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Effect of water cooking on antioxidant capacity of carotenoid-rich vegetables in Taiwan

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ABSTRACT

Carotenoid-rich green leafy vegetables including cilantro, Thai basil leaves, sweet potato leaves, and choy sum were selected to evaluate the effects of water cooking or boiling on their total carotenoid content (TCC), total phenolic content (TPC), and total antioxidant capacity (TAC). The percentage inhibition of peroxidation (%IP), Trolox equivalent antioxidant capacity (TEAC), and metal-chelating effect were used to evaluate TAC. The results indicated that TCC reached the maximum after boiling cilantro, Thai basil leaves, and sweet potato leaves for 10 minutes, 5 minutes, and 5 minutes, respectively, and choy sum remained almost unchanged after 30 minutes of boiling. Boiling cilantro and choy sum had a negative effect on their TPC, whereas there was a significant increase in TPC of Thai basil leaf and sweet potato leaf at 1 minute and 5 minutes of boiling, respectively. During water cooking, TAC of the vegetables did not demonstrate a consistent trend. However, TCC was a vital contributor to %IP, whereas TPC showed a strong association with TEAC. Our findings suggest that a boiling time of ≤ 5 minutes would be better for preserving or enhancing TCC and TPC as well as revealing a higher %IP, TEAC, or metal-chelating effect for the four vegetables investigated in this study.

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1. Introduction

Green leafy vegetables are rich in carotenoids, which have several proven and postulated health benefits [1]. Carotenoids mostly consumed in general diets are α -carotene, β -carotene, lutein, zeaxanthin, neoxanthin, violaxanthin, and lycopene. Among these carotenoids, β -carotene is the most important

provitamin A, which plays a pivotal role in human visual health promotion. Besides, a wide range of non-vitamin A active carotenoids, including lutein and zeaxanthin, are the primary components of human pigments found in both the macula and lens, which act as powerful antioxidants and high-energy blue light scavengers [2]. Lutein, zeaxanthin, and β -carotene are all powerful antioxidants. Higher blood

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concentrations of carotenoids obtained from plant foods show potential actions against cancer, stimulate the immune system, prevent cardiovascular diseases, and protect against age-related macular degeneration and cataract [3]. Consumption of vegetables containing higher amounts of these carotenoids is beneficial, therefore, our previous study analyzed the total carotenoid content (TCC; i.e., lutein, zeaxanthin, and β -carotene) in 25 fresh vegetables commonly consumed in Taiwan, and found cilantro, Thai basil leaves, sweet potato leaves, and choy sum contain the highest amount of TCC [4].

Although most vegetables are cooked prior to consumption, evidence is emerging that *in vivo* bioavailability of many bioactive compounds is enhanced as vegetables are cooked. Fresh raw plants can be prepared in various ways of domestic cooking to transform them into a ready to eat dish. Besides, cooking gives the final product more pleasant sensory characteristics, as well as being more digestible and microbiologically safer to eat. However, the nutritional value is increased or decreased depending on the cooking method. The cooking method not only affects the nutritional composition of the food, but also the level of available bioactive compounds. In Chinese cuisine, green leafy vegetables are often boiled, stir-fried, or deep-fried. According to Kao et al [4], both stir frying and deep frying caused a marked decrease in TCC, whereas water cooking preserved the majority of the carotenoids in vegetables. In the case of frying, a substantial loss of TCC could be explained by the lipophilic nature of carotenoids, with the consequent leaching of these molecules into oil and their instability at the high temperature during frying. On the contrary, no lutein was detected in the water after boiling vegetables in it [5]. Moreover, many studies have reported the increase of TCC in boiled vegetables as a consequence of better extraction efficiency [6], and therefore water cooking might be the better domestic cooking method for carotenoid-rich green leafy vegetables.

It is well known that most phenolic compounds are water soluble and are usually lost during boiling. Phenolics are also sensitive to other heat treatments such as frying [7], baking [8] and microwaving [9]. Nevertheless, an increase in total phenolics was found in boiled green beans, peppers, broccoli [10], tomatoes [11], and blanched artichokes [12]. The possible reasons were easier liberation of phenolics from softened matrix, and/or inactivation of polyphenol oxidase, which could be responsible for the loss of phenolics in raw plants [12]. It was suggested that a cooking method for each vegetable would be preferred to preserve the nutritional and physicochemical qualities [7]. Regarding carotenoid-rich green leafy vegetables such as cilantro, Thai basil leaves, sweet potato leaves, and choy sum, water cooking or boiling might be considered a suitable cooking method to preserve or even enhance the nutritional qualities of vegetables [4].

