

Evaluation of the physico-chemical and microbiological quality of tshui wine made from the fruit of *Grewia coriacea* Mast

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Abstract

This study focused on the variability of physico-chemical and microbiological quality properties of wine made from the traditional fruit juice of *Grewia coriacea* Mast) commonly known as "tshui", widely consumed and appreciated in Congo Brazzaville. Samples of sweet must (intermediate product) taken every 5 days for 30 days during alcoholic fermentation were used for the various physico-chemical and microbiological analyses. These analyses were carried out using standard physicochemical and microbiological methods. Physicochemical results showed that the wine obtained had a density of 1.04 ± 0.2 , a pH of 3.2 ± 0.4 , a total acidity of 1.3 ± 0.2 , a Brix degree of $13.7 \pm 2.1\%$, an alcohol degree of $5.2 \pm 1.6\%$, a vitamin C content of 1.7 ± 0.2 (Kcal/100 ml) and a temperature of 26.2 ± 0.5 °C. Polyphenols and anthocyanins in the wine increased as a result of the high alcohol content and yeast-induced acidification. Microbiological analysis revealed that yeasts, with 1.7×10^8 cfu/mL, were the dominant microflora in the wine, while the lactic acid bacteria isolated belonged to the *Lactobacillus* and *Leuconostoc* genera.

Keywords: Evaluation; Physicochemical and microbiological qualities; Tshui wine; Fermentation

1. Introduction

Fermentation has been used by mankind for millennia to obtain foods of improved nutritional value (V. Gotcheva, 2001). In the Congo, certain non-ligneous forest products are often transformed into beverages, the manufacture of which includes an essential alcoholic fermentation stage (J. J. Assiedu et al, 1991; A. V. D. A. Kühle et al, 2001; E. S. Naumova et al, 2003). This is the case of *Grewia coriacea* fruits called tshui-téké (figure 1) in the Republic of Congo. In fact, the Congolese population is extremely fond of tshui-téké juice, which is a beautiful purplish-red color. A centuries-old culture is based on the consumption of wine made from the fruit of *Grewia coriacea* Mast. Preparing a drink from *Grewia coriacea* fruit involves an extraction stage, followed by pasteurization and storage. The transformation of *Grewia coriacea* Mast fruit juice into wine is essentially a microbiological process. Its empirical technological process involves double fermentation: alcoholic fermentation combined with natural lactic fermentation (N. Maoura et al, 2006). Over the years, manufacturing conditions have remained unchanged. What's more, the production process suffers from a crucial lack of precision measuring instruments, good hygiene practices and technology such as where successive sweet musts are inoculated with the ferment from previous fermentations, with little knowledge of the actual nature of this

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ferment. The resulting wines are unstable and of poor quality. In addition, yeasts, mainly the *Saccharomyces cerevisiae* species, transform sugars into alcohol and carbon dioxide during alcoholic fermentation (J. P. Larpent, 1991). These microbial metabolisms determine the physico-chemical characteristics of the wine (alcohol content, pH, density...) and contribute to the production of secondary metabolites, notably polyphenols, which contribute to the structure of the wine and reveal its gustatory and sensory potential (J. P. Larpent, 1991). *Grewia coriacea* is one of the most widely consumed spontaneous edible fruits in Congo's main cities. *Grewia coriacea* fruits also have medicinal virtues with multiple therapeutic properties. Studies carried out on the *Grewia coriacea* species show its richness in anthocyanins, vitamin C, antioxidants and lipids (Okandze Mbama et al, 2018; A.B. Madiélé Mabika et al, 2016, M.G. Okiemy-Akeli et al, 2016; L. Attibayeba et al, 2010; L. Attibayeba et al, 2007). As with many fermented beverages consumed in the Republic of Congo, little is known about their nutritional value and organoleptic qualities. With the aim of improving the organoleptic, nutritional and microbiological qualities of this traditional product, we proposed in this work to produce *Grewia coriacea* wine and to study the variability of these physicochemical properties and microbial biodiversity.

2. Materials and methods

2.1. Study setting

The *Grewia coriacea* wine production trials were carried out in the period from June to August 2021 at INRSITT, located in the Cité Scientifique ex-ORSTOM in Brazzaville. Physicochemical analyses were carried out at the Food Technology Laboratory, while microbiological analyses were carried out at the Laboratoire de Microbiologie et Biochimie des Substances Naturelles of the Faculty of Science and Technology.

2.2. Plant material

The plant material consisted of *Grewia coriacea* Mast fruits purchased at the Texaco market in arrondissement 5 (Ouenzé), Brazzaville.



Figure 1 *Grewia coriacea* Mast fruits

SARIS Congo white sugar purchased at the Texaco market; and yeast (*Saccharomyces cerevisiae*).

2.3. Methods

2.3.1. Wine-making process

The tshui wine-making process is shown in Figure 2. The process is divided into three main stages: fruit treatment, chaptalization and fermentation.

