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Effect of Spontaneous Fermentation on the Biochemical and Microbiological Quality of Tamarind-Based (*Tamarindus indica* L.) Beverages

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A B S T R A C T

In Côte d'Ivoire, tamarind is used to produce a very popular beverage called 'tomidji', whose nutritional and microbiological quality is unsatisfactory. This study was conducted to enhance the nutritional and microbiological quality of tamarind-based beverages by combining spontaneous fermentation with the addition of ingredients. For this purpose, various tamarind-based beverages were produced, with or without the addition of ginger and/or turmeric. These bevrages were then left to ferment for 48 h. During fermentation, the biochemical and microbiological characteristics were determined using standardised methods. The results showed that the beverage produced exclusively from tamarind observed a more acidic pH, with a value of 2.1. Those produced with the addition of ingredients recorded pH values between 2.27 and 2.78. After 12 h of fermentation, an increase in reducing sugar content was observed in beverages T (from 0.10 to 0.21 mg/mL), TT (from 0.08 to 0.80 mg/ml) and TG (from 0.12 to 0.80 mg/mL). Furthermore, the tannin content in the TG beverage was found to have decreased significantly after 12 h of fermentation (from 0.749 to 0.351 mg GAE/mL). During fermentation, the number of lactic acid bacteria increased in all beverages, with values ranging from 2.0 $\times 10^3$ to 6.0 $\times 10^7$ CFU/mL. In contrast, yeast and mould counts decreased significantly, as did those of aerobic mesophilic flora (AMF). AMF counts decreased from 8.8 $\times 10^5$ to 1.0 $\times 10^1$ CFU/mL. With an initial yeast load of 2.102 CFU/mL, yeast was undetectable after 12 hours of fermentation. Weissella confusa was the only species of lactic acid bacteria identified during this fermentation. Finally, indicator or potentially pathogenic bacteria, namely Staphylococcus, E. coli, coliforms, Sulphite-reducing anaerobic bacteria (SRB) and Salmonella were not found in any of the beverages.

Introduction

The tamarind is a sweet and tart tropical fruit that is rich in macronutrients and micronutrients. Of all fruits, tamarind has the highest protein, carbohydrate and mineral content. It is also an excellent source of vitamin C and essential amino acids (Aka *et al.,* 2025; Samarou *et al.,* 2022; Pinar, 2014). Tamarind processing has evolved across different continents, with methods adapted to local preferences and the types of products in demand (Grollier *et al.,* 1998). In Africa, it is used in the production of beverages for nutritional, medicinal and therapeutic purposes (Bakayoko *et al.,* 2024a, Komutarin *et al.,* 2004). Devi and Boruah (2020) reported several medicinal properties of tamarind pulp, including antimalarial, antidiarrhoeal, antioxidant and antimicrobial properties.

In Côte d'Ivoire, tamarind pulp is used to produce a very popular beverage called 'tomidji', (Yao *et al.,* 2021; Samarou *et al.,* 2022). The production of this beverage is mainly carried out by women, whose primary motivation is to improve their family income, sometimes at the expense of the quality of the product offered to consumers. Yao *et al.,* (2021) reported that the physicochemical and microbiological quality of these beverages was unsatisfactory. Furthermore, the presence of antinutritional compounds such as phenolic compounds, tannins and L-Dopa (Pugalenthi *et al.,* 2004) in tamarind could render the drink unfit for consumption.

Fermentation is a process that improves both the nutritional value and sensory properties of food (Kaur *et al.,* 2019; Djéni *et al.,* 2008). It also contributes to the detoxification and elimination of certain undesirable compounds, including antinutritional factors present in raw foods, such as phytates, tannins and polyphenols (Gadaga *et al.,* 1999). Fermentation is generally carried out by microorganisms such as lactic acid bacteria, particularly *Enterococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus*, and *Pediococcus*, and yeasts and molds, mainly *Debaryomyces*, *Kluyveromyces*, *Saccharomyces*, *Geotrichium*, *Mucor*, *Penicillium*, and *Rhizopus* species (Sharma *et al.,* 2020; Blandino and Al-Aseeri, 2003). Furthermore, the addition of ingredients like pineapple, ginger, mint positively affects the concentration of micronutrients in fruit pulp-based juices (Ganou *et al.,* 2020). Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is one of the most commonly consumed dietary condiments in the world (Surh *et al.,* 1999). The evidence for the effectiveness of ginger as an antioxidant, anti-inflammatory agent, antinausea compound, and anticancer agent as well as the protective effect of ginger against other disease conditions are reviewed (Ali *et al.,* 2008; Borrelli *et al.,* 2005). Turmeric (*Curcuma longa* L.) is reported to possess numerous medicinal properties, including antioxidant, anti-protozoal, anti-tumor, anti-inflammatory and antivenom activity (Saha *et al.,* 2022; Tanvir *et al.,* 2017)

