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Production and Characterization of Juice from Mucilage of Cocoa Beans and its Transformation into Marmalade

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Abstract: More than 550,000m^3 of juice from mucilage of cocoa beans are produced and abandoned in farms each year. This cloudy substance is composed of 85.3% of moisture. Production and transformation into marmalade were made. High performance liquid chromatography was used to identify reducing sugars and organic acids and gas liquid chromatography was used for minerals identification. Physical parameters were also determined. The results of analyses showed that the pH of the juice from cocoa beans was 3.14 and its glucose content was very high with around 214.2±6.2g/L. The total soluble solids were 16.17^o Brix. The crude proteins and ascorbic acid contents of this natural syrup were evaluated at 7.2±0.21g/L and 18.3±7.5mg/L, respectively. Analyses also revealed that potassium and calcium contents of the cocoa beans syrup were 950±16.32mg/L and 171.5±34.1mg/L, respectively. Other minerals like sodium, magnesium and phosphorus are lower. This juice was high in citric acid at 9.1±0.6mg/L, malic acid at 3.6±0.5mg/L and acetic acid at 2.28±0.7mg/L. It was lower in fumaric acid, oxalic and lactic acid. Marmalade was produced with cocoa bean juice with additional sugar and cocoa placenta (11.5%) to the mucilage (44.72%). The output of the manufacture was 46.2%. Cellulose and fat contents were 5.36±0.43% and 5.23±0.15%, respectively. Total soluble solids were 67.14^o Brix. Sensory evaluation was conducted on 1-5 point hedonic scale. The results of sensory rating were statically analyzed with student t-test. Analyses did not show any significant difference ($p = 0.5$) in taste, color and consistency compared with a commercial apricot marmalade. Appearance and acceptability were found significantly different ($p = 0.5$). On a 1-5 rating scale, the acceptability of cocoa marmalade (3.56±0.7) was fairly lower than that of commercial marmalade (3.96±0.5). Considering the output of manufacture, more than 239.2 tons of marmalade are expected to be produced each year.

Key words: Cocoa, juice, marmalade, sensory evaluation, organoleptic quality

Introduction

Cocoa (*Theobroma cacao* L) is known worldwide for its beans. They contain about 50% of fat (Asiedu, 1991). Cocoa is very important ingredient in several kinds of foods, such as chocolate, cakes, biscuit, child-foods, ice creams and sweet consumed in developed countries (Guehi *et al.,* 2007). The fat from cocoa also called cocoa butter is particularly used in cosmetics, pharmaceutical industries.

In the time, the cocoa's economy of the most developing and particularly Côte d'Ivoire based primarily on their agricultural resources is strongly dependent on the standards and the often rigorous and rigid quality standards fixed by the developed countries (Guehi *et al.,* 2007).

Otherwise, for several reasons, namely the expensive price of cocoa butter, chocolate industries are currently envisaging partial substitution of cocoa butter by other vegetal fats (Hoda *et al.,* 2006; Osborn et Akoh, 2002; Lipp *et al.,* 2001). Because of these opportunities about to replace cocoa butter by others fats, cocoa's economy could be negatively affected, serious drawbacks should affect smallholder farmers and raw cocoa bean quality could go to lower.

In order to circumvent such situation, cocoa traders should work in favour of the wealthy by exploiting by-products namely shells and juice. Indeed, during cocoa harvesting, cocoa beans are collected in tanks for fermentation. From mucilaginous pulp embedding beans, a sweetened and vicious juice pours in abundance.

Nowadays, several policies are being directed in cocoa by-products valorization. These researches are mainly related to cocoa pods and the mucilaginous juice, widely used. In Nigeria and Ghana, cocoa pods were used for soap fabrication (Antonio *et al.,* 1993). These pods were also used for poultry feeding (Wood and Lass, 1985) for tropical region where conventional animal foods are highly in concurrence with those of human being. The juice has been successfully used in Brasilia for alcoholic beverage, vinegar (Samsiah *et al.,* 1991) and jelly fabrication (Wood and Lass, 1985).

