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Vinegar production trial from cashew apple (*Anacardium occidentale*) using thermo-tolerant Acetobacter strains with high acetic acid yield in non-optimized small scale conditions

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Abstract

This study aims to evaluate whether or not selected acetic bacteria strains could optimally perform fermentation at high temperature with ordinary laboratory materials without adjusting growth temperature. Vinegar production was carried out by controlled successive alcoholic and acetous fermentation in batch mode. Progress of fermentation was monitored by using various biochemical tests. Cashew apple vinegars quality was determined by sensory analysis. The selected acetic acid bacteria strains produced maximum acid after the 2nd day of acetic fermentation. The produced vinegars had an acidity of 5.34±1.07-5.86±0.07 acetic acid g/100mL, productivity 0.74±0.15-0.81±0.01 g/Lh and pH 3.15±0.09- 3.19±0.07 respectively. The end of the fermentation was noticed by a decrease in acidity that occurred after the 3rd day. Sensory evaluation rated the vinegar acceptable in terms of astringency, pungency and acidity when compared to commercial samples. Thus, the use of thermotolerant bacteria to prepare commercial-grade vinegar from cashew apple, commodities without economic value, has potential to scale up to domestic use. .

Keywords: thermotolerant, acetic acid bacteria, vinegar, sensory analysis, cashew apple

Résumé

L'objectif de cette étude est d'évaluer la capacité des souches de bactéries acétiques sélectionnées à croitre de façon optimale dans des conditions expérimentales qui ne nécessitent pas une régulation de la température pendant la phase d'acétification. La production de vinaigre s'est faite par une double fermentation alcoolique et acétique contrôlée en mode discontinu. Le suivi de la fermentation a été fait par des tests biochimiques. L'acceptabilité des vinaigres produits a été déterminée per une analyse sensorielle. Les souches de bactéries acétiques sélectionnées ont permis d'accumuler le maximum d'acide acétique dans des délais records en 72 heures de fermentation. Les vinaigres présentent une acidité de 5,34±1,07-5,86±0,07 acide acétique g/100mL, une productivité de 0,74±0,15-0,81±0,01 g/Lh et pH 3,15±0,09- 3,19±0,07. La fin de l'acétification est indiquée par le phénomène de suroxydation qui se traduit par une baisse en acidité à partir du 3ème jour. Les échantillons de vinaigre ont été jugés acceptables en termes de d'astringence, de notes piquantes et d'acidité comparativement aux échantillons du commerce. De ce fait, l'utilisation de bactéries acétiques thermo tolérantes à forte production d'acide pour la production de vinaigre avec la pomme de cajou, produit sans valeur marchande, a le potentiel d'être exploité à l'échelle artisanale.

Mots-clés: thermotolérant, bactéries acétiques, vinaigre, analyse sensorielle, pomme de cajou.

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Introduction

Cashew (*Anacardium occidentale*) is a tropical evergreen tree grown for the cashew nut industry. The peduncle, or cashew apple is a waste byproduct of the cashew nut harvest (Touré et al., 2015; Das and Arora 2017). It is rich in vitamins, polyphenols, sugars, minerals, aminoacids and dietary fiber (Soro et al., 2017) and can be considered as a functional food. Due to their high moisture (85-90%) and high sugar content (55-65%, dry basis), the cashew apples are prone to rapid microbial spoilage (Sivagurunathan et al., 2010). With a national production of 750 000 t (2016), Côte d'Ivoire is among the world's leading exporters of raw nuts (Soro et al., 2017). The weight of the leftover cashew apple is about 10 times higher than that of the harvested nuts (Attri, 2009), indicating that, almost 7 million tons of underutilized cashew apples. Fermentation, however, can be a viable solution to avoid this loss. This includes alcoholic and acetic, as well as various other types of fermentation. Cashew wine can be produced by alcoholic fermentation of peduncle juice and then submitted to acetic fermentation where alcohol molecules are oxidized into acetic acid molecules by the action of acetic bacteria, resulting in characteristic vinegar taste (Tesfaye et al., 2002).

Vinegar is defined as "the liquid adequate for human consumption, produced from an agricultural raw material that has in composition starch and sugars, by a double fermentation process, alcoholic and acetic, and that contains adequate quantities of acetic acid" (FAO/WHO, 1987). Vinegar is mainly a 4-6% diluted acetic acid solution with the ability to slow microbial growth and contributes to the sensorial properties of foods. It is thus utilized as seasoning or preservative and serves as well as the basis for simple remedies for people and animals (Ho et al., 2017).

