EFFECT OF CONVENTIONAL COOKING METHODS; BLANCHING, BOILING, AND STIR-FRYING ON VITAMIN C, TANNIN, AND INOSITOL PHOSPHATES; PENTA AND HEXAPHOSPHATES CONTENTS IN SELECTED THAI VEGETABLES

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE (NUTRITION)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2005

ISBN 974-04-6287-1

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was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Nutrition)
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1 June, 2005

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ACKNOWLEDGEMENTS

This thesis can't be succeeded smoothly without grateful helps from many people. In the first, I would like to thank my major advisor Asst. Prof. Ratchanee Kongkachuichai, who give me everything including learning, teaching, and opportunities that make me a better person. Due to her kindness and efforts to my thesis, I can feel the relationship between teachers and students. She also makes me understand the meaning of the word "teacher".

The next special person that I would like to thanks is Assoc. Prof. Pongtorn Sugpuarg, my co-advisor who gives me understanding about how to work and always find the way out for me when I have a problem.

Third, I would like to thank Miss Rin Charoensiri, my co-advisor who helps me in everything, not only in my thesis's working but also in my labatory room' life. She makes me feel better when I feel weak. And she's always glad with me when I feel happy. From my heart, I would like to say thank you to you.

Very special thank goes to Assoc. Prof. Nopamon Sritongkul for her kindness in giving examination and suggestions that make my thesis complete.

Moreover, special thank is extended to Prof. Voranant Sukpiphat, who gave good opportunities to all students in nutrition major I want to say thank you to you, too.

I would like to thank my friends in Ramathibodi hospital and institute of nutrition, especially, Miss Temsiri Chaidet and Miss Kanokporn Rungsimunwong, two persons who never let me stand alone and always be by my side even good times or bad times. If I don't have your helps, I can't have this successful day. You are the friends in need.

I am grateful to all the lecturers and staffs of the Ramathibodi hospital and institute of nutrition for their valuable advice and knowledge that they are always willing to give me.

Finally, I would like to thank my family that always supports and encourages me. Their warm love gives me power to struggle all the obstacles until this successful day. Thank you.

Weenanan Somsub

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M. Sc. (NUTRITION)

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ABSTRACT

The effect of blanching, boiling and stir-frying on the amount of inositol penta and hexakisphosphates (IP5 and IP6) contents in selected Thai leaves and tender tips and flower vegetables were determined using ion-pair reverse-phase chromatography. In addition, vitamin C and tannin contents were measured by spectrophotometer. Results indicated that vitamin C content of raw and cooked vegetables ranged from 0.50 to 85.59 mg/100g and 0.22 to 46.48 mg/100g, respectively. Stir-fried Pagwanpa, Pagwanban and Cowslip creeper flower were excellent sources of vitamin C with an average about 67.70 mg/100g. The tannin content ranged from 3.14 to 1353.22 mg/100g of tannic acid equivalents for raw and 1.80 to 679.22 mg/100g of tannic acid equivalents for cooked vegetables. The high concentration of tannin was found in lead tree (Yod–kratin) with approximately 1353.22 mg/100g for raw and 679.22 mg/100g tannic acid equivalents for blanched. The phytate content in leaves and tender tips and flower vegetables ranged from 4.49 to 52.32 mg/100g in raw and 3.89 to 50.74 mg/100g in cooked, respectively. The neem tree was found to be the highest source of phytate (52.32 mg/100g). After cooking, blanched Neem tree and stir-fried Pagwanban had the greatest amount of phytate with approximately 38.39 and 50.74 mg/100g. The vitamin C content significantly decreased by all conventional cooking methods, while concentrations of tannin and phytate were not significantly reduced in all cooked samples. All cooked leaves and tender tips and flower vegetables resulted in 18 to 95 % vitamin C loss compared to raw, whereas higher tannin and phytate retention was observed with approximately 44 to 93 % for tannin and 58 to 79 % for phytate, respectively. Results in this study indicated that among three conventional cooking methods (blanching, boiling and stir-frying), boiling may be the best and most suitable for household use to reduce anti-nutritional factors, particularly tannin and phytate in vegetables.

KEY WORDS : VITAMIN C/ TANNIN/INOSITOL PHOSPHATES/ VEGETABLES/COOKING

104 P. ISBN 974-04-6287-1

ผลของการปรุงสุกด้วยวิธีการลวก การต้ม และการผัดต่อปริมาณวิตามินซี แทนนิน และไฟเตท ในผักบางชนิดในประเทศไทย (EFFECT OF CONVENTIONAL COOKING METHODS; BLANCHING, BOILING, AND STIR-FRYING ON VITAMIN C, TANNIN, AND INOSITOL PHOSPHATES; PENTA AND HEXAPHOSPHATES CONTENTS IN SELECTED THAI VEGETABLES)

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อวิเคราะห์ผลของการลวก ต้ม ผัด ที่มีต่อปริมาณของวิตามินซี แทนนิน และไฟเตท ในผักบางชนิดที่บริโภคในประเทศไทย พบว่าปริมาณวิตามินซีที่วิเคราะห์โดยวิธีสเปคโตรโฟโตมิเตอร์ในตัวอย่างดิบมีค่าอยู่ในช่วง 0.50–85.59 มิลลิกรัมต่อ 100 กรัม โดยผักหวานบ้านเป็นแหล่งที่ดีของวิตามินซี โดยมีค่าเฉลี่ยอยู่ประมาณ 85.59 มิลลิกรัมต่อ 100 กรัม เมื่อนำผักไปผ่านกระบวนการปรุงพบว่าปริมาณวิตามินซีลดลงอยู่ในช่วง 0.22–46.48 มิลลิกรัมต่อ 100 กรัม โดยผักหวานบ้านที่ผ่านการปรุงโดยการผัดจะมีปริมาณวิตามินซีสูงสุด คือ 67.70 มิลลิกรัมต่อ 100 กรัม จากการวิเคราะห์หาปริมาณแทนนินโดยวิธีสเปคโตรโฟโตมิเตอร์พบว่า ในผักดิบมีค่าอยู่ในช่วง 3.14–1353.22 มิลลิกรัมต่อ 100 กรัมในขณะที่ผักที่ผ่านกระบวนการปรุงจะมีค่าอยู่ในช่วง 1.80–679.22 มิลลิกรัมต่อ 100 กรัม โดยผักที่มีปริมาณแทนนินสูงสุดคือกระถิน ซึ่งมีปริมาณแทนนินในตัวอย่างดิบคือ 1353.22 มิลลิกรัมต่อ 100 กรัม และ 679.22 มิลลิกรัมต่อ 100 กรัม เมื่อผ่านการลวกปริมาณไฟเตทที่วิเคราะห์โดยวิธี iron-pair reverse-phase chromatography พบว่าในผักดิบมีปริมาณไฟเตทอยู่ในช่วง 4.49-52.32 มิลลิกรัมต่อ 100 กรัมโดยสะเดามีปริมาณไฟเตทสูงสุด คือ 52.32 มิลลิกรัมต่อ 100 กรัม ในผักที่ผ่านกระบวนการปรุงมีปริมาณไฟเตทอยู่ในช่วง 3.89–50.74 มิลลิกรัมต่อ 100 กรัม โดยสะเดาลวกและผักหวานบ้านผัดมีปริมาณไฟเตทสูงสุดคือ 38.39 มิลลิกรัมต่อ 100 กรัม และ 50.74 มิลลิกรัมต่อ 100 กรัมตามลำดับ วิตามินซีมีการลดลงอย่างมีนัยสำคัญในแต่ละวิธีการปรุง คือ ผักประเภทใบและยอด รวมทั้งผักประเภทดอก มีการลดลงของปริมาณวิตามินร้อยละ 18-95 เมื่อเปรียบเทียบกับผักดิบ ในขณะที่การปรุงไม่มีผลต่อการลดลงของปริมาณแทนนินและไฟเตท โดยพบว่าเมื่อผ่านขบวนการปรุงแทนนินในผักยังคงเหลืออยู่ร้อยละ 44-93 และไฟเตทเหลืออยู่ร้อยละ 58-79 อย่างไรก็ตามผลของการศึกษานี้แสดงให้เห็นว่าการต้มมีผลต่อการลดลงของปริมาณแทนนินและไฟเตทซึ่งเป็นตัวขัดขวางการดูดซึมสารอาหารมากกว่าวิธีการปรุงอาหารวิธีอื่น

104 หน้า ISBN 974-04-6287-1

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LIST OF ABBREVIATIONS

IP5	Inositol pentaphosphates
IP6	Inositol hexaphosphates
°C	degree celcius
min	minute
rpm	revolutions per minute
mg	milligram
g	gram
ANOVA	analysis of variance
ml	millilitre

CHAPTER 1
INTRODUCTION

Iron deficiency becomes the public health problem in developing countries, because their staple foods are mainly rice, cereal, grains and vegetables more than animal product (1). Rice, cereal, grains and vegetables are the major source of nonheme iron that poorly absorbed because of the present as strong inhibitor of mineral absorption (particular, iron, zinc, copper and calcium). Specially, iron absorption is affected by both enhancers and inhibitors present in the diet. One of potent enhancers is ascorbic acid and two common inhibitor of iron absorption are polyphenolic compounds (tannin) and phytate which are found in many plant foods (2).

Polyphenolic compounds such as tannin was found in tea, coffee and certain vegetables, bind nonheme iron effect to iron absorption. Presumably tannin form complexes with iron in the intestinal lumen reducing iron bioavailability (3). The tannin contributes strongly to the low bioavailability of iron in vegetable diet. It is well establish that tannin, polyphenolic compound, reduces iron absorption when include in the diet at high levels (4, 5). In addition, in 1991 Tuntawiroon *et al*., also reported that tannin was found in Thai vegetable (Yod kratin) strongly inhibited and decreased iron absorption more than ≥ 50% when compared to control meal which without Yod kratin added (6). In addition, 2002, Glahn *et al*., demonstrated that maximal inhibited iron uptake more than 57 to 80% by tannic acid at a ratio of 1:1 or less.

Phytate (myo-inositolphosphate) is a naturally occurring constituent of plant seed, cereal, bean, some fruit and vegetables where it acts as a storage form of phosphate. It is of considerable nutritional interest because it has been showed to

strongly bind or chelate divalent minerals in the digestive trace as well, making them unavailable for absorption particularly calcium, iron, zinc and copper (7, 8). Sandberg *et al*., 1986 suggested that the cooking or food processing such as cooking, fermentation, autoclave and milling process can reduce or eliminate the amount of phytic acid by altered the inositol hexaphosphates to other degradation forms e.g., penta, tetra, tri, di and monophosphate (9). Several studies indicated that phytate specially IP5 and IP6 are major inhibitors in cereal foods and strongly inhibit iron and zinc absorption by forming insoluble complexes with iron even under acidic conditions. Therefore, it reduces physiological availability of dietary minerals, particularly phytate/zinc molar ratios or phytate/calcium molar ratios of the diet are a useful predictor of iron and zinc and calcium bioavailability and high ratios have been negatively associated with growth in children (7, 8, 10, 11, 12, 13, 14, 15). The studied by Saha, Weaver and Mason (1994) showed that the absorption of radiolabelled iron on rats decreased significantly when molar ratios of phytate to iron (phytate/iron) were above 14 in wheat-flour based diet (16). Inaddition, Glahn, 2002 demonstrated that maximal inhibition of iron uptake by phytic acid occurred at ratio of 1:10 in an in vitro digestion/caco-2 cell model. A recently study by Fredlund in 2002, also showed that diet formula with a phytate/zinc ratio for 5.7:1 or higher significantly lower zinc absorption in human (17). Moreover, WHO in 1996, categorized phytate:zinc molar ratio into three levels: phytate:zinc molar below 5 are designated as high zinc availability (45-55%); ratio within the range 5-15 as moderate availability (30-35%); and ratio above 15 as low availability (10-15%) (18). However, other hydrolytic formed such as IP1, IP2 and IP3 have less capacity to bind minerals or the complex formed are more soluble, they may be reduced the negative effect on minerals absorption (19, 20). Therefore, the prediction of minerals bioavailability from different foodstuffs or meal might need to know the actual amounts of enhancer (vitamin C) or inhibitors (tannin and phytate) content to improve the quality or bioavailability of their meal particularly in developing countries.

It is widely accepted that ascorbic acid or vitamin C is one of the potent enhancer compounds, which increases rate iron absorption (21). Vitamin C enhances

iron absorption by reducing ferric ion (Fe^{+3}) to ferrous state (Fe^{+2}) that more bioavailability than ferric ion or chelating ferric ion (22). Various investigators demonstrated that the addition of ascorbate-rich vegetable, such as cauliflower to a meal has been showed to increase the absorption of nonheme iron (23-27). Furthermore, ascorbic overcome the inhibitory effect of tannin acid addition to meal (28). However, the heated process might completely destroy not only for anti-nutritional factors but also for vitamin C. Therefore, the information on enhancer and inhibitors content and the effect of cooking process upon their concentration in foods are necessary for predicting the total available of nutrients in a meal, particularly iron, zinc and calcium. Unfortunately there is little information regarding on the phytate and tannin content of raw and cooked Thai vegetables. Therefore, the objective in this study was to determine the effect of conventional cooking (blanching, boiling and stir-frying) on moisture, vitamin C, myo-Inositol phosphates; penta and hexaphosphates (IP5 and IP6) and tannin contents in selected Thai vegetables.

CHAPTER 2
OBJECTIVE

The Objectives in This Study :

General objective:

To determine the effect of blanching, boiling and stir-frying on vitamin C, *myo*-inositol phosphates; penta and hexaphosphates (IP5 and IP6) and tannin contents in selected Thai vegetables.

Specific objectives :

1. To determine the vitamin C, *myo*-inositol penta and hexaphosphates and tannin contents in raw and cooked Thai vegetables.
2. To determine the effect of conventional cooking (blanching, boiling and stir-frying) on vitamin C, *myo*-inositol phosphates; penta and hexaphosphates (IP5 and IP6) and tannin contents in selected Thai vegetables.
3. To determine the effect of fermentation on vitamin C, *myo*-inositol phosphates; penta and hexaphosphates (IP5 and IP6) and tannin contents in mustard green model.

CHAPTER 3
LITERATURE REVIEW

3.1 Iron

Iron deficiency, and more particularly iron deficiency anemia, is generally accepted as being the widest spread nutritional deficiency disease in the world. The World Health Organization (WHO) estimate that 46 and 48 % of children with 5-14 years old and pregnant woman all over the world, respectively, are anemic (29). Iron is an essential micronutrient. As an integral part of hemoglobin, it is required for the transport of oxygen and carbon dioxide in blood. Iron is also a component of several tissue enzymes, such as cytochroms that are critical for energy production, and enzymes involved in the immune system (30).

3.2 Distribution of iron in the body

The body of a healthy adult contains between 3 and 4 g of iron (approximate 50 mg/kg) (31). As present in hemoglobin, it is required for the transport of oxygen and carbon dioxide critical for cell respiration. Iron is also a component of various tissue enzymes, such as the cytochroms that are critical for energy production and enzymes necessary for immune system functioning and stored intracellular via proteins such as ferritin and hemosiderin, which are found in the liver, spleen and bone marrow (32).

3.3 Function of Iron

The main function of iron can be drive into three parts as follow:

(a) Transport and storage of oxygen.

(b) Cofactor of Enzymes and Other Proteins.

(c) Formation of red blood cell.

3.3.1 Transport and storage of Oxygen

Iron (as Fe^{2+}) within the metalloproteins hemoglobin and myoglobin can bind to oxygen molecules. Homoglobin can transport oxygen molecules through the blood while myoglobin store oxygen within muscles (41). Hemoglobin is a multi-subunit protein that comprising four subunits each which one heme group and associate iron ion attached. We can found hemoglobin in the red blood cells. As red blood cells pass through the capillaries of the lungs, oxygen from the lungs becomes bound to the hemoglobin molecules and then the transport their cargo of oxygen-bearing hemoglobin when the tissues require.

Myoglobin is a single-subunit protein that is structure similar to the individual subunits of hemoglobin, myoglobin can found only in the muscle when the body need an immediate supply of oxygen such as during exercise.

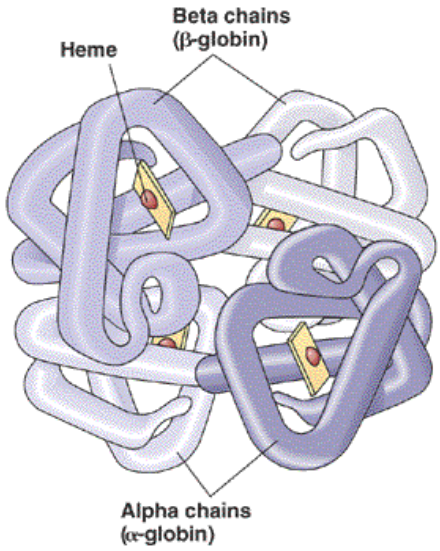


Figure 1. Hemoglobin

Source: Thomson learning. Inc., 2002

3.3.2 Cofactor of Enzymes and other protein

The iron-containing heme group is also a part of several protein related in the release of energy during the oxidation of nutrient and the trapping of that energy with adenosine triphosphate (ATP) (31). And iron also play the role as a cofactor bound to several nonheme enzymes that required for the functioning of cell (32, 33).

For example, conversion of beta carotene to the active form of vitamin A, synthesis of collagen and detoxification of drugs and other toxic compounds in the liver and intestine.

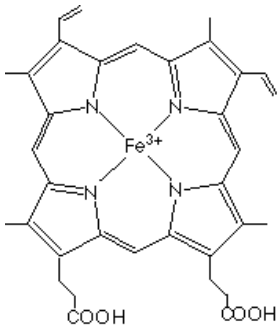
3.3.3 Formation of Red blood cells

Iron is obviously required for the formation of red blood cells because the iron-containing protein hemoglobin is a major component of red blood cells (erythrocytes). The iron is stored as hemosiderin and ferritin in the liver and spleen or is returned to the bone marrow in order to incorporation a new hemoglobin molecule (34).

3.4 Metabolism of Iron

3.4.1 Digestion

Iron present in food in two forms i.e. heme and nonheme iron (35). Nonheme iron in food also present as insoluble ferric ion (Fe^{3+}). Nonheme iron is solubilized by the acid gastric juice and reduced the ferrous state in stomach. There are many components in the small intestine also affect iron bioavailability (40). In the part of heme iron, it is absorbed by the intestinal mucosa in the from of intact heme complex. However, the heme complex largely found in the diet as hemoglobin or myglobin must first be released from the globins prior to absorption. Carpente (1992) show that the release of heme from globin in the deodenum was due to the action of duodenal enzymes, especially trypsin (45).



