Application of a Validated Method for the Determination of Provitamin A Carotenoids in Indonesian Foods of Different Maturity and Origin

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Provitamin A carotenoids were measured using a validated high-performance liquid chromatography method in a number of selected leafy vegetables of different maturity and origin, sweet potatoes, and mangos from Indonesia. Mean provitamin A content per 100 g of edible portion was 492 retinol equivalents (RE) for water spinach (*Ipomoea aquatica*; $n = 8$), 640 RE for spinach (*Amaranthus viridis*; $n = 8$), 1776 RE for cassava leaves (*Manihot utulissima*; $n = 8$), 992 RE for papaya leaves (*Carica papaya*; $n = 9$), 1889 RE for sweet shoot leaves (*Sauropus androgynus*; $n = 4$), 289 RE for jointfir spinach (*Gnetum gemon*; $n = 2$), 6 RE for sweet potato (*Ipomoea batatas*; $n = 22$), and 250 RE for mango (*Mangifera indica*; $n = 11$). Provitamin A content was significantly higher in dark green (mature) leaves (24% on average) than in young leaves. Leafy vegetables collected on the market had significantly lower levels of provitamin A compared to those of field samples (19% on average). Provitamin A content for most, but not all, vegetables was lower than values reported in Indonesian food tables.

Keywords: *Carotenoids; vegetables; maturity; Indonesia; provitamin A; validation; high-performance liquid chromatography*

INTRODUCTION

Vitamin A deficiency presents a serious public health problem in many parts of the world, South East Asia (including Indonesia) being the most afflicted area (WHO, 1991). It is estimated that, in developing countries, 70−90% of the vitamin A intake is derived from plant foods, especially vegetables and fruits (FAO/WHO, 1988). This indicates the need for provitamin A data of plant foods produced by validated methods that can separate individual carotenoids.

Many of the data on the provitamin A content of foods incorporated in food tables have been obtained by methods which do not exclude other carotenoids (Simpson, 1983; Beecher and Kachik, 1984; West and Poortvliet, 1993) or limit proper evaluation of the data presented due to lack of information of the methods used (Mangels et al., 1993; West and Poortvliet, 1993). Such methods include extinction measurements of plant extracts, conventional open column chromatography (AOAC, 1990, which actually measures total carotenes, except lycopene), and high-performance liquid chromatography (HPLC) methods which have been inadequately evaluated (Simpson, 1983; Rodriguez-Amaya et al., 1988; West and Poortvliet, 1993).

Apart from analytical methodology, a number of factors, including genetic variety, light duration and intensity, temperature, maturity, season, postharvest storage, fertilizers, and diurnal fluctuations determine β-carotene content in plant foods. For instance the β-carotene content increases during ripening of fruits or growth of carrots (Mozafar, 1994). For leafy vegetables, reported effects of maturity on provitamin A content are limited and less conclusive. Ramos and Rodriquez-Amaya (1987) found an increase in provitamin A content in lettuce and endive during maturation. Sweeney and Marsh (1971a) found that the β-carotene content of spinach decreased with increasing maturity of the vegetable.

Several methods for carotenoid measurement have been published over the past decade, which all differ in isolation techniques and HPLC systems. Recently these methods have been reviewed extensively (Packer, 1992; Schiedt and Liaanen-Jensen, 1995). Several studies have focused on the distribution and identification of carotenoids in the elution profile using off-line spectroscopic techniques such as NMR and mass spectroscopy (Khachik et al., 1986, 1992). More recently the use of on-line UV−vis photodiode-array detection, enabling the production of three-dimensional spectra for individual carotenoids within 1 s, contributed much to a rapid identification of eluted carotenoids and thus to the potential of method validation (De Leenheer and Nelis, 1992; Ben-Amotz, 1995). The concept of validation in quantitative measurements of carotenoids in food however involves an adequate description of all stages of the analytical process and testing of performance, including between-laboratory measurements (Greenfield and Southgate, 1992). Here we describe a method that has been tested thoroughly for extraction efficiency, recovery, and long-term repeatability and which has been shown to produce valid results for lutein, zeaxanthin, α-carotene, β-carotene, and lycopene, in an interlaboratory certification study in the European Union

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Standards, Measurement and Testing program (Scott et al., 1996). The method has been used for the determination of the provitamin A content in a number of frequently consumed leafy vegetables and fruits of different maturity and different origin from Indonesia.

