Antioxidant properties of selected fresh and processed herbs and vegetables

Eric Wei Chiang Chan, Yuen Ping Tan, Sze Jia Chin, Li Yi Gan, Kor Xian Kang, Chin Hong Fong, Hui Qi Chang, Yee Chern How

Faculty of Applied Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia

Submission Date: 8-8-2013

ABSTRACT

Introduction: Although the antioxidant properties (AOP) of herbs and vegetables are well known, the effects of different processing methods are poorly studied. In this study, the effects of blanching, microwave, freezing, brining, and pickling on the AOP of selected herbs and vegetables were analysed and evaluated for the first time. **Methods:** The AOP based on phenolic content (total phenolic content, total flavonoid content, and caffeoylquinic acid content), and antioxidant activity (free radical scavenging activity, ferric reducing power, and ferrous ion chelating ability) were analysed using the Folin-Ciocalteu, aluminium chloride, molybdate, DPPH radical scavenging, potassium ferricyanide, and ferrozine assays, respectively. Processing methods were blanching, microwave, freezing, brining, and pickling. **Results:** Of the 10 fresh herbs assessed, leaves of *Anacardium occidentale* displayed the strongest AOP followed by *Polygonum minus* and *Cosmos caudatus*. Although vegetables are low in phenolic content and weak in primary antioxidant activity, they are strong in secondary antioxidant activity. Blanching, microwave, and freezing treatments had variable effects on the AOP of herbs, which included significant loss or gain and relatively unchanged. Brining with salt and pickling with vinegar over a three-week period led to declines in the AOP of vegetables. The effect of pickling was more adverse as the process involved pasteurisation. **Conclusion:** Herbs are rich in phenolics with potent primary antioxidant activity. Vegetables are strong in secondary antioxidant activity. The effects of processing on the AOP largely depend on the type of herbs and vegetables.

Keywords: Herbs, Vegetables, Blanching, Microwave, Freezing, Brining, Pickling.

1. INTRODUCTION

In Malaysia, the consumption of raw herbs and vegetables is considered a traditional healthy diet because of their potential health-promoting properties.[1] Locally known as ulam, they are served as a side dish or as ingredients in specialty dishes in many Malay restaurants and eateries throughout the country. Ulam may also be blanched, sautéed, curried or fried. It is believed that the regular intake of ulam can assist in preventing degenerative diseases, delaying the sign of aging, and improving general health. When eaten raw, ulam is often dipped in hot and spicy sauce to enhance flavours.

It is generally believed that processed or cooked herbs and vegetables are lower in nutritional value than raw samples because some of their phytochemicals and enzymes tend to be degraded by heat or leached into the cooking water. Some studies have shown the effect of thermal treatment in reducing phenolic content and antioxidant activity of herbs and vegetables that were subjected to blanching, boiling, and freezing processes.[2]

In most Asian countries (e.g. China, Japan, and Korea), vegetables are preserved and consumed regularly. Yet there are hardly any studies on their antioxidant properties (AOP) compared to fresh vegetables. Although much work has been done on the effects of cooking on the

***Corresponding address:**
E-mail: chanwc@ucsiuniversity.edu.my (E.W.C. Chan)

DOI: 10.5530/fra.2014.1.7

AOP of vegetables, there is limited research on the effects of brining and pickling of vegetables.

When brining vegetables, salt is used as a preservative and softening agent.[3] Besides enhancing their flavour, salt has an antimicrobial effect due to its capacity to reduce water activity. However, brining can reduce the nutritional value of preserved vegetables because of the elimination of water-soluble vitamins and minerals. The brining process involves fermentation by lactic acid bacteria. When pickling vegetables, vinegar is used followed by pasteurisation.[4] The vinegar provides a sour and acidic taste, while the pasteurisation process destroys spoilage microbes and inhibits fermentation.

Although the AOP of herbs and vegetables are well known, the effects of different processing methods are poorly studied. In this study, the antioxidant properties of selected fresh herbs and vegetables were analysed and evaluated. For the first time, the effects of blanching, microwave, freezing, brining, and pickling on their AOP were assessed with comparison to those of fresh samples.

