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Research Article

Vitamin C, folate, and phytochemical compounds of organically versus inorganically grown fruits and vegetables commonly consumed in Thailand

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ABSTRACT

Currently, organic plants are very popular among consumers due to lack of pesticide residues and their being good sources of bioactive compounds. However, only a few reports have compared bioactive compounds in organically versus inorganically grown plants. Consequently, this study's objective was to determine vitamin C, folate, carotenoid and flavonoid contents in 5 varieties of vegetables (kale; *Brassica albroglabra*, cabbage; *Brassica oleracea var. capitata*, carrot; *Daucus carota subsp*, tomato; *Solanum lycopersicum* and yardlong bean; *Vigna unguiculata ssp. Sesquipedalis*) and 5 varieties of fruits (pineapple; *Ananas comosus*, papaya; *Carica papaya*, long kong; *Lansium parasiticum*, rambutan; *Nephelium Lappaceum* and watermelon; *Citrullus lanatus*) that had been grown organically and inorganically . Results showed that vitamin C and folate contents in inorganic plants, particularly, carrot, yardlong bean, pineapple, papaya, and watermelon were slightly higher than those of organic ones. While carotenoid contents in organic Chinese kale, tomato, and yardlong bean were significantly higher than those of inorganic ones. However, vitamin C, total carotenoid and flavonoid contents were not significantly different between organic and inorganic plants, except for Chinese kale. It is noted that, nearly all the selected vegetables and fruits in present study were good sources of vitamins and phytochemicals, especially Chinese kale showed excellent amounts of vitamin C, folate, carotenoid and flavonoids while watermelon contained good amount of lycopene. Therefore, regularly consumed fruits and vegetables especially Chinese kale and watermelon as a plant-based diet might lower the risk of chronic disease due to the presence of high amount of bioactive compounds in them.

1. INTRODUCTION

Currently, people are greatly interested in improving their health, especially when an unhealthy diet is associated with the onset of non-communicable diseases (NCDs). Consequently, fruits and vegetables that are rich in natural antioxidants are receiving greater attention, since evidence have shown that consuming such foods are associated with preventing or delaying the onset of NCDs and maintaining a healthy weight[1,2]. Several bioactive compounds found in fruits and vegetables, such as vitamin C, polyphenol, carotenoids, and flavonoids have shown a strong inverse correlation with many chronic diseases, including cancer, hypertension, diabetes, cardiovascular disease, and neurological disorders[3-7]. Consumers, however, are also becoming more aware of the adverse health effects of toxic residues in fruits and vegetables that are used in cultivation, such as chemical fertilizers, herbicides, and pesticides. Consequently, the demand for organic fruits and vegetables is increasing among consumers, health educators, farmers, and food retailers. Consumers, especially, believe that organically grown fruits and vegetables are free of pesticide residues and are of better quality, healthier, and more nutritious compared to conventionally grown produce.

It is hypothesized that since organic fruits and vegetables cultivated without the use of insecticides or chemical pesticides, protect themselves against the environment by increasing their own protective or chemical substances in order to defend against free radical reactive oxygen species (ROS) in the environment, ultra violet light (UV), as well as insect pests, diseases, and bacteria that could damage or destroy cells[8]. Some studies have found no significant differences in nutrient content and bioactive compound values between organically and conventionally grown fruits and vegetables, since it can be affected by geographic location, local soil, climactic conditions, seasonality, maturity at time of harvest, and post-harvest storage practices[9].

In Thailand, no data are available comparing nutrient content and bioactive compounds between organic and inorganic fruits and vegetables. Consequently, this study compared nutrients (folate and vitamin C content) and antioxidant content (8 forms of flavonoids and 6 forms of carotenoids) in 5 varieties of certified organic and inorganic fruits (pineapple, *Ananas comosus*; papaya, *Carica papaya;* long kong, *Lansium parasiticum*; rambutan, *Nephelium Lappaceum*; watermelon, *Citrullus lanatus,*) and 5 varieties of vegetables (cabbage, *Brassica oleracea var. capitata*; carrot, *Daucus carota subsp. Sativus*; Kale, *Brassica albroglabra*; tomato, *Solanum lycopersicum*; yardlong bean, *Vigna unguiculata ssp. Sesquipedalis*).

2. MATERIALS AND METHODS

2.1. Materials

Ten varieties of organically and inorganically grown fruits and vegetables were selected based on common consumption in Thailand. Each variety was weighed to approximately 3 kilograms and was collected from July 2016 to July 2017. Six representative samples of conventionally (inorganically) grown fruits and vegetables were purchased from traditional distribution trade centers in Thailand, locally known as Talaad Thai and Talaad Simummuang, as well as from Ratchaburi province. Certified organic samples were obtained from the Health Society Company, which is an organic farm network that has received the certification standard for organic produce (IFOAM certified) by the participatory guarantee system (PGS). During transportation all the sample were kept in ice box with lid covered and bring to laboratory in the same day of purchased and immediately analyzed the moisture and vitamin C content**.** Table 1 gives the names and numbers of each studied variety.

Table 1. Name, scientific name and number of vegetable and fruit samples

English name	Scientific name	Number of sample
Vegetables		6
Chinese kale (organic and inorganic)	*Brassica albroglabra*	6
Cabbage (organic and inorganic)	*Brassica oleracea var.capitata*	6
Carrot (organic and inorganic)	*Daucus carota subsp.sativus*	6
Tomato (organic and inorganic)	*Solanum lycopersicum*	6
Yardlong bean (organic and inorganic)	*Vigna unguiculata ssp.sesquipedalis*	6
Fruits		6
Pineapple (organic and inorganic)	*Ananas commosus*	6
Papaya (ripen; organic and inorganic)	*Carica papaya*	6
Long kong (organic and inorganic)	*Lansium parasiticum*	6
Rambutan (organic and inorganic)	*Nephelium lappaceum*	6
Water melon (organic and inorganic)	*Citrullus lanatus*	6

Inorganic vegetables and fruits were purchased from three traditional distribution module trade centers in Thailand (n= 6 of each variety). Organic vegetables and fruits were obtained from the certified organic farm network of Health Society Company in various provinces of Thailand (n=6 of each variety).

