



## Fermented *Parkia biglobosa* seeds as a nitrogen source supplementation for bioethanol production from cashew apple juice

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### ABSTRACT

Nutritional requirements in the fermentation process are key parameters for optimal yeast development and ethanol production. Natural nutritional supplements rich in nitrogen, phosphorus, sulfur, and micro-elements can improve the performance of yeasts and offer a sustainable, cost-effective, and environmentally friendly alternative to synthetic chemicals. This study aimed at investigating the effect of a natural yeast nutrient (fermented *Parkia biglobosa* seeds) on bioethanol production from cashew apple juice by *Saccharomyces cerevisiae*. The proximate and mineral compositions of fermented seeds were evaluated. Their powder was added to yeast medium at a concentration of 4–12 g/L. The behavior of two yeast strains (*Angel brand super alcohol* (S1) and *Angel brand thermal-tolerant alcohol* (S2)) was inspected. Titratable acidity, pH, °Brix, and density were evaluated during 144 h of fermentation. Sugar consumption was maximal after 72 and 48 h of fermentation for S1 and S2 yeast strains, respectively. The best ethanol yields of 0.19 and 0.22 g/g were obtained with S1 and S2 yeast strains, respectively, using 12 g/L of nutrients for the first and without nutrient supplementation for the second (control sample). The non-conventional nutrients from fermented *P. biglobosa* seeds seem to be favorable for ethanol production using only S1 yeast strain.

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**Keywords:** Fermented *Parkia biglobosa* seeds, *Saccharomyces cerevisiae*, fermentation, cashew apple juice, bioethanol.

## INTRODUCTION

All over the world, the demand for bioethanol keeps growing due to its economic importance and wide spectrum of use for applications such as biofuels, disinfectants, solvents, as a precursor of other organic chemicals, and as an ingredient of many alcoholic beverages (Onuki et al., 2016). A multitude of various food- or non-food-based substrates (sugar, starch, cellulose, and algae) are used in bioethanol production technologies. Increasingly, research is looking at the utilization of alternative feedstocks such as agricultural and forest residues, municipal wastes, and lignocellulosic and algal biomass, for bioethanol production (Sarkar et al., 2012; Zabed et al., 2014). This generally has a dual purpose: firstly, renewable fuel production, and secondly, the reduction of environmental pollution by the valorization of residual biomass or wastes. However, currently available technologies for cellulosic ethanol production are very expensive and less efficient due to the low ethanol titer of fermentation broth and the high steam energy consumption in its distillation (Tesfaw and Assefa, 2014). Industrial bioethanol production is based on starch or sugar-based crops that give the best yields. Fermentation carried out by a variety of micro-organisms (e.g., fungi, bacteria, yeast) has a central role in bioethanol production. *Saccharomyces cerevisiae* yeast is one of the best fermentation microorganisms used. Furthermore, many parameters, including temperature, pH, oxygen, initial sugar concentration, and nutrient supplementation, directly influence the viability of yeasts, the specific rate of fermentation, and the ethanol yield (Zaman et al., 2008; Parrondo et al., 2009; Onuki et al., 2016).

In this study, considering the overall state cited above, we focus on the use of a natural yeast nutrients during ethanol fermentation. Through its use, we aimed at substituting conventional chemical nutrients ( $\text{CO}(\text{NH}_2)_2$ ,  $\text{NH}_4\text{Cl}$ ,  $(\text{NH}_4)_2\text{HPO}_4$ ,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{MnCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , etc.) and to make bioethanol

production cheaper and more environmentally friendly. Yeast nutrients play an important role in the fermentation process. Nutrients constitute a source of energy because they contain vital elements (e.g., H, O, C, N, P, S). They also provide multiple micro-elements (e.g., K, Mg, Ca, Na, Mn, Fe, Zn, etc.) and vitamins (e.g., thiamine ( $\text{B}_1$ ), pyridoxine ( $\text{B}_6$ ), biotin ( $\text{B}_7$ ), riboflavin, nicotinic acid ( $\text{B}_3$ ), pantothenic acid ( $\text{B}_5$ ), etc.), which significantly impact yeast growth, stress tolerance, ethanol yield, and secondary synthesis of fermentation metabolites (Venkateshwar et al., 2010; Perli et al., 2020; van Dijk et al., 2020). Moreover, nitrogen compounds such as amino acids and proteins stimulate yeast growth and improve fermentation kinetics. They are more efficient than ammoniacal nitrogen alone and can be used as an alternative ammoniacal nitrogen and carbon source (Ribéreau-Gayon et al., 2006). Despite their usefulness, the assimilation of nutrients by yeast does not always improve growth. Nutrient requirements of microorganisms vary from one strain to another (Ribéreau-Gayon et al., 2006; Parrondo et al., 2009). Yeast cells respond differently to the amount and quality of external nutrients and make optimal use of accessible nutrients by adapting to nutritional deficiencies (Zaman et al., 2008).

