International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 1494-1498

Received: 01-07-2017
Accepted: 02-08-2017

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Nutrient fortified king coconut water as an isotonic thirst quenching beverage for sports men and women

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Abstract
The main objective of the study was to develop an isotonic thirst quenching beverage to provide maximum effectiveness to the consumers by changing the natural composition of king coconut (*Cocos nucifera aurantiaca*) water. A preliminary study was carried out using three variables; amount of carbohydrates (6, 7, 8 w/v%), electrolytes (20, 25, 30 mmol/L) and volume of king coconut water (50, 100, 150, 200, 250 mL) and 9 carbohydrate-electrolyte samples were selected out of 45 samples using a sensory analysis. Tonicity of the samples were identified by the osmolality parameter. Since all selected samples were in the hypertonic range, the least hypertonic sample (6% sugars (CHO), 20 mmol/L Sodium, 100:150 mL ratio of king coconut water to deionized water) was chosen to develop two isotonic range products. The final product was selected by a sensory analysis. Chemical composition of the selected final product can be concluded as 5.4% carbohydrates, 0.09% protein, 0.01% total fat, 0.1% total ash, no crude fiber, 22.05 kcal/100g total energy, 20 mmol/L Sodium, 24 mmol/L Potassium, 11 mmol/L Magnesium and 6.75 mmol/L Calcium. The osmolality of the final product was 289 mOsm/kg. Final carbohydrate-electrolyte drink showed microbiological stability up to two months under refrigerated conditions.

Keywords: sports drink, king coconut, isotonic, osmolality

Introduction
Sports drinks are designed to provide the right balance of carbohydrate and fluid, to ensure that they are emptied quickly from the stomach and are rapidly absorbed from the small intestine. The main purpose of sports drinks is to provide water, energy and electrolytes in an appetizing and readily available form. Sports drinks are projected towards athletes and other individuals exposed to active physical activities in order to keep them hydrated and delay exhaustion. It contains optimal levels of carbohydrates as a source of energy, certain salts e.g. sodium chloride, sodium citrate, potassium chloride, potassium citrate and some other minerals such as Magnesium and Calcium to achieve target value of electrolyte content and some vitamins like Vitamin A, C, E [1, 2]. Fluid loss during strenuous, long duration exercise is commonplace and can result in thermal stress, impaired cognition and cardiovascular function, accelerated fatigue, and impaired exercise performance [3, 4]. Sports drinks are specially formulated beverages that help people to rehydrate during and after exercise. Beverage formulated for rehydration and recovery generally have two main aims; to replace the water and electrolytes lost in sweat secreted during exercise and to replace the carbohydrate utilized from liver and muscle store during exercise. Sports drinks should contain 40-80 gL-1 (optimally 60- 80 gL-1) carbohydrate and more than 0.4 gL-1 (optimally > 0.6 gL-1) sodium [5]. Moreover, considering its absorption, osmolality of drink is very essential. Sports drinks mostly are designed as isotonic [1]. Isotonic and slightly hypotonic solutions with osmolality 200–330 mOsmkg-1 are used to prepare sports drinks. Osmolality is intensely influenced by the proportion of monosaccharides, disaccharides or polysaccharides besides carbohydrate content as well as electrolyte content [5]. Sodium in sports drinks play a relatively minor role in the absorption process because sodium is quickly added to the intestinal lumen by intestinal secretion [6]. The osmolality of a beverage is directly related to the solute composition of the beverage. Increasing the amount of carbohydrates or electrolytes in a drink also increases the osmolality of the drink. Research studies indicate that sports drinks should be either hypotonic or isotonic to ensure rapid gastric emptying and intestinal absorption [7]. Net fluid absorption occurs only from isotonic and hypotonic solutions. When hypertonic solutions are consumed.

