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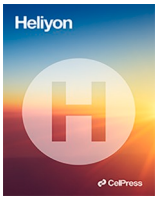

Research article

Physicochemical, nutritional, antioxidant properties and stability monitoring of coconut (*Cocos nucifera* L.) water from two localities in Cameroon

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ABSTRACT

The nutritional value of a food is linked to the quality and quantity of the nutrients it contains. It offers a major advantage in establishing a food table composition (FTC) which is a tool that provides information on the quantity of nutrients contained in a food. Furthermore, certain natural beverage are not taken into account in the FTC. This is the case of the water of *Cocos nucifera* nuts, although widely consumed around the world. The aim of this study was to valorise the water of *Cocos nucifera* nuts (mature and mid-mature) to contribute to the enrichment for the cameroonian FTC. Physicochemical, nutritional and antioxidant parameters were assessed by standard methods. Physicochemical analyses showed that mid-mature nuts from Edea and Bafia, have respectively an average of 0.2 ± 0.04 and 0.19 ± 0.05 mL of water/g of nut, with soluble dry extract of 4.3 ± 0.28 and 5 ± 0.0°B for a pH of 5.01 ± 0.01 and 5.11 ± 0.0. The total titratable acidity was of 0.113 ± 0.0 and 0.117 ± 0.0 mg citric acid per 100 mL water. The mean contents of total and reducing sugars, proteins, free amino acids and lipids in the same samples were 5.49 ± 0.05 and 5.56 ± 0.04; 5.09 ± 0.6 and 4.99 ± 0.7; 0.12 ± 0.0 and 0.15 ± 0.0; 0.06 ± 0.0 and 0.09 ± 0.0; 0.07 ± 0.0 and 0.10 ± 0.0g/100 mL of water, respectively. These data showed that from mid-maturity to full maturity, there was a significant increase ($p < 0.05$) in pH, lipid, protein, free amino acid, and phenolic contents and decrease in water volume, total titratable acidity, total and reducing sugars contents. In general, mineral contents increased significantly ($p < 0.05$), while total antioxidant power decreased with maturity. As for stability, degradation processes are more intense at room temperature than in the refrigerator.

1. Introduction

Due to the wide range of food products, adopting a balanced and diversified diet would not be possible without the use of food composition tables. This is a tool that provides information on the amount of energy, macronutrients and micronutrients contained in foods [1]. In the 20th century, methods of analysis and development of food composition tables were improved in many countries. In addition, the founding of INFOODS (International Network of Food Data Systems) in 1984 provided a major breakthrough for food composition tables, whose data are used for research on diet, health, reproduction, growth, and development. Nationally and

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internationally, food composition data are used to assess the nutritional value of foods consumed at the individual and population levels [2]. Nutrient levels in foods vary from country to country due to differences in cultivars, soils, climate, agricultural practices, and recipes for compound dishes. Therefore, each country should have a program designed for its own food composition data [3]. Although Cameroon has an institution in charge of developing food composition databases, it only has an inventory of about 342 foods with known nutritional composition. This food composition database essentially considers only cooked and commonly consumed foods, while omitting non-conventional food resources which are generally unprocessed products such as wild fruits and vegetables, plant nectars, and natural beverages [4]. According to the FAO, several non-conventional food sources are consumed worldwide and are particularly rich in minerals and vitamins and sometimes confer high amounts of calories to the body. They are normally consumed fresh, as a snack or as a complementary food or beverage [5]. Indeed, there are many sources of natural beverages including *Cocos nucifera* water. *Cocos nucifera* L. is an exotic fruit of the *Cocos nucifera* palm. It is a tropical perennial plant native to Southeast Asia [6]. Indeed, the water and kernel are the edible and economically profitable parts of the coconut [7]. In immature nuts, the water constitutes a delicious and nutritious drink, consumed in the tropics for its high content of sugars, minerals and vitamins. When mature, it loses its delicious taste to the kernel [8]. Studies indicate that regular consumption of coconut water controls blood pressure in hypertensive patients [9]. Coconut water is used in many countries as an oral rehydration solution, for the treatment of cholera, gastroenteritis and childhood diarrhea [10]. It is also an excellent drink for diabetics due to its ability to regulate blood sugar [11]. Similarly, Campbell-falck et al. [12] showed that this water could be used as intravenous rehydration fluid in patients living in rural areas. In Ivory Coast, Coulibaly et al. showed that coconut water is mainly consumed for its fortifying, digestive and refreshing properties. Coconut water is also good sources of nutrients and bioactive compounds. These phytochemical and biochemical characteristics depend on the variety of coconut [13]. Other study in Sry Lanka show that water of different variety of tender coconut have antioxidant properties [14]. All these observed activities depend largely on the macro- and micronutrient composition of coconut water, which itself differs according to the country or locality where the nuts are harvested. The objective of this study was to evaluate the nutritional composition, antioxidant capacity and stability of coconut water from two localities in Cameroon (Edea and Bafia).

2. Methods

2.1. Plant material

The plant material consisted of coconut water of hybrid variety N°PB111 (Cameroon Red Dwarf x WAT) obtained from coconuts aged six months (semi-mature nuts) and 12 months (mature nuts) after emergence of the inflorescence. They were collected in the cities of Bafia and Edea which represent two areas of intense coconut production in Cameroon.

2.2. Sampling method

In this study, coconuts of two different stages of maturity were used. In September 2020, we collected five randomly selected coconut trees from five areas and per city, five mid-ripe nuts (six months) which is the most common stage of coconut water consumption and five mature nuts (twelve months) each, at a rate of 25 nuts per area and per stage of maturity. The harvested fruits were carefully transported to the Food Sciences and Metabolism laboratory of the University of Yaounde 1 where water extraction was carried out. We obtained 25 samples for each stage of maturity.

2.3. Extraction of Cocos nucifera water

Fresh water of mature *Cocos nucifera* was obtained after opening the hard exterior of the fruit. The fleshy part was then carefully opened at one end and the water was poured into a beaker. For *Cocos nucifera* mid-maturity, water was obtained after trimming the top end of the fruit following the protocol described by Prades et al. [15]. Water volume in each nut was taken and yields was estimated based on nut mass.

