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Use of Chitosan and *Moringa oleifera* Leaf Extract as Bioconservation Agents for *Tchapalo*, a Traditional Beer from Côte d'Ivoire

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A B S T R A C T

Tchapalo is a traditional fermented drink widely consumed in West Africa. However, like many other traditional beers, *tchapalo* has a relatively short shelf life after production. The aim of this study was therefore to assess the effect of adding the natural preservatives *Moringa oleifera* and chitosan on the microbiological and physico-chemical stability of *tchapalo* during storage. For this purpose, four samples of *tchapalo* were collected from female brewers in two districts of Abidjan (Abobo and Yopougon). The samples were treated with concentrations of 0.5% and 1% chitosan, and concentrations of 2% and 3% *Moringa oleifera* leaf powder. The samples were then stored at room temperature, and microbiological and physico-chemical analyses were carried out at various time intervals (0, 1, 3, 5, 8 and 14 days). The results showed that the initial microbial loads in the tchapalo were 1.7×10^9 CFU/mL for aerobic mesophilic flora, 1.1×10^8 CFU/mL for yeasts, 8.6×10^3 CFU/mL for lactic acid bacteria, 7.2×10^2 CFU/mL for acetic acid bacteria and 4.0×10^1 CFU/mL for enterobacteria. These concentrations decreased to below 1 CFU/mL from the first day of storage through to the fourteenth day, following treatment with chitosan at concentrations of 0.5% and 1%. With *Moringa oleifera*, the reduction in microbial loads was more gradual, with levels falling below 1 CFU/mL from the fifth day of storage. In terms of physico-chemical properties, the titratable acidity and refractometric dry matter content were stabilised by the chitosan treatment. Chitosan is therefore an effective natural preservative for extending the shelf life of *tchapalo* by at least 8 days.

Introduction

In Africa, cereals are often used to produce various alcoholic drinks through fermentation processes. These drinks are produced in several countries and have different names depending on the country: *ikagage* in Rwanda, *pito* or *burukutu* in Nigeria and Ghana, *dolo* in Burkina Faso, *amgba* in Cameroon, *doro* or *chibuku* in Zimbabwe, *tchoukoutou* or *tchakpalo* in Togo and Benin, and *tchapalo* in Côte d'Ivoire (Djè *et al.,* 2008; Glover *et al.,* 2009; Lyumugabe *et al.,* 2012; Kayodé *et al.,* 2007).

Tchapalo is produced by the alcoholic fermentation of sweet wort made from sorghum, maize or millet malt. This beer is characterised by its cloudy appearance, its low alcohol content, and the presence of suspended solids and yeast. *Tchapalo* is very popular among the Ivorian population and plays a central role in many ceremonies and popular festivities, notably weddings, christenings, initiation rites and funerals (Djè *et al.,* 2008). Furthermore, *tchapalo* is commonly consumed during work in the fields, offering refreshment and fostering a sense of conviviality. It is also offered to visitors as a gesture of hospitality and welcome, demonstrating its role in strengthening social bonds within communities (Aka *et al.,* 2010; Oyewole et Isah, 2012). In the past, *tchapalo* production was concentrated in the north of Côte d'Ivoire and was intended for daily family consumption (N'Guessan, 2009; Amané *et al.,* 2012).

Nowadays, production has shifted from a domestic scale to commercial production. It has become a genuine income-generating economic activity, particularly in Abidjan, the economic capital, where there are over a hundred production and sales outlets known as 'tchapalodromes'. (Koffi *et al.,* 1993; Aka *et al.,* 2008). Furthermore, various therapeutic properties are traditionally attributed to tchapalo. Several authors report that this drink is used in traditional medicine for its supposed laxative, antimalarial and anti-haemorrhoidal effects (Amané *et al.,* 2005; Aka *et al.,* 2010). This beer is accessible to a large part of the population due to its relatively low price (Djè *et al.,* 2008; N'Guessan, 2009).

However, like many other traditional beers, *tchapalo* has a relatively short shelf life. According to Attchelouwa *et al.,* (2017), its shelf life at room temperature generally does not exceed two days; this constitutes a major constraint on its marketing and distribution. Furthermore, the short shelf life of *tchapalo* increases the burden on female producers, who are forced to produce fresh batches daily in order to meet demand and limit losses caused by the rapid deterioration of the product. This situation results in a heavy workload and constitutes a major challenge to the development of this activity.

Various methods have been proposed to extend the shelf life of beverages. These include heat treatment (pasteurisation), filtration and chemical treatment (the addition of artificial preservatives) (Ellis *et al.,* 2005; Osseyi *et al.,* 2011). Some of these methods can be complex and costly for small-scale producers to implement. Furthermore, the preservation of traditional beverages using chemical agents, such as sodium metabisulphite, can lead to adverse effects, including respiratory tract irritation and potentially life-threatening anaphylactic symptoms (Vally *et al.,* 2009).

Consequently, natural methods are being used more and more. Indeed, the authors have shown that extracts of *Moringa oleifera* reduce the growth of coliforms and moulds, thereby improving the microbiological stability of beverages (Ayirezang *et al.,* 2016). Other studies have reported that the addition of *Cymbopogon citratus* (lemongrass) essential oil has been shown to stabilise traditional beers produced in Benin (Dahouenon-Ahoussi *et al.,* 2012; Konfo *et al.,* 2012).

