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The biochemical characteristics of coconut (*Cocos nucifera* L.) water during germination

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ABSTRACT

The purpose of this study was to determine the biochemical characteristics of coconut water during germination. The three cultivars most used worldwide were studied; West African Tall 'WAT', Malaysian Yellow Dwarf 'MYD' and improved 'PB121^{+}' hybrid. Germinated 'MYD' and 'PB121^{+}' had the greatest physical characteristic (water weight). As for the chemical parameters, the amounts of total sugar fluctuated from 25.60 mg/ml to 50.85 mg/ml while the amount of ashes and pH varied from 0.25% to 0.47% and 6.36 to 6.97 respectively. Coconut water from the 'WAT' and from the 'PB121^{+}' hybrid which is richer in sugars and ashes is appropriate for vinegar making. It could be also used as an additive in food for children suffering from mineral deficiencies.

Keywords: Biochemical parameter, cultivar, nut, hybrid.

INTRODUCTION

Coconut is an oleaginous plant cultivated in the tropical countries. Its world area is 11,857 million ha (Amrizal, 2003). Ivorian coconut fields cover 50.000 ha of land and 80% of them are located along the coastal area. Coconut represents the mains export crop for the majority of farmers (Konan et al., 2006). The kernels of mature nuts and the water of immature nuts are the most exploited elements.

The oil content of copra varies up to 70% according to varieties' (Assa et al., 2006). This oil represents a principal valorization of the coconut. Indeed, this oil is used for food and oleochemistry (Van der vossen et al., 2007).

Coconut water is a liquid contained in the cavity of fruit (Jayaleskmy et al., 1988). Several studies have examined the coconut's physicochemical parameters with the aim of improving its usage. Studies by Assa (2007) on immature coconut water have shown that it

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is a delicious and nutritive drink. The immature coconut water can be used for rehydration and can cure children gastric diseases (Campos et al., 1996). Assa (2007) worked on the physicochemical characteristics and organoleptic of coconut water during maturation. Akualou (2001) studied on the physicochemical and microbial parameters of mature coconut water. The transformation and conservation techniques of coconut water are less developed in Ivory Coast. Investigations of Assa et al., (2006) showed that the transformation technologies of coconut water are unknown by farmers. During the manufacture of copra, the mature coconut water is not used and represents a residue. In Ivory Coast, there have been no studies done on the coconut water of the germinated nuts. Thus, the knowledge of the biochemical parameters of coconut water of the germinated nuts could allow its valorization and increase the farmer's income.

It could also complete the data post-harvests on coconut. This study looks at the biochemical characteristics of coconut water of the germinated nuts from the varieties most used worldwide, in order to propose better ways of marketing them.

MATERIALS AND METHODS

Plant material

The plant materials used consisted of mature fruits aged 14 months from the 'PB121^{+}' hybrid, Malaysian Yellow Dwarf 'MYD' and West African Tall 'WAT' cultivars, which are respectively the female and male parents of 'PB121^{+}'. Coconuts were harvested from trees aged 25 years (Malaysian Yellow Dwarf), 28 years (West African Tall) and 11 years (PB121^{+}). These three types of coconut palm are the most cultivated in Ivory Coast, and are the most popular cultivars worldwide. This material was collected at Marc Delorme Research Station of the National Agronomic Research Centre (CNRA), located in Port-Bouet, Ivory Coast.

Methods

Eight coconut palms per cultivar were chosen and divided into 2 sets of 4 coconuts per cultivar. Each set was a replicate within each cultivar, and comprised 4 bunches of mature fruit at 14 months. From each set, 4 groups of 8 nuts each were made up. The 8 nuts of each group came from all four bunches, that is to say, 2 nuts from each bunch. The 8 groups per cultivar were used as coconut water samplings in the first nut analyses.

The first analysis of nuts was carried out less than 24 hours after harvest. The nuts were then stored under a shed at the ambient temperature for one month before the second analysis was performed. The remaining nuts were put in a seedbed at the nursery. One and three months after being placed in the seedbed, the third and fourth analyses were respectively carried out.

For each analysis, one cultivar group was selected per set. The eight nuts from each group constituted the representative sample for the set. Thus, 8 coconut water samples were treated per cultivar.

