

ผลจากการปรุงประกอบอาหารและการย่อยอาหารในหลอดทดลองต่อปริมาณสารประกอบฟีนอลิกและฤทธิ์การต้านอนุมูลอิสระของพืชจากป่าชุมชน

The Effect of Traditional Cooking and *In Vitro* digestion on Total Phenolic and Antioxidant Activity of Selected Thai Local Plants

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บทคัดย่อ

การศึกษาผลกระทบต่อปรุงโดยการต้มและการย่อยอาหารในหลอดทดลองของพืชจากป่าชุมชนที่ได้คัดเลือก 4 ชนิด ได้แก่ ลูกสำรอง ใบกุ่มน้ำ ใบชะมวง และใบชะครามต่อปริมาณสารประกอบฟีนอลิก ฟลาโวนอยด์ และฤทธิ์ต้านอนุมูลอิสระ (DPPH, FRAP และ ABTS) พบว่าก่อนต้มใบชะมวงมีฤทธิ์ในการต้านอนุมูลอิสระ DPPH สูงสุด ใบชะครามมีฤทธิ์ในการต้านอนุมูลอิสระ ABTS สูงสุด และใบกุ่มน้ำมีฤทธิ์ในการต้านอนุมูลอิสระ FRAP สูงสุด หลังผ่านการต้ม 60 นาที พบว่าใบกุ่มน้ำมีฤทธิ์การต้านอนุมูลอิสระสูงสุด นอกจากนี้ปริมาณสารประกอบฟีนอลิกและฟลาโวนอยด์ของใบกุ่มน้ำ ใบชะมวง และใบชะครามหลังการต้มมีค่าลดลง แต่อย่างไรก็ตามความสามารถในการต้านอนุมูลอิสระยังคงมีเหลืออยู่ อย่างไรก็ตามลูกสำรองที่ผ่านการต้มมีผลในเชิงบวกต่อปริมาณสารประกอบฟีนอลิกและฟลาโวนอยด์ โดยเวลาต้มที่ 60 นาที ลูกสำรองมีปริมาณสารประกอบฟีนอลิกและฟลาโวนอยด์สูงสุด หลังผ่านกระบวนการย่อยในหลอดทดลอง ลูกสำรอง ใบกุ่มน้ำ และใบชะมวงมีปริมาณสารประกอบฟีนอลิกและฟลาโวนอยด์เพิ่มขึ้นอยู่ในช่วง 4%-15% ยกเว้นใบชะคราม ฤทธิ์การต้านอนุมูลอิสระของพืชทั้งหมดด้วยวิธี DPPH และ ABTS เพิ่มขึ้น แต่อย่างไรก็ตามวิธี FRAP มีค่าลดลง จากการทดลองพบว่าปรุงอาหารและการย่อยอาหารมีผลต่อการเปลี่ยนแปลงของความเข้มข้นทางชีวภาพและความสามารถในการต้านอนุมูลอิสระของพืช

คำสำคัญ: ลูกสำรอง ใบกุ่มน้ำ ใบชะมวง ใบชะคราม การต้านอนุมูลอิสระ

Abstract

The effect of traditional cooking and *in vitro* digestion were investigated in four Thai local plants—Sum rong (*Scaphium scaphigerum* Wall.), Kum nam (*Crateva magna* Lour.), Cha muang (*Garcinia cowa* Roxb.), and Cha kram (*Suaeda maritima* L.)—on total phenolic content, total flavonoid content, and antioxidant activity (DPPH, ABTS and FRAP assay). The results showed that before cooking, Cha muang had the highest antioxidant activity in DPPH, Cha kram had the highest antioxidant activity in ABTS, and Kum nam had the highest antioxidant activity in FRAP.

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Surprisingly, after boiling for 60 minutes, Kum nam had the highest antioxidant activity in DPPH, ABTS, and FRAP. Furthermore, total phenolic and total flavonoid content after cooking of Kum nam, Cha muang, and Cha kram were decreased. However, cooked Sum rong had positive effects on total phenolic and total flavonoid content, and with 60 minutes cooking, Sum rong has the highest content in phenolics and flavonoids. Additionally, four vegetables after cooking also had antioxidant activity, consisting of DPPH, ABTS, and FRAP. After *in vitro* digestion, the total phenolic and total flavonoid content of Sum rong, Kum nam, and Cha muang were increased in range 4%-15% except for Cha kram. Moreover, the antioxidant activity in DPPH and ABTS after digestion of four plants was increased. However, the FRAP value of four plants after digestion was decreased. These results demonstrated that cooking condition and *in vitro* digestion effect on the polyphenols, flavonoids, and antioxidant activity.