2.4. Stability monitoring of Cocos nucifera water

For stability monitoring, a 50 mL sterile syringe was inserted into the perforation and the aspirated water was poured directly into 50 mL Falcon tubes. One and a half litres of freshly extracted commercial coconut water (noted sample X) was purchased from a nut seller in the city of Yaounde to compare its stability to collected samples.

2.5. Physicochemical analyses

2.5.1. Measurement of pH and total titratable acidity

The pH was determined using a pH meter (Hanna Instruments) according to the method described by AOAC [16]. The total titratable acidity was determined by titration of acids contained in 10 mL of *Cocos nucifera* water by NaOH (0.1N) using phenolphthalein at a pH 8.1 [16]. The total titratable acidity was expressed as gram of citric acid per 100 mL of coconut water.

2.5.2. Measurement of brix degree (°B)

Soluble solids concentration was measured using a pocket pal 1 refractometer (Atago, °B±0.1 %, Japan) and expressed as a

percentage (°Brix). Before taking readings, the refractometer was normalized with distilled water and adjusted to a reading of 0°brix. Two drops of coconut water were placed on the refractometer's glass prism and the reading recorded. The reading was corrected to a standard temperature of 20 °C by adding 0.28 % to obtain the % SSC at 27 °C. SSC was determined according to the following formula: SSC% = (refractometer reading × dilution factor) + 0.28 [17].

2.5.3. Proximal analysis of water from Cocos nucifera nuts

Dry matter contents were determined by steaming at 105 °C. A 10 mL volume of coconut water was introduced into an aluminum container and the whole was weighed M1 and oven-dried at 105 °C until a constant mass was obtained. Each time the samples were removed from the oven, they were placed in a desiccator for cooling, to prevent reabsorption of moisture from the air prior to weighing. After drying, the total dry residue or dry matter (DM) was weighed (M2), and the dry matter was calculated.

Total lipids were determined by differential solubility in hexane using liquid-liquid extraction method. Three consecutive extractions were carried out. For the first extraction, 20 mL of hexane were added to 20 mL of coconut water, the mixture was then vigorously shaken and allowed to settle. To 20 mL of the decantate from the first extraction were added 10 mL of hexane for the second extraction. The same operation was repeated for the third extraction. After lipid extraction, the hexane was evaporated on a rotary evaporator. The tared extraction flask was dried in an oven at 105 °C for 30 min. At the end of this operation, the lipid vial was weighed. At the end of this operation, the lipid vial was weighed.

Total sugar content was determined by the phenol colorimetric method after extraction. Briefly, 0.5 ml coconut water was added to a test tube; 10 ml 1.5 N sulfuric acid was added and sealed with a stopper fitted with an exhaust tube. The mixture was boiled for 15 min and allowed to cool to room temperature. Next, 10 ml 70 % ethanol, 0.5 ml 2 % zinc sulfate and 0.5 ml 10.6 % potassium ferrocyanide were added and the resulting mixture filtered into a 50 ml flask. For total sugar estimation, 1.0 mL the solution was mixed with 1.0 mL of 5 % phenol and 1.0 mL of distilled water for 1 min; 5.0 mL of concentrated H_2SO_4 was then added, shaken for 3 min. After settling down for 30 min, the resulting solution was cooled by water for 20 min and then measured at 480 nm by UV–Vis technique using a spectrophotometer (ZUZI/421150). The blank sample was prepared in the same procedure without coconut water. The concentration of total sugar was calculated using calibration curve obtained by replacing coconut water with D-glucose (10–80 μg range).

Reducing sugars was determined by the DNS colorimetric. Reducing sugars was extracted from 1 ml coconut water, 20 ml distilled water. the mixture was boiled for 1h; then cooled. defecate with 0.5 ml zinc sulfate (0.02 g/ml) and 0.5 ml potassium ferrocyanide (0.106 g/ml) then filtered into a 50 ml flask. 2 ml of solution was mixed with 1.5 ml DNS solution and 6.5 ml distilled water for 1 min, then heated to 80 °C over a water bath for 10 min and cooled with water. The resulting solution was measured at 540 nm using a spectrophotometer (ZUZI/421150). The blank sample was prepared using the same procedure.

The crude protein content was obtained after mineralization of the samples according to the Kjeldahl method [16]. After sulfuric mineralization of 1 mL in the presence of a catalyst, the ammoniac generated was neutralized by NaOH ($CuSO_4$). After that, the ammonia in the coconut water sample was distilled out of the boric acid till it turned a blue green color. After titration with 0.1 N HCl solutions, the distillate was colorless. The percent total nitrogen and crude protein were calculated using a conversion factor of 6.25.

Total free amino acid content was determined by the method of Kendall (1963) In a test tube, 100 μL of coconut water was taken and 10 mL of 80 % butan-1-ol added. The mixture was left at room temperature for 12 h 200 μL of the solution was taken to which 1 mL of freshly prepared ninhydrin and 2 mL of 80 % ethanol were added. The blank consisted of 200 μL of distilled water. The mixture was heated at 100 °C for 10 min. After cooling, absorbances were read at 550 nm using a spectrophotometer (ZUZI/421150) [18].

The vitamin C content of coconut water was determined by direct titration with iodine. Each 5 mL of fresh plant juice was transferred to a 50 mL Erlenmeyer flask. Five millilitres of 2 N sulfuric acid was added, mixed, diluted with 5 mL water and 0.6 mL T S. starch was added as an indicator. The solution was directly titrated with 0.1 N iodine previously standardized with standard primary arsenic trioxide. A blank titration was performed prior to titration of each sample (n = 5). Each mL of 0.1 N iodine is equivalent to 8.806 mg ascorbic acid [19].

Minerals (Ca, Mg, Na, K and Fe) were analyzed by atomic absorption spectrophotometry [16].