Determination of biochemical parameters

Coconut water weight was measured by using a 1/100 precision scale (*Sartorius*, Washington, USA). Dry matter content of the coconut water (% MS) was measured in an oven (Memmert, Berlin, Germany) at 104 °C for 4 hours (AOAC, 2000). The amounts of total and reducing sugars were evaluated respectively with the sulphuric phenol method (Dubois et al 1956) and with the 3.5 dinitro-salicylic acid (DNS) methods (Bernfeld 1955) by using a spectrophotometer (*Spertronic Genesis 5*, JASCO-V-530, Tokyo, Japan). The amount of ash was obtained after total incineration in a furnace muffle (VOLCA V50, Berlin, Germany) at 550 °C for 4 hours (BIPEA, 1976).

The pH and titratable acidity were determined according to AFNOR methods (1973). The refractometric dry extract was measured by a manual refractometer (DIGIT-032, Bruxelles, Belgium). The amount of proteins was obtained by direct reading with a spectrophotometer (*Spertronic Genesis 5*, JASCO-V-530, Tokyo, Japan*)* at 280 Nm (Assa et al., 2002).

Statistical analysis

The data were subject to an analysis of variance (ANOVA) using the software SPSS 16.0 for windows. Mean and standard deviations were calculated and, when *F*-values were significant at the $p < 0.05$ level, the mean difference was separated using the Newman Keul’s test. These analyses were carried out to compare the 3 cultivars.

RESULTS AND DISCUSSION

Coconut water weight

Coconut water weight of ‘WAT’ and the ‘PB121^{+}’ hybrid were identical statistically from 0 to 2 months of germination. Then it significantly dropped at the end of germination to 37.18 g and 52.84 g respectively for the ‘WAT’ and ‘PB121^{+}’. However, for ‘MYD’, coconut water weight significantly dropped during germination. Thus, coconut water weight of ‘MYD’ significantly decreased from 126.64 g (0 month of germination) to 9.77 g at the end of germination (Table 1).

Statistical analysis indicated a significant difference between the three cultivars at each stage of germination. Thus, at the beginning of germination, the ‘PB121^{+}’ hybrid and its female parent ‘MYD’ provided coconut water weight statistically identical and higher. At the end of germination, coconut water weight of ‘PB121^{+}’ hybrid and its male parent ‘WAT’ were statistically identical and higher. Indeed, at 4 months of germination, ‘MYD’ provided the lowest coconut water weight (Table 1).

Dry matter content

For ‘WAT’, the dry matter content of the coconut water was constant statistically until the 2 months of germination before significantly increasing to 6.28% at the end of germination. That of the ‘PB121^{+}’ hybrid, significantly dropped from 4.88% (0 month of germination) to reach 3.54% at the end of germination. With regards to ‘MYD’, the values did not statistically vary during germination ($P > 0.05$).

The analysis of variance shows a significant difference between the three cultivars at each stage of germination (Table 1).

The dry matter content of coconut water for ‘PB121^{+}’ hybrid drops during germination, except that of ‘WAT’ which increases at the end of germination. The decrease in the dry matter content noticed for ‘PB121^{+}’ hybrid is related to the intensification of nut respiration. This depends on factors such as temperature, moisture and oxygen quantity, sugars decompose in water, carbon dioxide and heat (Niquet and Lasseron, 1989). However, the heat production during respiration can cause warming that result in a loss of dry matter (FAO, 2007).

Ash content

The ash content of the coconut water for ‘WAT’ and ‘MYD’ was statistically identical during germination (Table 1). Thus, for ‘WAT’, that content was included between 0.40% (0 month of germination) and 0.37% (4 months of germination). ‘MYD’ provided content that fluctuated between 0.32% and 0.26% from 0 to 4 months of germination.

As for ‘PB121^{+}’ hybrid, the ash content of the coconut water significantly dropped from 0.47% (0 month of germination) to 0.33% (1 month of germination). Then, it did not statistically vary until the end of germination where it was 0.25%.

At 0 and 1 month of germination, the ash contents of the coconut water differentiated the three cultivars. Beyond this period, the analysis of variance did not show a significant difference between the three cultivars. The male parent ‘WAT’ and the ‘PB121^{+}’ hybrid provided the highest ash contents. However, at the end of the germination, the ash contents were equal statistically for the hybrid ‘PB121^{+}’ and his two parents.

