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Production of Vinegar Mango Using *Acetobacter tropicalis* CRSBAN-BVA1 and CRSBAN-BVK2 Isolated from Burkina Faso

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Abstract

Production and quality of vinegar from mango juice was evaluated using a two steps production procedure. The first step of fermentation was done using *Saccharomyces cerevisae* KVL013 for 7 days. The second step, an acetic fermentation was realized using two acetic acid bacteria: *A. tropicalis* CRSBAN-BVA1 and *A. tropicalis* CRSBAN-BVK2 for 21 days. Several parameters of the vinegar produced such as physico-chemical and sensory properties were determined. Microbial density during each step was monitored. Results showed that pH, alcoholic and acetic acid contents of vinegar were respectively 2.97%, 7% and 4.54% g/ml respectively by using *A. tropicalis* CRSBAN-BVA1 and 3.02%, 7% and 4.32% g/ml with *A. tropicalis* CRSBAN-BVK2. Sensory evaluation revealed that the vinegar was acceptable to the panellists. Results of microbial density showed that the maximum concentration of cell biomass produced was 4.32×10^8 and 4.25×10^8 CFU/ml respectively for CRSBAN-BVA1 and CRSBAN-BVK2.

Keywords

Mango, *Saccharomyces cerevisae*, *Acetobacter tropicalis*, Fermentation, Vinegar

1. Introduction

Vinegar is the product of the oxidation of substrates that contain ethanol [1],

derived from a two steps of fermentation process using agricultural raw materials. The two-step process consists of the anaerobic conversion of sugars to ethanol by Yeast, usually *Saccharomyces* genus followed by the aerobic oxidation of ethanol to acetic acid by bacteria, usually *Acetobacter* genus [2]. This last step is facilitated by acetic acid bacteria (AAB). Several species of *Acetobacter* have been described as the main vinegar producer such as, *A. aceti*, *A. pateurianus*, *A. peroxydans*, *A. orleaniensis*, *A. lovaniensis*, *A. estuniensis*, *A. malorum*, *A. cerevisiae and A. oeni* [3].

Vinegar was used as a food preservative because its acidity retards growth of microbial, contributes to the sensory properties of a number of foods [4] and several useful applications in human living such as cleaning, disinfection [5]. It was also recognized as having a number of health benefits due to therapeutic compounds [6].

Vinegar having many uses, however most of the vinegar marketed in sub-Saharan Africa is produced from the dilution of acetic acid of petrochemical origin and often does not meet the food standards that recommend a natural product obtained by double fermentation (alcoholic and acetic) from raw materials of agricultural origin [7].

Vinegar can be produced from many kinds of sources like grapes, apples, and some many other tropical fruits like pineapples, dates, oranges, mangoes [8].

Since mango production is very important in West Africa and especially in Burkina Faso, Significant quantities (30% - 50%) of mangoes are lost every year after harvest [9] [10]. It is necessary to exploit this biomass for local acetic acid production. Some work has been carried out on the transformation of these mangoes into bioethanol and biogas [11] [12], but the level of transformation is still low. Mangoes are particularly biotransformable and highly perisable fruits, because of their high-water content and the presence of carbohydrate. The sugars adhering to the mango fruit are ideal for fermentation. This ability allows processing and storage of agro-perishable foods to preserve or to valorize them over a long period of time.

Due to the abundant production of mango in Burkina Faso high rate of post-harvest lost is observed. To reduce this phenomenon, the present study was conducted to produce vinegar from mango fruits using indigenous acetic acid bacteria. In addition, the quality of the vinegar produced was assessed.

2. Materials and Methods

2.1. Sources of Fruits and Microorganisms

Mangoes Amelie variety was used from Banfora district of Burkina Faso. Yeast strain and acetic acid bacteria were isolated from experiments in the Laboratory of Microbiology and Microbial Biotechnology, University Joseph Ki-Zerbo (Burkina Faso). *Saccharomyces cerevisiae* KVL013 was used for alcoholic fermentation and *Acetobater tropicalis* CRSBAN-BVA1, *Acetobater tropicalis* (CRSBAN-BVK2) were used for acetic fermentation.