Fermented *Parkia biglobosa* seeds (*soumbala*, *aftin*, *dawadawa*, *netetu*, all local West-African dialects) were used in this study as yeast nutrients due to their high protein, fat, and mineral contents (Oladunmoye, 2007; Boateng et al., 2014; Aremu et al., 2015; Makalao et al., 2015). Commonly fermented *P. biglobosa* seeds are produced by traditional uncontrolled fermentation of seeds and used as food condiment in African countries including Burkina Faso, Benin, Nigeria, Ghana, and Senegal among others (Oladunmoye, 2007; Boateng et al., 2014; Soetan et al., 2014; Aremu et al., 2015; Camara et al., 2016). Despite the existence of different approaches to increase the ethanol yield and productivity, the effect of some alternative nutrients on yeast fermentation activity remains unknown.

*S. cerevisiae* is a major industrial yeast of first-generation ethanol (Tesfaw and Assefa, 2014; Ruchala et al., 2020). Its fermentation substrate is cashew apple juice (*Anacardium occidentale* L.), a potential source of fermentable sugars (3.85–4.63% (w/v) of glucose, 3.90–4.52% (w/v) of fructose) which is very poorly valued because of its astringency due to the presence of tannins (Deenanath et al., 2013; Padonou et al., 2015; Gbohaïda et al., 2016; Soro et al., 2017). Previous evaluation of the chemical composition and nutritional profile of the cashew apple juice showed its richness in vitamin C (104.0–293.5% (w/v)) and mineral elements (3609.93–4361.28 mg/L of potassium, 121.60–413.44 mg/L of magnesium, 218.03–234.01 mg/L of sodium, 120.24–601.20 mg/L of calcium, 35.10–44.80 mg/L of iron, 3.55–4.47 mg/L of zinc, 3.16–19.75 mg/L of manganese, 6.25–22.00 mg/L of iodine), which makes it a good substrate for use in agro-resource bioconversion into bioethanol by fermentation (Dedehou et al., 2015; Padonou et al., 2015; Soro et al., 2017; Agbagnan Dossa et al., 2018; Toure et al., 2019). The aim of this research was to investigate the effect of natural yeast nutrients from fermented *P. biglobosa* seeds on bioethanol production from cashew apple juice by *S. cerevisiae*.

## MATERIALS AND METHODS

### Sugary substrate

Cashew apples, false fruits of *A. occidentale*, were collected from various trees from the Parakou area in the central region of Benin. The juice of the different varieties of cashew apple was extracted by mechanical pressing using a screw press, filtered, and sterilized by autoclaving at 121°C for 20 min (Popov et al., 2010; Lin et al., 2012; Gbohaïda et al., 2016). After cooling to ambient temperature, the sterilized juice was used as a sugary substrate for bioethanol fermentation.

### Nutrient additive

Fermented *P. biglobosa* seeds were purchased from Bingo Market, Ouagadougou, Burkina Faso. The seeds had been crushed in

an electrical mill and sieved through a 1 mm sieve. The required amount of seed powder was measured out and sterilized in an oven at 121°C for 20 min. The sterilized powder was stored aseptically at 4°C in a plastic container until further use.

### Biochemical characterization of fermented *P. biglobosa* seeds

The mineral content (Ca, Mg, K, Fe, Zn) of the naturally fermented *P. biglobosa* seeds was measured. Calcium and magnesium were measured by the titrimetric method (Rodier, 2009), while total iron was determined by UV-Visible spectrophotometry (HACH DR/2400, USA) according to the analysis methods of the French Association for Standardization (AFNOR, 1986). In addition, potassium, sodium, copper, and zinc concentrations were measured by flame atomic absorption spectrometry (VARIAN SPECTRA 110, USA). The fermented seed powder was reduced to ashes after cremation at 550°C for 4 h (NABERTHEM, C290, Germany); ashes were digested for 30 min in a mixture of 1M nitric acid and 3N hydrochloric acid. The filtrates were used to determine the mineral content following the standard NF EN 14082 and ISO 15587-2 methods. The moisture content was measured according to the method of the Association of Official Agricultural Chemists (AOAC, 1990). The dry matter was determined after drying for 24 hours at 110°C. The Kjeldahl method (Wolf, 1968) was used to determine the protein content. After mineralization, distillation, and titration, the crude protein content was determined from the nitrogen content. This nitrogen rate, with a coefficient of 6.25, was converted into the protein content (AOAC, 1990).

### Microorganisms and inoculum preparation

The microbial strains used in this study were two variants of *S. cerevisiae* dry yeast from the Chinese company «Ryan Wu/Angel Yeast Co., Ltd»: *Angel brand super alcohol* (S1) and *Angel brand thermal-tolerant alcohol* (S2). The inoculum was prepared by separately introducing 0.5 g of each of the dry

(lyophilized) yeast strains into 4.5 mL of buffered peptone water. They have been activated at 30°C for 30 min.

### Must preparation

To acclimatize yeasts to the sugary substrate, pre-fermentation was carried out by incorporating the inoculum in 1/10 of the total volume of sterilized cashew apple juice to be fermented while leaving the mixture under stirring at  $30 \pm 2^\circ\text{C}$  for 30 min in an aerobic and aseptic environment. Then the mixture was added to 9/10 of the remaining sterilized juice volume.