The ash contents of coconut water for ‘PB121^{+}’ hybrid dropped during germination. The drop could be due to the use of potassium by haustorium for the young seedling metabolism (Manciot et al., 1979). Similar results were obtained by Jayalekshmy et al. (1988**).** These authors showed a fall of the potassium contents in the coconut water during maturation. They revealed that potassium is the abundant mineral in coconut water.

Protein content

The protein contents of the coconut water of ‘WAT’ significantly increased from 2.15% (0 month of germination) to 3.20% at the end of germination.

‘MYD’ and the ‘PB121^{+}’ hybrid provided contents which did not statistically vary during germination. At the end of germination, the highest protein contents were obtained (Table 2).

A significant difference is noticed at 2 and 4 months of germination between the three cultivars. At 4 months of germination, the female parent ‘MYD’ and the ‘PB121^{+}’ hybrid provided statistically higher and constant proteins contents. But, at 0 month of germination, these contents were identical statistically for the ‘PB121^{+}’ hybrid and his two parents.

The proteins of the coconut water are minor components (Campos et al., 1996). The proteins contents of the coconut water for ‘WAT’ increase during germination. These results have been supported by Assa et al., (2006), Jayalekshmy et al. (1988) and Agnememel (2007) on the mature of coconut water. During the kernel formation, the amino acid contents of coconut water increase. In parallel, the proteins contents of kernel drop. Thus, this increase in proteins contents of coconut water can be explained by a hydrolysis of kernel proteins. Indeed, the amino acids of kernel proteins would be implied in the synthesis of the coconut water lipids (Assa, 2007). They intervene in the formation of acétyl-CoA. This biochemical process would continue during germination.

Sugar content

The amounts of total sugar (TS) of each cultivar were constant statistically during germination (Table 2).

Thus, for ‘WAT’ the amounts of total sugar were included between 36.05 mg/ml and 43.27 mg/ml respectively from 0 to 4 months of germination.

As for ‘MYD’ the amounts of total sugar were 24.35 mg/ml (0 month of germination) and 25.60 mg/ml (4 months of germination).

For the ‘PB121^{+}’ hybrid, the values were 50.85 mg/ml, 56.43 mg/ml, 43.01 mg/ml and 40.06 mg/ml respectively at 0, 1, 2 and 4 months of germination.

At the beginning of germination, the amount of total sugar for the ‘PB121^{+}’ hybrid was highest. But, at the end of germination, it had statistically the same values with his two parents (Table 2).

The amounts of reducing sugar (RS) of coconut water for ‘MYD’ and the ‘PB121^{+}’ hybrid significantly dropped during germination (Table 2). Thus, for ‘MYD’, the values dropped from 15.47 mg/ml to 2.02 mg/ml between 0 and 4 months of germination. For ‘PB121^{+}’ hybrid, these amounts fluctuated between 25.01 mg/ml (0 month of germination) and 4.26 mg/ml (4 months of germination).

With regards to ‘WAT’, the amounts of reducing sugar of the coconut water significantly dropped from 10.95 mg/ml to 5.14 mg/ml (1 month of germination).Then, these amounts did not statistically vary until the end of germination where they were 4.85 mg/ml.

At 0 month of germination, the amounts of reducing sugar for ‘PB121$^{+'}$ hybrid were higher. But, at the end of the germination, the ‘PB121$^{+'}$ hybrid and its male parent ‘WAT’ statistically provided equal and higher proportions (Table 2).

The drop of reducing sugar contents of the coconut water for the three cultivars during germination could be due to their use by kernel and haustorium for their development. The developing kernel might, therefore, be utilizing the sugars of coconut water as precursors for the lipidic synthesis by glycolytic way (Jayalekshmy et al., 1988). This biochemical phenomenon would continue during the germination. Thus, the haustorium would use the coconut water and kernel sugars for its development and the young seedling metabolism. The coconut in germination would use only sugars present in the coconut water (Balasubramaniam et al., 1973).

pH

For each cultivar, the pH of the coconut water of was statistically constant during germination (Table 3).

Thus, for ‘WAT’, the pH of coconut water was 6.55 (0 month of germination) and 6.73 at the end of germination.

