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Nutrients and bioactive compounds of Thai indigenous fruits ☆

Kunchit Judprasong, Somsri Charoenkiatkul *, Parunya Thiyajai, Monruedee Sukprasansap

Institute of Nutrition, Mahidol University, Putthamonthon 4 Rd., Salaya, Phutthamonthon, Nakhon Pathom, Thailand

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ABSTRACT

This study determined the nutritional potential of Thai indigenous fruits in terms of nutrients, bioactive compounds, and antioxidant activities. Three indigenous fruits were collected at two conservation areas in Kanchanaburi province, Thailand. The results showed that *Phyllanthus emblica* L. exhibited the highest levels of vitamin C (575 ± 452 mg/100 g), total phenolics (TP) (3703 ± 1244 mGAE/100 g), and antioxidant activities, as measured by DPPH, FRAP and ORAC assays. Compared to the other two fruits, *Antidesma velutinosum* Blume contained higher levels of most nutrients and dietary fibre (15.6 ± 5.9 g/100 g), as well as carotenoids (335 ± 98 μg/100 g) and phytosterols (22.1 ± 3.9 mg/100 g). *Spondias pinnata* (L.f.) Kurz was high in total phenolics (3178 ± 887 mGAE/100 g) and antioxidant activity. Moreover, high correlations were found between TP and antioxidant activities ($r > 0.9$). These Thai indigenous fruits are potentially good sources of nutrients, bioactive compounds, and antioxidant activities. Conservation and utilisation should be promoted for food security and consumption as part of a healthy diet.

1. Introduction

Thailand is located in a tropical rainforest zone and is home to a great diversity of plants that grow naturally without the use of chemical substances. Traditionally, indigenous plants have been an important food source for local people living in and around forested areas. Research into the health benefits of fruits and vegetables has grown rapidly, not only in terms of their being sources of food but also medicine (Lizcano, Bakkali, Ruiz-Larrea, & Ruiz-Sanz, 2010). Furthermore, a healthy diet is rich in vegetables and fruits that are sources of dietary fibre, minerals, vitamins, and bioactive compounds, which are major sources of antioxidants. Epidemiological studies have shown that high intake of fruits and vegetables can prevent non-communicable diseases, such as atherosclerosis, diabetes, and cancers (Hung et al., 2004). Well-known antioxidants in plants are vitamins A, C, and E; carotenoids; phytosterols; as well as phenolic compounds (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).

Considering the biodiversity of Thailand's tropical forests, very little information exists on the health and nutritional benefits of many indigenous plants. Consequently, a survey of edible indigenous fruits at two conservation areas in Kanchanaburi province, Thailand, was carried out under the umbrella of the Plant Genetic Conservation Project, initiated by Her Royal Highness Princess Maha Chakri Sirindhorn in 2009. The survey's objective was to determine the nutritional potential of Thai indigenous fruits in terms of nutrients, bioactive compounds, as well as antioxidant activities.

2. Materials and methods

2.1. Food sampling and samples preparation

The survey was conducted in different locations and at different times in two conservation areas in Kanchanaburi province, namely, Srinakarind dam and Wang-khamen Mountain, Thailand, during January 2009 to March 2011. Three highly available, indigenous fruits were collected for study, including the whole fruits without seeds of *Phyllanthus emblica* L. or *Emblica officinalis* (Amla; Indian gooseberry; Thai name, Ma-Kham-Pom) ($n = 5$), the whole fruits of *Antidesma velutinosum* Blume (Thai name, Ma-Mao) ($n = 6$), and peeled fruits without seeds of *Spondias pinnata* (L.f.) Kurz (Hog plum; Thai name, Ma-Kok-Pa) ($n = 3$). The samples were kept in dark plastic bags and then transported via an ice-box to a laboratory at the Institute of Nutrition, Mahidol University. The fruits were then washed with tap water, followed by washing twice with deionized water. The edible part was homogenised using a food processor (Mara®, Thailand) and divided into two portions. The first portion was immediately analysed for moisture and vitamin C before being stored in an acid-washed screw-capped plastic bottle at −20 °C and reserved for other nutrient analyses. The second portion was freeze-dried, ground, vacuum-packed in laminated

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* Corresponding author. Tel.: +66 2800 2380x320; fax: +66 2441 9344.
E-mail address: somsri.chr@mahidol.ac.th (S. Charoenkiatkul).

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aluminium foil bags, and stored at −20 °C for determination of bioactive compounds and antioxidant activities.

2.2. Reagents

Deionized water (resistivity 18.2 MΩ cm^{-1}) was obtained by means of a Millipore water purification system (Millipore RiOs-DITM134, Bedford, MA, USA). Standards of lutein, zeaxanthin, and α-carotene were purchased from Carotenature (Lupsingen, Switzerland). 2′-Azobis (2-amidinopropane) dihydrochloride, 2,4,6-tripiridyl-s-triazine (TPTZ), Folin–Ciocalteu reagent, standards of quercetin, kaempferol, isorhamnetin, myricetin, apigenin, luteolin, hesperitin, chlorogenic acid, caffeic acid, 5-α cholestane, campasterol, β-sitosterol, stigmasterol, cholesterol, β-cryptoxanthin, lycopene, and β-carotene were obtained from Sigma (St. Louis, MO, USA). Ferulic acid and naringenin standards were purchased from Aldrich chemistry (Milwaukee, WI, USA). Trolox (6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, tertiary butylhydroquinone (tBHQ), trimethylchorosilane, and hexamethyldisilazane were obtained from Fluka (Buchs, Switzerland). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) 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 has been accredited by and complies with ISO 17025:2005 for proximate compositions, minerals, and vitamins. All values are presented per 100 g fresh sample.

