

Mineral Nutrients in Inflorescence Sap Produced from Various Coconuts (*Cocos nucifera* L.) Cultivated in Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Author ODMJ designed the study and wrote the first draft of the manuscript. Author KNY performed the statistical analysis and filled the submitted and revised manuscripts. Authors KKJL and BGHM expertized the study. Author ARR assisted results interpretations and submissions. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate mineral features correlated to coconut inflorescence sap produced from various varieties for contributing in quite valorization.

Study design: Four coconut varieties studied, namely Malayan Yellow Dwarf (MYD), West African Tall (WAT), and PB121⁺ and PB113⁺ hybrids. Coconut sap produced and sampled per variety. Sap samples processed into ashes and submitted to mineral analysis using energy dispersive spectrometer.

Place and Duration of Study: Marc Delorme Research Station for coconut (National Centre of Agronomic Research) and Laboratory of Biochemistry and Food Sciences (Félix HOUPHOUËT-

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BOIGNY University), Abidjan, Côte d'Ivoire, between 2014 and 2015.

Methodology: Three coconut palms chosen for each variety and coconut sap produced using unopened inflorescence of rank 8. Sap harvested daily at 7 PM and 5 AM, and sampled by homogeneous volume mixture of both daily productions. Three days sampling implemented, leading to 12 coconut samples at overall. Sap samples processed into ash with oven. Minerals in ashes assessed using Energy Dispersive Spectrometer coupled with Scanning Electronic Microscope (EDS-SEM) device. Outcomes valued after analytical validation of the method used. Statistical treatment of Data using SPSS and STATISTICA softwares.

Results: Ash contents varying ($P = .01$) between 0.18% and 0.27% (w/w). Saps of MYD (0.26%) and PB113⁺ (0.27%) were richer in ash compared to WAT and PB121⁺. Thirteen mineral nutrients measured in the coconut sap, 8 macroelements (K, Cl, Si, Na, Mg, P, S and Ca) and 5 oligoelements (Fe, Cu, Mn, Zn and Br). Macroelements recorded contents between 1.25 mg/100 g and 90.65 mg/100 g and oligoelements displayed contents varying from few traces to 0.70 mg/100 g ($P < .05$). Coconut sap produced from MYD correlated to greatest mineral properties, with large presence of K, P, Fe, Si, Na, S, and Mg. PB113⁺ hybrid also revealed highly significant sap minerals. Oppositely, sap of WAT resulted in lower mineral contents.

Conclusion: Regarding the nutritional interests for minerals, the consumption of coconut sap, especially that of MYD and PB113⁺ varieties, could result in successful contribution in food balance for populations and the trading of this foodstuff could provide substantial incomes for coconut farmers.

Keywords: Coconut cultivar; inflorescence sap; minerals; valorization.

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is a tropical perennial crop originating from coastal regions of Indian and Pacific Oceans [1]. This culture is widely planted over the world, with total acreage beyond 12 million ha [2]. In Côte d'Ivoire, the coconut culture is implemented on 50,000 ha of lands mainly located on the coastline where it remains one of the main livelihoods for numerous local farmers [3,4]. The Ivorian coconut culture provides about 55,000 tons of copra each year, which is the first copra yield for African countries. Copra is usually processed into oil and is yet the main coconut valorization in Côte d'Ivoire. However, the competition resulting from other plants oils, with often more nutritional advantages, does not result in suitable prices for the copra. Thus, despite the great research efforts for increasing the production yield [5], the coconut culture still provides lower incomes to farmers. Yet, the coconut is a "plant of life" all parts (from roots to leaves) of which are useful and can account particular valorization. Thanks to this interests, and facing the concerns in the trading of copra, the major coconut countries such as Philippines and India, have successfully experimented the coco sap production using the young unopened inflorescences [6]. The coconut inflorescence sap is consumed as drink (*toddy*) and is also processed into coco syrup and coco sugar. These foodstuffs are still undergoing great

promotion for resulting in significant incomes for the coconut industry [7,8].

