



## **Physicochemical Characteristics of Coconut Water from “*in vitro* Culture” (*Cocos nucifera* L.) According to Fruit Maturation Stage and Storage Period**

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### **Authors' contributions**

*This work was carried out in collaboration between six authors. Authors DZAB and AAB designed the study, collected samples from the field and performed the laboratory tests, and produced a draft of the manuscript. Author KBR checked the data for validity and carried out the analyses of the study. Authors ARR, KLP and KJL further managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** To reduce expenses and losses related to the dissemination of the coconut, PB121 hybrids were developed using “*in vitro* culture” technique. But this type of coconut hybrid planted in 1984 is not yet fully investigated. The aim of this study was to assess the physical and chemical characteristics of water from mature coconuts of this type of PB121 hybrid according to nut maturation stage and storage period.

**Methodology:** The ordinary (*in situ*) PB121 was used as control material.

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**Results:** The results showed a decrease of the coconuts weight (1276.26 g to 876.08 g) and coconuts water weight (163.19 g to 140.66 g) from the 1<sup>st</sup> to 4<sup>th</sup> week of storage regardless of their ripeness stage. Water chemical parameters such as sugar content (27.66 mg/ml to 17.03 mg/ml), acidity (270 meq/100 g to 70 meq/100 g) and dry matter (5.06 % to 3.79 %) also declined during this period. On the other hand, the ash content and pH increased first and then decreased from the second week of storage.

**Conclusion:** The results show an overall similarity between the two types of hybrids. However, water of "in vitro culture" PB121 hybrid is more acidic than that of ordinary PB121. It can therefore be used for drinking or be used to make vinegar.

*Keywords: Coconut; in vitro; maturation; water storage.*

## 1. INTRODUCTION

The coconut palm (*Cocos nucifera* L.) is a tropical perennial plant native to South-east Asia and South Asia [1] which is grown on 12 million hectares through the world. The Ivorian coconut plantation covers 50,000 hectares with an annual production of 55,000 tones of copra [2]. Over 80 % of the plantation is located on the littoral where it is the main cash crop of the populations. In this part of the country, the coconuts are the main product of palm crops [3].

The coconut is a seeded drupe that shows, at ripeness, from outside to inside a smooth and waxy skin, a fibrous mesocarp or husk, a woody endocarp or shell, a white kernel and a liquid called coconut water filling the 3/4 of the central cavity. An embryo is housed in the kernel as the earliest germ pore [4]. Quantitatively, the mature coconut is composed of 35 % of husk, 12 % of shell, 28 % of kernel and 25 % of water [5].

The coconut palm is called "tree of life" for its multiple uses [6]. Among the products of coconut, water from the nut is a delicious and nutritious beverage consumed with its pleasant taste. It contains sugars and mineral elements [7]. This liquid has many health benefits. Indeed, it can be used as a substitute for industry soft drinks that can cause diseases such as diabetes [8]. Its nutritive properties are widely recognized in infantile nutrition [9]. It is used for oral rehydration of children with gastroenteritis. It contains little vegetable fats and helps to detox the body [10]. Coconut water is used to produce vinegar and fermented beverage. During the maturation of coconuts, there are significant changes in the chemical composition of coconut water. Thus, at ripeness, it loses its delicious taste in favor of the kernel [11].

However, coconut production and its shipping are expensive. These high costs are associated with the high weight and the perishable nature of the coconuts. Given these constraints that limit production and shipping of seeds, the road to production in vitro plantlets was explored by research. Coconut palm production in vitro was initiated with PB121. The evaluation of agronomic and morphological characters of PB121 from "in vitro culture" showed no significant differences in behavior with those produced by standard techniques (in situ or ordinary PB121) [12]. However, the physicochemical and technological characteristics of coconuts from "in vitro culture" are still unknown. Moreover, we observe that the coconut samples from coconuts palm grown in vitro decay faster than those of ordinary hybrids during the storage period [12].

This study aims to determine the physicochemical characteristics of water of PB121 from “in vitro culture” compared to those of ordinary PB121 to propose appropriate recovery ways. It will show if the “in vitro culture” technique has an effect on the coconut water.

