Original article

Comparison of nutrient composition of ripe and unripe fruits of *Nypa fruticans*

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Comparison of nutrient composition of ripe and unripe fruits of *Nypa fruticans*.

Abstract – Introduction. *Nypa fruticans* is one of the mangrove plants in Malaysia. Leaves of the plant are traditionally used for thatching, while the sap is useful for producing an alcoholic drink, sugar and syrup, as well as vinegar. At present, *N. fruticans* fruit is considered as an underutilized fruit. Its flesh (endocarp) is considered nutritionally inferior. Hence, our study aimed to determine the proximate composition and total phenolic content of ripe and unripe flesh of *N. fruticans* to explore its food uses as a source of nutrients. **Materials and methods**. Determination of proximate content followed the AOAC methods, while total phenolic content was determined using the Folin-Ciocalteu reagent assay. **Results and discussion**. The results showed significant differences for all the proximate values (moisture content, ash, carbohydrate, crude protein, crude fat, and insoluble and soluble fiber) between the ripe and unripe flesh of the fruit. The flesh of ripe fruit also had higher ($P < 0.05$) total phenolic content than its unripe counterpart. **Conclusion**. The ripe and unripe flesh of *Nypa fruticans* could potentially be used as functional food ingredients in the future.

Peninsular Malaysia / *Nypa fruticans* / fruits / developmental stages / maturity / proximate composition / phenolic compounds

Comparaison de la composition nutritionnelle des fruits mûrs et immatures de *Nypa fruticans*.

Résumé – Introduction. *Nypa fruticans* est l'une des plantes des mangroves malaisienne. Les feuilles de la plante sont traditionnellement utilisées pour couvrir les toits, tandis que la sève est utilisée pour la production de boissons alcoolisées, de sucre ou de sirop et de vinaigre. À l'heure actuelle, le fruit de *N. fruticans* est considéré comme un fruit sous-utilisé. Sa chair (endocarpe) aurait une basse valeur nutritionnelle. Notre étude a cherché à déterminer la composition globale et le contenu en phénols totaux de la pulpe de fruits mûrs et immatures de *N. fruticans* pour explorer son utilisation en aliment source de nutriments. **Matériel et méthodes**. La détermination de la teneur globale de la pulpe a été effectuée en utilisant les méthodes de l'AOAC, tandis que la teneur en composés phénoliques totaux a été déterminée en utilisant un dosage avec le réactif de Folin-Ciocalteu. **Résultats et discussion**. Les résultats ont montré des différences significatives entre pulpe mûre et immature pour tous les paramètres de la composition globale du fruit (humidité, cendres, glucides, protéines brutes, matières grasses brutes, fibres solubles et insolubles). La chair du fruit mûr a présenté aussi une teneur en composés phénoliques totaux plus élevée ($P < 0,05$) que celle de la chair non mûre. **Conclusion**. La pulpe mûre et immature de *Nypa fruticans* pourrait potentiellement être utilisée à l'avenir comme un aliment fonctionnel.

Malaisie péninsulaire / *Nypa fruticans* / fruits / stade de développement / maturité / composition globale / composé phénolique

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1. Introduction

Mangrove forests occupy 14.65 million hectares over the world [1] with an economic value of 200,000 USD to 900,000 USD per hectare [2]. Besides the economic benefits, the mangrove forests also play an important role in human livelihood as a source of food, timber, medicine and fuel [3]. Malaysia has the third largest mangrove forest in the Asia-Pacific region, at approximately 645,852 ha [4]. Most of the mangrove forests are dominated by several species such as *Nypa fruticans, Sonneratia caseolaris, Avicennia alba, Rhizophora apiculata, R. mucronata* and *Bruguiera gymnorhiza* [5].

Trees of *Nypa fruticans* are widely grown on the west coast of Peninsular Malaysia. It bears a huge globular cluster of the palm fruit. Young palm fruit (*figure 1*) has sweet edible sap which is used as a beverage. There are also several other uses of the *N. fruticans* tree. For example, leaves of *N. fruticans* are traditionally used as roof material (thatching) and woven products such as hats and baskets. The young leaves of *N. fruticans* are used to produce tobacco wrappers [6, 7]. The sap of *N. fruticans* is used to produce an alcoholic drink, sugar, syrup and vinegar. Sometimes, the young seed of *N. fruticans,* called "attap chi" in Malaysia, is served as a dessert ingredient and in local ice confections due to its sweetness [8]. *N. fruticans* is also used in traditional medical remedies. According to Teo *et al.*, young shoots, burned roots and leaves, and decayed wood of *N. fruticans* are traditionally used for treating headaches, toothaches and herpes [7].

