Journal of Food and Nutrition Research, 2024, Vol. 12, No. 11, 502-507
Available online at http://pubs.sciepub.com/jfnr/12/11/5
Published by Science and Education Publishing
DOI:10.12691/jfnr-12-11-5

Selection of Potential Cashew Apple Yeast Starters from the Foro Sub-Prefecture in Northern Côte d'Ivoire

Soumahoro Souleymane[1,*], Kouame Maimouna Liliane[1], Anon Attoh Hyacinthe[1], Yeo Sibirina Abdoulaye[1], Toure Abdoulaye[2], Sessou Philippe[3], Soro Yadé René[4]

[1]Laboratory of Biochemistry, Microbiology and Valorization of Agricutural Resources, Agropastoral Management Institute, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire
[2]Laboratory of Biotechnology and Valorization of Agroresources and Natural Substances, Training and Research Unit of Biological Sciences, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire
[3]Laboratory for Applied Biology Research, Polytechnic School of Abomey-Calavi, Cotonou, Benin
[4]Laboratory of Biotechnology, Faculty of Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire
*Corresponding author: souleysoum@yahoo.fr

Received October 15, 2024; Revised November 17, 2024; Accepted November 24, 2024

Abstract The aim of this study was to add value to cashew apples from northern Côte d'Ivoire. Yeasts were isolated from cashew apples and screened to select potential starters. Following screening, the best strains underwent biochemical identification by MALDI TOF mass spectrometry to determine the species involved. The results enabled us to select three (3) strains with the best properties compared with the other strains tested. These three strains belong to the Saccharomyces cerevisiae species, according to their MALDI-TOF MS profiles. These highly interesting technological properties point to the potential use of these three strains as starters in biotechnological applications.

Keywords: *cashew apple, Saccharomyces cerevisiae, Screening, starter, yeast*

Cite This Article: Soumahoro Souleymane, Kouame Maimouna Liliane, Anon Attoh Hyacinthe, Yeo Sibirina Abdoulaye, Toure Abdoulaye, Sessou Philippe, and Soro Yadé René, "Selection of Potential Cashew Apple Yeast Starters from the Foro Sub-Prefecture in Northern Côte d'Ivoire." *Journal of Food and Nutrition Research*, vol. 12, no. 11 (2024): 502-507. doi: 10.12691/jfnr-12-11-5.

1. Introduction

The cashew tree (Anacardiumouest L.) has two fruit parts: the nut, which is the seed contained in a hard shell, and the cashew apple, a fleshy pseudo-fruit to which the nut is attached. Cashew nuts are traded internationally. Indeed, it is one of the world's most important agro-industrial products. Global cashew nut production is estimated at over 3.7 million tonnes, with Côte d'Ivoire, the world leader in the production [1]. In Côte d'Ivoire, the surface area of cashew orchards is estimated at over 1,400,000 ha belonging to more than 420,000 producers, with production rising from 702,000 tonnes of raw cashew nuts in 2015 to 1,024,000 tonnes in 2022 [2]. The cashew apple is 9 to 10 times the weight of the nut, corresponding to over 6 million tons of cashew apples. Almost all of this production is lost at the point of harvest because it is not used industrially in Côte d'Ivoire due to its astringency and certain taboos [3]. Abandoning apples in the fields is a source of environmental pollution. Cashew apples are very rich in vitamin C and fermentable sugars [4,5]. This makes it a suitable substrate for yeast. Thus, the search for new yeast strains from natural sources such as cashew apple could be of interest to the food industry. These new strains could offer interesting properties, such as improved tolerance to environmental stresses or enhanced production of compounds of interest to the food industry [6]. Other studies have shown that yeasts can be used to produce fermented beverages with unique flavor profiles [7,8]. The aim of this study is to characterize cashew apple yeasts with a view to using them for biotechnological applications.

2. Materials and Methods

2.1. Material

The biological material was yellow cashew apples harvested at maturity (Figure 1) from a farmer's field at Foro in the sub-prefecture of Tioroniaradougou (Korhogo Department, Côte d'Ivoire). The apples were taken to the laboratory of the Peleforo GON COULIBALY University in KORHOGO for microbiological analysis.