2.5.4. Evaluation of total phenolic compounds

Total phenolic compounds were extracted and quantified by the Folin-Ciocalteu colorimetric method described by Gan et al. [20]. In fact, to 50 μL of each *Cocos nucifera* water sample, 2 mL of 2N Folin-Ciocalteu reagent was added. After shaking and 5 min of incubation, 2 mL of 10 % (p/v) sodium carbonate solution was added. The whole set was incubated at room temperature and in the dark for 120 min. The absorbance of the blue colour of the resulting solutions was read at 765 nm against the blank using a spectrophotometer (ZUZI/421150). The total phenolic compound contents of each sample were determined using gallic acid as a standard and were expressed as mg GAE/mL *Cocos nucifera* water.

2.6. Assessment of antioxidant potentials in vitro

2.6.1. Assessment of the total antioxidant capacity (TAC)

This was done by the method described by Prieto et al. [21]. For this purpose, 200 μL of each *Cocos nucifera* water sample were introduced into the 5 mL test tubes and mixed with 2000 μL of a reagent consisting of H_2SO_4 (0.6 M), Na_2PO_4 (28 mM) and ammonium molybdate (4 mM). The tubes were then closed and incubated at 95 °C for 90 min. The absorbance was measured at 695 nm against the blank after cooling using a spectrophotometer (ZUZI/421150). The blank and control tubes consisted of 200 μL of distilled water and 200 μL of gallic acid (0.02 mg/mL) respectively mixed with 2000 μL of the above reagent. TAC was expressed as mg gallic acid

equivalent per mL *Cocos nucifera* water (mg GAE/mL).

2.6.2. Evaluation of the reducing power of ferric ions (FRAP)

The antioxidant potential of the different extracts was evaluated by their capacity to reduce iron III and iron II following the method described by Benzié and Strain [22]. Briefly, the FRAP reagent was prepared from 20 mmol/L iron(III) chloride solution, 10 mmol/L TPTZ solution in 40 mmol/L HCl and 300 mmol/L sodium acetate buffer (pH 3.6) in a volume ratio of 1:1:10, respectively. 0.1 mL of each *Cocos nucifera* water sample, 3 mL of freshly prepared FRAP reagent was added, and then the mixture was incubated 5min of incubation at room temperature and in the dark. The absorbance of the reaction medium was read at 593 nm against the blank. The overall antioxidant potential was determined from the calibration line using the regression equation of the calibration line. Antioxidant potential was expressed as mg Fe (II)/mL *Cocos nucifera* water. Gallic acid was be used as standard.

2.6.3. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test

The method described by Lopes-Lutz et al. [23] was used to evaluate the ability of different *Cocos nucifera* water samples to trap the DPPH radical. One millilitre of different diluted samples at 10 %, 20 %, 30 %, 40 % and 50 % was added in 3 mL of methanolic solution of DPPH 0.3 mM. After homogenization, the whole was placed in the dark for 30 min at room temperature. At the end of this time, the absorbance was measured with a spectrophotometer at 517 nm against the blank composed of the methanol/water mixture (80 %). Gallic acid was used as standard. The control consisted of a methanolic solution of DPPH (0.004 %) and the methanol/water mixture (80 %). The percentage of DPPH radical inhibition (I%) was calculated using the following formula:

$$\text{Percentage of inhibition (I\%)} = \frac{(\text{Abs of control} - \text{Abs of sample})}{(\text{Abs of control})} \times 100$$

where: Abs is absorbance.

Afterwards, a curve of % DPPH was plot using OriginPro 8 software and IC50 value was obtained. The scavenging activity was expressed as IC_{50} (mL of coconut water/mL).

2.7. Monitoring the stability of Cocos nucifera water

Immediately after extraction, the physical and chemical parameters (pH, Brix degree, sugar, lipid and protein contents) were measured and 16 tubes for each sample were placed at 4 °C and 6 were left at room temperature. The turbidity of the water (by observation of the cloudy appearance, the appearance of air bubbles in the tube) were done every 24 h and the scoring was done using crosses according to the cloudiness of the water. Similarly, the contents of total sugar, lipids, proteins, as well as pH and Brix degree were measured every 24 h. The experiment was done against a control which consisted of commercial coconut water and stopped when some parameters became constant.

2.8. Statistical analysis

Quantitative variables were analyzed using Graph Pad Prism version 9.0.0 and SPSS version 20.0.0 for Windows. The Shapiro wilk test was used to determine the data distribution of our variables; when the distribution was normal, t-tests and ANOVA were used. When the distribution was abnormal, Man Whitney-U-test or Friedman's test was used at the 5 % significance level.

3. Results

3.1. Physicochemical characterization of mature and mid-mature Cocos nucifera nuts

3.1.1. Determination of mean water volume per nut mass unit

Table 1 below shows the masses and water volumes of semi-mature and mature coconuts from Edea and Bafia.

The average volume of water per nut, just like the average ratios of water volume and nut mass were calculated. At the mid-mature stage, the average volumes of water contained in the nuts were respectively 156.45 ± 5.45 mL and 146.25 ± 1.27 mL for the nuts harvested at Edea and Bafia. At maturity, the whole nuts harvested in Edea and Bafia had respectively 85.05 and 90.68 mL of water. Reduced to the unit of mass, the values obtained showed that Edea mid-mature coconut (EMM) and Edea mature coconut (EM) had an

Table 1
Masses and water volumes of semi-mature and mature coconuts from Edea and Bafia.

	EM	BM	EMM	BMM
Mass of whole coconut (g)	860.25 ± 53.5^{a}	809.75 ± 65.56^{a}	781.75 ± 47.87^{b}	756.5 ± 46.4^{b}
Mass of shelled coconut (g)	473.25 ± 76.11^{a}	401.75 ± 13.04^{a}	–	–
Average volume of water (mL)	85.05 ± 2.11^{a}	90.68 ± 2.66^{b}	156.45 ± 5.45^{c}	146.25 ± 1.27^{d}
Average volume of water per unit mass of nut (mL/g)	0.098 ± 0.01^{a}	0.11 ± 0.05^{b}	0.2 ± 0.04^{c}	0.19 ± 0.05^{d}

Values assigned different letters in the same row are significantly different ($p < 0.05$). EMM: Edea mid-mature coconut water; EM: Edea mature coconut water; BMM: Bafia mid-mature coconut water; BM: Bafia mature coconut water.

average ratio of 0.20 ± 0.04 and 0.19 ± 0.05 mL/g respectively. The same, no significant difference ($p > 0.05$) was observed between the two values obtained. Furthermore, a significant difference ($p<0.05$) was observed between the averages ratios obtained when going from nuts harvested in Edea to those from Bafia whatever the degree of maturity.