The total flowering period of *N. fruticans* is between 8.2–9.6 months, and divided into seven stages [9]. In the third stage of its cycle, the female flower starts to form in a single spherical shape, yellow in color, and is found at the tip of the inflorescence trunk. At the same time, the male inflorescence is positioned below the female inflorescence. In the fifth stage (about two months old), young or unripe fruits start to grow and the fruits are oval in shape and lighter brown in color compared with ripe fruits, which are a darker brown color (*figure 1*). In total, the fruits take 180 days to turn from young into ripe fruits (from the fifth to the sixth stage).

Figure 1.
Unripe and ripe fruit of *Nypa fruticans*.

Several studies have been carried out to maximize the utilization of *N. fruticans*. Tamunaidu and Saka have characterized the chemical properties of various parts of *N. fruticans* such as the front, leaf, husk and shell [10]. While, Prasad *et al.* reported a higher total phenolic content (TPC) of unripe fruit of *N. fruticans* [(6.08 ± 0.10) mg·g^{-1}] than the ripe fruit [(0.22 ± 0.10) mg·g^{-1}], and the better antioxidant properties of the unripe fruit [11]. A few years back, one Malaysian company identified the potential use of ethanol from nypa palm to replace fossil fuel which helps in preventing ozone depletion[1]. At present,

[1] The Star Online, 2007, April 10. Malaysian company says bio-fuel from nipah can help halt global warming. http://www.thestar.com.my/story.aspx?file=%2f2007%2f4%2f10%2fbusiness%2f20070410184839&sec=business (access Nov. 2012)

N. fruticans fruit is still considered as an underutilized palm. Most of the time, the exocarp and mesocarp of the globular fruit are discarded during collection of the sap as the flesh (endocarp) of the fruit is considered nutritionally inferior. Hence, our study aimed to determine the proximate composition and total phenolic content of ripe and unripe flesh of *N. fruticans* to further utilize them as a source of nutrients.

2. Materials and methods

2.1. Sample preparation

The samples of this study were ripe and unripe flesh of *N. fruticans*. Maturity of the fruit was determined based on the size and color of the fruit as well as the texture of the flesh. The globular fruits were collected fresh from Parit Buntar, Perak, Peninsular Malaysia, and immediately transported to the Universiti Putra Malaysia (UPM). After reaching the nutrition laboratory, tap water was used to wash the fruits; the cleaned fruits were air-dried at room temperature. The fruits were stored in a freezer at –20 °C. For sample preparation, they were taken out of the freezer and thawed to room temperature. The fruits were cut and the flesh of the globular fruits was collected and stored at –80 °C. The flesh was freeze-dried to remove water. After freeze-drying (Virtis, New York, USA), the samples were ground into smaller pieces or powder form and stored under –20 °C before further analysis.

2.2. Chemicals

All chemicals used were of analytical grade. Anthrone reagent, glucose standard, Kjeltab (7 g potassium sulfate, 0.8 g copper (II) sulfate), Tashiro's indicator and phosphate buffer were from Fisher Scientific (Pennsylvania, USA). Petroleum ether, ethanol (78%), ethanol (95%), acetone, α-amylase, protease, amyloglucosidase, celite, methanol, Folin-Ciocalteu reagent and sodium bicarbonate were from Sigma Chemical (Missouri, USA). Sodium hydroxide, perchloric acid, sulfuric acid, hydrochloric acid and boric acid were purchased from J.T. Baker (New Jersey, USA).

2.3. Determination of proximate composition

The moisture content of samples was determined using the direct drying method [12]. About 10 g of homogenized sample was positioned on a dried aluminum dish with a cover and placed in an oven at 105 °C overnight. The process was repeated until a constant weight was obtained. The initial weight and constant weight after drying were recorded to determine the total moisture lost. All samples were analyzed in triplicate.