To date, no studies have been conducted to extend the shelf life of tchapalo. The aim of this study was therefore to evaluate the effect of adding *Moringa oleifera* leaf extract and chitosan on the microbiological and physico-chemical stability of tchapalo during storage at room temperature.

The aim of this study was to evaluate the effect of adding *Moringa oleifera* and chitosan on the microbiological and physico-chemical stability of *tchapalo* during storage. The results indicate that both preservatives tested significantly improve the microbiological and physico-chemical stability of *tchapalo*. However, chitosan appears to be the most effective preservative for extending the shelf life of *tchapalo* for at least eight days.

Materials and Methods

Sample collection

Samples of *tchapalo* were collected from two producers in the Yopougon and Abobo districts of Abidjan (Côte d'Ivoire). From each producer, 3 litres of tchapalo were

collected in sterile bottles and transported to the laboratory in a cool box containing dry ice. Once at the laboratory, the tchapalo was divided into 100 mL portions and distributed among 25 sterile 150 mL vials.

A portion was used for physico-chemical and microbiological analyses of the fresh *tchapalo*. The sampling procedure was repeated twice at each production site.

Preparation of the preservatives and adding to the *tchapalo*

The *Moringa oleifera* leaves were harvested and placed in a clean polyethylene bag. The leaves were thoroughly washed under running water, then air-dried at room temperature (30–31 °C) and a relative humidity of 20–30 per cent (Danso *et al.,* 2014) for 72 hours. The dried leaves were ground into a fine powder using a blender (Philips HR2000/16) for 3 minutes, then the Moringa powder was collected in a clean, airtight bowl and stored in the refrigerator at 5 °C. Next, 50 g of dried Moringa leaf powder was added to 500 ml of distilled water, which was sealed and stored for one week in the refrigerator (5 °C), with the contents shaken periodically to ensure a homogeneous mixture. The extract was filtered through muslin cloth, then boiled for 30 minutes. Finally, the extract was added to the *tchapalo* at concentrations of 2 % and 3 %.

For chitosan, 1 g was dissolved in 99 ml of 1 % glacial acetic acid to obtain a 1 % stock solution. The resulting solution was homogenised and stored at room temperature for 24 hours. This solution was then added to the *tchapalo* at concentrations of 0.5 % and 1 %.

The samples were stored at room temperature (27.7±1.7°C), then were removed successively at various time intervals (1 day, 3 days, 5 days, 8 days and 14 days) for analysis.

Microbiological analyses

Successive decimal dilutions of the various samples were prepared, and then the aerobic mesophilic flora (AMF), yeasts, lactic acid bacteria, acetic acid bacteria and enterobacteria were counted on their respective media. For AMF, 1 mL of the sample was added to 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).

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).

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

Physico-chemical analysis

The pH was measured using a digital pH meter (P107 Consort, Consort NV, Belgium). Total titratable acidity (TTA) was determined by titrating 5 mL of the sample with 0.1 M NaOH using phenolphthalein as an indicator and was expressed as percentage lactic acid. Total soluble solids (TSS), expressed as °Brix, were measured using a hand-held refractometer (Atago, Japan).

Statistical Analysis

Statistical analyses were performed using R software (version 4.5.1; R Core Team, 2025). Physicochemical parameters were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for multiple comparisons. Microbiological data were analyzed using the Kruskal–Wallis test. Differences were considered statistically significant at $p < 0.05$. Principal component analysis (PCA) was performed to investigate the relationships among treatments based on the physicochemical and microbiological variables and to identify the variables contributing most to sample discrimination.

Changes in microbial counts

Changes in GAM count

The GAM count observed in fresh *tchapalo* was 1.7×10^9 CFU/mL (Table 1). This count showed slight variations after eight days of storage of the *tchapalo* without a preservative. It ranged between 7.7×10^9 and 1.3×10^9 CFU/mL. After the 14th day of storage, the GAM count decreased significantly to 1.1×10^7 CFU/mL.

In the *tchapalo* to which preservative had been added, a decrease in total bacterial count was observed after one day of storage, and this decrease was greater with chitosan than with Moringa leaves. For the *tchapalo* treated with 0.5 % and 1 % chitosan, the total bacterial count was less than 1 after one day of storage (Table 1). The values observed up to 14 days of storage were below 3.6×10^1 CFU/mL.

In the *tchapalo* to which Moringa leaf powder had been added, the counts decreased significantly ($p < 0.05$) from 1.7×10^9 CFU/mL to 1.5×10^6 CFU/mL for the 2% concentration and to 5.6×10^5 CFU/mL for the 3% concentration. These counts decreased gradually to reach 1.0×10^3 CFU/mL for the 2 % concentration and 8.3×10^2 CFU/mL for the 3 % concentration.

Changes in yeast count

In fresh *tchapalo*, a yeast count of 1.1×10^8 CFU/mL was obtained (Table 2). In the absence of a preservative, the yeast concentration remained relatively stable after eight days of storage, at 1.0×10^6 CFU/mL.

In samples treated with 0.5% and 1% chitosan, yeast counts dropped to levels below 1 CFU/mL during the 14-day experiment.

With 2% and 3% *Moringa* leaf powder, counts decreased significantly after 3 days of storage. The values recorded were 2.2×10^5 CFU/mL and 2.5×10^4 CFU/mL for the 2% and 3% concentrations respectively. These counts then decreased to levels below 1 CFU/mL from the 8th day of storage.

