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Evaluation of Organic Acids and Sugars Contents During the Production of "Tchapalo", a Traditional Sorghum Beer in Côte D'ivoire

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Abstract: The evaluation of organic acids contents during tchapalo production was investigated. Samples of mash of sorghum, cooked sediment, wort, sour wort, sweet wort and tchapalo were analysed by HPLC. Titratable acid, pH, soluble solids, reducing sugars and total sugars were also tested. The predominant organic acid detected always in the mash was propionic acid (2.07±3.51 g LG^{1}). Contents of soluble solids, reducing sugars and total sugars were 2.7±0.8%, 1.78±1.48 and 13.75±1.32 g LG^{1}, respectively. During spontaneous fermentation, lactic acid and sometimes acetic acid were the only organic acids produced causing the increase of titratable acidity of the sour wort. Reducing sugars consumed was compensated by that released after the hydrolysis of total sugars. Majority of organic acids and sugars were concentrated in the sweet wort by the cooking. Then lactic acid was again produced together with citric acid during alcoholic fermentation; contents of sugars decreased a lot. Therefore in tchapalo, lactic acid (14.56±3.58 g LG^{1}) was predominant followed by propionic (3.45±1.98 g LG^{1}), citric (1.96±0.75 g LG^{1}) and malic (1.73±1.17 g LG^{1}) acids and contents of soluble solids, reducing sugars and total sugars were 8.5±2.2%, 33.86±20.0 and 88.42±48.4 g LG^{1}, respectively.

Key words: Organic acids, sugars, fermentations, traditional beer, tchapalo processing

INTRODUCTION

Tchapalo is a traditional alcoholic beverage from sorghum grains produces by women. Initially these women produced it in the northern part of Côte d'Ivoire, but today they produce this beverage throughout the whole country, particularly at Abidjan where it knows a remarkable success (Yao *et al*., 1995; N'Da and Coulibaly, 1996; Sawadogo-Lingani *et al*., 2007). This success is due not only to its relatively low price and therapeutic properties but also to its nutritional value that contributed significantly to improve the diet of consumers. These qualities have given to tchapalo a lot appraise product by population (Yao *et al*., 1995). Tchapalo production, like many other indigenous fermented beverages is characterized by a series of complex physical, biochemical and microbiological changes affecting the principal components of sweet wort and tchapalo. It involves two successive steps of fermentation which occur at ambient conditions (Van der Aa Kühle *et al*., 2001; Nanadoum *et al*., 2005). The primary, spontaneous fermentation is carried out by microorganisms inherent in the raw material, containers and the surrounding environment (Togo *et al*., 2002). This stage confers the souring taste and storage longevity. The second, alcoholic fermentation is performed by the inoculation of the sweet wort with dried yeast harvested from previous tchapalo and gave the final product. These fermentations are difficult to control and productions come down often in deplorable hygienic conditions.

Organic acids are naturally present in foods or they are synthesized during biochemical metabolic processes or bacteria metabolism (Akalin *et al*., 2002; Soyer *et al*., 2003; Karadeniz, 2004). They have important roles in beverages because they affect the organoleptic properties, stability, nutrition, acceptability and in maintaining quality (Santalad *et al*., 2007). Their presence and concentration determine tartness, other flavor attributes and the progress of fermentation. Organic acids also improved the safety or preserving of traditional beverages (Soomre *et al*., 2002). In Côte d'Ivoire, the production of organic acids during tchapalo processing

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is not identified even though, the production of this beverage is based on sensorial tests (e.g., taste, flavor and color). The importance of these compounds is especially pertinent in assessing factors contributing of the flavor characteristic of sweet wort and tchapalo. The present study was to obtain the database of organic acids production during the different steps of tchapalo production.

MATERIALS AND METHODS

Tchapalo processing: Tchapalo processing started by the malting of sorghum grain, sun-drying and milling to give malted sorghum flour. This flour was mixed out with water containing a sticky substance. The mixture obtained called mash was separated from supernatant and sediment. The sediment was cooked during 2-2 h 30 min; later mixed with the supernatant to give wort. The wort was left for a spontaneous fermentation during the night to give after percolation the sour wort. The sour wort was cooked during 4-6 h to give sweet wort which is cooled and inoculated with dried yeast harvested from previous tchapalo for alcoholic fermentation during 9-12 h. The product obtained after alcoholic fermentation is tchapalo (Fig. 1).

