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Nutrients value and antioxidant content of indigenous vegetables from Southern Thailand

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ABSTRACT

Evidence from epidemiological studies has strongly suggested that diets rich in fruits and vegetables play a vital role in disease prevention. The aim of this study was to determine nutrient and antioxidant content for 15 varieties of indigenous vegetables and fruits collected from Southern Thailand. The data indicated that indigenous vegetables provided small to moderate amounts of macronutrients and minerals. The highest content of β-carotene was found in Indian lettuce (*Lactuca indica*; 3575.54 μg/100 g), whereas water dropwort (*Oenanthe javanica*; 7439.11 μg/100 g) had the highest lutein content. Ripe cashew apple (*Anacardium occidentale*; 178.34 mg/100 g) and Spanish joint fir (*Gnetum gnemon*; 109.43 mg/100 g) were excellent sources of vitamin C. Mon-pu (*Glochidion perakense*) and young cashew leaves (*Anacardium occidentale*) were rich sources of β-carotene, lutein, total polyphenol, especially gallic acid, and had relatively high ORAC and FRAP activities. In conclusion, Thai indigenous vegetables provide diverse natural bioactive compounds that may contribute health benefits to the consumer.

1. Introduction

Currently, much interest has focused on the vital role of many plants that are considered to be able to prevent chronic diseases, especially plants that are rich in natural antioxidants. Epidemiological data have suggested that consuming diets rich in fruits and vegetables can reduce the risk of various chronic diseases in humans (Dauchet, Amouye, Hercberg, & Dallongeville, 2006). This effect has been proposed to be due to their content of diverse bioactive compounds that possess antioxidant activities and includes tocopherols, ascorbic acid, carotenoids, polyphenols, phenolics and anthocyanins which contribute to the functionality of fruits and vegetables in health promotion and prevention of chronic diseases (Nugroho, Malik, & Pramono, 2013; Andarwulan et al., 2012).

Southern Thailand is located in tropical zone that is characterised by high humidity and rain throughout the year. Therefore, this region has a diverse abundance of indigenous vegetables that are important in local diets and have been used as a source of traditional medicines for the treatment of arthritis, diabetes, constipation and hypertension (Nugroho, Malik, & Pramono, 2013; Andarwulan et al., 2012). Indeed, Thai indigenous vegetables have been reported to be good sources of antioxidants, and many also contain with anti-bacterial, anti-inflammatory, anti-mutagenic, and anti-carcinogenic compounds (Povichit, Phrutivorapongkul, Suttajit, Chaiyasut, & Leelapornpisid, 2010). Therefore, the promotion of higher consumption of these indigenous vegetables among people may represent a natural and sustainable alternative for improving the health of individuals residing in the region. However, less information about the nutritive values, antioxidant content, and activity of indigenous vegetables in Thailand is limited. The primary aim of this study was to determine the nutritive content, antioxidant content, and antioxidant activity of 15 varieties of indigenous vegetables and fruits from Southern Thailand. It would be anticipated that results of nutritive and antioxidant contents and activity from indigenous plants investigated will serve as a database for the Thai food composition tables and for the continued development food based dietary guidelines for promoting consumption of vegetables for the Thai population.

2. Materials and methods

2.1. Sample collection

Southern Thailand comprises 14 provinces, namely Chumphon, Surat Thani, Ranong, Nakhon Si Thummarat, Phatthalung, Trang,

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Satun, Krabi, Phang Nga, Phuket, Songkhla, Pattani, Yala and Narathiwat. Thirteen varieties of the commonly consumed fresh indigenous vegetables and two varieties of fruits were collected from six of the provinces where these foods are available. Two to three kg of each food were purchased from each of three representative markets in each province. Samples were transported on ice in a cool box to Food Chemistry Laboratory, Institute of Nutrition, Mahidol University, Nakhon Pathom province within 24 h. Information on the individual vegetables and fruits, photographs, and their provinces of origin is presented in Table 1.

2.2. Sample preparation

Upon arrival three sets of the same variety of a vegetable (or fruit), collected from three markets in a particular province, were individually washed with water to remove dirt and contaminants, and rinsed with deionized water. The edible portion of each set was prepared and separately homogenised (Ace homogenizer, NISSEI Ltd., Tokyo, Japan) in a dark room at 25 °C. Approximately 500 g of each of homogenised samples were pooled and re-homogenised to obtain a single, uniform composite sample. Samples from the other five provinces were treated similarly. Thus a total of six composite samples were obtained for each vegetable or fruit.

The same protocol was followed with all the other collected vegetables and fruits. Moisture and vitamin C contents were determined in duplicate immediately after samples were homogenised and pooled. The homogenised samples were next divided into two portions and stored in acid-washed polythylene bottles at −20 °C until analysis. One bottle was used to determine the amounts of specific antioxidants (total polyphenols, vitamin E, β-carotene, lutein, cyanidin, peonidin) and the antioxidant activity by ORAC and FRAP assays. The second bottle was used for proximate and mineral compositions.