2. MATERIALS AND METHODS

2.1 Herbs and vegetables

Selected herbs were *Anacardium occidentale* L. (cashew), *Centella asiatica* (L.) Urban (pennywort), *Citrus hystrix* DC. (kaffir lime), *Cosmos caudatus* Kunth (wild cosmos), *Hibiscus sabdariffa* L. (roselle), *Oenanthe javanica* (Blume DC.) (water dropwort), *Piper betle* L. (betel), *Piper caninum* Blume (wild betel), *Piper sarmentosum* Roxb. (wild pepper), and *Polygonum minus* Huds. (smartweed). Selected vegetables were *Agaricus bisporus* (Lange) Imbach (button mushroom), *Allium cepa* L. (onion), *Allium sativum* L. (garlic), *Capsicum annuum* L. (chilli), *Zea mays* L. (baby corn), and *Zingiber officinale* Roscoe (ginger). The fresh herbs and vegetables were purchased from markets in Kuala Lumpur. Photographs of some of the ulam herbs studied are shown in Fig. 1.

2.2 Extraction

Fresh and processed samples (1 g) were finely powdered with liquid nitrogen in a mortar using a pestle. The fine powder of samples was extracted with 50 ml of methanol with continuous swirling (120 rpm) in an orbital shaker for 1 h at room temperature. Extracts were then filtered through Whatman No. 1 filter paper into Buchner flasks by vacuum filtration, and subsequently transferred into falcon tubes to be stored at −20°C in a freezer for further use.

2.3 Processing methods

Blanching of herbs involved immersing 1 g of sample in 50 ml of boiling water for 30 s. The blanched samples retained on the sieve were wiped dry and extracted while

Fig. 1. Photographs of some of the ulam herbs (top row) and vegetable (bottom row) studied.

the blanching water was kept for analysis of phenolic content.

Microwave treatment of herbs involved placing 1 g of sample in a microwave oven (230–240 V, 50 Hz) at the centre position for 30 s. Upon removal from the oven, water vapour formed on the beaker was removed using tissue paper to avoid re-absorption of water by the samples.

Freezing of herbs involved placing 15 g of sample in a freezer (−20°C) for 24 h. Frozen samples were taken out of the freezer and allowed to thaw for 30 min, prior to extraction.

Brining of vegetables involved immersing 100 g of sample into a 250 ml Schott bottle containing 100 ml of 15% brine solution for three weeks. Each sample was cut into 2 × 2 cm cubes and the brine solution was prepared by dissolving 15 g of sodium chloride in 100 ml of ultra pure water. The bottles were capped and stored at room temperature for three weeks. Analysis of the AOP of brined vegetables was conducted weekly.

Pickling of vegetables involved immersing 100 g of sample into a 250 ml Schott bottle containing 100 ml of vinegar. Each sample was cut into 2 × 2 cm cubes and pasteurised by heating in a water bath for 10 min at 70 °C. The vinegar was brought to boil before pouring into the Schott bottle. The bottles were capped and stored at room temperature for three weeks. Analysis of the AOP of pickled vegetables was conducted weekly.

2.4 Antioxidant assays

Fresh and processed herbs and vegetables were analysed for phenolic content (total phenolic content, total flavonoid content, and caffeoylquinic acid content), and antioxidant activity (free radical scavenging activity, ferric reducing power, and ferrous ion chelating ability). The following AOP protocols have been described in our previous publications.[5,6]

Total phenolic content (TPC) of plant samples was determined using the Folin-Ciocalteu (FC) assay. FC reagent (1.5 ml, 10 times dilution) and sodium carbonate (1.2 ml, 7.5%, w/v) were introduced into test tubes containing 300 µl of extracts. Absorbance was measured at 765 nm after incubating for 30 min in the dark. TPC was expressed as gallic acid equivalent (GAE) in mg/100 g.

Total flavonoid content (TFC) of plant samples was assessed using the aluminium chloride assay. Extracts (1 ml) were introduced into test tubes containing 4 ml of water. Sodium nitrite (0.3 ml, 5%) and aluminium chloride (0.3 ml, 10%), and sodium hydroxide (2 ml, 1 M) were then added, followed by 2.4 ml of water, making up to 10 ml. After incubating at room temperature for 10 min, absorbance of the mixtures was determined at 415 nm against a sample blank of 1 ml with 9 ml of water. TFC was expressed as quercetin equivalent (QE) in mg/100 g.

Caffeoylquinic acid content (CQAC) of plant samples was quantified using the molybdate assay. Molybdate reagent was prepared by dissolving 16.5 g sodium molybdate, 8.0 g dipotassium hydrogen phosphate, and 7.9 g potassium dihydrogen phosphate in 1 litre of water. To each plant extract (0.3 ml), 2.7 ml of the reagent was added and incubated for 10 min. Absorbance was measured at 370 nm against a sample blank of 0.3 ml with 2.7 ml of water. CQAC was expressed as mg chlorogenic acid equivalent (CGAE)/100 g.

