

## Full Length Research Paper

# Bioethanol production from *Curcubita pepo* and *Opilia amentacea* fruits using four strains of *Saccharomyces cerevisiae*

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Ethanol is commercially produced by fermenting agricultural products that contain high sugar or starch contents. In this study, the juices of *Opilia amentacea* and *Curcubita pepo* were used to produce bioethanol. A mixture of Urea ( $\text{CON}_2\text{H}_4$ ), monosodium glutamate ( $\text{C}_5\text{H}_8\text{NNaO}_4$ ), potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), and magnesium sulfate heptahydrate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) is used to promote batch ethanol production with four strains of *Saccharomyces cerevisiae* as the fermenting organisms. The results obtained have shown that the nutrients mixture significantly affected kinetic parameters and enhanced bioethanol production. Subsequently, the highest outputs of  $60.72 \pm 0.68$  and  $50.93 \pm 1.61$  g ethanol/kg were obtained respectively with *O. amentacea* and *C. pepo*. In the same time,  $460.97 \pm 8.66$  g ethanol/kg were got as maximum output from sucrose (NG).

**Key words:** Fruit juices, enrichment, *Saccharomyces cerevisiae*, fermentation, bioethanol.

## INTRODUCTION

Growing environmental concerns and increasing petroleum products prices have recently stimulated interest in optimizing fermentation processes for large-scale production of alternative fuels such as bioethanol (Wyman, 2001). The yeast "*Saccharomyces cerevisiae*" is the major industrial ethanol producer, because it is generally acknowledged as a safe microorganism that can produce ethanol by fermentation up to 20% (v/v) from carbon sources (Cot et al., 2007). Typically, ethanol fermentation is carried out under Normal Gravity (NG) technology, which is defined

as the preparation and fermentation of mashes containing 20-24% of dissolved solids (Thomas et al., 1996).

Ethanol can be produced from many different raw materials such as corn grain (in USA), sugarcane (in Brazil), tapioca and molasses (in Thailand) (Khongsay et al., 2010). In Togo, ethanol is commonly produced in artisanal way by using palm wine, corn, sorghum or millet as raw materials for local beer brewing. Ethanol is also produced in industrial scale with sugarcane molasses and cassava chips at Anié (Togo) by a Chinese company

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called "Sinto". The Actual challenge is to use less agricultural value products/residues or wastes which are not competing with common food production.

Therefore more interest are devoted to ethanol production by developing and implementing advanced process technologies (Mielenz, 2001). Thus, wild fruits from different plants with potentially higher level of sugars can be used as an alternative low-cost feedstock (Mazmanci, 2011).

In this study, bioethanol production from two types of local fruits (*Opilia amentacea* and *Curcubita pepo*) is compared to sucrose fermentation using four strains of *S. cerevisiae*. The cells growth and effects on main fermentation kinetic parameters were investigated.

## MATERIALS AND METHODS

### Microorganism and inoculum preparation

Four strains of *S. cerevisiae* were used as fermentative yeasts. The first strain of *S. cerevisiae* called "Safelevure" (noted S1) is generally used in bakery and in the artisanal production of local beers in Togo. The three other ones (noted S2, S3 and S4) obtained from "Ryan Wu" Company, are the first new biotechnology products in China which has been widely used in the production of drinking alcohol, dehydrated alcohol and fuel ethanol. 1.00 g of each strain of active dry yeast was introduced into 250 mL of a preculture medium which had the following composition (in g/L): Yeast extract, 10.00; Tryptone, 20.00; Glucose (Prolabo®), 20.00 and NaCl (Prolabo®), 9.00. After homogenization, the liquid mixture is incubated at the room temperature (30-32°C) with continuous stirring using a magnetic stirrer. After 24 h of incubation, the active cells were then harvested and used as inoculum for ethanol production (EP) (Novidzro et al., 2013).

### Raw materials

*O. amentacea* (Opiliaceae) and *C. pepo* (Cucurbitaceae) fruits were harvested in June 2010 in Agou-Akplolo forests, located on the foot of Mount Agou in Togo. The juices were extracted with distilled water and autoclaved at 121°C during 15 min to prevent contamination as well as to enhance their sugar concentration (Mazmanci, 2011). Then the samples were centrifuged and filtered. Filtrates were kept at -23°C against bacterial contaminations until their use for reducing sugars analysis and alcoholic fermentation.

### Ethanol production media

*O. amentacea* and *C. pepo* fruit juices containing total soluble solids of 20°Bx supplemented with/without a mixture of four nutrients were used as EP media. Referring to the literature, four nutrients were mixed in following proportions (Al-Obaidi et al., 1987; Acourene and Tama, 2001; Novidzro et al., 2013) and used as additional supplements to study their effects on EP with the mashes. Their contents in the fermentation broth were (g/L): Urea (CON<sub>2</sub>H<sub>4</sub>), 2.00; Monosodium Glutamate (C<sub>5</sub>H<sub>8</sub>NNaO<sub>4</sub>), 1.00; Potassium Dihydrogen Phosphate (KH<sub>2</sub>PO<sub>4</sub>), 0.25 and Magnesium sulfate heptahydrate (MgSO<sub>4</sub>.7H<sub>2</sub>O), 0.25. A comparison is made with sucrose syrup in the same conditions.

