

**Composition and Physicochemical Properties of Fresh and Freeze-Concentrated Coconut
(*Cocos nucifera*) Water**

**Seow Eng Keng^a, Azhar Mat Easa^a, Ahmad Munir Che Muhamed^b, Cheong-Hwa Ooi^b, and *Tan
Thuan Chew^a**

^aFood Technology Division, School of Industrial Technology,
Universiti Sains Malaysia,
11800 USM, Penang, MALAYSIA

^bAdvanced Medical and Dental Institute,
Universiti Sains Malaysia,
13200 Kepala Batas, Penang, MALAYSIA

Corresponding author; Thuan Chew,

^aFood Technology Division, School of Industrial Technology,
Universiti Sains Malaysia,
11800 USM, Penang, MALAYSIA
Email: thuanchew@usm.my

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ABSTRACT

The aim of this study was to produce a double-strength freeze-concentrated coconut water. Coconut water obtained from green (GC) and mature coconuts (MC) with initial total soluble solids (TSS) of approximately 6 and 4 °Brix, respectively, were used for the production of a double strength coconut water (12 and 8 °Brix, respectively) using a simplified freeze-concentration process. The freeze-concentrated samples were significantly ($P < 0.05$) higher in sugars, acidity, minerals, protein, crude fat, and total phenolic compounds as compared to the fresh coconut water. Sensory evaluation indicated no significant difference ($P > 0.05$) in consumers' acceptability score of the freeze-concentrated coconut water when compared with fresh coconut water. Upon reconstitution (to initial TSS), reconstituted freeze-concentrated samples retained the same acceptability as of fresh coconut water. This suggests that both the freeze-concentration and reconstitution processes had no significant ($P > 0.05$) changes to consumers' acceptability score. Hence, freeze-concentrated coconut water could be a better rehydration drink than fresh coconut water; providing more nutrients without affecting its acceptability.

Keywords: Coconut water, freeze-concentration, composition, physicochemical properties, sensory acceptability

ABSTRAK

Tujuan kajian adalah untuk menghasilkan air kelapa sejuk beku-pekat dengan kekuatan ganda dua. Air kelapa yang diperoleh daripada kelapa muda (GC) dan kelapa matang (MC) dengan jumlah pepejal larut (TSS) awal lebih kurang 6 dan 4 °Brix, masing-masing, digunakan untuk menghasilkan air kelapa dengan kekuatan ganda dua (6 dan 4 °Brix, masing-masing) dengan menggunakan proses kepekatan sejuk beku dipermudah. Sampel sejuk beku-pekat mempunyai kandungan gula, keasidan, mineral, protein, lemak kasar, dan jumlah sebatian fenolik yang lebih tinggi secara signifikan ($P < 0.05$) berbanding dengan air kelapa segar. Penilaian sensori menunjukkan perbezaan yang tidak signifikan ($P > 0.05$) dalam skor penerimaan pengguna bagi air kelapa beku-pekat berbanding dengan air kelapa segar. Hasil daripada pembancuhan semula (ke TSS awal), air kelapa sejuk beku-pekat dicampur semula mempunyai penerimaan pengguna yang setara dengan air kelapa segar. Hal ini menunjukkan bahawa kedua-dua proses pemekatan sejuk beku dan pencampuran semula tidak mengubah skor penerimaan pengguna secara signifikan ($P > 0.05$). Oleh yang demikian, air kelapa sejuk beku-pekat boleh merupakan minuman rehidrasi yang lebih baik berbanding dengan air kelapa segar; membekalkan nutrien yang lebih banyak tanpa mempengaruhi keboleherimaannya.

Kata Kunci: Air kelapa, kepekatan sejuk beku, komposisi; sifat fizikokimia, keboleherimaan sensori

INTRODUCTION

Coconut water has become a well-known drink and is gaining popularity in beverage industry due to its high nutritional value (Santoso *et al.*, 1996; Yong *et al.*, 2009; Tan *et al.*, 2014) and potential therapeutic properties (Campbell-Falck *et al.*, 2000; DebMandal and Mandal, 2011). In sports application, coconut water has been proposed as a natural alternative to commercial rehydration drink due to its ability to rehydrate and replenish electrolytes lost during exercise (Saat *et al.*, 2002; Cappelletti *et al.*, 2015). The sweetness of coconut water caused less nausea and fullness, as well as no stomach upset when consume in a larger volume as compared to water. However, the low sodium content in coconut water has shown to have a fairly low rehydration index. With that in mind, Ismail *et al.* (2007) investigated the effectiveness of sodium-enriched coconut water (SCW) as a rehydration drink after exercise-induced dehydration. It was found that SCW performed at par with commercial sports drink for whole body rehydration, but with better fluid tolerance. Hence, low concentrated coconut water, *i.e.* double strength coconut water, could serve as a better rehydration drink compared to sodium-enriched coconut water, since it can provide more energy (higher sugars content) and electrolytes (higher minerals content).