2.1.1. Sample preparation

Upon arrival at the laboratory, six sets of the same variety of fruit or vegetable from the trade centers and the Health Society Company were individually washed with tap water to eliminate any contaminants and rinsed again with deionized distilled water. The edible portion of each vegetable or fruit set was prepared and homogenized separately (Ace homogenizer, NISSEI, Ltd, Tokyo, Japan) in a dark room at 25ºC. Approximately 300 g of each homogenized sample were pooled together to obtain a single sample. Consequently, a total of six representative composite samples were obtained for each variety of vegetable and fruit.
Individual samples from the six representative samples of the same variety (inorganic and certified organic) were then analyzed in duplicate. Moisture and vitamin C contents were determined immediately after the samples were homogenized and pooled. The homogenized samples were divided into three portions and stored in acid-washed polyethylene bottles. One bottle was used to determine antioxidant content (total polyphenol, 8 forms of flavonoids; ferulic acid, myricetin, quercetin, luteolin, naringenin, hesperetin, kaemferol and apigenin and anthocyanidins; cyanidin and peonidin). The second bottle was used for folate. The third bottle was used to determine 6 forms of carotenoids (lutein, zeaxanthin, beta-cryptoxanthin, alpha-carotene, beta-carotene and lycopene).

2.2. Methods

2.2.1. Moisture content determination

To determine moisture content, each sample was dried in a hot air oven at 100 ± 5ºC until constant weight was obtained according to AOAC method 950.46 (Latimer)[10].

2.2.2. Vitamin C determination

Each sample's vitamin C content was analyzed using HPLC according to a modification of the procedure of Brause *et al.*,[11] In brief, 3-10 g of each homogenized sample was weighed into a 50 ml volumetric flask, then 3 ml of 10% metaphosphoric acid (MPA; Merck Darmstadt, Germany) was immediately added and diluted with deionized water to the required volume. The diluted sample was then filtered (Whatman #1, Whatman International Ltd., Maidstone, England). The pH of all sample filtrate was adjusted to 5.0-5.25 with 4 M sodium hydroxide or 10% MPA (w/v) prior to the addition of 10 mg dithiothreitol (DTT, Sigma-Aldrich, St.Louis, MO, USA). The solution was mixed and incubated in the dark at room temperature (25ºC) for 1 h. All sample solutions were filtered (Whatman #42, Whatman International Ltd., Miadstone, England) and then re-filtered through a 0.45 µm syringe membrane filter (Chrome Tech® Milford, MA, USA) before injection for the HPLC analysis. Vitamin C content was analyzed using HPLC system equipped with a Waters 515 pump (Water Corporation, Milford, MA, USA) and Jasco UV 975 detector (Jasco International, Co., Ltd, Tokyo, Japan). Vitamin C was separated using a Zorbax 5µm ODS column (250 x 4.6 mn) with an analytical guard column C-130B (2 x 20 nm). The mobile phase was 5% K_2PO_4 at a flow rate of 0.8 ml/min. Vitamin C was monitored at 254 nm. The results were reported as milligram of ascorbic acid per 100g of fresh weight (mgAA/100g). The HPLC chromatogram of standard vitamin C was shown in Figure 1.

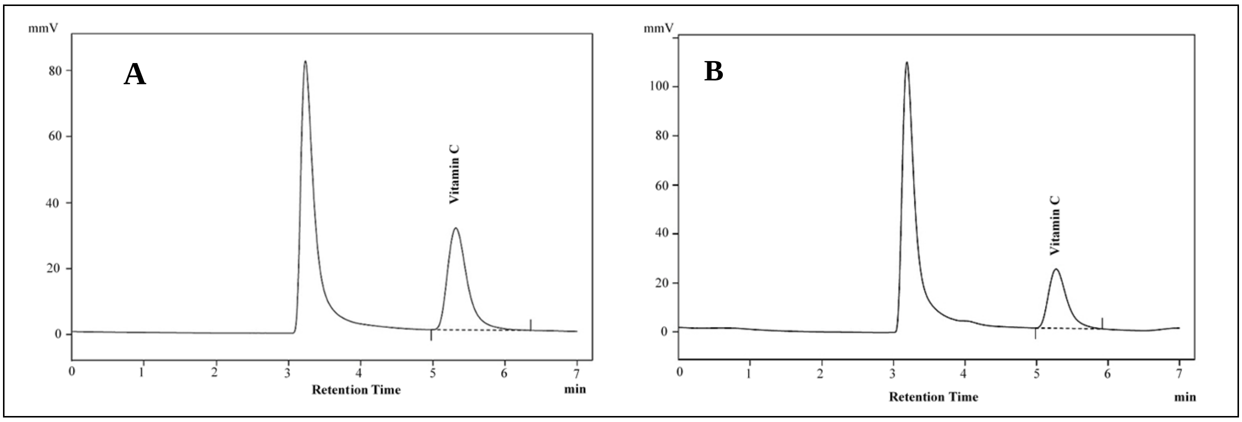

Figure 1. HPLC chromatogram of standard vitamin C **(A)** and chromatogram of vitamin C content in Chinese kale **(B)**.

2.2.3. Folate content determination

Folate analysis was performed according to the procedure of Li *et al.*,[12] and DeVries *et al.*,[13,14]. Briefly, each homogenized sample (3-5g) was weighed into an Erlenmeyer flask into which 100 ml of 0.1 M phosphate buffer, pH 7.8 was added. Each sample tube was then wrapped in aluminum foil and placed in an autoclave at 120ºC for 10 min, and then cooled at room temperature. Protease (2 mg/ml) per *g* sample was added and the sample tube was placed in an incubator (model 6500, Fisher Scientific) at 37ºC for 3 h. Each sample tube was returned to the boiling water bath for 5 min to inactivate protease, followed by cooling at room temperature. Thereafter, 1 ml (20 mg/ml) of α-amylase per *g* sample was added, followed by incubation for 2 h at 37ºC. The pH of the sample was adjusted to 7.8 and 4 ml of chicken pancreas conjugase solution was added, followed by adding 0.5 ml of toluene, and incubated at 37ºC for 16 h. The sample tube was returned to a boiling water bath for 5 min to inactivate the conjugate. The sample tube was cooled at room temperature and diluted to appropriate volume with deionized water, then the solution was filtered through Whatman No.4 filter paper into an Erlenmeyer flask. Thereafter, a pipetted appropriate volume of the clear sample solution was adjusted to pH 6.2 with acid or alkaline solution. Finally, the clear solution was pipetted at 0.5, 1 and 2 ml, and diluted to a final volume (2 ml) with phosphate buffer pH, 6.2, followed by adding 2 ml of folate assay medium (Difco™ Folic acid *Casei* Medium, Becton, Dickinson & Co., Sparks, MD 21152, USA) and mixed thoroughly. The sample tube was placed in an autoclave at 110ºC for 10 min and rapidly cooled down to room temperature using an ice water bath. After this period, each sample tube was inoculated with one drop of lactobacillus *case*i ATCC 7469 and mixed thoroughly. The sample tube was then returned to the boiling water bath at 100ºC for 5 min to stop bacterial growth. Growth was measured by turbidimetric method at the wavelength of 630 nm.

