International Food Research Journal 23(6): 2426-2435 (December 2016)
Journal homepage: http://www.ifrj.upm.edu.my

Blanching process increases health promoting phytochemicals in green leafy Thai vegetables

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Article history

Received: 5 September 2015
Received in revised form: 24 February 2016
Accepted: 7 March 2016

Keywords

Chlorophylls
Carotenoids
Polyphenols
Antioxidant capacity
Green vegetables
Cooking

Abstract

Different methods of cooking affected nutritional levels of vegetables, which were found to be based on habit and society. Ten commonly consumed green leafy vegetables; thorny tree, dill, chinese chive, peninsular, asiatic pennywort, betel vine, garden parsley, vietnamese coriander, swamp morning glory and sweet basil were investigated for the effects of Thai domestic blanching and streaming on chlorophylls, carotenoids, total polyphenol content (TPC) and total antioxidant capacity (TAC). Blanching for 30 s significantly ($P < 0.05$) enhanced contents of chlorophylls, lutein, β-carotene, TPC and TAC in most tested vegetables. A significant correlation ($P < 0.05$) among chlorophylls and β-carotene ($r = 0.888$), lutein and β-carotene ($r = 0.784$), pheophytin and TAC ($r = 0.615$, DPPH and $r = 0.568$, FRAP), TPC and TAC ($r = 0.965$, DPPH and $r = 0.959$, FRAP) were also found. After 5 min streaming, TPC and TAC showed similar patterns which were higher and lower in some cooked vegetables compared to fresh samples. Chlorophylls significantly changed to pheophytins during streaming. Alteration of pheophytins enhanced TAC as a significant correlation was obtained (r = 0.727, DPPH) and r = 0.713, FRAP) in streamed vegetables. Results demonstrated that 30 s blanching better preserved and also promoted more nutrients and antioxidant properties in green leafy vegetables than streaming.

Introduction

Green leafy vegetables are a rich source of phytochemicals and antioxidants such as chlorophylls, carotenoids and polyphenols (Oboh, 2005; Nanasombat and Teckchuen, 2009; Chandrika *et al*., 2010; Steiner-Asiedu *et al*., 2014). In the recent years, there has been increasing interest in plant phytochemicals because of epidemiological evidences supporting that high consumption of vegetables and fruits reduces risk of chronic diseases such as cancer and cardiovascular events (Riccioni, 2009). Major sources of chlorophylls and carotenoids for human consumption are from green vegetables with many types of preparations including fresh and cooked. Previous studies reported that vegetables with various cooking methods such as boiling, streaming, frying, stir-frying and microwaving, resulted in changes of phytochemical contents and antioxidant capacities (Turkmen *et al*., 2006; Ferracane *et al*., 2008; Azizah *et al*., 2009; Yuan *et al*., 2009; Porter, 2012). More step of cooking could lead to more depletion of nutritional properties of vegetables. For example, high loss of chlorophylls, vitamin C, soluble proteins and soluble sugars could be caused by stir-frying followed by boiling (Yuan *et al*., 2009). In contrast, antioxidant activity of cooked artichokes (boiling, streaming and frying) and broccoli (boiling and streaming) significantly increased after cooking. These results indicated that cooking methods affected nutritional levels of vegetables (Ferracane *et al*., 2008; Miglio *et al*., 2008).

Differences of cooking procedures are found to be based on habit and society. Some previous studies focused on effects of boiling, streaming, microwaving, frying, and stir-frying methods (Turkmen *et al*., 2006; Yuan *et al*., 2009; Steiner-Asiedu *et al*., 2014) but lacked of investigation on blanching of green leafy vegetables. In food industry, blanching is a pre-treatment step prior to canning or freezing and aims to inactivate enzyme activity and preserve vegetables. For Thailand, blanching and streaming are domestic and common cooking methods for vegetables that usually found in Thai cuisine. Few information is available for green leafy vegetables grown in Thailand and cooked by blanching and streaming. Therefore, the aim of this study was to investigate the effects of domestic blanching and streaming on

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Table 1. Thai green leafy vegetables used in this study

Common names/Thai names	Botanical names	Abbreviations
Asiatic pennywort/ Boa-Bok	*Centella asiatica* (Linn.) Urban	AP
Betel vine/ Cha-Poo	*Piper betel* Linn.	BV
Chinese chive/ Kui-chai	*Allium tuberosum* Roxb.	CC
Dill/ Phak-Chee-Laao	*Anethum graveolens* Linn.	DL
Garden parsley/ Phak-Chee-Farang	*Eryngium foetidum* Linn.	GP
Peninsular/ Saranae	*Mentha cordifolia* Opiz ex Fresen	PN
Swamp morning glory/ Phak-bung	*Ipomoea aquatica* Forsk.	SM
Sweet basil/ Horapha	*Ocimum basillicum* L.	SB
Thorny tree/ Cha-Om	*Acacia pennata* subsp. *Insuavia*	TT
Vietnamese coriander/ Phak-Paew	*Polygonum odoratum* Lour.	VC

contents of phytochemicals including polyphenols, chlorophylls, lutein and β-carotene in 10 commonly consumed green leafy vegetables; thorny tree, dill, chinese chive, peninsular, asiatic pennywort, betel vine, garden parsley, vietnamese coriander, swamp morning glory and sweet basil. Results of antioxidant activities of these vegetables were also reported.

