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Comparison of Pineapple Fruit Characteristics of Plants Propagated in Three Different Ways: By Suckers, Micropropagation and Somatic Embryogenesis

YAPO Edwige Sopie Salomé[1,2], KOUAKOU Kouakou Laurent[2], BOGNONKPE Jean Pierre Irénée[2], KOUAME Patrice[1] and KOUAKOU Tanoh Hilaire[2]*

[1]*Université d'Abobo-Adjamé, UFR des Sciences et Technologie des Aliments, Laboratoire de Technologie, Alimentaire, 02 BP 801 Abidjan 02, Côte d'Ivoire*
[2]*Université d'Abobo-Adjamé, UFR des Sciences de la Nature, Laboratoire de Biologie et Amélioration, des Productions Végétales, 02 BP 801 Abidjan 02, Côte d'Ivoire*

Abstract

Pineapples are traditionally propagated by suckers. The advantage of using tissue-cultured plants for pineapple production has been demonstrated in recent years. Despite the large-scale use of micropropagated plants in the subtropical regions, little information is available on the nutritional quality of the fruit. Some morphological states (weight and length of fruit, diameter of fruit and heart of fruit), physico-chemical aspects (pH, titratable acidity, soluble solids and water contents), biochemical contents (vitamin C, total phenols, total dietary fiber, total free amino acid, lipids, protein and minerals) and sensorial attributes (appearance, color, firmness, odor, sweetness, tartness and acceptability) were studied. This, in order to assess nutritional properties and consumer acceptability of fruits (FR) derived from conventionally-propagated sucker, fruits (FM) derived from in vitro propagation plant and fruits (FE) derived from somatic embryogenesis regenerated plants. Significant differences were found in weight, length, diameter, heart diameter and commercial interest of the different fruits, and also between other chemical parameters (pH, soluble solids, titratable acidity, total dietary fiber, total sugars, protein, amino acid, phenol and vitamin C). Values of the ratio between soluble solids and acid were the lowest in the fruit FR, while they were the highest in the fruit FE compare to the fruit FM. In addition, the mineral analyzes show that K, Ca, Mg and P are the major minerals found in pineapple fruits and Al, Fe, Na and Zn are present in trace. A taste panelist preferred the tartness of the fruit FE as well as sweetness and tartness of fruit FM. The quality of the fruits coming from in vitro culture appeared to be the best. Plants resulting from tissue culture can be recommended for large-scale farming.

Keywords: *Ananas comosus*; In vitro culture; Fruit; Pineapple; Physico-chemical composition; Sensory analysis

Introduction

The pineapple [*Ananas comosus* (L.) Merrill] has long been one of the most appreciated fruit from tropical and subtropical area, because of its attractive flavor and refreshing sugar-acid balance. Pineapple has long been an important cash crop. It is produced in more than 80 countries among which is Cote d'Ivoire, where pineapple represents the second most planted fruit and a good cropping option for the coastal area. The cropped varieties grown for human consumption are Smooth Cayenne, Red Spanish, Perolera, Pernambuco and Queen. Smooth Cayenne accounts for approximately 70% of world pineapple production [1] and is the progenitor of the most important varieties used for juice and fruit consumption. It is also the main pineapple variety currently grown in Cote d'Ivoire.

The increasing demand of pineapple in the world requests an intensification of the cultivation which rely on the availability of planting material. The traditional method of propagation by suckers is laborious, with a very low multiplication rate and a slow regeneration cycle of new suckers [2]. Furthermore, the prevalence of diseases and pests on pineapple planting materials (suckers) has generated the need for clean planting materials in large quantities for both small- and large-scale farmers. Pineapple being a monocotyledonous, self-incompatible highly heterozygous plant with a 2-year time between successive fruit generations, conventional breeding to improve fruit quality is difficult. Actually, this approach has been generally unsuccessful to develop new varieties [3]. Alternatively, different methods have been developed to produce plant material free of disease, such as in vivo and in vitro multiplication.