Figure 1. Ripe cashews

2.2. Methods

2.2.1. Isolation and Identification of Yeasts

Twenty-five (25) grams of cashew apples are diluted in 225 ml of peptone salt solution (0.1% (w/v) bactopeptone and 0.85% (w/v) NaCl). The solution thus prepared constitutes the stock solution, which undergoes successive decimal dilutions (10^{-1} to 10^{-4}) with tryptone salt solution. A volume of 100 µ L of each dilution has been streaked onto MYGP agar (3 g/L yeast extract, 3 g/L malt extract, 5 g/L bactopeptone and 10 g/L glucose) containing 100 mg/L chloramphenicol (Sigma). After inoculation, Petri dishes have incubated and yeast strains have morphologically been identified after 3 days of incubation at 30°C, then yeast cells have been observed fresh under a precision optical microscope (Zeiss Microlmaging GmbH 37081, Germany) at objective ×100. Presumptive yeast isolates were stored in cryotubes containing MYGP broth supplemented with 20% glycerol at -20°C for subsequent testing [9].

2.2.2. Yeast Screening

Depending on the test to perform, strains are grown overnight at 25 ◦C either on MYGP agar or in MYGP broth and then the cultures are used to inoculate either MYGP broth or specific media. For the latter, each strain is harvested by centrifugation (5000 rpm for 10 min), washed once in NaCl 0.9% (w/v) solution, re-suspended to Optical Density (OD) 600 of 1.0 in the same solution [10]. Subsequently, each strain has been spotted (5 µL) in duplicate onto specific media.

2.2.2.1. Screening of High Fermentative-capacity Yeasts

The fermentative capacity of yeast strains isolated from cashew apples has been studied according to the method of [11] with a slight modification. From pre-culture of 24 hours, pure yeasts culture has been suspended in saline tryptone to get an optical density of 0.7 at 600 nm and 100 µL of this suspension have been used to inoculate 10 mL of YPG medium containing a Durham tube into essay tube. Then, the culture has been incubated at 30°C for 6 days, without agitation. Fermentative capacity has also been determined by measuring the gas production in Durham test. Yeasts usually oxidize sugars into ethanol with production of gas [12]. This volume of gas and ethanol produced is related to the fermentative strength of strain [13].

2.2.2.2. Catalase Activity

The method described by [14] was used to demonstrate the catalase activity of yeast isolates. Yeast biomass, sampled with a 1 µl sterile loop, was added to a drop of 3% (v/v) H_2O_2. The development of bubbles has indicated positive activity.

2.2.2.3. Acetic Acid Production

A loopful (1 µL) of biomass of each strain has been streaked onto Chalk agar (yeast extract 3 g/L, glucose 10 g/L, calcium carbonate 3 g/L, agar 15 g/L) plates and has incubated for 7 d at 25°C [15]. The presence and extent of a clear halo around the yeast biomass has indicated the rate of acetic acid production.

2.2.2.4. Protease Activity

The method described by [16] was used to identify the protease activity of yeast isolates. One hundred (100) ml of MYGP medium (3 g/L malt extract, 3 g/L yeast extract, 5 g/L bacteriological peptone, 10 g/L glucose, 5 g/L NaCl and 20 g/L agar) were prepared and sterilized for 15 min in an autoclave at 121°C. Separately, 100 ml of skimmed milk solution (10% w/v) were prepared and autoclaved at 121°C. The two media were mixed and poured into Petri dishes. Isolates were then spot-inoculated and incubated at 30°C in the oven for 48 hours. The presence of a clear zone around the colony indicates the presence of protease activity.

2.2.2.5. H_2S production

To assess hydrogen sulphide production, 10 µL of each strain from the previously prepared YPD liquid were inoculated on the BIGGY medium and kept for 2–3 days at 24 C, respectively. Visual scale was used as a function of the increasing level of H_2S produced [17,18].

2.2.2.6. Growth at Various Temperatures and pH

Yeast strains have been grown in standard liquid medium containing 0.05% yeast extract; 0.3% casein peptone; 1% glucose at pH 5.6. To assess the influence of temperature on the growth of yeast isolates, 10 ml of standard liquid medium contained in a test tube has been inoculated with 100 µL of yeast pre-culture, OD600 = 0.7. The cultures have then been incubated for 72 h at temperatures ranging from 30 to 50°C. The influence of pH variations on the growth of yeast isolates has been analyzed in the same medium at different pH values (2.5; 4; 5 and 7) and has been incubated at 30°C. Yeast isolate growth has been determined by measuring the turbidity of the culture medium at 600 nm using a spectrophotometer [10].

