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Cashew apples: Physico-chemical analysis and occurrence of yeasts in plantations north of Côte d'Ivoire for processing into bioethanol and fermented beverages

Souleymane SOUMAHORO ¹, Maimouna L KOUAME ¹, Wilfried K YAO ^{2,*}, Abdoulaye TOURE ³ and Yadé R SORO ²

¹ Laboratory of Biochemistry, Microbiology and Valorization of Aggroresources, Agropastoral Management Institute, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire, Korhogo BP 1328.

² Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, Faculty of Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire, 22 BP 582 Abidjan 22.

³ Laboratory of Biotechnology and Valorization of Aggroresources and Natural Substances, Faculty of Biological Sciences, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire, Korhogo BP 1328.

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Abstract

The cashew apple is a pseudo-fruit that, once removed from the nut, is abandoned on plantations because the apple is not valued. The aim of this research is therefore to add value to the cashew apple by isolation yeasts with a view to fermenting this substrate to produce bioethanol and fermented beverages. Cashew apples from farmers' plantations in northern Côte d'Ivoire were subjected to physico-chemical analysis and yeast isolation. These isolated yeasts were screened for fermentative capacity and the influence of glucose, NaCl and ethanol on growth. The results indicate that cashew apples have an acid pH of 3.77 ± 0.03 with a high water content of $89.58 \pm 0.49\%$. In addition, vitamin C content was 13.15 ± 0.12 mg/100 g and the Brix value was 8.6. Forty-one (41) yeasts were isolated and were able to produce CO₂ in liquid medium with different production levels. In the end, seven (7) isolates were selected as potential starters as they were able to resist the influence of NaCl, glucose and ethanol on growth.

Keywords: Cashew apples; Physico-chemical; Yeasts; Potential starters

1. Introduction

The cashew (*Anacardium occidentale* L.) is a significant perennial crop of Anacardiaceae family. Originating in Brazil, it was introduced to Africa and Asia by Portuguese explorers in the sixteenth century. Mainly in tropical and subtropical climates, it is cultivate [1]. Côte d'Ivoire, India, and Cambodia are the top three producers, with global production estimated to be above 3.8 million tons [2]. Approximately half of the world's production is generated by West Africa who is principally an agricultural region.

The cashew nut has evolved to be a vital strategic component for the economy as a whole as well as the agriculture industry [3]. The product is a significant source of income for smallholder farmers in Côte d'Ivoire, the third-biggest cashew nut exporter in the world and its biggest producer (1.2 million tons), and is a factor in the economic and social success of this country. The cashew nut sector actually generated over 300 billion CFA francs [4].

The interest in cashew nuts lies in the fact that they contain a number of interesting nutrients. Cashews are rich in fat, protein and fibre. These are food components that are very rich in macronutrients, micronutrients, vitamins, minerals

* Corresponding author: Wilfried K YAO

and a number of active metabolites [5, 6]. Consumption of cashew nuts has been associated with a reduced risk of diseases such as cancer, diabetes, cardiovascular and respiratory diseases [4].

Although, the cashew tree is mainly grown for its nuts, which are of nutritional and economic interest, but it also produces the apples (false fruit) to which the nuts are attached. It is a fleshy, very juicy fruit that can be consumed raw or in the form of fresh juice, jam or other beverages. Cashew apples have a high nutritional value, particularly due to their high vitamin C content [7]. It is rich in polyphenolic compounds (tannins), carotenoids, fibre, vitamins, certain amino acids, organic acids, sugars (glucose and fructose), antioxidants and mineral elements essential [8]. Due to its content of polyphenolic compounds, the cashew apple has a significant astringency. Cashew apple is considered as a good source of nutrients [9].