Free radical scavenging (FRS) activity of plant samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Different dilutions of extracts (1 ml) were added to 2 ml of DPPH (5.9 mg per 100 ml methanol). Absorbance was measured at 517 nm after 30 min. FRS based on IC_{50} was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg ascorbic acid (AA)/100 g of sample. AEAC was calculated as $IC_{50\ (ascorbic\ acid)}/IC_{50\ (sample)} \times 10^5$ where IC_{50} of ascorbic acid was 0.00387 mg/ml.

Ferric reducing power (FRP) of plant samples was determined using the potassium ferricyanide assay. Different dilutions of extracts (1 ml) were added to 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%, w/v), and the mixture was incubated at 50°C for 20 min. After adding trichloroacetic acid solution (2.5 ml, 10%, w/v), the mixture was separated into aliquots of 2.5 ml, and diluted with 2.5 ml of water. Ferric chloride solution (500 ml, 0.1%, w/v) was added to each diluted aliquot. After incubation for 30 min, absorbance was measured at 700 nm. FRP was expressed as mg GAE/100 g. The calibration equation for gallic acid was $y = 16.767x$ ($R^2 = 0.9974$), where y is the absorbance and x is the GA concentration in mg/ml.

Ferrous ion chelating (FIC) ability of plant samples was measured using the ferrozine assay. Different dilutions of extracts (1 ml) were mixed with $FeSO_4$ (0.1 mM, 1 ml) and with ferrozine (0.25 mM, 1 ml). After 10 min of incubation, absorbance was measured at 562 nm and FIC was calculated as chelating ability in % = $(1 - A_{sample}/A_{control}) \times 100$. FIC ability was expressed as median chelating

efficiency concentration (CEC_{50}) in mg/ml or the effective concentration of extract to chelate ferrous ions by 50%.

2.5 Statistical analysis

All analyses were undertaken in triplicate (n = 3) and results were expressed as means ± standard deviations (SD). Analysis of variance (ANOVA) was analysed using the Tukey Honestly Significant Difference (HSD) test, based on significant difference of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Fresh herbs

Of the 10 fresh herbs analysed for phenolic content (TPC, TFC, and CQAC) and primary antioxidant activity (AEAC and FRP), leaves of *A. occidentale* and *C. asiatica* displayed the highest and lowest values, respectively (Table 1). Based on TPC and/or AEAC values, the species were ranked as 'high' for *A. occidentale*, *P. minus*, and *C. caudatus*; 'moderate' for *P. caninum*; and 'low' for *P. betle*, *C. hystrix*, *O. javanica*, *P. sarmentosum*, *H. sabdariffa*, and *C. asiatica*. The classification used was 'high' for > 2000 mg GAE/100 g and/or > 2000 mg AA/100 g; 'moderate' for 1000–2000 mg GAE/100 g and/or 1000–2000 mg AA/100 g; and 'low' for < 1000 mg GAE/100 g and/or < 1000 mg AA/100 g. Of the three species ranked as 'high', the outstanding TFC (922 ± 53 mg GE/100 g) and CQAC (1270 ± 65 mg CGAE/100 g) of *C. caudatus* were noteworthy. In terms of FIC ability, which is a secondary antioxidant activity, strongest CEC_{50} was observed in *C. hystrix* (1.2 ± 0.2 mg/ml) and *A. occidentale* (1.9 ± 0.2 mg/ml).

Applying the same classification for the AOP of eight fresh western culinary herbs,[5] none qualified as 'high' with only *Rosmarinus officinalis* (rosemary), *Thymus vulgaris* (thyme), and *Origanum majorana* (marjoram) ranked as 'moderate'. Complementing findings of this study, TPC, FRP and DPPH radical scavenging activity of methanol extract of *A. occidentale* were the strongest amongst 30 species of herbs and leafy vegetables tested.[7] Similarly, ranking based on TPC of aqueous extracts of some Malaysian herbs was of the order: *P. minus* > *C. caudatus* > *O. javanica* > *C. asiatica*.[8] Out of 12 Malay traditional herbs, highest DPPH radical scavenging activity was also reported in *A. occidentale* (IC_{50} of 15 ± 0.1 μg/ml) and *C. caudatus* (IC_{50} of 20 ± 0.1 μg/ml).[9]

The outstanding AOP of *A. occidentale* reported in this study may be attributed to its phenolic constituents. Major flavonoids identified in leaf shoots of two varieties of *A. occidentale* were kaempferol 3-*O*-glucoside, kaempferol 3-*O*-arabinofuranoside, quercetin 3-*O*-glucoside, and quercetin 3-*O*-galactoside.[10]

