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Analytical Methods

Nutrients and bioactive compounds in popular and indigenous durian (*Durio zibethinus* murr.)

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ABSTRACT

This study identified nutrients, fatty acids, bioactive compounds and antioxidant activities of two popular varieties (Mon-thong, Cha-ni) and two indigenous varieties (Kra-dum and Kob-ta-kam) of durian. Each of variety was collected from 3 gardens in Nonthaburi province, Thailand. At optimal ripeness, the edible part was separated, homogenised or freeze dried, as fresh or dry samples for further analysis using standard methods. All durian varieties contained a considerable amount of dietary fibre (7.5–9.1 g/100 g dry matter, DM) and high amounts of carbohydrate and sugar (62.9–70.7 g and 47.9–56.4 g/100 g DM respectively). Cha-ni, Kra-dum and Kob-ta-kam varieties had monounsaturated (MUFA) (6.1–7.8 g/100 g DM) > saturated (SFA) (4.2–5.7 g/100 g DM) > polyunsaturated fatty acid (PUFA) (0.8–1.5 g/100 g DM), whereas the Mon-thong variety had SFA > MUFA > PUFA (5.1, 4.0, 1.1 g/100 g DM, respectively). The Kob-ta-kam variety showed greater potential for health benefits in terms of carotenoids and β-carotene (2248 μg and 1202 μg/100 g DM respectively). Phenolic compounds and antioxidant capacity were not significantly different among each variety, though the Cha-ni variety had the lowest. This study provides data on nutrients, bioactive compounds and antioxidant activities of indigenous and popular durian varieties that could be used for consumer education as well as for incorporation into the food composition databases.

1. Introduction

Epidemiological studies have shown a negative association between the high intake of fruits and vegetables and cardiovascular diseases (Hu, 2003) as well as certain cancers (Riboli & Norat, 2003). Vegetables and fruits are well-known as good sources of dietary fibre, minerals, vitamins, and bioactive compounds. The antioxidant property in plants derives mainly from bioactive compounds such as carotenoids, phytosterols, as well as phenolic compounds and vitamins C and E. (Brewer, 2011). Phenolics are the most abundant compounds in plants, and many studies have demonstrated a strong relationship between these compounds and antioxidant activities (Chang et al., 2007).

Native to Southeast Asia and known as the king of fruits, durian (*Durio zibethinus* murr.) is popular in many countries including Thailand. Nonthaburi province, a suburb of Bangkok, is known as the best place for growing durian in Thailand; however, the cultivated area is decreasing due to urbanisation and environmental change. Although some cultivars have been grown in other areas of Thailand, durian from Nonthaburi remains popular.

Ho and Bhat (2015) presented composition and nutritional value information for durian mainly among popular commercial varieties. Information for some popular durian varieties in Thailand, mostly from Rayong and Chantaburi provinces in the eastern region of the country, also have been reported (Charoensiri, Kongkachuichai, Suknicom, & Sungpuag, 2009; Haruenkit et al., 2007, 2010). However, the database on durian in the ASEAN food composition tables (Puwastien, Burlingame, Raroengwichit, & Sungpuag, 2000) is limited. Only one item of mixed varieties was complied from Malaysia, the Philippine and Thailand. Nutrition data for specific varieties of indigenous durian from Nonthaburi have not been reported, despite their popularity. For further conservation, healthy consumption and export promotion purposes, accurate nutrition data for indigenous durians are needed. Consequently, four common varieties of durian, both popular varieties and indigenous varieties, were studied in terms of nutrients, fatty acids, bioactive compounds, as well as antioxidant activities.

2. Materials and methods

2.1. Food sampling and samples preparation

Food sampling and samples preparation followed standard guidelines (Greenfield & Southgate, 2003). Four common varieties

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of durian, selected by their gardeners (Mon-thong, Cha-ni, Kradum and Kob-ta-kam) (*D. zibethinus* murr.), were collected from 3 gardens in Nonthaburi province during April to May 2011. Mon-thong and Cha-ni represented popular varieties, while Kradum and Kob-ta-kam represented indigenous varieties.

The fruits were transported to the Institute of Nutrition, Mahidol University laboratory and kept at room temperature. Based on their local wisdom, the gardener provided recommendations on optimum ripeness (3–7 days after harvested depending on varieties) together with the character of flesh loss that would adhere to the thick shell (Haruenkit et al., 2010). The edible part was cut into pieces by plastic knife, and then divided into two portions. The first portion was homogenised using a food processor (Mara®, Thailand) and kept in acid-washed screw-capped plastic bottles. Homogenised samples were immediately analysed for moisture and vitamin C before being kept at −20 °C for other nutrient analyses. The second portion was freeze-dried, ground, vacuum-packed in laminated aluminium foil bags, and stored at −20 °C for determination of bioactive compounds and antioxidant activities.