In Côte d'Ivoire, excepting the walnut, few attempts aim the valorization of other coconut parts. The rare works achieved for the coconut sap assessed the yield and the biochemical parameters, especially macronutrients. The investigations resulted in optimal coconut sap production using the inflorescence at stage of two months before opening. Moreover, the coconut sap is with great carbohydrates content, especially sucrose [9] and can be highly more profitable than the walnuts [10].

However, mineral elements are not investigated in the sap originating from various coconut varieties, although some values are reported and reveal predominance of potassium [11]. Yet, mineral nutrients have importance in numerous metabolic functions [12,13]. Indeed, zinc has fundamental role for the brain [14], whereas the iodine deficiency results in diseases as goiter [15]. Many mineral elements, as calcium, iron, magnesium, etc. have physiological actions in muscular contraction, blood oxygenation and coagulation, nervous impulses conduction, blood acid-base balance ensuring, and appropriate immune and heart functions. Some minerals, such as calcium, support the building of bone, teeth or muscles tissues, while others are main components or activators of enzymes and hormone molecules. Mineral elements represent

4% human body weight [16] and are provided by the food. This survey aims to assess comparative mineral elements in the sap of some of the most widespread coconut varieties for resulting in best uses and diversifying valorization of this crop.

2. MATERIALS AND METHODS

2.1 Plant Material

The coconut sap was produced using unopened inflorescences (or spathes) of the four more widespread coconut varieties from Côte d'Ivoire, namely Malayan Yellow Dwarf (MYD), West African Tall (WAT), and improved hybrids PB121⁺ (crossing MYD x improved WAT) and PB113⁺ (crossing Cameroon Red Dwarf x improved Rennel Island Tall) planted on experimental parcels located at the Marc Delorme Research Station for Coconut of the National Centre of Agronomic Research. This research site is representative of the general climatic conditions in the Abidjan region, with 151.62±143.06 mm rain/year. The rainfall is lower (< 100 mm) between December and March and from August to September. But it's more important during the other months, reaching peak of 443 mm in June. The site also reveals stable hygrometry and temperature around 88%/year and 26°C/year, respectively, with 188.97±46.69 hours of sunstroke per year.

2.2 Methods

2.2.1 Sampling

The survey has been worked between 2014 and 2015 from 3 healthy and adult palms (more than 9 years of age) per coconut variety. From the leaves crown upon each palm tree, the spathe of rank 8 was considered for the sap extraction as described by Konan et al. [17]. Thus, the spathe was tied, trained to drooping position, bled, and then put into a plastic can washed beforehand into water boiling at 100°C. The can was covered with a muslin cloth treated with hypochloride acid for maintaining the sap quality during the full exudation. The sap was collected twice a day, at 7 AM and 5 PM, in sterilized sampling pots, and kept at -20°C before analyses. The sampling consisted of homogeneous mixing of the sap produced each day from the 3 coconut palms per variety. Three days sampling was achieved, leading to 12 sap samples (1 sample * 4 varieties * 3 days) investigated at overall.

2.2.2 Coconut sap samples mineralizing

The sap samples were mineralized using incinerating method [18]. Ten (10) g of coconut sap were put into a muffle oven (Nabertherm, Germany) at 550°C for 8 hours incineration, allowing full destruction of the organic matter. The ashes were then cooled into desiccator, weighed on a three digits scale, and the resulted ash contents were determined using the following formula:

$$\text{Ash Content (g/100 g)} = W_1 * 100 / W_0$$

With: W_0 , weight of the raw sap sample (10 g); W_1 , weight of the ash

2.2.3 Minerals determination

2.2.3.1 Operating conditions for determining minerals from the coconut sap

The mineral analysis was performed from the ashes through an Energy Dispersive Spectrometer device, coupled with a Scanning Electronic Microscope (SEM-FEG Supra 40 VP Zeiss 2008, Zurich, Switzerland) operating at variable pressure. The apparatus was equipped with an X-ray detector (Oxford instruments) bound to a flat shape of the EDS microanalyser (Inca cool dry, without liquid nitrogen). The operative conditions of the EDS-SEM device were:

- **Zoom:** 10x to 1000000x; • **Resolution:** 2 nm; • **Variable voltage:** 0.1 KeV à 30 KeV.