Keywords: Sum rong, Kum nam, Cha muang, Cha kram, Antioxidant activity

Introduction

In recent years, people have turned more attention in health, especially the role of fruit and vegetables in human health. Several epidemiological studies have indicated that a high intake of fruits and vegetables is associated with a reduced risk of a number of chronic diseases, such as atherosclerosis and cancer¹. Dillard and German reported that grapes can help to prevent form atherosclerosis, because they are rich in resveratrol, moreover, red pepper and ginger have ability to be an anti-cancer¹. Interestingly, not only vitamins and minerals are contained in fruits and vegetables but essential amounts of polyphenols are also present, (polyphenols are a group of phytochemicals recognized as the most abundant antioxidants in our diet, such as artichoke, leek, and broccoli^{2,3}) Previous study reported that phenolic compounds play an important role in antioxidant activity and have been correlated to lower incidence for cardiovascular diseases, cancer, aging, and age-related degenerative processes, besides the continuously consumption of fruit and vegetables containing phytochemicals can help the prevention of diseases³. Therefore, natural antioxidant in vegetables are involved in defenses against many chronic diseases such as diabetes, atherosclerosis, cancer, and cardiovascular diseases⁴.

Most dietary vegetables are eaten after cooking in different ways according to the recipes and the culinary traditions of various countries. Thai local vegetables consist of many species (more than 150 species), some of them which can be consumed raw, but some of them can be consumed after cooking processes, such as boiling,

microwaving, and frying^{5,6}. There are many cooking methods that are used such as steaming, microwaving, frying, stir-frying which have both profound positive and negative effects on the quantity and quality of phytochemical compounds in comparison with fresh ones^{5,7}. These cooking processes would bring about a number of changes in physical characteristics and chemical composition of vegetables^{7,8}. Additionally, during vegetable processing, antioxidant breakdown and their leaching into surrounding water may influence qualitative changes on the antioxidant activity of the vegetables; for example the antioxidant activity of kale⁸ and white cabbage⁹ were decreased after the boiling process, however, the antioxidant activity of broccoli¹⁰ was increased after boiling. In other studies conducted on various vegetables including gourd vegetables showed that total polyphenol content and antioxidant activity of the cooked vegetables could be higher or lower in comparison to fresh vegetables^{11,12}. Nevertheless, cooking time is also correlated with the content of the reduction of antioxidant, with longer cooking time decreasing the antioxidant capacity. The effect of variation of different cooking methods especially thermal treatment have been reported that the total phenolic content and antioxidant capacity decreased in kale, spinach, cabbage and shallots¹⁰. Moreover, another study examined choy sum showed that boiling it for 30 minutes led to continuous decrease in antioxidant in TEAC method¹³.

The digestion of food in humans can affect the functional properties of bioactive compounds in foods, such as functional properties of phenolics, flavonoids and antioxidant activity after digestion^{14,15}. The change in

bioavailability of phenolics and flavonoids in dietary compounds can be caused by the transition from acidic gastric to the mild alkaline intestinal environment¹⁶. Recent research has elucidated the functional properties of phenolics, flavonoids, anthocyanins and antioxidant activity after *in vitro* gastrointestinal digestion of vegetables, fruits, and their extracts¹⁷. Bioavailability is the proportion of food ingestion that can be absorbed and utilized in the body and can be affected by many factors, for example, structural properties of the phytochemicals, pH alterations, and enzyme activity in the gastrointestinal tract¹⁷. A number of *in vitro* methods have been used to determine the bioaccessibility and/or bioavailability of individual antioxidant compounds such as carotenoids, tocopherols, or polyphenols in order to isolate compounds which remain stable and active throughout the digestion and absorption processes¹⁸⁻²². Previous studies reported that the change of pH effected the change of polyphenols and flavonoids in grape, which greater in intestine than stomach^{14,23}. Many studies reported that thermal processing can enhance the bioavailability of nutrients after digestion, for example the bioavailability of cooked carrots provide more carotenoid than when raw^{20,21}. Furthermore, cooked carrot, amaranth and fenugreek leaves with water at 15 psi for 10 min showed a significant increase in beta-carotene capacity after *in vitro* digestion²². Another study has also reported that cooked herbs showed significant increases in antioxidant activity after digestion¹⁹. Nevertheless, some cooked plants showed significantly decreased bioavailability when compared to uncooked¹⁸. However, very little information is available in the literature regarding the antioxidant activity of Thai local vegetables after traditional cooking and *in vitro* digestion. Therefore, the objectives of this study were to evaluate four selected Thai local vegetables regarding the effect of cooking and *in vitro* digestion on total phenolic and total flavonoid, and antioxidant activity.

Materials and Methods

Sample preparation

Four Thai local plants were selected randomly based on local peoples likely consumption of them as an

ingredient in their local dishes. The plants were purchased from local markets. The edible parts of plants were chopped and then blended into small pieces. All prepared samples were freeze dried and blended into fine powder before being stored in zip lock bags at room temperature for further processing.

Cooking method

The cooking method was based on previous study with minor modifications²⁴. Cooking was performed by traditional methods; boiling method using electric stove and tap water (1:20 (w/v) ratio). The raw plants (20 grams) were transferred into pots after the temperature reached the boiling point (100 °C), thereafter raw plants were cooked for 5, 10, 15, 30, and 60 min. After cooking, the plants were drained for 10 min and cooled on ice. Then, cooked plants (20 grams) were homogenized for 1 min with 100 mL water using a blender. After that, the mixed homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was collected for further analysis (total phenolic and flavonoid contents and antioxidant activities).