The pHs of the mashes were adjusted then in 4.5 ± 0.2. The EP media were transferred into a capped plastic flask of 1000 mL with a final working volume of 250 mL and then autoclaved at 121°C for

15 min to prevent the final mixtures against contamination. The sterilized mashes were used as fermentation media after cooling down (Novidzro et al., 2013).

### Fermentation conditions

Each inoculum, prepared previously, was introduced into 10 % (v/v) of each EP medium so that the concentration of viable cells at the beginning of the fermentation must be equal to 2.8 × 10<sup>6</sup> viable cells/mL. After a step of pre fermentation during 12 h, the 90% of the staying mashes were added to each pre fermented EP medium. The fermentation was operated in batch mode and performed in a shaker at 100 rpm (Khongsay et al., 2010) in the laboratory at room temperature of 30 ± 2°C for 10-15 days. Data were expressed as the mean of four experiments. Samples were taken at appropriate time intervals for tests.

### Analytical methods

The fruits juices have been characterized to determine chemical groups present in the mesocarps of the fruits such as polyphenols, via the reaction with ferric chloride (Rafael et al., 2005), and flavonoids via the reaction "with cyanidine" (Brock et al., 2006; Konkon et al., 2008).

The cell numbers in the fermentation broth were determined by direct counting method with methylene blue staining on Motic/B1 Series/System Microscopes instrument using Malassez hemacytometer, while the total soluble solids (TSS) were determined by *Fabre Mesurelec* hand-held refractometer ((RAM 0-80% 106), respectively as described by Khongsay et al. (2010). The pH was measured by pH-meter (CRISON/PH 25).

The fermentation broth was centrifuged at 13,000 rpm for 10 min (Laopaiboon et al., 2008). The supernatant was then determined for the total residual sugars by phenol sulfuric acid method (Mecozzi, 2005) and the reading absorbance was 490 nm, using a calibration curve produced with glucose in DMS 300 UV VISIBLE Spectrophotometer. The reducing sugars were determined by Bertrand Method and sucrose in fruit juices by the following formula (Acourene and Tama, 2001):

$$[\text{Sucrose}] = ([\text{Total Sugars}] - [\text{Reducing Sugars}]) \times 0.95 \quad (1)$$

Percentage of total sugars consumption rate (SCR: %) was calculated as the ratio of the consumed mass of total sugars to the initial mass of total sugars (Laopaiboon et al., 2008). Ethanol concentration was analyzed by high-performing liquid chromatography (using *Chrompack pump; Chromapack/RI Detector; Merck/D-2500/Chromato-Integrator* and *C18 (25 cm x 4.6 mm) column*). The volumetric ethanol productivity (Q<sub>p</sub>: g/L/h) and the percentage of conversion or yield efficiency (E<sub>y</sub>:%) were calculated by the following equations (Khongsay et al., 2010):

$$Q_p = \frac{P}{t} \quad \text{and} \quad E_y = \frac{Y_{p/s} \times 100}{0.54} \quad (2)$$

where, **P (g/L)** is the final ethanol concentration produced, **t (h)** the fermentation time giving the highest ethanol concentration during batch fermentation on EP media under NG conditions and **0.54** the maximum theoretical ethanol of sucrose consumption.

The ethanol yield (**Y<sub>p/s</sub>: g/g**) is calculated by the following formula (Swain et al., 2007):

$$Y_{p/s} = \left[ \frac{\text{Mass of product (Ethanol) formed}}{\text{Mass of substrate (Total sugars) consumed}} \right] \quad (3)$$

Statistical analysis was carried out via Turkey test by one-way and

**Table 1.** Characteristics of raw fruits juices of *Curcubita pepo* and *Opilia amentacea*.

Characteristic	<i>Curcubita pepo</i> juice	<i>Opilia amentacea</i> juice
Volume (mL)	1100.0 ± 4.0	1200.0 ± 5.0
Total soluble solid (°Bx)	20.0 ± 0.1	20.0 ± 0.1
Total sugars (g/L)	101.1 ± 2.4	122.7 ± 2.3
Reducing sugars (g/L)	56.9 ± 3.0	114.1 ± 2.7
Sucrose (g/L)	42.0 ± 0.6	8.2 ± 0.4
Polyphenols test	+	++++
Flavonoids Test	++	++++

+, slightly positive test; ++, positive test; +++++, very positive test.

two-way ANOVA using MSTATC.EXE software. All the experiments were performed in four times and the results were expressed as mean ± standard deviation (SD) of the four experiments.

## RESULTS AND DISCUSSION

### The fruits juices biochemical composition

The characteristics of raw juices extracted from 1000 g of each fruit are shown on Table 1. To extract juices of identical initial concentration of 20.0 ± 0.1 Bx, the volume of water used varied from one fruit to another. *O. amentacea* juice appeared on Table 1, as the richest substratum in total sugars, with a concentration of 122.7 ± 2.3 g/L corresponding to a quantity of total sugars of 147.2 ± 2.8 g/kg of fruit while the juice of *C. pepo* could be distinguished like the weakest in total sugar, with its concentration of 101.1 ± 2.4 g/L in total sugars, equivalent to a quantity of 111.2 ± 2.6 g/kg of fruit. The quantities of total sugars in the fruit juices were low by comparison to sucrose syrup (230 g/L) with the same initial concentration of 20.0 ± 0.1°Bx.