The purpose of this study was to investigate the effects of water cooking on the changes in TCC, total phenolic content (TPC), and total antioxidant capacity (TAC) for carotenoid-rich vegetables including cilantro, Thai basil leaves, sweet potato leaves, and choy sum. Based on this study, the optimal cooking time to minimize the loss of bioactive compounds will be defined and the relationship between each antioxidant component and antioxidant capacity will thus be established.

2. Materials and Methods

2.1. Chemicals

The (all-E)-isomers of the lutein and β -carotene standards were products of Sigma Chemical Co. (St. Louis, MO, USA). The (all-E)-isomer of the zeaxanthin standard was purchased from Extrasynthese (Genay, France). The internal standard, β -apo-8-carotenal, was the product of Fluka Analyticals (Seelze, Germany). All extraction and high-performance liquid chromatography (HPLC) solvents (LiChrosolv, gradient grade) were from Merck KGaA (Darmstadt, Germany). All other chemicals used were of analytical grade and from Sigma Chemical Co.

2.2. Preparation of vegetables

The vegetables investigated were Thai basil leaf (*Ocimum basilicum* var. *thyrsoflora*), cilantro (*Coriandrum sativum* L.), choy sum (*Brassica rapa* var. *parachinensis*), and sweet potato leaf (*Ipomoea batatas* L.). The preparation process was as described previously [4]. All vegetables were purchased from a local market in Taichung, Taiwan. Within 24 hours the samples were prepared in the common manner (e.g., hard stems and blemishes removed) and then washed, wiped, cut into almost equal small pieces (~ 1.0 cm²), and mixed well.

2.3. Boiling of vegetables

The boiling of vegetables was carried out as described previously [4]. Two hundred milliliters of water was brought to boil in a 500-mL beaker. The beaker was covered to prevent water loss due to evaporation. Ten grams each of cilantro, Thai basil leaves, sweet potato leaves, and choy sum were boiled separately for 0 minutes, 1 minute, 5 minutes, 10 minutes, 20 minutes, and 30 minutes. The samples were drained for 30 seconds. After boiling, all the samples were cooled rapidly on ice, packed in polyethylene bags with nitrogen gas added and kept at 4°C. The determination of TAC and antioxidant compounds was carried within 24 hours after cooking.

2.4. Determination of TAC and TPC

2.4.1. Preparation of vegetable extracts

Extractions were carried out as previously described by Ferracane et al [12], with a few modifications. The extraction procedures were carried out under dim light to prevent photodegradation. Ten grams each of raw or boiled samples in a centrifuge tube (50 mL) with a stopper were homogenized under nitrogen flow for 1 minute, with 40 mL 60% ethanol, in a homogenizer (Model 01-01200; PRO Scientific, Oxford, CT, USA) and centrifuged at 1000g for 5 minutes, and the supernatant was collected. The precipitate was re-extracted by adding 20 mL 60% ethanol, homogenizing for a further 1 minute, and centrifuged at 1000g for 5 minutes. This ethanol extraction was repeated four times, and the resulting supernatants were combined and dried under vacuum, at a temperature below 30°C. The residue was then redissolved by ultrasonic agitation to a final volume of 20 mL in 60% ethanol, and this ethanol extract was used for the following analyses.

2.4.2. Ferric thiocyanate method to determine the percentage inhibition of peroxidation

The percentage inhibition of peroxidation (%IP) of the ethanol extracts of the uncooked and cooked vegetables was determined by the ferric thiocyanate method [13], with some modifications. The vegetable extracts were further diluted with ethanol to 50 times their volume. The mixture of 0.5 mL diluted vegetable extract, 2.5 mL of linoleic acid solution (0.02 M) in absolute ethanol, and 2.0 mL phosphate buffer (0.2 M, pH 7.0) was mixed in a test tube with a screw cap and then incubated in an oven at 37°C in the dark. To 0.1 mL of this mixture, 4.7 mL 75% ethanol and 0.1 mL ammonium thiocyanate (30 g/100 mL) were added. At exactly 3 minutes later, 0.1 mL 0.02 M ferrous chloride in hydrochloric acid (3.5 g/100 mL) was added to the reaction mixture, and the absorbance was read at 500 nm every 24 hours until the absorbance of the control reached a maximum. The control and the standards were subjected to the same procedures as the sample. However, for the controls, only the extracting solvent was added, whereas, for the standards, 0.5 mL vegetable extract was replaced with 0.5 mL of 50 µg/mL butylated hydroxytoluene (BHT), vitamin C, and vitamin E. %IP was calculated by the following equation: %IP = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control reaction and A_1 is that in the presence of the samples or BHT, vitamin C, or vitamin E.