Nevertheless, these results seem very limited and practically unexploitable at wide scale. This situation is favoured by the lack of complete and detailed information about chemical and physical characteristics of the indicate derivated products. Cocoa bean juice could be used in industrial processes for beverages manufacture such us pineapple, orange and other many tropical fruit juices if its production is supported and improved in the on-farm processing of cocoa. In addition cocoa bean juice can be used for alcoholic drinks, due to its high sugar contents (Redgwel *et al.,* 2003; Sandra *et al.,* 2007). Several micro-organisms namely yeasts like *Saccharomyces cereviciae* and *Hanseniaspora guillermondii* and bacteria like *acetic acid bacteria* (AAB), *lactic acid bacteria* (LAB) are present in the juice (Nielsen *et al.,* 2007) can favour the production of others products by alcoholic fermentation. Côte d'Ivoire, Ghana and Indonesia are the three countries with the largest amount of land in cocoa production worldwide and they are also the three largest cocoa producers. They produced 14,05,000, 736,000 and 41500 metric tons during marketing year (2003/2004), respectively (ICCO, 2004). The amount of cocoa juice produced each year is estimated to 300,000, 160,000 and 90,000m^3. This juice is used in a small quantity by farmers for beverage consumption and the mucilage coating placenta is sucked then the placenta is thrown in farms during cocoa pods opening processing. Although ivorian farmers hope in further higher price of beans, they should reorganize cocoa industry by adding value on cocoa bean juice in industrial area.

The purpose of this study is to transform cocoa bean juice into marmalade. This was carried out following three steps: determination of chemical and physical properties, possible preparation of marmalade from cocoa bean juice and evaluation of sensory properties of the final product.

Materials and Methods

Cocoa bean juice extraction: Cocoa bean juice was collected immediately after cocoa pods opening from three big cocoa farms around Abidjan (South Côte D'Ivoire) in September, 2007. Fresh cocoa beans were packed in a basket and placed at around one a meter up from the ground. Baskets are slightly bent in order to collect the juice in bottle. Juice was immediately stored in cool box during the transport to the laboratory.

Chemical analysis: Total soluble solids were measured with a refractometer (JP SELECTA, Spain) graduated from 0-30 and results were expressed in degree Brix. The pH was measured with a pH meter (107 Consort, Belgium) using an agitator (JP SELECTA, Spain). The determination of ash content and moisture was made using the constant weight method. Crude cellulose content was measured by Soest method (AOAC, 1976). Sugars and organic acids contents were assessed by the HPLC while minerals were evaluated using atomic absorption spectrophotometry. The HPLC system (shimadzu corporation, Japan) used, was equipped with a pump (shimadzu LC-6Aliquid Chromatograph), a detector (shimadzu, SPD-6A UV spectrometric detector) and an integrator (shimadzu C-R 6A Chromatopac).

Fat and ascorbic acid contents were estimated using a Soxhlet extraction apparatus and 2, 6 dichlorophenol indophenol, respectively (AOAC, 1976). Titratable acidity was determined using volumetric measurement (AOAC, 1976). Protein content was evaluated with a folin phénol reagent (Lowry *et al.,* 1951).

Cocoa juice marmalade preparation: The marmalade was prepared following Unipectin method using 915g of fresh cocoa bean juice and 165g of crushed placenta. The mixture was boiled in order to evaporate water. When total soluble solids of the mixture reached 25%, 750g of sugar was added. When the marmalade reached 60% of dry matter, the heating was reduced for eight minutes while mixing continue. A drop of marmalade was placed in a glass in order to verify its cooking. (As the cooking of marmalade is reached when the drop in the bottom of the glass is easily removed). The marmalade was then placed in hot sterile jars and kept until the following day.

Sensory evaluation of cocoa marmaladel: For sensory analysis, the marmalade was stored at ambient temperature for a month production and then it was compared to commercial apricot marmalade for many properties such as taste, color, flavor, consistency and overall acceptability.

A portion (5g) of sample was served in randomly coded plastic plates to 25 untrained assessors. Each attribute was rated on 1-5 point hedonic scale where 1 designed "very bad" and 5 "very good" (SSHA, 1990). For consistency, an additional 5g of each sample was smeared on a slice piece of bread (Egbekun *et al.,* 1998).

Statistical analysis: The results of sensory test were statically analyzed using the t-test software STATSOFT STATISTICA 99th edition.

