EFFECT OF DIFFERENT COOKING METHODS ON THE BIOACCESSIBILITY OF POLYPHENOLS AND ANTIOXIDANT CAPACITIES OF *PAKWAN BAN (SAUROPUS ANDROGYNUS)*

จุฬาลงกรณ์มหาวิทยาลัย
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ผลของวิธีการปรุงอาหารในรูปแบบที่แตกต่างกัน ต่อปริมาณสารโพลีฟีนอลพร้อมใช้สำหรับการดูดซึมหลังการย่อยและความสามารถในการต้านอนุมูลอิสระของผักหวานบ้าน (*Sauropus androgynus*)

นางสาวไอมาส อารัมสารี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของวิธีการปรุงอาหารในรูปแบบที่แตกต่างกันต่อปริมาณสารพฤกษเคมีและความสามารถในการต้านอนุมูลอิสระของใบผักหวานบ้าน (*Sauropus androgynus*) ก่อนและหลังการย่อยในระบบทางเดินอาหารจำลองในหลอดทดลอง โดยระยะเวลาที่เหมาะสมในการปรุงอาหารแต่ละวิธี ถูกเลือกจากปริมาณสารฟีโนลิกทั้งหมด ความสามารถในการต้านอนุมูลอิสระโดยการรีดิวซ์เฟอร์ริก (FRAP) คุณสมบัติด้านสี ความแข็ง และการยอมรับด้านเนื้อสัมผัสโดยรวมของใบผักหวานบ้านที่ผ่านการปรุงด้วยเวลาต่าง ๆ กัน ในที่สุดวิธีการปรุงผักหวานบ้านด้วยวิธีการต้ม นาน 5 นาที และวิธีปรุงโดยใช้เตาไมโครเวฟ ความแรงไฟ 800 วัตต์ นาน 90 วินาที ถูกเลือกนำมาเปรียบเทียบกับผักหวานบ้านดิบ ผลการทดลองพบว่า ผักหวานบ้านที่ปรุงด้วยวิธีไมโครเวฟแสดงความสามารถในการต้านอนุมูลอิสระจากการทดสอบด้วยวิธี FRAP และวิธีทดสอบความสามารถต้านอนุมูลอิสระ DPPH มากกว่าที่พบในผักหวานบ้านที่ปรุงด้วยวิธีการต้มและผักหวานบ้านดิบอย่างมีนัยสำคัญ ($P<0.05$) โดยการปรุงอาหารด้วยวิธีการต้มและวิธีไมโครเวฟ ทำให้ปริมาณสารพฤกษเคมีพร้อมใช้ในการดูดซึมหลังการย่อย ได้แก่ สารฟีโนลิกทั้งหมด สารฟลาโวนอยด์ทั้งหมด และสารเบต้าแคโรทีน รวมถึงความสามารถในการต้านอนุมูลอิสระจากการทดสอบด้วยวิธี FRAP และวิธี DPPH ของใบผักหวานบ้าน เพิ่มขึ้นอย่างมีนัยสำคัญมากกว่าที่พบในผักหวานบ้านดิบที่ไม่ผ่านการปรุง ($P<0.05$) นอกจากนี้ การเติมน้ำมันปาล์มปริมาณร้อยละ 10 (ปริมาตรต่อน้ำหนัก) ระหว่างการปรุงอาหาร ทั้งในผักหวานบ้านดิบ วิธีการต้ม และวิธีไมโครเวฟ ช่วยเพิ่มปริมาณสารฟีโนลิกทั้งหมด สารเบต้าแคโรทีน และความสามารถในการต้านอนุมูลอิสระ (ทั้งวิธี FRAP และ วิธี DPPH) ที่พร้อมใช้หลังการย่อยในระบบทางเดินอาหารจำลองในหลอดทดลอง ได้อย่างมีนัยสำคัญเมื่อเทียบกับผักหวานบ้านที่ปรุงด้วยวิธีเดียวกันแต่ไม่ได้เติมน้ำมันปาล์ม ($P<0.05$) ผลการวิจัยนี้จึงอาจสรุปได้ว่า การปรุงผักหวานบ้านด้วยวิธีไมโครเวฟและการเติมน้ำมันปาล์มลงไประหว่างการปรุงอาหารช่วยเพิ่มปริมาณสารพฤกษเคมีพร้อมใช้สำหรับการดูดซึมหลังการย่อยและคงความสามารถในการต้านอนุมูลอิสระของผักหวานบ้านได้ดีกว่าวิธีการต้มและการรับประทานผักหวานบ้านดิบ

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IMAS ARUMSARI: EFFECT OF DIFFERENT COOKING METHODS ON THE BIOACCESSIBILITY OF POLYPHENOLS AND ANTIOXIDANT CAPACITIES OF *PAKWAN BAN (SAUROPUS ANDROGYNUS)* . ADVISOR: KITTANA MAKYNEN, Ph.D., 147 pp.

This study was aimed to assess the effect of different cooking methods on the amounts of phytochemical compounds and antioxidant capacities of sweet leaf (*Sauropus androgynus*) before and during *in vitro* simulated gastrointestinal digestion. The optimum cooking time of each cooking method was prior selected according to the total phenolic compounds, the ferric-reductase antioxidant power (FRAP) activity, the color characteristics, the hardness, and the texture preference of cooked sweet leaf samples. Consequently, the sweet leaf samples cooked by boiling for 5 mins or microwaving at 800 watts for 90 sec were compared with raw sweet leaf. The results showed that the microwave cooking significantly demonstrated the higher antioxidant capacities (both FRAP and DPPH radical scavenging activities) of sweet leaf samples than the raw and the boiling methods ($P < 0.05$). The boiling and microwave cooking methods significantly increased the bioaccessibility of phytochemicals: total phenolic compounds, total flavonoids, beta-carotene contents, and the antioxidant capacities (FRAP and DPPH assay) of sweet leaf than those found in the raw leaves ($P < 0.05$). Furthermore, the addition of 10% palm oil (v/w) during all cooking methods (raw, boiling, and microwave cooking) significantly increased the bioaccessibility of total phenolic compounds, beta-carotene content, and the antioxidant capacities (FRAP and DPPH assay) compared with those without palm oil addition, respectively ($P < 0.05$). In conclusion, microwaving and addition of palm oil during cooking exhibited the increased bioaccessibility of total phenolic compounds, beta-carotene content and antioxidant capacities than boiled and raw sweet leaf.

Department: Nutrition and Dietetics Student's Signature ....
Field of Study: Food and Nutrition Advisor's Signature ....
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CHAPTER I

INTRODUCTION

1.1 Background and Rationale

Nowadays, the consumption of phytochemicals has been associated with the reduced chronic diseases. Functional foods that contain significant amount of bioactive components may provide desirable health benefits and play important roles in the prevention of chronic diseases (Liu, 2003). For example, carotenoids help protecting cellular systems from oxidative damage and stimulate DNA repair (Lorenzo et al., 2009). Phenolic compounds have shown various health beneficial effects, including anti-diabetic (Akkarachiyasit et al., 2010), anti-hyperlipidemic (Ngamukote et al., 2011), anti-inflammatory (Nichols and Katiyar, 2010), and anti-bacterial activities (Daglia, 2012). Whereas many epidemiological, meta-analysis, and *in vitro* studies reported the association of polyphenols with the prevention of chronic diseases, there are still limitations in the intervention studies. The reasons of limitation including the differences in dosing, the interaction with the food matrix, and the differences in polyphenols bioaccessibility that causes severe losses during digestion (Bohn, 2014).

Green leafy vegetables are good source of phytochemicals, especially carotenoids (Raju et al., 2007). *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 (Somdee et al., 2016). Sweet leaf contains many vitamins with high contents, earning it the name "multivitamin plant" (Agrawal et al., 2014). In 100 grams of sweet leaf, it contains beta-carotene (5.6 mg), thiamin (0.5 mg), riboflavin (0.21 mg), and vitamin C (244 mg) (Ching and Mohamed, 2001, Padmavathi and Rao, 1990). Aside from its multivitamin content, the sweet leaf is also rich in polyphenols and exhibits the antioxidant properties. It contains total polyphenols approximately 20 mg gallic acid equivalent (GAE)/g dry weight (Wong et al., 2006) and demonstrated high antioxidant capacities than other 10 vegetables (Andarwulan et al., 2012). Sweet leaf can be consumed as raw vegetable in salad, boiled, or stir-fried vegetable.

Cooking and addition of some food ingredients can improve the bioaccessibility of phytochemicals (D'Archivio et al., 2010, Bohn, 2014). Boiling and microwave cooking are example of conventional cooking methods. Heat treatment that involved in cooking methods promotes the conversion of high molecular weight phenolic acids (glycoside and esters) to low molecular weight phenolic acids (aglycone). Several studies recommended microwave cooking as the cooking method that might prevent loss of polyphenols during cooking since this method kept the cleaved polyphenols inside cell membrane (Spector, 2014, Jiménez-Monreal et al., 2009, Yamaguchi et al., 2001). Moreover, cooking facilitates polyphenols release during digestion via

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modification of polyphenols from glycoside to aglycone which is more biologically active (Kaulmann et al., 2016, Bohn, 2014, Mtolo et al., 2017).

One of food ingredients that might improve the bioaccessibility of polyphenols is the cooking oil. Addition of cooking oil during cooking was found to increase total phenolic content in vegetable via the creation of stable emulsion that favors the stability of polyphenols (Ortega et al., 2009). However, there is no research about the bioaccessibility of phytochemicals and antioxidant capacities of sweet leaf among the different cooking methods with the addition of cooking oil. Therefore, the aim of this study was to determine the effect of different cooking methods with and without cooking oil on the bioaccessibility of phytochemical compounds and antioxidant capacities of sweet leaf.

1.2 The Objective of the Study

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This study aims to assess the effect of different cooking methods on the bioaccessibility of phytochemical compounds and antioxidant capacities of the sweet leaf (*Sauropus androgynus*).

1.3 Research Questions

1) How do the different cooking methods affect the amount of phytochemical compounds and the antioxidant capacities of sweet leaf before simulated digestion?
2) How do the different cooking methods affect the bioaccessibility of phytochemical compounds and the antioxidant capacities of sweet leaf during simulated digestion?
3) How do the cooking oils affect the phytochemical compounds and antioxidant capacities of sweet leaf during simulated digestion?

1.4 Hypotheses

1) The content of phytochemical compounds and the antioxidant capacities of cooked sweet leaf (boiling and microwave cooking methods) before simulated digestion are different from those of the raw sweet leaf.
2) The bioaccessibility of phytochemical compounds and the antioxidant capacities of cooked sweet leaf (boiling and microwave cooking methods) during simulated digestion are different from those of the raw sweet leaf.
3) Addition of cooking oils in raw and cooked sweet leaf with different cooking methods (boiling and microwave cooking) improves the bioaccessibility of

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phytochemical compounds and the antioxidant capacities during simulated digestion.

1.5 Conceptual Framework

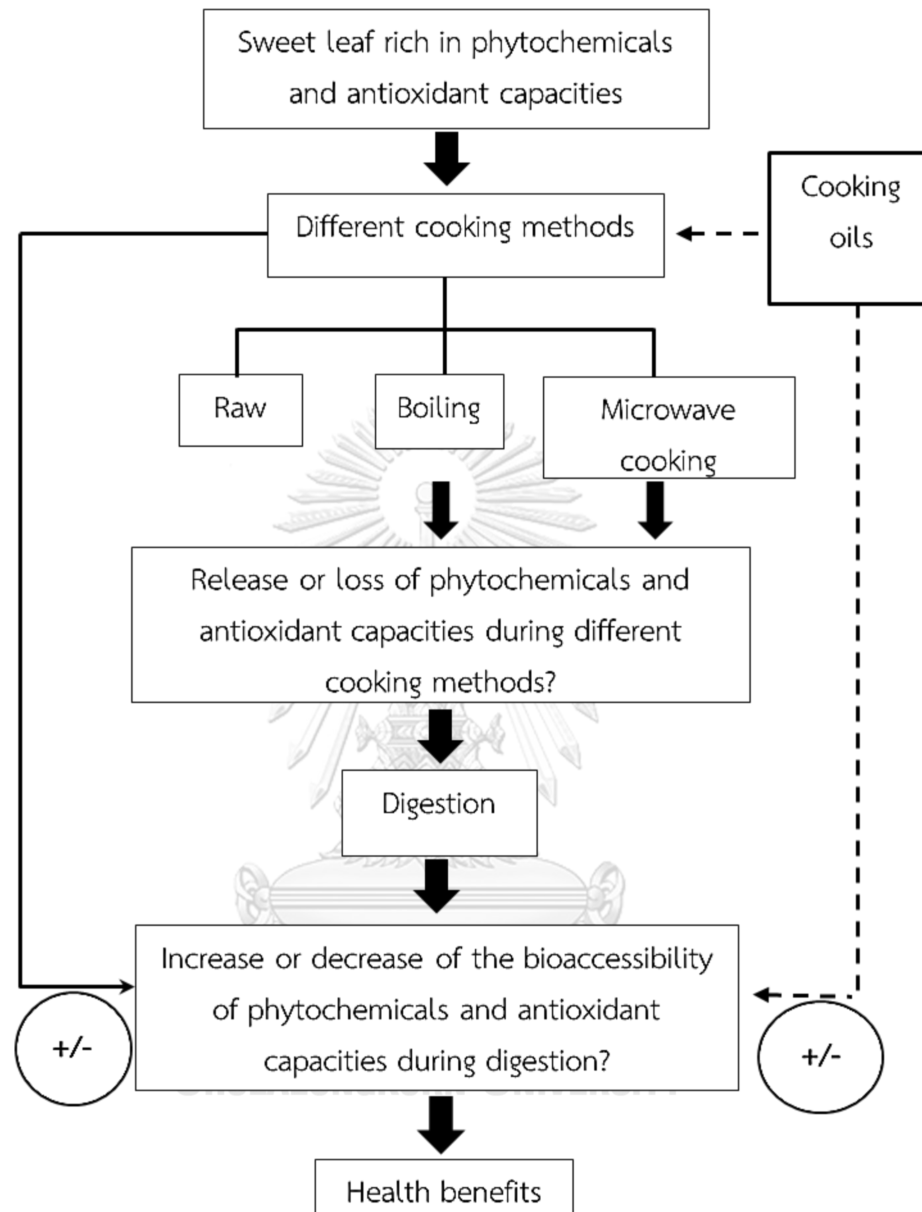

Figure 1.1 Conceptual framework

CHAPTER II

LITERATURE REVIEW

2.1 Phytochemicals

Phytochemicals are bioactive non-nutrient compounds found in plants such as fruits, vegetables, grains, and other plant foods. Recently, there is such growing interest in fruits and vegetables as the source of phytochemicals which play role as natural antioxidant and has been linked to the reduction of oxidative stress (Liu, 2003).

Accumulation of Reactive Oxygen Species (ROS) increases the oxidative stress and is linked to aging and the development of age-related diseases. Oxidants as free radicals can be generated through normal intracellular metabolism in mitochondria and peroxisomes. Numerous external agents also can trigger ROS production. Increased ROS can be detrimental and lead to the aging, diseases, and cell death. The impairment caused by ROS is the result from random damage to protein (protein degradation, fragmentation, peroxidation, modification, and inactivation), lipid (lipid peroxidation and fatty acid loss), and DNA (base modification, single or double strand breaks) (Finkel and Holbrook, 2000).

The endogenous antioxidants defenses maintain the vital cellular and biochemical function in organism. Changing the balance of oxidants-antioxidants towards the increasing of oxidants may lead to oxidative damage. Plants sources also

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provide natural phytochemical compounds such as tocopherols, carotenoids, ascorbic acid, polyphenols, etc. which exhibit antioxidant activities in several mechanisms such as acting as radical scavengers and metal-chelating agents (Kohen and Nyska, 2002).

The most exciting findings have been achieved with antioxidant vitamins and its precursor that are found in dark, leafy green vegetables, and yellow/orange fruits and vegetables. It was estimated that it could be more than 100 different phytochemicals in just a single serving of vegetable (Surh, 2003).

Based on their biosynthetic origins, the phytochemicals can be classified as carotenoids, polyphenols, alkaloids, nitrogen-containing compounds, and organosulfur compounds as shown in figure 2.1.

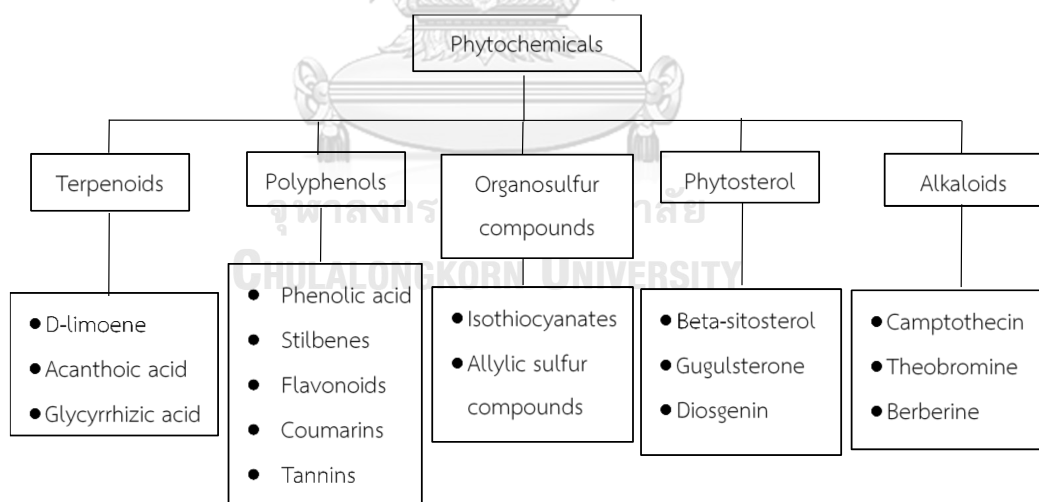

Figure 2.1 Classification of phytochemicals
(Somani et al., 2015)

2.1.1 Carotenoids

Carotenoids are yellow-red polyene pigments widely distributed in nature and some of them (alpha-carotene, beta-carotene, and beta-cryptoxanthin) are pro-vitamin A. They are synthesized *de novo* by all photosynthetic organisms. The most characteristic structural feature of carotenoids is the conjugated polyene chain. Functional group of carotene structure can be hydroxyl, methoxy, cyclic ethers, keto, aldehyde, carboxylic acid, lactones, acylesters, glycosides, glycoside esters, and sulfates.

According to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature of carotenoids, the *cis-trans* isomerism is still used to denote the carotenoids. The *cis-* isomerism indicates that the functional groups are on the same side of the carbon chain whereas the *trans-* isomerism indicates that the functional groups are on the opposing sides of the carbon chain. The *trans*-carotenoids are usually the naturally occurring stereoisomer and thermodynamically most stable. It can be isomerized to *cis*-carotenoids because of the light and heat (Krinsky et al., 2009). The isomerizations of carotenoids occur in the relocation of single or double bond on one isomerism form into another (figure 2.2).

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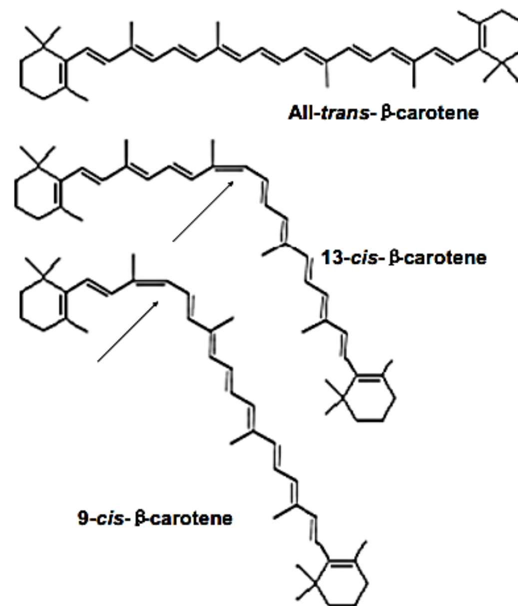

Figure 2.2 Different structures of *trans*- and *cis*- carotenoids

* The arrow pointing at the *cis*- isomerization (Khoo et al., 2011)

According to the oxygen content, carotenoids can be divided into the oxygen-containing carotenoids; such as lutein, zeaxanthin, and β-cryptoxanthin which are known as xanthophylls while the oxygen-free carotenoids; such as alpha-carotene, beta-carotene, gamma-carotene, and lycopene, are known as carotenes (figure 2.3).

