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Fermentation of Cocoa Juice (*Theobroma cacao* L.) and Roselle (*Hibiscus sabdariffa* L.) Extracts into a Wine-Like Alcoholic Drink

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ABSTRACT

An undistilled alcoholic wine-like drink was prepared by fermenting cocoa juice and roselle extracts with wine yeast, *Saccharomyces cerevisiae* var. *bayanus*. The wine was strongly reddish because of roselle extracts, acidic in taste [total titrable acidity (TTA) = 8.025 ± 0.9 g tartaric acid/100 ml], and high in ethanol (11.02 ± 0.9%) concentration. The roselle-cocoa wine-like alcoholic drink had a mean polyphenol value of 962 ± 24.4 mg/l, with little variation in volatiles acidity (0.25 ± 0.00). Methanol content was lower than the limit of 1000 g/hl absolute alcohol (AA) and the higher alcohol values satisfy the qualitative demands of 140 g/hl AA. Isovaleric acid was found in high concentrations compared to other fruit-based commercial wines. Substances which can cause health hazards (estragol) or organoleptic faults (acetaldehyde, ethyl acetate, ethyl lactate, and diethyl succinate) were lower than their respective recommended limits. High concentrations of iron were also recorded (1.24 ± 0.87 mg/l) and were attributed to roselle extracts. Besides, low concentrations of Cu (0.69 ± 0.03 to 0.93 ± 0.03), Pb (2.5 ± 0.0 to 3.7 ± 0.6) and Ca (6.0 ± 1.0 to 7.5 ± 1.7) were recorded and may not pose a threat to potential consumers. Sensory evaluation rated the cocoa juice wine as quite acceptable as an alcoholic drink. Significant differences ($P < 0.01$) exist between the cocoa-roselle wine-like drink and commercial grape wine in taste, aroma, flavor and aftertaste probably because of high polyphenol content in the former. A standardization of the process is required to achieve better control of the levels of the above compounds with regard to the observed variations in composition.

Keywords: aromatic compounds, cocoa, fermentation, polyphenols

INTRODUCTION

Theobroma cacao (Sterculiaceae), an important tropical rain forest species, is growing for its oil-rich seed, to produce cocoa and cocoa butter (Figueira *et al.* 1993). Cocoa seeds are a major cash crop of the tropical world, but prices fluctuate widely and economic hardships occur when prices are low. Despite this, only about 10% of the fresh weight of the fruit is commercialize, although several promising commercial products could be obtained from the cocoa tree (Allary 2000; Aregheore 2002). One strategy to increase income for cocoa growers is to identify and commercialize new products that will not interfere with the main seed crop. Amongst these products is cocoa juice. Although the nutritional value is poor, many reports are available on the beneficial effects of cocoa juice on human health (Vinson and Proch 1999). Roselle (*Hibiscus sabdariffa* L.) or guinea sorrel is also an annual not well exploited tropical plant cultivated mainly for its attractive edible calyces (Cisse *et al.* 2009a). The calyx, which may be green, light or dark-red, is used as a vegetable or in food and fruit beverages as biological colorants (Tsai and Ou 1996; Wong *et al.* 2002; Cisse *et al.* 2009b). Juice is primarily extract from the red-colored calyces, which is a good source of anthocyanin (Babalola *et al.* 2001). More recently, the nutritional attributes of roselle and potential health benefits of extracts from the calyx were highlighted (Adegunloye *et al.* 1996; Onyenekwe *et al.* 1999; Babalola *et al.* 2001; Ismail *et al.* 2008).

The objective of this study is to develop a new red wine product using both cocoa juice and roselle extract as raw material and wine yeast, *Saccharomyces cerevisiae* var. *bayanus* as starter culture. The physicochemical parameters of wine are screen and sensory qualities are compared to other commercial fruits wines.

MATERIALS AND METHODS

The project was conducted at the Institute of Agricultural Research for Development (IRAD) laboratory of Coffee and Cocoa Technology from July 2007 to August 2008.

Cocoa juice

Cocoa juice was extracted from mature cocoa pulp (*Cocoa theobroma* L.) from Cameroon. Juice extraction was performed in a semi-continuous cocoa pulp extractor provided by a horizontal cylinder with a perforated wall. Rotating blades mounted on the central axis remove the pulp from the seed. Throughput was 99-180 kg/hr. About 50 l of clear yellowish cocoa juice where extracted and clarified according to the enzymatic method described by Djokoto *et al.* (2006) using *Saccharomyces cerevisiae* (ATCC 52712) extracted pectinase.

Roselle extracts

Dry red roselle (*Hibiscus sabdariffa*) calyces were purchase in a local market. The hot water extraction (HWE), describe by Wong *et al.* (2003) was use for roselle juice extraction. Extraction was perform using 20 mg dried ground calyces/l of distilled boiling water (100°C) for 20-25 min as described by Aina and Odipe (2006).

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Preparation of yeast starter

The starter culture was prepared by modifying the method described by Juroszek *et al*. (1987) using 1 g/l of wine yeast (*Saccharomyces cerevisiae* var. *bayanus*), which is made into slurry with some of the aliquots to be fermented and mixed into the main portion to which sugar at 23°C Brix is added.

Must preparation and fermentation

Cocoa juice was mixed with roselle extracts at 20, 30, 40, and 50% v/v (roselle extract/cocoa juice). The Brix of the mix was then brought up to 23°C Brix by adding sugar cane saccharose bought from a local market. The fermenting vessel filled to about ¾ full, plugged with cotton wool with cork through which the fermenting lock was inserted. Then 450 ppm of sodium metabisulphite, 0.67% ammonium sulfate and 1 g/l of citric acid were added to the must. Fermentation was allowed to proceed in the room at a fixed temperature of 30°C for 168 hr in a 7-l pilot fermentation tank (New Brunswick®) with an automatic controlled temperature and oxygen pressure enclosed system to avoid fluctuation.

Maturation of the wine

The fermented liquor was filtered through a 0.45-mm Millipore filter and racked into a clean bottle filled up to the neck and lightly covered with cotton wool. This was then allowed to age for about 28 weeks during which racking was done at 8-week intervals at 28°C. After storage, physicochemical parameters were carried out in triplicate and mean values were recorded.