2.2. Production of Mango Juice

Mango fruits were washed, peeled and pressured. Juice was obtained from the peeled mangoes by mechanical pressure. Juice was pasteurized at 90˚C during 15 mins to prevent microbial contamination and to concentrate sugar. Brix degree result was determined using a refractometer (ATAGO N1). After cooling at room temperature, the juice was distributed in 1000 mL sterile plastic bottle.

2.3. Preparation of Inoculum

Preparation of inoculum was realized according to the modified method [13]. A preculture of 24 hours was carried in YEPD medium for *Saccharomyces cerevisiae* KVL013 and GYC medium for *Acetobacter tropicalis* CRSBAN-BVA1 and *Acetobacter tropicalis* CRSBAN-BVK2. Cells of each strain were picked and dissolved in 100 mL of saline water. The inoculum of yeast and acid bacteria was adjusted each at 10^6 cells/mL.

2.4. Production of Mango Vinegar

Mango vinegar was produced by the method [14] with modification as shown in **Figure 1**.

2.4.1. Alcoholic Fermentation

Alcoholic fermentation was conducted in plastic bottles of capacity 1000 mL.

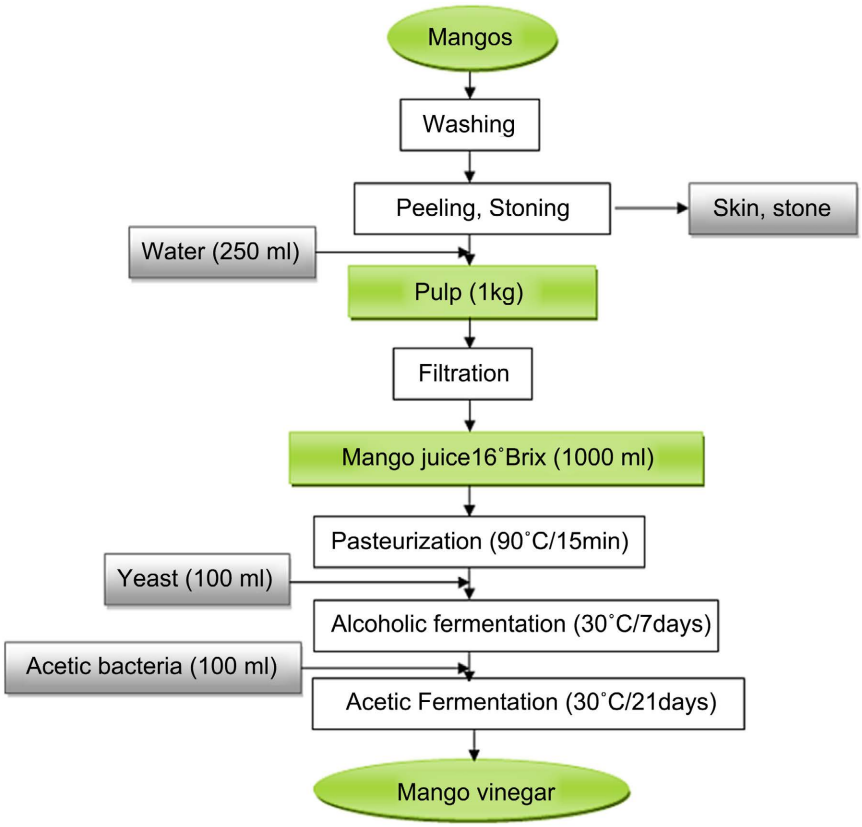

Figure 1. Process of mango vinegar production.

The bottles containing 1000 mL of mango juice (16˚ Brix) were sealed at 30˚C with 10 mL of 10^6 cells/mL of *Saccharomyces cerevisae* KVL013 for one week to allow alcoholic fermentation.

2.4.2. Acetic Fermentation

After one week of alcoholic fermentation, the alcoholic juice containing 7% ethanol was subsequently used for acetic fermentation process with 10^6 cells/mL of *Acetobacter tropicalis* CRSBAN-BVA1 and Acetobacter *tropicalis* CRSBAN-BVK2. The bottles were sealed and fermentation was performed at 30˚C for 21 days.

2.5. Chemical Analysis

2.5.1. Determination of pH

The pH was determined according to Association of Analytical Chemists [15]. Ten (10) g of vinegar was weighed into a beaker, mixed thoroughly in 100 mL of distilled water and centrifuged for 20 mins at 200 rpm. The supernatant was then decanted and the pH was determined with a standard pH-meter.