2.2.2.7. Determination of Ethanol by Distillation

Ethanol assay was carried out at the end of fermentation. Fermented molasse was distilled to ethanol, using the powerful Quickfit/FC3/13 column distiller, 85 cm long and 4.45 cm in diameter (Fischer Scientific, Sweden) A temperature of 79°C was maintained at the top of the column until all the alcohol in the fermented juice was evaporated and condensed. Ethanol content was determined using an alcoholmeter (Biobase, china). Three independent experiments were carried out.

2.2.2.8. Identification of the Yeast Isolates

The yeast cultures isolated from the cashew apple extract were prepared as per the method developed by [19]. Direct colony transfer - formic acid method was adopted for preparing the isolated yeast cultures in feeding the MALDI-TOF MS. Young 24 hours grown east isolates (CAP 1 to CAP 9) were smeared separately on a polished steel MSP 96 target plate using a tooth pick on which 1 µL of 70% formic acid was added, and thin smear was prepared allowed for air drying. Followed by it, saturated-cyano-4-hydroxycinnamic acid (HCCA) matrix (1 µL) prepared using 50% acetonitrile and 2.5% trifluoroacetic acids solution was overlaid onto the air-dried yeast isolates on the target plate at room temperature. The target plate was placed into the plating chamber of the MALDI-TOF MS and shuttered for performing the identification of the yeasts.

2.2.3. Statistical Analysis

All measurements have been performed in triplicate. Statistical analyzes of the data have been performed through Statistica version 7.1 software. Means have been compared through Tuckey's HSD test with a significance level of only 5% ($p < 0.050$).

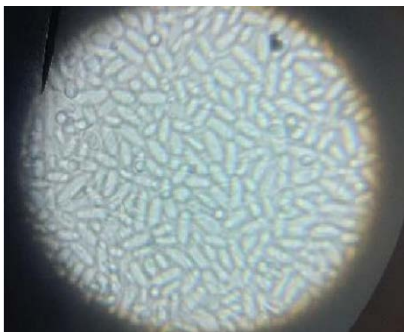
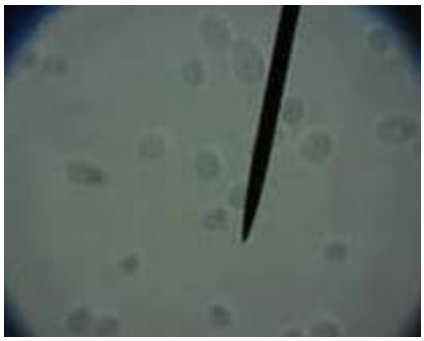
3. Results and Discussion

3.1. Results

3.1.1. Morphology of Yeast Involving in Fermentation of Cashew Apples

forty-one (41) yeast isolates have been isolated from cashew apples. Table 1 shows the morphology, color, and texture of the colonies of the different strains grown on MYGP agar.

Table 1. Colony characteristics of yeast strains grown on MYGP agar

Strains	Colony Morphology, Color, and Texture	Optical microscopy of representative strains
YAN8, YAN9, YAN11, YAN20, YAN31, YAN32, YAN33, YAN34, YAN37, YAN38 and YAN40	Convex, White, Matt/ opaque	
YAN1, YAN2, YAN3, YAN4, YAN5, YAN6, YAN7, YAN10, YAN12, YAN13, YAN14, YAN15, YAN16, YAN17, YAN18, YAN19, YAN21, YAN22, YAN23, YAN24, YAN25, YAN26, YAN27, YAN28, YAN29, YAN30, YAN35, YAN36, YAN39 and YAN41	Convex, Cream colored, Smooth/Glossy	

3.1.2. Fermentative Capacity of Yeast Strains

Among the 41 yeast isolates tested for their fermentative capacity, only nine (9) isolates or 21.95% showed a high fermentative capacity with a CO2 volume equal to 7.5 cm3. While ten (10) isolates or 24.39% produced CO2 moderately with values ranging from 1 to 4 cm3 and twenty-two (22) isolates or 53.66% produced CO2 weakly with values from 0 to 1 cm3 (Table 2). Highly fermentable isolates (9) were selected to study their biochemical properties.