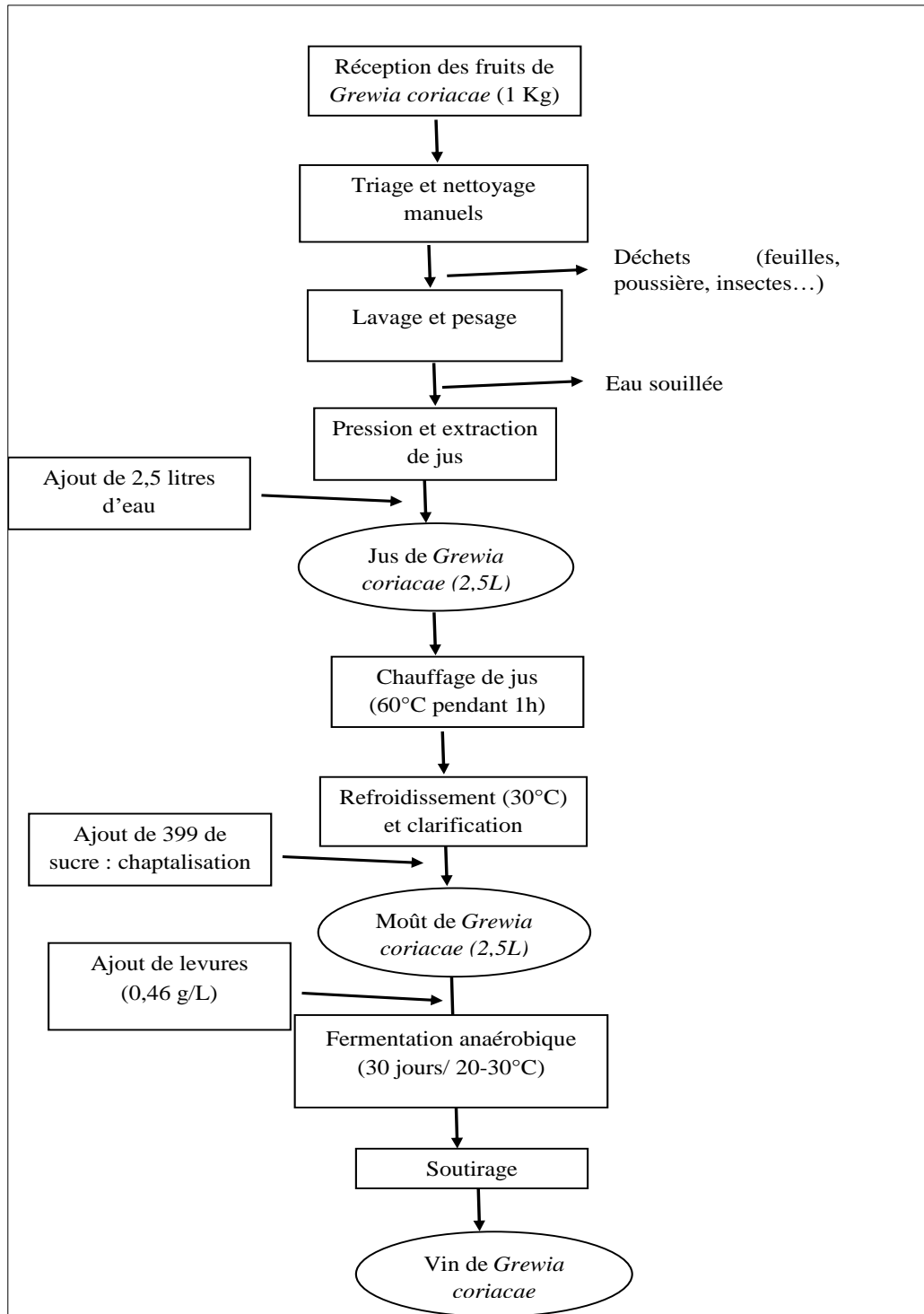


Figure 2 Wine-making process

2.4. Fruit processing and juice extraction

A 1 kg quantity of fresh, sorted fruit was weighed, then rapidly washed under a jet of water to remove any contamination. The fruit is then mechanically pressed by hand to separate the stones from the shells. A volume of 2.5 L of water is then added to the shells, and the mixture is brought to the boil at a temperature of 60°C for 1 hour. The mixture is cooled and filtered to remove insoluble particles. The filtrate obtained constitutes the must. Initial density and residual sugar content measurements were carried out on the must.

2.4.1. Chaptalization

Chaptalization is the process of adding sugar (sucrose) to the must to increase the alcohol content.

To make a 12% vol. wine, we measured the potential alcoholic strength of the must, which corresponds to 2.5% vol. This value must be subtracted from the desired final alcohol content, i.e. $12 - 2.5 = 9.5\%$ vol. additional alcohol content.