3.1.2. pH, total titratable acidity and brix degree

The pH, total titratable acidity and brix degree in the different samples were recorded in Table 2 below.

The data obtained showed that pH and total titratable acidity parameters were inversely proportional. Depending on the stage of the nut, a decrease in acidity and an increase in pH are observed when moving from the mid-mature to the mature state. No significant difference ($p > 0.05$) was observed between pH of the water of the mid-mature coconuts harvested both in Bafia (BMM: 5.11 ± 0.0) and the mid-mature coconuts in Edea (EMM: 5.01 ± 0.01). Of the same opinion no significant difference ($p > 0.05$) was observed between these two waters with mature nuts. As for total titratable acidity, water from mid-mature nuts harvested in Bafia had a significantly higher value ($p<0.05$) than that from the mid-mature nuts harvested in Edea (0.113 ± 0.0 mg citric acid/100 mL). The Brix values showed that for the water of the mid-mature nuts, they vary significantly from 4.3 ± 0.3 to 5 ± 0.0 respectively for the water of the nuts harvested in Edea and Bafia. On the other hand, for mature nuts harvested in the same cities, these values are similar (3.62 ± 0.17°B).

3.2. Nutritional characterization of Cocos nucifera water

3.2.1. Macronutrients composition of Cocos nucifera water

The following Table 3 represents the macronutrients composition of *Cocos nucifera* water. It was about dry matter, total sugars, reducing sugars, total lipids, total proteins and total amino acids.

This table showed that the dry matter of *Cocos nucifera* nut water decreases significantly ($p < 0.05$) with maturity. No significant difference ($p > 0.05$) was observed between EMM (6.53 ± 0.64 %) and BMM (6.86 ± 0.11 %) mid-mature nuts. The same was observed for mature EM (4.96 ± 0.9 %) and BM (5.24 ± 0.73 %) nuts. Total and reducing sugar contents decreased significantly ($p < 0.05$) with maturity. No significant difference was observed between the water of the mid-mature nuts from the two localities; the same was observed for the water of the mature nuts. Total sugar concentrations were 5.49 ± 0.05 and 3.24 ± 0.02 g/100 mL for EMM and EM respectively; 5.56 ± 0.04 and 3.11 ± 0.03 g/100 mL for BMM and BM. As for reducing sugars, BMM and BM had contents of 4.99 ± 0.7 and 2.95 ± 0.2 g/100 mL; and varied from 5.09 ± 0.6 to 3.04 ± 0.1 g/100 mL for EMM to EM respectively. Results obtained show that lipid content of the water contained in *Cocos nucifera* increases significantly ($p < 0.05$) with the maturity level for nuts harvested in Bafia. Thus, they were 0.07 ± 0.0 and 0.10 ± 0.0g/100 mL of water for EMM and BMM respectively, and 0.216 ± 0.0 and 0.251 ± 0.01g/100 mL of water for EM and BM. There was a significant ($p < 0.05$) increase in protein and amino acid contents of *Cocos nucifera* water with nut maturity. For protein, these contents were 0.122 ± 0.0 and 0.155 ± 0.0 g/100 mL for EMM and BMM, and 0.253 ± 0.0 and 0.284 ± 0.01 g/100 mL for EM and BM, respectively. The water in EM had the best amino acid contents (0.17 ± 0.0 mg/100 mL) followed by BM (0.13 ± 0.0 mg/100 mL), BMM (0.095 ± 0.0 mg/100 mL) and EMM (0.065 ± 0.004 mg/100 mL).

3.2.2. Some minerals and vitamin C contents of Cocos nucifera water

The following Table 4 represents some minerals (calcium, magnesium, sodium, potassium, iron) and vitamin C contents of *Cocos nucifera* water.

EM showed significantly ($p < 0.05$) the highest contents of calcium (23.25 ± 0.03 mg/100 mL), magnesium (7.37 ± 0.12 mg/100 mL), sodium (16.83 ± 0.04 mg/100 mL) and potassium (32.61 ± 0.0 mg/100 mL). For iron and vitamin C, the best levels were obtained significantly ($p < 0.05$) with BMM (8.99 ± 0.8 mg/100 mL and 19.08 ± 0.24 mg/100 mL respectively).

3.3. Total phenolic compounds and in vitro antioxidant activities of Cocos nucifera water

The total phenolic compounds (TPC), total antioxidant capacity (TAC), iron III reducing capacity (FRAP) and free radical scavenging capacity (DPPH) of waters were recorded in Table 5 below.

It showed that all samples contain phenolic compounds and this increase significantly with the maturity of *Cocos nucifera* nut. However, EM has significantly ($p<0.05$) the best content (0.07 ± 0.0 mg GAE/mL *Cocos nucifera* water), followed by BM (0.03 ± 0.0 mg GAE/mL water), BMM (0.01 ± 0.0 mg GAE/mL water) and finally EMM (0.003 ± 0.0 mg GAE/mL water). EMM presented significantly ($p < 0.05$) the best capacity to reduce ferric iron (7.21 ± 0.25 mg Fe^{2+}/mL) and scavenge DPPH radical with an IC_{50} of

Table 2
pH, titratable acidity and brix degree of coconut water from Bafia and Edea.

	pH	Total titratable acidity (mg citric acid/100 mL)	Brix degree (°B)
EMM	5.01 ± 0.01^{a}	0.11 ± 0.0^{a}	4.30 ± 0.28^{a}
BMM	5.11 ± 0.003^{a}	0.117 ± 0.0^{b}	5 ± 0.0^{b}
EM	5.66 ± 0.01^{b}	0.08 ± 0.0^{c}	3.62 ± 0.17^{c}
BM	5.68 ± 0.0^{b}	0.076 ± 0.0^{d}	3.62 ± 0.17^{c}

Values assigned different letters in the same column are significantly different ($p < 0.05$). EMM: Edea mid-mature coconut water; EM: Edea mature coconut water; BMM: Bafia mid-mature coconut water; BM: Bafia mature coconut water.

