

Original Research Article

Physico-chemical analysis and isolation of yeasts from wild fruits *Cola cordifolia* in the north of Côte d'Ivoire: selection of potential starters

ABSTRACT

Aims: Valorization of the wild fruit *Cola cordifolia* through physico-chemical analysis and yeast isolation

Study design: Original research.

Place and Duration of Study: This study was carried out at the Laboratory of biochemistry, microbiology and valorization of agrosources of the Institute of Agropastoral Management at the Peleforo Gon Coulibaly University between January and June 2024.

Methodology: *Cola cordifolia* fruits were collected from farmers plantations in the town of Korhogo to be investigated. For this purpose, physico-chemical parameters were determined. Microbiological analyses were also carried out. They consisted in isolation and identification yeasts from the pulp of *Cola cordifolia*, testing their fermentative capacity, and then subjecting isolates with a high fermentative capacity to the influence of various parameters such as glucose and ethanol.

Results: The results of physico-chemical analysis revealed that the fruit had an acid pH (5.74 ± 0.08) with a high moisture content, a vitamin C content of $6.40 \pm 1.65 \text{ mg/100g}$ and a high brix degree (25 ± 0 °B). In addition, microbiological analyses enabled us to select five (5) isolates with the best growth rates compared with the other isolates tested. So, these isolates could therefore be used as potential starters for biotechnological applications.

Conclusion: *Cola cordifolia* fruits would be of interest in the development of yeast culture collections useful for controlled fermentations.

Keywords: *Cola cordifolia*, Yeasts, Characterization, Selection, Starters.

1. INTRODUCTION

Fermentation is one of the most ancient methods known for preparing and preserving food [1]. It was used long before microorganisms and their role in the fermentation process were discovered [2]. Fermented foods were first developed on farms and were produced naturally, in an uncontrolled way, bringing into competition a varied flora whose development was guided only by the physico-chemical variations of the environment [2]. The aim of fermented foods was to improve flavour and texture, extend shelf life and, in some cases, reduce the risk of contamination [3]. Nowadays, fermentation processes play an important role in food technology in both developed and developing countries, particularly in Côte d'Ivoire.

Côte d'Ivoire is the biggest fruit-producing country in West Africa, exporting large quantities to the European market. Some of the fruit is marketed in Côte d'Ivoire, while others are destined solely for local consumption, with no resulting profit. However, a large quantity is lost because it is highly perishable [4]. Although some alternatives to direct consumption have already been developed (jams, fruit concentrates, fruit juices, nectars, purées, etc.), a large quantity of fruit is still left in the fields to be disposed of as waste. It is a little-known fruit and is therefore little used by the local population from a nutritional and industrial point of view, and its consumption remains seasonal. Production is abundant, but the fruit is less valued. Processing by fermentation has been proposed and applied in some countries as an alternative for most wild fruits. The resulting product is usually a fruit wine, often distilled, with a variable alcohol concentration, or fruit vinegar [5].

Obtaining these products requires the use of starter cultures in the food fermentation industry, the aim of which is to predict and safeguard product quality [6]. So, these microbial starters play a significant role in the fermentation process [7]. In addition, the inoculation of microbial starters, namely yeast, has been widely used in the food industry to obtain a product of predictable quality, including in the production of alcoholic beverages such as wine and beer [6]. Due to its high carbohydrates level, *Cola cordifolia* could be a suitable substrate for the research and selection of an interesting fermentative flora for the food industry. Consequently, better use of *Cola cordifolia* by transformation into various finished products through bioconversion into ethanol could limit environmental pollution and create new economic activities that would create jobs [8]. Consequently, making better use of *Cola cordifolia* by transforming it into various finished products through bioconversion into ethanol could limit environmental pollution and create new economic activities that create jobs [8]. The aim of this work is to contribute to the valorization of *Cola cordifolia* fruit for use as a substrate for alcoholic fermentation.

