Journal of Agricultural Science and Food Technology
Vol. 1 (3), pp. 22-33, August, 2015
Research Paper
ISSN: 2465-7522
http://pearlresearchjournals.org/journals/jmbsr/index.html

Optimization of Cocoa Pulp Juice Fermentation with Yeast Starter Cultures of Cocoa Heap Fermentations

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Accepted 31 July, 2015

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ABSTRACT

The Cocoa Research Institute of Ghana (CRIG) produces crude ethanol from cocoa sweatings by spontaneous fermentation and distillation. The crude ethanol is further refined and blended into gin and brandy for the local market. The operation is sub-optimal and results in waste of raw materials, energy and time. Following a research carried out on the biodiversity and population of microorganisms involved in heap fermentation, pure cultures of the yeasts became available thus prompting the need to further optimize the process of alcohol production from cocoa pulp juice using starter cultures prepared from microorganisms isolated from cocoa heap fermentations. In these experiments, 10 yeast strains and a combination of some of these strains were used as starter cultures for the fermentation of cocoa pulp juice to produce ethanol. Results from metabolite analyses of this study revealed that *Saccharomyces cerevisiae* and *Issatchenkia orientalis* gave maximum ethanol production of 1571.7 mM and 1396.8 mM within 36 and 48 h of fermentation, respectively as compared to the spontaneous fermentation which gave maximum ethanol production of 503.2 mM within 48 h. *Pichia kluyveri* which gave the lowest ethanol production (324.9 mM) produced the maximum mannitol concentration of 249.0 mM.

Key Words: Cocoa sweatings, Metabolite analysis, HPAEC-PAD, GC-MS, Sensory evaluation.

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INTRODUCTION

Raw cocoa beans are sheathed in an aromatic mucilaginous pulp, which constitutes about 40% of the seed fresh weight. This mucilaginous pulp is spongy and contains the sap (pulp juice) known in the industry as ‘sweatings’. It is made up of about 82 to 87% water. Cocoa sweatings is rich in sugars (10 to 15%), salts (8 to 10%), pectin (2 to 3%), organic acids (1 to 2%) and proteins (0.6%) (Roelofsen, 1958; Lopez, 1986; Schwan and Wheals, 2004) with a relatively low pH (3.0 to 4.0) primarily due to high concentration of citric acid (1 to 3%) (Thompson et al., 2001; Ardhana and Fleet, 2003; Schwan and Wheals, 2004). The relatively high content of pectin (2 to 3%) and other polysaccharides (1 to 2%) makes the pulp viscous limiting diffusion of air (Schwan et al., 1995; Schwan and Wheals, 2004). Of the sugars present, about 60% is sucrose and 39% is a mixture of glucose and fructose.

The concentration of sucrose, glucose and fructose is a function of cultivar and fruit age, with unripe pods containing higher proportions of sucrose and ripe pods containing mainly fructose and glucose (Roelofsen, 1958; Lehrian and Patterson, 1983; Thompson et al., 2001).

Cocoa fermentation occurs largely on the mucilaginous pulp. The reaction is spontaneous (allowing naturally

occurring microorganisms to ferment the pulp sugars) and involves a succession of microorganisms. The primary colonizers are yeasts, followed by lactic acid bacteria (LAB), acetic acid bacteria (AAB) and *Bacillus species*. During the early and mid-phase of the spontaneous fermentation of raw cocoa beans yeasts produce ethanol from carbohydrates and assimilate citric acid under anaerobic and low pH conditions and cause depectinization of the pulp, consequently the pulp liquefies and the juice drain off allowing air to diffuse into the heap (Schwan and Wheals, 2004; Schwan et al., 1995). As air diffuses into the heap, microaerophilic LAB, citrate-fermenting, acid-tolerant and ethanol-tolerant *Lactobacillus plantarum* and *Lactobacillus fermentum* strains dominate this phase (Cleenwerck et al., 2007; Nielsen et al., 2007).

In this micro-aerobic environment citrate and sugars are converted into acetic acid, lactic acid, and mannitol, causing a slight increase in pH of the pulp (Cleenwerck et al., 2007). As the fermenting mass becomes progressively more aerobic, *Acetobacter pasteurianus* grow to be the main AAB species but additionally, *Acetobacter syzygii*, *Acetobacter ghanensis*, *Acetobacter tropicalis*, and *Acetobacter senegalensis* are present (Cleenwerck et al., 2007; Cleenwerck et al., 2007; Nielsen et al., 2007). The AAB metabolize the ethanol initially formed by the yeasts to acetic acid. This process is exothermic and causes the temperature of the heap to rise to 45 to 50°C limiting the growth of many microorganisms. The increased temperature, in combination with increased pH and aeration is associated with growth of aerobic spore-forming *Bacillus species* (Thompson et al., 2001). The assessment of the microbial community of both the heap and tray fermentations in the past 7 years in Ghana resulted in the isolation and characterization of various species of yeast. Among these are *Saccharomyces cerevisiae, Candida krusei, C. carpophila, C. orthopsilosis, C. quercitrusa, Pichia kluyveri, P. membranifaciens, P. mexicana, P. manshurica, P. caribbica*, *Hanseniaspora quilliermondii, H. opuntiae, Issatchenkia orientalis (*now *Pichia kudriavzevii), Saccharomycodes ludwigii* and *Kodamaea ohmeri* (Camu, 2007; Nielsen, 2006; Kumi, 2006; Daniel et al., 2009). Less frequently encountered species documented are *C. carpophila, C. orthopsilosis, Kodamaea ohmeri, Meyerozyma (Pichia) caribbica, Pichia manshurica, Saccharomycodes ludwigii, and Yamadazyma (Pichia) mexicana* (Daniel et al., 2009). *S. cerevisiae* is the most often detected and abundant yeast species during cocoa bean fermentations potentially due to their rapid growth, pectinolytic activity, and ethanol tolerance, followed by *Pichia kudriavzevii* (formerly *Issatchenkia orientalis*) and *Pichia membranifaciens*, generally after an initial fermentation phase dominated frequently by *H. guilliermondii* (Schwan et al., 1995; Ardhana and Fleet, 2003; Jerpersen et al., 2005; Nielsen et al., 2005, 2007). Knapp (1920) reported the possibility of producing alcoholic beverages from cocoa 'sweatings' on commercial scale in the early 20th century through controlled fermentation and distillation processes.