Physicochemical analyses

pH was determined using a Kent EIL 7020 model pH meter. The pH of the fermented liquor was taken in triplicate after maturation (28 weeks).

Residual sugar content was preceded by determination of glucose and fructose using the enzymatic method described by McCloskey (1978), after maturation (28 weeks).

Titrable acidity (as percentage (w/w) tartaric acid) was determined according to the Association of Analytical Chemists (1990) method. Acidity was determined by titration with 0.1 N NaOH, solution and expressed as percentage tartaric acid; bromothymol blue was used as an indicator.

Total polyphenols were assayed colorimetrical using the Folin-Dennis Ciocalteau reagent as described by Juan Mangas *et al.* (1993) and the results were expressed as mg/l of gallic acid.

Micronutrients and **mineral profile** of the inorganic elements Fe, Ca and Cu were determined by flame atomic absorption spectrometry and Pb by graphite furnace atomic absorption spectrometry (Soufleros *et al*. 2004).

Volatile acidity was determined using the Mathieu method (Ribereau-Gayon and Peynaud 1962), by titration of the volatile acids separated from the wine by steam distillation and titration of the distillate.

Volatile compounds In order to determine volatile compounds, gas chromatography was performed. Acetaldehyde, methanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol were analyzed by the official method of OIV (1994), slightly modified, with direct injection of the diluted distillate into a Carbowax 400+Hallcomid M. 1801 classic column, as described by Soufleros *et al.* (2004). Higher esters (ethyl acetate, 3-methyl butyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl decatetranoate, hexyl acetate, ethyl lactate, diethyl succinate and phenyl-ethyl acetate), fatty acids (butyric, isobutyric, isovaleric, hexanoic, octanoic, decanoic and dodecanoic), and higher alcohols (1-hexanol, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol and 2-phenylethanol) were analyzed by the official method of OIV (1994), slightly modified, after their extraction from the samples by a mixture of solvents, as described by Soufleros *et al.* (2004). The wine was injected into a capillary column (DB Wax 57 CB).

Total ethanol content was preceded by a spectrophotometric micro-method for the determination of ethanol after distillation of wine that was made alkaline by a suspension of calcium hydroxide (Andrea *et al.* 2004).

Sensory analyses

4 different coded samples of cocoa-roselle red wines (20, 30, 40 and 50%) and two commercial wines Castillo de Liria (rack 1994) and Baron De Ley "Finca Monasterio" 2003, purchased from the local market, were presented to a panel of experienced tasters comprising 10 members of regular wine drinkers. The taste test was carried out when the wines were mature for about six months. The sensory analysis procedure suggested by Rivella (1987) was used. Testing was conducted in accordance with the internationally approved criteria for sensory analysis of wines (Union International des Oenologies 1986).

Procedure

Each taster was given an evaluation form for each of the wine samples. The form included four sensory attributes:

(1) Taste.
(2) Aroma.
(3) Visual examination (color and overall appearance).
(4) Overall acceptability (harmony).

Panellists were asked to assess the wines in terms of the listed attributes using a nine-point hedonic scale with nine representing like extremely and one indicating dislike extremely. Tasting was carried out in a highly illuminated tasting room. Tasters were provided with water to rinse their mouth after each round of tasting and were prevented from communicating to each other to avoid undue bias. Each taster was served with 25 ml of each wine sample in a different coded form to allow for replications and mean values were recorded. Data were subjected to analysis of variance and means were separated using Duncan's multiple range test with $P < 0.05$ (Steel and Torrie 1980; Gomez and Gomez 1985).

RESULTS

Chemical composition of cocoa-roselle wine

Table 1 shows the mean values of the chemical compounds in cocoa-roselle wines fermented with *Saccharomyces cerevisiae* yeast strains and matured for 6 months. The variables related to fermentation vigor were ethanol production and sugar remaining after fermentation. Wines were fermented to dryness; the remaining concentrations of reducing sugar were less than 1.3 ± 0.5 g/l in all wines preparations close to the value of *Passadouro Red 2000*, a Portuguese red wine, which is 1.3 g/l (Quinto 2007).

pH varies from 2.5 ± 0.00 (must at 50%) to 3.6 ± 0.6 (must at 20%) (**Table 1**). We noted a correlation (r=0.89) between pH and the amount of roselle extracts added.

Total acidity of the wines was relatively high (from 7.2 ± 0.6 to 8.8 ± 0.10 mg/l), typical of roselle acidity. The total volatile acidity varied around 0.25 ± 00.00 and was close to the recommended value of 0.22 g/l (EC Council Regulation No 1493/1999). However, some significant differences could be noticed regarding total polyphenol content which varied from 921 ± 25 (20%) to 1002 ± 67 mg/l (50%).

Methanol: The mean concentration for this compound was 727.95 ± 97 g/hl AA varying from 361.0 ± 49 g/hl AA in the must at 50% (v/v) roselle extract (thus 50% cocoa juice) to 949.2 ± 75 g/hl AA in the must of 20% (v/v) roselle extract (thus 80% cocoa juice) (**Table 2**). However, none of above-mentioned samples gave a higher concentration of methanol than the mentioned limit of 1000 g/hl although the 80% cocoa juice sample had a value close to this limit.

1-Hexanol: this is an alcohol derived only from raw material and not from fermentation (Soufleros *et al.* 2001).

Table 1 Comparative study of the chemical compounds obtained by microvinification of cocoa juice and Roselle (*Hibiscus sabdarifa*) extracts by *Saccharomyces cerevisiae* yeast strains.

Sample	Total titrable acidity (mg/l)	Volatile acidity (g/l)	Ethanol (%)	pH	Residual total sugars (g/l)	Total polyphenols (mg/l)
20%V/V	7.2 ± 0.6	0.3 ± 0.00	10.9 ± 0.9	3.6 ± 0.6	1.2 ± 0.6	921 ± 25
30%V/V	7.7 ± 0.10	0.2 ± 0.00	10.8 ± 0.5	3.6 ± 0.4	1.3 ± 0.2	953 ± 64
40%V/V	8.4 ± 0.14	0.3 ± 0.00	10.9 ± 0.7	2.9 ± 0.0	1.5 ± 0.1	972 ± 39
50%V/V	8.8 ± 0.10	0.2 ± 0.00	11.5 ± 0.2	2.5 ± 0.0	1.3 ± 0.3	1002 ± 67

Table 2 Comparative study of the alcohols and acetaldehydes obtained by vinification of cocoa juice and *Hibiscus sabdarifa* extracts by *S. cerevisiae* yeast strains.