Materials and Methods

Materials

Ten species of freshly harvested local Thai green leafy vegetables (Table 1) were purchased from local markets in Khon Kaen Province, Thailand, on the day of processing. Only leaves of vegetables were cooked by two cooking methods in triplicates. To obtain more homogeneous samples, each vegetable was prepared in batches of 150 g. Each batch was then divided into four equal portions. One portion was retained raw, and others were cooked.

Chemicals

Chlorophyll a (Chl *a*), chlorophyll *b* (Chl *b*), β-carotene, lutein, gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picryhydrazyl, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic (Trolox) were purchased from Sigma-Aldrich (St Louis, MO, USA). Folin-Ciocalteu was purchased from Fluka (Milwaukee, WI, USA). Chlorophylls derivatives, pheophytin a (Pheo a) and pheophytin b (Pheo b) were prepared by acidification of fresh green vegetables with acetic acid and then heated at 100°C for 20 min then, extracted (Ferruzzi *et al*., 2001) and purified by thin layer chromatography (Quach *et al*., 2004). Purity was confirmed by HPLC to be greater than 90% (Kao *et al*., 2011). All HPLC grade solvents were purchased from Merck.

Cooking procedures

For blanching, vegetables were added to boiling tap water in a covered pot (1:5, vegetable/water) and cooked on a moderate flame for 30 s. Then samples were drained off. For streaming, a single layer of fresh vegetables was steamed in a domestic closed vessel using a stream basket suspended above a small amount of boiling water. Cooking time was 5 min starting from when the sample was suspended above boiling water. After cooking, samples were rapidly cooled on ice, cut into small pieces and freeze-dried at -50°C for 48 h. The freeze-dried sample was finely ground, kept separately in sealed bags and stored at -20°C.

Determination of chlorophylls and carotenoids contents

Chlorophyll derivatives and carotenoids in fresh and cooked vegetables were extracted and analyzed according to the method described by Huang *et al.* (2008) and Loranty *et al*. (2010), respectively. All experiments were performed under dim light to prevent pigments degradation. Chlorophylls and carotenoids content were determined based on standard chlorophylls and carotenoids, consisting of Chl *a*, Chl *b*, Pheo *a*, Pheo *b*, lutein and β-carotene. Amounts of chlorophylls and carotenoids were calculated by using linear equation following linear least square regression analysis obtained from each standard and contents were expressed as mg/g dry weight (DW).

Preparation of vegetables extracts for total polyphenol content and total antioxidant capacity

Vegetables extracts for TPC and TAC were prepared using the method of Pérez-Jiménez *et al.* (2008) with some modifications. Grounded sample 0.1 g was weighted, then 4 mL of acidic methanol/ water (50:50, v/v; pH2) was added, and the tube was shaken at room temperature for 1 hour. The tube was centrifuged for 10 min and the supernatant was recovered. Four milliliters of acetone:water (70:30, v/v) was added to the residue, and shaking and centrifugation were repeated. Methanolic and acetonic extracts were combined and stored at -20°C until analysis.

Determination of total polyphenol content

TPC was measured by the method of Herald, *et al*. (2012). Briefly, 25 µL of test sample or standard was mixed with 25 µL Folin-Ciocalteu solution (dilute 1:1 v/v with distilled water) and 75 µL of distilled water. The mixture was left at room temperature for 6 min before the addition of 100 µL of sodium carbonate solution (75 g/L). The reaction was kept in the dark at room temperature for 90 min and the absorbance was measured at 765 nm. Gallic acid (25-400 µg/mL) was used as a standard. The TPC values were expressed as gallic acid equivalent (GAE) (mg GAE/g DW).

Determination of total antioxidant capacity

TAC of vegetables extract were determined by FRAP and DPPH assay. FRAP reagent was prepared by mixing 0.3 M acetate buffer (pH 3.6) with 20 mM ferric chloride hexahydrate and 10 mM TPTZ in 40 mM HCl (10:1:1). Trolox (25-400 µmol/L) was used as a standard. TAC values were expressed as micromoles of Trolox per gram of dry weight (µmol Trolox/g DW). The procedure of FRAP assay followed the method of Firuzi *et al.* (2005) with minor modification. Briefly, either vegetable extract or standard was incubated with FRAP reagent in the dark at 37°C for 30 min and the absorbance at 595 nm was determined by a microplate reader.

DPPH radical scavenging activity was used to determine antioxidant activity of vegetable extracts as reported by Herald *et al*., (2012) with minor modification. Vegetable extract or standard (25 µL) was incubated with 0.3 mM DPPH solution in the dark at room temperature for 30 min and the absorbance at 517 nm was determined by a microplate reader.

