

Effect of blanching pre-treatment on antioxidant activities and involved compounds in fresh daylily (*Hemerocallis fulva* L.) flowers

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RESEARCH ARTICLE

Abstract

Effect of two blanching methods (blanching in steam or in hot water), on antioxidant properties of daylily flowers were determined with antioxidant assays, including 1-diphenyl-2-picrylhydrazyl free radical scavenging, superoxide anion scavenging activity, reducing power and nitric oxide radical-scavenging. Antioxidant compounds, such as phenolic composition, ascorbic acid and β -carotene were also analysed. Results showed that the phenolic compounds, β -carotene, ascorbic acid and antioxidant activities in daylily flowers were affected significantly by blanching pre-treatments. Blanching enhanced antioxidant activities and (+)-catechin content, and resulted in great reduction of β -carotene, ascorbic acid, but steam blanching had the higher antioxidant activity and related compound contents than those of hot water blanching. A significant positive correlation between antioxidant activity and (+)-catechin content was observed in this work. Overall, steam blanching would be a better choice for daylily flower pre-treatment than blanching in hot water.

Keywords: blanching, daylily flowers, antioxidant activity, antioxidant compounds

1. Introduction

Daylilies (*Hemerocallis fulva* L.) are primary natives of Asia where they have long been used as medicine and as flavour vegetable. It is also one of the most economically important flowering herbaceous perennial nursery crops in the USA (Tomkins *et al.*, 2001). The extracts of the daylily flowers have been shown to have antioxidant activities (Fu *et al.*, 2009) and inhibition of cancer cell proliferation (Cichewicz *et al.*, 2004). Pharmacological studies have shown that daylilies can facilitate neurological changes in sleeping mice (Uezu, 1998) and impact motor activity in rats as a result of alteration to the normal levels of several central nervous system neurotransmitters (Hsieh *et al.*, 1996).

Both fresh and dried daylily flowers are widely consumed as important components in traditional eastern Asian cuisine (Cichewicz *et al.*, 2002). Irrespective of their consumption forms, daylily flowers were usually blanched before direct consumption or further drying. Blanching

was a primary step in vegetables processing, which could be briefly described as the process of heating vegetables to a temperature high enough to destroy enzymes present in the tissue. It stopped the enzyme action, settled the colour, and shortened the drying and dehydration time. Especially for daylily flower, blanching was a necessary step to ensure safety for the plant toxicity of colchicine, the main active alkaloids existed in daylily flowers. Due to the improperly edible methods of daylily flower, many poisoning cases were reported every year in human and animals, which were at risk for gastrointestinal distress and acute renal failure (Milewski and Khan, 2006; Poppenga, 2010). Blanching process would destroy structure of colchicine and eliminate its toxicity (Hong *et al.*, 2003). It was usually carried out in hot water or in steam, and to avoid the collapse of flowers and influence the sensory quality. Blanching in short time (less than 2 min) was usually employed in traditional processing of fresh daylily flowers.

Even if a lot of information about the effect of blanching on antioxidant capacity can be found in vegetables (Yao and Ren, 2011) and fruits (Rossi *et al.*, 2003), there was still a dearth of which in daylily flowers. The objectives of this study was to assess the effect of hot water and steam blanching for 90–100 s on antioxidant properties of fresh daylily flowers. Phenolic composition, ascorbic acid and β -carotene of these samples were also determined and compared.

2. Materials and methods

Chemicals

Butylated hydroxyanisole (BHA), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, β -carotene, were purchased from Sigma Aldrich (St. Louis, MO, USA). Authentic standards of phenolic compounds were purchased from Sigma and Fluka. All other chemicals used were of analytical grade.