Many yeast species have been identified from cocoa bean fermentation but the activity of these wild yeast strains in natural fermentation is inadequate and limits the amount of ethanol produced per unit time probably due to the limited starting inoculum. Artificial inoculation of cocoa fermentations has been used to improve the production of sweatings (Sanchez et al., 1985; Buamah et al., 1997) hence it will be possible to increase the production of ethanol if the starting inoculum is increased. CRIG in the 1970s experimented and established general procedures for the conversion of cocoa pulp sugars into ethanol through fermentation and distillation (Adomako et al., 1995). In the CRIG's procedure, the pulp juice is collected by pressing fresh raw cocoa beans packed in large tanks mounted on wooden stands as described by Adomako and Takrama (1996) and spontaneous fermentation carried out in 220-liter metal drums. Spontaneous fermentation occurs due to naturally occurring microorganisms. These naturally occurring microorganisms involves a mixture of organisms that are not characterized hence may vary from one batch to the other. As a result undesirable secondary metabolites are produced that make the crude cocoa alcohol to be characterized with extreme astringency and bitterness which are not found in the raw cocoa sweatings and as such attributable to the fermentation process.

The fermented pulp juice also needs to undergo triple distillation to make the alcohol potable for formulation into alcoholic beverages such as gin and brandy. The procedure is very tedious, expensive and non-economical on large scale production.It is, therefore, important to have a method that will increase the quantity of ethanol produced, shorten the duration of fermentation, with a view to eliminating the extreme astringency and bitterness associated with the alcohol produced by spontaneous fermentation. Based on properties important for cocoa bean fermentation, namely sucrose, glucose, and citrate assimilation capacity, pH, ethanol, and heat-tolerance, ten yeasts strains isolated from Ghanaian cocoa bean fermentation were selected and made into starter cultures (Camu, 2007). In this work, ten yeast strains were used in single culture or dual mixed cultures in the large-scale (120 liters batch) fermentation of cocoa pulp juice (sweatings) to produce ethanol that was blended into Cocoa Gin and Brandy and evaluated for consumer acceptability.

MATERIALS AND METHODS

Preparation of Cultures for Fermentation

The cultures for the fermentation were prepared using broth containing 20 g /L of glucose (Sigma-Aldrich, St. Louis, MO) and 5 g/L of yeast extract sterilized by autoclaving at 121^{o}C for 15 min and was done over a period of three days. On the first day, 10 ml of the broth was inoculated with a colony of the yeast strain and incubated at 30^{o}C for 24 h. On the second day, 2 ml of the 10 ml culture was transferred into a fresh 100 ml broth and incubated at 30^{o}C for 24 h. Ten milliliters of the 100 ml culture mentioned above was transferred into another fresh 1 L broth and incubated at 30^{o}C for 24 h. All chemicals and reagents used in culture preparation were microbiological or analytical grade and those used in chromatography were HPLC grade.

Pulp Juice Fermentation and Distillation

About 120 L of cocoa pulp juice were put in metal drums fitted with taps from which samples were drawn. The initial pH and temperature were recorded and samples were taken before each metal drum was inoculated with the 1 L of the different strains of yeast (*S. cerevisiae, I. orientalis, P. kluyveri, P. carribica, P. mexicana, P. manshurica, C. carpophila, S. ludwigii, K. ohmeri* and *C. orthopsilosis*) and allowed to ferment for 5 days. A combination of yeasts strains (*S. cervisiae*/*I. orientalis*, *S. cerevisiae*/*C. carpophila* and *K. ohmeri*/ *C. carpophila*) were also used to ferment the pulp juice. Samples were taken at 6 h interval till the end of fermentation for metabolite analysis. After the fermentation period, 100 L of the fermented cocoa pulp juice were then distilled using the 40 gallon Jacob Carl stainless steel/Copper bottom pot Distillation Plant (CARL GmBH, Eislingen, Germany). The alcohol concentration of each fermentation process was recorded.

