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Exploration of Autochthonous Fungal Population and its Bioactive compounds Elucidation from the Three Different Coconut Water Samples

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ABSTRACT

Coconut (*Cocos nucifera* L.) water is a refreshing drink consumed mostly directly from the fruit. However, in recent times, consumers in Accra prefer to have it transferred into plastic bags for later consumption; this favours a high risk of bacterial contamination. Since it is rich in nutrient, it may become unwholesome with possible high bacteria loads. Tender coconut water is a refreshing drink with electrolytes (ionic mineral) similar to human plasma. This refreshing drink is filled with many healthy natural nutrients which can enhance the body's metabolism and immunity and is used more as a health supplement. Initially these three kinds of coconut water interestingly present various diversity of the fungal organisms with different morphological in nature. Mainly those organisms were predominantly developed on two experimental coconut water such as tender and mature coconut water except king coconut water. Though, totally nine numbers of mouldy appearance white colour fungi were seen on mature coc. water and this similar kind of fungi five number also present in tender coco. Water too. But this kind of observed fungi didn't develop on the third water sample (King coco. water) Data not shown. Furthermore, other kind of morphologically different round shaped pale green colour fungi organisms also been noticed on both two samples (mature and tender coc. water) but it was absent in king water. In addition small thickened pure dark green colour fungi developed on the three coconut water samples viz., 11 in mature, 8 in tender and 5 in king coc. Water respectively. From the present result clearly showed among the three different kinds of coconut king coconut water showed maximized chemical as well as protein composition has been noticed than the control also other two varieties of coconut water. Although, exceptionally small white colour organisms were present. As a result of this coconut water imply that should it be contaminated shortly after two or three days of incubation, the early consumption of the water (before one and half hours), will most likely avert the risk of bacterial infection. In mature coconut water possessed totally nine compounds, of these nine bioactive compounds peak maximum showed n-Hexadecanoic acid with its retention time (43.41) as well as abundance (93) was and 1- Methyl -2-octenoic acid as a minimum revealed compound denoting abundance and its retention time such as 33.0 and 10.

Keywords: Coconut Water; Fungal Growth, Bioactive constituents, GCMS

ARTICLE INFO

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1. Introduction

Coconut water is now entering the nanoworld. A high-quality NiFe₂O₄ nanosized powder was prepared by a new route using a natural proteic solution of coconut water and metal ions (De Paiva *et al.*, 2009). The water of the green coconut (*Cocos nucifera* L.) fruit, also referred to as coconut juice, is a natural drink common in the tropics (Fonseca *et al.*, 2009; Walter *et al.*, 2009; Debmandel and Mandal, 2011). Coconut water has been called the fluid of life due to the medicinal efficiency as well as health benefits especially promoting digestion and cleaning the urinary regulations. Recently, Luzviminda, 2013 reported that the coconut water is very effective in treating kidney and urethral stones. Mainly it is a significant urinary antiseptic that helps in eliminate the poison in case of mineral poisoning aids absorption of drug taken by the individual making their peak concentration in the blood. It is mostly grown in the southern part of the country. The coconut fruit is sold openly while the coconut water and endosperm are mostly consumed fresh and directly from the fruit. However, in recent times, some consumers prefer to transfer these into plastic bags so that it may be transported or/and stored refrigerated for several hours before consumption reported by Adolf *et al.*, (2012). During this transfer, the water is most likely to be exposed, with a high possibility of contact with pathogenic bacteria. The liquid endosperm inside a young coconut is known as coconut water. It is fat free and low in calories. Sodium, potassium, phosphorus, chloride and magnesium are the main minerals found in coconut water, besides vitamin-C and sugars (Carpenter *et al.*, 1964; Magda, 1992; Campos *et al.*, 1996).

Coconut water presents anticarcinogenic properties (Sylviano *et al.*, 1992) and can be used as dehydrating solution administrated in oral and intravenous form, the later incase of severe dehydration (Magat and Agustin, 1997; Falck *et al.*, 2000). It has a great demand especially during the hot season. It is very effective especially for diarrhoea attacked people and excellent tonic for old and sick. The processed green coconut water increased the availability of coconut water and the producers sell it at reasonable price. The present investigation was undertaken with a view to preserve green coconut water which will be easily transported and increase availability all over the country.