Plant material and preparation

The flowers of daylily grown at the pilot farm in Shandong Agricultural Engineering College were harvested at their commercial maturity based on colour and shape. Harvest was about 27–35 days after tassel. All flowers were transported to the lab within 30 min after harvested and divided into three portions before being subjected to three different blanching treatments: non-blanching (raw), blanching in hot water (98 °C) for 90–100 s and blanching in steam for 90–100 s (steam blanching). All the samples were cooled at room temperature and freeze-dried in a lyophiliser (Savant VLP-200; Ideal Vacuum Products, LLC, Albuquerque, NM, USA) for 12 h and ground with a blender and sieved through a 100-mesh screen to obtain daylily flower powder, then sealed in air-tight polyester bottles and stored at -20 °C for further analysis.

Ethanol extraction

Extraction was prepared by macerating 10 g of powder with 100 ml of 70% ethanol. Mixture was kept in a rotary shaker overnight and then filtered through Whatman no. 1 (Sigma Aldrich). The filtrate was evaporated in rotary vacuum evaporator using a water bath (45 °C) and SHB-III water-circulation multifunction vacuum pump (Zhengzhou Great Wall Scientific Industry and Trade Co., Zhengzhou, China P.R.). Ethanol extract was obtained when the residues were further freeze-dried.

Reducing power

The reducing power of daylily flowers was determined by the method of Fu *et al.* (2009). Different amounts of extracts (final concentrations was 25, 50, 75 and

100 μ g/ml, respectively) were mixed with 2.5 ml of 0.2 mol/l phosphate buffer (pH 6.6) and 2.5 ml of 0.03 mol/l potassium ferricyanide [$K_3Fe(CN)_6$]. Aliquots (2.5 ml) of 0.6 mol/l trichloroacetic acid were added to the mixture, which was then centrifuged for 10 min at 1000 $\times$ g (Hitachi SCR20BC, Tokyo, Japan). The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 6 mmol/l $FeCl_3$. The absorbance was measured at a wavelength of 700 nm in a spectrophotometer. BHA was used as a positive standard.

DPPH radical scavenging activity

Effect of extract on DPPH free radical was measured based on the method of Fu *et al.* (2009). Positive control was prepared by mixing 2 ml of ascorbic acid (0.05 mg/ml) and 3 ml of DPPH (0.04 mg/ml), whereas negative control was prepared by mixing 2 ml distilled water with 3 ml of DPPH. 2 ml of the extract (final concentrations was 25, 50, 100, 150 μ g/ml, respectively) was added to 3 ml of DPPH. The mixture was gently homogenised and left to stand at room temperature for 30 min. Absorbance was read using a spectrophotometer at a wavelength of 520 nm. The ability of extract to scavenge DPPH free radical was calculated using the following equation.

$$\text{Scavenging activity (\%)} = \frac{A(-ve) - A_s}{A(-ve) - A(+ve)} \times 100\%$$

Where, A_s is the absorbance of the sample, $A(-ve)$ and $A(+ve)$ are the absorbance values of negative and positive controls, respectively.

Nitric oxide radical scavenging assay

The nitric oxide radical-scavenging activity of daylily flower was determined by the method of Hazra *et al.* (2008). At physiological pH, nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions, which may be quantified by the Griess Illosvoy reaction. The reaction mixture contained 10 mmol SNP, phosphate buffered saline (pH 7.4) and test solution in a final volume of 3 ml. After incubation for 150 min at 25 °C, 1 ml of sulfanilamide (0.33% in 20% glacial acetic acid) was added to 0.5 ml of the incubated solution and allowed to stand for 5 min. Then 1 ml of naphthylethylenediamine dihydrochloride (NED) (0.1% w/v) was added and the mixture was incubated for 30 min at 25 °C. The pink chromophore generated during diazotisation of nitrite ions with sulphanilamide and subsequent coupling with NED was measured at a wavelength of 540 nm against a blank sample.