Acetic acid is produced both synthetically and biologically by bacterial fermentation. Synthetic production mainly depends on petroleum-derived stocks such as methanol, acetaldehyde, butane or ethylene. The biological way accounts for only about 10 percent of world production (Ragsdale et al., 2008). It remains important for vinegar production, because many of the world food purity laws stipulate that vinegar used in foods must be of biological origin. Acetic acid bacteria (AAB) are the main microorganisms used for that purpose. They belong to the family of Acetobacteraceae, which includes several genera and species. Currently, they are classified into nineteen genera. The main species responsible for vinegar production belong to the genera Acetobacter, Gluconacetobacter, Gluconobacter and Komagataeibacter because of their high capacity to oxidise ethanol to acetic acid and high resistance to acetic acid released into the fermentative medium (Nakano and Fukaya, 2008; Fernández-Pérez et al., 2010). Some genera, namely Acetobacter spp, can further oxidise the produced acetic acid to CO2 and H2O in the absence of ethanol (Gullo et al, 2014). AAB are strictly aerobic microorganisms, Gram-negative or Gram-variable, catalase-positive and oxidase-negative, ellipsoidal to rod-shaped cells that can occur singly, in pairs or chains. They are also mesophilic microorganisms, and their optimum growth temperature is between 25 and 30 °C. The optimum pH for their growth is 5.0–6.5, but they can also grow at lower pH values (Sengun and Karabiyikli, 2011). Earlier processes used for making biological vinegar were the Orleans process (which is also known as the slow process), the quick process (which is also called the generator process), and the submerged culture process (Adams, 1998). The quick process and submerged culture process are used for commercial vinegar production today.

Global warming in these years has brought indoor temperature increase beyond 30 °C even in night time in many countries. That is the serious challenge to not only vinegar fermentation but also other fermentation industries, since they need a huge amount of cooling water to maintain the optimum fermentation temperature. In submerged cultures, a large amount of heat is generated during fermentation and thus cooling costs become rather expensive (Ndoye et al., 2006).

It is with that perspective that vinegar fermentation at higher temperatures has been investigated with isolated acetic acid bacteria thermotolerant strains (Kanchanarach et al., 2010. Konaté et al; 2015). Indeed, strains of acetic acid bacteria capable of growing optimally at high temperature are undoubtedly more suitable for low cost technologies.

In this paper, we assessed the acid production from selected thermotolerant acetic bacteria strains performing in non-optimized and low cost conditions and analyzed the quality of the yielded product.

Materials and Methods

Materials

Cashew apples were collected in the northern area of Côte d'Ivoire, notably from Sinematiali (geographic coordinates 9° 35' North, 5° 23' West) and Ferkessedougou (9° 35' 37" North, 5° 11' 50" West). Three selected acetic bacteria strains namely *Acetobacter pasteurianus*, (BA1) *Acetobacter* sp, (T6HS14) *Acetobacter Okinawensis* (T3G2) were provided by the laboratory of Biotechnology of University Félix Houphouet-Boigny (Abidjan) for acetic acid production. These strains were isolated from palm wine (Konaté et al., 2015) and fermented cocoa beans (Soumahoro et al., 2015). The strains were screened for their abilities to produce acid at high temperature such as 37 to 40°C and were then identified by biochemical typing methods. Commercial baker's yeast Saccharomyces cerevisiae strain was used for alcohol production.

Preparation of the must

The cashew apples were washed in bleached water (2%) for decontamination and thoroughly rinsed with tap water. The fruits were sliced into small pieces and finely mashed using hand held immersion blender (Moulinex, France). The juice was then extracted manually by sieving with a clean muslin cloth. The extracted juice was coarsely clarified in order to reduce the astringency (dryness) linked to the tannins. The raw juice was put in the refrigerator (4°) for 6 h decantation and the clear supernatant is gently recovered. The clear juice was concentrated into syrup at 60°C in water bath (Codex Stan, 2005). Water was physically removed in an amount sufficient to increase the Brix level to a value at least 1.5 times the initial Brix. The syrup was conditioned into sterile bottles. For the purpose of this study, the syrup was diluted to give a must of 10°B total soluble solids when needed. The diluted must was bottled, corked and pasteurized at 65°C for 10 min for further use.

cashew must alcoholic fermentation

Preparation of yeast culture

The yeasts inoculum was prepared by suspending 2 g of the commercial yeast into 20 mL of sterile distilled water (37°C). Then a preferment was prepared by using the yeast suspension to seed 1/10 (150 mL) of the total must volume to be used (1.5 L). The mixture was held at room temperature on a rotary shaker for 24 hours at 150 rpm. The preferment with an OD600nm comprise between 0.8 and 1 was used to inoculate the remaining 9/10 of the must (1350 mL) contained in 2 L Erlenmeyer flask. The bottles were loosely closed with carded cotton. The mixture was incubated at ambient temperature. Alcoholic fermentation was monitored until total soluble solids (TSS), dropped to the lowest constant value. The TSS was measured with a refractometer.

