

Comparison of the Basic Nutritional Characteristics of the First Extract and Second Extract of Coconut Milk

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ABSTRACT: Coconut milk is the aqueous extract of grated coconut kernel. In traditional cooking in the South Asian region, grated coconut kernel is hand-pressed with water to obtain a viscous white coconut milk known as first extract (FE). The coconut kernel remaining after the first extraction is further extracted with a fresh portion of water to obtain second extract (SE), which is less viscous. In the present study, the nutritional composition and the effect of FE and SE on the serum lipid profiles was evaluated. The results indicate that the lipid and protein contents of SE is equivalent to a 3 fold diluted solution of FE. However, the levels of sugars and phenolic contents of the SE cannot be achieved by the same fold of dilution of FE. There is no significant difference ($p < 0.05$) in the lipid profiles of the rats fed with SE after 21 days.

KEYWORDS: Coconut kernel, Coconut milk, Nutrient composition, Phenolic content, Lipid profiles

I. INTRODUCTION

Coconut milk is the aqueous emulsion of coconut kernel, which is prepared by hand or machine pressing fresh grated coconut kernel. Coconut milk has many culinary applications. Coconut milk is consumed directly or with cooked food. In the preparation of vegetable, fish or meat curries, coconut milk is added as the liquid medium for boiling such food. Coconut milk is chilled, enzymatically treated or fermented to separate coconut oil, which is also used in cooking. Coconut milk can also be spray dried to obtain coconut milk powder, which can easily be reconstituted with water either at 30°C or 100°C (not necessary) to obtain a homogeneous coconut milk solution.

Nutrient components of coconut milk include lipids, sugars, proteins and several other minor compounds. Coconut milk is different from animal milk such as cow's milk. While cow's milk has equal amounts of oil and proteins, coconut milk has ten times more oil than proteins [1]. Results of few studies on the chemical and nutritional aspects of coconut milk have been reported. The fat percentage of coconut milk decreases with the volume of water added during the extraction of coconut milk and there is no remarkable difference in the fat content of coconut milk extracted with cold water (27-30°C) and hot water (88-93 °C) [2]. The total fat content varies from 5.88% to 21.83% in coconut milk depending on the concentration of coconut milk. Total solid and total fat contents of coconut milk, coconut cream and dried coconut powder have been reported [3].

A study with alloxan induced diabetes in Sprague-Dawley rats indicates that coconut kernel protein reduces the diabetes related pancreatic damage in treated rats compared to the control [4]. In addition, inclusion of coconut protein and L-arginine into ethanol fed rats produced lower levels of total cholesterol, LDL + VLDL cholesterol, triglycerides

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and atherogenic index in the serum. The major factor responsible for the hypolipidemic effect of coconut protein is the high content of L-arginine [5].

A careful observation of the literature indicates that the nutritional and chemical information about coconut milk is sporadic and absolutely meagre. Consumption of coconut milk as FE and SE is becoming more and more widespread among the consumers. However, reports on the detailed comparison of the nutritional properties of FE and SE are almost nonexistent. Here, we report the results of a detailed investigation of the composition of FE and SE and the effect of consumption of FE and SE on the lipid profiles in rats.

II. MATERIALS AND METHODS

Chemicals

Authentic phenolic standards for HPLC analysis and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, MO). Fatty acid methyl ester standards for GC analysis were purchased from Fluka (Buchs, Switzerland). All other chemicals were purchased from either Sigma or Fluka.

Sample collection and preparation

Coconuts of 12–14 months maturity from 'ordinary tall' coconut cultivars were used for the study. Coconut milk was prepared by hand pressing scraped coconut endosperm with water. The FE (thick coconut milk) was prepared by pressing grated coconut kernel with distilled water 1:1 (w/v). The SE (thin coconut milk) was produced following the same protocol by adding an equal volume of distilled water to the coconut kernel remaining after the first extraction. The viscous slurry resulting from hand pressing the coconut kernel was squeezed through a cheesecloth to obtain FE and SE.