Superoxide anion scavenging activity

The superoxide anion scavenging activity was measured using the xanthine/xanthine oxidase method (Fu *et al.*, 2009). Extracts (final concentrations were 40, 80, 160 µg/ml, respectively) were separately added to a 1.0 ml mixture of 0.4 mmol/l xanthine and 0.24 mmol/l nitro blue tetrazolium chloride (NBT) in 0.1 mol/l phosphate buffer (pH 8.0). 1.0 ml of a xanthine oxidase solution (0.049 units/ml), diluted in 0.1 mol/l phosphate buffer (pH 8.0), was added and the resulting mixture incubated in a water bath at 37 °C for 40 min. The reaction was terminated by adding 2.0 ml of an aqueous solution of 69 mmol/l sodium dodecylsulphate and the absorbance of NBT was measured at a wavelength of 560 nm. L-ascorbic acid was used as the positive standard.

Determination of ascorbic acid and β-carotene

Sample flowers were homogenised for 1 min at maximum speed in a blender. The homogenate (2.0 g) was added to 20 ml of 4.5% metaphosphoric acid and vortexed. Extracts were centrifuged at 1,640×g for 15 min at 20 °C. The supernatant was filtered through a Whatman no. 1 filter and diluted to 25 ml with the 4.5% metaphosphoric acid. Ascorbic acid concentrations were measured according to the methods of Asami *et al.* (2003). Analysis was performed using a Waters 515 HPLC pump equipped with a Waters 486 tunable absorbance detector (Waters, Milford, MA, USA). Reverse-phase separation was attained using an Agilent Technologies (Palo Alto, CA, USA) Zorbax 5 µm Eclipse XDB-C₁₈ (4.6 × 250 mm). The mobile phase was Nanopure water brought to pH 2.2 with sulfuric acid. The flow rate was 0.5 ml/min, and the detection wavelength was 245 nm. Sample aliquots were filtered through a 0.45 µm poly (tetrafluoroethylene) filter prior to injection.

The extraction of carotenoids was carried out according to the method described by Deepa *et al.* (2007). The separation and quantification of β-carotene was carried out using C₁₈ reverse-phase column (Zorbax, 5 µm, 250 × 4.6 mm; Agilent Technologies) and binary gradient elution system (acetone-H₂O, 75:25) initially maintained for 5 min, changing linearly to 95:5 in 5 min and kept for 10 min. The flow rate was 1.5 ml/min, and the sample injection volume was 20 µl. At the end of analysis the column was washed with acetone for 3 min and conditioned with the initial proportion for 10 min. Detection of β-carotene was monitored at a wavelength of 450 nm.

Analysis of phenolic compounds by HPLC

Extraction of phenolic and individual phenolic compound was analysed according to the method of Fu *et al.* (2009). HPLC analysis of phenolic compounds was performed on a Waters 2695 system controller connected to a photodiode array detector Waters 2996, which was set to a scanning

range from 240 to 450 nm with a 2.4 nm resolution. The column was Inertsil ODS-3 (5 µm, 250×4.6 mm; GL Sciences, Tokyo, Japan) protected by a guard column (Easyguard C₁₈, Diamonsil™; Dickma Co., Beijing, China P.R.). Sample (20 µl) filtrated through 0.22 µm low-extractable polyvinylidene fluoride membrane was injected. The chromatographic conditions were applied that a gradient of two solvents of A (acetic acid-water, 5:95) and B (methanol for HPLC, 100%) was programmed as: 0 min (95% A + 5% B), 17 min (88.5% A + 11.5% B), 28 min (88.3% A + 11.7% B), 57 min (28.3% A + 71.7% B), 60 min (95% A + 5% B), with a flow rate of 0.8 ml/min. Phenolic compounds were identified by comparing retention time and UV (ultraviolet rays) spectral matching with authentic standards, and quantitative analysis was performed in triplicate using external calibration curves and content of phenolic compounds was expressed as mg/100 g fresh weight.

Statistical analysis

Data were reported as mean ± SD for triplicate determinations. Analysis of variance and least significant difference tests (SPSS for Windows, SPSS Inc., Chicago, IL, USA) were conducted to identify differences among means, while a Pearson correlation test was conducted to determine the correlations among means. Statistical significance was declared at $P < 0.05$.

