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VARIATION OF ELECTROLYTES, AMINO ACIDS AND REDUCING SUGARS IN COCONUT WATER OF DIFFERENT AGES FROM AN INLAND REGION OF BANGLADESH

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ABSTRACT
Coconut water, extracted from the fruit of *Cocos nucifera* L., is a popular drinks throughout the tropics. The variable nature of the composition of the drinks had been established multiple times before, with regards to age, location and genetic variation. However, in Bangladesh, such studies were not carried out before. Thus, this study aims to compare electrolytes, amino acids and carbohydrates concentration in coconut water of different age collected from an inland region of the country. To determine electrolyte concentration atomic absorption spectrophotometry and Mohr titrimetric method were applied. Determination of carbohydrate and amino acid concentration required two separate high performance liquid chromatography (HPLC) methods. Regarding electrolytes concentration, rise of potassium, calcium and chloride ion concentration and fall of sodium and magnesium ion concentration were apparent. Potassium ion was the most abundant cation (50.88-67.56 mEq/L) while steep rise of magnesium ion concentration from 4 month to 6 months (4.14±0.17 to 12.72±2.52 mEq/L) was observed. Fructose and dextrose concentrations also escalated with coconut age. Amino acid concentrations varied as well. Histidine (0.43 g/100ml), arginine (0.053 g/100ml) and arginine (0.142 g/100ml) were the most abundant amino acids found in water 4, 6 and 8 months old coconuts. Overall, the trends of variation of components show intake of coconut water can bring different physical outcomes to different consumers and therefore, must be chosen carefully for patients with electrolytic imbalance and other medical complications.

1. Introduction

As a tropical tree, the global distribution and familiarity of the coconut tree (*Cocos nucifera* L.) is overwhelming. The tree conquered from East Indies to West Indies, from the remotest pacific islands to neo-tropical realm (Nayar, 2006). The consumable part of the tree is the coconut fruit. Numerous products can be extracted from the fruit such as coconut water, coconut milk, coconut meat, coconut apple, etc. Among them, coconut water had been more popular and subjected to extensive research due to its unique composition: a fine admixture of solution with electrolytes, carbohydrates, amino acids, vitamins, lipids, organic acids, enzymes, phytochromes (Yong et al., 2009).

The coconut water is drinkable and a popular source of hydration in tropical countries (Santoso et al., 1996). The electrolytic composition of coconut water is different from blood regarding the concentration of potassium (K+), sodium (Na+) and chloride (Cl-) ions. Yet, it provides essential ions to the body in cases of dehydration when taken orally (Saat et al., 2002). This replenishing drink had been popular to tropics and it is also commercialized in various countries, sometimes as sports drinks (Petroianu et al., 2004; Kalman, et al., 2012). Apart from the electrolytes, the coconut water was found to contain different carbohydrates: sucrose, glucose, fructose, galactose, mannose, arabinose, mannitol and xylose and different essential amino acids (del Rosario et al., 1984; Santoso et al., 1996). Moreover, coconut water also contains higher ratio of arginine, alanine, cysteine and serine, greater than those found in cow's milk (Santoso et al., 1996, Prades et al., 2012).

The proportion of nutrients in coconut water varies as the fruit grows old. The immature coconut, in which the endosperm is not well developed, is the most popular refreshing drinks in the tropics. The best time to harvest a coconut for drinking is at age 6-7 months, just as the jelly-like endosperm begins to form. At this stage the water has maximum sweetness and low acidity (Rolle, 2007; Chuku et al., 2014). This is due to the fact that as coconuts mature, their composition and physicochemical properties alter with the white kernel lining the inner shell becoming opaque and hard (Tan et al., 2014). For example, Child et al. (1950) reported that the sugar content of it become concentrated as the water volume decreases with age. Additionally, they reported that after a certain age, the taste of coconut water changes due the rise of pH value, turbidity and minerals concentration. The quantity of electrolytes in coconut water will be varied in Bangladesh due to the reasons such as geographical variation, temperature and humidity (Santoso et al., 1996). Genetic variation and variation among cultivars are another two prominent examples of content differences in coconut water (Santoso et al., 1996; Solangi et al., 2011).

Many research works had been done to determine the exact amount of minerals in coconut water (Saat et al., 2002; Solangi et al., 2011; Waziri et al., 2013). Few researches showed a variation of mineral components in ripe and unripe coconut (Vigliar et al., 2006; Adegoke et al., 2012). Many works describe composition of carbohydrates, but only few describes the variation in the maturation stage (Vigliar et al., 2006; Prades et al., 2012). On the other hand, there had been occasional variation in determination of protein and amino acid compositions from various authors (Santoso et al., 1996; Campbell-Falck et al., 2000). There has been very little research on coconut water quality from Bangladesh. Some of them focused on arsenic contents of coconut water (Safiullah et al., 2013). However, no article had been published to describe the electrolytes, carbohydrates and amino acid composition of coconut water of any varieties in the geographical area of Bangladesh.

