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Changes of Antioxidant Capacity and Phenolics in *Ocimum* Herbs after Various Cooking Methods

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Abstract

Five different cooking methods - blanching, boiling, steaming, sautéing and high temperature (121°C) cooking - were applied to *Ocimum* herbs. Four *Ocimum* species - *O. americanum* (hairy basil), *O. tenuiflorum* (holy basil; syn. *O. sanctum*), *O. basilicum* (sweet basil) and *O. gratissimum* (wild basil) - were used to determine the effect of heating on their antioxidant capacity, total phenolic content, and presence of phytochemicals. Cooking with excess water, blanching, and boiling resulted in a reduction in both antioxidant capacity and phenolic content. HPLC chromatograms revealed that rosmarinic acid leached from the sweet basil leaves into the cooking water, in which sinapic acid was also detected. Meanwhile sautéing, as well as steaming at atmospheric and high pressures respectively, generally enhanced the antioxidant capacity of *Ocimum*, which was related to an increase in phenolic content. Similar chromatograms were detected in fresh, atmospheric steamed, and sautéed leaves, although the intensity varied. A major compound of the studied *Ocimum* herbs, rosmarinic acid, although found to increase in sautéed leaves, was substantially minimized in leaves steamed under pressure.

Discipline: Food
Additional key words: antioxidant capacity, basil, heating, rosmarinic acid, sinapic acid

Introduction

The effects of thermal treatment on the antioxidant and antiradical properties of fruits and vegetables have been broadly studied. This has been shown to induce an increase in the main phenolic substances in blood orange juice, such as anthocyanins and total cinnamates (Lo Scalzo *et al*., 2004). Thermal treatment initiated the isomerization of lycopene when carrot homogenate was heated above 100 °C; the ratio of all-*trans* to total-*cis*-isomers changed from 90:10 to 40:60 (Mayer-Miebach *et al*., 2005). Moreover, Viña *et al*. (2007) reported that blanching by immersing Brussels sprouts in water (100 °C) induced increased radical scavenging activity values.

Herbs from the genus *Ocimum* are used in Asian, Mediterranean and African cuisines, as well as in medicines. The most frequently studied plant in this genus is *O. basilicum*, sweet basil. *O. sanctum*, holy basil, is the second most often studied *Ocimum* herb. Other *Ocimum* herbs which have been documented are *O. americanum*, *O. gratissimum*, *O. campechianum* (syn. *O. micranthum*), *O. minimum*, *O. selloi* and *O. kilimandscharicum* (Grayer et al., 2002; Silva et al., 2008).

In Thailand, common culinary herbs from this genus are *O. basilicum* (sweet basil), *O. tenuiflorum* (holy basil; syn. *O. sanctum*), *O. americanum* (hairy basil), and *O. gratissimum* (wild basil). These *Ocimum* herbs are served either fresh or cooked. Cooking methods applied are boiling, steaming or frying.

It has been reported that cooking processes can bring about changes in the antioxidant property and chemical composition of vegetables (Turkmen *et al*., 2005; Sikora *et al*., 2008; Moreno *et al*., 2007). Home cooking temperatures caused either losses or increases of antioxidant components determined in some vegetables (Gazzani *et al*., 1998; Lo Scalzo *et al*., 2007; Moreno *et al*., 2007; Roy *et al*., 2007; Sikora *et al*., 2008; Wachtel-Galor *et al*., 2008). With regard to *Ocimum* herbs, Juntachote & Berghofer (2005) reported that the antioxidant

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activity of holy basil extract was heat-stable.

Since *Ocimum* herbs are commonly consumed and cooked in many ways, we aimed to study the changes in antioxidant capacity, total phenolics, and chemical composition after five various heat cooking methods: blanching, boiling, steaming, sautéing, and heating to 121 °C.

Materials and methods

1. Plant materials and chemicals

Four species of *Ocimum* genus were studied: *O. americanum* (hairy basil), *O. tenuiflorum* (holy basil), *O. basilicum* (sweet basil) and *O. gratissimum* (wild basil). Samples were purchased from local markets in Bangkok, Thailand. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2-(N-Morpholino)ethanesulfonic acid monohydrate (MES), rosmarinic acid, caffeic acid, ferulic acid, sinapic acid and apigenin were purchased from Sigma-Aldrich (St. Louis, MO). Folin-Ciocalteu phenol reagent was obtained from Merck (Darmstadt, Germany). Other reagents used in this study were of analytical grade.