Sampling: Sampling was carried out on mash, cooked sediment, wort, sour wort, sweet wort and tchapalo during each tchapalo production. Samples were collected from three areas (Abobo, Attecoubé and Yopougon) in the district of Abidjan. They were collected in sterile small bottles, labelled and then transported to the laboratory in a box containing a freezing pack. Four productions samples were collected from each area. A total of seventy-two samples have been taken.

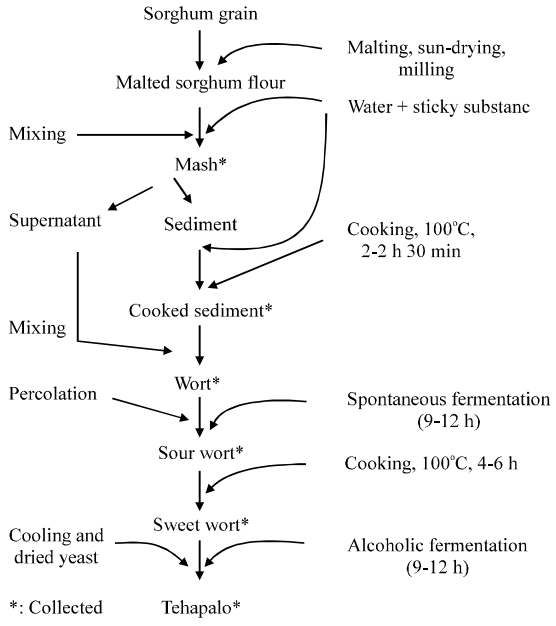

Fig. 1: Diagram of tchapalo processing

Determination of pH, titratable acidity and soluble solid: Value of the pH was determined with a pH-meter (pH-meter P 107, CONSORT, Bioblock, France). Titratable acidity was determined using the standard method described by Amoa-Awua *et al*. (1996). The titratable acidity was expressed as g lactic acid LG^{1}. The soluble solid content of samples was determined as Brix using a hand refractometer (ATAGO, N-20E, Japon). Two independent measurements were made on each sample.

Samples preparation for HPLC and organic acid standards: Samples were centrifuged at 4000 rpm for 30 min. Supernatants were filtered through a 0.45 µm Millipore membrane filter (Sartorius AG, Go¸ttingen, Germany) and then stored at -20°C until analysis. Aliquots of sample (20 µL) were injected into the HPLC. Standards solutions obtained from organic acids (oxalic, citric, tartaric, malic, lactic, acetic, fumaric and propionic acids) were prepared individually with double distilled water and filtered through a 0.45 µm Millipore membrane filter.

HPLC analyses conditions: Analyses were carried out with an ion- exclusion ORH-801 column (300×6.5 mm) (Interchrom, France) preceded by a Universal Guard Cartritge-Holder column. The High-Performance Liquid Chromatograph system (LC-6A, Shimadzu corporation, Japan) was equipped of a Shimadzu LC-6A pump. Column effluents were monitored by an UV detector (SPD-6A, Shimadzu Corporation, Japan) set at 210 nm. The mobile phase (0.004N H_2SO_4) used at a flow rate of 0.8 mL $minG^{1}$ was filtered through a 0.45 µm Millipore membrane filter (Sartorius AG, Go¸ttingen, Germany). Each sample was injected in duplicate. Organic acids were identified by comparing their retention times with those of standards.

Determination of total sugars and reducing sugars: Total sugars were determined by the phenol sulphuric acid method according to Dubois *et al*. (1956). The reducing sugars were quantified as previously described by Bernfeld (1957). Two independent measurements were made on each sample and results were expressed in g LG^{1}.

Statistical analysis: The data were analysed using one-way Analysis of Variance (ANOVA) (statistica, 99ème edition). Duncan's multiple range test was used to compare the means when a significant variation was established by ANOVA at the significance level (" = 0.05).