In the present study, approaches have been made to evaluate the concentration of those components of the coconut water of Bangladesh and variation of the composition regarding different ages of maturity of the fruit has been observed.

2. Materials and Methods

2.1. Materials

2.1.1. Instruments

To measure osmolarity, a osmometer was used (the Advanced Micromometer 330, USA). The pH was calculated by a pH meter (Mettler-Toledo, Malaysia). The quantitative determination of electrolytes (Ca, Mg, Na, and K) were done using the single beam atomic absorption spectrometer (AAS) by using atomic absorption spectrophotometer (Bulk Scientific model 7000, Shimadzu, Japan). For analysis of amino acids and carbohydrates, a glass column of 1.5 X 30 cm and a rotavapor was employed for the preparation of the sample with different aged coconut water. Amino acids were quantified with a Waters 2695 Alliance HPLC system (Waters Inc., Milford, CT, USA) which consisted of a 9012Q pump, 9100 autoinjector and 9075 fluorescence detector. Separation was carried out in a Waters Nova-Pack reversed phase C18 column, 4 µm particle size, 150 X 3.9 mm i.d. A specific Nova-Pack guard column was placed between the autoinjector and column. Waters 2695 Alliance HPLC system (USA) was also used for carbohydrates analysis. A differential refractometer (Waters R-401, USA) was used as detector. All the chromatographic information was reprocessed in Star Work-station (version 4.5) supplied by Varian. All the reagents used were of analytical grades.

2.1.2. Samples

Three coconut trees (all tall variety of green coconuts) in Narinda, Dhaka, Bangladesh were kept in close observation for maturing coconuts in 2018 and samples were collected in different seasons for coconuts of 4 months, 6 month and 8 months of age (n=20 for each). Coconut water were collected and filtered without contamination by the same process as described in Alchoubassi et al., 2020.

2.2. Determination of Electrolytes

The concentration of Na+, K+, Mg2+, Ca2+ were determined by flame AAS analysis. Before analysis blank, standard and sample solution were prepared. For blank solution of Na+, 1000µl (1ml) 10% cesium chloride (CsCl2) was transferred into a 100ml conical flask and then added together with 0.1M nitric acid (HNO3) up to mark. Furthermore, 1ml of each solution of 10% CsCl2, 0.1% KCl, 0.2% KCl were transferred to 100ml conical flask separately for preparing blank solution of K+, Mg2+, Ca2+ respectively and then water for injections (WFI) was added up to mark of each conical flask. The three standard solution for each ion of Na+, K+, Mg2+, Ca2+ were taken into 100 ml conical flask where 0.1, 0.2 and 0.4 ppm (1ml, 2ml, 4ml) for Na+ ion, 0.2, 0.4, 0.8 ppm (2ml,4ml, 8ml) for K+ ion, 0.1, 0.2 and 0.4 ppm (1ml, 2ml and 4ml) for Mg2+ ion and 0.5, 1 and 2 ppm (5ml, 10ml and 20ml) for Ca2+ ion respectively and then added the same ingredients that used to prepare blank solution of each to adjust up to mark. To prepare the sample, 40µl, 20µl, 100µl, 500µl filtered coconut water were taken into 100ml conical flask separately for determination of Na+, K+, Mg2+, Ca2+ respectively and then the same ingredients were added to prepare blank solution of each to adjust up to mark. The dilution factor of Na+, K+, Mg2+, Ca2+ were 2500, 5000, 1000, 200 times simultaneously.

To briefly describe the overall method, the sample first drawn to flame to constitute gaseous phase by ionization process. The calibration curve is obtained from three different concentrations of standard solutions and absorption verses concentration curve is prepared to analyze the samples. Ideal sample injection quantity is 10 to 20ml. After preparations of blank, standard and sample solution using the brands A, B, and C for blank, standard and sample respectively by employing the experimental conditions of Ca, Mg, Na and K ion quantification. In order to evaluate accurately, sample and standard curves were compared.

On the other hand, Cl- concentration were determined by Mohr titrimetric method as described by Sawyer et al., 2003.

2.3. Determination of Amino Acids

Preparation of Samples and Derivatisation. Filtered coconut water was taken into a glass beaker and shaken at a rapid speed. Derivatisation for fluorescence was done by 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate according to a method described earlier (Cohen, 2011).