Coconut water, as a tropical fruit juice, is highly valued and consumed in tropical areas since it is tasty and has desirable

nutritional and therapeutic properties. The total world coconut cultivation area was estimated in 1996 at 11 million hectares (ha), and around 93% was found in the Asian and Pacific regions (Punchihewa and Arancon, 2005). Indonesia, the Philippines, and India are the largest producers of coconut in the world. Coconut (*Cocos nucifera* Linn.) fruit is filled with the sweet clear liquid “coconut water” when the coconut is about 5 to 6 months old. Coconut water has been called the “fluid of life” due to its medicinal benefits such as oral rehydration, treatment of childhood diarrhea, gastroenteritis and cholera (Carpenter *et al.*, 1964; Kuberski 1980). It is high in electrolyte content and has been reported as an isotonic beverage due to its balanced electrolytes like sodium and potassium that help restore losses of electrolytes through skin and urinary pathways. Coconut water was claimed as a natural contender in the sports drink market with its delicate aroma, taste and nutritional characteristics together with the functional characteristics required in sports drink (Food and Agricultural Organization (FAO), 2005).

2. Material and methods

Ten approximately 1µl samples per three different coconut water were examined according to the guidelines proposed by Cappucino Manuel, (2004). A 10 g portion of each sample was suspended in 90 ml Na₄P₂O₇·10 H₂O to disperse organic colloids; further dilutions were made in NaCl (0.9%). The final dilution (1: 20 000) was plated (1 ml per plate) on two replicates: five of potato-dextrose agar (PDA), three of carboxy-methyl cellulose agar (CMC) and three of PDA supplemented with cycloheximide (CX) to retard the growth of all fungi, allow the isolation of slow-growing colonies and focus on fungi Plates were incubated at 37°C. The number of colony forming units per g of dry weight (CFU/g dwt) was noticed both for the total mycoflora and for each species or morphotype. Fungi were identified conventionally according to their macroscopic and microscopic features.

Physico-chemical analysis

The physico-chemical analysis was done initially at the time of harvesting. Then this was continued up to 6 months of storage with one month interval for different parameters. Total soluble solids content was determined with a hand refractometer and expressed as percentage. Percentage titrable acidity was estimated according the standard methods of AOAC (1984) where as pH was determined using a digital pH meter as described by Ranganna, (1966).

Mineral content was measured by atomic absorption spectrophotometer.

3. Results and discussion

Maximum and minimum amount of minerals present in the experimental various coconut water Sample(s) shown on the table-1. From the present result revealed that the maximum amount of minerals observed in King Coconut water such as Na- 4.53, K- 3.11, Ca - 62.30, Mg-12.03 and P-9.64 respectively. Despite, another two kinds of coconut water showed significantly reduced amount of such an experimental minerals than the control. In addition after seven days incubated coconut water possessed the remarkable decreased in nature of chemical content was noticed. Though, one of the other criteria of TSSH was one to three fold increased when compared with fresh coconut water of mature, king and tender coconut water. Meanwhile, it was increased tssh expressed on the three kinds of coconut water such as king, mature and tender comparatively with its control. From the present result clearly showed among the three different kinds of coconut king coconut water showed maximized chemical as well as protein composition has been noticed than the control also other two varieties of coconut water.

Apart from the fig-1 denoted clearly when the three different kinds coconut water were incubated after seven days (1 week) the following remarkable observation has been made. Initially these three kinds of coconut water interestingly present various diversity of the fungal organisms with different morphological in nature. Mainly those organisms were predominantly developed on two experimental coconut water such as tender and mature coconut water except king coconut water. Though, totally nine numbers of mouldy appearance white colour fungi were seen on mature coc. water and this similar kind of fungi five number also present in tender coco. Water too. But this kind of observed fungi didn't develop on the third water sample (King coco. water) Data not shown. Furthermore, other kind of morphologically different round shaped pale green colour fungi organisms also been noticed on both two samples (mature and tender coc. water) but it was absent in king water. In addition small thickened pure dark green colour fungi developed on the three coconut water samples viz., 11 in mature, 8 in tender and 5 in king coc. water respectively. Although, exceptionally small white colour organisms were present. As a result of this coconut water imply that should it be contaminated shortly after two or three days of incubation, the early consumption of the water (before one and half hours), will most likely avert the risk of bacterial infection. In other words, consuming the coconut water directly from the fruit has the lowest potential risk of bacterial infection.

