Polyphasic comparison of yeast population's dynamics in box and heap fermentation of cocoa bean in Cameroon

POLYPHASIC COMPARISON OF YEAST POPULATION'S DYNAMICS INVOLVED IN BOX AND HEAP FERMENTATIONS OF COCOA BEANS IN CAMEROON

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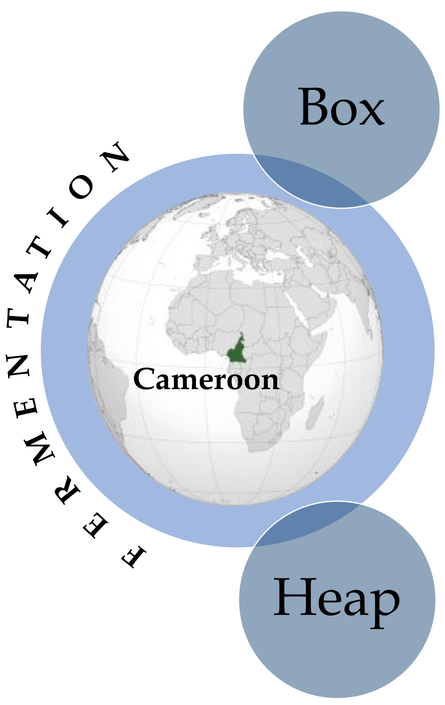
The use of starter cultures during food fermentation has been extensively studied preliminary, in order to obtained quality cocoa. Recent studies are going on to determine the influence of started cultures on the fermentation process to improve traditional practices with special attention on yeasts. This microorganism has been used to improve the pectinolytic activity in order to drain the pulp during fermentation of foodstuff such as, wine, jam, alcohol, syrup and marmalades (Buamah *et al.,* 1997; Dzogbefia *et al.,* 1999; Leal *et al.,* 208; Samah *et al.,* 1992; Sanchez *et al.,* 1985). *Saccharomyces cerevisiae* has been used as the preferred starter culture for most food and beverage fermentation processes (Schwan *et al.,* 1995) and has been received the ''generally recognized as safe'' (GRAS) and ''qualified presumption of safety'' (QPS) status. This study aimed to determine the dynamics of yeast populations and the role of these microorganisms during fermentation including, the changes in temperature, pH, chemical compounds, and activation of certain metabolites during box and heap fermentation of cocoa beans in Cameroon.

METHODOLOGY

Table 1. General methodology of cocoa fermentation study

Time	0 2 4 5 6	Analysis
	*Saccharomyces cerevisiae* (S)	Physical Microbiological Chemical
	S. cerevisiae and *Torulaspora delbrueckii* (ST)	
	Control	



SAMPLE PREPARATION

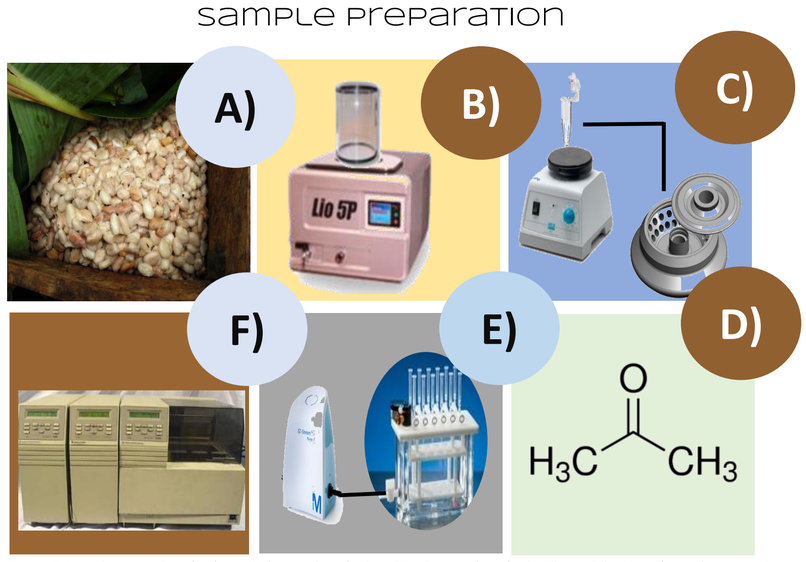


Figure 2. Sample preparation for fermented cocoa bean/pulp. **A)**Fresh cocoa bean/pulp, **B)** Liophilization of samples previusly pestle, **C)** Defeated ground samples were vortex and centrifugate, **D)** Removal of proteins through acetone:water:formic acid (70:29.5:0.25), **E)** Removal of polyphenols throught C18 cartridge, **F)** High-performance liquid chromatography