2.5.2. Analysis of Alcoholic Content

Alcohol produced during the production was determined by the densimetric method [15]. A volume of 50 mL of the mango wine was taken and added in 50 mL of distilled water. The mixture was distilled and 50 mL of the distillate was recovered and carried to boiling in an ebulliometer. Density of the distillate was measured using a densimeter. Concentration of ethanol content was confirmed by HPLC.

2.5.3. Analysis of Acetic Acid Content

Content of acetic acid was determined every 48 h by titration of 1 mL of fermented sample with sodium hydroxide 0.5N. The acidity of vinegar expressed in degree of acetic acid was calculated using formula [16]:

Acetic acid (g/100 mL) = Volume of NaOH (mL) used in titration × 0.03 × 2.

2.6. Microbial Density Analysis

Microbial density was followed using standard plate count method. Gelose Sabouraud added with Chloramphenicol was used for the enumeration of yeasts. The chloramphenicol makes it possible to inhibit the development of the bacteria present in the wine. Culture was incubated at 30˚C for 24 hours. The acetic bacteria (BA) were counted using GYC medium (10% glucose, 1.0% yeast extract, 2.0% calcium carbonate, 1.5% agar, pH 6.8) supplemented with 100 mg. l^{-1} of Pimaricin to inhibit the growth of yeasts and moulds. Culture was incubated at 30˚C for 48 hours.

2.7. Sensory Evaluation

Final vinegar was evaluated by 30 panellists of 20 - 40 years of age, including students and staff of Research Center in Biological Food and Nutrition Sciences

(CRSBAN). The panellists were selected for participation on the basis of their preference. The evaluations took place in the mid-morning between 9:00 and 10 h:30 mins. This was conducted at room temperature of 30˚C under white light.

Samples were served in clean transparent glasses which had been labeled with 3 digit random numbers. Questionnary and water for mouth rinsing between each tasting were provided. Commercial vinegar (Mango vinegar, cider vinegar, colorless acetic acid vinegar and coloured acetic acid vinegar) was used as reference. The mango vinegar was evaluated for their appearance, taste and general acceptability. The results were analysed using Analysis of Variance (ANOVA) [17].

2.8. Statistical Analysis

Questionnary was carried out using Sphynx software. XLSAT version 02.27444 was used for statistical analysis of the data. Difference between the results about sensory profil was given using test ANOVA. All Statistical tests were performed at 5% significance level.

3. Results and Discussion

3.1. Chemical Parameters

3.1.1. pH

A reduction progressive of pH was observed during the fermentation and varied from 4.09 to 2.97 for the CRSBAN-BVA1 strain and 4.16 to 3.02 for the CRSBAN-BVK2 strain (**Figure 2(a)** and **Figure 2(b)**). Increase in production of acetic acid was leading a decrease in pH of vinegar. pH of vinegar is related to the amount of acidity present [13]. This decrease in pH could be explained by the presence of certain organic acids such as acetic acid, citric acid.

3.1.2. Brix Degree

In raw mango juice of 16˚ Brix, the sugar concentration was become 6˚ Brix after alcoholic fermentation (Alc) by the strain *Saccharomyces cerevisiae*. A decrease of sugar concentration (2.5˚ and 3˚) was also noticed after the acetic fermentation (Acf) by the strains CRSBAN-BVA1 and CRSBAN-BVK2 (**Figure 3**). Decrease in the sugar level is explained by the metabolism of sugars by the yeast during alcoholic fermentation. Decrease in sugar levels after acetic fermentation could be explained by metabolism of residual yeasts after their elimination by centrifugation in fermented mango juice [18].

3.1.3. Alcoholic Content of Vinegar

Production of ethanol during alcoholic fermentation by the strain *Saccharomyces cerevisiae* was presented in **Figure 2(a)** and **Figure 2(b)**.