2. MATERIAL AND METHODS

2.1. Materials

Cola cordifolia fruits were collected from farmers plantations in the town of Korhogo in northern Côte d'Ivoire (9° 27' 41" North, 5° 38' 19" West). These fruits were transported at Laboratory of biochemistry, microbiology and valorization of agrosources at Peleforo GON COULIBALY University (Korhogo, Côte d'Ivoire) in stomacher bags for physico-chemical analysis and yeast isolation.



A

B

Fig 1: *Cola cordifolia* fruits (A) and seeds (B)

2.2. Methods

2.2.1. Physico-chemical analysis of *Cola cordifolia* fruits

2.2.1.1. Determination of moisture content and dry matter in *Cola cordifolia* fruits

Method used to determine moisture content and dry matter was that described by AOAC[9]. It was based on dehydrating the samples by oven drying to a constant weight. Five (5) grams of *Cola cordifolia* pulp samples were weighed into a glass capsule of known mass (m_0). The capsule containing the sample (total mass m_1) was placed in an oven set at 105 °C for 24 h and then placed in a desiccator to cool. After cooling, the sample and capsule were weighed together (m_2). Moisture content (H) expressed as a percentage of wet sample mass was determined by the following relationship:

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

Dry matter (DM) content, expressed as a percentage of the raw sample mass was determined by the formula below:

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

2.2.1.2. pH of *Cola cordifolia* fruits

pH was determined using a pH meter that measures the electromotive force using an electrode sensitive to hydrogen ions (H⁺). This determination was carried out using the AOAC method[9]. Ten (10) grams of *Cola cordifolia* pulp was grinded and homogenised in 100 mL of distilled water, then filtered through Whatman No. 4 filter paper. The glass electrode of the pH meter (HANNA) was immersed in the mixture. The pH value was displayed on the screen of the pH meter, which had been calibrated previously.

2.2.1.3. Vitamin C content of *Cola cordifolia* fruits

Vitamin C content was determined using the method described by Pongracz[10]. The principle of this method consists of stabilizing vitamin C with metaphosphoric acid/acetic acid, then oxidising it with 2,6-dichlorophenol indophenol (2,6-DCPIP) which is reduced. 10 g of ground pulp (m_s) were solubilised in 40 mL of metaphosphoric acid/acetic acid (2%; w/v). The mixture was centrifuged at 3000 rpm for 20 min. Supernatant was collected in a 50 mL flask and made up to the mark with boiled distilled water. After cooling, a 10 mL volume of the contents of the flask was removed and placed in an Erlenmeyer flask (test sample). The assay sample was titrated with a 0.5 g/L solution of 2,6-DCPIP (2,6-dichlorophenol indophenol)

until the colour changed to a persistent pink for 30 s. 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:

$$\text{Vitamin C (\%)} = \frac{(0.5 \times V \times 10^{-3}) \times 5}{m_e} \times 100$$

2.2.1.4. Soluble dry extract of *Cola cordifolia* fruits

Soluble dry extract, expressed in Brix, was measured using an ATC refractometer in accordance with the manufacturer's recommendations. To do this, 10 g of *Cola cordifolia* pulp were ground and a drop of this juice was placed on the plate of the refractometer prism. The value was read directly from the refractometer ocular.

2.2.2. Isolation and identification of yeasts from *Cola cordifolia* fruits

2.2.2.1. Isolation of yeasts from *Cola cordifolia* fruits

Ten (10) grams of *Cola cordifolia* pulp were homogenised in 90 ml of peptone water solution followed by decimal serial dilutions. Then, 100 µL of each dilution was streaked onto MYGP agar (3 g/L yeast extract, 3 g/L malt extract, 5 g/L bacto peptone and 10 g/L glucose) containing 100 mg/L chloramphenicol. Finally, the Petri dishes were incubated at 30°C for 72 hours. Presumptive yeast isolates were stored in MYGP broth supplemented with 20% glycerol at -20°C for subsequent tests [11].