There are various applications capable to concentrate liquid product, such as by mean of evaporation (Galici *et al.*, 2009; Hongvaleerat *et al.*, 2008), membrane filtration (Couto *et al.*, 2011; Galaverna *et al.*, 2008), and

freeze-concentration process (Lo *et al.*, 2007; Belén *et al.*, 2013; Miyawaki *et al.*, 2016). High temperature treatment can affect the flavour quality and the phenolic compounds in coconut water can be oxidized and lead to yellow discoloration (Ito *et al.*, 2003; Tan *et al.*, 2015). Thereby, freeze-concentration process was selected in this study to produce freeze-concentrated coconut water with minimal changes to its quality and consumers' acceptability. Freeze-concentration process allows the removal of water from a solution by forming a high-purity ice-crystals and separated to leave a concentrated liquid. Due to the nature of the low temperature used, undesirable chemical and biochemical changes can be avoided as well as minimising the damage to product quality (Miyawaki *et al.*, 2016).

To our knowledge, simplified freeze-concentration process was used to produce freeze-concentrated sugar-cane juice (Lo *et al.*, 2007), however none on coconut water. The aim of this study was to evaluate the freeze-concentration process to produce freeze-concentrated coconut water with double its original total soluble solids content. This would be evaluated against fresh coconut water. By controlling the freeze-concentration process, the soluble components, mainly sugars, and minerals, occurring within the fresh coconut water can be increased without the use of excessive heat such as that applied during the process of evaporation.

MATERIAL AND METHODS

Chemicals and reagents

All chemicals used in this study were of analytical grade, except for methanol, glucose, fructose, and sucrose, which are HPLC grade. All the chemicals were purchased from Sigma-Aldrich Co., St. Louis, USA. Bradford Protein Assay kit was purchased from Bio-Rad Laboratories, Hercules, USA.

Coconuts

Coconut fruits of two different maturity stages were used in this study. Green coconuts (GC) used were of 8–9 months old and contained a soft thin (2–4 mm) layer of coconut flesh. Mature coconuts (MC) used were of ≥ 12 months old and contained a hard thick (~10 mm) layer of coconut flesh. All coconuts (from coconut variety Malayan Tall) were purchased from a local commercial dealer (Anba Coconuts) located at Abu Siti Lane, Penang, Malaysia, between the month of January and April, 2015.

Extraction of coconut water

Surface of the coconut husks was cleaned with distilled water and 1% bleach (Clorox) before a stainless-steel knife was used to perforate the fruit mesocarp. Coconut water was manually extracted from the coconut fruit and filtered through muslin cloth. The filtered coconut water from several fruits (5 to 10 coconuts with similar maturity age) was pooled in a 2 L Schott bottle and kept temporary in an icebox filled with ice packs. All coconut water prepared was processed on the same day of purchase and extraction. Three batches of coconut water were prepared for each maturity stage.

Production of freeze-concentrated coconut water

Two types of freeze-concentration of coconut water were prepared; freeze-concentrated coconut water prepared from GC (FGC) and MC water (FMC). Freeze-concentration of coconut water was carried out according to the method as described by Lo *et al.* (2007) with slight modifications. Extracted coconut water was filled into polypropylene plastic casings (18 cm \times 12 cm) that was then sealed and subjected to a rapid chilling treatment to -18°C using an air blast freezer (Irinnox, Italy). Each plastic casing can be filled with 200 mL of coconut water. The process of rapid chilling from ambient temperature to -18°C took around 30 min to complete. At the end of this process the core temperature of the coconut water-ice mixture was -8°C .

The coconut water-ice mixture was then transferred into a domestic freezer (-18°C ; Sharp, Malaysia) and stored for 24 h, whereby the hardening and completion of ice formation occurred. At this stage, separation of ice and unfrozen phase consisted mainly of freeze-concentrated coconut water was evident. A small opening

was made at one end of the plastic casing to allow the flow of the concentrated coconut water into a volumetric flask. This process of thawing was performed at room temperature and stopped once the total soluble solid (TSS) of the thawed coconut water reached double the initial TSS value. The TSS reading was taken every 5 min and the thawing process took 50 min to complete. The production trials were repeated three times to yield standardised freeze-concentrated coconut water with predefined, *i.e.* double, TSS value.

Assessment of physicochemical properties of coconut water

Total soluble solids (TSS)

TSS was expressed as °Brix and measured with a digital refractometer (Hanna Instruments Inc., H196801, Cluj-Napoca, Romania) at 25°C (Tan *et al.* 2014).

pH

The pH of coconut water was determined using pH electrode (Mettler-Toledo, Inlab Semi Micro Electrode, Greifensee, Switzerland) attached to a pH meter (Mettler-Toledo, S40 SevenMulti™, Greifensee, Switzerland). Calibration on the pH meter with buffer solution of pH 4.01, 7.00, and 9.21 was done prior to measurements.

Turbidity

Turbidity of the coconut water was determined using a turbidimeter (Hach, 2100P, Loveland, USA) as described by González-Neves *et al.* (2014). Calibration on the turbidimeter with Formazin Turbidity Standard solutions of <0.1, 20, 100, and 800 NTU was done prior to measurements. Turbidity value was expressed in Nephelometric Turbidity Units (NTU).

Assessment of composition of coconut water

Protein content

Protein content of the samples was determined according to Bradford Method as described by Tan *et al.* (2014). Bovine serum albumin (BSA) was used as standard. Measurement of absorbance was carried out using UV-Vis spectrophotometer (Shimadzu, UV-1650 PC, Nakagyo-ku, Japan) at wavelength of 595 nm. Relative measurement of protein concentration can be obtained by comparing with the BSA standard curve.