The carbohydrate content of samples was determined by using the Clegg anthrone method [12]. Exactly thirteen milliliters of 52% perchloric acid reagent were added to digest the sample into simpler compounds. Anthrone was prepared by dissolving 0.1% anthrone in a sulfuric acid solution (sulfuric acid:water at a ratio of 2.3:1.0, v/v) before use. Five mL anthrone reagent were added to the sample and it was incubated in a boiling water bath for exactly 12 min. Water was used as a blank. The mixture was analyzed by using a spectrophotometer (Secomam, France) at 630 nm against the blank. Glucose (0–100 $mg \cdot L^{-1}$) was used to construct a standard curve for quantification and the results were expressed as g of glucose·100 g^{-1} fresh weight of sample.

The Kjeldahl method was used to determine the crude protein in samples [12]. About 1 g homogenous sample was digested with 15 mL sulfuric acid and two Kjeldahl tabs, and the solution was incubated at 420 °C for 1 h. The resulting digest was mixed with 30 mL of 4% boric acid containing a few drops of Tashiro's indicator, 80 mL of distilled water and 50 mL of 40% sodium hydroxide (NaOH) in a distillation unit. The solution was titrated with 0.2 N hydrochloric acid (HCl) until the original purplish color was obtained. The measured nitrogen content was converted to protein content by using a conversion factor of 6.25.

A Soxhlet apparatus was used to extract and determine the fat content in samples [13]. Exactly 10 g of sample was put into an extraction thimble and placed in the Soxhlet

extractor. The sample was extracted with 150 mL of petroleum ether on the apparatus for 8 h to 16 h. The petroleum ether was removed by evaporation and the extracted fat was dried in an air oven at 100 °C for 1 h. The residue crude fat was weighed.

Soluble and insoluble fibers in samples were determined by using the enzymatic gravimetric method based on AOAC methods [12]. About 1.0 g sample was digested with 0.1 mL of α-amylase at pH 6.0 and incubated at 100 °C for 15 min. Next, the solution was adjusted to pH 7.0 and digested by adding 10 mL of 0.275 N NaOH and incubated at 60 °C for 30 min. Again the solution was adjusted to pH 4.0–4.6 and digested by adding 0.1 mL of amyloglucosidase. The solution was incubated at 60 °C for 30 min, then filtered. The filtrate was reserved for soluble fiber determination, while the residue was washed with 10 mL distilled water and 10 mL 95% ethanol. The residue in the crucible was washed again with 15 mL of acetone. Lastly, the crucible was dried overnight at 105 °C. Three replicates of the sample were used to determine protein and ash, and total soluble fiber was calculated. For soluble fiber determination, the reserved filtrate was added to four volumes of 95% ethanol which was preheated to 60 °C. The solution was filtered and the residue was washed with 20 mL 78% ethanol three times, then twice with 10 mL of 95% ethanol. After that, the residue in the crucible was washed with 15 mL of acetone. The crucible was dried overnight at 105 °C and weighed.

The direct ashing method was used to determine the ash content of samples [12]. Exactly 5 g fresh sample was added to a crucible. The crucible was placed into a muffle furnace overnight at 550 °C. Later, the sample was weighed and these steps were repeated until a constant weight was obtained.

2.4. Determination of total phenolic content

Folin-Ciocalteu reagent was used to determine the total phenolic content in samples [14]. The sample was extracted using 20 mL 80% methanol and agitated at 200 rpm at 50 °C for 2 h. After filtration, one hundred µL of the extract were added to 5 mL of ten-fold diluted Folin-Ciocalteu reagent and left to stand for 5 min. Next, we added 1.5 mL of 6% (w/v) sodium carbonate solution to the mixture and allowed it to stand at room temperature for 90 min. Absorbance of the solution was measured at 725 nm using a spectrophotometer (Secomam, France). Data were expressed as mg gallic acid Eq·100 g^{-1} fresh weight and dry weight. A standard calibration curve was plotted with the concentration of 0.02–0.2 gallic acid mg·mL^{-1}.

2.5. Statistical analysis

We used SPSS version 17.0 software to analyze our data, which was expressed as mean ± standard deviation for triplicate determinations (n = 3). An independent t-test was used to compare means of proximate nutrient contents (ash, crude protein, crude fat, carbohydrate, and total soluble and insoluble fibers) and total phenolic content of the ripe and unripe fruit samples. The confidence interval was set to 95% and the P-value for statistical significance was defined as $P < 0.05$.