Table 1 Phenolic content and antioxidant activity of leaves of selected fresh herbs

Fresh herb (common name)	Phenolic content			Antioxidant activity		
	TPC	TFC	CQAC	AEAC	FRP	CEC_{50}
Anacardium occidentale (cashew)	3890 ± 336	347 ± 48	1090 ± 70	6620 ± 513	3260 ± 235	1.9 ± 0.2
Polygonum minus (smartweed)	2330 ± 283	392 ± 54	206 ± 36	2510 ± 264	2230 ± 149	5.4 ± 0.4
Cosmos caudatus (wild cosmos)	1880 ± 84	922 ± 53	1270 ± 65	2390 ± 300	1110 ± 50	3.9 ± 0.3
Piper caninum (wild betel)	1340 ± 74	212 ± 6	200 ± 17	995 ± 68	764 ± 72	3.4 ± 0.7
Piper betle (betel)	664 ± 15	75 ± 12	143 ± 13	442 ± 69	403 ± 19	4.8 ± 0.1
Citrus hystrix (kaffir lime)	664 ± 82	234 ± 35	76 ± 9.3	213 ± 18	207 ± 25	1.2 ± 0.2
Oenanthe javanica (water dropwort)	621 ± 63	231 ± 27	521 ± 62	762 ± 72	192 ± 12	4.5 ± 0.1
Piper sarmentosum (wild pepper)	379 ± 24	243 ± 43	105 ± 24	130 ± 20	135 ± 7.0	2.7 ± 0.2
Hibiscus sabdariffa (roselle)	298 ± 27	160 ± 15	295 ± 34	146 ± 18	158 ± 2.0	12 ± 1.0
Centella asiatica (pennywort)	247 ± 30	79 ± 20	227 ± 29	218 ± 39	147 ± 22	6.3 ± 0.4

Data on phenolic content and antioxidant activity are means ± standard deviations. Abbreviations and units: TPC = total phenolic content (mg GAE/100 g), TFC = total flavonoid content (mg QE/100 g), CQAC = caffeoylquinic acid content (mg CGAE/100 g), AEAC = ascorbic acid equivalent antioxidant capacity (mg AA/100 g), FRP = ferric reducing power (mg GAE/100 g), CEC_{50} = median chelating efficiency concentration (mg/ml), GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent, and AA = ascorbic acid. Lower CEC_{50} values mean stronger ferrous ion chelating ability.

The potent AOP of *P. minus* may be due to high contents of flavonoids e.g. rutin, catechin, quercetin, isohamnetin, and kaempferol.[11] The aqueous extract of *P. minus* at 200 ppm had better ferric reducing ability than butylated hydroxytoluene (BHT) but comparable to butylhydroxyanisole (BHA).[8]

The outstanding TFC and CQAC of *C. caudatus* complemented findings of an earlier study that its very high AEAC was attributed to phenolic compounds of proanthocyanidins, quercetin glycosides, caffeoylquinic acids, and (+)-catechin.[12]

3.2 Processed herbs

Blanching in hot water for 30 s resulted in a significant decrease in the phenolic content and antioxidant activity of *A. occidentale* and *P. betle* compared to fresh samples (Table 2). Declines in values of the blanched herbs ranged from 20–31% and 30–60%, respectively. Blanching of *C. caudatus* resulted in significant declines in TPC, TFC, and AEAC, with CQAC and FRP remaining unchanged. Values of blanched *P. minus* remained relatively unchanged with the exception of TFC, which showed a significant increase of 48%. In terms of susceptibility to blanching, AOP ranking was of the order: *P. betle* > *A. occidentale* > *C. caudatus* > *P. minus*.

Findings of this study are consistent with those of earlier research, which reported that blanching of vegetables generally caused declines in AOP. On the contrary, some studies have also reported gains and/or loss in AOP of vegetables following blanching. Asparagus, burdock, carrot, eggplant, and green chilli blanched for 5 min resulted in 10–120% increase in FRS activity.[13] Onion, radish and spinach had declines of 56–80%. Green leafy vegetables blanched for 5 min showed an increase in phenolic content of up to 200%, but loss in FRS activity and ferric reducing ability of up to 52% and 80%, respectively.[14] The ferric reducing power and quercetin content of blanched rhizomes of *Curcuma mangga* were significantly higher than those of fresh rhizomes.[15]

Loss in AOP during blanching could be due to the large surface area of the herbs in contact with the boiling water, which caused solubilisation and leaching of phenolic compounds into the water.[13] In this study, the TPC, TFC, and CQAC of blanching water were 5–20% for *A. occidentale*, 66–93% for *C. caudatus*, 2–17% for *P. minus*, and 2–47% for *P. betle*, suggesting the amount of antioxidants leached varied with the species.