MATERIALS AND METHODS

Chemicals and Standards. The source of chemicals was as follows: tetrahydrofuran (THF), ethanol, anhydrous sodium sulfate, and calcium carbonate from Merck (Darmstadt, Germany); methanol from Labscan Ltd (Stillorgan Industrial Park Co., Dublin, Ireland); hexane from Rathburn Chemicals Limited (Walkerburn, Scotland); butylated hydroxytoluene (BHT) from Sigma Chemical Co. (St Louis, MO 63178). Tetrahydrofuran, methanol, and hexane were HPLC grade; α-carotene, β-carotene, lycopene, and lutein standards were purchased from Sigma Chemical Co.; zeaxanthin, 15,15′-*cis*-β-carotene and β-cryptoxanthin standards were a gift from Hoffmann La Roche Ltd (Basel, Switzerland).

Standard solutions of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, *cis*-β-carotene, and lycopene were prepared from ampuled stock standards in THF (containing BHT, 0.01% w/v). The carotenoid concentrations of the standards were measured in a Zeiss M4 QIII spectrophotometer (Carl Zeiss, Oberkochen/Württemberg, Germany), using extinction coefficients, $E^{1\%}_{1\ cm}$ of 2550 ($\lambda_{max} = 445$ nm) for lutein and 2540 (450 nm) for zeaxanthin in ethanol. The extinction coefficients used for β-cryptoxanthin, *all-trans*-α carotene, *all-trans*-β-carotene, and lycopene in hexane were 2386 (452 nm), 2800 (444 nm), 2592 (453 nm), and 3450 (472 nm), respectively (De Ritter and Purcell, 1981). To obtain complete solubilization, an ultrasound bath (Eurosonic 22, Wilten Woltil, De Meern) was used for 10 min. The concentrations of the individual carotenoids were determined by correction with the purity of each compound, measured by HPLC. Calibration standards were shown to be stable for 6 weeks at −20 °C in the dark.

HPLC Instrumentation. The HPLC system (Spectra System) was equipped with two pumps (P4000), a solvent degasser (SCM400), a temperature-controlled autosampler (AS3000), a UV−visible forward optical scanning detector (Spectra Focus SM5000A), interface (SN4000), and control and integration software (PC1000, version 2.5), all manufactured by Thermo Separation Products Inc. (355 River Oaks Parkway, San Jose, CA 95134). A reversed phase Vydac 218 TP 54 column (250 × 4.6 mm i.d.) from The Separations Group (Hesperia, CA, 92345) was used, containing silica polymerically modified with C18 (300 Å pore diameter, 5 μm particle size). The metal frits (cylindrical metal "sieves" fitted at the entry and outlet of the column, holding the column packing but allowing a free flow of mobile phase) in the column were replaced by PAT (Peek Alloyed with Teflon) frits from the same manufacturer, to minimize carotenoid degradation on the column, which was shown to occur especially for lycopene when the original column, fitted with metal frits was used. A guard column (10 × 4.6 mm i.d.), packed with material similar to that in the analytical column, was attached to the inlet of the analytical column.

The optimized mobile phase consisted of a mixture of methanol and THF (98:2 v/v), isocratically pumped with a flow rate of 1.0 mL/min. The temperature of the autosampler was kept at 4 °C, while the column temperature was maintained at 20 °C using a waterjacket around the column. This mobile phase composition and column gave both an acceptable retention time and an acceptable resolution for critical carotenoid pairs (α-carotene/β-carotene and lutein/zeaxanthin). The selection of column and mobile phase (methanol with THF as modifier) was based on an evaluation of the performance of 65 reversed phase columns and different mobile phase systems by Epler et al. (1992). This study revealed that methanol-based mobile phases generally showed higher recoveries than acetonitrile-based mobile phases, while polymeric columns were shown to have better selectivity for structurally similar carotenoids.