Heme

Figure 2. Heme

Source: (149)

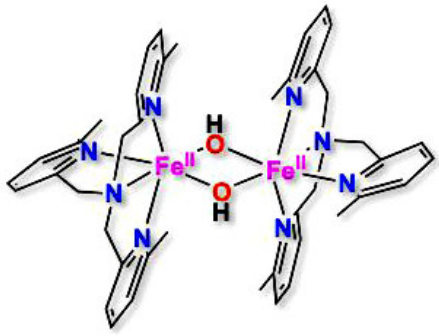


Figure 3. Nonheme

Source: (150)

3.4.2 Absorption

The mainly absorption of iron occurred in the duodenum and jejunum. The absorption of iron from heme and nonheme occurred by different mechanism. Heme iron is released from hemoglobin during digestion in the small intestine. The globin moiety and other protein present in the diet appear, during digestion, to yield residues that inhibit heme polymerization and maintain it in free, absorbable from the heme appears to bind to specific receptor in the luminal intestinal surface. The heme moiety is then catabolized within the mucosal cell by heme oxygenase (35). Nonheme iron is the largest component of dietary iron. Once nonheme food iron enters the alimentary canal, it is acted upon by the gastric juices containing pepsin and hydrochloric acid. Because the acid appears to be important in reducing ferric to ferrous iron. As iron enters the duodenum and pit rises, the ferric iron is rapidly precipitated as ferric oxyhydroxides, whereas the ferrous iron remains relatively soluble as pit rises (36). Uptake in to the mucosal cell and with ferrous salts being better absorbed than ferric salts. However, rate of iron absorption appears to be under controlled by the body's need (35).

Base on a large number of predominantly single-meal studies, food can be classified as having low, intermediate, and high iron bioavailability, as shown in Table 1 (37).

Table 1. Relative bioavailability of nonheme iron in a number of foods

Foods	Low	Intermediate	High
Cereal	Maize	Corn flour	
	Oatmeal	White flour	
	Rice		
	Sorghum		
	Whole wheat flour		
Fruits	Apple	Cantaloupe	Guava
	Avocado	Mango	Lemon
	Banana	Pineapple	Orange
	Grape		Papaw
	Peach		Tomato
	Pear		
	Plum		
	Rhubarb		
Vegetables	Eggplant	Carrot	Beetroot
	Legumes	Potato	Broccoli
	Soy flour		Cabbage
	Isolated soy protein		Cauliflower
			Pumpkin
			Turnip
Beverages	Tea	Red wine	White wine
	Coffee		
Nuts	Almond		
	Brazil		
	Coconut		
	Peanut		
	Walnut		
Animal proteins	Cheese		Fish
	Egg		Meat
	Milk		Poultry

Ref: Bothwell *et al*., 1989

3.4.3 Transportation

Iron is transported by a protein transferrin. Transferrin binds iron that is either released from intestinal epithelium into the blood circulation is secreted from macrophages after the degradation of hemoglobin. And, transferrin distributes iron throughout the body to erythrocyte precursors in the bone marrow for new hemoglobin synthesis (34).

3.4.4 Storage

Most storage iron is held in the liver, bone marrow and spleen, although a small quantity of ferritin occurs within the blood plasma. Storage iron concentration in the body varied depending on gender and iron status (34).

3.4.5 Excretion

Some iron is lost in urine, perspiration, and faces, within dead cells shed from the skin; and in clippings if nails and hair. The greatest loss is in faces. Iron lossed in fecal about 0.7 mg/day. However women must also replace the significant amounts of iron lost in menstruation (35).

3.5 Food Sources of Iron

Liver are the rich source of iron. However, liver have not a popular dietary item and have also high cholesterol content. The iron in eggs is concentrated almost in the yolk, but on 2% of this iron is absorbed by the body. In contrast, iron in meat product is absorbed up to 30%. Other reasonable sources of iron include cereal products and vegetables, but they are poorly absorbed because they contain nonheme iron (34, 38).

3.6 Thai Dietary Reference in Take for Iron

Iron requirement vary throughout an individual’s lifetime. It’s depended on gender, age, nutrition and state of health (39). The estimates of daily amounts of iron have been provided in the Thai Dietary Reference In take for Thais 2003 (DRI) (40), were considered as a reference levels and used as guidelines to determine optimal Iron intake.

Table 2. Thai Dietary Reference Intake (Thai DRI) for Iron Through the life cycle.

Group	**Age**	**DRI (mg/day)**
	(months)	
Infant	0-11	9.3
	(years)	
Children	1-3	5.8
	4-5	6.3
	6-8	8.1
Adolescents		
Boy	9-12	11.8
	13-15	14.0
	16-18	16.6
Girl	9-12	19.1
	13-15	28.2
	16-18	26.4
Adults		
Male	19-70	10.4
	71^{+}	10.4
Female	19-50	24.7
	51-70	9.4
	71^{+}	9.4
Pregnent		60[a]
Lactating		15

[a] Supplement

Ref:Thai RDI 2003

3.7 Iron Bioavailability

Bioavailability is the fraction of the ingested nutrient that utilized for normal physiological function and storage. This definition recognizes that one of the major determinants of bioavailability is that proportion which is absorbed from the gastrointestinal tract (41). The factors of iron absorption have various enhancers and inhibitors.

3.7.1 Enhancers

(a) Meat, Fish, and Poultry

The enhancing effect of meat, fish, and poultry (MFP) iron absorption is well known. MFP contained animal tissue protein that increases the absorption of iron. The enhancing effect to meat on iron absorption, know as the meat factor or meat effect, may be related to the potential ability of sulfhydryl-containing amino acids or peptides to chelate nonheme iron and thereby facilitate intestinal absorption. In vitro studies suggest that sulfhydryl content of meat protein may play a key role in the reduction of Fe^{3+} to more bioavailable Fe^{2+} (42), and in vivo studies in man showed enhanced nonheme iron absorption in the presence of cystein (43, 44).

Martizes (1970) indicated that addition of either fish or an equivalent amount of synthetic amino acid to 100g fish the result show doubled iron absorption from black beans (45). Reddy and cook (1991) show that adding 80g ground beef to a standard meal consisting of bun, french fries, and milk shake doubled iron absorption from 1.96 to 3.90, percent (46).

The effect of meat on iron absorption in adults has mostly been documented with the use of single-meal radioisotope studies the test a single meat dose ranging from 40 to 100g (47, 48). And in an infant study in which the addition of 25 g meat to vegetable meal increased fractional nonheme iron absorption 1.5 fold (49). In 2003, Baech *et al*. showed that small amounts (> 50g) of pork meat significantly increased nonheme iron absorption in a dose dependent manner from meal with a high content of inhibitors and a low content of promoter for iron absorption (50).

(b) Ascorbic Acid

Ascorbic acid is a strong enhancer of nonheme iron absorption. It may promotes food iron absorption by several interdependent mechanisms. First, ascorbic acid may promote acid conditions within dietary iron is efficiently solubilized. Second reducing ferric iron to its better absorption ferrous from. Third, ascorbic acid may forming chelates with iron in stomach and maintaining the solubility of nonheme iron when the food enters the alkaline environment of the small intestine (51). The studied by Reddy (1991), indicated that iron absorption from a semi synthetic meal increased three-fold after adding 75 mg ascorbic acid and four fold after adding 100 mg ascorbic acid (46).

Many studies exhibited that ascorbic acid can improve iron absorption even in the presence of inhibitors such as phytates in cereals and soya, tannins in tea, and calcium. Hamdgoui *et al*. (1990) reported that the addition of 20 mg ascorbic acid in the mixed meal with the tea decoction increased the nonheme iron absorption from the mixed meal by more than 100 % (52). Morever, Siegenberg *et al.* (1991) showed that more than or equal 50 mg ascorbic acid was able to overcome the effects of any meal containing more than 100 mg tannic acid (28). The studies by Brazaca, 2003 also demonstrated that addition of orange juice in legume sample increase iron absorption in in vitro model (53). Martinez, 2004 suggested that ascorbic acid showed significant positive effect on dialyzable iron by influent on pH (54).

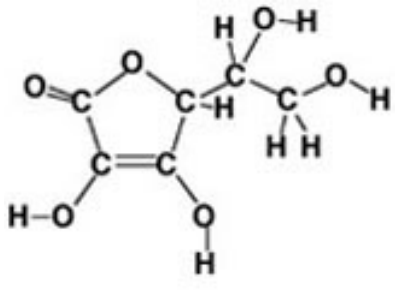


Figure 4. Ascorbic acid (Vitamin C)

Source: (151)

(c) Other

Organic acid such as citric acid, malic acid, tartaric acid, and lactic acid also enhance iron absorption. Some of sugar such as fructose and lactose but not glucose or galactose also have some enhancing effect on iron absorption (55, 56).

3.7.2 Inhibitors

(a) Polyphenols

Phenolic compound is foods originate from one of the main classes of secondary metabolites in plant (57). Chemically, phenolics can be defined as substances processing an aromatic ring bearing one or more hydroxyl substitutents, including their functional derivatives. Phenolic compounds range in size from small monomeric phenolic acids (e.g., gallic acid, caffeic acid) to large polymerized polyphenolic molecules. Vegetable tannins are defined as large polyphenols. Molecular weight (MW) 500-5000, which have the property of precipitation proteins in aqueous media (58). Via this mechanism they might interfere with protein utilization in animal and possibly also in human (59).

The tannins are structurally divided into two subgroups, condensed and hydrolysable tannin (58). The condensed tannin are polymers of flavanols (e.g, catechin), and the hydrolysable tannins contain gallic acid, or related compounds, esterified to a carbohydrate. Tannins are also considered as potent enzyme inhibitors due to interference of iron absorption to form Fe-phenolic complex formation in the gastro-intestinal lumen making the iron less available for absorption. Gillooly *et al.* (1983) found that the addition of a pure phenolic compound (tannic acid) to broccoli meal dramatically reduced iron absorption from 29.7 to 15% in man (22).

Morck *et al.* (1983) studied the consumption of black tea and coffee has been show to strongly inhibit Fe absorption from composite meals (60). And studies by Brune *et al.* (1989) suggested that phenolic compounds processing galloyl groups are mainly responsible for inhibition of iron absorption. They also found a relationship between the content of galloyl groups in foods and the degree of inhibition of iron absorption. However phenolic compounds with at least two adjacent hydroxyl groups, i.e., baring catechol or galloyl groups, may have excellent iron-

binding properties (4). They observed no inhibition of Fe uptake when catechin was added to the test meal. The studied of Tuntawiroon *et al*.(1991) demonstrated that a serving of Yod kratin (leaves of the lead tree, *Leucaena glauca*), a vegetable consumed widely in Thailand and that is high in phenolics, reduced Fe absorption from a composite meal of rice, fish and vegetables by almost 90 % (6).

In addition, Harrel *et al.* (1999) studies the effect of polyphenol-containing beverages on Fe absorption from a bread meal were estimated in adult human subject from the erythrocyte incorporation of radio-Fe. The results showed that all beverages were potent inhibitors of Fe absorption and reduced absorption from the bread meal by 50-70 %, whereas beverages containing 100-400 mg total polyphenols/serving reduced Fe absorption by 60-90 % (3).

In 2001, the studied in young women subjects consumed test meal presence of a phenolic-rich extract from green tea and rosemary resulted in decreased nonheme iron absorption. Iron absorption decreased from 12.1 to 8.9% in the presence of green tea extract and from 7.5 – 6.4 % in the presence of rosemary extract. (61).

Morover, Glahn *et al*. (2002) studied the effect of tannic acid on iron uptake in an in vitro digestion/Caca-2cell. They suggested that Tannic acid was a more potent inhibitor of nonheme iron uptake, as maximal inhibition (97.5%) of iron uptake occurred at a ratio of 1:1 or less (62). In 2004, Afsana *et al*., studied the effect of tannin on iron absorption in rat. The resulted showed that feeding diet containing more than 10 g TA/kg diet reduced the hemoglobin concentration (Hb), hematocrit (Ht) and Fe concentration (63).

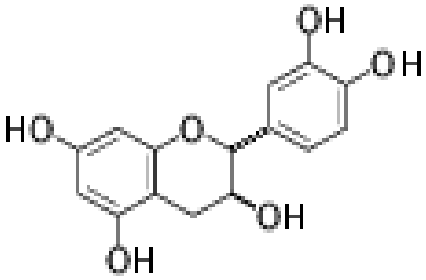


Figure 5. Catechin

Source: (152)

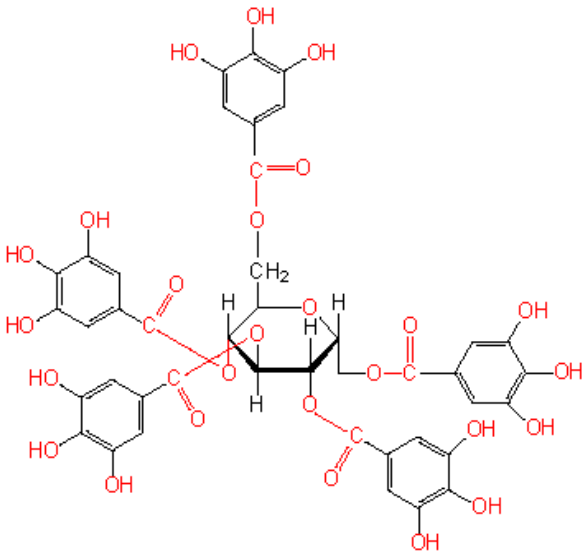


Figure 6. Tannin

Source: (153)

(b) Phytate

Phytic acid is an important constituent of food crops such as cereals, legumes, and oilseeds. The salt from, phytate commonly exists in cereals, legumes, and other crops, where it serves several physiological function, especially seed germination. Phytate is the major storage form of phosphorus and represents more than 80 % of the total phosphorus in cereals, and other seed crops. Phytate was considered as an anti-nutrient because it is a strong chelator of divalent minerals such

as calcium, magnesium, zinc, and iron, and binds with these mineral and decreases their bioavailability (64).

Several in vitro and in vivo studies in animal and human clearly indicate that phytate decrease mineral bioavailability by forming complex with these minerals (65, 66). Many of the phytate mineral complexes are insoluble and, therefore, many became unavailable for absorption under normal physiological conditions. Because phytate are ionic in nature, they also react directly with charged groups of proteins or indirectly with the negatively charged groups of proteins mediated by a positively charged mineral ion such as iron. The resultant phytate-protein and phytate-mineral-protein complexes may also adversely influence protein digestion and bioavailability (67, 68).

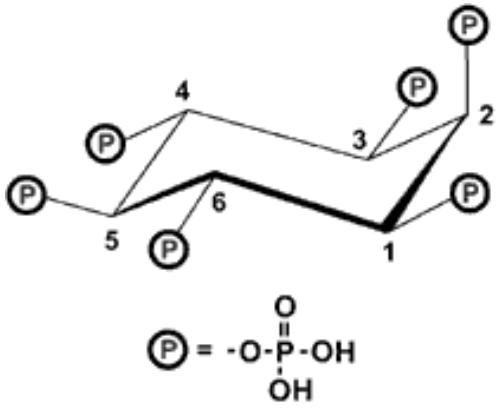


Figure 7. Phytic acid
Source: (154)

(c) Oxalate

Oxalate is salt derived from oxalate acid. Oxalate can combine with irons and change iron into an insoluble form that unavailable for absorption. Oxalic acid is an organic acid found in such foods as spinach, rhubarb and chocolate. The study of Gillooly *et al*. (1983) showed that the addition of 1 g calcium oxalate to cabbage reduced iron absorption by 61 percents, no relationship between oxalic acid content and iron adsorption emerged when three vegetables containing large amounts of oxalate were examined iron absorption was poor from spinach and beet root greens (22).

(d) Calcium

Calcium is metallic element, taken into the body as a constitute of certain foods, especially diary products that are the most efficient source of calcium. Concerning of iron absorption, numerous studies have been shown that calcium, even in small amount, had an inhibitory effect on iron absorption (69, 70).

Hallberg, 1991 showed that calcium had a inhibitory effect on iron absorption. Iron absorption was reduced by 50 – 60 % at doses of 300 - 600 mg Ca (71). And the studies in 1992, Hallberg *el al*. suggested that inhibitory effect of calcium on iron absorption is situated within the intestinal mucosal cells (72).

3.8 Effect of Cooking on Vitamin C

Changes in vitamin content during cooking process have been reported by many studies. Heating is the most common method for preparing food, but vitamin C can be easily destroyed by heating since vitamin C is easily oxidized. Klein, 1982, note that the amount of water used in cooking have significantly reduced vitamin C contents (73). In the fact that vitamin C easily oxidized, particular on exposure to heat and leached in to the cooking water or soaking water (74). In 1988, Fenema suggested that steam blanching might result in less loss if it is followed by cooling that dose not involve water (i.e., air cooling). In addition, the reduction of vitamin C content including leaching of nutrients, exposure to air, the nature of the food, its reaction in the cooking environment (acid or alkaline medium), the time and degree of heating. Moreover, the short of cooking times and small amounts of water are used, more vitamin C will be retained in any cooking methods (75).

3.9 Effect of Cooking on Tannin

Processing normally affects inhibitor of iron such as tannin content, which in turn can enhance or reduce the bioavailability of proteins and mineral (76). The studied of Habiba, 2001 demonstrated that tannin in peas were reduced from 2.06 mg/g in raw seed to 1.53 mg/g in microwave cooked peas and found that pressure cooking brought about a higher reduction (17.5 – 20.9 %) in tannin than that observed by ordinary cooking (12.1-17.5 %) (77). It is common that many vegetables are

cooked by a simple boiling process or microwave process before use. Therefore, cooking process would certainly bring about a number of changes in physical characteristics and chemical composition of vegetables (78). In 1995, Mosha *et al.* indicated that after conventional cooking and microwave blanching methods, the level of tannic acid was significantly reduced after cooking process (79). Racchi, 2002 reported that boiling method significantly reduced antioxidant activity (phenolic compounds) in mushroom juice (80). In addition, Zhang and Hamuzz, 2004 reported that tannic acid or phenolic compound in vegetables show to be heavily lost during prolong cooking time, whereas other processes, such as conventional blanching or stir-frying, percent tannin loss would be lower (81).

3.10 Effect of Cooking on Phytate

Various food processing and preparation methods reduce phytate content. The decrease in phytic acid content by soaking, germination and fermentation is due to the activation of intrinsic phytase (82). The speed of the decrease is dependent on temperature, pH and the occurrence of activators and inhibitors, which vary among plant materials (83).