Changes in lactic acid bacteria count

The lactic acid bacteria count observed in fresh *tchapalo* was 8.6×10^3 CFU/mL. This count did not show any significant variation ($p > 0.05$) during the 14 days of storage at room temperature. The values ranged from 9.2×10^3 to 3.2×10^3 CFU/mL (Table 3).

The lactic acid bacteria count remained below 1 CFU/mL from the first to the 14th day of storage following treatment with different concentrations of chitosan (0.5% and 1%).

With the addition of different concentrations of *Moringa* leaf powder, the lactic acid bacteria counts remained relatively stable between the first and third days. The values ranged from 1.5×10^4 to 2.4×10^4 CFU/mL. These counts were less than 1 CFU/mL from the fifth day of storage.

Changes in acetic acid bacteria count

The acetic acid bacteria count in fresh *tchapalo* was 7.2×10^2 CFU/mL. It increased significantly to 8.1×10^4 CFU/mL after three days of storage without preservatives. It then fluctuated without any significant difference being observed until the 14th day of storage. The values ranged from 1.4×10^5 to 2.3×10^5 CFU/mL (Table 4).

The acetic acid bacteria count remained below 1 CFU/mL from the first to the 14th day of storage following the addition of 0.5% and 1% chitosan.

However, the acetic acid bacteria count remained relatively stable after eight days when 2% and 3% *Moringa* was added to the *tchapalo*.

The values ranged from 1.1×10^3 to 5.5×10^3 CFU/mL. On the 14th day of storage, the counts were less than 1 CFU/mL.

Changes in the enterobacteria count

The enterobacteria count was 4.0×10^1 CFU/mL in fresh *tchapalo* and 2.5×10^1 CFU/mL after one day of storage without a preservative. This count decreased below 1 CFU/mL from the third day of storage onwards (Table 5).

For all samples to which a preservative had been added, the counts were below 1 CFU/mL from the first day of storage.

Changes in physico-chemical parameters

Changes in pH

The pH of fresh *tchapalo* was 3.35. This value remained relatively stable during eight days of storage of the *tchapalo* without preservatives. However, a significant decrease was reported on the 14th day, with a value of 2.5 (Figure 1).

The pH followed a similar trend in the samples to which 0.5% and 1% chitosan had been added, as well as in those treated with 2% and 3% *Moringa* leaf powder.

Indeed, the values remained relatively stable until the eighth day, then significantly decreased on the 14th day.

For chitosan, the values obtained on the final day of storage were 2.75 and 2.77 for 0.5 % and 1 % respectively. For *Moringa*, values of 2.7 and 2.61 were obtained for concentrations of 2 % and 3 % respectively.

Changes in titratable acidity

The titratable acidity of fresh *tchapalo*, measured at 1 %, did not change significantly after five days of storage without a preservative. On the contrary, it increased to 1.4 % after eight days of storage, then reached 1.6 % on the 14th day (Figure 2).

Following the addition of chitosan as a preservative, the titratable acidity increased after one day of storage. The values increased to 1.58 % and 1.66 % respectively for concentrations of 0.5 % and 1 %. Thereafter, the titratable acidity remained relatively stable until the 14th day of storage.

The titratable acidity increased significantly after five days of storage in the *tchapalo* to which *Moringa* leaf powder had been added. A value of 1.64 % was obtained for the 2 % concentration, with a value of 1.53 % for the 3 % concentration. The values remained relatively stable for the remainder of the experiment.

Changes in total soluble solids

A total soluble solids (TTS) value of 8.18°Brix was obtained for fresh *tchapalo*. In the absence of a preservative, this value decreased significantly to 6.4°Brix after five days of storage. This decline continued until the 14th day, with a value of 5.68 measured (Figure 3). In contrast, the TTS increased significantly after the first day of storage when chitosan was added to the *tchapalo*. The values increased from 8.18°Brix to 9.23°Brix and 9.58°Brix respectively for concentrations of 0.5% and 1%. Thereafter, the values remained relatively stable until the end of the experiment.

With *Moringa*, the TTS value showed little variation ($p>0.05$) during the first eight days of storage. The values fluctuated between 7.3 and 7.6°Brix for the 2 % concentration and between 7.2 and 7.73 for the 3 % concentration. However, a significant decrease was observed, with values of 6.23 and 6.2 respectively for the 2 % and 3 % *Moringa* concentrations at the end of the experiment.

Results of principal components analysis

The first two axes of the Principal Component Analysis (PCA) explained 98.56 % of the total variability in the data, with Axis 1 contributing 78.05 % and Axis 2 contributing 20.51 %. This high proportion of explained variance indicates that the factorial plan formed by these two axes accurately reflects the information contained in the data.

Axis 1 primarily distinguishes the chitosan-treated samples from the control sample (Figure 4). The chitosan-treated samples are negatively correlated with this axis, while the control sample is positively correlated with it. This axis also contrasts the TTS and titratable acidity, which are negatively correlated, with the counts of yeast, lactic acid bacteria and AMF, which are positively correlated (Figure 5).

This pattern suggests that the chitosan-treated samples are characterised by high values of TTS and titratable acidity, associated with low microbial loads. Conversely, the control samples exhibit higher microbial activity, accompanied by a reduction in TTS and titratable acidity.