2. Cooking preparation and sample extraction

Only edible *Ocimum* parts were collected for analysis. Each material sample weighed exactly 10.0 g; treatments were performed in triplicate. Samples were heat-treated as follows:

a) Blanching: samples were cooked for 3 min in 1.5 L of boiling water in a stainless steel vessel.
b) Boiling: samples were boiled for 15 min in 1.5 L of boiling water in a stainless steel vessel.
c) Steaming: samples were placed in a steamer and cooked for 15 min.
d) Sautéing: 10 g samples were cooked in a shallow pan for 1 min with approximately 5 ml of soybean oil.
e) Pressure cooking: samples were placed in an autoclave and cooked at 121 °C for 20 min.

Excess water or oil from blanching, boiling and sautéing was neatly drained or removed from the treated sample. The heat-treated leaves were kept at -70 °C until use. Fresh leaves of each species were determined and assigned as controls.

A sample was homogenized (IKA Ultra-Turrax, TP18/10) in methanol (1:10 w/v) for 2 min, and filtered after standing for 20 min. The filtrate volume was adjusted to 100 ml and maintained at -20 °C until analyzed.

3. DPPH radical scavenging capacity

The reaction mixture contained 900 µl of DPPH-MES solution and 100 µl of diluted sample extract. The absorbance at 515 nm was measured with a spectrophotometer (Shimatzu, UV mini 1240) after 60 min. A series of extract concentrations – 1/5, 1/10, 1/20, 1/40, 1/80, 1/160 and 1/320 – was prepared. An appropriate dilution for radical-antioxidant interaction can be indicated when the DPPH scavenging capacity of each sample peaks (Bondet *et al*., 1997). Absolute methanol was used as a control.

Scavenging stable free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH), DPPH method, is a frequently used technique to determine antioxidant activity. There are many forms used to express the relative activity of the tested substances such as % of antioxidant activity, IC_{50} values (the concentration to inhibit 50%) or antioxidant capacity by comparing the antioxidant activity of the sample to that of a standard compound, Trolox or vitamin C. Meanwhile, the concentration of DPPH scavenged by antioxidants can be directly determined by applying Beer's law, which is the linear relationship between absorbance and concentration of an absorbing species. A result was expressed as the mmol g^{-1} wet weight of the corresponding plant material, which represents the amount (mmol) of DPPH radicals which were scavenged by antioxidants in plant extract equivalent to a 1 g sample on a wet weight (ww) basis:

DPPH scavenging capacity (mmol DPPH/g ww)
$= (C_{515} - S_{515}) \cdot v \cdot 11.2^{-1} \cdot m^{-1} \cdot 1000^{-1}$
C_{515} = absorbance at 515 nm of the control
S_{515} = absorbance at 515 nm of the sample
v = volume (ml) of the reaction mixture
11.2 is the molar absorption coefficient of DPPH (mM^{-1}) at 515 nm in methanol
m = amount of plant tissue (g), wet weight, used for an assay

When determined by this method, it is found that 2 mmol Trolox or vitamin C can scavenge 1 mmol DPPH. Thus, the results of this study can be converted to Trolox equivalent antioxidant capacity (TEAC, mmol trolox / g ww) or Vitamin C equivalent antioxidant capacity (mmol vitamin C / g ww) by dividing the DPPH scavenging capacity value by 2.

4. Determination of total phenolic content

Total phenolic compounds were determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965). The results were expressed as mg gallic acid equivalent (GAE) per g of sample, wet weight.

5. HPLC analysis of phenolic compounds

A high-performance liquid chromatography (HPLC) system (JASCO, Gulliver series) was used, comprising a vacuum degasser, quaternary pump, autosampler, thermostated column compartment, and photodiode array detector (DAD). A 100 mm × 4.6 mm i.d. TSK-GEL Super-

ODS (TOSOH Bioscience, Montgomeryville, PA) was maintained at 40 °C. The method used 100% acetonitrile (eluent A) and 0.5% aqueous formic acid (eluent B) with a set analysis time at 30 min. Linear gradient 10-40% acetonitrile was used at a mobile phase flow rate of 1 ml/min, with a sample injection volume of 50 µl, and scanning between 220 and 420 nm.