2.2.4. Determination of 6 carotenoid forms

Samples were extracted following the method of Speek *et al.*, in a dark room to protect carotenoids from degradation[15]. In brief, samples were saponified by placing a 2-10 g homogenized sample into a brown round-bottom flask. This was followed by adding 10 ml of a freshly prepared aqueous solution of 10% ascorbic acid (Unilab # A79, Australia) and 50 ml of 2M ethanolic potassium hydroxide (KOH) (Merck # 105033, Darmstadt, Germany). This solution was refluxed in a boiling water bath for 30 min. Samples were then cooled to room temperature. After adding 70 ml of hexane (J.T. Baker # 9309-03, 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 separating funnel containing 50 ml of 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 (NaCl; UNIVAR # A465, Ajax Finechem, Newzeland) and with a consecutive 100 ml water until it becomes alkali-free. An aliquot was collected and evaporated in a rotary evaporator (Buchi, Switzerland) under vacuum in a 37°C water bath. The standard carotenoids in this study included Lutein (0133, Carote Nature, Germany), zeaxanthin (0119, Carote Nature, Germany), β-cryptoxanthin (0055, Carote Nature, Germany), lycopene (0031, Carote Nature, Germany), α-carotene (0007, Carote Nature, Germany) and β-carotene (C9750, Sigma, USA). Analyses of carotenoids were performed using an Alliance 2695, Waters HPLC connected with Waters 486 UV/VIS detector (Waters Corporation, USA). The residue of each sample were dissolved in 1 ml methylene chloride (CH_2Cl_2: J.T. Baker # 9324-68, USA) and 2 ml mobile phase. Separation of carotenoids in each sample was performed using a C18 column (Vydac 201TP, C_{18} 4.6 x 250 mm, 5 µm column, Grace division, USA) with a guard column (Vydac 201TP, cartridge C18 4.6 x 12.5 mm, 5µm, Grace division, USA) at a flow rate of 0.7 ml/min at a controlled temperature at 30°C and monitored at 450 nm. The mobile phase consisted of HPLC-grade acetonitrile (CH_3CN; Lab Scan # LC 1005, Thailand): HPLC-grade methanol (CH_3OH; Lab Scan # LC 1115, Thailand): methylene chloride (CH_2Cl_2; J.T. Baker # 9324-68, USA): triethylamine (TEA; Fluka # 90342, Switzerland): and ammonium acetate (CH_3COONH_4; Merck # 1.01116, Darmstadt, Germany) at a ratio of 90:8:2:0.085:0.085 (v/v/v/v and w/v). The results of carotenoid contents in all fresh vegetable and fruit samples were analysed in duplicate and expressed as micrograms/100 g fresh weight (µg/100g).

2.2.5. Determination of 8 flavonoid forms

Flavonoid content was determined using a modified procedure following Merken & Beecher.,[16]. Briefly, an approximately 3-5 g homogenate sample was hydrolyzed in 40 ml of 62.5% aqueous methanol containing 0.5 g/l *tert*-butylhydroquinone (Sigma-Aldrich, St. Louis, MO, USA) and 10 ml of 6 N HCl (Merck, Darmstadt, Germany) at 90˚C in a shaking water bath for 2 hours (Memmert, Duesseldorf, Germany). After cooling to room temperature, 100 µl of 1% ascorbic acid (Ajax Finechem, Victoria, Australia) was added and each sample was diluted to 50 ml with methanol (J.T. Baker, PA, USA) and sonicated for 5 minutes (Branson 2510, Danbury, CT, USA). The solution was filtered through a 2.5 µm pores filter (Whatman International Ltd., Maidstone, England). Before being injected for HPLC analysis, the solution was passed through a 0.2 µm PTFE syringe filter (Chrom Tech®, Milford, MA, USA). The standard flavonoids in this study included ferulic acid (Fluka, New York, USA), myricetin (Fluka, St. Quentin Fallavier, France), quercetin (Fluka, Buchs, Switzerland), luteolin (Fluka, Rehovot, Israel), naringenin (Fluka, New York, USA), hesperetin (Fluka, Gillingham, England), kaemferol (Fluka, Neu-Ulm, Germany), and apigenin (Fluka, Neu-Ulm, Germany). Flavonoid determination was performed using an HPLC system consisting of a quaternary gradient pump (Agilent G1315A, Agilent Technologies, CA, USA), vacuum degasser (Agilent G1379A, Agilent Technologies, CA, USA), autosampler (Agilent G1329A, Agilent Technologies, CA, USA), Zorbax Eclipse XDB-C18 column (250 x 4.6 mm, 5 µm) with a guard column (12.5 x 4.6 mm, 5 µm) of the same stationary phase (Agilent Technologies, CA, USA), a thermo-stated column control with temperature at 30ºC (Agilent G1316A, Agilent Technologies, CA, USA), and diode array detector (Agilent G1315B, Agilent Technologies, CA, USA) at wavelengths of 210, 280, 325, 338, and 368 nm. Elution was carried out at a flow rate of 0.6 ml/min using the following gradients of water (A), methanol (B), and acetonitrile (C), each containing 0.05% (w/w) trifluoroacetic acid (Merck, Darmstadt, Germany) with solvent parameters as 0-5 min: 90-85% A, 6-9% B, 4-6% C; 5-30 min: 85-71% A, 9-17.4% B, 6-11.6% C; 30-60 min: 71-0% A, 17.4-85% B, 11.6-15% C; 60-61 min: 0-90% A, 85-6%B, 15-4% C; and 61-66 min: 90% A, 6% B, 4% C. The flavonoids peak identification was done by comparing the retention time and the UV spectrum of unknown peaks to the authentic standards using ChemStation software (Agilent G1379A, Agilent Technologies, CA, USA). The results were expressed as micrograms per 100g of fresh weight. The HPLC chromatogram 8 form of standard flavonoids as showed in Figure 2.