### Fermentation process

Bioethanol batch fermentation experiments were carried out using 1.5 L plastic (PE) bottles previously disinfected with ethanol 96%, each containing 0.5 L of juice. Fermentation was initiated by the addition of the nutrient additive at different concentrations (0, 4, 8, and 12 g of fermented *P. biglobosa* seed powder per liter of substrate) at pH 4.35  $\pm$  0.01 and  $30 \pm 2^\circ\text{C}$  for about six (06) days with an initial Brix degree of  $13.9 \pm 0.0$  °Bx. A blank (sterilized cashew apple juice) without the addition of growth factor or yeast strain was also used.

### Fermentation parameters

Fermentation parameters were monitored by measuring physico-chemical parameters such as the pH, titratable acidity (TA, g acetic acid/L), Brix degree (°Bx), and density of must samples withdrawn at 24 h intervals. The pH of the must was measured with a pH meter (HANNA Instruments HI8014, USA) that was calibrated with buffer solutions of pH 4.0 and 7.0 (Zoecklein et al., 1999). TA was determined on 5.0 mL of must by the titrimetric method with sodium hydroxide (0.1N) and phenolphthalein as the pH indicator. The Brix degree is a measurement of the apparent concentration of sugar and is expressed as a percentage by weight (% wt). One Brix degree equals 1 gram of sucrose in 100 grams of solution (Son et al., 2009; Margalit, 2012). The total soluble dry

matter content (Brix degree) of the substrates was evaluated using a MISCO PA201 (Palm ABBE™, USA) digital refractometer. The density of the must provides an approximate indication of the ethanol content (Chen et al., 2003; Ribéreau-Gayon et al., 2006). It was estimated gravimetrically with an uncertainty of 1 mg using an analytical balance and a 50 mL pycnometer. The calibration was performed with distilled water.

### Distillation and characterization of bioethanol

At the end of fermentation, ethanol in must samples was separated by fractional distillation. The distillation was performed using a Wilmad-LabGlass, LG-5890-130 Vigreux distilling column 900 mm 29/32. Only the fraction distilled at  $78 \pm 2^\circ\text{C}$  at the head of column was recovered for further analysis. The ethanol content was evaluated gravimetrically using the AOAC (1990) method. The specific gravity and kinematic viscosity of bioethanol were determined by the ASTM D 4052 and ASTM D 445 methods, respectively.

### Calculations

The sugar concentration (1) was calculated based on °Bx and density of the must (Son et al., 2009; Margalit, 2012). The amount of sugar consumed (2), absolute ethanol yield (3), fermentation efficiency (4), and volumetric ethanol productivity (5) were calculated using the following equations (Abdullah et al., 2015):

$$\text{Sugar concentration (g/L)} = 10^\circ\text{Bx} \times \text{density} \quad (1)$$

$$\text{Sugar utilisation (\%)} =$$

$$\frac{\text{Original sugar (g)} - \text{Residual sugar (g)}}{\text{Original sugar (g)}} \times 100 \quad (2)$$

$$\text{Absolute ethanol yield (g/g)} =$$

$$\frac{\text{Ethanol content (g/L)}}{\text{Total utilized sugar (glucose, saccharose, fructose) (g/L)}}$$

$$(3)$$

Fermentation efficiency (%) =

$$\frac{\text{Actual ethanol yield}}{\text{Theoretical ethanol yield}} \times 100 \quad (4)$$

Volumetric productivity (g/L h) =

$$\frac{\text{Maximal ethanol concentration (g/L)}}{\text{Fermentation time (h)}} \quad (5)$$

### Statistical analysis

All ethanol fermentation tests and characterization procedures were performed in triplicate. The errors are reported as the standard deviation of the mean and were calculated using Microsoft Excel 2013. The error bars shown in the figures are based on standard deviation values. A two-way analysis of variance (ANOVA) with an interaction nutrient concentration/yeast strain was implemented to determine the ethanol content in the distillate (g/L) and absolute ethanol yield (g/g) values. The means were compared by Tukey's test at a significance level of  $p=0.1$  using R software.

## RESULTS

### Composition of fermented *P. biglobosa* seeds

The mineral composition and nitrogen, protein, dry matter, and moisture contents of the fermented *P. biglobosa* seeds used in this study are shown in Table 1.

The available nitrogen was  $6.67 \pm 0.01\%$ , while the crude protein content was  $41.70 \pm 0.04\%$ . Moreover, calcium was found to be the most abundant mineral at  $4403.26 \pm 25.87$  ppm, followed by potassium and magnesium at  $2633.23 \pm 21.59$  and  $1894.63 \pm 42.96$  ppm, respectively. The values for iron and zinc ( $75.33 \pm 4.16$  and  $63.39 \pm 0.55$  ppm), respectively, were not negligible.