2.2.2.2. Identification of yeast from *Cola cordifolia* fruits

Morphological identification system of the yeast species was studied using the conventional methods described by Barnett et al. [12] and also conventional methods of the Biolog identification system.

2.2.3. Screening of potential yeasts starters isolated from *Cola cordifolia* fruits

2.2.3.1. CO₂ production by yeast isolates

The fermentative capacity of yeast strains was studied according to the method of Dung and Phong [13] with a slight modification. From preculture of 24 h, pure yeast culture was resuspended in saline tryptone to get an optical density of 0.7 at 600 nm and 100 µL of this suspension were used to inoculate 10 mL of YPG medium containing a Durham tube into an assay tube. Then, the culture was incubated at 30°C for 6 days without agitation. Fermentation capacity was also determined by measuring gas production in the tube. Under anaerobic conditions, yeast oxidises sugars to ethanol, producing CO₂. This volume of CO₂ and ethanol production is related to fermentative strength of every strain [14].

2.2.3.2. Influence of glucose and ethanol concentration on the growth of yeast isolates

The influence of glucose and ethanol on the growth of yeast isolates was evaluated using a liquid medium containing 0.05% yeast extract; 0.3% casein peptone with different sugar concentrations. Glucose was added at 10%, 20%, 30% and 40%. Ethanol was added at the following concentrations: 0%, 5%, 8%, 10%, 12% and 14%. 10 mL of the liquid medium in a test tube was inoculated with 100 µL of yeast preculture, OD₆₀₀=0.7 and incubated at 30°C for 3 days. After incubation time, the growth was determined by measuring the optical density at 600 nm at spectrophotometer (Piway Medical Labs, Singapore) [15].

2.2.4. Statistical analysis

All the analyses were performed in triplicate and data were analyzed using Excel and SPSS Statistics 20.0. Means were compared using Duncan test with a significance level of 5% (p < 0.050).

3. RESULTS AND DISCUSSION

3.1. Determination of the physico-chemical parameters of *Cola cordifolia* fruits

Physico-chemical parameters of the pulp are presented in Table 1. *Cola cordifolia* has an acidic pH (5.74), which is in accordance with the results of Magaia et al. [16] who indicate that the pH of wild fruit is naturally acidic and between 3 and 6. pH is associated with the total acidity of the pulp, due to the presence of organic acids such as citric, malic, oxalic and tartaric acids [17]. This acid pH of the fruit could limit their contamination by pathogenic bacteria. In addition, fruits with acidic properties are recommended for the production of juice and jam, indicating the potential of the fruits from *Cola cordifolia* in industrial production [18]. The soluble dry extract or Brix degree of *Cola cordifolia* is 25°B. These results are similar to those reported by Serpen [19], who showed that the grapes had a Brix level of between 15 and 26. These data presented shows that this fruit has a high Brix level and therefore a high concentration of sugars. This fruit could therefore be used to produce various juice concentrates. The moisture content of *Cola cordifolia* is very high (72.68%) while that of the dry matter is low (27.32%). These results are in conformity with those of Ekué et al. [17], who showed that most fleshy fruits have a fairly high moisture content, reaching up to 79.19% in the northern region of Côte d'Ivoire. In addition, study by Fernandez-ruiz et al. [20] mentioned that the majority of the African wild fruits reviewed provide high moisture values (around 70-90%). This high moisture content limits the storage time of these fruits, which makes them highly perishable. However, to use *Cola cordifolia* fruits appropriately, it would be interesting to dry it so that it can be preserved for a long time. Vitamin C content of *Cola cordifolia* is around 6.45 mg/100g. This value is below the vitamin C content of certain wild African fruits such as *Adansonia digitata* and *Balanites aegyptiaca* with values of 337 and 89.6 mg/100g dry weight respectively [21]. However, the low vitamin C content of *Cola cordifolia* fruit is not negligible. Vitamin C, also known as ascorbic acid, is a water-soluble vitamin that is essential for the body to function properly. It is a powerful antioxidant found only in fruit and vegetables, whose benefits include boosting the immune system, collagen formation, iron absorption and cardiovascular protection [21]. The amount of Vitamin C and soluble dry extract allow *Cola cordifolia* to be considered excellent sources of bio-elements.