Phytic acid degraded during boiling and streaming at temperatures around 100 °C, but degradation is greatest in processes in which phytase is activated (84, 85). Decrease of phytic by deep fat frying at about 180 °C and chemical hydrolysis of myo-inositol hexaphosphate to lower ester when autoclaving (121 °C, 60 min) (86, 87) illustrates the heat instability of phytic acid at higher temperatures. Significant decreases in phytic acid may also be due to discarding leached soluble phytates with cooking water (88). Kingsley found that the content of phytates were 43 % lower in cooked African oil beans than in raw beans (11.2 and 6.4 g/kg dry matter, respectively) (89). Decrease in phytates after cooking broad beans, butter beans and lentils were from 0.96 to 0.48, 1.5 to 1.33 and 0.86 to 0.34 %, respectively (90). Lease and de Boland *et al.* (91, 92), who studied the effect of autoclaving on phytate content, suggest that the rate of phytate destruction is low when it is associated with the protein and/or cations in natural products. Toma and Tabekhia similarly noted that cooking rice in domestic tap water resulted in no significant loss of phytic acid, whereas cook it in distilled deionised water reduces the phytic acid content by two-thirds (93). This

different can be attributable to the ability of phytic acid to form salt complexes. In addition, Elhardallou and Walker proposed that the smaller decrease of phytate content found in cooked broad beans, compare to butter beans and lentils, was due to the formation of relatively large amount of insoluble phytate complexes (90).

In maize, the loss of phytic acid during thermal treatment at a higher moisture content results in a greater loss than that under a similar dry thermal treatment (94). Mosha *et al.* showed that both conventional and microwave blanching of vegetables resulted in a significant reduction of phytic acid (79). Using a conventional 10 min blanching method, phytic acid contents were reduced from 1.01 to 0.27, 3.97 to 1.04, 2.16 to 1.0, 0.36 to 0.12 and from 0.33 to 0.18 mg/100g fresh weight in cabbage, collard, turnip, sweet potato and peanut greens, respectively, by an HPLC method. Lower decreases were observed for shorter blanching times. Microwave blanching caused reduction on the same level. It was concluded that the decrease in phytic acid content could be partly due to leaching (88, 79).

Cooking autoclaving and extrusion cooking have been shown to result in significant phytate losses. Bishnoi *et al.* found that soaking, pressure cooking, and especially pressure cooking of soaked as well as soaked-dehulled peas, decreased phytic acid contents. Proportional losses in the latter case ranged from 41 to 51 % (95). In extrusion cooking about 25 % of phytic acid is hydrolysed into penta- and tetraphosphates (15, 96). Within the studied processes, losses were highest during germination. Khalil and Mansour found germination to be more effective way to decrease phytic acid content of faba beans (decrease 54 %) than either cooking (decrease 31 %) or autoclaveing (lower 41 %), determined by the method of Wheeler and Ferrel (97, 98).

Fermentation of food changes or creates unique flavours, changed textural properties and improves nutritional quality and digestibility. Kingley (89) found that the concentration of phytates was 76 % lower in fermented African oil beans than in raw beans (11.2 *vs* 2.7 g/kg dry matter, respectively). In some traditional Indian fermented foods (fermented and steamed dhokla) almost all phytic acid may be hydrolyzed, although in most foods 50% or less of the phytate remains (99)

3.11 Method for Analysis of Phytate

In the early, the quantitative analysis of phytate (InsP6) was based on precipitation with ferric chloride, or purification using anion-exchange chromatography. A disadvantage of these methods is the lack of specificity in distinguishing between InsP6 and its degradation products. Because inositolphosphates with three to five phosphate groups ($InsP_3$-$InsP_5$) as well as $InsP_6$ have been shown to be nutritionally significant (100-104). It is importance to have a reliable method for the determination of the individual inositol phosphates. The development of ion-pain HPLC procedures and capillary electro migration methods became possible to study $InsP_6$ and some of its hydrolysis products during food processing and digestion.

3.11.1 Precipitation Method

Precipitation methods are based on the principle that InsP6 forms an insoluble stable complex with ferric ion in dilute acid and, presumably, is the only phosphate compound with that property (105). The phosphorus content in the precipitate can be determined after wet ashing or hydrolysis, giving a direct measure of the $InsP_6$ content. A certain ratio between iron and InsP6 is required for quantitative precipitation. In direct methods, stoichiometric relationship between $InsP_6$ and unprecipitated ferric ions is determined. However, the precision in this method may be impaired by the presence of lower inositol phosphates that also complex with iron. Other compounds able to bind to iron, such as polyphenols, are also coprecipitated (106).This methods, furthermore, have a low sensitivity and can thereby not detect low amounts of $InsP_6$ (107).

3.11.2 Ion Exchange Methods

This method simplicity and low cost, ion exchange chromatography is often used for processing a large number of samples, though it is not a rapid procedure. The InsP6 value from the iron-precipitation method was indicated to be higher than values from ion exchange method. However, contamination of InsP6 with the lower inositol phosphates and nucleotides seemed to interfere with this method

(108, 109). In fact, measurements of $InsP_6$, according to AOAC, were found to correlate to the sum of $InsP_3$, $InsP_4$, $InsP_5$ and $InsP_6$ determined by HPLC ion-pair chromatography, suggesting that these inositol phosphates were included in InsP6 determination by the AOAC method (64). Another disadvantage of the method it does not detect low level of $InsP_6$.

3.11.3 HPLC method

A number of high-performance liquid chromatography (HPLC) techniques have been developed for the analysis of InsP6 in foods (130-132). These methods separate InsP6 from inositol by using reversed phase octadecyl (C-18) stationary phases and aqueous potassium dihydrogen phosphate or sodium acetate mobile phases. A disadvantage of these and other contemporary procedures is elution of $InsP_6$ on the solvent front. InsP6 is only weakly retained on the column resulting in extremely poor solution.

To purify samples prior to analyses by HPLC, a strong anion exchange resin was used (76, 77) after extraction with hydrochloric acid (HCl). The main advantages of this rather time-consuming step are effective concentration of inositol phosphates, removal of inorganic phosphate, and elution of most impurities. HCl is removed by evaporating of extract to dryness. Sandberg & Anderinne, 1986, Lehrfeld, 1989 recommended that ion-exchange liquid chromatography and ion-pair HPLC methods are shown to be suitable and accurately for analysis of various inositol phosphates forms (IP1 to IP6) that are important for nutritional studies (64).

CHAPTER 4
MATERIALS AND METHODS

4.1 Samples Collection

Selected vegetables in this study were selected base on the Fourth National Nutrition Survey of Thailand in 1995, which indicated vegetable commonly consumed in Thailand as shown in Table 3 (113-115). They were freshly purchased from five representative markets in Bangkok area during January to December, 2003 as show in Table 4. At each market, 0.5–3.0 kg samples were bought from three representative outlets.

Table 3 Vegetables commonly consumed in Thailand[1,2]

English name	Thai name	Scientific name	Cooking method
Leaves and tender tips vegetables:			
Acacia pennata, leaves and tender tips	Cha-oom	*Acacia insunavis*	Blanching
Bitter cucumber, leaves and tender tips	Yod-ma-ra	*Monordica charantia* Linn.	Blanching, Boiling, Stir-frying
Cassia, leaves and tender tips	Kee-lhek	*Cassia siamea* Britt.	Boiling
Indian pennywort, leaves	Bai-bao-bok	*Centella asiatica (L.)Urb.*	Blanching

Table 3 Vegetables commonly consumed in Thailand[1,2] (*Continued*)

Food item	Thai name	Scientific name	Cooking method
Leaves and tender tips vegetables: Leadtree, leaves and tender tips	Kra-tin	*Acacia farnesiana* Willd.	Blanching
Mustard greens, leaves	Pak-kard-kheaw	*Brassica Juncea Czern. & Cross*	Fermentation
Neem tree, leaves and tender tips	Sa-dao	*Azdirachta indica* A.	Blanching
Paco (Oke fern), leaves and tender tips	Pak-good	*Asystasiella neesiana Lindau.*	Blanching, Stir-frying
Pagwanban**,** leaves and tender tips	Pak-wan-ban	*Sauropus andogynus (L) Merr.*	Blanching, Boiling, Stir-frying
Pagwanpa, leaves and tender tips	Pak-wan-pa	*Melientha suavis* Pierre.	Blanching, Boiling, Stir-frying
Paksien, pickled	Pak-sein	*Cleom gynandra Linn.*	Fermentation
Sesbania, leaves and tender tips	Yod-care	*Sesbania granddiflora (L.) Poir.*	Blanching, Boiling

Table 3 Vegetables commonly consumed in Thailand[1,2] (*Continued*)

English name	Thai name	Scientific name	Cooking method
Flower vegetables:			
Banana, flower	Hua-plee	*Musa sapientum Linn.*	Boiling
Cowslip creeper, flower	Dok-kha-jorn	*Telosma minor Craib*	Blanching, Boiling, Stir-frying
Sesbania, yellow, flower	Dok-sa-noh	*Sesbania javanica Miq.*	Blanching, Boiling, Stir-frying

[1]Food Composition Tables

[2]The fourth National Nutrition Survey of Thailand by the Nutrition Division, Department of Health, Ministry of Public Health, Thailand, 1992 and 1995, respectively

Table 4 Name of five local markets in Bangkok where the food samples were purchased

Local Markets	District
Huay khwang	Huay khwang
Salanamron	Bangkkok-noi
Saphan khwai	Chatuchak
See yan	Bang chue
Thonburi	Bang plad

4.1.2 Sample Preparation

All samples were freshly bought from five representative markets in Bangkok area. At each market, 1-3 kg samples purchased from three representative outlets and pooled to obtain one sample per each and immediately analyzed for moisture and vitamin C on the same day.

Before analysis, all vegetables were washed with tap water several times to remove adhering slime. They were then prepared individually using common household practice. The edible parts of each sample were separated and washed again with deionized water and drained on stainless steel sieve until dry. Each sample was then divided into four portions. One portion was analyzed as a fresh sample and other portion was prepared and cooked by either boiling, blanching or stir-frying according to common household methods. The samples, fresh or cooked were individually homogenized in an electrical blender (Moulinex Master Chef model 375).

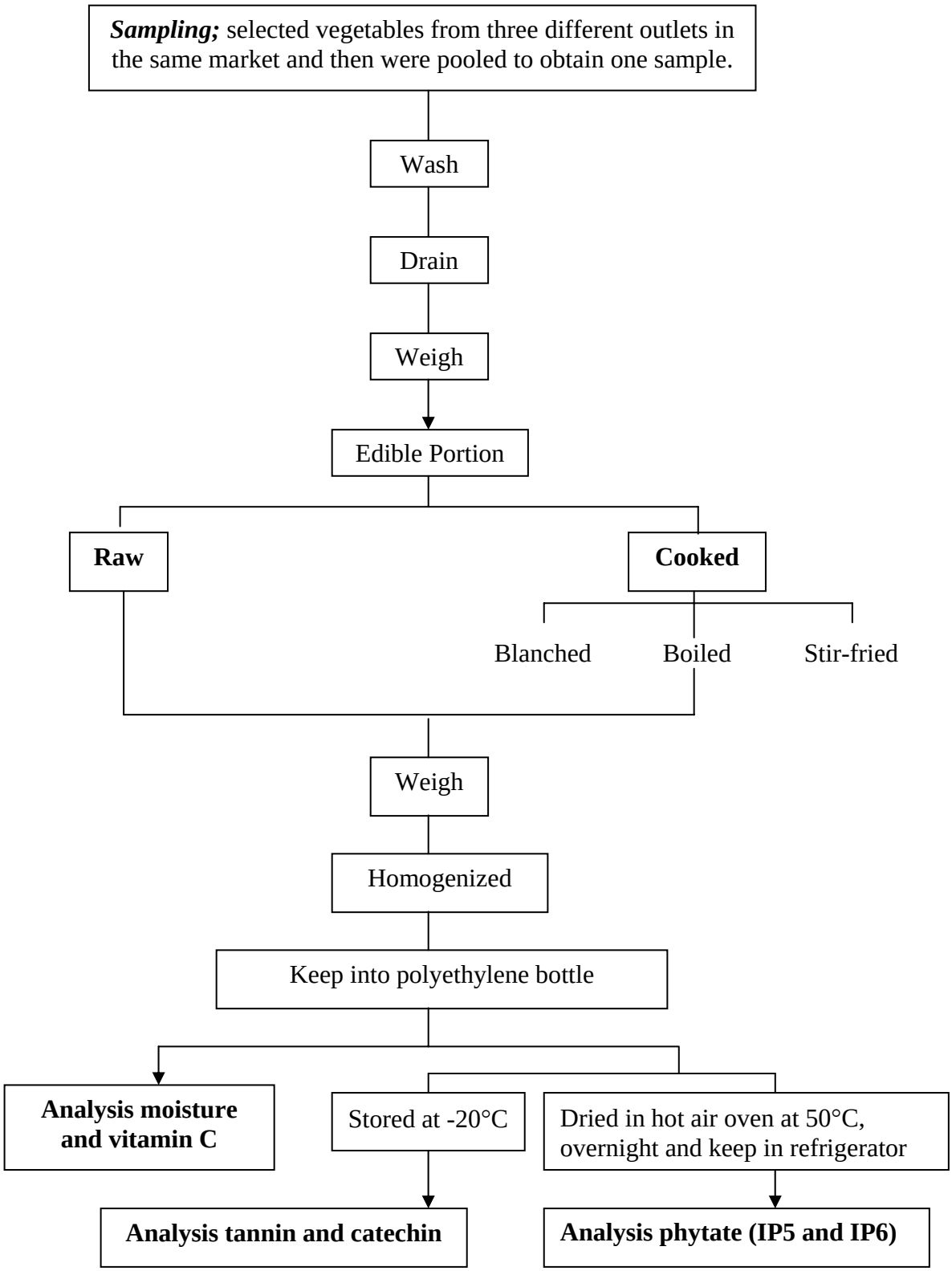


Figure 8 Schematic picture of sample preparation

4.1.3 Cooking Method

After the samples were separated for cooked portions, they were then cooked under method household habits. Blanching, Boiling and stir-frying methods were suggested that commonly used for preparing vegetable in household cooking (116).

(a) Blanching Method

Approximated 100 grams of edible portion of vegetables were immersed in aluminum containers that contain 500 ml of boiling deionized water for 1 to 3 minutes (depended on the nature of each sample). Then the samples were placed on the sieve until cool at room temperature and weight.

(b) Boiling Method

Approximated 100 grams of edible portion of vegetables were put on aluminum containers that contain 500 ml of boiling deionized water and then cover with a lid until the samples become tender (3 to 5 minutes depended on the nature of each sample). Then the samples were placed on the sieve until cool at room temperature and weight.

(c) Stir-frying Method

Approximated 100 grams of edible portion of vegetables were heated 5 g of soybean oil and continuously stir until crisp-tender. Times for cooking were depended on the nature of each sample (Approximately 1-5 minutes). Then samples were placed on the dish and allowed to cool before weight.

(d) Fermentation Method

Fresh vegetables were cleaned, trimmed, mixed with salt and then dehydrated under shad for 5-6 h. The vegetables were then squeezed and packed in the clay jar that contains boiled water. Add salt and palm sugar and closed the jar in order to set condition, natural lactic acid fermentation (117). The fermentation took about 4 days. The pickles were kept and prepared for all chemical analysis.

4.2 Analytical Method

All vegetables were analyzed in duplicate for moisture, vitamin C, tannin, catechin and phytate (IP5 and IP6) contents.

4.2.1 Moisture Determination

Moisture content of sample was determined by drying the sample in hot air oven at 100±5 °C until constant weigh was obtained (AOAC, 1990; 925.45). As seen in Appendix 3 (118).

4.2.2 Vitamin C Determination

Vitamin C content in the vegetables was extracted with metaphosphoric acid and determined by fluorescence spectrometer (AOAC, 2000; 967.22). As seen in Appendix 4 (119).

4.2.3 Tannin and Catechin Assay

Tannin and catechin content were determined by extracting the tannin and catechin with 50 % dimethylformamide (DMF) in acetate buffer and read the absorbance at 578 and 680 nm by Spectrophotometer as according to the method of Brune *et al*., 1991. The calculation of tannin or catechin equivalent was used linear regression equations for the standard curves. As seen in Appendix 5 (120).

4.2.4 Phytate (IP5 and IP6)

The phytate (IP5 and IP6) contents in all samples were determined by following the Appendix 6. Samples were extracted by hydrochloric acid with appropriate ratio as according to the method of Hotz *et al*., 2001 (121). Refrective index detector (RI) was used in HPLC system for analysis of phytate (IP5 and IP6) content in the samples.

4.2.5 Calculation of True Retention and Dry Basis Content for Total Vitamin C, Moisture, Tannin, Catechin and Phytate (IP5 and IP6) Contents in Selected Vegetables (TR%)

Percent true retention was calculate by the following equation of Murphy, *et al*., 1975 (122).

$$\% \text{ True retention (TR)} = \frac{\text{nutrient content per g of cooked food x g of food after cooked x100}}{\text{nutrient content per g of raw food x g of raw meat}}$$

$$\% \text{ Loss nutrient} = 100 - TR$$

$$\text{Nutrient (mg/100g of dry basis)} = \frac{\text{Nutrient (µg/g of wet basis)}}{100 - \% \text{ moisture}} \times 100$$

4.2.6 Identification the level of Vitamin C, Tannin and Phytate (IP5 and IP6) Contents in Selected Vegetables

Criteria for identification the level of vitamin C, tannin and phytate (IP5 and IP6) contents were based on the Quartile deviation. The data of vitamin C, tannin and phytate (IP5 and IP6) contents were divided into three levels; high medium and low level by using SPSS for Windows version 10.0 software**.**

4.3 Quality Control System

In-house control materials were used for the quality control of analysis data. Tang® natural fresh orange was used as control material for vitamin C. Homogenized sample of rice (Chao Hom Nil)) was prepared as an in-house control sample for tannin. Soybean flour used as an in-house control sample for phytate (IP5 and IP6). They were kept in refrigerator at 8°C and used as a quality control sample for determination in this study. The assigned value was developed from 10 values of vitamin c, tannin, catechin and phytate (IP5 and IP6) contents obtain from single sample analysis on three different days. The sample was analyzed for vitamin c, tannin, catechin and phytate (IP5 and IP6) contents in each unknown samples. A quality control chart maintained the quality of analysis. The accepted value was within mean $\pm$ 2 SD.

4.4 Statistical Analysis

Reference intervals of mean value including standard deviation (SD) were used to represent total vitamin C, moisture, tannin, catechin and phytate (IP5 and IP6) contents in this study. For identify effect of conventional cooking method on phytate, tannin, catechin and vitamin C contents were computed by the One-way ANOVA with Scheff's test and independent t-test. Using SPSS for Windows version 10.0 software performed all computations.