2.4.3. Trolox equivalent antioxidant capacity assay

The antioxidant capacity was determined using the procedures described by Arnao et al [14] and Scalzo et al [15], with a few modifications. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) is a chromogen (colorless) that is converted to blue-green colored ABTS⁺ radical cation by an oxidative reagent. ABTS⁺ can also be reduced to its colorless form by an antioxidant. The absorbance was measured spectrophotometrically at 734 nm as a function of concentration. The scavenging percentage of ABTS⁺ was calculated relative to Trolox, a water-soluble analog of vitamin E used as an antioxidant standard. The ABTS⁺ solution was prepared by mixing 0.25 mL 1 mM ABTS, 0.25 mL 0.5 mM H₂O₂, 0.25 mL peroxidase (44 U/mL) and 1.5 mL deionized water. The mixture was then incubated at 25°C for 1 hour. After the addition of 0.25 mL vegetable extract, the mixture reacted at room temperature for 10 minutes, and the absorbance was read at 734 nm. Quantification was based on the standard curve of Trolox. Antioxidant capacity was presented as micrograms of Trolox equivalent per gram of fresh sample weight.

2.4.4. Metal chelating effect – Fe²⁺–ferrozine chelating assay

The chelating activity on Fe²⁺ was measured as reported by Dinis et al [16], with some modifications. Vegetable extract (0.25 mL) was added to a mixture of 25 µL FeCl₂ (2 mM) and 0.8 mL absolute ethanol. The reaction was initiated by the addition of 0.05 mL 5 mM ferrozine solution. The mixture was shaken vigorously and then allowed to rest at room temperature for 10 minutes, and the absorbance at 562 nm was read. The results were given as a percentage of inhibition of ferrozine–Fe²⁺ complex formations. The results were calculated using the formula in Equation 1. In this formula, the control and the standards were subjected to the same procedures as the sample, except that only the extracting solvent was added for the control, whereas for the standards, 0.25 mL

vegetable extract was replaced with 0.25 mL of 50 µg/mL or 100 µg/mL EDTA.

$$\text{Metal – chelating effect (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad [1]$$

where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the samples or standards.

2.4.5. Determination of TPC

TPC was determined by the Folin–Ciocalteu colorimetric method described by Gao et al [17], with some modifications. Fifty microliters of each ethanol extract of uncooked or cooked vegetables was mixed with 0.5 mL Folin–Ciocalteu reagent and 1 mL deionized water, and incubated at room temperature for 3 minutes. Following the addition of 2.5 mL sodium carbonate (20 g/100 mL) to the mixture, TPC was determined after 1 hour incubation at room temperature. The absorbance of the resulting blue color was measured at 765 nm. Quantification was based on the standard curve of gallic acid. The results were expressed as micrograms of gallic acid equivalents per gram of fresh sample weight.

2.5. Determination of TCC

The determination of carotenoids content was carried out as described previously [4], except the extracting solvent was replaced with 60% ethanol. The analytical chromatographic separations were performed on an HPLC system (LC-10AT; Shimadzu, Kyoto, Japan) equipped with a photodiode array detector (SPD-M 20A; Shimadzu). The data were processed using the Shimadzu CLASS-VP version 6.1 chromatography data system. All extracts were analyzed in duplicate over a reversed-phase 5 µm polymeric C30 carotenoid column (4.6 mm internal diameter × 250 mm; YMC Inc., Waters, Milford, MA, USA). A linear HPLC gradient was composed of mobile phase (A), a mixture of methanol and methyl tertiary butyl ether (85:15, v/v) and (B), a mixture of methanol and methyl tertiary butyl ether (6:94, v/v). After injecting 10 µL of sample, (B) was increased from 0% to 100% over 45 minutes, at the expense of (A) from 100% to 0%. The flow rate was 1 mL/min, and the HPLC runs were monitored at 450 nm. Carotenoid concentration in samples was quantified using the calibration curves. The calibration curves for (all-E)-isomers of lutein, zeaxanthin, and β-carotene were obtained by area measurement of pure reference compounds at various concentrations. Regarding the (Z)-isomers of carotenoids, the (Z)-isomers were quantified based on the calibration curves of their corresponding (all-E)-isomers of carotenoid standards because their extinction coefficients were similar [18]. A fixed concentration of β-apo-8-carotenal (5 µg/mL) was used as an internal standard to adjust the carotenoids content in raw and thermally processed vegetables. Total carotenoids content was obtained by summing each carotenoid isomer evaluated.