‘MYD’ provided the pH included between 6.06 and 6.75 respectively from 0 to 4 months of germination.

As for the ‘PB121$^{+'}$ hybrid, the pH of coconut water was 6.58, 6.94, 6.97 and 6.36 respectively at 0, 1, 2 and 4 months of germination.

At 0 and 4 months of germination, the pH of ‘PB121$^{+'}$ hybrid’s coconut water and its two parents were statistically identical (Table 3).

At 0 month of germination, the coconut water for ‘MYD’ is slightly acid. These results have been supported by Assa (2007). However, our values of pH obtained during germination for the three cultivars are higher than those obtained by this same author. The differences may be because we did not work in the same conditions. Indeed, the nuts are exposed in nursery to the environmental factors. The pH of the coconut water is slightly acid and varies according to the stage of maturity, variety and environment (Benavent and Sanchovzalls, 1992).

Titratable acidity

The titratable acidity of coconut water for ‘MYD’ and ‘PB121$^{+'}$ hybrid significantly dropped 1 month of germination before statistically increasing at the end of germination (Table 3).

Thus, for ‘MYD’, the value dropped from 33.28 meq g/100 ml to 14.96 meq g/100 ml (1 month of germination). Then, it increased to 24.12 meq g/100 ml at the end of germination.

As for ‘PB121$^{+'}$ hybrid, this acidity dropped from 34.37 meq g/100 ml to 21.27 meq g/100 ml before increasing to 22.39 meq g/100 ml at the end of germination.

With regards to ‘WAT’, titratable acidity significantly dropped to 14.21 meq g/100 ml (1 month from germination). Then, this acidity did not statistically vary until the end of germination where it was 17.09 meq g/100 ml.

At 4 months of germination, titratable acidity of coconut water for ‘PB121$^{+'}$ hybrid and his male parent ‘WAT’ were statistically equal and higher. But, at the beginning of germination, the titratable acidity of coconut water for hybrid ‘PB121$^{+'}$ was higher (Table 3).

Table 1: Variation of weight, dry matter and ash contents of coconut water of 3 cultivars during germination.

Parameters	**Stage of germination (month)**	**Cultivars**			
		WAT	**MYD**	**PB121⁺**	**P inter cultivar**
Water weight (g)	0	99,18 ± 50,25^{aB}	126,64 ± 42,71^{aA}	137,95 ± 49,39^{aA}	**0,001**
	1	77,98 ± 29,83^{aB}	106,65 ± 37,79^{bA}	122,97 ± 52,82^{aA}	**< 0,001**
	2	77,75 ± 45,29^{aB}	80,95 ± 57,68^{cB}	115,47 ± 47,52^{aA}	**< 0,001**
	4	37,18 ± 33,94^{bA}	9,77 ± 25,93^{dB}	52,84 ± 56,80^{bA}	**< 0,001**
	P intra cultivar	**< 0,001**	**< 0,001**	**< 0,001**	
DM (%)	0	4,86 ± 0,26^{bA}	3,77 ± 0,32^{aB}	4,88 ± 0,60^{aA}	**< 0,001**
	1	4,89 ± 0,32^{bA}	3,69 ± 0,32^{aC}	4,42 ± 0,53^{bB}	**< 0,001**
	2	4,99 ± 0,29^{bA}	4,16 ± 0,76^{aB}	4,21 ± 0,09^{bB}	**< 0,001**
	4	6,28 ± 1,57^{aA}	4,09 ± 0,41^{aB}	3,54 ± ,70^{cB}	**< 0,001**
	P intra cultivar	**< 0,001**	**0,209**	**<0,001**	
Ash (%)	0	0,40 ± 0,08^{aAB}	0,32 ± 0,08^{aB}	0,47 ± 0,07^{aA}	**0,002**
	1	0,39 ± 0,10^{aA}	0,31 ± 0,07^{aB}	0,33 ± 0,13^{bAB}	**0,050**
	2	0,39 ± 0,10^{aA}	0,30 ± 0,07^{aA}	0,28 ± 0,13^{bA}	**0,097**
	4	0,37 ± 0,16^{aA}	0,26 ± 0,05^{aA}	0,25 ± 0,11^{bA}	**0,112**
	P intra cultivar	**0,965**	**0,266**	**0,003**	

P: Test probability, **MYD**: Malaysian Yellow Dwarf, **WAT**: West African Tall, **PB121⁺**: improved hybrid PB121**, DM**: Dry Matter.
Averages followed by the same tiny letter in the same column are not significantly different for each parameter.
Averages followed by the same capital letter in the same line are not significantly different for each parameter.