Table 2. CO2 production of yeast isolate from cashew apples

Volume of CO2 (cm3)	Number of isolates	Percentage (%)	Fermentative capacity
7.5 cm^3	9	21.95	High level
[1 – 4 cm^3]	10	24.39	Middle level
[0 – 1 cm^3]	22	53.66	Low level

3.1.3. Biochemical properties of High Fermentative Capacity Yeasts Involving Cashew Apples Fermentation

All the yeasts isolates involving cashew apples fermentation tested have the catalase and H_2S production capacity. But the level of production is different from one yeast isolate to another. About protease activity, seven (7) have produced with high activity for three (3) isolates (YAN 2 YAN 36, YAN 41). While only tree (3) yeasts isolates (YAN 9, YAN 20, YAN 39) are able to produce acetic acid (Table 3).

Table 3. Biochemical properties of yeast isolates involving cashew apples fermentation

Strains	Acetic Acid Production [a]	Catalase activity [b]	Protease Activity [c]	H_2S Production [d]
YAN2	-	+++	+++	+
YAN9	+	++	-	++
YAN11	-	++	+	+
YAN20	+	++	++	++
YAN35	-	++	+	+
YAN36	-	+++	+++	+
YAN39	+	++	++	+
YAN40	-	++	-	++
YAN41	-	+++	+++	+

[a] Halo: -, none; +, low. [b] Development of bubbles: +, low; ++, medium, +++, high. [c] Activity: −, no halo; +, small diameter; ++, medium diameter; +++, large diameter. [d] Biomass color: ++, black brown; +, brown.

3.1.4. Growth Capacity of the High Fermentative Yeasts Isolate Involving Cashew Apples Fermentation at Different pH

The results show a significant difference in isolate growth at different pH levels (2.5, 4 and 7). However, at pH 5, isolate growth showed no significant difference (Table 4). All isolates tested showed good growth at pH 4. At this pH, isolate YAN36 had the best growth (0.966) and isolate YAN had the lowest growth (0.691)

Table 4. Impact of pH on the growth of selected yeast isolates

Strains	pH 2.5	pH 4	pH 5	pH7
YAN2	0.327 ± 0.01d	0.917 ± 0.02b	0.843 ± 0.02a	0.772 ± 0.01a
YAN9	0.564 ± 0.01ab	0.864 ± 0.03c	0.745 ± 0.01b	0.672 ± 0.01b
YAN36	0.510 ± 0.05bc	0.966 ± 0.01a	0.721 ± 0.05b	0.644 ± 0.04b
YAN41	0.467 ± 0.00c	0.880 ± 0.00c	0.743 ± 0.02b	0.658 ± 0.02b
YAN20	0.592 ± 0.01a	0.785 ± 0.01d	0.628 ± 0.00c	0.610 ± 0.00bc
YAN11	0.454 ± 0.02c	0.819 ± 0.01d	0.709 ± 0.01b	0.622 ± 0.01bc
YAN35	0.082 ± 0.00f	0.697 ± 0.01e	0.750 ± 0.00b	0.540 ± 0.02cd
YAN39	0.157 ± 0.00e	0.693 ± 0.00e	0.604 ± 0.05c	0.548 ± 0.07cd
YAN40	0.341 ± 0.01d	0.691 ± 0.01e	0.561 ± 0.02c	0.510 ± 0.01d
Pr > F	0.000	0.000	0.000	0.000
Significant	Yes	Yes	Yes	Yes

In the same column, means with different letters (a, b, c, d, e, f) are significantly different (Turkey's HSD test, $p < 0.05$).

3.1.5. Growth Capacity of the High Fermentative Yeasts Isolate Involving Cashew Apples Fermentation at Different Temperature

Table 5. Impact of temperature on the growth of selected yeast isolates

Strains	30°C	35°C	40°C	45°C
YAN2	0.751 ± 0.02b	0.944 ± 0.01a	0.543 ± 0.02b	0.227 ± 0.01a
YAN41	0.865 ± 0.01a	0.927 ± 0.02a	0.416 ± 0.02cd	0.145 ± 0.02c
YAN36	0.740 ± 0.01b	0.925 ± 0.04a	0.657 ± 0.03a	0.178 ± 0.03b
YAN35	0.749 ± 0.04b	0.830 ± 0.01b	0.354 ± 0.04de	0.000 ± 0.00d
YAN40	0.742 ± 0.03b	0.514 ± 0.02d	0.461 ± 0.01c	0.000 ± 0.00d
YAN20	0.761 ± 0.00b	0.443 ± 0.03e	0.362 ± 0.03de	0.000 ± 0.00d
YAN39	0.764 ± 0.03b	0.668 ± 0.03c	0.313 ± 0.03e	0.000 ± 0.00d
YAN9	0.571 ± 0.03c	0.827 ± 0.02b	0.412 ± 0.01cd	0.000 ± 0.00d
YAN11	0.726 ± 0.01b	0.801 ± 0.00b	0.326 ± 0.01e	0.000 ± 0.00d
Pr > F	0.000	0.000	0.000	0.000
Significant	Yes	Yes	Yes	Yes

In the same column, means with different letters (a, b, c, d, e) are significantly different (Turkey's HSD test, $p < 0.05$).