In vitro multiplication provides a crucial advantage for the propagation and the genetic improvement of the pineapple. The first objective of researchers has been the provision of healthy plants to farmers. But different performances were observed inside the same variety in relation with the replication technique used: propagation or micropropagation. In addition, the possibility of the regeneration of the plant through tissue culture appears to be the basis to gain good results in performance and taste. Youmbi et al. [4] showed that the vitroplants of banana gave a high yield potentiality of with good morphological fruits characteristics. Although plants from in vitro regeneration can be homogeneous, this culture can cause somaclonal variations that may affect fruit quality [5]. Therefore, the characterization of the genetic variability of cultured plants tissue and their relation to fruit quality appears to be important [6,7]. The physical and chemical development of pineapple fruit has been also extensively studied [8-11]. Quality of the pineapple fruits depending on agronomic factors, chemical

***Corresponding author:** KOUAKOU Tanoh Hilaire, Université d'Abobo-Adjamé, UFR des Sciences de la Nature, Laboratoire de Biologie et Amélioration des Productions Végétales, 02 BP 801 Abidjan 02, Côte d'Ivoire, Tel: (+225) 20304279; Fax: (+225) 20304302; E-mail: tanohilaire@yahoo.fr

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constituents and origin of the suckers [12-14]. To our knowledge, no report was published on physicochemical and sensory characteristics of pineapple fruits in the relation with the initial mode of plant propagation.

The aim of the present study was to determine and compare the physicochemical properties, mineral composition and sensory characteristics of pineapple fruits resulting from plants obtained by micropropagation, somatic embryogenesis and conventional propagation. Correlations between these parameters of fruits and pineapple seedlings origin were discussed.

Material and Methods

Plant materials

Pineapple (*Ananas comosus* L. cv. Smooth Cayenne, clone CI-9) fruits of Ivory Coast were obtained by classic propagation, micropropagation and somatic embryogenesis procedures [15-17]. Fruits were collected in the experimental fields located at the University of Abobo-Adjamé (Abidjan, Ivory Coast). The site is situated between 6°51' N and 5°18'W. Rainfall average in Abidjan is about 2000 mm per year, with annual temperatures ranging between 21 and 29°C. The experiment was laid out in a randomized block design with three replications in experimental plots of 6m × 5m size. The spacing between the seedlings is 25cm on the line and 40cm between the lines. The recommended agronomic treatments for the commercial culture of smooth Cayenne were applied. The pineapple fruits were manually harvested between 150 and 158 days after flowering initiation. Fruits, with similar characteristics of ripening (skin color), were selected. Three types of pineapple fruits were therefore used:

- FE: Fruit coming from plants regenerated through somatic embryogenesis;

- FM: Fruit coming from plants regenerated by micropropagation;

- FC: Fruit coming from plants regenerated by classic propagation.

Each type of fruit was peeled, crushed then filtered on sieve, in order to separate fibers and juices. The juices, obtained were stored at -80°C until use.

Morphological analysis

The weight of pineapple fruits was determined. Fruits having higher or equal weight to 800g have been considered as interesting commercial fruits and their rate expressed in percentage was calculated. Length and diameter of fruits were measured with a ribbon meter. The pineapples flesh was removed and the heart's diameter was measured.

Physico-chemical analysis

pH measurement: pH of pineapple juice samples was evaluated with pH meter Consort C830. The measurement was a mean of triplicates.

Soluble solids: The total soluble solids of pineapple juice samples were measured using digital refractometer Digit-032 at 20°C. Results were reported as degrees Brix.

Titratable acidity: The titratable acidity, expressed in percent of citric acid, was determined by the titrametric method. The pineapple juice contains a number of organic acids (malic and citric acids), readily neutralized by strong bases and can be titrated versus standard bases such as sodium hydroxide (NaOH). A 10g sample of pineapple juice was weighed and titrated to the end point [18] using a standard solution of 0.1 N of NaOH. The end point was determined using pH meter and phenolphthalein indicator. 0.5 mL of phenolphthalein indicator was added to the sample and titrated to faint pink end point. The volume of 0.1 N of NaOH used was recorded. The total acidity can then be calculated using equation: % acid = [Volume of 0.1 N of NaOH (mL) × 0.64]/10. The result is expressed as percentage of citric acid (g citric acid/100 g fresh weight). The ratio [(total soluble solids)/(titratable acidity)] was calculated to determine the concentration of pineapple juice.

Water content: Ten (10) g of pineapple juice were placed in a vacuum dryer at 105 ± 2°C. With intervals of regular time, the sample was weighed, after cooling, until obtaining a constant weight. The water content is considered to be the evaporated part before the weight stability.

Biochemical analysis

Vitamin C and Total phenols concentrations: Vitamin C and total phenol concentration were determined with the method of Georgé et al. [19]. 10 mL of pineapple juice was homogenized with 30 mL of methanol 80%. The mixture was centrifuged at 1560 × g for 10 min. The supernatant was collected. Vitamin C and total phenol were measured using Folin reagent method in the presence of a range of standard ascorbic acid and gallic acid at 760 nm, respectively.