These results show an increasing production of ethanol at 72 hours of fermentation whose ethanol concentration was between from 1% to 7% g/l. Reduction progressive of ethanol concentration was observed after 72 hours. At the end of fermentation, final ethanol concentration was 1.5% g/l. Maximum concentration

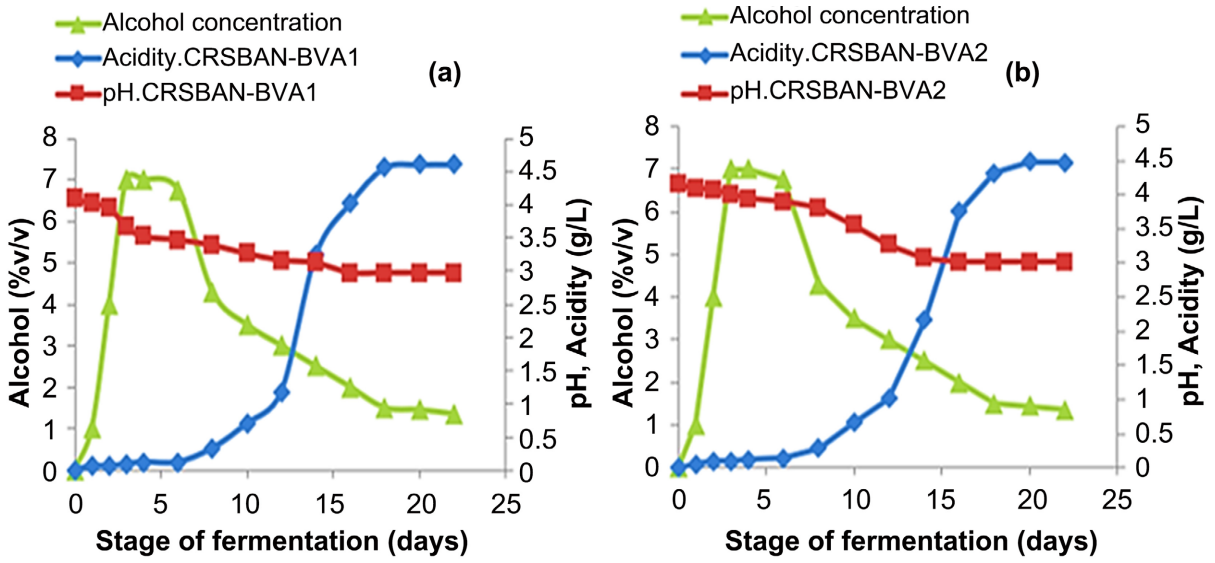

Figure 2. Evolution of alcohol, pH and acidity during fermentation.

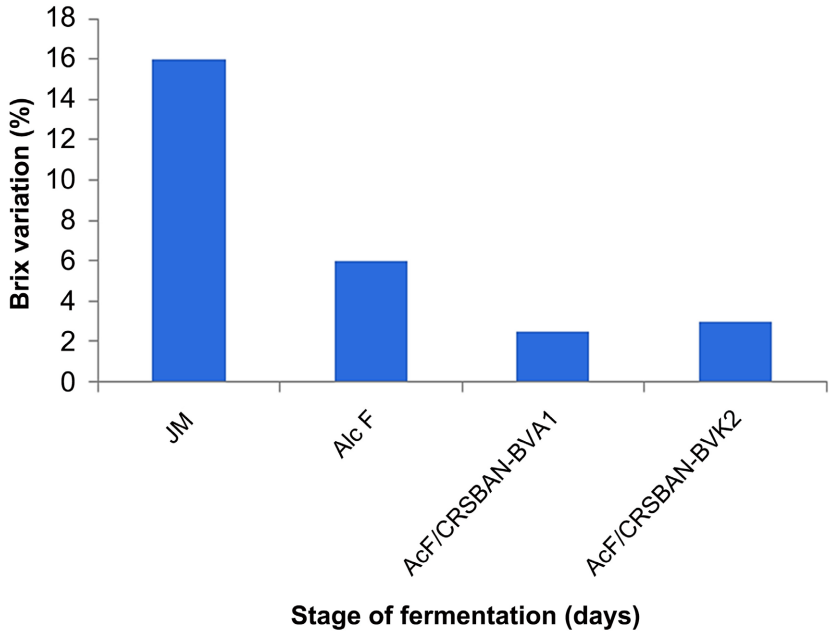

Figure 3. Brix degree evolution during fermentation.

of ethanol was obtained in 72 hours and was of 7% (g/l). This concentration was higher than those values which was 6.4 g/l [19] but lower than other values which was 8.9 g/l and 22.4 g/l [14] [20]. This variability production of alcohol could be due to the metabolic capacity of yeasts or the adaptability of yeasts with various sugar concentrations during fermentation [20] [21].