With a concentration of 114.1 ± 2.7 g/L, or a quantity of 136.9 ± 3.2 g/kg of fruit, *O. amentacea* was also the fruit which had the most elevated content in reducing sugars. The one of *C. pepo* was only of 56.9 ± 3.0 g/L, or a quantity of 62.6 ± 3.3 g/kg of fruit. But, about the sucrose content, *C. pepo* was the best, with a concentration of 42.0 ± 0.6 g/L, or a quantity of 46.2 ± 0.7 g/kg of fruit, against a concentration of 8.2 ± 0.4 g/L and quantity of 8.2 ± 0.4 g/kg of fruit, for *O. amentacea*. Probably, with these contents in sugars presented in this study, *O. amentacea* would be the most favorable substratum to the alcohol production. Therefore, the same total soluble solids does not mean the same reducing sugar contents of which different juices should be stated.

The pHs of the fruit juices were 5.35 ± 0.08 and 3.25 ± 0.06, respectively for *C. pepo* and *O. amentacea*. These values could contribute to a better conservation of juices, then for good alcohol production. Indeed, it had been proven that, in fermentation broth, an acidic pH allowed to avoiding contaminations or the bad reactions, due to the presence of other micro-organisms, such as the bacteria (Ban et al., 1988).

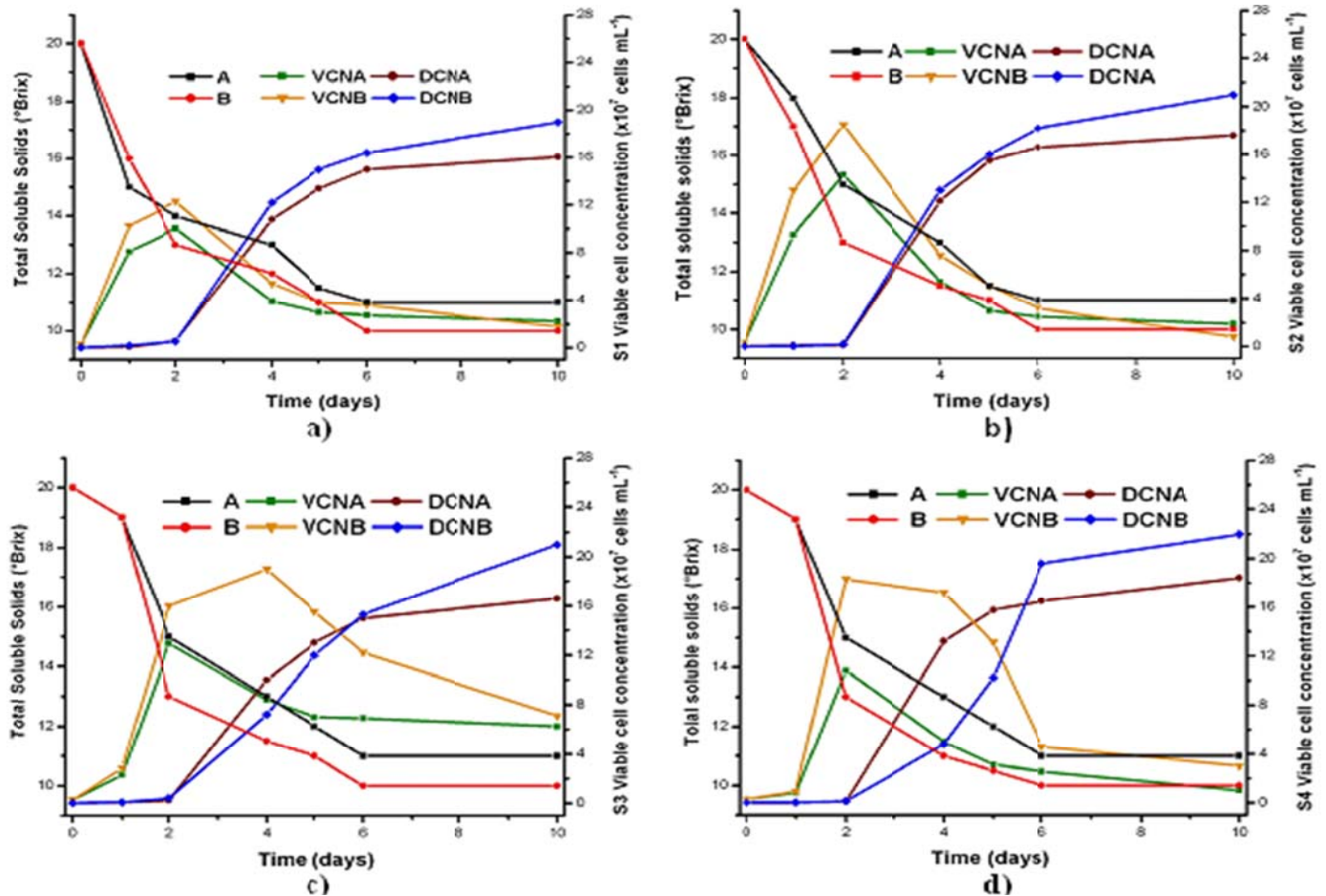
The two phytochemical tests revealed that *O. amentacea* juice contained more phenolic compounds (Polyphenols and flavonoïds) in relation to the one of *C. pepo*. However, phenolic compounds were considered as poisons to yeasts growth, so they could have negative impacts for alcohol production (Almeida et al., 2007).

