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Effect of Temperature-Time Couple on the Quality of Isongo, A Burundian Traditional Banana Wine

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Abstract
The banana wine submitted to different pasteurization temperatures was kept for two weeks at room temperature and the quality parameters were analyzed prior to pasteurization, after one week and after two weeks. The observations were made on the physico-chemical, microbiological parameters and organoleptic qualities.The results of the analyses carried out lead us to conclude that the banana wine produced by craftsmen and consumed in Burundi is poorly prepared but that it could acquire many physico-chemical, microbiological and organoleptic qualities if their manufacture were well conducted. Among the temperature and time couples studied, pasteurization at 64°C for 8 minutes gives good qualitative results after two weeks of storage. It is for this reason that the treatment of the banana wine by heat (64°C during 8 minutes) after fermentation is recommended to correct deficiencies that appear during the conservation of this product.
Keywords: Banana wine, temperature-time couple, physico- chemical, microbiological parameters, organoleptic qualities
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1. Introduction

Banana is a crop adapted to low altitude of tropical regions (Central America, Caribbean, West Africa, Indonesia, Australia) where it is mostly grown for export. The high altitude banana varieties, found in the highlands of East Africa, constitute a specific group that is relatively less well known (Bigirimana et Njejimana, 1999, Mujaayezu, 1990, Semajeje,1986).

In Burundi, the banana is cultivated in all natural regions except for the highlands of Mugamba and Bututsi where the altitude exceeds 2000 m. There are four types of banana varieties in Burundi: cooking bananas, wine bananas, dessert bananas and plantains (, Kanyaruguru, 1999 ,Bagankunda, 1987).

The wine varieties are by far the most frequent, followed by the cooking varieties, the dessert varieties and the plantains. The first are used to manufacture beer and wine. Its production is simple and is known all over the country and it is one of the products accessible to the low income population, because of its selling price and the production techniques used (Nzigamasabo et Nimpagaritse, 2009).

Nevertheless, the poor conditions of manufacture and storage of banana wine, present various risks of contamination likely to affect the health of consumers. It is therefore strongly recommended to manufacture banana wine in a hygienic way in order to ensure the health of the consumers.

Isongo, a Burundian, Traditional banana wine is a beverage obtained by fermenting banana juice using sorghum flour as yeast source. It is made by hand and uses traditional tools which constitute a favorable ground for the proliferation of microorganisms. Since hygiene and sanitation are not respected and preservation treatments are not applied, this result in wine of questionable quality which can have implications on the health of consumers (Irambona, 2008). In Burundi, there are no studies made on the methods of preservation of the quality of banana wine by heat that is why in the framework of our work, we were interested in the treatment of banana wine by heat which is one of the techniques of preservation of the quality of food products.

This study aims to compare the effects of different pasteurization temperatures on the physico-chemical, microbiological and organoleptic qualities of wine during different storage times. Thus, the banana wine was subjected to different temperature-time

couples calculated using the following formula: $t = t^* . 10^{-\frac{T - T^*}{z}}$

Where: t = processing time, t* = reference treatment time, T* = reference temperature (60°C), T = temperature to which the product will be subjected and is chosen arbitrarily, z =It is a thermo resistance factor.

2. Material and Methods

2.1. Banana collection and processing

Wine banana varieties were purchased on the local market and transported to the laboratory for processing and analysis. The traditional processing techniques as described by Nzigamasabo et Nimpagaritse (2009) were used. Briefly, green bananas were ripened for 3–5 days in a covered warm place with bananas leaves to insure uniform

temperature. The ripe bananas were mixed with spear grass. Juice was then extracted by squeezing the mixture with their hand. The process of squeezing was continued until all the pulp is exhausted of juice. The juice was filtered through grass held in a bottle.

