Scientific African 6 (2019) e00123

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Phytochemical compounds, antioxidant activity and non-enzymatic browning of sugars extracted from the water of immature coconut (*Cocos nucifera* L.)

L.A. Akpro[a,b,*], G.A. Gbogouri[a], B.R. Konan[b], A.E. Issali[c], K.J.L. Konan[c], K.D. Brou[a], G.J. Nemlin[d]

[a] *Laboratory of Nutrition and Food Safety, UFR STA, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Cote d'Ivoire*
[b] *Laboratory of Biotechnology and Tropical Products Processing, UFR STA, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Cote d'Ivoire*
[c] *Laboratory of Technology and oil, service of plants selection, protection and improvement. Marc Delorme Research Station, National Center for Agronomic Research (CNRA), Regional Direction of Abidjan, 07 BP 13 Abidjan 07, Cote d'Ivoire*
[d] *National Center for Agronomic Research (CNRA), General Direction of CNRA, Abidjan, Cote d'Ivoire*

ARTICLE INFO

Article history:
Received 7 January 2019
Revised 15 July 2019
Accepted 6 August 2019

Key words:
Coconut water sugar
Phytochemical compound
Antioxidant activity
Non-enzymatic browning
Immature coconut

ABSTRACT

The aim of the study was to evaluate the phytochemical compounds and the antioxidant activity of sugars extracted from the coconut water. The energy value and the products of the non-enzymatic browning had also been studied. The phytochemical screening of white sugars, brown sugars and syrups, had showed the presence of polyphenol compounds (340 and 581.33 mg of galate eq./ 100 g), flavonoids (10–25.9 mg/100 g), alkaloids (2.5–8.6 g/100 g), phytates (20–26.9 mg/100 g) and tannins (35 and 50 mg/100 g). The white sugar had shown a high potential of iron reducing and of scavenging of free radicals from DPPH, compared to the brown sugar and to the syrup. In fact, the capacity of the white sugar to reduce the iron ranged from 35 to 55 mg of Trolox/100 g against from 5 to 14.79 mg of Trolox/100 g approximately for the brown sugar. Similarly, the inhibition of the DPPH radical ranged from 35.09 to 39.64% for the white sugar, against 23 to 26% for the brown sugar.

The absorbance increases linearly and significantly with the time/temperature couple at 280 nm and 480 nm. Sugars obtained from the hybrids ($PB121^+$, $PB113^+$) and from the tall coconut (WAT) presented the highest browning degrees, in contrast with the MYD and EGD sugars which displayed the lowest optical densities. The carbon/nitrogen ratio was higher in sugars of EGD, MYD and $PB121^+$, and particularly higher in white sugars than in brown sugars and syrups. The energy value is more important in the brown sugars and syrups than in the white sugars. These phytochemical compounds could confer to the sugars of coconut water curative and preventive effects as antioxidants.

* Corresponding author at: Laboratory of Nutrition and Food Safety, UFR STA, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Cote d'Ivoire.
E-mail address: akprolathroanselme@gmail.com (L.A. Akpro).

https://doi.org/10.1016/j.sciaf.2019.e00123

Introduction

Food insecurity and hunger affect more than one billion people worldwide with negative implications for the health, the productivity, and the well-being of vulnerable members of our global population. African indigenous plants have the potential to play a central role in the fight against the hunger, the food insecurity and the health concerns in Sub-Saharan African. The understanding of the historical and current uses of African indigenous plants, during the periods of food shortage, hunger and disease, is essential to the developing of effective programs and policies, to promote the sustainable production and consumption of these local edible plants for population's health [1].

The coconut water is an opalescent liquid found in the cavity of the coconut fruit. It is sterile and used as a refreshing drink. The coconut is known for its many virtues. It is known to be a powerhouse of health benefits. It helps to treat many diseases such as throat infections, tapeworms, gonorrhea, digestive problems, influenza, lice, giardia, bronchitis, and many other ailments. It reinforces the immune system and has antifungal, antiviral, anti-parasitic and antibacterial properties [2]. The coconut can be consumed under various forms, such as the raw coconut, the coconut oil, the coconut milk, the coconut butter, the coconut water etc. It improves the insulin secretion in the body and boosts the utilization of blood glucose [3]. In fact, the coconut consumption also helps to control diabetes by having a positive impact on the hormones that control blood sugar in the body [4]. Due to this, the rise in blood sugar levels slow down and this also helps to lower the glycemic cravings of a person. The coconut is also known to cause quick digestion and has a positive impact on other symptoms that are associated with bowel and digestive disorders. It is also known to help with the absorption of nutrients and minerals in the body, while also providing the number of dietary fibers that your body needs. The consumption of coconut, by its rich content in fiber, relieves the stress on the pancreas, contributing to the reducing of the diabetes risk. In recent years, the coconut water has become a very trendy beverage [5].

Among the known biological potential of plants, the antioxidant activity is of increasing interest [6]. This is due to the important role played by the antioxidant compounds, found in plants and their fruits, in the treatment and prevention of oxidative stress diseases [6–8]. In addition, liquids from some fruits such as citrus fruit [9], grape juice [2], and black grapes [10] have these same antioxidant properties. These substances, although not belonging to any group of conventional substances, perform many functions in the body [11–14]. Would sugars extracted from coconut water be sources of phytochemicals capable of inducing antioxidant activity? The interest of this activity is that it constitutes an anti-carcinogenic source, as it has been demonstrated experimentally and epidemiologically by Pamplona [2]. Sugars of the coconut water being derived from coconut fruit; it seemed to us ideal to test their composition in non-nutritive substances. This study aims to evaluate the phytochemical compounds, the products of non-enzymatic browning and the antioxidant activity of sugars extracted from immature coconut water during Lathro's research work.