Ten (10) mg ashes were spread on contact surfaces prepared with adhesive carbon for mineral analysis. The mineral elements were acquired with following parameters: zoom, 50 x; Probe diameter, 30 nm to 120 nm; probe energy, 20 KeV and 25 KeV; work distance (WD), 8.5 mm. The chemical composition was investigated in triplicate.

2.2.3.2 Validation of the measuring method for mineral elements

For validating the mineral analysis by EDS-SEM device, the linearity, repeatability, reproducibility, yields of extraction, and limits of detection and quantification were valued according to standard processes [19]. For each mineral, the linearity of the measuring was valued with 5 standard contents points between 25% and 125%. Then, an external standard mineral solution at 0.5

mg/100 g was prepared for respective 20 and 10 essays of repeatability and reproducibility. Also, 5% mineral content was added to standard samples for determining the yield of mineral extraction in 10 essays.

2.2.4 Statistical analysis

The validation parameters were assessed using the coefficient of determination of the standard lines (linearity) and the relative standard deviations (repeatability and reproducibility); whereas the percentage of mineral measured from the 25 µg added allowed estimation of the mineral extraction yield. From sap minerals, data were statistically treated with Statistical Program for Social Sciences (SPSS 22.0 for Windows) and Statistica (Statistica 7.1) softwares. The statistical analyses consisted in a one-way analysis of variance (ANOVA) at 5% significance with the coconut variety as source of variation. Thus, minerals means were compared using Student Newman Keuls post-hoc test. In addition, Multivariate Statistical Analysis (MSA) was performed through Principal Components Analysis (PCA) and Hierarchical Ascending Clustering for correlating sap minerals to coconut varieties.

3. RESULTS

3.1 Ash Content of the Coconuts Saps

The ashes represent 0.18 ± 0.02 to 0.27 ± 0.03 g/100 g sap. The samples from MYD and PB113⁺ provide respective mean ash contents of 0.26 and 0.27 g/100 g, which are significantly higher ($P = .01$) than the data from WAT (0.18 g/100 g). With an average of 0.23 g/100 g, the

PB121⁺ hybrid displays ash content intermediate to MYD and WAT (Fig. 1).

3.2 Validation Parameters for Minerals Measuring with EDS-SEM Device

The main validation data from mineral analysis essays are reported in Table 1. For the linearity, the coefficients of determination (R^2) recovered from the standard lines are included between 0.987 and 0.997. The relative standard deviations (RSD) from the 20 repeatability tests fluctuate between 1.08% and 1.96%, whereas the 10 reproducibility essays lead to RSD values from 2.34% to 4.92%. The minerals are detected over values of 0.011 mg/100 g to 0.061 mg/100 g and their minimal quantifiable amounts are between 0.014 mg/100 g and 0.080 mg/100 g. Regarding minerals added, the mean yields of extraction oscillate between 92.89% and 97.35%, showing 2.65% to 7.11% extraction defaults for the minerals assessed (Table 1).

3.3 Mineral Composition of the Coconuts Saps

From the ashes, the coconuts saps record 13 mineral nutrients which 8 macroelements and 5 oligoelements, in various contents ($P < .05$).

3.3.1 Mineral macroelements of the coconut sap

Macro mineral elements are constituted of potassium (K), chlorine (Cl), sodium (Na), magnesium (Mg), phosphorous (P), silicon (Si), sulfur (S), and calcium (Ca). They are found in various contents ($P < .05$) ranged from 1.25 ± 0.33 to 90.65 ± 11.20 mg/100 g in the coconuts saps studied (Table 2).