2.6. Statistical analysis

All results were statistically analyzed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). Differences between variables were tested for significance using analysis of variance. All data were the mean values of three replicates and the data were analyzed for significance at the $p < 0.05$ level.

3. Results and discussion

3.1. Effect of boiling time on antioxidant compounds

It is well documented that both phenolic compounds and carotenoids are important natural antioxidants in vegetables. Therefore, it is necessary to determine their concentration and antioxidant capacity and to make comparisons among vegetables. It has been reported that the solvent used in extraction would be very important. Variations in phenolic content have been observed in extracts from the same vegetable, but using different extracting solvents [19]. Mao et al [20] observed that the phenolic content and antioxidant capacity of day lilies were higher after ethanol extraction than in a water extract. Thus, considering the antioxidant capacity, the ethanol extracts from both raw and boiled vegetables were used in the present study. Fig. 1 illustrates the effects of boiling time on the TCC and TPC in extracts of different vegetables. The TCC of the vegetables except choy sum increased

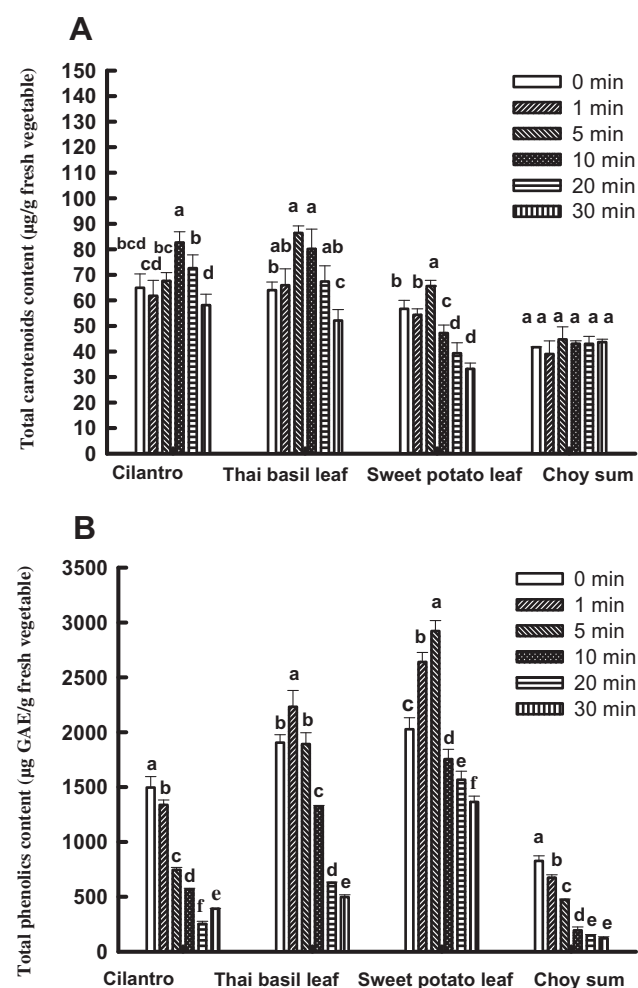


Fig. 1 – Effects of boiling time on total carotenoid content (A) and total phenolic content (B) in extracts of different vegetables. Data are means \pm standard deviations of three replicate determinations. Columns with different letters for each vegetable are significantly different ($p < 0.05$).

with the increase in boiling time until it reached a maximum and then decreased progressively. As for the choy sum, boiling did not affect TCC in its extract after 30 minutes cooking (Fig. 1A). This result suggests that cooking has different effects on different vegetables. As noted by Miglio et al [7], the same cooking procedure might affect the antioxidants of various vegetables differently. Several studies have reported that the increase of TCC in boiled vegetables may be partly caused by the destruction of cell walls and subcellular compartments upon boiling, which causes the release of antioxidants [21]. Hence, the structural matrix of the cell wall is likely to be the determining factor controlling the ability of cells to retain or degrade carotenoids and other phytochemicals [22].