Material and methods

Material

Preparation of coconut extracts

Three types of sugar (brown, white and syrup) were made from water of immature coconuts (aged of 8 and 9 months) were used in this study. These sugars were produced by Lathro et al. [15], after several tests of manufacture at the laboratory. Five varieties such as: the Tall West African (WAT), the Equatorial Guinea Green Dwarf (EGD), the Malaysia Yelow Dwarf (MYD), the Port-Bouët 121 improved ($PB121^{+}$) and the Port-Bouët 113 improved ($PB113^{+}$) were used to product sugars. After the harvest, the coconuts were treated less than 24 h by a phase of extraction with water, followed afterward by that of the production of sugars.

Chemicals products

Aluminum chloride, sodium acetate, sulfuric acid (H_2SO_4), potassium permanganate (KMnO4), chloridric acid (HCl), wade reagent, sodium phytate, acetic acid in ethanol, ammonium hydroxide, Folinciocalteus, sodium bicarbonate (Na_2CO_3) were the chemicals products used in this work.

Methods

Compounds quantifications

Determination of the total polyphenols content. The total polyphenols content was assayed using the Folinciocalteu's method [16]. 100 µL of sugar diluted at 1/5 in distilled water, was placed in a test tube, and 500 µL of Folinciocalteu's solution diluted at 1/10 in distilled water, were added. After 2 min, 400 µL of Na2CO3 at 20% (w/v) were added and then the whole were heated in a water bath (40 °C) for 5 min. The mixture was allowed to stand in a dark place for 30 min and thereafter, the absorbance was read using a spectrophotometer (Thermo Fisher Scientific, Madison WI 53,711 USA) at 760 nm against the Gallic acid (acid 3, 4, 5-trihydroxybenzoïc), a phenolic compound used as control.

Determination of the total flavonoids content. The method of Meda et al. [17] was used for the determination of the flavonoids. 0.5 mL of the supernatant obtained from the extraction of the polyphenols is removed and it was added successively: 0.5 mL of distilled water, 0.5 mL of aluminum chloride (10%, w/v), 0.5 mL of sodium acetate (1 M) and 2 mL of distilled water. The mixture was kept at the room temperature for 30 min and the absorbance was read using a spectrophotometer (mark: Thermo Fisher Scientific, Madison WI 53,711 USA) at a wave length of 415 nm against the blank. In parallel, the same procedure was repeated for the standard solution of Quercetin.

Determination of oxalates content. The oxalates were evaluated according to the method of Day and Underwood [18]. 2 g of sugar were homogenized in 25 mL of H_2SO_4 (3 M) with the magnetic stirring for 1 h. The resulting mixture was filtered with a Wathman filter paper. The filtrate is hot-titrated with a solution of KMnO4 (0.05 M) until the pink turn.

$$\text{Oxalates}(\text{mg}/100\text{g}) = [(2,2 \times \text{Veq} \times 100)/\text{m}_\text{e}]$$

Veq: volume with equivalence, $m_{e:}$ mass of the sugar

Determination of phytates content. According to the INRA method [19], 1 g of the coconut water sugar was homogenized in 20 mL of HCl (0.65 N) with the magnetic stirring for 12 h at the room temperature. Then, the mixture was centrifuged at 12,000 rpm for 40 min. 0.5 ml of the supernatant was removed, to which 3 ml of the Wade reagent was added. The mixture was allowed to stand for 15 min and the absorbance (OD) was read by using a spectrophotometer (Thermo Fisher Scientific, Madison WI 53,711 USA) at a wave length of 490 nm against a control. A calibration range was made under the same conditions with sodium phytate at 10 µg/mL. It is expressed in mg of sodium phytate equivalent/100 g.

$$\text{Phytate}(\text{mg}/100\text{g}) = [(\text{OD}_{490} \times 4)/(0,033 \times \text{m}_\text{e})]$$

m_e: mass of the sugar

Determination of alkaloids content. According to the method of Harborne [20], 5 g of the coconut water sugar were weighed and homogenized in 200 mL of a solution of 20% acetic acid in ethanol. The resulting solution was incubated for 4 h and then filtered through a Wathman filter paper. The filtrate obtained was concentrated by means of a boiling bath boiling up to a quarter of the initial volume. A concentrated solution of ammonium hydroxide was gradually added to the filtrate until a precipitate was obtained. Finally, the precipitate is filtered and dried in an oven at 80 °C before being weighed.

$$\text{Alkaloids}\ (\%) = [(\text{m x}\,100)/\text{m}_\text{e}]$$

m = mass of dried precipitate, m_e= mass of the sugar

Determination of the non-enzymatic browning products. The intermediates and the products derived from the non-enzymatic browning (Maillard reaction) were determined by absorbance at 280 nm and 480 nm, respectively. The measures were carried out on an extract obtained, by mixing 0.5 g of powdered sugar and 10 mL of distilled water for 10 min. The suspension was stirred for 15 min and centrifuged at 5000 rpm for 15 min. The supernatants were recovered and the absorbance at 280 nm (Ab-280) and 480 nm (Ab-480) were read by using a spectrophotometer (Thermo Fisher Scientific, Madison WI 53,711 USA). The values of the optical density correspond to the degree of the browning of the sugars, highlighting the quantity of the formed products.

Determination of the antioxidant capacity.