Samples. Edible portions of water spinach (*Ipomoea aquatica*), spinach (*Amaranthus viridis*), cassava leaves (*Manihot utulissima*), papaya leaves (*Carica papaya*), sweet shoot leaves (*Sauropus androgynus*), jointfir spinach (*Gnetum gemon*), sweet potatoes (*Ipomoea batatas*), and mangos (*Mangifera indica*) were purchased in August 1994 from different markets and fields (Parung and Anyar) in the Bogor district of West Java, Indonesia. Young and mature leaves were included for each type of vegetable and sampling location. Maturity of the leaves was determined by visual inspection of the color (dark green, mature; light green, young), by stiffness and by position of leaves along the stem. After collection, vegetables were transported in a coolbox containing ice to the Nutrition Research & Development Centre in Bogor. An amount of 100 g of the raw edible portion of the leafy vegetable was packed into plastic sample bags, frozen at −20 °C, and shipped on dry ice to Wageningen for analysis. Since it was planned to carry out an intervention trial in order to evaluate the effect of intake of green leafy vegetables on vitamin A status, it was decided to make an estimation of the effect of traditional cooking on carotene content. Two water spinach samples were stir-fried according to local custom by heating 100 g of the vegetable with 200 mL of water and 20 mL of oil for 4 min. The drained samples were packed and treated as mentioned before. Sweet potatoes and mangos were sent directly to the laboratory without any prehandling or special cooling.

Sample Extraction. Green leafy vegetables were homogenized in frozen condition. Sweet potatoes and mangos were peeled, and the seed in each mango was removed prior to homogenization. Homogenization was carried out using a mixer (model B-400, Büchi Laboratoriumtechnik AG, Flawil, Switzerland). Two grams of the homogenized sample was extracted in duplicate with Na_2SO_4 (4.0 g), $CaCO_3$ (0.5 g), and tetrahydrofuran containing 0.01% BHT (30 mL) in a 100 mL measuring cylinder, using a rod mixer (Polytron PT 20 OD, Kriens/Luzern, Switzerland) at moderate speed (speed 4) for 1 min. The extract was filtered through a glass funnel fitted with Whatman paper no. 54 (diameter 11 cm). The residue was re-extracted until the extract was colorless (usually two additional extractions with 30 mL of THF were sufficient). Completeness of extraction using this procedure was shown for lutein, α-carotene, and β-carotene by varying the sample amount (1, 2, 4, and 8 g), while other extraction conditions, except the amount of Na_2SO_4, were kept constant. Linearity for the extraction yield for lutein, α-carotene, and β-carotene was shown by Pearson's correlation coefficients which were all >0.99. The volume of the combined filtrates was reduced to near dryness at 35 °C under nitrogen in a rotary evaporator (Büchi, Flawil, Switzerland). The concentrated filtrate was transferred to a 25 mL volumetric flask and brought to volume with THF/methanol (1:1 v/v). The sample solution was filtered through an HPLC filter (Acrodisc, diameter 3 mm, 0.45 μm pore size), and 10 μL of the filtered solution was injected into the HPLC system. All sample preparations and extractions were done in duplicate under subdued yellow light (wavelength band 480−700 nm). Extractions were carried out in a fume hood. Moisture was determined in all samples according to Osborne and Voogt (1978) using a vacuum oven (Salvis AG, Reussbühl, Luzern, Switzerland), to improve comparison between young versus mature and field versus market samples.