Despite its high nutritional potential, and like many tropical fruits, the cashew is not very valorized in producing countries because a large proportion of its production is abandoned at the point of harvest, resulting in enormous post-harvest losses. In 2015, almost 6 million tons were produced. However, this was not exploited in Côte d'Ivoire [10]. Processing cashew apples into these various co-products is an opportunity to diversify producer's sources of livelihood, thereby helping to improve their livelihoods. Cashew apples are rich in fermentable sugars, making them an ideal substrate for the production of bioethanol by yeasts. As a fundamental microorganism in the production of many fermented products such as bread, beer, wine and dairy products [11], yeasts plays a crucial role in the food industry. Yeast also performs alcoholic fermentation of the sugars present in fruits to produce ethanol, its main compound [12]. The species '*Saccharomyces cerevisiae*' is the most commonly used because of its efficiency and robustness [13]. However, the research for indigenous yeast strains is of particular interest as they may offer interesting properties, such as improved tolerance to environmental stresses or enhanced production of compounds of interest to industry [14]. Studies by Barros *et al.* [15] have shown that yeasts isolated from cashew apple juice can be used to produce bioethanol and fermented beverages with unique flavour profiles. In Côte d'Ivoire, very few studies were carried out on the isolation of cashew apples yeasts for the production of bioethanol and fermented beverages. This study aims to contribute to a reduction in post-harvest losses through a selection yeasts with interesting potential.

2. Material and methods

The biological material used in this study consisted of mature cashew apples collected from farmers' plantations (Figure 1) in Tioroniaradougou, Korhogo Department (9° 27' 41" North, 5° 38' 19" West). Physico-chemical and microbiological analyses were carried out on these cashew apples, which were acheminated directly to the Laboratory of biochemistry, microbiology and valorization of agrosources of the Agropastoral Management Institute at the Peleforo Gon Coulibaly University of Korhogo (Côte d'Ivoire).



Figure 1 Cashew apples collected from farm plantations

2.1. Physico-chemical analyses of cashew apples

2.1.1. Dry matter and moisture content measurements in cashew apples

AOAC [16] described the method used to determine the dry matter and moisture content. The samples were oven dried to a consistent weight in order to dehydrate them. A glass capsule with a known mass (m_0) was filled with five (5) grams of cashew apples. The capsule containing the sample (total mass m_1) was cooled in a desiccator after being exposed to

a 24-hour oven at 105°C. After cooling, the samples and capsules were weighed (m_2) again. Using the following below, the moisture content (H) as a percentage of the mass was calculated:

$$H(\%) = \frac{(m_1 - m_2)}{(m_1 - m_0)} \times 100$$

The following equation was used to determine the dry matter (DM) content, which was expressed as a percentage of the raw mass of the sample:

$$MS(\%) = 100 - H(\%)$$

2.1.2. Cashew apples pH and titrable acidity

A mass of ten (10) grams of cashew apple ground, was homogenized in 100 milliliters of distilled water and filtered. The pHmeter (HANNA, Germany) glass electrode, which had previously been calibrated, was submerged in the liquid and the pH value on its screen [16]. Three (3) drops of phenolphthalein indicator were added to 10 mL of the filtrate obtained beforehand. A solution of NaOH (0.1N) contained in a burette is poured dropwise into the homogenized mixture until a pink colour is obtained. Titrable acidity is expressed in percentage of citric acid (%).

2.1.3. Total soluble solid (TSS) of cashew apples

Using an ATC refractometer, a drop of cashew apple juice was placed on the plate to test TSS, which was expressed in Brix degree (°B). The refractometer's ocular was used to read the value directly [17].

2.1.4. Amount of vitamin C in cashew apples

Cashew apples vitamin C content was evaluated using Pongracz *et al.* [18] method. To 40 mL of metaphosphoric acid/acetic acid (2%; w/v) were added 10 g ground mango pulp (me). The solution obtained was centrifuged at 3000 rpm for 20 min. Supernatant was collected in 100 mL flask and made up to the mark with boiled distilled water. Ten (10) mL volume of the contents cooled was removed and placed in an Erlenmeyer flask. Assay sample was titrated against a 0.5 g/L solution of 2,6-DCPIP until the colour changed to a persistent pink. 2,6-DCPIP solution was previously calibrated with a 0.5 g/L vitamin C solution. So V (mL), the volume of 2,6-DCPIP poured to equivalence. Vitamin C content as a percentage by mass of fresh sample is determined by the following relationship:

$$\text{Vitamin C (\%)} = \frac{(0.5 \times V \times 10^{-3}) \times 5}{m_e}$$

2.2. Cashew apples microbiological analysis

2.2.1. Isolation and biochemical characterization of yeasts

For yeast isolation, the stock solution consisting of 10 g of crushed cashew apples and 90 mL of peptone water solution was used to streak MYGP agar (3 g/L malt extract, 5 g/L bactopectone, 3 g/L yeast extract and 10 g/L glucose) supplemented with chloramphenicol (100 mg/L). MYGP agar were incubated at 30°C for 48 hours. After incubation, the yeasts were biochemically identified using standard Biolog Identification System Techniques in addition to macroscopic and microscopic characteristics [19].

2.2.2. Technological properties of yeasts isolated from cashew apples

Study of the fermenting capacity of yeasts

CO₂ production by yeasts strains was evaluated according to the method described by Koffi *et al.* [20]. The aim of this method is to determine the fermentative capacity of yeasts strains through by their CO₂ production capacity. Under anaerobic conditions, yeasts oxidise fermentable sugars to ethanol, producing gas (CO₂). The rate of CO₂ production correlates with the strain's fermentation capacity of the strain and is related to the amount of ethanol produced. In this

method, the hemolysis tube was used in place of the Durham bell for the standard Wickerham protocol [21] to collect the maximum amount of CO₂ released. The prepared YPG media were then sterilized in an autoclave at 121°C for 15 minutes. The broth liquid contained in the tubes was inoculated with a yeast suspension (OD 600 nm = 0.7) previously prepared in tryptone salt (TS). The cultures were incubated in an oven at 30°C for 72 hours without shaking, and the height of the gas produced in the hemolysis tube was measured after this incubation period. The volume of CO₂ released is given by the equation relationship:

$$\text{Volume of CO}_2 \text{ released (cm}^3\text{)} = \pi r^2 h$$

r: radius of the hemolysis tube

h: height of gas in the hemolysis tube

Stress conditions effects (glucose, ethanol and sodium chloride) on yeast growth

A liquid medium containing 0.3% casein peptone and 0.05% of yeast extract was used to assess the effects of glucose, ethanol and sodium chloride (NaCl) on the growth of yeast isolates at varying concentrations [22]. At 10%, 20%, 30%, and 40%, glucose was completed. The following concentrations of ethanol were applied: 0%, 5%, 8%, 10%, 12%, and 14%. Concerning NaCl, it was added at 2.5%, 5% and 10%. A test tube containing 10 mL of liquid medium was inoculated with 100 µL of yeast preculture (OD₆₀₀=0.7) and incubated for three days at 30°C. The yeasts growth was assessed using a spectrophotometer (Pioway medical Labs, Singapore) measuring the optical density at 600 nm after incubation period [23].

2.3. Data Analysis

For data processing, all measurements were performed in three experiments according to the parameter studied. Anova using SPSS version 20.0 software tested statistical differences between isolates and measured parameters. Means were compared using Duncan's test at 5% significance level.

3. Results and discussion

Table 1 describes pH, titrable acidity moisture content, dry matter, total soluble solids (TSS) and vitamin C of cashew apples. Moisture content and dry matter content are two correlated parameters. The moisture content of cashew apples is very high at 89.58%, while the dry matter content is low at around 10.42%. Studies by Singh *et al.* [24] in cashew apples from India presented moisture and dry matter contents of 85.62% and 14.36% respectively. The high water content of cashew apples would favour their deterioration after harvest by microorganisms present in the environment. On the other hand, this high water content could allow a large amount of juice to be extracted, which would be an advantage in the preparation of beverages [24].