## **2. MATERIALS AND METHODS**

### **2.1 Plant Material**

The plant material was composed of water coconuts of 12 (rank 24) and 13 (rank 25) months old from PB121 hybrid planted in 1984 at Marc Delorme Research Station of the National Agronomic Research Centre (Abidjan, Côte d'Ivoire). Hybrid PB 121 came from the crossing of the Malayan Yellow Dwarf (MYD) and the West African Tall (WAT). The study focused on the hybrid cultivated in vitro (PB121V) using zygotic embryos of nuts of ordinary PB121 (PB121O) which served as control. The coconuts have been harvested in 2007 on the experimental plot 064.

### **2.2 Methods**

The coconut trees were selected from those which have never fell ill. Six palms have been selected randomly by type of hybrid. Twelve palms were treated for the two hybrids. Three batches of harvesting are carried out successively. Each batch was composed of 4 palms (2 PB121O and 2 PB121V). On each palm, two bunches of ranks 24 and 25 respectively bearing mature coconuts of 12 and 13 months old were harvested. In all, 8 bunches per batch (4 palms x 2 bunches) have been collected.

On each bunch, two coconuts have been sampled randomly for the study of their water physicochemical characteristics. Sixteen coconuts (8x2) were utilized. Within each batch, water of 4 coconuts of the same rank taken on one hybrid is mixed to form one sample for analysis. Four samples were processed by analysis.

The first analysis was designated T0. It was made less than 24 hours after harvest each batch. Thus, per analysis and type of hybrid, 4 nuts of the same bunch were weighed, dehusked and broken to collect water. Water was filtered, weighed and used for biochemical analysis. The other nuts were stored outside on the ground. They were analyzed 1 week (T1), 2 weeks (T2), 3 weeks (T3) and 4 weeks (T4) after storage. Analyses were performed on 3 batches of harvest containing (4x3) 12 samples of water per treatment. Six (6) nuts were collected from rank 24 (13 months old) and 6 others from rank 25 (13 months old). In all, 60 samples of coconut water (5 treatments x 12 samples) from 240 nuts were analyzed.

#### **2.2.1 Determination of physicochemical parameters**

Coconut water and whole nut weight was measured by using a 1/100 precision scale (*Sartorius*). Dry matter content of the coconut water (% MS) was measured in an oven at 104°C for 4 h [13]. The amounts of total and reducing sugars were evaluated respectively with the sulphuric phenol method [14] and with the 3,5-dinitrosalicylic acid (DNS) methods [15] by using a spectrophotometer (*Spertronic Genesis 5*). The non-reducing sugar content was obtained by difference between total sugars and those of reducing. The amount of ash was obtained after total ashing in a furnace muffle at 550°C for 4 h [16].

The pH and titratable acidity were determined according to AFNOR methods [17]. The dry extract refractometric was measured by a manual refractometer. The amount of proteins was obtained by direct reading with a spectrophotometer (*Spertronic Genesis 5*) at 280 Nm [18].

### **2.2.2 Statistical analysis**

The data were subject to an analysis of variance (ANOVA) on three criteria (type of hybrid, ripeness stage and storage period) using the software SPSS 16.0 for windows. Mean and standard deviations were calculated and, when *F*-values were significant at the ( $p = .05$ ) level, the mean difference was separated using the "Newman-Keul's" test. These analyses were carried out to compare the 2 cultivars.