Fixed and volatile acid determination in cashew apple wine

The methods described by AOAC (2007) were used in the determinations of fixed and volatile acid. A quantity of 25 mL of the test sample, placed in a porcelain capsule, was carefully evaporated on a hot plate until the volume had reduced to 5-10 mL. Twenty-five milliliters of hot distilled water were added and the solutions were again evaporated to a final volume of 5-10 mL. The process was repeated two more times after which the residue was cooled and diluted to 50 mL with distilled water. This was titrated with 0.1N NaOH using phenolphthalein as indicator. The fixed acid was expressed as g malic acid/100 mL wine by using the titratable formula. The volatile acid was determined by subtracting the fixed acid value from the titratable acid value. The volatile acid was expressed as g acetic acid/100 mL.

Acetic Acid fermentation

The selected acetic bacteria strains were activated in carbonate broth (yeast extract (0. 5 %), glucose (0. 05 %) and peptone (0. 3%) at room temperature on a rotary shaker at 150 rpm, in tubes containing 20 mL medium.

Successive alcohol and acetous fermentation was carried out following the model set by Konaté et al (2015). Inoculation of acetic acid bacteria in the wine for acetification was done upon completion of alcoholic fermentation, meaning after 24 hours in this study. Five hundred (500) mL vials were used and 150 mL of cashew wine was seeded with 1 mL of bacterial suspension (D0600=0, 7) and incubated at room temperature (28-30°C) on a shaker at 150 rpm. Fermentation was stopped when constant or decreasing acid rates were noticed. Other series of test were conducted, to evaluate pH effect on fermentation rate, by using commercial grade CaCO3 (4g/L) for deacidification of the wine.

pH of the wine was thus adjusted from 3.8 up to 4.5 and submitted to acetous fermentation. The test was repeated three times. The products were characterized using their physicochemical profile.

Chemical analysis

Total and reducing sugars were determined by the 3,5DNS acid method (Bernfeld, 1955; Dubois et al., 1956). A pH meter (HANNA) was used to measure the pH. Total soluble solids, expressed in °Brix, was determined using a refractometer (C2 Comeca SA). Crude proteins were estimated by AOAC (1990) official methods.

The actual alcohol content of the finished wine and vinegar was determined with enzymatic kit using alcohol dehydrogenase (ADH) and NAD as cofactor (Sigma-Aldrich, Darmstadt, Germany). Ethanol concentration was determined by a coupled enzyme reactions, which results in the production of NADH. The NADH level, measured at OD340 nm (UV) was proportional to ethanol concentration present in the solution (Koffi et al., 2018).

Acid production was monitored daily by 5mL sample titration with NaOH 0.1N using phenolphthalein as indicator. The acidity of vinegar expressed in degrees of acetic acid was defined as the mass, in gram, of pure acetic acid in 100 g vinegar. The acid was determined by subtracting the volatile acid value of the wine from the titratable acid value. The volatile acid was expressed as g acetic acid/100 mL.

Polyphenolic compounds were estimated according to Singleton et al., (1999), comparing absorbance to a gallic acid equivalents standard curve. Flavonoid content was estimated by the method previously described by Meda et al. (2005) using quercetin as standard. Tannin compounds were determined by the method of Bainbridge et al. (1996) with tannic acid as standard.

Evaluation of vinegar density and test for solubility

The vinegar density which is the weight per volume, important to control purity of sample, was determined according to the method described by Raji et al. (2012). Hundred milliliters of vinegar was weighed and the density was determined by dividing the weight (grams) by the volume (milliliters). The vinegar solubility in three solvents, namely water, ethanol and acetone was determined.

Sensory evaluation

The vinegars produced from the non-deacidified (natural) cashew apple wine were submitted to sensory evaluation by hedonic and descriptive analysis. The vinegars produced were pasteurized using a water bath at 65°C for 30 min) followed by decantation to discard bacterial cells and sediments. The partially clarified vinegars were bottled and stored at room temperature.

Hedonic test

Hedonic sensory analysis of the cashew wine vinegars was evaluated by a panel of 30 members including students and staff of the laboratory of Agriculture, Biotechnology and Valorization of Biological Resources, from University of Felix Houphouet-Boigny. The untrained panel evaluated the samples on the basis of acidity, taste, aroma, appearance and overall acceptability by Hedonic Rating Test (0 - 10 scale and averaged). The samples were served at an ambient temperature in clear plastic glasses. Potable water was available for rinsing the mouth between testing samples. The results were analyzed using statistical analysis.