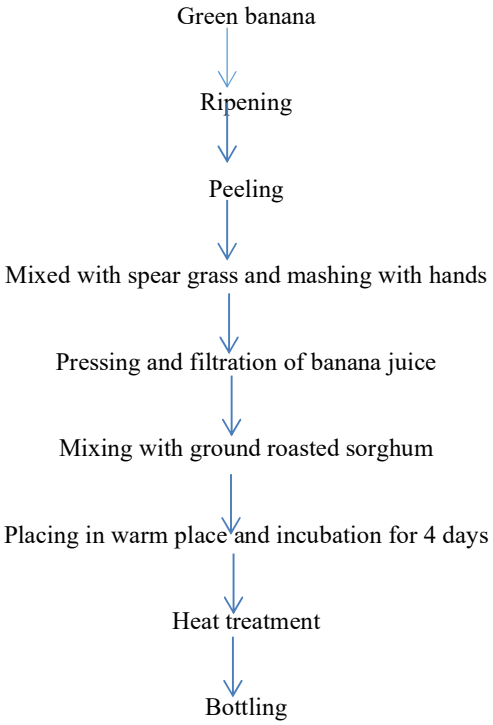

Figure 1. Flow chart of banana wine manufacture

2.2. Determination of physicochemical Characteristics

After the fermentation the pH, titratable acidity, fixed acidity and alcoholic degree were determined using official methods of analysis of AOAC (2012).

2.3. Microbiological analysis

2.3.1. Material sterilization

All materials used for microbial contents analysis were washed with water, dried and sterilized in autoclave at 121°C for 15 minutes apart for Petri dishes and pipettes which were sterilized using ethanol 70%. All techniques were done aseptically around the Bunsen burner. All of working tables were cleaned and disinfected with ethanol 70% so as to prevent cross contamination that could occur during operations.

2.3.2. Culture medium preparation

PCA was used for the determination of the total Aerobic Mesophilic Flora. 23,5 g was suspended in 100 ml of water, heated until completely dissolved and autoclaved at 121°C for 15 minutes, cooled to 45-50^{0}C and poured carefully into Petri dishes (Bunani et al, 2020, Kavishe et Matemu, 2015).

Mac Conkey agar was used for isolation and differentiation of total and fecal coliforms, staphyloccus , salmonella and shigella. 50 g was suspended in 1000 ml of water, heated until completely dissolved and autoclaved at 121°C for 15 minutes, cooled to 45-50^{0}C and poured carefully into Petri dishes (Bunani et al, 2020, Kavishe et Matemu, 2015).

CSA (clostridium Selective Agar) used for the isolation of clostridia, 44g was suspended in 1000 ml of water, heated until completely dissolved and autoclaved at 121°C for 15 minutes, cooled to 45-50^{0}C and poured carefully into Petri dishes (Bunani et al, 2020, Kavishe et Matemu, 2015).

PDA was used for isolation and differentiation of mold and yeast. 39 g was suspended in 1000 ml of water, heated until completely dissolved and autoclaved at 121°C for 15 minutes, cooled to 45-50^{0}C and poured carefully into Petri dishes. Chloramphenicol was used to suppress the growth of bacteria (Bunani et al, 2020, Kavishe et Matemu, 2015).

2.3.3. Serial dilution

Dilutions were very important in order to get a number of colonies that could be countable. Seven test tubes were

filled with 1 ml of sterilized traditional banana wine as shown below on Fig. 1. By using a micropipette, 1ml of the original sample was taken and put in 9 ml of peptone water (dilution 10^{-1}). After mixing, 1ml was taken from that tube and then put in the next tube (dilution 10^{-2}). The same process was repeated up to the tube number seven.

2.3.4. Inoculation

The inoculation has been done by putting 1000 µl of the diluted suspension samples into Petri dishes by using micropipette. Then the samples were spreaded on the agar media by using a spreader.