EQUIPMENT CONDITIONS

Table 2. Non-volatile compounds parameters used to quantified and identified organic acids

COMPOUNDS	SPECIFICATIONS	
• Oxalic • Lactic • Citric • Succinic • Gluconic • Formic • Malic • Tartaric • Piruvic • Fumaric	**Equipment**	HPLC, SpectraSystem P4000, Thermo Scientific
	Column	Aminex Ion Exclusion HPX-87H 300 x 7.8 mm column
	Mobile phase	Sulfuric acid 0.013N
	Flow rate	0.8 ml/min
	Detector	UV/VIS (210 nm)

Table 3. General parameters used to quantified and identified volatile compounds

COMPOUNDS	SAMPLE	
• Alcohols • Esters • Terpen • Aldehydes • Ketones • Acids • Phenol • Other	Dilution	40% NaCl
	HHS-SPME PARAMETERS	
	Fiber	50/30 um (DVB/CAR/PDMS)
	Exposition time	30 min
	GC-MS PARAMETERS	
	Column	DB-Wax ETR (30 m × 0.25 mm i. d. × 0.25 µm)
	Injection temperature	Helium at 1.0 ml/min
	Oven program	40°C x 5 min (5°C m^{-1}) 200°C x 0 min (10°C m^{-1}) 240°C x 5 min (5°C m^{-1}) 200°C x 30 min

RESULTS & DISCUSSIONS

PHYSICAL AND MICROBIOLOGICAL ANALYSIS

Table 4. Average temperature, pH and cell counts during box and heap fermentation of cocoa bean-pulp

Box		Fermentation time				
		0	48	96	120	144
pH	S	3.55 ± 0.03	3.88 ± 0.16	4.15 ± 0.11	3.96 ± 0.18	4.07 ± 0.16
	ST	3.54 ± 0.01	4.00 ± 0.16	4.20 ± 0.15	4.18 ± 0.26	4.18 ± 0.05
	C	3.55 ± 0.03	3.88 ± 0.17	4.15 ± 0.11	3.96 ± 0.18	4.07 ± 0.16
Temperature (°C)	S	26.73 ± 0.62	36.20 ± 1.53	41.88 ± 1.78	43.70 ± 2.94	43.97 ± 0.42
	ST	26.48 ± 0.34	35.10 ± 2.46	41.73 ± 2.06	42.78 ± 3.68	44.80 ± 0.70
	C	26.73 ± 0.62	36.20 ± 1.53	41.88 ± 1.78	43.70 ± 2.94	43.57 ± 0.42
Yeast (Log CFU)	S	6.66 ± 0.68	7.16 ± 0.23	<4.00 ± 2.31	4.65 ± 2.70	2.51 ± 0.04
	ST	6.63 ± 0.63	7.05 ± 0.06	<4.00 ± 0.00	4.98 ± 1.13	1.03 ± 0.04
	C	6.65 ± 0.46	7.52 ± 0.37	4.41 ± 0.47	2.00 ± 1.15	1.98 ± 0.04
LAB (Log CFU)	S	6.38 ± 0.31	8.16 ± 0.13	5.10 ± 2.45	5.83 ± 2.43	3.73 ± 0.35
	ST	7.13 ± 0.22	7.84 ± 0.27	4.33 ± 1.53	8.39 ± 0.30	5.19 ± 0.16
	C	7.01 ± 0.75	7.87 ± 0.40	<3.00 ± 0.00	6.49 ± 0.59	5.37 ± 0.24
AAB (Log CFU)	S	6.36 ± 0.24	7.00 ± 0.16	5.04 ± 2.36	5.11 ± 2.46	5.31 ± 0.04
	ST	6.21 ± 0.37	6.93 ± 0.18	5.10 ± 2.42	7.31 ± 0.24	3.53 ± 0.04
	C	6.28 ± 0.26	7.31 ± 0.26	<3.00 ± 0.00	5.75 ± 0.50	4.97 ± 0.02