Crude fat content

Crude fat content in coconut water samples was determined by AOAC Method 934.01, with slight modifications (AOAC, 1995). The weight of an empty round bottom flask after 1 h of oven-drying and 30 min of cooling in desiccators was recorded. Coconut water (10 mL) was placed in a separating funnel and mixed well with 10 mL of petroleum ether. The separating funnel was inverted slowly and the stopcock was opened to release the pressure. A few drops of the mixture were placed on the filter paper (Whatman Filter Paper Grade 41). Extraction was considered complete when there was no trace of oil observed on the filter paper. The petroleum ether was then collected in round bottom flask and induced to the Soxhlet extractor to extract fat. The solvent was siphoned and removed from the flask until almost dried. The flask was then heated in an oven at 105°C (Memmert, UM 600, Schwabach, Germany) for 1 h and kept in desiccators for 30 min before the weight of the flask was measured. The crude fat content was calculated and expressed in % (w/v) using the following equation:

$$\text{Crude fat content} = [(w_1 - w_2)/V] \times 100\% \quad (1)$$

where, w_1 is the weight of flask with fat (in g), w_2 is the weight of empty flask (in g), and V is the volume coconut water used (in mL)

Total phenolics content (TPC)

TPC of coconut water was determined using Folin-Ciocalteu method as described by Tan *et al.* (2014). Coconut water (1 mL) was placed in a 100 mL volumetric flask followed by 70 mL of distilled water and 5 mL of Folin & Ciocalteu's phenol reagent (10 times dilution). Mixture was incubated for 5 min at room temperature before adding 15 mL of 7.5% (w/v) sodium carbonate and topped up to 100 mL with distilled water. Mixture was incubated for 2 h at room temperature. Measurement of absorbance was carried out in UV-1650 PC UV-Vis spectrophotometer at wavelength of 765 nm. TPC was expressed as gallic acid equivalents (GAE) using units of mg/L (mg GAE/L).

Stock solution of gallic acid at 500 mg/L was prepared in deionised water. Working standard solutions of the mixture were prepared by dilution of stock solution to give final concentrations of 20, 40, 60, 80, and 100 mg/L in deionised water. All the standard solutions were treated as those of coconut water prior to absorbance measurement at wavelength of 765 nm.

Sugars content

Glucose, fructose and sucrose contents in coconut water was determined using HPLC as described by Tan *et al.* (2014). Approximately 50 mL of coconut water was transferred into a 250 mL conical flask containing 2 g of Amberlite MB-150 ion exchange resins. The mixture was allowed to rest (with occasionally swirling) for 15 min to allow the ion exchange resins to bond with both the cations and anions present in the coconut water. After 15 min, the mixture was filtered with muslin cloth and a portion of the filtrate (5 mL) was filtered through a pre-activated (with 5 mL of methanol followed by 5 mL of deionised water) Sep Pak C₁₈ (Waters Corporation, Milford, USA). The filtrate was then diluted 25 times with deionised water and filtered through membrane filter 0.45 µm (Whatman, 13 mm GD/X PTFE Filtration Media, Wycombe, UK) before being injected into the loop (volume: 20 µL) of the system.

Stock solution of sugars (consists of fructose, glucose, and sucrose) at 1 mg/mL were prepared in deionised water. Standard solutions of the mixture were prepared by dilution of stock solution to give final concentrations of 0.2, 0.4, 0.6, and 0.8 mg/mL in deionised water. All the standard solutions underwent cleaning steps (Amberlite MB-150 ion exchange resins, Sep Pak C₁₈, and membrane filter 0.45 µm) similar to those of coconut water prior to injection. Quantification of fructose, glucose and sucrose content in coconut water was performed using HPLC system equipped with a pump system (Waters Corporation, Waters 515 HPLC Pump, Milford, USA) and a refractive index detector (Waters Corporation, Waters 2414, Milford, USA). Separations of these sugars were carried out using Waters Sugar-Pak 1 column 300 mm × 6.5 mm, (Waters Corporation, Milford, USA) at 90°C. The flow rate was 0.5 mL/min and mobile phase used was 0.0001 M Ca-EDTA.

Minerals content

The concentrations of calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and iron (Fe) were determined using a flame atomic absorption spectrophotometer (Perkin Elmer, 4100ZL, Waltham, USA). Sample (1 mL) was mixed with concentrated nitric acid (6 mL) and 30% (v/v) hydrogen peroxide (1 mL) before digestion for 15 min using a microwave digester (Milestone Ethos One, High Performance Microwave Digestion System, Shelton, USA). After digestion, the digests were diluted accordingly with deionised water. For Na determination, 0.1% (w/v) potassium chloride was added during the dilution process. For Ca and Mg determination, 0.5% (w/v) lanthanum oxide was added during the dilution process. All diluted samples were then introduced to flame atomic absorption spectrophotometer and the absorbance obtained was recorded.

Sensory evaluation of freeze-concentrated coconut water

Seven-points scale hedonic test has been applied to evaluate and measure the degree of overall acceptance and degree of satisfaction for the fresh (as control), freeze-concentrated, and reconstituted (from concentrates) coconut water samples. Reconstitution of freeze-concentrated was carried out using potable water to achieved the desired TSS value.