The effect of boiling vegetables on TPC in their extracts is shown in Fig. 1B. The boiling of cilantro and choy sum had a negative effect on their TPC. It was evident that the loss of TPC increased with prolonged boiling time. In the cases of Thai basil leaf and sweet potato leaf, there was a significant increase in TPC in their extracts at the initial stage of boiling for 1 minute and 5 minutes, respectively. Subsequently, the TPC decreased as the boiling time increased. The initial increase in TPC might have been due to the liberation of phenolics from the intracellular proteins, changes in plant cell structure, matrix modifications, or the inactivation of the polyphenol oxidase [12]. By contrast, as suggested by Miglio et al [7], water cooking has a detrimental effect on polyphenols in vegetables, resulting in a complete loss of phenolic compounds, likely due to diffusion into the boiling water. In addition, Wachtel-Galor et al [23] found that with a boiling time of 5–10 minutes, the TPC in vegetable extracts decreased whereas it increased in cooking water.

In addition to carotenoids and phenolic compounds, green leafy vegetables are recognized as an excellent source of vitamin C, which appears to be the bioactive phytochemical most affected by boiling, because of its high water solubility and low resistance to heat treatment. The loss of vitamin C during boiling was primarily caused by leaching into the cooking water, because this vitamin was found in the cooking water [24]. In addition, the loss could be partially ascribed to thermal and enzymatic degradation [25]. Chuah et al [24] concluded that using minimal cooking water and reducing cooking time resulted in improved ascorbic acid retention.

Hence, the present study demonstrated that a boiling time of ≤ 5 minutes would be better for preserving or enhancing the antioxidants, for both carotenoid and phenolics in the four vegetables investigated in this study.

3.2. Effect of boiling time on %IP

The antioxidants present in the vegetable extracts may have various functional properties, such as free radical scavenging, peroxide decomposition, and metal chelating, or any combination thereof [26]. Chen et al [26] found that there were many differences among the test systems for determining the antioxidant capacity, and therefore they recommended using at least two different methods. In the present study, three assays based on different chemical mechanisms were used, namely the ferric thiocyanate test for the %IP, the Trolox equivalent antioxidant capacity (TEAC), and the metal-chelating effect. Fig. 2 illustrates the effects of boiling time on the %IP and TCC