In vitro digestion

In vitro gastrointestinal digestion was performed by selecting the best time of selected Thai local plants which contain high amount of antioxidant content. The model of the *in vitro* digestive was based on the method of Pasukamonset and colleague with minor modifications²⁵, and the process of *in vitro* digestion will be done in 2 phases. Phase I, the gastric phase consisted of 3 mL of porcine pepsin solution (40 mg/mL in 0.1 N HCl) and the pH was adjusted to 2.0±0.1 and incubated at 37 °C for 1 h in a shaking water bath. Phase II, the small intestinal phase was started by adjusting the pH of the gastric digestion to 5.3 with a combination of 100 mM NaHCO₃ and 1.0 N NaOH. Then, adding the small intestine enzyme solution containing 3 mg/mL of pancreatin and 2 mg/mL of bile acid. The final sample was adjusted pH to 7.2±0.1 volume standardize to 20 mL with 0.1 M PBS and incubated at 37 °C for 2 h in shaking water bath. After completion of the small intestinal phase, the samples were centrifuged at 3000 g for 30 min at 4 °C. The supernatant was collected, filtered and measured for antioxidant

activities.

Total phenolic content

The total phenolic content was determined based on previous study with minor modifications²⁶. Both raw and cooked plants and after *in vitro* digestion were extracted with 100 mL of water, homogenization using blender for 1 min. The supernatants (10 µL) were mixed with 90 µL of Folin-Ciocalteu reagent and incubated in the dark at room temperature for 5 min. After incubation, 100 µL of sodium carbonate was added and incubated in the dark at room temperature for 5 min. Then, the absorbance was measured at 750 nm using a spectrophotometer. Total phenolic content was expressed as mg gallic acid/100g fresh vegetables using the linear equation based on standard calibration curve of the gallic acid (0.02-1 mg/mL).

Total flavonoid content

The total flavonoid content was determined based on previous study with minor modifications²⁶. Both raw and cooked plants and after *in vitro* digestion were extracted with 100 mL of water by homogenization using a blender for 1 min. 10 µL of supernatants were added to 1.5 mL microtube, followed by 30 µL of 5% sodium nitrate. After incubation for 5 min, 30 µL of 10% AlCl₃ solution was added. Then, 200 µL of sodium hydroxide and 240 µL of deionized water were added. The mixture was mixed using a vortex mixture and 150 µL of components were pipetted into a microplate. The absorbance was measured immediately at 540 nm using a spectrophotometer. Total flavonoid content was expressed as mg catechin/100g fresh vegetables using the linear equation based on calibration curve of catechin (0.02-1 mg/mL).

Antioxidant activities

1,1-diphenyl-2-picrylhydrazyl radicals scavenging activity (DPPH)

The free radical scavenging activity was estimated using the DPPH method with minor modifications²⁷. Both raw and cooked plants and after *in vitro* digestion were extracted with 100 mL of water by homogenization using a blender for 1 min. For antioxidant assay, 10 µL of supernatants were added to 90 µL of DPPH solution (0.2 mM in ethanol) and incubated in the dark at room

temperature for 30 min. The decrease in the solution absorbance was measured at 515 nm using a spectrophotometer. Ascorbic acid (0.002-0.1 mg/mL) was used and was prepared using the similar procedure. The DPPH radical scavenging activity was expressed as mg ascorbic acid/100g fresh vegetables.

2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)

The ABTS radical-scavenging activity was determined according to Chayaratanasin et al. with minor modification²⁷. Both raw and cooked plants and after *in vitro* digestion were extracted with 100 mL of water by homogenization using a blender for 1 min. For antioxidant assay, the radical cation (ABTS^{•+}) was prepared by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. The mixture was incubated in the dark at room temperature for 12-16 hours. For measurements, the ABTS^{•+} solution was diluted with 0.1 M PBS, pH 7.4 to an absorbance of 0.700±0.020 at 734 nm. 10 µL of supernatants were added to 90 µL of ABTS^{•+} solution and incubated in the dark at room temperature for 6 min. The decrease in the solution absorbance was measured at 734 nm using a spectrophotometer. The ABTS scavenging activity was expressed as mg Trolox/100g fresh vegetables.

Ferric ion reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) was determined according to Chayatanasin et al. with minor modification²⁷. Both raw and cooked plants and after *in vitro* digestion were extracted with 100 mL of water by homogenization using a blender for 1 min. For antioxidant assay, the FRAP reagent was prepared from 0.3 M sodium acetate buffer solution (pH 3.6), 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) in 40 mM HCl, and 20 mM FeCl₃ in a ratio of 10:1:1 (v/v/v). The supernatants (10 µL) were mixed with 90 µL of FRAP reagent and incubated in the dark at room temperature for 30 min. The decrease in the solution absorbance was measured at 593 nm using a spectrophotometer. The antioxidant activity was expressed as mg ferrous sulfate/100g fresh vegetables based on calibration curve of ferrous sulfate (0.02-1 mg/mL).

Statistical analysis

The data were expressed as mean \pm standard deviation (SD) of three replicates. The results were analyzed using one-way ANOVA. Differences between means were evaluated using Duncan's new multiple range test and statistical significance was tested at $P < 0.05$. The comparison between two groups was analyzed using paired sample t-test and statistical significance was tested at $P < 0.05$.