Alcohols other than ethanol (in g/hl absolute alcohol)	20% v/v	30% v/v	40% v/v	50% v/v
Methanol	949.2 ± 75	804.9 ± 82	796.7 ± 64	361.0 ± 49
Butanol-1	3.03 ± 0.1	4.73 ± 0.11	6.34 ± 0.09	9.31 ± 0.16
2-methyl 1-propanol	82.59 ± 11.44	42.29 ± 10.37	38.97 ± 8.23	34.59 ± 9.17
2-methyl 1-butanol	51.49 ± 6.55	46.93 ± 4.35	44.49 ± 6.22	36.64 ± 7.24
3-methyl 1-butanol	162.4 ± 21.9	154.5 ± 22.1	146.1 ± 19.9	134.1 ± 23.7
1-Hexanol	1.12 ± 0.09	1.05 ± 0.09	0.90 ± 0.05	0.88 ± 0.07
cis-3-Hexen-1-ol	1.86 ± 0.07	1.25 ± 0.1	0.62 ± 0.02	0.43 ± 0.08
trans-3-Hexen-1-ol	0.70 ± 0.03	0.62 ± 0.09	0.58 ± 0.1	0.50 ± 0.07
trans-2-Hexen-1-ol	0.09 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.02 ± 0.00
2-phenyl-ethanol	3.02 ± 0.09	3.68 ± 0.07	3.94 ± 0.1	4.11 ± 0.05
Total higheralcohols (g/hlAA)	279.8 ± 76.1	264.2 ± 62.7	245.2 ± 48.0	232.9 ± 58.4
Acetaldehydes (g/hl AA)	134.5 ± 20.4	170 ± 33.7	210 ± 27.6	270 ± 32.9

Table 3 Comparative study of the acids and aromatic compounds obtained by microvinification of cocoa juice and *Hibiscus sabdarifa* extracts by *S. cerevisiae* yeast strains.

Acids (in g/hl absolute alcohol)	20% v/v	30% v/v	40% v/v	50% v/v
Butyric acid	0.19 ± 0.06	0.80 ± 0.07	2.56 ± 0.9	7.67 ± 1.3
Isobutyric acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.00
Isovaleric acid	14.43 ± 3.6	13.52 ± 3.1	15.02 ± 3.3	16.27 ± 2.5
Hexanoic acid	0.12 ± 0.01	0.45 ± 0.05	0.57 ± 0.08	2.11 ± 0.99
Octanoic acid	0.04 ± 0.00	0.21 ± 0.06	0.22 ± 0.03	0.36 ± 0.01
Decanoic acid	0.16 ± 0.08	0.19 ± 0.01	0.20 ± 0.05	0.35 ± 0.07
Dodecanoic acid	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.28 ± 0.01
Aromatic compounds				
Anethole, trans	567.3 ± 32.4	251.1 ± 23.3	230.6 ± 22.9	209.9 ± 25.1
Anisaldehyde	44.37 ± 4.1	16.62 ± 5.7	13.14 ± 2.6	12.59 ± 3.1
Estragol	0.79 ± 0.05	0.54 ± 0.07	0.49 ± 0.08	0.44 ± 0.03
Eugenol	0.33 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.00 ± 0.00

It imparts a fruity, liquorice and even toothpaste flavor profile to both wines and distillates (Ferreira *et al*. 1999). This substance is considered as a compound favorable to the organoleptic characteristics of wines and distillates (Soufleros and Bertrand 1987) if its concentration is between 0.5 and 10 g/hl AA; otherwise, a grassy flavor is imparted making the wine product unpleasant to both smell and taste (Tourliere 1977). 1-Hexanol has a mean value of 0.98 ± 0.4 and a maximum value of 1.12 g/hl AA (**Table 2**), which is considered to give a positive effect to the aroma of the cocoa-roselle–based wine-like alcoholic drinks.

2-Phenyl-ethanol: **Table 2** shows that cocoa-roselle–based wine-like alcoholic drinks presented values from 3.02 ± 0.09 (20% roselle extract) to 4.11 ± 0.05 (50% roselle extract). These values are close to those given for *Castillo de Liria* and *Barron de la Vale* but very low compared to *Tsipouro* wine values (Soufleros and Bertrand 1987). They also vary positively with the amount of roselle extract (**Table 2**) thus this can likely indicate the influence of roselle extracts on these compounds. Only *cis*-3-hexenol is found in a relatively high mean concentration of 1.86 ± 0.07 g/hl AA. The other two hexanols exist in trace amounts. The mean concentration of acetaldehyde was found to be 196.1 ± 28.6 g/hl AA (**Table 2**), ranging from 134.5 ± 20.4 to 270 ± 32.9 g/hl AA. The above values meet the official limits (73–500 g/hl AA) adopted by the European Council (Reg. 1576/89) (Official Journal of EEC 1989) for fruit wines. Cocoa-roselle–based wine-like alcoholic drinks has values generally close to *Passadouro Red 2000*, at 293 g/hl (Quinto 2007).

Table 4 shows that ethyl decatetranoate occurred at a very low concentration; hence, its effect is not important. The concentrations of the ethyl compounds (ethyl hexanoate, ethyl octanoate and ethyl decanoate), in most cases, were in accordance with the values reported for wines (Official Journal of EEC 1989). Isoamyl acetate, hexyl acetate and phenyl-ethyl acetate constitute the acetic acid esters, which are mostly responsible for the flower and fruity aroma of distillates (Ferreira *et al*. 1999; Silva and Malcata 1999). As can be seen from **Table 4**, isoamyl acetate has the highest concentration with a mean of 0.5 g/hl AA.

Volatile acids: The results presented in **Table 3** shown that the cocoa-roselle–based wine-like alcoholic drinks had a high concentration of isovaleric acid, quantitatively the most important compound in this group, and similar to isobutyric acid.