The aim of this study was to enhance the nutritional and microbiological quality of tamarind-based beverages by combining spontaneous fermentation with the addition of ingredients.

Materials and Methods

Plant materials

Tamarind pods (Tamarindus indica) were harvested between February to March from farmers in Korhogo, in the Poro region of Côte d'Ivoire. The rhizomes of turmeric (*Curcuma longua* L) and ginger (*Zingiber officinale*) were procured from the farmers in Divo, Lôh-Djiboua region of Côte d'Ivoire. All the materials were transported to the Nutrition and Food Security Laboratory at Nangui Abrogoua University for subsequent study.

Methods

Preparation of tamarind beverages

The tamarind pods were sorted and shelled, then macerated in 37 °C water for 15 minutes. The ingredients used for the different formulations (turmeric and ginger rhizomes) were sorted, washed and cleaned, then grated separately. Three tamarind-based beverages were prepared using these ingredients. One beverage was produced exclusively from tamarind (T), by adding 800 ml of water to 250 g of macerated tamarind pulp. Next, the tamarind-turmeric and tamarind-ginger beverages consisted of 90% tamarind pulp (225 g) and 10% turmeric or ginger (25 g). Finally, the tamarind, turmeric and ginger drink contained 90% tamarind pulp (225 g), 5% turmeric (12.5 g) and 5% ginger (12.5 g). The resulting beverages were filtered using a 5 mm sieve, and then salt was added at a rate of 20 g/L. The beverages were placed in jars, which were then hermetically sealed for 48 h of fermentation. Samples were taken at 0, 12, 24, 36 and 48 h for biochemical and microbiological analysis.

Determination of biochemical characteristic

The pH was determined using a pH meter according to the AOAC method (1990). The reducing sugar content of the fermented beverages was determined using the Bernfeld method (1955). The tannin concentration was determined using the method described by Bainbridge *et al.,* (1996).

Microbial flora characterization

Preparation of the initial solution

This method involved diluting 10 mL of each beverage with 90 mL of buffered peptone water, followed by successive dilutions. To prepare the 10^{-1} dilution, for example, 1 mL of the initial suspension was taken under aseptic conditions and mixed with 9 mL of buffered peptone water. Subsequent dilutions were made using the same technique up to the 10^{-x} dilution.

Enumeration of microorganisms

For mesophilic aerobic bacteria, 1 mL of the sample was added to a 90 mm Petri dish containing medium plate count agar (PCA). The Petri dishes were then incubated at 30 °C for 72 h (norme ISO 4833-1, 2013).

The enumeration of lactic acid bacteria was performed on MRS agar after the Petri dishes had been incubated for 48 h at 30°C (norme ISO 15214, 1998).

For yeasts and moulds 0.1 mL of decimal dilutions and the mother suspension were deposited on the surface of Sabouraud chloramphenicol agar and carefully spread using a sterile rake. The dishes were then incubated at 30 °C for 72 h (norme ISO 21527-1, 2008).

Staphylococcus was counted on Baird Parker medium to which 5% potassium tellurite egg yolk was added. The plates were then incubated in an oven at 37 °C for 24 h (norme ISO 6888-2, 2021).

Coliforms were enumerated on VRBL agar (Biokar, France), which was incubated for 48 h at 37 °C (norme ISO 4832, 2006).

Escherichia coli were enumerated on Rapid E. coli agar (Merck, Darmstadt, Germany), which was incubated for 24 h at 37 °C (norme ISO 16140, 2004).