It has been established that 16.83 grams of fermented sugars provide 1 degree of alcohol (S. Dequin, 2008; I.P. Regnault, 1990). For 2.5 L of must, we added 399.71 g of sugar (i.e. $9.5 \times 16.83 \times 2.5$).

2.4.2. Fermentation

Fermentation is an essential stage in wine-making, transforming sugars into alcohol, with simultaneous production of CO₂ and development of various floral, fruity or organoleptic compounds. For this purpose, we used the commercial yeast strain *Saccharomyces cerevisiae* at a dose of 0.46 g/L, available as active dry yeast. This yeast needs to be activated in a few milliliters of must for 30 minutes to obtain a good result.

The experimental semi-pilot set-up consisted of a 10 L fermenter, equipped with all the instruments needed to monitor fermentation progress (densimeter, pH-meter, refractometer, thermometer).

2.5. Fermentation took place over 30 days, and samples were taken every 5 days during fermentation.

2.5.1. Analytical technique

Analysis of physico-chemical parameters

The physicochemical analyses of the must samples taken were of two kinds. On the one hand, we used classic or standard basic methods of direct reading and titrimetry, and on the other, chromatographic and spectrophotometric measurements.

pH was measured by direct immersion of the electrode of an electronic pH meter (HANNA, Japan) in the must contained in a beaker containing 20 ml of sample at 25°C. Temperature was measured with a thermometer (CONSOR C931, Bioblock Scientific, France) and Brix with a portable digital refractometer (PR-101, Atago, USA) at room temperature. Total acidity (TA) was determined using the Bremond (1957) method. Density was determined using a densimeter. Vitamin C concentration was determined using the method of Tomohiro (1990). Detection of alcohols (ethanol, methanol and propanol) was carried out by gas chromatography (Shimadzu G M-9AM) fitted with a capillary column from tshui wine distillate. Alcohol content (TA) was determined using the method of Le Coq (1965).

Determination of total polyphenols during fermentation

- Total polyphenols were determined using the colorimetric method of Folin-Ciocalteux (V.L. Singleton & J. A. Rossi, 1965) with slight modifications.
- A calibration line was constructed with different concentrations of gallic acid: $y = 0.005x + 0.0142$ (1) with a correlation $R^2 = 0.9512$.
- x being the concentration of polyphenols in mg gallic acid equivalent per gram of dry matter (GA/gDM) and y the optical density.
- Absorbance is measured with a spectrophotometer at 725 nm against a methanol solution used as a blank.

Determination of anthocyanins during fermentation

Anthocyanins were determined by the subtractive method, using the reaction with sodium bisulfite. The quantity of anthocyanins was determined by the standard made with different concentrations of catechin, whose relationship obtained from the calibration curve is written :

$y = 0.0041x + 0.0126$ (2) with a correlation $R^2 = 0.9923$.

x being the anthocyanin concentration in mg catechin equivalent/g dry matter (EC/g Ms) and y the optical density.

The absorbance of the mixture obtained is measured directly with a UV-visible spectrophotometer at 510 nm.

Enumeration of lactic acid bacteria and yeasts

The classical bacteriological method was used in this study (C. M. Bourgeois and J. Y. Leveau, 1991; J. P. Guiraud, 1998; B. Bahiru et al, 2006). It involved aseptically adding 1 mL of each sample to 9 mL of a sterile 0.9% NaCl solution, then homogenizing. Successive dilutions were made (10⁻¹ to 10⁻⁷) and 0.1 mL of an aliquot of an appropriate dilution was inoculated in duplicate by surface spreading onto the following culture media:

- Man Rogosa Sharpe agar (J. C. De Man et al, 1960), incubated at 30°C for 48 h under anaerobic conditions to enumerate *Lactobacillus* and *Pediococcus*.
- Mayeux agar (1962), incubated at 25°C for 24 to 48 h to enumerate *Leuconostoc*.
- Bile Esculin Azide agar (BEA, ISO 7899/1) incubated at 37°C for 48 h to enumerate *Enterococcus*.
- Chalmers agar incubated at 25°C for 48 h to enumerate *Lactococcus*.
- sabouraud + chloramphenicol agar incubated at 25°C for 3 to 5 days to enumerate yeasts.

Characteristic colonies were counted on the basis of morphological and biochemical criteria (colony color, mobility, freshness, Gram staining, cell shape and size, absence of sporulation, cytochrome oxidase, absence of catalase for lactic acid bacteria, catalase production and budding for yeasts).

2.6. Statistical analysis

Data analysis was carried out using the one- and two-factor ANOVA method(s) with STATISTICA software (Stat., Soft, Inc, 1995). Statistical differences with a probability value below 0.05 ($P < 0.05$) are considered significant. When the probability is greater than 0.05 ($P > 0.05$), statistical differences are not significant.

3. Results and discussion

3.1. Variation in physico-chemical parameters during fermentation

The physico-chemical analysis focused on monitoring and measuring some of the parameters listed in Table 1. These are the final average values obtained at the end of the fermentation process.

