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RESEARCH ARTICLE

Study of changes organic acids in cocoa mucilaginous juice during alcoholic fermentation

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Abstract

The aim of this contribution is to be able to describe the movement of organic acids in fermented cocoa mucilaginous juice. Organic acids (acetic, citric, lactic, malic, oxalic) values during cocoa mucilaginous juice fermentation are investigated in this study.

The juice went through the different ways of fermentation. Spontaneous fermentation was conducted by natural yeast content and controlled fermentation was inoculated with baker yeast (*Saccharomyces Cerevisiae*). Organic acids were identified and quantified using high-performance liquid chromatography

The differences of average mass concentrations of acetic, malic and citric acids were statistically relevant with higher decreasing or increasing during spontaneous fermentation.

INTRODUCTION

Cocoa producing countries in West Africa such as Côte d'Ivoire and Ghana and in some Asian countries such as Indonesia and Malaysia produce the largest amount of cocoa beans.

Côte d'Ivoire, Ghana and Indonesia produced 1,405, 000, 736,000 and 41500 metric tons respectively during year 2003-2004 (Ostovar et Keeney, 1973). Each year, the amount of pulp solution produced by these countries is estimated 550 000 m^3. Unfortunately, only small quantity of this sweet cocoa juice is consumed as beverage and the major part is abandoned in farms.

The valorization of cocoa juice could produce additional financial benefits for small farmers and other storekeepers in the cocoa production and supply cocoa network and so reduce the negative drawbacks due to the drop in cocoa beans price at the international market. During cocoa bean fermentation, forty percent of cocoa juice can be removed without major consequences cocoa fermentation process. It represents more than 220 000 m^3. This amount of juice could be used in several products such as alcohol beverage and vinegar pin Brasilia (Samsiah et al., 1991).

Nowadays, several policies are being directing in cocoa by-product valorization. These researches are mainly related to cocoa pods and mucilaginous juice. Cocoa juice has already been used in alcohol production by coca juice fermentation. The amount of alcohol obtained is average 8.4% in controlled fermentation and 4% in spontaneous fermentation (Anvoh et al., 2010). It could be used further in wine production. Cocoa fermentation is a complex biochemical process, during which yeasts metabolize sugars and other constituents as substrates for their growth by converting them into ethanol (Mosso et al., 1999).

During alcoholic fermentation, next to alcohol, several other products are also produced like phenolic and organic compounds. Organic acids largely participate in the constitution, stability and sensorial qualities of the wine. (Tita et al., 2006). Chemical composition is one of the most important quality criteria for fruit product. Organic acids having important effects on characteristic fruit flavor play a major role in quality criteria such as stability, microbiological control color and flavor of the product **(**Fuleki et al, 1993; Romero and Munoz, 1993). They are also employed to determine fruit maturity (Wang, Gonzalez, Gbur and Alesage, 1993; Lamikanra et al., 1995).

The total content of organic acid in wine affects the drinks acidity, whereas the levels of specific organic acid can directly influence the flavor and taste of the drink. Therefore, organic acids profiles are monitored to determine the concentration of various organic acids at the end of cocoa fermentation.

The present work aims to compare the movements of organic acids (malic, citric, acetic, oxalic and lactic) produced during spontaneous and controlled fermentation of cocoa juice in order to establish a database of organic acid distribution in further cocoa wine production.

MATERIALS AND METHODS

Material

Cocoa bean juice was collected immediately after cocoa pods opening from three industrial farms around Abidjan (South of Côte d'Ivoire) in September 2014. Fresh cocoa beans were packed in a basket at about a meter up from the ground. The basket was slightly tilted in order to collect the juice in bottles. Juice was immediately stored in cool box at 4°C during the transport to the laboratory before storing it in a freezer at low temperature (-22°C).

Alcoholic fermentation process

Before beginning the fermentation process, fresh cocoa juice was analyzed to determine certain chemical characteristics such as pH, moisture content, titratable acidity, sugar content and inorganic component. In order to conduct spontaneous fermentation, fresh cocoa juice was put at different stable temperatures (28, 30, 35, 41°C) in a stirred bain-marie.