CHAPTER 5
RESULTS

5.1 Moisture Content Analysis

The moisture contents of fresh and cooked vegetables are showed in Table 5. The results showed that moisture of raw and cooked leaves and tender tips vegetables ranged from 75.01 to 91.95 % in raw and 70.99 to 92.99 % in cooked vegetables respectively while the moisture content of raw and cooked flower vegetables ranged from 88.13 to 91.95 % and 78.02 to 94.16 % respectively. The results for the effect of conventional, boiled, blanched or stir-fried on the content of moisture as showed in the Table 5. In conventional boiling or blanching methods, the moisture contents in all cooked vegetables seem to be unchanged, whereas the vegetables which were cooked by stir–frying had more decrease in moisture content than other methods.

Table 5 Moisture content in raw and cooked selected vegetables[12]

Food item	**Cooking method**	**Time (min)**	**% Moisture content *(n=5)***
Leaves and tender tips:			
Acacia pennata,	Raw		80.24 ± 3.03^{a}
leaves and tender tips	Blanching	2	87.49 ± 1.29^{b}
Bitter cucumber,	Raw		91.40 ± 1.33^{a}
leaves and tender tips	Blanching	1	92.46 ± 1.36^{a}
	Boiling	4	92.99 ± 0.98^{a}
	Stir-frying	3	83.52 ± 4.03^{b}
Cassia,	Raw		76.59 ± 1.62^{a}
leaves and tender tips	Boiling	4	87.05 ± 0.63^{b}

Table 5 Moisture content in raw and cooked selected vegetables[12] *(continued)*

Food item	Cooking method	Time (min)	% Moisture content *(n=5)*
Leaves and tender tips:			
Indian pennywort,	Raw		87.87 ± 1.75^{a}
leaves	Blanching		90.86 ± 1.74^{b}
Leadtree,	Raw	1	75.88 ± 3.30^{a}
leaves and tender tips	Blanching		78.87 ± 3.29^{a}
Neem tree,	Raw	1	75.01 ± 1.49^{a}
leaves and tender tips	Blanching		81.67 ± 0.77^{a}
Paco (Oke fern),	Raw	2	89.28 ± 0.96^{a}
leaves and tender tips	Blanching		90.95 ± 0.61^{a}
	Stir-frying	1	80.31 ± 2.99^{b}
Pagwanban,	Raw		82.72 ± 3.35^{a}
leaves and tender tips	Blanching	2	89.32 ± 1.68^{b}
	Boiling	4	89.84 ± 0.96^{b}
	Stir-frying	3	70.99 ± 2.90^{c}
Pagwanpa,	Raw		80.75 ± 3.07^{a}
leaves and tender tips	Blanching	1	84.75 ± 1.00^{ab}
	Boiling	2	85.30 ± 0.80^{b}
	Stir-frying	1	72.25 ± 3.12^{c}
Paksien, pickled	Fermentation	2	88.86 ± 0.97
Sesbania,	Raw		79.20 ± 2.76^{a}
leaves and tender tips	Blanching	2	86.35 ± 1.84^{b}
	Boiling	5	86.63 ± 1.70^{b}

Table 5 Moisture content in raw and cooked selected vegetables[12]*(continued)*

Food item	Cooking method	Time (min)	% Moisture content *(n=5)*
Flower:			
Banana, flower	Raw		91.95 ± 0.22[a]
	Boiling	5	94.16 ± 0.10[a]
Cowslip creeper, flower	Raw		88.67 ± 0.80[a]
	Blanching	3	91.92 ± 0.31[b]
	Boiling	5	92.06 ± 0.21[b]
	Stir-frying	5	79.72 ± 1.23[c]
Sesbania, yellow, flower	Raw		88.13 ± 1.02[a]
	Blanching	1	92.71 ± 0.47[b]
	Boiling	3	92.99 ± 0.34[b]
	Stir-frying	2	78.02 ± 1.38[c]

[1]Values are shown in mean ± SD of duplicate analysis.

[2]Values within the same column with different superscripts are significant difference by one-way ANOVA with Sheffe's test and independent t-test at $p < 0.05$

5.2 Vitamin C Content Analysis

The vitamin C content of raw and cooked in selected vegetables were shown in Table 6 and Figure 9. Wide variation of vitamin C contents were found in raw and cooked vegetables of this study which ranging from 0.50 to 85.59 mg/100g for raw and 0.22 to 46.48 mg/100g for cooked samples, respectively. When the Quartile deviation was used to set the concentration of vitamin C into three levels, particularly in high (more than 71.77 mg/100g), medium (9.64-71.77 mg/100g) and in low level (less than 9.64 mg/100g). The results showed that high concentrations of uncooked all vegetables were found in pagwanpa, pagwanban and sesbania (72.4-85.59 mg/100g of edible portion). Meduim level was found in neem tree, cowslip creeper, cassia, acasia pennata, paksien, pickled, indian pennywort, sesbania, flower, and leadtree which ranging from 10.77–71.53 mg/100g of edible portion as showed in Table 5. Low level of vitamin C was found in bitter cucumber (6.23 mg/100g of edible portion), paco (4.15 mg/100g of edible portion) and banana, flower (0.50 mg/100g of edible portion). Comparison on the vitamin C content among uncooked vegetable found that stir-fried pagwanpa, pagwanban and cowslip creeper, flower are good source of vitamin C content (64.42–70.80 mg/100g) when compared to the other items.

The effects of conventional cooking methods on vitamin C in selected vegetables are shown in Table 6. Cooking resulted in reducing vitamin C content. This reducing varied according to cooking method and time of exposure. The greatest decreasing (23.93–94.64 %) of vitamin C loss was observed in boiling method whereas in blanching and stir-frying was found from 14.42-93.71 % (Figure 9).

Table 6 Effect of cooking on the Vitamin C content of selected vegetables [1,2,3]

Food item	Cooking method	Time (min)	Vitamin C content (mg/100g wet weight)	Vitamin C content (mg/100g dry weight)	% retention Vitamin C	% Vitamin C loss
leaves and tender tips:						
Acacia pennata,	Raw		56.54 ±11.17[a]	292.41 ± 73.05[a]		
leaves and tender tips	Blanching	2	12.34 ± 2.85[b]	98.60 ± 19.54[b]	22.15 ± 4.97	77.85 ± 4.97
Bittercucumber,	Raw		6.23 ± 2.05 [a]	72.52 ± 22.30[a]		
leaves and tender tips	Blanching	1	1.13 ± 0.75[b]	14.73 ± 9.55[b]	18.04 ± 6.60[a]	81.96 ± 6.60[a]
	Boiling	4	0.34 ± 0.35[b]	4.66 ± 4.10[b]	5.36 ± 4.38[b]	94.64 ± 4.38[b]
	Stir-frying	3	1.66 ± 1.09[b]	9.57 ± 3.72[b]	22.57 ± 8.40[a]	77.43 ± 8.40[a]
Cassia,	Raw		62.01 ± 10.14[a]	264.18 ± 34.91[a]		
leaves and tender tips	Boiling	4	16.90 ± 5.93[b]	129.61 ± 41.55[b]	33.41 ± 11.59	66.59 ± 11.59
Indian pennywort,	Raw		22.83 ± 15.07[a]	182.08 ± 95.55[a]		
leaves	Blanching	1	11.42 ± 7.53[a]	120.82 ± 58.97[a]	50.81 ± 18.42	49.19 ± 18.42
Leadtree,	Raw		10.77 ± 3.88[a]	46.82 ± 21.32[a]		
leaves and tender tips	Blanching	1	5.37 ± 1.94[b]	26.97 ± 12.64[a]	53.23 ± 17.20	46.77 ± 17.20
Neem tree,	Raw		71.53 ± 1.07[a]	287.27 ± 21.56[a]		
leaves and tender tips	Blanching	2	46.48 ± 1.95[b]	253.77 ± 9.22[b]	77.70 ± 4.42	22.30 ± 4.42

Table 6 Effect of cooking on the Vitamin C content of selected vegetables [1,2,3] *(continued)*

Food item	Cooking method	Time (min)	Vitamin C content (mg/100g wet weight)	Vitamin C content (mg/100g dry weight)	% retention Vitamin C	% Vitamin C loss
leaves and tender tips:						
Paco (Oke fern),	Raw		4.15 ± 1.93[a]	39.74 ± 21.15[a]		
leaves and tender tips	Blanching	1	2.49 ± 1.41[ab]	27.09 ± 14.48[ab]	63.61 ± 14.10[a]	36.39 ± 14.10[a]
	Stir-frying	2	0.36 ± 0.41[b]	1.97 ± 2.33[b]	6.29 ± 5.26[b]	93.71 ± 5.26[b]
Pagwanban,	Raw		78.46 ± 31.90[a]	441.42 ± 102.12[a]		
leaves and tender tips	Blanching	2	12.23 ± 5.59[b]	115.41 ± 50.18[bc]	21.51 ± 10.33[a]	78.49 ± 10.33[a]
	Boiling	4	10.26 ± 4.66[b]	101.21 ± 43.52[b]	18.56 ± 8.45[a]	81.44 ± 8.45[a]
	Stir-frying	3	70.80 ± 19.80[a]	244.34 ± 68.89[c]	82.87 ±19.76[b]	17.13 ±19.76[b]
Pagwanpa,	Raw		83.59 ± 27.93[a]	426.78 ± 101.16[a]		
leaves and tender tips	Blanching	1	43.88 ± 10.91[ab]	287.12 ± 67.33[ab]	56.87 ± 5.96[ab]	43.13 ± 5.96[ab]
	Boiling	2	34.12 ± 9.25[b]	231.78 ± 61.68[b]	43.63 ± 6.75[a]	56.37 ± 6.75[a]
	Stir-frying	1	67.88 ± 37.60[ab]	236.69 ± 115.97[b]	70.59 ±23.46[b]	29.41 ±23.46[b]
Paksien, pickled	Fermentation		41.27 ± 4.73	372.90 ± 52.82		

Table 6 Effect of cooking on the Vitamin C content of selected vegetables [1,2,3] *(continued)*

Food item	Cooking method	Time (min)	Vitamin C content (mg/100g wet weight)	Vitamin C content (mg/100g dry weight)	% retention Vitamin C	% Vitamin C loss
Sesbania,	Raw		72.48 ± 35.23[a]	340.01 ± 150.06[a]		
leaves and tender tips	Blanching	2	24.26 ± 11.18[b]	174.95 ± 76.46[ab]	42.11 ± 8.34[a]	57.89 ± 8.34[a]
	Boiling	5	16.93 ± 7.78[b]	123.45 ± 53.75[b]	30.39 ± 10.68[a]	69.61 ± 10.68[a]
flower:						
Banana,	Raw		0.50 ± 0.29[a]	6.34 ± 3.76[a]		
flower	Boiling	5	0.22 ± 0.11[a]	3.78 ± 1.91[a]	50.36 ± 14.05	49.64 ± 14.05
Cowslip creeper,	Raw		66.29 ± 16.09[a]	586.92 ± 149.14[a]		
flower	Blanching	3	43.95 ± 11.27[a]	544.77 ± 143.10[ab]	81.98 ± 7.05[ab]	18.02 ± 7.05[ab]
	Boiling	5	40.93 ± 10.78[a]	515.02 ± 134.46[ab]	76.04 ± 5.16[a]	23.93 ± 5.16[a]
	Stir-frying	5	64.42 ± 13.31[a]	316.87 ± 59.61[b]	85.58 ± 3.35[b]	14.42 ± 3.35[b]
Sesbania, yellow,	Raw		14.87 ± 3.57[a]	124.32 ± 23.41[a]		
flower	Blanching	1	3.84 ± 1.87[b]	52.71 ± 25.17[b]	35.58±11.65[ab]	64.42±11.65[ab]
	Boiling	3	2.64 ± 1.27[b]	37.45 ± 17.52[b]	23.71 ± 7.49[a]	76.29 ± 7.49[a]
	Stir-frying	2	8.00 ± 3.14[ab]	36.70 ± 15.50[b]	49.37 ± 9.01[b]	50.63 ± 9.01[b]

[1]Data reported in mean + SD of duplicate analysis, n=5

[2]Values within the same column with different superscripts are significant difference by one-way ANOVA with Sheffe's test and independent t-test at $p < 0.05$.

[3]Median and range values are reported in Appendix A

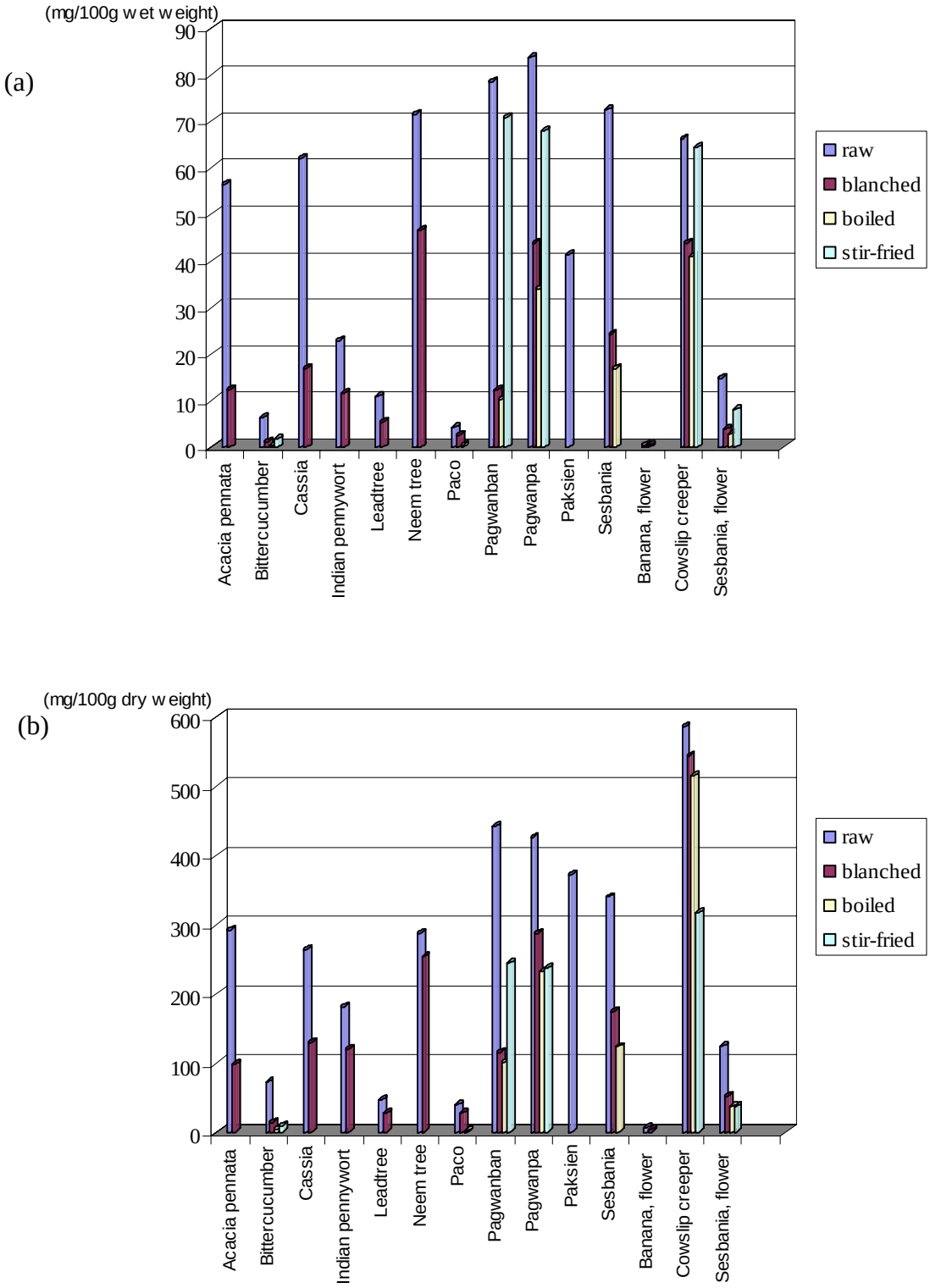


Figure 9 Effect of conventional cooking on vitamin C content in selected vegetables mg/100g wet weight (a) and dry weight (b)

5.3 Tannin Content Analysis

The tannin contents of raw and cooked in selected vegetables are shown in Table 7 and Figure 10. Analysis data from Table 7 showed that the values of tannin content in leaves and tender tips vegetables were higher than other item. The tannin content of selected vegetables ranged from 6.69 to 1353.22 mg/100g for uncooked and 1.80 to 800 mg/100g for cooked samples. Base on Quartile deviation, the levels of tannin content are divided into three levels. The high level of tannin was set to more than 404.95 mg/100g, medium range from 12.06-404.95 mg/100g and low its less than 12.06 mg/100g. The highest amount of tannin concentration was found in leafy and tips vegetables particularly leadtree, neem tree, and cassia which ranging from 443.15-1353.22 mg/100g of edible portion. Medium level was found in paco (392.21 mg/100g of edible portion), banana, flower (295.74 mg/100g of edible portion.), sesbania (62.51 mg/100g of edible portion), pagwanpa (53.16 mg/100g of edible portion), sesbania, flower (49.57 mg/100g of edible portion), acacia penata (32.86 mg/100g of edible portion), pagwanban (30.25 mg/100g of edible portion mg/100g of edible portion) and paksien, pickled (13.26 mg/100g of edible portion). Low level of tannin was found in cowslip creeper (8.46 mg/100g of edible portion), indian pennywort (6.69 mg/100g of edible portion) and bittercucumber (3.14 mg/100g of edible portion.). Consideration to the tannin content among the flower vegetables in this study showed that banana, flower had the greatest tannin content which approximately 295.74$\pm$20.62 mg/100g of edible portion, while the highest tannin content in leafy and tips vegetable items was found in leadtree (1353.22$\pm$526.70 mg/100g of edible portion). Among the cooking method, the results showed after cooking almost all vegetables were not significantly reduced in amount of tannin values, except, the tannin contents in neem tree and paco was significantly reduced by heat-treated process at $p < 0.05$.

Considering on the percentage of tannin loss between before and cooking condition in all vegetables are showed in Table 7. Tannin in raw all vegetables was ranged from 3.14 to 1353.22 mg/100 g of edible portion. Cooking results in reducing its content varied from 8.40 to 55.69 %. When three types of conventional cooking procedures were compared for their effect on the tannin content. The percentage of tannin loss in boiled and blanched were 22.77 to 55.28 % and 6.88-55.41 % higher

than in stir-fried (8.40 to 26.01%), except stir-fried leaves and tender tips vegetables; Paco (Oke fern) was the greatest tannin loss around 55.69 % as showed in Table 6. However, the reduction values were not significant difference among three types of cooking procedures at $p > 0.05$ (Table 7 and Figure 10).

