Effects of Heating Time on the Antioxidative Capacities of Citrus Fruit 

(*Citrus sinensis* (L.) Osbeck) By-products

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By-products of Orange (*Citrus sinensis* (L.) Osbeck) juice processing are that are rich in flavonoids. This study investigated changes in total phenolic and flavonoid concentrations and antioxidative activities of orange peel methanol extracts in response to various heating times (0, 30, 60, 90, 120, 150 and 180 min) at 100°C, after a 48-h drying treatment at 50°C. The total phenolic and flavonoid contents of the 0 min heat-treated peel methanol extract (T0) were 21.65 ± 1.42 GAE mg/g, and 4.77 ± 0.01 CE mg/g, respectively. The phenolic content increased as the heating time increased, as shown by the 180 min heating treatment (T180; 27.99 ± 0.38 GAE mg/g), but changes in total flavonoid content were not significant between different heating time treatments. Increasing the heating time of orange peels could improve the antioxidative activities (1,1-Diphenyl-2-picrylhydrazyl radical scavenging effect, ABTS·+ scavenging effect and reducing power) of methanol peel extracts, but there were no significant difference between the β-carotene bleaching inhibition of the different heating time treatments, excluding T30. According to correlation analysis, one possible factor of increasing antioxidative activities was the production of phenolic compounds during heating treatment.

Keywords: flavonoid, citrus, peel, antioxidative activity, phenolic content

Introduction

Rich bioactive compounds in citrus fruits, such as phenolic acids, flavonoids, limonoids, carotenoids and fibre are considered healthful components of the human diet (Bocco *et al.*, 1998; Gorinstein *et al.*, 2001; Schieber *et al.*, 2001). In 2008, Taiwan produced 250 thousand tons of orange (*Citrus sinensis* (L.) Osbeck), which were the highest amount of citrus fruits. In Taiwan, the wastes (peels) from orange juice processing was typically used as feed for animals or discarded, but orange peels are rich in bioactive compounds that could be further utilized.

In general, the waste from citrus juice processing has been used as a source for molasses, pectin, cold-pressed oil and limonene (Braddock, 1995). Recently, scientists studied various treatments for extracting bioactive compounds from citrus peels. These treatments include heat, far-infrared radiation, ultrasound-assisted extraction, fermentation, alkaline hydrolysis and enzyme treatments (Nicoli *et al.*, 1997; Niwa *et al.*, 1997; Bocco *et al.*, 1998; Lee *et al.*, 2003; Mandalari *et al.*, 2006; Rivas *et al.*, 2008; Khan *et al.*, 2010).

Heat treatment was the most commonly used method, but it changed the nutritional value, physical properties and microstructures of fruits, vegetables and their by-products (Que *et al.*, 2008; Akter *et al.*, 2010; Devahastin and Niamnuy, 2010). The traditional Chinese medicine, chen pi, was prepared from heat-treated *Citrus reticulata* peels and has been widely used to treat indigestion and inflammatory syndromes in the respiratory tract (Ou, 1999). Methanol extracts from the peels of heat-treated citrus fruit (*Citrus reticulata*) have antioxidative and anti-inflammatory activity (Ho and Lin, 2008). The peels of Ponkan (*Citrus reticulata* Blanco) and Tonkan (*Citrus tankan* Hayata) exhibit outstanding inhibitory effects on prostaglandin E2 (PGE2) and nitric oxide (NO) secretion (Huang and Ho, 2010).

Some studies indicated that heating temperature and time could affect the polyphenol content and antioxidative activities of citrus peels (Jeong *et al.*, 2004; Rehman 2006; Garau

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et al., 2007; Xu et al., 2007; Ho and Lin, 2008). Higher temperatures (> 100°C) destroyed the flavonoid compounds and decreased the antioxidative activities (Xu et al., 2007), and longer heating time increased the DPPH radical scavenging effect (Ho and Lin, 2008). Jeong et al. (2004) mentioned that heat treatments might release some low molecular weight phenolic compounds from, and increase the antioxidative activity of, citrus peels. Garau et al. (2007) also illustrated that the minor disruption of cell wall polymers, and peel pectic substance in particular, was promoted at around 50 and 60°C of dehydration, and the antioxidant capacity of the dehydrated citrus (Citrus aurantium v. Canoneta) by-products was significantly higher for orange peels than for pulp samples.