2.2.6. Quality control

In-house control materials (black rice flour, Tang® natural fresh orange powder, and whole milk powder) were used for quality control of all analyzed data. Black rice flour and Tang® were used as the daily control sample of antioxidants and vitamin C content while whole milk powder was used for total folate analysis.

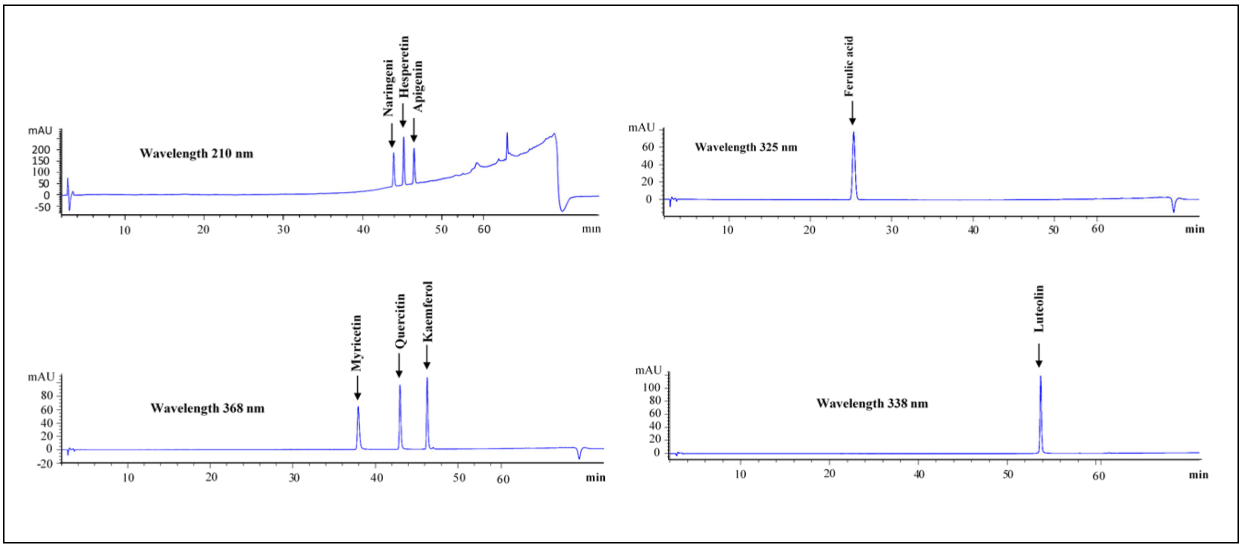

Figure 2. HPLC chromatogram 8 form of standard flavonoids.

3. STATISTISTICAL ANALYSIS

Data were expressed as mean ± standard deviation of duplicated analysis. Non-parametric Mann-Whitney U-test was applied to compare mean of nutrient content and bioactive compounds between organic and inorganic fruit and vegetable as same variety. The Statistical Package for Social Science (SPSS) for Windows version 19 (IBM Corporation, New York, USA) was used to analyze the data. Statistical significance was determined at a level of $p < 0.05$.

4. RESULTS AND DISCUSSION

4.1. Moisture Content

The moisture content of 10 varieties of vegetables and fruits ranged from 88.87% in Chinese kale (*Brassica albroglabra*) to 94.53% in cabbage (*Brassica oleracea var.capitata*) and 80.10% in rambutan (*Nephelium lappaceum*) to 91.12% in watermelon (*Citrullus lanatus*). Moisture content of the same variety of fresh vegetables and fruits grown under different systems (organic and inorganic) showed no significant differences (Table 2).

4.2 Vitamin C Content

Vitamin C is an essential phytonutrient and is one of the most important water soluble antioxidants found in fruits and vegetables[17,18,19]. It acts as an antioxidant to prevent free radicals from damaging tissues and inhibits LDL oxidation that can lead to atherosclerosis. Previous publications have shown that vitamin C may reduce the risk of chronic diseases including coronary heart disease, cancer, and various age-related chronic diseases. However, humans are incapable of synthesizing vitamin C due to the absence of the enzyme L-gluconolatone oxidase. Hence, this vitamin must be obtained from food.

Results of vitamin C analysis in fresh vegetables and fruits used in the present study are shown in Table 2 and Figure 1 showed an example of HPLC chromatogram of vitamin C in Chinese kale. Vitamin C content in vegetables ranged from 2.56 (inorganic carrot) to 64.25 (inorganic Chinese kale) mg/100g wet weight, while in fruits it ranged from 1.53 (inorganic long kong) to 48.12 (organic ripe papaya) mg/100 g edible portion. Inorganic Chinese kale had higher average value of vitamin C (64.25 mg/100g FW) than the organic samples (50.62 mg/100g FW) at $p < 0.05$.