Table 7 Effect of cooking on the Tannin content of selected vegetables [1,2,3,4]

Food item	Cooking method	Time (min)	Tannin content (mg/100g wet weight)	Tannin content (mg/100g dry weight)	% retention Tannin	% Tannin loss
leaves and tips:						
Acacia pennata,	Raw		32.86 ± 14.21[a]	171.03 ± 80.09[a]		
leaves and tender tips	Blanching	2	13.27 ± 2.65 [b]	107.34 ± 26.13[a]	44.59 ± 14.88	55.41 ± 14.88
Bittercucumber,	Raw		3.14 ± 1.11[a]	37.47 ± 16.41[a]		
leaves and tender tips	Blanching	1	2.06 ± 0.72[a]	27.53 ± 10.05[a]	70.68 ± 5.57[a]	29.32 ± 5.57[a]
	Boiling	4	1.80 ± 0.67[a]	26.12 ± 10.66[a]	63.45 ± 8.30[a]	36.55 ± 8.30[a]
	Stir-frying	3	3.08 ± 1.14[a]	20.86 ± 8.03[a]	85.54 ±11.05[b]	14.46 ± 11.0[b]
Cassia,	Raw		443.15 ± 31.01[a]	1905.35 ± 250.73[a]		
leaves and tender tips	Boiling	4	195.74 ± 70.91[b]	1506.22 ± 529.37[a]	54.95 ± 21.82	45.05 ± 21.82
Indian pennywort,	Raw		6.69 ± 2.77[a]	55.93 ± 22.94[a]		
leaves	Blanching	1	3.49 ± 1.43[a]	39.39 ± 16.73[a]	61.97 ± 9.74	38.03 ± 9.74
Leadtree,	Raw		1353.22 ± 526.70[a]	5703.07 ± 2514.19[a]		
leaves and tender tips	Blanching	1	679.22 ± 261.68[b]	3284.71 ± 1469.86[a]	53.53 ± 17.47	46.47 ± 17.47
Neem tree,	Raw		723.47 ± 214.09[a]	3172.53 ± 755.91[a]		
leaves and tender tips	Blanching	2	337.58 ± 130.47[b]	1832.81 ± 708.96[b]	54.88 ± 12.78	45.12 ± 12.78

Table 7 Effect of cooking on the Tannin content of selected vegetables[1,2,3,4] *(Continued)*

Food item	Cooking method	Time (min)	Tannin content (mg/100g wet weight)	Tannin content (mg/100g dry weight)	% retention Tannin	% Tannin loss
leaves and tips:						
Paco (Oke fern),	Raw		392.21 ± 12.91[a]	3687.08 ± 400.88[a]		
leaves and tender tips	Blanching	1	222.40 ± 44.34[b]	2445.59 ± 368.05[b]	62.21 ± 14.44[a]	37.79 ± 14.44[a]
	Stir-frying	2	188.58 ± 74.77[b]	976.60 ± 396.62[c]	44.31 ±18.46[b]	55.69 ±18.46[b]
Pagwanban,	Raw		30.25 ± 21.51[a]	163.22 ± 89.18[a]		
leaves and tender tips	Blanching	2	10.53 ± 6.14[a]	96.34 ± 48.04[a]	54.82 ± 32.27[a]	45.18 ± 32.27[a]
	Boiling	4	10.11 ± 6.69[a]	97.80 ± 57.29[a]	44.72 ± 30.62[a]	55.28 ± 30.62[a]
	Stir-frying	3	25.19 ± 17.72[a]	84.74 ± 56.70[a]	73.99 ± 20.32[a]	26.01 ± 20.32[a]
Pagwanpa,	Raw		53.16 ± 14.89[a]	283.44 ± 100.11[a]		
leaves and tender tips	Blanching	1	38.15 ± 21.57[a]	256.14 ± 156.53[a]	72.52 ± 22.97[a]	27.48 ± 22.97[a]
	Boiling	2	32.30 ± 18.56[a]	223.38 ± 135.85[a]	60.51 ± 20.86[a]	39.49 ± 20.86[a]
	Stir-frying	1	45.31 ± 17.22[a]	169.74 ± 83.04[a]	79.85 ± 23.44[a]	20.15 ± 23.44[a]
Paksein, pickle	Fermentation		13.26 ± 1.95	117.59 ± 21.53		

Table 7 Effect of cooking on the Tannin content of selected vegetables [1,2,3,4] *(Continued)*

Food item	Cooking method	Time (min)	Tannin content (mg/100g wet weight)	Tannin content (mg/100g dry weight)	% retention Tannin	% Tannin loss
Sesbania, ,	Raw		62.51 ± 58.08[a]	332.04 ± 366.94[a]		
leaves and tender tips	Blanching	2	41.84 ± 38.76[a]	326.01 ± 344.68[a]	83.41± 2.81[a]	16.59 ± 2.81[a]
	Boiling	5	27.11 ± 21.28[a]	218.22 ± 204.80[a]	61.41 ±10.91[a]	38.59 ±10.91[a]
flower:						
Banana,	Raw		295.74 ± 20.62[a]	3678.20 ± 284.18[a]		
flower	Boiling	5	197.18 ± 13.74[b]	3376.80 ± 212.64[a]	73.15± 0.97	26.85 ± 0.97
Cowslip creeper,	Raw		16.04 ± 6.55[a]	139.80 ± 47.33[a]		
flower	Blanching	3	8.46 ± 4.44[a]	104.46 ± 53.16[a]	62.68 ± 10.38[a]	37.32 ± 10.38[a]
	Boiling	5	6.22 ± 3.00[a]	77.68 ± 34.78[a]	46.40 ± 6.34[b]	53.60 ± 6.34[b]
	Stir-frying	5	15.40 ± 6.20[a]	75.25 ± 27.80[a]	81.83 ± 7.66[c]	18.17 ± 7.66[c]
Sesbania, , yellow,	Raw		49.57 ± 24.78[a]	422.00 ± 205.91[a]		
flower	Blanching	1	33.08 ± 16.92[a]	449.75 ± 224.62[a]	93.12 ± 2.81[a]	6.88 ± 2.81[a]
	Boiling	3	28.57 ± 15.50[a]	406.33 ± 216.42[a]	77.23 ± 9.95[b]	22.77 ± 9.95[b]
	Stir-frying	2	47.81 ± 23.63[a]	214.70 ± 102.95[a]	91.60 ± 4.36[a]	8.40 ± 4.36[a]

[1]Data reported in mean + SD of duplicate analysis, n=5

[2]Values within the same column with different superscripts are significant difference by one-way ANOVA with Sheffe's test and independent t-test at $p < 0.05$.

[3]Median and range values are reported in Appendix A

[4]Tannin content expressed in mg/100g of tannin equivalents

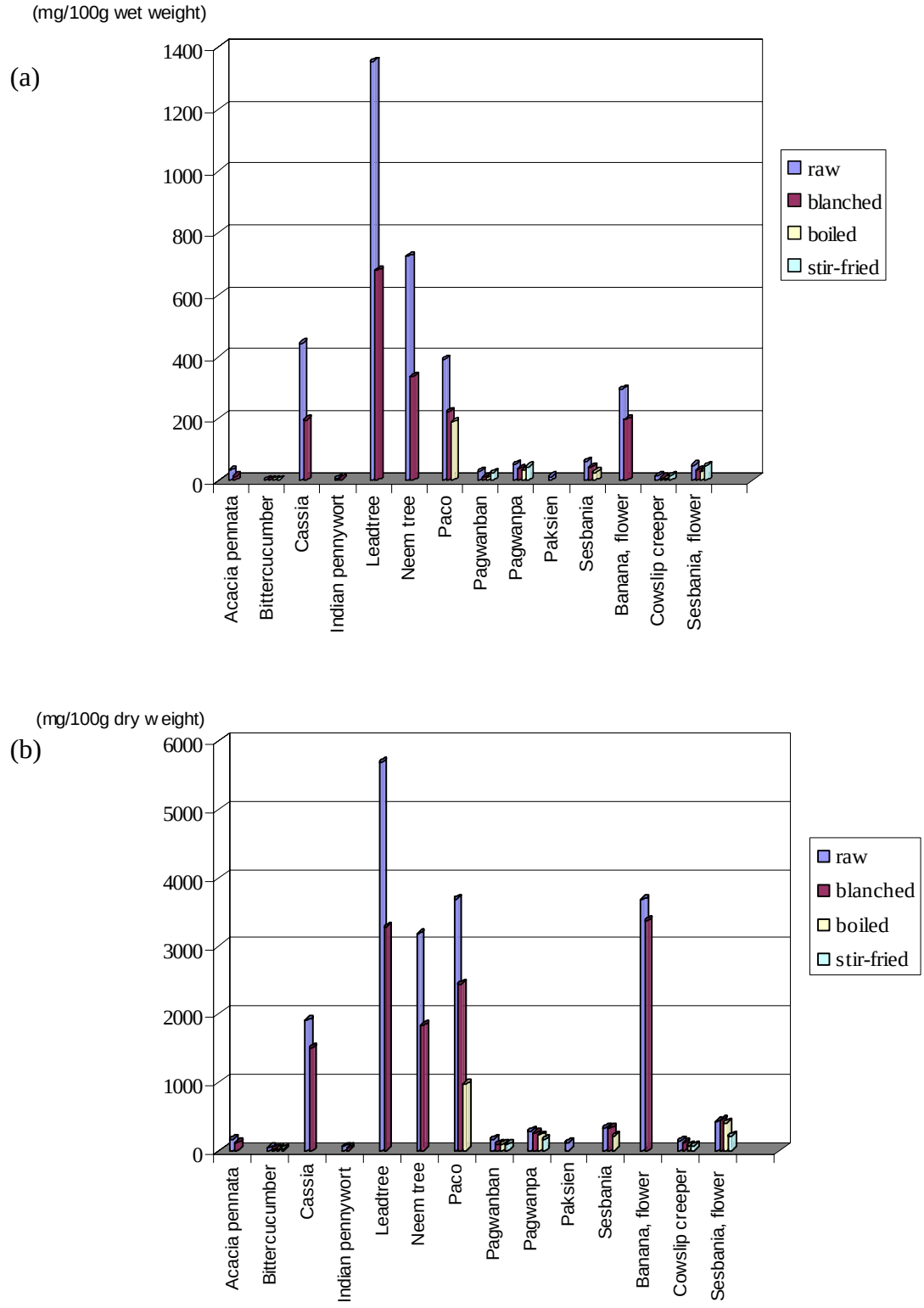


Figure 10 Effect of conventional cooking on tannin content in selected vegetables mg/100 tannin equivalent wet weight (a) and dry weight (b

5.4 Phytate Content Analysis

The phytate content of various raw and cooked vegetables by different cooking method are showed in Table 8-9 and Figure 11-12. Wide variations of phytate contents in raw and cooked vegetables ranged from 2.12 to 52.32 mg/100g in uncooked and 3.35 to 50.74 mg/100g in cooked, respectively. According to Quartile deviation, the data of phytate contents in raw samples are divided into three levels. High concentrations level of phytate (more than 36.98 mg/100g) was found in neem tree (52.32), pagwanban (48.76) and acacia pennata (42.06 mg/100 g of edible portion). Neem tree leafy vegetable was found to be the highest sources of phytate which approximately 52.32±5.96 mg/100 g of edible portion. The meduim level (7.95-36.98 mg/100g) was found in cowslip (35.29), cassia (29.29), paco (27.93), leadtree (27.49), pagwanpa (21.16), bitter cucumber (14.96), sasbania (13.09) and sesbania, flower (8.41). Low level of phytate (less than 7.95) was found in banana, flower (6.56), paksien, pickled (5.85), and indian pennywort (4.49 mg/100 g of edible portion).

Analysis the data in Table 9 indicated that effect of cooking on phytate content among raw and the types of cooking procedures were not significant difference at $p > 0.05$, except, sesbania, flower which found to be significantly effected by heated process at $p < 0.05$.

Two or three of home cooking procedures were compared for their effects on percent phytate retentions. The phytate contents in blanched and boiled in leaves and tender tips vegetable or flower vegetable were 74.19±4.39 % and 68.21±5.48 %, respectively, lower than in raw and stir-fried cooking when their compared in dry weight or percent retention as showed in Table 9 but the changes were not significant difference at $p > 0.05$. Except sesbania, yellow, flower vegetable, was significantly lower percent phytate loss (24.92±3.35 %), when compared to the among vegetables items $p < 0.05$ (Table 9 and Figure 12).

Table 8 Effect of cooking on the Phytate (IP5 and IP6) content of selected vegetables [1,2,3]

Food item	Cooking method	Time (min)	Wet basis (mg/100g)			Dry basis (mg/100g)		
			IP5	**IP6**	**Phytate**	**IP5**	**IP6**	**phytate**
leaves and tips:								
Acacia pennata,	Raw		3.93±1.16[a]	38.14±9.95[a]	42.06±10.52[a]	20.37±7.51[a]	198.59±70.61[a]	218.96±75.80[a]
leaves and tender tips	Blanching	2	2.54±1.02[a]	25.16±9.25[a]	27.69±9.95[a]	20.34±7.86[a]	198.27±59.41[a]	218.61±64.03[a]
Bittercucumber,	Raw		2.22±1.16[a]	12.74±7.52[a]	14.96±8.14[a]	26.79±14.97[a]	144.44±77.20[a]	171.23±86.40[a]
leaves and tender tips	Blanching	1	1.83±1.14[a]	9.65±5.52[a]	11.47±6.50[a]	25.70±17.92[a]	134.26±86.88[a]	159.99±102.59[a]
	Boiling	4	1.59±0.82[a]	8.58±5.40[a]	10.17±6.11[a]	23.64±14.00[a]	127.73±86.42[a]	151.36±98.33[a]
	Stir-frying	3	3.21±2.40[a]	12.24±4.48[a]	15.45±4.88[a]	23.97±17.45[a]	126.47±50.81[a]	150.43±60.29[a]
Cassia,	Raw		4.30±0.76[a]	24.98±5.49[a]	29.29±5.18[a]	18.31±2.37[a]	107.24±24.97[a]	125.54±23.29[a]
leaves and tender tips	Boiling	4	2.26±0.26[b]	13.51±2.65[b]	15.76±2.48[b]	17.47±2.20[a]	104.86±23.41[a]	122.23±22.96[a]
Indian pennywort,	Raw		1.18±0.14[a]	3.31±0.57[a]	4.49±0.44[a]	10.12±1.72[a]	29.32±4.77[a]	39.45±6.07[a]
leaves	Blanching	1	0.71±0.09[b]	2.65±0.46[a]	3.35±0.38[b]	7.99±1.78[a]	29.02±7.75[a]	37.00±8.77[a]
Leadtree,	Raw		4.24±0.59[a]	23.25±2.46[a]	27.49±2.65[a]	18.72±2.87[a]	98.50±8.50[a]	117.33±8.65[a]
leaves and tender tips	Blanching	1	3.05±0.32[a]	19.09±1.67[a]	22.14±1.72[b]	14.40±2.52[a]	91.41±10.22[a]	105.81±12.27[a]
Neem tree,	Raw		4.66±0.55[a]	47.67±5.92[a]	52.32±5.96[a]	18.74±3.02[a]	192.13±33.31[a]	210.87±34.96[a]
leaves and tender tips	Blanching	2	3.41±0.68[a]	34.98±10.81[a]	38.39±10.92[b]	18.55±3.26[a]	190.19±55.44[a]	208.74±55.55[a]

Table 8 Effect of cooking on the Phytate (IP5 and IP6) content of selected vegetables [1,2,3] *(Continued)*

Food item	Cooking method	Time (min)	Wet basis (mg/100g)			Dry basis (mg/100g)		
			IP5	**IP6**	**phytate**	**IP5**	**IP6**	**phytate**
leaves and tips:								
Paco (Oke fern)	Raw		3.06±2.76[a]	24.87±12.82[a]	27.93±14.85[a]	27.75±22.71[a]	226.23±108.76[a]	253.98±123.68[a]
, leaves and tender tips	Blanching	1	1.31±0.34[a]	19.33±13.78	20.64±13.94[a]	14.56±4.11[a]	211.35±142.19[a]	225.91±143.70[a]
	Stir-frying	2	2.06±1.19[a]	23.73±8.35[a]	25.79± 8.39[a]	14.49±3.01[a]	199.57±68.95[a]	214.06±68.33[a]
Pagwanban,	Raw		7.66±1.99[a]	41.10±13.00[a]	48.76±14.42[a]	44.75±11.10[a]	239.68±68.51[a]	284.42±76.19[a]
leaves and tender tips	Blanching	2	3.80±2.09[a]	28.12±6.84[a]	31.92±8.47[a]	38.03±25.50[a]	241.08±61.49[a]	279.11±85.37[a]
	Boiling	4	3.46±2.16[a]	24.51±5.51[a]	27.97±7.21[a]	36.41±25.58[a]	243.58±64.20[a]	277.61±82.31[a]
	Stir-frying	3	8.73±4.35[a]	42.00±11.20[a]	50.74±15.11[a]	37.59±15.98[a]	227.03±50.46[a]	264.62±63.50[a]
Pagwanpa,	Raw		3.12±1.08[a]	18.04±9.71[a]	21.16±10.53[a]	16.10±4.83[a]	95.29±51.23[a]	111.39±55.25[a]
leaves and tender tips	Blanching	1	2.14±0.85[a]	14.12±6.53[a]	16.26±7.36[a]	14.14±5.99[a]	92.96±44.80[a]	107.10±50.67[a]
	Boiling	2	2.01±0.84[a]	13.12±5.91[a]	15.12±6.62[a]	13.79±6.16[a]	89.52±41.52[a]	103.31±46.86[a]
	Stir-frying	1	4.40±2.29[a]	13.28±9.08[a]	17.68±10.47[a]	14.16±4.85[a]	88.54±58.72[a]	102.69±62.39[a]
Paksien,	Fermentation		1.49±0.24	4.36±0.77	5.85±0.82	13.88±2.47	41.75±7.44	55.45±8.77
pickled								

Table 8 Effect of cooking on the Phytate (IP5 and IP6) content of selected vegetables [1,2,3] *(Continued)*

Food item	Cooking method	Time (min)	Wet basis (mg/100g)			Dry basis (mg/100g)		
			IP5	**IP6**	**phytate**	**IP5**	**IP6**	**phytate**
Sesbania, leaves and tender tips	Raw		0.99±0.27[a]	12.09±2.93[a]	13.09±2.72[a]	4.71±0.74[a]	60.60±23.97[a]	65.31±23.39[a]
	Blanching	2	0.55±0.33[b]	7.20±1.21[b]	7.75±0.94[b]	3.95±1.99[a]	53.55±11.11[a]	57.50±9.41[a]
	Boiling	5	0.48±0.20[b]	6.37±2.05[b]	6.85±2.03[b]	3.63±0.45[a]	48.26±15.54[a]	51.89±15.47[a]
flower:								
Banana, flower	Raw		1.20±0.26[a]	5.35±1.58[a]	6.56±1.70[a]	14.92±2.97[a]	66.46±19.42[a]	81.37±20.60[a]
	Boiling	5	0.80±0.22[b]	3.47±1.28[a]	4.28±1.25[b]	13.76±3.65[a]	59.30±21.38[a]	73.60±20.55[a]
Cowslip creeper, flower	Raw		2.78±1.84[a]	32.51±17.57[a]	35.29±19.29[a]	23.94±14.75[a]	280.03±133.19[a]	303.97±146.96[a]
	Blanching	3	1.70±1.08[a]	18.13±11.78[a]	20.54±11.94[a]	21.07±13.33[a]	230.49±139.27[a]	251.56±140.89[a]
	Boiling	5	1.48±0.55[a]	18.21±9.69[a]	19.68±10.19[a]	18.52±6.63[a]	227.33±114.94[a]	245.85±120.88[a]
	Stir-frying	5	2.88±1.73[a]	30.71±15.08[a]	33.59±16.78[a]	18.68±11.76[a]	228.50±121.68[a]	247.18±133.44[a]
Sesbania, yellow, flower	Raw		2.64±0.77[a]	5.77±1.63[a]	8.41± 2.21[a]	22.19±5.56[ab]	48.48±12.45[a]	70.66±16.28[a]
	Blanching	1	1.54±0.29[b]	3.36±1.28[ab]	4.91±1.40[b]	21.06±2.91[b]	45.70±15.73[ab]	66.76±15.87[ab]
	Boiling	3	1.26±0.50[b]	2.62±0.75[b]	3.89±1.20[b]	17.84±6.70[b]	37.32±10.13[b]	55.17±15.96[ab]
	Stir-frying	2	2.58±0.64[ab]	5.60±1.62[a]	8.18±2.17[b]	15.17±2.77[bc]	37.48±5.62[b]	52.66±6.94[b]

[1]Data reported in mean + SD of duplicate analysis, n=5

[2]Values within the same column with different superscripts are significant difference by one-way ANOVA with Sheffe's test and independent t-test at $p < 0.05$.