The sensory test evaluation was being conducted in two sets of samples to avoid confusion among sensory panellist. For the first set, the sensory panellists were offered with 3 samples; GC (6.0 °Brix), FGC (12.1 °Brix), and reconstituted FGC with 6.0 °Brix (RFGC). Another 3 samples; MC (3.9 °Brix), FMC (7.9 °Brix), and reconstituted FMC with 3.9 °Brix (RFMC), were served to the same sensory panellists after the first set. The attributes that were being measured are appearance, colour, sweetness, aroma, sourness, saltiness, taste and overall acceptance. Total of 40 untrained sensory panellists from School of Industrial Technology's staffs and students participated in this evaluation. Prior to the sensory evaluation, all sensory panellists were given a brief information and instruction. The samples were coded using random numbers and arranged according to permutation numbers.

Statistical analysis

All measurements were performed using triplicate samples. Analysis of variance (ANOVA) and Tukey's test for multiple comparisons were used for analysing the data. SPSS version 20 (IBM, Armonk, USA) was used to complete the statistical analysis.

RESULTS AND DISCUSSION

Three batches of coconut water obtained from green (GC) and mature coconuts (MC), and freeze-concentrated coconut water prepared from GC (FGC) and MC water (FMC) were analysed for its physicochemical properties, composition and sensory properties. The yield of freeze-concentrated coconut water was estimated by comparing the weight of coconut water used and the amount of water obtained after the freeze-concentration process. Approximately 2 L of fresh coconut water produced approximately 1 L of freeze-concentrated coconut water. The exact yield of the freeze-concentrated coconut water could not be established since the TSS of freeze-concentrated coconut water was controlled at 2-times the initial TSS. After the freeze-concentrated coconut water was obtained, the unthawed ice remaining was discarded. The TSS of the unthawed ice from GC and MC water was approximately 0.8 and 0.5 °Brix, respectively. Despite differing in TSS value, the freeze-concentrated coconut water was visually similar to the fresh coconut water.

Physicochemical properties of freeze-concentrated coconut water

Table 1 shows that physicochemical properties of fresh coconut water were influenced by maturity and freeze-concentration process. Total soluble solid (TSS) indicates the sweetness of coconut water. The TSS of coconut water obtained from GC was significantly higher ($P < 0.05$) than that of MC water. This result is in agreement with the findings from Jackson *et al.* (2004) and Tan *et al.* (2014), who reported that TSS of coconut water decreases after maturity of coconut fruit exceeded 9–10 months old. It can be seen clearly that the simplified freeze-concentration process used in this study was able to double the initial TSS value of the GC and MC, from 6.0 to 12.1 °Brix and from 3.9 to 7.9 °Brix, respectively (Table 1). The water content trapped in the unthawed ice contributes to the increased in the TSS of the coconut water. Similar observation in TSS for freeze-concentrated sugar cane juice was reported by Lo *et al.* (2007). Other concentration approaches were shown to be able to increase the TSS of the fruit juice; such as blood orange juice using integrated membrane process (Galaverna *et al.*, 2008) and pineapple juice using osmotic evaporation (Hongvaleerat *et al.*, 2008).

Changes in pH were noticeable due to maturity as well as after the freeze-concentration process (Table 1). The pH of coconut water was found to increase with maturity. This trend compared favourably with those reported by Santoso *et al.* (1996), Jackson *et al.* (2004), Terdwongworakul *et al.* (2009), and Tan *et al.* (2014). During the ripening process of the coconut fruit, organic acid content (predominantly malic acid) of the coconut water was degraded and this accompanied by a steady increase in pH value from young to mature coconut water. After the freeze-concentration, freeze-concentrated coconut water was significantly lower ($P < 0.05$) in pH. Thus, the decrease in pH shows that the acidity of the coconut water is increasing as the organic acids content increases in concentration. The decrease in pH after freeze-concentration process is supported by the finding from Lo *et al.* (2007), where pH of sugar-cane juice decreased after the freeze-concentration. This might be due to the concentrating process, where part of the organic acids stays trapped in the concentrate (Galić *et al.*, 2009).

Table 1 Comparison of physicochemical properties and composition of coconut water obtained from green (GC) and mature coconuts (MC), and freeze-concentrated coconut water prepared from green (FGC) and mature coconuts (FMC).