Results

Results indicated that cocoa bean juice had a high nutritional value due to its contents in fat, carbohydrate, crude protein, mineral and vitamin.

The chemical and physical properties are summarized in Table 1. They showed that cocoa juice was very acidic. This acidity was revealed by a low pH (3.75) and a high titratable acidity at 170±6.28meq/L.

Citric acid was the most predominant organic acid, with an average value of 9.14±0.64mg/L. Malic and acetic acids contents were 3.6±0.5mg/L and 2.28±0.7mg/L,

respectively. The juice was very poor in oxalic acid (1.27±0.72mg/L), lactic acid (1.23±0.01mg/L) and fumaric acid (0.02±0.01mg/L) (Fig. 2). The total soluble solids were 16.17° Brix. The sweetness was mostly due to its high glucose contents averaging 214±6.42g/L. The juice was poor in saccharose which content was ranged from 21.31±3.21g/L (Fig. 1).
The mineral content was 3.76±0.84% and was represented by potassium (950±6.32mg/L), calcium (171.5±34mg/L) and magnesium (82.5±0.85mg/L); Sodium and phosphorus contents were 62.47±3.43mg/L and 30.5±3.77mg/L, respectively. The crude proteins content was estimated to 7.2±0.21g/L/L, respectively. Vitamin C content was 18.3±7.5mg/L and the fat content was estimated to 3.54±0.2%.
The nutrient composition of cocoa marmalade is reported in Table 2. Similarly to the juice, marmalade had a low pH value (3.14). The amount of Vitamin C decreased at 2.35±0.25mg/100g. Fat, brut cellulose and total soluble solids contents were 5.23%, 5.36% and 67.14%, respectively.
Table 3 showed the results of sensory tests. There was no significant difference (p = 0.05) in many properties such as astringency, taste, colour and consistency between cocoa juice marmalade and commercial apricot marmalade. However, the aspects and the acceptability of the two products were significantly different (p = 0.05). On 1-5 rating scale, the acceptability of cocoa marmalade (3.56±0.7) was fairly lower than that of commercial marmalade (3.96±0.5). The rating values of the aspect, colour, taste, astringency, flavour were also lower than those of apricot marmalade.

Discussion

Cocoa bean juice contains significant amount of several nutrients such as (sugar, minerals, fat and proteins). Compared to commonly consumed orange and pineapple juices, calcium content in cocoa juice is higher than orange and pineapple juices (Favier *et al.*, 1993). The contents of other minerals are similar to those found in these fruits.
The pH value of cocoa bean juice was slightly higher than that of orange juice (3.23-3.53) as reported Esteve *et al.* (2005).
The low pH and high total acidity were mostly due to the presence of several organic acids such as citric acid and acetic acid. These acids preserve the color, flavour and gustative characteristics of the juice according to Navarre (1998). So they could preserve cocoa juice of the bacterial contamination. Finally the low pH also contributes to the preservation of the microbiological quality of the juice by protecting it from non acidophil bacteria (Delanoë *et al.*, 1996).
Cocoa juice was high in sugar compared to that found in pineapple (12.5° Brix) (Regina *et al.*, 2006) and in

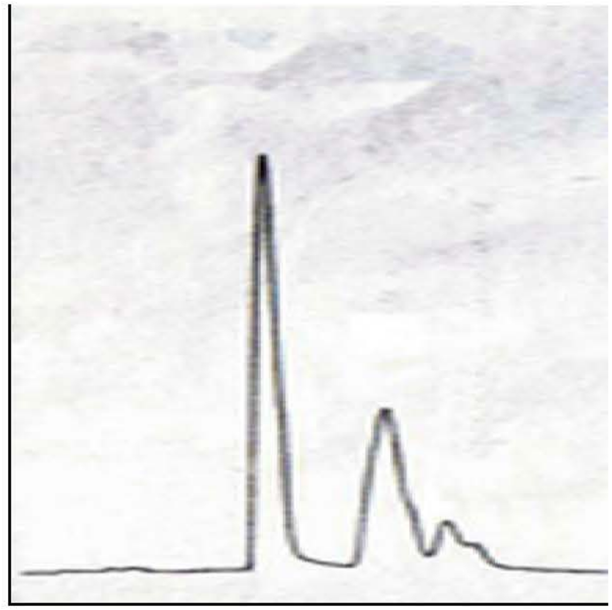

Fig. 1: Identification and quantification of reducing sugars in cocoa bean juice with HPLC method. a: Glucose; b: Saccharose.