Descriptive analysis

A descriptive analysis of flavor attributes was carried by 10 judges. The panelist was asked about the intensity of the flavor attributes, clarity; astringency; pungency; yeast aroma, fruity aroma, vinegar aroma. Panelists marked their perceived sense on an unstructured 10 cm straight line labeled "very weak =0" to "very strong=10" at the left and right end point (ISO 4121, 1987). The results were summarized in a radar figure.

Cider vinegar and chemical vinegar were used as positive and negative controls respectively. Respondents were acquainted with cashew apple juice and wine aroma.

Statistical analysis

Analysis of variance (ANOVA) and significant differences among the means were tested by LSD Fisher at the 95% confidence interval. Standard deviation was also reported. The results were declared significant when $p < 0.05$. For the descriptive statistics, the mean and standard deviations were reported.

RESULTS

Physicochemical characteristics of Cashew apple syrup and wine

The analytical results of cashew apple syrup and wine physicochemical characteristics are presented in Table 1. The level of total sugar in the must decreased from 651. 52±0.71 to 1.74±0.66 mg/100mL in the wine. Reducing sugar concentrations varied from 275. 42±0.70 to 1.30±0.47 and represented almost 99% of consumed sugars. The wine presented 4.85 ± 0.17 % (v/v) of alcohol content. The proteins decreased from 1. 67 in the must to 0.39 g/100 ml in the wine. Titratable and fixed acidity varied during the fermentation period from 0.24±0.02

to 0.73±0.04g malic acid/100 mL and 0.37±0.01 g to 0.79±0.03 g malic acid/100 mL /100 mL respectively. Conversely, pH dropped from 4. 32±0. 08 to 3. 88 ±0. 11 at the end of fermentation. During the fermentation, polyphenols increased from 125. 30±0. 36 to 152. 30±0. 50 mg/100mL. However, the tannins and flavonoids content remained unchanged in wine samples.

Table 1: Chemical analysis of cashew apple juice and wine

Parameters	Juice 10°B	Wine
pH	4.32±0.08	3.88 ±0.11
Alcohol %	-	4.85 ± 0.17
Soluble solids extract (°B)	10	4.11 ± 0.06
Titratable acidity	0.24±0.02*	0.73±0.04*
Fixed acidity	0.22±0.01*	0.61±0.03*
Volatile acidity (g acetic acid/100 mL)	0.02±0.01	0.12±0.01
Proteins g/100 ml	1.67±0.14	0.39±0.11
Total sugars mg/100mL	651.52±0.71	1.74±0.66
Reducing sugars mg/100 mL	275.42±0.70	1.30±0.47
Polyphenols mg/100 mL	125.30±0.36	152.30±0.50
Flavonoids mg/100 mL	11.14±0.40	10.62±0.30
Tannins mg/100 mL	46.9±0.14	35.17±0.53

*Equivalent malic acid g/100 mL

Acetic fermentation

The time course of acid production by the 3 selected of acetic acid bacteria strains in the regular wine (in straight lines) and the deacidified wine (discontinued lines) is shown in Figure 1. It is obvious from this figure that all three strains produced maximum acid after the 2nd day of fermentation meaning 72 hours in the regular cashew wine. The highest yield in acid production was delayed to 168 hours with the deacidified wine for BA1CaCO3 and 192 hours for T3G2 and T6HS14. The end of the fermentation was evidenced by the acidity decrease that occurred after the 3rd (72 h) and 9th day (216 h) in the regular and deacidified wines respectively.

The values corresponding to the highest yields in acid produced by the three strains are presented in Table 2. The maximum acid concentration accumulated with the regular wine was about 5. 86±0.07 % for all the acetic acid bacteria strains (p >0, 05). BA1caco3 and T6HS14caco3 recorded the lowest acid content. Analysis of the acetic acid productivity of the strains revealed the highest values with the regular wine (0.74±0.15 to 0.81±0.01 g/Lh). Concerning pH value, BA1CaCO3, T3G2CaCO3 and T6HS14CaCO3 displayed the highest values around 3.50 whilst, the samples from the regular wine presented values around 3.17. Evaluation of residual soluble solid extracts and alcohol showed no significant difference among the samples (p>0.05).