it is eventually reduced to the near isotonicity by intestinal secretions before net fluid absorption is obtained and it lowers the absorption rate and increases the risk of gastrointestinal discomfort [8]. Solutions containing 6% carbohydrates or less are absorbed more rapidly than solutions containing greater amounts of carbohydrates [9]. Besides all mentioned ingredients, beverage palatability also depends on kind of base used in its formula. King coconut water is a well-known source of sugars, amino acids, vitamins and minerals [10]. Its sugar content and mineral content makes it an ideal rehydrating drink after exercise. In relation to sport nutrition, king coconut water has been reported to provide hydrating effects similar to those of carbohydrate-electrolyte sport drinks [11, 12, 13]. King coconut (Cocos nucifera aurantiaca) is believed to be indigenous to Sri Lanka and it has been traditionally consumed as a refreshing beverage in a majority of coconut producing countries. Previous studies [14] have reported that, a tender king cocqnut (7-8 months old) has glucose 2-3 g/100 mL, fructose 2-3 g/100 mL, sucrose 0.1-g/100 mL, Potassium 1900-2500 mg/L, Chlorides 1200-1600 mg/L. Calcium 100-200 mg/L, Magnesium 45-55 mg/L, energy 17.4 kcal/100 mL, protein 0.1g/100 mL, fat 0.1g/100 mL and carbohydrates 4g/100 mL. The inclusion of glucose, sucrose and other complex carbohydrates in fluid replacement solutions have equal effectiveness in increasing exogenous carbohydrate oxidation delaying fatigue and improving performance [15]. A properly formulated beverage needs to cater voluntary fluid consumption, stimulation of rapid fluid absorption and speedy rehydration. Some of the medicinal properties of king coconut water are medium of oral rehydration, cooling the body, contains organic compounds containing growth promoting properties, diuretic, substitute for blood plasma, aids quick absorption of drugs etc [16]. The main aim of this research study was to develop a thirst quenching isotonic beverage as a sports drink by using nutrient fortified king coconut water by changing the natural composition of king coconut water and by adding deionized water, carbohydrate sources, electrolyte sources, antioxidants and required permitted preservative. Sensory analysis and osmolality are the main parameters that are taken into account to identify the best product.

Materials and Methods

King coconuts were washed in chlorinated water. Epicarp and parts of the mesocarp of the nuts were removed. King coconut water was measured into pre-sterile glass bottles, required amounts of ingredients were added and the total volume was brought up to 250ml with addition of deionized water as shown in Table 3.1. The bottles were immediately sealed with a cap and pasteurized at 80 0C by placing the bottles in the upright position in a boiling water bath for 20 minutes. A sensory evaluation was conducted for 45 different combinations of king coconut water solutions using 3 variables such as amounts of carbohydrates (6%, 7%, 8%); concentration of electrolytes (20, 25, 30 mmol/L) and volume of king coconut water (50, 100, 150, 200, 250 mL). A panel of 30 untrained judges were used for the Sensory evaluation. Taking a five-point hedonic scale into account, the sensory evaluation was conducted based on the combinations prepared by using the three variables as illustrated in Table 3.1. Sweetness, tartness, saltiness, mouthfeel and overall acceptability were the sensory attributes that were considered for the sensory evaluation. The best 9 samples were selected out of 45 samples at the end of the preliminary study as the best carbohydrate- electrolyte drink series.

Table 3.1: Variables used to prepare the king coconut water samples for the sensory analysis

Amounts of Carbohydrates (%)	Concentration of NaCl electrolytes (mmol/L)	Volume of king coconut water (mL)
6	20	50
7	25	100
8	30	150
		200
		250

Roduct development process

Analysis of osmolality of Carbohydrate-Electrolytes drinks:
Based on the results obtained from the preliminary study, the best carbohydrate-electrolyte drink series was selected and the osmolality was measured in each sample of the series (Freezing point depression osmometer – Model No: 3320) as shown in Table 3.2. Samples were graded as hypotonic, isotonic and hypertonic based on the osmolality values. The samples that belonged to the isotonic range or least hypertonic range were selected for further studies.

Table 3.2: Selected samples by the sensory evaluation

Product description			
CHO (%)	NaCl (mmol/L)	King coconut (mL)	Deionized water (mL)
6	20	100	150
6	25	250	0
6	30	250	0
7	20	200	50
7	25	250	0
7	30	50	200
8	20	250	0
8	25	200	50
8	30	250	0

Preparation of isotonic range samples:

New formulations for carbohydrate-electrolyte drinks were prepared by changing the sugar types and the osmolality of each sample was measured. Further modification was done with the addition of antioxidant (Vitamin C) and preservative (Potassium Sorbate). After the modifications, final osmolality was measured in each sample.

Sensory evaluation of the final samples

A panel of 30 untrained judges were used for the Sensory evaluation of the prepared samples. Taking a five-point hedonic scale into account, the sensory evaluation was conducted based on the sensory attributes; color, taste, odor, appearance, overall acceptability and the best sample was selected as the final product.

Chemical composition of the final product

Proximate analysis of the final product was done in three replicates under each testing parameter. Protein content of the king coconut water sample was determined by kjeldhal method [17]. Total fat content, Fiber content, ash content, moisture content were determined according to AOAC method [18]. Carbohydrate content of the king coconut water sample was determined according to the following formula [18].

% Available carbohydrate = 100 – (Protein% + Fat % + Fiber% + Ash% + Moisture %)

Total energy of the king coconut water sample was determined by the Atwater formula where fat, protein and

carbohydrate supplied were 4, 9 and 4 kcal/100g respectively [19].