Determination of the total fat content in coconut milk

Coconut fat was extracted from FE and SE to hexane using solvent extraction. Coconut milk (5.00 g) was mixed with hexane (10.0 mL) in a separating funnel and extraction was carried out thrice and the resultant hexane layers were collected. Hexane was removed from sample using a rotary evaporator and the weight of the remaining fat was measured.

Determination of sugar content in coconut milk

The total sugar and the reducing sugar contents in the FE and SE were determined using a previously described method [6].

Determination of total protein content in coconut milk

Coomassie dye-binding assay (Bradford assay) was used to determine the total protein content of FE and SE of coconut milk [7]. FE or SE (0.1 mL) was added to Coomassie dye reagent (5.0 mL) and mixed. The mixture was incubated at room temperature for 10 min. The absorbance was measured at 595 nm using a UV-visible spectrophotometer. Bovine Serum Albumin (BSA) was used as the standard for the calibration curve.

Determination of total phenolic content of coconut milk

For the Folin-Denis assay, DPPH radical scavenging activity and the determination of individual phenolic quantities, phenolic extracts were prepared as follows: Coconut milk (25 mL) was frozen and the lipids were removed by centrifugation (1080 g, 10 min). Then the aqueous layer was separated and methanol (2.0 mL) was added. Precipitated proteins were removed by centrifugation (1080 g, 10 min). The resultant extract (methanolic extract of phenolic substances, MEPS) was used for the analyses with appropriate dilutions where necessary. To MEPS (0.10 mL), water (0.90 mL) was added and mixed with Folin-Denis reagent (1.00 mL). After 3 minutes, Na₂CO₃ solution (35 %, 1.00 mL) was added. After 45 minutes, the mixture was centrifuged (1080 g, 10 min) and absorbance was measured at 745 nm using a UV/ Visible Spectrophotometer (GBC Cintra 202 double beam UV-Visible spectrometer, Dandenong, Victoria, Australia). Gallic acid was used as the standard for the calibration curve.

Identification of phenolic compounds in coconut milk

MEPS was purified by a reported procedure[8]. MEPS (2.0 mL) of FE or SE was concentrated under stream of nitrogen and acetonitrile (1.0 mL) was added to it. The mixture was washed three times with hexane (2.0 mL). Then the

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acetonitrile layer was separated and solvent was evaporated. The resultant residue was dissolved in methanol (0.4 mL) and filtered through a 0.45 μm filter unit prior to HPLC analysis. Sample (0.2 mL) was then concentrated again under a stream of nitrogen until all solvent evaporated and then the residue was redissolved in methanol (60 μL).

HPLC experiments were performed using an Agilent 1100- Infinity liquid chromatographic system (Agilent Technologies, Waldbronn, Germany) equipped with an Agilent 1200 diode array detector. A ZORBAX ECLIPSE Plus C18 column (Agilent Technologies, USA) (4.6 mm x 100 mm x 3.5 μm particle size) maintained at room temperature was used for this purpose. The mobile phase consisted of $1 \times 10^{-3} \text{ mol dm}^{-3}$ H_2SO_4 in deionized water (A) and methanol (B). The total running time was 35 min with a flow rate of 0.5 mL min^{-1} . The elution gradient began using 90% A/10% B. From 0 to 10 min, solvent B was increased to 55% and this composition (45% A/55% B) was continued for 10–20 min. From 20 to 30 min, solvent B was increased to 90% and this composition (10% A/90% B) was continued for 30–35 min. Sample (20 μL) was injected into the HPLC system. Authentic phenolic standards and phenolic compounds in MEPS were detected at a wavelength of 280 nm. The quantities of individual phenolic substances were determined using a reported method [9]. Standard curves for quantitative analysis of phenolic compounds were plotted using known concentrations of the authentic phenolic standards of identified phenolic compounds.