Parameters	Coconut Water Samples			
	GC	FGC	MC	FMC
Total soluble solids (°Brix)	6.0 ± 0.17 ^c	12.1 ± 0.12 ^a	3.9 ± 0.15 ^d	7.9 ± 0.09 ^b
pH	5.17 ± 0.06 ^c	5.00 ± 0.02 ^d	5.81 ± 0.03 ^a	5.67 ± 0.06 ^b
Turbidity (NTU)	7.52 ± 1.41 ^d	17.60 ± 2.62 ^c	50.65 ± 4.10 ^b	112.30 ± 5.17 ^a
Protein (mg/mL)	0.05 ± 0.013 ^c	0.11 ± 0.012 ^{ab}	0.08 ± 0.009 ^{bc}	0.15 ± 0.040 ^a
Crude fat (% w/v)	0.60 ± 0.032 ^d	2.64 ± 0.120 ^b	2.14 ± 0.259 ^c	4.21 ± 0.024 ^a
Total phenolics content (mg GAE/L)	62.56 ± 9.09 ^c	119.81 ± 6.76 ^a	55.67 ± 2.88 ^d	107.19 ± 4.52 ^b
Sugars Content				
Fructose (mg/mL)	29.76 ± 0.185 ^b	60.48 ± 3.881 ^a	16.20 ± 0.837 ^d	33.27 ± 3.916 ^c
Glucose (mg/mL)	28.93 ± 0.468 ^b	57.67 ± 4.286 ^a	16.08 ± 0.799 ^d	33.06 ± 3.703 ^c
Sucrose (mg/mL)	1.37 ± 0.475 ^d	2.54 ± 0.363 ^c	5.88 ± 0.020 ^b	11.66 ± 0.123 ^a
Minerals Content				
Potassium (mg/100mL)	372.10 ± 0.71 ^c	757.83 ± 4.04 ^a	195.20 ± 1.05 ^d	428.91 ± 8.15 ^b
Sodium (mg/100mL)	2.591 ± 0.01 ^d	5.00 ± 0.02 ^c	5.44 ± 0.09 ^b	7.90 ± 0.03 ^a
Magnesium (mg/100mL)	24.96 ± 0.60 ^d	46.08 ± 0.08 ^b	35.43 ± 0.12 ^c	55.74 ± 0.02 ^a
Calcium (mg/100mL)	9.97 ± 1.23 ^d	21.54 ± 1.03 ^c	22.77 ± 1.20 ^b	41.59 ± 0.28 ^a
Iron (mg/L)	0.20 ± 0.02 ^d	0.61 ± 0.04 ^b	0.41 ± 0.08 ^c	0.88 ± 0.05 ^a

Notes:

Comparison within the rows was shown in the table with the data written as mean ± standard deviation ($n = 3$). Means followed by the same superscript letter are not statistically significant at the 5% level.

Turbidity is the measurement of the cloudiness or haziness of coconut water due to the presence of suspended solids that are usually invisible to the naked eye. The turbidity of coconut water obtained from GC is significantly lower ($P < 0.05$) than the turbidity of MC water. This result agrees with the findings by Jackson *et al.* (2004) and Tan *et al.* (2014), who reported that turbidity increases with maturity of coconut fruit. Increase in turbidity could be due to increase in TSS (Jackson *et al.*, 2004) and/or oil content in the coconut water (Tanquenco *et al.*, 2007). In addition, the higher acidity (*i.e.* lower pH) of freeze-concentrated coconut water could cause the precipitation of compounds such as proteins, tannins, and polysaccharides. Moir *et al.* (2001) reported significant increase in cloudiness could be associated with the formation of precipitation due to increase in acidity. From this study, freeze-concentrated coconut water produced contains significantly higher ($P < 0.05$) TSS, acidity, and turbidity as compared to those obtained from fresh coconut water.

Compositions of freeze-concentrated coconut water

Similar effect is seen in compositions of coconut water, whereby compositions of coconut water were influenced by maturity and freeze-concentration process (Table 1). Trace amount of oil and protein were detected in all fresh coconut water, with significantly higher ($P < 0.05$) amount in freeze-concentrated coconut water. Based on the results obtained in this study, where the oil and protein contents in coconut water obtained from MC were significantly higher ($P < 0.05$) than those of GC, these values are consistent with the findings that the more mature the coconut fruit, the higher the oil and protein contents present in the coconut water (Jackson *et al.* 2004; Tanquenco *et al.* 2007; Tan *et al.* 2014). The freeze-concentrated coconut water has a significantly higher ($P < 0.05$) protein concentration than fresh coconut water. This could be due to the fact that protein becomes partially insoluble in water upon freezing, thus causes the polymerisation of protein molecules. At this stage, the proteins possibly interact with each other and form a cluster or agglomerate (Belén *et al.*, 2013).

The smaller molecules are more easily trapped inside the ice structure and larger molecules are less likely to be present in the ice fraction (Chen *et al.*, 2000). Thus, protein remains higher in concentrate of coconut water. The increase in protein content by freeze-concentrated of tofu whey can be observed in the findings from Belén *et al.* (2013).

Crude fat content in the coconut water increased in value as the maturity increases. The coconut water fat portion encompasses the jelly-like meat, thus the closer the coconut to the stage of meat formation the higher the fat content (Jackson *et al.*, 2004). The increase in the crude fat content of the freeze-concentrated coconut water may be due to the larger molecules of fat are less likely to be included in ice fraction and caused it to be accumulated at the concentrate juice in a way that could be similar to the way in which protein molecules.

TPC in GC was significantly higher ($P < 0.05$) than MC, while freeze-concentrated coconut waters showed significantly higher ($P < 0.05$) TPC than fresh coconut waters, which were about 2-fold higher, regardless of the maturity. The higher TPC content in freeze-concentrated coconut water could be associated to the availability of phenolics compound in coconut water that might be trapped inside the concentrate during the processing. Formation of ice crystal during the freezing process might cause the phenolics compound to become partially insoluble in water and remain in a higher amount in the concentrate. Consequently, coconut water with higher concentration of antioxidant content was successfully produced through the freeze-concentration process. This result tallies with the findings of increase in TPC in concentrated pineapple juice by using osmotic evaporation (Hongvaleerat *et al.*, 2008) and reverse osmosis (Couto *et al.*, 2011), as well as in wild elderberry juice concentrated juice (Galini *et al.*, 2009).