Soluble sugars: Ten (10) mL of each pineapple juice were homogenized with 20 mL of methanol 80%. The resulting mixture was centrifuged at 1560 × g during 10 min. The supernatant was adjusted to 50 mL with distilled water before use for analysis. Reduced sugars were determined by the method of Miller [20]. The yellow color of DNS (dinitrosalicylic acid) was also measured at 540 nm. As for the total sugars dosage, the measurement was carried out according to the method described by [21]. In presence of phenol and sulphuric acid, the solution turned to yellow-orange color which intensity is proportional to the concentration glucides. The intensity of this coloration was measured at 490 nm. A Mitton Roy spectrophotometer was used throughout the two previous investigations. The quantities of reducing and total soluble sugars were expressed in gram for 100 g of fresh weight. Glucose was used as reference at 200 µg/mL.

Total free amino acid or formol index: Total amino acid was performed according to method of [22]. Ten (10) mL of each pineapple juice was adjusted to pH 8.0 with sodium hydroxide 0.1N and 2.5 mL of formaldehyde 37% were added. The resulting mixture was adjusted to pH 8.0 with sodium hydroxide solution at 0.1N (volume V poured, expressed in mL). The concentration of assimilable nitrogen was calculated as follows: [nitrogen assimilable] (mg/L) = 28V.

Total dietary fibres: Total dietary fiber was assayed in 10 g fresh pineapple fruit [23] according to the enzymatic gravimetric of Prosky et al. [24].

Lipids content: Lipids were extracted following Marzouk and Cherif [25] method. 50 g of pineapple juice were homogenized in mixer with 50 mL of methanol. While agitating, 100 mL of chloroform were progressively added. The mixture was centrifuged at 1560×g during 15 min. The chloroform phase was concentrated with a rotary evaporator. After chloroform distillation, total lipids were obtained by weighing.

Total proteins: Nitrogen determination was carried out using the Kjeldahl method [26]. The protein content was obtained by multiplying the total nitrogen content with the international factor of conversion 6.25 [27].

Mineral analysis: Minerals were extracted by wet ashing methods. Total ash was obtained from 10g dry matter calcinations at 550°C and left until constant weight [22]. The sample (0.5g) was placed into beaker containing 10 mL of hydrochloric acid and filtered. The filtrate was adjusted to 50 ml with 1% hydrochloric acid. After recovering the minerals solution, the phosphorus (P) content was evaluated by colorimetric with the vanadomolybdic reagent. Optical density was readed at 430 nm in presence of standard range of dihydrogen potassium phosphate to 50 ppm of phosphorus. Magnesium (Mg) was determined by complexometry in presence of black Eriochrome and EDTA. Iron (Fe) was determined by spectrophotometry at 510 nm against a control without iron. Aluminum (Al), Calcium (Ca), plumb (Pb), potassium (K), Sodium (Na) and zinc (Zn) were determined using flame atomic absorption spectrophotometer (Jenway PFP7) with standard range of each element [22].

Sensory evaluation

Sensory analysis was carried out by a panel of 20 non-trained assessors, recruited among students, professors, and employees of Abobo-Adjamé University. Assessors evaluated the pineapple fruit quality using sorting-preference tests and hedonic scale. According to their preferences, assessors were asked to sort the samples from the less preferred (score 1) to the most preferred (score 5). A l-5 structured scale was used for appearance, color, firmness, odor, overall acceptability sweetness and tartness of small pieces of sliced pineapple fruit. Structured scales were used (Table 1): 1 to 3 structured scales was used for odor. Each assessor evaluated five samples of each cultivar, previously randomized to avoid position bias, and presented in recipients with lids, coded with random three digit-numbers.

Statistical analysis

Data were processed using Statistica version 6.0.1. Analysis of variance (ANOVA) was performed and mean was separated by Newman Keuls range test at $P \leq 0.05$. Significant differences were indicated by different letters in the same row. Analysis of variance was carried out on the individual accessions. Principal component analysis was performed to identify traits' contribution to observed variability in pineapple fruit pulp composition. Furthermore, accession by traits' interaction analysis to identify which traits of a specific accession are most prominent was accessed by GGE biplot analysis. The significance of treatment means was detected by least significant difference (LSD) at $P \leq 0.05$.