Table 2. Antioxidant content of organic and inorganic vegetables per 100 g of wet weight[1]

English name	Moisture (g)	Vitamin C (mg AA)	Folate (µg)
Vegetables			
Chinese kale (organic)	88.87 ± 2.74^a	50.62 ± 9.53^a	65.00 ± 1.41^a
Chinese kale (inorganic)	92.20 ± 1.25^a	64.25 ± 1.95^b	66.50 ± 3.54^a
Cabbage (organic)	94.22 ± 1.88^a	17.27 ± 0.01^a	53.50 ± 0.58^a
Cabbage (inorganic)	92.84 ± 0.62^a	19.72 ± 0.04^a	57.50 ± 7.78^a
Carrot (organic)	90.33 ± 2.17^a	3.23 ± 0.00^a	16.5 ± 1.73^a
Carrot (inorganic)	91.50 ± 0.84^a	2.56 ± 0.11^a	14.50 ± 2.12^a
Tomato (organic)	93.95 ± 0.19^a	20.52 ± 0.06^a	19.00 ± 0.00^a
Tomato (inorganic)	93.68 ± 0.40^a	22.80 ± 1.32^a	24.00 ± 2.82^a
Yardlong bean (organic)	91.55 ± 2.15^a	11.94 ± 0.24^a	53.00 ± 1.15^a
Yardlong bean (inorganic)	90.31 ± 0.58^a	9.08 ± 0.40^a	45.50 ± 0.71^a
Fruits			
Pineapple (organic)	84.81 ± 7.20^a	5.34 ± 0.07^a	5.50 ± 0.71^a
Pineapple (inorganic)	84.08 ± 3.99^a	5.01 ± 0.31^a	3.50 ± 0.71^a
Papaya (organic)	87.95 ± 0.84^a	48.12 ± 2.85^a	53.50 ± 0.71^a
Papaya (inorganic)	87.93 ± 0.51^a	44.75 ± 1.02^a	59.50 ± 4.95^a
Rambutan (organic)	79.28 ± 1.42^a	14.56 ± 0.19^a	3.00 ± 0.00^a
Rambutan (inorganic)	82.01 ± 2.07^a	11.63 ± 0.58^a	2.00 ± 0.00^a
Long kong (organic)	81.38 ± 0.41^a	1.61 ± 0.06^a	2.00 ± 0.00^a
Long kong (inorganic)	81.56 ± 0.49^a	1.53 ± 0.06^a	3.00 ± 0.00^a
Watermelon (organic)	91.12 ± 0.70^a	5.26 ± 1.21^a	0.80 ± 0.00^a
Watermelon (inorganic)	91.04 ± 0.32^a	4.05 ± 0.23^a	0.75 ± 0.07^a

[1]Data are shown as mean ± SD for the moisture, vitamin C, and folate content of each edible portion of fresh organic and inorganic vegetables and fruits derived from duplicate analysis (n = 6) . Mean values within the same column and variety with different superscript letters show significant differences between organic and inorganic growing systems at $p < 0.05$,by independent-samples Mann-Whitney U-test.

Similar results were reported by Ismail and Fun wherein Chinese kale samples produced under a conventional system presented higher values of vitamin C[20]. In addition, previous publications have shown higher vitamin C content in organic tomatoes, potatoes, and leafy vegetables compared to conventionally grown ones [21,22].

Differences in quality and nutrient composition of vegetable and fruit samples were observed in this study. Possibly, changes in the management of chemicals and agricultural practices, different weather conditions, or the growing system could have played a role in interfering with the influences and possible effects of the cultivation system[23]. In addition, present study's vitamin C content of all inorganic fruits and vegetables except cabbage were lower than those reported in the Thai Food Composition Tables and the Indian Food Composition [24,25]. Differences may be due to differences in the varieties of plants, fertilizers used, cultivation, soil, harvest time, post-harvest handling, and storage conditions can influence nutrient and antioxidants values in plants [18,19,26]. According to Thai dietary recommendations, consuming one serving of either organic or inorganic ripe papaya (130 g of edible portion) will meet the daily vitamin C requirement of adults and children, which is 60 mg/day. Foods that are ready to eat can be classified as a good source when it meets 10-19% of Thai dietary reference intake (Thai RDI) and as a rich source when it meets equal to or greater than 20% of the Thai RDI[27]. Consequently, a ripe papaya provides 100% of the daily requirement for vitamin C and thus is classified as a "rich" source of vitamin C.

In comparing the two different cultivation systems, no significant difference was observed for vitamin C content in fruits and vegetables. Overall there was a trend of higher vitamin C content in organically grown plants, particularly, yardlong bean and 5 varieties of fruits. This might be due to differences in the soil or fertilizer used in cultivation, since previous publications have suggested that the use of lower doses of nitrogen fertilizer in the form of nitrates[22].

4.3. Folate Content

Folates are water soluble vitamins that play an important role as essential coenzymes in the synthesis of purine and thymidine nucleotides for fetal development and health maintenance. Previous publications have indicated that an adequate intake of folate helps to reduce the risk of neural tube defects (NTDs), colon cancer, Alzheimer's, dementia, and cardiovascular diseases [28,29,30]. Present study gives valuable data on the total folate contents of fruits and vegetables commonly consumed in Thailand. Among the 5 varieties of vegetables studied here, inorganic Chinese kale had the highest level of folate at 66.50µg/100g wet weight, followed by organic Chinese kale (65.00 µg/100g wet weight), organic and inorganic cabbage (53.50 and 57.50 µg/100g wet weight), and organic yardlong bean (53.00 µg/100g wet weight). As for the 5 varieties of fruits, all of them except papaya had low folate content ranging from 0.75 (inorganic watermelon) to 5.50 (organic pineapple) µg/100g of edible portion. Organic and inorganic ripe papaya were found to be good sources of folate (53.50 and 59.50 µg/100g of edible portion). Regarding the folate content of different cultivars, no significant difference was observed (Table 2). When comparing the folate content of inorganic in the present study with previously reported results, it was found to be higher in cabbage and tomato and lower in carrot and yardlong bean. Among inorganic fruits, folate contents of ripe papaya and pineapple were higher, while watermelon was lower, than those reported in previous studies[31]. Unfortunately, no published data on folate content of organic fruits and vegetables were found to compare with the present study.

4.4. Carotenoid Content

Vitamin A is essential for normal vision, for maintaining the integrity of epithelial tissues, and for a wide variety of other metabolic functions. Studies from developing countries suggest that over 80% of the dietary intake of vitamin A comes from pro vitamin A (carotenoids). Carotenoids are synthesized as pigments by many plants and are found in green, orange, and yellow plant tissues. These carotenoids are composed of lutein, zeaxanthin, alpha-carotene, beta-carotene, beta-cryptoxanthine, and lycopene. Many researchers have suggested that eating pro vitamin A rich food such as green leafy vegetables and fruits may reduce the risk of chronic diseases, especially age related macular degeneration (AMD) and cataracts in elderly people since AMD is one of the major causes of blindness in elderly adults. In addition, previous publications have indicated that a diet rich in zeaxanthin and lutein is associated with a reduced risk of the development of cataract and macular degeneration [32-36].