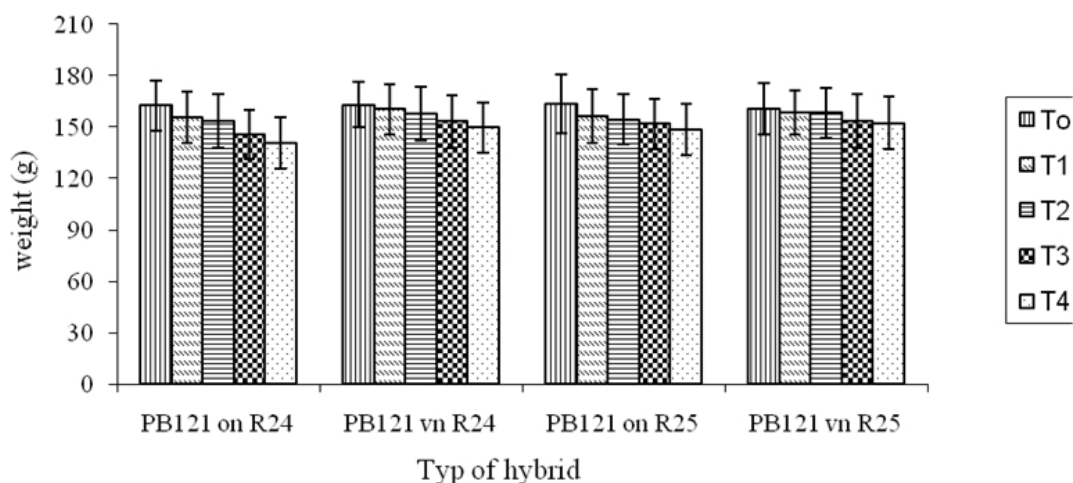
## **3. RESULTS AND DISCUSSION**

### **3.1 Coconut Water Weight**

Water weights of each coconut type (rank 24 and 25) decrease from T0 to T4. At rank 24, coconut water weight decreases from 162.65 g (T0) to 140.66 g (T4) and from 163.19 g (T0) to 149.77 g (T4) respectively for PB121O and PB121V. At rank 25, it decreases from 163.57 g (T0) to 148.8 g (T4) and from 160.82 g (T0) to 152.43 g (T4) respectively for PB121O and PB121V. From T0 to T4, water of coconut taken on rank 24 and 25 of hybrids PB121OR24, PB121OR25, PB121VR24 and PB121VR25 lost respectively 21.99 g, 14.77 g, 13.41 g and 8.38 g of their weight.

Statistical analysis is no indicated a significant difference between storage period and hybrids type (Fig. 1).

The decrease of coconut water weight during storage of studied hybrids could be due to its use for the completion of maturation and germination process. According [9], the constituents of coconut water are necessary for the development of kernel. There remains a small amount at maturity of "physiological liquid" in nuts, essential for germination [11]. This constitutes a reserve of nutrients necessary for the haustorium and seedling development [19]. Thus, in post-harvest process, water of mature nut is involved in the preparation for the germination. The involvement of coconut water and kernel in haustorium development has been also revealed by [12] and [19].



**Fig. 1. Variation of weight of coconut water during storage period and type of hybrid**

PB121on R24: coconut of rank 24 of ordinary hybrid Port Bouët 121.

PB121on R25: coconut of rank 25 ordinary hybrid Port Bouët 121.

PB121vn R24: coconut of rank 24 of hybrid Port Bouët 121 from “in vitro culture”.

PB121vn R25: coconut of rank 25 of hybrid 25 Port Bouët 121 from “in vitro culture”.

### 3.2 Dry Matter Content

The dry matter content decrease up to T4 for PB121O coconut water of rank 24 (from 5.06 % to 4.1 %), PB121V water of rank 24 (from 4.91% to 3.79%), PB121O R25 (from 4.90 % to 4.13 %) and PB121V water of rank 25 (from 5.05 % to 4.29 %). The dry matter content of PB 121O at rank 24 is the highest.

There is no significant difference between the hybrids and the ranks (Table 1).

### 3.3 Protein Content

At T0, protein content was statistically identical for the two hybrids types with a mean of 2.6 mg/ml. The protein contents increase significantly until T4. The values were 4.89; 4.47; 4.27 and 4.00 mg/ml respectively for coconut water of hybrid PB121O R24, hybrid PB121V at rank 24, and coconut water of hybrid PB121O and PB121V at rank 25. At the second storage week T2, protein content of coconut water of rank 25 decreases compared to those of rank 24, before increasing again. The protein content of coconut water of hybrid PB121O increases up to T4, with a maximum for coconut water of hybrid PB121O at rank 24 (Table 1).