Anethole and anisaldehyde are quantitatively the most important aromatic compounds delivered by anise (*Pimpinella anisum*) and other kinds of aromatic plants, although anisaldehyde occurs mostly from anethole's oxidation. It is known that the ratio of *trans*-anethole and anisaldehyde is 10: 1 for a natural extract. In the cocoa-roselle–based wine-like alcoholic drink samples that we analyzed, we found varying levels of these compounds. All of them contained very low quantities of the above substances (1.75–6.78 for *trans*-anethole and 0.50–1.48 g/hl AA for anisaldehyde); therefore, after organoleptic examination they appeared to be unflavored. Soufleros and Bertrand (1987) reported *trans*-anethole values ranging between 43.6 and 195.7 g/hl AA, and anisaldehyde values ranging between 4.3 and 24.9 g/hl AA for some commercial wines.

Estragol: It is an aromatic, strongly flavored and caustic

Table 4 Comparative study of the esters and minerals obtained by vinification of cocoa juice and *Hibiscus sabdarifa* extracts by *S. cerevisiae* yeast strains.

Esters (in g/hl absolute alcohol)	20% v/v	30% v/v	40% v/v	50% v/v
Hexyl acetate	0.62 ± 0.05	0.33 ± 0.08	0.28 ± 0.05	0.22 ± 0.09
Ethyl decatetranoate	0.02 ± 0.00	0.025 ± 0.00	0.0484 ± 0.01	0.13 ± 0.01
Isoamyl acetate	0.36 ± 0.09	0.20 ± 0.06	1.67 ± 0.1	0.78 ± 0.09
Ethyl acetate	116.1 ± 26.3	131.5 ± 33.4	501.4 ± 64.3	606.5 ± 55.7
Phenyl ethyl acetate	0.04 ± 0.00	0.68 ± 0.01	0.10 ± 0.09	0.21 ± 0.00
Ethyl lactate	5.49 ± 1.2	5.52 ± 1.1	8.05 ± 1.9	3.79 ± 0.9
ethyl decanoate	0.45 ± 0.08	0.45 ± 0.05	1.16 ± 0.07	0.49 ± 0.04
Ethyl hexanoate	0.75 ± 0.7	1.26 ± 0.9	0.35 ± 0.08	0.84 ± 0.04
Diethyl succinate	0.81 ± 0.01	0.69 ± 0.00	0.68 ± 0.07	0.36 ± 0.06
Ethyl octanoate 3	0.65 ± 0.05	0.50 ± 0.09	0.82 ± 0.04	0.55 ± 0.02
Minerals				
Fe (mg/L)	1.01 ± 0.2	1.19 ± 0.8	1.95 ± 0.7	2.63 ± 0.4
Cu (mg/L)	0.69 ± 0.03	0.75 ± 0.00	0.89 ± 0.05	0.93 ± 0.03
Ca (mg/L)	7.5 ± 1.7	5.7 ± 2.0	6.7 ± 0.9	6.0 ± 1.0
Pb (mg/L)	2.5 ± 0.0	2.9 ± 0.8	3.00 ± 0.2	3.7 ± 0.6

aldehyde, which mostly comes from sweet fennel (5–20% of essential oil), anise (1% of essential oil), anise star (5–6% of essential oil) (Liddle and Bossard 1984; European Commission 2001). Due to the properties of estragol, it is necessary to establish tolerance limits of this compound for cocoa-roselle–based wine-like alcoholic drinks. According to the Scientific Committee on Food (European Commission 2001), "estragol is a naturally occurring genotoxic carcinogen and for this reason it is desirable to adopt tolerated limits". However, "in the meantime a limit of 0.05 mg/kg (detection limit) for each person is recommended". Based on the above, the estimated average intake for consumers is 4.3 mg/day. The concentration of estragol in the cocoa-roselle–based wine-like alcoholic drinks is lower than (20 mg/l) proposed as a maximum for alcoholic beverages (Ministry of Finance and Economy of Greece 2002) and lower than that (100 mg/l) proposed by the European Confederation of Spirit Producers (2000) and also by the French Industry of Foods (1999). As it does not exceed the European limits, no hazards for consumers are present.

Eugenol: Better known in its pure form, eugenol is a flavorful compound found in carnation flowers and dentists use it as a topical anesthetic. According to Ferreira *et al.* (1999), it is an aromatic substance adding a somewhat liquorice, fruity peach-like, cinnamon aroma and flavor to wines and spirits. Eugenol concentration in the cocoa-roselle–based wine-like alcoholic drinks (**Table 3**) is very low compared to *aguardiente* (Rogerson and Freitas 2001) and *tsipouro* (Soufleros and Bertrand 1987).

Mineral content

In the case of the cocoa-roselle–based wine-like alcoholic drinks, we recorded a very high iron concentration varying from 1.01 ± 0.2 mg/l (20%) to 2.63 ± 0.4 mg/l (50%) (**Table 4**). Such levels may probably come from the roselle extract as there is a positive correlation (r = 0.974339) between iron levels and percentage of roselle extract in the must. This is because roselle calyx is known to be very rich in iron (Daramola and Asunni 2006). These values are higher than those given for most commercial wines and strong flavored aperitifs (Rodushkin *et al.* 1999).

Ca: Calcium is a nutrient important for the growth of plants and the creation of the skin and is mostly used by yeast. It is present in the cocoa-roselle–based wine-like alcoholic drinks due to dilution with water, or comes from cocoa juice and roselle extract as well. Ca levels in the cocoa-roselle–based wine-like alcoholic drinks (**Table 4**) were found to be high enough (vary from 5.7 ± 2.0 to 17.5 ± 5 mg/l) according to safety standards (Muntean *et al.* 1998) and compared to international beverages (Nascimento *et al.* 1999). Ca levels of 5.0 mg/100 g and above is necessary to maintain high quality throughout long-term storage of wines (Defra Gov UK 2006). Meanwhile, there are no recommendations for Ca levels in some commercial wines, as Ca deficiency has not been documented in many of them. This may not thus be a challenge for the cocoa-roselle–based wine-like alcoholic drinks standardization process.