Axis 2, which represents 20.51 % of the total variability, is mainly associated with samples treated with *Moringa oleifera* leaf powder, which are negatively correlated with this axis. Some acetic acid bacteria loads also show a negative correlation with this axis, suggesting that the *M. oleifera*-based treatment has a minor impact on acetic acid bacteria

Overall, PCA reveals a clear separation of the samples according to the treatment applied. Chitosan appears to be associated with improved physicochemical and microbiological stability of *tchapalo*, while samples treated with *M. oleifera* exhibit distinct characteristics, particularly with regard to acetic acid bacteria. Furthermore, different concentrations of the same preservative showed similar effects.

The aim of this study was to evaluate the effect of adding *Moringa oleifera* and chitosan on the microbiological and physico-chemical stability of *tchapalo* during storage. The total viable count observed in fresh *tchapalo* was 1.7×10^9 CFU/mL. This high initial total aerobic count reflects the high level of microbial activity

characteristic of traditional fermented beverages. Similar counts have been reported in *tchapalo* and other traditional African beers such as *dolo*, *pito* and *burukutu* (Attchelouwa *et al.,* 2017).

The initial yeast count observed in fresh *tchapalo* (1.1×10^8 CFU/mL) confirms the key role played by these microorganisms in the fermentation of this traditional drink. These yeasts play an active role in the conversion of fermentable sugars into ethanol, carbon dioxide and various aromatic compounds that contribute to the organoleptic characteristics of *tchapalo*. Yeast counts ranging from 10^6 to 10^9 CFU/mL have been reported in several traditional African beers, notably *tchapalo*, *dolo* and *pito* (Sawadogo-Lingani *et al.,* 2007; Attchelouwa *et al.,* 2017).

The initial lactic acid bacteria count observed in fresh *tchapalo* (8.6×10^3 CFU/mL) confirms the presence of this flora, which is characteristic of traditional fermented cereal-based drinks. Lactic acid bacteria play a vital role in the fermentation of *tchapalo* by producing organic acids, particularly lactic acid, which contributes to the acidification, microbiological stability and organoleptic properties of the drink (Sawadogo-Lingani *et al.,* 2007; Holzapfel, 2002).

An initial acetic acid bacteria count of 7.2×10^2 CFU/mL was observed in the fresh *tchapalo*. This count increased significantly after three days of storage to 8.1×10^4 CFU/mL. This could be attributed to ethanol metabolism. Indeed, during the storage of beverages, acetic acid bacteria can multiply and convert ethanol into acetic acid (De Roos and De Vuyst, 2018). Similar findings have been reported in several African fermented beverages, where the growth of acetic acid bacteria accompanies the deterioration phase of the product and contributes to an increase in volatile acidity (Sawadogo-Lingani *et al.,* 2007; Attchelouwa *et al.,* 2017).

The presence of an initial count of 4.0×10^1 CFU/mL of Enterobacteriaceae in fresh *tchapalo* indicates moderate contamination of the drink. Their presence in traditional fermented drinks is often linked to the microbiological quality of the raw materials, the water used, the processing equipment or the production environment (Holzapfel, 2002; Muyanja *et al.,* 2003).

The addition of chitosan at concentrations of 0.5 % and 1 % resulted in almost immediate inhibition of AMF, yeasts, lactic acid bacteria and acetic acid bacteria. The high sensitivity of these microorganisms to chitosan could be attributed to the mode of action of this biopolymer. Chitosan consists of protonated amine groups that interact with the negative charges present on the surface of bacterial cells. This interaction causes disruption of the cytoplasmic membrane, an increase in its permeability and the leakage of intracellular constituents, leading to a halt in growth and subsequently to cell death (Kong *et al.,* 2010). In acid-fermented beverages, chitosan is particularly effective due to its improved solubility and stronger interaction with microbial cells (Rhoades, 2006). Kong *et al.,* (2010) demonstrated that chitosan is particularly effective against food spoilage yeasts such as *Saccharomyces cerevisiae*, *Candida* spp. and *Zygosaccharomyces* spp. Furthermore, the elimination of acetic acid bacteria could be a significant advantage for the preservation of *tchapalo*, as these microorganisms are directly involved in increasing volatile acidity and in the development of the characteristic vinegary taste of spoiled fermented beverages (De Roos and De Vuyst, 2018).

When the *tchapalo* was treated with *Moringa oleifera* leaf extract, a regular decrease in total aerobic micro-organism counts was observed during storage. Furthermore, gradual decreases in the counts of yeasts, lactic acid bacteria and acetic acid bacteria were observed. Thus, the antimicrobial effect of *Moringa oleifera* proved to be more gradual. This antimicrobial activity is probably due to the presence of numerous bioactive compounds such as polyphenols, flavonoids, tannins, alkaloids and isothiocyanates. These molecules are capable of disrupting cell membranes, inhibiting certain metabolic enzymes and causing alterations in the energy-production mechanisms of microorganisms (Anwar *et al.,* 2007; Vergara-Jimenez *et al.,* 2017). Furthermore, Rahman *et al.,* (2009) demonstrated that the antimicrobial activity of *Moringa oleifera* extracts depends strongly on the concentration used, the contact time and the nature of the targeted microorganisms. Dahouenon-Ahoussi *et al.,* (2012) demonstrated that increasing the concentration of plant extracts significantly enhances the inhibition of yeasts and moulds in traditional foods and beverages.