3. Results

Reducing power

Reducing power is generally associated with the presence of reducing substances, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. The Fe³⁺-Fe²⁺ transformation was determined as the reducing capacity in this study. The reducing capacity of daylily flowers increased with the amount of extracts, and the highest reducing capacity was observed in steam blanched daylily flowers, but the lowest in raw material (Figure 1). The rank of reducing capacity in different treatment daylily flowers at the amount of 100 µg/ml, was steam blanching > hot water blanching > raw.

Scavenging effect on DPPH radical

The scavenging effects of extracts from daylily flowers tested on the DPPH radical were measured as shown in Figure 2. The scavenging activity of extracts on the inhibition of the DPPH radical was related to the concentration of extracts added and the activity increased with concentration for each treatment. The scavenging effect of extracts from daylily flower on the DPPH radical was 95.35, 86.24 and 73.47%, respectively, in steam blanching, hot water blanching and raw samples, at the concentration of 150 µg/ml. A significant difference was found among the treatments ($P < 0.05$).

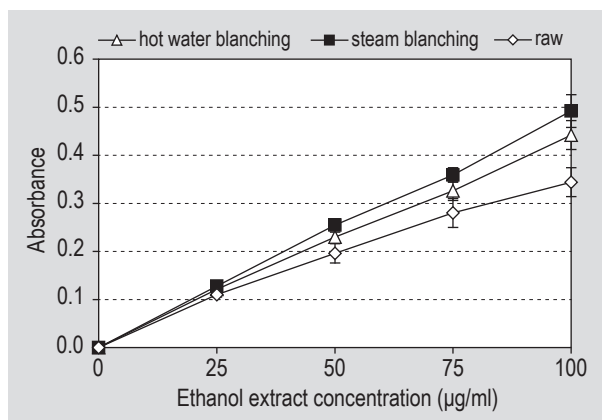


Figure 1. Reducing power of different amounts ethanol extract from steam blanching, hot water blanching and raw daylily flowers was used spectrophotometer detection of the Fe^{3+} - Fe^{2+} transformations. Each value represents mean $\pm$ standard deviation of three replicates.

Superoxide anion scavenging activity

Scavenging of superoxide anion radicals is of importance for protection against early events in oxidative damage. Superoxide anion radical scavenging activities increased with the amount of extract from daylily flowers (Figure 3). The scavenging effect of extracts from three treatments of daylily flower and vitamin C on superoxide anion radical was 95.68, 89.32, 74.55 and 60.82% for vitamin C, steam blanching, hot water blanching and raw, at the concentration of 160 $\mu\text{g/ml}$, respectively.

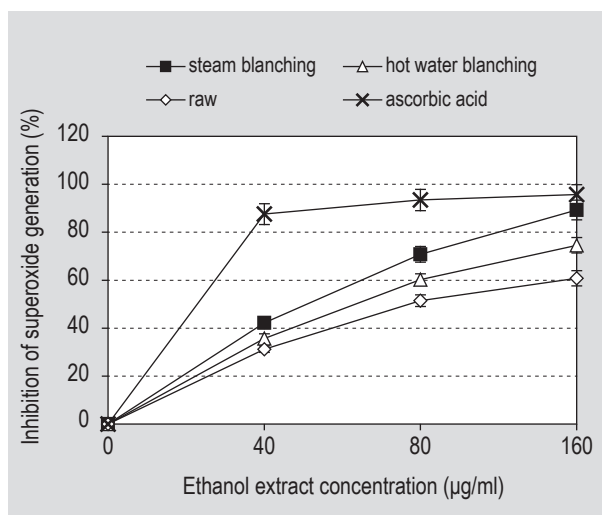


Figure 3. Superoxide anion scavenging activity of different amounts of ethanol extract from steam blanching, hot water blanching and raw daylily flowers and vitamin C was detected by the xanthine/oxidase method. Each value represents mean $\pm$ standard deviation of three replicates.