Table 3
Macronutrient composition of *Cocos nucifera* water.

	EMM	BMM	EM	BM
Dry matter	6.53 ± 0.64^{a}	6.86 ± 0.11^{a}	4.96 ± 0.9^{b}	5.24 ± 0.73^{b}
Total sugars (g/100 mL)	5.49 ± 0.05^{a}	5.56 ± 0.04^{a}	3.24 ± 0.02^{b}	3.11 ± 0.03^{c}
Reducing sugars (g/100 mL)	5.09 ± 0.6^{a}	4.99 ± 0.7^{b}	3.04 ± 0.1^{c}	2.95 ± 0.2^{d}
Total lipids (g/100 mL)	0.07 ± 0.0^{a}	0.103 ± 0.0^{b}	0.216 ± 0.0^{c}	0.251 ± 0.0^{d}
Total proteins (g/100 mL)	0.122 ± 0.0^{a}	0.155 ± 0.0^{b}	0.253 ± 0.0^{c}	0.284 ± 0.0^{d}
Total aminoacids (g/100 mL)	0.065 ± 0.0^{a}	0.095 ± 0.0^{b}	0.173 ± 0.0^{c}	0.13 ± 0.0^{d}

Values assigned different letters in the same row are significantly different ($p < 0.05$). EMM: Edea mid-mature coconut water; EM: Edea mature coconut water; BMM: Bafia mid-mature coconut water; BM: Bafia mature coconut water.

Table 4
Some mineral and vitamin C contents of mid-mature (EMM and BMM) and mature (EM and BM) coconut waters.

	EM	EMM	BM	BMM
Calcium (mg/100 mL)	23.25 ± 0.03^{a}	22.56 ± 0.06^{b}	21.95 ± 0.07^{c}	21.08 ± 0.07^{c}
Magnesium (mg/100 mL)	7.37 ± 0.12^{a}	6.28 ± 0.03^{b}	6.19 ± 0.05^{b}	6.43 ± 0.24^{b}
Sodium (mg/100 mL)	16.83 ± 0.04^{a}	11.13 ± 0.05^{b}	14.92 ± 0.0^{c}	12.41 ± 0.01^{d}
Potassium (mg/100 mL)	32.61 ± 0.0^{a}	31.20 ± 0.21^{b}	15.43 ± 0.0^{c}	11.68 ± 0.33^{d}
Iron (mg/100 mL)	1.28 ± 0.42^{a}	2.99 ± 0.0^{b}	4.92 ± 1.49^{d}	8.99 ± 0.8^{c}
Vitamin C (mg/100 mL)	10.52 ± 0.03^{a}	17.48 ± 0.03^{b}	16.44 ± 0.32^{c}	19.08 ± 0.24^{d}

The values assigned to different letters in the same row are significantly different ($p < 0.05$). EMM: Edea mid-mature coconut water; EM: Edea mature coconut water; BMM: Bafia mid-mature coconut water; BM: Bafia mature coconut water.

Table 5
Total phenolic compounds and antioxidant activities of the water of mid-mature (EMM and BMM) and mature (EM and BM) coconuts waters.

	EM	EMM	BM	BMM	GA
TPC (mg GAE/mL)	0.07 ± 0.0^{a}	0.003 ± 0.0^{b}	0.03 ± 0.0^{c}	0.01 ± 0.0^{d}	–
FRAP (mg Fe^{2+}/mL)	1.98 ± 0.03^{a}	7.21 ± 0.25^{b}	1.68 ± 0.03^{a}	5.96 ± 0.29^{c}	–
TAC (mgGAE/mL)	73.10 ± 4.72^{a}	103.94 ± 7.08^{b}	48.11 ± 2.37^{c}	130.10 ± 8.62^{d}	–
DPPH (IC_{50} mL/mL)	59.66 ± 4.14^{a}	19.78 ± 0.74^{b}	29.36 ± 2.51^{c}	26.09 ± 0.48^{d}	$6.75 \times 10^{-5} \pm 0.0$

Values assigned different letters in the same row are significantly different ($p < 0.05$). EMM: Edea mid-mature coconut water; EM: Edea mature coconut water; BMM: Bafia mid-mature coconut water; BM: Bafia mature coconut water; GA: Gallic acid.

Table 6a
Change in appearance at 4 °C.

	Turbidity 4°C				
	EM	EMM	BM	BMM	CCW
Day 0	*	0	*	0	**
Day1	*	0	*	0	**
Day2	*	0	*	0	**
Day3	*	0	*	0	**
Day4	**	*	**	*	***
Day5	**	*	**	*	***
Day6	**	*	**	*	****
Day7	***	**	***	**	*****
Day8	***	**	***	**	*****
Day9	***	**	***	**	*****
Day10	***	**	***	**	******
Day11	****	**	****	**	******
Day12	****	***	****	***	*******
Day13	****	***	****	***	******
Day14	****	***	****	***	*******
Day15	*****	***	*****	***	********

EMM: Edea mid-mature coconut water; EM: Edea mature coconut water; BMM: Bafia mid-mature coconut water; BM: Bafia mature coconut water; CCW: coconut water collected from a commercial in Yaounde.

19.78 ± 0.74 mg/mL although the latter was significantly ($p < 0.05$) higher than that of gallic acid (6.75×10^{-5} ±0.0) used as the reference antiradical agent. In terms of total antioxidant capacity, BMM showed the best activity (130.10 ± 8.62 mg GAE/mL).

3.4. Stability monitoring of Cocos nucifera water

The stability of *Cocos nucifera* water was monitored at room temperature (5 days) and at 4 °C to refrigerator (15 days). The physicochemical (turbidity, Brix degree and pH) and nutritional (total sugars, proteins, lipids) parameters of mid-mature (EMM and BMM) and mature (EM and BM) coconut waters collected respectively in the cities of Edea and Bafia were compared to those of a coconut water collected from a commercial in the city of Yaounde (CCW).

