

Original Research Article

Evolution of physicochemical parameters during storage of the inflorescence sap deriving from four Ivorian coconut (*Cocos nucifera* L.) varieties.

ABSTRACT

Aim: The study investigated the changes involving with the main physicochemical parameters of the coconut inflorescence sap deriving from MYD, WAT, PB 121⁺ and PB 113⁺ varieties during storage.

Place and duration: Marc Delorme Research Station for coconut, National Center of Agronomic Research, Abidjan, Côte d'Ivoire, between March and May, 2013.

Methodology: The coconut sap was collected from six coconut trees per variety, and then a sample was constituted with the mixture. Three samples of the coconut sap from each variety, resulting with three different days of sap collection, were stored at 35±2 °C. Then 10 parameters were assessed during 120 h (5 days).

Results: The results showed a significant effect of the storage duration upon the whole parameters. Indeed, important decreasing was concerning with the total sugars contents (from 16.08 to 0.93 g/100 mL), pH (7.32 to 4.17) and the relative density (1.055 to 1) of the coconut sap samples, without any differentiation between varieties. Also, the total soluble solids and the dry matter contents dropped respectively from 16.31 to 3.82% and 16.47 to 3.09 g/100 g. On the other hand, significant rising was recorded with the total titrable acidity (from 0.26 to 1.85 g/100 g) and the tannins contents (from 5.65 à 13.2 mg/L). Concerning with the reducing sugars and proteins, partial increasing in their contents led respectively to 6.60 to 8.29 g/100 mL and 2.34 to 2.84 g/100 mL after 24 hours of storage. The total polyphenols contents reached also peaks of 606.84 mg/L to 1316.24 mg/L after 36 h, before decreasing. The WAT differed from the three other varieties with highest contents of total polyphenols and tannins contents during the 120 hours of storage of the sap.

Conclusion: The study evidenced similar evolutions of the physicochemical traits during 5 days of natural fermentation of the sap resulting from various coconut types. Thanks to its highest polyphenol content, the sap deriving from WAT could be more nutritious.

Keywords: Coconut sap - varieties - storage - fermentation - sugars.

27 INTRODUCTION

28 The coconut (*Cocos nucifera* L.) is a tropical crop mainly cultivated for the production of nuts. When they are
 29 immature, the nuts contain a delicious and refreshing drink [1] and ~~at their full maturity stage~~, they are raw
 30 material for oil production for food, cosmetic and pharmaceutical industries. However, ~~the ant's~~ attacks and ~~the~~
 31 environmental constraints lead to decreases of the yield in nuts production. In addition, the severe crash of the
 32 price of coconut oil, resulting from competition with other oil crops such as oil palm and soya, limits
 33 continuously the plant's profitability. For the revival of ~~the~~ sector and to get numerous coconut farmers out of
 34 poverty, other diversified products, like coconut sap, seem to be promising perspectives [2]. The coconut sap
 35 (*Neera*) remains the most important value added product that has sought the attention of all the stakeholders in
 36 coconut [3]. Indeed, the coconut sap records great valorizations in the countries of South-eastern Asia
 37 (Philippines, India, Indonesia, etc.) which have the largest coconut farms in the world. The sap contains several
 38 nutrients that are mostly ~~with~~ carbohydrates (sucrose, glucose and fructose). There are also amino acids as
 39 glutamine, vitamins (C, B) and minerals ~~of which~~ potassium, phosphorus and iron [2; 4; 5]. Following the
 40 example of the sap ~~deriving~~ from other palm species, the coconut sap is often consumed directly by the local
 41 populations and is very appreciated ~~thanks~~ to its nutritious values. However, the most important valorizations of
 42 the coconut sap are ~~concerning~~ with the processing into syrup, sugar and distilled alcohol [6; 7]. The coco syrup
 43 and sugar ~~still~~ are among the foods with low glycemic index, lower than 50 [8]. Thus, these by-products record
 44 so high ~~value-added~~ that the production, processing and trading of the coconut sap seem more profitable than the
 45 empirical sale of the nuts [9].

46 Nevertheless, the shelf life ~~remains~~ a limiting factor for the valorization of the coconut sap. Indeed, the rich
 47 nutritious composition of the sap ~~let~~ it logically to a fast spoilage. The deterioration is caused by the
 48 microorganisms ~~involving with~~ both environmental and hygienic conditions of the sap production [10]. After the
 49 harvest of the sap, the glucides undergo spontaneous degradation leading to several chemicals as alcohols, acids,
 50 aldehyds, ketones and esters [11] characterizing loss of the stability of the product.

51 In Côte d'Ivoire, there isn't any report ~~with~~ the changes of the physicochemical parameters of the coconut sap
 52 after harvesting. Yet, the country owns numerous coconut varieties which differ with the physicochemical traits
 53 of their sap [4]. Considering the role of the palms saps in the diet of the populations, it is important to know
 54 more about the changes ~~occurring~~ during storage. This study investigates the ~~evolution~~ of the physicochemical
 55 parameters of the sap provided by four widespread coconut varieties.

56 MATERIAL AND METHODS

57 1. Plant material

58 The plant material was the sap deriving from inflorescences (spathes) of four coconut varieties; the Malayan
59 Yellow Dwarf (MYD), West African Tall (WAT) and the improved hybrids PB 121 (MYD x improved WAT)
60 and PB 113 (Cameroon Red Dwarf x improved Rennell Island Tall).