In mature coconut water possessed totally nine compounds, of these nine bioactive compounds peak maximum showed n-Hexadecanoic acid with its retention time (43.41) as well as abundance (93) was and 1- Methyl -2-octenoic acid as a minimum revealed compound denoting abundance and its retention time such as 33.0 and 10. Moreover second most

peak compound named as Hexadecanoic acid methyl ester also observed specific RT and abundant percentage was 37.77 and 87%. In addition four other compounds also present with various optimum level such as 2,5-Diphenyl Lignoceric acid and its RT and abundance was 56.34 and 49 also 3,7,11,15-Tetramethyl-2-hexadecen-1-ol besides it had RT value 37.52 and abundant range was 43% respectively. Furthermore, 3-Hydroxy butane ester, 5-ethyl-Pyrogallol as well as 3-Hydroxy butane ester illustrated at moderately in the mature coconut water.

In third coconut water sample was king coconut from the GCMS chromatogram revealed the following specific resulted product or bioactive compound present in the present experimental sample. Totally seven compounds are identified among the seven Ethyl Octanoate was a peak level and it's observed other criteria such as noted RT and abundant percentage was 3.61 and 98.64. Despite optimum range of compound also been noted named such as also including their RT as well as abundant range was followingly 3- Hydroxy Methional, RT-6.65, Ab-44.53%, 1,6 Di butanol RT-8.69, Ab-42.03, 2,6 Nonadienol RT 4.37, Ab- 39.7% and Di Ethyl Butanoate RT-3.61 and percentage of abundance 20.05 respectively. Though, from the present experimental sample a least amount of compound also been observed such as Methyl dodecanoate 8.69 was Retention time and 10.20 as a noted abundant percentage.

Discussion

It is a clear, colourless, sweet, naturally flavoured slightly acidic drink. Decades of research have shown that coconut water is a rich source of nutrient, among which are essential amino acids (lysine, leucine, cystine, phenylalanine, tyrosine, histidine, and tryptophan), palmitic and oleic acids and dietary minerals (Manimuthu *et al.*, 2015). The principal sugars in coconut water are glucose, fructose, and sucrose, while tartaric, citric and malic acids are its abundant organic acids. It also contains vitamin B1, vitamin B2 and vitamin-C. The results in physico-chemicals parameters showed that the total soluble solid of fresh coconut water was 5.2%, after first month it came down 4.0%. TSS changed in other treatments was very negligible during storage period at room temperature with seven days of incubation it was also agreed by (Thampan and Rethinam, 2006; Unagul *et al.*, 2007)

Due to fungal influence of the coconut water previously few researchers were discussed about the initial acidity of green coconut water was 0.19% and was slightly decreased during storage period in all treatments but the changed was higher in control because no heat treatment and no preservative were added. The pH of fresh coconut water was 5.10, but after first month of storage the changed was very negligible in all treatments already been discussed by (De Paiva *et al.*, 2009). Effect of mineral content in fresh and preserved coconut water Na, K, Ca, Mg and Fe was observed in fresh coconut water were 0.41%, 0.62%, 0.20%, 0.03% and 0.02% respectively but after six month of storage it became slightly decreased in all treatments by

Sylianco *et al.*, (1992) ; Magat and Agustin, 1997). Previously similar view of coconut water and its nutritional content was opined by (Magda, 1992) such as coconut water in its envelope is sterile and composed of both organic and inorganic compounds (almost all minerals found in food). Compared with other fruit juices, the dry weight of mature coconut water is very low: 5% to 6% versus 12% to 15% for apple juice. The main components of coconut water are soluble sugars but it also contains proteins, salts and a very small quantity of oil, which contributes to its very low food energy level, *i.e.*, 44 cal·L⁻¹. The vitamin C content varies from (20 to 40) mg·L⁻¹. This is not high compared with other fruits such as orange or acerola, but it is sufficient to prevent oxidation for a limited period (Dupaigne, 1971). The vitamin B group is present in coconut water with 0.64 µg·mL⁻¹ of nicotinic acid and 0.52 µg·mL⁻¹ of pantothenic acid