2.3.5.Incubation

The Petri dishes were put into incubator for 24 hours at 37°C to enable Aerobic Mesophilic Flora to grow on PCA, clostridia on CSA, fecal coliform, E.coli, staphylococcus, salmonella, and shigella on MCA. For Petri dishes of PDA, they were putted for 72 hours at 25-30°C to enable yeasts and mold to grow (Bunani et al, 2020, Kavishe et Matemu, 2015).

2.3.6.Description Of Colonies

In order to differentiate the microorganisms, the description of colonies is needed. For our study, the colonies were differentiated using the following characteristics:

Size: diameter in millimeter, *Form:* Punctiform, circular, filamentous, irregular, rhizoid, *Elevation:* flat,raised convex, pulvinate, umbonate, umbilicate, *Margin:* entire, undulate, lobate, *Color:* white, yellow, black, buff,orange, pink, *Density:* opaque, translucent, transparent,

Consistency: viscid, membranous, brittle, butyrins.

2.3.7.Enumeration

The enumeration of the microbial load was performed on the surface, after the appropriate incubation period for each microorganism. This was done using an electric colony counter with a magnifying glass and separating the colonies on the petri dishes with a pen marker.

2.4. Sensory Analysis

Sensory evaluation was carried out to know the acceptability of the wine by carrying out In- house consumer acceptability test using in-house panelists, according to the method described by **Nwobodo (2013)**. Sensory evaluation was carried out by 10 untrained panelists who were selected based on their availability, objectivity and being conversant with wine tasting. The sensory attributes evaluated were color, taste/ mouth feel, smell and clarity on a 5-point hedonic scale (where 1 represents dislike very much and 5 represents like very much). The wine samples were served in clean plastic cups to individual panelist in a booth in a well-lit Environment Where There Was No Interference For Bias Expression.

2.5. Statistical analysis

The experiments were conducted in triplicate and the results were expressed as mean with standard deviation. Statistical analysis of the data was performed using SPSS Package Program. Statistical significance was taken at 95% confidence interval when $p<0.05$. When Analysis of Variance (ANOVA) revealed a significant effect ($p<0.05$), the data means were compared by the least significant difference (Duncan's Multiple Range test) test.

3. Results and discussion

3.1. Physico chemical parameters

The physico-chemical analysis of banana wine treated at different temperature-time couples (or pasteurization) was focused on pH, alcoholic degree, volatile acidity, fixed acidity and total acidity. The results are presented in Table 1.

The pH of the wine was determined since it has a great influence on the properties of the wine, as well as on its biological and chemical stability. Acidity confers to the wine a better microbiological and physico-chemical stability by limiting the development of micro-organisms and by increasing the antiseptic fraction of the sulphur dioxide, it is also a pillar of the gustatory balance.

The results show a decrease in pH compared to the pH before heat treatment ($pH=3.82 \pm 0,028$). It is $3,78 \pm 0,012$ and $3,76 \pm 0,041$ for the wine treated at 60°C for 30 min respectively after 7 days and 14 days, that treated at 62°C for 16 min has a pH of $3,77 \pm 0,025$ and $3,74 \pm 0,043$ respectively after 7 and 14 days. While the one treated at 64°C for 8 min had a pH 3, $81 \pm 0,028$ and 3, $79 \pm 0,039$ respectively after 7 days and 14 days. The results also show that the pH of the banana wine treated at 62°C for 16 min has a lower pH than the other treatments, but there is was not a significance difference between different wines ($p<0,05$).

We also noted that the pH decreased during the storage time. The same trend was observed by Gavimash et al (2012). The pH of banana wine pasteurized at different temperature-time couples remain within the recommended norms of wines, i.e. pH varying between 3 and 5.

The result of the pH values in the experiment shows progressive decrease in pH value with storage time.

These results showed that the wine became lightly acidic as the storage time progressed. The drop in pH records the utilization of the sugar present in the must by the yeast. The finding from this study suggests that yeasts could strive under acidic pH (Akubor et. al., 2003) and that these yeasts were not completely inactivated during the pasteurization process.