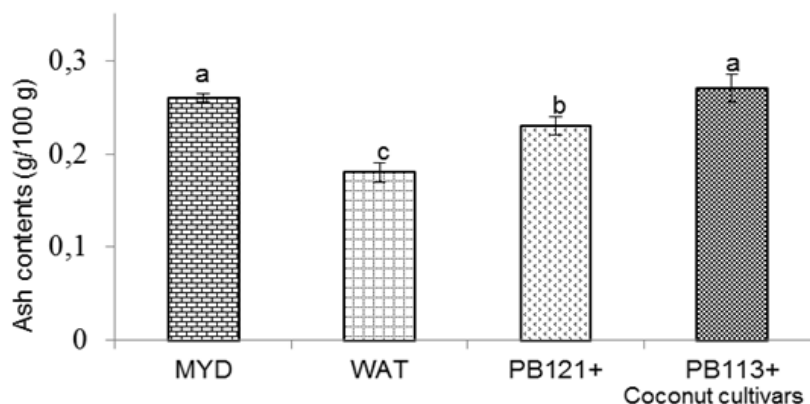


Fig. 1. Ash contents of the inflorescence sap produced from four coconut varieties

With respective means of 67.01 to 90.65 mg/100 g and 43.54 to 62.85 mg/100 g, K and Cl are the major coconut sap minerals. Both minerals are more concentrated from the PB113⁺ (90.65 mg K and 62.5 mg Cl per 100 g sap) and MYD (87.82 mg K and 56.39 mg Cl per 100 g sap). The Na, Mg, Si, P, S, and Ca result in contents of 1.25 to 15.14 mg/100 g sap. MYD records the greatest Na and Si contents with respective means of 9.35 and 15.14 mg/100 g, whereas Ca is more provided by PB121⁺ (5.45 mg/100 g). Regarding Mg, P, and S, the PB121⁺ hybrid and its female parent MYD show respective means of 4.87, 7.64 and 7.16 g mg/100 and 4.95, 6.99 and 6.60 g mg/100, which are over values resulting from WAT and PB113⁺ hybrid (Table 2).

3.3.2 Mineral oligoelements of the coconut sap

The oligoelements are constituted of iron (Fe), copper (Cu), zinc (Zn), manganese (Mn.) and bromine (Br). Their significant contents vary between 0.11±0.05 and 0.70±0.15 mg/100 g sap. However, excepted from Fe, mean contents of which oscillate from 0.12 to 0.39 mg/100 g, any other oligoelement is not significantly and simultaneously measured in the coconut saps, as revealed by traces below LOQ values of Cu, Zn, Mn, and Br from several samples shown in Table 3. Otherwise, when significantly measured, the oligoelements contents do not differentiate the saps provided by the four coconut varieties ($P > .05$).

Table 1. Validation parameters for the evaluation of minerals contents using energy dispersive spectrometer (EDS)

Minerals	Linearity		RSDRep (%)	RSDRepr (%)	LOD (mg/100 g)	LOQ (mg/100 g)	YExt (%)
	ESL	CD (R^2)					
K	Y = 382.1x	0.997	1.38	4.79	0.061	0.080	93.52
Cl	Y = 413.1x	0.987	1.96	4.92	0.060	0.075	92.89
Mg	Y = 145.2x	0.994	1.21	3.21	0.045	0.063	95.32
P	Y = 266.7x	0.998	1.49	3.47	0.040	0.047	96.85
S	Y = 59.58x	0.991	1.08	2.38	0.046	0.056	97.35
Ca	Y = 738.1x	0.99	1.55	2.34	0.051	0.071	94.88
Na	Y = 20.83x	0.993	1.32	3.48	0.035	0.042	97.01
Si	Y = 1,233.5x	0.989	1.36	4.52	0.054	0.065	96.32
Fe	Y = 2.285x	0.992	1.64	3.62	0.011	0.016	93.98
Cu	Y = 185.3x	0.991	1.38	2.5	0.011	0.014	95
Mn	Y = 36.49x	0.998	1.22	2.92	0.021	0.036	93.55
Br	Y = 1,305x	0.994	1.45	2.82	0.024	0.037	94.08
Zn	Y = 33.68x	0.997	1.36	3.32	0.030	0.041	94.82