Results and Discussion

Total phenolic content and total flavonoid content

The total phenolic content and total flavonoid content of the four selected Thai local plants (Sum rong, Kum nam, Cha muang, and Cha kram) are described in Table 1 and Table 2. The total phenolic content (Table 1) and total flavonoid content (Table 2) of each plant were significantly different ($P < 0.05$). The total phenolic content of raw plants ranking was: Cha kram $>$ Kum nam $>$ Sum rong $>$ Cha muang. The raw state of Cha kram had the highest total phenolic content (0.78 ± 0.03 mg gallic acid/100g fresh vegetables), while Cha muang had the lowest total phenolic content (0.11 ± 0.01 mg gallic acid/100g fresh vegetables). The total flavonoid content of raw vegetables ranking was: Sum rong $>$ Kum nam $>$ Cha muang $>$ Cha kram. Raw Sum rong had the highest total flavonoid content (1.49 ± 0.04 mg catechin/100g fresh vegetables), while Raw Cha muang had the lowest total flavonoid content (0.10 ± 0.01 mg catechin/100g fresh vegetables).

After the cooking process, the total phenolic content and total flavonoid content of four local plants were significantly decreased in the increasing in cooking time. However, total phenolic and flavonoid content of Sum rong were significantly increase in the increasing in cooking time. Previous study reported that longer cooking time was correlated with the content of the reduction of total phenolic content, besides the breakdown of phenolic compounds, the change in cell structure and food matrix can also cause the loss of phenolic content during cooking^{7,10}. Nevertheless, cooking temperature,

pressure, and time can lead to the change of chemical composition and physical structure of foods in order to increase the number of phenolic contents²⁸. Another previous study reported that the increase of phenolics and flavonoids triggered by the increased level of free flavonols²⁹.

Antioxidant activity

The antioxidant activity of the four selected Thai local plants is shown in Figure 1. The DPPH radical scavenging activity in the raw plants was in range 0.59-3.07 mg ascorbic acid/100g fresh vegetables. The raw Cha muang had the highest DPPH radical scavenging activity (3.07 ± 0.04 mg ascorbic acid/100g fresh vegetables), followed by Kum nam, Cha kram, and Sum rong. The highest ABTS scavenging activity in the raw plants was discovered in Cha kram (1.52 ± 0.03 mg Trolox/100g fresh vegetables), followed by Cha muang, Kum nam, Sum rong. The FRAP value of the raw plants was in range 0.74-2.16 mg FeSO_4 /100g fresh vegetables, and the rank of FRAP value of the raw plants was: Kum nam $>$ Cha kram $>$ Sum rong $>$ Cha muang. This study showed that the same cooking method produces different effects on antioxidant activity.

The antioxidant activity—DPPH (figure 1a), ABTS (figure 1b), and FRAP (figure 1c)—after cooking was decreased in the increasing in cooking time. Previous study reported that cooking duration affected antioxidant capacity due to the change of texture, color, structure, and nutrition value¹⁰. However, Sum rong showed the increased in antioxidant activity during the cooking process in the increasing in cooking time, comparing with the raw. Previous study reported that the increased of antioxidant capacity can cause by the reduction of the matrix softening and the formation of the new molecules²⁴. Lutz and colleague reported that boiled artichokes had increased their antioxidant activity³⁰. Furthermore, different vegetables contain different compounds, which some compounds able to tolerate with thermal but some are not^{10,31}. Thus, different vegetables cooked with the same cooking method may have different effects of antioxidant capacity.

Total phenolic content and total flavonoid content after *in vitro* digestion

The effect of *in vitro* digestion on total phenolic content and total flavonoid content of the four selected Thai local plants are described in Table 3. The phenolic content after *in vitro* digestion ranking was Kum nam > Sum rong > Cha muang > Cha kram. Kum nam had the highest total phenolic content (0.50 ± 0.02 mg gallic acid/100g fresh vegetables). However, *in vitro* digestion, Kum nam showed no significantly different in total phenolic content compared to cooked Kum nam. Cha kram had no presented in total phenolic content, however, cooked Cha kram showed the significantly different compared to *in vitro* digestion cha kram. The total flavonoid content after *in vitro* digestion ranking was Sum rong > Kum nam > Cha muang > Cha kram. Sum rong had the highest flavonoid content (1.97 ± 0.05 mg catechin/100g fresh vegetables) after *in vitro* digestion, besides Sum rong also showed the significant difference between cooked and *in vitro* digestion. In contrast, Cha kram had no presented in total flavonoid content, and it showed the significant difference between cooked and *in vitro* digestion.

The total phenolic content and total flavonoid content after digestion of three plants (Sum rong, Kum nam, and Cha muang) showed the increasing levels in both contents compared to cooked plants without digestion. In addition, Sum rong, Kum nam, and Cha muang were increased in phenolic content of 15%, 5%, and 4%, respectively, and were increased in flavonoid content of 33%, 14%, and 3%, respectively. However, only Cha kram showed no presented in phenolic and flavonoid content compared to cooked state without digestion. Previous study reported that bioavailability of active compounds, such as phenolics depended on various factors including chemical state of the compound, plant matrix and interactions with macromolecules e.g. enzymatic activity¹⁷. Another study reported that after digestion, broccoli showed significant decrease in flavonoid content, because the pancreatin digestion compounds is associated with flavonoids which could not cross the dialysis membrane³². However, the chemical reaction in near-neutral conditions and the degradation or isomerization could be catalyzed

by the presence of oxygen and the transition of metal ions, which can affect the increasing of phenolic content³³.

