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Physicochemical changes of raffia sap (*Raphia mambillensis*) contents during spontaneous fermentation

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The chemical content of a substance can influence its shelf life. Fermentation causes changes in freshly tapped sap and therefore makes its large scale use difficult. In order to study the effect of time on fermentation on the raffia sap property, its physico-chemical and microbiological characteristics were determined during five days. The results recorded for the harvest reveal an acidity, proteins and energy of about 67.7°D, 6.69 g/l, and 113.64 kcal/l respectively. Raffia sap acidity and protein content increase during fermentation when the energy value decreases. The microbiological analysis showed that this sap contains an average of 16.46 x 10^7 cfu/ml of total germs which increases during fermentation. This sap is therefore rich in useful and pathogenic micro-organisms. We noticed that this sap is very rich in lactic acid bacteria and yeasts which could be isolated and exploited for alcoholic and malolactic fermentations in order to obtain a very stable raffia sap.

Key words: Raffia sap, spontaneous fermentation, physicochemical property, lactic acid bacteria, yeast, nutrient content.

INTRODUCTION

The raffia sap commonly called raffia wine is a part of a series called "palm wine" which refers to all alcoholic beverages from the fermentation of sap of various palm trees. The raffia wine is an alcoholic traditional, sweet, effervescent beverage, usually consumed by the poor populations of Black Africa, Latin America and Asia (FAO, 1998).

In Cameroon, this sap is obtained from the palm tree *Raphia sp.* in the West region and particularly from the *Raphia vinifera* and *Raphia mambillensis* in the area of Dschang. It is largely consumed and presented in all traditional ceremonies. In response to humanitarian needs and environmental issues increasingly growing, the use of raffia sap increases more and more (Mbuagbaw et al., 2012). Indeed, it is used in almost all African tribes in particular during births, traditional rites, weddings, funerals. It is used in the treatment of venereal diseases, measles, and typhoid and is a significant contribution in the treatment of impotence. It also favors milk production in lactating women (Tachago, 2007; Mbuagbaw et al., 2012). This drink is rich in sugars, proteins, alcohol, minerals, and vitamins (Malaisse, 1997; Ogbonna et al., 2013) and contains a certain number of microorganisms (Okafor, 1972; Obire, 2005). With time, fermentation converts sugars to ethanol, then into lactic or acetic acid (Matthews et al., 2004), which is accompanied by a loss of sweet taste and palatability (Odunfa, 1985). Like other alcoholic traditional beverages, the fresh raffia sap has a very short life span; it cannot be preserved for more than one day; the sap becomes very sour which makes the product unacceptable by the consumers (Uzochuku, 2004; Mintah

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et al., 2011). The present work was undertaken to assess the physico-chemical and microbial evolutions of freshly harvested raffia sap during fermentation in order to understand the transformation that takes place in the sap and further set-up a process of conservation for this sap in Menoua division (West-Cameroon).

MATERIALS AND METHODS

Sample collection

The raffia sap used in this study was obtained from raffia palm plantations located at three different areas. Sixty-seven (67) samples of fresh raffia sap were collected (1500 ml each) from winegrower in Menoua division (West-Cameroon) during a three months period. Those samples were kept in clean sterile bottles and transported to the laboratory within 30 - 60 min of tapping. The samples were immediately distributed in three sterile bottles properly labelled and recorded; one for immediate analysis, another for the second day and the third bottle for the fifth day analysis. The last two bottles were preserved in the laboratory under sterile conditions at room temperature (approximately 25°C).

Analysis of samples for physico-chemical contents

Twenty milliliter (20 ml) portion of each sap sample were removed aseptically for each measurement. Moisture content and the density of the fresh wine were determined using the method described by AOAC (1995); acidity level was determined by titration of the sap with NaOH 1/9 N and pH using a pH-meter (SUNTEX TS-2) for the first, second, and fifth day. Each sample was repeated in triplicate.

