# Assessment of Cashew Apple Juice on Fermentative Activity of Two Lactic Bacteria in Production of Yoghurt

Abdoulaye Toure, Yadé Réné Soro, Armel Fabrice Zoro, Ledjou Arnaud Kogbou Djah, and Adama Coulibaly

***Abstract*— Cashew apple is not practically used such as cashew nut which is one of most traded agricultural export products from Northern Ivory Coast. This work focus on the use of cashew apple juice as food additive in production of yoghurt. The aim of this study is to evaluate physicochemical parameters of cashew apple juice and its effect on fermentative activity of two lactic bacteria (*Lactobacillus delbrueckii subsp bulgaricus* and *Streptococcus salivarius subsp thermophilus*). Various types of yoghurt were formulated with incorporation of different proportions (0%, 5%, 10%, 15%, 25%, 35% and 40%) of cashew apple juice in milk (v/v). Each formulation was fermented with lactic ferment containing the above two lactic bacteria. Effect of juice on fermentative activity of the two bacteria was carried out by pH, titratable acidity with duration of fermentation to obtain 80 Dornic degrees. Results of phytochemical analysis ($p<0.05$) showed that cashew apple juice have an acid pH with value of 3.96 ± 0.03 and titratable acidity of 0.41 ± 0.01 %. This juice contains a high level of moisture (77.48 ± 0.09 %) and vitamin C (124.74 ± 1.17 mg/100 ml) but a low concentration of reducing sugars (1.46 ± 0.03 g/100 ml). The study of effect of cashew apple juice incorporated into milk, showed that the formulations with 5% and 10% improved fermentative activity of lactic bacteria tested. These two formulations (5% and 10 %) reduced fermentation time (180 min) compared to control (220 min). This study suggests that cashew apple juice could be a food additive in production of yoghurts as a source of vitamin C and catalyst for fermentation. In perspective, we plan to assess toxicity and shelf of yoghurt with cashew apple juice.**

***Index Terms*— cashew apple juice, fermentative activity, Ivory Coast, lactic bacteria, physicochemical properties, yoghurt.**

## I. Introduction

Yoghurt is a very popular product around the world due to both its organoleptic and nutritional characteristics [1]. This fermented milk is obtain using fermentative activity of two lactic bacteria (*Lactobacillus delbrueckii subsp bulgaricus* and *Streptococcus salivarius subsp thermophilus*) [2]. In addition to fermentation, several species of lactic bacteria are used to give organoleptic and nutritional characteristics to yoghurt. Some strains of *S. salivarius subsp thermophilus* and *L. acidophilus* are used for their ability to produce less lactic acid and more exopolysaccharides [3]. Thus, in Europe and Northern America there is a trend towards probiotic, low-acid and low-fat yoghurt [1], [4]. In addition, there are high protein yoghurts and fruit yoghurts obtained by adding respectively serum proteins and pieces of certain fruits or their purees [1]. Also, to meet consumer requirements, manufacturers use food additives to improve organoleptic, technological and nutritional characteristics of their products [4]. But currently, tendency is to reject synthetic additives that are considered dangerous for health in favor of natural additives and safe for the consumer [5]. Therefore, this work focused on the use of cashew (*Anacardium occidentale*) apple juice as food additive in production of yoghurt. In fact, cashew production in Côte d'Ivoire has increased in recent years from 235 000 tons in 2006 to 738 000 tons in 2018 [6]. However, cashew apple is not practically used such as cashew nut which is one of most traded agricultural export products from Northern Ivory Coast. Various research works revealed that, cashew apple is a good source of vitamin C, polyphenols and carotenoids [7], [8], [9]. Also, in Benin, different methods of valorization of this by-product of cashew were carried out by production of juice, alcohol, wine, jam and other products [10]. In Ivory Coast, [11] showed that "baobab" (*Adansonia digitata*) and "néré" (*Parkia biglobosa*) flours used as food additives in formulation of yoghurt were improved fermentative activity of *L. delbrueckii subsp bulgaricus*. In order to contribute to valorization of cashew apple juice, the main purpose of this study is to use it as a food additive in production of yoghurt. It is therefore a question to determine some physicochemical parameters of cashew apple juice and its effect on fermentative activity of two lactic bacteria (*L. delbrueckii subsp bulgaricus* and *S. salivarius subsp thermophilus*).