Statistical analysis

All data were calculated as mean ± SD. One-way analysis of variance (ANOVA), Duncan's multiple range test and Pearson correlation were performed by SPSS (version 17.0, Chicago, IL, USA).

Results

Chlorophylls, carotenoids, total polyphenol content and total antioxidant capacity of fresh green leafy vegetables

Chlorophylls and carotenoids composition of 10 green leafy vegetables commonly consumed and grown in Thailand as herb and used to flavor many foods were shown in Table 2. Amount of Chl in fresh vegetables were found to be varied. Chl *a* was the predominant form and found at a higher content than Chl *b* in all freshly harvested vegetables. Chl *a* and *b* and total Chl (Chl *a+b*) differed between species (P < 0.001). The Chl *a/b* ratios were varied from 1.83 - 4.67. Total Chl content of all fresh vegetables were in the range of 4.07 - 15.79 mg/g DW. The highest value was found in dill (15.79 mg/g DW) followed by betel vine, garden parsley, and chinese chive, which were found to contain 14.65, 11.99, and 11.51 mg/g DW, respectively.

Chromatographic method by HPLC analysis demonstrated that green leafy vegetables were a rich source of lutein and β-carotene. β-carotene in raw vegetables varied from 0.37 mg/g DW in thorny tree to 0.80 mg/g DW in dill whereas lutein varied from 0.42 mg/g DW in vietnamese coriander to 1.37 mg/g DW in betel vine. Overall, among 10 fresh vegetables, dill was the richest source of Chl and β-carotene while betel vine was the richest source of lutein and contained high amount of total Chl.

Total polyphenol content of vegetable extracts were examined by Folin-Ciocalteu solution. As shown in Table 2, TPC of 10 green leafy vegetables were significantly different and ranged from 12.07-191.92 mg GAE/g DW. Vietnamese coriander showed the highest value at 191.92 mg GAE/g DW, followed by thorny tree, peninsular and betel vine, which were found to contain 93.69, 51.30 and 50.88 mg GAE/g DW, respectively.

Results from DPPH assay (Table 2) showed the highest antioxidant capacity in vietnamese coriander, followed by betel vine and asiatic pennywort which were found to contain 791.97, 275.34 and 267.66 µmol Trolox/g DW, respectively. For FRAP assay, vietnamese coriander also contained the highest reducing power followed by peninsular and asiatic pennywort which shown to be 414.62, 142.20 and 102.99 µmol Trolox/g DW, respectively.

Effect of domestic cooking methods on chlorophyll derivatives and carotenoids contents

Effects of blanching and streaming methods on contents of Chl *a* and b, Pheo *a* and *b* were summarized in Table 3. Changes in Chl *a* and *b* of green leafy vegetables between fresh and cooked were found to be statistically significant (P < 0.05). Alteration of Chl to Pheo during heating resulted from pheophytinization and it was found that Chl *a* was more sensitive to pheophytinization than Chl *b* during cooking. Pheo *a* and *b* contents increased in all vegetables after cooking and most of pheophytin formation occurred during streaming. Overall results showed that Chl *a* and *b* content of all vegetables were retained and/or enhanced after blanching.

Even with blanching, thorny tree, dill, chinese chive, garden parsley, and sweet basil, retained

Table 2. Chlorophylls, carotenoids, total polyphenol content and total antioxidant capacity of fresh green leafy vegetables [a]