3.4.1. Turbidity of Cocos nucifera water stored in the refrigerator (4 °C) and room temperature

Tables 6a and 6b showed the variation of turbidity of *Cocos nucifera* water at the refrigerator (4 °C) and room temperature (25 °C). This was reflected by a change in appearance depending on time.

Turbidity of *Cocos nucifera* water varied significantly ($p < 0.05$) with increasing storage time at both room temperature and in the refrigerator. After 15 days of refrigerated storage, the turbidity of these waters was similar to that on the 5th day of storage at room temperature. The commercial coconut water (CCW) was significantly more turbid than the other waters studied. At 25 °C, compared with CCW, whose turbidity level is (***), we can consider that coconut quality has deteriorated.

3.4.2. Percentage decrease in nutritional and physicochemical of Cocos nucifera water measured during the monitoring of the stability both in the refrigerator (4 °C) and room temperature

Tables 7a and 7b showed the percentages decrease in nutritional (lipids, proteins, total sugars) and physicochemical (pH and Brix degree) of *Cocos nucifera* water measured during the monitoring of the stability both room temperature and in the refrigerator (4 °C) after 5 and 15 days respectively.

The macronutrient contents of the different *Cocos nucifera* waters significantly ($p < 0.05$) decreased during storage. For the lipid contents, the percentage of decrease ranged from 26.76 ± 0.02 for EMM to 52.12 ± 0.0 % for CCW after 5 days of storage at room temperature. At 4 °C after 15 days of storage, these values ranged from 21.12 ± 0.01 for EMM to 54.4 ± 0.02 % for CCW. Regarding protein contents, CCW had the greatest percentage decrease at room temperature (57.13 ± 0.01 %) and at 4 °C (58.17 ± 0.09) followed by EM (43.08 ± 0.01 % at room temperature) and (45.05 ± 0.01 % at 4 °C). At room temperature, the percentage of reducing of total sugars were from 19.2 %; 6.66 %; 19.82 %; 10.25 % and 67.18 %. When the coconut waters were stored at 4 °C, the percentage of reducing were 9.95 %; 7.83 %; 14.14 %; 6.47 % and 59.86 % for EM, EMM, BM, BMM and CCW respectively. The pH and Brix degree decreased significantly ($p < 0.05$) with storage time at both room temperature and 4 °C. At room temperature and during the 5 days of storage, the pH for EM, EMM, BM, BMM and CCW with respective percentages of decrease of 18.19 %; 13.56 %; 16.02 %; 12.13 % and 32.88 %. The Brix degree decreased by 15.11 %; 13.1 %; 13 %; 11.81 % and 25.92 % respectively for EM, EMM, BM, BMM and CCW.

4. Discussion

Coconuts represent a commodity in the different regions where they are exploited. They are consumed both for their white flesh (kernel) and the water they contain. The maturity of the coconut is inversely proportional to the quantity of water it contains. Indeed, the more mature the nut becomes the less water it contains [24]. This would justify the high volume of water observed with mid-mature coconuts harvested both in Edea (EMM: 156.45 ± 5.45 mL) and in Bafia (BMM: 146.25 ± 1.27 mL). These values were lower than 308 mL and higher than 117 mL obtained by Appaiah et al. [24] with water from mid-mature and mature coconuts harvested in India respectively.

The pH measures the concentration of free protons, this differs from the titratable acidity in that, the latter represents the sum of free protons and undissociated acids in a solution. The pH and the titratable acidity of the water contained in the coconut evolve in an inverse way with the maturity. As the coconut matures, the pH increases and the acidity decreases. Mature coconut water from Edea (EM) is reported to contain more hydronium ions and organic acid residues such as amino acids, fatty acids and carbon dioxide (CO_2) dissolved in it compared to mature coconut water harvested in Bafia (BM). These compounds may be derived from metabolic reactions

Table 6b
Change in appearance at room temperature (25 °C).

	Tubidity at room temperature				
	EM	EMM	BM	BMM	CCW
Day0	*	0	*	0	**
Day1	*	0	*	0	***
Day2	**	*	**	*	****
Day3	***	**	***	**	*****
Day4	****	***	****	***	******
Day5	****	***	****	***	*******

EMM: Edea mid-mature coconut water; EM: Edea mature coconut water; BMM: Bafia mid-mature coconut water; BM: Bafia mature coconut water; CCW: coconut water collected from a commercial in Yaounde.

Table 7a
Percentage decrease in parameters measured during the monitoring of the stability of the water of half-mature (EMM and BMM) and mature (EM and BM) coconuts harvested respectively in the cities of Edea and Bafia and stored at room temperature (25 °C).

	EM	EMM	BM	BMM	CCW
Lipids (%)	37.5 ± 0.0	26.76 ± 0.02	42.23 ± 0.02	30.09 ± 0.01	52.12 ± 0.0
Proteins (%)	43.08 ± 0.01	29.5 ± 0.02	33.09 ± 0.02	29.16 ± 0.07	57.13 ± 0.01
Total sugars (%)	19.2 ± 0.01	6.66 ± 0.01	19.82 ± 0.01	10.25 ± 0.01	67.18 ± 0.0
pH (%)	18.19 ± 0.0	13.56 ± 0.0	16.02 ± 0.0	12.13 ± 0.0	32.88 ± 0.0
°B (%)	15.11 ± 0.02	13.1 ± 0.04	13 ± 0.02	11.81 ± 0.07	25.92 ± 0.01

Values assigned different letters in the same row are significantly different ($p < 0.05$). EMM: Edea mid-mature coconut water; EM: Edea mature coconut water; BMM: Bafia mid-mature coconut water; BM: Bafia mature coconut water; CCW: coconut water collected from a commercial in Yaounde.

Table 7b
Percentage of decrease of the parameters measured during the monitoring of the stability of mid-mature (EMM and BMM) and mature (EM and BM) coconut water harvested respectively in Edea and Bafia and stored in a refrigerator (4 °C).