### Evolutions of brix and yeasts population during fermentation period

The curves of Figures 1 and 2 showed the profiles of Brix and the number of the living and dead yeast cells variations, during the fruit juices fermentation. After the inoculation, there is a continuous dropping of the Brix for each strain. However, the dropping of the Brix show three phases: a lag phase at the beginning, followed by an acceleration phase, and then finishing by the deceleration phase. It is known that the Brix reduction is favorable to ethanol production under anaerobic conditions.

Simultaneously, a slow rhythm increase of the living cells number was observed as the fermentation begun. This was followed respectively by an acceleration phase, an exponential growth phase and finally, by a decline phase, characterized by a rapid decrease in viable cell numbers. These developments are in accordance with works achieved by Aiba et al. (1973). On the other hand, the presence of dead cells happened before a very quick intensified phase. Microbial growth in juices reveals four phases namely lag, exponential, stationary, and death phases. During lag phase, cells got progressively accustomed to the new environment and were growing (there is no increase in cell numbers). Exponential phase was the period when cell numbers increases rapidly. When the growth and death rates of cells were approximately equal, the cells entered the stationary phase. Finally, when the living environment of cells comes to accumulate toxic wastes and to face the lack of nutrients, these eventually results in death of the cells (Fugelsang and Edwards, 2007).

With enrichment of the media by nutrients mixture, the Brix reduction became important, and the cellular growth also rose significantly. Whatever the strain used in the non-enriched media, the reduction of Brix was slow and



**Figure 1.** The effects of the mixture of nutrients on the profiles of Brix and cell growth during batch ethanol fermentation of *Curcubita pepo* fruit juice: a, with S1; b, with S2; c, with S3 and d, with S4. A= pure juice; B = enriched juice; VCNA = Viable Cell Number in A; VCNB = Viable Cell Number in B; DCNA = Dead Cell Number in A and DCNB = Dead Cell Number in B.

weak, and the cellular growth always remained lower when compared to enrich experiment case. At the end of fruit juice fermentation, it is noteworthy that number of the dead cells in *C. pepo* experiment was higher than viable cells. The inverse phenomenon was observed for *O. amentacea*.

#### The maximum viable cell numbers during the fermentation

The yeasts proliferation varied from one medium to another (Table 2). The values of the maximal viable cells number of every strain were significantly different ( $P \leq 5.0\%$ ) in the enriched media as well as in the non-enriched media. Among the three different media investigated, *C. pepo* juice showed the best yeasts cells growth. So, the maximal viable cells number is obtained in the non-enriched medium with S2 ( $14.30 \pm 0.05 \times 10^7$  to  $0.02 \times 10^7$ /mL cells). But *O. amentacea* juice is the medium in which yeasts cell growth was the poorest.