Table 1 Physico-chemical parameters of the must during fermentation

parameters	Density	°Brix	pH	T°C	AT(%)	TA(%vol, alc)	Vitamin C (mg/100ml)
Samples	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD
Sweet must	1.04±0.2	13.7±2.1	3.2±0.4	26.2±0.5	1.3±0.2	5.2±1.6	1.7±0.2

X: Mean, SD: Standard deviation, T°C: Temperature, AT: Total acidity, TA: Alcohol content

3.1.1. Changes in must density during fermentation

Density is defined as the ratio, expressed as a decimal number, of the density of wine or must at 20°C to the density of water at the same temperature. The variation of this parameter in must before and during fermentation is shown in figure 3.

The density curve shows a decreasing trend over the course of fermentation, beginning with a latent phase during the first five days, due to the slow rate of fermentation. This phase corresponds to the gradual saturation of the medium with carbon dioxide. After 5 days, when carbon dioxide (CO₂) begins to be released, a rapid decrease in density can be observed. Indeed, the transformation of sugar into alcohol (of lower density than water) under the effect of yeast induces a decrease in density both through the disappearance of sugars and the generation of alcohol (J. Blom, M.J. De Mattos and L.A. Grivell, 2000). A steady, gradual decrease in density confirms that the fermentation process is proceeding well. The values obtained in this case range from 1.071 to 1.020.

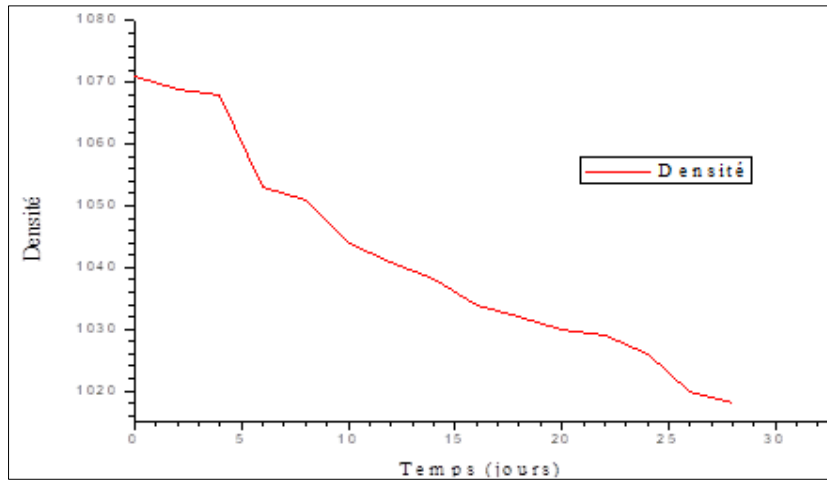


Figure 3 Evolution of density during fermentation

3.1.2. Evolution of Brix level and alcohol content in wine

The breakdown of soluble dry matter in "tshui" wine, as measured by Brix level, is shown in figure 4. Before chaptalization, the must had a residual sugar level of 2.5° Brix, which was corrected to 19.2° Brix by adding sucrose, in order to achieve the desired alcohol level of 12%vol. The saccharomyces yeasts used here, with their ability to transform fermentable sugars into alcohol and carbon dioxide, are responsible for the appearance of a downward-sloping curve. At the start of alcoholic fermentation (48 to 72 hours), there was a sharp drop in Brix value (15°), followed by a slow decrease between days 3 and 8. Rapid degradation of soluble dry matter continued from day 10 to day 30, stabilizing at a value of 4.3 on day 28.

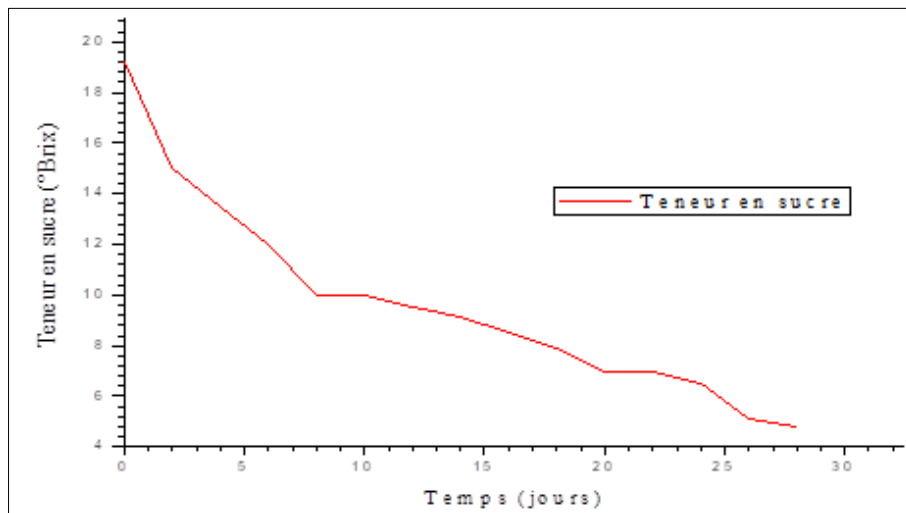


Figure 4 Evolution of sugar content during fermentation

The alcohol content corresponds to the quantity of fermentable sugars transformed by the yeast during fermentation. As the sugar content decreases, the alcohol content increases, as shown in figure 5.