3. Results and discussion

3.1. Nutrient composition

According to our results regarding the nutrient composition of ripe and unripe flesh of *N. fruticans*, the major component was moisture: (35.71 ± 1.19)% for the ripe flesh and (90.10 ± 0.95)% for the unripe flesh *(table I)*. In our study, the moisture content of ripe flesh of *N. fruticans* was lower than that reported by Osabor *et al.* at (41.96 ± 0.28)% [15]. Moisture content of food is an index of water activity, that indicates the stability and susceptibility to microbial contamination [16]. Based on this, unripe flesh of *N. fruticans* had very high moisture, thus potentially having a short life that could promote microbial growth as compared with its ripe counterpart. A significant difference ($P < 0.05$) was observed in crude carbohydrate content between ripe and

Table I.

Comparison of nutrient composition (g·100 g^{-1} fresh weight) of ripe and unripe flesh of *Nypa fruticans* studied in different geographical locations.

Location	Maturation	Moisture content	Ash	Carbohydrate	Protein	Fat	Insoluble fiber	Soluble fiber
Peninsular Malaysia (current study)	Ripe	35.71 ± 1.19	1.11 ± 0.08	21.40 ± 0.46	4.02 ± 0.17	4.33 ± 0.39	15.11 ± 0.67	15.50 ± 1.71
	Unripe	90.10 ± 0.95	0.78 ± 0.07	4.40 ± 0.36	0.97 ± 0.02	0.52 ± 0.06	0.88 ± 0.10	0.96 ± 0.24
Borneo Island [18]	Unripe	94.1	1.9	2.5	0.7	0.1	–	–
Thailand [17]	Unripe	88.5 ± 0.46	1.71 ± 0.36	2.62	1.43 ± 0.01	0.03 ± 0	5.18 ± 0.01	5.66 ± 0.04
Nigeria [15]	Ripe	41.96 ± 0.28	2.7 ± 0.01	51.08 ± 1.71	2.27 ± 0.01	0.94 ± 0.01	–	–

Results are mean of triplicate ± standard deviation.

unripe flesh of *N. fruticans*, at (21.40 ± 0.46)% and (4.40 ± 0.36)%, respectively. This indicates that the carbohydrate content of the flesh of *N. fruticans* increases as maturity progresses. The crude carbohydrate content of ripe flesh of *N. fruticans* reported by Osabor *et al.* [(51.08 ± 1.71)%] [15] was much higher than that in our study. However, the crude carbohydrate content of the flesh of *N. fruticans* reported by Bunyapraphatsara *et al.* (2.62%) [17], as well as by Hoe and Siong (2.5%) [18], was lower compared with that of the unripe flesh of *N. fruticans* found in our study. This might be due to differences in factors such as climate, soil, plant nutrition and storage conditions [19].

The protein content of ripe and unripe flesh of *N. fruticans* was low at (4.02 ± 0.17)% and (0.97 ± 0.02)%, respectively (*table I*). The percentage of crude protein content in unripe flesh of *N. fruticans* was close to the values reported by Bunyapraphatsara [17] and Hoe and Siong [18], which were (1.43 ± 0.01)% and 0.7%, respectively. The crude protein in ripe flesh of *N. fruticans* was relatively higher than that of coconut (3.9%) [20]. Furthermore, the protein content in ripe flesh of *N. fruticans* was also relatively higher than that in the flesh of some local fruits such as durian (2.7%), mangosteen (0.6%), mango (2.1%), papaya (0.5%), pineapple (0.5%) and rambutan (0.7%) [20]. The ripe flesh of *N. fruticans* consisted of (4.33 ± 0.39)% of fat, while the unripe flesh of *N. fruticans* contained significantly lower fat at only (0.52 ± 0.06)% ($P < 0.05$). As compared with palm fruits (*Elaies guineensis* from the same family), the fat content of *N. fruticans* flesh was very much lower. Furthermore, coconut also had a higher content of fat (33.9%) than both ripe and unripe flesh of *N. fruticans* [20].