Controlled fermentation was carried out with commercial and dry *S.cerevisiae* used in bakery industry. Fresh cocoa juice was pasteurized at 70°C for 15 minutes in a water bath then reactivated S. *cerevisiae* in a 10% solution of saccharose for one hour at ambiant temperature of between 28 and 30°C then added to the juice with 30 ghL^{-1} as inoculation ratio (Mosso et al., 1999). Biochemical and physical characteristics of fresh and pasteurized cocoa juice before fermentation were determined.

Biochemical analysis

organic acid contents were determined by HPLC (Reddy et Reddy, 2005)**.** HPLC system (shimadzu corporation, Japan) used was equipped with a pump (shimadzu LC-6A liquid chromatographic detector) and integrator (Shimadzu, C-R 6A Chromatopac). The column used was tansgenic organic acids (ICSep ORH-801), mobile phase was sulphuric acid (0,004 N) at 0.8ml/min debit. Detection was made at 210 nm at 35°C.

Statistic analysis

Data were analyzed by the analysis of variance (ANOVA) using the soft package STATISTICA v.9 (34). Least significant difference (LSD) test was used for post hoc comparison of significant treatement mean values.

Results

A representative organic acids in cocoa mucilaginous sample is shown in table 1. Citric acid (9.14 ± 0.6 mg/L) was the major acid in cocoa mucilaginous juice variety analyzed account for 50% or more of the total acidity. Malic acid is the second important organic acid present in cocoa juice with 3.60 ± 0.5 mg/L. Acetic, oxalic and lactic acid concentration are less important respectively 2.28 ± 0.7, 1.27 ± 0.7 and 1.23 ± 0.1 mg/l.

Fumaric acid was the lowest organic acid identified and the measured values correspond with this acid was 0.02 ± 0.0 mg/l.

Table 1: Major organic acids concentration contained in cocoa juice

Organic acids	Value (mg/L)
• Citric acid	9.14 ± 0.6
• Malic acid	3.60 ± 0.5

• Acetic acid	2.28 ± 0.7
• Oxalic acid	1.27 ± 0.7
• Lactic acid	1.23 ± 0.1
• Fumaric acid	0.02 ± 0.001

Acetic acid amounts

The results of acetic acid contents were shown in figure 1. This organic acid increased during cocoa juice fermentation. The increasing of acetic acid is more important during spontaneous from 4.1 ± 0.09 to 6.2 ±0.2 mg/l compared to that in controlled process (3.14 ± 0.09 to 3.6 ± 0.09 mg/l. During spontaneous fermentation 51 % of the acid is produced than only 16% in controlled fermentation.

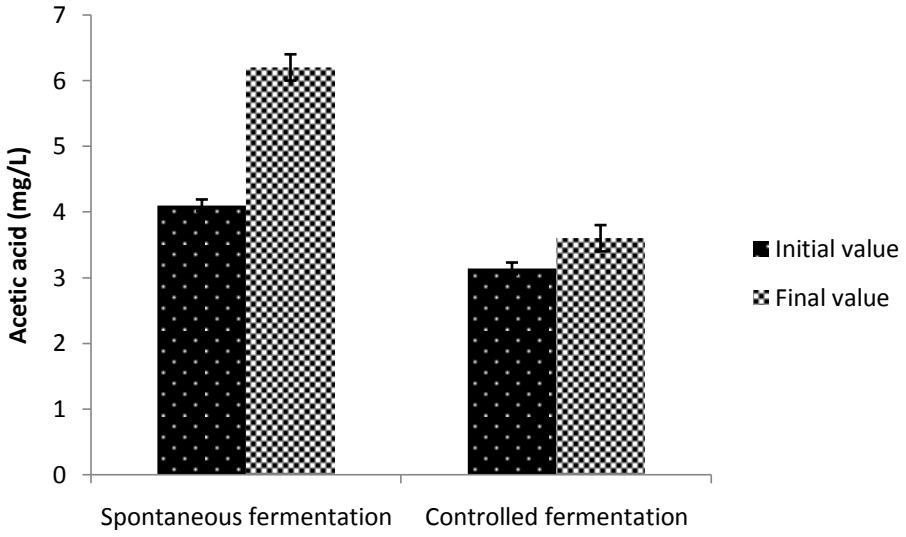

Figure1: Acetic acid amounts

Citric acid amounts

These results indicated that the concentration of citric acid goes lower from the beginning to the end of the fermentation whatever the types of fermentation. The decreasing rate is more important during spontaneous fermentation (40.5%) compared to 26%.