Table 1 Physico-chemical properties of cashew apples

Parameters	Unit	Mean ± SD
pH	-	3.77±0.03
Titrable acidity (TA)	%	1.54±0.05
Moisture content	%	10.42±0.23
Dry matter	%	89.58±0.49
Vitamin C	mg/100 g	13.15±0.12
Total soluble solids (TSS)	°Brix	8.6±0.0

The content and diversity of organic acids present in fruit are correlated with titrable acidity and pH values [24]. In our study, the pH of cashew apples was very acidic (3.77) with a titrable acidity of 1.54%. Titrable acidity and pH found in our studies are similar to those of Talasila *et al.* [25] who obtained low pH values ranging from 3.9 to 3.2 with a corroled titrable acidity (0.321) in juices. These conditions will be favorable for the growth yeasts who tolerate low pH values

[26]. Total soluble solid (TSS) in this study is summarized in Table 1. Total soluble solid are related to the quantity of sugars present in a solution. The amount of sugar increases with the Brix value. Several studies on cashew apples juice have reported a Brix level of between 7.4 and 13.8 [27, 28, 29]. This report suggests that cashew apples contain a large quantity of sugars, which would be suitable for processing into juice or jellies or fermentation to obtain fermented drinks or bioethanol [30]. An important nutrient, vitamin C is crucial for sustaining both human and animal physiological functions [31]. The cashew apples in our study have a low vitamin C content (13.15 mg/100 g) compared with several studies reported in the scientific literature. In fact, several studies indicate that the vitamin C content of cashew apples is between 50 and 300 mg/100 g [32, 33, 31]. The low vitamin C content of cashew apples could be explained by their exposure to the high temperatures found in farmers' plantations in Korhogo. The high temperatures could degrade the thermolabile vitamin C present in the fruits.

Forty-one (41) potential yeasts were isolated on MYGP agar based on morphological and microscopic characteristics. The isolation of indigenous yeasts is of particular interest because these new strains may have interesting properties, such as greater tolerance to environmental stress or improved production of compounds of interest to the food industry [34, 14]. In biotechnological process used in the food industry, fruits have been repertoried as sources for isolating novel yeasts species with desired flavor and fragrance properties [35].

The fermentative capacity of the yeasts was assessed by the production of CO₂ in liquid medium. On this basis, all the isolated strains were able to produce ethanol at different production levels according to the classification of Koffi *et al.* [20]. According to the volume of CO₂ released (0 to 7.5 cm³), three groups were established (Table 2). The first group consists of nine (9) isolates with very high fermentative capacity, producing a volume of CO₂ between 4 and 7.5 cm³, i.e. 21.95% of the isolates. Ten (10) isolates, i.e. 24.39%, produced CO₂ volumes between 2 and 4 cm³, considered as average producers. Finally, the remaining strains (22), i.e. 53.66%, produced low amounts of CO₂ with values between 0 and 2 cm³. Several studies have established the ability of yeast to produce ethanol [36, 20, 37, 38]. The first recognized property of yeast, and one that is widely used in industry, is the production of ethanol. Thus, our ethanol-producing isolates could be used in biotechnological processes for the production of bioethanol and fermented beverages [35].

Table 2 CO₂ production of yeasts strains isolated from cashew apples

Groups	Volume of CO ₂ (cm ³)	Number of isolates	Percentage (%)	Fermentative capacity
Group 1	[4-7.5 cm ³]	9	21,95	High level
Group 2	[2-4 cm ³ [10	24,39	Medium level
Group 3	[0-2 cm ³ [22	53,66	Low level

Isolates with a high fermentation capacity were selected for the influence of glucose, ethanol and NaCl on yeast growth. Figure 2 shows the influence of NaCl concentration on the growth of yeast isolates. A decrease in absorbance at 600 nm is observed with an increase in the concentration of NaCl in the liquid medium, until it reaches zero at 10%. However, up to 5% NaCl, all isolates showed growth, and this was not negligible. Thus, sodium chloride appears to be a limiting factor for growth and ethanol production by yeasts. Studies by Logothetis *et al.* [39] found that cell growth decreased with increasing NaCl concentration, and that 10% NaCl completely inhibited yeast growth. Rodríguez-Navarro and Ortega [40] asserted that the addition of NaCl to culture media had the effect of increasing the concentration of NaCl inside the cells, which implicitly had a negative impact on yeast growth and viability. Research carried out by Oda and Tonomura [41] has shown that when cultured in media with NaCl concentrations ranging from 0% to 3%, the leavening capacity of the baker's yeast *S. cerevisiae* decreases considerably. Isolates that showed an absorbance of more than 0.1 at 600 nm could be chosen as potential starters.