The increase of protein content in coconut water would come from the hydrolysis of proteins in the kernel during its formation. Indeed, during the kernel formation, amino acid content in coconut water increases. Meanwhile, there is a decrease of kernel protein content. The amino acids of kernel are involved in lipid synthesis of water during nut maturation [11]. They are involved in the formation of acetyl coenzyme A used primarily for lipid synthesis and secondarily for protein synthesis. This biochemical process would continue during storage and justify the gradual increasing of protein content of coconut water.

### 3.4 Dry Extract Refractometric

Statistical analysis is no indicated a significant difference between hybrids type. There is however, a significant difference between storage periods. Thus, at T0, the dry extract refractometric average is 5 %. From T2, values decrease significantly for hybrid PB121V coconut water from 4.8 % to 4.12 % (T4) while for hybrid PB121O water, the decreasing begins at T1 (4.7%) until T4 (4.1 %) (Table 1).

The dry extract refractometric of coconut water translates the solid compounds contents. The gradual decline may be due to the transformation of soluble compounds of coconut water such as sugar. The transformation of soluble constituents would be earliest in PB121O compared to PB121V which has not yet completed maturation during storing period. The decrease of dry matter content of coconut water could be explained by lower dry extract refractometric and biochemical phenomena related to nuts breathing. Indeed, whatever the storage conditions, respiration or oxidation always takes place. However, its intensity depends on factors such as temperature and humidity of plant material stored [24]. Thus, in the presence of oxygen, free sugars are transformed into water, dioxide carbon and heat. The heat causes temperature rises that would explain the decreases of dry matter and sugar content found in water of stored nuts. The phenomenon is more significant in the ordinary PB121 than that derived from "in vitro culture".

**Table 1. Variation of protein, dry matter and dry extract refractometric content during storage period and type of hybrid**

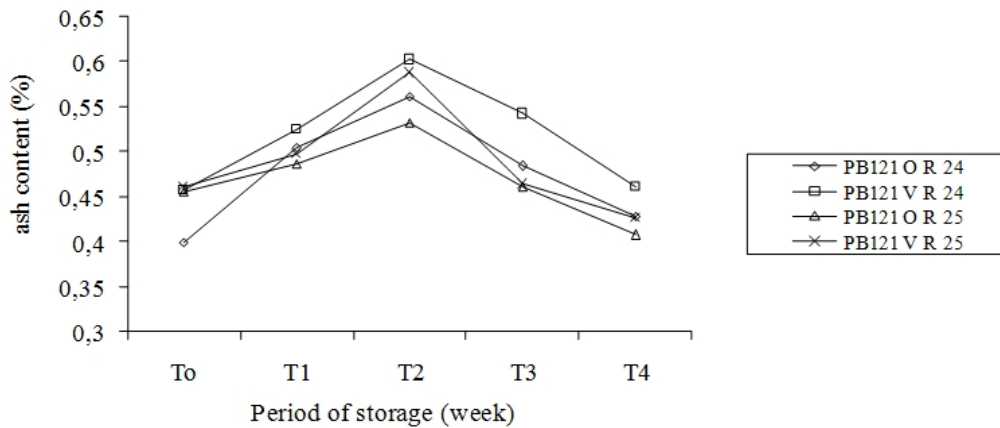
Types of hybrid	Treatment	Protein content (mg/ml)	Dry matter content (%)	Dry extract refractometric (%)
<b>PB121O R24</b>	T0	2.592	5.069	5
	T1	2.863	4.943	4.783
	T2	3.809	4.456	4.5
	T3	4.481	4.173	4.133
	T4	4.893	4.106	4.133
<b>PB121V R24</b>	T0	2.597	4.917	4.988
	T1	3.012	4.882	4.983
	T2	4.065	4.585	4.816
	T3	4.254	4.491	4.366
	T4	4.271	3.792	4.233
<b>PB121O R25</b>	T0	2.512	4.905	5
	T1	3.252	4.679	4.65
	T2	3.611	4.351	4.4
	T3	3.820	4.133	4.133
	T4	4.474	4.034	4
<b>PB121V R25</b>	T0	2.797	5.050	4.966
	T1	3.362	4.841	4.966
	T2	3.645	4.708	4.866
	T3	3.456	4.629	4.5
	T4	4	4.293	4.133
<b>CV</b>		16.6	7.6	5
<b>LSD</b>		0.982	0.573	0.382