[3]Median and range values are reported in Appendix A

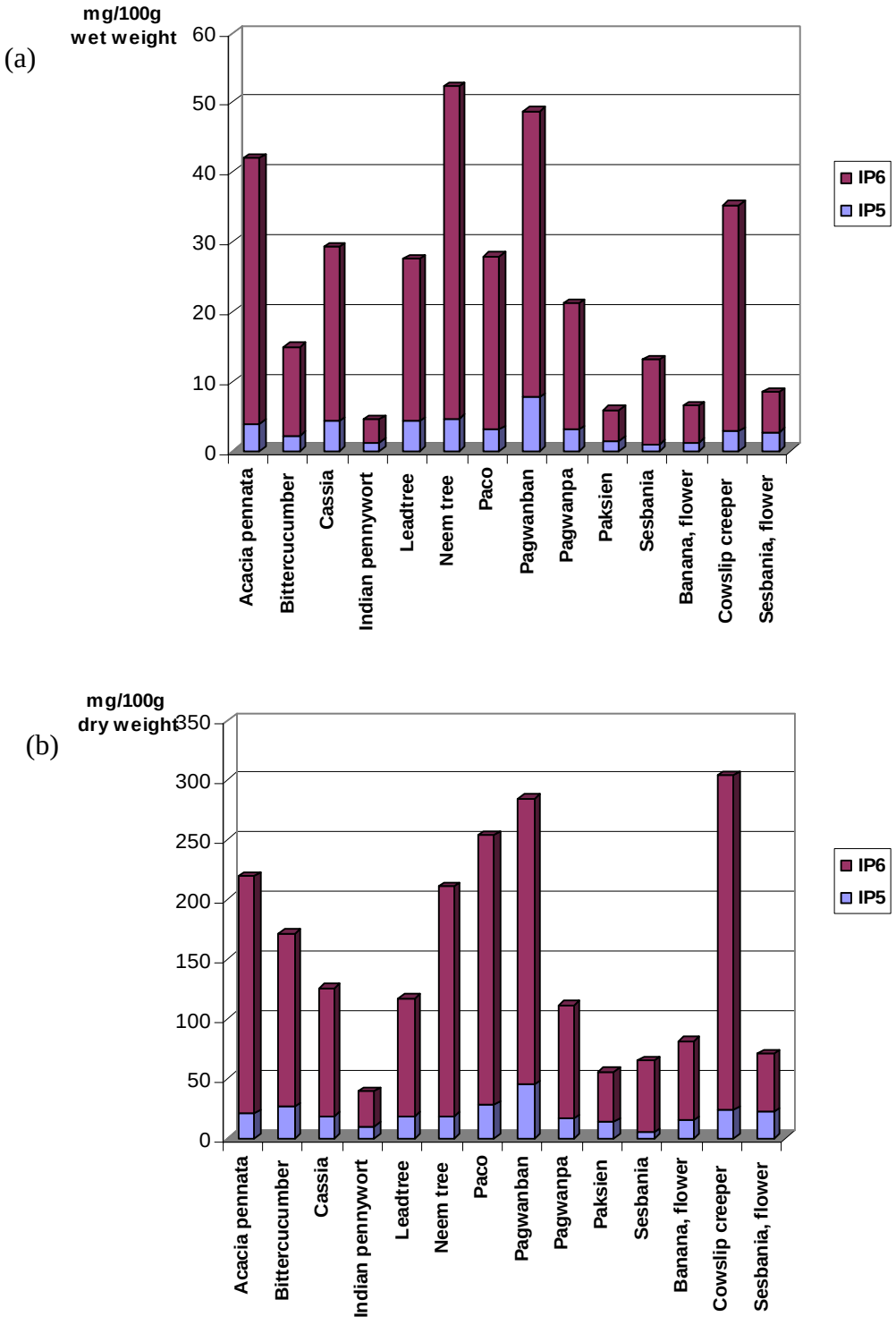


Figure 11 Phytate content in selected vegetables in raw mg/100 g wet weight (a) and dry weight (b)

Table 9 Effect of cooking on the Phytate content of selected vegetables [1,2,3]

Food item	Cooking method	Time (min)	Phytate content (mg/100g wet weight)	Phytate content (mg/100g dry weight)	% retention Phytate	% Phytate loss
leaves and tips:						
Acacia pennata, leaves and	Raw		42.06 ± 10.52[a]	218.96 ± 75.80[a]		
tips	Blanching	2	27.69 ± 9.95[a]	218.61 ± 64.03[a]	69.75 ± 10.69	30.25 ± 10.69
Bittercucumber, leaves and	Raw		14.96 ± 8.14[a]	171.23 ± 86.40[a]		
tips	Blanching	1	11.47 ± 6.50[a]	159.99 ± 102.59[a]	79.03 ± 19.61[a]	20.97 ± 19.61[a]
	Boiling	4	10.17 ± 6.11[a]	151.36 ± 98.33[a]	76.23 ± 22.55[a]	23.77 ± 22.55[a]
	Stir-frying	3	15.45 ± 4.88[a]	150.43 ± 60.29[a]	74.11 ± 10.89[a]	25.89 ± 10.89[a]
Cassia, leaves and tips	Raw		29.29 ± 5.18[a]	125.54 ± 23.29[a]		
	Boiling	4	15.76 ± 2.48[b]	122.23 ± 22.96[a]	65.67 ± 3.80	34.33 ± 3.80
Indian pennywort, leaves	Raw		4.49 ± 0.44[a]	39.45 ± 6.07[a]		
	Blanching	1	3.35 ± 0.38[b]	37.00 ± 8.77[a]	76.34 ± 2.31	23.66 ± 2.31
Leadtree, leaves and tips	Raw		27.49 ± 2.65[a]	117.33 ± 8.65[a]		
	Blanching	1	22.14 ± 1.72[b]	105.81 ± 12.27[a]	78.27 ± 4.03	21.73 ± 4.03
Neem tree, leaves and tips	Raw		52.32 ± 5.96[a]	210.87 ± 34.96[a]		
	Blanching	2	38.39 ± 10.92[b]	208.74 ± 55.55[a]	78.90 ± 8.00	21.10 ± 8.00

Table 9 Effect of cooking on the Phytate content of selected vegetables [1,2,3] *(Continued)*

Food item	Cooking method	Time (min)	Phytate content (mg/100g wet weight)	Phytate content (mg/100g dry weight)	% retention Phytate	% Phytate loss
leaves and tips:						
Paco (Oke fern), leaves	Raw		27.93 ± 14.85[a]	253.98 ± 123.68[a]		
and tips	Blanching	1	20.64 ± 13.94[a]	225.91 ± 143.70[a]	72.83 ± 14.28[a]	27.17 ± 14.28[a]
	Stir-frying	2	25.79 ± 8.39[a]	214.06 ± 68.33[a]	69.11 ± 15.22[a]	30.89 ± 15.22[a]
Pagwanban, leaves	Raw		48.76 ± 14.42[a]	284.42 ± 76.19[a]		
	Blanching	2	31.92 ± 8.47[a]	279.11 ± 85.37[a]	75.16 ± 8.80[a]	24.84 ± 8.80[a]
	Boiling	4	27.97 ± 7.21[a]	277.61 ± 82.31[a]	72.90 ± 18.41[a]	27.10 ± 18.41[a]
	Stir-frying	3	50.74 ± 15.11[a]	264.62 ± 63.50[a]	71.34 ± 1.85[a]	28.66 ± 1.85[a]
Pagwanpa, leaves	Raw		21.16 ± 10.53[a]	111.39 ± 55.25[a]		
	Blanching	1	16.26 ± 7.36[a]	107.10 ± 50.67[a]	75.09 ± 14.06[a]	24.91 ± 14.06[a]
	Boiling	2	15.12 ± 6.62[a]	103.31 ± 46.86[a]	69.15 ± 17.66[a]	30.85 ± 17.66[a]
	Stir-frying	1	17.68 ± 10.47[a]	102.69 ± 62.39[a]	68.28 ± 13.13[a]	31.72 ± 13.13[a]
Paksien, picked	Fermentation		5.85 ± 0.82	55.45 ± 8.77		

Table 9 Effect of cooking on the Phytate content of selected vegetables [1,2,3] *(Continued)*

Food item	Cooking method	Time (min)	Phytate content (mg/100g wet weight)	Phytate content (mg/100g dry weight)	% retention Phytate	% Phytate loss
Sesbania, , leaves and tips	Raw		13.09 ± 2.72[a]	65.31 ± 23.39[a]		
	Blanching	2	7.75 ± 0.94[b]	57.50 ± 9.41[a]	64.83 ± 6.75[a]	35.17 ± 6.75[a]
	Boiling	5	6.85 ± 2.03[b]	51.89 ± 15.47[a]	57.68 ± 9.35[a]	42.32 ± 9.35[a]
flower:						
Banana, flower	Raw		6.56 ± 1.70[a]	81.37 ± 20.60[a]		
	Boiling	5	4.28 ± 1.25[b]	73.60 ± 20.55[a]	68.85 ± 12.76	31.15 ± 12.76
Cowslip creeper, flower	Raw		35.29 ± 19.29[a]	303.97 ± 146.96[a]		
	Blanching	3	20.54 ± 11.94[a]	251.56 ± 140.89[a]	70.47 ± 18.10[a]	29.53 ± 18.10[a]
	Boiling	5	19.68 ± 10.19[a]	245.85 ± 120.88[a]	69.06 ± 12.43[a]	30.94 ± 12.43[a]
	Stir-frying	5	33.59 ± 16.78[a]	247.18 ± 133.44[a]	71.28 ± 2.56[a]	28.72 ± 2.56[a]
Sesbania, flower, yellow	Raw		8.41 ± 2.21[a]	70.66 ± 16.28[a]		
	Blanching	1	4.91 ± 1.40[b]	66.76 ± 15.87[ab]	75.41±11.08[ab]	24.59±11.08[ab]
	Boiling	3	3.89 ± 1.20[b]	55.17 ± 15.96[ab]	66.10 ± 15.04[a]	33.90 ± 15.04[a]
	Stir-frying	2	8.18 ± 2.17[b]	52.66 ± 6.94[b]	75.08 ± 3.35[b]	24.92 ± 3.35[b]

[1]Data reported in mean + SD of duplicate analysis n=5

[2]Values within the same column with different superscripts are significant difference by one-way ANOVA with Sheffe's test and independent t-test at $p < 0.05$.

[3]Median and range values are reported in Appendix A

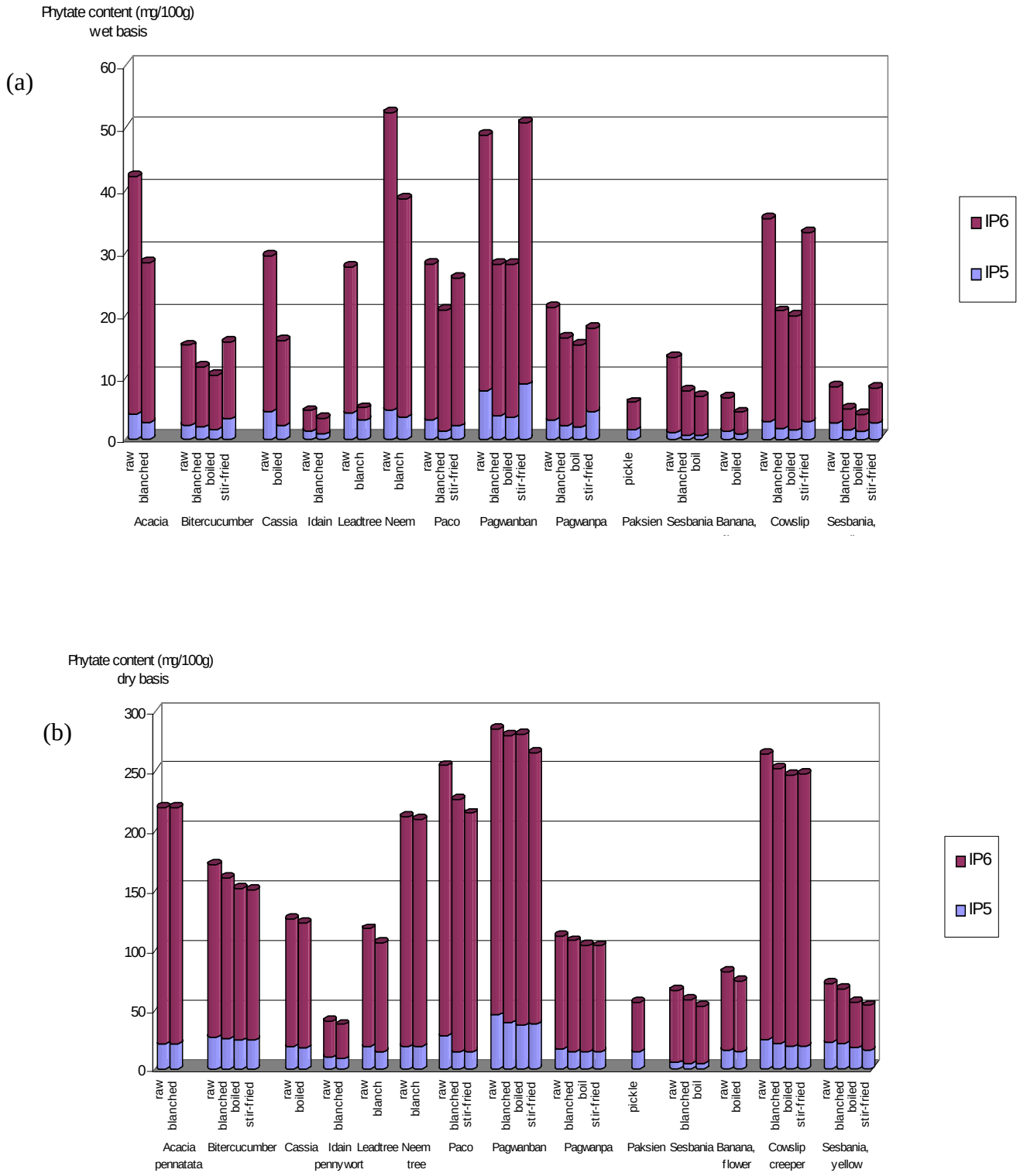


Figure 12 Effect of conventional cooking on phytate content in selected vegetables mg/100g wet weight (a) and dry weight (b)

5.5 Effect of fermentation on moisture, vitamin C, tannin and phytate (IP5 and IP6) contents in mustard green model

There are various food processing and preparing of methods that have to be claim to reduce nutrient particularly vitamin C and anti-nutritional factors (phytate and tannin). Fermentation is one of the foods processing to changes or creates unique flavors, textural properties and improves nutritional quality and digestibility. Therefore, the objective in this experiment was to evaluate the effect of fermentation (natural lactic fermentation) in selected Mustard green vegetables on vitamin C, tannin and phyate contents since mustard green are representative of pickled vegetables commonly consumed in Thailand.

The results of this study are summarized in Table 10. Mustard green in raw had the moisture content 93.12 and 92.88 %, vitamin C 42.81 and 33.32 mg/100g, tannin 14.55 and 12.78 mg/100g, and phytate content 2.12 and 2.06 mg/100g in raw samples. An effect of fermented process was compared for its effects on vitamin C, tannin and phytate contents. As the results showed in Table 10, concentrations of vitamin C and tannin were reduced more than 60 % for vitamin C and 43 % for tannin, respectively. Looking to the results of phytate, generally inositol hexa (IP6) or penta phosphate (IP5) forms make up over 90 % of total phytate in raw vegetables, cereal and grains but get degraded during processing or cooking. The data of IP 5 and IP 6 of Mustard green vegetables in this model showed that IP6 in raw (un-fermented) was around 2.06 to 2.12 mg/100 g of edible portion and IP5 was not detected. As expects after the fermentation of Mustard green vegetable for 4 days, IP6 in fermented sample was not detected (Table 10).