Vegetables	Chl *a* (mg/g DW)	Chl *b* (mg/g DW)	Lutein (mg/g DW)	β-carotene (mg/g DW)	TPC (mg GAE/ g DW)	TAC by DPPH (µmol Trolox/ g DW)	TAC by FRAP (µmol Trolox/ g DW)
AP	8.80 ± 0.33^{ab}	2.63 ± 0.08^{a}	0.90 ± 0.02^{d}	0.64 ± 0.02^{f}	43.55 ± 1.95^{c}	267.66 ± 0.48^{b}	102.9 ± 4.29^{d}
BV	11.34 ± 0.21^{f}	3.31 ± 0.07^{b}	1.37 ± 0.03^{g}	0.46 ± 0.01^{a}	50.88 ± 6.11^{d}	275.34 ± 73.33^{b}	74.44 ± 3.86^{a}
CC	8.96 ± 0.37^{a}	2.55 ± 0.10^{a}	0.80 ± 0.04^{b}	0.62 ± 0.02^{c}	13.47 ± 0.41^{ab}	152.42 ± 7.98^{a}	2.05 ± 0.86^{c}
DL	12.56 ± 0.37^{g}	3.23 ± 0.12^{b}	1.11 ± 0.03^{f}	0.80 ± 0.03^{g}	40.64 ± 1.10^{c}	182.50 ± 13.34^{a}	81.67 ± 4.36^{a}
GP	9.02 ± 0.26^{a}	2.97 ± 0.08^{c}	1.03 ± 0.03^{e}	0.59 ± 0.01^{bc}	12.07 ± 1.01^{a}	33.24 ± 12.33^{d}	41.43 ± 0.80^{b}
PN	8.94 ± 0.06^{a}	2.39 ± 0.01^{d}	0.85 ± 0.01^{c}	0.58 ± 0.01^{b}	51.30 ± 2.48^{d}	200.01 ± 10.16^{a}	142.20 ± 9.13^{e}
SM	8.31 ± 0.06^{c}	1.78 ± 0.02^{f}	0.68 ± 0.02^{a}	0.55 ± 0.01^{e}	42.19 ± 2.06^{c}	75.72 ± 17.00^{cd}	75.20 ± 5.51^{a}
SB	8.50 ± 0.17^{bc}	2.08 ± 0.02^{e}	0.66 ± 0.01^{a}	0.46 ± 0.01^{a}	16.82 ± 1.22^{b}	191.63 ± 16.22^{a}	54.58 ± 2.20^{b}
TT	4.15 ± 0.67^{d}	1.17 ± 0.04^{h}	0.60 ± 0.02^{i}	0.37 ± 0.02^{d}	93.69 ± 2.32^{e}	102.15 ± 28.21^{c}	19.54 ± 3.19^{c}
VC	2.63 ± 0.09^{e}	1.44 ± 0.01^{c}	0.42 ± 0.01^{h}	0.48 ± 0.01^{a}	191.92 ± 1.87^{f}	791.97 ± 10.36^{e}	414.62 ± 31.35^{f}

[a]Data are means ± SD of triplicate experiments. Mean values in same column with different letter are statistically significant between fresh vegetables (P < 0.05). AP=Asiatic pennywort, BV=Betel vine, CC=Chinese chive, DL=Dill, GP=Garden parsley, PN=Peninsular, SM=Swamp morning glory, SB= Sweet basil, TT=Thorny tree, VC= Vietnamese coriander.

their total Chl contents. Total Chl of others (asiatic pennywort, peninsular, and vietnamese coriander) increased after blanching, except betel vine and swamp morning glory which significantly lost total Chl contents in all types of cooking process. These results were contrary to streaming method which long heat treatment clearly caused a loss of Chl (P < 0.05) in most tested vegetables.

In comparison between fresh and cooked vegetables, lutein and β-carotene contents were found to be statistically significant (P < 0.05) in most vegetables, except for chinese chive which blanching and streaming did not affect carotenoids contents. In this study, heat treatment by blanching and streaming retained and/or significantly enhanced contents of lutein and β-carotene in most cooked vegetables and only those of Betel leave significantly decreased.

Effect of domestic cooking methods on total polyphenol content and total antioxidant capacity

Effects of blanching and streaming on TPC and TAC were investigated and shown in Table 4. After cooking, TPC and TAC showed similar patterns in most vegetables which were higher in some cooked vegetables compared to fresh samples. In four out of ten cooked vegetables (peninsular, asiatic pennywort, garden parsley, and sweet basil), TPC significantly increased compared with fresh samples. The highest increase of polyphenols was found in blanched and streamed sweet basil (793 and 630%, respectively), followed by blanched and streamed garden parsley (258 and 284%, respectively). While, TPC and TAC in thorny tree, and dill significantly decreased after cooking which polyphenol reduction ranged from 14-52%. However, these two cooking methods did not affect the phenolic content of chinese chive.

Correlation between total chlorophylls, total pheophytins, carotenoids and total polyphenol contents and total antioxidant capacity

A correlation between total Chl, Pheo *a+b*, carotenoids, TPC and TAC in 10 fresh and cooked green leafy vegetables were evaluated by Pearson method and shown in Table 5. In fresh vegetables, a significant correlation was found among Chl and lutein (r = 0.880, P < 0.01), and this correlation still retained in cooked vegetables. Moreover, correlation coefficient among Chl and β-carotene content in blanched vegetables (r = 0.888, P < 0.01) was higher than those in fresh vegetables (r = 0.638, P < 0.05). For antioxidant capacities, we observed the positive correlation among TPC and TAC in both fresh and cooked vegetables. As shown in Table 5, a higher correlation coefficient was found in blanched vegetables (r = 0.965, P < 0.01 for DPPH and r = 0.959, P < 0.01 for FRAP).

Based on the effect of heating, increased pheophytin in cooked vegetables could be resulted from pheophytinization. The significant correlation between Pheo *a+b* and TAC was found in both blanched and streamed vegetables. Moreover, data showed that correlation coefficients between Pheo *a+b* and TAC were higher in streaming process than blanching method.