Antioxidant activity after *in vitro* digestion

The antioxidant activity of the four selected Thai local plants after *in vitro* digestion is shown in Table 4. The DPPH radical scavenging activity after digestion was in range 0.66-3.41 mg ascorbic acid/100g fresh vegetables. Kum nam had the highest DPPH radical scavenging activity (3.41 ± 0.20 mg ascorbic acid/100g fresh vegetables), followed by Cha muang, Sum rong, and Cha kram. The percentage of the increase in DPPH radical scavenging activity of each plant after digestion was in range 35%-57%. The highest ABTS scavenging activity after digestion was discovered in Cha muang (9.69 ± 0.22 mg Trolox/100g fresh vegetables), followed by Kum nam, Cha kram, and Sum rong. The FRAP value after digestion was in range 0.26-0.35 mg FeSO₄/100g fresh vegetables, and the rank of FRAP value was: Kum nam > Cha muang > Sum rong > Cha kram.

The comparison of antioxidant activity of four plants between cooked and *in vitro* digestion, DPPH and ABTS reported a significant increase in all plants after digestion. However, FRAP value showed a significant decrease in all plants after digestion. Previous study reported that the increasing of antioxidant capacity after digestion caused by the nature of food matrix¹⁹, the change of enzymatic activity¹⁷, and chemical actions during the digestion process contribute to the extraction of bioactive molecules from the food matrix³³. In addition, the pH alteration can affect the increase of antioxidant activity, it has been reported that the antioxidant capacity of some fruits was increased after digestion because of the pH alteration, which pH can affect the change in structure of molecules³⁴. Nevertheless, FRAP is the only method that effects the decrease of antioxidant activity after digestion of four plants. The results from present investigation are in agreement with Neto and colleague who reported that the decreasing of FRAP value may due to the synergistic effect between phenolic compounds released from the plant matrix during digestion, which causes them to contribute the maintenance of reducing power activity at desirable levels³⁵.

Table 1 Effect of traditional cooking time on total phenolic content of selected Thai local vegetables

Type of Thai local vegetables	Raw (mg gallic acid/100g fresh vegetables)	Cooked (mg gallic acid/100g fresh vegetables)				
		5 min	10 min	15 min	30 min	60 min
Sum rong (<i>Scaphium scaphigerum</i> Wall.)	0.16±0.02 ^b	0.08±0.01 ^e	0.09±0.01 ^e	0.11±0.01 ^d	0.13±0.01 ^c	0.18±0.02 ^a
Kum nam (<i>Crateva magna</i> Lour.)	0.72±0.05 ^a	0.45±0.03 ^b	0.42±0.03 ^c	0.36±0.02 ^d	0.33±0.01 ^e	0.29±0.01 ^f
Cha muang (<i>Garcinia cowa</i> Roxb.)	0.11±0.01 ^a	0.05±0.01 ^b	0.03±0.01 ^c	0.03±0.01 ^c	0.02±0.01 ^d	0.02±0.01 ^d
Cha kram (<i>Suaeda maritima</i> L.)	0.78±0.03 ^a	0.26±0.02 ^b	0.22±0.02 ^c	0.20±0.01 ^d	0.18±0.01 ^e	0.16±0.01 ^f

Superscript are expressed as significant different in the same raw according to Duncan's test ($P < 0.05$).

Table 2 Effect of traditional cooking time on total flavonoid content of selected Thai local vegetables

Type of Thai local vegetables	Raw (mg catechin/100g)	Cooked (mg catechin/100g fresh vegetables)				
		5 min	10 min	15 min	30 min	60 min
Sum rong (<i>Scaphium scaphigerum</i> Wall.)	1.49±0.04 ^b	1.36±0.03 ^c	1.25±0.05 ^e	1.07±0.01 ^f	1.33±0.01 ^d	1.64±0.03 ^a
Kum nam (<i>Crateva magna</i> Lour.)	0.54±0.03 ^a	0.34±0.02 ^b	0.28±0.02 ^c	0.23±0.02 ^d	0.18±0.01 ^e	0.14±0.01 ^f
Cha muang (<i>Garcinia cowa</i> Roxb.)	0.10±0.01 ^a	0.09±0.01 ^b	0.07±0.02 ^c	0.05±0.01 ^d	0.03±0.01 ^e	0.01±0.01 ^f
Cha kram (<i>Suaeda maritima</i> L.)	0.05±0.01 ^a	0.02±0.01 ^b	ND	ND	ND	ND

ND = not detected. Superscript are expressed as significant different in the same row according to Duncan's test ($P < 0.05$).