A comparison of the two treatments shows that chitosan exhibits faster and more effective antibacterial activity than Moringa oleifera extract. This difference may be associated with the very nature of the mechanisms of action of the two preservatives.

Table.1 Changes in AM count (CFU/mL) during storage at room temperature of tchapalo treated with preservatives

Preservatives (%)	Storage time (days)					
	0	1	3	5	8	14
Control sample (0%)	$(1.7\pm 0.9) \times 10^{9a}$	$(1.3\pm 0.5) \times 10^{9a}$	$(2.1\pm 1.9) \times 10^{9é}$	$(1.9\pm 2.0) \times 10^{9a}$	$(7.7\pm 4.9) \times 10^{8a}$	$(1.1\pm 1.9) \times 10^{7b}$
Chitosan (0.5%)	$(1.7\pm 0.9) \times 10^{9a}$	< 1	$(3.6 \pm 2.1) \times 10^{1b}$	< 1	$(2.8 \pm 3.9) \times 10^{1b}$	< 1
Chitosan (1%)	$(1.7\pm 0.9) \times 10^{9a}$	< 1	< 1	$(1.7 \pm 2.4) \times 10^{1b}$	$(1.5 \pm 2.1) \times 10^{1b}$	< 1
***Moringa* (2%)**	$(1.7\pm 0.9) \times 10^{9a}$	$(1.5 \pm 2.1) \times 10^{6b}$	$(2.2 \pm 3.1) \times 10^{6b}$	$(1.1 \pm 1.4) \times 10^{5c}$	$(4.2 \pm 1.4) \times 10^{3d}$	$(1.0 \pm 5.6) \times 10^{3d}$
***Moringa* (3%)**	$(1.7\pm 0.9) \times 10^{9a}$	$(5.6 \pm 8.0) \times 10^{5b}$	$(1.3 \pm 1.7) \times 10^{6b}$	$(3.5 \pm 3.2) \times 10^{4c}$	$(1.9 \pm 2.5) \times 10^{4c}$	$(8.3 \pm 0.8) \times 10^{2d}$

The values are expressed as the mean ± standard deviation for four independent trials. Different letters on the same line indicate significant differences ($\alpha < 0.05$)

Table.2 Changes in yeast count (CFU/mL) during storage at room temperature of *tchapalo* treated with preservatives

Preservatives (%)	Storage time (days)					
	0	1	3	5	8	14
Control sample (0%)	$(1.1 \pm 0.7) \times 10^{8a}$	$(1.2\pm 1.4) \times 10^{8a}$	$(1.1 \pm 0.9) \times 10^{8a}$	$(7.6\pm 4.2) \times 10^{7a}$	$(9.7 \pm 5.2) \times 10^{7a}$	$(1.0 \pm 2.3) \times 10^{6b}$
Chitosan (0.5%)	$(1.1 \pm 0.7) \times 10^{8}$	< 1	< 1	< 1	< 1	< 1
Chitosan (1%)	$(1.1 \pm 0.7) \times 10^{8}$	< 1	< 1	< 1	< 1	< 1
***Moringa* (2%)**	$(1.1 \pm 0.7) \times 10^{8a}$	$(1.4 \pm 1.1) \times 10^{7a}$	$(3.6 \pm 3.8) \times 10^{5b}$	$(2.2 \pm 5.6) \times 10^{5b}$	< 1	< 1
***Moringa* (3%)**	$(1.1 \pm 0.7) \times 10^{8a}$	$(1.3 \pm 1.1) \times 10^{7a}$	$(7.2 \pm 4.7) \times 10^{4b}$	$(2.5 \pm 0.6) \times 10^{4b}$	< 1	< 1

The values are expressed as the mean ± standard deviation for four independent trials. Different letters on the same line indicate significant differences ($\alpha < 0.05$)

Table.3 Changes in lactic acid bacteria count (CFU/mL) during storage at room temperature of *tchapalo* treated with preservatives

Preservatives (%)	Storage time (days)					
	0	1	3	5	8	14
Control sample (0%)	$(8.6 \pm 8.9) \times 10^{3a}$	$(8.8 \pm 2.8) \times 10^{3a}$	$(5.1 \pm 3.6) \times 10^{3a}$	$(9.2 \pm 5.7) \times 10^{3a}$	$(3.2 \pm 3.5) \times 10^{3a}$	$(4.1 \pm 0.8) \times 10^{3a}$
Chitosan (0.5%)	$(8.6 \pm 8.9) \times 10^{3}$	< 1	< 1	< 1	< 1	< 1
Chitosan (1%)	$(8,6 \pm 8,9) \times 10^{3}$	< 1	< 1	< 1	< 1	< 1
***Moringa* (2%)**	$(8.6 \pm 8.9) \times 10^{3a}$	$(1.5 \pm 8.9) \times 10^{4a}$	$(1.7 \pm 2.4) \times 10^{4a}$	< 1	< 1	< 1
***Moringa* (3%)**	$(8.6 \pm 8.9) \times 10^{3a}$	$(1.8 \pm 2.1) \times 10^{4a}$	$(2.4 \pm 2.1) \times 10^{4a}$	< 1	< 1	< 1

The values are expressed as the mean ± standard deviation for four independent trials. Different letters on the same line indicate significant differences ($\alpha < 0.05$)