Table 2: Variation of proteins, total sugars and reducing sugars contents of the coconut water of 3 cultivars during germination.

Parameters	Stage of germination (month)	Cultivars			
		WAT	MYD	PB121⁺	P inter cultivar
Protein (mg/ml)	0	2,15 ± 0,23^{abA}	2,5 ± 0,31^{aA}	2,69 ± 0,08^{aA}	**0,426**
	1	2,46 ± 0,56^{bA}	3,08 ± 0,35^{aA}	2,73 ± 0,29^{aA}	**0,120**
	2	2,14 ± 0,53^{abB}	3,39 ± 0,81^{aA}	2,93 ± 0,50^{aAB}	**0,024**
	4	3,20 ± 0,29^{aB}	4,59 ± 1,89^{aA}	4,60 ± 2,39^{aA}	**0,004**
	P intra cultivar	0,030	0,110	0,138	
TS (mg/ml)	0	36,05 ± 16,18^{aAB}	24,35 ± 6,26^{aB}	50,85 ± 28,72^{aA}	**0,008**
	1	38,49 ± 19,55^{aA}	39,62 ± 15,63^{aA}	56,43 ± 26,01^{aA}	**0,076**
	2	43,54 ± 30,03^{aA}	30,04 ± 18,29^{aA}	43,01 ± 28,74^{aA}	**0,371**
	4	43,27 ± 13,84^{aA}	25,60 ± 12,20^{aA}	40,06 ± 26,70^{aA}	**0,336**
	P intra cultivar	0,772	0,070	0,461	
RS (mg/ml)	0	10,95 ± 4,23^{aB}	15,47 ± 6,12^{aB}	25,01 ± 9,63^{aA}	**< 0,001**
	1	5,14 ± 1,52^{bC}	10,56 ± 6,89^{abB}	16,84 ± 3,06^{bA}	**< 0,001**
	2	4,31 ± 3,01^{bB}	7,41 ± 3,95b^{cAB}	9,18 ± 4,89^{cA}	**0,018**
	4	4,85 ± 1,48^{bA}	2,02 ± 1,79^{cB}	4,26 ± 1,91^{dA}	**0,030**
	P intra cultivar	**< 0,001**	**0,001**	**<0,001**	

P: Test probability, **MYD**: Malaysian Yellow Dwarf, **WAT**: West African Tall, **PB121⁺**: improved hybrid PB121,. **TS:** Total Sugar, **RS**: Reducing Sugar.
Averages followed by the same tiny letter in the same column are not significantly different for each parameter.
Averages followed by the same capital letter in the same line are not significantly different for each parameter.

Table 3: Variation of pH, titratable acidity and dry extract refractometric of the coconut water of 3 cultivars during germination.

Parameters	Stage of germination (month)	Cultivars			
		WAT	MYD	PB121^{+}	P inter cultivar
pH	0	6,55 ± 0,88^{aA}	6,06 ± 0,79^{aA}	6,58 ± 0,95^{aA}	**0,120**
	1	6,69 ± 0,66^{aA}	6,95 ± 0,75^{aA}	6,94 ± 0,66^{aA}	**0,142**
	2	6,85 ± 0,66^{aA}	6,85 ± 0,66^{aA}	6,97 ± 1,32^{aA}	**0,784**
	4	6,73 ± 0,84^{aA}	6,75 ± 1,76^{aA}	6,36 ± 0,97^{aA}	**0,136**
	P intra cultivar	**0,500**	**0,371**	**0,061**	
Titratable acidity as citric acid (méq g/100ml)	0	28,48 ± 19,93^{aAB}	33,28 ± 12,42^{aB}	34,37 ± 20,42^{aA}	**0,038**
	1	14,21 ± 4,92^{bB}	14,96 ± 6,69^{cB}	21,27 ± 16,01^{cA}	**< 0,001**
	2	16,48± 5,50^{bB}	21,79 ± 5,60^{bA}	22,33 ± 9,35^{bA}	**0,001**
	4	17,09 ± 12,01^{bB}	24,12± 22,41^{bA}	22,39 ± 16,01^{cA}	**0,233**
	P intra cultivar	**< 0,001**	**< 0,001**	**< 0,001**	
Dry extract refractometric (%)	0	5,87 ± 2,39^{aA}	3,83 ± 0,47^{aB}	5,03 ± 0,54^{aA}	**< 0,001**
	1	5,27 ± 1,08^{bA}	3,75 ± 0,54^{aC}	4,46 ± 0,88^{bB}	**< 0,001**
	2	5,02 ± 0,87^{bA}	3,64 ± 0,79^{aC}	4,16 ± 0,96^{bB}	**< 0,001**
	4	4,83 ± 0,41^{bA}	3,34 ± 2,77^{aB}	3,01 ± 1,97^{cB}	**< 0,001**
	P intra cultivar	**< 0,001**	**0,585**	**< 0,001**	