Table 3 Effect of *in vitro* digestion on total phenolic content and total flavonoid content of selected Thai local vegetables

Type of Thai local vegetables	Time (min)	Phenolic (mg gallic acid/100g fresh vegetables)		Flavonoid (mg catechin/100g fresh vegetables)	
		Cooked	<i>In vitro</i> digestion	Cooked	<i>In vitro</i> digestion
		Sum rong (<i>Scaphium scaphigerum</i> Wall.)	60	0.18±0.02	0.33±0.03 [*]
Kum nam (<i>Crateva magna</i> Lour.)	5	0.45±0.03	0.50±0.02	0.34±0.02	0.48±0.02 [*]
Cha muang (<i>Garcinia cowa</i> Roxb.)	5	0.05±0.01	0.09±0.01 [*]	0.09±0.01	0.12±0.02
Cha kram (<i>Suaeda maritima</i> L.)	5	0.26±0.02	ND	0.01±0.01	ND

ND = not detected. * expressed as significant different in paired sample t-test ($P < 0.05$)

Table 4 Effect of *in vitro* digestion on antioxidant activity of selected Thai local vegetables

Type of Thai local vegetables	Time (min)	DPPH (mg ascorbic acid/100g fresh vegetables)		ABTS (mg trolox/100g fresh vegetables)		FRAP (mg FeSO ₄ /100g fresh vegetables)	
		Cooked	<i>In vitro</i> digestion	Cooked	<i>In vitro</i> digestion	Cooked	<i>In vitro</i> digestion
		Sum rong (<i>Scaphium scaphigerum</i> Wall.)	60	0.74±0.02	1.09±0.07 [*]	0.68±0.01	4.61±0.04 [*]
Kum nam (<i>Crateva magna</i> Lour.)	5	2.84±0.04	3.41±0.20 [*]	1.42±0.02	9.58±0.26 [*]	2.00±0.02 [*]	0.35±0.01
Cha muang (<i>Garcinia cowa</i> Roxb.)	5	1.01±0.02	1.40±0.09 [*]	1.48±0.02	9.69±0.22 [*]	1.15±0.04 [*]	0.34±0.03
Cha kram (<i>Suaeda maritima</i> L.)	5	0.26±0.02	0.66±0.04 [*]	0.44±0.03	5.50±0.05 [*]	0.41±0.02 [*]	0.26±0.02

* expressed as significant different in paired t-test ($P < 0.05$)