Coconut water resembles blood plasma in its contents. Its successful intravenous use has been documented (Falck *et al.*, 2000). During the Pacific War of 1941-45, coconut water was siphoned directly from the nut to wounded soldiers for emergency plasma transfusions (FAO, 2005). It is believed that coconut water could be used as an important alternative for oral rehydration and even so for intravenous hydration of patients in remote region due to its electrolyte content (Watter *et al.* 2009). Coconut water may also offer protection against myocardial infarction (Adolf *et al.*, 2012). In Jamaica coconut water is known as a heart tonic and is used to strengthen the heart and improve circulation. Research bears this out. Animal studies show that coconut water consumption improves the ratio of good cholesterol to bad and reduces plaque formation in arteries, thus reducing risk of heart attack and stroke. High blood pressure is one of the primary risk factors associated with heart disease and stroke (Campos *et al.*, 1996; Magat and Agustin, 1997; De Paiva *et al.*, 2009). The minerals

potassium and magnesium are known to help reduce high blood pressure. Human studies show that coconut water, which is a good source of both of these minerals, is effective in reducing high blood pressure and increasing circulation (Debmandal and Mandal, 2011). Studies also demonstrated that coconut water consumption reduces the risk of heart failure in heart disease patients (Dares *et al.*, 1999; Fonseca *et al.*, 2009). The evidence is so convincing that the FDA allows coconut water to carry the claim that it “may reduce the risk of high blood pressure and stroke (Rattan, 1994). Since, it is tasty and has desirable nutritional and therapeutic properties.

The total world coconut cultivation area was estimated in 1996 at 11 million hectares (ha), and around 93% was found in the Asian and Pacific regions (Punchihewa and Arancon, 2005). Indonesia, the Philippines, and India are the largest producers of coconut in the world. Coconut (*Cocos nucifera* Linn) Coconut water, as a tropical fruit juice, is highly valued and consumed in tropical.) fruit is filled with the sweet clear liquid “coconut water” when the coconut is about 5 to 6 months old. Coconut water has been called the “fluid of life” due to its medicinal benefits such as oral rehydration, treatment of childhood diarrhea, gastroenteritis and cholera (Carpenter *et al.*, 1964; Kuberski, 1980; Vigliar *et al.*, 2006). In spite of these seemingly opposing potentials of coconut water, there are limited reports of the survival and growth of pathogenic bacteria in coconut water. A report by Debmandal *et al.* (2011) in profounded the similar opinion about the microbes present in the coconut water modelling the growth of *L. monocytogenes* in coconut water presented data to show that fresh coconut water was favourable for the survival and growth of *L. monocytogenes* and that refrigeration at 10°C or 4°C retarded, but did not inhibit, growth of the bacterium in green coconut water.

Table 1: Chemical composition of various types of coconut water

Minerals (%)	Name of the various coconut (mg/100ml)			
	Control	Mature	King	Tender
Na	3.76	3.15	4.53	2.81
K	2.83	2.40	3.11	1.67
Ca	59.85	56.33	62.30	52.46
Mg	10.67	11.52	12.03	9.58
P	8.31	8.32	9.64	9.66
PH	5.0	5.1	5.9	5.5
TSSH	1.06	2.36	4.32	3.65
Protein	1.5	1.54	1.65	1.65

After 7 days treatment				
Na	2.13	2.13	3.73	1.57
K	1.01	1.20	1.65	0.68
Ca	50.35	46.32	58.67	43.61
Mg	7.36	9.31	7.65	5.83
P	5.2	5.31	6.94	6.32
PH	4.6	5.4	5.3	3.8
TSSH	3.21	5.01	4.6	4.25
Protein	1.67	4.3	1.87	1.66