*LSD: Little significant difference; CV: coefficient de variation*

### 3.5 Ash Content

The ash content varies significantly according to the types of hybrids and storage periods. At T<sub>0</sub>, the values are 0.40 %, 0.46 %, 0.45 % and 0.46 % respectively for PB121O R24, PB121V R24, PB121O R25 and PB121V R25. They increase significantly until T<sub>2</sub> with a maximum of 0.56 % for water of hybrid PB121V at rank 24. From T<sub>2</sub> to T<sub>4</sub>, the values decrease to 0.40 % for water of hybrid PB121O at rank 25. The water of PB121V at rank 24 is richer in ashes from T<sub>1</sub> to T<sub>4</sub> (Fig. 2).

Increasing of ash content of coconut water would come from the mineral reserves in other parts of the nuts that are husks, shells and kernels. These minerals and waters were absorbed by the plants through their roots in the soil [26]. According [9], potassium is the most abundant mineral in coconut water. It helps to regulate the osmotic pressure in cells. The results of our study show a similarity between PB121O and PB121V for most parameters studied with the exception of the high acidity and stability of sugar content in coconut from “in vitro culture”.



**Fig. 2. Variation of ash content of coconut water during storage period and type of hybrid**

PB121on R24: coconut of rank 24 of ordinary hybrid Port Bouët 121.

PB121on R25: coconut of rank 25 ordinary hybrid Port Bouët 121.

PB121vn R24: coconut of rank 24 of hybrid Port Bouët 121 from “in vitro culture”.

PB121vn R25: coconut of rank 25 of hybrid 25 Port Bouët 121 from “in vitro culture”.

### 3.6 Sugar Content

The total sugar contents are 27.40; 27.52; 27.66, and 27.97 mg/ml respectively for PB121O R24, PB121O R25, PB121V R24 and PB121V R25. From T<sub>0</sub> to T<sub>4</sub>, values decrease significantly until 17.03 mg/ml for water of hybrid PB121O at rank 24 (Fig. 3). In general, waters of coconuts taken on rank 24 are sweeter than those of rank 25. Comparing the two hybrids, water of hybrid PB121V is richer in sugars. The total sugar contents are significantly different according to the types of hybrids and storage periods.

The reducing sugar contents at T0 give 13.38, 12.66, 13.13 and 12.53 mg/ml respectively for water from PB121O R24, PB121V R24, PB121O R25 and PB121V R25. They decrease significantly up to 8.59; 9.17; 8.59 and 8.37 mg/ml, respectively for PB121O R24, PB121O R25, PB121V R24 and PB121V R25 at T4 (Fig. 4). The reducing sugar contents were higher for both the two types of hybrids studied at T0 with maximum values of 13.38 mg/ml at rank 24.

The non reducing sugar contents decrease from T0 to T4 for the 2 types of hybrids PB121. The decrease was greater in PB121O R24 (14.02 mg/ml to 8.76 mg/ml) and PB121O R25 (15.32 mg/ml to 9.43 mg/ml). Coconut water of hybrid PB121V is richer in non-reducing sugars than the control material whatever the rank and storage period. His values also decrease from 15.86 mg/ml (T0) to 1.14 mg/ml (T4) and from 16.43 mg/ml (T0) to 11.23 mg/ml (T4) respectively for coconuts of ranks 24 and 25 (Fig. 5).

Statistical analysis of the non-reducing sugar contents obtained showed a significant difference at the 5 % level between ranks and hybrids.

The sugars of coconut water are also used by kernel during its development for the synthesis of fat. According Assa [11], the sugar content of coconut water is inversely correlated with the increase in fat content of kernel during maturation, hence the decrease in sugar content of coconut water during storage of nuts. The results obtained show that the non-reducing sugars are the majority of sugars from mature coconut water regardless of the type of hybrid. The main sugars of mature coconut water are sucrose, glucose, sorbitol and fructose [25].