RESULTS AND DISCUSSION

Titratable acidity, pH and organic acids detected in intermediate products before spontaneous fermentation: A representative chromatogram of organic acids in a sample of sour wort analysed by HPLC is shown in Fig. 2. The peaks 1, 3, 4, 6, 7, 8 and 9 represent oxalic, citric, malic, lactic, acetic, fumaric and propionic acids, respectively. The Fig. 3 shows the pH, titratable acidity and organic acids which was detected in all analysis samples.

Titratable acidity and pH remained relatively constant from mash to wort and did not varied significantly ($p>0.05$) between productions and between areas. Oxalic (0.28±0.08 g LG^{1}), citric (0.43±0.27 g LG^{1}), malic (0.55±0.47 g LG^{1}), lactic (0.95±1.02 g LG^{1}), propionic (2.07±3.51 g LG^{1}) and fumaric (9.2±3.4 mg LG^{1}) acids were detected always in the mash of sorghum with a predominance of propionic acid (Fig. 3). The same organic acids were also found in the cooked sediment and their content did not changed significantly ($p>0.05$) in the wort obtained after the mixing of supernatant and cooked sediment. Occasionally detected organic acids are presented in Table 1. Acetic acid (0.62±0.14 g LG^{1}) was detected in 75% of the mash in Yopougon. On the other hand, it not was found in the samples of the other areas. The presence of acetic acid in the mash to wort may be due to the method employed by producers (N'Da and Coulibaly, 1996). Indeed, at Abobo and Attecoubé, the dried sorghum malt was milled a few times before the beginning of production and no acetic acid was detected in the mash to wort. On the other hand, at Yopougon, producers milled the dried sorghum malt one to 14 days before the beginning of production. Perhaps the conservation of the molested malt favoured the growth of microorganisms which produce acetic acid before the beginning of production. Tartaric acid was also occasionally found and the detection frequencies varied from one area to another (Table 1).

Evolution of pH, titratable acidity and organic acids during the spontaneous fermentation: During spontaneous fermentation, pH of the wort decreased from 5.67±0.42 to 3.8±0.24 in the sour wort and varied significantly ($p<0.05$) between productions and one area to another. Conversely, the titratable acidity increased from 0.17±0.05 to 0.56±0.18 g lactic acid LG^{1}. The content of lactic acid became 10 fold higher (7.75±2.5 g LG^{1}) and high proportion of fumaric acid (61.64%) was metabolised at the end of fermentation. The content of others organic acids did not vary significantly ($p>0.05$) in the sour wort

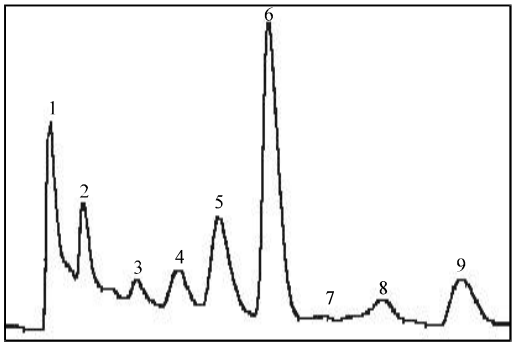

Fig. 2: Chromatogram of organic acids of a sour wort sample: 1 = Oxalic acid, 2, 5 = unknown, 3 = Citric acid, 4 = Malic acid, 6 = Lactic acid, 7 = Acetic acid, 8 = Fumaric acid, 9 = Propionic acid

Table 1: Presence and contents of acetic and tartaric acids during tchapalo production on different areas

			Step samples of tchapalo production					
Organic acids	Presence and contents	Areas	Mash	Cooked sediment	Wort	Sour wort	Sweet wort	Tchapalo
Acetic acid	Presence (%)	Abobo	0	0	0	75	0	0
		Attecoubé	0	0	0	50	0	0
		Yopougon	75	75	75	100	100	25
	Content (g LG^{1})	Abobo	0	0	0	1.42±1.62	0	0
		Attecoubé	0	0	0	0.23±0.11	0	0
		Yopougon	0.62±0.14	0.96±0.5	0.59±0.16	0.64±0.25	1.83±1.15	0.94±0.02
Tataric acid	Presence (%)	Abobo	100	0	50	0	0	0
		Attecoube	50	0	0	0	0	0
		Yopougon	75	0	75	75	0	0
	Content (g LG^{1})	Abobo	0.31±0.23	0	0.15±0.07	0	0	0
		Attecoubé	0.13±0.11	0	0	0	0	0
		Yopougon	0.25±0.08	0	0.28±0.13	0.11±0.09	0	0