Metabolite Analysis

Sample Preparation

Frozen samples of the pulp were used to prepare aqueous extracts for metabolite analysis. Twenty grams of the pulp was mixed with 80 ml of ultrapure water (MilliQ; Waters Corp., Milford, MA, USA) with an omnimixer (Phillips, Brussels, Belgium) for 5 min. The homogenate was centrifuged at 17,000 x g at 4^{o}C for 15 min and the supernatant was retained. The sediment was washed with 20 ml of ultrapure water and centrifuged, and the washing supernatants were combined with the first ones to provide aqueous extracts for further analyses. These extracts were clarified by filtration through 0.45-µm pore-size filters (Whatman) before further use.

High Pressure Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)

Concentrations of glucose, fructose, maltose and mannitol were determined by HPAEC-PAD (Dionex, Sunnyvale, CA) using a CarboPacPA10 column. The mobile phase, at a flow rate of 1.0 ml min^{-1}, consisted of ultrapure water (0.015 µS cm^{-1}; eluent A), 167 mM NaOH (eluent B), and 500 mM NaOH (eluent C) with the following gradient: 0.0 min, 87% A and 13% B; 40.0 min, 15% A and 85% B; 41.0 min, 100% C; 49.0 min, 100% C; 50.0 min, 87% A and 13% B; and 65.0 min, 87% A and 13% B. The samples (700 µl) were treated with acetonitrile (700 µl) to remove proteins.

Gas Chromatography-Mass Spectrometry (GC-MS)

Ethanol was measured by gas chromatography on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled to an Agilent 5973N mass spectrometer (Agilent Technologies). A capillary column (DB-WAXetr; Agilent Technologies) was used together with the following temperature programme: 0.0 min, 40^{o}C; 5.0 min 40^{o}C; 9.29 min, 100^{o}C; 10.37 min, 230^{o}C and 15 min, 230^{o}C.

Preliminary Sensory Evaluation

A consumer type of sensory evaluation was conducted for the blended gin and brandy using the alcohols obtained from cocoa pulp fermentation for the different strains of yeasts (*Pichia manshurica, P. kluyveri, P. caribbica, Kodamaea ohmeri, Saccharomycodes ludwigii, Issatchenkia orientals, Candida orthopsilosis S. cerevisiae/I. orientalis*) and that of the alcohol obtained from a spontaneous fermentation to show how the various products are likely to be accepted on the market. Twenty untrained personnel from CRIG were used in the evaluation. Taste and smell were the main sensory characteristics that were evaluated. For taste, the parameters considered were bitterness and biting effect that is usually induced by high acidity. For the smell, the question asked was whether the formulated gin or brandy smelled good or bad. Cocoa gin and brandy produced by the New Product Development Unit of CRIG were used as controls.

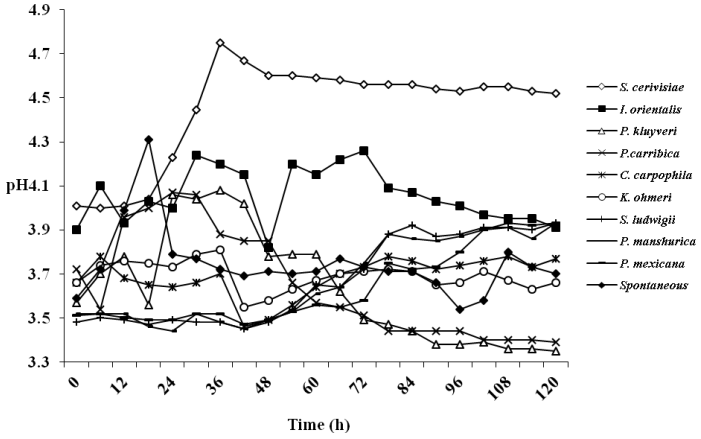

Figure 1. pH changes during cocoa pulp juice fermentation using different yeast strains: *S. cerevisiae, I. orientalis*, *P. kluyveri, P. caribbica, C. carpophila, K. ohmeri, S. ludwigii, P. Mexicana, P. manshurica* relative to the spontaneous fermentation.

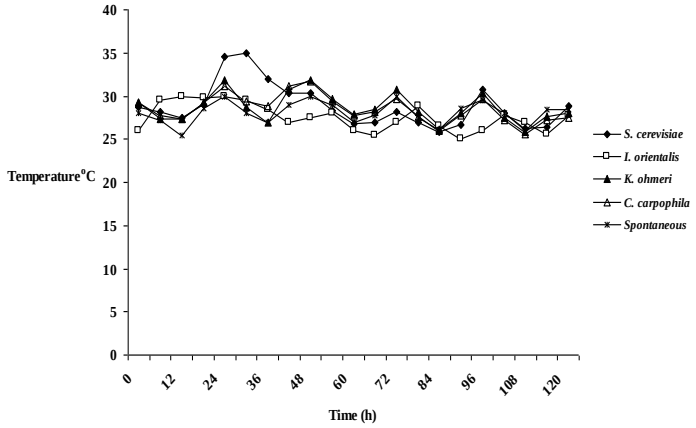

Figure 2. Temperature changes during cocoa pulp fermentation of four of the top fermenting yeast strains relative to the spontaneous process.

Data Analysis

Data analysis and graphics were made using Microsoft Excel Program.