Analysis of samples for microbial contents

The amount of micro-organisms was determined by serial dilutions and spread plate technique was used on appropriate selective media (Durgesh et al., 2008). Successive dilutions of the sap were prepared in screw test tubes and appropriate dilutions were poured into plates then enumerated for total plate count until scattered colonies were obtained. Ten milliliter (10 ml) of each sample was diluted with 90 ml of sterile buffered peptone water and well mixed. One milliliter (1 ml) of each dilution sample was inoculated in the following media: M17 Agar (Biolife) for the lactococci; the lactic acid bacilli on MRS Agar (Biokar Diagnostic); salmonellas and shigellosis on Salmonella-Shigellosis Agar (MERK) yeasts and moulds on Potato-Dextrose Agar; enteric bacteria on MacConkey Agar (SIGMA); staphylococci on Staphylococcus110 Agar (SIGMA) and total flora on plate count agar (SIGMA). All plates were incubated under aerobic conditions at room temperature (25 ± 1°C) and the colonies were counted using a colony counter (Stuart) after a period of 24 h to 72 h depending on the growth media. The mean number of colonies counted was expressed as log of colony forming units (cfu)/ ml. In order to check up the specificity of media, Gram stain test was done and colonies were observed under a light microscope with an appropriate dye to observed their shape and verify their membership in the considered group. Each experiment was performed on samples newly collected and those of the second and fifth days. Each sample was carried out in triplicate.

Analysis of samples for nutritional contents

Determination of total ash

Total ash was the residue obtained after incineration of the dried sample and represented as the mineral part of it. The method described by AOAC (1980) was adopted which consists of incinerating the sample in an oven (Hereaus) at 560°C in an oxidizing atmosphere until obtaining a residue of constant weight.

Determination of lipid content

Determination of fat quantity was done by Soxhlet extraction. The lipid was extracted in a Soxhlet extraction apparatus (Glassco) with hexane as solvent. The Soxhlet extraction procedure is a semi continuous process, which allows the buildup of the solvent for some time in the extraction chamber. A dried sample was crushed and introduced in an extraction thimble. The solvent surrounded the sample and was then siphoned back into the boiling flask. The lipid content was calculated at 0 % moisture by weight difference of the flask before and after complete extraction of the oil as described by IUPAC (1979).

Mass of lipid = (weight of the flask + extracted oil) − (weight of the flask)
Lipid content (%) = mass of lipid extracted (g)/sample weight (g) × 100).

Determination of total proteins

Determination of total protein was carried out by determining total nitrogen in each sample. Total nitrogen was determined by the Kjeldahl method as described by AOAC (1980). This method transformed organic nitrogen of the dried sap into mineral nitrogen by mineralization with concentrated sulfuric acid and a catalyst using ramp of mineralization all under hood. The nitrogen present in the sample was fixed in the form of $(NH4)_2SO_4$. After digestion, the solution was made alkaline by adding sodium hydroxide using an automatic distiller (Kjeltec System) to release ammonia. After distillation, the amount of ammonia was determined by acid-base titration with boric acid and the amount of protein was estimated using a conversion factor.

Determination of total carbohydrate

Total carbohydrates were determined using the current method of difference (AOAC, 1980). Total carbohydrate = [rate of dry matter - (ash + rate of lipid + rate of protein)] or
Total carbohydrate = [rate of organic matter - (rate of lipid + rate of protein)].

Statistical analysis

Data collected from physico-chemical, microbiological and nutritional content analyses of the sap at different stages of spontaneous fermentation were subjected to statistical analysis. All the results underwent the variance analysis (ANOVA) to the threshold of probability 0.01 and the test of Bonferroni was used to compare the averages by using software GRAPHPAD INSTAT.

RESULTS

The sap sources are shown in Table 1. After having transported the different sap samples collected in the laboratory, physical and chemical tests such as water content, density, Dormic acidity and pH were done on those samples.