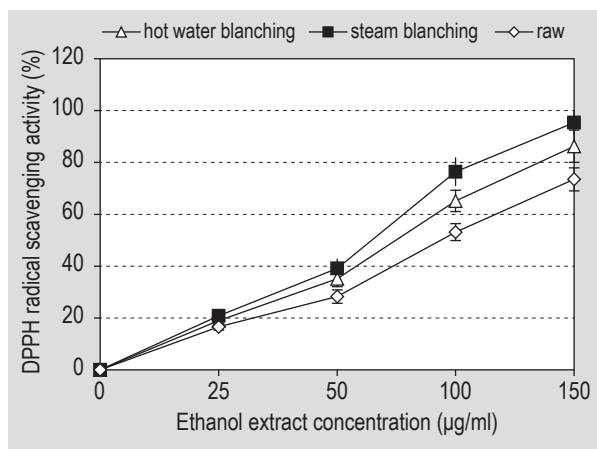


Figure 2. 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of different amounts of ethanol extract from steam blanching, hot water blanching and raw daylily flowers. Each value represents mean $\pm$ standard deviation of three replicates.

Nitric oxide radical scavenging activity

It is well known that nitric oxide plays an important role in various inflammatory processes, the toxicity of NO increased greatly when it reacted with superoxide radical, forming the highly reactive peroxynitrite anion (ONOO^-) (Majumdar, 2003). It can be seen from Figure 4 that the scavenging effect of extracts from three treatments of daylily flower on nitric oxide radical followed the order: raw > steam blanching > hot water blanching, significant difference was found among these three treatments ($P < 0.05$). Nitric oxide radical scavenging activity decreased by 10.49% and 23.11% for steam blanching and hot water blanching, respectively.

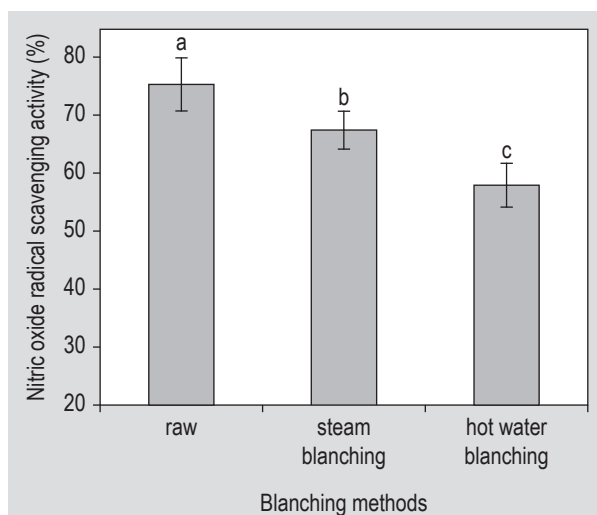


Figure 4. Nitric oxide radical scavenging activity of ethanol extract from steam blanching, hot water blanching and raw daylily flowers. Each value represents mean $\pm$ standard deviation of three replicates, different letters upper in bars mean significant difference ($P < 0.05$).

And it was different from the tendency of the above three antioxidant assays as the order was steam blanching > hot water blanching > raw, which indicated that different compounds were responsible for these activities.

Antioxidant compounds

Fruits and vegetables are good sources of natural antioxidants for the human diet, containing many different antioxidant components, such as carotenoids, vitamins, and phenolic compounds, which provide protection against harmful free radicals and have been strongly associated with reduced risk of chronic diseases and age-related functional decline in addition to other health benefits.

Ascorbic acid could chelate heavy metal ions, react with singlet oxygen and other free radicals, and suppress peroxidation, thus reducing the risk of arteriosclerosis, cardiovascular diseases, and some forms of cancer. In this experiment, blanching caused loss of ascorbic acid in daylily flowers (Table 1). Ascorbic acid content in raw samples was 1.31 mg/g, while it was 0.95 and 0.65 mg/g in steam blanching and hot water blanching samples, which caused a significant loss of 27.50 and 50.38%, respectively ($P<0.05$). A similar trend was found in fresh broccoli in which blanching in water for 90 s caused an ascorbic acid loss by 47.5% (Zhang and Hamauzu, 2004).