Table 3. Effects of domestic cooking methods on chlorophylls and carotenoids content of green leafy vegetables (mg/g DW)[a]

Vegetables/ cooking method	Chl *a*	Ch *b*	Pheo *a*	Pheo *b*	Lutein	β-carotene
Asiatic pennywort						
Fresh	8.80 ± 0.19^{a}	2.63 ± 0.04^{a}	0.30 ± 0.03^{a}	ND	0.90 ± 0.01^{b}	0.64 ± 0.01^{b}
Blanching	11.30 ± 0.39^{b}	2.89 ± 0.09^{b}	0.45 ± 0.02^{b}	ND	1.11 ± 0.03^{a}	0.77 ± 0.02^{a}
Streaming	6.51 ± 0.06^{c}	2.31 ± 0.02^{c}	1.87 ± 0.04^{c}	0.19 ± 0.01	1.05 ± 0.02^{a}	0.76 ± 0.01^{a}
Betel vine						
Fresh	11.34 ± 0.12^{a}	3.31 ± 0.04^{a}	0.13 ± 0.00^{a}	ND	1.37 ± 0.02^{a}	0.46 ± 0.01^{b}
Blanching	3.25 ± 0.42^{b}	1.39 ± 0.13^{b}	0.22 ± 0.01^{a}	ND	0.85 ± 0.03^{b}	0.42 ± 0.01^{a}
Streaming	5.42 ± 0.26^{c}	1.87 ± 0.00^{c}	1.55 ± 0.08^{b}	0.13 ± 0.00	1.04 ± 0.01^{c}	0.45 ± 0.01^{a}
Chinese chive						
Fresh	8.96 ± 0.21^{a}	2.55 ± 0.06^{a}	0.15 ± 0.00^{a}	ND	0.80 ± 0.03^{a}	0.61 ± 0.01^{a}
Blanching	8.69 ± 0.09^{a}	2.30 ± 0.02^{b}	0.26 ± 0.00^{b}	ND	0.82 ± 0.01^{a}	0.65 ± 0.02^{a}
Streaming	6.72 ± 0.18^{b}	1.89 ± 0.03^{c}	1.43 ± 0.00^{c}	0.17 ± 0.00	0.85 ± 0.01^{a}	0.65 ± 0.01^{a}
Dill						
Fresh	12.56 ± 0.21^{a}	3.23 ± 0.07^{a}	0.18 ± 0.00^{a}	ND	1.11 ± 0.02^{a}	0.80 ± 0.02^{b}
Blanching	12.80 ± 0.54^{a}	3.02 ± 0.13^{a}	0.41 ± 0.01^{b}	ND	1.44 ± 0.07^{b}	1.03 ± 0.03^{a}
Streaming	8.70 ± 0.43^{b}	2.32 ± 0.12^{b}	1.89 ± 0.01^{c}	0.19 ± 0.00	1.29 ± 0.06^{a}	0.95 ± 0.04^{a}
Garden parsley						
Fresh	9.02 ± 0.15^{a}	2.97 ± 0.05^{a}	0.43 ± 0.00^{a}	ND	1.03 ± 0.02^{a}	0.59 ± 0.01^{a}
Blanching	9.60 ± 0.17^{a}	2.85 ± 0.07^{a}	0.38 ± 0.01^{a}	ND	1.20 ± 0.02^{b}	0.73 ± 0.01^{b}
Streaming	6.28 ± 0.35^{b}	2.10 ± 0.12^{b}	1.78 ± 0.05^{b}	0.20 ± 0.01	1.03 ± 0.00^{a}	0.60 ± 0.01^{a}
Peninsular						
Fresh	8.94 ± 0.02^{a}	2.39 ± 0.00^{a}	0.16 ± 0.00^{a}	ND	0.85 ± 0.01^{a}	0.58 ± 0.00^{a}
Blanching	14.97 ± 0.02^{b}	3.74 ± 0.02^{b}	0.36 ± 0.00^{b}	ND	1.54 ± 0.01^{b}	0.97 ± 0.01^{b}
Streaming	8.29 ± 0.01^{c}	2.33 ± 0.00^{c}	2.30 ± 0.01^{c}	0.22 ± 0.01	1.22 ± 0.01^{c}	0.90 ± 0.01^{c}
Swamp morning glory						
Fresh	8.31 ± 0.04^{a}	1.78 ± 0.01^{a}	0.25 ± 0.00^{a}	ND	0.68 ± 0.01^{a}	0.55 ± 0.00^{b}
Blanching	5.51 ± 0.11^{b}	1.49 ± 0.03^{b}	0.22 ± 0.01^{b}	ND	0.88 ± 0.01^{b}	0.73 ± 0.02^{a}
Streaming	5.90 ± 0.09^{c}	1.76 ± 0.03^{a}	1.75 ± 0.01^{c}	0.17 ± 0.00	1.08 ± 0.00^{c}	0.75 ± 0.01^{a}
Sweet basil						
Fresh	8.50 ± 0.10^{a}	2.08 ± 0.01^{a}	0.17 ± 0.00^{a}	ND	0.66 ± 0.01^{b}	0.46 ± 0.01^{b}
Blanching	8.95 ± 0.27^{a}	1.90 ± 0.05^{a}	0.30 ± 0.00^{b}	ND	0.89 ± 0.04^{a}	0.68 ± 0.02^{a}
Streaming	6.22 ± 0.21^{b}	1.64 ± 0.07^{b}	1.71 ± 0.01^{c}	0.16 ± 0.00	0.89 ± 0.03^{a}	0.68 ± 0.02^{a}
Thorny tree						
Fresh	4.15 ± 0.04^{a}	1.17 ± 0.02^{b}	0.36 ± 0.01^{a}	ND	0.60 ± 0.01^{a}	0.37 ± 0.01^{a}
Blanching	3.81 ± 0.20^{a}	0.92 ± 0.05^{a}	0.32 ± 0.01^{b}	ND	0.60 ± 0.05^{a}	0.34 ± 0.02^{a}
Streaming	2.71 ± 0.18^{b}	0.84 ± 0.06^{a}	1.72 ± 0.01^{c}	0.19 ± 0.00^{b}	0.76 ± 0.04^{b}	0.36 ± 0.01^{a}
Vietnamese coriander						
Fresh	2.63 ± 0.05^{a}	1.44 ± 0.01^{a}	0.77 ± 0.04^{a}	ND	0.42 ± 0.00^{b}	0.48 ± 0.00^{b}
Blanching	8.50 ± 0.52^{b}	2.44 ± 0.08^{b}	0.63 ± 0.03^{a}	ND	0.71 ± 0.03^{a}	0.81 ± 0.03^{a}
Streaming	2.73 ± 0.36^{a}	1.42 ± 0.09^{a}	3.37 ± 0.08^{b}	0.31 ± 0.00	0.65 ± 0.04^{a}	0.74 ± 0.04^{a}