ESL, equation of standard lines; CD, coefficient of determination; RSDRep, relative standard deviation for repeatability; RSDRepr, relative standard deviation for reproducibility; LOD, limit of detection; LOQ, limit of quantification; YExt, mean yields of extraction

Table 2. Minerals macroelements contents (mg/100 g) in the coconut inflorescence sap produced from MYD, WAT, PB121⁺, and PB113⁺ varieties

Minerals	PB113 ⁺	PB 121 ⁺	WAT	MYD	F-value	P-value
K	90.65±11.20 ^a	76.99±8.70 ^{ab}	67.01±6.58 ^b	87.82±1.52 ^a	5.69	.02
Cl	62.85±7.78 ^a	43.54±3.88 ^b	46.19±5.64 ^b	56.39±0.62 ^a	8.98	.01
Si	8.46±1.10 ^b	10.75±2.36 ^b	9.84±1.44 ^b	15.14±2.25 ^a	7.18	.01
Na	4.29±0.43 ^c	6.84±0.95 ^b	3.17±0.32 ^c	9.35±0.49 ^a	63.91	<.001
Mg	2.84±0.36 ^b	4.87±0.87 ^a	2.41±0.17 ^b	4.95±0.08 ^a	23.19	<.001
P	7.14±0.93 ^a	7.64±1.11 ^a	4.15±0.61 ^b	6.99±0.85 ^a	9.38	.01
S	4.63±0.54 ^b	7.16±1.00 ^a	3.03±0.38 ^c	6.60±0.49 ^a	25.43	<.001
Ca	1.25±0.33 ^c	5.45±0.74 ^a	1.66±0.15 ^c	3.18±0.59 ^b	42.53	<.001

Per line, values (\pm Standard deviation) with the same lowercase scripts are statistically identical at 5% significance; F-value, value of the statistical Fisher Test of the ANOVA; P-value, value of the probability test of the ANOVA; Na, sodium; Mg, magnesium; Si, silicone; P, phosphorous; S, sulfur; K, potassium; Cl, chlorine; Ca, calcium

Table 3. Minerals oligoelements contents (mg/100 g) in the coconut inflorescence sap produced from MYD, WAT, PB121⁺, and PB113⁺ varieties

Minerals	PB113 ⁺	PB 121 ⁺	WAT	MYD	F _{-value}	P _{-value}
Fe	0.33±0.06 ^a	0.39±0.07 ^a	0.12±0.07 ^a	0.32±0.19 ^a	3.57	.07
Br	traces	0.70±0.15 ^a	0.58±0.05 ^a	< 0.37	63.6	.05
Cu	0.47±0.40 ^a	0.33±0.14 ^a	< 0.014	0.51±0.28 ^a	2.46	.14
Mn	0.13±0.1 ^a	0.15±0.11 ^a	0.27±0.13 ^a	< 0.036	3.4	.07
Zn	0.11±0.05	< 0.041	< 0.041	< 0.041		

Per line, values (\pm Standard deviation) with the same lowercase scripts are statistically identical at 5% significance; F_{-value}, value of the statistical Fisher Test of the ANOVA; P_{-value}, value of the probability test of the ANOVA; Fe, iron; Cu, copper; Zn, zinc; Mn, manganese; Br, bromine.

3.3 Multivariate Parameters

The Principal Components Analysis (PCA) is performed with the F1 and F2 components supporting 86.71% total variance. The F1 factor displays 7.35 of eigenvalue and expresses 55.42% variance and F2 records 4.07 of eigenvalue with 31.30% variance (Table 4).

Fig. 2A shows the gatherings of the studied sap samples and mineral traits. Thus, the MYD is correlated with major contents in K, P, Na, S, Si, Mg and Fe, while the PB113⁺ hybrid is more provided with Cl and Zn. Few mineral nutrients are with sap samples from PB121⁺ and WAT although both varieties are more provided in Ca and Br, and Mn and Cu, respectively. Such correlations are strengthened by the dendrogramme drawn using the unweighing pair group method with arithmetic means (Fig. 2B).