3.1.4. Acetic Acid Content of Vinegar

During acetic fermentation, a light production of acetic acid was observed at the first week of fermentation whose acetic acid produced during this stage varied from 0.18% to 1.08% (w/w) for the CRSBAN-BVA1 strain and from 0.24 to 1.02

for the CRSBAN-BVK2 strain. After this first week of fermentation, an optimal production of acetic acid was obtained with all the two strains and varied from 3.24% to 4.54% (w/w) for CRSBAN-BVA1 (**Figure 2(a)**) and 2.16% to 4.32% (w/w) for CRSBAN-BVK2 (**Figure 2(b)**).

Highest content (4.54%) of acetic acid was obtained with CRSBAN-BVA1 strain compared to that of CRSBAN-BVK2 strain which was 4.32%. These values are slightly lower than that obtained during the production of mango vinegar in Togo which was 4.70% [14]. 4.65% of acidity was obtained during the production of vinegar starting from mango juice [22].

In Côte d'Ivoire, an acidity content of 6% (w/w) was obtained during production of banana vinegar [19]. This difference between values could depend on the capacity of strains. Strain of *Acetobacter* that able to produce 1.7 g/l of acetic acid can be employed like choke in the production of vinegar especially in industrial production [23]. Artisanal production of the vinegar is generally slow and requires one lasted from 4 to 5 week for a complete fermentation [22].

3.2. Microbial Density

Biomass produced by yeasts and bacteria during the alcohol fermentation and acetic fermentation during vinegar production were presented in **Figure 4**. Curve presents an exponential phase of growth followed by a phase of decrease for all strains. Exponential phase of growth was observed in a shorter time (1st at 3rd day) with the yeast strain and the produced maximum biomass was 4.36×10^8 UFC/mL. It was maintained between seven days before decreasing. This phase corresponds to the maximum production of ethanol.

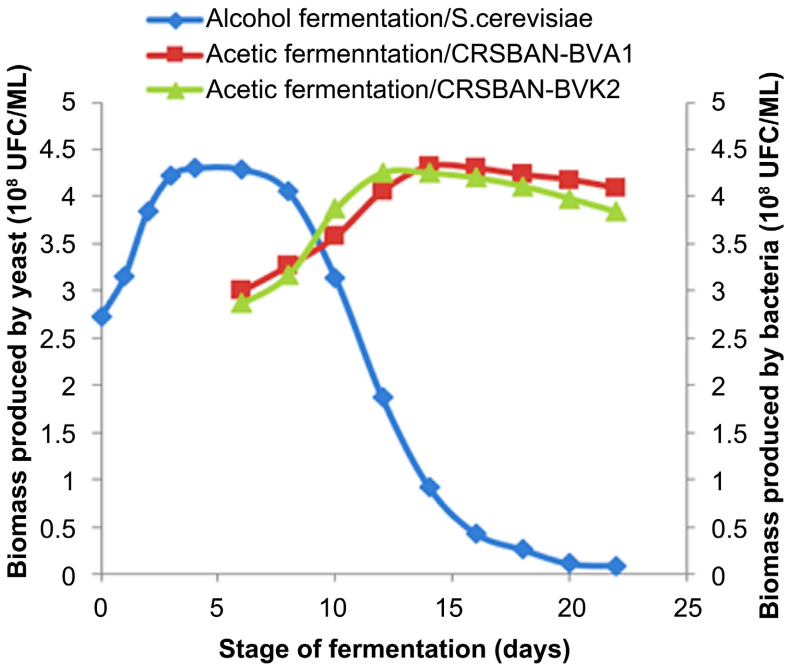

Figure 4. Biomass produced by yeast and bacteria during alcoholic and acetic fermentation.

The bacterial biomass concentration increased slightly at the start of fermentation up to the 8th day when an increasing production of biomass was observed faster until the 14th day followed by a slight tendency to decrease. The maximum concentration of biomass produced was 4.32×10^8 and 4.25×10^8 CFU/mL for CRSBAN-BVA1 and CRSBAN-BVK2.

