

Title page

Glucid factors of the inflorescence sap of four coconut (*Cocos nucifera* L.) cultivars from Côte d'Ivoire.

Abstract

Aim: To assess physicochemical characters related to the glucide contents of the sap provided by four coconut cultivars.

Study design: Thirty-six coconut saps from thirty-six healthy coconut trees inflorescences, replicated two times. Trees were randomly considered, cultivars were the most widespread coconut types in Côte d'Ivoire. The whole coconut saps consists to three batches of three samples per cultivar.

Place and duration: Station Marc Delorme for the coconut Research at Port Bouet, National Centre of Agronomic Research of Côte d'Ivoire during 2012 and 2013.

Methodology: Inflorescences saps from four coconut cultivars namely Malayan Yellow Dwarf, West African Tall, and PB 121⁺ and PB 113⁺ hybrids were investigated according to their carbohydrate amount. Nine coconut trees were randomly selected per cultivar on experimental fields, and their unopened inflorescences (spathes) of rank 8 have undergone the production of sap. The sap was harvested twice a day and samples from both harvestings were stored at -20 °C. Even quantities of the daily samples were mixed and a slight volume was taken from this mixture for analysis. Five traits (pH, contents of total soluble solids, dry matter, total soluble sugars and reducing sugars) related to glucidic richness of the coconut sap were assessed. Then the main carbohydrates components (sucrose, glucose, fructose, mannose and glycerol) were elucidated using a high performing chromatograph, and led to evaluation of the sweet index.

Results: The effect of coconut cultivars on pH and contents of total soluble solids, dry matter, total soluble sugars, sucrose, mannose, glycerol and sweet index was significant at $P = 0.05$. The sap of MYD recorded highest means of pH (7.32). This type of sap contained also highest contents of total soluble solids (16.31 %), dry matter (16.47 g/100 ml), total soluble sugars (16.08 g/100 ml), mannose (0.019 g/100 ml) and glycerol (0.094 g/100) contents and provided the greatest sweet index (16.00). Sucrose was the main carbohydrate of coconut sap. The MYD obtained sucrose content (12.24 g/100 ml) higher than WAT (9.40 g/100 ml). Hundred (100) ml of saps from PB 121⁺ and PB 113⁺ provided respectively 10.31 g and 10.38 g of sucrose, which intermediate both means above. The contents of reducing sugars, glucose and fructose were respectively between 2.41 and 3.73 g/100 ml; 1.63 and 1.84 g/100 ml and from 1.24 to 1.52 g/100 ml, but didn't differentiate saps originating with MYD, WAT, PB 121⁺ and PB 113⁺ cultivars. Reducing sugars were significantly involving with glucose ($r = 0.83$) and fructose ($r = 0.91$).

Conclusion: Glucide of coconut inflorescence sap is related to its contents of total soluble solids, dry matter and the whole carbohydrates. Thanks to their higher sucrose, glucose and fructose contents, the saps of the MYD, PB121⁺ and PB113⁺ cultivars could be suitable for the production of syrup, sugar and alcohol in order to improve benefits derived from coconut.

Keywords: Coconut Sap, inflorescences, cultivars, physicochemical characters, Côte d'Ivoire.

2. Introduction

Coconut (*Cocos nucifera* L.) is a widespread perennial crop of the whole intertropical and humid zone. It's cultivated on more than 12 millions of ha [1] and represents the main livelihood of more than 10 millions of

families [2]. All parts of the coconut are useful [3]. Nevertheless, the walnut is still being the most longstanding and widespread valorization of this plant. However, face to the decline of the profitability of the nuts products, the tapping of sap from inflorescences (or spathes) seemed to be alternative under promoting. Such uses of the coconut spathes, which are to the detriment of the production of nuts, are developed in many countries through Southeast Asia and in Latin America [4].