During fermentation, the moisture contents of raffia sap

Table 1. Sources for raffia sap samples.

Month	Source			
	Bamendou	Foreke	Foto	Total
1st	7	4	14	25
2nd	5	3	8	16
3rd	8	3	15	26
Total	20	10	37	67

Table 2. Physico-chemical properties in the raffia sap during five days of spontaneous fermentation.

Number of days of fermentation	Parameters			
	Water content (%)	Density (g/ ml)	Dormic Acidity (°D)	pH
0	96.11 ± 1.21	1.00 ± 0.02	67.6 ± 06.38	4.08 ± 0.19
2	97.12 ± 0.72	0.97 ± 0.01	90.6 ± 20.00*	3.40 ± 0.07*
5	97.17 ± 0.05	1.00 ± 0.04	113 ± 13.68*	3.21 ± 0.16*

*, Significant difference; n, 67.

were: 96.11 ± 1.21%; 97.12 ± 0.72% and 97.17 ± 0.059% respectively at harvest, second and fifth days of spontaneous fermentation at room temperature (approximately 25°C). This content increases between harvest and the second day and then seems to be stabilized the fifth day (Table 2). However, there is not any significant difference at $p > 0.01$ from one sample to another and during five days of fermentation.

Values of the density were about 1.005 ± 0.02, 0.97 ± 0.01 and 1.002 ± 0.04 g/ml. We noticed that this content decreased slightly during the second day, then increased on the fifth day (Table 2). However this variation of density from one day to another was not significant. We obtained the following pH average values: 4.08 ± 0.19; 3.4 ± 0.07; 3.21 ± 0.16 respectively at harvest, second and fifth days (Table 2). This pH decreases during fermentation and was significantly different at $p > 0.01$ between harvest, second day and fifth day.

The values of Dormic acidity were: 67.6 ± 6.38°D; 90.6 ± 20°D and 113.4 ± 13.68°D. Dormic acidity of this sap increased during fermentation, what would also justify the reduction in the pH. In fact high Dormic acidity translates the presence of the lactic acid in the wine. This acidity as for the pH was significantly different at $p > 0.01$ between harvest and the fifth day, what translates the very acid character observed at this stage of fermentation.

In order to understand all these physico-chemical changes in the sap during spontaneous fermentation, we analyzed its wild microflora. We noticed that the microflora of the fresh raffia sap of *R. mambillensis* is very dense and it contains useful micro-organisms (Figure 1) and pathogenic micro-organisms (Figure 2); its total microflora increasesd during the five days. The most abundant micro organisms in the fresh sap were the lactic bacteria (lactobacilli and lactococci), then come yeasts (Figure 1). Then, we had the faecal coliforms, the staphylococci, few salmonellas and shigellosis (Figure 2), and other non-determined micro-organism but however present in the total flora. We noticed a positive correlation between lactic acid bacteria, yeasts, salmonellas and shigellosis and the density of the sap; also between enteric bacteria, staphylococci, acidity and the water content. Sap acidity seems to be unfavourable to useful micro-organisms and favourable to pathogenic micro-organisms.

During fermentation, there was no significant difference at $p > 0.01$ between the lactic bacilli, the lactococci, yeasts and moulds, total coliform bacteria; however these kinds are significantly different from staphylococci and salmonellas and shigellosis. Evolution of sap nutrient content during fermentation was studied and the results are presented per liter of sap in Table 3. From this, the average rate of protein increased during the fermentation while the lipid decreased and was significantly different at $p < 0.05$ between the first and fifth days.

Total carbohydrates average decreased during the fermentation with a significant difference at $p < 0.05$ between harvest, second and fifth days. Average ash decreased in the fermented raffia sap. There was no correlation between proteins, fat and total flora. Proteins significantly increased the fifth day. In general, there was a decrease in dry matter, lipids, carbohydrates, ash, and energy with the fermentation time; this decrease was significant at the fifth day.