Results and Discussions

Morphological characteristics

Some morphological characteristics of the three pineapple fruit clones analyzed in the present study are summarized in Table 2. Pineapple fruits FR and FM are smaller in size and their heart diameter lower than fruits FE. The weight and commercial caliber of pineapple fruits decrease from the pineapple fruits FE followed by fruits FM and FR. This caliber is associated to a gain of organic matter. The weight of pineapple fruits FR respects the values cited by Singleton [28] for ripe pineapple fruit. These results seem to reveal the preponderant role of in vitro culture in the gain of organic matter observed with pineapple fruits FE and FM. It justifies the more significant commercial interest observed in these fruits. After obtaining the suckers by micropropagation, the leaves were used to induce somatic embryogenesis. In vitro culture allowing a youthfulness of seedlings [29], the longer time past by explants on the culture media during somatic embryogenesis would have permitted to obtain pineapple suckers having a more important

Characteristics	Scales				
	1	2	3	4	5
Appearance	good	fairly good	acceptable	slightly bad	bad
Color	bright yellow	pale yellow	slightly brownish yellow	brownish yellow	Brown
Firmness	very firm	firm	fairly firm	slightly firm	soft
Odor	characteristic	slightly characteristic	off-odors	-	-
Sweetness	very strong	strong	fair	low	no
Tartness	very strong	strong	fair	low	no
Overall acceptability	likes very much	likes slightly	accepts	dislikes slightly	dislikes

Table 1: Scales structures for sensory parameters.

Characteristics	Different types of pineapple fruits					
	FR		FM		FE	
	A	B	A	B	A	B
Fruit weight (g) (without crown)	600a	1200b	600a	1600c	700d	2600e
Length fruit (mm)	100a	150b	100a	150b	110a	170c
Fruit diameter (mm)	80a	110b	90a	114b	90a	134c
Heart diameter of fruit (mm)	30a	31b	30a	31a	2.6a	28ab
% FCIC	43a		53.7b		65c	

Fruits FR (fruits derived from conventionally-propagated sucker); fruits FM (fruits derived from *in vitro* propagation plant); fruits FE (fruits derived from embryogenesis regenerate plants); (A): minimum value; (B): maximum value; FCIC: fruits of commercial interest caliber. Different letters in the same row indicate significant differences at 5%. Mean value of three batches analyzed on triplicate.

Table 2: Morphological characteristics of pineapple fruits coming from different origin of suckers.

rejuvenation degree than those coming from the micropropagation. The use of vitroplants seemed to have increased the yield of pineapple fruits. Youmbi et al. [4] noted a significant effect of vitroplants on the fruit yield attributes and the good morphological characteristics in the banana cultivar. Moreover, the formation of embryos can be seen as the major way of rejuvenation in species as mentioned by Bonga [30]. Low heart diameter observed with pineapple fruits FE compared to fruits FM and FR seem to indicate a gain of flesh. It would mean that fruits FE contain more juice than fruits FM and FR. Consequently, the incidence of in vitro culture on physico-chemical and biochemical parameters must be established in order to appreciate the influence of rejuvenation on in vitro culture induction of suckers. It is wise to notify that pineapple seedlings resulting from the traditional propagation (PR) gave fruits showing the weakest morphology, certainly due to the ageing or degeneration of pineapple seedlings resulting from an intensive culture. That entailed the degradation of soils and the abusive use of chemicals.

Physico-chemical characteristics

The physico-chemical characteristics of three pineapple fruit clones analyzed are shown in (Table 3). Significant differences were found in the physico-chemical analysis of the three pineapple fruits, but there was no significant difference for water content (Table 2). The samples of fruits FR and FM analyzed have pH levels around 3.0 (pH 3.45 and 3.85 respectively), except the fruit FE with a pH of 4.14. Pineapple juice pH is also known to vary with growing location, harvesting time, fruit maturity and other factors which affect the fruit [10]. Every microorganism has a minimal and an optimal pH required for its growth. The excellent storing qualities of fruits are related to their respective pH, foods with low pH value (below 4.5) are usually not really spoiled by bacteria [31]. The acidity was the highest in the fruits FR while the soluble solids were the highest in the other fruits (FM and FR). The ratios between soluble solids and acid were 5.0 (fruit FR), 11.51 (Fruits FM) and 14.75 (fruits FE). Fruit qualities such as the fruit sugar and acidity were significantly influenced by the plant propagation methods. These results showed that the fruits FE were sweeter than the fruits FM followed and subsequently than fruit FR. The acidity and soluble solids values found for the fruit FM and FE felt within the range reported by Py et al. [32] and Cano et al. [33]. The selected parameters considered to predict the eating quality of pineapple fruits were soluble solids (SS), titratable acidity (TA), SS/TA (also known as the Brix/acid ratio), pH, color and translucency. If we considered the ratio SS/TA, often used for industrial classification of these products, the high levels found in the fruits FE and FM are caused by a low acidity in these products compared to the fruits FR. However, flesh SS was the only parameter found suitable as a year-round index of pineapple eating quality [11]. All pineapple fruits were constituted of 86% of water, thus showing the juicy state of smooth cayenne pineapple fruits. These results are higher than those reported by Reinhardt et al. [13]. It could mean that the pineapple fruits coming from plants obtained through in vitro culture are excellent for all type of cannery (juice, slices, etc.), and could be well accepted on foreign and domestic fresh fruit markets.