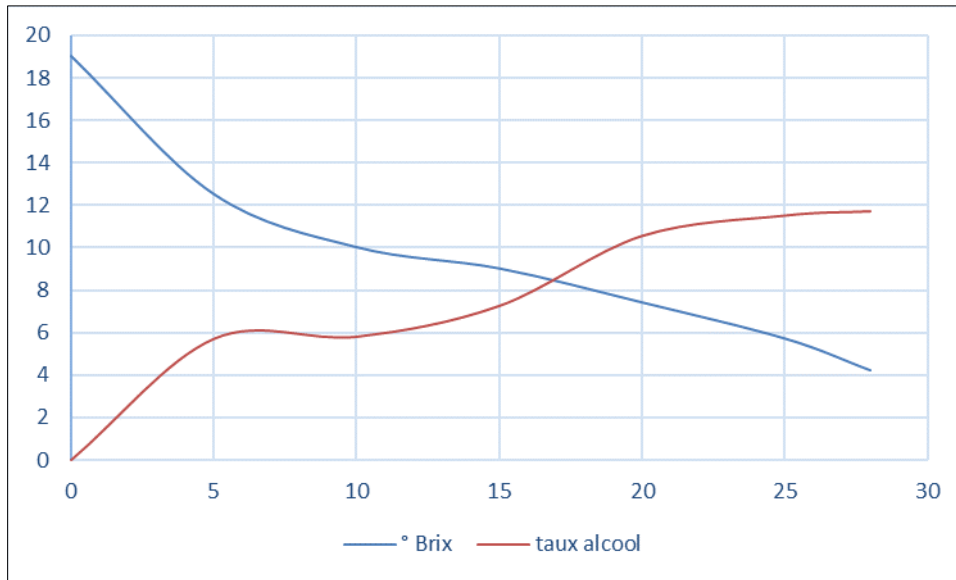


Figure 5 Evolution of sugar content and alcohol during fermentation

3.1.3. Variation in acidity, pH and temperature during fermentation

Acidity is a fundamental parameter in wine-making, where its level depends on the ripeness of the fruit. It can be increased or decreased during fermentation. In addition, it is often correlated with pH measurement, which gives a value for the acid-base content of samples.

Grewia coriacea fruits contain a large number of different organic acids (L. Attibayeba et al, 2007), each of which contributes to the acidity of the must. Total acidity, which averages 1.1% in sweet must, rises to 3.7% in wine.

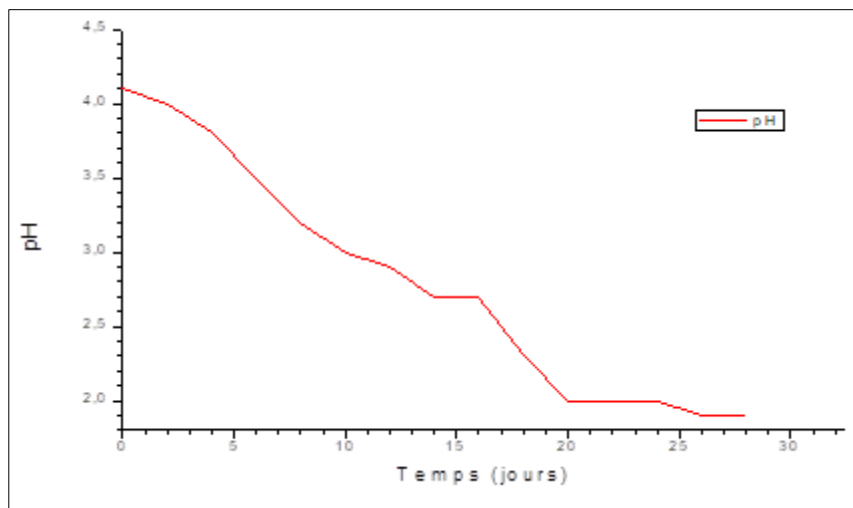


Figure 6 Evolution of pH during fermentation

Analysis of the pH curve during fermentation (figure 6) shows that pH decreases, and acidity increases. Acidity is caused by the fermentative activity of microorganisms, which break down glucose, the main substrate present in the medium, producing acids that acidify the medium. As the medium gradually becomes low in reducing sugars, acidity increases.