Results and discussion

1. Antioxidant capacity and polyphenols of fresh *Ocimum* herbs

Antioxidants in 100 g of fresh *Ocimum* herbs were able to scavenge DPPH radicals differently, ranging from 2.43 to 10.80 mmole. The order of antioxidant capacity was sweet basil (10.80 ± 0.80 mmole DPPH/100 g) > wild basil (4.80 ± 0.10 mmole DPPH/100 g) > holy basil (4.67 ± 0.33 mmole DPPH/100 g) > hairy basil (2.43 ± 0.47 mmole DPPH/100 g). The content of total phenolics ranged from 213.97 to 608.77 mg GAE/100 g, and ranked from high to low as sweet basil (608.77 ± 23.21 mg GAE/100 g), holy basil (539.36 ± 34.59 mg GAE/100 g), wild basil (390.67 ± 43.28 mg GAE/100 g), and hairy basil (213.97 ± 48.08 mg GAE/100 g).

From the HPLC chromatograms in Fig. 1, the major peaks, B1, C1 and D1, eluted at approximately 8 min for holy basil, sweet basil and wild basil was identified as rosmarinic acid through comparison with the authentic chemical (Sigma). Both samples showed the same retention time and UV spectra with λmax at 236, 290s and 328 nm. For fresh hairy basil, unlike the others, the major substances in the methanolic extract, A1 and A2, were eluted at 18.69 and 19.45 min respectively. Neither of the compounds has been identified to date. The peak B2 of holy basil eluted at 17.95 min demonstrated λmax at 236 and 280 nm, similar to the spectrum of peak C2 of sweet basil, which eluted at 21.67 min.

Previous studies reported that the phenolic compounds in sweet basil were cinnamic acid, caffeic acid, sinapic acid, rosmarinic acid, ferulic acid, carnosic acid,