The β -carotene content of daylily flowers was affected by blanching treatment: a loss of 16.67 and 27.08% was found

for steam blanching and hot water blanching, respectively (Table 1), but there was no significant difference between them ($P>0.05$).

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Previous work demonstrated that (+)-catechin, chlorogenic acid, rutin and quercetin were the main antioxidant compounds in fresh daylily flower extract (Fu *et al.*, 2009), so in this work, we investigated variations of these four active compounds in blanching treatments.

Changes of four antioxidant compounds in daylily flowers were different affected by blanching (Table 2). (+)-catechin, the major phenolic compounds (Fu *et al.*, 2009), increased by 23.4 and 14.9% for steam blanching and hot water blanching, respectively ($P<0.05$), while changes of chlorogenic acid, rutin and quercetin were minimal and no significant difference was found in most cases ($P>0.05$). Total phenolic content in extracts of daylily flowers was in the order of steam blanching > hot water blanching > raw and the amount was 7.17, 8.50 and 7.92 mg/g, respectively, and blanching treatments increased phenolic content by 18.55 and 10.46% for steam blanching and hot water blanching, respectively (Table 2).

Table 1. Contents of ascorbic acid and β -carotene content (mg/g) of blanched daylily flowers. Each value represents mean $\pm$ standard deviation of three replicates. Different letters in the same row mean significant differences ($P<0.05$).

Index	Treatment		
	Raw	Steam blanching	Hot water blanching
Ascorbic acid	1.31 $\pm$ 0.04a	0.95 $\pm$ 0.02b	0.65 $\pm$ 0.03c
β -Carotene	0.48 $\pm$ 0.04a	0.40 $\pm$ 0.03b	0.35 $\pm$ 0.03b

Table 2. Contents of individual phenolic compounds (mg/g) of blanched daylily flowers. Each value represents mean $\pm$ standard deviation of three replicates. Different letters in the same row mean significant differences ($P<0.05$).

Index	Treatment		
	Raw	Steam blanching	Hot water blanching
(+)-Catechin	5.76 $\pm$ 0.29a	7.11 $\pm$ 0.35b	6.62 $\pm$ 0.28c
Chlorogenic acid	0.39 $\pm$ 0.03a	0.36 $\pm$ 0.04a	0.29 $\pm$ 0.02b
Rutin	0.67 $\pm$ 0.04a	0.70 $\pm$ 0.04a	0.68 $\pm$ 0.03a
Quercetin	0.35 $\pm$ 0.03a	0.33 $\pm$ 0.02a	0.33 $\pm$ 0.03a
Total	7.17	8.50	7.92