Total Energy (kcal/100g) = Protein% x 4 + Fat% x 9 + Carbohydrate% x 4

Determination of Vitamin C content

Vitamin C content (Ascorbic acid content) of the king coconut water sample was determined by HPLC [18]. A standard Vitamin C (Ascorbic acid) solution with a concentration of 0.4mg/ml was used for the standardization of Indophenol dye solution.

Sorbic acid determination

Sorbic acid in king coconut water sample was determined by Reversed Phase Liquid Chromatography [18]. HPLC column (C_{18}, 250 mm×4.6 mm, 5 µm); Mobile phase (methanol - Phosphate buffer solution: 1+9 with pH 6.7); 1.2 mL/min Flow rate; 227 nm Wavelength for UV Detection; Column Temperature under room temperature; sample size of 10 µL were the chromatographic conditions used.

Determination of pH of the final product

The pH value of the Carbohydrate-electrolyte drink was measured using the pH meter (HQ11d Portable pH meter).

Determination of mineral content in the final product by Atomic Absorption Spectroscopy (AAS) Method

King coconut water sample was tested for Na, K, Ca and Mg by the Atomic Absorption Spectrometer (Thermo scientific/iCE 3000 AA05121002 v1.30) using the relevant standard stock solutions [18].

Microbiological analysis

The Coliform count and Yeast & mold count were analysed using petri films. Total Plate count was analysed using sterile nutrient agar [18].

Shelf life determination

Chemical, physical and microbiological parameters were checked once a week. The color and turbidity of the drink was checked once a week by opening a new bottle. Simple comparison tests were conducted weekly to check if there are any significant influences to sensory and visual qualities of the king coconut drink.

Statistical analysis

Sensory evaluation results of the preliminary study were obtained by the Kruskal-Wallis test using the software MINITAB® 17. For sensory evaluations, Kruskal-Wallis test followed by a pair wise comparison were performed between samples to obtain the average rank values to identify the best 9 samples out of 45 samples.

Results and Discussion

Osmolality of Carbohydrate-Electrolytes drinks and tonicity of the samples

Solutions with an osmolality similar to that of blood are called isotonic solution. Table 4.1 illustrates the osmolality values of the selected 9 samples and the tonicity of the samples are graded as isotonic (275-295 mOsm/kg H_2O); hypertonic (>295 mOsm/kg H_2O); hypotonic (<275 mOsm/kg H_2O) [20].

Table 4.1: Osmolality and tonicity of the selected best samples

Product description				Osmolality (mOsm/kg)	Tonicity
CHO (%)	NaCl (mmol/l)	King coconut (ml)	Deionized water (ml)		
6	20	100	150	306	Hypertonic
6	25	250	0	402	Hypertonic
6	30	250	0	408	Hypertonic
7	20	200	50	448	Hypertonic
7	25	250	0	462	Hypertonic
7	30	50	200	452	Hypertonic
8	20	250	0	525	Hypertonic
8	25	200	50	518	Hypertonic
8	30	250	0	543	Hypertonic

Osmolality of the further developed products

Table 4.2 illustrates the osmolality and the tonicity of the two further developed samples after the alterations made with the sugar types in the least hypertonic range sample (Sample with an osmolality of 306 mOsm/kg). Table 4.3 illustrates the osmolality and the tonicity of the products after the addition of antioxidant (Vitamin C) and preservative (Potassium Sorbate).

Table 4.2: Osmolality and tonicity of the further developed products

Sample	Alterations made with the sugar types (CHO%)	Osmolality (mOsm/kg)	Tonicity
A	6.5	287	Isotonic
B	7.5	284	Isotonic

Table 4.3: Omolality and the tonicity of the products after the addition of antioxidant (Vitamin C) and preservative (Potassium Sorbate).

Sample	Osmolality (mOsm/kg)	Tonicity
A	292	Isotonic
B	289	Isotonic

Results of the sensory evaluation of the final samples

Sensory evaluation results of the final samples were obtained and a statistical analysis was done by the Friedman test using the software MINITAB® 17. Based on the results obtained from the statistical analysis as shown in Table 4.4, both samples have a significant difference for all attributes. Sample B has the highest values for all attributes and it was selected as the final product.

Table 4.4: Sum of rank values for the sensory attributes of the final samples

Sum of rank values of sensory attributes					
Sample	Color	Taste	Odor	Appearance	Overall acceptability
A	37.5b	33.0b	32.5b	33.0b	30.0b
B	52.5a	57.0a	57.5a	57.0a	60.0a

*Different letters (a, b) within the same column indicate statistically significant differences among the samples

Results of chemical composition and pH of the final product

An electrolyte drink or an electrolyte drink base contain no less than 50 g/L and no more than 100 g/L total carbohydrates (dextrose, fructose, glucose syrup, maltodextrin, sucrose) and no more than 50 g/L fructose. As shown in Table 4.5, the final product contains 5.4 % carbohydrate content on wet basis. Protein, total fat and ash contents were detected in minor quantities. Fiber content was not detected and detected energy value was 22.05 kca/l100g (92.2572 KJ). Antioxidant (Vitamin C 400 mg/L) and preservative (Potassium Sorbate 153.64 mg/L) contents were within the standard ranges [22]. Vitamin C plays a role in free radical defense.