## II. Materials and Methods

### A. Biological materials

These materials consist of cashew apple collected in march 2019 from farm near Peleforo Gon Coulibaly University, milk powder (LP®, Ireland) purchased from Korhogo market (Ivory Coast) and lactic ferment (Yalacta®, France) provided by "Pharmacie de la rue du commerce" (Abidjan, Ivory Coast).

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### B. Chemicals

Reagents [(glucose, vitamin C, phenolphthalein, sodium hydroxide, 3.5-Dinitrosalicylic acid (DNS), metaphosphoric acid, acetic acid, dichlorophenol-indophenol (DCPIP)] were purchased from Sigma-Aldrich (Germany). Man Rogosa Sharpe (MRS) agar was from Sharlau (Espagne). All chemicals used in this study were of analytical grade.

### C. Production of cashew apple juice

Before juice extraction, cashew apple samples were treated according to method using by [12]. The treatment consisted to select the good quality of apples and wash them with distilled water. Finally, these apples were disinfected by dipping in bleach solution (8° Chl) diluted to 37% during 30 min before rinsed them with distilled water. Cashew apple juice was extracted according to [13] method with slight modification. The apples removed from their nuts ($M_0$) were crushed using a Blender (Nasco, China). The ground product obtained was pressed over a sieve to obtain raw cashew apple juice ($M_1$). The extraction yield (R) was determined by the following (1):

$$R\ (\%) = \frac{M1 \times 100}{M0} \qquad (1)$$

To ensure conservation of the raw juice of cashew apple, it was pasteurized following to method described by [13]. This juice was treated at 80 °C for 25 min. After cooling, the juice was put in a sterile glass bottle and then stored in a refrigerator at 4°C until used.

### D. Physicochemical analysis of cashew apple juice

pH, titratable acidity and moisture of cashew apple juice were performed using official methods [14]. The pH value was recorded by pH-meter (Hanna, Spain). Titratable acidity was determined by titrated 20 ml of juice by sodium hydroxide solution (NaOH, 0.1N). The moisture content of juice was obtained on dry matter basis by the difference of weight before and after drying 10 g of sample in an oven (Memmert, Germany) at 105°C until constant weight. Reducing sugars content of juice was determined according to [15]. To 1 ml of juice were added successively 0.5 ml of distilled water and 0.5 ml of DNS. The mixture was heated in a water bath at 100°C for 5 min. After cooling, 2 ml of distilled water were added to the mixture. The absorbance was measured at 540 nm using a spectrophotometer (PG Instruments, England). Value of reducing sugars of juice was determined using a calibration curve of glucose (0.1 mg/ml) as standard. Vitamin C of juice was dosed by titration following the method described by [16]. 10 ml of mixture [5 ml juice and 5 ml metaphosphoric acid-acetic acid (2%, w/v)] were titrated with dichlorophenol-indophenol (DCPIP) 0.5 g/l. Vitamin C concentration of cashew apple juice was calculated with following (2):

$$C_{Vit\ C} = \frac{C_I \times V_i}{V_s} \qquad (2)$$

$C_{Vit\ C}$: Concentration of Vitamin C,
Ci: Concentration of DCPIP solution,
Vi: Volume of DCPIP,
Vs: Volume of dosed solution.

### E. Influence of cashew apple juice on fermentative activity of lactic bacteria

Production of yoghurt was carried out according to the method of [17]. Various types of yoghurt were formulated with incorporation of different proportions (0%, 5%, 10%, 15%, 25%, 35% and 40%) of cashew apple juice in milk (v/v) (Table I).

Table I: Proportions of ingredients for different formulations

| Ingredients | Formulations | | | | | | |
|---|---|---|---|---|---|---|---|
| | F0 (0%) | F1 (5%) | F2 (10%) | F3 (15%) | F5 (25%) | F7 (35%) | F8 (40%) |
| Milk (ml) | 100 | 95 | 90 | 85 | 75 | 65 | 60 |
| Cashew apple juice (ml) | 0 | 5 | 10 | 15 | 25 | 35 | 40 |