Heap		Fermentation time				
		0	48	96	120	144
pH	S	3.55 ± 0.01	4.24 ± 0.17	4.48 ± 0.79	4.90 ± 0.97	5.30 ± 0.59
	ST	3.53 ± 0.01	4.32 ± 0.23	4.05 ± 0.40	4.52 ± 0.74	4.65 ± 0.62
	C	3.50 ± 0.05	3.87 ± 0.08	4.24 ± 0.29	3.99 ± 0.33	4.33 ± 0.39
Temperature (°C)	S	28.20 ± 1.15	38.17 ± 0.75	36.57 ± 1.80	40.07 ± 0.12	38.95 ± 1.95
	ST	27.37 ± 0.32	39.00 ± 2.21	36.57 ± 0.90	39.38 ± 0.32	40.60 ± 0.52
	C	26.27 ± 0.06	38.97 ± 0.32	39.37 ± 2.57	40.30 ± 0.56	39.13 ± 0.15
Yeast (Log CFU)***	S	6.30 ± 0.92	7.71 ± 0.15	7.12 ± 0.16	7.99 ± 0.29	5.59 ± 0.16
	ST	6.25 ± 0.85	7.71 ± 0.15	6.54 ± 1.41	7.43 ± 0.07	7.63 ± 0.04
	C	6.52 ± 0.71	6.62 ± 0.02	6.37 ± 0.04	7.20 ± 0.28	6.52 ± 0.03
LAB (Log CFU)***	S	6.64 ± 0.25	8.27 ± 0.19	8.33 ± 0.04	8.65 ± 0.28	8.24 ± 0.02
	ST	6.91 ± 0.29	8.07 ± 0.09	7.99 ± 0.01	8.51 ± 0.04	8.20 ± 0.03
	C	6.72 ± 0.03	7.15 ± 0.21	8.38 ± 0.04	9.09 ± 0.13	8.21 ± 0.04
AAB (Log CFU)***	S	6.17 ± 0.23	6.80 ± 0.14	8.32 ± 0.02	7.78 ± 0.18	7.54 ± 0.02
	ST	5.73 ± 0.04	7.10 ± 0.28	8.08 ± 0.11	7.66 ± 0.01	7.88 ± 0.03
	C	6.63 ± 0.04	5.64 ± 0.15	8.44 ± 0.02	8.59 ± 0.04	8.33 ± 0.04

Values are expressed as mean ± standard deviations from triplicate determinations. Abbreviations: **S:** *Saccharomyces cerevisiae* strain (ID67), **ST:** *S. cerevisiae* and *Torulaspora delbrueckii* strain (ID103) and **C:** Control.

CHEMICAL ANALYSIS

NON-VOLATILE

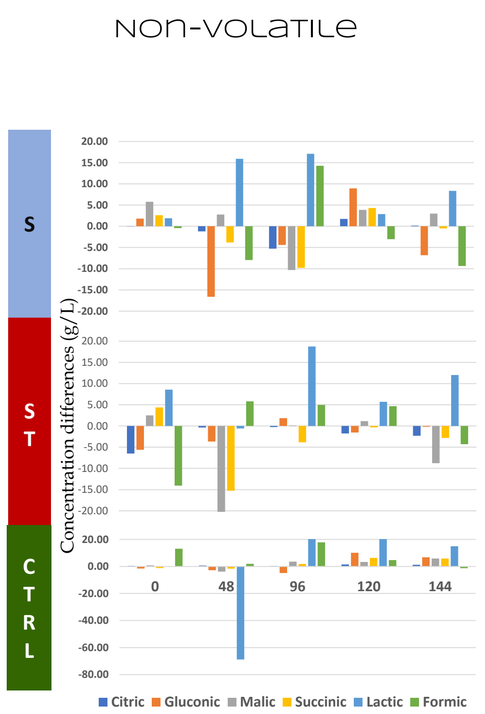


Figure 2. Difference of box and heap fermentation during cosoa fermentation (g/L)