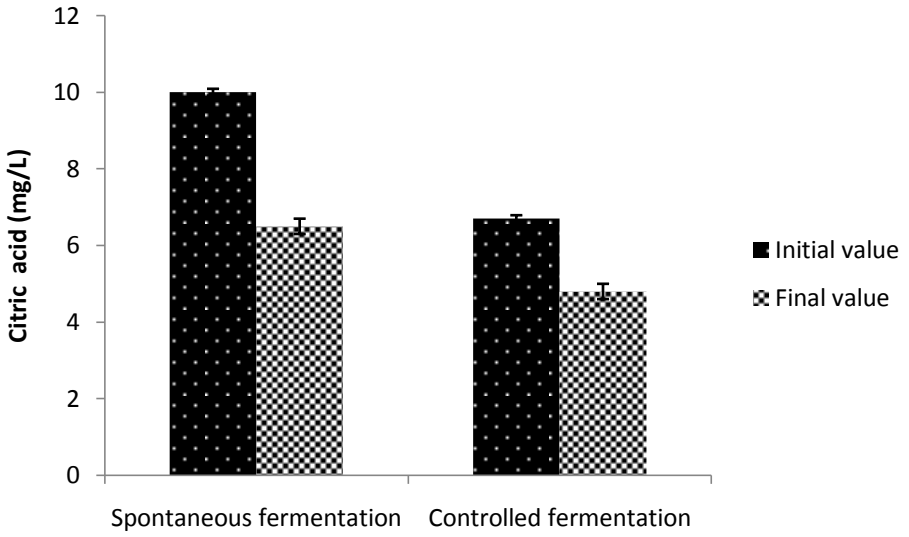

Figure 2: Citric acid amounts

Lactic acid amounts

Lactic acid is also produced during cocoa juice fermentation. The most production rate is obtained during spontaneous fermentation with 0.61±0.04 to 1.23 ± 0.13 mg/l meaning, the average increase to 10% in comparison with only 21% during controlled fermentation from 1.34 ± 0.1 to 1.63 ± 0.14.

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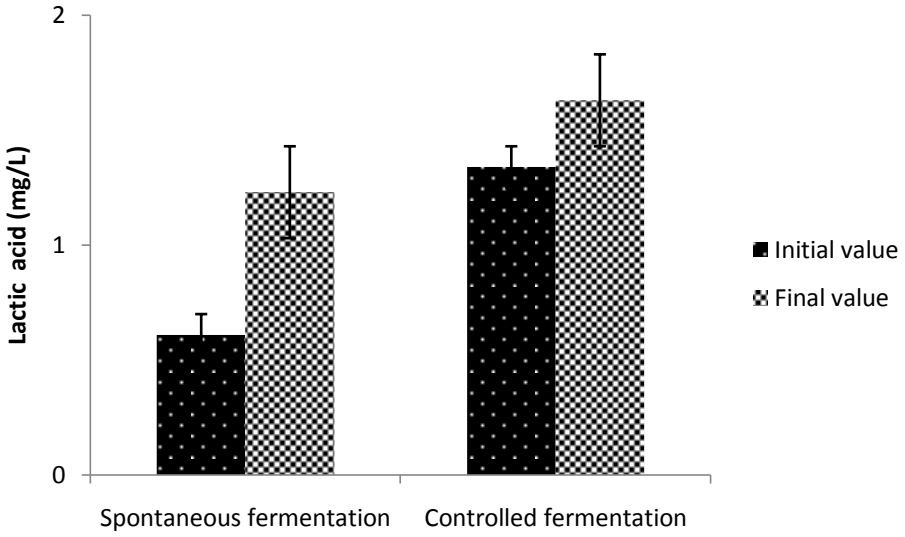

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Figure 3: Lactic acid amounts

Malic acid amounts

The concentration of malic acid in the relative value decreased on average to 25.06 % of its original value. The highest reduction occurred in spontaneous fermentation with the 3.64 ± 0.13 to 0.1 ± 0.01mg/L and 1.8 ± 0.34 to 1.51 ± 0.1).