4. Discussion

Generally, blanching can result in loss of antioxidant activity due to the large surface area of the vegetables in contact with water or steam during blanching, which might be due to the loss of activity of some antioxidant components. A loss of approximately 50% antioxidant activity is caused by blanching in processed vegetables (Hunter and Fletcher, 2002); more than 50% loss of antioxidant activity determined by β -carotene bleaching assay and DPPH free radical scavenging assay were found in five types of cabbages after blanching in water at 98 °C for 5, 10 and 15 min than fresh samples (Amin and Lee, 2005). Antioxidant compounds, such as ascorbic acid, total phenolic, and β -carotene contents decreased 48.87, 22.97 and 15.33%, respectively, after blanching in water at 90 °C for 2 min in persimmons peel (Akter *et al.*, 2010). In celery, hot water blanching for 10 min gave extensive reductions in total phenolic content by 37.8%, DPPH 21.0% and ABTS 27.7% on average, and steaming for 10 min had the modest effect (Yao and Ren, 2011). Furthermore, there was also the probability that most components with high antioxidant activity had high solubility in boiling water, such as ascorbic acid and some water soluble phenolic compounds (Gil *et al.*, 2000). However, some reports found that blanching would enhance the antioxidant activity, blanching for 30–60 s increased free radical scavenging activity in sweet potato leaves (Chu *et al.*, 2000); thermal processing of sweet corn caused antioxidant activity and total phenolics to increase by 44 and 54%, respectively, although 25% loss of ascorbic acid was observed (Dewanto *et al.*, 2002). Turkmen *et al.* (2005) found that antioxidant activity of the vegetables was increased by boiling. Steam blanching at 85 °C for 3 min improved the retention of antioxidant capacity in the final product of blueberries (*Vaccinium corymbosum* L.) from approximately 66.9–56.9% in not blanched berries to 88.6–95.7% in blanched ones (Giovannelli *et al.*, 2012). In this present work, blanching enhanced the antioxidant activity of daylily flowers measured by four assays, accompanied the increasing content of phenolic, although ascorbic acid and β -carotene loss was observed. This result may be attributed to the fact that short blanching time and high temperature. Yao and Ren (2011) also found that phenolic content and antioxidant activities were affected significantly by time of the thermal treatments. Report of Heras-Ramírez *et al.* (2012) indicated that shorter drying times and higher temperatures reduced the degradation reaction of polyphenols. Upon steam- and water-blanching of coriander leaves and fruits. The amounts of phenolics as well as antioxidant capacities were increased compared with the unheated control, whereas, prolonged heat treatment resulted in lower values than short-time blanching (Kaiser *et al.*, 2013).

The increase of antioxidant activity in this work might be attributed to the formation of compounds that have

antioxidant activity during the blanching treatment, especially for phenolic content, which was increased by 18.55 and 10.46%, respectively (Table 2). It was in agreement with the observation of Dewanto *et al.* (2002) that antioxidant activity and total phenolics increased by 44 and 54%, respectively, after thermal processing. In the present work, antioxidant activity increased accompanied by the phenolic increased, although ascorbic acid decreased remarkably, this results indicated novel compounds contributed to antioxidant activity were formed or transformed, especially for novel phenolic compounds. Heras-Ramírez *et al.* (2012) found that individual phenolic compounds were significantly increased ($P < 0.05$) in blanched pomace compared with the unblanched samples, the higher concentration of polyphenolic compounds may be the result of a release of bound polyphenolics from destructured tissues caused by the blanching conditions. Other studies also reported increase in polyphenolics caused by thermal treatments attributed to the release of bound polyphenolics from the plant tissue (Bao *et al.*, 2004; Turkmen *et al.*, 2005). Although other studies have reported decrease in individual polyphenolic contents for different vegetables and legumes, it should be noted that those studies were performed under more drastic conditions of temperature and time (Xu and Chang, 2009).

Our previous study showed that (+)-catechin was the most abundant phenolic compound in daylily flowers, accounting for about 74.11% of the sum of phenolic compounds, and (+)-catechin could be the main compound responsible for its antioxidant property (Fu *et al.*, 2009). In blanched daylily flowers, (+)-catechin was also the most abundant phenolic compounds (more than 80% in Table 2). A report in tea extracts carried out by Manzocco *et al.* (1998) that pasteurisation was found to cause an increase in antioxidant activity of teas, which was rich in (+)-catechin. Thus in blanched daylily flowers, (+)-catechin was the main compound responsible for the antioxidant activities.

5. Conclusions

Our data demonstrated that the phenolic compounds, β -carotene, ascorbic acid and antioxidant activities in daylily flowers were affected significantly by blanching treatments. Blanching enhanced the antioxidant activities and (+)-catechin content, resulted in great loss of β -carotene, ascorbic acid. Steam blanching had the higher antioxidant activity and compounds than those of hot water blanching. Thus, steam blanching may be recommended for daylily flowers pre-treatment for the further processing or consumption in view of health effects.

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