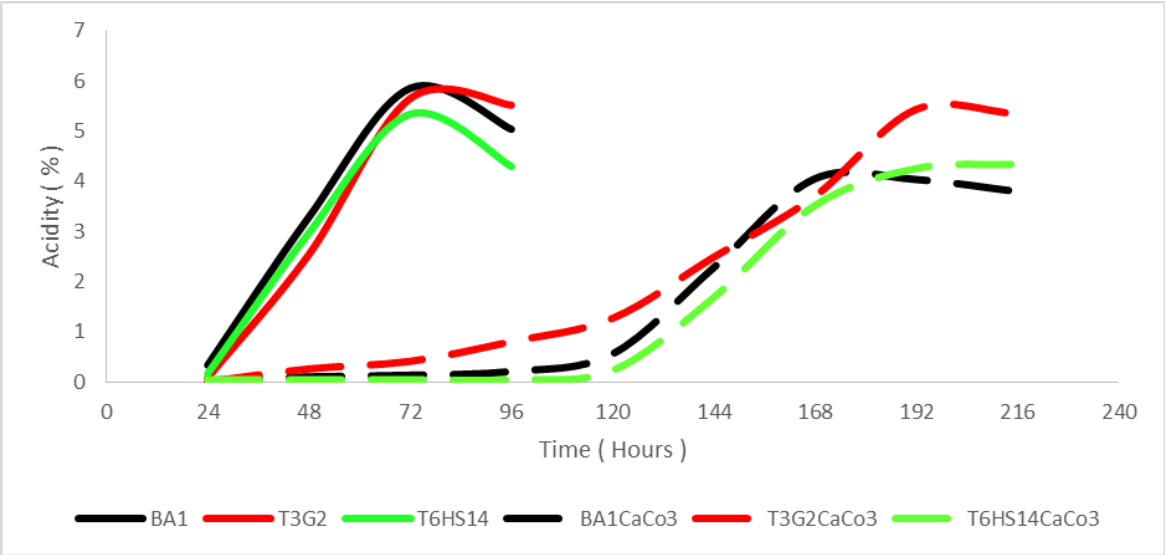

Figure 1: Time course of acid production by the three selected strains of acetic acid bacteria in a regular (pH 3.88 ±0.11) and a deacidified (pH 4.52±0.15) cashew apple wines.

The straight line curves depict the activity of BA1, T3G2 and T6HS14 strains in the regular cashew wine; the discontinued lines stand for the acetification of BA1$CaCo_3$, T3G2CaCo3 and T6HS14CaCo3 in the deacidified wine.

Table 2: Comparative physicochemical properties of vinegars yielded from deacidified and regular cashew apple wines

	Acidity (%)	Soluble solids extracts °B	pH	Residual alcohol (vinegar)	Productivity g/Lh
BA1	5.86±0.07^a	3.90±0.15^a	3.15±0.09^b	0.45±0.38^a	0.81±0.01^a
T3G2	5.65±0.18^a	3.67±0.31^a	3.19±0.07^b	0.51±0.36^a	0.78±0.03^a
T6HS14	5.34±1.07^a	3.78± 0.30^a	3.17±0.08^b	0.60±0.47^a	0.74±0.15^a
BA1$CaCO_3$	4.04±0.28^b	3.85±0.27^a	3.55±0.10^a	0.27±0.18^a	0.21±0.01^c
T3G2 $CaCO_3$	5.42±0.12^a	3.88±0.22^a	3.50±0.11^a	0.15±0.13^a	0.28±0.01^b
T6HS14 $CaCO_3$	4.25±0.35^b	3.48±0.15^a	3.53±0.17^a	0.24±0.19^a	0.22±0.02^c

Mean values with the same superscript in a column are not significantly different (P>0,05) according to the Fisher's LSD.

Quality analysis of the yielded vinegar from selected bacteria

The physicochemical characteristics of the vinegar produced are presented in Table (3). It was shown that vinegars were fully miscible in water, ethanol and acetone and their densities varied from 1.02±0.01 to 1.03±0.05 g.cm3. Moreover, the amounts of residual sugars went as low as 0.58±0.52 to 0.24±0.22 g/100mL. Presence of polyphenols, flavonoids and tannins were also evidenced. A decrease in polyphenols and tannins from the wine (152. 30±0. 50 mg/100mL and 35.17±0. 53 mg/100mL) to the vinegars was observed.

Table 3: Physicochemical properties of cashew vinegars.

Parameters	T3G2	T6HS14	BA1
Density g.cm-^{3}	1.02±0.04	1.02±0.01	1.03±0.05
pH	3.19±0.07	3.17±0.08	3.15±0.09
Acidity (g acetic acid/100 mL)	5.65±0.18	5.34±1.07	5.86±0.07
Solubility in water	fully miscible	fully miscible	fully miscible
Solubility in ethanol	fully miscible	fully miscible	fully miscible
Solubility in acetone	fully miscible	fully miscible	fully miscible
Total sugars g/100mL	0.37±0.21	0.58±0.52	0.24±0.22
Polyphenols mg/100 mL	90.97±0.57	93.69±0.12	79.89±0.92
Flavonoids mg/100 mL	10.21±0.14	10.25±0.14	14.94±0.25
Tannins mg/100 mL	14.40±0.22	16.63±0.17	23.44±0.34

The produced cashew apple vinegars from the selected strains of acetic bacteria are presented in Figure 2.
The picture shows that T6HS14 and T3G2 vinegar samples appeared cloudier than the BA1 sample.