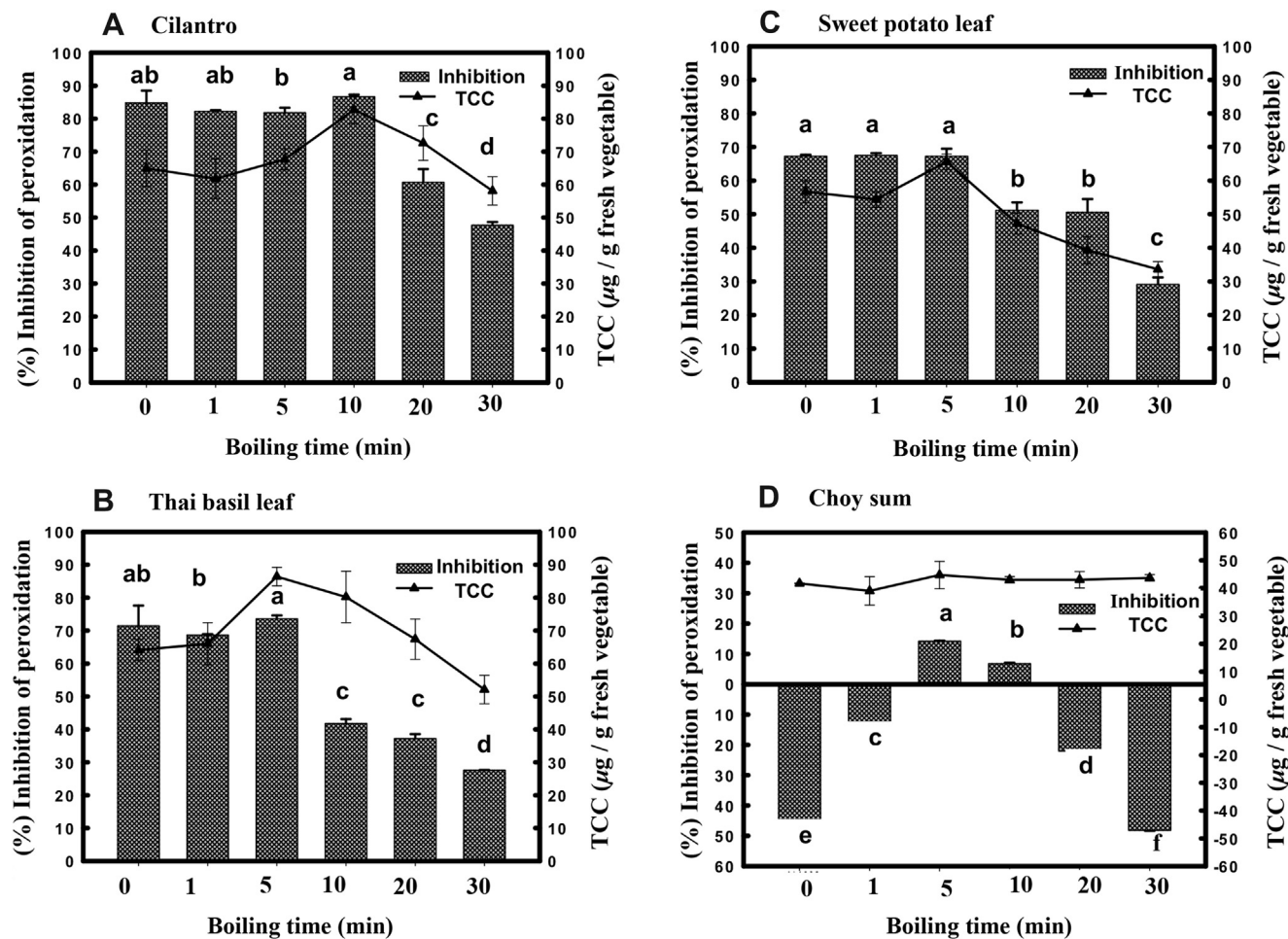


Fig. 2 – Effects of boiling time on the percentage inhibition of peroxidation and total carotenoid content in extracts of cilantro (A), Thai basil leaf (B), sweet potato leaf (C), and choy sum (D). Data are means \pm standard deviations of three replicate determinations. Columns with different letters for each vegetable are significantly different ($p < 0.05$).

in extracts of different vegetables during 30 minutes. A similar trend was observed among the extracts from cilantro, Thai basil leaves, and sweet potato leaves. Taking sweet potato leaf as an example (Fig. 2C), the %IP remained basically unchanged, and then declined steadily after 5 minutes of boiling. The duration required for both %IP and TCC to start decreasing for cilantro, Thai basil leaves, and sweet potato leaves was 10 minutes, 5 minutes, and 5 minutes, respectively (Fig. 2A–C). These findings revealed that carotenoids might play a crucial role in this antioxidant capacity, even though TCC did not correlate well with %IP. This could be attributed to other antioxidants such as phenolics, or possibly a combination of individual antioxidants, that exhibit synergistic effects. Choy sum, when only cooked for 5 minutes and 10 minutes, showed a positive %IP in their extracts, whereas a pro-oxidant effect was observed for other choy sum extracts (Fig. 2D). In addition, there was no significant difference in TCC, whereas TPC exhibited a time-dependent decrease upon 30 minutes boiling of choy sum (Fig. 1). This indicates that phenolics in choy sum extract might play an inhibitive role in lipid peroxidation, as previously stated. In addition, Brassica vegetables such as choy sum have been found to be rich in minerals [27].

Among the green leafy vegetables, the Brassica group are an excellent source of minerals, accumulating high levels of Ca, Fe, Cu, Mg, K, Zn, Na, and Mn in their plant tissue [28,29]. Thus, the minerals such as Fe, Cu, and Zn in the choy sum tissue might induce lipid oxidation forming peroxides and result in a negative %IP. By contrast, the increase of %IP resulted from antioxidants (e.g., carotenoids and phenolics) was less than the decrease of %IP due to the formation of lipid peroxides induced by the metal ions in the choy sum tissue.

3.3. Effect of boiling time on TEAC

Fig. 3 shows the effects of boiling time on TEAC and TPC in extracts of different vegetables. The TEACs for uncooked vegetables were as follows: sweet potato leaf > Thai basil leaf > cilantro > choy sum, with values of 1540 μg Trolox/g fresh vegetables, 1371 μg Trolox/g fresh vegetables, 1303 μg Trolox/g fresh vegetables, and 1260 μg Trolox/g fresh vegetables, respectively. This study demonstrated that the same cooking practices produce different effects on the antioxidant capacity of extracts from different vegetables. For choy sum, boiling led to a continuous decrease in its TEAC during the 30