Previous studies compared the coconut's profitability from the production of walnut to that involving tapping of sap. They mentioned the high incomes resulting from the production of coconut sap. According to [5], the coconut sap could procure 3 to 16 times higher incomes to the farmers than the trading of walnuts or coprah. Indeed, the coconut inflorescence sap (CIS) is a natural raw material used for the production of by-products with substantial value. Indeed, the SIC is a nutritious liquid with a high content of glucides, which are mainly consisting to sucrose [6]. Thanks to this advantage, the CIS is turned to coco syrup and sugar after partial or full water evaporation ([7], [3]). Besides, this quality predispose the SIC to rapid changes. It undergoes spontaneous and natural fermentation and leads prior to alcoholic beverage [8] then acid raw material for vinegar production [9].

However, characterization of the coconut sap hasn't ever been achieved in Côte d'Ivoire, even though this country wishes promoting of this raw material. Also, few works attempted on the compounds of CIS compare different coconut varieties. Moreover, many coconut types still presenting agronomic, morphological and genetic divergences [10]. In addition, the quality of such a substance could depend on the conditions surrounding with its production [3]. The present study investigates the glucides characters of the sap from the most widespread coconut cultivars in Côte d'Ivoire in order to other ways of their valorization.

2. MATERIAL AND METHODS

2.1 Plant material

The plant material was sap collected from spathes of four coconut cultivars cultivated in Côte d'Ivoire, namely improved hybrids PB 113 (Cameroon Red Dwarf x improved Rennell Islands Tall) and PB 121 (Malayan Yellow Dwarf x improved West African Tall) and local dwarf (Malayan Yellow Dwarf) and tall (West African Tall) cultivars.

2.2 Sampling

From each cultivar, three groups of three adult and healthy coconut trees were randomly selected at three experimental fields of the Marc Delorme Research Station of the National Center of Agronomic Research, Abidjan, Côte d'Ivoire. Thus, 36 coconut trees (3 coconut trees* 3 batches* 4 cultivars) were sampled. Then, the spathe of rank 8 of each tree was chosen for the coconut sap's production [10]. That led to the use of 36 spathes during this study. Spathes were severed with a slicing knife and the flowing exudate was collected into a polyethylene can previously sterilized with water boiled at 100 °C. The can was fastened to the spathe and both were covered with a sterile muslin cloth in order to preserve the hygienic quality of the sap. The sap was harvested twice a day, at 7 AM and 5 PM [10]. From each harvesting, a volume of 150 ml of sap by spathe was

sampled into sterile pots. Samples were immediately placed into an isotherm cool bag, transported to laboratory and stored -20 °C until analysis [6].

Before analysis, the two daily sap harvests from a spathe were mixed homogeneously, and then a volume of 200 ml was sampled. So, 36 samples of sap were analyzed during a sap harvesting season. The study was achieved along two seasons, in 2012 and 2013. Finally, 72 samples of CIS were investigated during these attempts.

2.3 Evaluation of physical characters related to the glucid richness of the SIC

The potential of hydrogen (pH) was determined by using a pH-meter (HANNA Instruments). The total soluble solids contents were read with a manual refractometer (DIGIT 032, Brussels, Belgium) and expressed in percentage. The dry matter was valued by drying samples of sap using an oven (Mettler, Berlin, Germany) at 104 °C [11] and a two digits scale.

2.4 Analysis of chemical characters linked to the glucid richness of the SIC

Sugars contents were achieved with spectrophotometer methods. The contents of total soluble sugars were analyzed by phenol and sulfuric acid method [12]. Reducing sugars contents were determined with the use of 3, 5 - dinitrosalicylic acid [13]. The main components of sugars were revealed by High-Performance Liquid Chromatography (Dionex Corp., Sunnyvale, CA) using a pulsed amperometric detector (Dionex ED50). Ten µl of CIS were diluted with 9,990 µl of deionized water, and then filtered on a Millipore (0.45 µm) polyethylene filter and injected on a column (Carbopac MA-1, Dionex, 4 x 250 mm). Sugars components were eluted with 0.8 M NaOH at 0.4 mL min⁻¹. External standard was mixture of pure reagents from Sigma-Aldrich Corporation, USA. Sugar components contents allowed evaluation of the sap's sweet index:

$$SI \text{ (g/100 ml)} = \text{Suc} + 1.5 * \text{Fru} + 0.8 * \text{Glu} + 0.3 * \text{Man}$$

With: SI, sweet index; Suc, sucrose content; Fru, fructose content; Glu, glucose content; Man, mannose content.