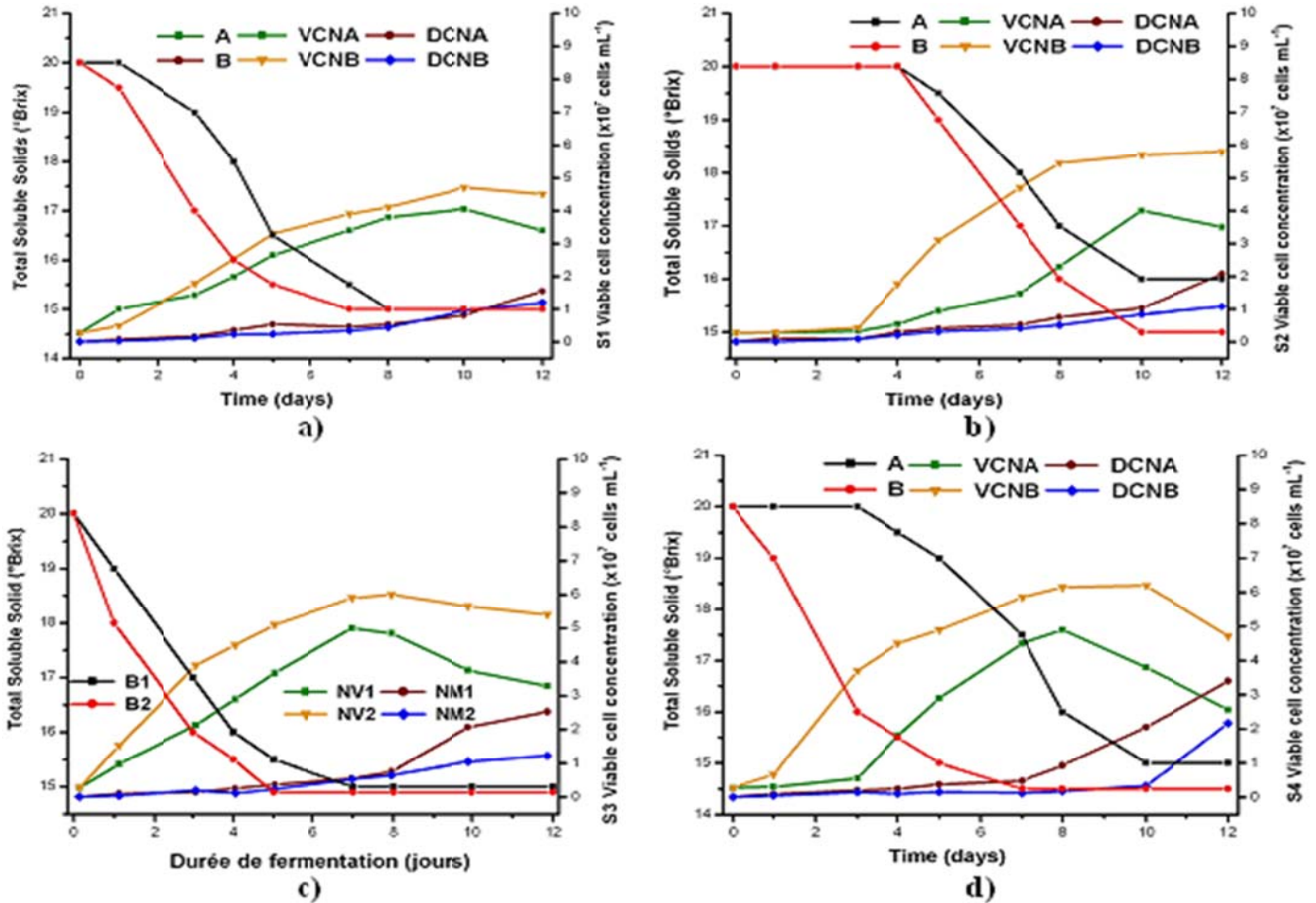
The possible existence of poisonous substances

inhibiting the development of the yeasts in this medium might likely explain the phenomenon, according to the phytochemical tests down.

On the whole, the growths of S2, S3 and S4 were better than those of S1. One can suggest that S2, S3, and S4 would be more effective than S1 for ethanol production. The weak alcoholic tolerance of S1 or other inhibiting factors could explain the weak performance of S1, although it has been proven that the presence of some by-products such as  $\text{CO}_2$ , organic acids and phenolic compounds (Almeida et al., 2007) not only disturb the development of the yeasts, but also inhibit their production capacity (Fugelsang and Edwards, 2007).

#### The main kinetic parameters during ethanol production fermentation

The main kinetic parameters of fermenting sucrose, *O. amentacea* and *C. pepo* fruits mashes were shown on Tables 3, 4 and 5, showing very interesting effect of nutrients on the fermentation.



**Figure 2.** The effects of the mixture of nutrients on the profiles of Brix and cell growth during batch ethanol fermentation of *Opilia amentacea* fruit juice: (a, with S1; b, with S2; c, with S3 and d, with S4. A= pure juice; B = enriched juice; VCNA = Viable Cell Number in A; VCNB = Viable Cell Number in B; DCNA = Dead Cell Number in A and DCNB = Dead Cell Number in B.

**Table 2.** The maximum viable cells numbers obtained in the fermentation broths.

EP medium	Maximum viable cells numbers (x107 cells.mL-1)			
	S1	S2	S3	S4
Non-enriched sucrose syrup	2.61 ± 0.01 <sup>c</sup>	8.05 ± 0.03 <sup>b</sup>	5.80 ± 0.07 <sup>b</sup>	7.61 ± 0.01 <sup>b</sup>
Enriched sucrose syrup	8.80 ± 0.06 <sup>b</sup>	18.70 ± 0.04 <sup>a</sup>	17.07 ± 0.08 <sup>b</sup>	14.60 ± 0.04 <sup>b</sup>
Non-enriched <i>Curcubita pepo</i> juice	10.05 ± 0.02 <sup>a</sup>	14.30 ± 0.05 <sup>a</sup>	12.95 ± 0.02 <sup>a</sup>	10.80 ± 0.04 <sup>a</sup>
Enriched <i>Curcubita pepo</i> juice	12.30 ± 0.02 <sup>a</sup>	18.50 ± 0.11 <sup>b</sup>	19.00 ± 0.02 <sup>a</sup>	18.30 ± 0.09 <sup>a</sup>
Non-enriched <i>Opilia amentacea</i> juice	4.05 ± 0.08 <sup>b</sup>	4.10 ± 0.04 <sup>c</sup>	5.00 ± 0.05 <sup>c</sup>	4.90 ± 0.02 <sup>c</sup>
Enriched <i>Opilia amentacea</i> juice	4.70 ± 0.03 <sup>c</sup>	5.79 ± 0.07 <sup>c</sup>	6.00 ± 0.04 <sup>c</sup>	6.40 ± 0.07 <sup>b</sup>

S1, *S. cerevisiae* "Safelevure" strain generally used in bakery and in the artisanal production of local beers in Togo; S2, S3 and S4 were obtained from "Ryan Wu" Company in China and has been widely used in the production of drinking alcohol, dehydrated alcohol and fuel ethanol. Values are in the mean ± standard deviation of 4 experiments. Mean followed by the same letter (a, b and c/a', b' and c') next to the values within a same column are not significantly different using Turkey's multiple range test at the level of 5%.