Orange (Citrus sinensis (L.) Osbeck) peel with a total flavonoid content of 35.5 CE mg/g, db (Wang et al., 2008) should have more potential antioxidative activity after heating treatment. However, existing research provides few information about the changes caused by heating time treatments for by-products. Therefore, this study investigated the effect of drying time on phenolic and flavonoid contents and the antioxidative capacity of orange peels.

Materials and Methods

Preparation of heated by-products extract  Oranges (Citrus sinensis (L.) Osbeck) grown in Gukeng Township, Yunlin County in central Taiwan, were harvested when the fruits were mature and the color of the fruits was yellow. The fruits were transported to local market at room temperature, and they were bought at the same day in January 2009. The weight of the oranges was around 200 ± 10 g. To simulate orange juice processing, oranges were separated to juices and by-products (included peel, segment wall, juice sac wall, central axis and seed) after squeezing by gauze, and by-products were further dried to as samples. The fruits were washed and directly cut into two pieces and squeezed to make juice using hand by squeezer (Juice Maker, XuanHe Machinery, Taichung, Taiwan). To simulate the chen-pi making, the by-products were dried in an oven at 50°C for 48 h (Ho and Lin, 2008). The dried by-products were heated at 100°C for 0, 30, 60, 90, 120, 150 and 180 min (T0, T30, T60, T90, T120, T150, and T180) and then were ground into a powder. Methanol was used as a solvent for phenolic compound extraction, which including flavonoids from fresh plant tissues (Yao et al., 2004). Each powder was extracted twice with a 10-fold volume of methanol for 20 h and filtrated with filter paper (No. 1, Advantec Toyo Kaisha, Japan). The filtrates were evaporated under a vacuum using a rotary evaporator at < 50°C to obtain the dried extracts, and the extracts were stored at −20°C until use.

Determination of total phenolic content  Total phenolic content was determined by a method modified from Julkunen-Titto (1985), and gallic acid was used as the standard. Deionised water (1 mL), Folin-Ciocalteu's phenol reagent (0.5 mL) and 20% Na2CO3 solution (2.5 mL) were added into the standard or sample solutions (50 μL). The mixtures were incubated at ambient temperature in the dark for 20 min, and the absorbance was measured at 735 nm (Spectro UV-Vis Auto Spectrophotometer, Labomed Inc., Culver City, CA, USA). Total phenolic content was calculated using a standard curve and expressed as mg gallic acid equivalents/g extract, dry basis (GAE mg/g, db).

Determination of total flavonoid content  The method reported by Zhishen et al. (1999) was modified and used to determine total flavonoid content. A 250 μL sample or standard (catechin) solution was mixed with 1.25 mL of deionised water and 75 μL of 5% NaNO2 for 6 min. The mixture was added to 10% AlCl3·H2O (150 mL) for 5 min, and then added to 0.5 mL of 1 M NaOH. The volume of total mixture was diluted to 2.5 mL with deionised water, and the absorbance was measured at 510 nm. A standard curve was generated with (+)-catechin, and the results were expressed as mg catechin equivalents/g extract, dry basis (CE mg/g, db).