HPLC analysis. The chromatographic conditions were as follows: flow 0.1 ml/min until 3 minute and then 1.5 ml/min, volume of injection 10 µl for each solvents. Solvents A contained sodium phosphate buffer (10 mM, pH 7.3), methanol, tetrahydrofuran (80:19:1) and solvent B contained sodium phosphate buffer (10 mm, pH 7.3), methanol (20:80). The gradient elution were set up as 100% of solvent A during 3.5 minutes, 0-15% of Solvent B in A for 6 mins, 15% of Solvent B isocratically for 5 mins, 15-30% of solvent B for 5 mins, 30-40%

of solvent B for 4 min and 40-80% of solvent B for 12 mins. Fluorimetric detection is carried out using excitation and emission wavelengths of 340 and 426 nm respectively.

2.4. Determination of Reducing Sugars

Carbohydrate analysis was done by HPLC coupled with a refractometer. The similar chromatographic conditions and method was followed as described in Trani et al., 2017.

3. Results and Discussions

3.1. Osmolarity and pH

The osmolarity and pH of the coconut water is shown in table 1. The osmolarity seemed to decrease from month 4 to month 8 gradually from 486-494 milliosmole/L to 345-353 milliosmole/L. A previous findings reported a decrease in osmolarity from 377.3 to 310.3 milliosmole/L in 8 month and 9 month old coconuts respectively (Neto et al., 1993). Similar results were obtained in other study of coconut from Brazil with median value of 419 milliosmole/L (Vigliar et al., 2006). On the other hand, the pH value of the coconut water decreased with age (table 1).

3.2. Concentration of Electrolytes

Among the cations found in coconut water, potassium ion (K+) is the most abundant regarding any age groups as shown in table 1. The more matured coconut is taken, the more the concentration decreased. Interesting findings were observed in case of Mg2+ which increased from an average of 4.14 milliequivalent/L (mEq/L) of 4 months of age to 12.72 mEq/L and 14.06 mEq/L in 6 months and 8 months aged coconut respectively. Small but significant amount of sodium and calcium ion were present in all samples with concentration rising with maturation. Sodium ion increased almost 4-fold from 4 month and 8 month of sample (1.98 to 8.03 mEq/L). The total cation concentration increased over time. Chlorine was the only anion measured. Its concentration declined from an average of 47.69 mEq/L of 4 month mature sample to 31.83 mEq/L of 6 month (table 1). According to Solangi, et al., 2011, certain genetic varieties of coconut showed similar trend of rise and fall in electrolyte concentrations. For instance, in that study coconut water of Sri Lankan tall variety had potassium concentrations decreasing from 32.94 mEq/L to 31.77 mEq/L in 6-7 month and 11-12 month respectively. In other different studies, potassium and sodium concentration of coconut water oscillated between 35.1-81.8 and 0.7-9.7 mEq/L respectively (Chavalittamrong, et al., 1982; Campbell-Falck et al., 2000). The extended range of concentrations of different observations can be assumed due to soil quality, genetic variation or maturation of the fruit (Jackson, et al., 2004; Uphade, et al., 2008). Coconut water is a good source of citrate and malate ions (Patel et al., 2018) and the anions of the salt form may shift towards those ions rather than chloride ion with increasing age.

Table 1. Variation of osmolarily, pH and electrolytes concentration in waters of differently aged coconuts

Properties	Values		
	4 month (n=20)	6 month (n=20)	8 month (n=20)
Osmolarity (milliosmole/L)	486-494	428-446	345-353
pH	4.63-4.65	5.55-5.58	5.64-5.70
K^+ (mEq/L)	67.56±2.19	63.87±1.11	50.88±1.81
Na^+ (mEq/L)	1.98±0.23	2.02±0.18	8.03±0.23
Mg^{2+} (mEq/L)	4.14±0.17	12.72±2.52	14.06±2.11
Ca^{2+} (mEq/L)	4.87±0.30	6.75±0.22	8.78±0.49
Cl^- (mEq/L)	47.69±0.99	42.76±1.31	31.83±0.49