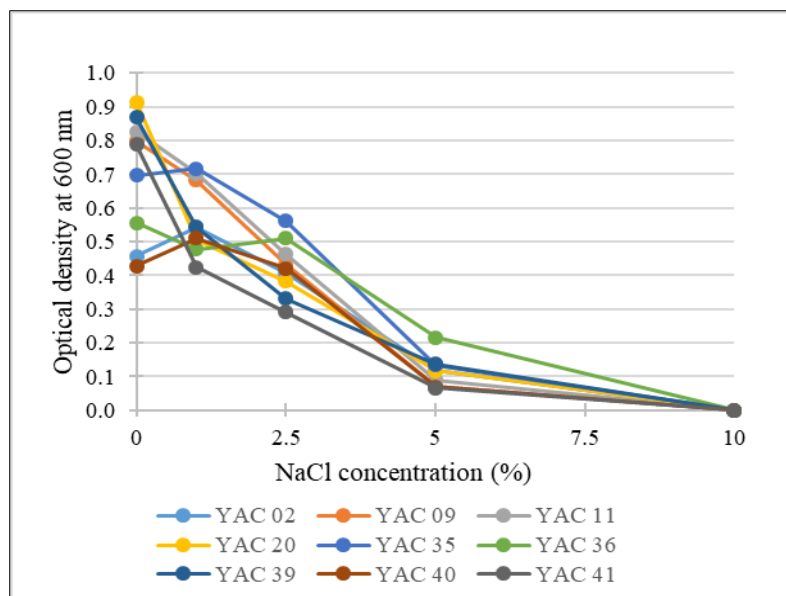


Figure 2 Impact of NaCl concentration on the growth of high-fermentation yeast isolates

Glucose is a substrate that can influence yeast growth and bioethanol production. In our study, all nine isolates were able to grow in the presence of different concentrations of glucose ranging from 5 to 30% in liquid medium (Table 3). Peak growth was observed at 20% glucose for all strains with optical densities (600 nm) reaching values of 3.69, 3.42 and 3.23 for isolates YAC 35, YAC 39 and YAC 02, respectively. Our results are consistent with those of Tadese *et al.* [42], who found a growth peak of 20% for yeasts isolated in Ethiopia. Furthermore, the study of Koffi *et al.* [20] revealed that *S. cerevisiae* and *Pichia kudriavzevii* yeasts isolated from natural cocoa fermentations in Côte d'Ivoire were able to grow in liquid media containing 50% glucose, with optical densities of 1.2 and 1.4 respectively. The ability of the yeast strains to resist the stress caused by the presence of glucose would be an advantage for our strains. Research by Tadese *et al.* [42] showed that *S. cerevisiae* 9Li2 was able to ferment 40 g/L glucose to produce 15 g/L ethanol at 40°C for 72 hours. Our different isolates are therefore potential starters for the production of bioethanol and fermented beverages from cashew apples.

Table 3 Impact of glucose content on the growth of high-fermentation yeast isolates

Yeasts strains	Optical density per concentration of glucose at 600 nm			
	5%	10%	20%	30%
YAC 02	0.97±0.17 ^b	1.15±0.25 ^b	3.23±0.19 ^a	0.82±0.02 ^b
YAC 09	1.00±0.21 ^c	1.41±0.05 ^b	1.67±0.03 ^a	0.40±0.01 ^d
YAC 11	0.83±0.04 ^c	1.23±0.04 ^b	2.39±0.07 ^a	0.45±0.01 ^d
YAC 20	0.98±0.09 ^c	1.78±0.09 ^b	2.84±0.07 ^a	0.25±0.01 ^d
YAC 35	1.07±0.06 ^c	1.34±0.20 ^b	3.69±0.08 ^a	0.93±0.02 ^c
YAC 36	0.86±0.03 ^{bc}	1.02±0.13 ^b	2.33±0.16 ^a	0.68±0.02 ^c
YAC 39	0.82±0.08 ^{bc}	1.01±0.08 ^b	3.42±0.19 ^a	0.77±0.02 ^c
YAC 40	1.32±0.16 ^c	2.25±0.07 ^b	2.64±0.04 ^a	0.25±0.01 ^d
YAC 41	0.65±0.08 ^c	0.89±0.05 ^b	2.91±0.15 ^a	0.88±0.04 ^b

The data is shown as means±SEM for n = 3. Duncan's test indicates that means with different letters in the same line are significantly different (p<0.05).