Biomass ranged from 1.2×10^{11} UFC/ml for *Saccharomyces cerevisia* and 4.32×10^8 and 4.25×10^8 UFC/mL for *Acetobacter tropicalis* (CRSBAN-BVA1 and CRSBAN-BVK2). These values are inferior with those reported by other auteurs which were obtained 1.2×10^9 UFC/mL [13] and slightly higher than that brought back, which was of, 10^8 UFC/ml during the production of the wine vinegar [24].

This difference could be in the adverse condition with strain or the performance of strain. Optimal biomasses are obtained at period or concentration out of ethanol and acetic acid are high in the medium [22].

3.3. Sensory Properties

Result of comparative sensory evaluation of vinegar Product with *Acetobacter tropicalis* (CRSBAN-BVA1, CRSBAN-BVK2) and commercial vinegar was presented in **Figure 5**. Sensory tests showed that vinegars product by fruit with strains CRSBAN-BVA1 and CRSBAN-BVK2 were moderately acidic by majority of panelists (51.70%; 45.10%). Mango vinegar and apple cider vinegar were also moderately acidic by minority (51.70%; 45.10%; 43.30% and 42%) of panelists whereas vinegars produced from acetic acid (colorless acetic acid vinegar, coloured acetic acid vinegar) had a strong acidity (52.7% and 51.40%) represented in **Figure 5(a)**. The yellow color of vinegar was found to be very attractive by 83.3%; 93.3% and 100% of panelists for CRSBAN-BVA1 vinegar, CRSBAN-BVK2 vinegar and Cider vinegar. Panelist (100%) was founded dark color with coloured acetic acid vinegar, mango vinegar and colorless acid acetic vinegar a clear color (**Figure 5(b)**). Tasters (51.70% and 46.7%) found that CRSBAN-BVA1 vinegar and CRSBAN-BVK2 vinegar had a slight sweet taste and had no aftertaste compared to mango vinegar and cider vinegar. Minority (26.7% and 35%) of tasters found these vinegars to have a sweet taste and an aftertaste. Other of panelists did not find a sweet taste in the colorless acetic acid vinegar and in the colored acetic acid vinegar (**Figure 5(c)**). Tasters showed that the CRSBAN-BVA1 vinegars and the CRSBAN-BVK2 vinegar exhibited a moderately noticeable fruity odor (mango aroma) compared to commercial vinegars where this odor was very weakly noticeable presented in **Figure 5(d)**.

The majority of panelists (53.3% and 51%) found CRSBAN-BVA1 vinegar and CRSBAN-BVK2 vinegar to be moderately cloudy. When with apple cider vinegar and mango vinegar, 50% and 40% of the panelists found that these vinegars were also moderately cloudy. Compared to the colorless and colored acetic acid vinegar, 76.70% and 60% of the panelists found that these acetic vinegars were not cloudy (**Figure 5(e)**).

Sensory tests showed that vinegars produced from *Acetobacter tropicalis* strains (CRSBAN-BVA1 vinegars and CRSBAN-BVK2 vinegar) were moderately acidic