With the DPPH assay. The scavenging activity (electron transfer capacity) against the stable DPPH (1, 1 diphenyl-2-picrylhydrazyl) of the plant extracts, was evaluated according to the method of Brand-Williams et al. [21], with minor modifications according to Choi et al. [22]. The free radical 1, 1 diphenyl-2-picrylhydrazyl (DPPH) is reduced by the antioxidant that has radical scavenging properties. The radical absorbs at 517 nm and this absorbance disappears during the reduction. Briefly, aliquots (50 µL) of the leaf extracts and of the pure compounds were mixed with 2 mL of 6×10^{-5} M methanolic solution of DPPH radical in a cuvette. The absorbance measures were started immediately and the decrease of the absorbance at 517 nm was determined after 16 min for all samples. The control was the absorbance of the radical without the antioxidant. Catechin and gallic acid solutions in methanol were also tested for purposes of comparison. All the determinations were carried out in duplicate and the inhibition percent of the DPPH radical was calculated according to the formula of Yen and Duh [23].

$$\%\mathbf{Inhibition} = [(\mathbf{Ac}(\mathbf{o}) - \mathbf{AA}(\mathbf{t})/\mathbf{Ac}(\mathbf{o})]\mathbf{x100}$$

Where Ac (o) is the absorbance of the control at $t = 0$ min and AA (t) is the absorbance of the antioxidant at $t = 16$ min

With frap method. The ferric reducing antioxidant power (FRAP) assay, developed by Benzie and Strain [24] modified and Meda et al. [17]. was used in this study. The assay is based on the reducing power of a compound or of an antioxidant. The reduction of the Fe^{3+} (ferric ion) to Fe^{2+} (ferrous ion) is taken as unit [24]. The $Fe2^{+}$ ion forms a blue complex with the 2, 4, 6-tripyridyl-s-triazine (TPTZ) which absorbs at 593 nm. High absorbance values at this wavelength indicate a higher reducing power of the antioxidant or of the polyphenol. The FRAP reagent was prepared by mixing one volume TPTZ (10 mM) in HCl (40 am) with 10 vol of acetate buffer (300 mM), at pH 3.6, and one volume of ferric chloride (20 mM).

Briefly, the FRAP reagent (1.5 mL) was placed in a cuvette and the blank reagent was taken at 593 nm. Sugar samples (50 µL, diluted 10 times) and 150 µL of deionized water were added. The readings were carried out immediately and 15 s later for 8 min. No additional absorbance changes occurred after 4 min. Therefore, the reading of the absorbances at 4 min was used for the calculations. A standard curve was prepared using different concentrations of FeS04.7H20 (100–1000 µM). All the solutions have been measured and analyzed for positive results. All the solutions were freshly prepared and the catechin and the gallic acid were also measured as positive controls and for the comparison purposes.

Determination of the carbon/nitrogen ratio and of the energy value of sugars. The ratio of carbon/nitrogen was estimated by the calculation of the relationship between the total sugar content and the protein content, according to Konan [25]. The energy value of coconut water sugars was measured according to the equation of Akpabio et al. [26]. The fatty maters, the proteins, the total sugars and the titrable acidity contents, were obtained by using respectively, the methods of AFNOR [27], Sedmark & Grosberg [28], Dubois et al. [29] and AOAC [30]

$$EV(Kcal/100g) = 9\,TMG + 4\,TST + 4\,TPR + 3\,TAT$$

EV: energy value in Kcal/100 g,
TMG: fatty matters,
TST: totals sugars content,
TPR: proteins content,
TAT: titrable acidity content

Statistical analysis

The data obtained from the phytochemicals compounds, the antioxidant activities, and of the non-enzymatic browning reactions were submitted to the one-way analysis of the variance (ANOVA). The analysis was performed by using the version 22 of the IBM SPSS statistics software. The significant differences between the means were allowed for $p < 0.05$, using the Tukey's post hoc differences test.

Results and discussion

Results

Contents of flavonoids, total polyphenols, tannins, phytates, oxalates and alkaloids

The contents of total polyphenols, flavonoids, tannins, phytates, oxalates and alkaloids derived from the varieties of sugars extracted from coconut were presented in Table 1. There was a significant difference ($p < 0.05$) between the total polyphenol contents of the extracted sugars. The values ranged from 340.32 mg of galate eq./100 g (PB121+) to 535.99 mg of galate eq./100 g (MYD) for the syrup; from 348.66 mg of galate eq./100 g (EGD) to 526.22 mg of galate eq./100 g (MYD) for the brown sugar; and from 428.05 mg of gelato eq./100 g (WAT) to 581.13 mg of galate eq./100 g (MYD) for the white sugar. The coconut variety MYD exhibits the highest levels of sugars while the $PB121^{+}$ contains the lowest. The brown sugar $PB113^{+}$ contained more flavonoids (25.95 mg/100 g), meanwhile the white sugar extracted from the same variety showed the lowest value of this compound. The results have also showed that the WAT syrup contained less of tannins (35.81 mg/100 g of syrup) than the others. A high level of tannins was found in the white sugar MYD (49.93 mg/100 g). For the phytates contents, there was no difference between the brown and the white sugars for all the coconut varieties, although the syrup sugar WAT contained less of phytates (20.98 ± 0.70 mg/100 g) and that the EGD brown sugar exhibited the highest value (26.95 ± 0.54 mg/100 g). The contents of oxalates and alkaloids were high in brown sugar WAT, with respectively, values of 36.67 ± 1.52 mg/100 g and of 8.60 ± 0.36 mg/100 g. However, for the syrup level, there was a significant difference ($p < 5\%$) with the contents ranged between 2.5% and 4.41%.

DPPH radical inhibition and frap of coconut water's sugars

The assay of the total antioxidant capacity (FRAP) and of the anti-radical capacity (DPPH) of the sugars extracted from the coconut water is presented in Table 2. The results have revealed that the white sugars extracted from all the varieties coconut had the highest antioxidant activity compared to others. The syrup sugar inhibited more of DPPH and iron-free radicals than the brown sugars. For the brown sugars, the percent of inhibition of the DPPH radical was greater in the varieties EGD, $PB121^{+}$ and $PB113^{+}$. The values ranged from 35.09% to 39.64% for white sugars, and from 23.84% to 26.65% for the brown sugars. All the sugar syrups capture with the same capacity the radical DPPH, with the exception of WAT syrup, which had an antioxidant capacity of 19.64%. The variances analysis showed a significant difference ($p = 0.003$) between

Table 1
Flavonoids, total polyphenols, tanins, oxalates, phytates and alkaloids contents.