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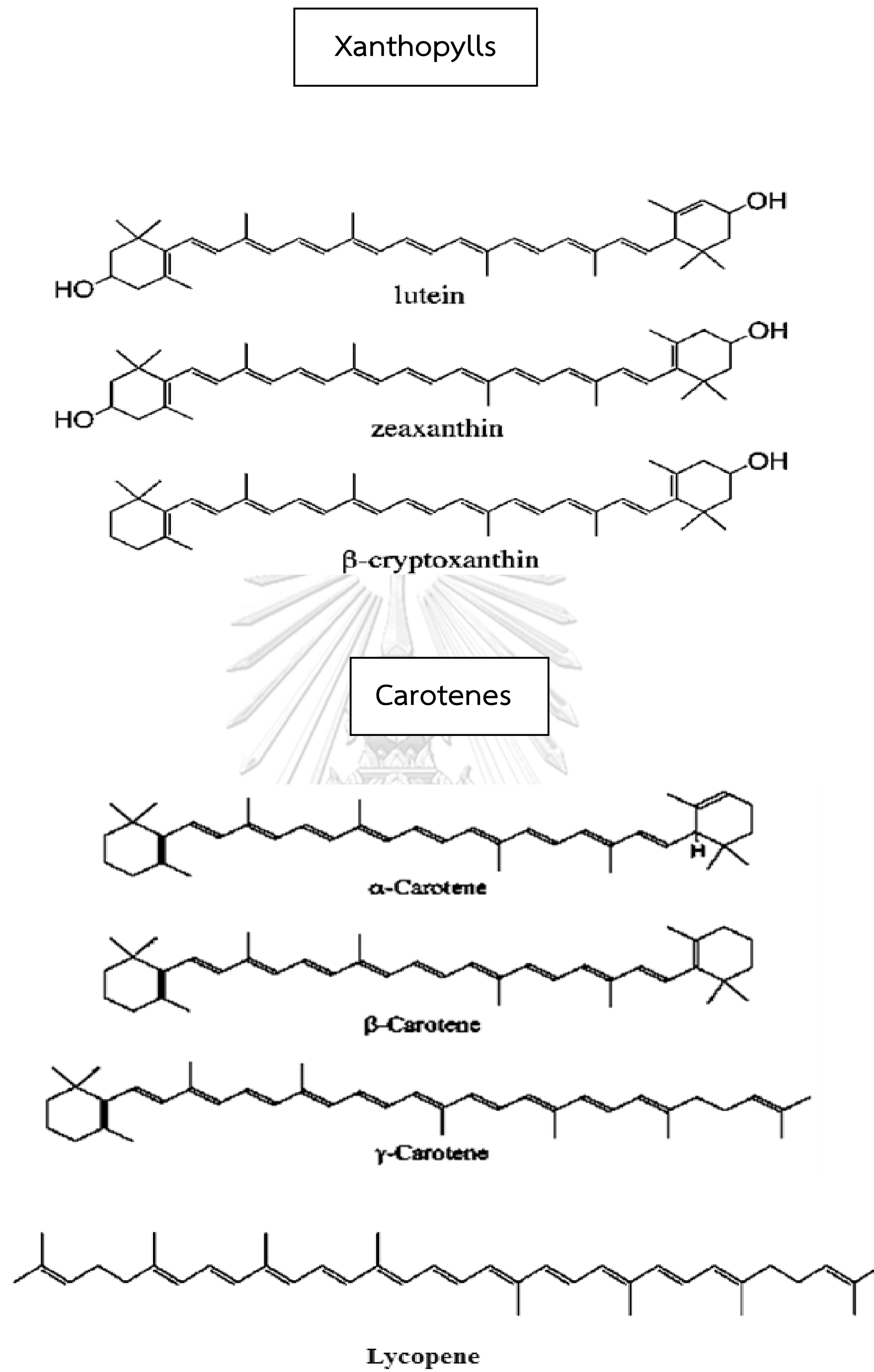

Figure 2.3 Structure of some xanthopylls and carotenes

[Adapted from Arvayo-Enríquez et al. (2013)]

2.1.1.1 The vitamin A biosynthetic pathway of pro-vitamin A carotenoids

Vitamin A (a group of fat-soluble retinoids, including retinol, retinal, retinoic acid, and retinyl esters) is essential for normal growth and development, immune system function, and vision. The pro-vitamin A carotenoids must be converted into retinol and other retinoids inside the body. The vitamin A biosynthetic pathway of all-trans-beta-carotene is shown in figure 2.4.

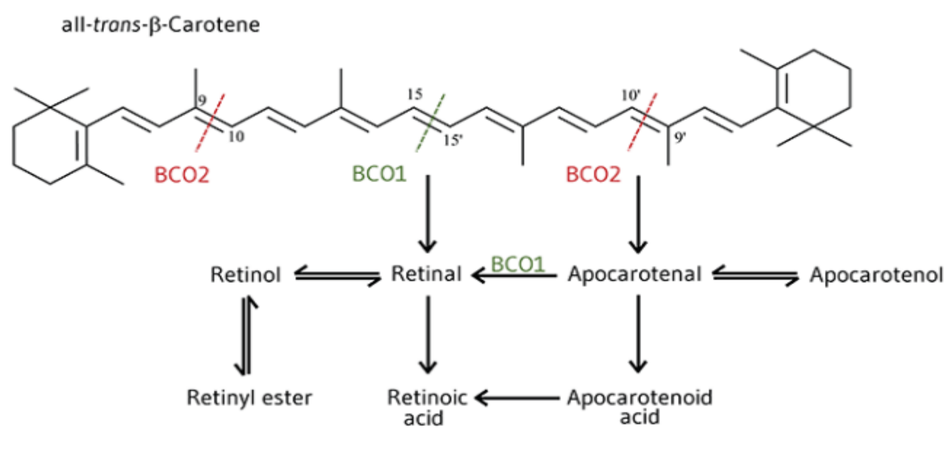

Figure 2.4 The vitamin A biosynthetic pathway of all-trans-beta-carotene (Higdon, 2016)

As shown in figure 2.4, the β,β-carotene-15,15′-oxygenase (BCO1) catalyzes the symmetrical cleavage of pro-vitamin A carotenoids, the beta-carotene, at the 15, 15’ double bond to produce one or two molecules of retinal. Retinal can be oxidized to retinoic acid or reduced

to retinol, and further converted to retinyl ester for storage or transport. Pro-vitamin A carotenoids may also be cleaved by the second putative carotene oxygenase (BCO2) at either the 9,10 or 9',10' double bond, leading to the rise in apocarotenals which can also be converted into apocarotenols or apocarotenoid acids. Apocarotenals and apocarotenoid acids can be further converted to retinals and retinoic acids (Higdon, 2016).

2.1.1.2 Health benefits of carotenoids

Carotenoids involve in reducing risk factor of certain cancer and eye diseases. The carotenoids that mostly studied are beta-carotene, lycopene, lutein, and zeaxanthin.

Although the correlation of carotenoids and other types of cancer have been evaluated, beta-carotene intake and/or beta-carotene level in plasma are mainly related to the reduced risk of lung cancer according to the 11 of 15 epidemiological studies. Case-control studies confirmed the positive effect of tomato consumption (lycopene-rich food) to reduce incidence of esophageal cancer. Lutein and zeaxanthin are related to the protective role against oxidative damage that caused by blue light in human retina (Johnson, 2002).

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2.1.1.3 Antioxidant activities of carotenoids

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. Auto-oxidation processes such as lipid peroxidation, are associated with free radical chain reaction that involve peroxyl radicals. Carotenoids act as chain-breaking antioxidant by rapidly and efficiently scavenging such free radicals by three general mechanisms as shown in figure 2.5.

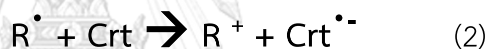

Figure 2.5 Anti-oxidative mechanisms of carotenoids

($R^{\bullet}$= Free radical; Crt= Carotenoids)

(Fiedor and Burda, 2014)

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As shown in figure 2.5, carotenoids scavenged free radical by electron transfer between carotenoid (Crt) and free radical ($R^{\bullet}$) resulting in carotenoid radical cation ($Crt^{\bullet +}$)(1) or carotenoid radical anion ($Crt^{\bullet -}$) (2); radical adduct formation between carotenoid and free radical to be

$RCrt^{\bullet}$(3); and hydrogen atom transfer from carotenoid to free radical which leads to the formation of neutral carotenoid radical (4).

2.1.1.4 Determination of carotenoids

A variety of methods have been employed to detect the carotenoids in food samples. Thin layer chromatography, High Pressure Liquid Chromatography (HPLC) and combination of HPLC with mass spectrometry including Matrix Assisted Laser Desorption/Ionization (MALDI-TOF) have been used for determination of carotenoids. The most commonly used method for identification and quantification of carotenoids utilizes HPLC combined with UV-visible absorption detection. Maximum absorbance of beta-carotene is detected at wavelength 458 nm. The normal-phase HPLC system is not suitable for carotenoids detection due to poor separation of non-polar molecules whereas the reversed-phase HPLC is preferred for carotenoid separation because carotenoids are non-polar molecules. Among the columns, C18 columns with isocratic or gradient elution are preferred. The C30 columns are also able to resolve carotenoids, however it requires 60 minutes or more for complete separation (Gupta et al., 2015).

The reversed-phase HPLC system is characterized by the polar mobile phase and the hydrophobic stationary phase. Typical stationary

phase of the reversed-phase system are hydrophobic and bonded to the surface of silica support particles. The composition of mobile phase in the reversed-phase HPLC can be modified by isocratic or gradient system. Initially, the mobile phase is designed to favor the adsorption of hydrophobic or less polar compounds to the stationary phase. Subsequently, the mobile phase composition is modified to favor the desorption of hydrophobic compounds from the stationary phase back to the mobile phase. Consequently, the compounds with different polarities will be eluted from the column at different retention time which can be detected with detector system. More polar molecules or hydrophilic compounds will be detected first followed by less polar molecules (Aguilar, 2004).

2.1.2 Polyphenols

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2.1.2.1 Classification of polyphenols

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Polyphenols are the most dominant antioxidants in the natural diet. Dietary consumption of total polyphenols could be as high as 1 gram/day, which is higher than all other classes of phytochemicals and dietary antioxidants, such as vitamin C (Scalbert et al., 2005).

Polyphenols are synthesized by plants and important for a protective mechanism against free radicals and UV radiation (Nimse and

Pal, 2015). It includes several classes such as phenolic acids, flavonoids, stilbenes, coumarins, and tannins (figure 2.6). Polyphenols exhibited antioxidant properties due to its hydroxyl group ability as electron donor.

Phenolic acids are one of the major classes of polyphenols. Phenolic acids are non-flavonoid polyphenols which can be divided into two main types; hydroxybenzoic acid and hydroxycinnamic acid derivatives. Hydroxybenzoic acid can be classified as the common structure of a large number of derivatives obtained from phenolic acids. The functional groups substituted to the *ortho-* or *para-* positions of phenolic rings have been shown to be more effective than those attached to the *meta-* position in terms of antioxidant activity (figure 2.7). Alkyl esters group (i.e methyl and propyl) is the frequent functional group seen in many subclasses of phenolic compounds (Farhoosh et al., 2016).

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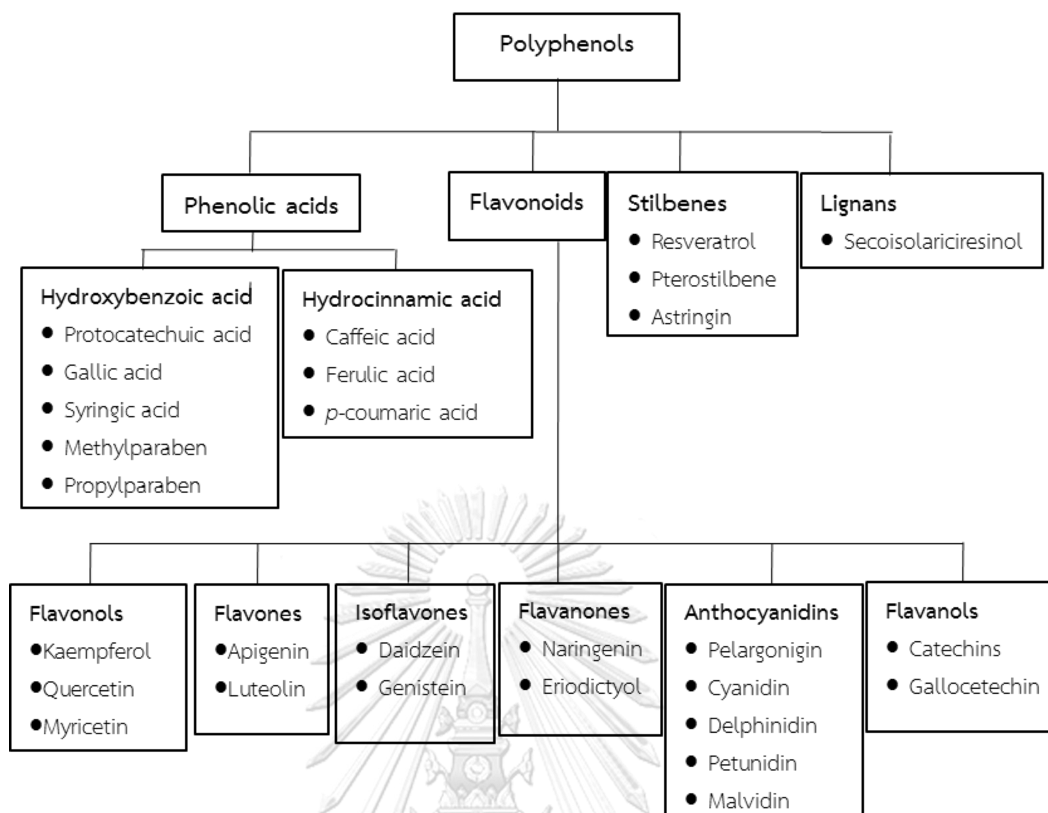

Figure 2.6 Classification of polyphenols

[Adapted from (Hardman, 2014)]

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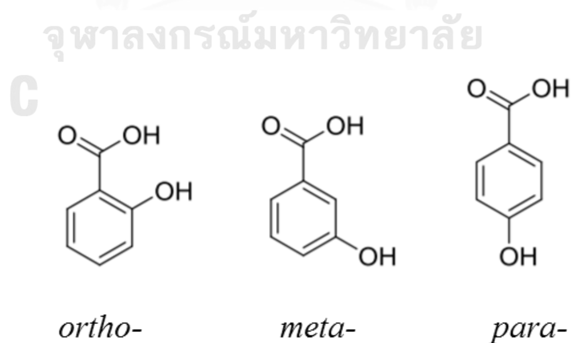
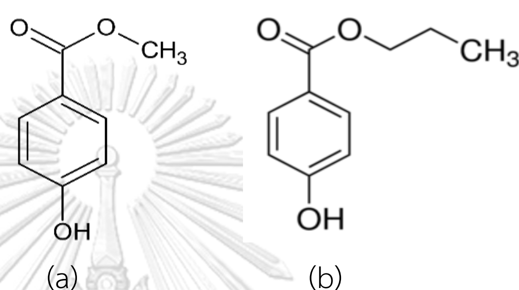


Figure 2.7 Common structure of *ortho*-, *meta*-, and *para*-hydroxybenzoic acid

(Booth et al., 2012)

Methylparaben and propylparaben, the phenolic compounds found in leaves part of sweet leaf, are the subclasses of phenolic acids which contain methyl- and propyl- group attached in *para*- position of hydroxybenzoic acid, respectively (figure 2.8).



(a) (b)

Figure 2.8 Structures of some derivatives of hydroxybenzoic acid (a) methylparaben and (b) propylparaben (Ma et al., 2002)

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Flavonoids contain 15-carbon skeleton which consist of two phenyl rings (A and B) and heterocyclic ring (C) (figure 2.9). Flavonoids classification differed by the hydroxylation pattern in heterocyclic ring (C). In addition to the phenolic acids and flavonoids, there are several non-flavonoid polyphenols found in food that considered important for human health such as resveratrol and lignans (Tsao, 2010). General structures of some polyphenols are shown in figure 2.9.

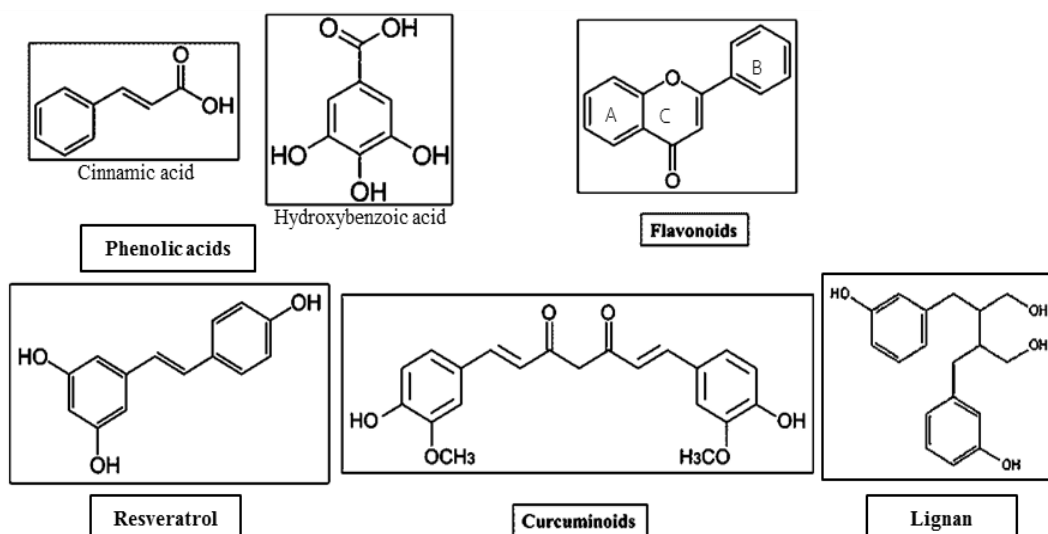

Figure 2.9 General structures of some polyphenols

(Lall et al., 2015)

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2.1.2.2 Health benefits of polyphenols

Epidemiological studies demonstrated the positive effect between the consumption of phenolic-rich diet and risk of chronic diseases. It is well explained that consumption of phenolic-rich foods and beverages may increase plasma antioxidant capacity. The increasing of plasma antioxidant regarding to the dietary polyphenols consumption can be associated either by the presence of polyphenols in plasma, by the sparing effect of polyphenols and other endogenous antioxidants, or by the effect of polyphenols on pro-oxidative food components such as iron. Consequently, the increasing plasma

antioxidant capacity might protect cell constituents against oxidative damage and finally reduce the risk of chronic diseases related to oxidative stress (Pandey and Rizvi, 2009).

Polyphenols also exhibited cardio-protective effect due to its ability on antioxidant, anti-platelet, anti-inflammatory activities as well as increasing high-density lipoprotein (HDL), and improving endothelial function. In addition, polyphenols demonstrated the anti-diabetic effect due to the inhibition of carbohydrate digestive enzymes (Adisakwattana et al., 2011, Benalla et al., 2010, Yoshikawa et al., 2001).

2.1.2.3 Antioxidant activities of polyphenols

Health benefits of polyphenols strongly associated to its antioxidant activity. Antioxidants are compounds that can prevent biomolecules (proteins, nucleic acids, polyunsaturated lipids, sugars) from undergoing oxidative damage through free radical mediated reactions. Inhibition of oxidizing chain reaction can occur through several mechanisms, including direct quenching reactive oxygen species, inhibition of enzymes, and chelation of metal ions.

The antioxidant activities of phenolic compounds related to its chemical structure, particularly the presence of hydroxyl groups. There

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are two main mechanisms involved with antioxidant activities of polyphenols (figure 2.10).

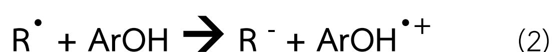

Figure 2.10 Anti-oxidative mechanisms of polyphenols

$R^{\bullet}$= Free radical; ArOH= Phenolic antioxidant

[Adapted from (Leopoldini et al., 2004)]

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As shown in figure 2.10, the first mechanism is H-atom transfer (1). Free radical ($R^{\bullet}$) removes a hydrogen atom from phenolic antioxidant (ArOH) and the phenolic itself becomes a radical ($ArO^{\bullet}$). Hydrogen bond, conjugation, and resonance of $ArO^{\bullet}$ structure make it a nonreactive phenolic radical. The second mechanism is the electron transfer (2). The phenolic antioxidant can give an electron to the free radical and make it more stable (Leopoldini et al., 2004).

2.1.2.4 Metabolic pathway of polyphenols

Structures of polyphenols affect their intestinal absorption. One of the important parameters is the molecular weight. Most of low molecular polyphenols (aglycone and esters form) are absorbed in the

small intestine via passive diffusion. The low urinary recovery of high molecular weight aglycone flavonoids such as theaflavin (molecular weight= 568) indicated its low absorption in gut.