During alcoholic fermentation, the pH of the must is constantly changing. Organic acids consumed or produced undergo dissociation and release hydrogen ions into the fermentation medium, thus influencing pH (J. P. Larpent, 1991). Alcohol, the main product of fermentation, also contributes to pH variation. During alcoholic fermentation, pH changes in three phases. The first phase is characterized by a decrease in pH from day 1 to day 14, when a minimum pH of 2.7 is reached. The second phase is also characterized by a decrease in pH until day 20. This pH decrease continues until fermentation

ceases. After day 20, a third phase is observed, during which the pH stabilizes at 1.9. Indeed, after 20 days, it seems that the must has a pH that is not optimal for the *Saccharomyces cerevisiae* species. According to Guiraud (1998), the optimal pH for growth of *Saccharomyces cerevisiae* species is between 3.5 and 5. Yeast growth is therefore inhibited.

Must pH seems to evolve linearly with alcohol content. The higher the alcohol content, the lower the pH. From an initial pH of 4.1 at 0% vol. to 1.9 at 11.7% vol. This shows that alcohol has a significant influence on pH values.

Several factors have been identified as being important in controlling fermentation, including temperature. The latter is said to have a primordial effect on fermentation kinetics, given that alcoholic fermentation is known to be an exothermic reaction, which therefore releases heat.

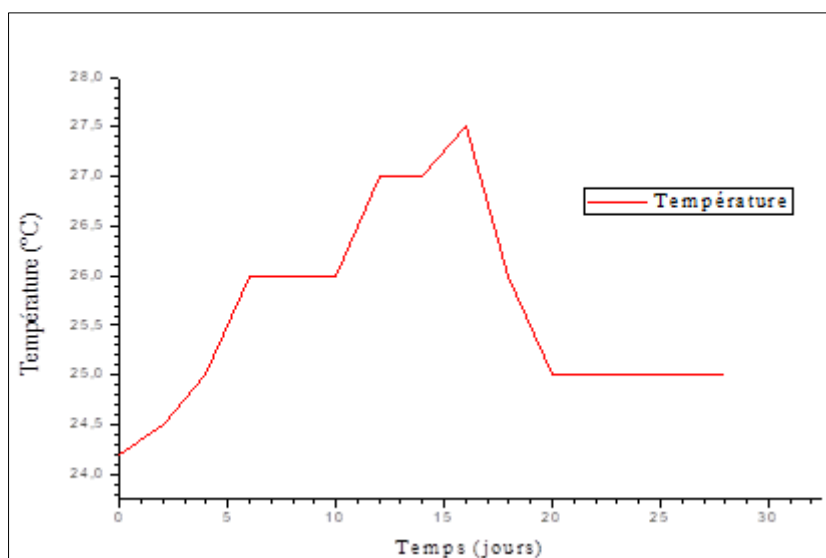


Figure 7 Temperature evolution during fermentation

Analysis of temperature measurements (figure 7) shows that temperature gradually rises from 24.2°C to 27.4°C on day 15, passing through two plateaus before remaining constant. The optimum growth temperature for yeast species *Saccharomyces cerevisiae* is between 25–28°C (I-V. Leveau et al, 1993 ; N. Gournier-Chateau et al, 1994 ; C.M. Bourgeois et al, 1996). This temperature range was reached during fermentation kinetics. Knowing that the critical temperature during fermentation is 27.7° C, we assume that at this temperature yeasts no longer reproduce and die, causing fermentation to slow down and then stop, hence the drop in temperature observed after 15 days, stabilizing around day 20 until the end of fermentation.

3.1.4. Evolution of vitamin C

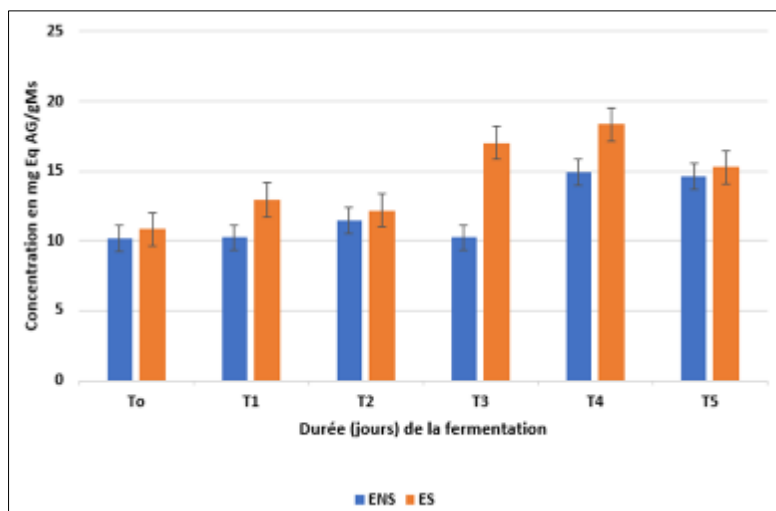
Vitamin C, otherwise known as ascorbic acid, is a natural antioxidant or powerful oxidation reducer. Its presence in wine not only enhances its nutritional value, but also preserves bouquet, aroma and freshness. It also delays oxidative breakdown. It has been estimated at 3.6 g/100 g in sweet must, while its value in wine is lower at 0.5 g/100 g. This can be explained by the fact that vitamin C is a heat-sensitive vitamin, and alcoholic fermentation is exothermic, hence its reduction.