61 2. Methods

62 2.1. Sampling

63 From each variety, six adult and healthy coconut palm trees were randomly chosen on experimental coconut
64 plots of the Marc Delorme Research Station of the National Center of Agronomic Research in Côte d'Ivoire.
65 Then, the spathes of rank 8 of the coconut trees have undergone the sap harvesting. Thus, 24 spathes (1 spathe x
66 6 trees x 4 varieties) were investigated during the work. The spathe was severed and its surface was introduced
67 into an empty plastic can through a circular opening allowing the sap gathering. The cans were previously
68 sterilized with hot water boiling at 100 °C. The can and the spathe were covered with a sterile muslin cloth in
69 order to preserve the hygienic quality of the sap. At each harvesting, the sap was immediately spilled into plastic
70 sterile bottles, placed into isotherm cool bag with ice sheets, sent to laboratory and kept at -20 °C until
71 analysis [5]. After each harvest, the continuous oozing of the sap was ensured by removal of 3 to 5
72 mm thickness from the surface of the spathe. The sap was harvested twice a day, at 7 AM and 5 PM
73 [12]. Later, the saps provided by the trees of each coconut variety were mixed and a sample of 1.5 L was
74 considered for analyses. Samples were stored at 35±2 °C [13] in an oven. Then the physicochemical parameters
75 of the sap were assessed after 0, 4, 8, 12, 24, 36, 48, 72, 96 and 120 h of storage. The investigations were
76 repeated during three different days of harvest and the whole study was achieved on 12 samples of coconut sap
77 (1 sample of 1.5 L x 4 varieties x 3 repetitions).

78 2.2. Determination of physical parameters

79 The dry matter content was determined by drying 10 g of sap sample into an oven (Memmert, Berlin, Germany)
80 at 105 °C [14]. The relative density was valued by measuring the mass of 1 mL of sap. Both needed the use of an
81 electronic two digits scale (Mettler BD 202, USA). The total soluble solids content was recorded with a
82 refractometer (DIGIT 032, Brussels, Belgium).

83 2.3. Determination of the chemical and nutritive parameters

84 The potential of hydrogen was valued with use of a pH-meter (HANNA Instruments).
85 The total titrable acidity was measured by titration (15) of the sap samples with solution of sodium hydroxide.
86 The total polyphenols content was determined using the folin ciocalteus method [16] improved by Boizot and

Charpentier [17]. Then the tannins content was deducted from the total polyphenols with the use of vanillin in acid solution [18]. The proteins contents were achieved according to the method described by Lowry *et al.* [19], using also folin ciocalteus reagent. The total soluble sugars contents were assessed with phenol and sulfuric acid method [20]. Reducing sugars contents were determined by using the 3, 5 - dinitrosalicylic acid [21]. All the analyses were achieved in triplicates.

3. Statistical analysis

The sap samples harvested through the three days of investigation were replications for each variety. The statistical analyses were achieved with SPSS software (SPSS 16.0 for windows, SPSS Inc.). They consisted in two factors analysis of variance (ANOVA). The factors were the type of variety and the duration of the coconut sap's storage. Means were classified by the Newmann Keuls test at 5% significance level. Then, correlations between parameters were assessed according to the Pearson index.

RESULTS

1. Assessment of the physicochemical parameters during the storage of the sap

The statistical analysis of the data showed significant effects of the storage duration ($P < .001$) on the 10 characters assessed from the sap provided by the four types of coconut (table 1).

1.1 Physical parameters

a) Dry Matter

Considering the four varieties, the dry matter content decreased statistically ($P < .001$) from 14.64-16.47 g/100 g to 3.09-5.55 g/100 g during 120 h of storage of the coconut saps. Indeed, the early 12 h of storage revealed steady dry matter of the sap provided by the MYD (16.47 to 13.86 g/100 g), WAT (14.86 to 12.82 g/100 g), PB 121⁺ (14.75 to 14.70 g/100 g) and the PB 113⁺ (14.64 to 14.06 g/100 g). However, the means recorded from the WAT were statistically lower than those of MYD, PB 121⁺ and PB 113⁺. After 24 h, the decreasing of the dry matter was significant for each variety until 120 h of storage. It led to 5.53, 3.09, 5.24 and 4.55 g/100 g respectively with the MYD, WAT, PB 121⁺ and PB 113⁺. This stage didn't show any difference between varieties from 24 h to 48 h. But after 48 h of storage, the PB 121⁺ and MYD varieties provided dry matter contents which are statistically superior to WAT and PB 113⁺ ones (figure 1).

b) Relative density

The relative density of the coconut saps lowered during the storage with means varying statistically from 1.055 to 1 ($P < .001$). However, the decreasing became significant after 8 h whereas the means were 1.047 (MYD and WAT) and 1.050 (PB 121⁺ and PB 113⁺). At the beginning (0-8 h) and the end (96-120 h) of storage, the relative

density were stable with values of 1.046 to 1.055 and 1 to 1.013, respectively. Moreover, apart from the values of 0 h and 120 h steps, similar means ($P=0.69$) were provided with the four varieties all along the storage (figure 2).

c) Total soluble solids

The total soluble solids contents remained stable in the first 12 h of storage where the means stayed between 16.31% and 12.82%. In this stage, the sap of WAT recorded less total soluble solids (14.69% to 12.85%) than those of PB 113⁺ (14.65% and 13.77%), PB 121⁺ (14.82% and 14.63%) and MYD (16.31% and 13.87%). From 24 h of storage, the decreasing was significantly faster until 96 h where values reached 4.95%, 7%, 8.27%, and 8.30% (respectively for WAT, PB 113⁺, MYD and PB 121⁺). The last 24 h of storage (96 h-120 h) gave statistically steady means of total soluble solids from each variety. Otherwise, between 24 h and 120 h of storage, MYD and PB 121⁺ provided more soluble solids than WAT and PB 113⁺ (figure 3).