Salmonella detection involved pre-enrichment by incubating the stock solution at 37 °C for 24 h, followed by enrichment consisting of inoculating 0.1 mL of the pre-enrichment broth into 10 mL of Vassiliadis Rappaport broth in sterile screw tubes. The seeded broths were then incubated at 42 °C for 24 h, after which isolation consisted of streaking Hektoen selective agar from the enrichment broths. Petri dishes containing the inoculated selective agar were then incubated at 37 °C for 24 h (norme ISO 6579-1, 2017).

The Sulphite-reducing anaerobic bacteria (SRB) counting method involved submitting the dilution to a 10-minute thermal treatment at 80°C in a thermostatic bath (Memmert, Germany) before introducing 5 mL of the inoculum into 20 mL of supercooled TSN in tubes (norme ISO 15213-1, 2023).

Identification of lactic acid bacteria

Isolation of lactic acid bacteria

Lactic acid bacteria strains were isolated at different times (0, 12, 24, 36 and 48 h) for each beverage (T, TT, TG and TCG). Five strains were randomly selected from countable plate of each sample.

Approximately 200 isolates (25 strains for each beverage) were obtained for further analysis.

Catalase test

The protocol consisted on transferring a loopful sample of bacteria into two or three drops of hydrogen peroxide 3% (w/v). When the formation of oxygen was observed by bubbles formation, the test was positive.

Gram stain

This method consisted on transferring a tiny sample of bacteria to a drop of sterile water in a slide and fix it with heat. Afterwards, the smear was flooded with crystal violet for 1 minute and the reagent was rinsed off gently. Lugol's iodine was added during 1 minute and it was gently washed. A decolorizer of 1:1 (v/v) mix of alcohol and acetone was added and quickly rinsed. Finally, safranin was added as a strain counter, during 1 minute and then gently washed. Afterwards, the smear was observed under optical microscopy with total magnifications of 400x and 1000x.

Species identification by MALDI-TOF mass spectrometry

The isolates to be identified and the reference strain (ATCC 8739) were cultivated on MRS agar for 12–18 h. Then, samples were prepared by transferring a small quantity of bacteria onto a VITEK MS-DS target plate using a sterile 1 µl loop. An on-target protein extraction step was then performed by overlaying the sample with 1 µL of 100% formic acid, after which it was allowed to dry at room temperature. Once dry, the spots were covered with 1 µl of matrix α-HCCA (α-cyano-4-hydroxycinnamic acid), prepared according to the manufacturer's instructions. Once the mixture had dried, the VITEK MS-DS slide was inserted into the VITEK MS PRIME instrument. Spectra were acquired using the default settings and compared with the database.

Statistical analysis

The results were processed using R software. An ANOVA followed by Tukey's post hoc tests were used to compare the means at the 5% significance level (p=0.05).

Results and Discussion

Evolution of biochemical characteristics of the Tamarind-based beverages

Evolution of pH

pH levels of the prepared beverages were acidic, ranging from 2.1 to 2.78 (Table 1). Beverage made exclusively from tamarind (T) was the most acidic. During fermentation, pH of this beverage increased significantly, rising from 2.1 to 2.42 after 36 h. pH of the TT beverage initially decreased from 2.78 to 2.52 after 12 h and then increased to 2.62 after 24 h. In contrast, pH of the TG and TTG drinks increased after 24 h, rising from 2.38 to 2.42 and from 2.27 to 2.56, respectively. These values then decreased, fluctuating between 2.43 and 2.47 for the TTG beverage. pH of the TG beverage remained relatively stable between 12 and 36 h, then decreased to 2.37 after 48 h.

Evolution of reducing sugar content

The reducing sugar content of the beverages ranged from 0.08 to 0.12 mg/mL (Table 2). After 12 h of fermentation, an increase in reducing sugar content was observed in beverages T (from 0.10 to 0.21 mg/mL), TT (from 0.08 to 0.80 mg/ml) and TG (from 0.12 to 0.80 mg/mL). In these three drinks, the reducing sugar content then decreased to 0.21 mg/mL for drink T, 0.19 mg/mL for drink TT and 0.07 mg/mL for beverage TG after 24 h. For beverage TTG, no significant variation was observed after 96 h of fermentation. The values fluctuated between 0.08 and 0.12 mg/mL.

Evolution of tannin content

The highest value (0.749 mg GAE/mL) of tannin content was recorded in the beverage prepared with tamarind and ginger (TG). Lower values, ranging from 0.471 to 0.509 mg GAE/mL, were observed in other beverages (Figure 1).