Analysis of hesperidin contents by HPLC  The method used for the analysis of phenolic acid and flavonoid composition of a citrus peel was based on the method reported by Lin et al. (2010). The Waters HPLC system consisting of a Model 600 controller pump system (Waters, MA, USA) equipped with an on-line degasser (Degasys DG-1310, Uniflows, Tokyo, Japan), a C18 column (250 mm × 4.6 mm i. d., 5 μm) (Hypersil GOLD, Thermo Fisher Scientific Inc., Waltham, MA) and a UV-Vis detector (Waters 486 Tunable Absorbance Detector, Waters, MA, USA). The flow rate was 0.8 mL/min, and the absorbance of sample was recorded at 280 nm. The elution was used a gradient solvent system that consisted of methanol (A) and 9% acetic acid (aqueous) (B), and was performed as follows: 0 min, 5% A; 5 min, 17% A; 25 min, 17% A; 40 min, 31% A; 76 min, 31% A; 80 min, 40% A; and 120 min, 40% A. Hesperidin was used as the standards and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Determination of the antioxidative activities of orange peel extracts  The antioxidative activities of the samples were determined by using four indicators: DPPH radical scavenging activity, ABTS+ radical scavenging activity, reducing power and inhibition of β-carotene bleaching. Each of these methods was described below.

1. DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging effect  DPPH radical scavenging activity was measured using the method from Epsin et al. (2000) with modifications. A 20 μL sample was dispensed into the wells of a 96-well plate. A 200 μL aliquot of a 0.2 mM DPPH solution pre-
pared with 100% methanol was added into each sample well. The absorbance of the plates was measured at 517 nm (A_{517}) using a microplate reader (Multiskan® Spectrum, Thermo, Vantaa, Finland) after incubation at 37°C in a dark room for 30 min. The DPPH radical scavenging effect was evaluated based on the percentage of DPPH radical scavenged using the following equation:

\[ S_{\text{DPPH}} = \{S_b - (S_c - S_s)\} / S_b \times 100 \]  \hspace{1cm} (1)

where \( S_{\text{DPPH}} \) is the DPPH radical scavenging effect expressed as a percentage, \( S_b \) is \( A_{517} \) of a blank treatment (100% methanol solution), \( S_c \) is \( A_{517} \) of a sample solution, and \( S_s \) is \( A_{517} \) of the background of a sample.

2. \textit{ABTS}⁺ radical scavenging effect The \textit{ABTS}⁺ radical scavenging effect was determined using the method reported by Scalzo et al. (2005), with modifications. Peroxidase, \( \text{H}_2\text{O}_2 \) and 2,2-azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) were mixed with deionised water to a final peroxidase activity as 4.4 U/mL, and \( \text{H}_2\text{O} \) and ABTS concentration as 100 mM and 50 μM, respectively. The solution was incubated at 30°C in a dark room for one hour and dispensed in 300 μL aliquots into a 24-well plate, which was previously loaded with 30 μL of sample solutions per well. After 2 min, the absorbance of the plate was determined at 734 nm (\( A_{734} \)) with a microplate reader. The \textit{ABTS}⁺ radical scavenging effect (\( S_{\text{ABTS}} \)) was calculated using the following equation:

\[ S_{\text{ABTS}} = \{A_b - (A_c - A_s)\} / A_b \times 100 \]  \hspace{1cm} (2)

where \( A_b \) is \( A_{734} \) of a blank treatment (100% methanol solution), \( A_c \) is \( A_{734} \) of a sample solution, and \( A_s \) is \( A_{734} \) of the sample background.

The \textit{ABTS}⁺ radical scavenging effect of these samples was used for calculating the TEAC. The antioxidant capacity of the compounds was expressed relative to that of Trolox equivalents (μM).

3. Reducing power assay The reducing power of the samples was determined using the modified method from Oyaizu (1986). A sample (250μL) was mixed with sodium phosphate buffer (250μL, 0.2M, pH 6.6) and potassium ferricyanide (250μL, 1%), incubated at 50°C for 20 min and 250μL of 10% (g/mL) trichloroacetic acid was added at the end of the incubation. The mixture was centrifuged at 3000 rpm for 10 min, and a 100μL aliquot of supernatant was mixed with 100μL deionised water and 25μL of 0.1% FeCl₃. Each solution was then dispensed into a 96-well plate. After 10 min, the absorbance was measured at 700 nm using a microplate reader.

4. Inhibition of \( \beta \)-carotene bleaching assay To determine the inhibition of \( \beta \)-carotene bleaching, a bleaching assay was performed as described by Siddharaju and Becker (2003).