Cu: The copper levels in the cocoa-roselle–based wine-like alcoholic drinks were found to be low compared to some commercial wines with values varying from 0.75 ± 0.00 to 0.93 ± 0.03 mg/l (**Table 4**). There seems to be no relationship between the must and Cu level in the wine. This can be explained by the fact that Cu, mostly found in grape wines, originates from treatment with $CuSO_4$ spreading procedures and from grape and wine contact with copper surfaces. Moreover, we did not use such processes for cocoa-roselle–based wine-like alcoholic drink production. Cu levels in cocoa-roselle–based wine-like alcoholic drinks (**Table 4**) are compared with the values reported in the literature for some artisanal drinks (Nascimento *et al.* 1999). Brazilian Legislation (Portaria No. 371/1974), which sets a limit of 5.0 mg/L (Official Journal of Brazil 1974) controls cu concentration. The same limit of 5.0 mg/L for Cu was also given by Latvia Republic (Reg. No. 101) (Official Journal of Latvia 2001). The daily intake of Cu from a normal adult's diet is between 1 and 3 mg, roughly corresponding to the intake levels recommended by most authorities (WHO 1984; Muntean *et al.* 1998). This indicates that cocoa-roselle–based wine-like alcoholic drinks, in most cases, are safe for consumption with regard to Cu.

Pb: Lead pollution is caused by factors like toxic rain, fertilizers, factory residues that initially pollute both air and water (Muntean *et al.* 1998). For the samples tested (**Table 4**) the mean value varied from 2.5 ± 0.0 to 3.7 ± 0.6 mg/l. These concentrations are far below the limits given for an adult as the maximum daily intake through food and drinks, which usually reaches 250–300 mg (Muntean *et al.* 1998). These same amounts were adopted from the authorities of Latvia Republic (Reg. No. 101) (Official Journal of Latvia 2001) as the upper limit of daily human intake.

Sensorial evaluation

The sensory qualities of the roselle–cocoa based wine-like alcoholic drinks and commercial wines are summarized in **Fig. 1**. The cocoa-roselle–based wine-like alcoholic drinks exhibited significant ($P < 0.05$) increase in scores for aroma (5-8) vs. (4-5) in commercial wines (**Fig. 1**). This can be linked to the aroma of cocoa juice and confirm the high levels of aromatic compounds recorded (**Table 3**). The taste exhibited records varying from 3 to 6 vs. 4 and 7 respectively for *Castillo de Liria* and *Baron De Ley*, respectively. This implies that cocoa wine present less taste than commercial wines. This may be due to high levels of acidity (**Table 1**). A strong correlation (r = 0.91) exists between the percentage of roselle extract and panelists' choice based on taste. This may indicate that the roselle extract is the main

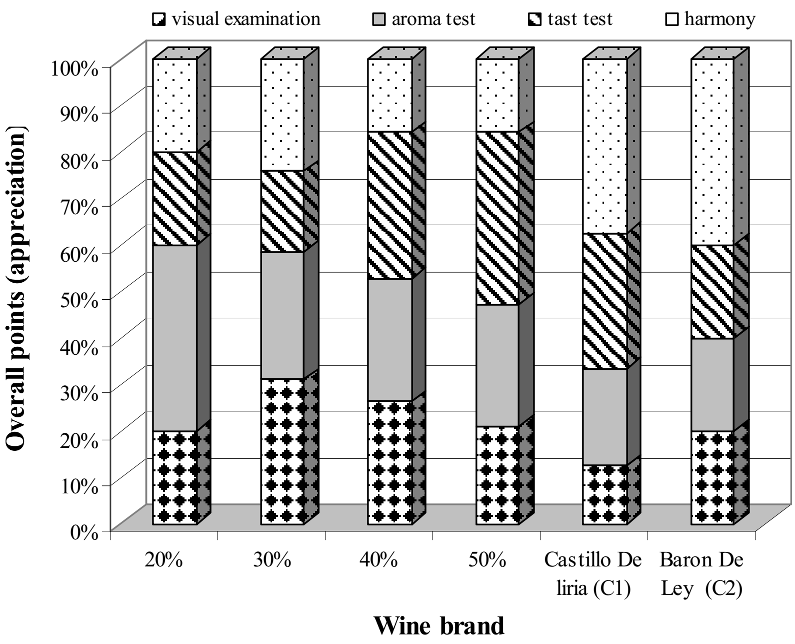

Fig. 1 Evaluation of sensorial properties of cocoa-roselle vine like alcoholic drink.

ingredient-driving consumer's choice in cocoa-roselle–based wine-like alcoholic drinks. Concerning appearance, roselle-based wine-like alcoholic drinks seem to be better than commercial wines.

Overall acceptability (harmony) indicates that the roselle-based wine-like alcoholic drinks are less accepts than their commercial counterparts are. This may also be due to the strong acidity recorded.

Irrespective of the percentage of roselle extract added, multiple regression analyses revealed an inverse significant ($P < 0.05$) relationship between aroma, taste, visual examination and overall acceptability of roselle–cocoa based wine-like alcoholic drinks Titrable acidity was also significantly ($r = 0.4$; $P < 0.05$) associated with taste (**Fig. 1**).

DISCUSSION

We can notice high ethanol production capacity 11.02 ± 0.22% from cocoa juice and Roselle extracts, which is above the newly recommended values of more than 8.5% for wines (Food Gov UK 2007) adopted by the EC (Reg. 1576/89). However, there were no significant difference ($P < 0.01$) amongst different percentages (20-50%, v/v) uses, this may be due the fact that we upload Brix to 23°C for all samples must before fermentation, thus initial sugars contents as well as fermentation conditions were similar.

The value of total titrable acidity is above the minimum level recommended by the legislation, which is 4 g/l (Food Gov UK 2007). This can be due to the high acid content of added roselle extract (Omemu *et al.* 2006).

The presence of methanol in wines at relatively high levels may be explained by the fact that methanol is formed by pectinolytic enzymes that split the methoxyl group from pectin present in cocoa juice; for this reason, the concentration of methanol in the wine increases with the amount of cocoa juice in the must as shown in **Table 2**. According to the European regulation (No. 1576/89) (Official Journal of EEC, 1989), the methanol concentration must be lower than 1000 g/hl AA. High concentrations of methanol may likely be due to the 4-to-6 month maturation period.