**Sugars consumption**

The sugar consumption rate (SCR: %) were respectively

79-89 and 76-87% (Table 2) in the enriched and non-enriched sucrose medium, depending on yeast strains. However, total sugars utilization in the fruit juices was

**Table 3.** The main fermentation kinetic parameters of ethanol production with sucrose mash ( $C_0=20^\circ\text{Brix}$ ).

Kinetic parameter	S1		S2		S3		S4	
	A	B	A	B	A	B	A	B
SCR (%)	75.98 ± 2.02	79.10 ± 1.48	86.72 ± 1.32	87.72 ± 1.79	80.01 ± 2.33	82.77 ± 1.55	87.39 ± 1.45	88.56 ± 2.51
t (h)	120	48	120	48	120	48	144	72
$P_{th}$ (g/L)	94.02 ± 2.50	97.88 ± 1.83	107.31 ± 1.63	108.54 ± 2.21	99.00 ± 2.88	102.42 ± 1.92	108.14 ± 1.79	109.58 ± 3.11
$P_{exp}$ (g/L)	74.82 ± 1.32	92.91 ± 2.48	90.66 ± 2.18	105.97 ± 1.99	85.94 ± 1.16	98.53 ± 2.04	93.73 ± 1.22	104.83 ± 3.44
Qp (g/L/h)	0.624 ± 0.011	1.936 ± 0.052	0.756 ± 0.018	2.208 ± 0.041	0.716 ± 0.010	2.053 ± 0.043	0.651 ± 0.008	1.456 ± 0.048
Yp/s (g/g)	0.428 ± 0.008	0.511 ± 0.014	0.455 ± 0.011	0.525 ± 0.010	0.467 ± 0.006	0.518 ± 0.011	0.466 ± 0.006	0.515 ± 0.017
Ey (%)	79.29 ± 1.40	94.57 ± 2.52	84.17 ± 2.02	97.27 ± 1.83	86.48 ± 1.12	95.85 ± 1.98	86.36 ± 1.12	95.31 ± 3.13

**Table 4.** The main fermentation kinetic parameters of ethanol production with *Curcubita pepo* fruit juice ( $C_0 = 20^\circ\text{Brix}$ ).

Kinetic parameter	S1		S2		S3		S4	
	A	B	A	B	A	B	A	B
SCR(%)	48.50 ± 2.44	72.82 ± 2.07	54.89 ± 1.78	85.28 ± 2.52	59.17 ± 1.49	88.80 ± 1.91	55.29 ± 2.56	83.75 ± 2.33
t (h)	120	72	120	72	120	72	144	96
$P_{th/c}$ (g/L)	26.38 ± 1.33	39.61 ± 1.13	29.86 ± 0.97	46.38 ± 1.37	32.18 ± 0.71	48.30 ± 1.04	30.07 ± 1.39	45.55 ± 1.26
$P_{exp}$ (g/L)	23.03 ± 1.00	37.66 ± 1.35	27.02 ± 1.12	45.19 ± 0.54	25.92 ± 1.37	46.3 ± 1.46	26.98 ± 1.12	44.27 ± 0.79
Qp (g/L/h)	0.192 ± 0.008	0.523 ± 0.019	0.225 ± 0.009	0.628 ± 0.008	0.216 ± 0.011	0.643 ± 0.020	0.187 ± 0.008	0.461 ± 0.008
Yp/s (g/g)	0.470 ± 0.020	0.512 ± 0.018	0.487 ± 0.020	0.524 ± 0.006	0.433 ± 0.023	0.516 ± 0.016	0.483 ± 0.020	0.523 ± 0.009
Ey (%)	87.06 ± 3.78	94.83 ± 3.40	90.26 ± 3.74	97.15 ± 1.16	80.31 ± 4.24	95.61 ± 3.01	89.45 ± 3.71	96.91 ± 1.73

S1, *S. cerevisiae* "Safelevure" strain generally used in bakery and in the artisanal production of local beers in Togo; S2, S3 and S4 were obtained from "Ryan Wu" Company in China and has been widely used in the production of drinking alcohol, dehydrated alcohol and fuel ethanol.

relatively low. Only 49-59 and 53-63% of total sugars are used in *C. pepo* and *O. amentacea* non enriched juices, respectively.

When the juices were enriched, these values were improved up 73 to 89% and 63 to 78%, respectively (Tables 3 and 4). All the obtained results showed that the enrichment of the medium provokes an increase of the sugar consumption rates.

Approximately 11-37% of initial total sugars content still remained at the end of enriched juices fermentation. Complete sugar utilization may be

achieved by optimizing aeration rate, agitation, and other nutrients supplementation, as already evoked by Laopaiboon et al. (2008).