Yeast isolates showed varying growth rates at different temperatures. Statistical analyzes reveal a significant difference in the growth of isolates. The result of the growth of the 9 best yeasts isolates with a high fermentation power at different temperature give two. The first group consists of yeast isolates (YAN40, YAN20 and YAN39) with shows the best growth at 30° C. The second group consists of yeast isolates (YAN2, YAN41, YAN36, YAN35, YAN9 and YAN11) which shows good growth at 35°C. Three isolates, YAN2, YAN41 and YAN36, grew at 45°C.

3.1.6. Identification and ethanol Production of Selected Yeast Isolates

Isolates YAN 2, YAN 36, and YAN 41 showed the best characteristics (high CO_2 production, no acetic acid production, high catalytic activity, high protease activity, low H_2S production, and good resistance to temperature and pH variation) for fermentation. These isolates have been selected for ethanol production and identified. The three selected isolates were all identified as *Saccharomyces cerevisiae* strains on the basis of their MALDI-TOF MS profiles. The ethanol production of selected isolates YAN2, YAN36, YAN41 was 6.39±0.12%, 6.44±0.09% and 6.42±0.09% respectively (Figure 2). Statistical analysis showed that there was no significant difference between the ethanol production of the three yeast strains.

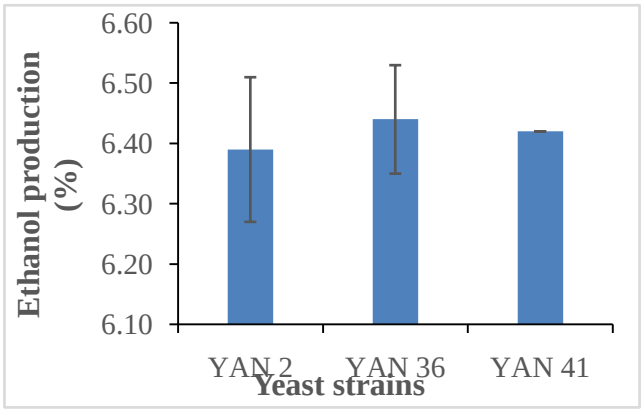


Figure 2. Ethanol production by selected yeast strains

4. Discussion

The study of technological properties is essential in the search for strains with exceptional properties [20]. A study of the fermentative power of yeasts isolated from cashew apples revealed that 21.95% of isolates had a high fermentative power with a volume of 7.5 cm3 of CO2 produced. These results differ from those of [10] who isolated yeasts with high fermentation capacity from *Cola Cordifolia* pulp, but with CO2 volumes of between 4 and 6 cm3.

As far as the study of pH is concerned, its variation had a significant influence on the growth of yeast isolates with high fermentation capacity. Indeed, the optimal pH for growth of the yeast strains isolated was pH 4. These results differ from those of [21] whose strains perform best at pH 3. Similarly, temperature variation influenced the growth of yeast isolates. Temperatures above 40°C had a negative influence on the growth of yeast isolates with high fermentation capacity. Indeed, temperature is the main parameter influencing the activity and physiology of

microorganisms [22]. It affects growth rate, CO2 production rate, ethanol production and cell viability [23].