1.2. Chemical and nutritive parameters

a) Sugars contents

Total carbohydrates

The 120 h of storage of the coconut sap revealed a significant decreasing of the total carbohydrates contents ($P<.001$). Still, the decrease remained insignificant during the earlier storage (12 hours). Meanwhile, the total carbohydrates consisted between 16.08 g/100 mL and 12.85 g/100 mL of the sap, considering the four varieties. The significant decreasing started at 24 h whereas the means were 12.11, 12.34, 12.47 and 12.58 g/100 mL with the WAT, MYD, PB 121⁺ and PB 113⁺, respectively. Beyond 24 h, the values declined significantly until 96 h and reached means of 1.26 g/100 mL to 1.82 g/100 mL. Between 96 h and 120 h of storage, the total carbohydrates contents stayed statistical constant from each variety. Moreover, during the 120 h of storage, the carbohydrates contents didn't differ statistically the saps provided by the four coconut varieties (figure 4).

Reducing sugars

The reducing sugars contents showed statistical divergence ($P<.001$) between durations of the storage. Indeed, the means were between 2.41 and 3.73 g/100 mL just after the harvest of the sap. But 4 h later, they lowered to 0.56, 1.81 and 1.87g/100 mL (respectively from the WAT, PB 113⁺ and the MYD) or stayed relatively constant to 2.45 g/100 mL (PB 121⁺). Then they increased until 24 h of storage and reached maximal means of 6.6, 6.94, 7.85 and 8.29 g/100 mL with the MYD, WAT, PB 113⁺ and the PB 121⁺, respectively. Beyond 24 h, the reducing sugars contents dropped regularly and significantly till recording slight means of 0.06 to 0.12 g/100 mL at 120 h (figure 5).

b) Proteins contents

The ~~whole treatments revealed various~~ proteins contents ($P < .001$) ~~fluctuating between 0.14 and 2.84 g/100 mL~~ without any ~~divergence~~ between the varieties. ~~Indeed,~~ the proteins contents were ~~statistically stable~~ until the 8th hour of storage, with means of 0.63 to 0.83 g/100 mL (MYD), 0.48 to 0.59 g/100 mL (WAT), 0.49 to 0.58 g/100 mL (PB 121⁺) and 0.40 to 0.55 g/100 mL (PB 113⁺). From 8 h, the proteins contents increased significantly until 24 h where they reached peak values of 2.34 to 2.84 g/100 mL ~~with~~ the four varieties. Beyond 24 h of storage, the figure 6 shows continuous dropping of the proteins contents until 72 h, before stabilizing ~~at~~ means of 0.29 to 0.57 g/100 mL (MYD), 0.14 to 0.82 g/100 mL (WAT), 0.30 to 0.61 g/100 mL (PB 121⁺) and 0.22 to 0.58 g/100 mL (PB 113⁺).

c) Total titrable acidity

Considering the four varieties, the total titrable acidity increased statistically from 0.26 to 1.85 g/100 g during the 120 h of storage, with relative stability stages. ~~Statistically, the acidity differentiated the four coconut varieties ($P < .001$).~~

With the MYD, the significant increasing of the total titrable acidity was observed between 4 h and 8 h (0.50 to 0.72 g/100 g), 12 h and 24 h (0.84 to 1.17 g/100 mL) and after 72 h (1.53 to 1.85 g/100 g).

~~Concerning with the~~ WAT, the increase of the acidity was significant from the beginning of the storage till 36 h (0.32 to 1.19 g/100 g), then within 48-72 h (1.27 to 1.40 g/100 g).

From the PB 121⁺, the total acidity ~~raised statistically~~ within the early four hours of ~~the~~ storage (0.31 to 0.61 g/100 g) and between 12 h and 120 h (0.77 to 1.65 g/100 g). On the other hand, between 4 h and 12 h, the acidity didn't show any obvious change.

The acidity recorded from the PB 113⁺ varied significantly from 0 h to 24 h (0.31 to 1.04 g/100 g) then between 48 h and 72 h (1.20 to 1.31 g/100 g). But within 24 h and 48 h (1.04-1.20 g/100 g) then from 72 h to 120 h (1.31-1.45 g/100 g) the variations were not statistically ~~important~~ (figure 7).

d) pH

The four types of sap provided the same pH values ~~by steps~~ ($P=0.12$). However, the pH of each coconut sap sample decreased statistically from 7.32 to 4.17 during the 120 h of storage ($P < .001$). From the first 8 h, the pH remained statistically steady, fluctuating between 6.97 and 6.83 (PB 113⁺), 7.10 and 6.67 (PB 121⁺), 7.18 and 6.93 (WAT) and between 7.32 and 6.6 (MYD). Thereafter, it decreased ~~statistically~~ until 96 h of storage where the means became 4.37 to 4.47. ~~Besides,~~ beyond 96 h, the pH didn't change anymore, providing values of 4.17 to 4.2 at 120 h of storage (figure 8).

e) Total polyphenols

Before the storage, the sap deriving from WAT contained more total polyphenols (565.8 mg/L) than that of MYD, PB 121⁺ and PB 113⁺ (254.2, 402.8 and 464.4 mg/L, respectively). During the storage, the WAT provided also highest values while the MYD recoded the lowest. This character differentiated commonly the durations of storage (P<.001). Indeed, from 0 h to 24 h of storage, the total polyphenols content remained statistically constant from each variety. Between 24 h and 36 h, it increased significantly to maximum of 606.84 mg/L (MYD), 1021.37 mg/L (PB 121⁺), 1045.58 mg/L (PB 113⁺) and 1316.24 mg/L (WAT). Then the means stayed highest until 48 h, before dropping to values varying between 292.02 mg/L and 601.14 mg/L at 96 h of storage. Between 96 h and 120 h, the total polyphenols contents didn't show any obvious change from each type of coconut sap (figure 9).

f) Tannins

The change of the tannins contents was specific to each variety (P<.001).

With the MYD, the tannins contents increased significantly from 0 h to 8 h (5.65 to 6.62 mg/L) before stabilizing till 24 h (6.71 mg/L). Later, they rose continuously and reached 9.95 mg/L at 120 h.

Concerning with hybrids, the significant increasing of the tannins contents appeared within the early 8 hours of storage, where the averages varied between 5.99 and 7.25 mg/L (PB 121⁺) and 6.19 and 7.57 mg/L (PB 113⁺). However from 8 h, both hybrids provided steady and similar values fluctuating between 7.25 and 8.38 mg/L.