[a]Data are means ± SD of triplicate experiments. Mean values in same column with different letter are statistically significant between fresh and cooked vegetables ($P < 0.05$).

Table 4. Effect of domestic cooking methods on total polyphenol content and total antioxidant capacities of green leafy vegetables[a]

Vegetables/ cooking method	TPC (mg GAE/ g DW)	TAC by DPPH (μmol Trolox/ g DW)	TAC by FRAP (μmol Trolox/ g DW)
Asiatic pennywort			
Fresh	43.55 ± 1.95[a]	267.66 ± 0.48[b]	102.99 ± 4.29[a]
Blanching	61.97 ± 2.23[b]	341.09 ± 15.21[a]	134.03 ± 6.29[b]
Streaming	58.13 ± 0.52[c]	323.85 ± 25.43[a]	117.10 ± 6.43[c]
Betel vine			
Fresh	50.88 ± 6.11[a]	275.34 ± 73.33[a]	74.44 ± 3.86[a]
Blanching	19.59 ± 1.02[b]	146.95 ± 24.45[b]	19.33 ± 1.77[b]
Streaming	47.34 ± 8.20[a]	258.38 ± 27.21[a]	82.48 ± 3.92[c]
Chinese chive			
Fresh	13.47 ± 0.41[b]	152.42 ± 7.99[a]	2.05 ± 0.86[b]
Blanching	12.66 ± 0.18[a]	149.53 ± 2.59[a]	3.97 ± 0.97[a]
Streaming	13.19 ± 0.12[ab]	155.85 ± 3.10[a]	3.73 ± 0.23[a]
Dill			
Fresh	40.64 ± 1.10[b]	182.50 ± 13.34[b]	81.67 ± 4.36[b]
Blanching	28.47 ± 4.09[a]	155.93 ± 10.45[a]	60.16 ± 2.43[a]
Streaming	32.71 ± 4.17[a]	168.07 ± 6.02[ab]	55.75 ± 3.46[a]
Garden parsley			
Fresh	12.07 ± 1.01[b]	33.24 ± 12.33[b]	41.43 ± 0.81[b]
Blanching	43.17 ± 2.46[a]	195.62 ± 16.01[a]	167.96 ± 2.69[a]
Streaming	46.40 ± 2.56[a]	219.97 ± 33.01[a]	164.68 ± 4.75[a]
Peninsular			
Fresh	51.30 ± 2.49[b]	200.01 ± 10.16[b]	142.20 ± 9.13[b]
Blanching	80.26 ± 4.15[a]	406.20 ± 30.32[a]	252.47 ± 9.99[a]
Streaming	84.34 ± 1.86[a]	431.27 ± 11.94[a]	238.81 ± 1.39[a]
Swamp morning glory			
Fresh	42.19 ± 2.06[a]	75.72 ± 16.99[a]	75.20 ± 5.51[a]
Blanching	32.31 ± 1.51[b]	71.21 ± 13.63[a]	56.84 ± 4.70[b]
Streaming	89.54 ± 1.69[c]	344.92 ± 9.67[b]	157.80 ± 1.81[c]
Sweet basil			
Fresh	16.82 ± 1.22[a]	191.63 ± 16.22[a]	54.58 ± 2.20[a]
Blanching	150.21 ± 2.35[b]	746.18 ± 9.70[b]	405.34 ± 5.13[b]
Streaming	122.72 ± 0.87[c]	685.85 ± 14.70[c]	331.96 ± 12.60[c]
Thorny tree			
Fresh	93.69 ± 2.33[a]	102.15 ± 28.21[a]	19.54 ± 3.19[b]
Blanching	44.54 ± 1.32[b]	74.13 ± 8.37[a]	13.67 ± 4.47[ab]
Streaming	80.49 ± 2.24[c]	69.85 ± 17.36[a]	11.03 ± 1.58[a]
Vietnamese			
coriander	191.92 ± 1.86[a]	791.97 ± 10.36[b]	414.62 ± 31.35[a]
Fresh	173.87 ± 4.33[b]	836.78 ± 4.76[a]	400.42 ± 35.36[a]
Blanching	150.57 ± 3.57[c]	860.39 ± 27.13[a]	393.16 ± 26.78[a]
Streaming			

