Effects of Different Cooking Methods and Palm Oil Addition on the Bioaccessibility of Beta-Carotene of Sweet Leaf (Sauropus androgynous)

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(Received June 5, 2019)

Summary Beta-carotene is one of phytochemicals which play role as natural antioxidant related to the reduction of oxidative stress that is linked to Non-communicable diseases (NCDs). Sweet leaf (Sauropus androgynous), one of the indigenous plants in Asia, contains high contents of beta-carotene. However, the bioaccessibility of beta-carotene in sweet leaf might be altered among the different cooking methods. Therefore, the effects of different cooking methods (raw, boiling, and microwave cooking) and addition of palm oil on the bioaccessibility of beta-carotene of sweet leaf were investigated before and during in vitro simulated gastrointestinal digestion. We found that the boiling and microwave cooking methods caused the lower beta-carotene contents in cooked sweet leaf compared to raw leaf. However, the addition of 10% (v/w) palm oil during cooking helped increasing the bioaccessible beta-carotene contents after digestion in all cooking methods, compared to those without palm oil addition (p,<0.05). In addition, the bioaccessibility of beta-carotenes was found to increase about 20% when the palm oil was added into the microwaved sweet leaf. The findings of this study suggested that the addition of 10% (v/w) palm oil during cooking could improve the bioaccessible beta-carotene contents in the sweet leaf, especially when the sweet leaf was cooked by microwave.

Key Words Beta-carotene, bioaccessibility, cooking methods, palm oil, Sauropus androgynous

Recently, the consumption of phytochemicals on a regular basis has been related with the reduced chronic diseases. Food sources that contain significant amount of bioactive components may provide desirable health benefits and play important roles in the prevention of chronic diseases (1). For example, carotenoids help protecting cellular systems from oxidative damage and stimulate DNA repair (2). Carotenoids exhibited the health benefits related to their antioxidant activity. The dietary carotenoids act as antioxidant by quenching single oxygen and by scavenging free radicals.

Green leafy vegetables are good source of phytochemicals, especially carotenoids (3). Sauropus androgynus (known as katuk/pakwan ban/cekur manis/sweet leaf) is one of green leafy vegetable growing wild in Southeast Asia. It is considered as palatable and nutritionally superior to other leafy vegetables (4). Sweet leaf contains many vitamins with high contents, earning it the name “multivitamin plant” (5). In 100 grams of sweet leaf, it contains 5.6 mg of beta-carotene (6) and demonstrated high antioxidant capacities than other 10 vegetables (7). Sweet leaf can be consumed as raw vegetable in salad, boiled, or stir-fried vegetable.

The bioaccessibility of beta-carotene is known to be limited due to the variation of compounds in the food matrix (8). Several study on different food matrix has been conducted to see the effect addition of certain compounds to improve the bioaccessibility of beta-carotene, including dietary fat (9–11).

Cooking and addition of some food ingredients might affect the beta-carotene content in sweet leaf. Cooking processes are reported to cause loss of beta-carotene content in vegetable. Heating triggered degradation of protein and beta-carotene complex which resulted in extreme water leaching (12). Regarding to its bioaccessibility, presence of oil might improve carotenoids bioaccessibility. Ingestion of fat along with carotenoids was thought to be crucial for the absorption of carotenoids (13). Sweet leaf is known to be source of beta-carotene which the bioaccessibility is known to be limited. There have been no study conducted to investigate the effect of cooking oil addition on the bioaccessibility of beta-carotene content in the sweet leaf. Therefore, this study aims to investigate different cooking methods (raw, boil-
Cooking Methods and Bioaccessibility of Beta-Carotene

MATERIALS AND METHODS

Sample preparation and cooking processes. Fresh sweet leaf (Sauropus androgynus) was purchased from a farmer in Nakhon Pathom, Thailand and the plant was identified by a botanist from the Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. The leaves were cleaned and homogenized using a kitchen blender before cooking. Raw (control) leaves of homogenized fresh sweet leaf were blanketeted under nitrogen and kept as the raw sample. The boiled sample involved cooking the homogenized sweet leaf in boiling distilled water (1:1, weight per volume, w/v) for 5 mins. The microwaved sample was prepared by mixing homogenized sweet leaf with distilled water (1:1, w/v) and cooking in a microwave oven at 800 W for 90 s.