The WAT recorded significant increasing of the tannins contents between 0 h and 4 h (6.45 to 7.03 mg/L), 8 h and 12 h (7.34 to 8.42 mg/L) and from 24 h to 96 h (9.41 to 12.88 mg/L). Besides, the tall variety provided higher tannins contents than the other varieties during the 120 h of storage of the coconut sap (figure 10).

2. Correlations between parameters

The values of Pearson indexes (*r*) indicate positive and negative significant correlations between the 10 characters assessed. Thus, the pH, the relative density and the contents of total soluble solids, total carbohydrates and dry matter were closely correlated during the 120 h of storage of the sap (*r* varying from 0.84 to 0.98). Also, the reducing sugars and the proteins contents changed similarly (*r*=0.81). The tannins contents were directly correlated with the total titrable acidity (*r*=0.65).

On the other hand, the tannins contents and the total titrable acidity were reversely correlated with the contents of total carbohydrates, dry matter, total soluble solids and the relative density and the pH of the coconut saps, with *r* fluctuating between -0.90 and -0.66 (table 2).

207

208 DISCUSSION

209 The continuous changes of the physicochemical parameters of the coconut sap could probably be attributed to
 210 effects of ~~the ferments~~ microorganisms. Till dates, numerous ~~of~~ authors reported the chemical processes
 211 explaining the fermentation process of the palm saps and the main by-products ~~resulting from~~ [11; 22]. Indeed,
 212 the carbohydrates of the coconut sap ~~revealed~~ a great proportion of sucrose [4]. ~~Prior~~ the sucrose is ~~inverted~~ into
 213 glucose and fructose, which are used later by the microorganisms for their growth ~~thanks to enzymes such as~~
 214 ~~hydrolases~~. However this hydrolysis ~~activity would be slight in the early times of the sap's storage because of the~~
 215 low presence of ferments. But the exponential growth of the ~~germs~~ enhanced and accelerated ~~logically~~ the
 216 consumption of the sugars. Concerning with the palms saps, three stages were specified along the natural

217 fermentation. They consist in an initial lactic, an intermediate alcoholic and a final acetic fermentation [23; 24].
 218 Thus, effects of the micro-organisms from the first stages improve those of the following ones [25]. Our results
 219 corroborate the works of Eze and Ogan [13] who reported a decrease of the total sugars contents from 11.61% to
 220 0.23% after 33 h of fermentation at 36-37 °C ~~of saps produced by oil palm~~. Otherwise, after four days, the stable
 221 total carbohydrates contents could explain the inhibition of the microorganisms' growth, after the sugars become
 222 scarce and the environment hostile by the lower pH.

223 The total carbohydrates contents were correlated to the density, the dry matter and the total soluble solids
 224 contents of the coconut saps. Indeed, sugars are the major macromolecules in the sap of the palms trees [4; 26].
 225 According to Miallet-Serra *et al.* [27], the dry matter of the juices, as the coconut sap, is mainly constituted of
 226 glucides. The consumption of the sap sugars ~~involves~~ a continuous ~~decreasing of the~~ relative density ~~from~~
 227 four varieties. After five days of storage, ~~the majority of sugars are consumed~~ so that the relative density of the
 228 sap ~~becomes~~ close to that of ~~the inner~~ water ~~whose value is~~ 1. The total soluble solids contents remain steady
 229 during the first 24 h of the storage because the macromolecules of the sap would not be significantly metabolized
 230 within this time. Besides, the WAT provided lower values of total soluble solids during the early storage. Among
 231 the four studied coconut varieties, the WAT provided the lowest sucrose content [4]. Thus the total soluble solids
 232 could depend on the sucrose at the beginning of the storage. But later, other molecules of sugars and proteins
 233 would significantly count for their contents.

234 The initial decrease of the reducing sugars contents could result from the use of the residual glucose in the sap at
 235 the time it was harvested. Thereafter, they increase until 24 h of storage, whereas the total sugars contents drop.
 236 According to Olive [28], after hydrolysis of sucrose, the yeasts would prefer the glucose ~~which undergoes~~

~~directly the glycolysis chain reactions.~~ In this case, the increase of the reducing sugars content would be induced by the accumulation of the fructose whose use appears after the rarefying of the sucrose and glucose contents. The results show the maximal reducing sugars contents after 24 h of storage. ~~That is opposite to the works of Xia et al. [23] who indicated the highest values (0.8%) after two to three days of fermentation of the sap produced by the same plant.~~ These authors worked with a fermentation temperature of 25 °C which could have delayed the hydrolysis capacity of the ferments. Afterwards, the dropping of the reducing sugars contents evidences the consumption of all sugars by the microorganisms. A natural occurrence of ~~microbial proteins~~ in the sap would enhance its spontaneous fermentation [29]. However, the increase of the proteins contents reveals a continual and significant synthesis of enzymes during the first 24 h of storage. After 36 h, the substrates become scarcer and there were a repression of the proteins synthesis ~~from the~~ microorganisms [28]. Consequently, the proteins contents decrease during the ~~remainder~~ fermentation process, ~~so that they record~~ positive correlation with the reducing sugars contents. Moreover, Lal et al. [30] reported that the non-alcoholic portion of the palm wine improves the inhibition action of the ethanol against the fructose 1, 6-diphosphatase and the glucose 6-phosphatase which are the key enzymes of the gluconeogenic pathway. Thus, the fermented coconut sap would be hypoglycemic and its intake could contribute to the prevention of the diabetes mellitus. The results showed a significant increase of the titrable acidity at the beginning of the storage. Indeed, the initial lactic fermentation of the coconut sap by the bacteria as *Lactobacillus* produces lactic acid that increases the titrable acidity of the solution. The activity of the lactic bacteria lowers the pH and promotes the growth of other germs as yeasts and moulds, whose optimal pH is between 5 and 6. Those yeasts are responsible of the alcoholic fermentation. Thus, the relative stabilization of the titrable acidity, could characterize stages of alcohol production. An increase of the titrable acidity was obtained until the 5th day of storage. Indeed, the acetic bacteria (genus *Acetobacter*) occur mainly at the last stage of fermentation. Their effects lead to production of acetic acid and increase the total acidity of the coconut sap which becomes sour and could later turn to vinegar [31]. From these results, the total titrable acidity of the coconut sap is constantly higher than the means reported by Eze and Ogan [13] with the oil palm sap. The intense acidification contributes to the development of astringent and bitter tastes characterizing the fermented coconut sap [10]. The raising of the total polyphenols contents results from the degradation of the polyphenols ~~bindings with plant sap's~~ other molecules. According to Xia et al. [23], the polyphenols would be bound with sugars, proteins, cellulose and starch ~~inner the sap~~. Thus, the continuous acid hydrolysis of those bonds during fermentation [32]