[a]Data are means ± SD of triplicate experiments. Mean values in same column with different letter is statistically significant between fresh and cooked vegetables ($P < 0.05$).

Discussion

Phytochemical data on amount of Chl, lutein, β-carotene, TPC, TAC and alteration after cooking of green leafy vegetables which wildly consumed as herb and food seasoning in Thailand have been limited when compared with other vegetables. Contents of phytochemical in plants were reportedly found to vary greatly among studies (Oboh, 2005; Turkmen *et al*., 2006; Ferracane *et al*., 2008; Miglio *et al*., 2008; Nanasombat and Teckchuen, 2009; Yuan *et al*., 2009; Chandrika *et al*., 2010; Porter, 2012). Results from our study (Table 2) showed that fresh dill contained 15.79 mg/g DW for total Chl and 0.80 mg/g DW for β-carotene. Lisiewska *et al*., (2004) reported that fresh dill leaves contained 1,440 and 50 mg/kg fresh matter for total Chl and β-carotene, respectively. For asiatic pennywort, Chandrika *et al*., (2010) reported the level of 980.2 μg/g DW for lutein content, which was in agreement with the level of 0.90 mg/g DW

Table 5. Correlation coefficients (r) between total chlorophylls (Chl *a+b*), total pheophytin (Pheo *a+b*), carotenoids, polyphenol content and total antioxidant capacities in 10 green leafy vegetables [a]

Variables	Chl *a+b*	Lutein	β-carotene	TPC	TAC by DPPH	TAC by FRAP
Pheo *a+b*						
Fresh	-0.739**	-0.559	-0.245	-0.058	-0.112	-0.293
Blanched	0.439	0.102	0.460	0.598*	0.615*	0.568*
Streamed	-0.315	-0.325	0.358	0.713*	0.727**	0.713*
Chl *a+b*						
Fresh	—	0.880**	0.638*	0.095		
Blanched	—	0.852**	0.888**	0.197		
Streamed	—	0.872**	0.698*	-0.536		
Lutein						
Fresh		—	0.389	0.140		
Blanched		—	0.784**	-0.148		
Streamed		—	0.569*	-0.488		
β-carotene						
Fresh			—	0.121		
Blanched			—	0.214		
Streamed			—	0.057		
TPC						
Fresh				---	0.850**	0.863**
Blanched				---	0.965**	0.959**
Streamed				---	0.857**	0.855**
TAC by DPPH						
Fresh					—	0.935**
Blanched					—	0.956**
Streamed					—	0.958**

[a]Data are expressed as correlation coefficients (r) among phytochemicals and antioxidant activity in fresh and cooked vegetables from two cooking methods.*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

from our study and 255.5 μg/g DW for β-carotene, lower than our results (0.64 mg/g DW). Variations in phytochemical contents of plants, as reported in several reports, are likely to depend on species, growth stage, season, age of leaves, pre and post-harvest treatment (Lisiewska *et al*., 2004; Bergquist *et al*., 2006; Mario *et al*., 2007) and difference in polarity of solvent extraction (Rahman *et al*., 2013).

Few data on Chl and carotenoids content of green leafy vegetables observed in previous studies cannot be compared. Nevertheless, we concluded that green leafy vegetables examined in this study were plants with high abundant of Chl, lutein and β-carotene similar to those in dill, (Lisiewska *et al*., 2004) spinach, (Bergquist *et al*., 2006; Turkmen *et al*., 2006) kale, (Lefsrud *et al*., 2007) wild and garden rocket (Žnidarčič *et al*., 2011). Plants with high amount of Chl also contained high level of lutein and β-carotene. Our results support the correlation among Chl and carotenoids contents (Table 5) as reported in Kale (Kopsell *et al*., 2004). This results indicated that it may be possible to use green pigment of Chl to estimate lutein and β-carotene content in green leafy vegetables.