Gain in AOP during blanching might be attributed to thermal destruction of cells releasing antioxidative compounds and to the inactivation of polyphenol oxidase, which inhibits polyphenol degradation.[13] The increment could also be due to the hydrolysis of flavonol glycosides to their respective aglycones,[15] and the breakdown of tannins to simple phenolic compounds,[14] which are expected to possess more potent antioxidant activity.

Microwave treatment did not affect the AOP of leaves of *A. occidentale* with TPC, TFC, CQAC, AEAC, and FRP having values comparable to fresh samples (Table 2). Leaves

Table 2 Percentage gain or loss in the phenolic content and antioxidant activity of leaves of selected herbs following blanching, microwave, and freezing

Processed herb (common name)	Treatment	Percentage gain (+) or loss (−) compared to fresh herb				
		TPC	TFC	CQAC	AEAC	FRP
Anacardium occidentale (cashew)	Blanching	−20*	−25*	−31*	−27*	−30*
	Microwave	+1	−8	+4	+10	+7
	Freezing	−21*	−15*	−6	−11	+11
Cosmos caudatus (wild cosmos)	Blanching	−26*	−34*	+2	−29*	−14
	Microwave	+27*	+22*	+60*	+38*	+26*
	Freezing	+24*	+33*	+27*	+61*	+19*
Polygonum minus (smartweed)	Blanching	−9	+48*	−2	−13	−11
	Microwave	+14	+68*	+10	+10	+4
	Freezing	−15	−25*	−28*	−50*	+13
Piper betle (betel)	Blanching	−43*	−47*	−43*	−60*	−30*
	Microwave	+74*	+19*	+30*	+69*	+85*
	Freezing	+80*	+47*	+40*	+70*	+90*

Abbreviations and units: TPC = total phenolic content (mg GAE/100 g), TFC = total flavonoid content (mg QE/100 g), CQAC = caffeoylquinic acid content (mg CGAE/100 g), AEAC = ascorbic acid equivalent antioxidant capacity (mg AA/100 g), FRP = ferric reducing power (mg GAE/100 g), GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent, AA = ascorbic acid, and * = significantly different at $p < 0.05$ compared to fresh herbs.

of *P. minus* showed slight gains in values of which only the increase in TFC was significant. However, leaves of *C. caudatus* and *P. betle* exhibited significant increase in values ranging from 22–60% and from 19–85%, respectively.

Data from this study are supported by earlier findings. Microwave treatment resulted in a significant increase in the AOP of leaves of *Morus alba* and *Thunbergia laurifolia*.[16] TPC, AEAC, and FRP of microwave-dried leaves of *M. alba* were 24%, 91%, and 30% higher than those of fresh leaves, respectively. TPC and AEAC of microwave-dried *T. laurifolia* leaves were 38% and 84% higher. The enhancement effects of microwave treatment on the AOP of plant samples have also been reported in green tea of *Camellia sinensis*.[17]

The enhanced AOP of microwave-dried herbs might be due to the release of bound phenolic compounds, brought about by the breakdown of cellular constituents.[18] Microwave energy could have increased the solubility of polyphenols by preventing them from binding to the leaf matrix.[17] Other contributing factors included rapid heat transfer and thermal inactivation of polyphenol oxidase activity in samples due to microwave irradiation.[19] Another likely cause for the increase in antioxidant activity following microwave-drying was the production of additional phenolic compounds from precursors already present in the samples.[18]

Freezing had variable effect on the AOP of leaves of *A. occidentale* and *P. minus* (Table 2). Significant declines were observed in TPC (21%) and TFC (15%) of *A. occidentale*. Loss in CQAC and AEAC, and gain in FRP were comparable. Declines in TFC (25%), CQAC (28%), and AEAC (50%) of *P. minus* were significant while TPC and FRP were relatively unchanged. However, frozen leaves of *C. caudatus* and *P. betle* exhibited significant increase in TPC, TFC, CQAC, AEAC, and FRP with values ranging from 19–61% and 40–90%, respectively.

The effect of freezing on AOP was reported to be largely dependent on the type of vegetables.[20] Notably, freezing increased the antioxidant activity in green vegetables of beans, zucchini, and peas, while decreasing or not affecting the activity in yellow or red vegetables of carrots, tomatoes, and pepper. Frozen vegetables were reported to share similar antioxidant values as fresh vegetables purchased from supermarkets.[21] The levels of antioxidant activities were much higher compared with canned and jarred vegetables. The effect of freezing on fresh red raspberries was reported.[22] Fruits were stored at 4°C (freezer) for 3 days and then at 18°C (refrigerator) for 24 h, mimicking the post-harvest route to the supermarket and to consumers. Anthocyanin levels were unaffected, vitamin C levels declined, and contents of phenolic acids and flavonols increased. Overall, there was no effect on the antioxidant capacity of the frozen fruits.