increase the total polyphenols contents which reached peaks means at 36 h. However, after 48 h, the ~~decreasing~~
~~of~~ total polyphenols content ~~would derive from their~~ enzymatic oxidization due to activation of the polyphenol
oxidase and phenolase according to the hypothesis of Konan *et al.* [33]. ~~The enzymes could have been~~
~~synthesized by microorganisms.~~ The works of Xia *et al.* [23] didn't show any obvious change of the coconut
sap's polyphenols content after reaching peaks values from 56 h of fermentation at 25 °C. In fact, our
investigations were carried out under 35±2 °C, which contributed to activate the enzyme synthesis by the
ferments. Besides, the results reveal a higher presence of total polyphenols in the sap deriving from WAT during
the storage. It could explain the greater resistance of the tall coconuts trees, than the dwarves and their hybrids,
against diseases. Indeed, the polyphenol compounds are anti-oxidizing agents protecting the biomolecules
against degradation. Moreover, they have anti-ageing function for the organisms [34, 35]. The tannins are part of
polyphenols compounds. The perpetual increasing of their content reveals the continuous degradation of the
polyphenols bindings. According to Onah *et al.* [36], the tannins would have protective effect on the
hemoglobin. So, the coconut sap could strengthen the immune reaction of the organism.

CONCLUSION

The assessment of the physicochemical parameters during the storage of the coconut sap showed a continuous
degradation of the total ~~sugars~~ which ~~are~~ the major macromolecules. Prior, the sugars are hydrolyzed into
reducing sugars, whose content increases partially, and then led to alcohols and acids by the action of ferments
microorganisms. Consequently, the titrable acidity of the coconut saps increases regularly during the storage.
Besides, the proteins content increased during the early storage, thanks to an increasing enzymes synthesis from
the microorganisms. However, the scarcity of the fermentable substrate stops the proteins synthesis. Otherwise,
the coconut saps grows richer with polyphenols compounds during the first two days of storage, with great
values from the WAT. Thus, the consumption of the coconut sap after two days fermentation could strengthen
the immune system. Beyond four days, the sap contains very little nutrients and its higher acidity could even turn
it unpleasant for drinking.

Competing interests:

Authors declare that the whole study results from an original work achieved by themselves and any other interest
does not exist.

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385 **Table 1:** Statistical data of physicochemical parameters of the inflorescence saps deriving from MYD, WAT,
386 PB121⁺ and PB113⁺ coconut varieties during the storage at 35±2 °C

Sources of			PARAMETERS									
variation	df		TCC	RSC	PRC	TSS	TTA	pH	DEN	DMC	TPC	TAC
Varieties	3	SS	2,18	16,62	0,66	77,31	0,43	0,48	0,00	38,21	2692788	89,75
		F-value	0,47	1,42	1,05	26,60	8,98	1,43	0,76	10,12	68,92	299,25
		P-value	0,70	0,24	0,37	<0,001	<0,001	0,24	0,52	<0,001	<0,001	<0,001
Durations	9	SS	3242,88	710,22	65,41	1048,98	21,93	127,84	0,03	1591,48	5581122	180,57
		F-value	234,61	20,25	34,78	120,31	153,20	127,78	39,87	140,52	47,61	200,68
		P-value	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
Varieties	x	SS	37,01	54,32	4,97	27,93	0,34	1,39	0,00	19,20	358718	63,17
		F-value	0,89	0,52	0,88	1,07	0,80	0,46	0,21	0,56	1,02	23,40
Durations		P-value	0,62	0,97	0,64	0,40	0,74	0,99	1,00	0,95	0,45	<0,001
Error	80	SS	122,87	311,74	16,72	77,50	1,27	8,89	0,01	100,67	1041940	8,00
Total	120	SS	14070,83	2617,15	203,60	17255,04	152,43	4061,49	127,72	16011,77	54522893	8140,03

387 SS, sum of squares; F-value, value of the statistical test; P-value, probability value of the statistical test; df,
388 degree of freedom.

389 TCC, total carbohydrates contents; RSC, reducing sugars contents; PRC, proteins contents; TSS, total soluble
390 solids contents; TTA, total titrable acidity; pH, potential of hydrogen; DEN, relative density; DMC, dry matter
391 contents; TPC, total polyphenols contents; TAC, tannins content.

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Table II: Matrix of correlations between physicochemical parameters during storage of the saps deriving from the four coconut varieties studied.