Table 4. Eigen values and variance supported by the factors (F) resulting from the principal component analysis

Components	F1	F2	F3
Eigen values	7.20	4.07	1.73
Variance (%)	55.42	31.30	13.29
Cumulated variance (%)	55.42	86.71	100.00

4. DISCUSSION

Processed from incineration of the biological raw matter, the ashes gather overall mineral components. They are nutrients absorbed by the plant roots with water from soil and sent to organs through the raw sap [20] according to their need. From the outcomes, these nutrients were more in inflorescence of MYD than WAT and the progeny PB121⁺ (or MYD x WAT). In fact, the Dwarf coconuts are generally less strong than Tall and Dwarf x Tall hybrids coconuts: they show lower development, and could require lower nutrients for maintaining their organs. The hypothesis is also supported by the higher macronutrients traits (carbohydrates,

proteins, fat) in the coconut sap resulting from dwarf varieties [9].

The R² values found from the calibrations essays were close to 1, forecasting the quasi-linear estimation of the mineral nutrients accordingly to their presence in the coconut sap. Also, the lower relative standard deviations (<5%) resulting from reproducibility and repeatability tests state on quite stability of the EDS-SEM method used. This assertion is evidenced by the recovery of the full minerals amounts as shown by the weak extraction defaults below 7.2% added minerals. Such characteristics are indications for quite reliability and precision of the data recorded from the mineral measures using the EDS-SEM method.

Thus, the study revealed a great presence of mineral macroelements, the main compounds of which are potassium and chlorine. Previous attempts reported the high potassium level from several coconut parts [21,22]. Our results also show potassium and magnesium contents over respective sodium and calcium contents. These statements reveal a heterogeneous mineral absorption by plants from the soil. Besides, the minerals are with diverse distribution from coconuts, especially from various plantations lands. For example, Debmalya and Mazundar [23] found potassium below sodium in coconut sap from the Indian coasts. The nutritional interests of potassium, sodium, magnesium and calcium are widely reported by authors. Indeed, the sodium-potassium (Na⁺/K⁺) and magnesium-calcium (Mg²⁺/Ca²⁺) pumps have active roles for carrying biomolecules as glucose, urea, fatty acids, etc., throughout the cytoplasmic membrane of cells. These active transports are achieved with the help of enzyme systems as energy-dependent ATP-ases. Mg²⁺ ions are also used as enzyme cofactors for the overall glycolysis reactions. These macroelements are therefore advantages for the coconut sap's food uses.