2.5 Statistical analysis

The spathes from groups of coconut trees were replications for cultivars. The statistical analysis evaluated differences between coconut cultivars using a One-way analysis of variance (ANOVA) and Newmann Keuls test for post hoc at P-value = 0.05. The correlations between all characters were valued according to the Pearson index. Statistical analyses were performed with use of SPSS software (SPSS 16.0 for windows). All experiments were achieved at least in triplicate.

3. RESULTS

3.1 Physical characters

Table 1 showed significant difference between the four coconut cultivars resulting from the physical characters assessed ($P < .001$).

The contents of total soluble solids varied statistically from 14.65% to 16.31%. Thus, the sap of MYD contained more total soluble solids (16.31 g/100 ml) than that of the three other cultivars which gave similar means of TSS (14.65 to 14.82 g/100 ml).

The pH of the sap of the coconut tree fluctuated between 6.97 and 7.32 and differed statistically the cultivars studied. The MYD provided the highest pH (7.32), while the PB113⁺ hybrid recorded the lowest value (6.97). The PB 121⁺ hybrid and its male parent gave the same value of pH (7.10 and 7.18, respectively).

The dry matter was statistically more abundant into the sap of MYD (16.47 g/100 g). For this character, the contents provided by the sap of the cultivars WAT, PB 121⁺ and PB 113⁺ (14.86 g/100 g, 14.75 g/100 g and 14.64 g/100 g, respectively) seemed statistically identical (Table 1).

Table 1: Averages of three physical characters linked to the glucide content of the sap produced by spathes of four coconut cultivars studied.

Characters	Means ± SD				F	P	CV (%)
	MYD	WAT	PB 121 ⁺	PB 113 ⁺			
TSS (%)	16.31±1.09 ^a	14.69 ± 0.81 ^b	14.82 ± 0.89 ^b	14.65 ± 0.83 ^b	16.76	<0.001	7.49
pH	7.32±0.17 ^a	7.18 ± 0.22 ^b	7.10 ± 0.2 ^b	6.97 ± 0.15 ^c	16.26	<0.001	3.15
DM (g/100 g)	16.47±1.46 ^a	14.86±0.96 ^b	14.75±1.12 ^b	14.64±1.07 ^b	14.79	<0.001	9.01

F, statistical value; P, value of probability test; CV, coefficient of variation. Per row, means ± SD (standard deviation) with the same superscript are statistically identical. PB 113⁺, improved PB 113; PB 121⁺, improved PB 121; WAT, West African Tall; MYD, Malayan Yellow Dwarf; TSS, total soluble solids; pH, potential of hydrogen; DM, dry matter content.

3.2 Chemical characters

Sugars were the main macromolecules dissolved into the coconut sap. They consisted to 14.14% to 16.08% of the sap, which represented 91.39% to 94.63% of the dry matter. Statistically, the total sugars content differentiated the coconut cultivars ($P < 0.001$). Indeed, the sap produced by MYD provided the highest total soluble sugars content (16.08 g/100 ml). The PB 121⁺, PB 113⁺ and WAT cultivars recorded statistically the same total soluble sugars contents in their sap (14.23 g/100 ml, 14.14 g/100 ml and 14.54 g/100 ml, respectively).

Concerning the reducing sugars content, it consisted to 2.41% to 3.73% of the CIS. But this character didn't differ ($P = .11$) the four coconut cultivars studied (Table 2).

Table 2: Total soluble sugars and reducing sugars contents of the coconut sap from the four cultivars studied.

Characters	Means ± SD				F	P	CV (%)
	MYD	WAT	PB 121 ⁺	PB 113 ⁺			
TS (g/100 ml)	16.08±0.7 ^a	14.23±0.76 ^b	14.14±1.10 ^b	14.54±1.14 ^b	15.10	<0.001	8.29
(g/100 g dm)	92.56±4.32	91.40±4.88	91.40±7.11	94.63±7.42			
RS (g/100 ml)	3.21±2.02 ^a	3.28±1.64 ^a	2.41±1.30 ^a	3.73±2.87 ^a	2.13	0.11	65.13
(g/100 dm)	19.96±12.56	23.05±11.52	1704±7.62	25.65±19.73			

F, statistical value; P, value of probability test; CV, coefficient of variation. Per row, means ± SD (standard deviation) with the same superscript are statistically identical. PB 113⁺, improved PB 113; PB 121⁺, improved PB 121; WAT, West African Tall; MYD, Malayan Yellow Dwarf; TAC, total titrable acidity; TS, total soluble sugars content; RS, reducing sugars content, dm, dry matter.