2.3. Determination of proximate analysis (macronutrient) and mineral content

To determine moisture content, each sample was dried in a hot air oven at 100 ± 5 °C to a constant weight according to AOAC method 950.46 (Latimer, 2012). Ash was determined by incineration at 550 °C, according to the method of AOAC 930.30 (Latimer, 2012). Protein content was analysed by the Kjeldahl method according to AOAC method, 991.20 (Latimer, 2012). The amount of protein contained in the indigenous vegetables was calculated from total nitrogen multiplied by 6.25. Crude fats were extracted in Soxtec Avanti 2055 (Foss, Denmark) with petroleum ether at 40–60 °C according to AOAC method 948.15 (Latimer, 2012). Total carbohydrates were calculated by subtracting the sum of the percentage moisture, total fat, protein and ash from 100%. For mineral composition, samples were digested by a wet ashing according to the AOAC method 984.15 (Latimer, 2012) and analysed by inductively coupled plasma-optical emission spectrometer (ICP-OES, PerkinElmer Optima 4200 DV, Waltham, MA, USA).

2.4. Determination of total polyphenol content

Total phenolic concentration (TPC) was determined using the Folin–Ciocalteau method (Brune, Hallberg, & Skanberg, 1991). Briefly, 2 g of homogenised samples were extracted by constant shaking at room temperature with 20 ml of 50% dimethyformamide (Sigma–Aldrich, St. Louis, MO, USA) in 0.1 M acetate buffer for 16 h. After extraction, samples were centrifuged (20 min, 25 °C, 3000 rpm) and supernatants were collected. The supernatants were diluted to appropriate volume with water. The 25 μl of diluted samples were added to 96-well plate, followed by 125 μl of 10% Folin–Ciocalteau (Merck, Darmstadt, Germany) and 100 μl 0.5 M sodium hydroxide to each well and mixed. Absorbance was measured at 750 nm using automated microplate reader (Sunrise™-Tecan, Victory, Australia) after standing for 15 min at 25 °C. Gallic acid (Sigma–Aldrich, St. Louis, MO, USA) was used as the standard with concentrations ranging from 0.00 to 80.00 ppm. Data were expressed as in milligram gallic acid (GAE) equivalents per 100 g of fresh weight (mg GAE/100 g).

2.5. Determination of vitamin C content

Vitamin C content was analysed using HPLC according to a modification of the procedure of Brause, Woollard, and Indyk (2003). Briefly, 3–10 g of homogenised sample was weighed into a 50 ml volumetric flask. Then, 3 ml of 10% metaphosphoric acid (MPA; Merck Darmstadt, Germany) was added and diluted with deionized water to volume and the solution was filtered (Whatman #1, Whatman International Ltd., Maidstone, England). pH of filtrate was adjusted to 5.0–5.25 with 4 M sodium hydroxide or 10% MPA (w/v) prior to addition of 10 mg dithiothreitol (DTT, Sigma–Aldrich, St. Louis, MO, USA). The solution was mixed and incubated in the dark at room temperature (25 °C) for 1 h. The mixture was filtered (Whatman #42, Whatman International Ltd., Maidstone, England) and re-filtered through a 0.45 μm membrane filter (Chrom Tech®, Milford, MA, USA) prior to injection for HPLC analysis. Vitamin C determination was performed using an HPLC system equipped with a Waters 515 pump (Waters Corporation, Milford, MA, USA) and Jasco UV 975 detector (Jasco International, Co., Ltd, Tokyo, Japan). Vitamin C was separated using a Zorbax 5 μm ODS column (250 × 4.6 mm) with an analytical guard column C-130B (2 × 20 mm) (Upchurch Scientific, USA). The mobile phase was 0.5% KH_2PO_4 at a flow rate of 0.8 ml/min. Vitamin C was monitored at 254 nm. The results were expressed as millgrams of ascorbic acid per 100 g fresh weight (mgAA/100 g).

2.6. Determination of vitamin E, β-carotene, and lutein contents

Samples were extracted according to the the method of Speek et al. (1985) in the dark to protect vitamin E, β-carotene, and lutein from degradation. In brief, samples were first saponified by placing a 3.0–5.0 g homogenised sample into a brown round-bottom flask. This was followed by addition of 10 ml freshly prepared aqueous solution of 10% ascorbic acid (Ajax Finechem, Victoria, Australia) and 50 ml 2 M ethanolic potassium hydroxide (KOH; Merck, Darmstadt, Germany). This solution was refluxed in a boiling water bath for 30 min and cooled to room temperature. After adding 70 ml of hexane (J.T. Baker, PA, USA), the samples were mixed by continuous shaking for 2 min. After separation of the two layers, the upper layer was transferred to a brown-glass separatory funnel containing 50 ml 5% (w/v) KOH solution. The samples were extracted twice with 35 ml of hexane. The combined hexane extract was washed with 100 ml of 10% (w/v) sodium chloride (Ajax Finechem, Victoria, Australia) and with a consecutive 100 ml aliquots of water until alkali-free. An aliquot was collected and evaporated in a rotary evaporator (Buchi, Flawil, Switzerland) under vacuum in a 37 °C water bath. Residue was dissolved in 1 ml of chloroform (RCI Labscan, Bangkok, Thailand) and 1 ml of methanol (J.T. Baker, PA, USA).

Analysis of vitamin E was performed using an HPLC system equipped with a Waters 515 pump (Waters Corporation, Milford, MA, USA) and Jasco UV 975 detector (Jasco International, Co., Ltd, Tokyo, Japan). Vitamin E was analysed using a C18 column (Water resolve C18 3.9 × 150 mm, Waters Corporation, Milford, MA, USA) and a mobile phase of methanol at a flow rate 0.6 ml/min and monitored at 295 nm. The results were expressed as milligrams/100 g of fresh sample (mg/100 g).