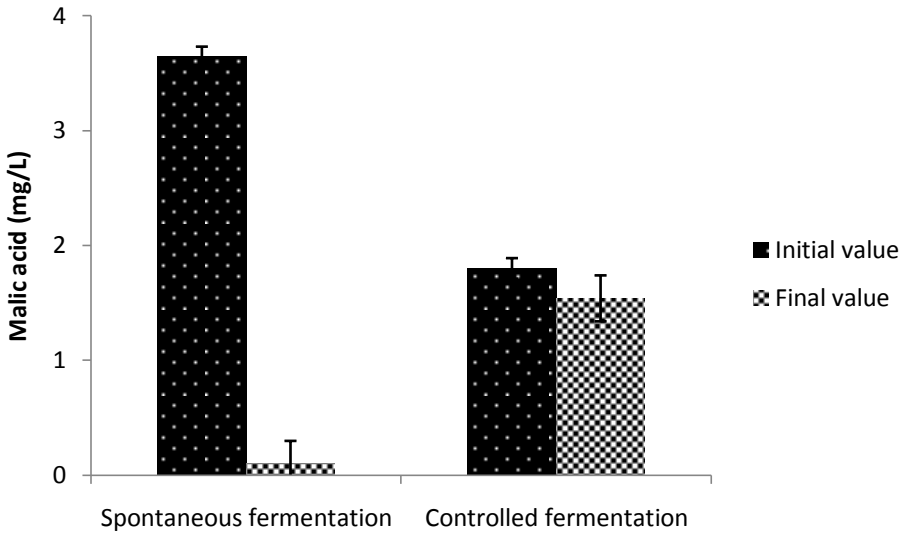

Figure 4: Malic acid amounts

Oxalic acid amounts

During cocoa juice, oxalic acid was produced. The average increase of concentration of oxalic acid. This increase of the concentration is the same in the two types of fermentation. The concentration value of oxalic acid is 1.7 mg/l.

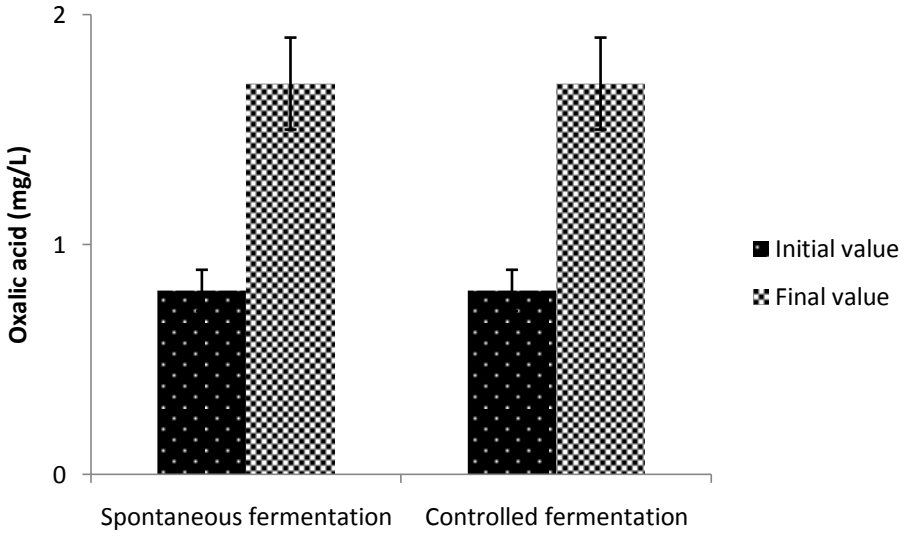

Figure 5: oxalic acid amounts

Organic acids comparison according to the type of fermentation

In our study, significant variation of amount of citric, acetic and malic acid was observed between the types of fermentation. The differences of average mass concentration of oxalic and lactic acid are not statistically relevant no significant differences between the controlled and the spontaneous processes.