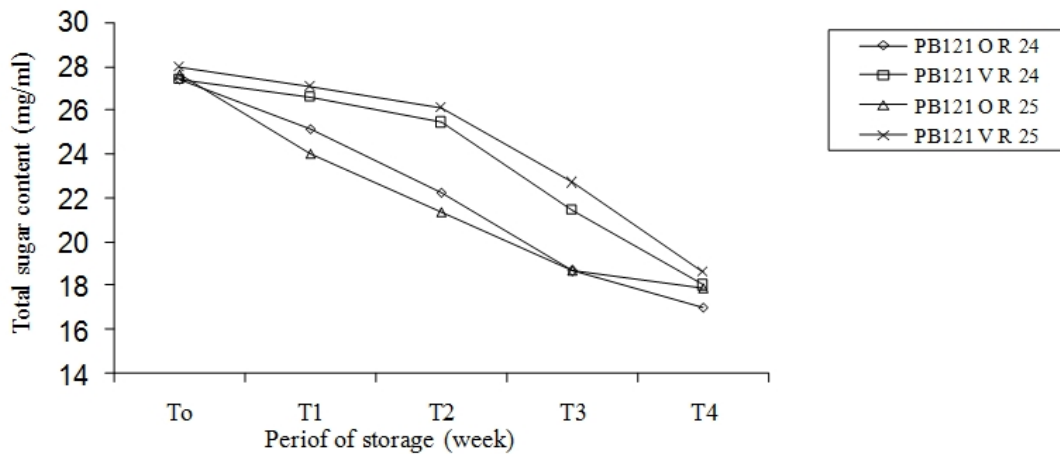
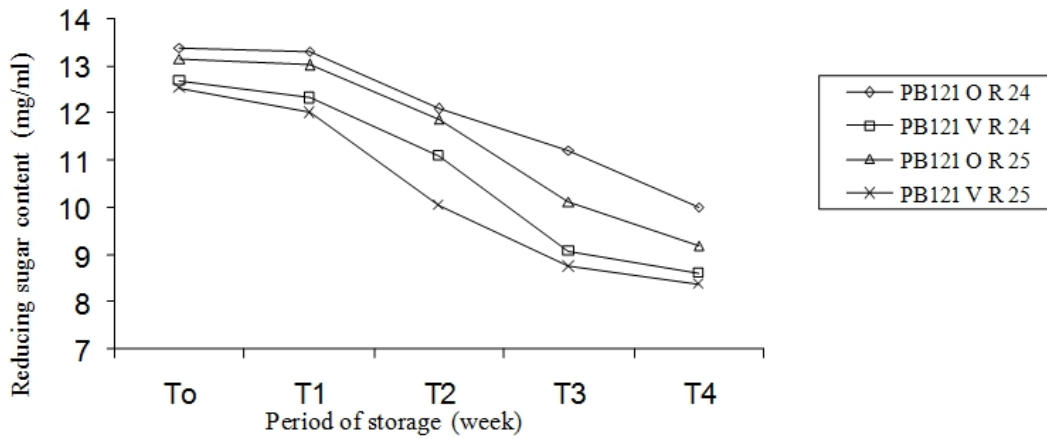


Fig. 3. Variation of total sugar content of coconut water during storage period and type of hybrid





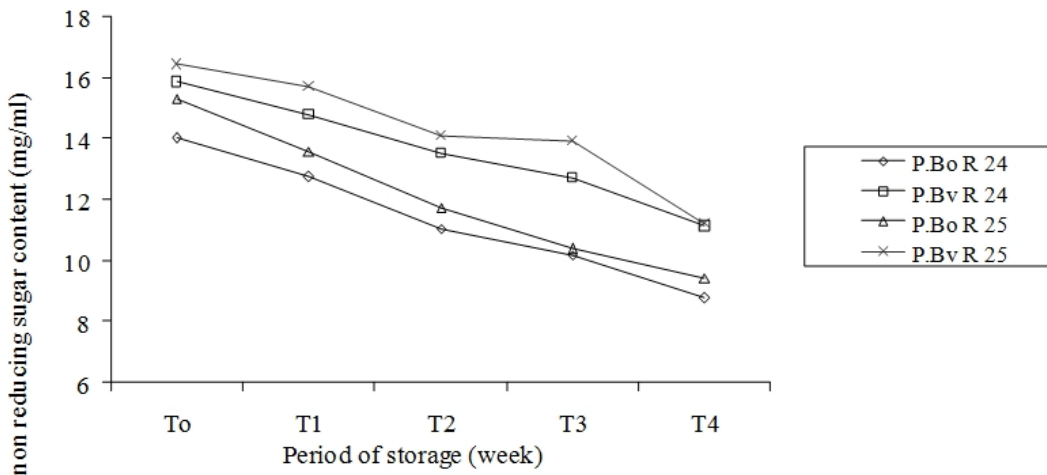
**Fig. 4. Variation of reducing sugar content of coconut water during storage period and type of hybrid**