The most important higher alcohols referred to cocoa-roselle wine-like alcoholic drink, are isobutyl alcohol, butanol-1 and amylic alcohols. The detected higher alcohols in roselle red wine constitute the group of compounds with the highest concentration, which gives wines flavoring aroma (Ferreira *et al.* 1999; Silva and Malcata 1999). In the normal process of wine production from grapes, the levels of these compounds are influence by several processing factors, such as carbohydrate source (grape, plum, and cherry) fermentation conditions, maturation techniques, etc, as noticed in some distillates liquor (Silva and Malcata 1998). Silva and Malcata (1999) observed the essential character of the higher alcohols. For this reason, the European legislation (Reg. No. 1576/89) (Official Journal of EEC 1989) requires that the minimum levels for these aromatic substances should not be higher than 140 g/hl AA. Generally, all our samples satisfy the demands mentioned above and have concentrations very far from desirable limits.

The acetaldehyde levels of cocoa roselle wine is within the official limits, this means that, generally, the cocoa-roselle bases wine like alcoholic drinks may be fermented and aged under conditions which do not support any spontaneous creation of hot spots caused by bacteria (Silva and Malcata 1998). Acetaldehyde is a compound coming from the fermented raw materials, which increases during aging (Silva and Malcata 1998). It is also consider mainly a result of spontaneous or microbial-mediated oxidation (Silva and Malcata 1999).

The group of esters seems containing the highest number of different compounds that contribute to the flavor of wines and account for a pleasant aroma, indicative of the quality of the wines, is the fatty acid esters (Silva and Malcata 1999; Soufleros *et al.* 2001). Ethyl hexanoate, ethyl octanoate and ethyl decanoate, which are produced during fermentation (Silva and Malcata 1998), generally increase during aging (Silva and Malcata 1999; Soufleros *et al.* 2001). Throughout maturation process, high temperatures can releases a significant amount of these esters from the yeast cells where they remain bound after fermentation (Caumeil 1983). These three compounds are quantitatively a small portion compared to the other volatile compounds, but they impart a highly profiled aromatic character for the spirits (Ferreira *et al.* 1999).

Trans-3-hexenol, *cis*-3-hexenol and *trans*-2-hexenol: These three hexanols are those that usually participate in the flavor profile of some wines and distillates (Silva and Malcata 1999). The cocoa-roselle bases wine-like alcoholic drinks has higher concentrations of *cis*-3-hexenol (**Table 2**), than *tsipouro* (Soufleros and Bertrand 1987) and *bagaceiras* (Silva *et al*. 1996; Silva and Malcata 1998, 1999) but significantly lower than *Castillo de Liria* probably because of the different initial material used for their production.

The other two acetates follow with values equal to 0.14 g/hl AA each. Ethyl acetate deriving mainly from bacterial spoilage of the marc distillates (Silva and Malcata 1998, 1999) and imparts an acidic character to the wine (Ferreira *et al.* 1999) when its concentration is higher than 180 g/hl AA (Soufleros and Bertrand 1987). Ethyl acetate is the ester with the highest concentration as can be observed in other works (Soufleros and Bertrand 1987). Ethyl lactate and diethyl succinate are produce mainly from bacteria. Ethyl lactate is characterized from yeast extract, wet and bakery profile, whereas diethyl succinate is characterized from fusel and flowery presentation (Ferreira *et al.* 1999). On the other hand, Soufleros and Bertrand (1987) note that ethyl lactate at concentrations not more than 10 g/hl AA stabilize the aroma and smoothness the firm character of certain substances. The concentration of ethyl lactate in our wines (**Table 4**) was lower than concentrations reported in the literature for other wines, as well as the recommended values of 10 g/hl AA. There is thus a need to increase this compound by a control of fermentation and maturation process of the wines. Diethyl succinate levels were similar to those recorded in *Castillo de Liria* and *Baron de Ley*, and were significantly higher comparing to those reported by Silva and Malcata (1999).

The average concentrations of fatty acids were find to be similar to other wines studied by Silva *et al.* (1996), Silva and Malcata (1998, 1999) and Soufleros and Bertrand (1987). Short-chain fatty acids (butyric, isobutyric and isovaleric) have an odor similar in strength to acetic acid (Silva and Malcata 1999; Soufleros *et al.* 2001) and significantly affect the aromatic character of the wine (**Table 3**) (Ferreira *et al.* 1999; Silva and Malcata 1999). Long-chain fatty acids, hexanoic, octanoic, decanoic and dodecanoic acid have a lesser impact on the flavor of wines (Silva and Malcata 1999; Soufleros *et al.* 2001).

In the case of the cocoa-roselle base wine-like alcoholic drinks, we record a very high iron concentration. The minerals in food and drinks might originate from the soil and fertilizers, the equipment containers and utensils used for food processing, storage or cooking, and the industrial activity (Karadjova *et al.* 1998; Muntean *et al.* 1998). Sometimes the minerals might also originate from contaminated water, which is use for diluting the must. However, minor elements can also result from the biological material used for must preparation.

CONCLUSION

The development of this wine-like alcoholic drink is clearly a new challenge for growing wine industries in tropical areas. This study extends the list of potential product from cocoa tree and highlight possibilities to use cocoa juice and Roselle to produce a wine like alcoholic drink. First results obtained and discuss above give great expectations for further cocoa wine development. However, the variation of chemical composition of wine shows that cocoa-roselle based wines needs standardization and more systematic production in order to ensure a homogenous and more qualitative product. Wine is well known for its social and sensory qualities and actions. It acts as a source of antioxidant substances such as flavonoids, anthocyanins, flavonols, cathechins, leuco anthocyanins and resveratrol. It will be thus interesting to speculate on the potential addition at this, the beneficial effects of cocoa juice that derive primarily from ingredients such as antioxidant substances (polyphenols, or flavonoids), nutritional compounds and xanthenes, mainly caffeine and theobromine. It is therefore interesting to speculate that the traditional use of cocoa juice and roselle extracts for wine production will be a challenge for cocoa growers and scientist as well.