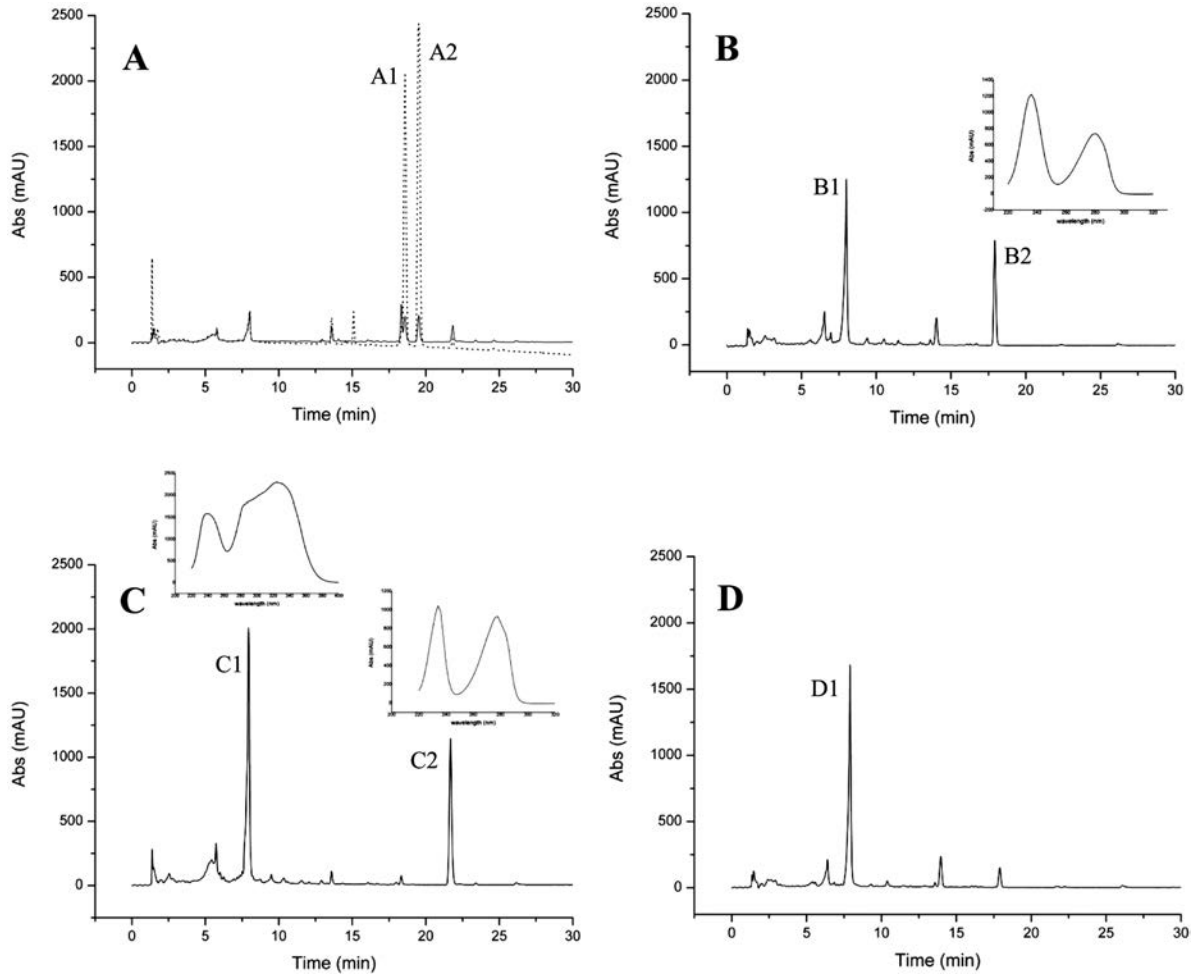

Fig. 1. HPLC chromatograms monitored at 280 nm (solid line) and at 240 nm (broken line) of fresh leaves of the studied *Ocimum* herbs: A) hairy basil; B) holy basil; C) sweet basil; and D) wild basil

catechol, cinnamyl and caffeoyl derivatives (Loughrin & Kasperbauer, 2001; Jayasinghe *et al*., 2003). In holy basil, the phenolic compounds found were ursolic acid, carnosic acid, eugenol, sinapic acid, rosmarinic acid, cirsilineol, cirsimaritin, isothymonin, apigenin and isothymusin (Kelm *et al*., 2000; Lukmanul Hakkim *et al*., 2007). In this study, none of the existing components in fresh samples could be identified as caffeic acid, ferulic acid, sinapic acid or apigenin, as indicated by retention time matching with its standard and the UV absorption spectrum.

2. Changes in the antioxidant capacity by heat cooking

Changes in the antioxidant capacity and/or total phenolic content of the heat-treated leaves were investigated. It was found that cooking either decreased or increased the antioxidant capacity. In general, blanching and boiling caused a reduction, whereas steaming and sautéing caused an enhancement (Fig. 2). This phenomenon resembled the findings of a previous study on the effects of various cooking methods (steaming, boiling and microwaving) on cabbage and choy sum (Wachtel-Galor *et al*., 2008). However, they reported that an increase in antioxidant capacity, as measured by ferric reducing/antioxidant power (FRAP) assay, was not due to an increase in total phenolic content. Unlike the present study, which was measured by the DPPH scavenging method, antioxidant capacity is directly related to total phenolic content.

3. Effects of various cooking methods

(1) Changes during sautéing

Among the five cooking methods, the heat treatments that led to an increase in the antioxidant capacity of *Ocimum* herbs, in descending order, were: sautéing, steaming, and high-pressure cooking at 121 °C. The phenolic contents in the sautéed leaves of tested *Ocimum* herbs substantially exceeded the level in fresh samples.