Before each experiment, a \( \beta \)-carotene-linoleic acid emulsion was freshly prepared. \( \beta \)-Carotene (2.0 mg) was dissolved in 10 mL chloroform. Linoleic acid (2.0 mg) and Tween 80 (200 mg) were dissolved in 1 mL of the \( \beta \)-carotene chloroform solution. The solution was then evaporated under a vacuum by a rotary evaporator at 45°C, to remove the chloroform. Deionised water (50 mL) was added to the mixture, and the mixture was vigorously shaken to form an emulsion. A 250μL aliquot of the emulsion was dispensed into each well of a 96-well plate, which was previously loaded with 30 μL of sample solution. The plate was incubated at 45°C, and the absorbance was measured at 470 nm using a microplate reader, after an incubation time that ranged from 20 to 120 min.

\textbf{Statistical analysis} The data were subjected to analysis of variance (ANOVA) and Duncan’s multiple range tests, which were administered to identify significant differences between the means at a level of \( p < 0.05 \). All treatments were performed two replicates. The determined results were reported as an average of three replicates.

\textbf{Results and Discussion}  \hspace{1cm} \textit{Changes in total phenolic and flavonoid compounds during heating times} Table 1 shows the changes in total phenolic and flavonoid contents of dried peels in response to different heating times. The total phenolic and flavonoid contents of \( T_0 \) citrus peel were 21.65 ± 1.43 GAE mg/g and 4.77 ± 0.09 CE mg/g, respectively. The total phenolic contents of different heating time treatments were variable, and at \( T_{180} \) the content reached the highest concentration (27.99 ± 0.38 GAE mg/g). The results were similar to those reported by Ho and Lin (2008), but there was not significant difference in the total flavonoid contents between heating treatments.

The total phenolic contents of 80% methanol Huyou

\textbf{Table 1.} Changes in total phenolic and flavonoid contents at different heating times.

<table>
<thead>
<tr>
<th></th>
<th>Total phenolic (mg/g, db)¹</th>
<th>Total flavonoid (mg/g, db)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_0 )</td>
<td>21.65 ± 1.43c</td>
<td>4.77 ± 0.09a</td>
</tr>
<tr>
<td>( T_{30} )</td>
<td>24.16 ± 2.53bc</td>
<td>4.11 ± 0.03a</td>
</tr>
<tr>
<td>( T_{60} )</td>
<td>21.35 ± 1.70c</td>
<td>4.29 ± 0.22a</td>
</tr>
<tr>
<td>( T_{90} )</td>
<td>26.59 ± 0.87ab</td>
<td>4.36 ± 2.55a</td>
</tr>
<tr>
<td>( T_{120} )</td>
<td>22.93 ± 0.98c</td>
<td>4.00 ± 0.38a</td>
</tr>
<tr>
<td>( T_{150} )</td>
<td>24.11 ± 1.75bc</td>
<td>5.47 ± 0.31a</td>
</tr>
<tr>
<td>( T_{180} )</td>
<td>27.99 + 0.38a</td>
<td></td>
</tr>
</tbody>
</table>

¹ mg gallic acid equivalent/g of dry weight.
² mg catechin equivalent/g of dry weight.
³ Dried basis.
⁴ Data are presented as means ± standard deviations (\( n = 3 \)).
⁵ Different letters for the individual extracts indicate that the values are significantly different (\( p < 0.05 \)).
(Citrus paradisi Changshanhyou) peel extracts decreased as the heating time increased to 120°C, with heating treatments at 90, 120 and 150°C for 30min (Xu et al., 2007). However, the total phenolic content of 70% ethanol Citrus unshiu peel extracts increased as heating temperature and time increased (at 50, 100 and 150°C), and several low molecular weight phenolic compounds were newly formed in the Citrus unshiu peel extracts heated at 150°C for 30 min (Jeong et al., 2004). Heating time and temperature changed the phenolic content of the citrus peel, and the trend of this change varied between citrus strains. Ho and Lin (2008) indicated the flavonoid contents increased as the heat time increased, but the total flavanone glycoside contents of the 150°C heating Huyou peel was lower than that of Huyou peel heated to 120°C (Xu et al., 2007). Also, the total flavanone glycoside content from Huyou peel heated for 90 min was lower than that heated for 60 min at 120°C. Therefore, a high temperature (> 100°C) treatment destroyed the flavonoid compounds but a low temperature (< 100°C) did not (Xu et al., 2007). To increase phenolic compounds, citrus peels heated at a low heating temperature (< 100°C) for long time (> 180 min) would be a better option.