RESULTS AND DISCUSSION

pH and Temperature Changes During Cocoa Pulp Fermentation

pH and temperature changes during the fermentation remained relatively constant for all the yeast strains (Figures 1 to 2). The pH and temperature changes for the experiments conducted using *S. cerevisiae* as starter cultures showed a trend that was in accordance with that observed by Ravelomanana et al. (1985). The temperature rose from an initial temperature of 28.7 to 34.9^{o}C after 24 h and gradually decreased to about 29^{o}C after 60 h (Figure 2). However, temperature changes for the rest of the yeast species and that of the spontaneous fermentation were relatively stable around 29^{o}C throughout the fermentation. This could be due to the fact that metabolism of the sugars by the other yeast species

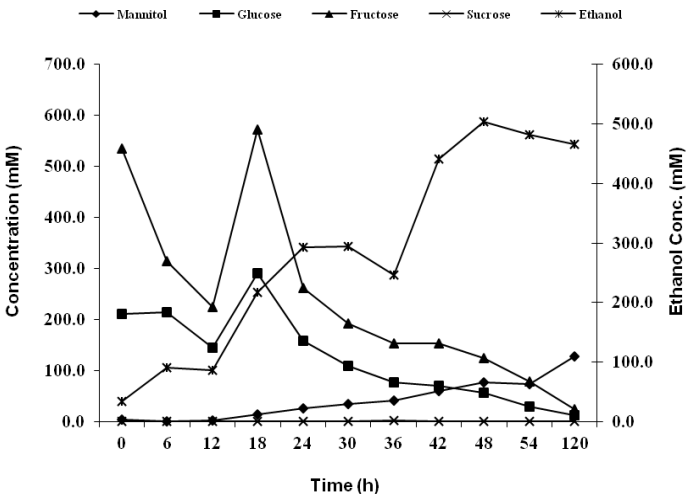

Figure 3. The utilization of glucose, fructose and sucrose and the production of ethanol and mannitol during cocoa pulp juice fermentation by spontaneous process.

and that of the spontaneous were too low to bring about the small increase in temperature as observed by Ravelomanana et al. (1985). The pH changes for the yeasts were also around 4.0 except for *S. cerevisiae* which was around 4.8 (Figure 1).

The rise in pH due to the activities of *S. cerevisiae* support the fact that, after the production of alcohol thereis always a drop in the pH of the fermenting pulp juice. Using combinations of yeast strains such as *S. cervisiae* and *I. orientalis* to ferment the pulp juice did not lead to an increase in temperature and pH (data not shown) as compared to the single strain fermentations. This could be due to the fact that there was not enough substrate for both yeasts to metabolize as a result of competition hence the relatively stable temperature. The conversion of glucose and fructose to ethanol is an exothermic process producing about 93.3 kJ/mol and as fermentation proceeds, pH and temperature also increase (Knapp, 1937; Forsyth and Quesnel, 1963; Carr, 1982; Schwan et al., 1995; Thompson et al., 2001).

Metabolite Analysis

The fermentation of the fresh cocoa pulp juice was conducted for five days using the various yeast strains. The spontaneous fermentation (Figure 3) was also carried out for 5 days where maximum alcohol production of 503.2 mM was achieved in 48 h with residual fructose or glucose concentration of 120 mM or less. A general observation from all the graphs, indicated that fructose or glucose concentration after 48 h was less than 120 mM (Figures 6, 8 and 10) and effectively zero in other cases (Figures 4, 5, 11 and 12) except in (Figure 9) where these values were about 270 mM. This trend indicates that extending sweatings fermentation (spontaneous or using starter cultures) beyond 48 h in most cases has no added advantages in enhancing alcohol yields as fermentable sugars become limiting soon after this period. Similar trends were observed in cocoa bean fermentation carried out by Camu (2007). Among the yeast strains used, *S. cerevisiae* was the best performer in terms of attaining high ethanol concentration within the shortest time of fermentation. This was followed by *I. orientalis*. *S. cerevisiae* recorded a maximum ethanol concentration of 1571.7 mM after 36 h of fermentation (Figure 4) while *I. orientalis* produced maximum ethanol concentration of 1396.8 mM after 48 h of fermentation (Figure 5). The sugar utilization patterns for *S. cerevisiae* and *I. orientalis* indicated that between 36 and 48 h of fermentation almost all the sugars have been converted to alcohol. This means that in fermenting cocoa pulp juice with either *S. cerevisiae* or *I. orientalis*, optimum fermentation could be obtained between 36 and 48 h. This fit into our expectation of producing alcohol within the shortest possible time but less than the five days. In a controlled alcoholic fermentation carried out using *S. cerevisiae* (Anvoh et al., 2010), alcohol production from cocoa pulp juice almost doubled (8.4%) compared to

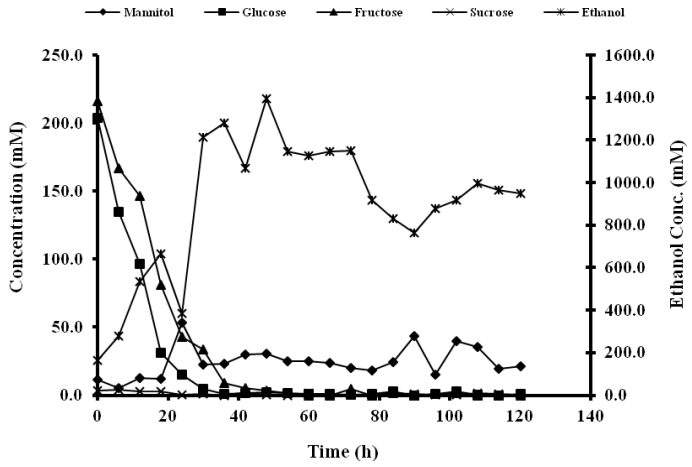

Figure 4. The utilization of glucose, fructose and sucrose and the production of ethanol and mannitol during cocoa pulp juice fermentation by *S. cerevisiae.*