Palm oil at 10% (v/w) were added to sweet leaf samples (raw, boiled, and microwaved). All sweet leaf samples were made up to the same total final volume by the addition of distilled water. All samples were blanketeted with nitrogen and stored at −20°C until analyses.

In vitro simulated gastrointestinal digestion. The homogenized sweet leaf samples were digested according to the in vitro simulated gastrointestinal digestion procedure adapted from Pasakamonset, Kwon (14). Briefly, 1 g of homogenized sweet leaf samples were incubated at 37°C in a shaking water bath for 1 h with 3 mL pancelline peptic solution (40 mg/mL in 0.1 N HCl), at pH 2.0±0.1 to initiate the gastric phase. Then, the small intestinal phase was started by increasing the pH to 4.5 before the addition of amylglucosidase solution (120 mg/mL). After 30 min at 37°C with shaking, the pH was increased to 5.3 before the addition of 9 mL of small intestinal enzyme solution containing pancreatic (3 mg/mL) and bile acid (12 mg/mL) in 100 mM NaHCO₃. The final volume was increased to 20 mL with bile salts solution and the pH was adjusted to 7.2±0.1. and then incubated with shaking at 37°C for 2 h. The supernatant (aqueous fraction) was collected after centrifugation of digesta (12,000 rpm, 5°C for 1 h), filtered through a 0.22 μm nylon filter and stored at −20°C for further analyses.

The determination of beta-carotene content. The extraction of sweet leaf samples was performed using the method described by Corte-Real, Desmarchelier (15). Briefly, 1 g sweet leaf samples or 4 mL aqueous fraction were soaked with 5 mL of methanol and 1 mL of 30% KOH for the saponification of chlorophylls. The sample mixture then vortexed for 1 min at room temperature. The mixture centrifuged for 5 min (1,300 rpm, 4°C) and the methanol parts were collected. The methanol part were extracted once with 9 mL of 1:1 (v/v) hexane : acetone solution. The pellets were also extracted once with 9 mL of 1:1 (v/v) hexane : acetone solution, vortexed for 1 min, sonicated for 5 min, and centrifuged at 1,300 rpm (4°C, 5 min). The hexane parts were collected from both methanol part and pellets. The residue of extract were re-extracted with 4 mL hexane and 2 mL of saturated NaCl (1 g NaCl in 2 mL distilled water), vortexed, sonicated, and centrifuged in the same way as the previous step to collect the supernatant. Finally, the residue parts were re-extracted again with with 2 mL of diethyl ether, vortexed, sonicated, and centrifuged in the same way as the previous step then collected the supernatants. All collected organic phases including hexane and diethyl ether part from the extraction were dried under a stream of nitrogen (45 min, 25°C) and kept at –20°C under nitrogen blanket until further analysis.

The extracts of sweet leaf samples were reconstituted with hexane. Reconstituted extract samples (100 μL) were injected into the reverse-phase HPLC system (Shimadzu, Japan) equipped with pump (LC-10AD) consisting of a guard column (Inertsil ODS-3 5 μm, 4.0×10 mm×2, GL Sciences, Japan), coupled with a reverse-phase C18 column (Inertsil ODS-3V 5 μm, 4.6×150 mm, GL Sciences, Japan) and equipped with UV-Visible spectrophotometer detector (SPD-10A, Shimadzu, Japan). The mobile phase consisted of solvent A [80% acetonitrile, 15% methanol, and 5% dichloromethane (v/v)] and solvent B [30% acetonitrile, 20% methanol, and 50% dichloromethane (v/v)]. The gradient method was the following: 5–70% solvent B (0–18 min); 70–5% solvent B (18–20 min); and 5% solvent B (20–22 min). UV-vis detection was performed at 458 nm (16). The results were presented as μg beta-carotene/g leaves.

RESULTS

The effect of different cooking methods and addition of 10% (v/w) palm oil on the beta-carotene content in sweet leaf samples before and after digestion is shown in Table 1. Before digestion, boiling and microwave cooking caused the significant reduction in the beta-carotene content of sweet leaf samples.

The addition of palm oil at 10% (v/w) increased the amount of bioaccessible beta-carotene in sweet leaves after digestion comparing to those of the same cooking method without oil addition. Furthermore, when 10% (v/w) of palm oil was added during the microwave cooking, the percentage of bioaccessibility of beta-carotenes in the microwaved sweet leaves was increased about 20%.