Table 3. Carotenoid contents of organic and inorganic vegetables per 100 g of wet weight[1]

English name	Lutein (µg)	Zeaxanthin (µg)	β-crypto-xanthin (µg)	Lycopene (µg)	α-carotene (µg)	β-carotene (µg)	Total carotenoids (µg)
Vegetables							
Chinese kale (organic)	2035.98 ± 273.30[a]	ND	53.32 ± 4.41[a]	ND	ND	2339.03 ± 695.44[a]	4428.33 ± 969.66[a]
Chinese kale (inorganic)	1699.55 ± 167.80[a]	ND	39.72 ± 2.17[b]	ND	ND	2158.87 ± 117.20[b]	3898.13 ± 105.28[b]
Cabbage (organic)	14.60 ± 0.39[a]	ND	ND	ND	ND	5.58 ± 0.43[a]	20.18 ± 0.50[a]
Cabbage (inorganic)	22.25 ± 5.60[b]	ND	ND	ND	ND	10.98 ± 5.28[a]	33.23 ± 10.88[b]
Carrot (organic)	119.05 ± 33.78[a]	12.40 ± 3.30	ND	ND	918.63 ± 68.17[a]	2872.90 ± 727.61[a]	3910.58 ± 564.79[a]
Carrot (inorganic)	114.27 ± 5.95[a]	ND	ND	ND	865.25 ± 40.97[a]	5290.20 ± 201.11[a]	6269.73 ± 240.72[b]
Tomato (organic)	83.67 ± 5.76[a]	1.20 ± 1.40	13.58 ± 1.75[a]	2960.70 ±736.98[a]	95.85 ± 17.68[a]	420.58 ± 31.10[a]	3575.58 ± 642.42[a]
Tomato (inorganic)	73.27 ± 5.65[a]	ND	9.17 ± 2.06[a]	1761.15 ±326.52[b]	55.30 ± 10.10[b]	282.80 ± 24.60[a]	2181.69 ± 339.45[b]
Yardlong bean (organic)	359.05 ± 23.29[a]	ND	17.32 ± 1.49[a]	ND	9.57 ± 0.57[a]	436.93 ± 29.18[a]	822.87 ± 13.68[b]
Fruits							
Pineapple (organic)	4.83 ± 1.12[a]	ND	ND	ND	ND	30.62 ± 2.71[a]	35.45 ± 3.79[a]
Pineapple (inorganic)	9.35 ± 1.51[a]	ND	14.45 ± 11.38	ND	ND	170.70 ± 58.19[b]	194.50 ± 70.59[b]
Papaya (organic)	ND	ND	958.85 ± 72.22[a]	2347.65 ± 318.33[a]	101.75 ± 11.69[a]	368.08 ± 70.31[a]	3766.33 ± 282.72[a]
Papaya (inorganic)	ND	ND	817.90 ± 143.33[a]	2435.40 ± 386.33[a]	108.82 ± 22.98[a]	311.03 ± 103.89[a]	3673.15 ± 632.72[a]
Rambutan (organic)	ND	ND	ND	ND	ND	ND	ND
Rambutan (inorganic)	ND	ND	ND	ND	ND	ND	ND
Long kong (organic)	0.85 ± 0.05[a]	ND	ND	ND	ND	1.88 ± 0.35[a]	2.73 ± 0.40[a]
Long kong (inorganic)	0.75 ± 0.05[a]	ND	ND	ND	ND	1.77 ± 0.47[a]	2.52 ± 0.48[a]
Watermelon (organic)	41.57 ± 27.17[a]	ND	60.43 ± 38.78[a]	4690.20 ± 1173.71[a]	214.07 ± 111.82[a]	395.92 ± 188.54[a]	5402.19 ± 1419.10[a]
Watermelon (inorganic)	35.70 ± 55.38[a]	ND	75.92 ± 23.94[a]	6110.20 ± 486.55[a]	293.62 ± 42.62[a]	549.13 ± 334.16[a]	7064.74 ± 778.01[a]
Yardlong bean (inorganic)	276.92 ± 40.39[a]	ND	11.55 ± 1.52[a]	ND	5.83 ± 0.97[a]	373.25 ± 15.41[a]	667.55 ± 57.73[a]

[1]Data are shown as mean ± SD for 6 forms of carotenoids for each edible portion of fresh organic and inorganic vegetables and fruits derived from duplicate analysis (n=6). Mean values within the same column and variety with different superscript letters show significant differences between organic and inorganic growing systems at $p < 0.05$, by independent-samples Mann-Whitney U-test. ND: not detected

Results on analysis of 6 forms of carotenoids in organic and inorganic fruits and vegetables are shown in Table 3. Regarding lutein content there were no significant differences between organic and inorganic fresh vegetables. Organic Chinese kale (2035.94 µg/100g FW) had the highest lutein content, followed by inorganic Chinese kale (1699.55 µg/100g FW), organic and inorganic yardlong bean (359.05 and 276.92µg/100g FW), and organic and inorganic carrot (119.05 and 114.27µg/100g FW). Organic and inorganic cabbage had the lowest lutein contents (14.60 and 22.25µg/100g FW). These values are not in agreement with those reported by USDA ; Kaulmann *et al.*,; Reif *et al.*,; Maurer; Jeffery *et al.*,[31, 37-40] . Zeaxanthin was only detected in organic carrot and tomato. As for fruits lutein content was relatively low and zeaxanthin was undetectable in all varieties.

When a compared to previous studies reported by the USDA; Khonsarn & Lawan;

Martinez-Valdivieso *et al.*, present study revealed lower amounts of lutein and zeaxanthin in all inorganic fruits, except for watermelon which showed higher amount [31,41,42].

Among the 10 varieties of fruits and vegetables in this study, both organic and inorganic ripe papaya proved to be excellent sources of β-cryptoxanthin (948.85 and 796.07μg/100g FW), whereas other fruits and vegetables ranged from not detectable to 75.92 μg/100g FW.