Table 1: Physico-chemical parameters of banana wine treated at different time-temperature couples

	Temperature-time Couple						
	Before pasteurization	Before pasteurization			14 days after pasteurization		
		60°Cduring 30 min	62°Cduring 16 min	64°Cduring 8 min	60°Cduring 30 min	62°Cduring 16 min	**64°Cduring 8 min**
pH	**3,82^{a} ± 0,028**	**3,78^{a} ± 0,012**	**3,77^{a} ± 0,025**	**3,81^{a} ± 0,028**	**,76^{a} ± 0,041**	**3,74^{a} ± 0,043**	**3,79^{a} ± 0,039**
Alcohol content (%)	**3,85^{a} ± 0,12**	**3,89^{a} ± 0,233**	**4,44^{c} ± 0,225**	**4,03^{b} ± 0,205**	**3,97^{b} ± 0,153**	**4,47^{c} ± 0,231**	**4,05^{b} ± 0,227**
Titratable acidity (g/l)	**4,6^{a} ± 0,137**	**4,6^{a} ± 0,137**	**5,2^{c} ± 0,615**	**4,9^{b} ± 0,041**	**5,3^{c} ± 0,085**	**5,0^{b} ± 0,143**	**4,7^{a} ± 0,707**
Fixed acidity (g/l)	**2,9^{a} ± 0,095**	**3,2^{c} ± 0,745**	**3,2^{c} ± 0,745**	**2,7^{b} ± 0,083**	**2,9^{a} ± 0,095**	**2,8^{a,b} ± 0,095**	**2,9^{a} ± 0,044**
Volatile acidity (g/l)	**1,4^{a} ± 0,096**	**1,4^{a} ± 0,096**	**2,0^{b} ± 0,136**	**2,2^{c} ± 0,144**	**2,6^{d} ± 0,065**	**2,2^{c} ± 0,124**	**1,9^{b} ± 0,141**

Values followed by different letters within rows are significantly different ($P < 0.05$). Mean ± SD (n = 3).

The alcohol in a wine is the result of the transformation, total or partial, of the sugar contained in the must. The alcohol content influences the pH, wine quality, conservation and market value. Its content depends on the initial sugar concentration of the must, the fermentation conditions that can slightly vary the yield of the conversion (Otgbayo, Akwa, Tanimola, 2020, Randrianantoandro and Andriamamisa, 2018)

Alcohol content increased with storage time for all pasteurization temperatures, it was the highest for the wine treated at 62°C for 16 min (4,47 ± 0,231) and he lowest for the wine pasteurized at 64°C for 8 min (4,05 ± 0,227). Significant difference (p>0,05) has been observed between these heat preserved wines.

The titratable acidity of wine is one of the essential constituents of its organoleptic as well as of its conservation. It is therefore important to follow it periodically throughout the winemaking process, especially during the alcoholic and malolactic fermentation but also until the bottling (Randrianantoandro et Andriamamisa, 2018). The total acidity is linked to all the acids present in the wine and translates the gustatory characteristics of the wine.

The banana wine treated at 60°C for 30 min had respectively a titratable acidity of 4, 6 ± 0,137 g tartaric acid/l banana wine after 7 days and 5,3 ± 0,085 g tartaric acid/l banana wine after 14 days. The wine treated at 62°C for 16 min has a titratable acidity of 5,2 ± 0,615g tartaric acid/l banana wine and 5,0 ± 0,143g tartaric acid/l banana wine after 7 and 14 days respectively. While the one treated at 64°C for 8 min has respectively 4,9 ± 0,041 g of tartaric acid /l of banana wine and 4,7 ± 0,707g of tartaric acid after 7 and 14 days.

There is a gradual increase of titratable acidity as the storage time progressed. As the production of alcohol is due to the activity of yeasts and other bacteria, these results show that the different time-temperature couples did not totally inactivate the microorganisms and that they continue to degrade the substrates with the production of alcohol and acids. Despite the observed significance difference between the samples, we notice that the time-temperature couple of 64C-8min is the best compared to the other time-temperature couples.