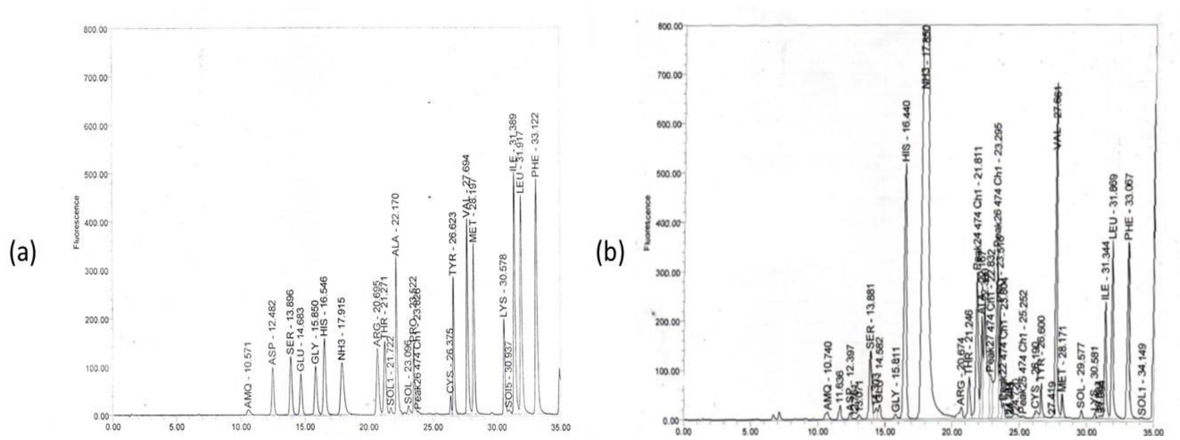
3.3. Concentration of Amino Acids

After HPLC-fluorometric analysis of coconut water, several amino acids in different concentration were observed as shown by the chromatograms of a representative sample in the figure 1.

Overall, amino acid concentration is comparatively higher in more matured coconut with an exception of histidine and leucine (table 2). In 4 month old coconut, histidine (0.430 g/100ml) was found as one of the more prominent amino acid. However, coconuts with 8 months of age contains more serine and glutamic acid 0.266 and 0215 g/100ml respectively. All the amino acids found in the coconut water appeared in previous studies (Young et al., 2009). Moreover, the overall protein content was found to be higher with maturation in a prior study (Jackson, et al., 2004).

Table 2. Variation of different amino acid contents in differently aged coconut water

Amino acids (g/100ml)	**Values**		
	4 months	6 months	8 months
Serine	0.075	0.129	0.266
Leucine	0.059	0.055	0.033
Glutamic Acid	0.032	0.114	0.215
Aspartic Acid	0.010	0.024	0.033
Glycine	0.005	0.011	0.019
Cysteine	0.030	0.035	0.077
Alanine	0.022	0.027	0.028
Proline	-	0.015	0.096
Valine	0.029	0.088	0.083
Arginine	0.034	0.053	0.053
Histidine	0.430	0.052	0.052
Isoleucine	0.036	0.033	0.062
Lysine	0.007	0.036	0.046



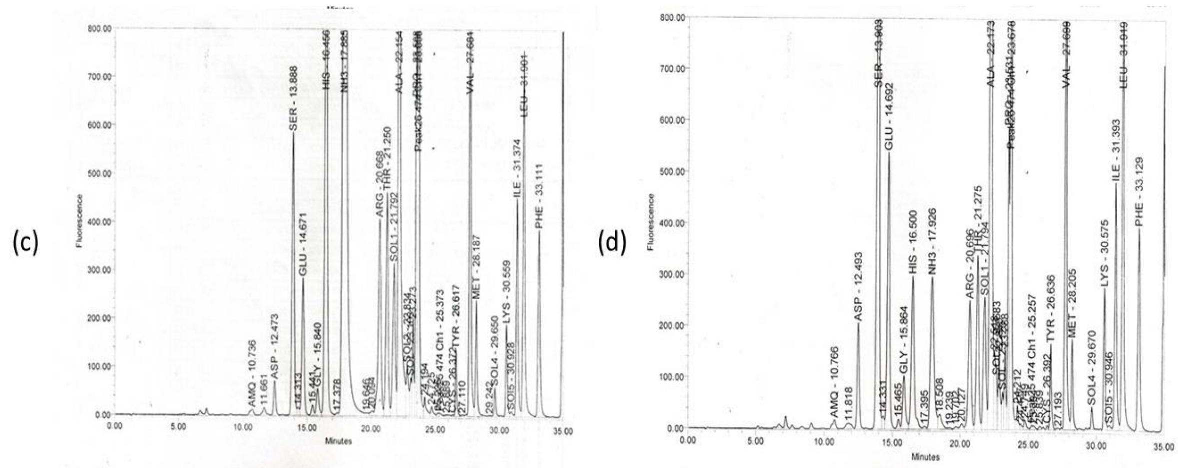
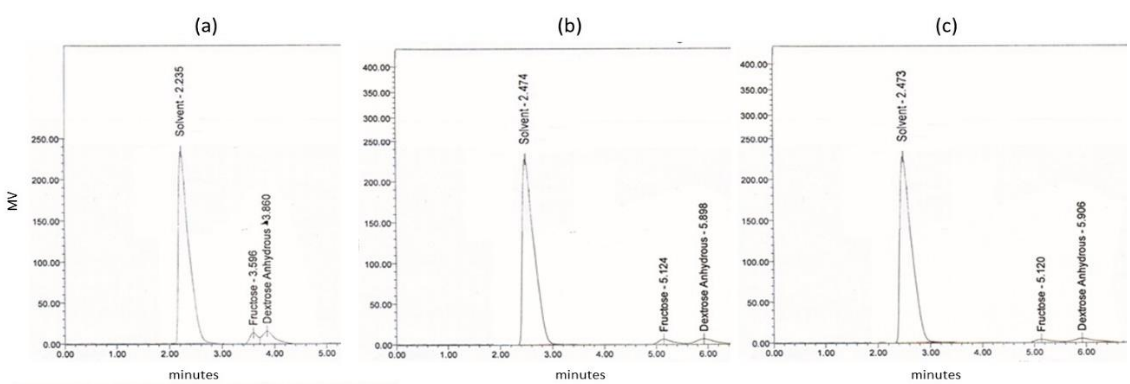