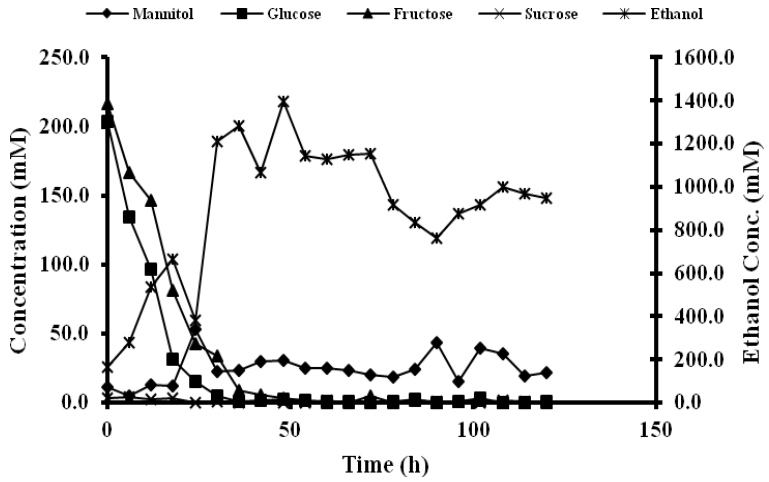

Figure 5. The utilization of glucose, fructose and sucrose and the production of ethanol and mannitol during cocoa pulp juice fermentation by *I. orientalis.*

4.3% by the spontaneous fermentation. *S. cerevisiae* and *I. orientalis* (*Candida krusei*) which recorded the maximum ethanol concentration is not unexpected because they are known to ferment sugars vigorously and as a consequence are the most numerous and frequently isolated yeast during cocoa bean fermentation (Schwan et al., 1995; Ardhana and Fleet, 2003; Jerpersen et al., 2005; Nielsen et al., 2005, 2007).

In comparing the metabolism of sugars of the combination of *S. cerevisiae* and *I. orientalis* to that of the individual yeast strains, one could conclude that their performance in terms of ethanol production fell below expectation. The maximum ethanol concentration using the two strains combined was 503.7 mM (Figure 6) which is comparable to that of the spontaneous fermentation. The low activity could be due to competition among the two yeast strains and also insufficient substrate for the two to metabolize and hence could not attain maximum activity. *P. kluyveri* had the lowest activity in terms of ethanol production. It gave a maximum ethanol

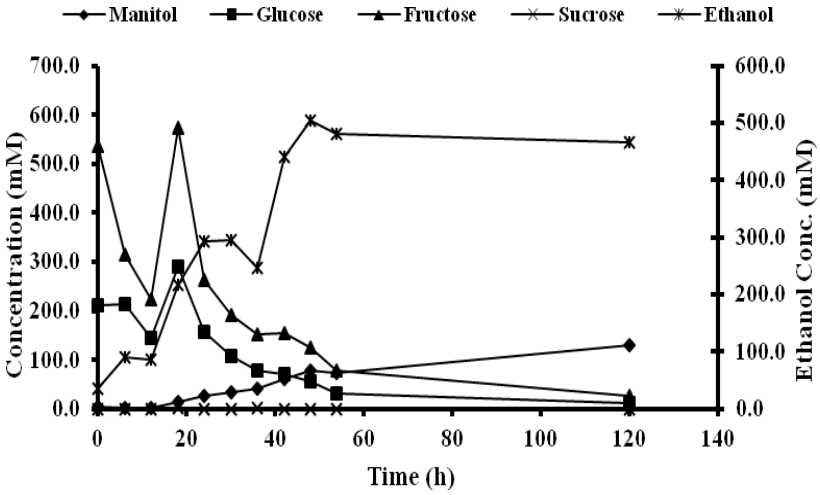

Figure 6. The utilization of glucose, fructose and sucrose and the production of ethanol and mannitol during cocoa pulp juice fermentation by *S. cervisiae/I. orientalis.*

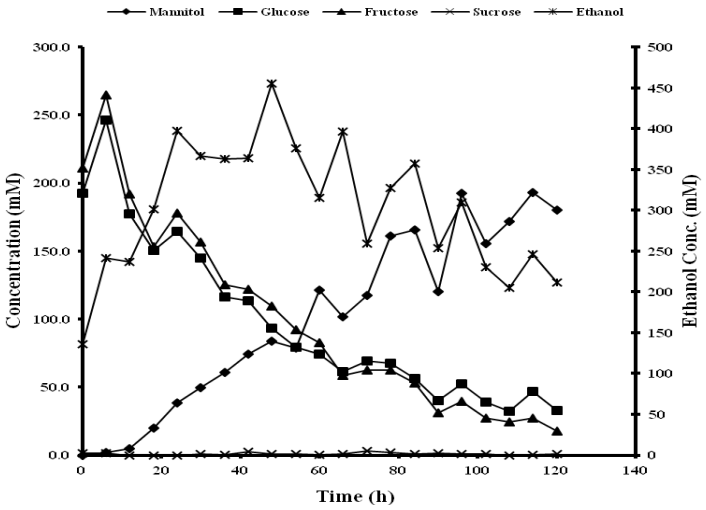

Figure 7. The utilization of glucose, fructose and sucrose and the production of ethanol and mannitol cocoa pulp juice fermentation by *P. caribbica.*