3.1.5. Evolution of total polyphenols and anthocyanins during fermentation

Polyphenols are molecules that support the main organoleptic properties of wines. They are widely present in wine, more so in red wines than in whites.

They are involved in color, either in their native form (anthocyanins, flavonols) or after oxidation (enzymatic browning).

Polyphenols and anthocyanins were analyzed on samples of unsweetened must and sweetened or chaptalized must. Their evolution is illustrated in figures 8 and 9.

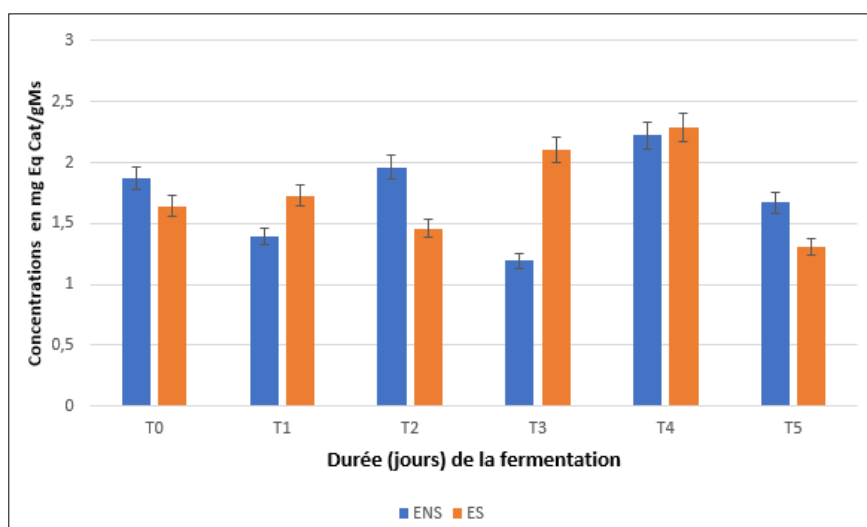


ENS: unsweetened sample, ES: sweet sample, T0= 5th day of fermentation, T1=10th day of fermentation, T2= 15th day of fermentation; T3=20th day of fermentation; T4= 25th day of fermentation, T5=30th day of fermentation.

Figure 8 Variation in polyphenol production during fermentation

Analysis of figure 8, showing total polyphenol concentrations, shows that these vary and increase over time from 10.82 (day 5) to 14.90 mg Eq AG/g (day 30) after 30 days of fermentation in the unsweetened sample. Total polyphenol production is much more pronounced from day 15 (15.05 mg Eq AG/g Ms), with a maximum concentration peak observed on day 25. In the sweetened sample (ES), total polyphenol concentration also varied from 10.82 to 18.30 mg Eq AG/g Ms, with two peaks, on day 20 (17.4 mg Eq AG/g Ms) and day 25 (18.3 mg Eq AG/g Ms) after fermentation. After the full fermentation period, the total polyphenol concentrations of the sweetened sample are higher than those of the unsweetened sample. These results show that the concentration of total polyphenols is inversely proportional to the sugar content. When microorganisms consume sugar, the medium becomes low in sugar and total polyphenols are produced. This corroborates N. Huynh t et al, (2014), who state that microorganisms are strongly involved in the production of total polyphenols. T. Petelinc et al. (2013) argue that *Saccharomyces cerevisiae* yeast play a key role in increasing polyphenol production, since added sugar is an important factor in their growth.

However, the increase in total polyphenols in unchaptalized must is explained by the natural sugar composition of *Grewia coriacea* fruit, which serves as a substrate for microorganisms in the fermenting environment. *Grewia coriacea* Mast fruits at maturity contain 0.4 to 1.7 g/L of carbohydrates (L. Attibayeba et al, 2007).



ENS: unsweetened sample, ES: sweet sample, T0= 5th day of fermentation, T1=10th day of fermentation, T2= 15th day of fermentation; T3=20th day of fermentation; T4= 25th day of fermentation, T5=30th day of fermentation.