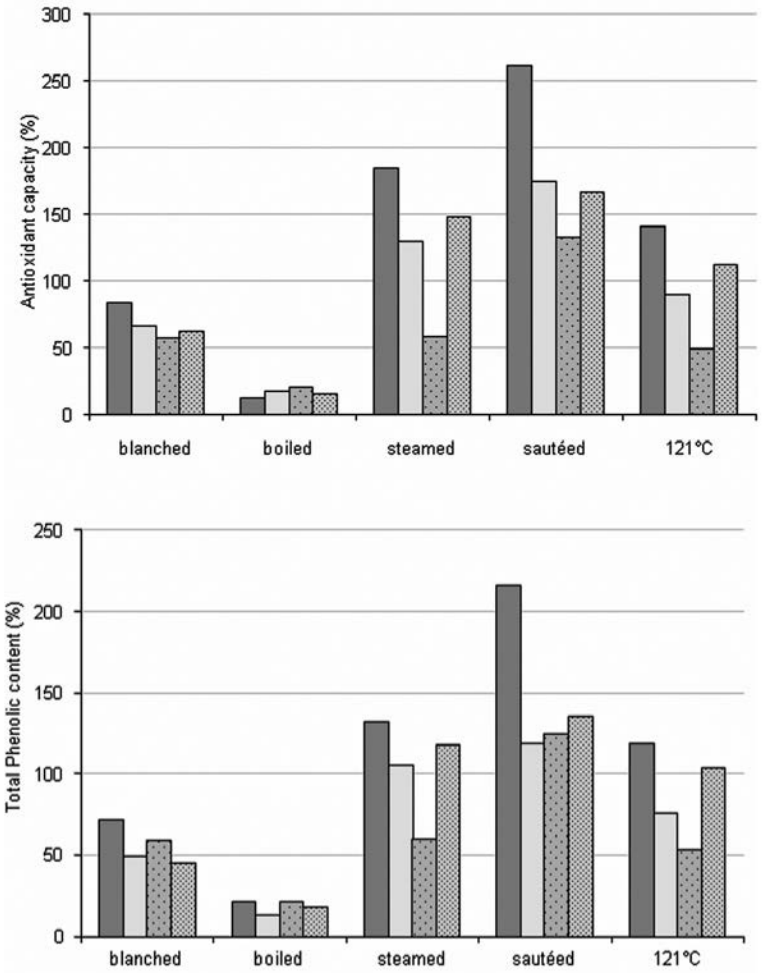

Fig. 2. Antioxidant capacity and total phenolic content remaining in leaves after various cooking methods compared to fresh *Ocimum* herbs

■ : O. americanum, □ : O. tenuiflorum, ▨ : O. basilicum, ▩ : O. gratissimum.

HPLC chromatograms illustrated that cooking by sautéing also dramatically enhanced the rosmarinic acid content. Chromatograms of all sautéed *Ocimum* herbs were similar to that of sweet basil (Fig. 3). This change is clearly exhibited in hairy basil, in which rosmarinic acid was tremendously increased (Fig. 4). This increase in rosmarinic acid content could play an important role, due to exhibiting stronger antioxidant activity than caffeic acid or chlorogenic acid (Guillot *et al*., 1996; Kim *et al*., 2006). The results of the present study were opposite to those of Moreno *et al*. (2007), who reported that total phenolics of 3.5 min stir-fried broccoli exhibited a reduction compared to uncooked broccoli. It cannot be absolutely concluded that cooking in oil for a short time would increase the total phenolic content in all kinds of vegetables. However, this tends to apply to *Ocimum* herbs.

(2) Changes during steaming and pressure cooking

When the leaves were treated with water vapor by atmospheric steaming, the total phenolic content of the studied *Ocimum* herbs was found to increase, except sweet basil (Fig. 2). However, the rise in measured phenolic content could not be unveiled by the HPLC chromatograms (Figs. 4, 5). In the case of antioxidant capacity, the substantial enhancement of rosmarinic acid may play an important role in scavenging DPPH radicals of hairy basil (Fig. 4). In the chromatograms of leaves of all examined *Ocimum* species heat-treated at 121 °C (Figs. 3, 4, 5), peaks with retention time of around 2 min, which were presumably small and hydrophilic phenols, were increased. This indicated that those compounds were formed during high-pressure cooking; and as explained by Oboh (2005) the tannins were broken down into simple phenolics.

(3) Changes during blanching and boiling

Declining antioxidant capacity and total phenolic content were found when the leaves were cooked in an excess amount of water, by either blanching or boiling (Fig. 2). Greater losses occurred in boiled leaves than blanched leaves. This finding agreed with Wachtel-Galor *et al*. (2008), who reported that antioxidant content fell with increasing cooking time, regardless of whether *Brassica* vegetables were cooked by boiling, microwaving or steaming. The reduction in total phenolic content in blanched and boiled leaves corresponded well with the peak height observed in HPLC chromatograms of all assayed *Ocimum* herbs, as presented in Figs. 3 and 4. A reduction in total phenolic content after boiling is common. Ismail *et al*. (2004) reported that 1-min blanching significantly decreased the total phenolic contents of spinach,