2.2. Reagents and standards

Deionised water was obtained by means of a Millipore water purification system with resistivity 18.2 MΩ cm^{-1} (Millipore RiOs-DITM134, Bedford, MA, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 98%), gallic acid (99%), and tert-butylhydroquinone (tBHQ, 99%) were obtained from Fluka (Buchs, Switzerland). Standards of lutein (⩾95%), zeaxanthin (⩾95%), and α-carotene (⩾95%) were obtained from Carotenature (Lupsingen, Switzerland). 2′-azobis (2-amidinopropane) dihydrochloride (AAPH, 97%), 2,4,6-tripiridyl-s-triazine (TPTZ, ⩾98%), Folin–Ciocalteau reagent, standards of quercetin (⩾95%), kaempferol (⩾97%), isorhamnetin (⩾95%), myricetin (⩾96%), apigenin (⩾95%), luteolin (⩾98%), hesperitin (⩾97%), chlorogenic acid (⩾95%), caffeic acid (⩾98%), β-cryptoxanthin (⩾97%), lycopene (⩾90%), and β-carotene (⩾95%) were obtained from Sigma–Aldrich® (St. Louis, MO, USA). Ferulic acid (⩾99%) and naringenin (98%) standards were purchased from Aldrich chemistry (Milwaukee, WI, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH, ⩾95%) radical was purchased from Wako (Wako company, Japan). All other chemicals and solvents used were of analytical and HPLC grade.

2.3. Nutrient determination

Nutrient analysis was conducted using standard AOAC methods (AOAC, 2005). All samples were analysed at the Institute of Nutrition laboratory, which conformed to ISO 17025:2005, the international standard for laboratory quality systems in terms of proximate compositions, minerals, and vitamins. The results of all measurements were presented as mean ± standard deviation in dry weight of the edible portion, except for moisture content.

2.3.1. Proximate composition

Total nitrogen followed AOAC method number 981.10 (AOAC, 2005) using the Kjeldahl method, and calculated into protein content using specific (Jones) factors, for instance, 6.25 for studied samples. Moisture content was determined by the drying method at 100 ± 2 °C until a constant weight, method No. 952.45 (AOAC, 2005). Crude fat content was analysed by acid digestion prior to continuous extraction using petroleum ether in Soxtec system, method No. 945.16 (AOAC, 2005). Ash content was determined by incinerating all organic matter at 550 ± 5 °C, method No. 945.46 (AOAC, 2005). Carbohydrate was calculated using the following formula: 100-moisture-protein-fat-ash; energy was calculated by Atwater factor (4 for protein and carbohydrate and 9 for total fat). Enzymatic gravimetric method was used for total dietary fibre analysis, method No. 991.43 (AOAC, 2005).

2.3.2. Minerals

Acid digestion in a closed Teflon vessel was employed for determination of magnesium, iron, copper, and zinc using an inductively coupled plasma optical emission spectrophotometer (ICP-OES), method No. 984.27 (AOAC, 2005). The acid solution, dissolved from ash residue after incinerated at 550 °C for 2 h, was analysed by flame atomic absorption spectrophotometer (AAS), method No. 975.03 (AOAC, 2005) for calcium, sodium, and potassium contents. The acid solution was also determined for phosphorus by the gravimetric method (AOAC, 2005).

2.3.3. Vitamins

Vitamins C and E were determined using the HPLC method (AOAC, 2005; Sanchez-Mata, Camara-Hurtado, Diez-Marques, & Torija-Isasa, 2000, method No. 992.03, respectively). For vitamin C, the sample was extracted in 3% metaphosphoric acid. The homogenate was filtered, and ascorbic acid separated by reversed-phase HPLC with UV detection at 248 nm and quantified against an external ascorbic acid standards. For vitamin E, the sample was saponified in ethanolic potassium hydroxide solution, extracted with hexane and tocopherol (vitamin E) was separated by reversed-phase HPLC with UV detection at 292 nm and quantified against an external tocopherol standards.

For carotenoid analysis, samples were saponified and extracted according to the procedures of Sungpuag, Tangchitpianvit, Chittchang, and Wasantwisut (1999) with slight modification. In brief, freeze dried samples (1–2 g) were saponified with 2 N potassium hydroxide (KOH) in the present of 10% ascorbic acid for 30 min. Thereafter, the samples were extracted with hexane and re-saponified with 5% KOH two times. The hexane phase was collected and washed with 10% sodium chloride. DI water was used for the final wash to obtain neutral pH. The extracts were evaporated at 40 °C. Dry samples were dissolved with mobile phase and filtered through a 0.2 μm PTFE syringe filter. Chromatographic separation of individual carotenoids was performed using reverse-phase column (0.5 μm Vydac 201TP54-C18 (4.6 × 250 mm, CA, USA)) with a photodiode array detector (Agilent Technologies, USA). A constant flow rate of mobile phase including acetronitrile:methanol:dichloromethane (80:11:9 v/v/v) containing 0.01% (v/v) triethylamine and 0.01% (w/v) ammonium acetate at 0.7 ml/min was maintained at ambient temperature. The chromatogram was monitored at 450 nm. Retention time and spectrum of unknown peaks were compared with the five authentic standards including lutein, zeaxanthin, β-carotene, α-carotene, β-cryptoxanthin and lycopene using ChemStation (Agilent Technologies, USA) (Judprasong, Charoenkiatkul, Thiyajai, & Sukprasansap, 2013).

2.3.4. Fatty acids

Fat and fatty acids were extracted using hydrolytic methods. Fat was extracted into petroleum ether, then methylated to fatty acid methyl esters (FAMEs) using boron trifluoride (BF_3) in methanol. Fatty acids were determined by capillary gas chromatography (GC) against C17:0 internal standard, method No. 996.06 (AOAC, 2005).

2.4. Bioactive compound analysis

2.4.1. Flavonoids and phenolic acids

Flavonoids and phenolic acids were determined based on Merken and Beecher (2000) with minor modification. A freeze-dried sample was hydrolysed with 62.5% methanol containing