In our present study, insoluble and soluble fibers in the ripe flesh of *N. fruticans* were high at (15.11 ± 0.67)% and (15.50 ± 1.71)%, respectively (*table I*). The total fiber was as much as (30.60 ± 1.78)%, that made up about a quarter of the proximate nutrient content. The insoluble and soluble fibers of the unripe flesh of *N. fruticans* were relatively lower compared with that of ripe flesh, at (0.88 ± 0.10)% and (0.96 ± 0.24)%, respectively. Total fiber of the unripe flesh of *N. fruticans* was generally low, only (1.85 ± 0.34)% of the flesh. However, Bunyapraphatsara *et al.* found lower levels of insoluble and soluble fibers in *N. fruticans* flesh: (5.18 ± 0.01)% and (5.66 ± 0.04)%, respectively [17], compared with the results of our samples. As compared with other local fruits, ripe *N. fruticans* flesh has relatively higher total fiber than durian (0.9%), mangosteen (5.1%), mango (0.4%), papaya (0.7%), pineapple (0.6%), and rambutan (0.3%) [20]. The high fiber content of ripe *N. fruticans* flesh allows the fruit to be recommended for prevention of constipation. The ash content of the ripe flesh of *N. fruticans* was significantly higher ($P < 0.05$) than that of the unripe flesh at (1.11 ± 0.08)% and (0.78 ± 0.07)%, respectively. Previous studies also reported that the flesh of *N. fruticans* fruit is rich in

minerals, especially potassium {(128.52 ± 0.64) mg·100 g^{-1} [15] and 120 mg·100 g^{-1} [18]}, and magnesium (97 mg·100 g^{-1}) [18].

3.2. Comparison of proximate nutrient composition of *N. fruticans* fruit from different geographical locations

The nutrient composition of the flesh of *N. fruticans* has been studied widely. The nutrient composition, especially proximate nutrient contents of the flesh, has previously been reported. The proximate contents of *N. fruticans* flesh from our study were compared with other studies from Borneo Island [18], Thailand [17] and Nigeria [15] (*table I*). Soluble and insoluble fibers were not determined by Hoe and Siong [18] or Osabor *et al.* [15]. The ripe flesh of *N. fruticans* from our study (Peninsular Malaysia) has the lowest moisture and ash contents compared with others (*table I*). The protein and fat contents of the ripe flesh of *N. fruticans* from Peninsular Malaysia were higher compared with fruits from other geographical regions, except for carbohydrate content. The ripe flesh of *N. fruticans* from Nigeria had carbohydrate content ~140% higher than the carbohydrate content of ripe flesh of *N. fruticans* from Peninsular Malaysia; while the unripe flesh of *N. fruticans* from Peninsular Malaysia had carbohydrate content about 1.5 times higher than that of the unripe flesh of *N. fruticans* from Borneo Island and Thailand. Among the *N. fruticans* fruits, unripe flesh from Thailand had the lowest fat content. Besides, ripe *N. fruticans* flesh from Peninsular Malaysia had about three times higher soluble and insoluble fibers compared with those of the unripe flesh of *N. fruticans* from Thailand, while the unripe flesh of *N. fruticans* from Peninsular Malaysia had a fiber content 5–6 times lower than that of the unripe flesh of *N. fruticans* from Thailand.

Unripe *N. fruticans* fruit commonly contains a collection of sap, where the moisture content can be as high as 95%. The sap has a sweet taste with high carbohydrate content, especially the reducing sugar. The unripe flesh of *N. fruticans* from Peninsular Malaysia could be sweeter than the unripe flesh from Borneo Island and Thailand as it contains higher available carbohydrate content (*table I*). Due to the different maturity stages of the *N. fruticans* fruits, there is a great variation in their proximate composition. Generally, the ripe flesh of *N. fruticans* from Peninsular Malaysia has higher nutritional qualities as compared with the fruits from other geographical locations.

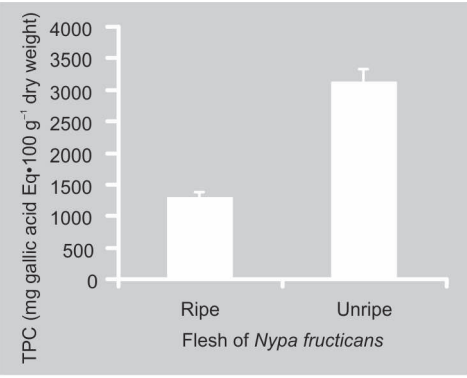

Figure 2.
Total phenolic content of ripe and unripe fruit of *Nypa fruticans*.