### Fermentation parameters

#### pH and TA variation during fermentation

The pH and TA variations that occurred during the fermentation process are presented in Figures 1 and 2. The blank and each of the two strains (S1 and S2) of *S. cerevisiae* showed different TA and pH dynamics in the musts. The pH of the blank (juice substrate) slowly

decreased throughout fermentation from an initial value of 4.35, ultimately reaching a value of 3.98 after 120 h. On the other hand, the pH of the S2 culture medium dropped drastically to 3.87 during 24 h of inoculation. The same trend was observed for all S2 musts containing nutrients (e.g., S2N4, S2N8, and S2N12), regardless of their concentration. The minimum pH for these was reached after 24 h of fermentation. The pH of the S1 (nutrient free) culture medium gradually decreased to a minimum of 3.90 after 72 h, while all S1 nutrient supplemented musts (e.g., S1N4, S1N8, and S1N12) reached a minimum pH after 48 h of fermentation. As shown in Figure 1, the minimum pH increased slightly to 4.00–4.12 in all musts except for the blank. However, the pH increased to a greater extent as the nutrient concentration increased.

The TA variations corresponded to changes in pH levels in the musts during ethanol fermentation (Figure 2). The TA in the blank gradually increased from an initial concentration of 2.8 g/L to 4.4 g/L after 144 h of fermentation. Otherwise, the S1 and S2 nutrient-supplemented musts reached the maximum TA level (4.1–4.2 g/L) at around 48 h and 24 h, respectively. Afterwards, there was a slight decrease in the TA content to about 3.7 g/L and 3.8 g/L for all musts except for the blank.

#### Sugar Utilisation

Figure 3 shows the trend of sugar utilisation in different cashew apple juice media during 144 h of inoculation. The initial concentration of sugars in the juice was 147.76 g/L (equivalent to 13.9 °Bx), and it slowly reduced in the blank to 54.5 g/L (5.5 °Bx) at the end of fermentation. A steep decrease in the sugar content was observed for all S2 musts from 24 to 48 h of fermentation, giving the minimum value of about 5.0 °Bx, equivalent to a sugar uptake of about 65%. The Brix degree changed less suddenly for the S1 mediums (e.g., S1, S1N4, S1N8, and S1N12), leading to the minimum sugar content of about 5.1 °Bx after 72 h of fermentation. However, as shown in Figure 3, the sugar uptake was

approximately the same in all musts at the end of 144 h of fermentation.

#### Must density evolution

The variation of must density during fermentation related to is presented in Figure 4. The S2 yeast strain was characterized by a fast drop in must density from 1.063 to about 0.996 within the first 24 h in all yeast mediums of this series, while in the S1 yeast strain mediums, the density decreased gradually to the same value of 0.996 within 72 h of fermentation.

#### Optimization Parameters

Qualitative and quantitative estimations of ethanol production are represented in Figures 5 and 6, respectively. The results showed that the highest significant absolute ethanol yield of 0.22 g/g was recorded for the S2 yeast strain grown in the medium without nutrient addition. The ethanol concentration in bioethanol obtained from this medium was 90.4% (v/v), equivalent to 712.9 g/L. The S2 yeast strain mediums supplemented with 4 and 8 g/L of nutrients and the S1 yeast strain

medium supplemented with 12 g/L of nutrients gave the same absolute ethanol yield of 0.19 g/g. The ethanol content of these mediums was 90.0%, 90.7%, and 83.4% (v/v), respectively.

Table 2 gives a comparison of bioethanol production from cashew apple juice using S1 and S2 strains of *S. cerevisiae* supplemented with different nutrients concentrations.

The two-way ANOVA for the nutrient concentration–yeast strain interaction gave a p-value of 0.074, which is significant compared to the threshold of 10%. Multiple comparisons of means (Tukey contrasts) of the ethanol content in the distillate as well as the absolute ethanol yield were similar to each other and are presented in Table 3.

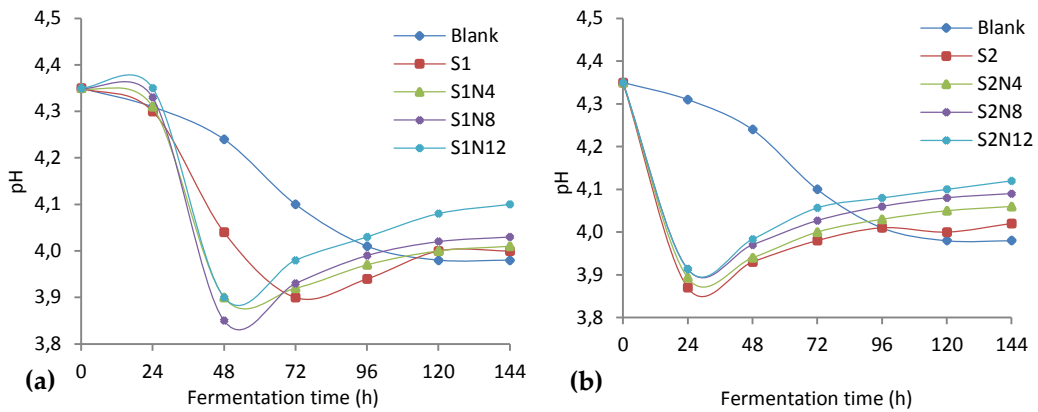
#### Characteristics of obtained bioethanol

The characteristics for the S1 and S2 yeast strain series distillates were as follows: 0.874 and 0.873 for specific gravity at 15 °C, and 1.62 mm<sup>2</sup>/s and 1.59 mm<sup>2</sup>/s for kinematic viscosity at 40 °C, respectively.