#### Fermentation time

Fermentation times were very low in enriched media, unlike those of non-enriched media. S1, S2, and S3 gave the same fermentation times which were always lower than those of S4. Moreover, the fermentation times were identical in

sucrose and *C. pepo* media. Comparatively, *O. amentacea* juices media showed high fermentation times. Possibly, the intensity of inhibitory substances in *O. amentacea* juice might slow down significantly the fermentation process. The cells growth (Figures 1 and 2) could support this possibility.

#### Final ethanol concentration ( $P_{exp}$ ) produced

The final  $P_{exp}$  values from sucrose media are two



**Table 5.** The main fermentation kinetic parameters of ethanol production with *Opilia amentacea* fruit juice  $C_0 = 20^\circ$ Brix).

Kinetic parameter	S1		S2		S3		S4	
	A	B	A	B	A	B	A	B
SCR. (%)	53.04 ± 1.52	62.74 ± 2.01	61.34 ± 1.77	76.89 ± 1.55	63.04 ± 1.28	74.89 ± 2.25	59.80 ± 1.12	78.19 ± 2.11
t (h)	192	120	192	120	192	120	240	144
$P_{th/c}$ (g/L)	35.01 ± 1.00	41.42 ± 1.33	40.49 ± 1.17	50.76 ± 1.02	41.61 ± 0.84	49.44 ± 1.49	39.48 ± 0.74	51.62 ± 1.39
$P_{exp}$ (g/L)	32.85 ± 1.44	35.31 ± 1.30	37.12 ± 1.27	48.98 ± 1.08	38.97 ± 1.75	47.55 ± 1.07	36.95 ± 1.47	50.60 ± 0.57
$Q_p$ (g/L/h)	0.171 ± 0.008	0.294 ± 0.011	0.193 ± 0.007	0.408 ± 0.009	0.203 ± 0.009	0.396 ± 0.009	0.154 ± 0.006	0.351 ± 0.004
$Y_{p/s}$ (g/g)	0.505 ± 0.022	0.459 ± 0.016	0.493 ± 0.017	0.519 ± 0.011	0.504 ± 0.023	0.517 ± 0.012	0.504 ± 0.020	0.527 ± 0.006
$E_y$ (%)	93.47 ± 4.10	84.94 ± 3.13	91.33 ± 3.12	96.15 ± 2.12	93.30 ± 4.19	95.82 ± 2.16	93.27 ± 3.71	97.67 ± 1.10

SCR (%) = Sugar Consumption Rate ; t = fermentation time;  $P_{th/c}$  = theoretical final ethanol concentration which must be produced;  $P_{exp}$  (g/L) = experimental final ethanol concentration produced;  $Q_p$  = volumetric ethanol productivity;  $Y_{p/s}$  = ethanol yield;  $E_y$  = yield efficiency; A = non enriched medium; B = enriched medium with urea ( $CON_2H_4$ : 2.00 g/L), monosodium glutamate ( $C_5H_8NNaO_4$ : 1.00 g/L), potassium dihydrogen phosphate ( $KH_2PO_4$ : 0.25 g/L) and magnesium sulfate heptahydrate ( $MgSO_4 \cdot 7H_2O$ : 0.25 g/L). S1, *S. cerevisiae* "Safelevure" strain generally used in bakery and in the artisanal production of local beers in Togo; S2, S3 and S4 were obtained from "Ryan Wu" Company in China and has been widely used in the production of drinking alcohol, dehydrated alcohol and fuel ethanol.

or three times higher than those of fruit juices. The maximum  $P_{exp}$  values with enriching approach are  $50.60 \pm 0.57$  g/L (equivalent to  $60.72 \pm 0.68$  g ethanol/kg of fruit) for *O. amentacea* fruits using S4 (Table 5) and  $46.3 \pm 1.46$  g/L (equivalent to  $50.93 \pm 1.61$  ethanol/kg) for *C. pepo* fruits using S3 (Table 4). The maximum  $P_{exp}$  value with sucrose medium enriched were  $105.97 \pm 1.99$  g/L (equivalent to  $460.97 \pm 8.66$  g ethanol/kg sucrose NG), reached with S2. From Tables 3, 4 and 5, it can be deduced that ethanol production with *O. amentacea* juice was slightly better than that with *C. pepo*. In addition, ethanol produced using S2, S3 and S4 gave better  $P_{exp}$  values relatively to those obtained with S1. It is known that high sugar consumption can lead to a good ethanol production (Khongsay et al., 2012 and Khongsay et al., 2010). So, the weak sugars quantity and the presence of poisonous substances such as phenolic compounds in fruit juices (Almeida et al., 2007), might likely have impacts on the outputs of ethanol production. Therefore, a preliminary detoxification process could contribute to the

improvement of ethanol production from these kinds of raw materials (Mazmanci, 2011). Unfermentable carbon source's existence in the juices might also explain why the ethanol yields were low in the juices.