Most flavonoids (except catechin and proanthocyanidin) are glycosylated (glycoside form) in food. The flavonoids in glycoside form exhibited higher intestinal absorption than the aglycone form. However, rhamnosides requires deglycosylations by the colonic microflora for better absorption (Scalbert et al., 2002). Different structure of glycosides and aglycones is shown in figure 2.11.

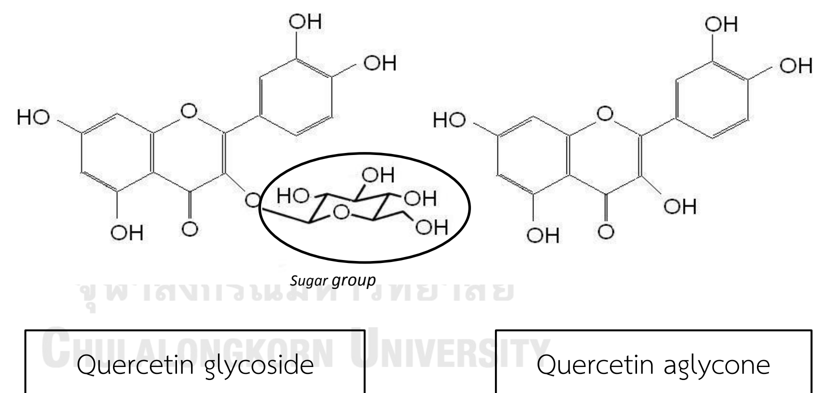

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Figure 2.11 Example of polyphenol glycoside and its aglycone

[Adapted from Manach et al. (2004)]

Polyphenols are mainly metabolized either in tissues or metabolized by colonic microflora. All polyphenols are conjugated to form *O*-glucuronides, sulphate esters, and *O*-methyl ether in small intestine and circulate in the blood stream to liver via hepatic portal

vein. All circulating polyphenols are glucuronidated and/or sulphated compounds, whereas no free aglycone polyphenols are found in plasma. These conjugated form are further metabolized in liver to methyl, sulphate, and glucuronic acid which can excrete via urine (Scalbert et al., 2002) (figure 2.12)

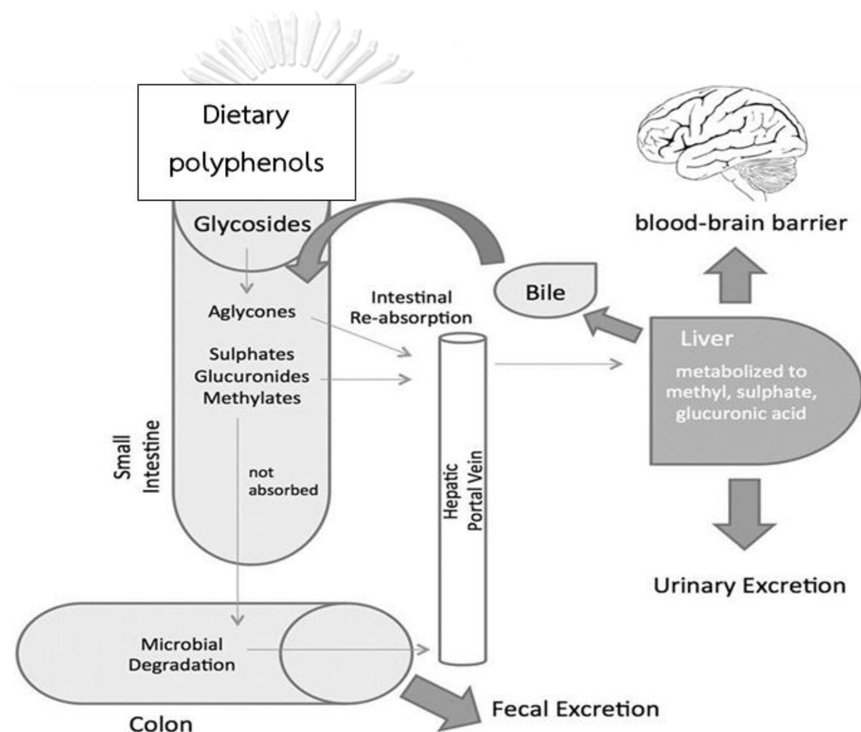

Figure 2.12 Metabolism pathway of dietary polyphenols

[Adapted from van Duynhoven et al. (2011)]

2.1.3 The bioaccessibility of dietary compounds

"Bioaccessibility" can be defined as the amount of compound solubilized in the small intestine and available for subsequent absorption. The definition of bioaccessibility comprises the release of compounds from food

matrices and their stability under the gastrointestinal condition (Tagliazucchi et al., 2010).

Determination of the bioaccessibility has been developed in the *in vitro* study. The *in vitro* bioaccessibility assay includes the sequences of mimicking oral, gastric, and intestinal phases of digestion. Different analytical approaches have been developed to measure the bioaccessibility of nutrients and bioactive compounds. The main features of *in vitro* gastrointestinal methods are temperature, shaking or agitation, and the chemical and enzymatic composition of saliva, gastric juice, duodenal juice, and bile juice (Fernández-García et al., 2009).

2.1.3.1 The bioaccessibility of carotenoids

To be absorbed intestinally, dietary carotenoids must be released from the food matrices and incorporated into mixed micelles (the mixture of bile salts and lipids). Cooking processes help releasing embedded carotenoids in the food matrices and increase their efficacy to be absorbed in small intestine. Furthermore, carotenoids absorption requires the presence of fat in meal. Ingestion of fat along with carotenoids was thought to be crucial for the absorption of carotenoids (Higdon, 2016).

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The different types of fat influenced the rate and extent of carotenoids absorption. The efficacy of carotenoids absorption was larger when carotenoids were digested along with lipids containing large fraction of long chain fatty acids comparing to the lipids dominant in medium or short chain fatty acids. According to the degree of unsaturation, lipids high in polyunsaturated fatty acids (PUFA) might promote the oxidation of carotenoid in the chyme fraction, resulting in less carotenoids available for absorption (Colle et al., 2012). Acidic pH in gastric phase significantly amplified the peroxidation of lipids. The lipid peroxidation in acidic pH during digestion lead to co-oxidizing many other dietary nutritional constituents, such as carotenoids (Kanner and Lapidot, 2001, Kenmogne-Domguia et al., 2014)

2.1.3.2 The bioaccessibility of polyphenols

The bioaccessibility of polyphenols is complicated due to many classes and forms of polyphenols existing. Most dietary polyphenols exist in the form of esters, glycosides, and polymers (Tagliazucchi et al., 2010). Their metabolites during digestion depend on their polarity, molecular weight, glycosylation, and esterification. Polyphenols might be cleaved into aglycone form during digestion. Polyphenols concentration could be increased during digestion because of the

releasing of non-extractable polyphenols (NEPP) that embedded inside the cell wall (Kaulmann et al., 2016).

Dietary fiber (such as hemicellulose), divalent minerals, viscous and protein-rich meal can reduce the bioaccessibility of polyphenols. Interestingly, digestible carbohydrates, dietary lipids, and additional antioxidants may enhance the bioaccessibility of polyphenols (Bohn, 2014).

The effect of dietary lipids addition on the bioaccessibility of polyphenols depends on the food sources of lipids and food components. Previous study reported that the *in vitro* digestion of black tea with high-fat milk demonstrated a significant decrease in the bioaccessibility of antioxidant activity. This report suggested a negative interaction between polyphenols and protein in high-fat milk. Polyphenols with high number of hydroxyl group have a high affinity for proteins resulting in protein-polyphenols complex formation leading to less antioxidant activity during gastrointestinal digestion (Bohn, 2014).

Nevertheless, some studies have shown that the addition of dietary fat increased the bioaccessibility of polyphenols, especially for the more non-polar ones such as aglycone polyphenols, which may require micellarization during digestion. Protective role of fat on the

bioaccessible polyphenols was mainly evident in procyanidin and phenolic acid. The presence of bile salts during intestinal digestion and interaction between fat and the polyphenols in micelles fraction created a stable emulsion that favors stability of polyphenols (Ortega et al., 2009). Therefore, the addition of fat such as cooking oils in food meal might resulted in the superior bioaccessibility of polyphenols (Kaulmann et al., 2016).

Another example of the antioxidants-rich food ingredients that may enhance the bioaccessibility of polyphenols is ascorbic acid-rich food such as citrus juices (grapefruit, lemon, lime, and orange). Citrus juices that added during the formulating green tea preparations were capable on stabilizing catechin free radicals that formed under pH conditions of the small intestine or indirectly synergistically sparing antioxidant activity from oxidative damage. Therefore, the finding suggested that addition of ascorbic acid-rich food such as citrus juice increases the digestive recovery of catechin and increases the amount of biaccessible catechin (Green et al., 2007).

Moreover, the bioaccessibility of polyphenols also can be affected by the pH of the gastrointestinal tract. Some polyphenols such as anthocyanins were more stable in gastric condition and tend to be

degraded in alkaline intestine condition, whereas flavanols and its glycosides were more stable in the mild alkaline pH of the small intestinal phase (Tagliazucchi et al., 2010). The summary of polyphenols bioaccessibility from previous studies is presented in table 2.1.

Table 2.1 The bioaccessibility of polyphenols during digestion

Polyphenols	Food source	Bioaccessibility after gastric digestion (%)	Bioaccessibility after intestinal digestion (%)	References
Gallic acid	Grape	95.4	56.7	(Tagliazucchi et al., 2010)
Caffeic acid		99.9	75	
Catechin		99.3	92.8	
Quercetin		99.1	94.2	
Resveratrol		102.3	30.5	
Total phenolic content	Cinnamon beverage	79	80	(Helal et al., 2014)
Quercetin-3-rhamnoside		94	53.3	
Kaempferol		109	90	
Coumaric acid		97	47	
Syringic acid		90	22.3	
Cinnamaldehyde		98	87	

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Cooking processes can increase the bioaccessibility and the release of polyphenols from food matrices. Heat treatment during

cooking is important to break the cell matrix and release the polyphenols which located in the vacuole of plant cells (figure 2.13) (Vats and Negi, 2013). It has been reported that the rupture of lignin-cellulose complex (lignocellulosic) in the plant cell wall by heat during cooking can increase the release of non-extractable polyphenols (NEPP) inside the cell walls which cannot be extracted from chemical extraction to the food matrices (Xu et al., 2007, Bohn, 2014).

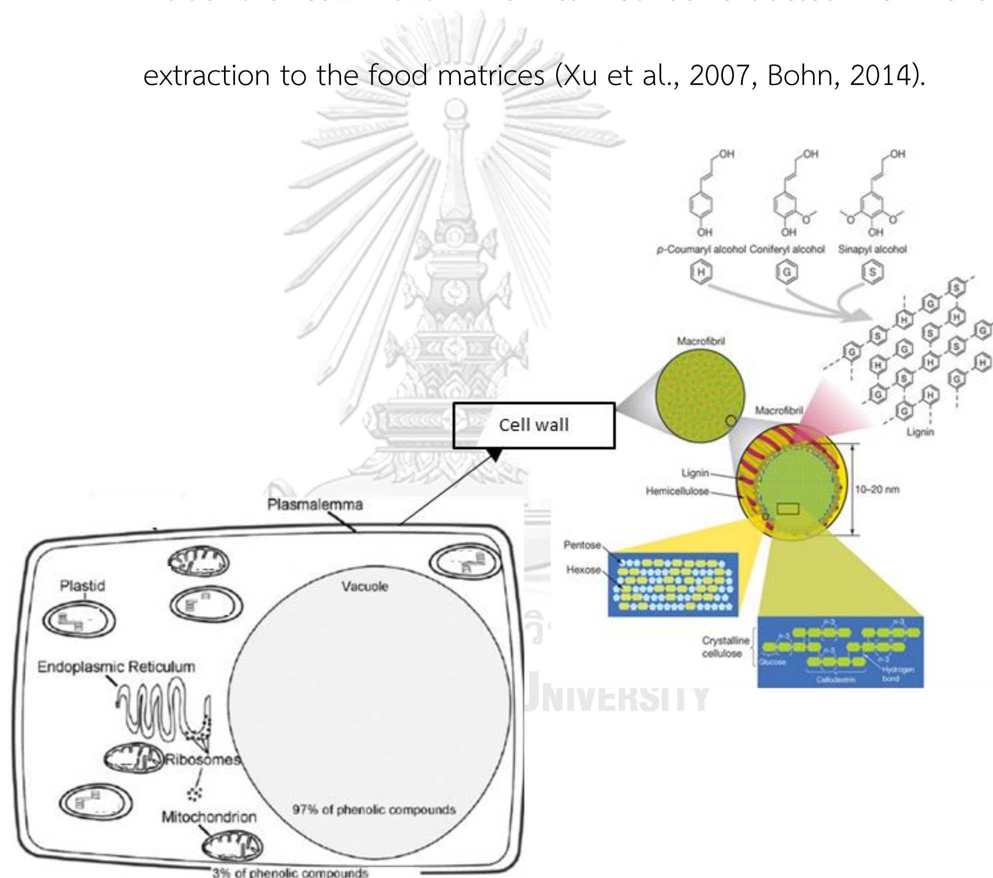

Figure 2.13 The internal localization of phenolic compounds in typical plant cell and the structure of lignin-cellulose complex in plant cell wall [Adapted from (Toivonen and Brummell, 2008, Rubin, 2008)]

2.2 *Sauropus androgynus* (Sweet Leaf)

Sauropus androgynus or sweet leaf (as called in Thailand: pakwan ban; Malaysia: Cekur manis; Indonesia: Katuk) is an indigenous vegetable in Southeast Asia. In Thailand, the demand of sweet leaf in the vegetable market has been increasing rapidly and it has become an export vegetable. Sweet leaf has dark green color and firm texture with a pleasant taste (Supapvanich et al., 2012). It can be consumed as raw vegetable in salad or cooked vegetable in many types of soups.

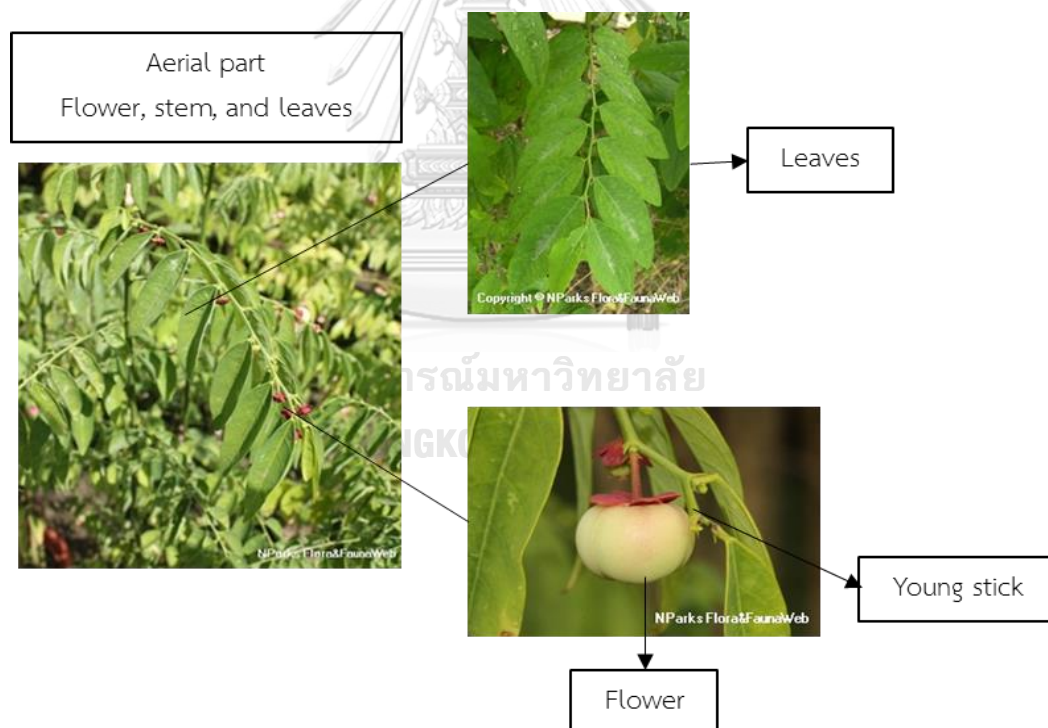

Figure 2.11 Morphology of *Sauropus androgynus* (sweet leaf)

[Adapted from NParks (2018)]

Sweet leaf was earned the name “multivitamin plant” regarding to its high content of vitamins (Padmavathi and Rao, 1990, Agrawal et al., 2014). As shown in table 2.2, it contains carotenoids (5.6 mg), thiamin (0.5 mg), riboflavin (0.21 mg), and vitamin C (244 mg) and also provides macronutrients such as protein (7.4 grams), fat (1.1 gram), and fiber (1.8 grams) per 100 grams of fresh leaves. According to its nutrition value and previous report, it indicated that sweet leaf is nutritionally superior to other leafy vegetables such as cabbage, drumstick leaves, and spinach (Padmavathi and Rao, 1990).

Table 2.2 Nutrition value of fresh sweet leaf

Nutrients	Amount (per 100 g)	References
Macronutrients		
Protein	7.4-7.6 g	(Padmavathi and Rao, 1990, Ching and Mohamed, 2001)
Fat	1.1 g	(Padmavathi and Rao, 1990)
Fiber	1.8 g	(Padmavathi and Rao, 1990)
Micronutrients		
Vitamin E	79.7 mg	(Ching and Mohamed, 2001, Padmavathi and Rao, 1990)
Vitamin C	244 mg	
Thiamin	0.5 mg	
Riboflavin	0.2 mg	

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2.2.1 Carotenoids content of sweet leaf

The major carotenoids in sweet leaf are alpha- and beta-carotenes. The amounts of alpha-carotene in sweet leaf ranged from 0.5 to 5.6 mg per 100 grams of fresh leaves (Padmavathi and Rao, 1990, Hulshof et al., 1997, M. Ogle, 2001) and the amounts of beta-carotene ranged from 1.6 to 10 mg per 100 grams of fresh leaves (Andarwulan et al., 2012, Hulshof et al., 1997, M. Ogle, 2001), respectively. The various concentration of carotenoids in sweet leaf depended on the cultivar and extraction methods.

2.2.2 Total phenolic content of sweet leaf

Aqueous extract of sweet leaf demonstrated its high antioxidant capacity and total phenolic content (Wong et al., 2006). Sweet leaf could contribute a significant amount of total phenolic and flavonoid content to the Indonesian diet (Andarwulan et al., 2012). Aqueous extract of sweet at concentration of 50 mg/mL exhibited the % inhibition of DPPH radical which ranged from 70 to 90% (Narayanaswamy and Balakrishnan, 2011).

The main polyphenols profile of sweet leaf is shown in table 2.3. Different extraction methods and different parts of plant might provide different bioactive compounds. The main polyphenol contents of leaves part in sweet leaf are the flavonoids: naringenin rutinoside and kaempferol

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glycoside; and the phenolic acids: methylparaben and propylparaben in the hydroxybenzoic acid subclass (table 2.3 and 2.4).

Table 2.3 The identified bioactive compounds of sweet leaf from previous studies

No	Part of plant	Extraction solvent	Bioactive compound(s)	References
1	Leaves	Ethanol	Methylparaben Propylparaben	(Samad et al., 2013)
2	Aerial part	Ethanol	3-*O*-beta-D-glucosyl-7-*O*-alpha-L-rhamnosyl-kaempferol 3-*O*-beta-D-glucosyl-(1⟶6)-beta-D-glucosyl-kaempferol 3-*O*-beta-D-glucosyl-(1⟶6)-beta-D-glucosyl-7-*O*-alpha-L-rhamnosyl-kaempferol	(Wang and Lee, 1997)
3	Aerial part	Methanol	Lignan diglycoside isolariciresinol 3-alpha-*O*-beta-piofuranosyl-(1⟶2)-*O*-beta-glucopyranoside Megastigmane glucoside, sauroposide	(Kanchanapoom et al., 2003)
4	Leaves	Aqueous ethanol (70%)	Naringenin rutinoside	(Senthamarai and Anusha, 2012)
5	Young sticks and leaves	Ethyl acetate and butanol	3-*O*-beta-D-glucosyl-(1⟶6)-beta-D-glucosyl-kaempferol	(Yu et al., 2006)

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Table 2.4 List of major components in the aqueous ethanol extract of sweet leaf (leaves part) analyzed by GC-MS (Samad et al., 2013)

Peak area (%)	Name of compounds	Type of compounds
31.75	9, 12, 15-Octadecatrienoic acid (Z,Z,Z)	Linolenic acid
6.19	Caffeine	Alkaloid
4.46	Methylparaben	Hydroxybenzoic acid
4.12	Propylparaben	Hydroxybenzoic acid
2.70	Hexadecanamide	Oleic acid amide
1.60	4-methyl-1,4-heptadiene	Alkaloid

2.2.3 Health benefits of sweet leaf

Several health benefits of sweet leaf were investigated due to its bioactive phytochemicals. The polyphenols contents in sweet leaf may act as hydrogen donor which provide health benefits as the antioxidants and also demonstrated the antibacterial, and anti-inflammatory properties (Madhu et al., 2014). The active compounds from the ethyl acetate extract of sweet leaf decreased the expression of cyclooxygenase-2 (COX-2), the pro-inflammatory enzyme; and also decreased prostaglandin E2 (PGE-2) and Nitric Oxide (NO) levels, the markers of inflammatory reactions. The COX-2 is an isoform of prostaglandin H synthase, an enzyme responsible for prostaglandins production. Activated inflammatory cells secrete NO, PGE-2, and cytokines

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when inflammation occur. Reducing those markers is important since uncontrolled inflammation may act as an etiologic for chronic diseases (Selvi and Baskar, 2014). In addition to anti-inflammatory property, the isolated compound (naringenin rutinoside) from the aqueous-ethanol extract of the sweet leaf also demonstrated the inhibition of the alpha-glucosidase activity, indicating its antidiabetic property (Senthamarai and Anusha, 2012).