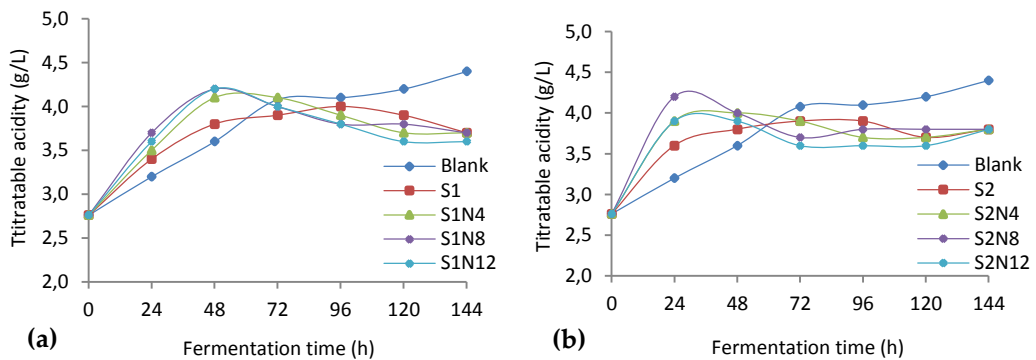
**Table 1:** Proximate and mineral compositions of fermented *P. biglobosa* seeds.

Components	Unit	Results	References*
Moisture	%	1.96	18.9
Dry matter	%	98.08	-
Protein	%	41.70 ± 0.04	36.8
N	%	6.67 ± 0.01	-
Ca	ppm	4403.26 ± 25.87	470
K	ppm	2633.23 ± 21.59	2504
Mg	ppm	1894.63 ± 42.96	748
Fe	ppm	75.33 ± 4.16	5.69
Zn	ppm	63.39 ± 0.55	2.8

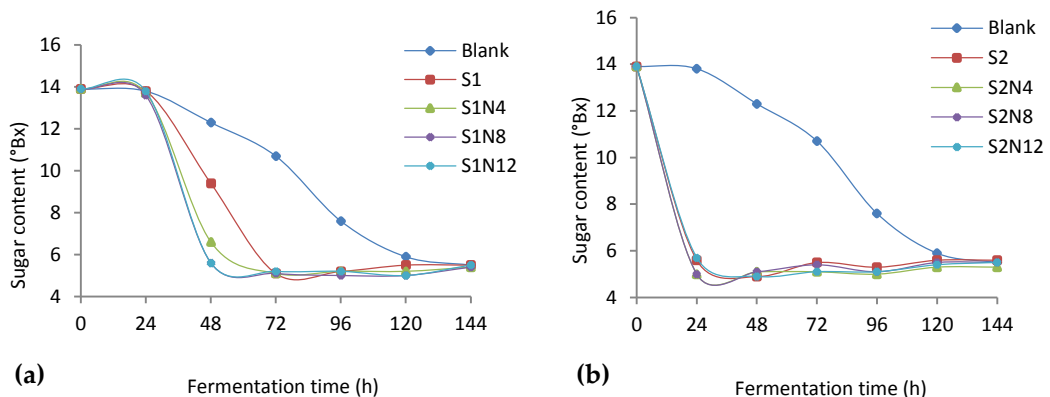
\* (Olandunmoye, 2007 ; Boateng et al., 2014 ; Aremu et al., 2015).



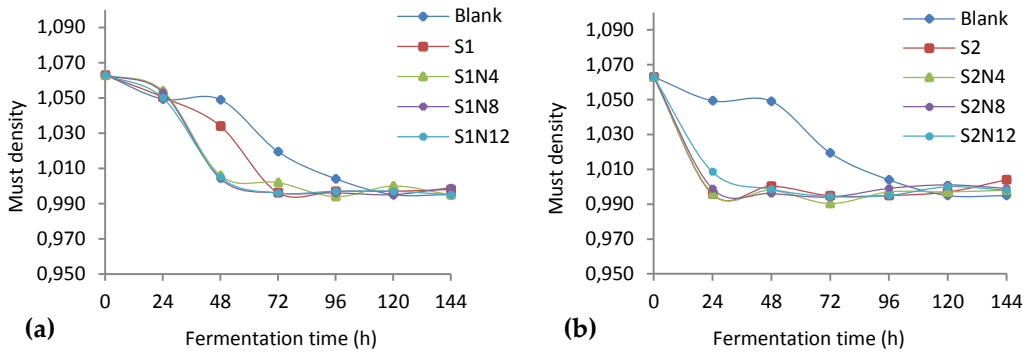
**Figure 1:** Effect of nutrients on the pH during the fermentation of cashew apple juice using *S. cerevisiae*: (a) Angel brand super alcohol yeast strain; (b) Angel brand thermal-tolerant alcohol yeast strain.