Table 4.5: results of the physicochemical characters of the final product

Tested parameter	Results
Protein content (% by mass on wet basis)	0.09
Total fat content (% by mass on wet basis)	0.01
Fiber (% by mass on wet basis)	Not detected
Ash (% by mass on wet basis)	0.10
Moisture (% by mass on wet basis)	94.40
Carbohydrate content (% by mass on wet basis)	5.40
Energy content (kcal/100g)	22.05
pH	4.50
Vitamin C content (mg/L)	400.00
Sorbic acid content (mg/L)	153.64

Mineral content of the final product

Table 4.6: Mineral content of the final product

Mineral	Amount (mg/L)	Amount (mmol/L)
Na	462	20
K	924	24
Mg	260	11
Ca	270	6.75

Sports drinks are used to replace electrolytes that are lost through sweat. Electrolytes lost through the sweat mainly comprise of sodium, potassium, calcium and chloride. The sodium in the sports drink contributes to palatability and also encourages drinking as well replacing that lost through sweat. Potassium affects heartbeat and aids muscle contraction. Calcium combines with other minerals within a protein framework to give structure and strength to bones and teeth, also calcium assists in blood clotting and it helps prevent loss of blood [21]. An electrolyte drink or an electrolyte drink base must contain no less than 10mmol/L of Sodium (Na) [22]. The developed sports drink contains a Na concentration of 20mmol/L (Table 4.6).

Results of the microbiological analysis

As shown in Table 4.7, yeast and mold count; and Coliform counts were not observed up to the end of the second month. Therefore, no fermentation was found until the end of the second month. Pasteurization, usage of pre-sterile bottles and the addition of preservatives have increased the microbial stability of the final product.

Table 4.7: Microbial count of the final product at the end of the first and second months

Method	Microbial count (CFU/mL) 1st Month	Microbial count (CFU/mL) 2nd month
Yeast and mold count	Nil	Nil
Coliform count	Nil	Nil

Shelf life determination results

Shelf life of the product was mainly determined by the color change and turbidity differences under two different temperatures (Table 4.8). As illustrated in Table 4.8, there were no color changes or turbidity up to the end of the 6th week under room temperature. At the end of the 7th week a very slightly whitish cloudiness was observed and at the end of the 8th week a slightly whitish cloudiness was observed. There were no color changes or turbidity up to the end of the 8th week under the refrigerated condition. All ingredients and desirable sensory attributes have to be preserved in sports drink during its shelf life. If only one ingredient or sensory attribute do not fulfil desired requirements, in spite of well microbiological findings, shelf-life should be shortened.

Table 4.8: Color and turbidity changes under room temperature and refrigerated conditions.

Product	End of the 1st week	End of the 2nd week	End of The 3rd week	End of The 4th week	End of The 5th week	End of The 6th week	End of the 7th week	End of the 8th week
Drink under room temperature	No color/ turbidity change	No color/ turbidity change	No color/ turbidity change	No color/ turbidity change	No color/ turbidity change	No color/ turbidity change	Very slightly whitish cloudiness	Slightly whitish cloudiness
Drink under refrigerated conditions	No color/ turbidity change	No color/ turbidity change	No color/ turbidity change	No color/ turbidity change	No color/ turbidity change	No color/ turbidity change	No color/ turbidity change	No color/ turbidity change

Conclusion

Sensory evaluation was the key point used to determine the overall quality and the acceptability of the final product. The final product formula is (Product B), 100ml of king coconut water; 150ml of deionized water; sugars (7.5%) as 2.0675g of glucose and 7.8217g of sucrose; 0.1g of Vitamin C; and 0.0384g of Potassium Sorbate. Chemical composition of the final carbohydrate-electrolyte drink is; 5.4 % carbohydrates, 0.09 % protein, 0.01 % total fat, 0.1 % total ash and no crude fiber. Total energy value of the final product is 22.05 kcal/100g (92.26KJ). Total electrolyte composition of the final product is; 20 mmol/L Sodium, 24 mmol/L Potassium, 11 mmol/L Magnesium and 6.75 mmol/L Calcium. Osmolality of the final product is 289 mOsm/kg and the final product pH is 4.5. The final product was microbiologically stable under two months of observation and the final product is very much stable for 2 months under refrigerated conditions.

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