Table 1. Physico-chemical parameters of *Cola cordifolia* fruit pulp

Parameters	Values	Methods
pH	5.74±0.08	Glass Electrode pH Meter [9]
Moisture (%)	72.68±0.64	Dehydration method [9]
Dry matter (%)	27.32±0.64	Dehydration method [9]
Vitamin C content (mg/100 g)	6.40±1.65	Titrimetry dosage method [9]
Soluble dry extract (°B)	25±0.00	R refractometer method

3.2. Isolation and biochemical characterisation of yeasts from *Cola cordifolia* fruits

Based on the macroscopic and microscopic observations of the colonies obtained, 56 presumptive yeasts were isolated from the pulp of *Cola cordifolia*. While awaiting molecular characterisation to identify the genus and species of the yeast isolated, the isolates were purified and then coded so that the first letter corresponded to the initial of the yeast name (Yeast) followed by the initial of the name of the fruit (*Cola cordifolia*) and finally the number assigned to the strain (YC1 to YC56). Several studies revealed that yeasts are widely distributed in various plants and localised especially on fruits and pulp. These studies

have enabled to isolate yeast strains from five different fruit samples of pineapple (*Ananas comosus*), watermelon (*Citrullus vulgaris*), mango (*Magnifera indica*), banana (*Musa acuminata*) and orange (*Citrus sinesis*) [22, 23]. Yeast isolates were identified representing three genera: *Candida*, *Saccharomyces* and *Kloeckera* [22, 23]. Isolation of the yeasts in our study could be explained by factors such as the favourable pH (acid pH), water activity and high sugar content in the fruits [20]. The results obtained in the current study shows that *Cola cordifolia* fruits are also a potential niche for the isolation of yeasts, among other sources.

3.3. Screening for ethanol production of yeasts isolated by *Cola cordifolia* fruits

Yeast isolates were tested for their ability to produce CO₂. On the basis of their fermentation capacity, these 56 yeast isolates were classified into three (3) groups according to the volume of CO₂ produced (Table 2). In fact, the ability of a strain to present better technological and functional performance is a key property in its selection as a potential starter [24]. Thus, among the 56 yeasts analysed for their fermentation capacity, 16 strains showed a high fermentation capacity with a CO₂ volume greater than 4 cm³. These results are similar to those of Koffi et al [25], who showed that among 743 yeasts isolated from cocoa fermentation in Côte d'Ivoire, 113 isolates were selected for their high fermentation capacity with a CO₂ volume greater than 4 cm³. These yeast strains are able to produce large quantities of ethanol because, during alcoholic fermentation, the quantity of carbon dioxide (CO₂) corresponds to the quantity of ethanol produced [14].

Table 2. CO₂ production by yeasts isolated from *Cola cordifolia* pulp

Fermentative capacity	Volume of CO ₂	Number of isolates
High level	[4 – 6 cm ³]	16
Middle level	[1 – 4 cm ³]	05
Low level	[0 – 1 cm ³]	35

3.4. Tolerance to glucose present in the liquid medium on yeast growth

The effect of glucose concentration on the growth of yeast isolates with high fermentation capacity is presented in Table 3. All the isolates showed growth at all the concentrations tested. However, growth varied according to glucose concentration, with a growth peak at 30% glucose. Isolate YC 42 showed the best growth with an optical density of 2.50 at 30% glucose. These results confirm those of Koffi et al [25] who indicate that certain yeast strains are able to resist 50% glucose in the culture medium. Based on the growth capacity of all yeast were considered tolerant on glucose, these isolates would be ideal candidates for ethanol production with high glucose concentrations.