Identification and Quantification. Carotenoids were quantified in the samples by comparing with external standards: six calibration levels were injected before and after each sample run. Samples were injected in duplicate, and elution of carotenoids was monitored at 450 nm. A solvent blank was included in each run. Carotenoids were identified by comparing their retention times with those of the standards and further by spectral analysis for peak purity measurement. The concentration of each carotenoid was determined from the average of the two calibration plots, in which the peak areas were plotted against the concentration of each compound. The detector responses were linear over the concentration range: Pearson's correlation coefficients were > 0.99 for all standards; the relative standard deviation of the regressions were <5%. 15,15′-*cis*-β-Carotene was not included in the standard mixture for quantification, but only for identification purposes, since

Table 1. Recovery and Repeatability of α-Carotene and β-Carotene Analysis Using Baby Food as a Quality Control Sample

compound	recovery[a] (%)	CV% within[b]	CV% between[b]
all-trans-α-carotene	94.8 ± 2.0	6.0	6.3
all-trans-β-carotene	97.4 ± 1.9	7.4	5.5

[a] Based on three separate measurements. [b] Calculated from 32 duplicate runs over a 1 year period.

extinction coefficients for this compound are not available. *cis*-β-Carotene was quantified from the standard line for *all-trans*-β-carotene, assuming the same detector response for both carotenoids. The quantitation limit was set at 2.5 times the minimal detectable level. The latter was defined as the amount of lutein, α-carotene, and β-carotene resulting in a peak-height three times the baseline noise. Provitamin A activity in the samples was expressed as retinol equivalents and calculated as follows (tagnames in square brackets, Klensin et al., 1989): (1/6)*all-trans*-β-carotene [<CARTB>] + (1/12)α-carotene [<CARTA>] + (1/12)*cis*-β-carotene (FAO/WHO, 1967).

Repeatability and Recovery. In each sample run, a control sample (homogenized baby food of the same lot obtained from Nutricia BV (Zoetermeer, The Netherlands) comprising 42% carrots (*Daucus carota*), 30% peas (*Pisum sativum*), 21% low fat milk, and 1% parsley (*Petroselinum crispum*)) was extracted in duplicate and injected into the HPLC system for monitoring the stability of the analytical procedure over time. For each sample run a new jar of baby food was opened. Prior to the sample runs, between-jar homogeneity was tested by analyzing the β-carotene content of six randomly chosen jars in one run. The result was evaluated against the within-run variability of the β-carotene content of 12 subsamples from the same jar of baby food. The amount of β-carotene in the control food was targeted within a 3 week period of stable analytical performance: 12 determinations in duplicate were carried out on 12 separate days, during which recovery of the analytical procedure was tested. Within-run and between-run coefficients of variation were calculated as a measure of repeatability of the analysis. Control samples were stored in the dark at +4 °C.

Statistical Analysis. Descriptive statistics (mean, standard deviation, and 95% confidence interval) of the provitamin A content were calculated, and differences between location groups and maturity groups were tested using analysis of variance. Stability of the control sample during 1 year of storage was evaluated by linear regression analysis.

RESULTS AND DISCUSSION

Recovery, Repeatability, and Stability of Control Sample. Table 1 shows the results of the recovery and repeatability experiments. As is shown by the high recovery and low variation, the method gave reliable results. In a recently performed certification study in the Standards, Measurement and Testing program of the European Union, the mean within-lab variations (combined within- and between-run variation) after exclusion of outliers, were 5.2% and 6.7% for *all-trans*-α-carotene and *all-trans*-β-carotene, respectively (Scott et al., 1996). These variations were calculated from results from two consecutive days. Our within-lab variation was about 50% higher, 8.7% and 9.2% for *all-trans*-α-carotene and *all-trans*-β-carotene, respectively, but calculated over a period of 1 year. The control sample used in our study was found to be stable for at least 1 year under the storage conditions used, making its use suitable for monitoring long-term analytical performance and hence monitoring long-term variations in β-carotene content of plant foods. The regression equation for the β-carotene content as a function of time during storage was as follows: β-carotene (μg/100 g) = 3737 − 0.26(time) [time in days], the 95% confidence interval for the time coefficient being [−1.1, +0.5].