DISCUSSION

Sap water content obtained at harvest (fresh sap) is similar to values reported by Malaisse (1997) with sap obtained from other palm tree species. Moisture content determines the shelf-life of sap. The lower the moisture content, the longer the expected shelf-life, thus moisture content is an important measure of sap quality as reported

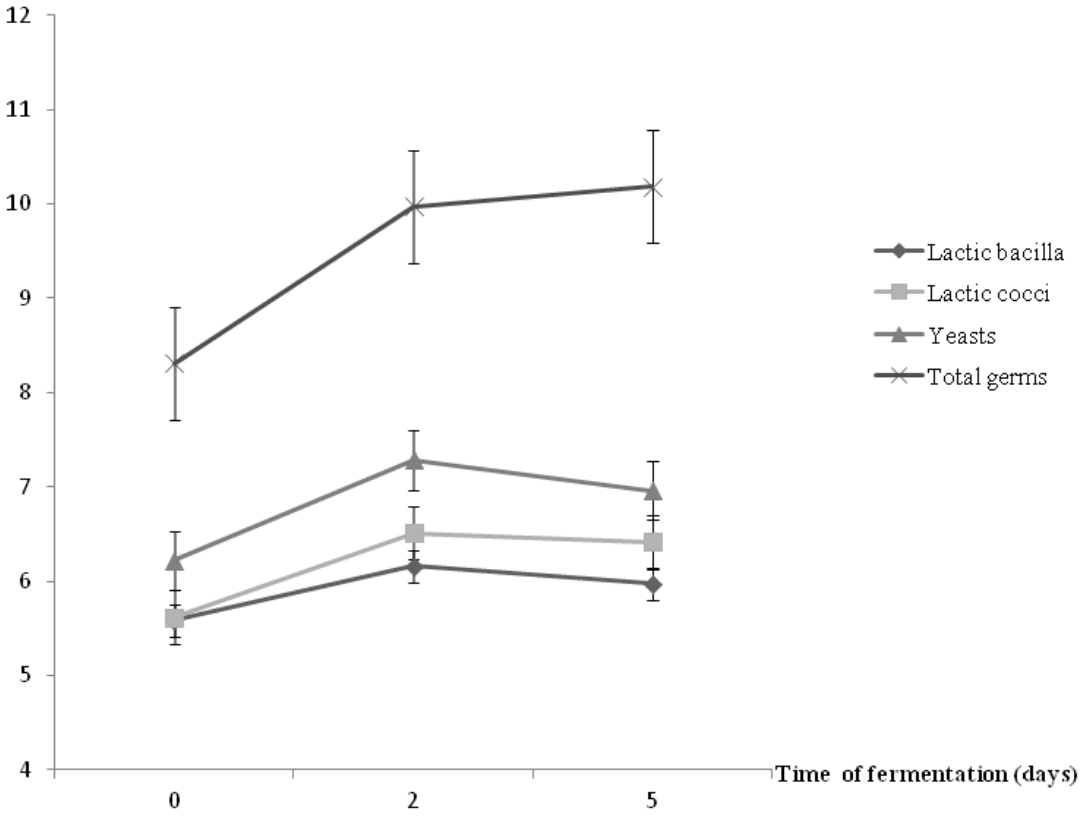

Figure 1. Evolution of useful microorganisms in the sap of *Raphia mambillensis.*