The biochemical properties important for optimal fermentation were evaluated. Three isolates (YAN2, YAN36, YAN41) showed interesting biochemical properties, namely high catalase activity, protease production and inability to produce acetic acid. Indeed, high catalase activity is a property that enables strains to cope with oxidative stress during fermentation [24]. The production of proteases by yeast strains is essential and plays a significant role in protein degradation. During fermentation, proteases released by yeast play a role in the degradation of proteins present in the fermentation medium [25]. Protease is also one of the yeast enzymes of interest, like esterases, glycosidases and cellulases, capable of hydrolyzing structural components [26]. The results are similar to those of [21] who showed a protease property of a Saccharomyces cerevisiae strain involved in cocoa fermentation. Moreover, acetic acid production by yeasts during alcoholic fermentation is not desirable [27]. The inability of selected isolates to produce acetic acid is therefore of great interest. Similarly, the production of hydrogen sulfite by strains is unfavorable to fermentation. Thus, yeast strains producing little or no H2S are particularly important, as there is no sulfite formation during fermentation. The difference in H2S production by yeast isolates obtained in this study has been confirmed by several authors [28,29]. The three selected isolates with the best characteristics are from the Saccharomyces cerevisiae species. However, [30] Gayathrya et al (2023) identified by MALDI-TOF MS, four yeast strains namely *Brettannomyces bruxellensis*, *Candida krusei*, *Candida tropicalis*, *Pichia norvegensis* that colonize cashew apple in India. This difference could be explained by the geographical location, variety and degree of ripeness of the apples.

With regard to ethanol production, the ethanol production of the Saccharomyces cerevisiae strains was 6.39 ± 0.12% for strain YAN2, 6.44 ± 0.09% for strain YAN36 and 6.42 ± 0.09% for strain YAN41. The ethanol yields of the three strains were statistically identical and close to those of [20] whose yields ranged from 5.39 to 6.81% for *Pichia manshurica*, *Hanseniaspora uvarum* and *Candida parapsilosis* species.

5. Conclusion

In all, 41 yeast strains were isolated from cashew apples, nine (9) of which were highly fermentative. High-fermenting isolates showed good growth at different pH levels and temperatures between 30 and 40°C. The biochemical properties of high-fermenting isolates vary depending on the isolate. Three isolates showed the best technological properties and were identified as Saccharomyces cerevisiae strains. These strains, with their interesting technological properties, hold great promise for biotechnological applications.

References

[1] FAO (2021). Food and Agriculture Organization. FAOSTAT Statistical database. Rome.

[2] FIRCA, Annual Report 2021, FIRCA and the cashew nut industry, https:// firca.ci/ blog/ le-firca-et-la-filiere-anacarde/. Accessed 09/29/2024.

[3] RONGEAD (2013). Knowing and understanding the international cashew market. Consulted on 10/10/2024. http:// www.rongead.org/ IMG/pdf/ Guide_RONGEAD.

[4] Assunção RB and Mercadante AZ (2003). "Carotenoids and ascorbic acid compositionfrom commercial products of cashew apple (Anacardiumouest L.)." *Journal of Food Composition and Analysis*, 16(6), 647-65.

[5] Soro D. (2012). Coupling of membrane processes for the clarification and concentration of cashew apple juice: performance and impacts on product quality. Doctoral thesis: Process engineering, University of Montpellier SupAgro, 156 p.

[6] Suh SO and Blackwell. M. (2005). The beetle gut as a habitat for new species of yeasts. In: F; E. Vega and M. Blackwell (ed): *Insect-fungal associations: ecology and evolution.* Oxford University Press. Pp 446-461.

[7] Romano P., Fiore C., Paraggio M., Caruso M. and Capece A. (2003). Function of yeast species and strains in wine flavor. International Journal of Food Microbiology, 86, 169–180.

[8] Coulibaly WH, Bouatenin KMJP, Kouamé AK, Camara F., Tra Bi YC, Toka DM, Joel S., Cot M. and Djè KM (2020). Use of non-Saccharomyces yeast strains as starter cultures to enhance fermented mango juice production, *Scientific African*, 6, 1-9.

[9] Samagaci L., Ouattara HG, Goualié, BG and Niamke, SL (2014). Growth capacity of yeasts potential starter strains under cocoa fermentation stress conditions in Ivory Coast. Emirates *Journal of Food & Agriculture*, 26(10), 861-870.

[10] Soumahoro S., Kouamé ML, Samagaci L., Soro YR and Lemaire M. (2024). Screening of Potential Yeast Starters from Cola cordifolia Fruit Pulp. *American Journal of Food and Nutrition*, 12(5), 127-133.

[11] Dung NTP and Phong XH (2013). Screening Thermo- and Ethanol Tolerant Bacteria for Ethanol Fermentation, American *Journal of Microbiological Research*, 1(2), 25-31.

[12] Hesclot H. and Vladescu B., Yeast in the food industries (1994).

[13] Dung NTP, Pornthap T. and Huynh XP (2012). Screening Useful Isolated Yeasts for Ethanol Fermentation at High Temperature, *International Journal of Applied Science & Technology*, 4, 65-71.