Provitamin A and β-Carotene Isomers in Plant Foods. Provitamin A content was found to be highest in cassava leaves (1776 RE/100 g) and sweet shoot leaves (1889 RE/100 g) (Table 2). Both types of sweet potatoes, described as Ubi Putih (11 RE/100 g) and Ubi Ungi (<1 RE/100 g), were shown to be poor sources of provitamin A. Considerable differences in provitamin A content were found in the mango types. Provitamin A content ranged from 32 RE/100 g in the Manalagi type to 550 RE/100 g in the Gedong type. Although for most types only two mangos were investigated, the variations found are indicative for the importance of cultivar differences in β-carotene content. Of the factors known that effect variability of β-carotene content in plants, genetic variability is next to analytical methodology probably the most important (Mozafar, 1994).

The only provitamin A compounds detected were α-carotene, β-carotene, and *cis*-β-carotene. There was more *cis*-β-carotene present in the samples than α-carotene, with the exception of mango, sweet shoot leaves, and jointfir spinach (Table 2).

We observed for *all-trans*-β-carotene content in the sample a coefficient of variation of 16% for cooked water spinach to 42% for papaya leaves (calculated from Table 2). This variation is based on analysis of both young and mature leaves and sweet potatoes of different stages of ripeness. Mercadante et al. (1991) found a coefficient of variation for β-carotene of 10−20% for green leafy vegetables of the same maturity and the same variety. Ramos and Rodriquez-Amaya (1987) found, similar as in our study, a coefficient of variation for β-carotene ranging from 15 to 41% in Brazilian leafy vegetables of different maturity.

The amount of *cis*-β-carotene in our study ranged from 12 to 16% of the total β-carotene content in the uncooked leafy vegetables. As expected, the stir-fried water spinach sample showed an increased amount of *cis*-β-carotene (Sweeney and Marsh, 1971b). No *cis* isomers were detected in mangos. Sweeney and Marsh (1971b) found that in raw vegetables about 20% of total β-carotene was present as *cis* isomers. Chandler and Schwartz (1987) reported that in raw green vegetables 20−28% of total β-carotene was present as *cis* isomers. The presence of *cis*-β-carotene in raw green vegetables was also reported by other authors (Bushway, 1986; Khachik et al., 1986; Quackenbush, 1987; Khachik et al., 1992). It has been suggested that at least part of the *cis* isomers detected in green plants are artifacts formed during the analytical process (O'Neil and Schwartz, 1995). On the basis of the retention time of the standard and on the presence of an absorbance peak in the near UV at 340 nm, we tentatively identified the *cis* peak in the vegetables initially as 15,15′-*cis*-β-carotene. However the *Q* ratio (ratio of the absorbance at wavelength maximum (450 nm) to absorbance at 340 nm (Quackenbush, 1987)), of the 15,15′-*cis*-β-carotene standard in methanol/THF (98/2, v/v) was 1.1. For the control sample the *Q* ratio of the *cis* peak of β-carotene in this solvent mixture was 2.6, indicating the presence of several coeluting isomers under the *cis* peak (Quackenbush, 1987; Chandler and Schwartz, 1987). By using optimized separation techniques for *cis* isomers of β-carotene on lime or Vydac columns, several authors report that in vegetable extracts 9-*cis*, 13-*cis*, and 15-*cis* are the predominant β-carotene isomers. In addition

Table 2. Carotenoid and Provitamin A Content of Selected Indonesian Vegetables and Fruit