DPPH radical scavenging assay

DPPH radical scavenging assay was carried out as described in a previous method [10]. MEPS (40 μL) were reacted with DPPH reagent prepared in methanol (0.8 mM, 30 μL) and added to methanol (240 μL) in a 96-well plate. The plate was shaken for 10 seconds and kept in the dark for 20 minutes. The absorbance at 517 nm was recorded and the inhibition percentages were calculated using the following formula: Inhibition (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the reaction mixture with solvent system instead of phenolic extract and A_1 is the absorbance of the reaction mixture with phenolic extract.

Determination of the fatty acid content of coconut milk

Methylation of fatty acids was carried out by a reported method [11]. The fatty acids were identified by comparison of the retention times of the signals with those of authentic fatty acid methyl ester standards. A gas chromatograph (Shimadzu GC-2010 Plus Gas Chromatograph, Japan) equipped with capillary column Rtx^R- WAX (crossbond with PEG 30 m x 0.32 mm, i.d. 0.25 μm) and flame ionization detector (FID) was used for this analysis. The injector and detector temperatures were set at 230 °C and 250 °C respectively. The carrier gas used was helium at a flow rate of 0.5 mL/min and analyses were performed on split mode (split ratio 100: 1). A temperature program of 130 °C (3 min), 130 °C to 210 °C at 45 °C/min, and 210 °C (12 min) was used throughout the study. The sample (1.0 μL) was injected into the GC system. Signals in the GC chromatogram were identified by comparison of the retention times with the retention times of authentic fatty acid methyl ester standards. Quantification of individual fatty acids were carried out by using an internal standard (heptadecanoic acid) and integration of the peak areas in the GC chromatograms.

Animal studies

Male weaning Wistar rats (weighing 110–120 g) were randomly selected from the Medical Research Institute, Colombo, Sri Lanka. They were then placed individually in cages and housed in a room with temperature range of 25 ± 2 °C with 12 h light-darkness cycle. Prior to the initiation of the experiment, the rats were acclimatized to the basal diet for fourteen days. Then the rats were randomly assigned to treatment groups (3 rats/ group). The control group was fed with a semi synthetic diet recommended by WHO. Second group was fed with the semi synthetic diet which contains 12 mL of FE per kg of feed. Third group was fed with semi synthetic diet which contains 12 mL of SE per kg of feed. Free access to the diet and water were provided to the rats for a period of 21 days. The rats were fasted for 14 h, anesthetized humanely under light anesthetic ether and blood was collected through the tail vein of each rat. The blood samples were centrifuged promptly (3000 g, 15 min) at room temperature. Separated top layer of serum from each sample was used for the analysis. The test kit method provided by Biolabo was used for the analysis of total cholesterol (TC), HDL cholesterol and triglyceride (TG) levels. LDL cholesterol was determined using the reported equation [12]: $\text{LDL} = \text{TC} - \text{HDL} - (\text{TG}/5)$

Ethical approval for the animal study using a rat model was obtained from Ethics Review Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura (Application Number -13/14).

Statistical Analysis

All analyses were run in triplicate unless otherwise indicated. Tukey's pairwise test was carried out after ANOVA for the determination of significant differences ($p < 0.05$) between the means. Chemical compositions were presented as mean \pm SD (standard deviation).