2.3 Cooking Methods

2.3.1 Principle of boiling as cooking method

Boiling is one of the conventional cooking methods commonly used in the daily life. “Boiling” applies the principle of convection; the flow of heat transfer through a liquid as medium. The heat that transfer from the heat origin (such as stove and fire) through the medium (water), cause a direct contact between heat and food such as the leaves of vegetable (Bastin, 2011). This boiling process might destroy the polyphenols content in the cooked vegetables.

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Degradation of polyphenols after boiling has been reported from previous studies. Boiling decreased the total phenolic content and antioxidant capacities of cabbage, broccoli, and choy-sum with a decrease more than 60% after 5 minutes of cooking. The depletion of total phenolic content after boiling

could be due to phenolic degradation during cooking (Wachtel-Galor et al., 2008). Moreover, heating citrus peel extract at high temperature longer than 30 minutes (at 120°C for 90 minutes) or higher temperature than 120°C (at 150°C for 30 minutes) can destroy flavanone glycoside (Xu et al., 2007). Furthermore, cooking with the stir frying (at 130°C for 5 minutes), and boiling method (for 5 minutes) caused the significant lost by 30% of total phenolic content in red cabbage (Xu et al., 2014).

2.3.2 Principle of microwave oven

Several studies recommended microwave cooking as the cooking method that might prevent loss of phytochemicals during cooking (table 2.5). Microwave oven works by converting the electricity from the power outlet into high-powered radio waves. These radio waves blast into food compartment, penetrate inside the food so that the molecules inside the food vibrate more quickly. Vibrating molecules produce heat and this heat rise up rapidly (Spector, 2014).

Previous studies explained that microwave heating retains the bioactive components in the cooked tissue. The antioxidant activity of vegetables cooked in the microwave oven was generally higher than those in boiling because microwave heating does not degrade antioxidant substance and keep

it inside the cell membrane of the vegetable (Jiménez-Monreal et al., 2009, Yamaguchi et al., 2001).

Table 2.5 Comparisons of phytochemical compounds and antioxidant capacities of some vegetables after boiling and microwave cooking

Vegetable	Conclusion	References
Kale	- DPPH radical scavenging activity → boiling > microwave method - Total phenolic content → microwave > boiling method	(Şengül et al., 2014
White cabbage	- DPPH radical scavenging activity →microwave > boiling method - Total phenolic content → boiling > microwave method	(Şengül et al., 2014)
Broccoli	2% total flavonols (by weight comparing to raw) were leached into the boiling water	(Price et al., 1998)
Red cabbage	Greater loss of anthocyanins in boiling method than that found in microwave cooking	(Xu et al., 2014)
Broccoli	- Beta-carotene contents → microwave > boiling method	(Zhang and Hamauzu, 2004)

* All information in this table were investigated in vegetables after cooking before digestion

2.3.3 The addition of cooking oils during cooking and the phytochemicals contents in food matrices

As previously mentioned in the topic of the bioaccessibility of carotenoids and polyphenols, the addition of cooking oils in the food matrices might improve the bioaccessibility of those compounds during gastrointestinal digestion and also affected the phytochemicals during cooking. Previous studies demonstrated that cooking oil increased total phenolic content in vegetable during cooking (Şengül et al., 2014).

Soybean oil and palm oil are the most common cooking oils used in Southeast Asia. They are economically affordable and represent different fatty acids profile. Previous report demonstrated that the oil containing high polyunsaturated fatty acids (PUFA) is more susceptible to oxidation during heating (Tabee et al., 2008).

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Table 2.6 shows that palm oil contains higher monounsaturated fatty acid (MUFA) than soybean oil, whereas soybean oil contains higher polyunsaturated fatty acid (PUFA) than palm oil. Therefore, the different cooking oils with different fatty acids pattern, such as palm oil and soybean oil, may affect the bioaccessible phytochemicals contents in food matrices before and during gastrointestinal digestion in different manners.

Table 2.6 Fatty acids profile of palm oil and soybean oil (Dubois et al., 2007)

Fatty acids	Types of oil	
	Soybean oil	Palm oil
Total SFA (%)	**15.7**	**50.4**
8:0 (%)	-	0.1
10:0 (%)	-	0.1
12:0 (%)	-	0.4
14:0 (%)	0.1	1.1
16:0 (%)	10.8	43.6
18:0 (%)	3.9	4.4
20:0 (%)	0.3	0.3
22:0 (%)	0.2	0.1
24:0 (%)	0.3	0.1
Total MUFA (%)	**24.2**	**39.4**
16:1 n-7 (%)	0.2	0.2
18:1 n-9 (%)	23.9	39.1
20:1 n-9 (%)	0.1	0.1
Total PUFA (%)	**59.8**	**10.5**
18:2 n-6 (%)	52.1	10.2
18:3 n-3 (%)	7.8	0.3

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CHAPTER III

MATERIALS AND METHODS

3.1 The Experimental Designs

This study was an experimental study consists of 3 phases as following:

1) The selection of the optimum condition for each cooking method
2) The determination of phytochemical compounds and antioxidant capacities before digestion (after cooking under the optimum condition)
3) The determination of phytochemical compounds and antioxidant capacities during digestion

The flowchart of experimental designs is shown in figure 3.1. The first phase was designed to determine the optimum condition of boiling and microwave cooking methods that provided the similar texture and sensory properties, with the highest antioxidant capacities in each cooking method. Consequently, the optimum type and minimum amounts of cooking oil addition that demonstrated the increasing in total phenolic content of food matrices after cooking were determined and chosen. The second and third phases were designed to determine the phytochemicals contents (polyphenols, flavonoids, and beta-carotene) and antioxidant capacities (FRAP and DPPH) of raw sweet leaf as control and cooked sweet leaf under optimum cooking conditions before digestion and during simulated digestion, respectively.

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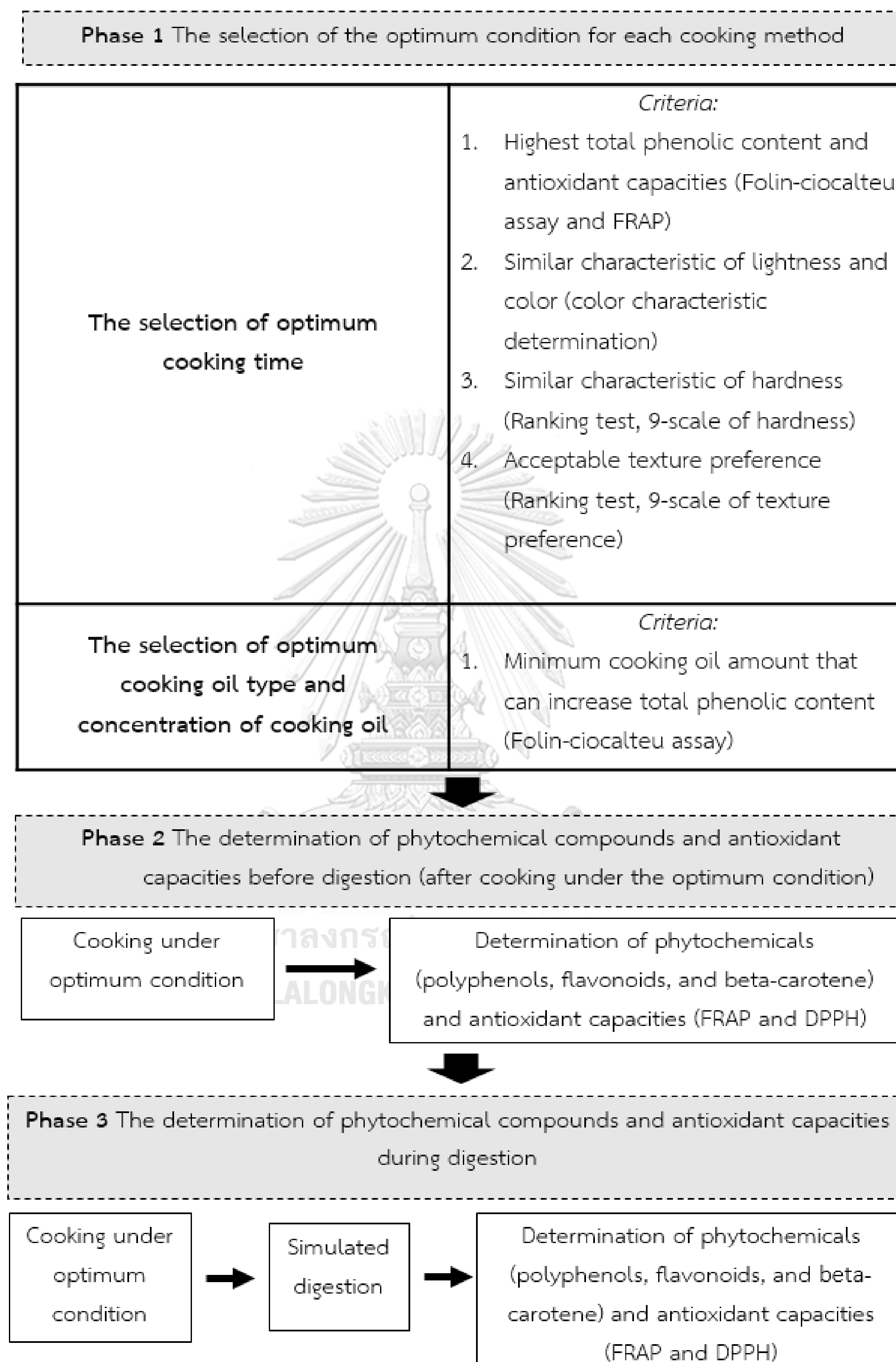

Figure 3.1 The flowchart of experimental design

3.2 Materials and Equipment

Table 3.1 Lists of chemicals used in this study

Chemicals	CAS Number	Company
Folin-Ciocalteu's phenol reagent	521-24-4	Sigma-Aldrich (USA)
Gallic Acid	5995-86-8	Sigma-Aldrich (USA)
Sodium carbonate (Na_2CO_3)	497-19-8	Ajax Finechem (Germany)
Iron (II) sulfate	7720-78-7	Ajax Finechem (Germany)
Iron (III) chloride	7705-08-0	Ajax Finechem (Germany)
2,2-diphenyl-1-picrylhydrazyl (DPPH)	1898-66-4	Sigma-Aldrich (USA)
2,4,6-tripyridyl-S-triazine (TPTZ)	3682-35-7	Sigma-Aldrich (USA)
Catechin	154-23-4	Sigma-Aldrich (USA)
Aluminum Chloride ($AlCl_3$)	7446-13-6	Ajax Finechem (Germany)
Sodium Nitrite ($NaNO_2$)	7632-00-0	Ajax Finechem (Germany)
Sodium hydroxide (NaOH)	1310-73-2	J.T.Baker (Malaysia)
Bile extract porcine	8008-63-7	Sigma-Aldrich (USA)
Pancreatin from porcine pancreas	8049-47-6	Sigma-Aldrich (USA)
Beta carotene	7235-40-7	Sigma-Aldrich (USA)

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Table 3.2 Lists of equipment used in this study

Equipment	Model	Company
Spectrophotometer	PowerWave XS2	BioTek (USA)
Analytical balance	ENTRIS224i-1S	Sartorius (Germany)
Colorimeter	ColorFlex EZ 45/0	HunterLab (USA)
Centrifuge	Z400K	Hermle (Germany)
	380R	Rotina (Germany)
Freezer (-20°C)	MDF 436	Sanyo (Japan)
HPLC system	LC-10AD	Shimadzu (Japan)
• Detector	SPD-10A UV-VIS detector	Shimadzu (Japan)
• Guard column	Inertsil ODS-3, 5μm (4.0 x 10 mm x 2)	GL Sciences (Japan)
• Column	Inertsil ODS-3V, 5μm (4.6 x 150 mm)	GL Sciences (Japan)
pH meter	Orion 4 star	Thermo scientific (USA)
Vortex	Genie 2	Scientific industries (USA)
Magnetic stirrer	C-MAG HS7	IKA (China)
Microwave oven	SHARP R-209	SHARP (Japan)
Auto-pipettes	BioPette	Labnet (USA)

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3.3 Phase 1: The Selection of the Optimum Condition for Each Cooking Method

3.3.1 The collection and preparation of plant

The fresh sweet leaf (*Sauropus androgynus*) was purchased from Nakhon Pathom, Thailand. The plant was identified by the botanist from Department of Botany, Faculty of Science, Chulalongkorn University (Voucher specimen: BCU015915). The leaves part of sweet leaf were collected and cleaned with tap water and air-dried. Then, the leaves were homogenized with the kitchen blender (Philips HR 2094; 750 watt) at 12,000 rpm for 10 seconds prior to cooking.

3.3.2 The selection of the optimum cooking time for each cooking method

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The optimum cooking time was determined by varying cooking time for boiling and microwave cooking. In boiling method, it was set up at 1 minute and 5 minutes of boiling duration. In microwave cooking method, it was set up at 60 seconds, 90 seconds, and 120 seconds of microwaving duration.

The cooking processes were conducted as following:

3.3.2.1 Raw

Homogenized fresh sweet leaf (50 grams) was mixed with distilled water (50 mL) and was kept at -20°C under nitrogen blanket as control until further analyses.

3.3.2.2 B o i l i n g

Boiling distilled water (50 mL) was prepared in the lid-covered beaker glass. Then, the homogenized fresh sweet leaf (50 grams) was added to boiling distilled water and kept boiling for 1 minute or 5 minutes. After that, the boiled sweet leaf samples were kept at -20°C under nitrogen blanket until further analyses.

3.3.2.3 Microwave cooking

Homogenized fresh sweet leaf (50 grams) was mixed with distilled water (50 mL) in microwavable container (material: polypropylene) and was wrapped with microwaveable plastic wrap. The homogenized sweet leaves were cooked in the microwave oven (Sharp R-209) at 800 watts for 60, 90, or 120 seconds. After that, the boiled sweet leaf samples were kept at -20°C under nitrogen blanket until further analyses.

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The optimum cooking time of each cooking method was selected according to four criteria. The first criterion was the total phenolic contents (Folin-ciocalteu assay) and antioxidant capacity (FRAP antioxidant activity). The second criterion was the color characteristic (colorimeter, Colorflex EZ-45/0, HunterLab, US). The third criterion was the hardness (9-scale ranking test of the hardness, involving 20 panelists) (Szczesniak, 2002). The fourth criterion was the texture preference (9-scale ranking test of texture preference, involving 20 untrained panelists) (Szczesniak, 2002).

All sweet leaf samples with different cooking time and cooking methods [raw, boiling (1 minute and 5 minutes), microwave cooking (60 seconds, 90 seconds, and 120 seconds)] were determined under four criteria as mentioned above.

a) The determination of total phenolic contents and FRAP antioxidant activity

Total phenolic content was determined using Folin-ciocalteu assay (detail of method as described in Phase 2). Cooking time that retained the highest total phenolic content was used as the first priority of criteria.

Antioxidant capacity was determined using FRAP antioxidant activity assay (detail of method as described in Phase 2). Cooking time

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that retained the highest FRAP antioxidant activity was considered as the second priority of criteria.

b) The determination of the color characteristics

The color characteristics of sweet leaf samples were determined using Hunterlab colorimeter (Colorflex EZ-45/0). The color characteristic of the samples were determined and quantified as: *L** (lightness; black= 0, white = 100); *a** (redness > 0, greenness <0); *b** (yellowness > 0, blue < 0); and *H** (hue angle; red= 0°, yellow= 90°, green= 180°, blue= 270°), when *H** was calculated with the following formula:

$$\tan^{-1}\frac{b*}{a*}$$

The *L*, a*,* and *b** data were presented as colorimetric unit. *H** value was presented as degree (°) unit. Similar color characteristic was determined from the value of *L* and H**.

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c) The determination of the hardness and the texture preference

The 9-scale ranking test involving 20 untrained panelists was performed to determine the hardness and texture preference according to the method described by Szczesniak (2002). The hardness was

defined as the force necessary to attain given deformation. In this test, panelists were asked to taste the samples and give the rank score of the hardness (1= "not hard at all" to 9= "very hard") and the texture preference (1= "dislike extremely" to 9= "like extremely"). Previous study demonstrated that 20 untrained panelists can be used in a ranking test if at least 15 of them give higher score of control group (in this current study, raw group) than test group (in this study, boiling and microwave cooking group) (Lawless and Heymann, 2010), therefore the number of 20 untrained panelists was used in this study.

The total phenolic content of sweet leaf after cooking was the prior consideration for the selection of the optimum cooking time. The cooking time that can retain the highest total phenolic content in each cooking method was chosen and the FRAP antioxidant activity was considered as the second priority. Consequently, the color characteristic and the hardness of sweet leaf samples under the optimum cooking time in each cooking method were considered. The cooking time of boiling and microwave cooking methods that provided the similar characteristic of L^* and H^*, and similar hardness levels were selected. Furthermore, all selected optimum cooking conditions of sweet leaf had to demonstrate the acceptable texture preference (scored more than 4.5 from overall 9-point ranking test).

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3.3.3 The selection of the optimum type and concentration of cooking oil for cooking sweet leaf

In this part, palm oil and soybean oil were chosen in the experiments because these two types of oil are the common cooking oil that used in Southeast Asia and represent the different fatty acids pattern of saturated, mono-unsaturated, and poly-unsaturated fatty acids. Palm oil represents the high-MUFA cooking oil and soybean oil represents the high-PUFA cooking oil.

Palm oil and soybean oil at different concentration [5%, 10%, and 15% (v/w)] were added to sweet leaf samples (raw, boiling, and microwave cooking conditions according to the previous selected optimum cooking time). The effect of these two types of cooking oils on the phytochemicals content of sweet leaf after cooking with different methods were determined. All sweet leaf samples were made up to the same total final volume by the addition of distilled water. All samples were blanketed with nitrogen and stored at -20°C until further experiments. The weight of sweet leaf and the volume of cooking oil and distilled water in each group at various concentrations of cooking oil are shown in table 3.3.

The optimum type and minimum amount of cooking oil which provided the highest total phenolic content of sweet leaf samples were selected for the next phase.

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Table 3.3 The composition of sweet leaf samples at the various concentrations of cooking oil

The concentration of cooking oil in sweet leaf sample (v/w)	Sweet leaf samples		Cooking oil (mL)	Added distilled water (mL)
	Leaves (g)	Distilled water (mL)		
0%	50	50	0	15
5%	50	50	5	10
10%	50	50	10	5
15%	50	50	15	0

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3.4 Phase 2: The Determination of Phytochemical Compounds and Antioxidant Capacities before Digestion (after Cooking under the Optimum Condition)

In this phase, the experiments were conducted under the optimum condition of each cooking method and the optimum amount of added selected cooking oil according to the results from Phase 1.

3.4.1 The preparation of sweet leaf samples and cooking processes

The fresh sweet leaf (*Sauropus androgynus*) was purchased from Nakhon Pathom, Thailand. The plant was cleaned and prepared for the experiments as described previously in the Phase 1. In one batch, 300 grams of

sweet leaf was homogenized before sampling into six equal portions (approximately 50 grams of homogenized sample per portion) for six cooking conditions. The sweet leaf samples were prepared as described previously with the optimum condition in each cooking method according to the results from Phase 1. The designated cooking methods included the six cooking conditions as shown in table 3.4.