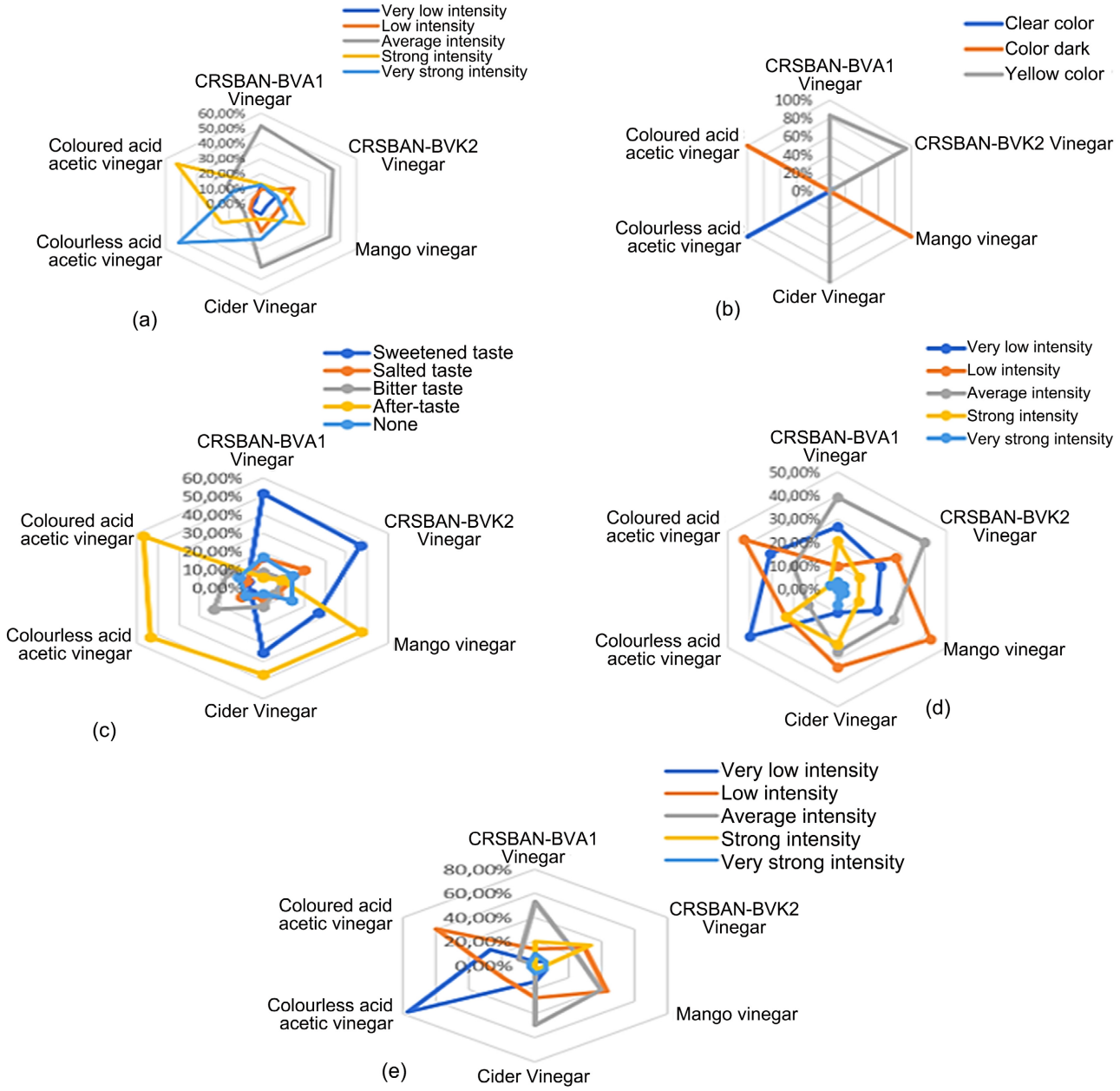

Figure 5. Perception of panelists on organoleptic quality of vinegar: (a) Taste; (b) Color; (c) Aftertaste; (d) Smell and (e) Appearance.

with an attractive mango odor and slightly sweet. These vinegars were favorably appreciated by the majority of the panelists compared to commercial vinegars. This slight sweet taste was much more appreciated by the majority of panelists. This shows that this vinegar meets the needs of the population. Banana vinegar and coconut vinegar product with *Acetobacter aceti* was generally accepted by panelist [18] [21]. That can be explained the fact these vinegar followed the process of double fermentation alcoholic and acetic compared to commercial vinegar.

4. Conclusion

The results of the present study revealed that strain *Acetobacter tropicalis* (CRSBAN-

BVA1 and CRSBAN-BVK2, was able to produce acetic acid (vinegar) from mango juice. These two strains, all isolated from fermented mango juice, each had an acidity of 4.54% and 4.32% for production duration 21 days. These two strains can therefore be proposed as a starter for the production of vinegar. Organoleptic tests showed that vinegar produced with two strains was an acceptable quality in comparison with commercial vinegars. The obtained results could possibly allow to develop an add value to mangos waste product by fermentation. Using mango water to make vinegar also reduces the cost and contributes to diversify the local vinegar market.

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Conflicts of Interest

The authors have not declared any conflict of interests regarding the publication of this paper.

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