Biochemical characteristics

In Table 4 are shown the vitamin C, total phenols, total sugars, reducing sugars, total dietary fibers, total proteins, amino acid and lipids contents of the three types of pineapple fruits. Significant differences

Characteristics	Type of pineapple fruit		
	FR	FM	FE
pH	03.45 ± 0.01a	03.85 ± 0.02b	04.14 ± 0.02c
Soluble solids (°Brix)	12.71 ± 0.12a	13.16 ± 0.17b	14.90 ± 1.08c
Titratable acidity (g citric acid/100 g f w)	02.51 ± 0.05a	01.23 ± 0.04b	01.01 ± 0.02c
Ratio (°Brix/Acidity)	05.00 a	11.51b	14.75 c
Water content (%)	86.13 ± 1.76a	86.49 ± 1.90a	86.97 1.49a

Fruits FR (fruits derived from conventionally-propagated sucker); fruits FM (fruits derived from *in vitro* propagation plant); fruits FE (fruits derived from embryogenesis regenerate plants); f.w (fresh weight). Different letters in the same row indicate significant differences at 5%. Mean value of three batches analyzed on triplicate.

Table 3: Physico-chemical characteristics of three types of pineapple fruits.

Characteristics	Type of pineapple fruit		
	FR	FM	FE
Vitamin C (mg ascorbic acid/g f.w)	11.93 ± 0.11a	12.03 ± 0.23b	13.66 ± 0. 18c
Total phenols (μg/g f.w)	126.00 ± 6.02a	145.00 ± 7.30b	157.00 5.25c
Total dietary fibres (mg/100 g f.w)	144.00 ± 5.01a	162.00 ± 6.17b	173.00 ± 0.001c
Total sugars (mg/g f.w)	11.00 ± 0.83a	13.07 ± 0.11b	13.65 ± 0.61b
Reducing sugars (mg/g f.w)	9.29 ± 0.04a	10.28 ± 0.05a	10.30 ± 0.05a
Total proteins (μg/g f.w)	653.75 ± 1.57a	700.81 ± 1.90ab	705.50 1.30b
Amino acid (μg/100 mL)	384.60 ± 9.60a	403.47 ± 8.92b	407.70 7.29b
Lipids (%)	11.11 ± 0.17a	11.39 ± 0.24a	11.42 ± 0.24a

Fruits FR (fruits derived from conventionally-propagated sucker); fruits FM (fruits derived from *in vitro* propagation plant); fruits FE (fruits derived from embryogenesis regenerate plants); f.w (fresh weight). Different letters in the same row indicate significant differences at 5%. Mean value of three batches analyzed on triplicate.

Table 4: Biochemical characteristics of three types of pineapple fruits.

were found in the vitamin C, total phenols and total dietary fibers, but there were no significant differences in reducing sugars and lipids with the three pineapple fruits. Total sugars, total proteins and amino acid showed lower contents in fruits FR whereas they were similar in fruits FM and FE. The vitamin C, total phenols and total dietary fibers contents were higher in fruit FE compared to fruit FM, but fruit FR had the lowest levels. Vitamin C was the highest in both the fruits coming from in vitro culture (FE and FM), and higher than the values reported by Pham et al. [34]. But, in this case the pineapple fruits were derived from conventionally propagated suckers. The total phenols (TP) content of fruits FE (157 µg/g f.w) was also higher than fruits FM (145 µg/g f.w), while fruits FR (127 µg/g f.w) had the lowest value. The obtained values of TP were similar to those of Gorinstein et al. [35]. TP play an important role on fruit aroma; savor and their antioxidant role that confer beneficial qualities for health. Recent researches indicate that antioxidants may contribute to the prevention of heart diseases and possibly some forms of cancer and other diseases [36-38]. The total dietary fiber (TDF) content ranged from 144 to 173 mg for the studied fruits. TDF was 11 units higher in the fruits FE compared to the fruits FM, while being of 18 units lower in fruits FR. TDF values obtained were similar to those of Vidal-Valverde et al. [39], but three times less than those reported by Gorinstein et al. [35]. The total sugars content of samples analyzed ranged from 11.00 mg/g f.w for fruits FR to 13.65 mg/g f.w for fruits FE; it is necessary to note that fruits FM have a value of 13.07 mg/g f.w which is not significantly different from that of fruits FE. These values are similar to those observed by Li and Schuhmann [40]. The results showed that the fruits FE and FM seem sweeter than the fruits FR. The reducing sugar content, which was identical between the three types of pineapple fruits, seems to reveal that the sweetened nature of fruits FE and FM is related to the presence of no reducing sugars. Indeed, Krueger and Krueger [9] reported that glucose and the fructose have smaller proportions in natural pineapples juices compared to sucrose, and Hodgson and Hodgson [10] revealed that sucrose is the main sugar in pineapple fruit. Amino acid and total proteins content of fruits FM and FE were higher than those of fruits FR (Table 3). However, these two biochemical parameters evolve in the same trend. The presence of amino acids in pineapple was identified by Dull [41]. Proteins are very long succession of amino acids. The quality of a protein was defined as effective when this protein meets the needs at the same time in nitrogen and in amino acids. The chemical index was 22% meaning that the pineapple's protein does meet perfectly the human needs of each of the essential amino acids, which are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine. Lipids results did not show significant differences between the three types of pineapple fruits. However, the lipids contents represent traces compared to other fruits. The physico-chemical and biochemical profile of the pineapple fruits shows that fruit derived from vitroplants (fruits FM and FE) seems better in term of taste and yield. That difference observed might be due to morphogenetic characters of plants derived from in vitro culture.