	TCC	RSC	PRC	TSS	TTA	pH	DEN	DMC	TPC	TAC
TCC	1,00									
RSC	0,39	1,00								
PRC	0,26	0,81	1,00							
TSS	0,91	0,41	0,23	1,00						
TTA	-0,86	-0,08	0,07	-0,81	1,00					
pH	0,91	0,14	0,05	0,84	-0,90	1,00				
DEN	0,88	0,24	0,06	0,87	-0,87	0,84	1,00			
DMC	0,95	0,41	0,23	0,98	-0,85	0,88	0,89	1,00		
TPC	-0,08	0,34	0,39	-0,17	0,19	-0,24	-0,15	-0,14	1,00	
TAC	-0,65	-0,12	-0,02	-0,81	0,65	-0,67	-0,66	-0,73	0,33	1,00

The bold values are significant at $P=.05$.

TCC, total carbohydrates contents; RSC, reducing sugars contents; PRC, proteins contents; TSS, total soluble solids contents; TTA, total titrable acidity; pH, potential of hydrogen; DEN, relative density; DMC, dry matter contents; TPC, total polyphenols contents; TAC, tannins content.

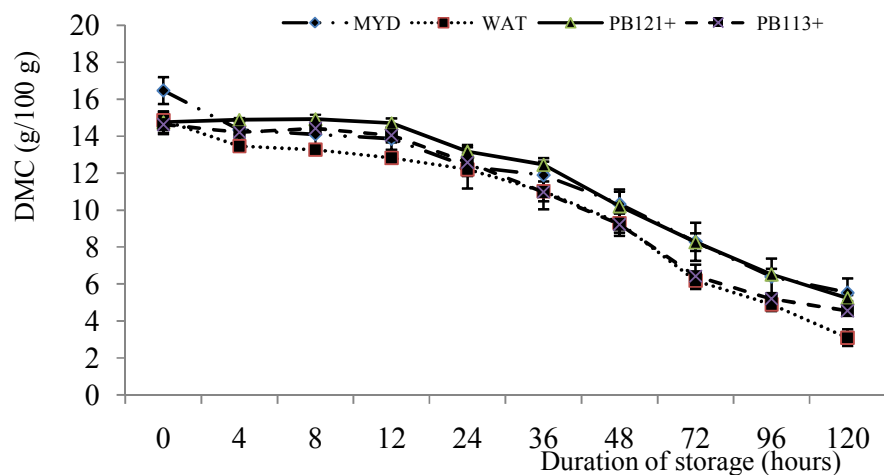


Figure 1: Evolution of the dry matter contents (DMC) of the sap deriving from MYD, WAT, PB 113⁺ and PB 121⁺ coconut varieties stored at 35±2 °C.

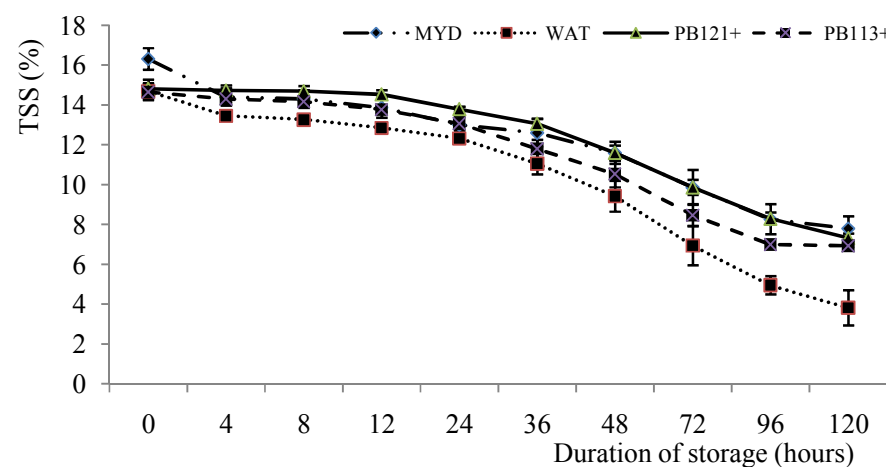


Figure 3: Evolution of the total soluble solids contents (TSS) during storage at 35±2 °C of the sap produced by MYD, WAT, PB 113⁺ and PB 121⁺ coconut varieties.

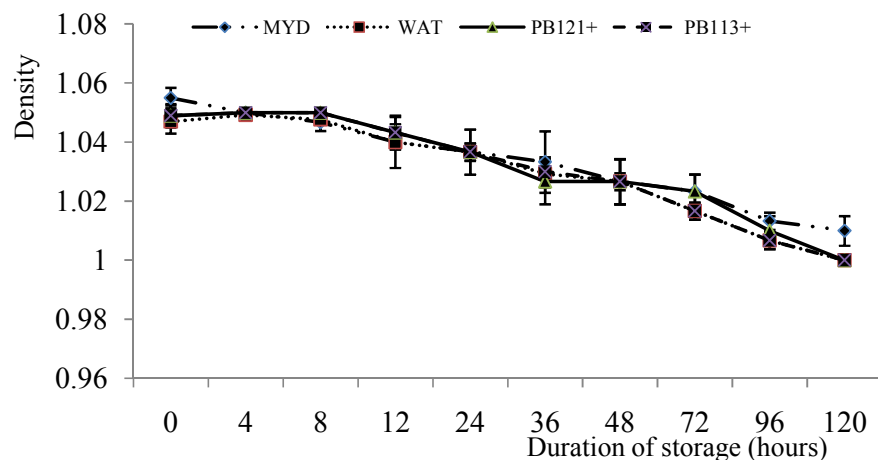


Figure 2: Changes of the relative density during storage at 35±2 °C of the sap resulting with MYD, WAT, PB 113⁺ and PB 121⁺ coconut varieties.