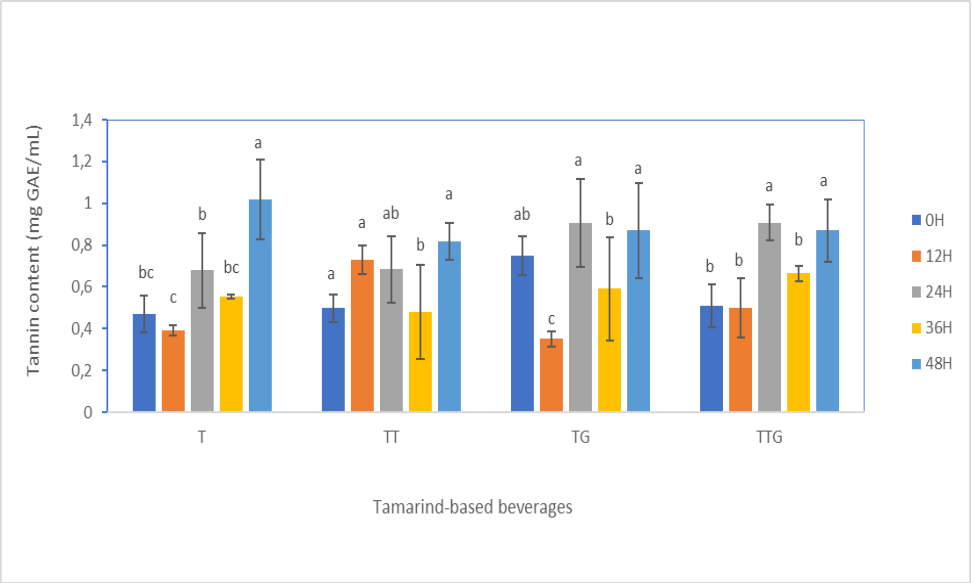
During fermentation, the tannin content fluctuated, characterised by alternating increases and decreases in tannin content. The tannin content in the TG beverage was found to have decreased significantly after 12 h of fermentation (from 0.749 to 0.351 mg GAE/mL). However, high concentrations of tannins (between 0.817 and 1.019 mg GAE/mL) were recorded at the end of the 48 h fermentation period. The highest value was observed in the beverage produced exclusively from tamarind (T).

Evolution of microorganisms

Table 3 shows the different microbial loads. In beverages T and TT, the AMF load increased significantly overall after 12 h of fermentation. The values increased from 7.0×10^4 to 1.5×10^6 CFU/mL for the T beverage and from 8.8×10^5 to 3.5×10^6 CFU/mL for the TT beverage. Then, they decreased steadily until the end of fermentation period (48 h). The final values observed were 1.0×10^1 CFU/mL and 4.9×10^2 CFU/mL for beverages T and TT, respectively. In beverages TG and TTG, the loads remained constant for up to 24 h and then decreased significantly. Thus, the values decreased from 3.8×10^5 to 5.4×10^3 CFU/mL, then to 5.0×10^1 CFU/mL after 48 h of storage for the TG beverage. For the TTG beverage, they decreased from 7.5×10^5 to 4.5×10^3 CFU/mL, then to 1.0×10^1 CFU/mL after 48 h.

Yeast was only observed in beverages at 0 h and 12 h, with loads ranging from 1.10^2 to 4.10^2 CFU/mL. Unlike yeast, lactic acid bacteria were detected at all fermentation times. Their loads increased significantly after 24 h of fermentation in all drinks.

Figure.1 Evolution of tannin content during the fermentation of tamarind-based beverages



T: Tamarind compound drink 100%, TT: Tamarind compound drink 90% and Turmeric 10%, TG: Tamarind compound drink 90% and Ginger 10%; TTG: Tamarind compound drink 90% and Turmeric5% and Ginger 5%
In the same column, values followed by the same letter do not differ significantly, Newman keul p=0.05