REFERENCES

Adegunloye BI, Olaniyi JO, Owolabi OA, Ajagbonna OP, Sofola OA, Coker HA (1996) Mechanisms of blood pressure lowering effect of the calyx extract of *Hibiscus sabdariffa* in rats. *African Journal of Medicure and Medical Sciences* **25**, 235-238

Aina JO, Shodipe AA (2006) Colour stability and vitamin C retention of roselle juice (*Hibiscus sabdariffa* L.) in different packaging materials. *Nutrition and Food Science* **36 (2)**, 90-95

Alary V (2000) *Les Cacaoculteurs camerounais face aux risques. Essai de modélisation* coll. «Bibliothèque du développement», L'Harmattan, Paris, 166 pp

Andrea D, Magrí AL, Fabrizio B, Sacchini A, Marini D (2004) Spectrophotometric micro-method for the determination of ethanol in commercial beverages. *Fresenius' Journal of Analytical Chemistry* **357 (7)**, 985-988

Aregheore EM (2002) Chemical evaluation and digestibility of cocoa (*Theobroma cacao*) byproducts fed to goats. *Tropical Animal Health and Production* **34 (4)**, 339-348

Association of Analytical Chemists (1990) *Official Methods of Analysis of the Association of Official Analytical Chemists* (15th Edn) Macmillan, Washington, DC, 341 pp

Babalola SO, Babalola AO, Aworh OC (2001) Compositional attributes of the calyces of roselle (*Hibiscus sabdariffa*). *Journal of Food Technology in Africa* **6 (4)**, 133-134

Cisse M, Dornier M, Sakho M, Mar Diop C, Reynes M, Sock O (2009a) La production de bissap (*Hibiscus sabdariffa* L.) au Sénégal. *Fruits* **64 (1)**, 1-14

Cisse M, Dornier M, Sakho M, Ndiaye A, Reynes M, Sock O (2009b) Le bissap (*Hibiscus sabdariffa* L.): Composition et principales utilisations. *Fruits* **64 (1)**, 179-193

Clydesdale FM, Main JH, Francis FJ (1979) Roselle (*Hibiscus sabdariffa* L) anthocyanin as colourants in beverage and gelation desserts. *Journal of Food Protection* **42**, 204-267

Daramola B, Asunni OA (2006) Nutrient composition and storage studies on roselle extract enriched deep-fat-fried snack food. *African Journal of Biotechnology* **5 (19)**, 1803-1807

David D, Dzogbefia VP, Oldham JH (2006) Rapid extraction of pawpaw juice with the application of locally produced pectic enzymes from *Saccharomyces Cerevisiae* ATCC 51712. *Food Biotechnology* **4 (14)**, 103-107

Defra.gov.uk (2006) *Section 6: Fruit, Wines and Hops* (2nd Edn), Willem Inc., United Kingdom, 234 pp

Djokoto D, Dzogbefia VP, Oldham JH (2006) Rapid extraction of pawpaw juice with the application of locally produced pectic enzymes from *Saccharomyces cerevisiae* ATCC 52712. *Food Biotechnology* **20**, 31-41

Du CT, Francis FJ (1973) Anthocyanins of roselle (*Hibiscus sabdariffa* L.). *Journal of Food Sciences* **38**, 810-812

Essenlen WB, Sammy GM (1973) Roselle: A natural red color in foods. *Food Product Development* **1**, 80-82

European Commission (2001) *Official Journal of the European Communities 22.6.2001 L 167/10* (2nd Ed), eur-lex.europa.books, Brussels, 123 pp

European Council Regulation (EC) (1999) *Regulation No 1493/1999 of 17 May 1999 on the Common Organisation of the Market in Wine* (2nd Edn), eur-lex.europa.books, Brussels 171 pp

European Union of Spirit Producers (2007) *Charter on Responsible Alcohol Consumption. European Spirits News* (4th Edn), European Spirits Publications, Brussels, 118 pp

European Union of Spirit Producers (2000) *Proposition to the European Council on Estragol Content as a Maximum for Alcoholic Beverages* (12th Edn), Macmillan, New York, 341 pp

Ferreira V, Hernández-Orte P, Escudero A, Lopez R, Cacho J (1999) Semi preparative reversed-phase liquid chromatographic fractionation of aroma extracts from wine and other alcoholic beverages. *Journal of Chromatography A* **864**, 77-88

Figueira A, Janick J, BeMiller JN (1993) New products from *Theobroma cacao*. In: Janick J, Simon JE (Eds) *Seed Pulp and Pod Gum. New Crops*, Wiley, New York, pp 475-478

Food gov UK (2007) *Notice No. 34 Notice to Wine Growers and Wine Producers Regional Wine Scheme 2006/2007* (6th Edn), Willem Inc., United Kingdom, 145 pp

French Industry of Foods (1999) *Proposition to the European Council on Estragol Content as a Maximum for Foods* (2nd Edn), eur-lex.europa.books, Brussels 153 pp

Gomez AK, Gomez AA (1985) *Statistical Procedure for Agricultural Research* (1st Edn), John Wiley and Sons, New York, 213 pp

Guymon JF, Ough CS (1972) Refermentation of high-alcohol high-sugar blending wine into distilling material for beverage brandy. *American Journal of Enology and Viticulture* **23 (3)**, 124-127

Ismail A, Ikram EHK, Nazri HSM (2008) Roselle (*Hibiscus sabdariffa* L.) seeds - nutritional composition, protein quality and health benefits. *Food* **2**, 1-16

Mangas J, Suárez B, Domingo B (1993) Automated determination of total polyphenols in apple juice. *Zeitschrift für Lebensmitteluntersuchung und – Forschung A* **197 (5)**, 424-426

Juroszek J-R, Feuillat M, Charpentier C (1987) Effect of the champagne method of starter preparation on ethanol tolerance of yeast. *American Journal of Enology and Viticulture* **38**, 194-198

Karadjova I, Zachariadis G, Boskou G, Stratis J (1998) Electrothermal atomic absorption spectrometric determination of aluminum, cadmium, chromium, copper, iron, manganese, nickel and lead in olive oil. *Journal of Analytical Atomic Spectrometry* **113**, 201-204

Liddle PAP, Bossard A (1984) The analysis of anethole and anethole-flavored beverages by gas chromatography. In: Nykanen L, Lethonen P (Eds) *Flavor Research of Alcoholic Beverages. Instrumental and Sensory Analysis*, Foundation for Biotechnical and Industrial Fermentation, Helsinki, 237 pp

McCloskey LP (1978) Enzymatic analysis for glucose and fructose. *American Journal of Oenology and Viticulture* **29 (3)**, 226-227