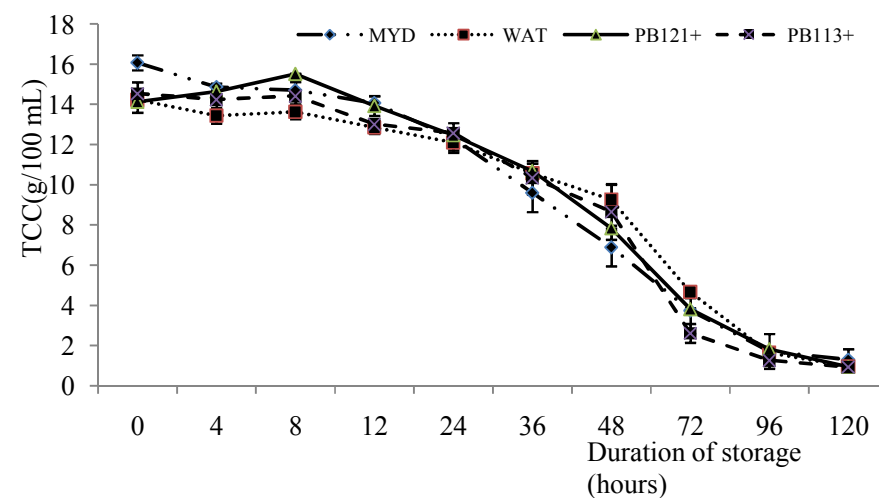


Figure 4: Changes of the total carbohydrates contents (TCC) during storage at 35±2 °C of the sap of MYD, WAT, PB 121⁺ and PB 113⁺ coconut varieties.

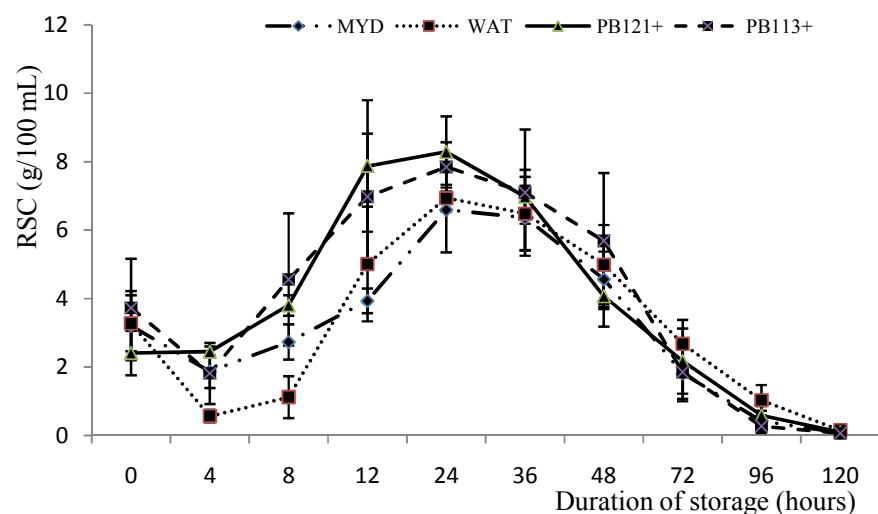


Figure 5: Evolution of the reducing sugars content (RSC) of the sap of the MYD, WAT, PB 121⁺ and PB 113⁺ coconut varieties during the storage at 35±2 °C.

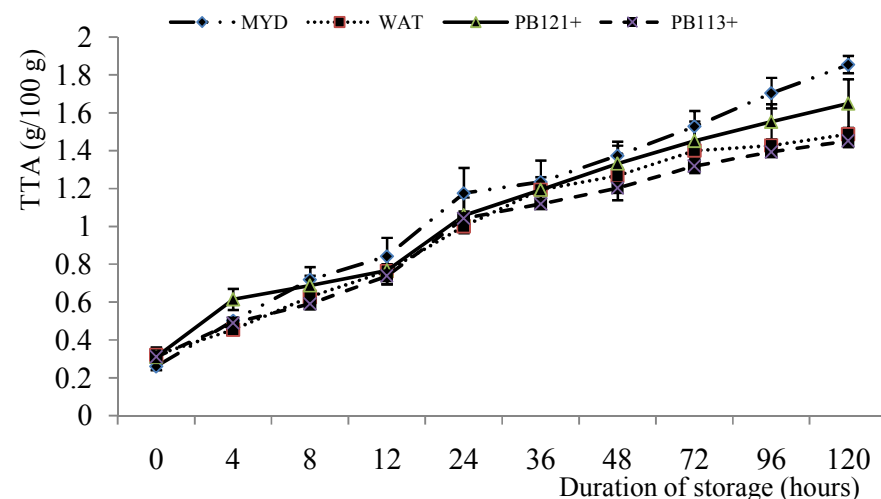


Figure 7: Total titrable acidity of the sap of MYD, WAT, PB 121⁺ and PB 113⁺ coconut varieties according to the duration of the storage at 35±2 °C.

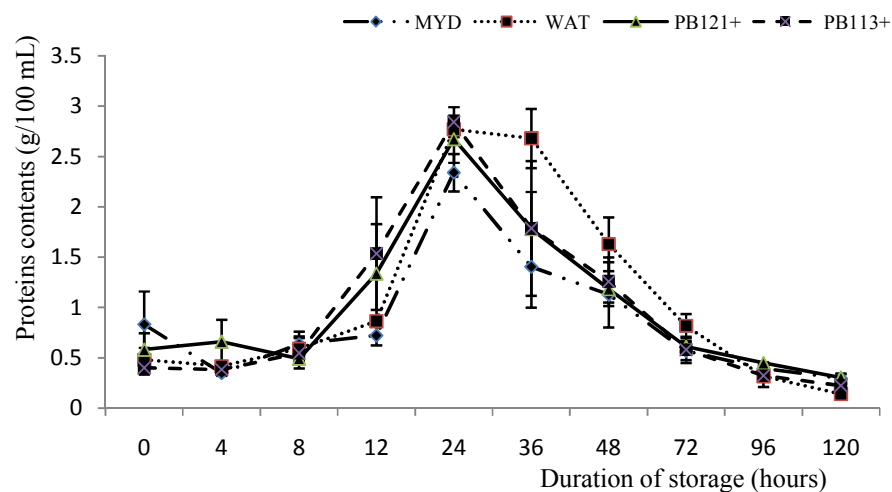


Figure 6: Changes of the proteins contents of the sap of MYD, WAT, PB 121⁺ and PB 113⁺ coconut varieties according to the duration of the storage at 35±2 °C.