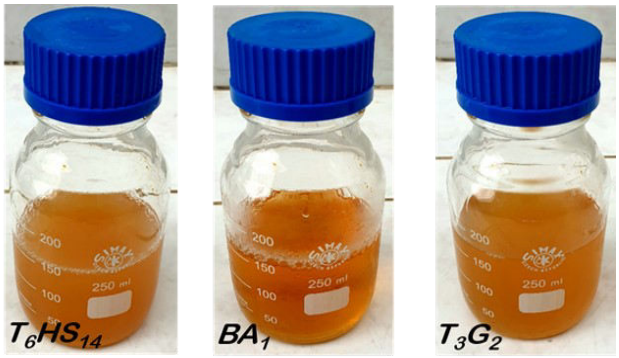

Figure 2: Cashew apple vinegars of three acetic acid bacteria strains

Regarding the sensory evaluation (Figure 3), the panelists had comparable likeness (P>0.05) for the cashew, cider and chemical vinegars in terms of acidity. Concerning the taste, no significant difference was registered between the samples except for T3G2 that displayed the lowest score (P<0. 05). Chemical and cider vinegars were found to be significantly superior (P<0. 05) to the three samples in terms of aroma. In terms of appearance, sample BA1 recorded the highest scores (P<0.05) along with the cider and chemical vinegars. For overall acceptability, cashew apple vinegars scored 6.17± 1.72 to 5.5 ± 1.25 on a total score of 10 which were comparable to the market vinegar samples.

Descriptive analysis scores were used to obtain sensory profiles as illustrated in Figure 4. The cashew apple vinegars of BA1, T6HS14 and T3G2 presented a more homogenous sensory profile for pungency, yeasty aroma, fruity aroma and perception of vinegar aroma. The cashew vinegars recorded lower scores when it came to fruity aroma; they registered higher scores in term of yeast smell. The standard samples, chemical and cider vinegars, exhibited mainly intra-group differentiation for clarity and yeast aroma from the experimental samples. Cider was clearly distinguished from the other ones for fruity aroma.