PB121O R24: water from coconut of rank 24 of ordinary hybrid Port Bouët 121.

PB121O R25: water from coconut of rank 25 of ordinary hybrid Port Bouët 121.

PB121V R24: water from coconut of rank 24 hybrid Port Bouët 121 from "in vitro culture".

PB121V R25: water from coconut of rank 25 of hybrid Port Bouët 121 from "in vitro culture".



**Fig. 5. Variation of non-reducing sugar content of coconut water during storage period and type of hybrid**

### 3.7 pH

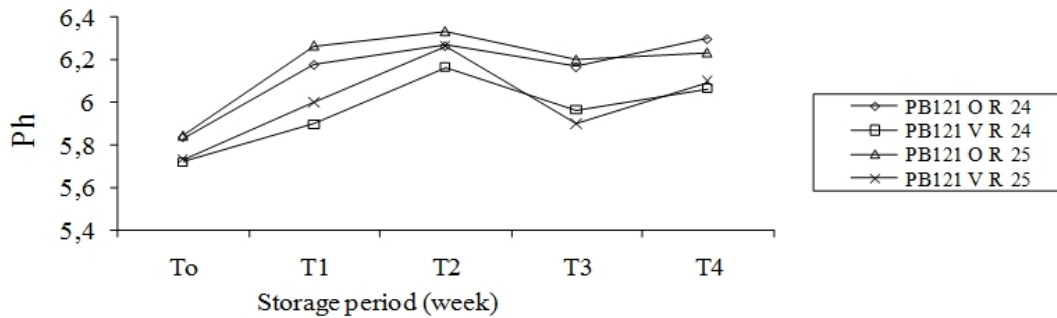
At T0, water pH (5.84) of hybrid PB121O taken on rank 24 and 25 are statistically identical. Concerning hybrid PB121V coconut water, the average pH is 5.7 at T0.

The pH values increased significantly up to 6.33 and 6.26 at T2 respectively for water of PB121O hybrid and hybrid PB121V taken on rank 25. At T3, they decrease to 5.96 for

PB121V R24 and 5.9 for PB121V R25. They increase again until T4 to maxima of 6.3 and 6.23 respectively for PB121O R24 and PB121O R25. Exception of T2, the pH obtained in the PB121O is higher than PB121V (Fig. 6). The variation of pH in coconut water of studied hybrids is different from that observed by [21].

Coconut water acidity is due to organic acids, free amino acids, fatty acid and carbon dioxide dissolved and coming from the tissue respiration [9]. The decrease of acidity suggests reducing of these various components in coconut water. Coconut water from hybrid PB121V would be richer in these compounds because it has proved considerably more acidic than that derived from hybrid PB 121O. Furthermore, immature coconut water is under hydrostatic pressure. This facilitates the dissolution of carbon dioxide and increases the concentration of acidic compounds [20]. This phenomenon justifies the high levels of acidic in nut water of rank 24 which is immature compared with those of rank 25.

This difference could be related to types of coconut, ripeness stages [22] or storage conditions. Indeed, during storage, nuts were exposed to environmental factors as arranged outside on the floor.



**Fig. 6. Variation of pH of coconut water during storage period and type of hybrid**

PB121O R24: water from coconut of rank 24 of ordinary hybrid Port Bouët 121

PB121O R25: water from coconut of rank 25 of ordinary hybrid Port Bouët 121

PB121V R24: water from coconut of rank 24 hybrid Port Bouët 121 from "in vitro culture".