#### Ethanol productivity ( $Q_p$ : g/L/h)

With sucrose, the best productivity ( $Q_p$ ) obtained was  $2.208 \pm 0.041$  g/L/h with S2, and the lowest one was  $1.456 \pm 0.048$  g/L/h with S4. Without nutrients, the best  $Q_p$  reached with S2 in sucrose mashes was  $0.756 \pm 0.018$  g/L/h, while the lowest one was  $0.624 \pm 0.011$  g/L/h obtained with S1 (Table 3). For *C. pepo* enriched juice, the best and the lowest  $Q_p$  obtained by S3 and S4 were  $0.643 \pm 0.020$  g/L/h and  $0.461 \pm 0.008$  g/L/h, respectively. But in the absence of enriching nutrients, *C. pepo* juice best productivity was  $0.225 \pm 0.009$  g/L/h with S2 and the lowest is  $0.187 \pm 0.008$  g/L/h by S4 (Table 4). In the case of *O. amentacea* enriched juices, it was S2 which

gave the best productivity of  $0.408 \pm 0.009$  g/L/h and the lowest one of  $0.294 \pm 0.011$  g/L/h was given by S1. Without the enrichment of *O. amentacea* juices, the best productivity of  $0.203 \pm 0.009$  g/L/h was given by S3, and the lowest one of  $0.154 \pm 0.006$  g/L/h by S4 (Table 5). So, to get high ethanol productivity, the fermentation time must be kept in a short time (Khongsay et al., 2010).

#### Ethanol yield ( $Y_{p/s}$ : g/g) and yield efficiency ( $E_y$ : %)

The experimental  $Y_{p/s}$  values ( $0.428 \pm 0.008$  g/g to  $0.527 \pm 0.006$  g/g) and the  $E_y$  values (79.29 ± 1.40 % to  $97.67 \pm 1.10$  %) (Tables 3 and 4), in relation to the final sugars consumptions, were slightly lower than what were hypothetically the theoretical predicted. The ethanol concentrations produced were also slightly lower than the calculated theoretical ethanol concentrations. Many factors explained this decrease in the expected

results. First, the production of by-products such as organic acids, other alcohols, ketones, etc. was harmful for the good productivity. Secondly, the use of parts of sugars by the yeasts to get energy, useful and compulsorily to their growth was also seen as a source of output loss (Ballerini and Alazard-Toux, 2006). The previous reported Yp/s of cane molasses (Reed, 2002), dried sweet potato chips/flour (Woolfe, 1992), and cassava chips/flour (Balagopalan et al., 1987) were 265 to 272 g ethanol/kg, 280 to 320 g ethanol /kg, and (420 to 450 g ethanol /kg), respectively.

In the present study, the maximum ethanol yields calculated were 41.296 g ethanol /kg for *O. amentacea* fruits and 36.134 ethanol g/kg for *C. pepo* fruits. Ameyapoh et al. (2006) produced bioethanol from *Manguifera indica* L. (Anacardiaceae) mash of 6°Bx with four strains of *S. cerevisiae* (AYS01, AYS02 AYS001 and L2056). They got outputs (g/L ethanol) of 7.83; 6.52; 5.21 and 8.65, respectively. On the other hand, beer production achieved by Dahouenon-Ahoussi et al. (2012) from *Sorghum bicolor* and *Musa acuminata* mashes, gave the output of 4.86%Vol, equivalent to approximately 37 g ethanol /L. Our results were better than those obtained by Ameyapoh et al., (2006) and Dahouenon-Ahoussi et al. (2012). However, they were lower than those obtained with *Washintonia robusta* fruit juice containing 105 g/L of reducing sugars, and which succeeded to a maximal production of ethanol of 25 g/L, equivalent to  $71.42 \pm 1.4$  g of ethanol/kg (Mazmanci, 2011).

The improvement of ethanol production yields by using other different microorganisms such as *Zymomonas mobilis* (Claassen et al., 1999), *Saccharomyces bayanus* (Castellar et al., 1998), *Saccharomyces pastorianus* (Fujii et al., 2001), *Kluyveromyces fragilis* (Szambelan et al., 2004) or genetically modified microorganisms (Ostergaard et al., 2000), will be investigated in the future.

## Conclusion

The outputs of bioethanol production obtained in this study were  $50.60 \pm 0.57$  g ethanol/L (equivalent to  $60.72 \pm 0.68$  g ethanol/kg of fruit) and  $46.3 \pm 1.46$  g/L (equivalent to  $50.93 \pm 1.61$  g ethanol/kg of fruit), respectively for *O. amentacea* and *C. pepo*. Moreover, all the other fermentation kinetic parameters measured in this study showed clearly that the media supplementation with the mixture of nutrients (urea, monosodium glutamate, potassium dihydrogen phosphate, and magnesium sulfate heptahydrate) improved significantly the batch ethanol production performances. However, the maximal concentration of ethanol produced with sucrose ( $105.97 \pm 1.99$  g/L) was comparatively more elevated. All the results we obtained suggested that S4 is the most suitable strain for ethanol production under normal gravity(NG). The use of these conditions can be very attractive in fuel ethanol production domains.

The use of *C. pepo* and *O. amentacea* fruits as alternative carbon sources for ethanol production was proposed. Ethanol production yields for the two fruits were very low. However, as these fruits were abundant and inedible, so their eventual exploitation for bioethanol production could be a source of added value for the local population.

This would decrease the use of edible products for fuel production. But, fruit juices detoxification and supplementation with little quantity of sucrose or other nitrogen sources and/or other nutrients as well as other fermentation processes should be further investigated to improve and complete sugar consumption under normal gravity and very high gravity conditions.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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