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Study of antioxidant activity and physicochemical properties of coconut milk (*Pati santan*) in Malaysia

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ABSTRACT

Coconut milk is the word used to describe the liquid obtained from the mechanical or manually press of the coconut meat usually with or without added water. In Malay word the coconut milk is known as 'Pati Santan' which usually soldas a fresh liquid form in the local market. The composition of coconut milk depends on the amount of water used for the extraction, affecting significantly moisture and fat content. The aim of this study was to determine the physicochemical properties and antioxidant activity based on TPC, FRAP, ORAC and DPPH of Malaysian coconut milk. Results of the study showed that coconut milk samples exhibited a significantly different (P<0.05) antioxidant activity in comparison of goat and cow's milk for all the assays except DPPH. Coconut's milk exhibited the highest AA in TPC, FRAP, DPPH and ORAC assays with mean value of 575.15 mg GA/100 g FW, 471.55 mg TE/100 g FW, 68.39 % and 784.47 umol TE/100g F.W, respectively. In contrast, the cow's milk exhibited the lowest mean value of 477.68 mg GA/100 g FW, 398.88 mg TE/100 g FW, 60.81 % and 361.96 umol TE/100g F.W, respectively).Results showed that physicochemical properties of Malaysian coconut milk were significantly different (P<0.05) among the comparative coconut milk samples of other tropical countries, with some samples more superior compared with others in one or more aspects. This result showed that Malaysian coconut milk possess high proteins content (3.40 ± 0.59) with lower amount of fats (15.44 ± 1.53).Thus, Pati Santan can be considered as an excellence source of antioxidants with health and medicinal applications of low content of fat.

Key words: Coconut milk (*Pati Santan*), Antioxidant activity, Physicochemical properties and Malaysia.

INTRODUCTION

Coconut (*Cocosnucifera L.*) belongs to the palm family (*Arecaceae*). Grown in abundance in Malaysia and southern Asia, Spanish explorers named the *cocos* meaning grinning face, because of the three little eyes. It is classed as a fruit and frequently confused for being a nut, the coconut is actually a one seeded drupe[1]. It is commonly used as an important source of coconut oil, milk and cream products, as well as, eaten fresh and processed into desiccated coconut flesh, coconut water and coconut milk[2]. In addition to this, coconut fruit immature can be used in several application, e.g. food, animal feed, soaps, detergents and cosmetics [3]. It gives also all that is necessary for living because nearly all parts can be used even the husks and leaves are used as materials in furnishings and decoration. Palm trees produce coconuts up to 13 times a year and although it takes a year for the coconuts to mature, a fully blossomed tree can produce between 60-180 coconuts in a single harvest[4].

Coconut palm is an economic plant which is cultivated in most tropical countries, is grown in more than 93 countries. According to the estimate given by the Asian Pacific Coconut Community (APCC), the global coconut

area in 2005 was 12 million ha[5]. It is a basic commodity grown on a very large scale, with an overall world output of about 50 million ton/year [6].Indonesia, Philippines and India are the major producers and account for about 75% of world production [5]. In Malaysia, the coconut palm is known as *kalpa,* it is the fourth important industrial crop after oil palm, rubber and paddy in terms of total planted area and it is one of the oldest agro-base industries.

Coconut milk is the word used to present the liquid obtained by manual or mechanical force of coconut meat [7]. It is the white, oil-in-water emulsion extracted from fresh coconut flesh with or without added water. It is made with finely grated coconut meat that is steeped in hot water and then filtered. Coconut milk is becoming an increasingly important raw material in home cooking as well as in the food processing industries [8]. It is estimated that 25% of the world coconut output is consumed as coconut milk [9]. It is a major and an essential ingredient in the preparation of a wide variety of food products such as curry, desserts, coconut jam spread, coconut syrup, coconut cheese, bakery products and beverages [9, 10]. It can also be used as a substitute for milk in some desserts such chocolate and other confection aries are exotically flavoured with coconut milk [8].