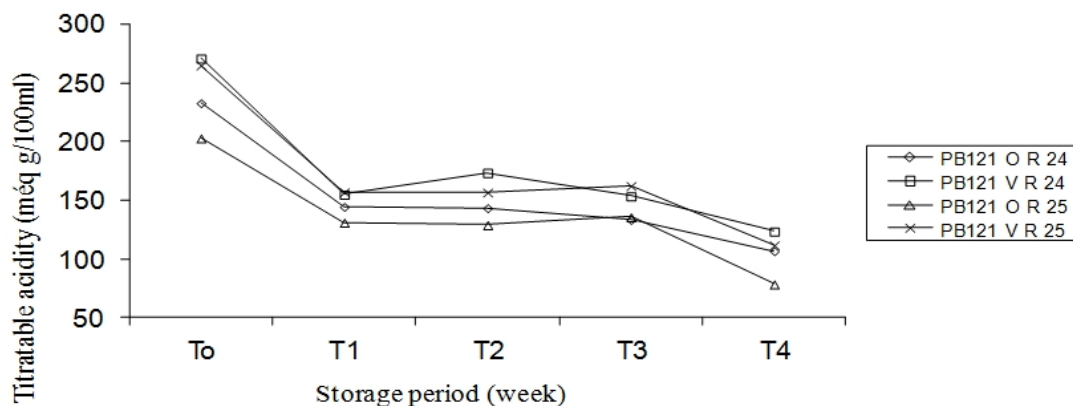
PB121V R25: water from coconut of rank 25 of hybrid Port Bouët 121 from "in vitro culture".

### 3.8 Titratable Acidity

At T0, water of hybrid PB121V coconut taken on rank 24 and 25, and the one of hybrid PB121O coconut are respectively constituted of 270 méq g/100 ml; 265 méq g/100 ml; 232 méq g/100 ml and 202 méq g/100 ml of acidity content. These levels decrease at T4 at the respective values of 120 méq g/100 ml; 110 méq g/100 ml; 100 méq g/100 ml and 70 méq g/100 ml. The decrease of water acidity content has the same shape however the type studied hybrid (Fig. 7).

Water pH and acidity, analyzed are inversely proportional from T0 to T2. This would indicate a low availability of weak acids in water of nuts stored up to 2 weeks. Indeed, the acidity reflects the strongly and lowly acids dissociated. While pH considers only strong acids. Between treatments T2 and T3, pH and acidity values are proportional. This could be related to the presence of many weak acids such as carbon dioxide that would influence the acidity

of nut water. In general, strong acids represent only 1 % of the acids, so they contribute little to the total acidity [23].



**Fig. 7. Variation of titratable acidity as citric acid of the coconut water during storage period and type of hybrid**

PB121O R24: water from coconut of rank 24 of ordinary hybrid Port Bouët 121

PB121O R25: water from coconut of rank 25 of ordinary hybrid Port Bouët 121

PB121V R24: water from coconut of rank 24 hybrid Port Bouët 121 from “in vitro culture”.

PB121V R25: water from coconut of rank 25 of hybrid Port Bouët 121 from “in vitro culture”.

#### 4. CONCLUSION

This study was conducted to determine the physicochemical characteristics of coconut water from PB121 cultivated “in vitro” according to ripeness stage and storage period. Ordinary PB121 produced in situ was used as control material. Nuts harvested at 2 ripeness stages were analyzed during storage period (0 to 4 weeks).

The results show a gradual decline of weight of whole nuts and their water during the 4 weeks of storage regardless of the type of hybrid and stage of maturity. Water chemical parameters such as sugar content, total solids and acidity decline when the pH and ash values increase before declining after the second week of storage.

Coconuts water from “in vitro culture” PB121 was more acidic than that of ordinary PB121. Moreover, the sugar content of ordinary PB121 is less stable. Water from PB121V is more concentrated in minerals and sugars.

The results suggest that nuts from PB121 cultivated “in vitro” have matured later. It would therefore be desirable to harvest at later stages, at rank 25 to prevent rot during storage. Water of PB121 from “in vitro culture” is acidic. It is therefore suitable for the manufacture of vinegar. It could also be advised, due to its high ash content, for the treatment of deficiencies in the body of minerals such as potassium.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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