concentration of 324.9 mM (Figure 9) after 120 h of fermentation and lower than that obtained by spontaneous fermentation (Figure 3). This means that at the end of fermentation, some sugars such as glucose and fructose were not fully metabolized. *P. caribbica* and *P. manshurica* also showed low activity.

The maximum ethanol concentration for *P. caribbica* was 450 mM recorded at the 48th h of fermentation (Figure 7) while *P. manshurica* recorded maximum ethanol concentration of 400 mM by the 60th h (Figure 11). *K. ohmeri, S. ludwigii* and *P. mexicana* showed a high maximum ethanol concentration of over 1000 mM. The maximum ethanol concentration for *K. ohmeri* was1095.3 mM (Figure 8). *Saccharomycodes ludwigii* recorded 1002.8 mM (Figure 12) whilst that of *P. mexicana* was 1002.6 mM (Figure 10). However, these concentrations were recorded after 96 to 120 h of fermentation and therefore did not fit into our premise of reducing the duration of fermentation. Mannitol is a polyhydric alcohol derived from mannose or fructose and present in fruits, leaves and other parts of various plants. It is widely used in the food industry because of its unique

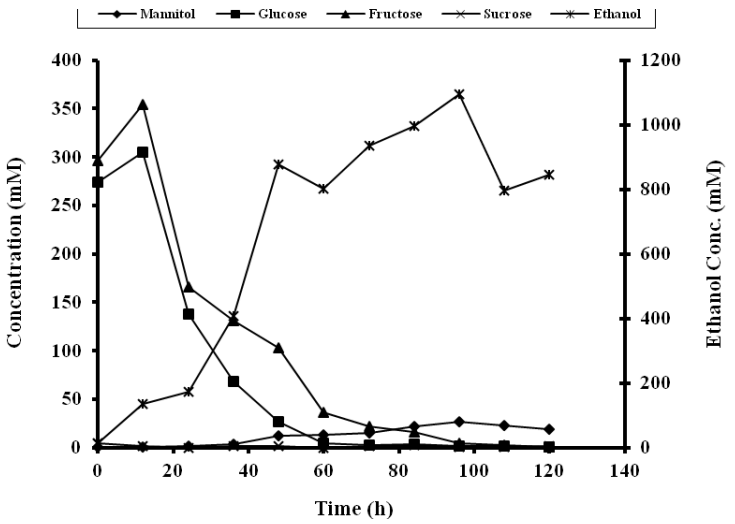

Figure 8. The utilization of glucose, fructose and sucrose and the production ethanol and mannitol during cocoa pulp juice fermentation by *K. ohmeri.*

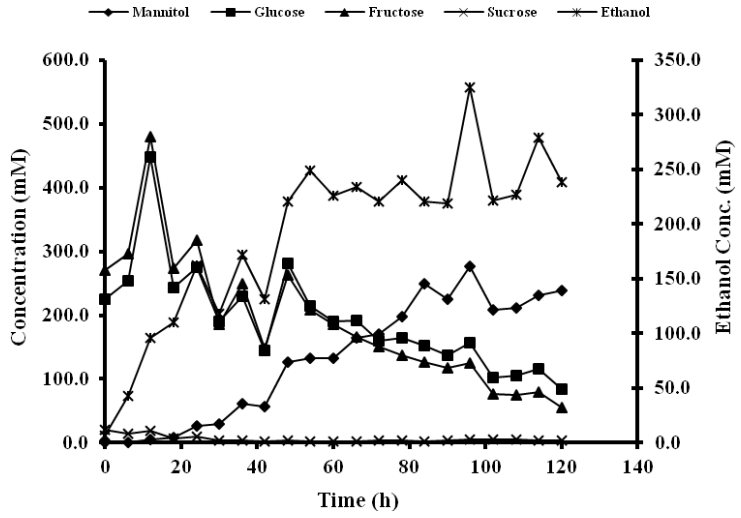

Figure 9. The utilization of glucose, fructose and sucrose and the production of ethanol and mannitol during cocoa pulp juice fermentation by *P. kluyveri.*

functional properties. As fermentation proceeded, the production of mannitol increased as observed by Camu (2007). *P. kluyveri* which gave the lowest ethanol concentration however recorded the maximum concentration of mannitol of 249.0 mM (Figure 9). *P. caribbica* also gave a maximum mannitol concentration of 192.9 mM (Figure 7).

Strength of Alcohol Produced and Preliminary Sensory Evaluation

The Cocoa Research Institute of Ghana has since the 1990s been distilling alcohol from spontaneous fermentation of cocoa pulp juice using the Jacob Carl Distillation Plant used for the distillation in this study

Table 1. Percentage alcohol produced for the different strains of yeast during the first distillation of fermented cocoa pulp juice.