	EM	EMM	BM	BMM	CCW
Lipids (%)	41.06 ± 0.0	21.12 ± 0.01	44.97 ± 0.04	26.21 ± 0.03	54.4 ± 0.02
Proteins (%)	45.05 ± 0.01	30.32 ± 0.02	35 ± 0.02	32.25 ± 0.02	58.17 ± 0.09
Total sugars (%)	9.95 ± 0.01	7.83 ± 0.07	14.14 ± 0.0	6.47 ± 0.0	59.86 ± 0.0
pH (%)	7.06 ± 0.0	2.99 ± 0.0	14.78 ± 0.0	7.4 ± 0.0	32.3 ± 0.0
°B (%)	10.46 ± 0.05	13.1 ± 0.03	16 ± 0.03	11.72 ± 0.02	24.48 ± 0.01

Values assigned different letters in the same row are significantly different ($p < 0.05$). EMM: Edea mid-mature coconut water; EM: Edea mature coconut water; BMM: Bafia mid-mature coconut water; BM: Bafia mature coconut water; CCW: coconut water collected from a commercial in Yaounde.

associated with coconut development. The results are consistent with those of Jackson et al. [25] who also observed an increase in pH in water from orange dwarf coconuts harvested in Jamaica. For mid-mature nuts, the values obtained were close to those obtained by Assa et al. [8] in the waters of NVE (5) and GOA (5.1) variety mid-mature nuts and higher than those obtained in the same study in the water of PB121+ hybrid nuts (4.9). Tan et al. [26] also observed a decrease in titratable acidity in coconut water with maturation with values of 0.089 and 0.06 mg malic acid per 100 mL of water from nuts aged about 7 and 12 months respectively. The Brix degree or soluble dry extract (SDE) of the analyzed waters, which represents the percentage by mass of sucrose of an aqueous solution of sucrose having the same refractive index as the product to be analyzed, under determined conditions of preparation and temperature, decreases with the maturity of the nuts. In the nut water samples analyzed, the values were 4.3 ± 0.28 and 5 ± 0.0 for EMM and BMM respectively and 3.62 ± 0.17 for EM and BM. These Brix values allow these waters to be classified as slightly sweet liquids according to the Codex Alimentarius (ESS<11°B). The value obtained in the mature coconut water was approximately equal to 3.9 % with the NJM variety coconut water, while the mid-mature coconut waters had a slightly lower soluble solids than the GOA variety coconut water (5.4 %) studied by Assa et al. [8].

Regarding macronutrients, a decrease in total and reducing sugar contents, but an increase in protein, free amino acids and lipids contents in the different batches of samples were observed with maturity. Indeed, during the ripening of the coconut, the carbohydrates present in the water are destroyed via glycolysis in pyruvic acid which, in the presence of coenzyme A is decarboxylated in acetyl coenzyme A, which participates in the synthesis of fatty acids, then of lipids which are the main constituent of the kernel [8]. Jackson et al. [25] also observed a reduction in total and reducing sugars in Grand Maypan coconut water (4.49–3.77 g/100 mL; 3.34 to 2.66 g/100 mL of water respectively for total and reducing sugars) aged 7 and 12 months harvested in Jamaica. Lipid contents in coconut water are extremely low, ranging from 0.07 to 0.216 for EMM and EM respectively, and 0.103 and 0.251 g/100 mL for BMM and BM. The increase in lipid, protein and amino acid contents would be due to the different synthesis reactions taking place in the water during this period. Appaiah et al. [27] had also observed an increase in lipid content in the water of coconuts purchased from Mysore, India. The values were 0.2 and 1.2 g/100 mL for immature and mature nut water, respectively. Our results (0.253 and 0.284 g/100 mL for EM and BM respectively) for protein contents are similar than those obtained by Coulibaly et al. [13] in the nut water of the coconut tree dwarf and street market from Ivory Coast market whose value was 0.291 and 0.269 g/100 mL respectively.

The mineral content of coconut water depends not only on the content of each mineral in the soil, but also on the coconut variety, cultivation techniques, and the presence or absence of watercourses around the plantations [28]. The samples had calcium contents of 23.25 ± 0.03 and 22.56 ± 0.06; 21.95 ± 0.07 and 21.08 ± 0.07 mg/100 mL, and magnesium contents of 7.37 ± 0.12 and 6.28 ± 0.03; 6.19 ± 0.05 and 6.43 ± 0.03 mg/100 mL of mature and mid-mature coconut water from Edea and Bafia respectively. Calcium is involved in bone formation and is necessary for blood clotting while magnesium is the cofactor of many enzymes involved in energy metabolism. Daily calcium requirements range from 200 to 1300 mg for children and 1000–1300 for adults, compared to 30–130 mg for children and 240–420 mg for adults for magnesium [29]. Although insufficient to cover the daily needs of an individual, the calcium and magnesium contents of these waters are not to be neglected.

Sodium contents in our waters were 16.83 ± 0.04 and 11.13 ± 0.05; 14.92 ± 0.0 and 12.41 ± 0.01 mg/100 mL of mature and mid-mature coconut water harvested in Edea and Bafia respectively. The contents of this mineral increase with maturity. It is necessary to

maintain the osmotic balance of body fluids, to control glucose absorption and to improve normal protein retention during growth. Excessive dietary sodium intake promotes high blood pressure or oedema in some individuals. The RDA value is between 1000 and 1500 mg/day for children, men and women [29]. The potassium and sodium contents of these waters justify their use in substitution with sports drinks (11.7 and 41 mg/100 mL); similarly, their low sodium content justifies their use in the management of hypertensive individuals. Potassium levels in our samples were 32.61 ± 0.0 and 31.2 ± 0.21; 15.43 ± 0.0 and 11.68 ± 0.33 mg/100 mL of water respectively in mature and mid-mature coconut waters harvested in Edea and Bafia. Potassium is an extracellular cation that plays an important role in humans. It is necessary for the functioning of the heart and plays an essential role in the contraction of skeletal and smooth muscles, making it normal for digestive and muscle function. It should be noted that the deficiency of this macroelement causes effects such as palpitation, abdominal restriction, nausea and constipation [30]. The values obtained in both mid-mature and mature coconut water are lower than the standard RDA range (400–3400 mg/100 mL), but also lower than those obtained by Tan et al. [26] in the water of large Malayan variety coconuts harvested in Malaysia whose values were 220.94 and 35.11 mg/100 mL of mid-mature and mature coconut water. The water samples analyzed had iron contents of 1.28 ± 0.42 and 2.99 ± 0.0, 4.92 ± 1.49 and 8.99 ± 0.8 mg/100 mL of mature and mid-mature nut water collected in Edea and Bafia, respectively. This mineral plays a major role as a constituent of hemoglobin, a protein in red blood cells responsible for the transport of respiratory gases [31]. These values are higher than those obtained by Chuku and Kalagbor [32] on coconut cultivars harvested in Nigeria and within the RDA range of 0.27–27 mg/100 mL as reported by the [33]. These waters are therefore good sources of iron for the human body.