III. RESULTS AND DISCUSSION

Total fat content of FE and SE is given in Table 1. Total fat content was calculated as a weight percentage in order to compare the fat contents of coconut milk with the reported values. Fat contents of different coconut milk extracts vary in the range of 4.14 – 8.71 g/100g [3]. Our results for total fat content of the FE falls within the same range. The results also show that the fat content of SE is about 3 fold less compared to FE. Protein content of SE is also about 3 fold less compared to FE according to Table 1. Based on the fat content and protein content, SE can be considered as a 3 fold diluted form of FE. However, Table 1 indicates that the total sugar and reducing sugar contents are only about 1.5 fold lower in SE compared to FE and total phenol contents are about 2.5 fold higher in the SE compared to FE, indicating that SE cannot be obtained by diluting FE. It is interesting to notice that the total phenolic content of SE is higher than that of FE. Aqueous fraction is used for total phenolic content determination. The aqueous volume of SE is roughly 1.5 fold higher than that of FE, which contributes to the higher total phenol content expressed in μg per 100 mL of SE. In the present study, aqueous methanolic extracts of the phenolic substances were used for the determination of phenolic contents. It has been reported that the total phenolic contents determined by colorimetric assays are interfered by some reducing substances such as amino acids [13]. To avoid such interferences, proteins were precipitated and the resultant clear methanolic extract was used for the determination of phenolic contents. For alcoholic extracts of plant materials, antioxidant activity correlates with the total phenolic contents [14]. In the calculation of DPPH radical scavenging activity, 1.5 fold difference in aqueous volume between FE and SE was factored for the calculation of adjusted DPPH radical scavenging activity. Higher DPPH radical scavenging activity (adjusted) can be attributed to the higher total phenolic content of SE (Table 1).

Table 1: Total fat, sugar, protein, polyphenol contents and antioxidant activity of FE and SE of coconut milk

Component	FE ^a	SE ^a
Total Fat (w/w%)	5.8 \pm 1.1	1.9 \pm 0.4
Total Sugar (mg/ml)	66.9 \pm 2.5	45.4 \pm 10.4
Reducing Sugar (mg/ml)	33.5 \pm 2.5	22.3 \pm 5.2
Proteins (mg/ml)	45.6 \pm 3.6	16.5 \pm 0.8
Polyphenol ($\mu\text{g}/100\text{ ml}$)	816 \pm 32	2040 \pm 219
DPPH radical Scavenging activity (%) (adjusted)	35.55 \pm 5.62	51.78 \pm 2.47

^aEach data point represents the mean of nine replicates \pm standard deviation

All the fatty acids present in FE and SE were clearly identified by the comparison of the GC retention times of the fatty acid methyl ester standards. Fatty acid composition of coconut oil extracted from FE and SE is given in Table 2. There is no significant difference in the percentage composition of individual fatty acids in the two extracts indicating that, only the quantity of fat is different in FE and SE while the quality of fat is same (Table 1 and Table 2).

Table 2: Fatty acid composition of FE and SE of coconut milk

Fatty acid	Percentage composition*	
	FE ^a	SE ^a
caproic (6:0)	< 0.20	< 0.20
caprylic(8:0)	9.8 \pm 0.4	10.5 \pm 0.4
capric(10:0)	7.4 \pm 0.3	7.7 \pm 0.3
lauric (12:0)	50.1 \pm 2.2	50.3 \pm 0.8
myristic(14:0)	18.9 \pm 1.1	19.1 \pm 0.5
palmitic(16:0)	6.8 \pm 0.7	6.4 \pm 0.7

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stearic (18:0)	2.3± 0.6	2.1± 0.1
oleic(18:1)	3.7± 0.6	3.2± 0.8
linoleic (18:2)	0.8± 0.1	0.6± 0.3

*Fatty acid compositions are not significantly different in FE and SE.