Figure 1. HPLC chromatograms of a representative sample of coconut water: (a) standard chromatogram, (b) chromatogram of 4 months old coconut, (c) chromatogram of 6 months old coconut and (d) chromatogram of 8 months old coconut.itle TNR 12 Normal

3.4. Concentration of Reducing Sugars

The representative chromatograms of HPLC-refractive index analysis for reducing sugars, e.g., dextrose and fructose are represented in the figure 2. Fructose concentration were found 2.30±0.16, 2.98±0.44 and 1.65±0.18 g/100ml in 4, 6 and 8 month old coconuts respectively. Similarly, dextrose concentration were 2.40±0.30, 4.02±0.39 and 2.65±0.08, fluctuating with increasing maturation.

Similar fluctuations of concentrations of total reducing sugar were previously observed by Child et al., 1950; with ultimate drop of sugar concentration in 18 month old coconut's water. Meanwhile, they reported total sugar concentration as higher in 10-14 month old coconut mainly due to increase of non-reducing sugar concentration. In fact, several sugars such as sucrose, glucose, fructose, mannitol, sorbitol, etc. appeared in literatures (del Rosario et al., 1984).



Peak Results (a)

	Name	RT	Area	Height	Amount	Units
1	Solvent	2.235	3215632	236579		
2	Fructose	3.596	138787	13354	2.313	g/100ml
3	Dextrose Anhydrous	3.860	253768	15481	2.403	g/100ml

Peak Results (b)

	Name	RT	Area	Height	Amount	Units
1	Solvent	2.474	5667808	384405		
2	Fructose	5.124	192597	10445	3.061	g/100ml
3	Dextrose Anhydrous	5.898	276059	10808	4.198	g/100ml

Peak Results (c)

	Name	RT	Area	Height	Amount	Units
1	Solvent	2.473	5676728	382477		
2	Fructose	5.120	100427	5476	1.596	g/100ml
3	Dextrose Anhydrous	5.906	175554	7135	2.670	g/100ml

Figure 2. HPLC chromatograms of a representative sample of coconut water for reducing sugar analysis: (a) chromatogram of 4 months old coconut, (b) chromatogram of 6 months old coconut and (c) chromatogram of 8 months old coconut

4. Conclusions

Coconut water is a popular refreshing drinks in the tropical world. There had been several works that explored the chemical and nutritional contents of this drink. Additionally the changes of coconut concentration due to soil quality, maturity and genetic variation was established. However, in Bangladesh, no such studies were found. Moreover, very few researches were available to explore the gradual changes of the coconut water contents from 2-6 months. The current study was aimed to fill this research gap. Samples of differently aged coconut were collected from a specific area of Dhaka and analyzed for electrolytes, amino acids and reducing sugar composition. The result obtained are in harmony with the previous studies in other settings. However, since the total concentration of cations (Na+, K+, Mg2+ and Ca2+) increased over time, gradual decrease of chloride ion concentration indicated an unknown anion-shifting in the salt concentration of the coconut water, probably going towards more citrate and malate ions. The current study had its constraints of not focusing on coconuts older than 6 months. It could neither be streamlined to a specific genetic diversity of coconut tree, nor could it be broadened the analysis to coconut trees in other regions of the country, especially coastal regions. The current authors hope that future studies would try to enlighten those research areas.

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