As for vitamin C, their contents were 10.52 ± 0.03; 17.48 ± 0.03; 16.44 ± 0.32 and 19.08 ± 0.24 mg/100 mL respectively for the mature and mid-mature nut waters harvested in Bafia and Edea. This micronutrient is necessary for the biosynthesis of collagen, an essential component of connective tissues involved in wound healing. It also plays an important role in immune function and promotes the absorption of hem iron present in plant foods [34]. Consumption of 100 mL of coconut water can meet the recommended dietary allowance for children, which is between 15 and 25 mg. Phenolic compound levels in our samples, expressed as mg GAE/mL, increased with maturity; values ranged from 0.003 ± 0.0 to 0.075 ± 0.0 mg GAE/mL for the mid-mature and mature nut waters harvested in Edea, and from 0.01 ± 0.0 to 0.03 ± 0.0 mg GAE/mL for the mid-mature and mature nut waters harvested in Bafia. It is a secondary plant metabolite that protects cells from oxidative stress-related damage. Appaiah et al. [27] had also observed an increase in phenolic compound content in coconut waters with maturity. The values obtained with mid-mature nut water were lower than that obtained by the same author in immature nut water purchased from a market in India (1.8 mgGAE/100 mL). In the same study, the same authors obtained phenolic compound contents of 4.3 ± 0.4 mg GAE/100 mL in mature nut water, lower than that obtained in mature nut water harvested in Edea, and higher than that of Bafia.

Accordingly, antioxidant activity is the ability of compound to diminish the production of oxidants or reactives species. The results obtained show that the total antioxidant capacity of the waters decreases with the maturity of the nuts; thus, 103.94 ± 7.08 and 73.1 ± 4.72; 130.2 ± 8.62 and 48.11 ± 2.37 mg GAE/mL were obtained for the mid-mature and mature nut waters harvested in Edea and Bafia respectively. The highest activity obtained with BMM (130.1 ± 8.62 mgGAE/mL) is three times lower than (420.99 ± 9.6 mgGAE/mL) obtained by Shayanthavi et al. on coconut water (Green Dwarf) in Sri Lanka [14]. Similarly, all the waters analyzed are capable of trapping DPPH radicals; the best inhibitory concentration was observed in the mid-mature coconut water harvested in Edea (IC_{50} = 19.79 mg/mL) followed by the mature coconut water harvested in Bafia with an IC_{50} of 26.09 ± 2.51, and then the mature coconut water of Bafia and Edea with the values of 29.36 ± 2.51 and 59.56 ± 4.14 mg/mL. As for the capacity of the different samples to reduce ferric ions to ferrous ions, the values obtained decreased with maturity; they ranged from 7.21 ± 0.25 to 1.98 ± 0.03 mg Fe^{2+}/mL and from 5.96 ± 0.29 to 1.68 ± 0.03 mg Fe^{2+}/mL respectively for the mid-mature and mature coconut waters collected in Edea and Bafia. The mid-mature sample from Bafia had the best TAC, while the sample from Edea had the best ability to scavenge free radicals and reduce ferric ions to ferrous ions. Phenolic compound levels increase in these waters with maturity, while total antioxidant capacity decreases [35,36]. This could be explained by the fact that not all phenolic compounds present in these waters act as antioxidants.

The study on stability monitoring showed a progressive decrease of both biochemical and physicochemical parameters measured. Indeed, during storage, the sugar contained in our samples underwent alcoholic fermentation, producing ethanol and CO_2. In an aqueous environment, CO_2 is transformed into carbonic acid; as the fermentation continues over the days, we see an increase in the CO_2 content and therefore in the carbonic acid, which leads to an increase in acidity and therefore a decrease in pH. At the same time, the oxidation of lipids and proteins contained in the medium is observed. The degradation of these macromolecules justifies the decrease of the soluble dry extract. It should also be noted that the degradation processes are more intense at room temperature than at 4 °C.

5. Conclusion

This work made it possible to determine the physicochemical, nutritional, antioxidant properties and stability monitoring of coconut (*Cocos nucifera* L.) water from two localities in Cameroon. It emerged from this study that the pH was slightly acidic for all these waters. Edea mid-mature coconut water and Bafia mid-mature coconut water contained more total and reducing sugars, while Bafia mature coconut water contained more lipids, proteins and amino acids. These waters contained phenolic compound and vitamin C which are antioxidants. Edea mature coconut water was richer in calcium, magnesium, sodium, and potassium while Bafia mid-mature coconut water contained more iron and vitamin C. Water from mid-mature nuts can be kept for a maximum of 2 days at room temperature and for a maximum of 4 days at 4 °C. All of this data can be taken into account to enrich the local food table composition.

CRediT authorship contribution statement

Pounde Djeumeni Hamilton: Writing – original draft, Methodology. **Kotue Taptue Charles:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Conceptualization. **Achu Mercy Bih Loh:** Writing – review & editing, Validation, Supervision. **Nantchouang Nankam Aristide Loïc:** Writing – original draft, Resources, Methodology. **Kansci Germain:** Writing – review & editing, Validation. **Fokou Elie:** Writing – review & editing, Validation.

Data availability

Data will be made available on request.

Ethics statement

This submission did not include human and animal participation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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