Table.1 Evolution of pH during the fermentation of tamarind-based beverages

Fermentation time (h)	Tamarind-based beverages			
	T	TT	TG	TTG
0	2.1±0.02^{a}	2.78±0.02^{c}	2.38±0.01^{a}	2.27±0.02^{a}
12	2.25±0.01^{a}	2.52±0.1^{a}	2.42±0.01^{b}	2.56±0.1^{c}
24	2.2±0.02^{a}	2.62±0.1^{b}	2.43±0.01^{b}	2.43±0.01^{b}
36	2.42±0.01^{b}	2.62±0.1^{b}	2.48±0.01^{b}	2.47±0.01^{b}
48	2.40±0.01^{b}	2.58±0.01^{b}	2.37±0.02^{a}	2.43±0.01^{b}

T: 100% tamarind based beverage, TT: 90% tamarind and 10% tumeric based beverage, TG: 90% tamarind and 10% ginger based beverage; TTG: 90% tamarind, 5% turmeric and 5% ginger.
In the same column, values followed by the same letter do not differ significantly, Newman keul p=0.05

Table.2 Evolution of reducing sugar content (mg/mL) during the fermentation of tamarind-based beverages

Fermentation time (h)	Tamarind-based beverages			
	T	TT	TG	TCG
0	0.10±0.09^{a}	0.08±0.00^{b}	0.12±0.04^{b}	0.08±0.05^{a}
12	0.21±0.01^{b}	0.80±0.03^{c}	0.80±0.03^{c}	0.09±0.01^{a}
24	0.21±0.03^{b}	0.19±0.02^{a}	0.07±0.01^{a}	0.09±0.01^{a}
36	0.11±0.02^{a}	0.08±0.01^{b}	0.12±0.02^{b}	0.12±0.02^{a}
48	0.10±0.01^{a}	0.8±0.01^{c}	0.11±0.01^{b}	0.12±0.01^{a}

T: Tamarind compound drink 100%, TT: Tamarind compound drink 90% and Turmeric 10%, TG: Tamarind compound drink 90% and Ginger 10%; TTG: Tamarind compound drink 90% and Turmeric5% and Ginger 5%.
In the same column, values followed by the same letter do not differ significantly, Newman keul p=0.05

Table.3 Changes in microorganism loads (CFU/mL) during the fermentation of tamarind-based beverages

Tamarind-based beverages	Storage time	AMF	Yeasts and moulds	Lactic acid bacteria	*Staphylococcus*	Coliformes	*E. Coli*	*Salmonella*	SRB
T	0	$(7.0\pm0.1)\times10^{4\,b}$	2.10^{2}	$(8.0\pm0.1)\times10^{3a}$	-	-	-	-	-
	12	$(1.5\pm0.2)\times10^{6\,a}$	1.10^{2}	$(1.4\pm0.1)\times10^{4a}$	-	-	-	-	-
	24	$(6.5\pm0.1)\times10^{4\,b}$	-	$(6.0\pm0.1)\times10^{5\,b}$	-	-	-	-	-
	36	$(2.5\pm0.1)\times10^{1\,c}$	-	$(8.0\pm0.1)\times10^{6c}$	-	-	-	-	-
	48	$(1.0\pm0.1)\times10^{1c}$	-	$(7.0\pm0.1)\times10^{5b}$	-	-	-	-	-
TT	0	$(8.8\pm0.2)\times10^{5\,b}$	2.10^{2}	$(6.0\pm0.1)\times10^{3a}$	-	-	-	-	-
	12	$(3.5\pm0.1)\times10^{6\,a}$	1.10^{2}	$(1.2\pm0.1)\times10^{4a}$	-	-	-	-	-
	24	$(6.5\pm0.1)\times10^{4\,c}$	-	$(8.0\pm0.1)\times10^{5b}$	-	-	-	-	-
	36	$(6.9\pm0.1)\times10^{2\,d}$	-	$(1.0\pm0.2)\times10^{7c}$	-	-	-	-	-
	48	$4.9\pm0.1)\times10^{2c}$	-	$(2.0\pm0.1)\times10^{7c}$	-	-	-	-	-
TG	0	$(2.7\pm0.1)\times10^{5\,a}$	4.10^{2}	$(2.0\pm0.1)\times10^{3a}$	-	-	-	-	-
	12	$(3.8\pm0.1)\times10^{5\,a}$	2.10^{2}	$(5.0\pm0.1)\times10^{3a}$	-	-	-	-	-
	24	$(5.4\pm0.2)\times10^{3\,b}$	-	$(3.0\pm0.1)\times10^{5b}$	-	-	-	-	-
	36	$(7.1\pm0.1)\times10^{1\,c}$	-	$(1.0\pm0.2)\times10^{7c}$	-	-	-	-	-
	48	$(5.0\pm0.1)\times10^{1c}$	-	$(2.0\pm0.1)\times10^{7c}$	-	-	-	-	-
TTG	0	$(3.9\pm0.1)\times10^{5\,b}$	3.10^{2}	$(3.0\pm0.1)\times10^{3a}$	-	-	-	-	-
	12	$(7.5\pm0.1)\times10^{5\,a}$	1.10^{2}	$(7.0\pm0.1)\times10^{3a}$	-	-	-	-	-
	24	$(4.5\pm0.2)\times10^{3\,c}$	-	$(7.0\pm0.1)\times10^{5b}$	-	-	-	-	-
	36	$(1.3\pm0.1)\times10^{1\,d}$	-	$(5.0\pm0.1)\times10^{7c}$	-	-	-	-	-
	48	$(1.0\pm0.2)\times10^{1c}$	-	$(6.0\pm0.1)\times10^{7c}$	-	-	-	-	-