Table 2: Means and standard deviations of soluble solids, reducing and total sugars Contents in different steps of tchapalo production samples

	Step samples of tchapalo production					
	Mash	Cooked sediment	Wort	Sour wort	Sweet wort	Tchapalo
Soluble solids (%)	2.7±0.8	13.5±1.7	7.9±1.0	9.7±1.6	14.8±1.8	8.5±2.2
Reducing sugars (g LG^{1})	1.78±1.48	55.23±22.8	36.51±20.4	41.31±16.13	90.33±41.8	33.86±20.0
Total sugars (g LG^{1})	13.75±1.32	217.33±71.98	129.66±102.4	77.54±46.7	262.75±128.4	88.42±48.4

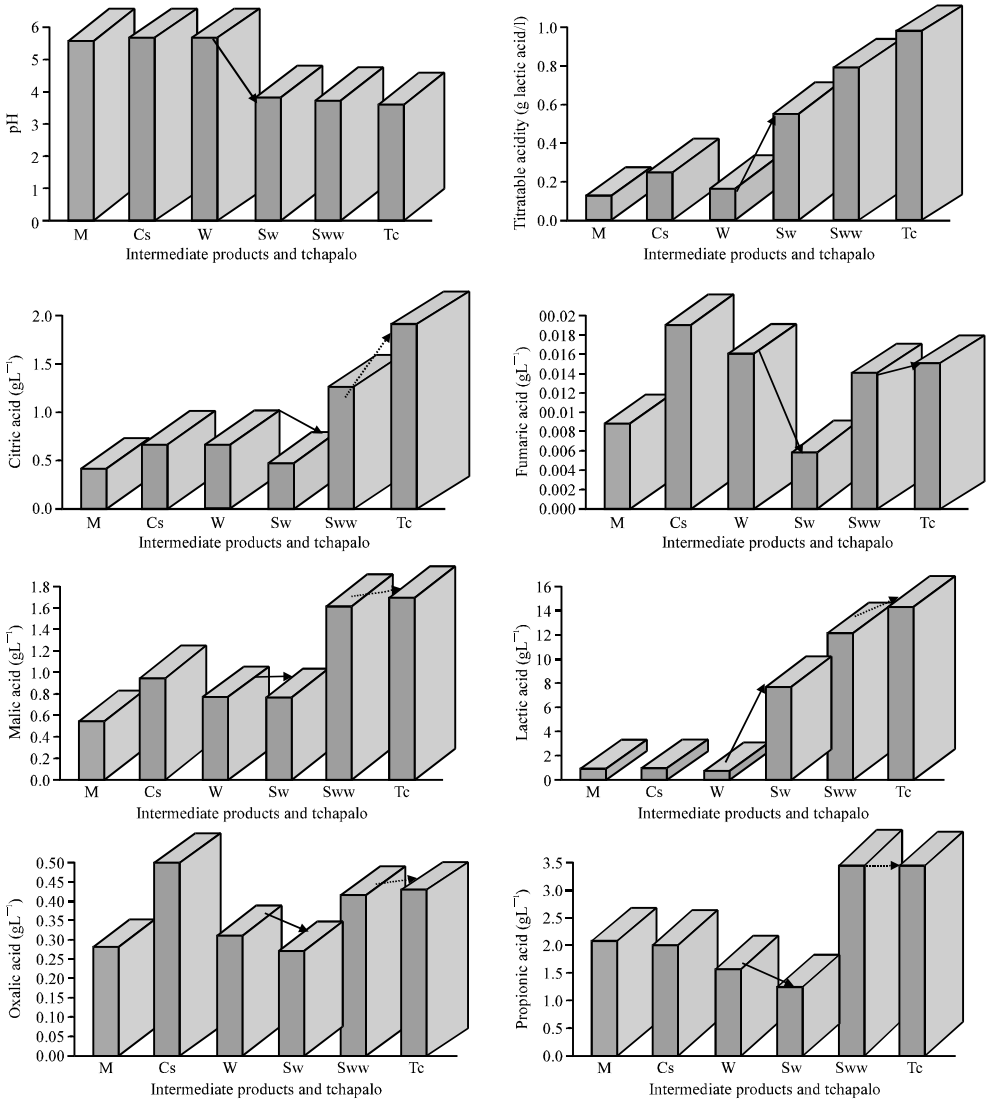

Fig. 3: pH, titratable acidity and Organic acids which were detected in all samples of intermediate and final products of tchapalo processing. M: mash, Cs: Cooked sediment, W: Wort, Sw: Sour wort, Sww: Sweet wort, Tc: Tchapalo,: spontaneous fermentation,: alcoholic fermentation

(Fig.3). Acetic acid appeared in the majority of sour wort samples in all areas with a concentration varying from 0.23±0.11 to 1.42±1.62 g LG^{1}) (Table 1). Therefore, lactic acid and sometimes small amounts of acetic acid were the only detected organic acids produced during the spontaneous fermentation.

These variations of acidity might be due to the activities of lactic acid bacteria, mainly Lactobacilli which were predominant microflora in the sour wort (result no shown). Many authors mentioned the presence of Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Enterococcus and Streptococcus in spontaneous lactic fermentation of sorghum products (Mohammed *et al*., 1991; Michodjèhoun-Mestres *et al*., 2005; Vieira-Dalodé *et al*., 2007). These lactic acid bacteria hydrolysed starch of sorghum leading to an acidified product particularly lactic acid that reduced pH. The low pH inhibited the growth of pathogenic microorganisms (Desmazeaud, 1996; Oyewole, 1997; Blandino *et al*., 2003). So, lactic acid production during the spontaneous fermentation increased the shelf-life of sweet sour, intermediate product drunk by women and children. Lactic acid conferred also the souring taste of wort (Van der Aa Kühle *et al*., 2001; Nanadoum *et al*., 2005) expected by producers. This souring taste was obligatory and important in this step because it determined the

further to process, organoleptic proprieties and the preserving of sweet wort. The content of lactic acid in sour wort observed by Nanadoum *et al*. (2006) was widely higher than our results. This result might be due to the difference in geographic regions, type of raw material, types of microorganisms and technologies used.