name			amount (μg/100 g of edible portion)			retinol equivalents (RE) per 100 g of edible portion[a]	
English	scientific	Indonesian	*all-trans*-α-carotene	*all-trans*-β-carotene	*cis*-β-carotene	fresh weight	dry weight[b]
water spinach, raw ($n = 8$[c])	*Ipomoea aquatica*	kangkung	14 ± 26[d]	2730 ± 1013	433 ± 217	492 ± 188	4560 ± 1908
water spinach, cooked ($n = 2$)	*Ipomoea aquatica*	kangkung	28 ± 40	3053 ± 481	1001 ± 177	595 ± 69	3002 ± 752
spinach, raw ($n = 8$)	*Amaranthus viridis*	bayam	69 ± 36	3527 ± 911	557 ± 172	640 ± 167	4551 ± 1201
cassava leaves, raw ($n = 8$)	*Manihot utulissima*	daun sinkong	38 ± 54	9912 ± 2503	1450 ± 422	1776 ± 447	7240 ± 953
papaya leaves, raw ($n = 9$)	*Carica papaya*	daun papaya	424 ± 355	5229 ± 2195	1023 ± 405	992 ± 422	5319 ± 1493
sweet shoot leaves, raw ($n = 4$)	*Sauropus androgynus*	daun katuk	1335 ± 878	10010 ± 2189	1312 ± 349	1889 ± 466	7910 ± 1042
jointfir spinach raw ($n = 2$)	*Gnetum gemon*	daun melinjo	1113 ± 410	1071 ± 376	208 ± 11	289 ± 98	1608 ± 416
sweet potato, raw ($n = 11$)	*Ipomoea batatas*	ubi putih	2 ± 3	58 ± 70	20 ± 28	11 ± 14	27 ± 34
sweet potato, raw ($n = 11$)	*Ipomoea batatas*	ubi ungi	nf[e]	5 ± 1	<1	<1	2 ± 1
mango ($n = 2$)	*Mangifera indica*	gedong	61 ± 86	3267 ± 2075	nf	550 ± 353	3397 ± 2394
mango ($n = 3$)	*Mangifera indica*	manalagi	nf	190 ± 123	nf	32 ± 21	174 ± 106
mango ($n = 2$)	*Mangifera indica*	indramayn	67 ± 5	1606 ± 166	nf	273 ± 27	1414 ± 121
mango ($n = 2$)	*Mangifera indica*	harum manis	55 ± 1	1080 ± 264	nf	185 ± 44	1361 ± 319
mango ($n = 2$)	*Mangifera indica*	golek	55 ± 3	1237 ± 626	nf	211 ± 105	1662 ± 949

[a] RE calculated as 1/6 *all-trans*-β-carotene + 1/12 *all-trans*-α-carotene + 1/12 *cis*-β-carotene [b] RE based on dry weight, calculated from the water content and retinolequivalents in the fresh sample [c] n = number of samples. [d] Mean ± standard deviation. [e] Not found.

Table 3. Provitamin A Content of Selected Raw Indonesian Vegetables According to Sampling Location and Maturity State

	provitamin A (RE/100 g of edible portion, based on dry weight)[a]					
	location[b]			maturity[c]		
	field	market		young	mature	
water spinach ($n = 8$)	5352 ± 2249(4)[d]	3768 ± 1328(4)	[−1613; 4781][e]	3249 ± 944(4)	5872 ± 1737(4)	[203; 5043][e]
spinach ($n = 8$)	4975 ± 720(4)	4126 ± 1539(4)	[−1230; 2928]	4116 ± 831(4)	4985 ± 1474(4)	[−1203; 2939]
cassava leaves ($n = 8$)	7498 ± 990(4)	6982 ± 981(4)	[−1189; 2221]	6503 ± 427(4)	7978 ± 697(4)	[475; 2475]
papaya leaves ($n = 9$)	6243 ± 1580(4)	4581 ± 1024(5)	[−388; 3712]	4387 ± 925(5)	6485 ± 1241(4)	[397; 3799]
sweet shoot leaves ($n = 4$)		7910 ± 1042(4)		8251 ± 804(2)	7569 ± 1464(2)	[−5764; 4399]

[a] Mean ± standard deviation: retinol equivalents (RE) calculated as (1/6)*all-trans*-β-carotene (μg) + (1/12)*all-trans*-α-carotene + (1/12)*cis*-β-carotene (μg). [b] Significantly different between field and market ($p = 0.017$). [c] Significantly different between young and mature ($p < 0.001$). [d] Number of samples in each location/maturity category. [e] 95% confidence interval for difference between field and market and between young and mature, respectively.

some di-*cis* isomers can be found (O'Neil et al., 1991; Khachik et al., 1992).