Mineral characteristics

The mineral compound recoveries can be observed in Table 5. The major elemental components present in pineapple fruits are K, Ca, Mg and P [9]. Al, Fe, Na and Zn are present in trace amount but Pb was not detected in pineapple fruits. From pineapple fruit elemental analysis, it can be seen that the concentrations of P, K, Ca, Mg, Na, Fe and Zn increased in the fruits FM and FE while Al decreased, compared to the fruits FR. It is noteworthy that mineral elements concentrations of pineapple fruits vary according to the initial mode of plant propagation. The physiological role of minerals is well documented. K is necessary for much enzymes activity to correct neuromuscular and cardiac processes, stomach acid secretion and aldosterone regulation. The daily requirement of K for an adult male and female is 390 to 585 mg [42]. Ca is a constituent of the skeleton and plays an important role in the blood pressure, the nerve impulse, the muscular contraction and the cardiac muscle operation [43]. The requirements in Ca are estimated at 500 mg per day for adult. P is a fundamental element for the cell because it constitutes a source. P is a fundamental element for the cell because it constitutes a source of energy quickly available. The phosphoric esters such as ATP, ADP and AMP are indeed implied in the bio-oxidation and the storage of energy during glycolysis and of the processes of phosphorylation.

Biological activity of calcium (Ca) and phosphorus (P) are dependent [44]. Mg is present in practically all tissues. For a suitable operation of the organism, Mg must be in balance in the human body with Ca, P, K and Na. Mg stimulates the formation of antibody, regularizes the cardiac rhythm and is a keystone of the assimilation of trace elements. Na plays an important role in acidity regulation and distribution water in the organism. Furthermore, Na intervenes in the

Characteristics	Type of pineapple fruit		
	FR	FM	FE
P	05.40 ± 0.01a	06.47 ± 0.02b	06.55 ± 0.04b
K	126.05 ± 4.60a	132.19 ± 3.74b	136.15 ± 4.20b
Ca	07.21 ± 0.25a	08.41 ± 0.17b	08.53 ± 0.27b
Mg	06.05 ± 0.13a	07.29 ± 0.15b	07.41 ± 0.20b
Na	0.82 ± 0.001a	01.15 ± 0.001a	01.20 ± 0.002a
Fe	0.168 ± 0.001a	0.172 ± 0.002a	0.170 ± 0.004a
Zn	0.057 ± 0.001a	0.089 ± 0.002b	0.105 ± 0.005b
Al	0.053 ± 0.004a	0.024 ± 0.001b	ND
Pb	ND	ND	ND

ND (not detected); d.w (dry weight); Fruits FR (fruits derived from conventionally-propagated sucker); fruits FM (fruits derived from *in vitro* propagation plant); fruits FE (fruits derived from embryogenesis regenerate plants). Different letters in the same row indicate significant differences at 5%. Mean value of three batches analyzed on triplicate.

Table 5: Mineral composition (mg/100 g d.w) of three types of pineapple fruits.