Phyto-chemical compounds	Sugars	Means ± Standard deviation [Ab]					Statistic data		
		Cultivars			Hybrids		F	P	CV (%)
		WAT	MYD	EGD	PB121+	PB113+	4.22	0.041	30.88
Total polyphenols (mg of galate equivalents/ 100 g)	Syrup	367.80±0.12^{jA}	535.99±0.25 g^{H}	468.35±0.06^{hG}	340.32±0.94^{eK}	437.19±0.61^{iG}	8.22	0.048	25.13
	Brown	361.08±1.03^{dA}	526.22±3.11^{aG}	348.66±0.05^{kL}	360.17±1.28^{dN}	435.53±0.51^{bG}	28.8	0.015	22.87
	White	428.05±2.68^{dB}	581.13±1.81^{aJ}	430.90±1.12^{cF}	437.78±0.13^{cH}	463.38±0.58^{bH}	1.95	0.047	29.01
Flavonoids (mg/100 g)	Syrup	17.78±0.37^{cH}	17.50±0.82^{cL}	18.99±0.71^{cP}	20.84±0.98^{aV}	14.33±0.44^{dB}	44.1	0.039	18.52
	Brown	19.62±0.91^{eI}	20.62±1.33^{eK}	24.17±1.33^{aQ}	24.37±1.54^{aW}	25.95±0.97^{aA}	3.45	0.025	26.75
	White	16.47±0.59^{hH}	20.90±0.21 g^{K}	16.40±1.47^{hR}	16.22± 0.78^{hY}	10.04±0.43^{iC}	18.25	0.001	20.08
Tanins (mg/100 g)	Syrup	35.81±0.65^{nH}	41.44±1.48^{mR}	37.71±0.83^{nM}	36.95±1.06^{nX}	39.90±0.15^{mU}	16.08	0.042	7.89
	Brown	40.00±1.52^{Cj}	42.13±2.06c^{bS}	46.08±1.87^{bL}	45.55±1.55^{bY}	41.46±3.12^{cbU}	46.01	0002	9.58
	White	40.81±0.71^{jJ}	49.93±0.14^{iT}	48.10±0.56 g^{N}	46.21±0.55^{hY}	46.56±0.67^{hV}	17.35	<0.001	14.54
Phytates (mg/100 g)	Syrup	20.98±0.70^{cO}	22.84±0.33^{bR}	24.44±0.62 g^{A}	22.04±0.87^{bR}	23.15±0.60^{bgM}	33.41	<0.001	10.25
	Brown	24.75±0.53^{hP}	24.80±0.65^{hS}	26.95±0.54^{iU}	24.04±0.99^{hX}	24.48±0.51^{hY}	74.15	<0.001	4.89
	White	23.99±0.31^{qP}	23.93±0.24^{qS}	26.28±0.71^{pU}	24.29±0.58^{qX}	24.49±0.50^{qY}	24.12	0.031	15.05
Oxalates (mg/100 g)	Syrup	31.00±1.00^{sD}	32.00±1.00^{sL}	30.00±1.00ts^{F}	31.67±1.55^{sW}	30.66±1.15^{tA}	9.58	0.152	31.85
	Brown	36.67±1.52^{aQ}	33.00±3.42^{bL}	34.00±1.00^{bQ}	33.33±1.53^{bW}	34.00±0.46^{bB}	9.00	0.053	24.45
	White	30.50±1.73^{eD}	24.27±0.65 g^{K}	27.30±0.97^{fM}	25.27±1.18^{fT}	26.56±1.28^{fC}	48.35	<0.001	11.87
Alkaloids (%)	Syrup	3.00±0.12 g^{H}	4.09±0.10^{fQ}	2.50±0.10^{hH}	3.10±0.11 g^{B}	4.41±0.15^{eP}	9.85	0.033	22.12
	Brown	8.60±0.36^{aG}	6.13±0.40^{bK}	7.26±0.99^{abG}	6.13±0.66^{bM}	7.90±0.40^{aS}	19.85	0.021	8.08
	White	3.52±0.25^{nH}	4.23±0.19rs^{Q}	3.55±0.39^{rL}	3.91±0.14^{rB}	4.07±0.06rs^{P}	55.10	0.001	41.15

In each line the means ± standard deviations with same letter indicate that there is no significant difference between the corresponding varieties for the variable considered. In the same column and for the same variable, the means ± standard deviations with different capital letters express a significant difference, F: statistical value associated with the ANOVA 2 test; P: value of the probability of the ANOVA test. CV: Coefficient of variability. WAT: West Africa Tall, MYD: Malaysia Yellow Dwarf, EGD: Equatorial Green Dwarf, PB121+: Port-Bouet 121+, PB113+: Port-Bouêt 113+.

Tabe 2
Anti-radical capacity (DPPH) and total antioxidant capacity (FRAP) of coconut water sugars.

Methods and sugars		Antioxidant activities by DPPH (%) and FRAP (mg Trolox/100 g)					
		WAT	MYD	EGD	PB121+	PB113+	*P*-value
White	DPPH	35.96±0.24^{hD}	35.09±1.15^{dP}	38.87±0.57^{bN}	37.89±0.51^{bM}	39.64±1.79^{bY}	0.001
Syrup		19.64±0.78^{Ik}	26.89±0.05^{hG}	25.84±0.45^{hC}	26.56±1.22^{hL}	26.78±0.38^{hF}	0.003
Brown		25.76±0.57^{aI}	23.88±1.35^{cB}	25.89±0.50^{aC}	23.84±0.70^{aP}	26.65±0.57^{aF}	0.047
White	FRAP	35.58±0.52^{hQ}	40.76±0.06^{hR}	43.06±0.43^{fY}	35.79±0.07^{hP}	55.58±0.57^{eU}	0.001
Syrup		10.43±1.95^{tS}	12.81±0.11^{sF}	16.79±0.75^{pG}	16.75±0.15^{rL}	26.48±0.62^{qC}	0.000
Brown		4.59±0.08^{fD}	6.76±0.06^{eT}	6.63±0.13^{eN}	12.64±0.25^{dB}	14.79±0.17^{cZ}	0.011

DPPH: 1, 1-diphenyl-2-picrylhydrazyl; FRAP: Ferric Reducing Ability of Plasma, measures the reducing power of iron expressed in mg Trolox/100 g. Ina line, the means ± standard deviations that have the same lowercase letter in superscript are statistically identic to 5% threshold. In a column and using the same method, the means ± standard deviations that have the same capital letter in superscript are statistically identic to 5% probability threshold.WAT: West Africa Tall, MYD: Malaysia Yellow Dwarf, EGD: Equatorial Green Dwarf, PB121 +: Port-Bouët 121+ improved, PB113+: Port-Bouët 113 improved.

the antioxidant activity in sugars and syrups. Indeed, the highest activities (table 2) were observed in syrups of the dwarf cultivars MYD and EGD and of the hybrids PB121+ and PB113+.