The volatile acidity is constituted by the part of the fatty acids belonging to the acetic series, which are found in the wines in the free state, and in the solified state. It is formed essentially by acetic acid, accompanied by small quantities of propionic and butyric acids. The volatile acidity is an important parameter of the quality of wines (Delanoë D. et al., 2007). It gives bouquet to the wine.

These acids are formed naturally in very small quantities during alcoholic and malolactic fermentations. They can also be formed accidentally as a result of bacterial development. But when the dose is too high, as in our case, the wine becomes cloudy and pitted. The determination of the volatile acidity of a wine also allows to know the sanitary state of the wines.

The wine treated at 60°C for 30 min has respectively 1,4 ± 0,096g and 2,6g of sulfuric acid /l after 7 days and 14 days, the one treated at 62°C for 16 min has 2,0 ± 0,136g and 2,2 ± 0,124g of sulfuric acid /l while it is respectively 2,2 ± 0,144 g and 1,9 ± 0,141g of sulfuric acid / l for the treatment at 64°C for 8 min.

Since it is wines with low volatile acidity that are recommended, it is the 64°C 8min treatment that is likely to preserve the quality of the wine because the low volatile acidity does not cause the wine to deteriorate in contact with air.

3.2. Microbiological Analysis Results

The purpose of microbiological analysis of wines is to monitor the alcoholic and/or malolactic fermentations and to detect the risks of microbial alterations. This then allows the detection of any anomaly, not only in the finished

product, but also during the different phases of its manufacture (OIV, 2015).

The results of microbiological analysis of pasteurized banana wine are given in Table 2 and concern the detection of total aerobic mesophilic flora, fecal coliforms including E. Coli, salmonella and shigella, clostridia, staphylococci, yeasts and molds.

Table 2: Microbiological parameters of banana wine treated at different time -temperature couples

		Temperature time couple					
	Before pasteurization	7 days after pasteurization			14 days after pasteurization		
		60°C during 30 min	62°C during 16 min	64°C during 8 min	60°C during 30 min	62°C during 16 min	64°C during 8 min
Total aerobic mesophilic flora	$6,089^{a} \pm 0,043$	$4,620^{b} \pm 0,006$	$4,620^{b} \pm 0,006$	$4,021^{c} \pm 0,096$	$3,954^{c} \pm 0,016$	$3,954^{c} \pm 0,016$	$3,556^{d} \pm 0,066$
Yeasts	$4,954^{a} \pm 0,049$	$4,484^{b} \pm 0,030$	$4,024^{c} \pm 0,045$	$3,903^{c} \pm 0,064$	$3,968^{c} \pm 0,012$	$3,863^{c} \pm 0,010$	$3,380^{d} \pm 0,040$
Fungi	$4,337^{a} \pm 0,024$	$3,845^{b} \pm 0,041$	$3,653^{c} \pm 0,029$	$3,342^{d} \pm 0,054$	$3,230^{d} \pm 0,104$	$3,041^{e} \pm 0,053$	$2,954^{e} \pm 0,057$
Fecal coliform : E. coli	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Salmonela and shigella	$3,079^{a} \pm 0,029$	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
Staphylloccus	$3,176^{a} \pm 0,090$	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
Clostridia	$2,103^{a} \pm 0,014$	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}

Values followed by different letters within rows are significantly different ($P < 0.05$). Mean ± SD ($n = 3$).