Coconut milk contains fat, water, carbohydrate, protein, and ash with the major components being water and fat[11]. As reported by [12, 11], coconut milk contains about 54% moisture, 35% fat and 11% solid non-fat and they also showed that fat content played an important role in the flow property of coconut milk [13]. Regular coconut milk is higher in fat and calories than cow's milk[2]. It is rich in proteins such as albumin, globulin, prolamin and glutein. Emulsifying agents help in increasing stability of food emulsions; examples of such are phospholipids, cephalin and lecithin which have been found in coconut milk[14]. However, it is recognized that the product which is highly susceptible to chemical and biochemical spoilage such as lipid oxidation [15].

Coconut endosperm containsa liquid portion, white coconut kernel and a thin brown outer skin of coconut kernel known as coconut testa. In addition to the method of extraction, the components of the endosperm may also play an important role in determining the final phenol content of coconut oil [16]. In which the substances are mainly responsible for the antioxidant properties of coconut milk [17]. Therefore, coconut milk is rich in antioxidants, which prevents free radical damage. Free radicals are associated with the development of many diseases, including cancer, cardiovascular disease, Alzheimer's disease and age-related dementia. Furthermore, antioxidants can help reverse previous damage and delay the aging process[18]. A glass of coconut milk while taking other antioxidant rich foods, such as pecans, raisins and cranberries, may boost the immunity while rebuilding the damaged cells in the body[18].

Coconut milk contains significant amounts of fat, but unlike other nuts, it provides fat that is mostly in the form of medium chain saturated fatty acids (MCFAs) that is abundant in mother's milk in particular, lauric acid[17]. This, converted in the body into a highly beneficial compound called monolaurin, an antiviral and antibacterial that destroys a wide variety of disease causing organisms. According to the National Center for Biotechnology Information, lauric acid has many germ fighting, antifungal and antiviral properties that are very effective at ridding the body of viruses, bacteria and countless illnesses[17]. Lauric acid may also reduce cholesterol and triglyceride levels, which lowers heart disease and stroke risks[18]. Hence, thought that consumption of coconut milk may help protect the body from infections and viruses. Furthermore, The fats that are present in coconuts are less likely to clog arteries, because the body does not store coconut fats which makes coconut milk a healthy alternative to cow's milk when it comes to preserving heart's health[18]. For instance, [19] stated that coconut yoghurt accords advantages in terms of nutrition and health, since it contains no cholesterol or lactose and only small quantities of saturated fatty acids; in addition to its advantage of low cost [20].

In Malaysia, coconut milk, called *Santan* in Malay, which is the milk obtained after adding water to freshly grated coconut flakes, and giving it a good press or squeeze, whereas, *Pati Santan* does not have any addition of water. It is a common ingredient in Malaysian and Southeast Asian cuisines, such as curries, soups, stews, candy and dessert preparations**.** [21]. The importance of coconut milk to Malaysian industries has prompted food scientists and food engineers in this country to develop new products from coconut milk for use as ingredients in household recipes both for the Malaysian market and for export [5]. In the present study, coconut milk from different local market in Malaysia was for analysis of antioxidant activity and physicochemical characteristics. Antioxidant activity of the local coconut milk was compare to goat and cow's milk and with other tropical countries in aspect of physicochemical properties. Therefore, this study was designed to determine the antioxidant activity and physicochemical properties of the Malaysian coconut milk.

EXPERIMENTAL SECTION

Sampling process: The coconut milk samples were bought from the fresh coconut milk sellers around Selongor in Malaysia. The samples were taken three times from three different days to obtained triplicate data. The samples were analysed as soon as possible to prevent nutritional changes.

DPPH radical scavenging activity: The method of [22] with minor modification was used to evaluate antioxidant activity through DPPH scavenging system. To prepare the stock solution, 40 mg was dissolved in 100 mL methanol. The solution was then stored at -20°C until use. By mixing 350 mL of the stock solution with 350 mL methanol, an absorbance of 1.0±0.01 unit was obtained using a spectrophotometer (Epoch, Biotek, USA) at 517 nm wavelengths. Approximately 100 µL of each fresh coconut milk extract with 1 mL methanolic DPPH solution was prepared and kept in the dark for 2 h to allow scavenging reaction to occur. The percentage of DPPH scavenging activity was calculated as:

DPPH scavenging activity (%) = [(A blank-A sample)/A blank]×100

where, A is the absorbance

Ferric Reducing Antioxidant Power (FRAP): FRAP assay was performed according to [22] with minor modification. FRAP reagent was prepared fresh using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate, 16 mL glacial acid made up to 1:1 with distilled water), 10 mM TPTZ (2,4,6-tris (2- pyridyl)-s-triazine) in 40 mM HCl and 20mMFeCl3•6H2O in the ratio of 10:1:1 to give the working reagent. Approximately 100 µL of extracted fresh coconut milk was added to 1 mL FRAP reagent and the absorbance was measured at 595 nm wavelength using a spectrophotometer after 30 min. Calibration curve of Trolox was set up to estimate the activity capacity of samples. Result was expressed as milligram of Trolox equivalents per 100 gram of fresh samples (mg TE/100 g of FW).

Total Phenol Content (TPC): Antioxidant activity through TPC was determined according to the method of [22] with minor modification. About 100 µL of extracted fresh coconut milk was added to 0.4 mL distilled water and 0.5 mL diluted Folin- Ciocalteu reagent. Samples with the reagent were left for 5 min and then 1 mL 7.5% sodium carbonate (w/v) was added. The absorbance was measured at 765 nm using a spectrophotometer after 2h. Calibration curve of gallic acid was plotted to evaluate the activity capacity of the samples (Fig. 1). Result was expressed as milligram of gallic acid equivalents per 100 gram of fresh sample (mg GA/100 g of FW).

Oxygen radical absorbance capacity (ORAC) The ORAC assay was conducted according to [23]. The ORAC assay was carried out on a fluorescence microplate reader (FLUOstar Omega, BMG LABTECH, Multi-Detection Microplate Reader, Germany). Peroxyl radicals were generated by AAPH, and fluorescence microplate reader was used at an excitation wavelength of 485 nm and an emission wavelength of 525 nm. Trolox was used as standard (50, 25, 12.5, 6.25, 3.12 mM). Proper dilutions of papaya extracts were made with ORAC buffer (potassium phosphate buffer, pH 7.4). For each ORAC run, a micro plate was prepared containing 25uµ of Trolox standards, buffer control, and sample dilutions, as well as 150ul of fluorescein (FL) solution. All ORAC analyses were performed at 37^0C with a 20 min incubation and 60 min run time. After the incubation, 25ul of AAPH was added to each well for a final volume of 200 uL. The results were calculated using the differences of areas under the FL decay curves between the blank and a sample and were expressed as micromole Trolox Equivalents per gram of sample (umol TE/g).

Physicochemical Properties of Coconut Milk: Samples were analyzed for physicochemical characteristics according to standard procedures. Data analysis was performed in triplicate. Protein content of the milk was measured by Kjeldhal method No. 920.105 and fat content by Mojonnier method [24]. Moisture content was evaluated by drying the samples at 105°C overnight in a Memmert Oven (Germany). Gravimetric method was used to determine ash content using a furnace at 550°C as described by [25]. Total Soluble Solids (TSS) was measured with a refract to meter at 20°C and pH of the fresh coconut milk sample was determined using a digital pH meter [26].Amino acid profile was deducted by High Performance Liquid Chromatography (HPLC) method of 6N HCl hydrolysate. Fluorescence detector was used with two mobile phase: AccQ tag eluent A, concentrate and AccQ tag eluent B, 60% acetonitrile [25]. Analysis of fatty acid profile was done by gas chromatography (GC) as per the AOCS method Ce1-62 [27]. Fatty acids present in oil of the coconut milk were first converted to fatty acid methyl esters (FAME) before injecting into GC column to obtain the fatty acid profile.

Statistical Analysis: Data were expressed as the mean of three independent experiments. Statistical comparisons of the results were subjected to one-way ANOVA using SPSS ver.20. Significant differences ($p<0.05$) among the different properties of the coconut milk were analyzed by Duncan' triplicates range test [28].