2.3.1. Proximate composition

Total nitrogen was determined using the Kjeldahl method, method no. 981.10 (AOAC, 2005) and calculated into protein content ($N \times 6.25$). Moisture content was determined by drying the sample in a hot air oven at 100 °C until a constant weight was obtained, method no. 952.45 (AOAC, 2005). Crude fat content was determined by acid digestion prior to continuous extraction using petroleum ether in Soxtec system, method no. 945.16 (AOAC, 2005). Ash content was analysed by incinerating all organic matter at 550 ± 5 °C, method no. 945.46 (AOAC, 2005). Carbohydrate per 100 g 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). Total dietary fibre was analysed using the enzymatic gravimetric method, method no. 991.43 (AOAC, 2005).

2.3.2. Minerals and vitamins

Microwave digestion was used for sample decomposition to determine 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 was used for calcium, sodium, and potassium analyses by flame atomic absorption spectrophotometer (AAS), method no. 975.03 (AOAC, 2005). Phosphorus was determined by the gravimetric method (AOAC, 2005). Vitamins C and E were determined using the HPLC method (Sanchez-Mata, Camara-Hurtado, Diez-Marques, & Torija-Isasa, 2000; AOAC, 2005, method no. 992.03, respectively).

2.4. Bioactive compound determination

2.4.1. Carotenoids

Samples were saponified and extracted according to the procedures of Sungpuang, Tangchitpianvit, Chittchang, and Wasantwisut (1999) with slight modification. In brief, freeze-dried samples were boiled with ethanolic potassium hydroxide with the addition of 10% (w/v) ascorbic acid, before being extracted with hexane and then evaporated to dryness. The residue was reconstituted with mobile phase and then filtered through a 0.2 μm PTFE syringe filter. Chromatographic separation of individual carotenoids was modified slightly from the method described by O'Connell, Ryan, and O'Brien (2007). It was carried out using an isocratic reverse-phase column, 0.5 μm Vydac 201TP54-C18 (4.6 × 250 mm, CA, USA) with a photodiode array detector (Agilent Technologies, USA). Mobile phase comprising acetronitrile:methanol:dichloromethane (80:11:9 v/v/v) containing 0.01% (v/v) triethylamine and 0.01% (w/v) ammonium acetate was used at a constant flow rate at 0.7 ml/min at ambient temperature. Qualitative and quantitative evaluation of chromatograms was monitored at 450 nm using ChemStation (Agilent Technologies, USA). Individual carotenoids (lutein, zeathantin, β-carotene, α-carotene, β-cryptoxanthin, and lycopene) were identified based on comparing retention time and the UV spectrum of unknown peaks to the authentic standards. Carotenoid contents were expressed as μg/100 g sample.

2.4.2. Phytosterols

Phytosterols were determined according to Dhanasattakorn (2002) with slight modification. A freeze-dried sample was digested with methanolic hydrochloric acid and then saponified with sodium hydroxide. Phytosterols were extracted with petroleum ether, dried, and dissolved using N,N′-dimethylformamide. The internal standard, 5α-cholestane, was added in the solution before derivatised with trimethylchorosilane and hexamethyldisilazane. The solution was injected into an Agilent™ 6890N GC equipped with an FID (Agilent Technologies, USA) and an HP-5 column (J&W, Scientific, USA). Phytosterols were identified by comparing the retention time (relative to 5α-cholestane) of samples with that of authentic standards (campesterol, β-sitosterol and stigmasterol) using ChemStation (Agilent Technologies, USA).

2.4.3. 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 0.5 g/L tBHQ and 6 N hydrochloric acid at 80 °C for 2 h. After cooling, 1% ascorbic acid was added. The mixture was transferred and the volume made up with methanol, then filtered through a 0.2 μm PTFE syringe filter. The HPLC analysis was performed using Agilent 1100 series (Agilent Technologies, USA) with a photodiode array detector and 5 μm Zorbax Eclipase XDB-C18 column (4.6 mm × 150 mm, Agilent Technologies, USA). The gradient mobile phase composed of HPLC water containing 0.05% (w/w) trifluoroacetic acid (TFA), methanol containing 0.05% (w/w) TFA, and acetronitrile containing 0.05% (w/w) TFA was used at a constant flow rate of 0.6 ml/min. Chromatograms were monitored at 338 nm and evaluated using ChemStation (Agilent Technologies, USA). Flavonoids (quercetin, kaempferol, isorhamnetin, myricetin, apigenin, luteolin, naringenin, and hesperitin) and phenolic acids (ferulic acid, chlorgenic acid, and caffeic acid) were identified by comparing the retention time and the UV spectrum of unknown peaks to the authentic standards. Flavonoid and phenolic acid contents were expressed as μg/100 g sample.

2.4.4. Total phenolics

Total phenolics content was determined by spectrophotometry using Folin–Ciocalteau reagent method (Lu et al., 2007) with slight modification. A freeze-dried sample was extracted with 70% ethanol and then reacted with a 10-fold diluted Folin–Ciocalteau reagent. Sodium carbonate at a concentration of 7.5% (w/v) was added, and the final volume was made up with deionized water.