Yeast strains	Percentage Alcohol Produced	
	First liter distilled	Bulked distillate
S. certevisiae	84	56
I. orientalis	86	54
P. kluyveri	74	10
P. caribbica	76	29
P. mexicana	85	54
P. manshurica	86	53
C. carpophila	85	53
S. ludwigii	85	54
K. ohmeri	80	47
C. orthopsilosis	84	33
S. cerevisiae + *I. orientalis*	85	30
Spontaneous Fermentation	85	30

*First Liter Distillate = % alcohol of the first liter at the start of distillation. *Bulked distillate = % alcohol after fermentation has been completed.

(Adomako et al., 1997). For example in 1997, 728 liters of alcohol at 82% strength was produced, blended and bottled for sale. Similarly in 1999, 1,110 liters of alcohol was produced and 657 liters was sold as technical grade alcohol and the remainder blended and bottled for sale as cocoa gin and brandy. In 2009, about 250 liters of cocoa gin and brandy were sold to the general public (Takrama et al., 2009). Alcohol produced from spontaneous fermentation is extremely astringent and bitter hence needs refining by triple distillation on the Jacob Carl Distillation plant before blending into gin and brandy. In this work, yeast strains were used to ferment cocoa pulp juice in order to reduce or eliminate the astringency and bitterness that characterized alcohol produced by the spontaneous fermentation and in addition eliminate the triple distillation refining step.

The percentage alcohol recorded in the bulked distillate from the Jacob Carl Distillation Plant is a measure of the strength of alcohol produced by the yeasts (Table 1). The first liter distillate distilled is usually a mixture of lower alcohols, for example, methanol (bp. 65^{o}C) and hence not added to the bulked alcohol which distils at 79^{o}C on the Jacob Carl Distillation Plant. The bulked distillate for the individual yeast strains showed that *S. cerevisiae* recorded the highest percentage alcohol of 56% and *P. kluyveri* recorded the lowest at 10%. *I. orientalis, P. mexicana* and *S. ludwigii* also produced alcohol of 54% whereas *P. manshurica* and *C. carpophila* produced alcohol of 53%. It is obvious that other yeast species apart from *S. cerevisiae* could be exploited for alcohol production from cocoa pulp juice. The combinations of yeast species which were expected to give a higher percentage of alcohol, however, did not (data not shown). The only combination which gave comparable results as the single strain fermentation was the *K. ohmeri /C. carpophila* mixture that gave an overall yield of 50% alcohol. Preliminary sensory evaluation conducted on the blended gin and brandy using alcohol fermented by the different yeast strains, *I. orientalis* and *P. manshurica* were adjudged the most bitter and worst smelling (Table 2 and 3). It is only the brandy and gin blended from alcohol from *K. ohmeri* that came close to the control sample (after triple distillation) in terms of taste and smell. Gin and brandy from alcohol using a combination of *S. cerevisiae* and *I. orientalis* was not bitter and did not have any smell. *I. orientalis* alone produced gin or brandy that was bitter and also had unpleasant smell so it was inferred that the good attributes for the combination came from *S. cerevisiae*.

Conclusion

pH and temperature changes during cocoa pulp juice fermentation followed classic fermentation kinetics showing an initial rise in pH and temperature followed by a fall as substrate became limiting for *S. cerevisiae*. All other yeast starter cultures did not elicit any marked changes in pH and temperature except *I. orientalis* and the spontaneous process which showed only modest rise and fall in pH. Furthermore, combinations of yeast strains such as *S. cervisiae* and *I. orientalis* did not produce the expected synergistic effect. Metabolite analyses of the yeast fermentations indicated that the major sugar components of sweatings, fructose and glucose, were depleted within 48 h in all cases which means extending

Table 2. Sensory evaluation of formulated using cocoa gin obtained from alcohol distilled of fermented cocoa pulp juice using different yeast strains.

Sample	Taste					Smell		
	Bitterness			Biting Effect				Does Not Smell
	Bitter	Not Bitter	Water-like	Biting	Not Biting	Good	Unpleasant	
Control	4	16	0	0	20	8	0	12
Spontaneous fermentation	0	16	4	0	20	0	8	12
P. manshurica	20	0	0	0	20	0	12	8
S. ludwigii	8	12	0	4	16	0	4	16
I. orientalis	12	8	0	0	20	0	12	8
P. kluyveri	0	8	12	8	12	0	4	16
P. carribica	0	20	0	0	20	0	4	16
K. ohmeri	4	16	0	4	16	4	4	12
C. orthopsilosis	4	16	0	0	20	0	8	12
S.cerevisiae/I. orientalis	0	16	4	4	16	0	8	12

Control = alcohol from triple distillation.

Table 3. Sensory evaluation of formulated cocoa brandy using alcohols distilled from fermented cocoa pulp juice using different yeast strains.