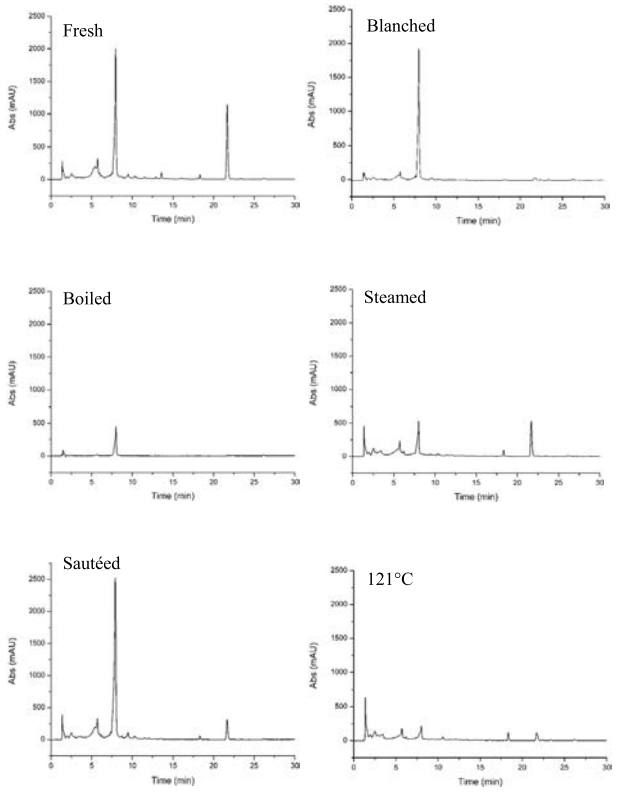

Fig. 3. HPLC chromatograms of *O. basilicum* (sweet basil) before and after various heat treatments, monitored at 280 nm

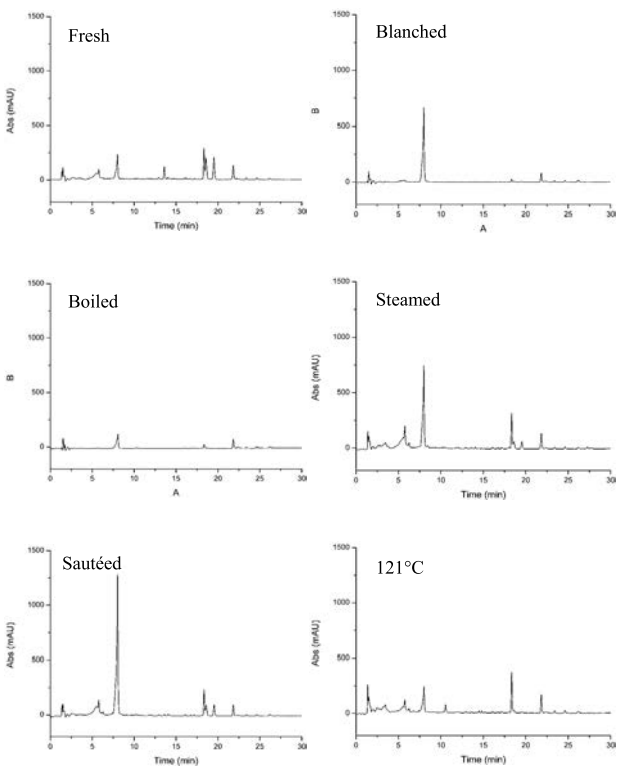

Fig. 4. HPLC chromatograms of *O. americanum* (hairy basil) before and after various heat treatments, monitored at 280 nm

swamp cabbage, kale, shallot and cabbage. Moreover, a loss of phenolics occurred when boiling squash, peas and leeks for 5 min (Turkmen *et al*., 2005). This was either due to the high heat sensitivity of phenolic compounds (Ismail *et al*., 2004), or to phenolic breakdown during cooking (Turkmen *et al*., 2005).

To better understand the loss of phenolics from boiled *Ocimum* leaves, an experimental boiling method was established. Ten grams of sweet basil was cooked in 100 ml of distilled water for 15 min. The antioxidant capacity and total phenolic content in 10 g of cooked leaves and cooking water adjusted to 100 ml were analyzed and compared to those in 10 g of fresh leaves. The results illustrated that phenolic compounds leaked into cooking water, and that more were induced during cooking. This is explained by the greater phenolic content in boiled leaves (20.39 mg GAE) combined with cooking water (109.50 mg GAE), accounting for 129.89 mg GAE, as opposed to the level in fresh leaves with 67.50 mg GAE. Phenolic compounds in cooking water seemed to be those with high antioxidant activity, which were able to scavenge DPPH 1.612 mmole. Phenolic compounds that existed in fresh leaves and remained in the cooked leaves scavenged 0.749 and 0.413 mmole of DPPH, respectively.