As for lycopene content, organic and inorganic watermelon (red colored pulp) proved to be excellent sources (4690.20 and 6110.20μg/100g FW), followed by organic tomato (red skin; 3172.43μg/100g edible portion), organic and inorganic ripe papaya (2347.65 and 2450.40 μg/100g FW), and inorganic tomato (1761.15μg/100g edible portion). Lycopene was not detected in other selected fruits and vegetables (Table 3).

Since few studies were found on the carotenoids content of organic plants, comparison with the present study was not possible. The highest value of beta-carotene was found in organic and inorganic carrot (3082.38 and 5290.20 μg/100g FW), followed by organic and inorganic Chinese kale (2339.03 and 2148.87μg/100g FW). Watermelon and ripe papaya showed the highest alpha- and beta-carotene contents as compared to other selected fruits in this study. The amount of alpha- and beta-carotene contents of fresh vegetables in this study were lower than those reported by the USDA[31] whereas higher values were found in papaya, pineapple, and watermelon as shown in Table 3. In addition, beta-carotene and lycopene content for long kong, papaya, rambutan, and watermelon in this study was in agreement with the data reported by Chareonsiri and Kongkachuchai [43]. Total carotenoid content of all organic vegetables were significantly higher than those of inorganic ones except for cabbage and carrot. As for fruits, papaya, longkong and watermelon did not show any significant difference in total carotenoids between the two cultivation systems. However, inorganic pineapple exhibited significantly higher total carotenoids than the organic samples and carotenoids were not detected in both organic and inorganic rambutan. A study by Mercadante & Rodriguez-Amaya[44] found that conventionally grown and organic kale of the same cultivar was significantly different in total carotenoids composition. Similarly, Walsh *et al.*,[45] indicated that vegetable cultivars grown on conventional farms using crop fertilizer may differ in carotenoid value than that of an organic farm. In the present study, carrot, Chinese kale, tomato, watermelon, and ripe papaya were excellent sources of total carotenoids.

4.5. Flavonoid Content

Epidemiological studies have shown that diets rich in fruits and vegetables are associated with longer life expectancy, possibly due to their being rich sources of vitamin and antioxidants compounds (phenolics, flavonoids, and carotenoids). Phytochemicals are bioactive non-nutrient plant compounds that have shown a remarkably high scavenging activity toward chemically generated radicals. They are effective in inhibiting oxidation of human low-density lipoproteins and thus have potential effects in preventing various human diseases, such as heart disease, cancer, stroke, diabetes, Alzheimer's disease, cataracts, and age-related function decline. Quercetin and other polyphenols have been shown to play a protective role in carcinogenesis by reducing the bioavailability of carcinogens. Ferulic acid (FA) is a major bioactive compound in fruits, vegetables and other plants that showed free radical scavenging effect which is comparable to that of superoxide dismutase. Therefore, FA has been claimed to reduce the risk of major chronic diseases through numerous biological effects such as anti-inflammatory, antimicrobial, antiallergic, hepatoprotective, anticarcinogenic, and vasodilatory activites[46,47]. In addition, a previous publication indicated that consumption of natural bioactive compounds like quercetin, myricetin, kaempferol, luteolin, and fisetin available in plants, help in decreasing systolic blood pressure, oxidative stress, LDL cholesterol and plasma cholesterol, improving dyslipidemia, preventing arteriosclerosis by inhibiting LDL oxidation and formation of platelets, suppressing body weight gain, and acting as an anti-inflammatory agent[48].

Table 4 showed the contents of 8 flavonoid forms in all tested vegetables and fruits. Organic and inorganic Chinese kale (1907.55 and 1313.38μg/100g FW) and organic and inorganic pineapple (4234.54 and 5606.16μg/100g FW) showed the highest content of ferulic acid, while organic and inorganic yardlong bean (2943.72 and 3143.56 μg/100g FW) and organic Chinese kale (1251.59 μg/100g FW) exhibited highest amount of quercetin.

Table 4. Flavonoids content of organic and inorganic vegetables per 100g of wet weight[1]

English name	Ferulic acid (µg)	Quercetin (µg)	Naringenin (µg)	Kaemferol (µg)	Total flavonoids (µg)
Chinese kale (organic)	1907.55 ± 590.47[a]	1251.59 ± 740.84[a]	ND	8573.94 ± 3364.07[a]	11733.08 ± 1315.77[a]
Chinese kale (inorganic)	1313.38 ± 512.54[a]	443.76 ± 113.27[b]	ND	3851.92 ± 798.49[b]	5609.06 ± 615.91[b]
Cabbage (organic)	ND	ND	ND	ND	ND
Cabbage (inorganic)	ND	ND	ND	ND	ND
Carrot (organic)	ND	ND	ND	ND	ND
Carrot (inorganic)	ND	ND	ND	ND	ND
Tomato (organic)	126.36 ± 4.74[a]	ND	2155.82 ± 239.74[a]	ND	2282.18 ± 236.55[a]
Tomato (inorganic)	69.22 ± 5.00[a]	ND	1869.18 ± 105.34[a]	ND	1938.40 ± 102.34[a]
Yardlong bean (organic)	393.63 ± 69.23[a]	2943.72 ± 121.18[a]	ND	ND	3337.35 ± 61.83[a]
Yardlong bean (inorganic)	413.75 ± 51.67[a]	3143.56 ± 874.42[a]	ND	ND	3557.31± 857.52[a]

[1]Data are shown as mean ± SD for 8 forms of flavonoids for each edible portion of fresh organic and inorganic vegetables and fruits derived from duplicate analysis (n=6). Mean values within the same column with different superscript letters show significant differences between organic and inorganic growing system at $p < 0.05$, by independent-samples Mann-Whitney U-test. ND: not detected. Myricetin, Lueolin, Hesperetin and Apigenin content was not detected in all the selected vegetable samples in present study.

Highest amount of kaeferol was found only in organic Chinese kale (8573.94 µg/100g FW), Naringenin was found only in tomato (2080.82 and 1869.18µg/100g FW). The HPLC chromatogram showed some of the flavonoids compounds found in Chinese kale and tomato as shown in Figure 3.