Mixture (milk and cashew apple juice) of each formulation was pasteurized at 85°C for 5 min. After cooling to 45°C, each formulation was inoculated at 4% with lactic ferment containing two lactic bacteria (*L. delbrueckii subsp bulgaricus* and *S. salivarius subsp thermophilus*). Effect of cashew apple juice on fermentative activity of lactic bacteria was carried out during incubation at 45°C by measuring titratable acidity (TA) and pH of mixture inoculated with lactic ferment over time. Titratable acidity was recorded by titrated 10 ml of each mixture inoculated with sodium hydroxide solution (0.111 mol/l) in presence of phenolphthalein [14]. Acidity expressed in Dornic degree (°D) was calculated with (3):

$$TA\ (°D) = C_{NaOH} \times V_{NaOH} \times M_{Lactic\ acid} \qquad (3)$$

TA: Titratable acidity (°D),
$C_{NaOH}$: Concentration of sodium hydroxide solution (mol/l),
$V_{NaOH}$: Volume of sodium hydroxide solution (l),
$M_{Lactic\ acid}$: Molecular mass of lactic acid (90 g/mol).
Effect of juice on fermentative activity of the two bacteria was evaluated by pH, titratable acidity with duration of fermentation to obtain 80 Dornic degrees.

### F. Statistical analysis

The statistical analyses were performed with Graph Pad Prism software version 8.0.2 (263). The variance analysis (ANOVA) was performed to determine differences between the averages according to method of Turkey at the 5% threshold ($p<0.05$ was considered significant). The results were expressed as averages with standard error on mean (mean ± SEM).

## III. RESULTS AND DISCUSSION

### A. Extraction yield and physicochemical parameters

The results of extraction yield and physicochemical parameters of cashew apple juice are summarized in Table II. The extraction yield of cashew apple juice is 75.84 ± 0.37%. The cashew apple juice is acid with pH and acidity of 3.96 ± 0.03 and 0.41 ± 0.01% respectively. It possesses high moisture content of 77.48 ± 0.09%. The cashew apple juice is rich in vitamin C (124,74 ± 1,17 mg/100 ml) and has a low content of reducing sugars (1.46 ± 0.03 g/100 ml).

The extraction yield of cashew apple juice of this study is lower than that obtained by [12] which is 82.20%. The difference of yield could be explained by the extraction method used. Indeed, according to [12], the most effective way for best extraction of juice, required the use of high pressure screw press. The dries matter content for cashew apple juice in this study is to higher than those obtained by [12] with rate of 14%. This high content of dries matter could be due to the non-clarification of the juice which contains several particles such as fibers and residual cakes). The pH of juice studied is almost identical to that obtained in Benin by [18] with pH values of raw cashew apple juice ranging from 3.9 to 4.0. Acidity of juice is lower than that of raw cashew apple juice studied in Nigeria by [19] with a titratable acidity of 0.48%. The non-pasteurization of juice by this author could be explained this difference of acidities. Indeed, the duration and temperature of the heat treatments affect the organic acids concentration of the juice. Vitamin C content of juice from Northern Ivory Coast is consistent with that (120.60 to 152.40 mg/100ml) of pasteurized cashew apple juice studies in Nigeria by [20]. These above vitamin C contents are lower than that (147.0 to 185.8 mg/100 ml) of unpasteurized juice studied by [18]. This difference could be explained by the reduction in the vitamin C content with the heat treatment applied to the pasteurized juices. However, the high vitamin C content of cashew apple juices gives them antioxidant properties. The consumption of 100 ml of cashew apple juice could cover the daily requirements (40 mg/day) of vitamin C as recommended by [21]. The cashew apple juice possesses high content of reducing sugar but it is half that of unpasteurized juice (3g/100 ml) studied in Benin by [22]. This could be due to the non-enzymatic browning between the reducing sugars and the proteins of the juice subjected to heat during pasteurization. Also, high reducing sugar content of cashew apple juice could be exploited for alcohol production [18]. Indeed, physicochemical composition of the juices is affected by several factors such as variety, agro-ecological zone, climate, cultural practices, level of maturity of the fruit and even it colors [18].

### *B. Effect of cashew apple juice on fermentative activity*

Effect of juice on fermentative activity of the two bacteria was evaluated by pH and titratable acidity with duration of fermentation to obtain 80 Dornic degrees. Figures 1 and 2 show respectively variation of pH and titratable acidity of different formulations after cashew apple juice added. For each formulation, the pH decreases and the acidity increases while the incorporation percentage of cashew apple juice increases. This acidity increased during fermentation was highlighted in the work of [11] with the used of "baobab" and "néré" flours as food additives in the production of yoghurt. This high acidity of formulation could be due to the original acidity of apple juice which is rich in organic acids and vitamin C [23], [24].