The major sugars that contribute to the sweetness of the coconut water are glucose, fructose, and sucrose (Campbell-Falck *et al.*, 2000; Tan *et al.*, 2014). The reducing sugars (*i.e.* fructose and glucose) were high, while the non-reducing sugar (*i.e.* sucrose) was low in GC (Table 1). Significant increase ($P < 0.05$) in sucrose content in parallel to the significant decrease ($P < 0.05$) of glucose and fructose were observed when comparing between GC and MC. These changes of sugars content might be due to the expense of fructose and glucose to form sucrose (Tan *et al.*, 2014).

There is a significant increase ($P < 0.05$) in each of the sugars content after the freeze-concentration process for both GC and MC. According to Prades *et al.* (2012), total sugars content is correlated to the total soluble solids. Hence, an increment in total sugars content of concentrated coconut water is observed as the total soluble solids increased. The result is in agreement with the finding by Galini *et al.* (2009), who reported that the increase in reducing sugars of wild elderberry juice after being concentrated. The increase in the sugars content might be due to the concentration process, whereby the sugar molecules are likely to remain in the concentrate after the water removal by physical mean.

Minerals are the second most constituents in coconut water, with K as the predominant mineral (Yong *et al.*, 2009; Solangi and Iqbal, 2011; Tan *et al.*, 2014). The K content in GC water is significantly higher ($P < 0.05$) than MC water, wherein the reverse trend is observed for Mg, Na, Ca, and Fe contents. In general, MC water has a significantly higher ($P < 0.05$) mineral content in comparison with GC water. There is a significant difference ($P < 0.05$) for all the samples of coconut water after freeze-concentration process. The result is in line with the findings with increase in P, Mg, K, and Ca contents of the concentrated pineapple juice (Couto *et al.*, 2011), and Mg and Na contents in freeze-concentrated tofu whey (Belén *et al.*, 2013). Interaction between the same molecules formed clusters during the freeze-concentration process, whereby big molecules were separated from the ice crystals and possibly remained in high concentration in the coconut water concentrate, resulting in the increase of minerals concentration. It has been reported that the level of minerals content in the coconut water depends on their presences in the soil, water irrigation, and the species of coconut cultivars (Solangi and Iqbal, 2011).

Sensory acceptability of freeze-concentrated coconut water

The appearance, aroma, sweetness, sourness, saltiness, and overall acceptance of the coconut water samples were evaluated. One feature of the freeze-concentrated coconut water samples, *i.e.* FGC and FMC, is its similar acceptability ($P > 0.05$) of its sensory attributes as compared to that of fresh coconut water, *i.e.* GC (Figure 1) and MC (Figure 2).

Although the composition and physicochemical properties of the freeze-concentrated coconut water samples were significantly higher ($P < 0.05$) than those of fresh coconut water (Table 1), however, sensory panellist showed similar acceptability ($P > 0.05$) between the freeze-concentrated and fresh samples. This suggests that the 2× increase in the composition and physicochemical properties was indistinguishable by sensory panellist. Study done by Lo *et al.* (2007) shows that sensory panellist showed similar acceptability for sugarcane juice with 25 and 30 °Brix values. The sensory panellists show differences in the acceptability when the sugarcane juice with 30 °Brix value was reconstituted to 15 and 20 °Brix values. Therefore, the freeze-concentrated samples could be a better rehydration drink compared to fresh ones, whereby it provides more nutrients (*i.e.* sugars and mineral contents) without any negative effect on the sensory acceptability.

In the reconstitution studies, freeze-concentrated coconut water samples (*i.e.* FGC and FMC) were reconstituted to the same TSS value as the single strength coconut water, *i.e.* fresh coconut water. Again, no significant ($P > 0.05$) differences were observed in the acceptability between the reconstituted freeze-concentrated (*i.e.* RFGC and RFMC) and fresh ones. However, when comparing the mean score for all the sensory attributes between samples from green (Figure 1) and mature coconuts (Figure 2), it was found that the maturity stages affect the preferences of the sensory panellists, whereby samples from green coconuts received higher mean score (>4) in all the sensory attributes as compared to those from mature coconuts with majority of the sensory attributes (*i.e.* sweetness, sourness, saltiness, and overall acceptability) received mean score of less than 4. Lower score of sweetness in mature coconut probably correlated with lower content of fructose and glucose in mature coconut when compared with green coconuts (Table 1). In general, panellist prefers the sweet taste of coconut water obtained from green coconuts.