RESULTS AND DISCUSSION

Antioxidant Activity of Coconut Milk: Table 1 shows the antioxidant activity results obtained by TPC, FRAP, DPPH and ORAC assays of coconut milk samples from different local markets of coconut milk sellers in Malaysia in comparison with goat and cow's milk. Statistical analysis by Duncan's test demonstrated that the difference antioxidant activity assays significantly affected ($P < 0.05$) by the coconut milk samples. Statistical analysis by Duncan's test also demonstrated that milk samples exhibited a significantly different ($P<0.05$) antioxidant activity among the three types of milk for all the assays except DPPH. Coconut's milk exhibited the highest antioxidant activity in TPC, FRAP, DPPH and ORAC assays with mean value of 575.15 mg GA/100 g FW, 471.55 mg TE/100 g FW, 68.39 % and 784.47 umol TE/100g F.W, respectively. In contrast, the cow's milk exhibited the lowest mean value of 477.68 mg GA/100 g FW, 398.88 mg TE/100 g FW, 60.81 % and 361.96 umol TE/100g F.W, respectively). Goat's milk contained higher antioxidant activity than cow's milk samples through all the assays with mean value of 544.08 mg GA/100 g FW, 481.69 mg TE/100 g FW, 67.44 % and 594.61 umol TE/100g F.W, respectively.

Table 1. Average of the antioxidant activity assays of coconut milk samples compare to goat and cow's milk in Malaysia

Sample	TPC (mg GA/100g)	FRAP (mg TE/100g)	ORAC (umol TE/100g)	DPPH (%)
Coconut	575.15 ± 2.26^{a}	610.19 ± 2.54^{a}	784.47 ± 3.60^{a}	68.39 ± 1.30^{a}
Goat	544.08 ± 1.83^{b}	481.69 ± 1.56^{b}	594.61 ± 2.25^{b}	67.44 ± 0.47^{ab}
Cow	477.68 ± 1.20^{c}	398.88 ± 1.54^{c}	361.96 ± 2.20^{c}	60.81 ± 0.43^{ab}

$^{a–c}$ Mean with different letters within the same column indicate significant difference ($P<0.05$).

Antioxidant compounds react with Folin-Ciocalteu reagent, and the reaction can be performed to measure the concentration of phenolic groups [29]. Therefore, deep blue coloration in milk samples indicates that high phenolic concentrations are present, whereas light blue coloration in milk samples indicates otherwise. FRAP assays depend on the mechanism that involves oxidation and reduction reactions, in which ferric ion is reduced to ferrous ion. This mechanism can be correlated with the table redox properties of antioxidant compounds in milk samples.

Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species [30]. Therefore, DPPH assays are used to determine the capacity of primary antioxidants in samples, in which these primary antioxidants react to scavenge free radicals from DPPH solution. Hence, the formation of the initiation chain of free radicals is inhibited, and the propagation chain is destroyed through the donation of a hydrogen atom or an electron. Consequently, free radicals can be modified to a more stable form of products [29, 31]. This result explains the discoloration of a milk samples from purple to yellow in DPPH solution. However, the superiority of the coconut milk in terms of exhibiting the highest level of antioxidant activity compared with the other milk samples may be attributed to the aforementioned traits. As reported by [17, 18], coconut milk is high antioxidants activity, that prevents free radical damage regarding to the abundance of phenol content in the coconut oil. This interpret the superiority of coconut milk in the antioxidant activity.

Table 2. Correlation coefficients of the antioxidant activities among coconut, goat cow's milk using different assays

	TPC	FRAP	ORAC	DPPH
TPC	-	-	-	-
FRAP	0.91	-	-	-
ORAC	0.97	0.84	-	-
DPPH	0.96	0.95	0.94	-

The correlation between the antioxidant assays was performed using the function CORREL in the Microsoft Excel program. All antioxidant assays results exhibited positive correlation (Table 2). TPC and ORAC assays showed the highest correlation among the tested milk samples ($R^2 = 0.97$). this correspond with several studies that also shown a highly positive correlation between FRAP, TPC, and DPPH assays [32, 33, 34, 35, 36, 37].

Physicochemical Properties: According to Malaysian Food Act 1983 and Regulations 1985, coconut milk shall be the emulsion extracted from fresh, sound, ripe kernel of the fruit of *Cocosnucifera*[39]. Consequently, the physicochemical characteristics of coconut milk in Malaysia have been investigated and compare to other tropical countries. Testing physicochemical properties of coconut milk, such as protein, fat, pH, moisture, ash, Brix (TSS)are important in studying the physicochemical composition as nutritional value attribute. Table 3 shows the

mean values of the physiochemical parameters of the coconut milk samples in Malaysia with comparison with Philippine, Thailand and Sri Lanka from other studies [11, 14, 38], respectively. Results showed that fat content in the Malaysian coconut milk samples was 15.44% and had significantly lower content ($P < 0.05$) than the other tropical coconut milk samples. By contrast Philippine coconut milk was the higher content of fat with percentage of 38%.