Table 10 Effect of fermentation on moisture, vitamin c, tannin and phytate content in mustard green[1,2]

Component (mg/100g)	**Mustard greens,** heart and leaves						**Mustard greens,** heart					
	Raw	*fermented*	*Raw*	*fermented*	% Retention	% Loss	*Raw*	*fermented*	*Raw*	*fermented*	% Retention	% Loss
	Wet basis		Dry basis				Wet basis		Dry basis			
Moisture (%)	93.12	92.11					92.88	97.70				
Vitamin C	42.81	19.88	622.24	240.30	32.93	67.07	33.32	18.96	467.98	259.73	39.86	60.14
Tannin	14.55	11.77	211.48	149.18	57.36	42.64	12.78	10.25	179.49	140.41	56.19	43.81
Phytate												
IP5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
IP6	2.12	nd	3.81	nd	nd	nd	2.06	nd	28.93	nd	nd	nd
Total phytate	2.12	nd	3.81	nd	nd	nd	2.06	nd	28.93	nd	nd	nd

[1]n=2

[2]nd = not detected

CHAPTER 6
DISCUSSION

Changes in moisture content of selected leaves and tender tips and flower vegetables in this study which were cooked by boiling or blanching seem to be unchanged after cooking while conventional stir-frying makes the moisture content significantly decreased $p < 0.05$. These results are in agreement with those of Charley, 1972, Tongchipianuit, 1996, Suttikomin, 2002 and Garcia-Arias, *et al*., 2003 which suggested that the greater loss of moisture content in conventional stir-fried condition might be due to a high temperature, no water added, and evaporation of water during the cooking process (123-126). The means average of vitamin C content in fresh leaves and tender tips vegetables and flower vegetables ranged from 85.59 mg/100g in pagwanpa to 0.50 mg/100g in flower vegetables. Comparison among the means of vitamin C concentration of different cooking methods in leaves and tender tips and flower vegetables are showed in Table 6, stir-fried pagwanpa, pagwanban, cowslip creeper, and flower vegetables are the excellent sources of vitamin C (64.42 to 70.80 mg/100g). Since the recommendation of dietary allowance vitamin C for human is 60 mg/day (40). It means that when consuming those vegetables only 100 grams, vitamin C requirement will be met. Comparison of means concentration of vitamin C of fresh leaves and tender tips and flower vegetables in this study with several previously published values demonstrated some similarities and variations (127, 128). However, the analysis values of vitamin C contents do not completely agree or close with other published values. This lack of agreement may be due to a number of factors, including seasonal variation and natural variation of the vegetables, transportation, and shelf time prior to purchase, preparing and cooking practices. In developing countries, one of their stable foods is vegetables that are contribution of mineral and vitamin to human nutrition, however, the contribution of vegetables to vitamin and minerals in human nutrition is limited due to the presence of anti-nutritional factors which render some of micro-nutrients unavailable for human nutrition (129). The most common anti-nutritional factors in leafy vegetables are phytic acid and tannic acid (130, 131).

Many pervious publications showed that phytic acid and tannic acid have strong negativity affected on bonding mineral unavailable, particularly, in 1992, Hurrel, *et al*., demonstrated that phytic acid intake of 4 to 9 mg/100g decreased iron absorption by 4-5 fold in humans (132). In addition, Tuntawiroon *et al*., 1991 also reported that tannic acid contained in some Thai vegetable (Yod-kratin) only 87.6 mg of a meal had strongly reduced iron absorption more than 50 % (6). In contrast, many publications demonstrated that heat treatments on the course of cooking can destroying or decrease anti-nutritional factors in many foods (125, 79). In this study showed that the level of tannin contents were higher in leaves and tender tips vegetables (3.14-1353.22 mg/100g of tannic acid equivalent). Especially, tannin content in lead tree was found to be the highest among other items. These results are in agreement with Suttikomin, 2002 which indicated that the amount of tannin contents were higher in leafy vegetables than fruit vegetables and other items as well as the publication of Mosha *et al*., 1995 suggested that vegetables with astringency taste contained more tannin content. Analysis data of tannin in this study agree with the suggestion of Mosha and colleagues in 1995 that astringency taste vegetables like fresh or cooked neem tree (leaves and tender tips) and leadtree (leaves and tender tips) contained the highest amount of tannin content (723.47 and 1353.22 mg/100g of tannic acid equivalents, respectively) when compared to other non-astringency taste vegetables (79). There is little information found in publication with regard to the tannin contents in leaves and tender tips or flower vegetables, except published data of Chanwitheesk *et al*., 2005 and unpublished data of Tuntawiroon. When comparing the analysis data in this study with Tuntawiroon and Chanwitheesuk *et a*l., 2005, the means of tannin contents in some young tender leaves or flower vegetables such as lead tree and neem tree are lower than the unpublished data of Tuntawiroon but higher than the publication of Chanwitheesuk and colleagues (134, 135). The differences among means of some analysis data of tannin in this study from Tuntawiroon and Chanwitheesuk and colleagues might be different from the environmental condition, soil, seasonal variation, the state of maturity of plant, different method of sampling and analysis, preparing or cooking practice, and genetic factors among the plant (79, 133, 136, 137). Phytate content was recognized to have negative correlation with mineral bioavailability. Analysis data in this study showed that phytate content was higher in

leafy vegetables especially in neem tree whereas the lower was found in indian pennywort. The comparison of the data in this study with previous study reported by Boontaveeyuwat *et al*., 1990 demonstrated that phytate contents in this study using HPLC technique were lower than the publication by Boontaveeyuwat in all food items due to the different in the cooking procedure and the method of phytate analysis (138). In this study, phytate was determined by an ion-pair high performance liquid chromatography analysis (HPLC) while Boontaveeyawat used the precipitation and an anion exchange method (139). Sandberg, 1995 suggested that the precipitation and an anion exchange method are not specific as they do not separate inositol hexaphosphates from lower inositol phosphates and thus overestimate the phytate content in processed foods (140). In addition, Phillppy *et al*., 1988 and 2004, Lehrfeld and Morris, 1992 suggested that the phytic acid in unprocessed food products mainly appear as inositol hexaphosphate (IP6), thus the precipitation method or AOAC anion-exchange method are useful to measure the phytic acid content in raw and unprocessed foods (109, 141, 142). These suggestions agree with the observation in our study, which found that phytate content in unprocessed soybean flour measured according to the AOAC method and HPLC technique were similar. The values obtained were in good agreement when the sample contained only IP5; inositol pentaphosphate and IP6; inositol hexaphosphate (1178.41$\pm$94.32 mg/100g for AOAC method and 1050.89$\pm$40.91mg/100g for HPLC technique). In addition, various investigators demonstrated that during the processing of foods for human consumption there is the potential for hydrolysis of phytate (IP6) to lower inositol phosphate forms by enzymatic and heat treatment (or thermal degradation) on the course of cooking process (79, 142). Since various publications have been shown that inositol phosphate in form inositol tris and tetra phosphates (IP3 and IP4) have no detrimental effect on mineral absorption (11, 100, 104). Therefore, using precipitation method or calorimetric method by AOAC, 2000; 967.22 for determination of phytate content in the form of phytate-phosphorus might be overestimated due to the calculation of phytic-phosphorus including the other forms of phytic acid, such as inositol tetraphosphate (IP4), inositol triphosphate (IP3) and inositol di-monophosphate (IP2 and IP1) (142). In fact the IP1 to IP4 do not inhibit mineral absorption, only IP5 and IP6 appear to interfere with biovailability of minerals (103). Sandberg & Anderinne,

1986, Lehrfeld, 1989 recommended that ion-exchange liquid chromatography and ion-pair HPLC methods are shown to be suitable and accurate for analysis of various inositol phosphates forms (IP1 to IP6) which are important for nutritional studies. Therefore, this study used HPLC technique for determination and quantification of inositol penta and hexakisphosphate (IP5 and IP6) from fresh and cooked vegetable products. Inositol penta and hexakisphosphate (IP5 and IP 6) had more inhibitory effect on micro-nutrients absorption than other inositol form; IP1, IP2, IP3 and IP4 (101, 162). According to Hurrel *et al*., 1992, the consumption of phytic acid more than 4 to 9 mg/100g decreased iron absorption by 4-5 fold in human (132). Tuntawiroon *et al*., 1991 demonstrated that the consumption of tannin more than 87 mg/one meal reduced iron absorption in that meal more than 50 % (6). Analysis data in this study might be concluded that the high consumption of blanched leaves and tender tips fresh neem tree and leadtree which contains high amount of phytate > 20 mg/100g and > 1000 mg/100g of tannic acid equivalents might be adversely affect on availability of mineral absorption.

Effect of conventional cooking method (blanching, boiling and stir-frying) on percent true retention and percent loss of nutrients (vitamin C) and non nutrients (phytate and tannin)

Bioavailability of iron in diet may depend upon ascorbic acid content, which is promoter, phytate and tannin, which are inhibitors of iron absorption. Therefore, the reduction or elimination of these anti-nutrients is important to improve iron absorption. This study, demonstrated the effect of cooking methods on the content of vitamin C, phytate (IP5 and IP6) and tannin in vegetables as showed in Table 6-9. The results obtained in the present study showed that the content of vitamin C significantly declined during conventional cooking methods, ranging from 14.42 to 94.64 % specially, the conventional boiling method seriously destroys vitamin C content in the samples. The greatest loss of vitamin C during the conventional boiling method of vegetables might be due to water soluble nature of vitamin C thus it could be lost through the water discarded after cooking. Fennema, 1997 also pointed out that the cooking procedure could result in significant losses of vitamin C (163). Yadav and Sehgal (1995, 1997) suggested that losses of ascorbic acid from vegetables including spinach and fenugreek during cooking procedures such as boiling, stewing, frying, blanching and pressure cooking, resulted in decreasing ascorbic acid with ranging from 36-83 % (164, 165). Similar finding was observed in this study, the conventional blanching, boiling and stir-frying resulted in reducing vitamin C content with approximately ranged from 18.02–94.64 %, particularly the boiling method which had the strongest effect to destroy the amount of vitamin C concentration by more than 94.64 % in all cooked vegetables. Moreover, Erdman and Klien, 1982 noted that the amount of water used in cooking, and to a lesser extent, the cooking time had affected vitamin C losses more than the source of energy or type of cooking (166). For the losses of vitamin C in stir-frying method might be due to the use of high temperature, the cooking time and frequent stirring which exposes the material to atmospheric oxidation. In all leaves and tender tips and flower vegetables, the tannin concentrations were not significantly ($p > 0.05$) reduced by conventional blanching, boiling and stir-frying. However, conventional boiling appeared to be more effective to destroy tannin in almost vegetables of this study, except tannin content in neem tree

and paco were significantly decreased (21.1 to 25.9 %) by heat-treated process at $p < 0.05$. Zhang and Hamuzz, 2004 reported that tannic acid or phenolic compound in vegetables showed to be heavily lost during prolong cooking time, whereas other processes, such as conventional blanching or stir-frying, percent tannin loss would be lower (167). In addition, Racchi *et al*. (2002) reported that boiling method significantly reduced antioxidant activity (phenolic compounds) in mushroom juice (168). This might be explained that tannic acid is not easily destroyed or eliminated in a short time during the heating process. It might be need a longer heating time for cooking or being largely leached into the cooking water in order to reduce tannin content (39). The effect of cooking on phytate content also showed similar trends. The results of this study indicated that there were no significant different in cooking methods on phytate contents where dry basis or percent retention was used to compare among uncooked and cooked samples. Except stir-fried sesbania, yellow, flower was significantly lower in percent phytate loss (24.92 %). The reduction of phytate varied according to cooking methods, ranged from 20.97-35.17 % for blanching, 23.77-42.32 for boiling and 24.92-25.90 % for stir-frying. Sanberg, *et al*.,1986 pointed out that cooking or food processing such as fermentation, autoclave and milling process could reduced phytate content. However, commom house-hold process, in its self, does not cause hydrolysis of phytate (169). It is possible that the elimination of phytate in conventional cooking might need a longer time for heat exposure. This is agree with Habiba, 2002 who found that the greatest reduction in pea seeds was observed after 40 minutes of ordinary cooking (170). And in the study of Mosha, 1995 also showed that both conventional and microwave blanching of vegetables resulted in a significant reduction of phytic acid when the cooking time was over 10 minutes (171). Although this study showed no significant different between tannin and phytate content in raw and cooked vegetables, all of them also found to be decreasing after cooking processes. Especially, the elimination of phytate by altered the inositol penta (IP5) and hexaphosphates (IP6) to lower form e.g., tetra, tri, di and mono phosphate should be considered. Because nutrition-related studies have indicated that it may be important to know relative amount of myo-inositol hexaphosphates (IP6), penta-, tetra-, tri-, di- and monophosphate ester, since even limited dephosphorylation of phytic acid can reduce its inhibitory effect on mineral absorption (172).

The analysis data in previous chapter found that anti-nutritional factors, particularly phytate in some leaves and tender tips or flower vegetable still remain high concentration even those vegetables were cooked by heat-treatment. Therefore, it was interesting to know that how the fermentation affect, vitamin C, tannin and phytate contents, in selected vegetable model to be reduce or eliminate inositol IP6 or IP5 into the other forms which did not interfere mineral bioavailability. Therefore the fermentation of mastard green vegetable was developed. Analysis data showed that after the fermentation of Mustard green vegetable for 4 days, vitamin C content was reduced more than 60 % whereas tannin lost about 42 to 44 % and IP6 in fermented sample was not detected. This results agree with Hag *et al*., 2001 found that phytate and tannin content of pearl millet after fermentation for 14 h were significantly decreased more than 50 % for phytate and 22.4 % for tannin (173). It could be explained that after fermentation, degradation of phytic acid may activate native phytases of plant origin or bacterial phytases may be activated and hydrolyses the phytic acid into the other forms or it could be due to the fermented process had several step in the procedure (i.e., washed, dehydrate under shade and soaked), results in decreased or changed in the form of phytate of fermented samples (173,174). These observations consist with our finding which found that IP6 and IP5 in the sample was not detected after fermented process when compared to raw sample (Table 10).

CHAPTER 7
CONCLUSION

The study showed that there were wide variations in the contents of vitamin C, tannin and phytate in the vegetables commonly consumed in Thailand. The vitamin C contents were high in pagwanpa, pagwanban, and sesbania whereas tannin and phytate concentrations were high in leadtree and neem tree. Vitamin C contents in all the food items were significantly reduced by conventional cooking methods, especially conventional boiling is strongly destroying vitamin C content. Tannin and phytate contents in almost vegetables were not significantly decreased by the conventional cooking methods. For fermentation Mustard green model, vitamin C content was reduced more than 60 % whereas tannin lost about 42 to 44 % and IP6 in fermented sample was not detected. Although this study showed no significant difference between tannin and phytate content in raw and cooked vegetables, all of them also showed similar trend decreasing after cooking process. The results indicated that among three conventional cooking methods, boiling may have the strongest effect on the reduction of nutrient (vitamin C) and anti-nutrient (tannin and phytate) in all vegetables.

Suggestion for Further studies

1. To determine phytate content (IP1-IP6) in Thai menu availably consumed.
2. To evaluate the effect of high heat treatment on degradation of phytate in process foods and ready to eat foods.
3. To determine phytate and tannin concentration in fresh fruits or fruit products.

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APPENDIX

APPENDIX A

Table 11 Moisture, vitamin C, tannin, catechin and phytate (IP5 and IP6) contents of vegetables (mg/100g of edible portion)[1,2,3]

Food item	Moisture (mg/100g)	Vitamin C (mg/100g)	Tannin (mg/100g)	Catechin (mg/100g)	IP5 (mg/100g)	IP6 (mg/100g)	Total phytate (IP5 and IP6) (mg/100g)
leaves and tender tips:							
Acacia pennata, Raw	80.24±3.03	56.64±11.17	32.86±14.21	38.22±16.35	3.93±1.16	38.14±9.95	42.06±10.52
	(75.03-86.24) 80.89	(45.63-72.70) 57.46	(22.74-57.46) 30.19	(22.97-61.27) 32.08	(2.76-5.46) 3.74	(27.60-54.61) 36.12	(32.33-60.08) 38.95
Blanching	87.49±1.29	12.34±2.85	13.27±2.65	21.71±6.12	2.54±1.02	25.61±9.25	27.69±9.95
	(85.75-88.63) 87.98	(9.64-16.90) 12.04	(10.30-16.33) 14.00	(15.77-31.21) 21.67	(1.78-4.08) 1.90	(15.92-39.65) 25.01	(17.70-43.73) 26.91
Bittercucumber, Raw	91.40±1.33	6.23±2.05	3.14±1.11	11.26±5.22	2.22±1.16	12.74±7.52	14.96±8.14
	(89.25-92.80) 91.59	(3.83-8.76) 6.64	(1.76-4.55) 3.31	(3.38-17.89) 12.27	(0.87-3.38) 2.21	(3.57-20.14) 14.54	(4.44-23.49) 17.92
Blanching	92.46±1.36	1.13±0.75	2.06±0.72	7.99±3.22	1.83±1.14	9.65±5.52	11.47±6.50
	(89.25-92.80) 91.59	(0.55-2.16) 0.69	(1.18-3.03) 2.15	(3.18-12.02) 8.03	(0.85-3.37) 1.22	(3.75-17.86) 7.80	(4.60-20.59) 9.02
Boiling	92.99±0.98	0.34±0.35	1.80±0.67	6.60±2.81	1.59±0.82	8.58±5.40	10.17±6.11
	(91.36-93.75) 93.33	(0.04-0.90) 0.19	(1.10-2.55) 2.00	(2.27-9.12) 6.31	(0.92-2.82) 1.14	(3.35-15.84) 5.78	(4.27-17.89) 6.92
Stir-frying	83.52±4.03	1.66±1.09	3.08±1.14	11.87±5.83	3.21±2.40	12.24±4.48	15.45±4.88
	(76.57-86.09) 85.40	(0.89-3.56) 1.24	(1.84-4.52) 3.45	(3.45-18.11) 12.54	(1.30-7.27) 2.18	(7.25-19.54) 11.26	(8.55-21.43) 14.62
Cassia, Raw	76.59±1.62	62.01±10.14	443.15±31.01	267.44±93.38	4.30±0.76	24.98±5.49	29.29±5.18
	(74.45-78.88) 76.67	(46.54-75.01) 64.36	(404.07-489.30) 446.15	(167.84-400.21) 241.12	(3.74-5/22) 4.59	(17.54-31.22) 26.14	(22.22-35.81) 29.61
Boiling	87.05±0.63	16.90±5.93	195.74±70.91	153.51±50.78	2.26±0.26	13.51±2.65	15.76±2.48
	(86.26-87.72) 87.06	(11.13-26.40) 16.26	(99.67-264.38) 192.52	(90.12-210.20) 141.21	(1.90-2.62) 2.29	(10.54-15.83) 14.85	(12.87-18.12) 16.75

Table 11 Moisture, vitamin C, tannin, catechin and phytate (IP5 and IP6) contents of vegetables (mg/100g of edible portion)[1,2,3] *(Continued)*