3.3 Main components of the soluble sugars

Chromatographic analysis revealed different proportions of fructose, glucose, glycerol, mannose and sucrose from carbohydrates of CIS (Table 3). Thus, sucrose was the major sugar component of CIS of the four cultivars. Sucrose contents varied from 9.40 g/100 ml to 12.24 g/100 ml. This disaccharide was statistically more abundant in the sap produced from MYD (12.24 g/100 ml) than that of WAT (9.40 g/100 ml). The hybrids PB 121⁺ (10.31 g/100 ml) and PB 113⁺ (10.38 g/100 ml) provided statistically even sucrose contents, intermediate of the MYD and WAT cultivars.

Glucose and fructose were minor components. Glucose content fluctuated between 1.63 and 1.84 g/100 ml, while fructose constituted 1.24 g to 1.52 g from 100 ml of sap. Nevertheless, the CIS from the whole cultivars studied recorded statistically the same contents of glucose and fructose.

Otherwise, slight contents of glycerol and mannose were noticed in CIS. The highest glycerol content was provided by MYD (0.094 g/100 ml). The sap resulted from the three other cultivars contained statistically identical amounts of glycerol, included between 0.05 and 0.058 g/100 ml. The CIS of MYD was also statistically richer in mannose (0.019 g/100 ml) than those of WAT (0.011 g/100 ml), PB 121⁺ (0.008 g/100 ml) and PB113⁺ (0.014 g/100 ml).

The sweet index, valued according to the carbohydrate components, differed the sap collected from MYD and those of the three other coconut types. Indeed, sweet index recorded with the sap produced by the dwarf coconuts (16) was statistically superior to those resulted from hybrids PB 121⁺ (13.68), PB 113⁺ (13.56) and tall WAT (13.03). These three cultivars provided statistically similar means of sweet index (Table 3).

Table 3: Contents of glucide components of the sap produced by spathes of the four coconut cultivars studied.

Glucide components	Means $\pm$ SD				F	P	CV (%)
	NJM	GOA	PB121 ⁺	PB113 ⁺			
Sucrose (g/100 ml)	12.24 $\pm$ 2.82 ^a	9.40 $\pm$ 2.26 ^b	10.31 $\pm$ 3.04 ^{ab}	10.38 $\pm$ 2.73 ^{ab}	2.66	0.048	27.07
Glucose (g/100 ml)	1.84 $\pm$ 0.24 ^a	1.72 $\pm$ 0.36 ^a	1.63 $\pm$ 0.22 ^a	1.64 $\pm$ 0.27 ^a	2.22	0.10	16.79
Fructose (g/100 ml)	1.52 $\pm$ 0.37 ^a	1.50 $\pm$ 0.31 ^a	1.38 $\pm$ 0.42 ^a	1.24 $\pm$ 0.33 ^a	2.55	0.07	26.28
Mannose (g/100 ml)	0.019 $\pm$ 0.007 ^a	0.011 $\pm$ 0.003 ^b	0.008 $\pm$ 0.002 ^c	0.014 $\pm$ 0.004 ^b	25.51	<0.001	46.99
Glycerol (g/100 ml)	0.094 $\pm$ 0.037 ^a	0.058 $\pm$ 0.014 ^b	0.058 $\pm$ 0.008 ^b	0.048 $\pm$ 0.013 ^b	32.94	<0.001	42.35
Sweet Index	16.00 $\pm$ 2.64 ^a	13.03 $\pm$ 2.26 ^b	13.68 $\pm$ 3.03 ^b	13.56 $\pm$ 2.64 ^b	3.41	0.025	20.21

F, statistical value; P, value of probability test; CV, coefficient of variation. Per row, means $\pm$ SD (standard deviation) with the same superscript are statistically identical. PB 113⁺, improved PB 113; PB 121⁺, improved PB 121; WAT, West African Tall; MYD, Malayan Yellow Dwarf.