Table 3. Physicochemical properties of fresh coconut milk in Malaysia comparative to other tropical countries (Philippine, Thailand and Sri Lanka)

	Malaysia	*Thailand	*Philippine	*Sri Lanka
Fat	15.44 ± 1.53	20.00 ± 1.46	38.00 ± 1.71	35.80 ± 2.27
Protein	3.40 ± 0.59	2.06 ± 0.06	3.50 ± 0.21	3.10 ± 0.71
Moisture	73.57± 0.24	74.60 ± 0.34	52.00 ± 1.06	57.00 ± 1.63
Ash	0.71 ± 0.01	0.64 ± 0.01	0.90 ± 0.03	0.84 ± 0.08
pH	5.60 ± 0.06	5.80 ± 0.04	6.00 ± 0.32	6.30 ± 0.14
Brix	7.50 ± 0.11	5.40 ± 0.08	9.00 ± 0.26	9.00 ± 0.31

*[11, 14, 37]

Proteins present in coconut milk play an important role on the stability of the emulsion and heating the coconut milk at higher temperature causes protein denaturation[40]. Coconut milk samples showed variance in total protein content between the different countries. Protein content in Malaysian coconut milk samples was higher 3.4% comparative samples except Philippine 3.5%. This result demonstrates that Malaysian fresh coconut milk is a rich source of protein. Variance values were also found in moisture content among all the comparative samples. Milk samples from Thailand were found to have highest moisture content (74.60 ± 0.34) followed by Malaysia, Sri Lanka and Philippine (73.57± 0.24, 57.00 ± 1.63 and 52.00 ± 1.06 respectively). As reported by [11, 12], coconut milk contained about 54% moisture, 35% fat and 11% solid non-fat and they also showed that fat content played an important role in the flow property of coconut milk [13].This study is in consistent with [39]research where the moisture content of fresh coconut milk were in range of 76.2% to 81.6% obtained from different Malaysian shops.
From the table, it can be shown there is significant difference ($P < 0.05$) amounts of ash among all the comparative coconut milk samples. Philippine's coconut milk had the highest ash content value (0.90±0.03), whereas Thailand's had the lowest (0.64±0.01). The pH of the coconut milk samples that were obtained from different sellers in Malaysia was measured at the same sampling day and the mean value was 5.80 ± 0.04. This result was within the pH values range of comparative samples from 5.60 ± 0.06 to 6.30 ± 0.14 with significant different ($P < 0.05$) values. In like situation, Brix value on Malaysian coconut (7.50 ± 0.11) was also within the concentration range of comparative samples from 5.40 ± 0.08 to 9.00 ± 0.31.

CONCLUSION

Coconut Milk products are prepared using a significant amount of comminuted coconut meat where most filterable fibres and residues are excluded, with or without coconut water, or with additional water, It being a refreshing beverage, provides important health benefits. The present study provides preliminary data on the physiochemical properties and antioxidant activity of coconut milk from local markets in Malaysia. Results showed that coconut milk exhibits a highest antioxidant activity with significant differences ($P < 0.05$) among goat and cow's milk. Malaysian coconut milk is also in different physicochemical properties such as protein, fat, Brix, ash content, pH, and moisture content in comparison with other tropical countries. This result showed that Malaysian coconut milk possess high proteins with lower amount of fats. These findings suggest that coconut milk could be considered as a promising food component that could prevent oxidative damage and reduce the risk of degenerative diseases. In addition, the antioxidant activity of these fractions from the coconut milk is of interest as a potential natural food antioxidant additive.

Recommendation
Future studies should be conducted to determine the factors that produce the desirable chemical composition for a specific purpose of the nutritional value. Breeding studies can also be carried out to produce coconut milk enriched with specific chemical compounds. Although coconut water is already well studied in terms of its chemical content, there may still be unknown solutes which contribute to its special biological effects. With the development of more advanced detection techniques, screening can be intensified to detect novel compounds of medicinal values present in coconut water.

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