The reducing power of iron is higher in the white sugar than in the syrup, which has also a high activity compared to the brown sugar. In fact, in the white sugar, the values of the antioxidant activity ranged from 35.58 mg Trolox/100 g to 55.58 mg Trolox/100 g, while the syrup sugar gave values ranged between 10, 43 and 26.48 mg Trolox/100 g. In addition, the values were even lower (4.59 to 14.79 mg Trolox/100 g) in the brown sugar of the coconut water; the highest proportions were determined in the brown sugar of the two hybrids PB121+ and PB113+ (12.64 and 14.79 mg Trolox/100 g respectively). In this case, the two dwarf coconut trees have activities that are statistically similar but different from the other cultivars studied (Table 2).

Intermediate and final products of the non-enzymatic browning

The variations of the intermediates (glycosylamine, cetosamine or aldosamine) and of the advanced products (melanoidin) of the non-enzymatic browning are presented in Fig. 1. Whatever the cooking method used, the primary products (glycosylamine, cetosamine) and secondary are increased linearly and significantly ($r > 0.76$, $p < 0.001$) with the cooking time. Thus, the PB121+ and PB113+ hybrid and the tall WAT had the highest degree of browning, while the sugars of the two dwarf ecotypes had the lowest browning. The browning observed during the cooking in the 5 varieties is rather of the non-enzymatic type. Upstream, the biochemical analysis has shown that the sugars of the PB121+, PB113+ and WAT ecotypes had significant levels of total phenols (300–500 mg of gallic acid/100 g). A significant linear correlation ($r = 0.86$,

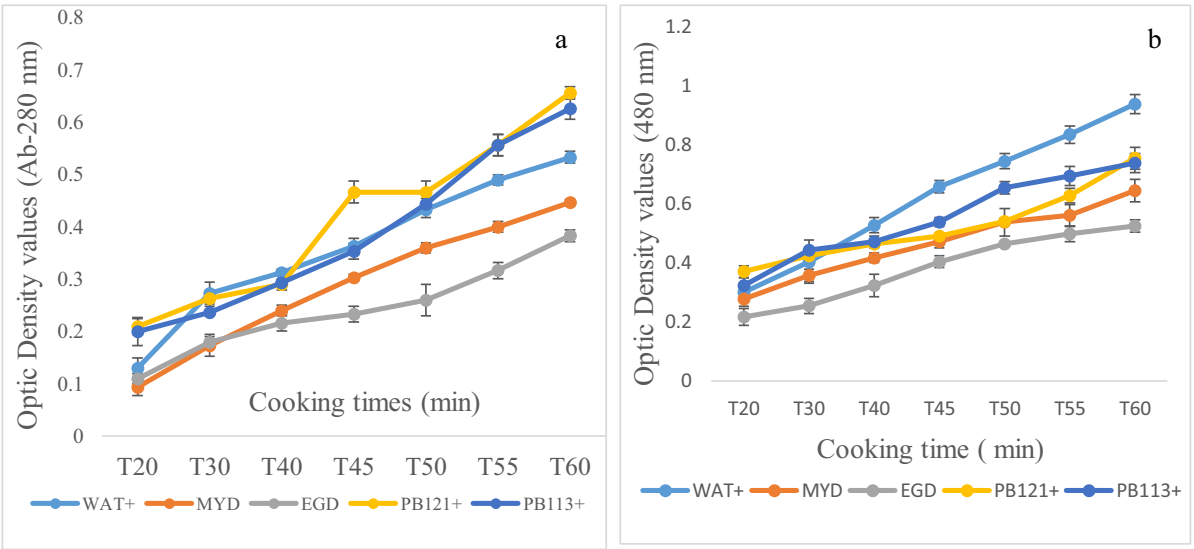

Fig. 1. Non-enzymatic browning degree.

Table 3
Carbon/Nitrogen ratios and energy values (kcal/100 g) of sugars.

Sugars		Cultivars			Hybrids	
		WAT^{+}	MYD	EGD	PB121^{+}	PB113^{+}
Syrup	C/N	2.78 ± 0.32^{Ay}	3.38 ± 0.45^{Wu}	3.38 ± 0.15^{Tu}	4.23 ± 0.09^{Qh}	2.22 ± 0.02^{Sd}
	EV	170.21 ± 0.7^{De}	182.08 ± 0.06^{Hf}	164.46 ± 0.10^{Fg}	170.91 ± 0.05^{Ge}	151.42 ± 0.51^{Gi}
Brown sugar	C/N	2.96 ± 1.51^{Ar}	3.90 ± 0.50^{Ff}	3.62 ± 0.08^{Vf}	4.57 ± 0.03^{Wt}	2.29 ± 0.04^{Sh}
	EV	171.71 ± 0.8^{Da}	186.36 ± 0.40^{Bp}	173.06 ± 0.61^{Ga}	173,39 ± 050^{Ha}	154.46 ± 0.70^{Tz}
White sugar	C/N	3.48 ± 0.09^{Ba}	4.29 ± 0.05^{Rb}	3.96 ± 0.005^{Rb}	4.30 ± 0.06^{We}	3.31 ± 0.01^{Hd}
	EV	150.47 ± 0.5^{Eb}	168.35 ± 0.06^{Zc}	156.46 ± 0.83^{Yd}	135.58 ± 0.72^{Re}	134.04 ± 0.63^{Pe}

In the same line, the means ± standard deviations that have the same Lower case letter in superscript are statistically identic to 5% threshold. In the same column the means ± standard deviations that have the same capital letter in superscript are statistically identic to 5% probability threshold. C/N: carbon/nitrogen, EV: Energy Value. WAT: West Africa Tall, MYD: Malaysia Yellow Dwarf, EGD: Equatorial Green Dwarf, PB121^{+}: Port-Bouet 121^{+} improved, PB113^{+}: Port-Bouët 113 improved.