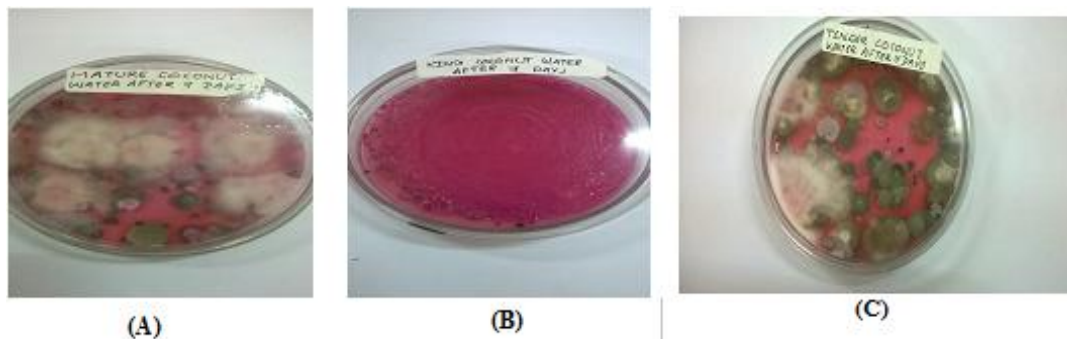


Figure 1: Autochthonous Fungal population of three various coconut water sample(s)



Figure 2: Total Autochthonous Bacterial Population from the three various coconut water sample(s)

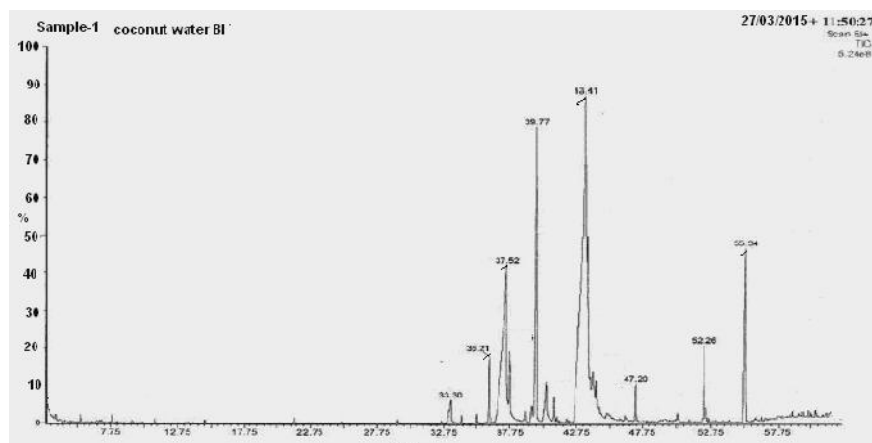


Figure 3: Compound elucidation of Mature Coconut water by GCMS Analysis

Table 2: Compounds elucidation of Tender Coconut water by GCMS Analysis

S.NO.	RETENTION TIME	COMPOUND(S) SEPARATED	ABUNDANCE (%)
1.	33.30	1- Methyl -2-octenoic acid	10
2.	36.21	3-Hydroxy butane ester	18
3.	37.52	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	43
4.	37.77	Hexadecanoic acid, methyl ester	87

5.	43.41	n-Hexadecanoic acid	93
6.	47.20	Hexadecanoic acid, ethyl ester	12
7.	52.26	5-ethyl- Pyrogallol	22
8.	56.34	2,5-Diphenyl Lignoceric acid	49