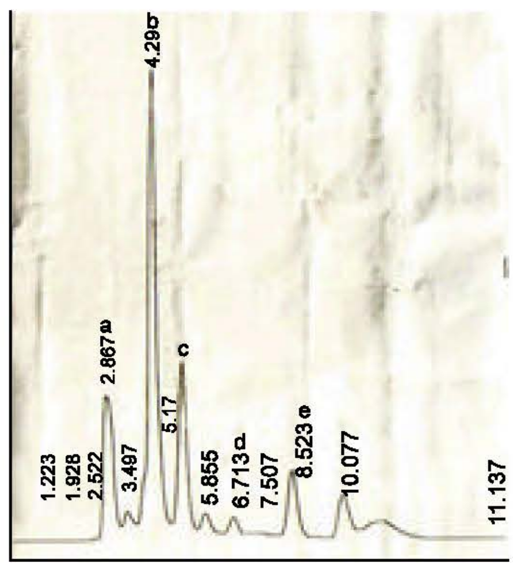

Fig. 2: Identification and quantification of organic acids in cocoa bean juice with HPLC method. a: oxalic acid; b: citric acid; c: malique acid; d: acetic acid; e: fumaric acid.

mandarin juice (52.2g/L of saccharose and 20.8g/L of glucose) (Ana *et al.*, 2004). These sugars have a key role by covering the astringency due to the organic acids. Fat and protein contained in cocoa bean juice were higher than those found in orange (3.12%) (Saïdani et Brahima, 2003) and (3.15-5%) (Latham, 2001), respectively.

Table 1: Physical and chemical characteristics of cocoa bean juice

Parameters	Means±SD
Moisture (%)	85.3±8.60
Total soluble solids (°Brix)	16.17±0.74
Glucose (g/L)	214.24±6.42
Saccharose (g/L)	21.31± 3.21
pH	3.75±0.81
Titratable acidity (meq/L)	170±6.28
Citric acid (mg/L)	9.14±0.64
Malic acid(mg/L)	3.6±0.50
Acetic acid (mg/L)	2.28±0.70
Oxalic acid (mg/L)	1.27±0.72
Lactic acid (mg/L)	1.23±0.01
Fumaric acid (mg/L)	0.02 ±0.01
Vitamin C (mg/1000mL)	18.3±7.50
Ash (%)	3.76±0.84
Total phosphorus (mg/L)	62.47±3.43
Calcium (mg/L)	171.5±34.01
Magnesium (mg/L)	82.5±0.81
Sodium (mg/L)	30.5±3.77
Potassium (mg/L)	950±16.32
Fat (%)	3,54±0,2
Total Proteins (g/L)	7.2±0.21

Means±SD of three determination

Table 2: Physical and chemical Properties of Cocoa Beans Juice Marmalade

Parameters	Means±SD
pH	3.14±0.4
Total soluble solids (°Brix)	67.14±0.23
Fat (%)	5.23±0.15
Cellulose (%)	5.36±0.43
Ascorbic acid (mg/g)	2.35±0.25
Output (%)	46.2

Means±SD of three determination

Table 3: Sensory Evaluation? Score Results of Marmalade Samples

Parameters	Cocoa Marmalade	Apricot Marmalade
Appearance	3.32±0.9[a]	3.71±0.91[b]
Colour	3.6±0.82[a]	3.87±0.45[a]
Taste	3.6±0.91[a]	4.33±0.48[a]
Astringency	3.48±1.00[a]	3.92±0.65[a]
Flavour	3.52±0.96[a]	3.87±0.74[a]
Consistency	3.84±1.03[a]	3.79±0.66[a]
Acceptability	3.56±0.71[a]	3.96±0.5[b]

[a,b]Means with the same letters within a column do not differ significantly (p = 0.05). *Range: 1 = very good, 5 = very bad.