$p < 0.05$) was observed between the total phenol content and the degree of browning of sugar during the cooking. In addition, a significant correlation was observed between the protein groups and the degree of browning. Moreover, there was a significant linear correlation ($r = 0.91$, $p < 0.05$) between the degree of browning and the reducing sugar content. In any case, it appeared that the browning was weak in the sugar of the dwarf ecotypes compared to the hybrids.

Carbon/nitrogen ratio and energy value of sugars in water of immature coconut

Table 3 shows the carbon/nitrogen ratio and the energy values of the sugars. The nitrogen/carbon ratios of the sugars are greater than 1. In fact, the values were ranged between 2.22 (PB113^{+}) and 4.23 (PB121^{+}) in the syrups, while those of the brown sugars were between 2.29 and 4.57. The white sugars values ranged from 3.31 (PB113^{+} hybrid) to 4.30 (PB121^{+} hybrid). The analysis of variance (ANOVA 2) has showed that there was a significant difference between the ratios of each sugar of the varieties studied. However, two coconut trees (EGD and MYD) provided statistically identical ratios for all the three categories of coconut water's sugars. For the energy value, it was between 151.42 and 182.08 kcal/100 g DM for the sugar syrup. The lowest energy values were determined in the hybrid sugar PB113^{+}, while the Malaysia Yellow Dwarfs (MYD) has the highest energy value in the different sugar categories. In addition, the energy value of the syrup derived from the water of the variety WAT, is statistically identical to that provided by the syrup of the hybrid PB121^{+}.

Discussion

Bioactive compounds were measured in various sugars extracted from the coconut water. The total polyphenols contents values were more significant in the brown sugars for all the studied coconut varieties. The differences between the sugars derived from the water of the coconut are statistically significant. The high level of total polyphenols was provided by the sugars of MYD and PB113^{+}, regardless of the sugar category. The observed differences could be attributed to the variety and to the phenolic compound composition of the raw sap. These differences in the variety are related to the genotypic

differences of the coconut trees, as shown by several researches. The varieties MYD, EGD, PB121$^+$ and WAT have biochemical and phytochemical compounds at different levels according to previous studies conducted on walnuts of several varieties, by Assa et al. [31], Prades et al. [32] and Kodjo et al. [33]

The flavonoids which are important antioxidant agents protect the biological macromolecules against the degradation [34]. They are more abundant in white sugars than in the syrups of coconut water. For instance, the WAT and MYD syrup have similar levels of flavonoids, while the other three varieties (EGD, PB121$^+$ and PB113$^+$) showed a significant difference. The similarities or the differences between the sugars could closely related to their composition in compounds less or non-polar. Parsa et al. [35] highlighted that the flavonoids have higher affinity links with the polar compounds which facilitates their total evaluation in food. For this reason, the white sugars of PB113$^+$, followed by those of the PB121$^+$ and the EGD varieties would be more filled with polar compounds such as steroidal fatty acids compared to the other two varieties. Flavonoids resulted from the coconut water sugars are below of the rates that were reported by Trinidad et al. [36] on brown sugars from coconut trees sap syrup.

According to Sefa-Dédeh and Agyir-Sackey [37], the distribution of the compounds such as oxalic and phytic acids in the plant kingdom varies from one family to another. In addition, the phytate compounds are able to form protein-phytate complexes during the transforming process of coconut water into sugar. This could justify the difference observed between the EGD sugars and those of the other coconut cultivars considered in the present study. In addition, the low phytate levels recorded by the syrups are related to the binding interactions between the phytates and the proteins, generated by the temperature/time couple during the heat treatment. This is in accordance with the work of Zaidi et al. [38], which states that excessive heat and alkaline treatments cause interactions between the phytates and the proteins, making their total evaluation difficult. The syrup was obtained after 10 min of heating at 60 °C after the brown sugar stage, hence the possibility of binding between these metabolites and the proteins which caused these low doses of phytates compared to the brown and white sugars. In contrast to phytates, the high levels of oxalates in sugar syrups and white but moderately low in brown sugars, were due to the fact that most of the oxalic acid in the plant is like of soluble complexes sodium and potassium oxalates. Also, a good correlation exists between the cooking treatment and the total oxalate contents. Losses of oxalate ranged between 20 and 26% could probably be attributed to the solubilization of certain quantities of soluble oxalates contained in coconut water under the effect of heat.

Alkaloids are cyclic compounds containing one or more nitrogen atoms. The result of the present study on this aspect confirms those of Dewick [39] who stated that the biological activity of alkaloids often depends on the amine function. This amine function is transformed into a quaternary system by the ionization at physiological pH. For brown sugars, the analysis of the ANOVA variance showed that there is no significant difference ($p > 0.5$) between the alkaloid contents. At this level too, there is a halving of the alkaloid rate following the application of heat which has made it possible to obtain the syrups compared with the white sugars. The decrease in the level of alkaloid compounds in brown sugar and syrup is explained by the heating effect which degrades the total nitrogen in the sugars and consequently, alkaloids which are also nitrogen compounds.