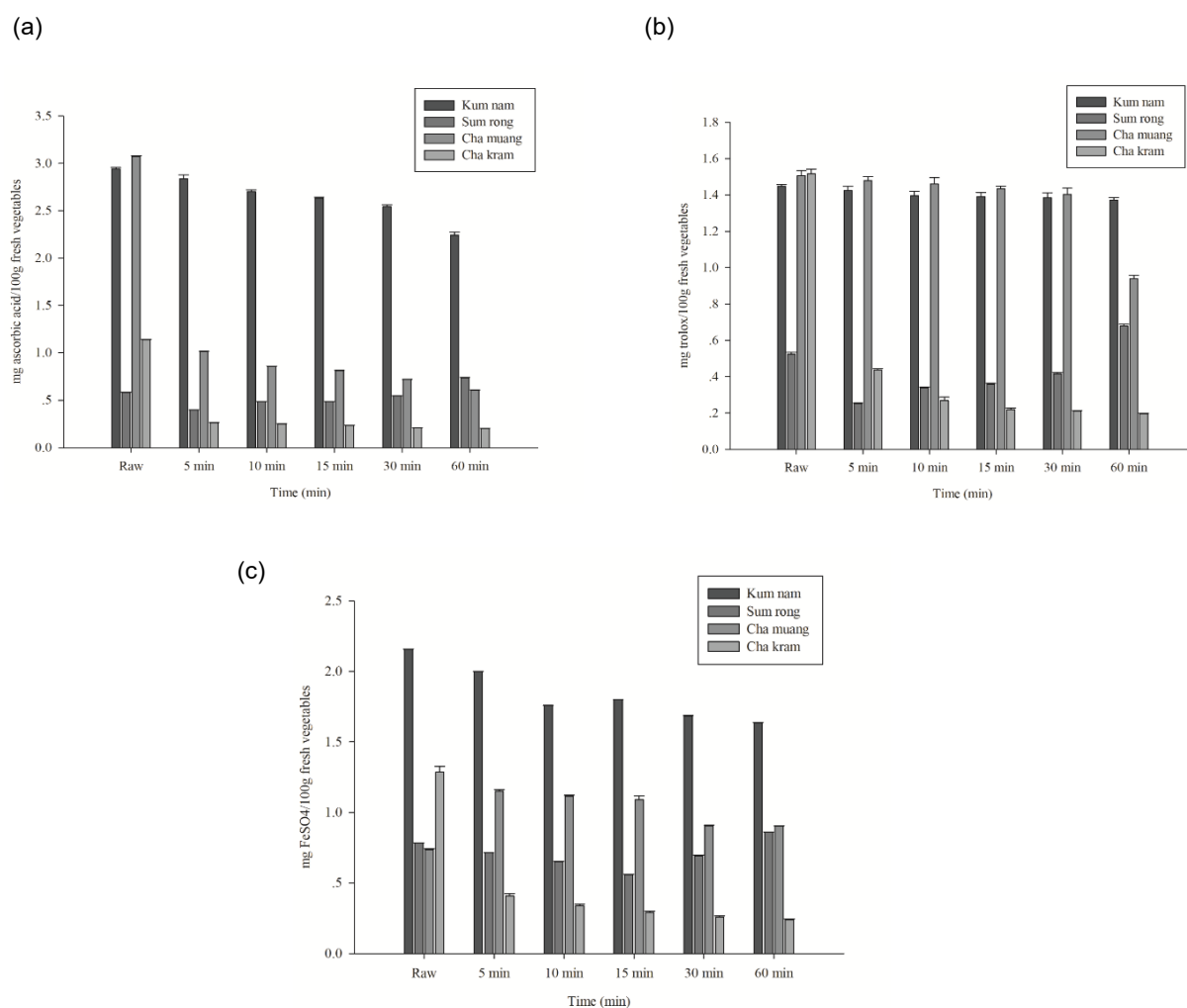


Figure 1 Effect of traditional cooking time on DPPH radical scavenging activity (a), ABTS scavenging activity (b), and FRAP activity (c) of selected Thai local vegetable

Conclusion

The findings from the present study show that the total polyphenols, flavonoid and antioxidant capacity of selected Thai local plants are significantly altered after cooking and digestion. The cooking process of four Thai local plants (Kum nam, Sum rong, Cha muang, and Cha kram) has shown an effect on total phenolic and total flavonoid content, and antioxidant activity. The phenolic and flavonoid content of Kum nam, Sum rong, and Cha muang presented after the cooking process, except Cha kram showed no content after being cooked for 10 minutes. Moreover, the DPPH and ABTS activity of all four plants was significantly increased after the cooking process, but the FRAP activity of all four plants was significantly decreased after the cooking process. After the *in vitro* digestion, the total phenolic and total flavonoid content, and antioxidant activity were raised in all four Thai local plants. In conclusion, our results demonstrated the association between cooking methods and health effects, such as phenolic, flavonoids, and antioxidant capacity after consumption. An intake of these plants may help to reduce risk of diseases caused by oxidative stress.

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References

- Dillard CJ, German JB. Phytochemicals: nutraceuticals and human health. *J Sci Food Agr*. 2000 Sep 15;80 (12): 1744-56.
- Septembre-Malaterre A, Remize F, Poucheret P. Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. *Food Res Int*. 2018 Feb 1;104: 86-99.
- Luna-Guevara ML, Luna-Guevara JJ, Hernández-Carranza P, Ruiz-Espinosa H, Ochoa-Velasco CE. Chapter 3 - Phenolic Compounds: A Good Choice Against Chronic Degenerative Diseases. In: Atta ur R, editor. *Studies in Natural Products Chemistry*. Elsevier; 2019;59: 79-108.
- Sarker U, Oba S. Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content. *Food Chem*. 2018 Jun 30;252: 72-83.
- Jiménez-Monreal AM, García-Diz L, Martínez-Tomé M, Mariscal M, Murcia MA. Influence of Cooking Methods on Antioxidant Activity of Vegetables. *J Food Sci*. 2009 Apr 1;74 (3): H97-H103.
- Nanasombat S, and Teckchuen, N. Antimicrobial, antioxidant and anticancer activities of Thai local vegetables. *J Med Plants Res*. 2009;3 (5): 443-9.
- Turkmen N, Sari F, Velioglu YS. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chem*. 2005 Dec 1;93 (4): 713-8.
- Şengül M, Yıldız H, Kavaz A. The Effect of Cooking on Total Polyphenolic Content and Antioxidant Activity of Selected Vegetables. *Int J Food Prop*. 2014 Mar 16;17 (3): 481-90.
- Faller ALK, Fialho E. The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. *Food Res Int*. 2009 Jan 1;42 (1): 210-5.
- Wachtel-Galor S, Wong KW, Benzie IFF. The effect of cooking on Brassica vegetables. *Food Chem*. 2008 Oct 1;110 (3): 706-10.
- Murador DC, Mercadante AZ, de Rosso VV. Cooking techniques improve the levels of bioactive compounds and antioxidant activity in kale and red cabbage. *Food Chem*. 2016 Apr 1;196: 1101-7.
- Tian J, Chen J, Lv F, Chen S, Chen J, Liu D, et al. Domestic cooking methods affect the phytochemical composition and antioxidant activity of purple-fleshed potatoes. *Food Chem*. 2016 Apr 15;197: 1264-70.
- Kao F-J, Chiu Y-S, Chiang W-D. Effect of water cooking on antioxidant capacity of carotenoid-rich vegetables in Taiwan. *J Food Drug Anal*. 2014 Jun