Table 3.4 The composition of sweet leaf samples in each cooking condition

Cooking conditions*	Sweet leaf samples		Cooking oil (mL)	Added distilled water (mL)
	Leaves (g)	Distilled water (mL)		
raw (control)	50	50	0	10
boiling	50	50	0	10
microwave	50	50	0	10
raw + palm oil (10% v/w)	50	50	10	0
boiling + palm oil (10% v/w)	50	50	10	0
Microwave + palm oil (10% v/w)	50	50	10	0

* Condition of cooking methods: boiling for 5 minutes; microwave at 800 watts for 90 seconds

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3.4.2 The determination of phytochemicals (total phenolic contents, total flavonoids, and beta- carotene) and antioxidant capacities (FRAP and DPPH) before digestion

3.4.2.1 The extraction method for the determination of total phenolic contents, total flavonoids, and antioxidant capacities

The extraction of sweet leaf samples was conducted according to the previous publication with slight modification (Hrncirik and Fritsche, 2004). Sweet leaf samples (1 gram) or palm oil and soybean oil (1 mL) were mixed in the solution consisting hexane (2 mL) and 60:40 (v/v) methanol:water (2 mL). The mixtures were vortexed vertically for 2 minutes, shaken at 100 rpm in the water bath for 50 minutes at room temperature, and centrifuged at 2,000 rpm for 10 minutes. The methanol parts were collected and filtered using Whatman paper no.1. The hexane phases then were re-extracted with 60:40 (v/v) methanol:water (1.5 mL) in the same way. All collected methanol extracts were dried using the rotary evaporator at 50°C. Then, dry extracts of samples (sweet leaf samples, palm oil, and soybean oil) were collected and kept at -20°C under nitrogen blanket and reconstituted in distilled water before further analyses.

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3.4.2.2 The determination of total phenolic contents

Total phenolic contents in sweet leaf extracts were determined using the Folin-Ciocalteu assay as described by Chusak et al. (2014). Reconstituted extract samples (50 µL) were mixed with the Folin-Ciocalteu's reagent (50 µL) and incubated at room temperature for 5 minutes before addition of 10% Na_2CO_3 (50 µL). After 30 minutes of the incubation at room temperature, the absorbances of mixtures were measured at wavelength 760 nm with a UV-vis spectrophotometer (BioTek, PowerWave XS2, Winooski, VT, USA). Gallic acid was used as the standard agent. The total phenolic contents in the samples were calculated from the calibration curve of standard gallic acid. The results were expressed as mg gallic acid equivalent (GAE)/g leaves.

3.4.2.3 The determination of total flavonoids contents

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Total flavonoids contents in sweet leaf extracts were determined using the aluminum chloride colorimetric method as described by Chusak et al. (2014) with slight modification. Briefly, reconstituted extract samples (100 µL) were mixed with 30 µL of $AlCl_3$ solution (10% w/v) and 200 µL of 1 M sodium hydroxide solution. After the incubation for 30 minutes at room temperature, the absorbances of mixtures were measured immediately at wavelength 430 nm with a

UV-vis spectrophotometer (BioTek, PowerWave XS2, Winooski, VT, USA). The total flavonoids contents in the samples were calculated from a calibration curve using catechin as the standard agent. The results were expressed as mg catechin equivalent (CE)/g leaves.

3.4.2.4 The determination of the antioxidant capacities

Antioxidant capacities of sweet leaf samples were determined as the ferric reducing antioxidant power (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity which were carried out according to the methods described by Chusak et al. (2014).

3.4.2.4.1 Ferric reducing antioxidant power (FRAP)

The FRAP reagent containing 0.3 M sodium acetate buffer solution (pH 3.6), 10 mM 2,4,6-trispyridyl-*s*-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3$ in the ratio 10:1:1 was freshly prepared. The mixtures of 10 μL reconstituted extract sample and 90 μL FRAP reagent were incubated for 4 minutes at room temperature. Then, the absorbances of the mixtures were measured at wavelength 593 nm with a UV-vis spectrophotometer (BioTek, PowerWave XS2, Winooski, VT, USA). The FRAP antioxidant activities of samples were calculated

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from a calibration curve using $FeSO_4$ as the standard agent. The results were expressed as mM $FeSO_4$ [Fe (II)] equivalent/g leaves.

3.4.2.4.2 DPPH radical scavenging activity

The $DPPH^{\cdot}$ solution (0.2 mM DPPH) was prepared freshly on the day of analysis. After mixing 100 µL of reconstituted extract samples and 100 µL of the $DPPH^{\cdot}$ solution, the mixtures were incubated at room temperature for 30 minutes in the dark and measured the absorbances at wavelength 515 nm with a UV-vis spectrophotometer (BioTek, PowerWave XS2, Winooski, VT, USA). The results were calculated from a standard curve using ascorbic acid as the standard agent. DPPH radical scavenging activities of samples were expressed as mg ascorbic acid/g leaves.

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3.4.2.5 The determination of beta-carotene content

3.4.2.5.1 The extraction method for the determination of beta-carotene content

The extraction of sweet leaf samples was performed using the method described by Corte-Real et al. (2018). Briefly, the sweet leaf samples (4 grams) were soaked with 5 mL of methanol and 1 mL of 30% aqueous KOH for the saponification

of chlorophylls. The sample mixtures were vortexed for 1 minute, sonicated at 37 kHz for 10 minutes in an ultrasonic bath, and then incubated in the dark for 20 minutes at room temperature. Consequently, the mixtures were centrifuged for 5 minutes (1300 rpm, at 4°C) and the methanol parts were collected. The collected methanol parts of samples were separated from the pellets and were extracted once with 9 mL of 1:1 (v/v) hexane:acetone solution. The pellet were also extracted once with 9 mL of 1:1 (v/v) hexane:acetone solution, vortexed for 1 minute, sonicated for 5 minutes, and centrifuged at 1300 rpm, 4°C for 5 minutes. Then, the hexane parts were collected from both methanol parts and pellets. The residue of extracts were re-extracted with 4 mL hexane and 2 mL of saturated NaCl (1 gram NaCl in 2 mL distilled water), vortexed for 1 minute, sonicated for 5 minutes, and centrifuged at 1300 rpm, 4°C for 5 minutes to collect the supernatant. Finally, the residue parts were re-extracted again with 2 mL of diethyl ether, vortexed for 1 minute, sonicated for 5 minutes, and centrifuged at 1300 rpm, 4°C for 5 minutes to collect the supernatant. All collected organic phases from the extraction were dried under a stream of nitrogen for 45 minutes at 25°C and kept at -20°C

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under nitrogen blanket until further analyses. Dry extracts of sweet leaf samples were reconstituted in hexane before the analysis of beta-carotene.

3.4.2.5.2 The HPLC condition for the determination of beta-carotene content

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 5µm, 4.0x10mmx2, GL Sciences, Japan), coupled with a reverse-phase C18 column (Inertsil ODS-3V 5µm (particle size), 4.6 x 150 mm (diameter x length), 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)] was used. The gradient method was the following: 5-70% solvent B (0-18 minutes); 70-5% solvent B (18-20 minutes); and 5% solvent B (20-22 minutes). The flow rate was 0.8 mL/minute, and the UV-Vis detection was performed at 458 nm wavelength (Zhong et al., 2016). The standard beta-

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carotene dissolved in hexane at the concentrations ranged from 0 μg/mL to 20 μg/mL, were used as external standard solutions to set up a calibration curve. The peak area of visible chromatogram of injected samples and standards were monitored at wavelength 458 nm for the calculation of beta-carotene concentration. The results were presented as μg beta-carotene/g leaves.

3.5 Phase 3: The Determination of Phytochemical Compounds and Antioxidant Capacities during Digestion

3.5.1 The *in vitro* simulated gastrointestinal digestion

The *in vitro* simulated digestion procedure was adapted from Pasukamonset et al. (2016) with slight modification. Homogenized sweet leaf samples (1 gram) were mixed with 3 mL of porcine pepsin solution (40 mg/mL in 0.1 N HCl), adjusted to pH 2.0±0.1, and incubated at 37°C in a covered horizontal-shaking water bath (Benchmark Scientific SB-12L) at 100 rpm for 1 hour. For the “gastric phase” samples, the volume of gastric digesta were adjusted to 20 mL and sampling aliquot 1.5 mL to centrifuge at 12,000 rpm for 1 hour, filter supernatant using 0.22 μm nylon filter, and collecting for further analyses as the aqueous fraction of the gastric phase.

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The intestinal phase continued from the previous gastric phase without the volume adjustment (different tubes from the “gastric phase” samples). The gastric digesta were adjusted to pH 4.5±0.1, followed by the addition of 0.15 mL amyloglucosidase solution (120 mg/mL). The mixtures were incubated under the same condition as the gastric phase for 30 minutes. Consequently, the mixtures were adjusted to pH 5.3 ± 0.1 before the addition of 9 mL of the small intestinal enzyme solution which containing pancreatin (3 mg/mL) and bile acid (12 mg/mL) in 100 mM $NaHCO_3$. After that, they were adjusted to pH 7.2±0.1, standardized the final volume to 20 mL, and incubated for 2 hours at 37°C in a covered horizontal-shaking water bath (Benchmark Scientific SB-12L) at 100 rpm. Then, the aliquot (1.5 mL) of intestinal digesta were collected as “intestinal phase” samples which were immediately centrifuged at 12,000 rpm for 1 hour, filtered supernatant using 0.22 µm nylon filter, and the aqueous fraction of the intestinal phase were collected.

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The aqueous fractions of the gastric and intestinal phases were stored under nitrogen blanket at -20°C until further analyses.

3.5.2 The determination of phytochemicals (total phenolic contents, total flavonoids contents, and beta- carotene content) and antioxidant capacities (FRAP and DPPH) during digestion

3.5.2.1 Sampling of aqueous fraction for the determination of total phenolic contents, total flavonoids, beta-carotene, and antioxidant capacities during digestion

The aqueous fractions of gastric and intestinal phases were collected and stored as described previously. Total phenolic contents, total flavonoids, and antioxidant capacities of the gastric and intestinal aqueous fractions of sweet leaf samples were determined using same methods as previously described in Phase 2.

3.5.2.2 The extraction method for the determination of beta-carotene in the aqueous fraction of digested sweet leaf samples

Aqueous fractions from gastric and intestinal phases were extracted and analyzed for the beta-carotene content using the same method and HPLC condition as previously described in Phase 2.

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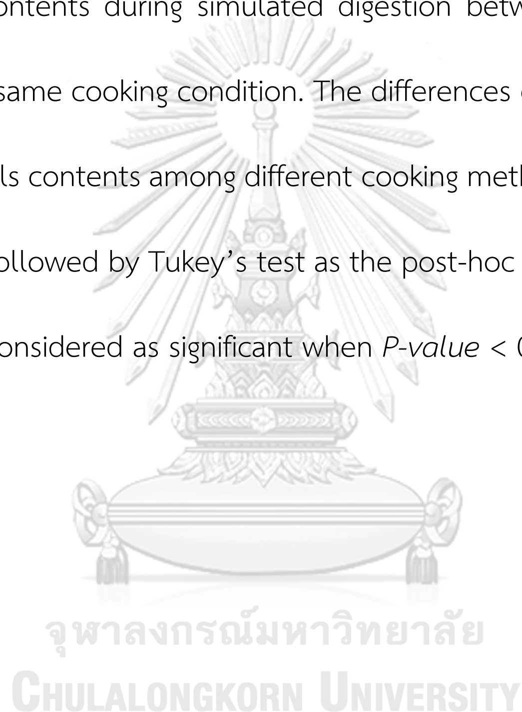
3.6 Statistical Analyses of Data

All data were statistically analyzed using SPSS Software version 16.0 (SPSS Inc, USA). The results were calculated from at least triplicate independent samples and data were presented as mean ± standard error of means (SEM). The student t-test was performed to determine any differences of the antioxidant activities and phytochemicals contents during simulated digestion between gastric and intestinal phases within the same cooking condition. The differences of the antioxidant activities and phytochemicals contents among different cooking methods were analyzed by the one-way ANOVA, followed by Tukey's test as the post-hoc multiple comparisons. The differences were considered as significant when *P-value* < 0.05.



CHAPTER IV

RESULTS

4.1 The Selection of the Optimum Condition for Each Cooking Method

The optimum cooking time of each cooking method were selected according to the four criteria with the following priorities: the antioxidant capacity (FRAP); the color characteristic; the hardness; and the texture preference, respectively.

4.1.1 The effect of different cooking time on the total phenolic contents and the antioxidant activity (FRAP)

The cooking time of each cooking method that retained more antioxidant activity was considered as the optimum cooking condition for next phase of experiments. The effect of different cooking time on the total phenolic contents and the antioxidant activity (FRAP) is shown in table 4.1.

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The results showed that the boiling methods (for 1 minute and 5 minutes) significantly decreased the total phenolic contents (TPC) of sweet leaf, whereas the microwave cooking (at 800 watts for 60, and 90 seconds) increased the amounts of TPC, compared to the raw sweet leaf. However, the longer duration of microwave cooking up to 120 seconds, still retained the amounts of TPC as same as the raw sweet leaf.

In addition, the microwave cooking of sweet leaf at 800 watts (up to 120 seconds) demonstrated the significant increases in the FRAP antioxidant activity compared to the raw sweet leaf, whereas the boiling methods significantly decreased the FRAP values when boiling for only 1 minute but interestingly, when boiling for 5 minutes, FRAP value was retained to the same level as found in the raw sweet leaf.

Table 4.1 The effect of different cooking time on the total phenolic contents and the antioxidant activity (FRAP) of sweet leaf samples

Cooking methods	Total phenolic contents (mg GAE/g leaves)	FRAP (mM Fe(II)/g leaves)
Raw (control)	22.89 ± 0.35[a]	188.96 ± 7.60[a]
Boiling for 1 minute	17.69 ± 1.45[b]	103.44 ± 4.36[b]
Boiling for 5 minutes	17.90 ± 1.34[b]	163.89 ± 18.78[a]
Microwave for 60 sec *	25.74 ± 1.20[c]	245.93 ± 8.40[c]
Microwave for 90 sec *	25.09 ± 0.38[c]	252.76 ± 16.01[c]
Microwave for 120 sec *	21.95 ± 0.82[a]	216.62 ± 10.54[d]

* Microwave at 800 watts; sec: seconds; GAE: gallic acid equivalent; FRAP: ferric-reductase antioxidant power; Data were presented as mean ± SEM (n=3).

[a,b] Different superscripts indicate the significant differences among different cooking methods within the same parameter (*P*-value < 0.05).

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The first prior criterion for the selection of optimum cooking time was the highest antioxidant activity (FRAP value). According to the results in table 4.1, all the microwave cooking methods with cooking time up to 120 seconds were the conditions that could retain or increase total phenolic contents and also increased FRAP antioxidant activity. However, the microwave cooking for 120 seconds tended to lower total phenolic contents and the FRAP antioxidant activity compared to the microwave cooking for 60 and 90 seconds. Hence, the optimum cooking time for microwave cooking method in this criterion were considered as duration of 60 and 90 seconds.

For the boiling method, both cooking duration for 1 minute and 5 minutes decreased TPC, but the boiling for 5 minutes could retain FRAP antioxidant activity while the boiling for 1 minute decreased FRAP value. Therefore, the optimum cooking time for boiling method in this criterion was considered as duration of 5 minutes.

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4.1.2 The effect of different cooking time on the color characteristics

The second criterion for selecting the optimum cooking time was color characteristic. Determination of color characteristic was performed using colorimeter. The results are shown table 4.2.

The similar color characteristics in this study was defined as the similar L^* and H^* values among the sweet leaf samples. The L^* value represented the

"lightness" when the value of "0" was defined as black and "100" was defined as white. The higher lightness value, the brighter green color of samples was observed, whereas the lower lightness value was defined as the dull green color. The *H** value represented the co-ordinate degree of hue parameter. If the angle of hue parameter was near 90°, it means the more yellow color of samples was observed. Meanwhile, if the angle of hue parameter was near or greater than 180°, it means the more dark green color of samples was observed.

The *a** and *b** values represented the greenness and the yellowness, respectively. Lower negative value of *a** (near to 0) means the lower intensity of green color of samples was observed. Positive value of *b** demonstrated the yellowness of samples. Higher positive value of *b** indicated higher intensity of yellow color of samples was observed. Both *a** and *b** values were presented as they were used for calculation of the *H** values.

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Table 4.2 The effect of different cooking time on the color characteristics (*L*, a*, b**, and *H** values) of sweet leaf samples

Cooking methods	*L**	*a**	*b**	*H** (°)
Raw (control)	81.52 ± 1.17^{a}	-0.01 ± 0.005^{a}	-4.22 ± 0.09^{a}	269 ± 0.53^{a}
Boiling for 1 minute	80.54 ± 0.98^{a}	-6.25 ± 0.09^{b}	1.24 ± 0.21^{b}	191 ± 1.84^{b}
Boiling for 5 minutes	41.62 ± 3.57^{b}	-2.77 ± 0.22^{c}	0.64 ± 0.04^{c}	167 ± 1.28^{b}
Microwave for 60 sec *	83.09 ± 0.95^{a}	-0.31 ± 0.21^{a}	0.11 ± 0.09^{c}	177 ± 3.07^{b}
Microwave for 90 sec *	44.81 ± 2.24^{b}	-6.36 ± 0.17^{b}	1.16 ± 0.03^{c}	169 ± 0.16^{b}
Microwave for 120 sec *	39.32 ± 3.06^{b}	-0.26 ± 0.12^{a}	1.97 ± 0.13^{b}	97 ± 3.26^{c}

* Microwave at 800 watts; sec: seconds; Data were presented as mean ± SEM (n=3).

*L** (lightness; black= 0, white = 100); *a** (redness > 0, greenness <0); *b** (yellowness > 0, blueness < 0); and *H** (hue angle; red= 0°, yellow= 90°, green= 180°, blue= 270°)

^{a,b} Different superscripts indicate the significant differences among different cooking methods within the same parameter (*P*-value < 0.05).

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The *a** and *b** values as shown in table 4.2 demonstrated that all boiling and microwave cooking methods shifted the color of sweet leaf samples from dark green to be brighter green, as the *b** values of all cooked sweet leaf samples were more yellow compared to the raw sweet leaf. In addition, the lightness value (*L**) of sweet leaf samples significantly decreased after boiling for 5 minutes; and microwave cooking for 90 seconds and 120 seconds, compared to the raw samples; indicating that boiling and microwave cooking

caused the color change from dark green in the raw sweet leaf to be brighter green in the cooked sweet leaf.

According to the results, considering at the *L** and *H** values, it may interpret that boiling sweet leaf samples for 1 minute resulted in the similar lightness (dark green color) to those of the microwave cooking for 60 seconds and the raw sweet leaf, whereas the boiling for 5 minutes demonstrated the same lightness of samples which was brighter color as found in the microwave cooking for 90 and 120 seconds. In addition, the boiling and microwave cooking with longer duration of cooking time, resulted in the less dark green color of sweet leaf. Considering at the *H** value, we found that the boiling and microwave cooking significantly caused more yellow color of the cooked sweet leaf samples, compared to the raw samples. However, the boiling (for 1 and 5 minutes) and microwave cooking (for 60 and 90 seconds) demonstrated no significant differences of the hue (yellow and dark green) color.

According to the TPC and FRAP values from previous experiments and the color characteristics from this experiment, the following cooking conditions: boiling for 5 minutes; and microwave cooking for 60, and 90 seconds, were further considered in the next criteria: the similar hardness and the acceptable overall texture preference.

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4.1.3 The effect of different cooking time on the hardness and the texture preference

The sweet leaf samples, with different cooking methods, were further evaluated for the hardness and the texture preference. The hardness was defined as the force necessary to attain given deformation. Total of 20 panelists evaluated the hardness and the overall texture preference of sweet leaf samples as shown data in table 4.3.

Table 4.3 The effect of different cooking time on the hardness and the texture preference of sweet leaf samples

Cooking methods	The hardness	Overall texture preference
Raw (control)	7.50 ± 0.34^{a}	1.89 ± 0.25^{a}
Boiling for 1 minute	5.59 ± 0.36^{b}	4.93 ± 0.25^{b}
Boiling for 5 minutes	2.90 ± 0.24^{c}	6.87 ± 0.27^{c}
Microwave for 60 sec *	5.58 ± 0.44^{b}	3.35 ± 0.29^{d}
Microwave for 90 sec *	3.82 ± 0.36^{c}	4.94 ± 0.30^{b}
Microwave for 120 sec *	3.13 ± 0.24^{c}	$5.94 \pm 0.31^{b,c}$

* Microwave at 800 watts; sec: seconds; Data were presented as mean ± SEM (n=20).

The scores of the hardness (1= “not hard at all” to 9= “very hard”) and the overall texture preference (1= “dislike extremely” to 9= “like extremely”)

^{a,b} Different superscripts indicate the significant differences among different cooking methods within the same parameter (*P*-value < 0.05).

According to the results shown in table 4.3, the hardness of cooked sweet leaf samples significantly decreased after cooking, compared to the raw samples, which indicated the softer textures of sweet leaf after the boiling and the microwave cooking methods. The longer cooking time resulted in the decreased hardness of cooked sweet leaf. The boiling for 5 minutes demonstrated the similar hardness to those of microwave cooking for 90 and 120 seconds while the boiling for 1 minute demonstrated the similar hardness to that of microwave cooking for 60 seconds. Nevertheless, the boiling for 1 minute and the microwave cooking for 60 seconds were not considered as the optimum cooking condition because of their high hardness levels.