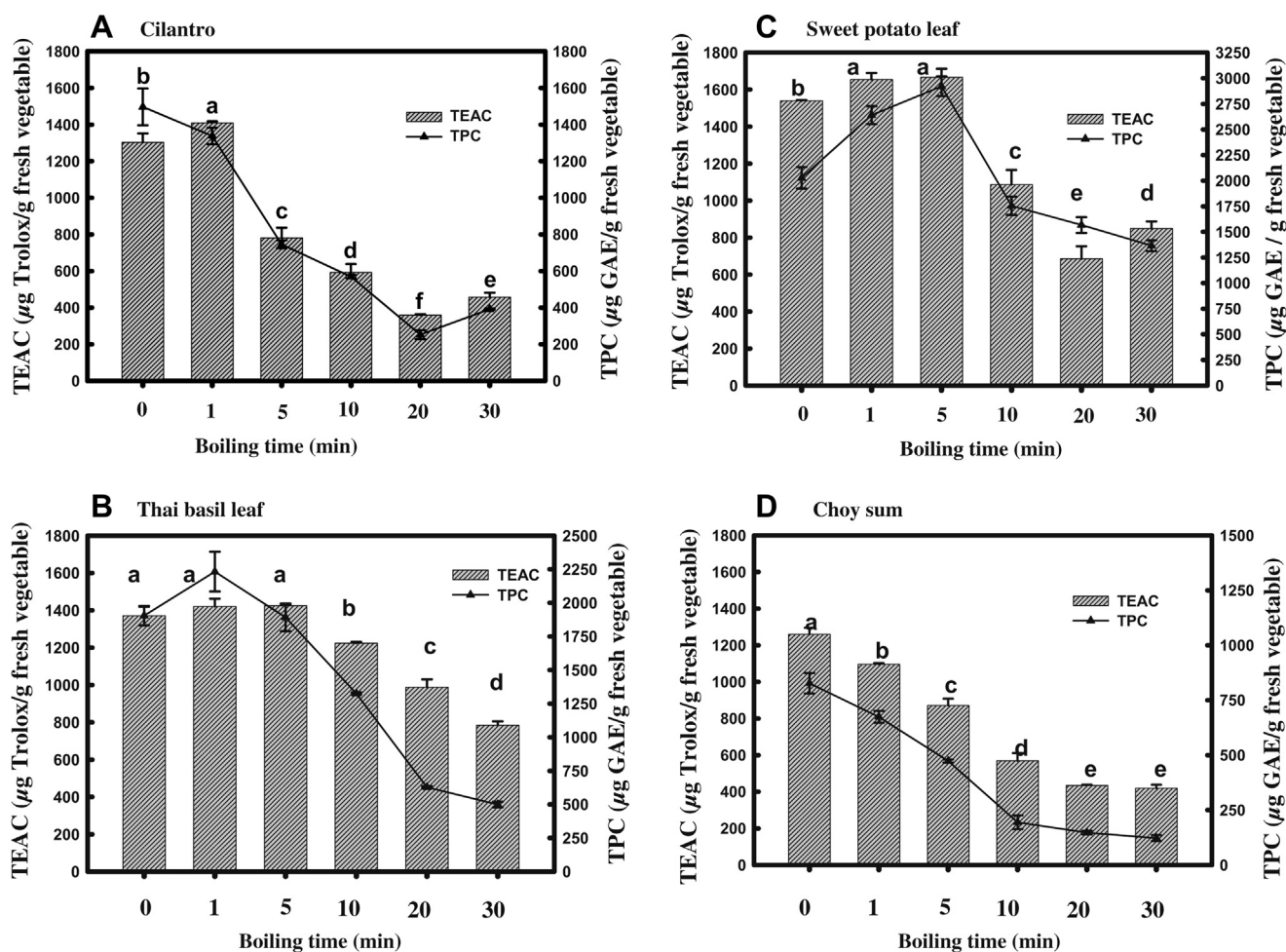


Fig. 3 – Effects of boiling time on Trolox equivalent antioxidant capacity and total phenolic content in extracts of cilantro (A), Thai basil leaf (B), sweet potato leaf (C), and choy sum (D). Data are means ± standard deviations of three replicate determinations. Columns with different letters for each vegetable are significantly different ($p < 0.05$).

minutes of boiling. However, in the case of cilantro and sweet potato leaf, the TEAC values increased at the initial stage of boiling and then decreased steadily. For Thai basil leaf, the antioxidant capacity remained basically unchanged for the first 5 minutes of boiling, which was then followed by a progressive decrease as the boiling time continued. The results also indicate that the TEAC values decrease with the decline in phenolic compounds during cooking. These findings show that long-term boiling has a detrimental effect on TEAC antioxidant capacity and phenolic concentration of the vegetables. Furthermore, a strong association was observed between the TEAC and TPC. The correlation coefficients (R^2) between the TEAC value and TPC for cilantro, Thai basil leaf, sweet potato leaf, and choy sum were 0.97, 0.93, 0.81, and 0.99, respectively. These results suggested that phenolic compounds were the major contributor to the TEAC antioxidant capacity, in agreement with Stratil et al [30].