The antioxidant capacity of food products is largely dependent on its phytochemical composition as well as conditions for handling of in vitro tests. The antioxidant capacity was evaluated according to the two methods, namely the iron reducing power (FRAP) method and that of the DPPH (1, 2-diphenyl-1-picrylhydrazyl) free radical scavenging. The results showed that the white sugars from the coconut water of cultivars of PB113$^+$, WAT and PB121$^+$ give the more important anti-radical activity. A significant difference ($p < 0.05$) was also observed in the syrups and the brown sugars. Indeed, the trapping powers of the DPPH radical are almost similar in all syrups except the WAT syrup which has a lower activity. Concerning the brown sugar, results of the anti-radical capacity differentiate the whole of varieties by forming two groups. The similar result was observed by Heignem et al. [40] who have shown that the responsibility for the reducing powers of a substance linked to hydroxyl groups in phenolic compounds and flavonoids. In other words, the significant differences in antioxidant activity can be explained by contents of polyphenol, flavonoid and alkaloid, unequally distributed in the studied sugars. Polyphenol compounds are involved in cardiovascular health through their vasodilatory and anti-inflammatory effects [40]. Like vegetable leaves such as cabbage [2], fruits such as orange, lemon, grapefruit [2,6], sugars extracted from coconut water contain significant amounts of polyphenol compounds and other phytochemicals that are anti-carcinogenic and anti-inflammatory.

Conclusion

Phytochemical compounds of sugars were extracted from the immature coconuts, and the degree of the non-enzymatic browning was evaluated. It may be noted that the sugars extracted from the coconut water were sources of polyphenol compounds that confer important antioxidant activities. The intermediate and final levels of the non-enzymatic browning (Maillard reaction) do not distinguish the sugars from the immature coconut water consumed. Instead, they have given to various sugars, a pleasant color and smell. The consumption of these sugars extracted from the immature coconut water could have a positive impact on cardiovascular diseases and protect against the free radicals and the oxidative stress.

Declaration of Competing Interest

The authors certify that they have NO affiliation with any organization or entity having a financial or non-financial interest in the subject or material discussed in this manuscript. There is no conflict of interest on this work.

CRediT authorship contribution statement

G.A. Gbogouri: Writing - review & editing. **B.R. Konan:** Writing - review & editing. **K.J.L. Konan:** Writing - review & editing.

Acknowledgment

The authors would like to thank the Biocatalysis and Bioprocesses Laboratory of Nangui Abrogoua University for their technical support. Our particulars thanks are addressed to DISSEKA kwitony Williams for his technical assistance to the research, and to Dr. OUSSOU N'Guessan Baptiste, Dr. DIAGOU Jean-Baptiste and Mr YEPIE Haudrey for her assistance in English translation and them helping in revision. This study has also been funded by the IDRC–CANADA and the CNRA in Côte d'Ivoire.