HPLC may help in understanding changes in substances during boiling (Fig. 6). Of the two major compounds detected in uncooked sweet basil leaves, the substance eluted at 7.99 min (rosmarinic acid) seemed less sensitive to heat, and more easily diffused into cooking water. This corresponded to the report by Fecka & Turek (2007), indicating that rosmarinic acid was one of the polyphenolic compounds most readily found in tea infusions. The other compound that eluted at 21.67 min was concluded to be heat-sensitive because it was not detected in the cooked leaves and cooking water, and seemed to be easily released from leaves since it nearly disappeared within 3 min of blanching (Fig. 3). In the cooking water, three major peaks were observed: peaks eluted at 2.46 min (244, 294s, 328 nm), at 5.74 min (245, 295s, 330 nm) and at 7.92 min (238, 290s, 328 nm). Thus, we concluded that existing components were released from plant tissues into cooking water rather than from compound breakdown during boiling. The substances in the cooking water of sweet basil were identified by comparing chromatographical properties and UV spectra with authentic chemicals. The substances eluted at 5.75 and 7.92 min were sinapic acid and rosmarinic acid, respectively.

4. Conclusion

The results obtained in this study suggest that heat treatment does not always diminish the antioxidant capacity of vegetables. It depends more on the cooking method, temperature, and cooking time. For *Ocimum* herbs, sautéing was the best cooking method for enhancing antioxidant capacity and total phenolic content, followed by steaming and high-pressure cooking. HPLC-DAD chromatograms disclosed changes in phenolic compounds after cooking *Ocimum* leaves. Depletion of the antioxidant capacity of *Ocimum* leaves after boiling was due to a reduction in phenolic content, and not to the decomposition of active phenolics. Enhancement of the antioxidant capacity of sautéed *Ocimum* leaves resulted from the formation of more antioxidative phenols during heat treatment.

These results demonstrate the need to investigate the effects of heat on chemical composition changes of individual vegetables, due to the different phytochemicals present.

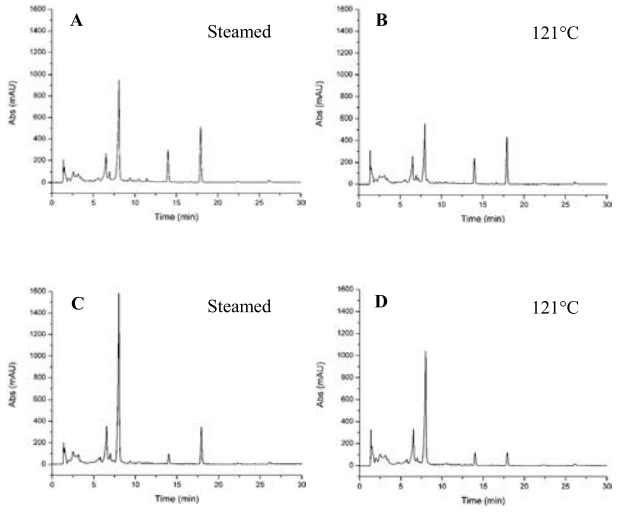

Fig. 5. HPLC chromatograms of (A and B) *O. tenuiflorum* (holy basil), and (C and D) *O. gratissimum* (wild basil), after steaming and high-pressure cooking, monitored at 280 nm

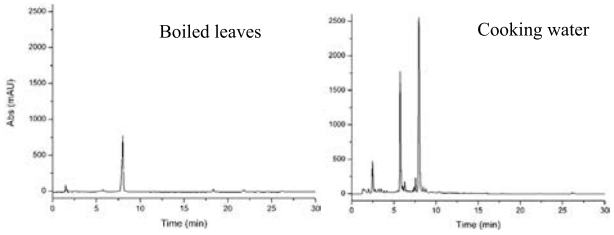

Fig. 6. HPLC chromatograms of *O. basilicum* (sweet basil), boiled leaves and cooking water, monitored at 280 nm

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