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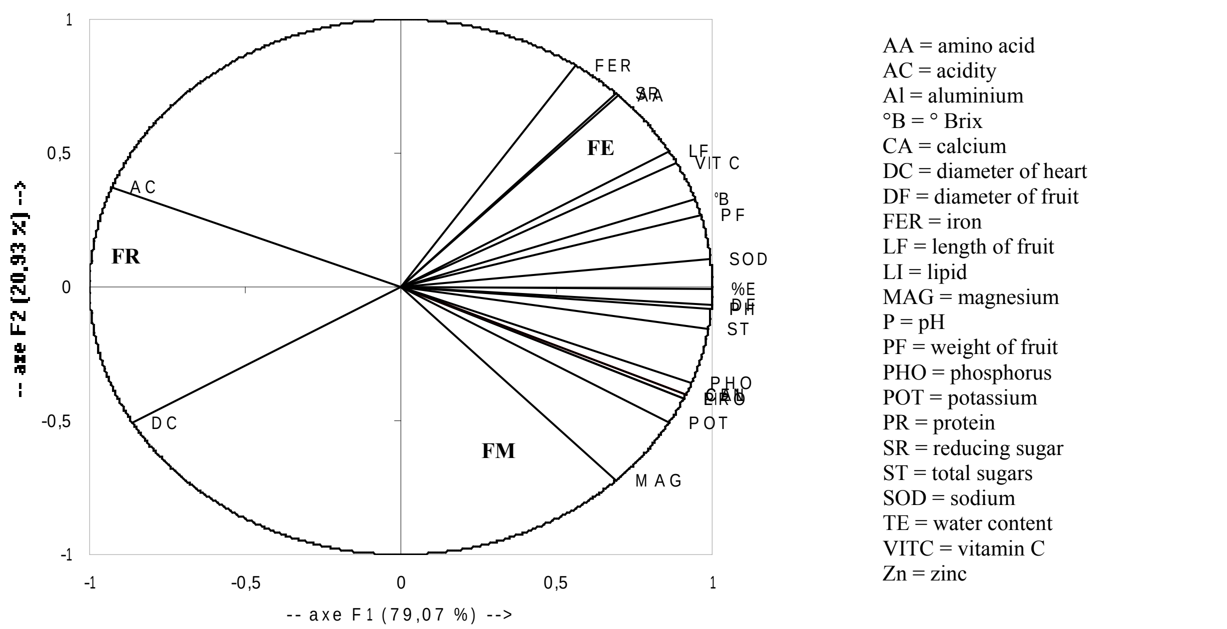
muscular contraction and control the blood pressure. The needs daily of Na are 1 to 2 g per day [45]. So, it is advised to eat pineapple fruit because they have a low content of Na. Fe content in pineapple fruits derived from in vitro culture plants was similar to fruits control (0.168-0.17 mg/100 g d.w). These values are lower than Fe content in spinach, which was 4 mg/100 g d.w. Fe enters in the formation of hemoglobin and enzymes, which play a crucial role in many metabolic reactions and is one of the most important elements in nutrition and good health. Al produced values between ND-0.024 mg/100 g d.w. Excessive consumption of Al causes Alzheimer's disease [46]. Pb was not detected in all pineapple fruits. It has been reported to be extremely dangerous to human health [47]. Zn supports the health of immune system, normal synthesis of proteins and health of men reproductive organs. Results indicate that consumption of pineapple fruits is beneficial to human health and should be encouraged.

After the minerals investigation, it is interesting to notify that a consumption of 500 mg of pineapple fruits can fill the daily needs of mineral such as K and P. It is wise to note that pineapple fruits coming from suckers resulting from in vitro cultures had significantly more important minerals contents than pineapple fruits (control) derived from traditional propagated suckers. However, to satisfy all needs of minerals with human organism, it is necessary to have a balanced food by consuming other fruits and vegetables. To discern the effect of the seedlings mode propagation on the pineapple fruits quality, it appeared necessary to achieve a principal component analysis (PCA). PCA achieved from the chemical parameters shows that the first two axes express 100% of variability. PCA (Figure 1) shows significant correlations between some variables considered and the type of pineapple fruit. Only lipid, Al, Zn, protein and water content showed no significant correlations with any of the type of fruits. The PCA, based on the correlations mentioned above, allows reduction of the number of explanatory variables of the experimental results. PCA permitted to classify the pineapple fruits in three homogeneous groups. The first group composed of the fruits FR is characterized by acidity and diameter of the heart; the second group represented by the fruit FM is characterized by the availability of Mg, P, K, Ca and lipid. Finally, the third group comprizing fruits FE is characterized by the variables length of fruit, weight of fruit, amino acid, reducing sugar, vitamin C, Fe and Brix degree. Fruits FM were closer to fruits FE. They are characterized by high levels pH, diameter of the fruit, total sugars and Na content. The discriminating analysis of the qualitative variables revealed that the acidity characterizing the fruits FR obtained from traditional propagated pineapple suckers, whereas the fruits FM and FE produced by in vitro cultured suckers characterized by the degree of Brix, total sugars, vitamin C, K and weight of the fruits. This could indicate that in vitro culture which causes a rejuvenation of pineapple suckers seems to increase the fruits quality. Al, Zn, lipid, protein and water are not useful parameters for characterization of pineapple fruits. The statistical analysis elucidated the variables Brix, vitamin C, K, pH, Na, total sugars, weight and diameter of fruit as useful quality indices for pineapple fruits.