Cocoa juice can not be considered as a veritable source of vitamin C in comparison with that found in orange (77±3mg/L) or pineapple (26.5ml/L) (Yurena *et al.*, 2006).

Cocoa marmalade had a comparable nutritional value as commercial marmalade. The highest consistency of cocoa marmalade could be attributed to the pectin contained in cocoa fresh juice. During marmalade preparation, the pectin formed a gel, resulting in an increased consistency of the product according to Emaldi *et al.* (2006).

The lower sugar content also induced the lower output of the marmalade manufacturing. Marmalade cooking needs 50% of sugar. This amount was higher than the quantity of sugar added during the cocoa marmalade formulation which used 41% of sugar. The addition of placenta also increases the amount of the crude cellulose content in cocoa marmalade which was higher than the marmalade cooked with lupines fruits which containing 0.2-0.8% of crude cellulose (Villarreol *et al.*, 1996).

Due to its higher nutritional value and the quantity, cocoa bean juice should be valorized with two objectives: for beverage consumption like tropical juice (orange, pineapple) and for marmalade preparation. On the basis of the current (2003-2004) cocoa production and the outcome of the manufacture (46.2%), more than 140, 74.6 and 42 tons of marmalade are expected to be produced by Côte d'Ivoire, Ghana and Indonesia respectively. This production (239.2 tons) would increase if other cocoa producing countries (Brazil and Nigeria) were considered.

Beside the beans, cocoa juice should be involved in cocoa industries' policies. Like cashew nut valorized in Tanzania, India and Brazil (Lautier *et al.,* 2001) and pineapple industry restructured in Martinique (Saudubray *et al.,* 2006), the perspective of cocoa manufacturing is necessary. Cocoa juice could stand out presently because the world market for certified citrus (fresh and juice) offers interesting prospects. It is low and represents only 0.3 percent of the world juice consumption and 0.1 percent of world juice production (Pascal Liu, 2004). So the exportation of 550 millions of liters of cocoa juice or 192 tons of marmalade should comply with new requirements for food security.

Conclusion: Valorization of fresh cocoa mucilaginous juice results from economical considerations. This juice production with its diversity of products (beverage and marmalade) seems to be able to constitute an interesting source of incomes for cocoa producing countries. Cocoa juice has a nutritional value (quantity and nutrient content) comparable to that of some tropical fruits and cocoa marmalade has an acceptable quality like some commercial products such as apricot marmalade.

Nevertheless, these new products should be more studied in order to find a long storage for the beverage and increase the input of the marmalade production. This perspective is important because cocoa juice is presently a by-product of cocoa industry with a strong economic potential.

References

Ana, G.P., L. Pilar, O. Jesus, J.R. Jose and S. Carlos, 2004. Changes in vitamin C and flavour components of mandarin juice due to curing of fruits. Food Chem., 91: 19-24.

Asiedu, J.J., 1991. Transformation des produits agricoles en zone tropicale CTA Karthala, page: 335.

AOAC., 1976. Official methods of analysis, 13th ed. Washington, DC: Association of official Analysis chemists.

Antonio, F., J. Jules and N.B. James, 1993. New products Theobroma Cacao. Seed pulp and pod gum. New Crops, 475-478.

Delanoë, D., C. Maillard and M. Dominique, 1996. Le Vin: De l'analyse à l'élaboration 4è éd. Lavoisier, page: 179.

Egbekun, M.K., E.O. Nda-Suleiman and O. Akinyeye, 1998. Utilization of fluted pumpkin fruit (Telfairia occidentalis) in marmalade manufacturing. Plant Food Hum. Nutr., 52: 171-176.

Emaldi, U., M. Jafet, S. Nassara and S. Carla, 2006. Pulpa del fruto del cardon dato (stenocereus grisous, cactaceae) como materia prima para la elaboracion de marmelada, Arch. Latinoamericanos Nutr., 46: 83-89.

Esteve, M.J., A. Frigola, C. Rodrigo and D. Rodrogo, 2005. Effect of storage periode under variable conditions on the chemical and physical composition and colour of spanish refrigerated oranges juices. Food Chem. Tox., 43: 1413-1422.