Table II: Physicochemical properties of apple juice ($p<0.05$)

| Parameters | Values |
|---|---|
| Yield (%) | 75.84 ± 0.37 |
| pH | 3.96 ± 0.03 |
| Acidity (%) | 0.41 ± 0.01 |
| Moisture (%) | 77.48 ± 0.09 |
| Dries matter (%) | 22.52 ± 0.09 |
| Reducing sugars (g/100 ml) | 1.46 ± 0.03 |
| Vitamin C (mg/100 ml) | 124.74 ± 1.17 |

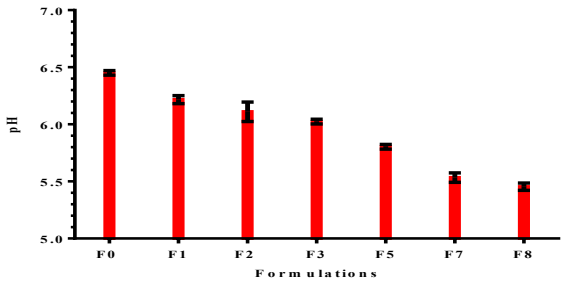


Fig 1. pH of different formulations after cashew apple juice added ($p<0.05$).

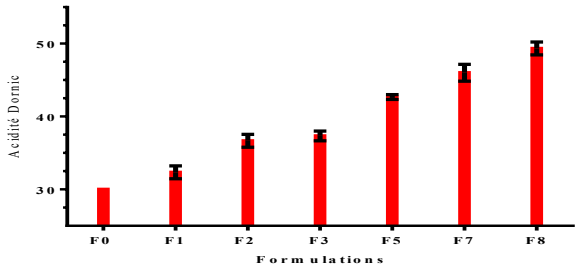


Fig 2. Titratable acidity of different formulations after cashew apple juice added ($p<0.05$).

Table III presents the duration of fermentation ($p<0.05$) to obtain 80 Dornic degrees for each yoghurt formulated by incorporation of cashew apple juice into milk. The formulations with 5 and 10 % of cashew apple juice show a significant reduction of fermentation time (180 min) compared to the control (220 min). This reduction in production time of yoghurt F1 (5%) and F2 (10%) could be due to the intake of vitamin C, reducing sugar and free amino acids necessary for the growth of lactic bacteria. In fact, during the latency time, bacteria produce compounds, such as vitamins and amino acids, which are necessary for their growth. Therefore, these compounds which were to be synthesized by the bacteria are brought by cashew apple juice [25], [26], [27]. However, all increases in the percentage of incorporation of the cashew apple juice into the milk above 10% (F3, F5, F7, F8) significantly increase it fermentation time compared to the control. This could be explained by the low initial pH with the presence of many organic acids preventing any activity of *S. salivarius subsp thermophillus* [26], [27]. According to [28], any high acidity would significantly inhibit the development of this species of lactic bacteria. Indeed, for lactic acid production, *L. delbrueckii subsp bulgaricus* needs to be synergy with *S. salivarius subsp thermophillus* which is inhibited and would increase latency time, hence fermentation.

Table III: Duration of fermentation of each formulation of yoghurt with cashew apple juice at 80°D..

| Formulations | Rates of incorporation (%) | Fermentation time (min.) |
|---|---|---|
| F0 | 0 | 220 ± 0.16 |
| F1 | 5 | 180 ± 0.23 |
| F2 | 10 | 180 ± 0.26 |
| F3 | 15 | 240 ± 0.08 |
| F5 | 25 | 315 ± 0.12 |
| F7 | 35 | 355 ± 0.33 |
| F8 | 40 | 355 ± 0.29 |

## IV. CONCLUSION

This study focused on some physicochemical parameters of cashew apple juice and its effect as food additive on fermentative activity of two lactic bacteria (*L. delbrueckii sub sp bulgaricus* and *S. salivarius subsp thermophilus*) in yoghurt production. It allows to highlight that this juice have high acidity with richness in vitamin C and reducing sugar which contribute significantly to the growth of lactic bacteria. Therefore, incorporation of cashew apple juice to milk at 5 to 10 % as food additive in yoghurt production, reduced effectively fermentation duration by 40 minutes. These results constitute one of the ways for valorization of cashew apple from Northern Ivory Coast which could improve producers' financial resources. However, combination of cashew apple juice and milk consomption must be *in vivo* studied on experimental animals to highlight toxicity of yoghurt produced.

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