VOLATILE

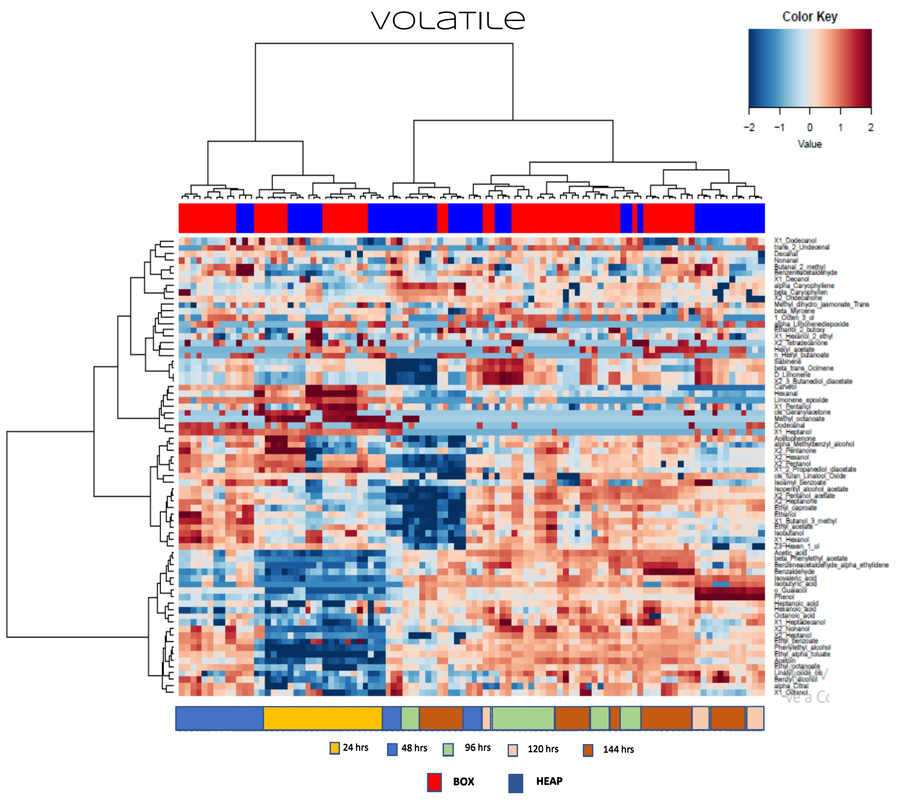


Figure 3. Volatile profile of box and heap fermentation during inoculated and non-inoculated cocoa fermentation (µg/kg)

PHYSICAL

The physical behavior during cocoa bean/pulp fermentation has already been investigated, indicating similar behaviors during fermentation. For example:
•Lower temperatures during fermentation (around 40-43 °C), slower increased in temperature from box fermentation compared with heap and maximal fermentation temperatures reached at 70 h
•Expected high initial counts of all microorganisms (yeast, LAB and AAB) are in accordance with previous studies. Similar yeast dynamics were recently observed by da Veiga *et al.,* (2013), reporting the highest peak of yeast counts at 36 h

NON-VOLATILE

Microbial communities are involved in the activation of important metabolic pathway to produce organic acids, during cocoa fermentation. For example, the non-oxidative phosphate pathway (formic acid) and the oxidative pathway of the TCA (succinic, malic, citric, fumaric, gluconic) have been demonstrated to activate them by yeast and/or bacteria.
This is the first study reporting formic, gluconic, formic, fumaric and tartaric acid. The organic acids mention before, play an important role during fermentation and it will help us to understand the mechanism of action of microbial communities during fermentation

VOLATILE

Seventy-two volatile compounds were identified by SPME-HS/GC-MS for all fermentation courses. Alcohols (20), esters (14) and terpenes (13) were the main present groups.
The most aboundant volátile's group compounds in terms of relative percentage were alcohols, esters and acids. Being the most aboundant the alcohol group.
Moreover, previuos studies have been identifying lower amount of volátile compounds during cocoa fermentation compared to what is reported above (Rodriguez-Campos *et al*., 2011)

CONCLUSION

A polyphasic approach applied in this study allowed us to compare and understand the potential of different yeast strains as starter cultures for enhanced two different fermentation setups. The combination of microbial analyses and metabolite analyses of a defined starter culture, provided us an overview of the differences between population dynamics in fermentation processes. As shown in the present study, minor physical and chemical differences were observed between the inoculated and spontaneous fermentations. Although, the aroma profile differed from fermentation process which indicated the effect of starter cultures. Future research is needed to assess inoculation densities of well-chosen starter cultures during fermentation in commercial scale heap, box and tray processes to standardized end products with respect to color, flavor and taste profiles of desirable