Table 3. Effect of glucose concentration on the growth of yeast isolates with high fermentation capacity

Yeasts strains	Optical density at 600 nm per glucose concentration			
	10 %	20 %	30 %	40 %
YC 03	0.63±0.04 ^c	0.72±0.04 ^b	0.87±0.02 ^a	0.73±0.07 ^b
YC 05	0.67±0.03 ^d	0.75±0.01 ^c	0.96±0.02 ^a	0.86±0.02 ^b

YC 06	0.80±0.02 ^a	0.62±0.02 ^c	0.84±0.03 ^a	0.74±0.03 ^b
YC 08	0.27±0.01 ^d	0.82±0.02 ^b	0.88±0.01 ^a	0.71±0.03 ^c
YC 16	0.21±0.07 ^c	1.00±0.04 ^b	2.11±0.13 ^a	0.95±0.02 ^b
YC 21	0.70±0.09 ^b	0.96±0.05 ^a	1.05±0.07 ^a	0.58±0.01 ^b
YC 28	0.56±0.04 ^d	0.88±0.01 ^b	1.00±0.03 ^a	0.62±0.01 ^c
YC 40	0.66±0.03 ^d	0.76±0.04 ^b	0.98±0.01 ^a	0.69±0.02 ^c
YC 41	0.78±0.02 ^c	1.62±0.10 ^b	2.23±0.06 ^a	0.56±0.04 ^d
YC 42	1.74±0.04 ^c	2.20±0.02 ^b	2.51±0.07 ^a	0.85±0.01 ^d
YC 46	0.56±0.01 ^d	0.74±0.03 ^c	2.14±0.03 ^a	0.95±0.06 ^b
YC 49	0.56±0.02 ^d	1.27±0.08 ^b	2.11±0.04 ^a	0.94±0.01 ^c
YC 51	0.54±0.03 ^c	0.98±0.01 ^a	1.00±0.02 ^a	0.63±0.03 ^b
YC 53	0.85±0.03 ^b	0.64±0.04 ^c	1.39±0.08 ^a	0.58±0.03 ^d
YC 54	0.25±0.01 ^d	0.64±0.03 ^b	0.77±0.03 ^a	0.47±0.01 ^c
YC 56	0.75±0.01 ^b	0.62±0.03 ^c	1.08±0.00 ^a	0.55±0.04 ^d

Data are represented as means±SEM (n=3). Mean with different letters in the same line are statistically different (p<0.05) according to Duncan's test

3.5. Tolerance to ethanol present in the liquid medium on yeast growth

Ethanol produced can be a limiting parameter for yeast growth during alcoholic fermentation. Table 4 shows the effect of ethanol concentration on the growth of yeast isolates with high fermentation capacity. This study shows that the growth of yeast isolates varies according to the ethanol concentration in the medium. From 0 to 10% ethanol in the culture medium, good growth was observed in the various isolates, with a growth peak at 5%. Above 10% ethanol, the growth of most of the yeast isolates is very low and drops to almost zero, with the exception of five isolates that grew relatively well when the medium was supplemented with 14% ethanol. Our work is in agreement with those of Pilap et al. [26], who showed that yeast strains isolated from acidic tropical fruits can tolerate ethanol concentrations in the range of 4-8% (v/v), although their cell viability was decreased upon an increased ethanol concentration. In fact, when the quantity of ethanol in the medium increases, metabolic activity is affected. The result is a drop in growth rate, cell viability and ethanol production capacity [27]. Isolates YC16, YC21, YC42, YC46 and YC49 showed the best growth profiles under different stress conditions (glucose and ethanol). The growth of these yeast isolates under stress conditions confirms the ability of these yeast strains to be used as starters in many food processes, particularly in the alcoholic fermentation of fruit.