The effect of ethanol concentration on yeast growth is shown in Table 4. All isolates were tolerant up to 14% ethanol with the exception of strains YAC 09 and YAC 11 which were only tolerant to 12% ethanol. The 16% ethanol concentration completely inhibited the growth of all isolates. Our results are in agreement with those of Dung *et al.* [43]

who observed yeast tolerance up to 12% ethanol. However, several studies in the scientific literature have reported high yeast resistance to ethanol stress below 16% [44, 38, 42]. Excessive ethanol concentrations cause cellular stress in yeast metabolism, which lowers cell viability and growth rate [45], ultimately resulting in low output. However, yeasts that can tolerate stresses and continue to be alive throughout the fermentation process are needed for industrial applications [46, 47]. Isolates (YAC 02, YAC 20, YAC 35, YAC 36, YAC 39, YAC 40 and YAC 41) with a tolerance to 14% ethanol could therefore be proposed as potential starters for processing cashew apples into bioethanol or fermented beverages.

Table 4 Impact of ethanol content on the growth of high-fermentation yeast isolates

Yeasts strains	Optical density per concentration of ethanol at 600 nm					
	5%	8%	10%	12%	14%	16%
YAC 02	0.86±0.02 ^d	2.32±0.07 ^a	0.98±0.03 ^c	1.42±0.06 ^b	0.81±0.06 ^d	0.0±0.0 ^e
YAC 09	2.16±0.07 ^a	0.87±0.05 ^b	0.52±0.01 ^d	0.65±0.03 ^c	0.03±0.00 ^e	0.0±0.0 ^e
YAC 11	1.90±0.06 ^a	0.97±0.05 ^b	0.62±0.03 ^c	0.39±0.04 ^d	0.0±0.00 ^e	0.0±0.0 ^e
YAC 20	2.24±0.14 ^a	2.35±0.06 ^a	0.81±0.05 ^b	0.76±0.01 ^b	0.51±0.03 ^c	0.0±0.0 ^d
YAC 35	2.64±0.13 ^a	1.74±0.01 ^b	0.83±0.04 ^c	0.65±0.05 ^d	0.43±0.04 ^e	0.0±0.0 ^f
YAC 36	2.89±0.03 ^a	1.60±0.06 ^b	0.92±0.07 ^c	0.89±0.03 ^c	0.70±0.10 ^d	0.0±0.0 ^e
YAC 39	3.54±0.13 ^a	2.55±0.09 ^b	1.27±0.15 ^c	0.89±0.06 ^d	0.69±0.05 ^e	0.0±0.0 ^f
YAC 40	2.32±0.15 ^a	1.21±0.02 ^b	0.73±0.01 ^c	0.53±0.04 ^d	0.19±0.02 ^e	0.0±0.0 ^f
YAC 41	2.62±0.02 ^a	1.50±0.07 ^b	0.87±0.08 ^c	0.79±0.04 ^c	0.52±0.01 ^e	0.0±0.0 ^f

The data is shown as means±SEM for n = 3. Duncan's test indicates that means with different letters in the same line are significantly different (p<0.05).

4. Conclusion

The aim of this study was to valorize cashew apples for possible processing into bioethanol and fermented beverages. To this end, cashew apples were biochemically characterized. The results show that cashew apples have an acidic pH (3.77), which correlates with their titrable acidity. They have a high water content with a significant amount of vitamin C (13.15 mg/100 g). A study of the technological properties and fermentative stresses of yeasts isolated from cashew apples allowed the selection of seven isolates, namely YAC 02, YAC 20, YAC 35, YAC 36, YAC 39, YAC 40 and YAC 41, as potential starters for the processing of cashew apples, thus adding value to the Ivorian cashew industry.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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