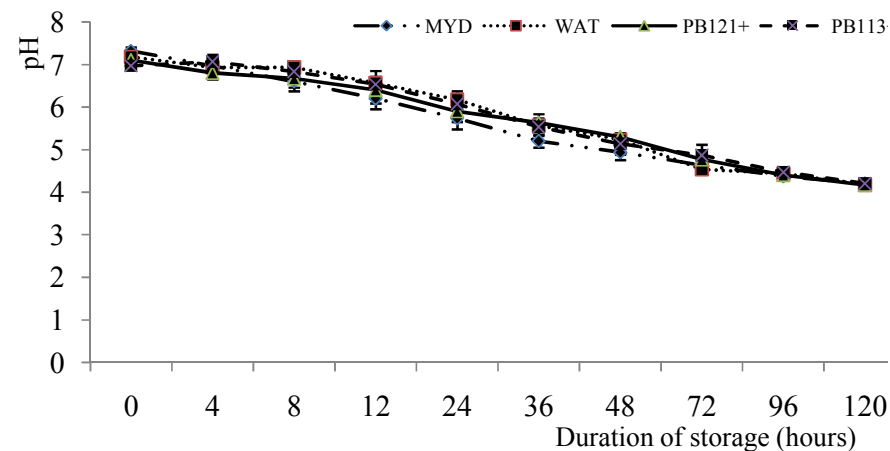


Figure 8: Potential of hydrogen (pH) of the sap deriving from MYD, WAT, PB 121⁺ and PB 113⁺ coconut varieties during the storage at 35±2 °C.

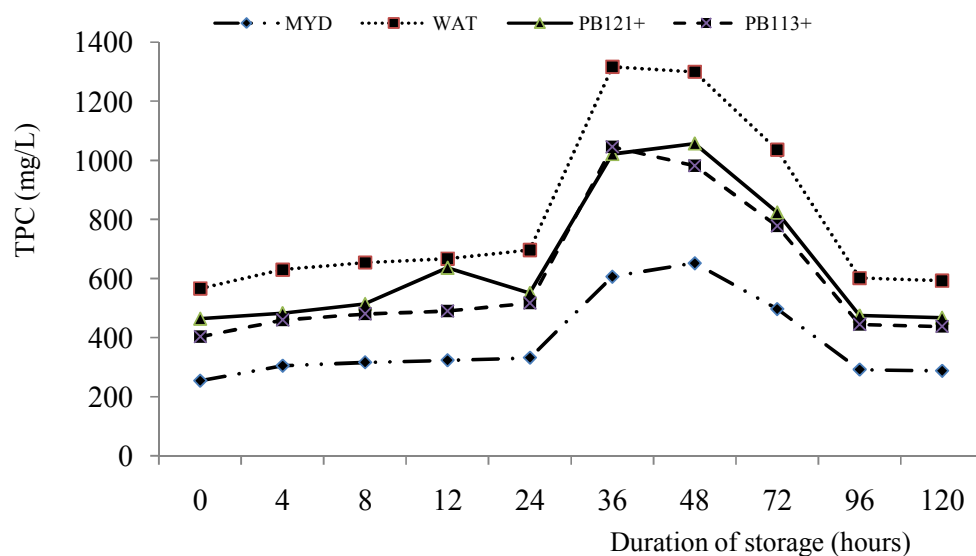


Figure 9: Variation of the total polyphenols contents (TPC) during the storage at 35±2 °C of the sap of MYD, WAT, PB 121⁺ and PB 113⁺ coconut varieties.

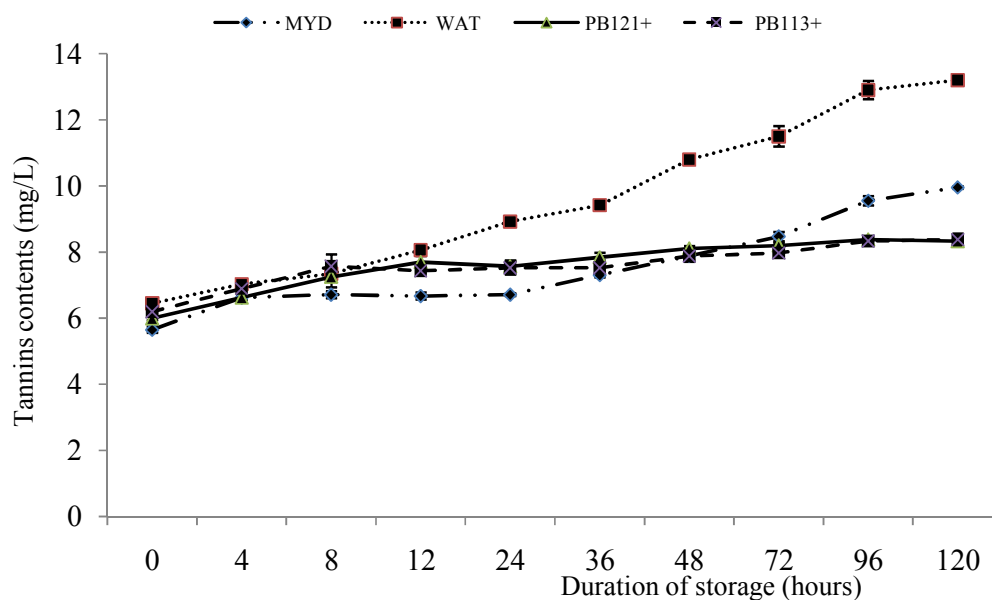


Figure 10: Evolution of the tannins contents of the sap of MYD, WAT, PB 121⁺ and PB 113⁺ coconut varieties according to the duration of the storage at 35±2 °C.