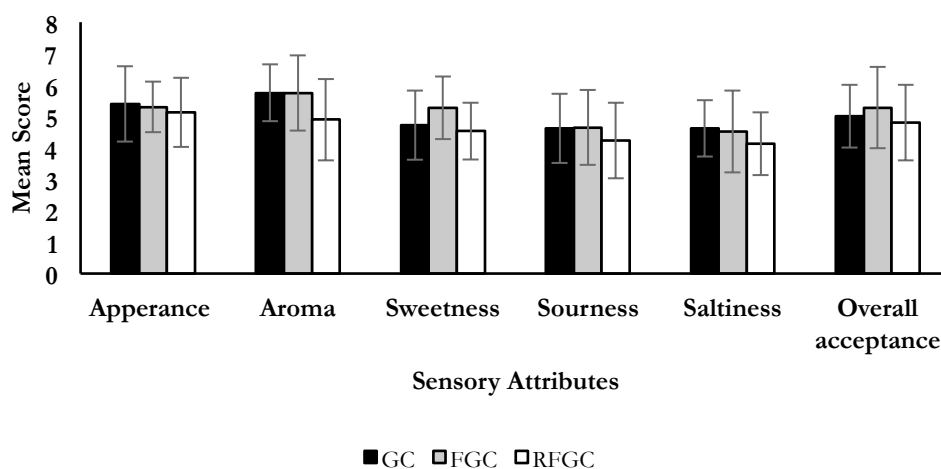


Figure 1 Sensory acceptability of coconut water obtained from green coconut (GC), freeze-concentrated coconut water prepared from GC (FGC), and reconstituted FGC at 6 °Brix (RFGC). No significant difference at $P > 0.05$ level according to Tukey's multiple-range between the 3 samples for all the sensory attributes evaluated.

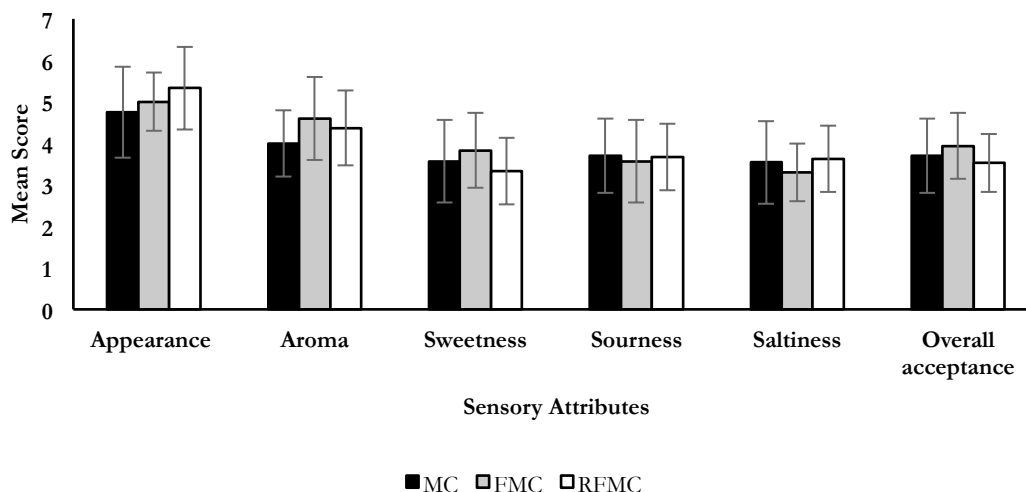


Figure 2 Sensory acceptability of coconut water obtained from mature coconut (MC), freeze-concentrated coconut water prepared from MC (FMC), and reconstituted FMC at 4 °Brix (RFMC). No significant difference at $P>0.05$ level according to Tukey's multiple-range between the 3 samples for all the sensory attributes evaluated.

CONCLUSION

A simplified freeze-concentration process was successful in producing double strength freeze-concentrated coconut water samples; FGC and FMC with TSS of 12 and 8 °Brix, respectively. The freeze-concentrated coconut water samples differed in all of the composition and physicochemical properties as compared to the fresh coconut water samples. However, the freeze-concentrated samples showed no significant difference in the sensory acceptability when compared to fresh and reconstituted coconut water samples. This freeze-concentration process showed the potential to serve as a processing method to produce double-strength coconut water without affecting the sensory acceptability, making it an ideal rehydration drink.

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REFERENCES

- AOAC (1995). Official Methods of Analysis. 16th eds. Association of Official Analytical Chemists International.
- Belén, F., Benedetti, S., Sánchez, J., Hernández, E., Auleda, J. M., Prudêncio, E. S., Petrus, J. C. C. & Raventós, M. (2013). Behaviour of functional compounds during freeze concentration of tofu whey. *Journal of Food Engineering* **116**: 681–688.