Table.4 Changes in acetic acid bacteria count (CFU/mL) during storage at room temperature of *tchapalo* treated with preservatives

Preservatives (%)	Storage time (days)					
	0	1	3	5	8	14
Control sample (0%)	$(7.2 \pm 1.8) \times 10^{2a}$	$(5.1 \pm 2.2) \times 10^{3ab}$	$(8.1 \pm 1.3) \times 10^{4b}$	$(1.4 \pm 3.7) \times 10^{5b}$	$(2.1 \pm 0.9) \times 10^{5b}$	$(2.3 \pm 2.5) \times 10^{5b}$
Chitosan (0.5%)	$(7.2 \pm 1.8) \times 10^{2}$	< 1	< 1	< 1	< 1	< 1
Chitosan (1%)	$(7.2 \pm 1.8) \times 10^{2}$	< 1	< 1	< 1	< 1	< 1
Moringa (2%)	$(7.2 \pm 1.8) \times 10^{2a}$	$(2.2 \pm 6.1) \times 10^{3a}$	$(3.6 \pm 3.7) \times 10^{3a}$	$(1.9 \pm 0.3) \times 10^{3a}$	$(1.4 \pm 2.1) \times 10^{3a}$	< 1
Moringa (3%)	$(7.2 \pm 1.8) \times 10^{2a}$	$(1.1 \pm 7.4) \times 10^{3a}$	$(5.5 \pm 7.5) \times 10^{3a}$	$(2.8 \pm 1.6) \times 10^{3a}$	$(1.0 \pm 1.2) \times 10^{3a}$	< 1

The values are expressed as the mean ± standard deviation for four independent trials. Different letters on the same line indicate significant differences ($\alpha < 0.05$)

Table.5 Changes in enterobacteria count (CFU/mL) during storage at room temperature of *tchapalo* treated with preservatives

Preservatives (%)	Storage time (days)					
	0	1	3	5	8	14
Control sample (0%)	$(4.0 \pm 5.6) \times 10^{1a}$	$(2.5 \pm 2.6) \times 10^{1a}$	< 1	< 1	< 1	< 1
Chitosan (0.5%)	$(4.0 \pm 5.6) \times 10^{1}$	< 1	< 1	< 1	< 1	< 1
Chitosan (1%)	$(4.0 \pm 5.6) \times 10^{1}$	< 1	< 1	< 1	< 1	< 1
Moringa (2%)	$(4.0 \pm 5.6) \times 10^{1}$	< 1	< 1	< 1	< 1	< 1
Moringa (3%)	$(4.0 \pm 5.6) \times 10^{1}$	< 1	< 1	< 1	< 1	< 1

The values are expressed as the mean ± standard deviation for four independent trials. Different letters on the same line indicate significant differences ($\alpha < 0.05$)

Figure.1 Changes in pH during storage at room temperature of *tchapalo* treated with chitosan (A) and *Moringa* (B)

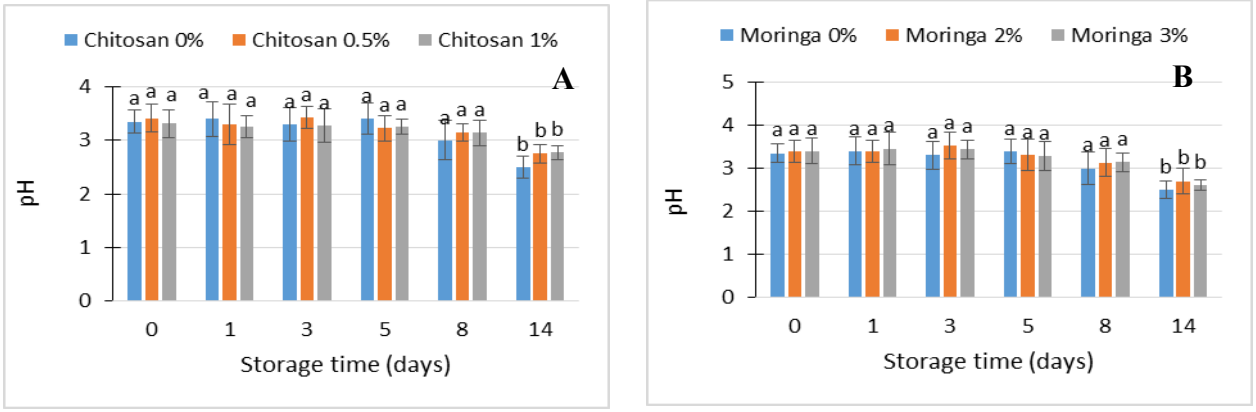


Figure.2 Changes in titrable acidity during storage at room temperature of *tchapalo* treated with chitosan (A) and *Moringa* (B)