3.3. Total phenolic content

Total phenolic contents (TPCs) of ripe and unripe flesh of *N. fruticans* showed a significant difference ($p < 0.05$) (*figure 2*). TPC of the unripe flesh of *N. fruticans* [(3136.6 ± 202.2) mg gallic acid Eq·100 g^{-1} dry weight] was two times higher than that of the ripe flesh [(1293.4 ± 93.2) mg gallic acid Eq·100 g^{-1} dw]. According to Velioglu *et al.*, TPC of selected plant materials such as fruits, vegetables and grain products ranged from (169 to 10548) mg·100 g^{-1} dw [21], while TPC of selected vegetables (spinach, kale and cabbage) was in a range of (886 ± 52) mg·100 g^{-1} to (7167 ± 73) mg gallic acid Eq·100 g^{-1} dw [22]. Prasad *et al.* also reported a significantly lower TPC for the ripe and unripe flesh of *N. fruticans* compared with the values found in this study [11] (*table II*). Although the fruit of *N. fruticans* was obtained from Peninsular Malaysia, the different geographical location might be the main factor for the variation in TPC of *N. fruticans* fruit. Other factors such as the maturity degree of the ripe and unripe flesh of *N. fruticans* as well as the different amount of Folin-Ciocalteu reagent used in our current study and the study of Prasad *et al.* [11]

Table II.

Comparison of total phenolic content (mg·100 g^{-1} fresh weight) of unripe and ripe flesh of *Nypa fruticans* from different geographical locations.

Maturity	Current study (Peninsular Malaysia)	Prasad *et al.* Kedah, Peninsular Malaysia [11]
Unripe	832	608
Ripe	310	22

might also contribute to the variation in TPC. The high TPC of the ripe and unripe flesh of *N. fruticans* can be used as a potential source of nutrients and antioxidants in various food applications.

4. Conclusions

Our study revealed that the ripe flesh of *N. fruticans* fruit can be a good source of macro-nutrients, especially fiber. The unripe flesh of *N. fruticans* fruit had higher total phenolics than the ripe fruit, that can be used as an alternative source of antioxidants. Due to the high nutritional quality of both ripe and unripe flesh of *N. fruticans*, the flesh of *N. fruticans* fruit could be served as a raw ingredient for various food uses in the future.

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Comparación de la composición nutricional de frutas maduras e inmaduras de *Nypa fruticans*.

Resumen – **Introducción**. *Nypa fruticans* es una planta de los manglares de Malasia. Las hojas de la planta se utilizan tradicionalmente para cubrir los tejados, mientras que su savia se utiliza para la producción de bebidas alcohólicas, azúcar, almíbar o vinagre. Hoy en día, la fruta de *N. fruticans* se considera una fruta infrautilizada. Su carne (endocarpio) al parecer posee un bajo valor nutricional. Nuestro estudio estaba encaminado a determinar la composición global y el contenido en fenoles totales de la pulpa de frutas maduras e inmaduras de *N. fruticans* para explorar su uso como fuente de nutrientes. **Material y métodos**. La determinación del contenido global de la pulpa se realizó con métodos de la AOAC, mientras que el contenido en compuestos fenólicos totales se determinó con una dosificación del reactivo de Folin-Ciocalteu. **Resultados y discusión**. Los resultados mostraron diferencias significativas entre la pulpa madura e inmadura en el caso de todos los parámetros de la composición global de la fruta (humedad, cenizas, glúcidos, proteínas brutas, materias grasas brutas, fibras solubles e insolubles). La carne de la fruta madura también presentó un contendido en compuestos fenólicos totales más elevado ($P < 0,05$) que el de la carne inmadura. **Conclusión**. La pulpa madura e inmadura de *Nypa fruticans* podría utilizarse potencialmente en el futuro como un alimento funcional.

Malasia Peninsular / *Nypa fruticans* / frutas / etapas de desarrollo / madurez / composición aproximada / compuestos fenólicos