^aEach data point represents the mean of three replicates ± standard deviation

Phenolic compounds such as phenolic acids and flavonoids are important as natural antioxidants. Due to the polarity of phenolic substances, more phenolic substances are partitioned in the aqueous fraction compared to water insoluble fraction of coconut milk [15]. FE can be considered as a less polar medium compared to SE due to higher fat and protein contents of FE. Under reverse phase conditions used in the HPLC experiments, phenolic substances elute in the order of increasing polarity. Higher total phenolic content as detected by colorimetric assay is further confirmed by the presence of higher quantities of phenolic substances in the SE (Table 3). The main origin of phenolic substances in coconut oil and coconut milk is coconut testa, the thin brown outer skin of white coconut kernel. Comparison of the reported information about phenolic substances of coconut oil [15] with the results of present study indicates that the identity of individual phenolic compounds are almost same in coconut oil and coconut milk even though coconut milk contains higher quantities of individual phenolic compounds. Amino acid content of coconut milk has been reported [16]. However, phenolic compounds in coconut milk have not been reported or compared for different types of coconut milk. Consumption of foods containing high amount of natural phenolic substances is beneficial to the health by reducing the risk of coronary heart diseases [16].

Table 3: Phenolic composition in FE and SE of coconut milk

Phenolic compound	HPLC retention time (min)	Amount (µg/100ml)*	
		FE ^a	SE ^b
Gallic acid	4.9	3.0 ±1.2	10.0 ± 0.1
Catechin acid	8.1	50.2±5.0	21.8 ± 3.3
Chlorogenic acid	9.3	4.0 ±0.7	13.1 ± 0.5
p-hydroxybenzoic acid	9.9	3.3 ± 1.0	13.8 ±0.3
Caffeic acid	10.2	4.6 ± 0.5	15.4 ± 2.1
Syringic acid	11.7	4.1 ± 0.7	2.5 ± 0.5
Ferulic acid	12.0	6.3 ± 1.0	22.5 ± 2.2
p-coumaric acid	13.6	2.5 ± 0.3	4.1 ± 0.4
Elagic acid	15.5	0.4 ± 0.1	2.0 ± 0.8

* Each data point represents the mean of three replicates ± standard deviation

^{a,b} Indicates that the values of the two columns (FE and SE) are significantly different for each phenolic compound

Table 4 gives the lipid levels of rats fed with diets containing FE and SE compared to a control with no added FE or SE in the diet. One of the major concerns of the consumers of coconut milk is the saturated fat content. About 95 % of the fat content of coconut milk is saturated fat (Table 2). However, 70% of the saturated fat includes medium and short chain triglycerides (Table 2), which have a less chance of deposition compared to longer chain fatty acids due to quicker metabolism [17]. Our results indicate that there is a significant difference (p<0.05) in TC and TG levels in rats after 21 days compared to day zero for the FE. However, the results for the SE indicates that the contents of SE does not have a significant influence on increasing lipid levels (Table 4). This may be attributed to the fact that SE contains a higher amount of phenolic substances and a lower fat content (Table 1-3). However a previous report indicates that the rats fed with coconut milk at 25%, 50% and 100% concentrations did not display significant concentration-dependent increase of serum lipids [18].

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Table 4:Lipid profiles of the rats fed with diets containing FE and SE of coconut milk

	Control*		FE*		SE*	
	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
TC (mg/dL)	83±1 ^a	86±4 ^a	87±1 ^a	98±3 ^b	87±2 ^a	85±1 ^a
TG (mg/dL)	167±13 ^a	175±2 ^a	179±4 ^a	207±6 ^b	178±5 ^a	175±3 ^a
HDL (mg/dL)	31±3 ^a	34±2 ^a	30±3 ^a	31±1 ^a	33±1 ^a	33±1 ^a
LDL (mg/dL)	19±1 ^a	17±4 ^a	21±3 ^a	26±3 ^a	18±2 ^a	17±3 ^a

*Values are means of three rats ± standard deviation. Letters ^a and ^b were used to compare statistical significance in rows within columns against control, FE and SE (p < 0.05).

IV. CONCLUSION

FE and SE of coconut milk display similarities as well as differences in their nutritional characteristics. FE is a higher calorie food due to the presence of higher amounts of lipids, sugars and proteins. SE is a lower calorie food with a higher phenolic content. Even though there is a statistically significant increase in TC and TG levels resulting from the consumption of FE compared to the consumption of SE, further studies are required to assess the health effects of consuming FE.

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