Changes in hesperidin content during heating times
There were many flavonoids in orange peels, and hesperidin was the general most content (Wang et al., 2008). Hence, hesperidin was determined during heating times. Hesperidin amounts of samples were changed during heating times and shown in Figure 1. The hesperidin contents of samples were significantly decreased at T30 and T90, and there were not significant among T60, T120, T150 and T180. This result revealed that hesperidin could be destroyed during heating treatment (100°C) and heating time (< 60min). Xu et al. (2007) mentioned that higher temperatures (> 100°C) destroyed the flavonoid compounds and decreased the antioxidative activities. In our study, the trend of hesperidin changes was similar to Xu et al. (2008) but the changes of antioxidative activities were opposite trend. It was an interesting phenomenon. We speculated that hesperidin was destroyed to lead the antioxidative activities to decrease, and some compounds were produced to increase the antioxidative activities. We found some unknown compounds in hesperidin determination. However, effects of these compounds were needed to further investigate.

Changes in antioxidative capacities during different heating times
Changes in the DPPH radical scavenging activity, ABTS⁺ radical scavenging activity, reducing power and inhibition of β-carotene bleaching of the samples during different heating times were described below.

1. DPPH radical scavenging effect
The changes in the DPPH radical scavenging effect of treatments at different heating times are shown in Figure 2. The DPPH radical scavenging effects increased as the concentration of extracts.
The DPPH scavenging effect (Kuljarachanan et al., 2009). Kuljarachanan et al. mentioned that vitamin C could be susceptible degradation during heating at higher temperature, but phenolic compounds are more stable under the same conditions. Furthermore, Jeong et al. (2004) and Ho and Lin (2008) indicated the DPPH radical scavenging effect increased as heating time increased. The EC50 values for the DPPH radical scavenging effect were 371 – 117 μg/mL when heated at 100°C for 0 – 180 min (Ho and Lin, 2008), but these values were lower than the those in this study. The difference may increased. The DPPH radical scavenging effects ranged from 54.59% to 75.99% in 2.5 mg/mL and from 88.78% to 94.92% in 5 mg/mL, and T180 had the highest scavenging effect. The half maximal effective concentrations (EC50) of extracts by the DPPH scavenging effect are shown in Table 2. The EC50 values decreased as heating time increased, and the EC50 value of T180 was the lowest value (1.19 ± 0.12 mg/mL).

The DPPH scavenging effect of lime (Citrus aurantifolia Swing) residues decreased when the drying time increased, and higher drying temperatures resulted in higher losses of DPPH scavenging effect (Kuljarachanan et al., 2009). Kuljarachanan et al. mentioned that vitamin C could be susceptible degradation during heating at higher temperature, but phenolic compounds are more stable under the same conditions. Furthermore, Jeong et al. (2004) and Ho and Lin (2008) indicated the DPPH radical scavenging effect increased as heating time increased. The EC50 values for the DPPH radical scavenging effect were 371 – 117 μg/mL when heated at 100°C for 0 – 180 min (Ho and Lin, 2008), but these values were lower than the those in this study. The difference may

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**Table 2.** EC50 values of orange peel extracts by DPPH radical method, ABTS + radical method, reducing power and inhibition bleaching effect.