References

[1] S. Lorraine Cordeiro, The role of African indigenous plants in promoting food security and health, Am. Chem. Soc. 1127 (2013) 273–287.
[2] G.D. Pamplona-Roger, Guide des aliments et leur pouvoir curatif, Bibliothèque Education et Santé 1 (2011) 159–179.
[3] Y.J. Bae, Y-K. Bak, B. Kim, M-S. Kim, J-H. Lee, M-K. Sung, Coconut-derived D-xylose affects postprandial glucose and insulin responses in healthy individuals, Nutr. Res. Pract. 5 (6) (2011) 533–539, doi:10.4162/nrp.2011.5.6.533.
[4] A.L. Anselme, G.G. Albarin, K.K. Jean-Louis, G.J. Brice, N.G. Jean, Glycemic index of sugars extracted from immature coconut water: case of coconut palms (*Cocos nucifera* L.) WAT, myd and PB121^{+}, J. Biosci. Med. 6 (2018) 99–110, doi:10.4236/jbm.2018.68008.
[5] N. Satheesh, N.B.L. Prasad, Producton of fermented coconut water beverages, Asian J. Food Agro-Ind 6 (05) (2013) 281–289.
[6] G.M. Cole, G.P. Lim, F. Yang, B. Teter, A. Begum, Q. Ma, M.E. Harris-White, A. Frautschy, Prevention of Alzheimer's disease: omega-3 fatty acid and phenolic anti-oxidant interventions, Neurobiol. Aging 26 (2005) S133–S136.
[7] R.H. Liu, Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals, Am. J. Clin. Nutr. 78 (2003) 517S–520S.
[8] E. Riboli, T. Norat, Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk, Am. J. Clin. Nutr. 78 (2003) 559S–569S.
[9] E. Middleton Jr., C.T. Kandaswami, Potential health-promoting properties of citrus flavonoids, Food Technol. 48 (1994) 115–119.
[10] G. Mazza, Anthocianins in grapes and grape products, Crit. Rev. Food Sci. Nutr. 35 (1995) 341–371.
[11] M.H. Adamu, J.O. Abayeh, O.M. Agho, L.A. Abdullahi, A. Uba, U.H. Dukku, M.B. Wufem, An ethnobotanical survey of bauchi state herbal plants and their antimicrobial activity, J. Ethnopharmacol. 99 (2005) 1–4.
[12] S.P. Leite, J.R.C. Vieira, P.Y.S. Medeiros, R.M.P. Leite, V.L.M. Lima, H.S. Xavier, E.O. Lima, Antimicrobial activity of *indigoferasuffruticosa.* evid. based complement, Altern. Med. 3 (2) (2006) 1–5.
[13] B. Singh, B.K. Chandan, N. Sharma, V. Bhardawaj, N.K. Satti, V.N. Gupta, B.D. Gupta, K.A. Suri, O.P. Suri, Isolation, structure elucidation and in vivo hepatoprotective potential of trans-Tetracos-15-enoic acid from *indigofera*tinctorialinnee, Phytotherapyresearch 20 (2006) 831–839.
[14] Bakasso Sahabi, Etudes Phytochimiques Et Potentialités Biologiques De Cinq Espèces D'i*ndigofera (FABACEAE)* Utilisées En Medecine Traditionnelle Au Burkina Faso. Thèse unique De Doctorat, Université de Ouagadougou, 2009, p. 54.
[15] L. A. Anselme, K. K. Jean-Louis, G. G. Albarin, B. K. Roger, Y. S. Didier, Physicochemical coconut water assessment and of the microbiological quality of its sugar extracted of five coconuts ecotypes at the marc delorme station, côted'ivoire, Int. J. Appl. Biol. Pharm. 1 (9) (2018) 24–31.
[16] V.L. Singleton, R. Orthofer, R.M. lamuela-Raventos, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, Methods Enzymo 299 (1999) 152–178.
[17] A. Meda, C.E. Lamien, M. Romito, J. Millogo, O.G. Nacoulma, Determination of the total phenolic, flavonoid and proline contents in burkina faso honey, as well as their radical scavenging activity, Food Chem. 91 (2005) 571–577.
[18] R.A. Day, A.L. Underwood, Quantitative Analysis, 5, Porentice Hall Pub, 1986, p. 701.
[19] INRA, Importance Des Phytases Et Phytates Dans L'alimentation Des Monogastriques, 7, POINTILLART A. 1994. INRA, PARIS, 2009, pp. 29–39.
[20] J.B. Harborne, Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, 1973, pp. 221–232.
[21] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of Free Radical Method to Evaluate Antioxidant Activity, 28, Lebensmittel-Wissenschaft und-Technologie, 1995, pp. 25–30.
[22] W. Choi Chang, K. Choi Bong, C. Sei Kim, S.H. Soon wang, J. HyeAhn, Y. Min Lee, K. Soo Kim, Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison, Plant Sci. 163 (6) (2002) 1161–1168.
[23] G.C. Yen, P.O. Duh, Scavenging effect ofmethanolic extracts of peanut hulls on free radical and active oxygen species, J. Agric. Food Chem. 42 (1994) 629–632.
[24] F.F. Benzie Iris, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power": the frap assay, AnalyticalBiochemistry 239 (1996) 71–77.
[25] K. N'guessan Ysidor, Evaluation de la production et caractérisations biochimique et sensorielle de la sève de quatre (4) cultivars de cocotier (Cocos nucifera L.) en côte d'Ivoire, Thèse de Doctorat en Biochimie-Science des aliments de l'Université de Cocody, Côte d'Ivoire, 2015, p. 47.
[26] U.D. Akpabio, A.E. Akpakpan, U.E. Udo, U.C. Essien, Physicochemical characterization of exudates from raffia palm (Raphiahookeri), Adv. Appl. Sci. Res. 3 (2) (2012) 838–843.
[27] AFNOR, Recueil De Normes françaises, Corps gras, Graines oléagineuses, Produits Dérivés, AFNOR Ed., Paris, France, 1986, p. 157.
[28] J.J. Sedmak, S.E. Grossberg, A rapid, sensitive, and versatile assay for protein using coomassie brilliant blue G250, Anal. Biochem. 79 (1977) 544–552.
[29] M. Dubois, K. Gilles, J. Hamilton, P. Rebers, F. Smith, Colorimetric method for the determination of sugars and related substances, Anal. Chem. 280 (1956) 350–356.
[30] AOAC, Official Methods of Analysis, 15th Edition, Association of Official Analytical Chemists, Arlington, 1990.
[31] R.R. Assa, J.L. Konan, N.G. Agbo, A. Prades, J. Nemlin, Caractérisation physicochimiques de l'eau des fruits de quatre cultivars de cocotier (*Cocos nucifera* L.) en côte d'Ivoire, Agronomie Africaine 19 (2007) 7–14.
[32] A. Prades, M. Dornier, N. Diop, J.P. Pain, Coconut water uses, composition and properties: a review, Fruits 67 (2012) 87–107.
[33] N.F. Kodjo, J.L.K. Konan, G.D. Ginette, S. Yao, A. Kouassi, N. Sébastien, Caractéristique physico-chimique des composantes de noix immature et mature de l'hybride de cocotier (*cocos nucifera L.*) nain jaune de malaisie x grand vanuatu cultivé en côte d'Ivoire, J. Animal Plants Sci. 27 (1) (2015) 4193–4206.
[34] Q. Xia, R. Li, S. Zaho, W. Chen, H. Chen, B. Xin, Y. Huang, M. Tang, Chemical composition changes of postharvest coconut inflorescence sap during natural fermentation, Afr. J. Biotechnol. 10 (66) (2011) 14999–15005.
[35] D. Parsa, F. Muhammad, D. Amara, K. Zaman, M. Rebecca, R. Amina, W. et Usama, Evaluation of antioxidant potential and comparative analysis of antimicrobial activity of various extracts of cucurbita pepo L. leaves, J. Agric. Sci. Food Technol. 3 (6) (2017) 103–109.
[36] P.T. Trinidad, C.M. Aida, S.S. Rosario, R.E. Rosario, Glycemic index of commonly consumed carbohydrate foods in the Philippines, J. Funct. Foods 2 (2010) 271–274.

[37] S. Sefa-Dedeh, K.E. Agyir-Sackey, Chemical composition and the effect of processing on oxalate content of cocoyam *xanthosoma sagittifolium* and *colocasia esculentacormels*, Food Chem. 85 (2004) 479–487.
[38] F. Zaidi, N. Hassissene, H. Allouache, M. Kichou, S. Ourdani, K. Rezki, M.M. Bellal, J. Li, J. Liu, S. Tao, Effects of tannic acid on the food intake and protein digestibility of root voles, ActaTheriologicaSinica 23 (1) (2003) 52–57.
[39] P.M. Dewick, Medicinal Natural Products: A Biosynthetic Approach, Edition 2, John Wiley & Sons, 2002, pp. 291–398.
[40] C.G.M. Heignem, G.R.M.M. Haenon, J.A.J.M. Vekemans, A. Bast, Peroxynitrite scavenging of flavonoids: structure activity relationship; environ, Toxicol. Pharmacol. (10) (2001) 199–206.