3.3 Fresh vegetables

Compared to fresh herbs, the phenolic content of fresh vegetables were significantly lower (Table 3). TPC ranged from 56 ± 4.7 mg GAE/100 g (baby corn) to 154 ± 10 mg GAE/100 g (garlic), TFC ranged from not detected (button mushroom) to 33 ± 7.6 mg QE/100 g (ginger), and CQAC ranged from 7.3 ± 1.7 mg CGAE/100 g (garlic) to 44 ± 8.6 (ginger). Based on antioxidant activity, AEAC was significantly lower than herbs with values ranging from 17 ± 1.0 mg AA/100 g (garlic) to 249 ± 25 mg AA/100 g (ginger), but CEC_{50} was significantly higher

Table 3 Phenolic content and antioxidant activity of selected fresh vegetables

Fresh vegetable (common name, part studied)	Phenolic content			Antioxidant activity	
	TPC	TFC	CQAC	AEAC	CEC_{50}
Allium sativum (garlic, bulb)	154 ± 10	8.3 ± 0.6	7.3 ± 1.7	17 ± 1.0	0.3 ± 0.0
Zingiber officinale (ginger, rhizome)	140 ± 17	33 ± 7.6	44 ± 8.6	249 ± 50	2.2 ± 0.7
Capsicum annuum (chilli, fruit)	86 ± 8.9	15 ± 3.3	8.6 ± 1.0	95 ± 7.0	1.6 ± 0.3
Allium cepa (onion, bulb)	79 ± 4.8	32 ± 2.7	29 ± 2.0	21 ± 2.5	1.5 ± 0.1
Agaricus bisporus (mushroom, cap)	59 ± 8.2	ND	12 ± 1.1	52 ± 4.4	1.2 ± 0.1
Zea mays (baby corn, cob)	56 ± 4.7	15 ± 2.9	38 ± 2.2	31 ± 2.0	2.2 ± 0.2

Data on phenolic content and antioxidant activity are means ± standard deviations. Abbreviations and units: TPC = total phenolic content (mg GAE/100 g), TFC = total flavonoid content (mg QE/100 g), CQAC = caffeoylquinic acid content (mg CGAE/100 g), AEAC = ascorbic acid equivalent antioxidant capacity (mg AA/100 g), CEC_{50} = median chelating efficiency concentration (mg/ml), GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent, AA = ascorbic acid, and ND = not detected. Lower CEC_{50} values mean stronger ferrous ion chelating (FIC) ability.

with values ranging from 2.2 ± 0.7 mg/ml (ginger) to 0.3 ± 0.0 mg/ml (garlic). Ranking based on AEAC was of the order: ginger > chilli > button mushroom > baby corn > onion > garlic. Ranking of CEC_{50} was of the order: garlic > button mushroom > onion ~ chilli > ginger.

It should be noted that AEAC and CEC_{50} are primary and secondary antioxidant activities, respectively. AEAC (in terms of FRS) measures the hydrogen and electron-donating ability of primary antioxidants that prevent oxidative damage by directly scavenging free radicals.[23] FIC (in terms of CEC_{50}) measures the ability of secondary antioxidants to chelate metal ions. These secondary antioxidants act indirectly by preventing the formation of free radicals through the Fenton's reaction.

Although vegetables are low in phenolic content (TPC, TFC, and CQAC) and weak in primary antioxidant activity (AEAC), they are strong in secondary antioxidant activity (CEC_{50}). The poor correlation between phenolic content and CEC_{50} indicates that phenolic compounds might not be the chelators of metal ions.

Garlic and onion were good ferrous ion chelators among the selected vegetables in this study. Garlic had significantly higher TPC and CEC_{50} than onion. TFC, CQAC, and AEAC of onion were significantly higher. A recent study however reported that TPC and FIC of onion were higher than garlic but FRS activity was higher in garlic.[24] The discrepancy might be due to the differences in extraction method and solvent used as reported in *Citrus limon*.[25]

3.4 Processed vegetables

Brining with salt led to declines in TPC and AEAC of garlic, onion, baby corn, and button mushroom over a three-week period (Table 4). Garlic with the highest TPC declined significantly by 43% in week 2 and by 62% in week 3. TPC and AEAC of button mushroom showed significant declines of 20−25% and 63−65%, respectively. Slight declines of 2−5% in TPC was observed in baby corn while AEAC declined 32% in the first week. Interestingly, onion exhibited significantly higher AEAC in weeks 2 and 3. Pickling with vinegar, similarly, led to significant declines in TPC and AEAC of garlic, onion, ginger, and chilli over a three-week period. Steep declines of 68−69% in TPC was observed in garlic while all selected vegetables displayed significant declines in AEAC.

