be intrinsic to the bean [32]. In this study, the highest concentrations of all chemical groups of volatile compounds were found at three and four fermentation days, after that a decrease was observed when fermentation time increased. The decrease of the contents of all aroma compounds during the fermentation could be explained by their conversion into other organic compounds such as lactic and acetic acids due to the growth and activity of both lactic and acetic bacteria. Our results were different from those reported by Rodriguez-Campos *et al.* [25] who found that total alcohols, aldehydes, ketones, acids and pyrazines concentrations increased significantly with an increase in fermentation time. According to these authors, the high contents of these compounds were detected after six or eight fermentation days. The highest concentrations of all volatile compounds after three and four fermentation days observed in our work confirm that it is not necessary to extend the fermentation process to seven and eight days.

The effect of selected yeast inoculum concentration on the volatile profile of raw cocoa beans indicated that *S. cerevisiae* strain used at 0.5g.kg⁻¹ 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 competition between yeasts in the use of the substrate [35]. Besides, *S. cerevisiae* strain inoculated at 0.5 g. kg⁻¹ promoted more high concentrations of desirable volatile compounds such as, butanol 3-methyl, 2-Phenylethanol, 3-Methyl-1-butanol, Butanol 3-methyl, 2-Pentanone, Acetoin and methyl acetate than the rate 1 g

.kg⁻¹. 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 our study were similar to those obtained by Sandhya *et al.* [22] from 10 % inoculum of starter consortia regulated microbial succession, led to consistent fermentation and to the development of high qualitative characteristics of the raw cocoa.

The results about the effect of yeast inoculum concentration on the sensory quality of chocolate indicated that chocolates made from cocoa beans inoculated at 0.5g of yeast per kg of fresh beans 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 g of yeast per kg of fresh beans 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 rate 0.5g of yeast per kg of fresh beans contributed better to the sensory quality of cocoa beans than the rate 1g of yeast per kg of fresh beans. Our results were similar with those 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 %). All these results confirm that yeasts have a greater impact on the sensory qualities of cocoa beans and final chocolates [11, 16, 20, 27, 34]. Although the volatile profile of cocoa 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

The inoculation at different concentrations of strain of high aroma potential of *S. cerevisiae* isolated from wine fermentation as a starter culture for cocoa fermentation had the effect on the volatile profiles and their relative concentrations of raw cocoa material, which influence the sensory properties of resulted chocolates compared to spontaneous fermentation. The results showed that diverse chemical compounds families were found in raw cocoa beans. *S. cerevisiae* strain used at 0.5 g.kg⁻¹ produced desirable chocolate key aroma compounds such as esters (methyl acetate) and alcohols (butanol 3-methyl, 2-phenylethanol) than when it was used at 1g.kg⁻¹. Sensory

profiling concluded that the rate 0.5 g of selected yeast per kg of fresh beans led to the chocolate with better fruity notes and higher scores of general quality than chocolate produced from 1 g of yeast per kg of fresh inoculated cocoa beans. *S. cerevisiae* strain used at 0.5 g.kg⁻¹ contributed better to the formation of volatile profile as aromatic potential yeast than the rate 1 g.kg⁻¹.

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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 (*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, 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 flavor precursors during spontaneous fermentation of fine flavor Trinitario cocoa beans. *European Food Research and Technology*. 2019; doi.org/10.1007/s00217-019-03307-y.
- 8 Crafacek 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. Curing 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, Kaasik 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 Schwan RF, Pereira GV, Fleet GH. “Microbial activities during cocoa fermentation,” in *Cocoa and Coffee Fermentations*, eds R. F. Schwan and G. H. Fleet (Boca). 2014.
- 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 interactive 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 Afoakwa EO. *Chocolate Science and Technology*. Wiley-Blackwell Publishers Oxford UK. 2010; pp 3-22.
- 29 Serra-Bonvehi J. Investigation of aromatic compounds in roasted cocoa powder. *European Food Researches and Technology*. 2005; 221: 19–29.
- 30 Frauendorfer F, Schieberle P. Identification of the key aroma compounds in cocoa powder based on molecular sensory correlations. *Journal of agricultural and food chemistry*. 2006; 54(15): 5521-5529.
- 31 Frauendorfer F, Schieberle P. Changes in key aroma compounds of Criollo cocoa beans during roasting. *Journal of Agricultural and Food Chemistry Science*. 2008; 56: 137-152.
- 32 Ho VTT, Zhao J, Fleet G. Yeasts are essential for cocoa bean fermentation. *International Journal of Food Microbiology*. 2014; 174: 72–87.

- 33 Belitz HD, Grosch W, Schieberle P. Coffee, tea, cocoa. *Food chemistry*. 2004; 938-970.
- 34 Chagas Juniora GCA, Ferreira 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 Aculey PC, Snitkjaer P, Owusu M, Bassompierre P, Takrama J, Nørgaard L, Petersen MA, Nielsen DS. Ghanaian cocoa bean fermentation characterized by spectroscopic and chromatographic methods and chemometrics. *Journal of Food Science*. 2010; 75: 300-307.
- 37 Crafac 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 Aprotosoie 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 Deucher 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.