- 1;22 (2): 202-9.
14. Bouayed J, Hoffmann L, Bohn T. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. *Food Chem.* 2011 Sep 1;128 (1): 14-21.
 15. Ti H, Zhang R, Li Q, Wei Z, Zhang M. Effects of cooking and *in vitro* digestion of rice on phenolic profiles and antioxidant activity. *Food Res Int.* 2015 Oct 1;76: 813-20.
 16. Gibson RS, Perlas L, Hotz C. Improving the bioavailability of nutrients in plant foods at the household level. *P Nutr Soc.* 2007;65(2):160-8.
 17. Celep E, İnan Y, Akyüz S, Yesilada E. The bioaccessible phenolic profile and antioxidant potential of *Hypericum perforatum* L. after simulated human digestion. *Ind Crop Prod.* 2017 Dec 15;109: 717-23.
 18. Baker I, Chohan M, Opara EI. Impact of cooking and digestion, *in vitro*, on the antioxidant capacity and anti-inflammatory activity of cinnamon, clove and nutmeg. *Plant Food Hum Nutr.* 2013 Dec 1;68 (4): 364-9.
 19. Chohan M, Naughton DP, Jones L, Opara EI. An investigation of the relationship between the anti-inflammatory activity, polyphenolic content, and antioxidant activities of cooked and *in vitro* digested culinary herbs. *Oxid Med Cell Longev.* 2012.
 20. Hedrén E, Diaz V, Svanberg U. Estimation of carotenoid accessibility from carrots determined by an *in vitro* digestion method. *Eur J Clin Nutr.* 2002 Apr 4;56: 425.
 21. Hornero-Méndez D, Mínguez-Mosquera MI. Bioaccessibility of carotenes from carrots: Effect of cooking and addition of oil. *Innov Food Sci Emerg.* 2007 Sep 1;8 (3): 407-12.
 22. Van Buggenhout S, Alminger M, Lemmens L, Colle I, Knockaert G, Moelants K, et al. *In vitro* approaches to estimate the effect of food processing on carotenoid bioavailability need thorough understanding of process induced microstructural changes. *Trends Food Sci Tech.* 2010 Dec 1;21 (12): 607-18.
 23. Tagliacuzzi D, Verzelloni E, Bertolini D, Conte A. *In vitro* bio-accessibility and antioxidant activity of grape polyphenols. *Food Chem.* 2010 May 15;120 (2): 599-606.
 24. Pellegrini N, Miglio C, Del Rio D, Salvatore S, Serafini M, Brighenti F. Effect of domestic cooking methods on the total antioxidant capacity of vegetables. *Int J Food Sci Nutr.* 2009 Jan 1;60 (sup2): 12-22.
 25. Pasukamonset P, Kwon O, Adisakwattana S. Alginate-based encapsulation of polyphenols from *Clitoria ternatea* petal flower extract enhances stability and biological activity under simulated gastrointestinal conditions. *Food Hydrocolloid.* 2016 Dec 1;61: 772-9.
 26. Adisakwattana S, Ruengsamran T, Kampa P, Sompong W. *In vitro* inhibitory effects of plant-based foods and their combinations on intestinal α -glucosidase and pancreatic α -amylase. *BMC Complem Altern M.* 2012 Jul 31;12 (1):110.
 27. Chayaratanasin P, Barbieri MA, Suanpairintr N, Adisakwattana S. Inhibitory effect of *Clitoria ternatea* flower petal extract on fructose-induced protein glycation and oxidation-dependent damages to albumin *in vitro*. *BMC Complem Altern M.* 2015 Feb 18;15 (1): 27.
 28. Koç M, Baysan U, Devseren E, Okut D, Atak Z, Karataş H, et al. Effects of different cooking methods on the chemical and physical properties of carrots and green peas. *Innov Food Sci Emerg.* 2017 Aug 1;42: 109-19.
 29. Stewart AJ, Bozonnet S, Mullen W, Jenkins GI, Lean MEJ, Crozier A. Occurrence of Flavonols in Tomatoes and Tomato-Based Products. *J Agr Food Chem.* 2000 Jul 1;48 (7): 2663-9.
 30. Lutz M, Henríquez C, Escobar M. Chemical composition and antioxidant properties of mature and baby artichokes (*Cynara scolymus* L.), raw and cooked. *J Food Compos Anal.* 2011 Feb 1;24 (1): 49-54.
 31. Bernhardt S, Schlich E. Impact of different cooking methods on food quality: Retention of lipophilic vitamins in fresh and frozen vegetables. *J Food Eng.* 2006 Nov 1;77 (2): 327-33.

32. Vallejo F, Gil-Izquierdo A, Pérez-Vicente A, García-Viguera C. In Vitro Gastrointestinal Digestion Study of Broccoli Inflorescence Phenolic Compounds, Glucosinolates, and Vitamin C. *J Agr Food Chem.* 2004 Jan 14;52 (1): 135-8.
33. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Change of phenolics, carotenoids, and antioxidant capacity following simulated gastrointestinal digestion and dialysis of selected edible green leaves. *Food Chem.* 2018 Mar 7;245: 371-9.
34. Chen G-L, Chen S-G, Zhao Y-Y, Luo C-X, Li J, Gao Y-Q. Total phenolic contents of 33 fruits and their antioxidant capacities before and after in vitro digestion. *Ind Crop Prod.* 2014 Jun 1;57: 150-7.
35. Neto JLL, de Almeida TS, de Medeiros JL, Vieira LR, Moreira TB, Maia AIV, Ribeiro PRV, de Brito ES, Farias DF, Carvalho AFU. Impact of bioaccessibility and bioavailability of phenolic compounds in biological systems upon the antioxidant activity of the ethanolic extract of *Triplaris gardneriana* seeds. *Biomed Pharmacother.* 2017 Apr 1;88: 999-1007.