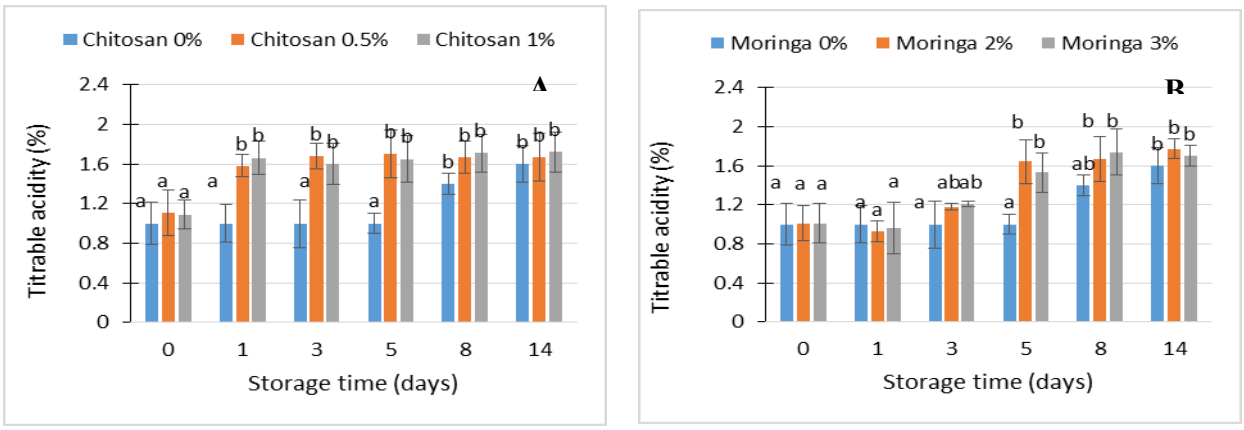


Figure.3 Changes in TTS during storage at room temperature of *tchapalo* treated with chitosan (A) and *Moringa* (B)

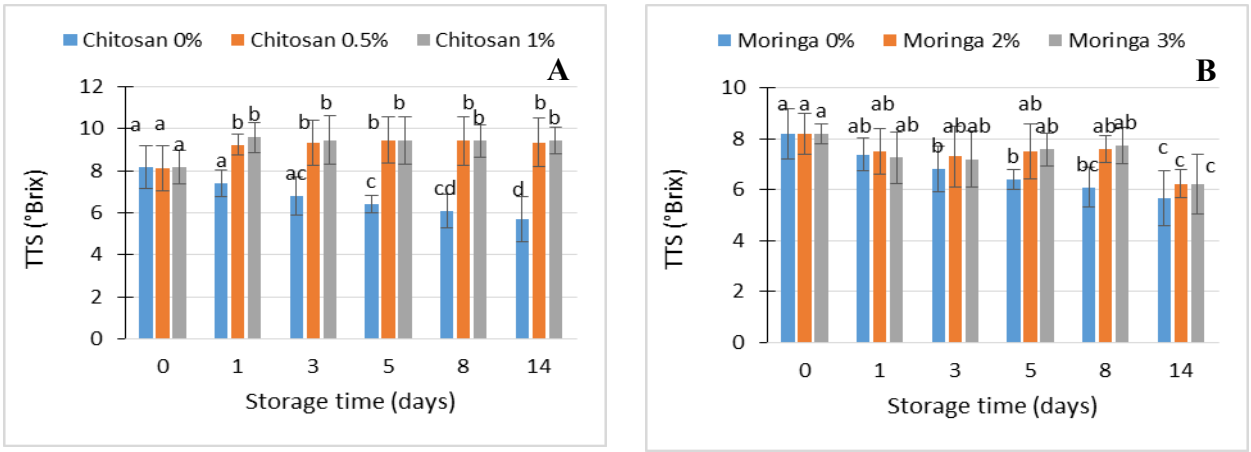


Figure.4 Plot of individuals derived from the principal component analysis

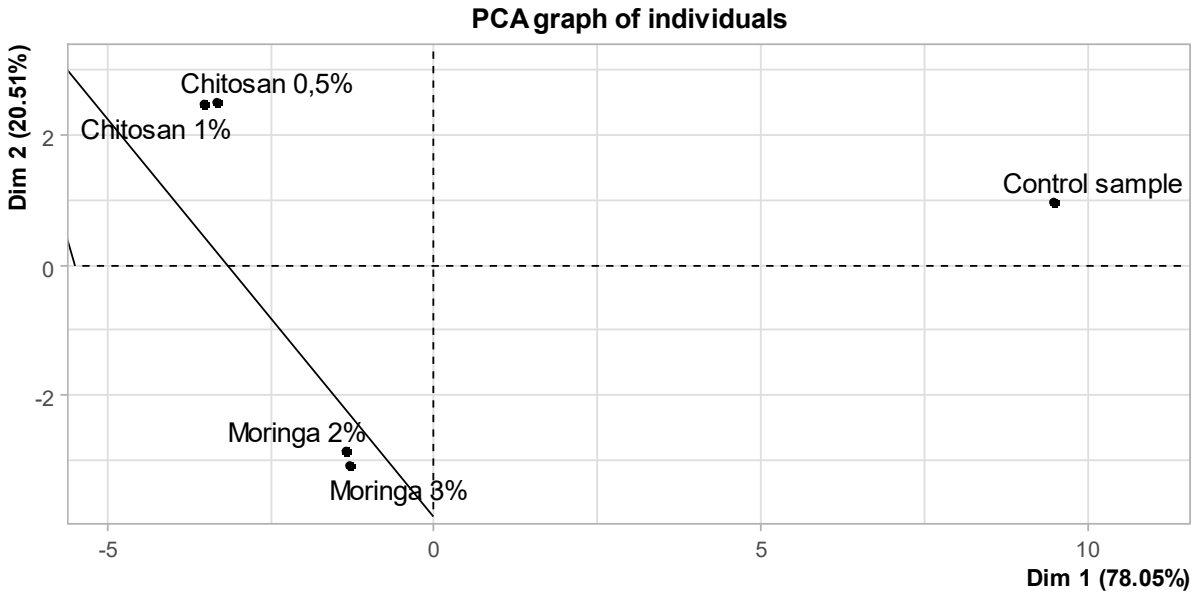
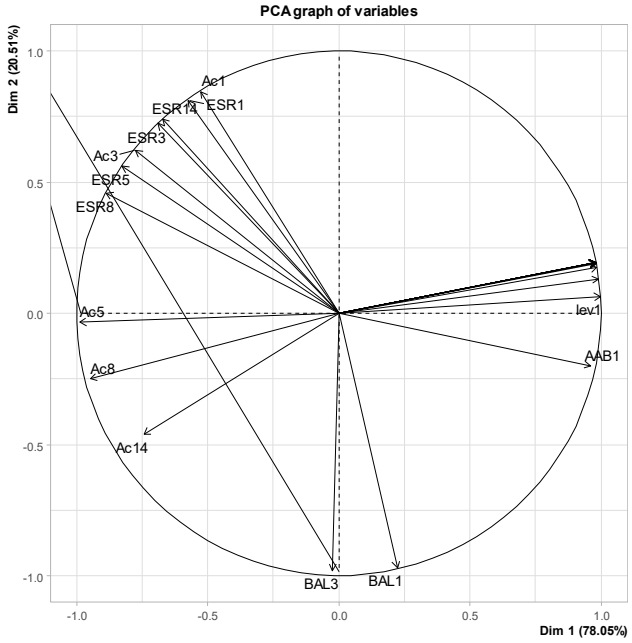