P: Test probability, **MYD**: Malaysian Yellow Dwarf, **WAT**: West African Tall, **PB121^{+}**: improved hybrid PB121.
Averages followed by the same tiny letter in the same column are not significantly different for each parameter.
Averages followed by the same capital letter in the same line are not significantly different for each parameter.

The acidity of coconut water might be due to organic acid, free amino acids, fatty acid and carbon dioxide dissolve during tissue respiration (Jayalekshmy et al., 1988). These compounds would come from the metabolic reactions in the fruits, such as the lipids, proteins and carbohydrates oxidation. Thus, the increase in the content of these compounds would have involved the acidity of coconut water at the end of germination.

Dry extract refractometric

The refractometric dry extract of coconut water for 'PB121^{+}' hybrid significantly dropped from 5.03% (0 month of germination) to 3.01% (4 months of germination). For 'WAT', this extract significantly dropped to 5.27% (1 month from germination). Then, it did not statistically vary until the end of germination where it was 4.83% (Table 3). As for 'MYD' the refractometric dry extract of coconut water was statistically constant during germination. Indeed, the values included between 3.83% and 3.34% respectively from 0 to 4 months of germination.

Statistical analysis

Statistical analysis indicated a significant difference between the three cultivars at each stage of germination. At 0 month of germination, the refractometric dry extract of coconut water for male parent 'WAT' and 'PB121^{+}' hybrid were statistically identical and higher. But, at the end of germination, refractometric dry extract of coconut water for female parent 'MYD' and 'PB121^{+}' hybrid were statistically equal and lowest (Table 3).

The refractometric dry extract of coconut water translates the solid compounds contents. It is correlated positively (r=0.65) with the dry matter content. That can be accounted by the fact that the dry matter of coconut water is made up of macromolecules and minerals. The soluble sugars are the majority compounds of coconut water (Compos et al., 1996). The proteins, lipids and the other components are in minor quantities. Thus, the drop in refractometric dry extract of coconut water for all the cultivars during germination could be due to the drop of the sugar contents. Similar results have been confirmed by Kamala and Velayudham (1978). The chemical parameters of coconut water for the 'PB121^{+}' hybrid and its two parents varied differently. For 'PB121^{+}' hybrid, titratable acidity, sugar and ashes contents were higher than the two parents. Comparing the hybrid to its parents in this way reveals the heterosis of hybrid. But, pH and the protein contents for 'PB121^{+}' hybrid and its two parents were identical statistically.

Conclusion

This study was carried out to determine the biochemical characteristics of coconuts in germination. The work focussed on coconut water of the three most widely grown coconut trees in Ivory Coast: 'MYD', 'WAT' and 'PB121^{+}' hybrid. The results showed that, coconut water of the three cultivars was slightly acidic during germination. The male parent 'WAT' and 'PB121^{+}' hybrid provided the highest ash contents. The proteins were the minor components of coconut water of the three cultivars. But, sugars were very abundant in coconut water. Thus, at the beginning of germination, the highest contents were provided for 'PB121^{+}' hybrid (50.85 mg/ml).

In terms of usage, Coconut water from the 'WAT' and from the 'PB121^{+}' hybrid which is richer in sugars and ashes is appropriate for vinegar making. It could also be used as an additive in food for children suffering from mineral deficiencies.

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