These findings suggested that beta-carotene content in sweet leaves can be altered by the cooking methods such as boiling and microwave cooking. The addition of palm oil (10% v/w) demonstrated the improvement of bioaccessible beta-carotene content after digestion in both raw and cooked sweet leaves.

DISCUSSION

Non-communicable diseases (NCDs) including heart disease, stroke, cancer, diabetes, and chronic lung diseases contribute for almost 70% of all deaths worldwide. Reducing the risk factors (such as the tobacco use, the physical inactivity, the unhealthy diet and the harmful use of alcohol) is the main focus of the prevention of NCDs (17).
Table 1. The effect of different cooking methods on the bioaccessibility of beta-carotene during the simulated digestion.

<table>
<thead>
<tr>
<th>Cooking conditions*</th>
<th>Beta-carotene content (µg/g leaves)</th>
<th>% Bioaccessibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before digestion</td>
<td>After digestion</td>
</tr>
<tr>
<td>Raw (control)</td>
<td>35.58±2.18*</td>
<td>7.46±0.81*</td>
</tr>
<tr>
<td>Boiling</td>
<td>15.80±0.74b</td>
<td>5.94±0.52a</td>
</tr>
<tr>
<td>Microwave</td>
<td>14.12±0.87b</td>
<td>3.67±0.20b</td>
</tr>
<tr>
<td>Raw+10% (v/w) palm oil</td>
<td>36.12±1.89*</td>
<td>9.53±1.33c</td>
</tr>
<tr>
<td>Boiling+10% (v/w) palm oil</td>
<td>19.63±0.95c</td>
<td>6.96±0.48d</td>
</tr>
<tr>
<td>Microwave+10% (v/w) palm oil</td>
<td>15.66±0.80b</td>
<td>7.18±0.45d</td>
</tr>
</tbody>
</table>

* Boiling for 5 min; Microwave at 800 watts for 90 s; Data were presented as mean±SEM (n=3).

The effect of different cooking methods on beta-carotene contents after digestion

Interestingly, even though the releases of beta-carotene after digestion from the cooked sweet leaf samples were lower than the raw sweet leaf that might be explained by the lower beta-carotene content in cooked samples since beginning before digestion; the percentage of bioaccessible beta-carotene in the cooked sweet leaf samples tended to be higher than raw sample. Thus, it may suggest that the cooking methods such as boiling and microwaving helped improving the beta-carotene bioaccessibility. These findings may be due to the mechanisms of particle size reduction during cooking which may contribute to the increased releases of phytochemicals from plants to the food matrices after digested by gastrointestinal enzymes (25).

The effect of the palm oil addition in different cooking methods on beta-carotene contents in sweet leaves

The addition of 10% (v/w) palm oil to the sweet leaf samples demonstrated an increase of the beta-carotene contents before digestion only in the boiled sweet leaf samples, not in the raw and microwaved sweet leaf samples. However, we found that the palm oil significantly increased the bioaccessibility of beta-carotene in both raw and cooked sweet leaves, compared to those of the same cooking method without oil addition. This finding was in line with the previous study which demonstrated that the addition of sunflower or palm oil at 10% (w/w) increase the percentage recovery of the bioaccessible beta-carotene fraction to 39–94% (26). This result may be explained as the requirement of dietary lipids for the efficient carotenoids bioaccessibility by several mechanisms. Firstly, the dietary lipids facilitate the transfer of carotenoids from food matrix to the lipid droplets during gastric phase. Secondly, the pancreatic lipase and bile salts act as emulsifiers that disrupts the large oil droplets to form small droplets and efficiently incorporate to the mixed micelles that further absorbed into the enterocytes (27).

In conclusion, this study suggested that the conventional cooking methods such as microwave cooking and boiling for only the short duration time with the addition of limited amount of cooking oil were recom-
mended to prevent loss of beta-carotene contents of sweet leaf to optimize the culinary aspect of sweet leaf for further health benefits.

Disclosure of state of COI
No conflicts of interest to be declared.

Acknowledgments
This research was supported by H.M. King Bhumibol Adulyadej 72nd Birthday Scholarship, Graduate School, Chulalongkorn University and Grant for International Research Integration: Chula Research Scholar. Rachadaphiseksomphot Endowment Fund, The Asia Research Center, Chulalongkorn University.

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