The synthesis of acetic acid during spontaneous fermentation in the majority of samples indicated that heterofermentative lactic acid bacteria were involved. This confirms our previous results which have shown that homofermentative and heterofermentative lactic acid bacteria were implicated in spontaneous and alcoholic fermentation steps of tchapalo production. Sawadogo-Lingani *et al*. (2007) have also found that *L. fermentum* was the dominant lactic acid bacteria species throughout the entire process to final dolo and pito wort, West African traditional sorghum beers.

Evolution of pH, titratable acidity and organic acids during the alcoholic fermentation: Before alcoholic fermentation, sour wort was cooked during 4 to 6 hours. That lead at end of the cooking to a sweet product called sweet wort. The majority of organic acids were concentrated in this sweet wort obtained (Fig. 3). Contents of lactic, propionic, malic, citric, oxalic and fumaric acids became 12.38±2.97, 3.46±1.14, 1.65±0.97 1.28±0.65, 0.41±0.17 and 14.19±6.0 mg LG^{1}, respectively. However, the pH (3.71±0.17) did not vary significantly ($p>0.05$).

During the alcoholic fermentation, the pH decreased to reach 3.63±0.15 in tchapalo and did not varied significantly ($p>0.05$) between productions but varied between areas ($p<0.05$). Titratable acidity of tchapalo (0.99±0.14 g lactic acid LG^{1}) was higher than that of sweet sour (0.8±0.15 g lactic acid LG^{1}) and did not varied significantly between productions and between areas. Changes in pH and titratable acidity during alcoholic fermentation were the result of the increase of citric and lactic acids contents (Fig. 3). Contents of propionic, malic, oxalic and fumaric acids did not vary significantly ($p>0.05$). Probably these organic acids did not consume or did not produce sufficiently during the alcoholic fermentation. At the end of the alcoholic fermentation, the obtained tchapalo contained 14.56±3.58 g LG^{1} of lactic acid which constituted the predominant organic acid. It was followed by propionic (3.45±1.98 g LG^{1}), citric (1.96±0.75 g LG^{1}) and malic (1.73±1.17 g LG^{1}) acids. These organic acids increased the shelf-life of tchapalo. Sanni *et al.* (1999) have also found lactic and malic acids in seketé, pito and burukutu, some traditional alcoholic beverages in Nigeria.