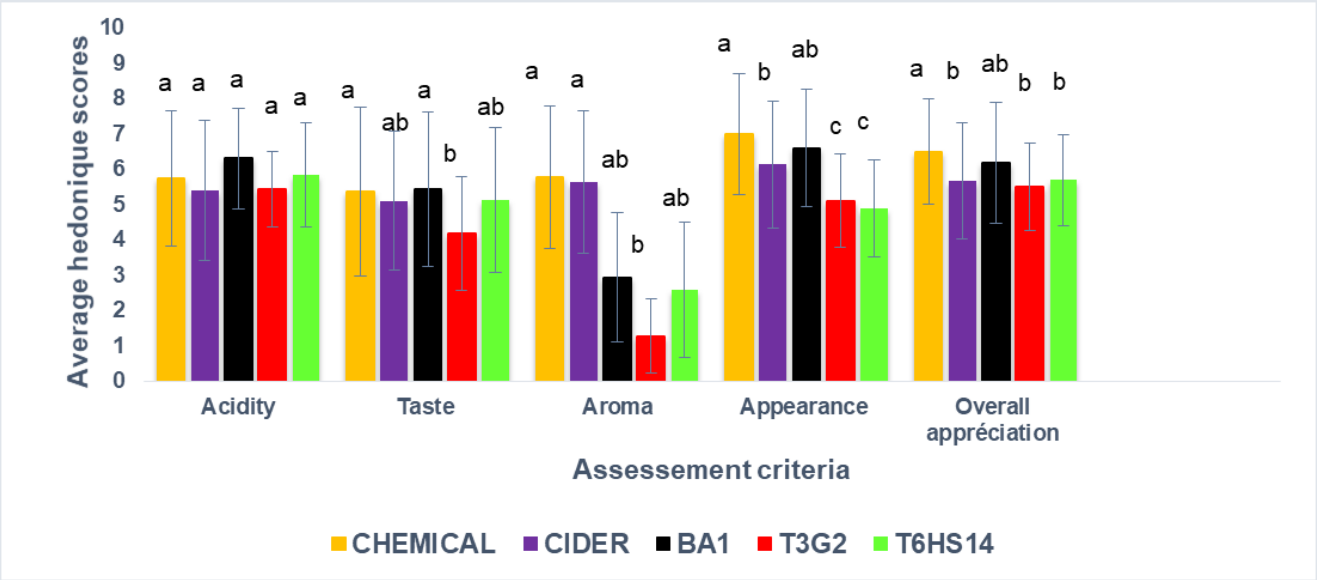

Figure 3: Hedonic sensory analysis of cashew apple vinegars

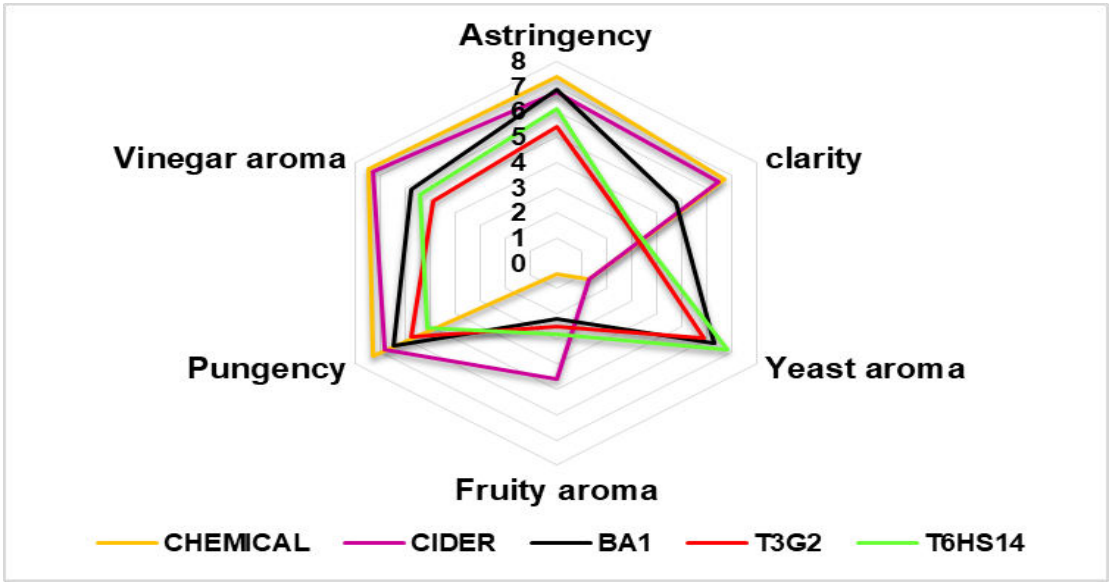

Figure 4: Descriptive sensory profile plot of mean attribute values for five vinegars samples (BA1, T6HS14, T3G2, chemical and cider)

Discussion

In this study, the abilities of acid production from selected thermotolerant acetic bacteria strains performing in non-optimized conditions, has been assessed and the quality of the yielded vinegar was analyzed.

The total sugar in the cashew apple must obtained was about 6.5 ±0.71 % and resulted in a wine with 4,85 ± 0,17 % of alcohol content. The low content of sugar observed at the end of alcoholic fermentation, indicated that the yeast have utilized the major proportion of the reducing and non-reducing sugar present in the must. The heat treatment on cashew apple juice for syrup production may have acted to release free fermentable sugars (Shafiei et al., 2010), hence making them available for yeast growth. Generally wine fermentation would last 500 h (15°C) to 184 h (28°C) to reach dryness (less than 2 g sugar/l) under static conditions (Molina et al., 2007).

The relatively short time of the completion of alcoholic fermentation (24 h) obtained in our study might be attributed to the fact that the must was pitched with actively growing yeast in the preferment. ndeed, the aeration for about 24 h may have contributed to the growth of the yeast because oxygen speeds up the synthesis of membrane and metabolites needed for growth (Maldonado et al., 1975). In this study, no supplementation with nutritional additives in the must was needed as generally requested to enhance the performance of yeasts (Chniti, et al., 2014). During the alcoholic fermentation, an increase in polyphenol content (from 125. 30±0. 36 to 152. 30±0. 50 mg/100mL) was observed. This phenomenon is diversely explained by authors. Plata et al. (2003) asserted that phenols might be formed during fermentation while Sacchi et al. (2005) stated that fermentation increases phenol extraction. Altogether, the presence of phenolic compounds in cashew wine is beneficial because, tannins, flavonoids and polyphenols, are reported to have bioactive properties (de la Rosa et al., 2018). Indeed, they are able to protect cell membranes from free-radical mediated oxidative damage involved in diverse pathologies.