Favier, J., J.I. Ripert and M. Feinberg, 1993. Répertoire général des aliments Tome 3. Table de composition Fruits exotiques Fruits de cueillette d'Afrique Tec et Doc. Lavoisier CIQUAL-CNEVA., page: 207.

Guehi, T.S., M. Dingkuhn, E. Cros, G. Fourny, R. Ratomahenina, G. Moulin and A. Clement, 2007. Identification and lipase-producing abilities of moulds isolated from Ivorian raw cocoa beans. Res. J. Agric. Biol., Sci., 3: 838-843.

Hoda, H.M.F., A.A.M. Magda, K.M.E.A.S. Abdel and N.L. Shereen, 2006. Cocoa substitute: Evaluation of Sensory Qualities and Flavour Stability. Eur. Food Res. Tech., 223: 125-131.

ICCO, 2004. Organisation internationale du cacao. Bulletin trimestriel de statistique du cacao. Volume XXX, N°4. Cacaoyère, 2003/2004, page: 38.

Latham Michael, C., 2001. La nutrition dans les pays en développement Rome, page: 513.

Lautier, E., M. Dornier, D.S. Filho and M. Reynes, 2001. Les produits de l'anacardier : caractéristiques, voies de valorisation et marchés. Fruits, 56: 235-248.

Lipp, M., C. Simoneau, F. Ulbert, E. Anklam, C. Crews, P. Brereton, W. de Greyt, W. Schwack and C. Wiedmaier, 2001. Composition of Genuine cocoa butter and cocoa butter equivalents. J. Food Comp. Anal., 14: 399-408.

Lowry, O.H, N.J. Rosebrough, Fara and R.J. Randall, 1951. Protein measurement with a folin phénol reagent. J. Biol. Chem., 193: 265-275.

Navarre Colette, 1998. L'œnologie, 4ième éd. Lavoisier Tec et Doc., page: 354.

Nielsen, D.S., O.D. Teniola, L. Ban-Koffi, M. Owusu, T.S. Andersson and W.H. Holzapfel, 2007. The microbiology of Ghanaian cocoa fermentation analysed using culture-dependent and culture-independent methods, 114: 168-186.

Osborn, H.T. and C.C. Akoh, 2002. Enzymatically modified Beef Tallow as a substitute for cocoa butter. J. Food Sci., 67: 2480-2485.

Pascal Liu, 2004. FAO. Commodity and trade policy research working paper N°5, page: 26.

Wood, G.A.R. and R.A. Lass, 1985. Cocoa. 4th ed. Longman, England UK., page: 620.

Redgwel, R.J., V. Trovato and D. Curti, 2003. Cocoa bean carbohydrates: roasting induced changes and polymer interactions. Food Chem., 80: 511-516.

Regina, K.G., E.B. Roy, M.D.A. José and C.D.M. Hilary, 2006. Technological aspect restructuring concentrated pineapple pulp. Food Sci. Tec., 759-765.

Saïdani, M. and M. Brahima, 2003. Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange. Phytochem., 62: 1283-1289.

Samsiah, S., Y.Q. Lan and C.E. Chong, 1991. Development of food product products from cocoa pulp abd sweating. In: Cocoa conference: Challenges in the, 90s, Kuala Lumpur, Malaysia, 25-28.

Saudubray, F., J.P. Horry, M.P.A., Alphonsine, D. Cros and A. Soler, 2006. Management de la qualité dans la filière ananas en Martinique. Fruits, 61: 39-53.

Sandra, L.G., L. Gérard, L.P. Jose, B. Michel and G. Jean-Pierre, 2007. Study on the microbiology and biochemistry of cocoa fermentation in the Dominican Republic. Int. J. Food Micr., 114: 124-130.

SSHA, 1990. Evaluation sensorielle. Technique et documentation, Lavoisier, page: 328.

Yurena, H., L. Gloria and G. Monica, 2006. Determination of Vitamin C in tropical fruit: A comparative evaluation of methods. Foods Chem., 96: 654-664.

Villarreol, M., E. Biolley, G. Larenas, E. Wittig, V. Diaz and Y. Munoz, 1996. Caracterizacion quimica, sensorial y clinica de mermelada de lupino. Arch. Latinoamericanos de Nutr., 46 : 234-237.