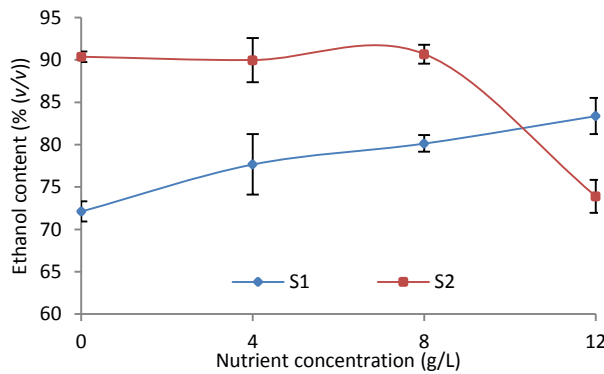
**Figure 2:** Effect of nutrient on the titratable acidity during the fermentation of cashew apple juice using *S. cerevisiae*: (a) Angel brand super alcohol yeast strain; (b) Angel brand thermal-tolerant alcohol yeast strain.



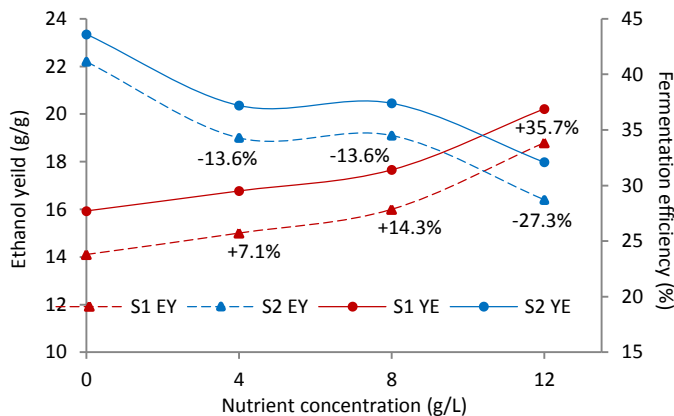
**Figure 3:** Sugar utilisation in the enriched musts using *S. cerevisiae*: (a) Angel brand super alcohol yeast strain; (b) Angel brand thermal-tolerant alcohol yeast strain.



**Figure 4:** Density variation in the musts supplemented with different nutrient concentrations using *S. cerevisiae*: (a) *Angel brand super alcohol yeast strain* and (b) *Angel brand thermal-tolerant alcohol yeast strain*.



**Figure 5:** Ethanol content (% (v/v)) in the bioethanol distillates. Points are expressed as means; error bars represent standard deviations (n = 3).



**Figure 6:** Absolute ethanol yield (EY) and fermentation efficiency (YE) resulting from the fermentation of cashew apple juice supplemented with different nutrient concentrations using S1 and S2 yeast strains of *S. cerevisiae*.



**Table 2:** Optimized parameters of bioethanol production from cashew apple juice using S1 and S2 strains of *S. cerevisiae* supplemented with different nutrient concentrations.

Nutrient concentration (g/L)	Ethanol content (% v/v)	Ethanol yield (g/g)	Fermentation efficiency (%)	Productivity (g/Lh)	Total sugar consumed (%)
<b>Blank</b>					
	90.0 ± 3.4	0.09 ± 0.01	18.5 ± 2.9	4.9 ± 0.2	62.7 ± 0.4
<b>S1</b>					
0	72.1 ± 1.2	0.14 ± 0.00	27.7 ± 0.2	4.0 ± 0.1	63.1 ± 0.4
4	77.7 ± 3.6	0.15 ± 0.06	29.5 ± 2.3	4.3 ± 0.2	63.4 ± 0.3
8	80.1 ± 1.0	0.16 ± 0.04	31.4 ± 7.2	4.4 ± 0.1	63.3 ± 0.4
12	83.4 ± 2.1	0.19 ± 0.04	36.9 ± 8.1	4.6 ± 0.1	63.2 ± 0.4
<b>S2</b>					
0	90.4 ± 0.6	0.22 ± 0.01	43.6 ± 1.8	5.0 ± 0.0	64.0 ± 0.3
4	90.0 ± 2.6	0.19 ± 0.00	37.2 ± 0.6	4.9 ± 0.1	62.8 ± 0.1
8	90.7 ± 1.1	0.19 ± 0.01	37.4 ± 1.0	5.0 ± 0.1	62.8 ± 1.0
12	73.9 ± 2.0	0.16 ± 0.02	32.1 ± 3.2	4.0 ± 0.1	63.1 ± 0.3

Data are means ± standard deviation (n=3).