Figure.5 Graph of the variables from the principal component analysis



Chitosan acts directly on cellular structures, whereas the antimicrobial compounds in Moringa must first be released and diffused into the medium before they can exert their full effect (Kong *et al.,* 2010; Leone *et al.,* 2015).

The titratable acidity of fresh *tchapalo* was 1 %, confirming the acidic nature of this traditional fermented drink. In fermented drinks, this acidity is mainly due to the accumulation of lactic acid produced by lactic acid bacteria, as well as the presence of acetic acid resulting from the activity of acetic acid bacteria (Blandino *et al.,* 2003; Sawadogo-Lingani *et al.,* 2007). During the storage of *tchapalo* without preservatives, the increase observed from the eighth day (1.4 %) and then on the fourteenth day (1.6 %) may reflect the continued

microbial metabolic activity within the *tchapalo*. This trend is consistent with the significant growth of acetic acid bacteria observed on the third day, as well as with the persistence of lactic acid bacteria throughout the storage period. These microorganisms may continue to produce organic acids, leading to an increase in titratable acidity (Attchelouwa *et al.,* 2017; De Roos et De Vuyst, 2018).

The TTS of fresh *tchapalo* was 8.18 °Brix. This value primarily reflects the soluble sugar content, but also the presence of other dissolved compounds such as organic acids, soluble proteins, peptides and certain minerals derived from sorghum and the malting and fermentation processes. The values obtained are comparable to those reported for several traditional fermented cereal-based beverages from West Africa (Sawadogo-Lingani *et al.,* 2007; Attchelouwa *et al.,* 2017).

In the control sample without preservatives, the TTS decreased significantly from 8.18 to 6.4 °Brix after five days of storage, then continued to decline until reaching 5.68 °Brix on the fourteenth day. This trend probably reflects the continuation of fermentation activity during storage. Indeed, the yeasts and certain bacteria present in the tchapalo continue to metabolise residual sugars to produce ethanol, organic acids, carbon dioxide and various secondary metabolites.

The gradual consumption of fermentable carbohydrates thus leads to a decrease in the concentration of soluble solids (Steinkraus, 1996; Blandino *et al.,* 2003).

In the samples treated with chitosan, titratable acidity and TTS remained relatively stable up to the fourteenth day. This stability of TTS and titratable acidity is consistent with the microbiological results obtained, which showed a significant reduction in yeasts, lactic acid bacteria and acetic acid bacteria following the addition of chitosan. By limiting the growth of these organic acid-producing microorganisms, chitosan reduces subsequent fluctuations in titratable acidity and thus contributes to the physicochemical stabilisation of the product (Rhoades, 2006; Kong *et al.,* 2010). This effect has also been observed in several foods preserved with chitosan, where the inhibition of the fermentative microflora slows the progression of parameters related to acidification (Dutta *et al.,* 2009).

In the samples treated with *Moringa oleifera*, the titratable acidity increased significantly after five days of storage. Furthermore, the TTS values decreased significantly on the fourteenth day. These results could be due to the persistence of yeasts, lactic acid bacteria and acetic acid bacteria in the samples to which *Moringa oleifera* had been added during storage. These microorganisms probably continued to produce organic acids, thereby leading to an increase in titratable acidity.

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Data availability

The datasets supporting the conclusions of this article are available from the corresponding author upon reasonable request

Author contributions

RAN Yao: Methodology, Investigation, data analysis and writing—original draf; KC Attchelouwa: Methodology, data curation and writing—original draft; MB Tano: Supervision, writing—review and editing. FK N'guessan: Conceptualization, resources, methodology, supervision reviewed and approved the final version of the manuscript.

Declarations

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Consent to Participate Not applicable.

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