Ministry of Finance and Economy of Greece (2002) *Document to Interest in Relation to Estragol Content as a Maximum for Alcoholic Beverages* (3rd Edn), eur-lex.europa.books, Brussels, 153 pp

Mounigan P, Badrie N (2007) Physicochemical and sensory quality of wines from red sorrel/roselle (*Hibiscus sabdariffa* L.) calyces: Effects of pretreatments of pectolase and temperature/time. *International Journal of Food Science and Technology* **42**, 469-475

Muntean N, Laslo R, Chitulescu R, Muntean E (1998) Heavy metals' content in some food products. *Institute of applied food Sciences* **12 (3)**, 56-87

Nascimento RF, Bezerra CWB, Furuya SMB, Schultz MS, Polastro LR, Lima NBS, Frango DW (1999) Mineral profile of Brazilian cachaças and other international spirits. *Journal of Food Composition and Analysis* **12**, 17-25

Office International de la Vigne et du Vin (OIV) (1994) Recueil des méthodes internationales d'analyse des boissons spiritueuses, des alcools et de la fraction aromatique des boissons. OIV (Ed), Paris, 289 pp

Official Journal of Brazil (1974) *Portaria No. 371, 18/09/1974* (1st Edn), eur-lex.europa.books, Brussels 179 pp

Official Journal of EEC (1989) *No L. 160, 12/6/1989* (5th Edn), eur-lex.europa.books, Brussels 243 pp

Official Journal of EEC (1990) *No L. 105, 25/4/1990* (6th Edn), eur-lex.europa.books, Brussels 632 pp

Official Journal of Greece (2001) *No 281, 18/12/2001* (8th Edn), eur-lex.europa.books, Brussels 532 pp

Official Journal of Latvia (2001) *No. 101, 6/3/2001* (6th Edn), eur-lex.europa.books, Brussels 149 pp

Omemu AM, Edema MO, Atayese AO, Obadina AO (2006) A survey of the microflora of *Hibiscus sabdariffa* (roselle) and the resulting "Zobo" juice. *African Journal of Biotechnology* **5 (3)**, 254-259

Onyenekwe PC, Ajani EO, Ameh DA, Gamaniel KS (1999) Antihypertensive effect of roselle (*Hibiscus sabdariffa*) calyx infusion in spontaneously hypertensive rats and a comparison of its toxicity with that in Wistar rats. *Cell Biochemistry and Function* **17**, 199-206

Purseglove JW (1977) *Tropical Crop: Dicotyledons* (Vol 1), Longman, Harlow, United Kindom, pp 370-373

Quinta do Passadouro (2007) Composition of the best red wine from Portugal: The Passadouro 2000. Available online: http://www.quintadopassadouro.com

Ribereau-Gayon J, Peynaud E (1962) *Analysis de Vino*, Aguilar (Ed), Madrid, 107 pp

Rivella E (1987) Proposition de procédures pour l'analyse sensorielle des vins dans les concours. *Revue Francaise d'Oenologie* **27**, 13-16

Rodushkin I, Odman F, Appelblad PK (1999) Multi-element determination and lead isotope ratio measurement in alcoholic beverages by high-resolution inductively coupled plasma mass spectrometry. *Journal of Food Composition and Analysis* **12**, 243-257

Rogerson FSS, de Freitas VAP (2001) Quantitative analysis of volatiles present in wine spirits used for port production. In: Garamon ED, Fraklin TH (Eds) *ASEV 52nd Annual Meeting*, San Diego, CA, 258 pp

Silva ML, Malcata FX (1998) Relationships between storage conditions of spirits obtained hereform. *American Journal of Enology and Viticulture* **49 (1)**, 56-64

Silva ML, Malcata FX (1999) Effects of time of grape pomace fermentation and distillation cuts on the chemical composition of grape marcs. *Zeitschrift fuer Lebensmittel-Untersuchung und-Forschung A* **208**, 134-143

Silva ML, Malcata FX, de Revel G (1996) Volatile contents of grape marcs in Portugal. *Journal of Food Composition and Analysis* **9**, 72-80

Soufleros E, Bertrand A (1987) Etude sur le "Tsipouro", eau-de-vie de marque traditionnelle de Grèce, précurseur de l'ouzo. *Connaissance de la Vigne et du Vin* **21 (2)**, 93-111

Soufleros E, Bertrand A (1991) La production artisanale du Tsipouro à Naoussa (Grèce). In: Bertrand A (Ed) *Les Eaux-De-Vie Traditionnelles d'Origine Viticole*, Lavoisier-Tec & Amp, Paris, 290 pp

Soufleros EH, Mygdalia AS, Natskoulis P (2004) Characterization and safety evaluation of the traditional Greek fruit distillate "Mouro" by flavor compounds and mineral analysis. *Food Chemistry* **86 (4)**, 625-636

Steel RGD, Torrie JH (1980) *Principles and Procedures of Statistics: A Biometrical Approach* (2nd Edn), McGraw-Hill Inc., New York, 647 pp

Tourlière S (1977) Commentaires sur la présence d'un certain nombre de composes accompagnant l'alcool dans les distillats spiritueux rectifiés et eaux-de-vie. *Industries Alimentary Agriculture* **94**, 565-574

Tsai PJ, Ou ASM (1996) Colour degradation of dried roselle during storage. *Food Science review* **23**, 629-640

Union International des Œnologues (1986) Union International des Œnologies, *XIX Congrès International de la Vigne et du Vin*, Santiago, Elsevier Science B.V., Amsterdam, pp 222-248

Vinson JA, Proch J, Zubik L (1999) Phenol antioxidant quantity and quality in foods: Cocoa, dark chocolate and milk chocolate. *Journal of the Science of Food and Agriculture* **47**, 4821-4824

Wong PK, Yusof S, Ghazali HM, Che Man YB (2002) Physicochemical characteristics of roselle (*Hibiscus sabdariffa* L.). *Nutrition and Food Science* **32**, 68-73

Wong P-K, Yusof S, Ghazali HM, Che Man YB (2003) Extraction of roselle juice. *Journal of the Science of Food and Agriculture* **83 (12)**, 1273-1278

World Health Organization (1984) *Guidelines for Drinking-Water Quality*, Garamouth, Geneva, 245 pp