[14] Bautista GJ, Rodríguez-Gómez F., Barrio E., Querol A., Garrido-Fernandez A. and Arroyo-López FN (2011). Exploring the yeast biodiversity of green table olive industrial fermentations for technological applications. *International Journal of Food Microbiology*, 147, 89–96.

[15] Lemaresquier H., Gainvors A., Lequart C., Charlemagne B., Frezier V. and Belarbi AB (1995). Selection of wine yeasts with clarifying activity: the different techniques used to characterize yeasts, interest in selecting a productive yeast. *Rev. Oenol*, 154, 23–29.

[16] Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I. and Ciani, M. (2011). Selected non-Saccharomyces wine yeasts in controlled multistarter fermentations with Saccharomyces cerevisiae. *Food Microbiology*, 28(5), 873–882.

[17] Mendes-Ferreira, A., Mendes-Faia, A. and Leão, C. (2002). Survey of Hydrogen Sulphide Production by Wine Yeasts. *Journal of Food Protection*, 65(6), 1033–1037.

[18] Hamdouche Y., Guehi T., Durand N., Kedjebo KB, Montet D. and Meile JC (2015). Dynamics of microbial ecology during cocoa fermentation and drying towards the identification of molecular markers. *Food Control*, 48, 117-122.

[19] Schulthess B., Brodner K., Bloemberg GV Zbinden R., Böttger E. C and Hombach M. 2013. Identification of Gram-positive cocci by use of matrix-assisted laser desorption time of flight mass spectrometry: comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. *Journal of Clinical Microbiology*, 51, 1834-1840.

[20] Holzapfel WH (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. *International journal of Food Microbiology*, 75 (3), 197-212.

[21] Koffi O., Samagaci L., Goualie B. and Niamke S. (2018). Screening of potential yeast starters with high ethanol production for a small-scale cocoa fermentation in Ivory Coast. *Food and Environment Safety*, 7 (2), 113-130.

[22] Watson K. (1987) "Temperature relations." In "The Yeast. Vol. 2: Yeasts and the Environment," Academic Press Inc; 2nd Revised edition, AH Rose and JS Harrison, pp 5-40.

[23] Aldiguier AS, Alfenore S., Cameleyre X., Goma G., Uribelarrea JL, Guillouet SE and Molina-Jouve C. (2004). Synergistic temperature and ethanol effect on Saccharomyces cerevisiae dynamic behavior in ethanol bio-fuel production. *Bioprocess and Biosystems Engineering*, 26(4), 217-222.

[24] Bisson LF, Karpel, JE, Ramakrishnan, V., Joseph L. (2007). Functional Genomics of Wine Yeast Saccharomyces cerevisiae. *Advances in Food and Nutrition Research*, 65-121.

[25] Amiour I. Bendjeddou H. (2022). Yeast-derived proteases produced by fermentation on solid media. Master's thesis, Université Frères Mentouri Constantine I, 91 pages.

[26] Maturano YP, Assaf, LAR, Toro ME, Nally, MC, Vallejo M., De Figueroa LIC, Combina M. and Vazquez F. (2012). Multi-enzyme production by pure and mixed cultures 49 of Saccharomyces and non-Saccharomyces yeasts during wine fermentation. *International Journal of Food Microbiology, 155* (1-2), 43-50.

[27] Lambrechts M. and Pretorius I. (2000). Yeast and its Importance to Wine Aroma - A Review. *South African Journal of Enology and Viticulture*, 21, 97-129.

[28] Barbosa C., Lage P., Esteves M., Chambel L., Mendes-Faia, A. and Mendes-Ferreira, A. (2018). Molecular and phenotypic characterization of *Metschnikowia pulcherrima* strains from Douro wine region. *Fermentation*, 4(1), 1-19.

[29] Garavaglia J., de Cassia de Souza Schneider R., Camargo Mendes SD, Welke JE, Alcaraz-Zini, C., Bastos Caramão, E. and Valente, P. (2015). Evaluation of *Zygosaccharomyces bailii* BCV 08 as a co-starter in wine fermentation for the improvement of ethyl esters production. *Microbiological Research*, 173, 59–65.

[30] Gayathrya G., Maheshwarib P., Artheec R., Asangeethad and Kjothilakshmi. (2023). Identification of spoilage yeasts in cashew apple (Annacardiumouest L.) using MALDI-TOF MS. (2023). *Journal of Agriculture and Ecology*. 17, 34-37.