Table 2: Organic acid values at the end of fermentations

Parameters	Spontaneous fermentation	Controlled fermentation

Citric acid (mg/L)	5,1± 0,4[a]	4,3 ± 0,4[b]
Acetic. acid (mg/L)	6,2 ± 0,1[a]	3,6 ± 0,2[b]
Lactic acid. (mg/L)	1,2 ± 0,1[a]	1,3 ± 0,2[a]
Oxalic acid (mg/L)	1,7 ± 0,1[a]	1,3± 0,1[a]
Malic acid (mg/L)	0,1± 0,01[a]	1,5± 0,1[b]

Results are means values of triplicate analyses ± S.D. Means within row designated by different letters are significantly different by the LSD test $P \leq 0.05$

Discussion

This study should help us to study the evolution of major organic acids during the cocoa mucilaginous juice fermentation that occur on further cocoa wine production. The results are compared with those refereed in literature for other types of wine and fermentation. Statistically differences were observed with the final values of citric acetic and malic acids. Despite the decreasing of citric acid content in both of processes of alcoholic fermentation, the concentration of this product was lower (4.87%) in the spontaneous compared to the controlled fermentation. The increasing of acetic acid was also more important during spontaneous process. This difference was due to the high diverse microorganism concentration found in the cocoa juice (Made and Graham, 2003, Camu et al., 2008).

The decreasing of citric acid during cocoa juice fermentation was already described by Sandra et al., 2007. It is due to the citric acid consumption by some microorganism such as *Candida inconspica and Hanseniaspora guillermondii* during their growth (Neilsen et al., 2007). Acetobacter transformed this compound as source of nutriment into acetic acid production**.** Oxalic and lactic acids were also produced during this fermentation in spontaneous fermentation. Lactic acid was produced by lactobacillus but in controlled fermentation, it was produced by *Saccharomyces cerevisiae* probably during malolactic fermentation was as previously described by Navarre (1998).

Bacteria of lactic fermentation degraded malic acid into lactic acid during malolactic fermentation (Gary et Justin., 2004). The malolactic fermentation causes the degradation of malic acid from wine and also its replacement with lactic acid which is better evaluated in sensory way.

According to the results obtained, cocoa mucilaginous juice had almost the same characteristics like other must (grape, apple, tropical juice) used in wines production. During cocoa mucilage juice fermentation, there are sugar consumption, alcohol production (Anvoh et al., 2010) and organic acids movements.

Nutritionally, Wine produced from cocoa mucilage juice could have same nutritional characteristics like dark chocolate. En deed, chocolate is made from the seed of cocoa. It is a major source of flavonols with highest antioxidant levels (Engler 2006; Vinson et al., 2006). It contains most of the necessary compounds and minerals to prevent major chronic heart condition, cancer and other age related diseases (Rein et al., 2000, Hammerstone et al., 2000, Cooper et al., 2008).

Arts et al. (1999) reported that dark chocolate contains catechins (a group of flavan-3-ol flavonoid compounds) at an average concentration of 0.535 mg/g; four times that of tea (0.139 mg/g). It is higher in antioxidant than red wine (Lee et al., 2003). By chocolate, cocoa consumption has shown to have the greater biological effects human health. Cocoa reduced platelet adhesion (Hermann et al., 2006) and also been shown to inhibit plasma lipid oxidation (wiswedel et al., 2004; Mursu et al., 2004).

So cocoa mucilage wine could have higher antioxidant level than red wine. However, the comparison from cocoa antioxidant with red wine was done on dark chocolate. Alcohol effect on flavanoids absorption should be shown because some compounds can slow down the intestinal absorption of flavonoids such us milk as demonstrated Serafini et al., (2010). If it is proved true that cocoa mucilage wine is higher in antioxidant than red wine, cocoa mucilage juice should be not only be fermented for alcohol production but as supposed Anvoh et al., (2010), this by product could also be used as raw material like grape, apple for cocoa wine production. That could increase the income of smallholders of cocoa producing chain by creating distilleries.

This perspective could reduce the utilization of tropical fruits such as orange and pineapple in ethanol production and allow diversifying the local fermented beverages.

Conclusion

This study demonstrated that the fermentation of cocoa mucilaginous juice into alcohol was followed by variation of organic acids amount. This variation was deeply observed during spontaneous fermentation than controlled process. Additional studies are needed to examine the movement of tartaric acid and other phenol compounds.

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