Furthermore, it was found that the cooked sweet leaf with the boiling, and microwave cooking methods, caused to the higher score of the overall texture preference than that of the raw sweet leaf. The boiling (for 1 and 5 minutes), and the microwave cooking of sweet leaf (for 90 and 120 seconds) demonstrated the acceptable texture preference (more than 4.5 of total 9-point ranking scale).

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Therefore, according to the consideration of the optimum cooking time under the four criteria as mentioned previously, the boiling for 5 minutes and the microwave cooking at 800 watts for 90 seconds were selected to compare with the raw sweet leaf as control for further experiments. The

summary of the qualified characteristics of sweet leaf that cooked with different cooking methods according to the 4 criteria is shown in table 4.4.

Table 4.4 The summary of the qualified characteristics of sweet leaf that cooked with different cooking methods according to the four criteria

Cooking methods	Total phenolic content and antioxidant activity		Color	Hardness	Texture
	TPC	FRAP			
Raw (control)	a	a	A,a	a	a
Boiling for 1 minute	b	b	A,b	b	b,x
Boiling for 5 minutes	b	a	B,b	c	c,x
Microwave for 60 sec *	c,x	c,x	A,b	b	d
Microwave for 90 sec *	c,x	c,x	B,b	c	b,x
Microwave for 120 sec *	a	a	B,c	c	b,c,x

* Microwave at 800 watts; sec: seconds; TPC: Total phenolic contents

Same letters indicate the similar characteristics in the same parameters;

Color: A,b represented L^* and H^*, respectively

"x" indicates the better values than that of control (raw); no color boxes indicated the unqualified characteristic in that criterion, compared to control; The optimum cooking time of each cooking method were selected according to the priorities and the number of qualified characteristics (dark orange-colored boxes). Raw sample was used as control.

4.2 The Selection of the Optimum Type and Concentration of Cooking Oil for Cooking Sweet Leaf

After the optimum cooking times of each cooking method were selected, the optimum type and concentration of cooking oil were further investigated. Soybean oil and palm oil were chosen for screening because they were common cooking oils used in Southeast Asia with the different fatty acids profiles. The various concentrations of cooking oils [5%, 10%, and 15% (v/w)] were added to the sweet leaf samples during cooking with the optimum condition (boiling for 5 minutes, and microwave cooking at 800 watts for 90 seconds, compared to the raw sweet leaf). The total phenolic contents of sweet leaf samples after the addition of various concentrations of soybean oil and palm oil during cooking with different methods (raw, boiling and microwave cooking) under the optimum cooking conditions are shown in table 4.5.

Total phenolic contents of soybean oil and palm oil that used in this study were 0.54 ± 0.03 mg GAE/mL, and 0.86 ± 0.09 mg GAE/mL, respectively. Therefore, when the maximum volume of cooking oils at 15% (v/w) were added into the sweet leaf samples, the highest concentration of total phenolic contents from soybean oil and palm oil in the food matrices approximately ranged from 0.08 mg GAE to 0.13 mg GAE/g leaves, respectively. Thus, the significant increase of total phenolic contents in the sweet leaf samples that occurred after the addition of some specific

concentrations of cooking oils during cooking should not come from the natural phenolic contents in these two types of cooking oils.

The addition of palm oil, started from 10% (v/w), significantly increased the total phenolic contents (TPC) of the sweet leaf samples in all cooking methods (raw, boiling, and microwave cooking), whereas the soybean oil needed to reach 15% (v/w) to demonstrate the same increased amounts of TPC as found in palm oil addition. Therefore, the 10% (v/w) of palm oil addition to sweet leaf samples was selected for further experiments in the next phases because it was the minimum amount of cooking oil that can increase the total phenolic contents in all cooking methods.

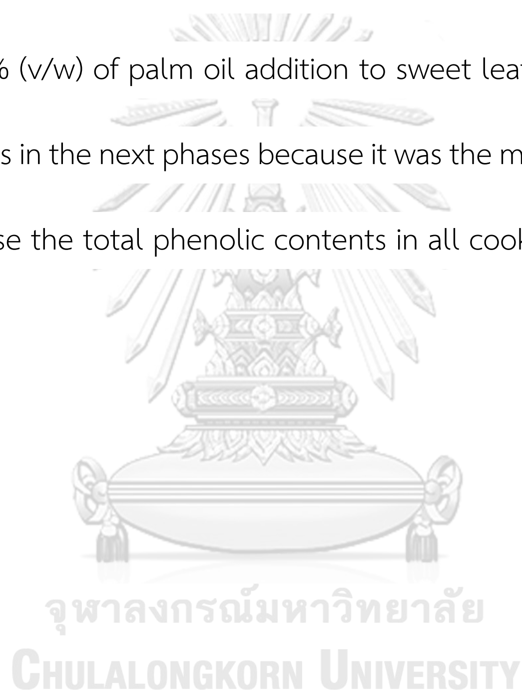
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Table 4.5 The total phenolic contents of sweet leaf samples that cooked under the optimum conditions of each cooking method with the addition of various concentrations of 2 different cooking oils

Cooking conditions*	Total phenolic contents (mg GAE/g leaves)
Raw (control)	23.42 ± 0.38^{a}
Raw + 5% (v/w) Palm oil	22.20 ± 0.42^{a}
Raw + 10% (v/w) Palm oil	26.04 ± 0.47^{b}
Raw + 15% (v/w) Palm oil	26.87 ± 0.18^{c}
Raw + 5% (v/w) Soybean oil	19.68 ± 0.57^{d}
Raw + 10% (v/w) Soybean oil	22.89 ± 0.46^{a}
Raw + 15% (v/w)Soybean oil	29.67 ± 0.34^{e}
Boiling (control)	12.94 ± 0.40^{a}
Boiling + 5% (v/w) Palm oil	14.03 ± 0.17^{a}
Boiling + 10% (v/w) Palm oil	17.86 ± 0.06^{b}
Boiling + 15% (v/w) Palm oil	20.62 ± 0.50^{c}
Boiling + 5% (v/w) Soybean oil	10.88 ± 0.42^{d}
Boiling + 10% (v/w) Soybean oil	15.34 ± 0.41^{a}
Boiling + 15% (v/w) Soybean oil	21.24 ± 0.15^{c}
Microwave cooking (control)	28.65 ± 0.55^{a}
Microwave cooking + 5% (v/w) Palm oil	31.52 ± 0.94^{a}
Microwave cooking + 10% (v/w) Palm oil	37.56 ± 0.19^{b}
Microwave cooking + 15% (v/w) Palm oil	41.40 ± 0.72^{c}
Microwave cooking + 5% (v/w) Soybean oil	34.10 ± 0.68^{d}
Microwave cooking + 10% (v/w) Soybean oil	34.32 ± 0.63^{d}
Microwave cooking + 15% (v/w) Soybean oil	36.63 ± 0.55^{b}

* Boiling for 5 minutes; Microwave at 800 watts for 90 seconds; GAE: Gallic acid equivalent. Data were presented as mean ± SEM (n=3). ^{a,b,c,d} Different superscripts indicate the significant differences of total phenolic contents within the same cooking method analyzed by one-way ANOVA (*P*-value < 0.05).

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4.3 The Effect of Different Cooking Methods on Total Phenolic Contents and Antioxidant Capacities before Digestion (After Cooking under the Optimum Condition)

After the sweet leaf samples were cooked under the optimum conditions of each cooking method, the phytochemical compounds (total phenolic contents, total flavonoids content, and beta-carotene content) and the antioxidant capacities (FRAP antioxidant capacity and DPPH radical scavenging activity) were determined before simulated digestion (table 4.6).

The results showed that the boiling for 5 minutes significantly reduced the total phenolic contents and beta-carotene contents; also tended to slightly decrease in the total flavonoids content and FRAP antioxidant capacity, but surprisingly demonstrated the significant increase in the DPPH radical scavenging activity. Microwave cooking at 800 watts for 90 seconds, demonstrated the retained amounts of total phenolic contents and the significant increases in the total flavonoids content, and the FRAP and DPPH antioxidant activities, whereas the decrease of beta-carotene contents was observed.

After the addition of 10% (v/w) palm oil, it seemed that all those parameters were remained or increased compared to their respective groups without oil addition.

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Table 4.6 The effect of different cooking methods on the phytochemicals contents and antioxidant capacities of sweet leaf samples before simulated digestion

Cooking conditions*	Total phenolic contents (mg GAE/g leaves)	Total flavonoids (mg CE/g leaves)	FRAP activity (mM Fe(II)/g leaves)	DPPH activity (mg ascorbic acid equivalent/g leaves)	Beta-carotene content (μg beta-carotene/g leaves)
Raw	26.11 ± 0.87^{a}	1.31 ± 0.02^{a}	176.9 ± 7.2^{a}	18.66 ± 0.61^{a}	35.58 ± 0.73^{a}
Boiling	18.66 ± 0.16^{b}	0.99 ± 0.02^{a}	162.8 ± 13.3^{a}	21.49 ± 1.22^{b}	15.80 ± 0.28^{b}
Microwave	29.12 ± 0.88^{a}	1.94 ± 0.04^{b}	254.9 ± 14.4^{b}	30.62 ± 0.62^{c}	14.12 ± 0.46^{b}
Raw + 10% (v/w) Palm oil	30.78 ± 0.59^{c}	1.36 ± 0.02^{a}	251.7 ± 8.9^{b}	16.86 ± 1.22^{a}	36.12 ± 0.37^{a}
Boiling + 10% (v/w) Palm oil	20.18 ± 0.54^{d}	1.36 ± 0.01^{a}	$206.4 \pm 19.4^{a,b}$	18.12 ± 0.12^{a}	19.63 ± 0.16^{c}
Microwave + 10% (v/w) Palm oil	40.06 ± 0.64^{e}	2.76 ± 0.04^{c}	262.7 ± 2.8^{b}	29.03 ± 0.49^{c}	15.66 ± 0.16^{b}

* Boiling for 5 minutes; Microwave at 800 watts for 90 seconds; GAE: gallic acid equivalent; CE: catechin equivalent; Data were presented as mean ± SEM (n=3). ^{a,b,c,d,e} Different superscripts indicate the significant differences (P<0.05) among different cooking conditions within the same parameter.

4.4 The Effect of Different Cooking Methods on the Bioaccessibility of Total Phenolic Contents during the Simulated Digestion

The releases of total phenolic contents during simulated digestion are shown in figure 4.1. There were no significant differences of bioaccessible total phenolic contents in the gastric phase among different cooking methods without the addition of palm oil. Interestingly, in the intestinal phase, the releases of total phenolic contents in the cooked sweet leaf (boiling and microwave cooking) were significantly higher than in the raw sweet leaf. The loss of total phenolic contents after intestinal digestion also improved in the cooked sweet leaf samples than in raw leaves.

Total phenolic contents (TPC) of the raw and boiled sweet leaf samples with 10% (v/w) palm oil addition in the gastric phase were significant lower than cooking without oil addition, whereas microwave cooking with palm oil still preserved TPC during gastric phase. When the palm oil were added during cooking, no differences of total phenolic contents in the gastric phase between the raw and boiled sweet leaf were observed, whereas the microwave cooking exhibited the significant higher level than those of the raw and boiling groups. Interestingly, the addition of palm oil in the sweet leaf samples with all cooking methods exhibited the significant increases of the total phenolic contents after 2 hour incubation of intestinal phase compared to the gastric phase. Moreover, the addition of palm oil also caused the significant increases

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of bioaccessible total phenolic contents after the intestinal phase in all cooking methods compared to their respective groups without palm oil addition.

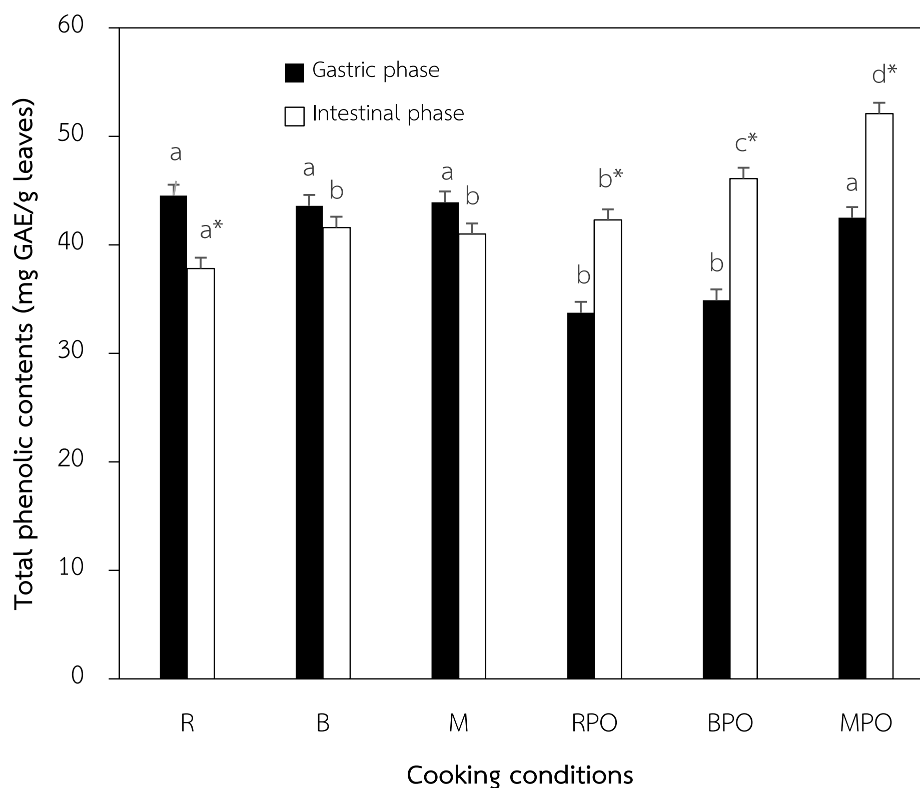

Figure 4.1 The effect of different cooking methods on the bioaccessibility of total phenolic contents during the simulated digestion.

Data were presented as mean ± SEM (n=3); GAE: Gallic acid equivalent.

[a,b,c,d] Different superscripts indicate the significant differences among different cooking conditions within the same phase of digestion analyzed by one-way ANOVA followed by Tukey's test. (*P*-value <0.05)

* Significant difference between gastric and intestinal phase, analyzed by student t-test (*P*-value< 0.05).

R: Raw; B: Boiling for 5 minutes; M: Microwave at 800 watts for 90 seconds;

RPO: Raw + 10% (v/w) palm oil; BPO: Boiling for 5 minutes + 10% (v/w) palm oil;

MPO: Microwave at 800 watts for 90 seconds + 10% (v/w) palm oil

4.5 The Effect of Different Cooking Methods on the Bioaccessibility of Total Flavonoids Contents during the Simulated Digestion

The releases of total flavonoids contents during simulated digestion are shown in figure 4.2. Without the addition of palm oil during cooking, there were no significant differences of the bioaccessible total flavonoids contents in the gastric phase between the raw sweet leaf and microwaved sweet leaf samples, whereas the significant increase of the bioaccessible total flavonoids content in the gastric phase was found in the boiled sweet leaf sample compared to the raw samples. Interestingly, in the intestinal phase, the bioaccessible total phenolic contents in the cooked sweet leaf samples (boiling and microwave cooking) were significantly higher than that found in the raw sweet leaf. However, the boiling method without the addition of palm oil, significantly lower the bioaccessibity of total flavonoids during the intestinal phase compared to the gastric phase.

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The addition of 10% (v/w) palm oil during cooking demonstrated no significant differences of the bioaccessible total flavonoids contents in the gastric phase within the same cooking methods without the oil addition. More than that, the addition of palm oil did not improve the bioaccessibiliy of total flavonoids contents after 2 hour incubation of intestinal phase within the same cooking methods. These findings suggested that the addition of palm oil might affect other classes of polyphenolic compounds instead of flavonoids.

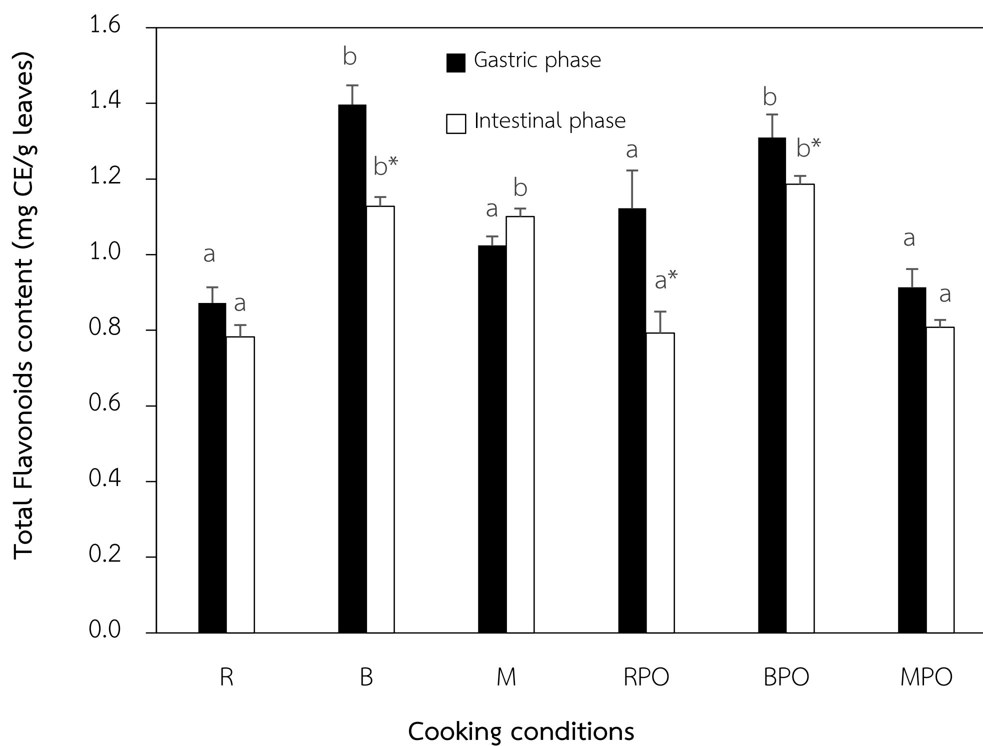
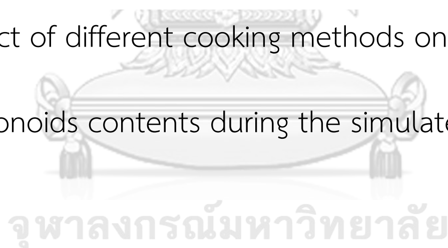


Figure 4.2 The effect of different cooking methods on the bioaccessibility of total flavonoids contents during the simulated digestion.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Data were presented as mean ± SEM (n=3); CE: Catechin equivalent.

[a,b] Different superscripts indicate the significant differences among different cooking conditions within the same phase of digestion analyzed by one-way ANOVA followed by Tukey's test. (*P*-value<0.05)

* Significant difference between gastric and intestinal phase, analyzed by student t-test (*P*-value< 0.05).

R: Raw; B: Boiling for 5 minutes; M: Microwave at 800 watts for 90 seconds;

RPO: Raw + 10% (v/w) palm oil; BPO: Boiling for 5 minutes + 10% (v/w) palm oil;

MPO: Microwave at 800 watts for 90 seconds + 10% (v/w) palm oil

4.6 The Effect of Different Cooking Methods on the Bioaccessibility of Antioxidant Capacities during the Simulated Digestion

4.6.1 The effect of different cooking methods on the bioaccessibility of FRAP antioxidant activity in the gastric and intestinal phases

Figure 4.3 shows the bioaccessibility of the antioxidant capacities in the gastric and intestinal phases among different cooking methods assessed by FRAP assay. Among the cooking methods without the palm oil addition, the FRAP values during the gastric and intestinal phases of the cooked sweet leaf samples (boiling and microwave cooking) were significant higher than those of the raw sweet leaf samples. The microwaved sweet leaf samples without oil demonstrated the highest bioaccessibility of the gastric and intestinal FRAP activities compared to raw and boiled sweet leaf samples. The significant decreases of FRAP activities were found after 2 hour incubation of the intestinal phase compared to the gastric phase, in all cooking methods. These findings suggested that FRAP activities of the sweet leaf samples declined in the condition of intestinal phase which not depend on the cooking methods.

Among the cooking methods with the addition of 10% (v/w) palm oil, the gastric and intestinal bioaccessible FRAP activities of the cooked sweet leaf samples (boiling and microwave cooking) were significant higher than those

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of the raw sweet leaf samples; and the microwave cooking method demonstrated the highest FRAP activities in the gastric and intestinal phases. The addition of palm oil caused the significantly increasing in the intestinal bioaccesible FRAP activities of sweet leaf samples in all cooking methods.