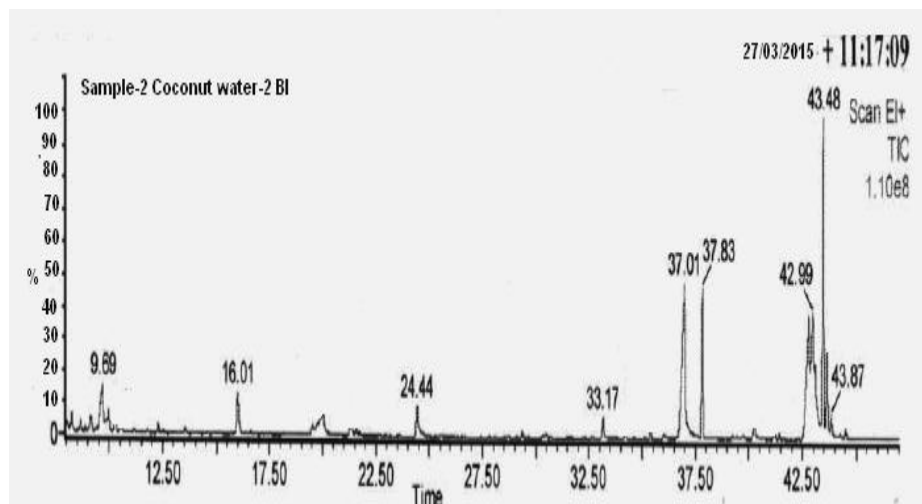


Figure 4: GCMS Chromatogram for King Coconut water

Table 3: Compounds elucidation of King Coconut water by GCMS Analysis

S. NO.	Retention Time	Compound(S) Separated	Abundance (%)
1.	9.69	2- Butyl Hexane	19
2.	16.01	3,5,7 Tri Hydroxy Nonanol	17
3.	24.44	1,2 Diocta Decanoic acid	12
4.	33.17	- Phenyl Limonene	11
5.	37.01	2,6 –Diphenyl octanal	53
6.	37.83	2, 4, 6 trimethyl undecanol	54
7.	42.99	2-phenylethanol	43
8.	43.48	Hexadecanoic benzothiazole	100
9.	43.87	Octane-3- one - 2-heptanol	0.05

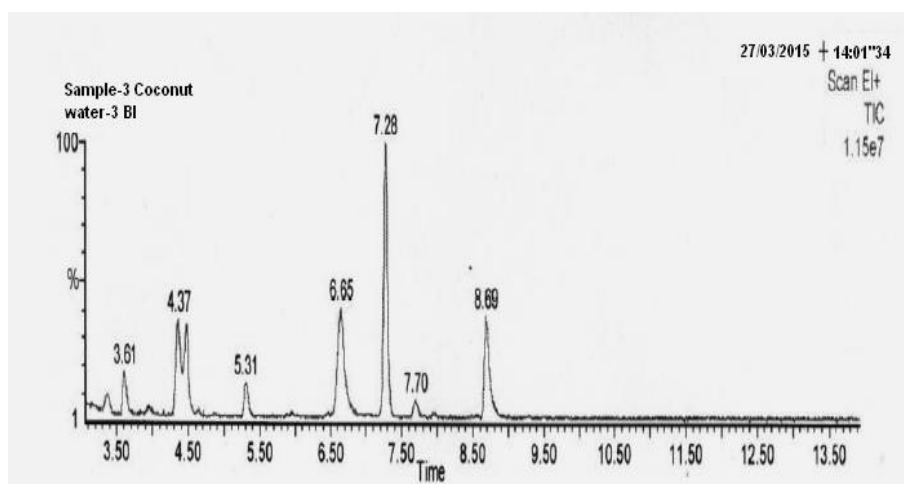


Figure 5: Chromatogram Analysis of King Coconut water

Table 4: Compounds elucidation of Mature Coconut water by GCMS Analysis

S. NO.	RETENTION TIME	COMPOUND(S) SEPARATED	ABUNDANCE (%)
1.	3.61	Di Ethyl Butanoate	20.05
2.	4.37	2,6 Nonadienol	39.7

3.	5.31 6.65	Phenyl-benzoic acid 3- Hydroxy Methional	18.71 44.53
4.			
5.	7.28	Ethyl Octanoate	98.64
6.	7.70	Methyl dodecanoate	10.20
7.	8.69	1,6 Di butanol	42.03

4. Conclusion

The present study may conclude about the coconut water from three different coconut varieties exactly showed that the mineral composition fresh and after seven days incubation ultimately fluctuation such as after seven days incubated coconut water possessed the remarkable decreased in nature of chemical content was noticed compare than the freshly analyzed coconut water samples of the King, Mature and tender coconut water. Though, one of the other criteria of TSSH was one to three fold increased when compared with fresh coconut water of mature, king and tender coconut water. Meanwhile, it was increased tssh expressed on the three kinds of coconut water such as king, mature and tender comparatively with its control. From the present result clearly showed among the three different kinds of coconut king coconut water showed maximized chemical as well as protein composition has been noticed than the control also other two varieties of coconut water. From the GCMS study clearly denoted the bioactive compound elucidation, mainly similar number of nine compounds were elucidated both on King and tender coconut water followed by seven total compounds were identified for mature coconut water. The elucidated compounds were named as n-Hexadecanoic acid, Hexadecanoic benzothiazole and Ethyl Octanoate elucidated for Tender, King and Mature coconut water respectively. These analytes were potentially important for our human health as well as necessary nutritional valuable secondary metabolites present on these three coconut water.

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