Figure 9 Variation in anthocyanin production during fermentation

Figure 9 shows the variability of anthocyanin concentration in musts. In both cases, anthocyanin concentration fluctuates during alcoholic fermentation. In the unsweetened sample (ENS), anthocyanin concentration ranged from 1.39 (day 20) to 2.22 mg Eq Cat/g Ms (day 25). At the start of fermentation (day 5), anthocyanin content was 1.7 mg Eq Cat/g Ms, a value close to that quantified in mature *Grewia coriacea* Mast fruit (M.G. Okiemy-Akeli, 2016), where concentration can reach 1.7 g/100g dry matter. At the end of fermentation, anthocyanin concentration decreased, reaching a value of 1.7 mg Eq Cat/g Ms (day 30). In the sweet sample (ES), anthocyanin concentration ranged from 1.31 (day 30) to 2.39 mg Eq Cat/g Ms (day 25). The finding here is that anthocyanin concentration in the sweet must is highest during the first 25 days of fermentation. This may be due to the inhibition of yeast growth as pH and temperature drop. Decreases can also be explained by their involvement in numerous reactions, as suggested by some authors (P. Ribéreau-Gayon, 1982; T.C. Somers, 1971; V. Cheynier et al, 1997; I. Romero-Cascales et al, 2005). Furthermore, according to Margherita Squadrito et al (2010), anthocyanin profiles vary mainly due to polyphenol oxidase (PPO), yeast and lactic acid bacteria.

3.1.6. Identification of alcohols in tshui wine

From a chemical point of view, wine is essentially a hydroalcoholic solution made up of numerous constituents, some of which are present in very low concentrations but which may nevertheless be of great organoleptic importance.

Gas chromatography is currently used in oenology to analyze wines. In our case, gas chromatography has enabled us to identify alcohols other than ethanol, namely methanol and propanol.

The results of this analysis revealed the presence of only ethanol in the tshui wine samples analyzed. Statistical analysis showed non-significant variability ($P > 0.05$) in ethanol content, with an average value of 5.2% vol. alc. (alcohol volume). Tshui wine distillate is colorless, with a sour taste and characteristic aroma.

3.2. Enumeration of fermentative flora

The enumeration of the various flora in the samples enabled us to obtain results expressed in Colony Forming Units (CFU) per milliliter of "tshui" wine, and are given in Table II. These values are the averages of three analyses.

Table 2 Load of fermentative microflora present in tshui wine (cfu/mL)

Type	Yeast	<i>Lactobacillus</i>	<i>leuconostoc</i>	<i>Enterococcus</i>	<i>Lactococcus</i>	<i>Pediococcus</i>
Tshui wine	$1.7 \times 10^8 \pm 3.6.10^7$	$1.9 \times 10^3 \pm 2.1.10^3$	$3.7 \times 10^2 \pm 4.6.10^3$	0	0	0

Analysis of Table II shows that the yeast load (1.7×10^8 cfu/mL) is higher than that of *Lactobacillus* (1.9×10^3 cfu/mL) and *Leuconostoc* (3.7×10^2 cfu/mL). On the other hand, *Enterococcus*, *Lactococcus* and *Pediococcus* gave zero results.

In the tshui wine, yeasts are the dominant flora according to the results. These yeasts certainly possess α -amylase activity, like those involved in cassava processing (D. R. Djoulde, 2004). The predominance of yeasts is probably due to the fact that they are added by inoculation of the fermenting agent (*Saccharomyces cerevisiae*) into the sweet must to ensure alcoholic fermentation (A. K. Yao et al, 1995 ; A. K. N'da and S. Coulibaly, 1996; N. Maoura et al, 2006), while lactic acid bacteria are supplied by the environment and the equipment used. Our results corroborate those of Solange Aka et al. (2008), who observed that lactic acid and yeast flora are present in tchapalo. The lactic acid bacteria present in tshui wine consist of *Lactobacillus* and *Leuconostoc*. These low-numbered bacteria are not accidental contaminants, as some authors mention their presence in traditional African beverages (J. J. Assiedu, 1991; N. Maoura et al, 2006 ; C. I. Iwuoha and O. S. Eke, 1996; A. I. Sanni et al, 1999). These bacteria are part of the ferment's natural environment. This low load could be due to the increased alcohol content produced by yeast during alcoholic fermentation (K. C. THOMAS et al, 2001). These lactic acid bacteria, which could be the same as those responsible for the lactic acid fermentation of the must, are probably involved in the significant hydrolysis of non-fermentable sugars during alcoholic fermentation. The consequence of this consumption of sugars is a reduction in the wine's brix and density.

4. Conclusion

Tshui wine is an alcoholic beverage of the mixed acid type. This work represents a first contribution to the valorization of tshui wine produced from *Grewia coriacea* Mast fruit. The must samples taken during fermentation and analyzed have statistically constant physico-chemical and microbiological properties, indicating regularity in the quality of the wine produced. The wine produced has an average ethanol content of 5.2% vol alc, 1.3 mg/100 mL vitamin C, contains neither methanol nor propanol, but does contain microorganisms that can alter its quality during storage. It would therefore be interesting to identify the yeasts and lactic acid bacteria involved in lactic and alcoholic fermentation, and

to study their technological properties. Analysis of physico-chemical parameters shows that density, pH and sugar levels evolve in the same way, and inversely proportional to temperature. Knowing the physico-chemical composition and microbiological quality of a food is an important step in its development. In our case, checking and monitoring the parameters of "tshui" wine could help improve its quality. It can serve not only as a guideline for preservation, but also on the conditions required for better hygienic quality.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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