Sensory evaluation

The sensory evaluation test was done to determine the quality and consumer acceptance of the pineapple fruits derived from in vitro propagation plant, somatic embryogenesis regenerated plants as well as fruits derived from conventionally-propagated sucker. The results from the taste panel are shown in Table 6. The average score by the taste panelists showed an acceptability of the fruits appearance. Concerning color, fruit samples was in the range of dark to bright yellow. Panelists classified the three pineapple fruits as firm. A slightly characteristic was found in odor analysis of the three samples fruits. Low to fair sweetness was found in the fruit FR, and a nearly fair sweetness in fruits FM and



FR = fruits derived from conventionally-propagated sucker; FM = fruits derived from *in vitro* propagation plant; FE = fruits derived from embryogenesis regenerate plants.

Figure 1: Principal components analysis of the composition of pineapple fruits through the mode of suckers' propagation. Biplot of first two principal components.

Characteristics	Type of pineapple fruit		
	FR	FM	FE
Appearance	3.00 ± 0.11a	3.20 ± 0.27a	3.00 ± 0.24a
Color	2.60 ± 0.23a	2.50 ± 0.14a	2.60 ± 0.18a
Firmness	2.20 ± 0.15a	2.10 ± 0.21a	2.20 ± 0.20a
Odor	1.60 ± 0.25a	1.50 ± 0.15a	1.50 ± 0.21a
Sweetness	3.70 ± 0.27a	2.80 ± 0.14b	2.60 ± 0.17b
Tartness	2.00 ± 0.22a	3.20 ± 0.19b	3.80 ± 0.22c
Overall acceptability	2.20 ± 0.18a	1.80 ± 0.20b	1.60 ± 0.14b

Fruits FR (fruits derived from conventionally-propagated sucker); fruits FM (fruits derived from *in vitro* propagation plant); fruits FE (fruits derived from embryogenesis regenerate plants); f.w (fresh weight). Different letters in the same row indicate significant difference at 5%; Mean value of three batches analyzed on triplicate

Table 6: Sensory analysis of pineapple fruits.

FE. Panelists also found out a strong tartness in fruit FR and a low to fair tartness in fruits FM and FE. With regard to the consumer overall acceptability of the fruits, FE received a higher rating than the fruit FM, whereas fruit FR got the lowest rating. These results would suggest that the consumer panel used other sensations than odor and flavor to rate these fruits. For the statistical analysis, the only significant differences detected by the taste panel were the sweetness and the tartness. There was least preference for flavor and taste; this could be due to the origin of the pineapple suckers and seem to influence the plant's metabolism [48]. The differences in soluble solids and phenols were not detected by panelists. The results from Tables 2-5 show lower quality parameters for fruit FR; fruit FE has the most interesting characteristics. The results of this work show the interest of fruits derived from in vitro culture. The positive incidence of plant regenerated by somatic embryogenesis on the total quality of pineapple fruits could be explained by the morphogenetic potentialities [4]. The knowledge on the nutritional density of the fruits products can ensure the consumers on their nutritional safety concerning the fruits derived from in vitro culture. In the future selection programs, the new challenges are the production and the sustainability of the quality of the fruits. However, it would be important to say that the nutritional quality of the fruit could be increased by the use of seedling genetically transformed with suitable characteristic. Indeed, one of the major problems of pineapple in Cote d'Ivoire is the high level of acidity. Fruits derived from in vitro culture plant such as micropropagation and somatic embryogenesis have lowest acidity and highest sugars content than the fruits derived from conventionally-propagated plant. The difference in nutrients could be explained by the rejuvenation process during *in vitro* culture or could be due to somaclonal genetic [49].

Conclusion

Pineapple fruits produced from in vitro plant can provide nourishment to consumers with satisfactory physical and chemical attributes. The mode of suckers' multiplication has an influence on the pineapple fruits quality. However, these suckers can be used for only one cycle of culture to limit contaminations and always have renewed suckers. Additional testing will be conducted during the next phase with a check of the genetic homogeneity of in vitro regenerated plant materials.

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