Among the 10 varieties of vegetables and fruits, organic Chinese kale (11733.08 µg/100g FW) had the highest amount of total flavonoids, followed by inorganic Chinese kale (5609.06 µg/100g FW), organic and inorganic pineapple (4234.54 and 5606.16µg/100g FW), organic and inorganic yardlong bean (3408.63 and 3827.31µg/100g FW), and organic and inorganic tomato (2210.82 and 1938.40µg/100g FW), respectively. In present study ferulic acid contents of organic and inorganic tomato (130.00 and 69.22 µg/100g FW) and carrot (not detected) were lower than those reported by Kumar & Pruthi[46] (290 – 6000 µg/100g FW in tomato and 1200-2800 µg/100g FW in carrot). Differences in the values of ferulic acid may be due to various factors, including particular variety, fertilizer, climatic conditions, soil, or geographical original[46]. Myricetin was not detected in all fresh vegetables and fruits in the present study, while Franke *et al.*,[49] reported myricetin contents in cabbage, tomato, yardlong bean, papaya, and pineapple to be lower than 40 µg/100g FW. They also reported quercetin content in yardlong bean at only 410.65µg/100g FW which was lower than the results of present study.

It is noteworthy that the 5 varieties of fruits selected for this study were not sources of flavonoids, since only quercetin was found in organic and inorganic pineapple (4234.54 and 5606.16 µg only organic Chinese kale exhibited significantly higher amount of total flavonoids than the inorganic ones (p<0.05), whereas other plants did not show significantly difference in total flavonoids content (Table 4). Our results are consistent with those of Dangour *et al.*,[50] and Smith-Spangler *et al.*,[51] which indicated that there was no significant difference in nutrient composition between organic and conventional crops. Analysis of data for 8 forms of flavonoids indicated that fresh fruits and vegetables in this study were not good flavonoid sources.

5. CONCLUSION

Five varieties of fruits and five varieties of vegetables grown organically and inorganically were selected for the present study as it were commonly consumed in Thailand.

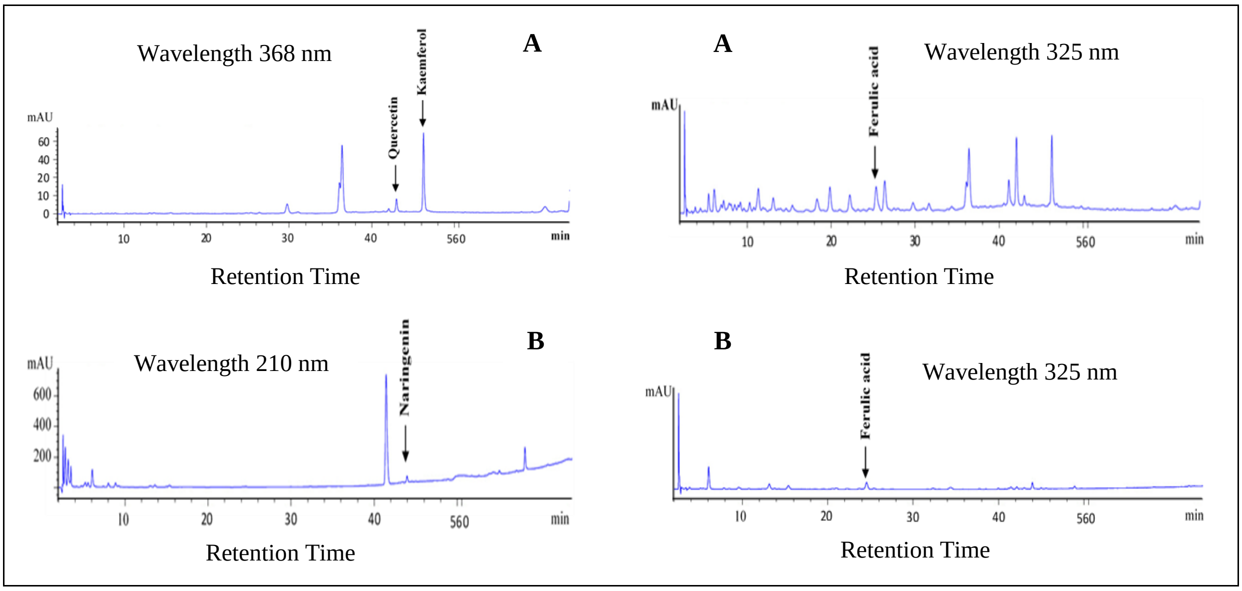

Figure 3. HPLC chromatogram some of flavonoids found in Chinese kale; *Brssica albroglabra* (A) and tomato*; Solanum lycopersicum* (B)

Results showed that overall, there was no significant difference in the content of antioxidants between fruit and vegetable samples grown in organic or inorganic systems. However, inorganic Chinese kale, cabbage and tomato had higher amounts of vitamin C and folate than those from organically grown system, whereas organic fruit samples in present study showed higher amount of vitamin C and folate than those from the inorganic growing system. Other antioxidants, such as carotenoids and flavonoids in most of the fruits and vegetables in this study, showed no significant differences between the two growing systems, except total carotenoids in vegetables. It should be noted that Chinese kale showed excellent amounts of both vitamin and bioactive compounds while watermelon showed good amount of lycopene. Therefore, watermelons and Chinese kale, good sources of bioactive compounds, are likely to be the interesting fruit and vegetable for further research in terms of their functional properties on health promotion. However, this study faced many limitations and confounding factors in comparing nutrients and phytochemical substances in fruits and vegetables grown organically and inorganically. Most notably, samples could not be obtained from the same source or from those cultivated in similar soils, climate conditions, harvest times or postharvest handing practices. However, this study attempted to minimize such limitations by randomly purchasing inorganic fruits and vegetables from representative retail and wholesale markets that are frequented by consumers. Therefore, the results of this study may be used as a guideline for purchasing organic vegetables and fruits by consumers and for generating a new database on organic vegetables and fruits for inclusion in the Thai food database.

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Conflict of interest
The authors declare that there is no conflict of interest.

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Ethical approval
In 2016, the university did not have rules or any issues to request research ethics in plants. And also the vegetable and fruit in this study are generally daily consumed by the consumer and it is not indigenous vegetables.

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