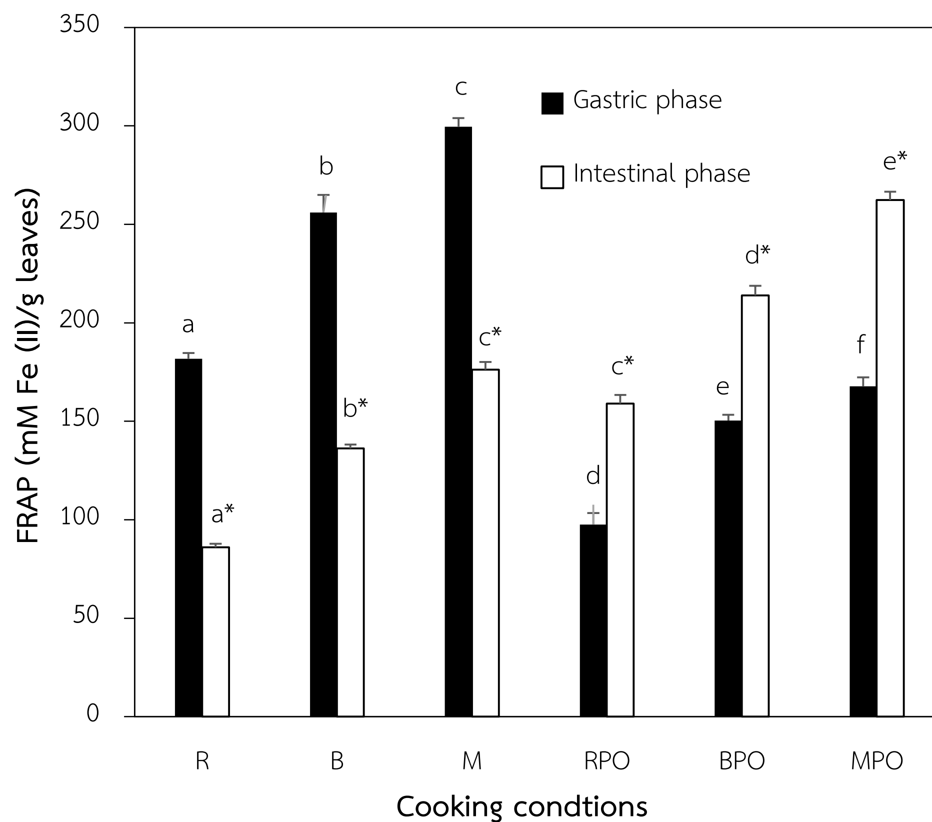

Figure 4.3 The effect of different cooking methods on the bioaccessibility of FRAP antioxidant activity during the simulated digestion.

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Data were presented as mean ± SEM (n=3); FRAP: Ferric-reducing antioxidant power.

[a,b,c,d,e,f] Different superscripts indicate the significant differences among different cooking conditions within the same phase of digestion analyzed by one-way ANOVA followed by Tukey's test. (*P*-value<0.05)

* Significant difference between gastric and intestinal phase, analyzed by student t-test (*P*-value< 0.05).

R: Raw; B: Boiling for 5 minutes; M: Microwave at 800 watts for 90 seconds;

RPO: Raw + 10% (v/w) palm oil; BPO: Boiling for 5 minutes + 10% (v/w) palm oil;

MPO: Microwave at 800 watts for 90 seconds + 10% (v/w) palm oil

4.6.2 The effect of different cooking methods on the bioaccessibility of DPPH scavenging activity in the gastric and intestinal phases

Figure 4.4 shows the bioaccessibility of the antioxidant capacities in the gastric and intestinal phases among different cooking methods assessed by DPPH assay. Among the cooking methods without the palm oil addition, the DPPH scavenging activities during the gastric phase of the cooked sweet leaf samples (boiling and microwave cooking) were significant higher than those of the raw sweet leaf samples. The microwaved sweet leaf samples without oil demonstrated the highest DPPH scavenging activity during the gastric and intestinal phases compared to raw and boiled sweet leaf samples. The significant decreases of DPPH scavenging activities were found after 2 hour incubation of the intestinal phase compared to the gastric phase, in all cooking methods. Fortunately, the addition of 10% (v/w) palm oil in all cooking methods significantly improved the DPPH scavenging activities of the sweet leaf samples after 2 hour incubation of intestinal phase although the addition of palm oil caused the significant decreases in the DPPH scavenging activities during the gastric phase in all cooking methods. These findings suggested that DPPH scavenging activities of the sweet leaf samples declined in the condition

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of intestinal phase which not depend on the cooking methods but they could be improved by the addition of palm oil during cooking.

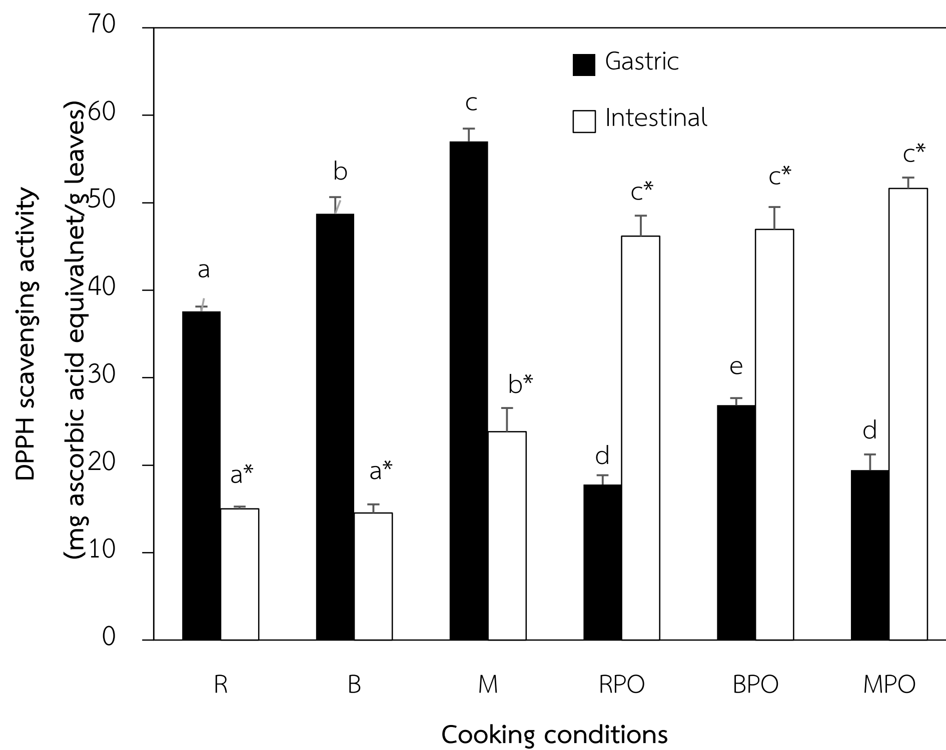

Figure 4.4 The effect of different cooking methods on the bioaccessibility of DPPH scavenging activity during the simulated digestion.

Data were presented as mean ± SEM (n=3); DPPH: 2,2-diphenyl-1-picrylhydrazyl.

[a,b,c,d,e] Different superscripts indicate the significant differences among different cooking conditions within the same phase of digestion analyzed by one-way ANOVA followed by Tukey's test. (P-value <0.05)

* Significant difference between gastric and intestinal phase, analyzed by student t-test (P-value < 0.05).

R: Raw; B: Boiling for 5 minutes; M: Microwave at 800 watts for 90 seconds;

RPO: Raw + 10% (v/w) palm oil; BPO: Boiling for 5 minutes + 10% (v/w) palm oil;

MPO: Microwave at 800 watts for 90 seconds + 10% (v/w) palm oil

4.7 The Effect of Different Cooking Methods on the Bioaccessibility of Beta-Carotene During the Simulated Digestion

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 4.7. Before digestion, the boiling and microwave cooking methods caused the significant reduction in the beta-carotene content of sweet leaf samples. The addition of palm oil at 10% (v/w) helped to increase the beta-carotene content in boiled sweet leaf samples but no improvement was found in the microwaved sweet leaf.

After the simulated gastrointestinal digestion, the bioaccescible beta-carotene contents of microwaved sweet leaf samples significantly decreased compared to raw sweet leaf and it tended to decrease in boiled sweet leaf sample. The addition of 10% (v/w) palm oil during cooking significantly increased the bioaccescible beta-carotene contents after digestion in all cooking methods, compared to 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 leaf was increased about 20%. These findings suggested that the addition of 10% (v/w) palm oil during cooking could improve the bioaccessibility of beta-carotene in the sweet leaf samples, especially when the sweet leaf samples were cooked by microwave.

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Table 4.7 The effect of different cooking methods on the bioaccessibility of beta-carotene during the simulated digestion

Cooking conditions*	**Beta-carotene content (µg/g leaves)**		**% Bioaccessibility**
	Before digestion	**After digestion**	
Raw (control)	35.58 ± 0.73^{a}	7.64 ± 0.01^{a}	21
Boiling	15.80 ± 0.28^{b}	5.94 ± 0.17^{a}	38
Microwave	14.12 ± 0.46^{b}	3.67 ± 0.34^{b}	26
Raw + 10% (v/w) palm oil	36.12 ± 0.37^{a}	9.53 ± 0.66^{c}	26
Boiling + 10% (v/w) palm oil	19.63 ± 0.16^{c}	6.96 ± 0.24^{d}	35
Microwave + 10% (v/w) palm oil	15.66 ± 0.16^{b}	7.18 ± 0.65^{d}	46

* Boiling for 5 minutes; Microwave at 800 watts for 90 seconds; Data were presented as mean ± SEM (n=2).

^{a,b,c,d} Different superscripts indicate the significant differences among different cooking conditions in the same column analyzed by one-way ANOVA followed by Tukey's test. (*P*-value <0.05)

CHAPTER V

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 for the prevention of NCDs (WHO, 2018).

Fruits and vegetables are good source of phytochemicals. Recently, there has been growing interest in fruits and vegetables as the dietary source of phytochemicals which play role as natural antioxidant and has been linked to the reduction of oxidative stress that is linked to aging and development of chronic diseases (Liu, 2003).

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The epidemiological and meta-analysis studies have been demonstrated that the consumption of fruits and vegetables can reduce the incidence of several chronic diseases such as cardiovascular disease, stroke, and type 2 diabetes. Several *in vitro* studies, including studies on cell lines or animal trials, also had shown the positive effect of fruits and vegetables consumption on the reduced oxidative stress and the decreased inflammation markers. While many epidemiological and *in vitro* studies mainly showed the effect of phytochemicals consumption on the reduced chronic diseases, the intervention studies have generally not confirmed these beneficial

effects. The proposed reasons for this discrepancy including the endogenous factors (digestive enzymes) and the dietary factors (certain food preparation methods that may affect dietary composition and structure) (Bohn, 2014).

The digestion process starts in mouth with the amylase as the predominant enzyme. Due to the short interaction time, the impact of oral digestion on the polyphenol release from food matrices is assumed to be low. Most of polyphenols release on the gastric condition which involving pepsin enzyme (resulted in finely ground digesta) and low pH value (as the diffusion of polyphenols from the food matrices into aqueous phase is due to the reduced ionic interaction). In the small intestine condition, pH increases and the secretion of pancreatic enzymes and bile acids are activated. The enzymes and substances involving in the intestinal digestion such as phospholipase, sterol esterase, amylase, carboxypeptidase, trypsinogen, chymotrypsinogen, lipase, and bile salts are active under alkaline pH condition in the small intestine. However, the increased pH in the small intestine was shown to degrade the polyphenols especially anthocyanin upon opening at the C ring (Bohn, 2014).

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Since most of vegetables are not consumed as raw food, an understanding on the effect of cooking methods on the release of polyphenols before and after digestion is important. Heat treatment during cooking can be double-edge sword in terms of the polyphenols bioaccessibility. The cooking methods with heat increase the risk of the degradation and the oxidation of polyphenols. Interestingly, heat treatment facilitates

the polyphenol release during the digestion due to the disruption of cell wall (Bohn, 2014). Thus, this study assessed the effect of different cooking methods (boiling and microwave cooking) on the bioaccessibility of phytochemical compounds and antioxidant capacities of the sweet leaf (*Sauropus androgynus*).

5.1 The Effect of Different Cooking Times on the Color Characteristics of Sweet Leaf

Color characteristics of sweet leaf samples were determined using Hunter L, a, b color space where "L" indicated lightness; "a", the red (+) or green (-) coordinate; and "b", the yellow (+) or blue (-) coordinate (Nielsen, 2017). The visualization of color space is shown in figure 5.1.

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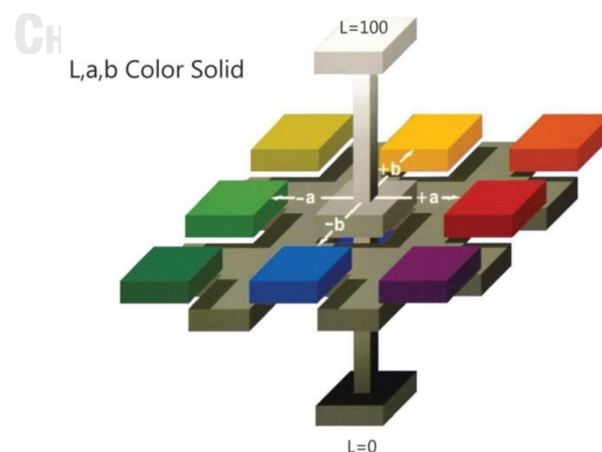

Figure 5.1 Hunter L, a, b color space

(HunterLab, 2015)

The *L** value represents "lightness" with the value of "0" defined as black and the value of "100" defined as white. Higher lightness value in sample means the sample has brighter green color while lower lightness value means the sample has dull green color. The *H** value represents the coordinate degree of hue. For example, if the *H** angle near 90°, it means the sample getting more yellow. Meanwhile, if the *H** angle near or greater than 180, it means the sample getting more dark green.

The intensity of green color is not only affected by the plant pigment, but also by the light scattering and the reflectance of the green surface. After moderate time of cooking, the air between cells was removed and expulsed, resulting in the higher intensity of green color. This explanation correlated to the results from this study that found more negative value of *a** (far from 0) of the cooked sweet leaf samples indicating that the higher intensity of green color were observed. In addition, we found that the boiling and microwave cooking methods shifted the color of sweet leaf samples from dark green to be brighter green, as evidences by the *b** values of all cooked sweet leaf samples were more yellow compared to the raw sweet leaf. Moreover, the lightness value (*L**) of sweet leaf samples significantly decreased and the shift of the hue angle (*H**) to yellow also noticed after the prolonged cooking time, indicating the color change from dark green in the raw sweet leaf to be brighter green in the prolonged cooked sweet leaf, which may explain by the degradation of chlorophyll occurred which resulting in the lower intensity of green color (Miglio et al.,

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2008), and the shifting the color towards the yellow value which related to the formation of pheophytin during cooking process. Pheophytin is formed by the exchanged of Mg^{2+} by H^{+} in the center of the porphyrin ring of the pigment. The formation of pheophytin was previously reported in the change the color of cooked vegetable from bright green to brown (Pellegrini et al., 2010).

5.2 The Effect of Different Cooking Times on the Texture Characteristics of Sweet Leaf

Ranking test involving 20 panelists was performed to identify the texture characteristics of the raw and cooked sweet leaf samples in this study. Ranking test was applied using 9-scale of hardness. The hardness scale was ranged from 1= "not hard at all" to 9= "very hard". In a ranking test, the participants were asked to rank a number of products in the descending or ascending order according to the preference or liking (hardness, sweetness, freshness, etc) (Svensson, 2012). The hardness was defined as the force necessary to attain a given deformation (Szczesniak, 2002).

The hardness of sweet leaf reduced as it was cooked by longer cooking time. It was in line with the previous study demonstrating that the cooking of carrots caused a decrease in the force needed to shred the vegetable. It indicated that cooking causing a decrease of firmness and consequently softening of the vegetable (Miglio et al., 2008).

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The texture preference was ranged from 1= “dislike extremely” to 9= “like extremely”. In this scale, the acceptable texture preference was picked from the sweet leaf samples that have higher score than median (> 4.5 of total 9 scales). According to the results of this study, the longer cooking time caused in the higher acceptability. For example, the boiling for 5 minutes demonstrated higher acceptability of the cooked sweet leaf samples than the boiling for 1 minute. It indicated that the reduced hardness by the cooking in a longer time resulting in the higher acceptability in the cooked sweet leaf.

5.3 The Effect of Different Cooking Methods on the Total Phenolic Contents, Total Flavonoids Contents, Beta-carotene Contents, FRAP Antioxidant Activity, and DPPH Radical Scavenging Activity of Sweet Leaf before Digestion

At the beginning of the study, the optimum cooking condition of sweet leaf samples in each cooking method were determined. The sweet leaf samples were cooked with various cooking time duration for boiling (1 minute and 5 minutes) and microwave cooking (60 seconds, 90 seconds, and 120 seconds). The results showed that the total phenolic contents of sweet leaf samples tended to decrease when they were cooked with longer cooking time. The different cooking principles in each cooking method may have an effect on the phytochemical compounds and antioxidant activities of vegetables due to the antioxidant release, the destruction of antioxidant

compounds, or the creation of redox-active metabolites during cooking (Şengül et al., 2014). The results from current study was in line with the previous study which reported that antioxidants and polyphenols destroyed with longer duration of cooking, for example, the antioxidant activities in broccoli, red pepper, pumpkin, cabbage, and kale decreased after cooking more than 5 minutes (Wachtel-Galor et al., 2008, Azizah et al., 2009, Hwang et al., 2012).

Interestingly, the microwave cooking increased or retained more total phenolic contents of sweet leaf samples than those found in the boiling method. Several studies recommended the microwave cooking as the cooking method that might prevent loss of polyphenols during cooking. The principle of microwave cooking was the penetration of the wave blast into the food compartment so that molecules inside the food vibrating more quickly and producing heat inside out. Therefore, the microwave heating does not directly degrade the antioxidants as it still keep the phytochemicals and antioxidants inside the cell membrane. Moreover, heat transfer in the microwave cooking is under the radiation principle, whereas the boiling method transfers heat to the food compartments with the convection principle which involving heat transfer via liquid as the medium (Spector, 2014, Jiménez-Monreal et al., 2009, Yamaguchi et al., 2001). The visualizations on the principle of microwave oven and the boiling cooking method are shown in figure 5.2 and figure 5.3, respectively.

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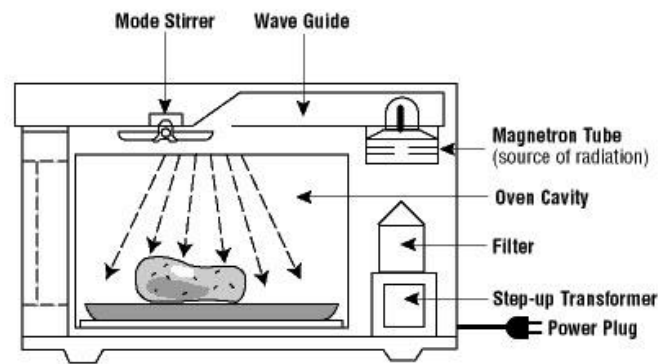

Figure 5.2 The principle of microwave oven cooking

(UAF, 2018)

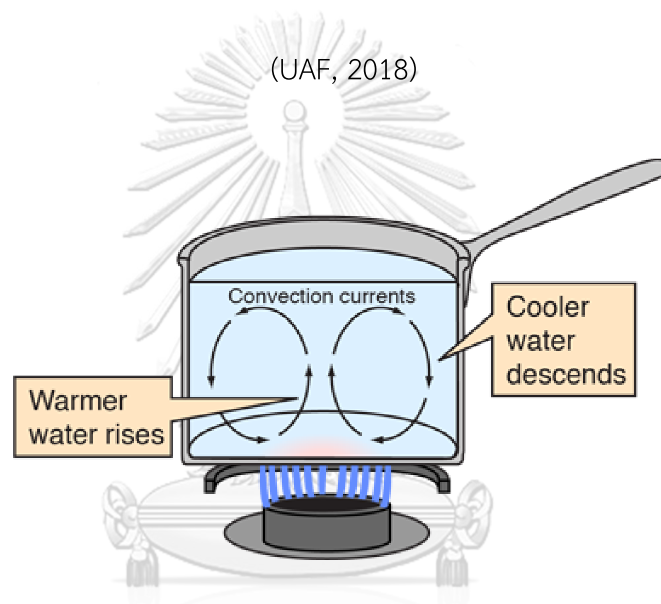

Figure 5.3 The principle of boiling cooking method

(GSU, 2018)

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Moreover, the results from this study also agreed with the previous study which demonstrated that the boiling method decreased the total phenolic contents and the antioxidant capacities of cabbage, broccoli, and choy-sum with a decrease more than 60% after 5 minutes of cooking. The depletion of total phenolic contents after cooking with the boiling method could be due to the phenolic degradation because of heat transfer from liquid medium during cooking (Wachtel-Galor et al., 2008). In addition,

during the boiling, there might be the chemical reactions which make the free phenolic compounds react with other compounds such as protein and forming irreversible covalent bond that is not hydrolyzed by extraction. It was confirmed in previous study as the free phenolic acids were reduced after boiling (Shahidi and Yeo, 2016).