The microbiological analysis shows that before pasteurization the banana wine contains a high microbial load of total aerobic mesophilic flora (logUFC,6,089±0,043) , yeasts (logUFC,4,954±0,049) and fungi (logUFC,4,337±0,024). This is quite understandable insofar as these microorganisms come from the manufacturer, the material and the ingredients used as ferment in particular sorghum. As the conservation time progresses, there is a progressive decrease of these microorganisms and this for all the couples time temperature. After 14 days storage time, the total aerobic mesophilic flora were $3,954 \pm 0,016$, $3,954 \pm 0,016$ and $3,556 \pm 0,066$ in wine treated respectively at 60°C for 30 min, 62°C for 16 min and 64°C for 8 min. The yeasts found were respectively $3,968 \pm 0,012$, $3,863 \pm 0,010$ and $3,380 \pm 0,040$ while fungi stood at $3,230 \pm 0,104$, $3,041 \pm 0,053$ and $2,954 \pm 0,057$.

After 14 days of conservation, the wines treated at 64C for 8 min contained fewer microorganisms than the other wines (table 2). As mentioned by Bunani et al (2020), Bourgeois,Mescle et Zuccay, (1996), the alcohol content and the low pH would be at the origin of the decrease of these microorganisms.

Salmonella, shigella, staphyloccocus and costridia known to be pathogenic bacteria were present before pasteurization but were subsequently inactivated by these thermal treatments; they were not detected neither at the 7th nor at the 14th day of storage.The microbiological analysis allows us to conclude that the final product is perfectly safe for human consumption since the values obtained for the different evaluation criteria are below the accepted standards.

3.3.Sensory Analysis Results

The sensory analysis shows that the panelists liked the color, taste and smell of the banana wine very much and no significant difference ($p>0,05$) was detected between the different heat treatments. The color is yellowish, with a pleasant taste not very sweet with an aftertaste of banana and a good smell less pungent.

Table 3 : Organoleptic parameters after 7 and 14 days storage.

Organoleptic parameters	Samples			
	Before pasteurization	Wine pasteurized at 60C during 30 min	Wine pasteurized at 62C during 16 min	Wine pasteurized at 64C during 8 min
Color and clarity	Yellowish $4,5^{a}\pm0,2$	Yellowish $4,4^{a}\pm0,1$	Yellowish $4,5^{a}\pm0,1$	Yellowish $4,4^{a}\pm0,2$
Taste	pleasant, slightly sweet taste, an aroma with an aftertaste of banana $4,6^{a}\pm0,1$	pleasant, slightly sweet taste, an aroma with an aftertaste of banana $4,5^{a}\pm0,1$	pleasant, slightly sweet taste, an aroma with an aftertaste of banana $4,6^{a}\pm0,2$	pleasant, slightly sweet taste, an aroma with an aftertaste of banana $4,5^{a}\pm0,1$
Smell	Pleasant odor and less pungent $4,5^{a}\pm0,2$	Pleasant odor and less pungent $4,5^{a}\pm0,2$	Pleasant odor and less pungent $4,4^{a}\pm0,1$	Pleasant odor and less pungent $4,5^{a}\pm0,2$

Values followed by different letters within rows are significantly different ($P < 0.05$). Mean ± SD (n = 3).
5= Strongly liked, 4= liked, 3= Moderately liked, 2= disliked, 1= Strongly disliked
Values = Mean ±SD (n=3)

The conservation beyond two weeks is accompanied by the alteration of the organoleptic qualities. Given that the wines are subjected to thermal treatments of pasteurization, the origin of these deteriorations would be the material of conservation.

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CONCLUSION

In Burundi, the majority of people consume banana alcoholic beverages. The food processors are not well educated and many lack education in food processing, hygienic and food handling. This may lead to high contamination of the banana alcoholic beverages. The objective of this work was to make banana wine at different temperature-time couples, to study the quality of the produced wines and to select the best temperature-time couple. The treatment of banana wine at different time-temperature pairings and the monitoring of their physico-chemical, microbiological and organoleptic qualities at different storage times reveals that the wines are of good quality. The results of microbiological and organoleptic analysis do not show any difference between the time-temperature combinations used. On the other hand, differences were observed in the physical and chemical parameters. Based on the results obtained, we can conclude that treating wine at 64oC for 8 min is the best temperature time couple that can be recommended for banana wine manufacturers.

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