T: Tamarind compound drink 100%,TT: Tamarind compound drink 90% and Turmeric 10%, TG: Tamarind compound drink 90% and Ginger 10%; TTG: Tamarind compound drink 90% and Turmeric5% and Ginger 5% In the same column, values followed by the same letter do not differ significantly, Newman keul p=0.05

AMF: aerobic mesophilic flora; SRB: Sulphite-reducing anaerobic bacteria

Table4 Homology between lactic acid bacteria isolated from different fermentation times and the reference strain *Weissella confuse*

Fermentation time (h)	Isolates	Reference identity	Identity (%)	Reference
0	S1	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S2	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S3	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S4	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S5	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
12	S1	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S2	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S3	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S4	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S5	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
24	S1	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S2	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S3	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S4	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S5	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
36	S1	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S2	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S3	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S4	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S5	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
48	S1	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S2	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S3	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S4	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2
	S5	*Weissella confusa*	99,9	VITEK® MS Data Base V3.2

Figure.1 Plant materials used in the preparation of beverages

A: Tamarind pods; B: Turmeric rhizomes; C: Ginger rhizomes

Specifically, they increased from 8.0 × 10^3 to 6.0 × 10^5 CFU/mL for beverage T and from 6.0 × 10^3 to 8.0 × 10^5 CFU/mL for beverage TT. As for beverages TG and TTG, the loads increased from 2.0 × 10^3 to 3.0 × 10^5 CFU/mL and from 3.0 × 10^3 to 7.0 × 10^5 CFU/mL, respectively. These bacteria were observed up to 48 hours of fermentation, at loads between 7.0 × 10^5 and 6.0 × 10^7 CFU/mL.

No indicator or potentially pathogenic bacteria, such as *Staphylococcus*, *coliforms*, *E. coli,* Sulphite-reducing anaerobic bacteria (SRB) or *Salmonella*, were detected in the beverages or during fermentation.

Identified species of lactic acid bacteria

All isolates showed 99.9% homology with the reference strain *Weisella confusa* from the VITEK® MS Data Base V3.2 (Table 4). Thus, *Weissella confusa* is the only species identified in beverages and during the 48 h of fermentation.

This study was conducted to enhance nutritional and microbiological quality of tamarind-based beverages by combining spontaneous fermentation with the addition of ingredients. The beverage made exclusively from tamarind (T) was the most acidic, with an average value of 2.1. The highly acidic nature of the drink is due to the high concentration of acidic compounds in the pulp. Grollier *et al.,* (1998) reported that the pulp contains significant amounts of organic acids (12 to 30% of dry matter), 98% of which is tartaric acid. Aka *et al.,* (2025) reported pH values between 2.3 and 2.8. Similar values (2.95) were reported by Hamacek *et al.,* (2013). The differences in pH values compared to our study could be due to the agroecological origin of tamarind. The beverages obtained after adding ginger and turmeric were less acidic (pH between 2.27 and 2.78) than those prepared exclusively with tamarind. These drinks could be more suitable for consumption. Several studies have shown that the highly acidic pH of beverages can cause mouth or stomach irritation, damage tooth enamel and have an unpleasant taste for the consumer **(**Sato *et al.,* 2021; Rajeev et Lewis, 2020; Seow *et al.,* 2005).