During the acetification in batch fermentation, the selected acetic acid bacteria worked rapidly in an appreciable lag time at room temperature (28-30°C). The Acetobacter strains BA1, T3G2 and T6HS14 generated mostly acetic acid at a rate of 0.81±0.10 g l−1 h ($P > 0.05$). Acetic acid accumulation reached a maximum level of about 5.86±0.07 % at the third day of fermentation. Ghosh et al. (2012) also reported similar findings with thermotolerant *Acetobacter aceti* strain for palm juice vinegar, using batch method at laboratory scale. The maximum acid yield (6.81 g acetic acid/100 mL) was reached in 72 h. These levels of acid produced are comparable, with those obtained in a number of studies that used mesophilic strains in continuous fermentation. For instance, Lowor et al. (2016) recorded the maximum acid content, 6.99±0.03 % on the 27th day of fermentation of cashew wine by maintaining the optimum growth temperature, 25 – 30°C, throughout fermentation period with mesophilic strain. It is known that in submerged cultures, heat generation is unavoidable because acetic acid fermentation is an exothermic reaction. Consequently, fermentation breakdown due to temperature variation is generally avoided by heating and cooling (Gullo et al., 2014). Thermotolerant bacteria are indeed useful for vinegar fermentation at higher temperatures which reduce cooling water expenses.

Beside temperature, pH is also another physical parameter that affect the growth of acetic acid bacteria. In literature, the optimum pH for their growth is 5.5 -6.3 (Ghosh et al., 2012). It is assessed that at low pH of wine (3.4 – 3.8) the growth of some strains can be inhibited (Sengun and Karabiyikli, 2011). Concerning the selected acetic acid bacteria strains, their maximum growth was observed at pH 5. However, adjusting the pH of the wine from 3.8 up to 4.5 in this study neither improved the rate of production nor increased the yield of acetic acid. The higher acid concentrations were mainly observed with the "natural" pH of the cashew wine (3.8 ±0.11).

This pH is similar in value to the pH of the cashew wine (3.84±0. 04) used for vinegar production by Lowor et al. (2016). The end of the acetification was signaled by the overoxidation which occurred on the 4th day of fermentation leading to a decrease in acidity.

Generally, the properties of the vinegar produced in this study were similar to the standard vinegar properties stated by Raji et al., (2012). Though the acid content measured, 5.86±0.07 %, was slightly higher than 4.5% reported by the authors, it fell within the range required by most countries. The decrease in polyphenols and tannins from the wine to the vinegars is consistent with the findings of Su and Silva (2006) who reported that acetification can significantly decrease total phenolics content. As regards the recorded densities, they compared well with the standard value of 1.047 g.cm-3 reported by Raji et al. (2012).

The main difficulty of tasting vinegar product is the pungent sensation produced by acetic acid, its major component. The strong yeast aroma characteristic of alcoholic fermentation observed in this study masks the perception of other aroma, namely the fruity aroma, of the experimental cashew apple vinegar samples. Thus, the low scores recorded during aroma assessment of the cashew vinegars may be ascribed to their yeast aroma imparted by the wine. Indeed, the contribution of yeast fermentation metabolites to the aromatic profile of wine is well documented (Suomalainen and Lehtonen, 1978). It is reported that Saccharomyces cerevisiae produces different concentrations of aroma compounds depending of fermentation conditions and must treatments. Moreover, it is recognized that not all S. cerevisiae strains are suitable for the fermentation process due to their production of undesirable off-flavors (Carrau et al., 2008). Sample BA1 was clearly distinguished from the T6HS14 and T3G2 samples as to appearance, more precisely on clarity. On examination of the physicochemical characteristics it seemed that acid accumulation in BA1 sample, although it was not statistically different from the others, could have affected the quality of the vinegar. The experimental cashew apples vinegars had acceptable sensory qualities in terms of astringency, pungency and acidity when compared to the commercial samples.

Conclusion

The present study showed that the selected thermotolerant strains of acetic acid bacteria could work optimally by sustaining high temperature fluctuation. Acetic acid production rate and yield were not hindered despite the lack of a cooling system. Using thermotolerant acetic acid bacteria is thus proven to be efficient to produce a cost-effective vinegar at domestic scale. This study showed also that ordinary household materials could be used to commercially exploit the underutilized cashew apples in Côte d'Ivoire through the production of vinegar. Vinegar which is a useful product, should increase the income of farmers. Batch fermentation was used as a trial process for vinegar production in order to investigate conditions of fermentation in the early stages of this experiment. However, growth conditions in a bioreactor under continuous mode need to be investigated for process optimization and standardization. Continuous system is one of the typical method used in fermentation industries because it is economical and efficient. Moreover, appropriate yeast strains for a pleasant wine aroma could be tested to ensure the organoleptic quality of the cashew apple vinegar.

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