Provitamin A activity of *cis*-β-carotene is lower compared to the *all-trans* form. For 9-*cis*-β-carotene and 13-*cis*-β-carotene provitamin A activity was estimated to be 38% and 53%, respectively, of that of *all-trans*-β-carotene (Zechmeister, 1949). Although the provitamin A activity of *cis*-β-carotene in this study was calculated as 50% of *all-trans*-β-carotene, the impact of this assumption on total provitamin A content is small.

Provitamin A in Foods of Different Maturity and Origin. No significant differences in provitamin A content were found between vegetables sampled in different areas (Parung and Anyar) of West Java (data not shown).

Mature leafy vegetables tended to have higher levels of lutein, α-carotene, *all-trans*-β-carotene, *cis*-β-carotene, provitamin A, and total carotenoids (as measured by total absorption at 450 nm) compared to young leafy vegetables. On average, the provitamin A content in mature leaves was 24% higher than in young leaves. Table 3 shows the data for the provitamin A content and 95% confidence intervals for the difference between means for the separate vegetables. Market vegetables tended to have lower levels of lutein, α-carotene, *all-trans*-β-carotene, *cis*-β-carotene, provitamin A, and total carotenoids compared to those in field samples (Table 3). On average, the provitamin A content of market vegetables was 19% lower compared to leafy vegetables collected in the field. The decreased carotenoid content in market samples can be attributed to postharvest degradation of carotenoids due to photobleaching (Young and Britton, 1990).

Comparison with Provitamin A Content in Food Tables. A comparison of the provitamin A content of vegetables analyzed in our study with the reported values of these vegetables in Indonesian food tables shows a lower provitamin A content of water spinach, spinach, papaya leaves, and jointfir spinach in our study, but not for sweet shoot leaves and cassava leaves (Table 4). For sweet potatoes and mangos of the type investigated, no data are available in the Indonesian

Table 4. Provitamin A Content of Selected Vegetables and Fruit from Indonesia

	provitamin A (RE/100 g of edible portion)[a]	
name	our result	Indonesian food tables
water spinach	492 [335, 649][b]	945[c]
spinach	640 [500, 780]	914[c]
cassava leaves	1776 [1402, 2150]	1650[c]
papaya leaves	992 [668, 1316]	1369[d]
sweet shoot leaves	1889 [1147, 2631]	1555[c]
jointfir spinach	289 [0, 1167]	1500[c]

[a] Based on fresh weight: retinol equivalents (RE), calculated as (1/6)β-carotene (μg) + (1/12)α-carotene (μg) + (1/12)*cis*-β-carotene (μg). [b] Mean and 95% confidence interval. [c] Hardinsyah and Briawan, 1990. [d] Nio, 1992.

food composition tables. The data on provitamin A content in Indonesian food tables mentioned here are based on measurements of extinctions of plant extracts (Hardinsyah and Briawan, 1990). Although the lower values for provitamin A found in our study using HPLC could be expected from a methodological point of view, it is interesting to notice that the data for both sweet shoot leaves and cassava leaves were comparable to the data in the Indonesian food table. This stresses the importance of factors other than analytical methodology in determining levels of carotene in vegetables. Studies in Indonesia showed a marginal vitamin A status in pregnant women as measured by serum retinol concentration, although vitamin A intake (mainly from green leafy vegetables) was adequate according to Indonesian food tables (Suharno et al., 1994). This suggests either a low bioavailability of the ingested carotenoids from their matrix or an overestimation of the provitamin A content of vegetables in food tables, or both. Recently it was shown that increased consumption of dark green leafy vegetables did not improve vitamin A status of breastfeeding women, indicating low bioavailability of β-carotene from complex matrices (de Pee et al., 1995). The results presented in the current study indicate that overestimation of the provitamin A content of green leafy vegetables partly may account for the finding of Suharno et al. (1994).

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