A recent study compared the AOP of fresh and commercially pickled papaya.[26] Pickling was done using vinegar, salt, sugar, and water. The study reported similar results of declines in AOP. Fresh papaya had TPC of 142 ± 12 mg GAE/100 g and FRS activity of 4.3 ± 0.3 mg/ml. For pickled papaya, TPC was only 45 ± 4.7 mg GAE/100 g and FRS activity was not detected.

Overall, the effect of pickling was more adverse as the process involved pasteurisation. The loss of AOP might be attributed to the thermal degradation of phytochemicals, loss of antioxidant enzyme activities, and enzymatic degradation of phenolic compounds.[18] The pasteurization process might have caused the leaching of antioxidants from the vegetables into the vinegar solutions.

4. CONCLUSION

Herbs are rich in phenolics with potent primary antioxidant activity. Although vegetables are low in phenolic content and weak in primary antioxidant activity, they are strong in secondary antioxidant activity. Blanching, microwave, and freezing treatments had variable effects on the AOP of the herbs, which included significant loss or gain and relatively unchanged. Brining with salt and pickling with vinegar led to declines in the AOP of vegetables over a three-week period. The effect of pickling was more adverse as the process involved pasteurisation. The

Table 4 Percentage loss or gain in phenolic content and antioxidant activity of selected vegetables following brining with salt and pickling with vinegar over a three-week period

Vegetable	Treatment	Percentage loss (−) or gain (+) compared to fresh vegetable					
		TPC (mg GAE/100 g)			AEAC (mg AA/100 g)		
		Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
Garlic	Brining	−10	−49*	−62*	−12	−47*	−59*
Onion		−25*	−37*	−35*	−29*	+25*	+30*
Baby corn		−5	−2	−2	−32*	−19*	−6
Mushroom		−20*	−22*	−25*	−65*	−65*	−63*
Garlic	Pickling	−69*	−68*	−69*	−71*	−71*	−71*
Onion		−30*	−30*	−30*	−67*	−57*	−62*
Ginger		−21*	−18*	−30*	−73*	−74*	−77*
Chilli		−17*	−21*	−26*	−69*	−79*	−82*

Abbreviations: TPC = total phenolic content and AEAC = ascorbic acid equivalent antioxidant capacity (mg AA/100 g), GAE = gallic acid equivalent, AA = ascorbic acid, and * = significantly different at $p < 0.05$ compared to fresh vegetables.

effects of processing on the AOP are largely dependent on the type of herbs and vegetables.

5. CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

ACKNOWLEDGEMENTS

This study formed part of the research projects on the antioxidant properties of fresh and processed herbs and vegetables conducted by final year students. The support of the Faculty of Applied Sciences, UCSI University is gratefully acknowledged.

Abbreviations

AA	Ascorbic acid
AEAC	Ascorbic acid equivalent antioxidant capacity
ANOVA	Analysis of variance
AOP	Antioxidant properties
BHA	Butylhydroxyanisole
BHT	Butylated hydroxytoluene
CEC	Chelating efficiency concentration
CGAE	Chlorogenic acid equivalent
CQAC	Caffeoylquinic acid content
DPPH	2,2-diphenyl-1-picrylhydrazyl
FC	Folin-Ciocalteu
FIC	Ferrous ion chelating
FRP	Ferric reducing power
FRS	Free radical scavenging
GAE	Gallic acid equivalent
HSD	Honestly significant difference
QE	Quercetin equivalent
SD	Standard deviation
TFC	Total flavonoid content
TPC	Total phenolic content