According to the data from the different phases of experiments in this study, total phenolic contents of the raw sweet leaf samples ranged from 23-26 mg GAE/g fresh leaves. It was in the range of the total phenolic contents of sweet leaf that reported from previous studies which ranged from 13-56 mg GAE/g fresh leaves differed by the extraction method, and the cultivar and the harvesting time of the sweet leaf (Somdee et al., 2016, Khalid and Babji, 2018, Rak, 2018, Wong et al., 2006). The major phenolic compounds in the leaves of sweet leaf are alkyl esters of *p*-hydroxybenzoic acid, which are derivatives of phenolic acids (Samad et al., 2013, Farhoosh et al., 2016); also naringenin rutinoside and kaempferol glycoside, which are in the subclasses of flavonoids (Senthamarai and Anusha, 2012, Yu et al., 2006).

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Phenolic acids in plant mainly present in the esters and glycosides form. After the synthesis at endoplasmic reticulum, they then transported through vesicles (small lipid bilayer) to vacuole (soluble-bound phenolic acids) and cell wall matrix (insoluble-bound phenolic acids) (figure 5.4). The high concentrations of phenolic acids inside the vacuole lead to a low pH condition of their storage. In the plant cell wall, phenolic acids are bound with insoluble macromolecules such as pectin, cellulose,

arabinoxylan, and structural protein. Phenolic acids can be released from the food matrices in the gastrointestinal tract by enzymes and pH condition (Shahidi and Yeo, 2016).

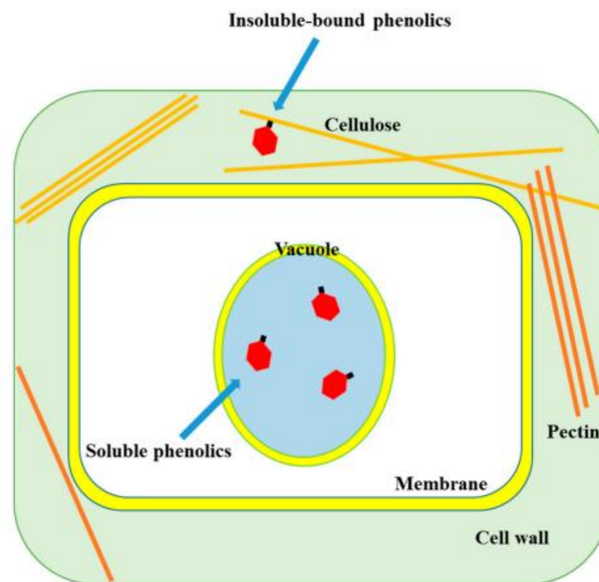

Figure 5.4 Localization of phenolic compounds in plant cell

(Shahidi and Yeo, 2016)

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According to the previous report, heat treatment promoted the conversion of high molecular weight phenolic acids (glycoside and esters) to low molecular weight phenolic acids (aglycone) in citrus peel extract which exhibited more antioxidant activity (Xu et al., 2007, Xiao, 2017). This might be beneficial in the microwave cooking method since the cleaved phenolic acids will be kept inside the cell membrane. However, the heat source in the boiling method comes from the medium (water) that cause a direct contact between heat source and the leaves (as explained previously

in the convection principle) (Bastin, 2011) that might degrade the polyphenols inside the membrane.

In this current study, the beta-carotene content of the raw sweet leaf samples was 35.58 ± 0.73 μg beta-carotene/g fresh leaves. It was in the range of the beta-carotene contents of the raw sweet leaf that reported from previous studies which ranged from 19-53 μg beta-carotene/g fresh leaves (M. Ogle, 2001, Othman et al., 2015, Sripanidkulchai et al., 2005).

In this study, we found that beta-carotene contents of the cooked sweet leaf samples (boiling and microwave cooking) significantly decreased comparing to the raw sweet leaf samples, similarly to the previous study which also reported the loss of beta-carotene after boiling (Hart and Scott, 1995). This phenomenon was ascribed to the breakdown of the cellulose structure of the plant cell and to the denaturation of carotenoid-protein complexes. In addition, the stability of carotenoids in foods are varied by the characteristic of food matrices, such as their chemical composition and the size of the particles (Palermo et al., 2014). We also found that the beta-carotene contents were reduced after microwave cooking. It may be caused by the degradation of chloroplast during food particle motion in the microwave heating leading to the release of beta-carotenes that be synthesized and stored in chloroplasts (figure 5.5) into the food matrices. Because the localization of beta-carotene in plant cells was different from that of the total phenolic contents, the effect of the boiling and

microwave cooking methods on the released contents of these two types of phytochemicals in the sweet leaf samples were possibly different. It is possible that some of phenolic compounds in the sweet leaf samples still remained and can be kept inside the plant cell wall while beta-carotenes which localized in the chloroplasts were degraded during the microwave cooking.

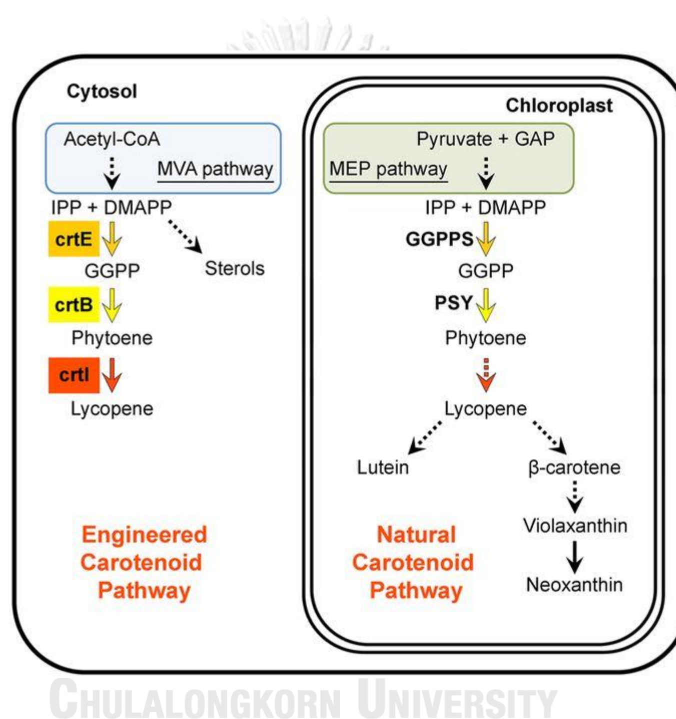

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Figure 5.5 The synthesis pathway and the localization of carotenoids in plant cell (Majer et al., 2017)

The cooking methods did not affect only the phytochemicals content but also the antioxidant capacities of the sweet leaf samples. We found that the ferric-

reductase antioxidant power (FRAP) activity of the boiled sweet leaf samples tended to reduce comparing to the raw samples, whereas the microwave cooking significantly increased the FRAP antioxidant activity. However, the DPPH radical scavenging activity of the cooked sweet leaf samples (both boiling and microwave cooking) significantly increased compared to the raw sweet leaf.

Although the cooking methods such as boiling may reduce some phytochemicals content and some of the antioxidant capacities of sweet leaf, the microwave cooking showed more preferable results than raw and boiling methods. These findings suggested that the microwave cooking was the recommended cooking method that might prevent loss of polyphenols and increase the antioxidant capacities of sweet leaf compared to the raw leaves.

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5.4 The Effect of the Cooking Oil Addition in the Different Cooking Methods on the Phytochemicals Contents and the Antioxidant Capacities of Sweet Leaf before Digestion

The effect of the addition of palm oil and soybean oil during cooking with the optimum condition of each cooking method on the phytochemical contents and the antioxidant capacities of the sweet leaf samples were determined in this study. The results found that the addition of palm oil, started from 10% (v/w), significantly increased the total phenolic contents (TPC) of the sweet leaf samples in all cooking

methods (raw, boiling, and microwave cooking), whereas the soybean oil needed to reach 15% (v/w) to demonstrate the same increased amounts of TPC as found in palm oil addition. Moreover, the addition of 10% (v/w) palm oil retained or increased the flavonoids contents; beta-carotenes contents; FRAP antioxidant activity; and DPPH radical scavenging activity of the sweet leaf samples before digestion.

The cooking oils might increase total phenolic contents in the vegetables during cooking via several mechanisms. Firstly, the interaction between fatty acids and polyphenols creates a stable emulsion that favors the stability of polyphenols in the food matrices. Another study also demonstrated that the cooking with oil increased the antioxidant activities of red radish, broccoli, and white cabbage (Şengül et al., 2014).

5.5 The Effect of Different Cooking Methods on the Bioaccessibility of Phytochemical Compounds and the Antioxidant Capacities of Sweet Leaf during the Simulated Digestion

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After the determination of phytochemical compounds and the antioxidant capacities of the sweet leaf samples before digestion, the raw and cooked sweet leaf under the optimum cooking condition were subjected into the *in vitro* simulated digestion consisting gastric phase and intestinal phase. The total phenolic contents of the raw and cooked sweet leaf samples were released into the gastric digesta within 1 hour incubation of the gastric phase with no significant differences among the cooking

methods. However, the total phenolic contents of the raw sweet leaf samples significantly decreased after 2 hours incubation of intestinal phase, whereas the loss of total phenolic contents after intestinal phase was significantly improved in the cooked sweet leaf (boiling and microwave cooking) as their total phenolic contents after the intestinal phase were not different from the gastric phase. The amount of total phenolic contents after *in vitro* simulated gastrointestinal digestion was significantly higher in the cooked sweet leaf samples than that of the raw leaves.

The flavonoids contents of the raw and cooked sweet leaf samples were also released into the gastric digesta within 1 hour incubation of gastric phase. The significant loss of the flavonoids contents occurred after 2 hours incubation of intestinal phase only in the boiled sweet leaf samples even though the intestinal bioaccessible flavonoids in the boiled samples was not different from that of microwaved samples and higher than that of raw samples.

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In the previous study, it demonstrated that about 15% total polyphenols was lost during the transition from the acidic gastric environment to mild alkaline intestinal environment (Tagliazucchi et al., 2010). Interestingly, in the intestinal phase, the bioaccessible total phenolic contents in the cooked sweet leaf samples (boiling and microwave cooking) were significantly higher than that found in the raw sweet leaf. This finding may be supported by the previous study which explained that the cooking process disrupted the plant cell walls, facilitating the polyphenols release during

digestion via the modification of polyphenols from glycoside to aglycone form which is more biologically active (Kaulmann et al., 2016, Bohn, 2014, Mtolo et al., 2017). In addition, the heat treatment during cooking process also takes role on the modifying of cell wall and protein complexes to increase their extractability during the *in vitro* digestion (Ribas-Agustí et al., 2017).

For the bioaccessible beta-carotene contents after the *in vitro* gastrointestinal digestion, we found that the cooked sweet leaf samples released the beta-carotene contents into the digesta lower than that of the raw sweet leaf sample. These results were in line with the previous study which reported that the release of beta-carotene in the cooked carrot could be lower due to the lower content in the initial state before digestion (Hornero-Méndez and Mínguez-Mosquera, 2007). Interestingly, even though the releases of beta-carotene after digestion from the cooked sweet leaf samples were lower than raw leaves, the % bioaccessibility of digested beta-carotene in the cooked sweet leaf samples tended to be higher than raw samples. It may suggest that the cooking methods such as boiling and microwaving helped improvement of the beta-carotene bioaccessibility.

In green vegetables, the carotenoids are associated intimately with proteins of the light-harvesting complex in the thylakoid membranes of the chloroplast. The processing and cooking methods could soften the plant tissue through the swelling of the cell wall, the dissolution of pectin, and the cell separation. The chopping and

mastication of vegetables caused the crushing and the shearing of the tissues and cells. Those mechanism of particle size reduction may contribute to the increased release of phytochemicals from plants to the food matrices (Hornero-Méndez and Mínguez-Mosquera, 2007).

The cooking methods also affected the bioaccessible antioxidant capacities of the sweet leaf samples during the gastrointestinal digestion. We found that the FRAP antioxidant activity in the gastric phase of the cooked sweet leaf samples (boiling and microwave cooking) were significantly higher than that of raw leaves. Even though the loss of FRAP antioxidant activity occurred in the transition between the gastric and the intestinal phases, the intestinal FRAP antioxidant activity values of the cooked sweet leaf samples were still significantly higher than that of raw leaves. Among the cooking methods, the microwave cooking was the best that significantly retained more FRAP antioxidant activities of sweet leaf samples during the gastric and the intestinal phases than the boiling and raw methods, respectively.

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However, the DPPH radical scavenging activities of the cooked sweet leaf samples (both boiling and microwave cooking) in the gastric phase were significantly higher than that of the raw sweet leaf. Similarly to the FRAP antioxidant activity, the loss of DPPH radical scavenging activities also occurred in the transition between the gastric and the intestinal phases.. Among the cooking methods, the microwave cooking was still the best cooking method that significantly retained more DPPH radical

scavenging activities of sweet leaf samples during the gastric and the intestinal phases than the boiling and raw methods, respectively.

5.6 The Effect of 10% (v/w) Palm Oil Addition in Different Cooking Methods on the Bioaccessibility of Phytochemical Compounds and Antioxidant Capacities of Sweet Leaf during the Simulated Digestion

Although we found that the addition of 10% (v/w) palm oil could retain or increase the flavonoids contents; beta-carotenes contents; FRAP antioxidant activity; and DPPH radical scavenging activity of the sweet leaf samples before digestion, the releases of those compounds and antioxidant capacities were not the same. During the gastric phase, the bioaccessible total phenolic contents of raw and cooked sweet leaf samples with the 10% (v/w) palm oil addition were significantly lower than or the same as found in the sweet leaf samples that cooked with the same methods without oil addition. However, all sweet leaf samples with the addition of palm oil demonstrated the significant increases in the bioaccessibility of total phenolic contents in the intestinal phases. It indicates that the palm oil at the designated amount might keep and delayed the release of polyphenols from the sweet leaf samples, and/or protected them from the degradation during the gastric digestion by forming the hydrophobic polyphenols complex or stable emulsion with lipids that favors the stability of polyphenols in the food matrices. These hypotheses may be supported by

the previous study which demonstrated that under the acidic pH, the phenolic acids which containing alkyl-ester at the lower pH value, exhibited higher hydrophobicity (Farhoosh et al., 2016). Moreover, the bile salts and lipid digestive enzymes in the intestinal phases may disrupt the food matrix by digesting the lipids (Ortega et al., 2009). In addition, the lipids from oil may increase polyphenol bioaccessibility because of the incorporation of apolar polyphenols in the food matrices which oil can be sufficiently emulsified during the intestinal digestion (Kaulmann et al., 2016).

However, we found that the addition of 10% (v/w) palm oil in the raw and boiled sweet leaf samples significantly lower the bioaccessibity of total flavonoids during the intestinal phase compared to the gastric phase but the bioaccessible total flavonoids contents of the sweet leaf samples not different from those found in the same cooking methods without oil addition.

Although the addition of 10% (v/w) palm oil in the sweet leaf samples with different cooking methods showed an increase in the beta-carotene contents in the boiled sweet leaf samples, no changes were observed in the raw and microwaved sweet leaf samples. After the gastrointestinal digestion, the palm oil significantly increased the bioaccessibility of beta-carotene in the sweet leaf samples in all cooking methods, compared to those of the same cooking method without the 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) increased the percentage recovery of the

bioaccessible beta-carotene fraction to 39-94% (Hedrén et al., 2002). It may explain by the fact that the dietary fat is required for the efficient carotenoids bioaccessibility via several ways. 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 hydrolyzed by lipase. In addition, dietary lipids also help carotenoids to be efficiently incorporate to the mixed micelles that further absorbed into the enterocytes (Huo et al., 2007).

The addition of cooking oil also affected the bioaccessible antioxidant capacities of the raw and cooked sweet leaf samples during the gastrointestinal digestion. During the gastric phase, the bioaccessible FRAP antioxidant activities and DPPH radical scavenging activities of raw and cooked sweet leaf samples with the 10% (v/w) palm oil addition were significantly lower than those found in the sweet leaf samples that cooked with the same methods without oil addition. However, all sweet leaf samples with the addition of palm oil demonstrated the significant increases in the bioaccessibility of both FRAP antioxidant activities and DPPH radical scavenging activities in the intestinal phases which were higher than those of the same cooking methods without oil addition. These findings really indicated that the addition of palm oil at the designated amount helped improving the bioaccessibility of antioxidant capacities of the raw and cooked sweet leaf during gastrointestinal digestion.

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CHAPTER VI

CONCLUSION

The cooking methods and the additional food ingredients such as the cooking oil are the important factors which affecting the stability and the bioaccessibility of phytochemicals and antioxidant capacities of sweet leaf before and during digestion. This study demonstrated that cooking sweet leaf prior to consumption provided the better bioaccessibility of total phenolic contents, total flavonoid contents, beta-carotene contents; and the antioxidant capacities: FRAP antioxidant activity and DPPH radical scavenging activity, than consuming it as raw leaves.

Microwave cooking at 800 watts for 90 seconds was the optimum cooking method that provided the best bioaccessibility of phytochemicals and antioxidant capacities, comparing to raw leaves and boiling for 5 minutes. Nevertheless, prolonged cooking time may lead to the degradation of phytochemicals and reducing the antioxidant capacities of sweet leaf. Moreover, the addition of limited amount of palm oil at 10% (v/w) during cooking helped increasing the bioaccessibility of total phenolic contents, beta-carotene contents, and the antioxidant capacities, comparing to the respective groups without the oil addition.

In conclusion, this study suggested that the conventional cooking methods such as microwave cooking and boiling for only the short duration time with the

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addition of limited amount of cooking oil were recommended to prevent loss of polyphenols and increase the antioxidant capacities of sweet leaf to optimize the culinary aspect of sweet leaf for further health benefits.

APPENDICES

APPENDIX I: VOUCHER SPECIMEN OF PLANT IDENTIFICATION

แบบขอใช้บริการพิพิธภัณฑ์พืช ศ.กสิน สุวตะพันธุ์ และหน่วยปฏิบัติการวิจัยพรรณไม้ประเทศไทย
ภาควิชาพฤกษศาสตร์ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

เลขที่ 5 / 2561 วันที่ 17 ม.ค. 2561

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3. วัตถุประสงค์การขอใช้บริการ

- [x] ตรวจระบุชื่อวิทยาศาสตร์ของพันธุ์ไม้ จำนวน 1 ตัวอย่าง ผักหวานบ้าน
- [ ] สอบถามข้อมูลพันธุ์ไม้
- [x] ทำ Voucher Specimen จำนวน 1 ตัวอย่าง
- [ ] อื่นๆ

ไร่ชื่อ: นครปฐม กำแพงแสน จ.นครปฐม
15/01/2018

ลงชื่อ Imas Arumsari
( )
ผู้ขอใช้บริการ

แบบรายงานผล 23/01/2018

เลขที่ 5 / 2561 วันที่ 23 มกราคม 2561

รายการงานที่ทำ Plant Identification & voucher specimen

ผลการตรวจวิเคราะห์ / สืบค้นข้อมูล

Vernacular name : Phak wan baan, Phak wan
Common name : Sweet leaf bush Family : Phyllanthaceae
Scientific name : *Sauropus androgynus* (L.) Merr.
Herbarium number : 015915 (BCU)
Collector number : Imas Arumsari 1

ค่าบริการ 200 บาท (-Two hundred baht-)

ลงชื่อ P. Klinratana
(Parinyanoot Klinratana)
ผู้ให้บริการ

APPENDIX II: PHOTOS DURING EXPERIMENTS

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Sauropus

After cleaning

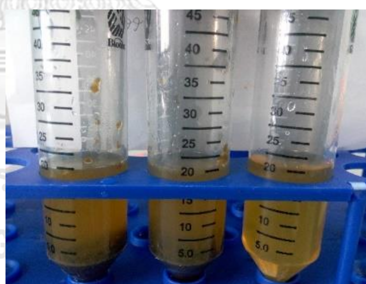
Homogenizing

After homogenizing

Sample: Boiling

Sample: Microwave cooking

Digesta: Gastric phase



Digesta: Intestinal phase

Beta-carotene extract

Sensory analysis

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