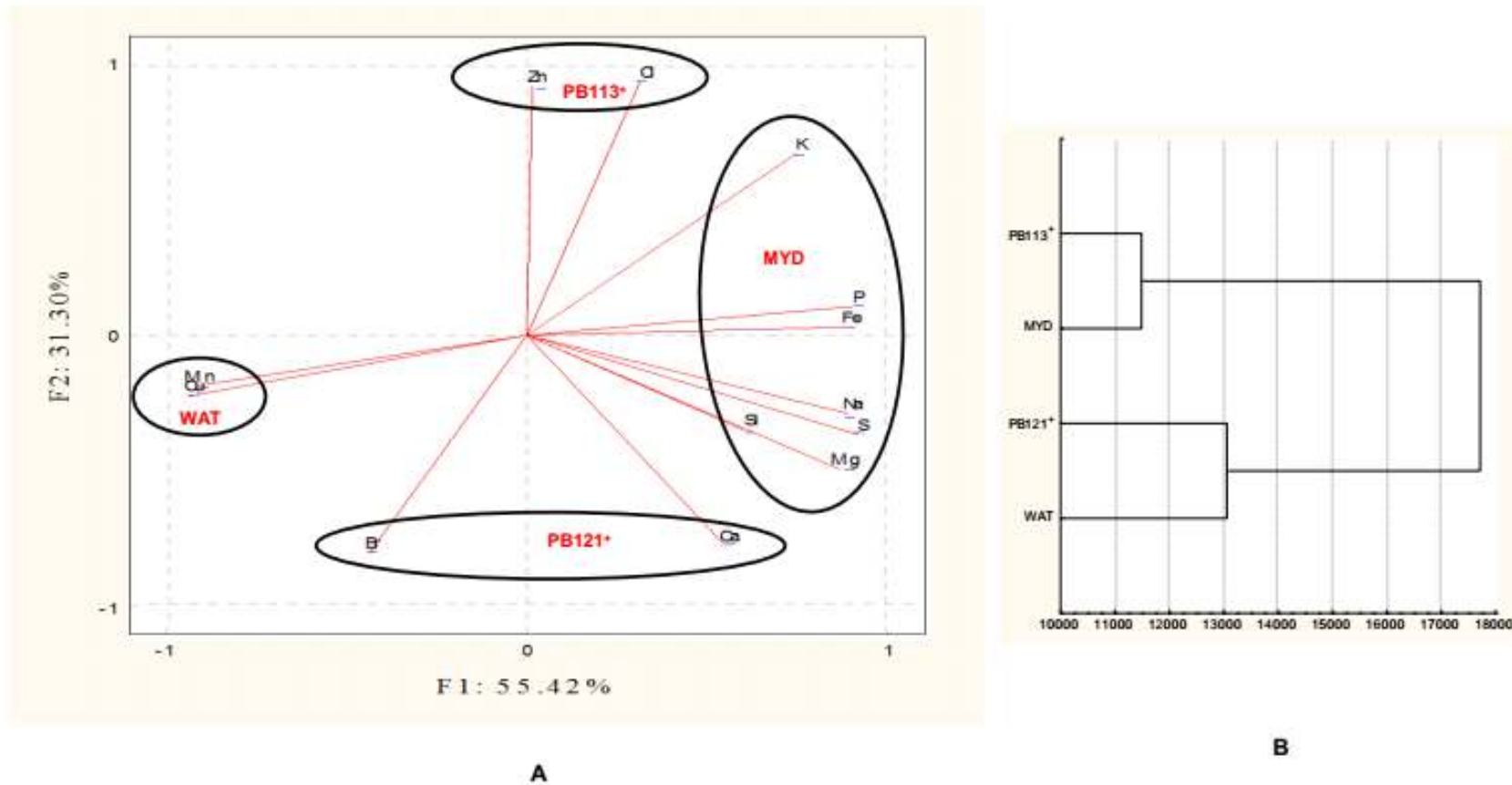


Fig. 2. Correlations between the minerals traits and the coconut saps in the F1-F2 factorial draw of the PCA (A) and dendrogramme of Hierarchical Ascending Clustering of the sap samples performed from the unweighted pair group method with arithmetic means (B)

The mineral oligoelements in the coconut sap are copper, iron, manganese and zinc, concentrations of which state on similar root trapping ability from coconut varieties, provided that the oligoelement is available in the cultivated soil. Indeed, the oligoelement found in the sap deriving from various coconut cultivars resulted in similar contents. Fe²⁺ and Zn²⁺ ions are known to be involved in the blood red globules synthesis [24]. Iron is also co-factor for hemoglobin in the trapping of oxygen, inherent stage in the blood oxygenation. Thus, the consumption of coconut sap as drink could result in the blood purification. Among the four assessed coconut types, the MYD provided sap with more mineral traits correlating the great ash content derived from the dwarf variety.

5. CONCLUSION

The coconut sap revealed more ash contents from PB113⁺ and MYD varieties than PB121⁺ and WAT. Thirteen mineral nutrients are found in the ashes, major of which are potassium and chlorine. There are also oligoelements as iron, copper and zinc. The work evidenced various mineral characteristics of the coconut saps relating to varieties. Regarding the physiological well-being supported by the mineral elements for health, the consumption of the coconut sap as drink could be profitable for populations and coconut farmers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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