During fermentation, pH recorded significant variations in all beverages. These variations could be due to the activity of microorganisms. Indeed, fermenting bacteria are capable of breaking down various organic acids (malate, citrate, pyruvate, fumarate, tartrate, gluconate) with or without a source of glucose in order to maintain a pH favourable to fermentation (Hegazi and Abo-Elnaga, 1980). The reducing sugar content increased significantly during the first 12 h of fermentation, then decreased. This increase could be due to the hydrolysis of the starch contained in the beverages. During fermentation, reducing sugars first appear through the enzymatic hydrolysis of polysaccharides (starch, dextrins, cellulose) or disaccharides (sucrose) into simple sugars, then gradually disappear as they are consumed by microorganisms to produce energy and fermentation metabolites (Timmermans *et al.,* 2023). Regarding antinutritional compounds, the tannin content in the beverage TG was found to have decreased significantly after 12 h of fermentation (from 0.749 to 0.351 mg GAE/mL). These results are consistent with those of Ly *et al.,* (2017) who reported that the fermentation with *Saccharomyces cerevisiae* in kernel flour for 12 h is comparatively more effective than 24 h to lower tannins content and improve nutritive value. Furthermore, reducing the tannin content could improve the digestion and nutrients absorption, as authors have reported that tannins may inhibit digestion and absorption of nutrients, causing constipation and digestive tract disorders of monogastric animals (Pugalenthi *et al.,* 2004; De Caluwé *et al.,* 2010).

From a microbiological perspective, the AMF and yeast and mould load decreased during fermentation, while an increase in the lactic acid bacteria load was observed. The activity of lactic acid bacteria during fermentation could explain the significant decrease in the AMF and yeast and mould load in beverages. According to Ibrahim *et al.,* (2021), natural antimicrobial compounds synthesised by lactic acid bacteria (LAB) generally have an inhibitory effect on pathogens and significantly limit the action of organisms responsible for food spoilage. A high level of lactic acid bacteria in fermented beverages would therefore improve their hygienic quality. *Weissella confusa* was the only species of lactic acid bacteria identified during this fermentation. This species is associated with the fermentation of various foods (Tuccillo *et al.,* 2022). The genus *Weissella* was identified by Bakayoko *et al.,* (2024b) as lactic acid bacteria involved in fermentation of tamarin. These authors also identified species belong to the genus *Pediococcus*, *Lactobacillus* and *Lacococcus.* For insistance*, Weissella confusa* identified in our study, is a species that has been widely studied for its ability to synthesise functional dextran for use in various food applications. Dextran produced by *W. confusa* improves the quality of several plant-based products (Wang *et al.,* 2022). The safety evaluation of the *W. confusa* strain revealed that it exhibited no haemolytic activity, was susceptible to various antibiotics and did not produce biogenic amines, indicating an extremely low pathogenic risk. Furthermore, *W. confusa* exhibited enhanced acid and bile tolerance, indicating its probiotic properties (Liu *et al.,* 2025).

Staphylococcus, *E. coli*, coliformes, Sulphite-reducing

anaerobic bacteria (SRB) and *Salmonella* were not found in any of the drinks. According to Aka-Gbezo *et al.,* (2016) this absence could be due to the acidic pH of beverages. This could also be explained by compliance with Good Hygiene and Manufacturing Practices (GHP/GMP) during beverage production.

In conclusion, the combination of spontaneous fermentation and ddition of ingredients improved biochemical and microbiological characteristics of tamarind drink. The addition of ginger and turmeric reduced acidity of the beverage and made it more suitable for consumption. Spontaneous fermentation also inhibited AMF, yeasts and moulds.

It was more effective in reducing tannin content after 12 h for the beverage produced from tamarind and ginger. *Weissella confusa* is the only species of lactic acid bacteria identified during spontaneous fermentation. This species could therefore be used for controlled fermentation of tamarind-based beverages.

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Author Contributions

Agbo Adouko Edith: Conceived the original idea and designed the model and wrote the manuscript. Brou Kouakou: Major role is supervised the work. Attchelouwa Kouadio Constant: Software usage, and formal data analysis. Anoh Ettien Raïssa Inès Stéphanie: Conceptualization, supervision, validation, text editing, and data organization.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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