Various studies (Nanasombat and Teckchuen, 2009; Tsai, 2011) demonstrated that natural phenolic compounds in plants were major contributors to antioxidant capacities. The significantly positive correlation was found between TPC and TAC in fresh vegetables of this study agreed with previous study which found in Desmodium species, (Tsai, 2011) kesum, turmeric and ginger (Maizura *et al*., 2010). It was reported that the highest antioxidant content was found when the plant present low Chl content (Simão *et al*., 2013). Total phenolic content of in vietnamese coriander, thorny tree, asiatic pennywort, dill and garden parsley reported by Nanasombat and Teckchuen (2009) were 52.0, 45.3, 7.8, 12.0 and

3.7 µg GAE/mg DW, which much lower than in this study. The inconsistency with previous studies may be because of difference in polarity of solvent extraction and different solvent extraction which the mixture of water and organic solvent resulted higher amount of TPC than 100% water or organic solvent (Metrouh-Amir *et al*., 2015).

The effects of two domestic cooking methods (blanching and streaming) on phytochemical compounds were examined and shown in Table 3-4. Different cooking processes affected physicochemical qualities of plants especially nutritional compounds (Oboh, 2005; Turkmen *et al*., 2006; Ferracane *et al*., 2008; Miglio *et al*., 2008; Yuan *et al*., 2009; Porter, 2012). Previous studies reported that Chl contents decreased after heat treatment as compared with raw vegetables (Turkmen *et al*., 2006; Yuan *et al*., 2009) and it was reported that blanching time varying from 120 s - 180 s showed a significant degradation of total Chl compared to raw material (Petzold *et al*., 2014). In contrast to this study, It was found that 30 s of blanching resulted in retaining or enhancing of Chl, lutein, and β-carotene in almost vegetables as shown in Table 3 and 4. This could be explained by short time of heat treatment by 30 s blanching had a little effect on the breakdown of organelles that contained pigments in plants, resulting in no degradation of phytochemical in processed sample. In summary, the difference from previous study was probably due to a rapid short-time blanching which could then be considered to be a good domestic cooking procedure for green leafy vegetables as presented in Table 5. The significant relationship between total Chl and lutein which found in fresh still remained in cooked vegetables. Moreover, the higher correlation between total Chl and β-carotene (r = 0.888, P < 0.01), and lutein β-carotene (r = 0.784, P < 0.01) was found in blanched vegetables.

Present study showed that cooking methods affected the contents of chlorophylls, particularly streaming procedure that caused a great loss of Chl *a* and Chl *b* in almost vegetables except blanched vietnamese coriander. Pheophytin derivatives content increased in all vegetables after cooking and found most in streamed vegetables. Non-phenolic fraction which was identified as chlorophyll-related compounds, Pheo *a* and *b*, have been reported as a potent antioxidant compound by reducing reactive oxygen species (DPPH) (Higashi-Okai *et al*., 2000). Moreover, pheophytins demonstrated scavenging capacities and dose-dependent effect by DPPH assay, which antioxidant activity of pheophytins were stronger significantly than that of Chl (P < 0.05) (Hsu *et al*., 2013). As found in both blanched and streamed vegetables, the positive correlation between Pheo and TAC reached statistically significant (P < 0.01) (Table 5). This might be concluded that conversion of Chl to Pheo after cooking might increase antioxidant capacity, especially in streamed green vegetables. It was found in streamed thorny tree and vietnamese coriander that TAC still remained with higher value after cooking even phenolic contents significantly lost. These results demonstrated that antioxidant capacities in cooked vegetables contributed by both phenolic compounds and Pheo.

Interestingly, antioxidant power of all cooked vegetables still retained and enhanced in most cooked vegetables except blanched betel vine and dill. Cooking with water caused a leaching effect of antioxidant and this effect increased with cooking time (Porter, 2012). Enhancing of TAC in short-time heat treatment might also be contributed by phenolic compounds as we found the significant correlation (P < 0.001) between TPC and TAC in both cooking procedures. Alteration of TPC after cooking could be due to disruption of vegetables tissue by heating which released more soluble polyphenols by conversion of insoluble phenolic compounds to more soluble form. In addition, vegetables rich in polysaccharide could be responsible for this evidence (Faller and Fialho, 2009).

Conclusions

Blanching and streaming had significant effects on Chl, lutein, β-carotene and antioxidant capacities. A significant finding of this study was blanching for 30 s in hot water enhanced nutritional compounds such as Chl, lutein, β-carotene, phenolic compound and antioxidant power. While both fresh and blanched vegetables showed significantly positive correlations between phytochemicals and between phytochemicals vs. antioxidant capacities, the correlations in blanched vegetables were consistently greater. It could be concluded that 30 s of blanching better preserved and also promoted nutrient and antioxidant properties in green leafy vegetables. Moreover, conversion of Chl to Pheo during streaming also enhanced antioxidant activity in vegetables.

Acknowledgments

This work was supported in part by grants from the Research Scholarship Year 2010 from the Graduate School, Khon Kaen University, Regional Medical Sciences Center 10 Ubon Ratchatani and National Research Council of Thailand.

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