Table 4. Effect of ethanol concentration on the growth of yeast isolates with high fermentation capacity

Yeasts strains	Optical density at 600 nm per ethanol concentration					
	0%	5%	8%	10%	12%	14%
YC 03	0.44±0.01 ^b	0.82±0.03 ^a	0.36±0.03 ^c	0.16±0.02 ^d	0.02±0.00 ^e	0.00±0.00 ^e
YC 05	0.53±0.05 ^b	0.63±0.04 ^a	0.15±0.03 ^c	0.14±0.01 ^c	0.00±0.00 ^d	0.00±0.00 ^d
YC 06	0.48±0.03 ^b	0.63±0.03 ^a	0.26±0.04 ^c	0.14±0.02 ^d	0.12±0.01 ^d	0.03±0.00 ^e
YC 08	0.58±0.00 ^b	0.75±0.04 ^a	0.13±0.02 ^c	0.16±0.02 ^c	0.00±0.00 ^d	0.00±0.00 ^d
YC 16	0.40±0.01 ^c	0.66±0.00 ^a	0.57±0.01 ^b	0.36±0.01 ^d	0.15±0.01 ^f	0.17±0.01 ^e
YC 21	0.46±0.03 ^b	1.01±0.02 ^a	0.18±0.03 ^c	0.16±0.00 ^{cd}	0.14±0.00 ^{de}	0.11±0.00 ^e
YC 28	0.84±0.03 ^b	0.85±0.02 ^a	0.15±0.01 ^c	0.15±0.04 ^c	0.11±0.01 ^c	0.00±0.00 ^d
YC 40	0.43±0.03 ^b	0.87±0.03 ^a	0.35±0.03 ^c	0.17±0.02 ^d	0.11±0.01 ^e	0.00±0.00 ^f
YC 41	0.54±0.03 ^b	0.73±0.03 ^a	0.34±0.02 ^c	0.17±0.00 ^d	0.16±0.02 ^d	0.04±0.01 ^e
YC 42	0.82±0.00 ^b	1.54±0.38 ^a	0.15±0.02 ^c	0.12±0.02 ^c	0.06±0.00 ^c	0.10±0.00 ^c
YC 46	0.77±0.00 ^b	1.07±0.03 ^a	0.15±0.00 ^c	0.15±0.02 ^c	0.11±0.01 ^d	0.11±0.00 ^d
YC 49	1.44±0.04 ^b	1.54±0.02 ^a	0.36±0.03 ^c	0.15±0.02 ^d	0.09±0.01 ^d	0.14±0.02 ^e
YC 51	0.45±0.03 ^b	0.85±0.02 ^a	0.16±0.00 ^c	0.18±0.02 ^c	0.05±0.00 ^d	0.01±0.00 ^e
YC 53	0.66±0.04 ^a	0.55±0.02 ^b	0.17±0.00 ^c	0.13±0.01 ^d	0.10±0.01 ^e	0.00±0.00 ^f
YC 54	0.32±0.02 ^c	1.94±0.04 ^a	0.81±0.08 ^b	0.84±0.02 ^b	0.17±0.00 ^d	0.00±0.00 ^e
YC 56	0.98±0.05 ^a	0.27±0.02 ^b	0.18±0.00 ^c	0.12±0.02 ^d	0.09±0.01 ^d	0.00±0.00 ^e

Data are represented as means±SEM (n=3). Mean with different letters in the same line are statistically different (p<0.05) according to Duncan's test

4. CONCLUSION

Physico-chemical analyses of the *Cola cordifolia* fruit showed that it has an acid pH with a high moisture content, a vitamin C content of around 6.40 mg/100g and a high Brix degree of 25 °B. Microbiological analyses revealed fifty-six (56) pure isolates. A study of the technological properties allowed us to select five (5) isolates named YC16, YC21, YC42, YC46 and YC49 which showed the best performance. These isolates showed improved growth in the face of various stress factors, including glucose and ethanol. These five (5) isolates could therefore be used as starters in biotechnological applications.

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