REFERENCES

1. Reihani SFS, Azhar ME. Antioxidant activity and total phenolic content in aqueous extracts of selected traditional Malay salads (ulam). *Int Food Res J*. 2012; 19(4):1439−1444.
2. Ismail A, Marjan ZM, Foong CW. Total antioxidant activity and phenolic content in selected vegetables. *Food Chem*. 2004; 87:581–586.
3. Albarracín W, Sánchez IC, Grau R, Barat JM. Salt in food processing; usage and reduction: a review. *Int J Food Sci Tech*. 2011; 46: 1329–1336.
4. Barrett DM. Pickling. In: Atkins D, Robertson S, eds. *Encyclopedia of Food Science, Food Technology and Nutrition*. vol. 6. New York: Academic Press; 1993.
5. Chan EWC, Kong LQ, Yee KY, Chua WY, Loo TY. Antioxidant and antibacterial properties of some fresh and dried Labiatae herbs. *Free Rad Antiox*. 2012; 2(3):20–27.
6. Chan EWC, Kong LQ, Yee KY, Chua WY, Loo TY. Rosemary and sage outperformed six other culinary herbs in antioxidant and antibacterial properties. *Int J Biotechnol Wellness Indust*. 2012; 1(2):142–151.
7. Sulaiman SF, Sajak AAB, Ooi KL, Supriatno, Seow EM. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. *J Food Compos Anal*. 2011; 24: 506–515.
8. Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS. Antioxidative activities of water extracts of some Malaysian herbs. *ASEAN Food J*. 2007; 14(1):61−68.
9. Abas F, Lajis NH, Israf DA, Khozirah S, Umi Kalsom Y. Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. *Food Chem*. 2006; 95: 566–573.
10. Mohd Shukri MA, Alan C. Analysis of phenolics in *Anacardium occidentale* shoot extracts using a reversed-phase high performance liquid chromatography tandem mass spectrometry (RP-HPLC-MS). *J Trop Agric Food Sc*. 2010; 38(2):221–230.
11. Nanasombat S, Teckchuen N. Antimicrobial, antioxidant and anticancer activities of Thai local vegetables. *J Med Plant Res*. 2009; 3(5):443–449.
12. Shui GH, Leong LP, Wong SP. Rapid screening and characterisation of antioxidants of *Cosmos caudatus* using liquid chromatography coupled with mass spectrometry. *J Chromatogr B*. 2005; 827:127−138.
13. Yamaguchi T, Mizobuchi T, Kajikawa R, et al. Radical-scavenging activity of vegetables and the effect of cooking on their activity. *Food Sci Technol Res*. 2001; 7(3):250–257.
14. Oboh G.. Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. *Swiss Soc Food Sci Technol*. 2005; 38:513–517.
15. Pujimulyani D, Raharjo S, Marsono Y, Santoso U. The effect of blanching on antioxidant activity and glycosides of white saffron (*Curcuma mangga* Val.). *Int Food Res J*. 2012; 19(2):617–621.
16. Chan EWC, Lye PY, Eng SY, Tan YP. Antioxidant properties of herbs with enhancement effects of drying treatments: A synopsis. *Free Rad Antiox*. 2013; 3(1):2–6.
17. Gulati A, Rawat R, Singh B, Ravindranath SD. Application of microwave energy in the manufacture of enhanced-quality green tea. *J Agric Food Chem*. 2003; 51:4764–4768.
18. Chan EWC, Lim YY, Wong SK, et al. Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chem*. 2009; 113:166–172.
19. Rodriquez-Lopez JN, Fenoll LG, Tudela J, et al. Thermal inactivation of mushroom polyphenol oxidase employing 2450 MHz microwave radiation. *J Agric Food Chem*. 1999; 47(8):3028–3035.
20. Danesi F, Bordoni A. Effect of home freezing and Italian style of cooking on antioxidant activity of edible vegetables. *J Food Sci*. 2008; 73(6):109–112.
21. Hunter KJ, Fletcher JM. The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. *Innov Food Sci Emerg Technol*. 2002; 3:399–406.
22. Mullen W, Stewart AJ, Lean MEJ, Gardner P, Duthie GG, Crozier A. Effect of freezing and storage on the phenolics, ellagitannins, flavonoids, and antioxidant capacity of red raspberries. *J Agric Food Chem*. 2002; 50:5197–5201.
23. Lim YY, Lim TT, Tee JJ. Antioxidant properties of several tropical fruits: A comparative study. *Food Chem*. 2007; 103:1003–1008.
24. Siti Fairuz CO, Said Zahir I, Mustapha SK, Aisyah MR, Kamarul RK. Antioxidant study of garlic and red onion: A comparative study. *Pertanika J Trop Agric Sci*. 2011; 34(2):253–261.
25. *Akhila* S, Bindu AR, Bindu K, Aleykutty NA. Comparative evaluation of extracts of *Citrus limon* Burm peel for antioxidant activity. *J Young Pharmacists*. 2009; 1(2):136 −140.
26. Nurul SR, Asmah R. Evaluation of antioxidant properties in fresh and pickled papaya. *Int Food Res J*. 2012; 19(3):1117–1124.