3.4 Correlations between the glucidic characters assessed

Values of Pearson indexes (r) showed positive significant links between several physicochemical characters (Table 4). Thus, the total soluble solids content was correlated to the dry matter content (r = 0.88). This one

depended on the amount of total soluble sugars of the CIS ($r = 0.77$). Also, the contents of total soluble solids and total soluble sugars were hardly closed ($r = 0.83$). Otherwise, the higher the reducing sugars content are the levels of glucose ($r = 0.85$) and fructose ($r = 0.91$) contents. Mean significant correlations engaged glycerol and mannose contents ($r = 0.56$), then glucose and fructose contents ($r = 0.56$). In addition, the great amount of sucrose ensured higher sweet index ($r = 0.90$).

Table 4: Matrix of correlations between physicochemical characters related to the glucide contents of the sap of the four coconut cultivars studied.

Characters	pH	DM	TSS	Gly	Man	Glu	Fru	Suc	RS	TS	SI
pH	1										
DM	0,48	1									
TSS	0,47	0,88	1								
Gly	0,23	0,18	0,24	1							
Man	0,14	0,22	0,17	0,56	1						
Glu	0,20	0,12	0,11	0,18	0,13	1					
Fru	0,09	0,09	0,08	0,12	-0,03	0,56	1				
Suc	0,26	0,12	0,19	0,34	0,33	-0,21	-0,42	1			
RS	0,17	0,13	0,12	0,22	0,08	0,85	0,91	-0,35	1		
TS	0,45	0,77	0,83	0,25	0,29	0,02	0,00	0,31	0,03	1	
SI	0,35	0,19	0,26	0,44	0,38	0,13	0,01	0,90	0,09	0,34	1

Bold values are significant at $P = 0.05$. pH, potential of hydrogen; DM, dry matter content; TSS, total soluble solids content; Gly, glycerol content; Man, mannose content; Glu, glucose content; Fru, fructose content; Suc, sucrose content; RS, reducing sugars content; TS, total soluble sugars content; SI, sweet index.

4. Discussion

The great difference of assessed physicochemical characters could be originated with the divergence between the four coconut genotypes. Thus, higher contents of total soluble solids and dry matter of the sap collected from MYD could be specificity of the dwarf coconut cultivars. Indeed, a part of the sap may directly result from both hydric and mineral absorption by plant's roots from soil. The second part is synthesized during photosynthesis and water transpiration involved by leaves ([14], [15]). So, the sap would transport and procure the main nutrients to all the plant organs, such as inflorescences [10]. These produce the fruits, nuts, and ensure their development, thanks to the coconut water that may have been resulted from the sap. However from the "dwarf" cultivars, the coconut water of the walnuts is generally richer in dry matter and total soluble solids than those of the tall cultivars and their hybrids [16]. That may have involved highest contents of TSS and DM in the sap of the dwarf coconut trees (MYD). However, the TSS and DM contents of the sap were clearly superior to those provided by the coconut water ([17], [18]). All soluble compounds of the CIS may not be engaged into the coconut water. Significant ratio of the nutrients could contribute to the nuts bearing and development of the other parts of the nuts.

Besides, the dry matter is especially concerning with the nonvolatile macromolecules of the CIS. That could explain the significant positive correlation between DM and TSS. The total soluble solids content of the CIS is close to that recorded from the sap of *Borassus flabellifer* which fluctuated between 11 and 17% [19]. Also, the dry matter content of the coconut cultivars studied doesn't differ from the statement of [20] with the sap produced by *Elaeis guineensis*.

The pH values confirmed the CIS as a nearly neutral raw material. This was previously indicated by [6] on the same palm plant. However, divergence appeared between the coconut cultivars. The PB 113⁺ and PB 121⁺ hybrids and the WAT could have produced saps with more hydronium ions (H₃O⁺) amount than the sap of MYD. These results exceed the pH (4.5) mentioned by [21] from sap of oil palm. Differed palm species would not provide the same pH value to their sap.