3.4. Effect of boiling time on metal-chelating effect

Fig. 4 shows that the chelating effect in uncooked vegetables ranked as follows: cilantro > sweet potato leaf > Thai basil

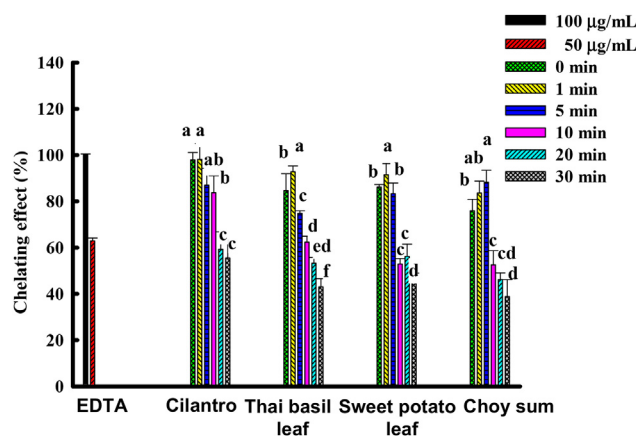


Fig. 4 – Effects of boiling time on the metal chelating effect in extracts of different vegetables. Data are means ± standard deviations of three replicate determinations. Columns with different letters within each vegetable are significantly different ($p < 0.05$).

leaf > choy sum, with values of 97.9%, 86.1%, 84.6%, and 75.9%, respectively. After boiling for 1 minute, the chelating effect in the extracts from Thai basil leaves and sweet potato leaves increased significantly to 92.7% and 91.5%, respectively; however, further cooking decreased the effect. For choy sum, the boiling time required for the chelating effect to reach a maximum was 5 minutes, and prolonged heating decreased the effect. Zhou and Yu [31] observed that the chelating activities of vegetable extracts were correlated with their TPC, however, in our research, the chelating activity was not well correlated with TPC, except for Thai basil leaf, because the correlation coefficients (R^2) between the chelating effect and TPC for cilantro, Thai basil leaf, sweet potato leaf, and choy sum, were 0.79, 0.94, 0.73, and 0.69, respectively. Yuan et al [32] demonstrated that compounds with structures containing two or more of the following functional groups: $-\text{OH}$, $-\text{SH}$, $-\text{COOH}$, $-\text{PO}_3\text{H}_2$, $\text{C}=\text{O}$, $-\text{NR}_2$, $-\text{S}-$, and $-\text{O}-$, could show metal chelation activity. Thus, molecules including organic acids such as citric, malic, tartaric, oxalic, succinic, lipoic, and phytic acid are noted to chelate transition metal ions [32]. Accordingly, the lack of significant correlation between TPC and the chelating effect may be related to the existence of chelating molecules other than phenolics in the extracts of vegetables. In addition, the increase of chelating effect at the beginning of the boiling stage can be attributed to the liberation of chelating molecules from the food matrix. This is based on the fact that, as stated previously [21], cooked vegetables have a better extractability. However, the subsequent loss in chelating effect is likely due to the fact that the hydrophilic chelating compounds, such as phenolic, citric, tartaric, and succinic acid, leach into the boiling water [23], and this leaching increases with the cooking time.

4. Conclusions

The present study indicates that boiling time has a determining effect on the levels of bioactive components and antioxidant capacities of vegetables. For carotenoid-rich green leafy vegetables, boiling might be the most suitable cooking treatment. Nevertheless, it is vital to use less boiling time to minimize the loss of water-soluble antioxidants, such as phenolics and vitamin C. Moreover, vegetable species exert a strong influence on the effect of boiling time upon antioxidant capacities. In this investigation, the four vegetables did not demonstrate a consistent trend under the same cooking conditions, which could be ascribed to morphological features, specific components, and antioxidants profile of vegetables.

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