<table>
<thead>
<tr>
<th></th>
<th>DPPH (mg/mL)</th>
<th>ABTS + (mg/mL)</th>
<th>Reducing power (mg/mL)</th>
<th>Inhibition bleaching (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>2.32 ± 0.04a</td>
<td>1.73 ± 0.08a</td>
<td>1.73 ± 0.04a</td>
<td>1.70 ± 0.05b</td>
</tr>
<tr>
<td>T30</td>
<td>2.07 ± 0.09b</td>
<td>1.69 ± 0.07a</td>
<td>1.16 ± 0.18b</td>
<td>3.25 ± 0.09a</td>
</tr>
<tr>
<td>T60</td>
<td>2.03 ± 0.09b</td>
<td>1.55 ± 0.03b</td>
<td>0.95 ± 0.04c</td>
<td>1.72 ± 0.06b</td>
</tr>
<tr>
<td>T90</td>
<td>1.58 ± 0.01c</td>
<td>1.56 ± 0.05b</td>
<td>0.76 ± 0.03d</td>
<td>1.65 ± 0.21b</td>
</tr>
<tr>
<td>T120</td>
<td>1.66 ± 0.09c</td>
<td>1.45 ± 0.06c</td>
<td>1.19 ± 0.03b</td>
<td>1.90 ± 0.02b</td>
</tr>
<tr>
<td>T150</td>
<td>1.58 ± 0.07c</td>
<td>1.42 ± 0.06c</td>
<td>0.99 ± 0.07c</td>
<td>1.70 ± 0.33b</td>
</tr>
<tr>
<td>T180</td>
<td>1.19 ± 0.12g</td>
<td>1.30 ± 0.01d</td>
<td>0.52 ± 0.12e</td>
<td>1.51 ± 0.11b</td>
</tr>
</tbody>
</table>

1 Data are presented as means ± standard deviations (n = 3).
2 Different letters for the individual extracts indicate that the values are significantly different (p < 0.05).
be due to the variation in citrus species. These results indicate that a higher heating time improves the DPPH radical scavenging effect of orange peels, and Chen et al. (2011) mentioned the similar results. We speculated that heat treatment would destroy some compounds, liberate some compounds or produce a new product though the interacting between components, and the effect of DPPH radical scavenging would be affected.

2. ABTS•⁺ radical scavenging effect Table 3 shows the changes in the TEAC of orange peel extract (10 mg/mL) at different heating times. The TEAC value increased as the heating time increased. The TEAC of T₁₈₀ was significantly higher than the other treatments. Lin et al. (2008) indicated that heat treatment could increase the TEAC value of kumquat peels. The EC₅₀ values of extracts from the ABTS•⁺ radical scavenging effect were determined for various concentrations of treated peel extracts, are shown in Table 2. The EC₅₀ values would gradually decrease as heating times increased, and T₁₈₀ had the lowest EC₅₀ value (1.30 mg/mL). Xu et al. (2007) indicated that the ABTS•⁺ effect in huyou (Citrus paradisi changshanhuyou) peel extract increased with heating time (30, 60 and 90 min at 120°C) and temperature (90, 120 and 150°C). Our result indicates a heating time affects the ABTS•⁺ radical scavenging effect in orange peels during heating at 100°C.

3. Reducing power The changes in reducing power in the various treated orange peels are shown in Figure 3. In this assay, the transformation of Fe³⁺ into Fe²⁺ in the methanol extracts was measured, and the yellow colour of the test solution changed from various shades of green or blue (Amarowicz et al., 2004). The reducing power at T₀ gradually increased as the concentration increasing to 7.5 mg/mL. A heating treatment increased the reducing power of peel extracts, and the reducing power at T₁₈₀ was significantly higher than in the other treatments. The reducing power of the Kinnow (Citrus reticulata Blanco cv Kinnow) rotary extract peel

Table 3. Changes in TEAC of orange peel extracts (10mg/mL) at different heating times.