The total sugars content of the sap could also depend on the coconut ecotypes. Besides, sugars remained main components of the dry matter; that enhance hard correlation between these two characters. The sap we studied is richer in total soluble sugars than that investigated by [22] from this palm plant. The difference may have involved with not only the ecotypes of coconut cultivars, but also the method used for the analysis and the environmental effect. From these authors, sap was filtered on a Watman paper whereas this step was achieved with a muslin cloth in our study. Watman paper could have deducted sugars residues from the sap, and led to the misestimating of the real sugars content of the CIS. Our results are however comparable to those of [9], stated about 14%.

From CIS, majority of sugars was with the sucrose, a non reducing disaccharide. This statement specifies every palm plants. Thus, sucrose would be the major carbohydrate reserve into the sap conducting organs (xylem and phloem) of the coconut tree. The sucrose contents of the PB 121⁺ and PB 113⁺ hybrids still close to those of MYD and WAT cultivars. On account of this, such character may have been inherited, with co dominance impact, from both Dwarf and Tall coconut genotypes. Our results corroborate the attempts of [6] whose sucrose content of CIS was 11.6 g/100 ml. Otherwise, sucrose is more abundant in the sap than the whole coconut products, such as coconut water and kernel, whatever their development stages [18]. During formation of the water, the sucrose from the sap would not be entirely intaken.

Even contents of fructose and glucose were revealed in the SIC of the four coconut cultivars studied. The contents of both monosaccharides enhance the final reducing sugars content of the SIC, because they seemed the main reducing sugars of CIS. Their presence in the plant may have resulted from two biochemical processes. The first source is concerning with enzymatic hydrolysis of sucrose, during fermentation stages. Indeed, the sap exudate undergoes a spontaneous fermentation by microorganisms occurring from the environment [23], using the sap's sucrose. Briefly, this phenomenon provides glucose and fructose into the sap. Thereafter, they're also metabolized, leading to alcohol and acid compounds. The second origin of reducing sugars would be a physiological synthesis by the plant. Thanks to their photosynthesis system, the coconut tree can perform molecules with higher atoms of carbon (C6) from C3 molecules [24]. This endogenous biological process may have produced other hexose, like mannose, among the reducing sugars of the coconut sap. The reducing sugars are substrate for enzymatic browning and Maillard reactions which could happen during production of coco

syrup and coco sugar from coconut sap [19]. The four coconut cultivars provided saps which still are as richer with reducing sugars as oil palm [20].

The presence of glycerol is processed from glycopyruvic fermentation of the reducing sugars (glucose, fructose and mannose) due to yeasts. Simultaneously, this oxidization could lead to other volatile organic compounds, such as acids, aldehyds, ketones and esters, which contribute to the astringency and aroma of the coconut sap ([25], [3]).

The highest sweet index of the sap produced by the MYD resulted from its substantial sucrose content. The sweet index and sucrose content are hardly closed. Thanks to this value of sweet index, the sap of the Dwarf coconut could be more delicious than that produced from spathes of Tall and hybrids coconuts. However, whatever the coconut cultivar, the sweet index of the sap overcomes the record from the coconut water whose maximum value was 7.5 g/100 ml [26].

Conclusion

These works showed a neutral pH for the sap of the four coconut cultivars studied. The dry matter of CIS contained major glucide components. These macromolecules were more abundant with the MYD cultivar. However, sucrose was main carbohydrate type, and it differentiated MYD to WAT. The PB 121⁺ and PB 113⁺ hybrids had intermediate means from this character. Furthermore, the similar contents of glucose and fructose revealed the same stage of synthesis of these monosaccharides into the sap of the four coconut cultivars. Thus, the sap produced by MYD, PB 121⁺ and PB 113⁺ could provide the same yield of productions of alcohol, syrup and sugar, thanks to their highest sucrose content. Nevertheless, the sap of the MYD could be more delicious for drinking.

Competing interests

All authors declared no competing interest with any information of this manuscript.

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Abbreviations:

CIS: coconut inflorescence sap; MYD: Malayan Yellow Dwarf; PB 121⁺: improved PB 121 hybrid; PB 113⁺, improved PB 113 hybrid; WAT, West African Tall.