While tartaric acid was not detected in tchapalo, acetic acid was found in 25% of the tchapalo samples at Yopougon with a content of 0.94±0.02 g LG^{1} (Table 1). The absence of acetic acid in most of tchapalo samples was conform to certain authors which mentioned that the traditional beer must not contain acetic acid because it was source of spoilage in traditional beverages (Odunfa, 1985; Sanni *et al*., 1999). Therefore it is very important to select lactic acid bacteria and yeasts isolated in steps of tchapalo production and use them to starter culture.

Organic acids such as citric, malic, lactic, oxalic, fumaric and acetic were also found in the different type of modern beers at different concentration. These differences in the concentration were related to the fermentation process as well as variation in the brewing procedure (Moll, 1991; Cortacero-Ramírez *et al*., 2005; Santalad *et al*., 2007).

Evolution of soluble solids, reducing and total sugars during tchapalo production: The content of soluble solids of the mash and the cooked sediment were 2.7±0.8 and 13.53±1.7% respectively and that of the wort obtained was 7.91±1%. During the spontaneous fermentation, the content of soluble solids of the wort increased slightly and became 9.69±1.6% in sour wort. Conversely, content of total sugars decreased from 129.66±102.4 g LG^{1} in wort to 77.54±46.7 g LG^{1} in sour wort. The content of reducing sugars did not varied significantly ($p>0.05$) during this step (Table 2). The major components in sorghum are sugars, mainly starch. The cooking of sediment (Fig. 1) leaded to the hydrolysis and gelatinization of starch. The supernatant (Fig. 1) contained the mobilised enzymes of the malting sorghum grains and amylolitic microorganisms that hydrolysed the gelatinized starch during the spontaneous fermentation (Michodjèhoun-Mestres *et al*., 2005; Nanadoum *et al*., 2006). The result was the increase of content of soluble solids. The decrease of content of total sugars was the result of the utilization of reducing sugars as a carbon source by lactic bacteria to produce lactic acid and eventually by associated yeast to produce ethanol. The content of reducing sugars did not varied significantly during spontaneous fermentation because their consumption was compensated by the hydrolysis of the gelatinized starch. This hydrolyse continued during the long cooking of sour wort leading to water evaporation and enormously concentration of soluble solids, reducing sugars and total sugars in sweet wort (N'Da and Coulibaly, 1996). The sweet wort obtained was consumed by children and women and contributed significantly to improve their diet.

During the alcoholic fermentation of the sweet wort by the added dried yeast, content of sugars decreased a lot: the content of soluble solids decreased from 14.76±1.83% in the sweet wort to 8.5±2.2% in the tchapalo, total sugars varied from 262.75±128.4 g LG^{1} to 88.42±48.4 g LG^{1} and reducing sugars decreased from 90.33±41.8 g LG^{1} to 33.86±40.0 g LG^{1} (Table 2). Contrary to spontaneous fermentation, the amylolytic activities during alcoholic fermentation did not compensate the consumption of reducing sugars. Indeed, the added of dried yeasts increased the load of yeast that utilized reducing sugars as a carbon source to produce ethanol (Mugala *et al*., 2003). Studies carried out on the traditional sorghum beers from West Africa were shown that these beverages were dominated by a variety of strains of *Saccharomyces cerevisiae* (Van der Aa Kühle *et al*., 2001; Nanadoum *et al*., 2005).

CONCLUSION

The main organic acids synthesized during tchapalo production were lactic, acetic and citric acids with a predominance of lactic acid. The others organic acids detected in tchapalo were that of the mash of sorghum which were concentrated during the processing. Reducing sugars consumed to produce lactic acid was compensated by that released after the hydrolysis of total sugars during spontaneous fermentation. The content of soluble solids, as well as that of total and reducing sugars decreased a lot to produce ethanol during alcoholic fermentation. Sugars and the detected organic acids contribute to the organoleptic quality of tchapalo.

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