**Table 3:** Multiple comparisons of means (Tukey contrasts) of the ethanol content and absolute ethanol yield.

Nutrient concentration (g/L)	Yeast strain	p-value
0	S1 and S2	0.10
0 and 12	S2	0.31
0 and 12	S1	0.71

## DISCUSSION

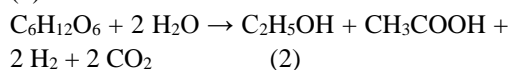
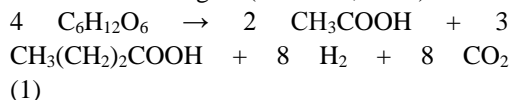
The present study investigated bioethanol production from cashew apple juice using two strains of *S. cerevisiae* and the natural yeast nutrient from fermented *P. biglobosa* seeds. Variable concentrations (0, 4, 8, and 12 g/L) of nutrients were supplemented into the fermentation medium. The first series consisted of the *Angel brand super alcohol* active dry yeast strain, and the second series consisted of the *Angel brand thermal-tolerant alcohol* active dry yeast strain.

The results indicate the great nutritional level of fermented *P. biglobosa* seeds. The protein and mineral contents were significantly higher than those given in the literature (Oladunmoye, 2007; Boateng et al., 2014; Aremu et al., 2015). Obviously, the physico-chemical and nutritional characteristics of the

seeds strongly depend on their origin and processing (Oladunmoye, 2007; Boateng et al., 2014; Soetan et al., 2014; Aremu et al., 2015). The macro- and microelements contribute to bioethanol fermentation. In fact, nitrogen is necessary for metabolism and yeast growth in the fermentation media. It allows yeast to accelerate the utilisation of sugars and the production of ethanol (Laopaiboon et al., 2009; Zahari et al., 2014; Abdullah et al., 2015). Minerals include calcium (Ca), potassium (K), magnesium (Mg), and many more trace metal ions are components of the yeast cell membrane and help maintain fermentation metabolism activities (Venkateshwar et al., 2010). Supplementing fermentation with complex nutrients would have beneficial effects such as an increase yeast cell mass and a reduction in the decline in the fermentation

rate due to the accumulation of ethanol (Tamunaidu et al., 2013). Fermented *P. biglobosa* seeds are relatively cheap and are available in tropical areas. Therefore, this product might be a very profitable and convenient nutrient substance for yeast growth during ethanol fermentation.

In order to estimate the effect of nutrients supplementation on cell activities, fermentation kinetics were tracked daily by measuring the pH, TTA (g/L), Brix degree (°Bx), and density. The increase in TTA followed by a drop in pH during ethanol fermentation was observed. This phenomenon might be due to the formation of weak carboxylic acids (Eqs. 1 and 2), which are by-products formed when the yeast cells metabolize the sugars (Lin et al., 2012).



According to Lin et al. (2012), pH can also be used as an indicator of the products formed in the fermentation process. For example, at pH of 5.5–6.0, the main products are ethanol and butyrate, whereas for pH values lesser than 5.0, the main product is acetic acid. Popov et al. (2010) mentioned that there is an optimal pH for each enzyme. This pH is acidic because of the acidophilic nature of the yeast itself. However, when the extracellular pH is not at its optimal level, the yeast cells themselves try to balance the hydrogen ions in order to maintain the optimal intracellular pH. Lin et al. (2012) found that the optimum pH range for *S. cerevisiae* used in ethanol fermentation is 4.0–5.0.

Sugars are the major fuel source of most organisms and play an important role in metabolism. Microorganisms such as *S. cerevisiae* use glucose as a nutrient for their growth. Sugar degradation via fermentation involves glycolysis through which one molecule of glucose is metabolized by microorganisms and two molecules of the three-carbon compound pyruvate are produced. Under anaerobic conditions,

pyruvate is further reduced to ethanol with the release of CO<sub>2</sub>. Only fermentable sugars (glucose, fructose, sucrose and maltose) are converted to ethanol (Dodić et al., 2009; Kuila and Sharma, 2018).

The result shows that the S2 yeast strain exhibited faster sugar depletion activity than that of S1. In the case of S2 series musts, sugar depletion slightly depended on the nutrient concentration and was complete after 48 h of fermentation for all S2 musts. In the S1 series musts, the time taken to achieve depletion of sugars was about 72 h. A similar observation for growth of the S1 yeast strain in cashew apple juice substrate supplemented or non-supplemented with urea was reported by Gbohaïda et al. (2016). However, the S2 yeast strain used in their study appears to be less effective than that of S1. We also observed, that the addition of nutrients to S1 culture medium accelerated the start of the fermentation process, giving a lower sugar content for nutrient-supplemented musts in the first 48 h of fermentation. However, the sugar levels became the same for all S1 musts after 72 h of fermentation.

At this point, it was concluded that the S1 yeast strain used in this study assimilates the nutrient supply and improves the rate of sugar utilisation. Otherwise, the S2 yeast strain is not quite sensitive enough to the presence of natural nutrients from fermented seeds of *P. biglobosa*. Yeasts have strain-specific capabilities. The nutrient requirement might be different for different yeast strains (Ribéreau-Gayon et al., 2006).