- Campbell-Falck, D., Thomas, T., Falck, T. M., Tutuo, N. & Clem, K. (2000). The intravenous use of coconut water. *American Journal of Emergency Medicine* **18**: 108–111.
- Cappelletti, M., Ferrentino, G., Endrizzi, I., Aprea, E., Betta, E., Corollaro, M. L., Charles, M., Gasperi, F. & Spilimbergo, S. (2015). High pressure carbon dioxide pasteurization of coconut water: A sport drink with high nutritional and sensory quality. *Journal of Food Engineering* **145**: 73–81.
- Chen, P., Chen, X. D. & Free, K. W. (2000). An experimental study on the spatial uniformity of solute inclusion in ice formed from falling film flows on a subcooled surface. *Journal of Food Engineering* **39**: 101–105.
- Couto, D. S., Cabral, L. M. C., Da Matta, V. M., Deliza, R. & Freitas, D. D. G. C. (2011). Concentration of pineapple juice by reverse osmosis: physicochemical characteristics and consumer acceptance. *Ciência e Tecnologia de Alimentos* **31**: 905–910.
- DebMandal, M. & Mandal, S. (2011). Coconut (*Cocos nucifera* L.: Arecaceae): In health promotion and prevention. *Asian Pacific Journal of Tropical Medicine* **4**: 241–247.
- Galaverna, G., Silvestro, G. D., Cassano, A., Sforza, S., Dossena, A., Drioli, E. & Marchelli, R. (2008). A new integrated membrane process for the production of concentrated blood orange juice: Effect on bioactive compounds and antioxidant activity. *Food Chemistry* **106**: 1021–1030.
- Galić, A., Dragović-Uzelac, V., Levaj, B., Bursać Kovačević, D., Pliješćić, S. & Arnautović, S. (2009). The polyphenols stability, enzyme activity and physico-chemical parameters during producing wild elderberry concentrated juice. *Agriculturae Conspectus Scientificus* **74**: 181–186.
- González-Neves, G., Favre, G. & Gil, G. (2014). Effect of fining on the colour and pigment composition of young red wines. *Food Chemistry* **157**: 385–392.
- Hongvaleerat, C., Cabral, L. M. C., Dornier, M., Reynes, M. & Ningsanond, S. (2008). Concentration of pineapple juice by osmotic evaporation. *Journal Food Engineering* **88**: 548–552.
- Ito, R., Yamamoto, A., Kodama, S., Kato, K., Yoshimura, Y., Matsunaga, A. & Nakazawa, H. (2003). A study on the change of enantiomeric purity of catechins in green tea infusion. *Food Chemistry* **83**: 563–568.
- Ismail, I., Singh, R. & Sirisinghe, R. G. (2007). Rehydration with sodium-enriched coconut water after exercise-induced dehydration. *The Southeast Asian Journal of Tropical Medicine and Public Health* **38**: 769–785.
- Jackson, J. C., Gordon, A., Wizzard, G., McCook, K. & Rolle, R. (2004). Changes in chemical composition of coconut (*Cocos nucifera*) water during maturation of the fruit. *Journal of the Science of Food and Agriculture* **84**: 1049–1052.
- Lo, W. M., Chua, L. S. T., Al-Kharkhi, A. F. & Azhar, M. E. (2007). Evaluation of freeze-concentrated sugarcane juice. *Journal of Tropical Agriculture and Food Science* **35**: 121–129.
- Miyawaki, O., Gunathilake, M., Omote, C., Koyanagi, T., Sasaki, T., Take, H., Matsuda, A., Ishisaki, K., Miwa, S. & Kitano, S. (2016). Progressive freeze-concentration of apple juice and its application to produce a new type apple wine. *Journal Food Engineering* **171**: 153–158.
- Moir, C. J., Andrew-Kabilafkas, C., Arnold, G., Cox, B. M., Hocking, A. D. & Jenson, I. (2001). Spoilage of Processed Foods: Causes and Diagnosis. Waterloo: The Food Microbiology of the Australian Institute of Food Science and Technology Incorporate. ISBN no.: 978-0957890701.

- Prades, A., Dornier, M., Diop, N. & Pain, J. P. (2012). Coconut water uses, composition and properties: a review. *Fruits* **67**: 87–107.
- Saat, M., Singh, R., Sirisinghe, R. G. & Nawawi, M. (2002). Rehydration after exercise with fresh young coconut water, carbohydrate-electrolyte beverage and plain water. *Journal of Physiological Anthropology and Applied Human Science* **21**: 93–104.
- Santoso, U., Kubo, K., Ota, T., Tadokoro, T. & Maekawa, A. (1996). Nutrient composition of kopyor coconuts (*Cocos nucifera* L.). *Food Chemistry* **57**: 299–304.
- Solangi, A. H. & Iqbal, M. Z. (2011). Chemical composition of meat (kernel) and nut water of major coconut (*Cocos nucifera* L.) cultivars at coastal area of Pakistan. *Pakistan Journal of Botany* **43**: 357–363.
- Tan, T. C., Cheng, L. H., Bhat, R., Rusul, G. & Easa, A. M. (2014). Composition, physicochemical properties and thermal inactivation kinetics of polyphenol oxidase and peroxidase from coconut (*Cocos nucifera*) water obtained from immature, mature and overly-mature coconut. *Food Chemistry* **142**: 121–128.
- Tan, T. C., Cheng, L. H., Bhat, R., Rusul, G. & Easa, A. M. (2015). Effectiveness of ascorbic acid and sodium metabisulfite as anti-browning agent and antioxidant on green coconut water (*Cocos nucifera*) subjected to elevated thermal processing. *International Food Research Journal* **22**: 631–637.
- Tanqueco, R. E., Rodriguez, F. M., Laude, R. P. & Cueno, M. E. (2007). Total free sugars, oil and total phenolics content of stored coconut (*Cocos nucifera* L.) water. *Philippine Journal of Science* **136**: 103–108.
- Terdwongworakul, A., Chaiyapong, S., Jarimopas, B. & Meeklangsaen, W. (2009). Physical properties of fresh young Thai coconut for maturity sorting. *Biosystems Engineering* **103**: 208–216.
- Yong, J. W. H., Ge, L., Ng, Y. F. & Tan, S. N. (2009). The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. *Molecules* **14**: 5144–5164.