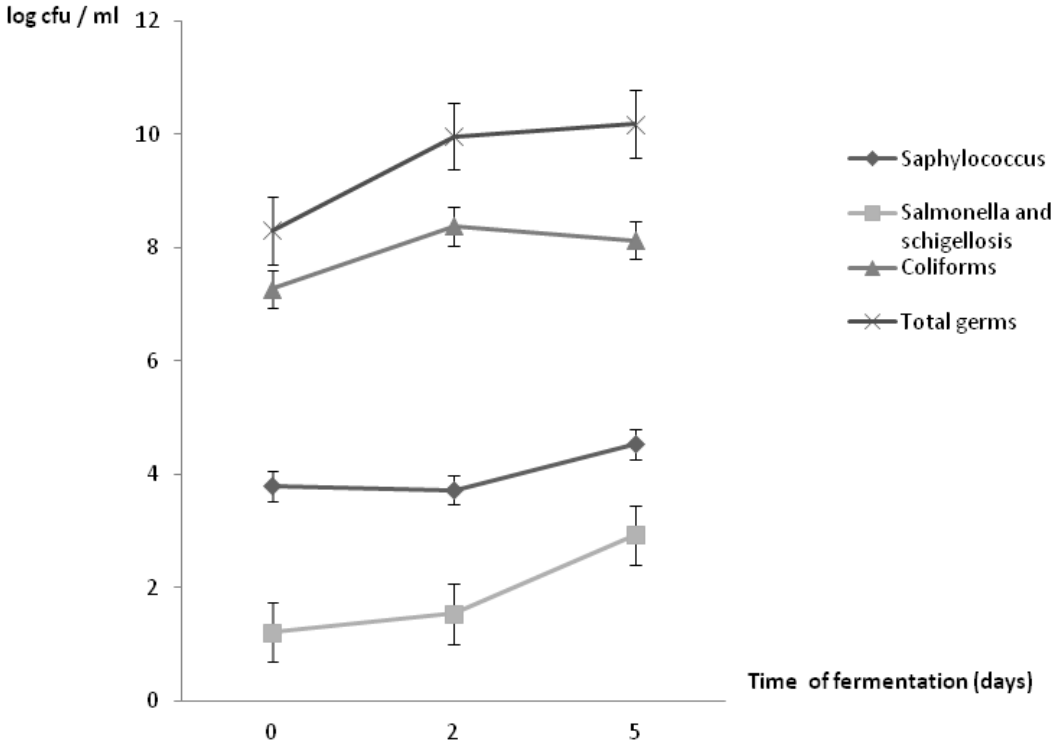

Figure 2. Evolution of pathogenic microorganisms in the sap of *Raphia mambillensis.*

Table 3. Nutritive composition of *Raphia mambillensis* sap during spontaneous fermentation.

Number of fermentation days	Parameter					
	Dry matter (g / l)	Protein (g / l)	Fat (g / l)	Carbohydrates (g / l)	Ash (g / l)	Energy (Kcal / l)
0	34.06 ± 0.51	6.69 ± 1.41	1.96 ± 0.47	17.31 ± 2.86	8.10 ± 1.23	113.64 ± 21.31
2	30.37 ± 0.74	7.01 ± 0.96	1.94 ± 0.39	13.51 ± 1.47	7.91 ± 0.70	99.54 ± 13.23
5	29.25 ± 0.16	8.85 ± 2.04 *	0.95 ± 0.51 *	11.84 ±2.02 *	7.71 ± 1.09 *	94.01 ± 20.83 *

* Difference is significant ($p < 0.05$) between the first and fifth days. n = 67

by Mintah et al. (2011). Increase in water content the second day corresponded to a reduction in the dry matter which could be due to substrate degradation such as ethanol to sugars with CO_2 production as reported by Matthews et al. (2004).

Between the second and the fifth day, there could be conversion of ethanol produced in lactic acid or acetic as described by Odunfa (1985) and Matthews et al. (2004). Density decrease observed the second day could be due to alcohol production which is lighter than water; between the second and the fifth day, there could be alcohol transformation into acetic acid and lactic acid. pH value obtained for the first day of spontaneous fermentation at room temperature is similar to that obtained by Malaisse (1997). According to this author, the chemical characteristics of the palm sap vary during the bleeding, making the comparisons sometimes delicate as indeed evidenced by several studies (Okafor, 1975; Mintah et al., 2011; Karamoko et al., 2012; Mbuagbow and Noorduyn, 2012; Ogbonna et al., 2013).