Sample	Taste					Smell		
	Bitterness			Biting Effect				Does Not Smell
	Bitter	Not Bitter	Water-like	Biting	Not Biting	Good	Unpleasant	
Control	15	5	0	0	20	5	5	10
Spontaneous fermentation	5	15	0	5	15	0	15	5
P. manshurica	10	10	0	5	15	0	15	5
S. ludwigii	10	5	5	0	20	0	10	10
I. orientalis	20	0	0	0	20	0	15	5
P. kluyveri	0	15	5	10	10	0	10	10
P. carribica	5	10	5	0	20	0	10	10
K. ohmeri	0	20	0	10	10	5	10	5
C. orthopsilosis	5	10	5	5	15	0	15	5
S.cerevisiae/I. orientalis	5	10	5	10	10	0	5	15

*Control = alcohol from triple distillation.

fermentation beyond 48 h would have no added advantage in enhancing alcohol yield. Ethanol production, however, increased sharply in the first 36 h for best performers but a more gradual increment taking up to 60 h was observed in other yeast cultures before levelling off or slightly decreasing.

Generally, mannitol production was very low but consistent in all yeast strains tested with *K. kluyveri* being the most promising producer. *S. cerevisiae* produced the highest amount of ethanol within the shortest time of fermentation followed by *I. orientalis*. Bulked distillation on the Jacob Carl Distillation Plant, where 100 liters of ferment were distilled, six yeast cultures individually recorded between 53 to 56% (v/v) alcohol strengths. Sensory evaluation of blended gin and brandy indicated that *I. orientalis* produced the most bitter principles, *P. manshurica*, the worst smells, and *K. ohmeri* was linked to good flavour notes. Among the six best alcohol producers, however, two, (*I. orientalis* and *P.manshurica*) may be rejected because they produced bitter by-products and unpleasant smells. It was thus concluded from this study that *S. cerevisiae*, *P. mexicana*, *S. ludwigii*, *C. carpohila* and *K. ohmeri* emerged best cultures for large-scale alcohol production with acceptable sensory attributes devoid of bitterness, astringency and unpleasant smells and having the shortest duration for fermentation.

RECOMMENDATIONS

Saccharomyces cerevisiae, *P. Mexicana, S. ludwigii*, *C.*

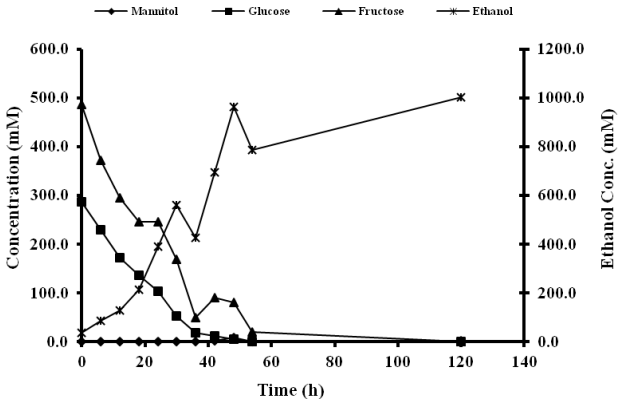

Figure 10. The utilization of glucose, fructose and sucrose and the production of ethanol and mannitol during cocoa pulp juice fermentation by *P. Mexicana.*

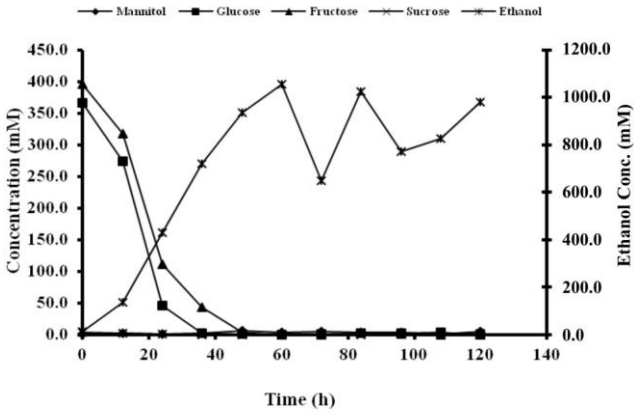

Figure 11. The utilization of glucose, fructose and sucrose and the production of ethanol and mannitol during cocoa pulp juice fermentation by *P. manshurica.*

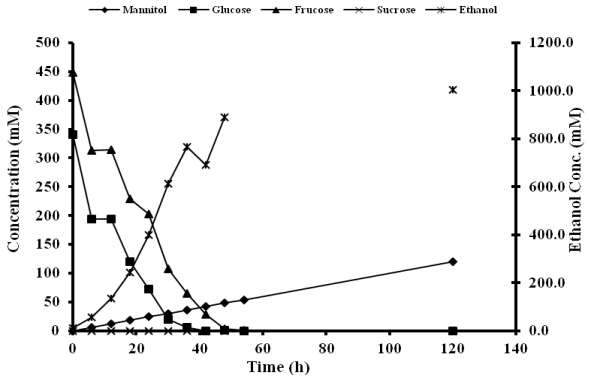

Figure 12. The utilization of glucose, fructose and sucrose and the production of ethanol and mannitol during cocoa pulp juice fermentation by *S. ludwigii.*

carpohila and *K. ohmeri* starter cultures are recommended for use in controlled fermentation processes in large-scale production of alcohol from sugars present in cocoa pulp juice. *P. kluyveri* starter cultures may be explored for mannitol production.

ACKNOWLEDGEMENT

This project was funded by Barry Callebaut, Belgium. The authors also appreciate Barry Callebaut, Ghana, and colleagues at CRIG for their immense contribution towards the completion of this project. This paper is published with the permission of the Executive Director of CRIG.

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