The density variation in musts during the 144 h fermentation process was controlled gravimetrically. In fact, there was decrease in the mass of the must due to sugar consumption, liberation of carbon dioxide, and ethanol formation throughout fermentation (Ribéreau-Gayon et al., 2006). We noted that the density of S2 yeast strain series musts decreases faster compared with those of S1 yeast strain series. This phenomenon proves the greater fermenting activity of the S2 yeast strain in the framework of this experiment and independently of nutrient addition. The

variation in density for both yeast strain series is in agreement with the changes in other fermentation parameters such as the pH, TTA, and sugar content, as discussed above.

In this study the important optimization parameters such as ethanol yield, ethanol content, ethanol productivity, fermentation efficiency, and sugar consumption were considered. The ethanol yield increased progressively as nutrients were added to the S1 yeast strain series. This was contrary to the S2 yeast strain medium, where the nutrient addition compromised the ethanol production. The S1 yeast strain medium supplemented with 12 g/L of nutrients (S1N12) showed an upsurge in ethanol yield of up to 35.7% compared to the nutrient free yeast medium. Furthermore, the best quantity/quality ratio of bioethanol was obtained for the S2 medium without nutrients ( $r = 0.0024$ ), while in the S1 yeast strain series, the best ratio was obtained with the S1N12 medium ( $r = 0.0023$ ). The obtained results indicate that the S1 and S2 strains of *S. cerevisiae* behave differently in relation to nutrient addition. Adding nutrients does not necessarily improve the fermentation parameters or the yield of ethanol using different yeast strains. The statistical analysis indicates that the ethanol content in the distillate and absolute ethanol yield obtained from S1 and S2 nutrient free yeast strain mediums were significantly different ( $p=0.1$ ). The type and specificity of a yeast strain define its efficiency in the production of ethanol under the given experimental conditions. The increase in nutrient supplementation to 12 g/L in the S2 yeast strain medium seemed to have a reductive effect on the ethanol yield and ethanol content, while it was favorable for the S1 medium. As showed in Table 2, the control blank also produced a small amount (0.09 g/g) of ethanol after 144 hours of fermentation. In fact, an alcoholic spontaneous fermentation of natural juice is possible due to an accidental microbial contamination of the must and favored by an ambient temperature of about 30°C (Jain et al., 2003). An increase in the titratable acidity from 2.8 to 4.4 g/L of the control blank at the end of fermentation

compared to that of the yeast strain mediums (3.7 g/L) indicates that the sugars in the natural juice were more widely converted into carboxylic acids (Yadav and Chakravarty, 2013).

The main source of uncertainty for the calculated ethanol yield (g/g) came from the final volumes of the distillates recovered by distillation from the musts. In fact, the final volumes of the musts were less than those of the juice at the start of fermentation due to the collection of aliquots for the monitoring of fermentation kinetics. They were also slightly different from each other due to some additional replications during the monitoring of fermentation kinetics.

In order to make a more general comparison between the efficiency of two *S. cerevisiae* strains, two types of bioethanol were obtained by mixing of the distillates derived firstly from the musts of S1 yeast strain series, and secondly from those of S2 yeast strain series. According to our results, the better performance with respect to the density and viscosity of produced bioethanol was attributed to the S2 strain.

## Conclusion

In this study, the final absolute ethanol content in bioethanol obtained from cashew apple juice during 144 h of fermentation using the S1 yeast strain with an increased natural nutrient concentration ranged from  $72.1 \pm 0.0\%$  (v/v) to  $83.4 \pm 2.1\%$  (v/v), while with the use of the S2 yeast strain, it was between  $90.4 \pm 0.0\%$  (v/v) and  $73.9 \pm 2.0\%$  (v/v). We can clearly see that two opposite trends appeared: the first showed the favorable effect of nutrient addition on ethanol production, and the second trend was the decrease of this with natural nutrient supply. Consequently, the fermented *P. biglobosa* seeds seem to be a good alternative natural nutrient for the S1 yeast strain of *S. cerevisiae*. Over 35% more ethanol was obtained from the must with a nutrient concentration of 12 g/L compared to that without nutrient supplementation. It is also noteworthy that the high nutrient concentration negatively affected ethanol production in the

case of the S2 yeast strain of *S. cerevisiae*. Nevertheless, the efficiency of ethanol fermentation using different natural nutrients has to be better investigated in terms of its impact on cell growth and microbial activity. Their nutritional properties, availability, and low cost could make them effective components for green ethanol production.

### COMPETING INTERESTS

The authors declare no conflict of interest.

### AUTHORS' CONTRIBUTIONS

SNY conceived the concept idea, designed the experiments, performed the experiments, interpreted the experimental results, performed the calculations, and prepared the manuscript. CPAD contributed to the design and execution of the experiments, performed the sample analyses, assisted in interpretation of the experimental results, supervised the research, and prepared the manuscript; VG contributed to the design of experiments; REN performed the experiments; IM contributed to the execution of the experiments; BHB performed the experiments and prepared the manuscript.

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