The microbiological analysis reveals that the most abundant micro-organisms in the fresh raffia wine are the lactic bacteria and the yeasts as underscored by Mattews et al. (2004). According to the same authors, the yeasts are responsible for the alcoholic fermentation of the sap and the lactic bacteria are responsible for malolactic fermentation (transformation of the L-malic acid into L-lactic). Raffia wine naturally contains micro-organisms (useful and pathogenic) as had already been described by certain authors such as Okafor (1972, 1975) and Obire (2005) for wine samples collected in Nigeria. At this stage of work, we recommend that raffia sap be consumed the first two days following the harvest because after this period it contains most pathogenic micro-organisms.

Nwachukwu et al. (2006) and Ogbulie et al. (2007) studied the effects of micro organisms on saps from other species and showed that these saps host a number of natural micro organisms that are responsible for their instability. Since these micro organisms catalyze the fermentation process, which leads to nutrient degradation or loss of sensory qualities of palm wine, loss due to the sour taste of fermented palm wine by the acids produced during the fermentation process as noticed by Malaisse (1997) and Ukhum et al. (2005).

The sap analysis showed that the average protein content increased during fermentation, which was due to cell death during fermentation. However, in our results, there is no correlation between changes in the rate of protein and that of the total flora during the fermentation. The protein average of fresh raffia sap was higher than the values obtained by Malaisse (1997) and Ghogomu (2004); this could be due to the influence of climatic and soil factors that influence the nutritive composition of sap samples.

Lipid decrease is due to degradation of raffia sap during fermentation by microorganisms. The energy value decreases, which is due to the reduction in the rate of reducing sugars which would be transformed into alcohol and acid. But if we had taken into consideration the increasing level of alcohol in the raffia sap from the work of Tachago in 2007 with the same species, the energy increased. The raffia sap naturally hosts microorganisms (pathogens and useful) as already described by Okafor in 1975 for sap samples collected in Nigeria. These pathogens originate from contact with the sap from the bark of the tree and plant debris (pulp rotten banana trunk) used to seal the entrance to the bud sectioned (Okafor, 1975; Uzochukwu, 2004; Ogbulie, 2007; Tachago, 2007).

The most abundant microorganisms in the raffia sap were lactic acid bacteria and yeasts as was underlined in Matthews et al. (2004) in red wine. Lactic acid bacteria, yeasts, salmonella and shigella decreased on the second day and subsequently increase as the density of the fifth day; we can say at this stage that these organisms have reached their stationary phase of growth. According to several authors such as Matthews et al. (2004), the yeasts are responsible for the alcoholic fermentation of a sap; lactic acid bacteria are responsible for malolactic fermentation (conversion of L-malic acid into L-lactic acid). Enteric bacteria and Staphylococcus increased until the fifth day as the acidity and water content. The sap acidity seems to be favourable to pathogenic micro-organisms and unfavourable to yeasts and lactic acid bacteria which are micro organisms with beneficial effects as reported by Tiepma et al. (2010). Sap acidity which seems to favour the growth of yeast and lactic acid bacteria led us to the perspective of isolating, characterizing for technological properties, identifying, and re-

seeding them in sterilized sap in order to control the fermentation.

Conclusion

A study of the impact of time of fermentation on raffia sap (*R. mambillensis*) property, its physico-chemical and microbiological characteristics during five days were carried out. The results of our study suggest that, the nutritional components as well as the microbiological content of the raffia sap from *R. mambillensis* change during spontaneous fermentation at room temperature. Wild microflora is very dense and includes both useful and harmful microorganisms with the majority being lactic acid bacteria and yeasts which could be isolated and exploited for alcoholic and malolactic fermentations, as well as for probiotic effects. In five days, the sap becomes very acidic and the most abundant acid was lactic acid. At the current state of our knowledge, these results constitute a database for studying the impact of fermentation period on the raffia sap and contribute to the creation of a stabilization process of this sap without completely changing its organoleptic qualities.

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