<table>
<thead>
<tr>
<th>Treatment (10 mg/mL)</th>
<th>TEAC (μM)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>6.22 ± 0.21c²</td>
</tr>
<tr>
<td>T₃₀</td>
<td>6.83 ± 0.18b</td>
</tr>
<tr>
<td>T₆₀</td>
<td>6.74 ± 0.02b</td>
</tr>
<tr>
<td>T₉₀</td>
<td>6.87 ± 0.19b</td>
</tr>
<tr>
<td>T₁₂₀</td>
<td>6.80 ± 0.07b</td>
</tr>
<tr>
<td>T₁₅₀</td>
<td>6.96 ± 0.26b</td>
</tr>
<tr>
<td>T₁₈₀</td>
<td>7.55 ± 0.24a</td>
</tr>
</tbody>
</table>

¹ Data are presented as means ± standard deviations (n = 3).
² Different letters for the individual extracts indicate that the values are significantly different (p < 0.05).

Fig. 3. Changes in reduce power of concentrations at positive controls (ascorbic acid (●) (+)-catechin (○)), and different heating times 0 min (▼), 30 min(▲), 60 min (■), 90 min (□), 120 min (●), 150 min (○) and 180 min (▲).
tition effects of extracts were higher than ascorbic acid at concentrations of 5 and 7.5 mg/mL. This indicates that heating orange peel extracts results in a better inhibition effect in comparison than with the effects from ascorbic acid. Phenolic compounds are known to inhibit lipid oxidation by donating hydrogen atoms to free radicals (Sánchez-Moreno et al., 1998). The EC50 values for extracts with an inhibition effect are shown in Table 2. Guimaraes et al. (2010) mentioned that the EC50 values for inhibition effect of the volatile and polar fraction of fresh, sweet orange peels were 18.44 and 0.26 mg/mL, respectively. In this study, the highest value for EC50 was at T30, and the EC50 values were not significantly different between the other treatments. This result indicates that the compounds, which could inhibit β-carotene bleaching, are destroyed by heating to 100°C for 30 min, and some compounds, which could inhibit β-carotene bleaching, are produced during longer heating times. However, the details of the reaction need to be further investigated.

5. Correlation assays
The correlation coefficients between the EC50 for antioxidative activity and total phenolic content, as measured by DPPH, TEAC, reducing power and inhibition bleaching effect, were −0.821, −0.572, −0.735 and −0.120, respectively. Also, the correlation coefficients between the EC50 for antioxidative activity and total flavonoid content were −0.365, −0.339, −0.096 and −0.426. However, the

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**Fig. 4.** Changes in β-carotene bleaching inhibition of concentrations at positive controls (ascorbic acid (●)), and different heating times 0 min(○), 30 min(▼), 60 min(△), 90 min(■), 120 min(□), 150 min(◆) and 180 min(◇).
correlation coefficients between the EC$_{50}$ for antioxidative activity and hesperidin content were 0.776, 0.799, 0.921 and 0.299. According to these results, the phenolic compounds of methanol peel extract played an important role in the effect of DPPH, TEAC and reducing power, but they could not affect the inhibition bleaching effect. The effect of the total flavonoid content that influenced the changes in antioxidative activities was lower than the effect from the total phenolic content. In this result, decreased hesperidin content could lead the antioxidative activities (DPPH, TEAC and reducing power) increase. The heat treatment may release some low molecular weight phenolic compounds from citrus peels (Jeong et al., 2004). We suggest that low molecular weight phenolic compounds can improve the antioxidative activities. Nevertheless, the compounds that could affect the inhibition of β-carotene bleaching require further investigation.

**Conclusion**

The results from this study indicated that heating times affected the changes in antioxidative activities of orange (*Citrus sinensis* (L.) Osbeck) by-products. An increase in the heating time for by-products results in an increase in antioxidative activities. The by-products from orange juice processing could be heated and ground to a powder for use as a food additive. This supplementation would enhance the nutritional value of the food since the powder contains high-fiber content and antioxidative properties, both of which are known to promote good health. The by-products from orange peels might be considered a healthy addition to the human diet. There was not evidence to prove the antioxidative effects in vivo, so the effects in vivo were needed to further investigation.

**References**