Food item	Moisture (mg/100g)	Vitamin C (mg/100g)	Tannin (mg/100g)	Catechin (mg/100g)	IP5 (mg/100g)	IP6 (mg/100g)	Total phytate (IP5 and IP6) (mg/100g)
leaves and tender tips:							
Indian pennywort, Raw	87.87±1.75	22.83±15.07	6.69±2.77	7.52±2.27	1.18±0.14	3.31±0.57	4.49±0.44
	(85.76-90.52) 90.44	(12.57-49.26) 16.31	(2.15-9.32) 6.78	(5.12-11.21) 6.88	(1.04-1.41) 1.13	(2.32-3.78) 3.45	(3.73-4.91) 4.60
Blanching	90.86±1.74	11.42±7.53	3.49±1.43	5.31±2.04	0.71±0.09	2.65±0.46	3.35±0.38
	(88.76-93.52) 90.44	(6.29-24.63) 8.16	(1.07-4.66) 4.12	(3.11-8.47) 4.98	(0.63-0.85) 0.68	(1.86-3.02) 2.76	(2.70-3.70) 3.47
Leadtree, Raw	75.88±3.30	10.77 ± 3.88	1353.22±526.70	nd	4.24±0.59	23.25±2.46	27.49±2.65
	(71.61-78.86) 76.85	(5.05-14.57) 12.54	(804.39-2054.43)1076.85		(3.36-4.99) 4.23	(20.26-26.30) 22.67	(23.62-30.53) 27.13
Blanching	78.87±3.29	5.37±1.94	679.22±261.68	nd	3.05±0.32	19.90±1.67	22.14±1.72
	(74.61-81.86) 79.85	(2.53-7.29) 6.27	(402.20-1027.21) 550.11		(2.70-3.45) 3.10	(17.23-21.04) 19.56	(19.98-24.14) 22.83
Neem tree, Raw	75.01±1.49	71.53±1.07	723.47±214.09	132.84±73.86	4.66±0.55	47.67±5.92	52.32±5.96
	(73.66-77.50) 74.58	(70.32-73.12) 71.23	(425.10-949.11) 754.80	(56.26-240.99) 145.66	(4.00-5.33)4.50	(40.32-53.33) 49.86	(44.68-58.67) 54.36
Blanching	81.67±0.77	46.48±1.95	337.58±130.47	100.32±53.56	3.41±0.68	34.98±10.81	38.39±10.92
	(80.99-82.86) 81.50	(43.87-48.97) 46.89	(200.01-500.12) 287.89	(45.12-180.12)109.22	(2.62-4.24) 3.23	(23.49-47.28) 39.26	(26.11-50.51) 42.24
Paco (Oke fern), Raw	89.29±0.96	4.15±1.93	392.21±12.91	168.52±80.03	3.06±2.76	24.87±12.82	27.93±14.85
	(88.30-90.80) 89.11	(1.82-6.49) 3.37	(375.42-410.56) 389.56	(120.21-310.45) 140.65	(1.74-7.99) 1.82	(6.33-40.45) 26.63	(8.12-48.44) 28.38
Blanching	90.95±0.61	2.49±1.41	222.40±44.34	133.81±51.93	1.31±0.34	19.33±13.78	20.64±13.94
	(90.34-91.84) 90.84	(1.00-4.33) 1.97	(181.12-283.94) 198.63	(71.39-211.01) 118.74	(0.80-1.69) 1.26	(2.40-39.85) 16.63	(3.64-41.54) 17.43
Stir-frying	80.31±2.99	0.36±0.41	188.58±74.77	121.24±61.52	2.06±1.19	23.73±8.35	25.79±8.39
	(76.41-84.15) 79.36	(0.02-1.05) 0.28	(139.04-314.19) 149.56	(52.10-220.10) 116.97	(0.10-3.06) 2.68	(10.21-32.53) 25.12	(12.89-35.22) 25.22

Table 11 Moisture, vitamin C, tannin, catechin and phytate (IP5 and IP6) contents of vegetables (mg/100g of edible portion) [1,2,3] *(Continued)*

Food item	Moisture (mg/100g)	Vitamin C (mg/100g)	Tannin (mg/100g)	Catechin (mg/100g)	IP5 (mg/100g)	IP6 (mg/100g)	Total phytate (IP5 and IP6) (mg/100g)
leaves and tender tips:							
Pagwanban, Raw	82.72±3.35	78.46±31.90	30.25±21.51	106.86±42.65	7.66±1.99	41.10±13.00	48.76±14.42
	(77.75-86.97) 83.49	(47.54-124.38) 72.59	(12.61-60.90) 16.93	(33.59-137.67)127.38	(5.40-10.58) 7.42	(25.41-53.45) 45.21	(32.83-64.03) 51.66
Blanching	89.32±1.68	12.23±5.59	10.53±6.14	64.84±30.73	3.80±2.09	28.12±6.84	31.92±8.47
	(86.66-91.03) 89.90	(6.81-21.03) 11.18	(4.21-18.60) 11.08	(13.75-87.87)74.20	(2.27-6.81) 2.46	(20.06-37.25) 29.19	(22.33-42.45) 31.64
Boiling	89.84±0.96	10.26±4.66	10.11±6.69	52.80±23.13	3.46±2.16	24.51±5.51	27.97±7.21
	(88.43-90.93) 90.15	(6.91-17.93) 7.92	(3.51-20.53) 7.86	(13.56-72.03) 57.45	(1.33-6.50) 2.47	(18.08-30.12) 27.25	(19.41-35.02) 30.46
Stir-frying	70.99±2.90	70.80±19.80	25.19±17.72	158.60±128.24	8.73±4.35	42.00±11.20	50.74±15.11
	(66.79-73.78) 71.69	(44.16-100) 69.18	(10.25-45.12) 14.52	(14.65-290.12)200.12	(5.40-15.56) 6.45	(29.56-54.69) 45.21	(34.96-70.25) 51.66
Pagwanpa, Raw	80.75±3.07	83.59±27.93	53.16±14.89	88.10±24.55	3.12±1.08	18.04±9.71	21.16±10.53
	(75.90-83.42) 80.60	(47.60-109.98) 90.64	(30-70.12) 55.66	(54.21-111.29)100.22	(1.88-4.53) 3.35	(9.45-34.12) 17.04	(11.33-38.65) 20.33
Blanching	84.75±1.00	43.88±10.91	38.15±21.57	68.83±19.59	2.14±0.85	14.12±6.53	16.26±7.36
	(83.14-85.64) 84.76	(27.72-56.60) 46.61	(20-69.02) 25.12	(38.22-90.12) 70.58	(1.14-3.25) 2.05	(6.54-22.29) 15.84	(7.68-25.55) 17.89
Boiling	85.30±0.80	34.12±9.25	32.30±18.56	65.36±19.27	2.01±0.84	13.12±5.91	15.21±6.62
	(84.17-86.20) 85.52	(1.88-42.91) 35.78	(14-55.12) 28.50	(35.11-85.12) 69.23	(1.03-2.96) 2.04	(6.81-20.12) 12.99	(8.13-23.08) 15.67
Stir-frying	72.25±3.12	67.88±37.60	45.31±17.22	93.41±25.67	4.40±2.29	13.28±9.08	17.68±10.47
	(69.50-76.38) 70.31	(27.36-107.11) 64.78	(29-70.85) 48.00	(54.21-112.41)110.12	(1.30-7.27) 4.15	(4.91-27.08) 11.26	(7.42-32.94) 18.53

Table 11 Moisture, vitamin C, tannin, catechin and phytate (IP5 and IP6) contents of vegetables (mg/100g of edible portion) [1,2,3] *(Continued)*

Food item	Moisture (mg/100g)	Vitamin C (mg/100g)	Tannin (mg/100g)	Catechin (mg/100g)	IP5 (mg/100g)	IP6 (mg/100g)	Total phytate (IP5 and IP6) (mg/100g)
leaves and tender tips:							
Paksien, pickled							
	88.86±0.97	41.27±4.73	13.26±1.95	10.92±1.64	1.49±0.24	4.36±0.77	5.85±0.82
	(87.52-90.01) 88.82	(34.96-46.12) 40.35	(11.25-15.91) 12.79	(8.28-12.32) 11.04	(1.28-1.89) 1.43	(3.13-5.07) 4.56	(4.63-6.77) 5.90
Sesbania, raw	79.20±2.76	72.43±35.23	62.51±58.08	27.77±19.32	0.99±0.27	12.09±2.93	13.09±2.72
	(76.40-83.52) 78.32	(25.23-109.41) 85.23	(19.40-159.50) 30.07	(6.85-54.91) 20.04	(0.61-1.25) 1.02	(8.47-16.43) 12.45	(9.69-17.04) 13.31
Blanching	86.35±1.84	24.26±11.18	41.84±38.76	16.53±11.14	0.55±0.33	7.20±1.21	7.75±0.94
	(84.47-88.47) 85.96	(7.01-36.73) 27.83	(12.93-106.33) 20.05	(3.23-30.96) 12.61	(0.30-1.12) 0.50	(5.55-8.53) 7.65	(6.67-8.83) 7.98
Boiling	86.63±1.70	16.93±7.78	27.11±21.28	14.12±9.66	0.48±0.20	6.37±2.05	6.85±2.03
	(84.78-89.09) 86.02	(4.20-24.09) 17.27	(11.42-62.33) 15.48	(2.84-28.12) 10.96	(0.40-0.57) 0.47	(4.12-9.50) 6.21	(4.59-9.96) 6.72
Flower:							
Banana, raw	91.95±0.22	0.50±0.29	295.74±20.62	139.55±41.63	1.20±0.26	5.35±1.58	6.65±1.70
	(91.75-92.23) 91.82	(0.25-0.88) 0.41	(275.37-318.33) 284.67	(98.58-188.25)120.55	(0.98-1.50) 1.06	(3.13-7.32) 5.32	(4.12-8.80) 6.38
Boiling	94.16±0.10	0.22±0.11	197.18±13.74	101.19±28.09	0.80±0.22	3.47±1.28	4.28±1.25
	(94.05-94.32) 94.13	(0.10-0.36) 0.25	(183.53-212.22) 189.87	(70.12-135.12) 95.12	(0.55-1.04) 0.72	(1.89-4.75) 3.57	(2.93-5.79) 4.29

Table 11 Moisture, vitamin C, tannin, catechin and phytate (IP5 and IP6) contents of vegetables (mg/100g of edible portion) [1,2,3] *(Continued)*

Food item	Moisture (mg/100g)	Vitamin C (mg/100g)	Tannin (mg/100g)	Catechin (mg/100g)	IP5 (mg/100g)	IP6 (mg/100g)	Total phytate (IP5 and IP6) (mg/100g)
Flower:							
Cowslip creeper, raw	88.67±0.80	66.29±16.09	16.04±6.55	58.20±9.55	2.78±1.84	32.51±17.57	35.29±19.29
	(87.49-89.45) 88.92	(47.08-85.54) 69.61	(11.46-27.33) 14.98	(46.67-70.12) 55.12	(1.01-5.25) 2.12	(18.34-54.21) 21.30	(19.73-58.35) 23.42
Blanching	91.92±0.31	43.95±11.27	8.46±4.44	41.57±11.64	1.70±1.08	18.83±11.78	20.54±11.94
	(91.52-92.32) 91.92	(30.24-57.29) 45.00	(6.21-16.40) 6.41	(25.27-54.17) 43.16	(0.74-3.44) 1.19	(6.80-36.08)13.51	(7.54-38.13) 15.82
Boiling	92.06±0.21	40.93±10.78	6.22±3.00	38.99±12.41	1.48±0.55	18.21±9.69	19.68±10.19
	(91.71-92.26) 92.10	(27.92-52.22) 43.35	(4.59-11.58) 4.97	(20.87-55.12) 39.79	(0.71-2.07) 1.44	(9.11-32.61) 13.52	(9.82-34.68) 14.74
Stir-frying	79.72±1.23	64.42±13.31	15.40±6.20	63.25±15.03	2.88±1.73	30.17±15.08	33.59±16.78
	(78.74-81.63) 79.09	(46.70-77.65) 70.34	(9.16-25.46) 15.21	(37.73-76.67) 65.41	(1.38-5.25) 2.12	(18.34-49.02) 21.30	(19.73-54.27) 23.42
Sesbania, yellow, raw	88.13±1.02	14.87±3.57	49.57±24.78	6.22±1.21	2.64±0.77	5.77±1.63	8.41±2.21
	(87.36-89.42) 87.44	(9.74-18.62) 14.57	(8.30-73.80) 58.15	(4.36-7.29) 6.52	(1.79-3.56) 2.27	(3.81-8.25) 5.89	(6.06-11.60) 8.16
Blanching	92.71±0.47	3.84±1.87	33.08±16.92	3.49±1.03	1.54±0.29	3.36±1.28	4.91±1.40
	(92.15-93.21) 92.92	(1.69-5.88) 3.81	(4.78-49.21) 39.41	(2.36-4.87) 3.74	(1.10-1.85) 1.52	(1.95-4.83) 3.78	(3.43-6.60) 4.89
Boiling	92.99±0.34	2.64±1.27	28.57±15.50	2.70±0.50	1.26±0.50	2.62±0.75	3.89±1.20
	(92.58-93.40) 93.05	(1.02-3.98) 2.32	(3.87-43.54) 29.18	(2.33-3.54) 2.46	(0.43-1.79) 1.36	(1.72-3.54) 2.55	(2.15-5.33) 3.96
Stir-frying	78.02±1.38	8.00±3.14	47.81±23.63	8.98±2.48	2.58±0.64	5.60±1.62	8.18±2.17
	(75.89-79.26) 78.54	(3.50-11.80) 8.52	(7.70-66.01) 59.45	(5.42-12.11) 9.11	(1.87-3.35) 2.27	(3.97-7.67) 6.11	(5.98-11.01) 8.61

[1]Mean ± SD, range (minimum – maximum) and median
[2] n = 5
[3]Tannin and catechin contents expressed in mg/100g of tannin equivalents and catechin equivalents, respectively

APPENDIX B

Table 12 Effect of cooking on the Catechin content of selected vegetables [1,2,3,4]

Food item	Cooking method	Time (min)	Catechin content (mg/100g wet weight)	Catechin content (mg/100g dry weight)	% retention Catechin	% Catechin loss
leaves and tips:						
Acacia pennata,	Raw		38.22 ± 16.35[a]	192.54 ± 77.62[a]		
leaves and tender tips	Blanching	2	21.71 ± 6.12 [b]	178.28 ± 65.00[a]	72.77 ± 11.92	27.23 ± 11.92
Bittercucumber,	Raw		11.26 ± 5.22[a]	139.73 ± 76.93[a]		
leaves and tender tips	Blanching	1	7.99 ± 3.22[a]	112.58 ± 52.44[a]	73.64 ± 15.44[a]	26.36 ± 15.44[a]
	Boiling	4	6.60 ± 2.81[a]	99.45 ± 49.27[a]	67.65 ± 16.53[a]	32.35 ± 16.53[a]
	Stir-frying	3	11.87 ± 5.38[a]	81.55 ± 39.94[a]	86.24 ± 5.43[a]	13.76 ± 5.43[a]
Cassia,	Raw		267.44 ± 93.38[a]	1155.52 ± 438.44[a]		
leaves and tender tips	Boiling	4	153.51 ± 50.78[a]	1155.97 ± 376.39[a]	71.55 ± 19.32	28.45 ± 19.32
Indian pennywort,	Raw		7.52 ± 2.27[a]	65.00 ± 30.77[a]		
leaves	Blanching	1	5.31 ± 2.04[a]	62.87 ± 38.74[a]	81.88 ± 8.11	18.12 ± 8.11
Leadtree,	Raw		nd	nd		
leaves and tender tips	Blanching	1	nd	nd	nd	nd
Neem tree,	Raw		132.84 ± 73.86[a]	541.42 ± 286.87[a]		
leaves and tender tips	Blanching	2	100.32 ± 53.56[a]	539.54 ± 264.65[a]	77.07 ± 12.37	22.93 ± 12.37

Table 12 Effect of cooking on the Catechin content of selected vegetables[1,2,3,4] *(Continued)*

Food item	Cooking method	Time (min)	Catechin content (mg/100g wet weight)	Catechin content (mg/100g dry weight)	% retention Catechin	% Catechin loss
leaves and tips:						
Paco (Oke fern),	Raw		168.52 ± 80.03[a]	1566.08 ± 682.89[a]		
leaves and tender tips	Blanching	1	133.81 ± 51.93[a]	1477.30 ± 543.96[ab]	81.90 ± 2.10[a]	18.10 ± 2.10[a]
	Stir-frying	2	121.24 ± 61.52[a]	615.43 ± 297.46[b]	67.03 ± 20.32[a]	37.97 ± 20.32[a]
Pagwanban,	Raw		106.86 ± 42.65[a]	610.57 ± 236.16[a]		
leaves and tender tips	Blanching	2	64.84 ± 30.73[a]	615.57 ± 312.62[a]	72.02 ± 15.63[a]	27.98 ± 15.63[a]
	Boiling	4	52.80 ± 23.13[a]	512.59 ± 219.76[a]	62.74 ± 12.38[a]	37.26 ± 12.38[a]
	Stir-frying	3	158.60 ± 128.24[a]	477.49 ± 318.60[a]	78.19 ± 11.54[a]	21.81 ± 11.54[a]
Pagwanpa,	Raw		88.10 ± 24.55[a]	469.25 ± 163.97[a]		
leaves and tender tips	Blanching	1	68.83 ± 19.59[a]	447.97 ± 110.36[a]	73.94 ± 5.30[a]	26.06 ± 5.30[a]
	Boiling	2	65.36 ± 19.27[a]	441.30 ± 117.44[a]	73.42 ± 9.82[a]	26.58 ± 9.82[a]
	Stir-frying	1	93.41 ± 25.67[a]	345.16 ± 119.79[a]	77.96 ± 6.56[a]	22.04 ± 6.56[a]
Paksein, pickle	Fermentation		10.92 ± 1.64	96.02 ± 10.31		

Table 12 Effect of cooking on the Catechin content of selected vegetables [1,2,3,4] *(Continued)*

Food item	Cooking method	Time (min)	Catechin content (mg/100g wet weight)	Catechin content (mg/100g dry weight)	% retention Catechin	% Catechin loss
Sesbania, ,	Raw		27.77 ± 19.32[a]	130.22 ± 80.79[a]		
leaves and tender tips	Blanching	2	16.53 ± 11.14[a]	117.63 ± 68.35[a]	73.20 ± 10.29[a]	26.80 ± 10.29[a]
	Boiling	5	14.12 ± 9.66[a]	104.06 ± 64.40[a]	64.29 ±9.39[a]	35.71 ± 9.39[a]
flower:						
Banana,	Raw		139.55 ± 41.63[a]	1738.02 ± 527.29[a]		
flower	Boiling	5	101.19 ± 28.09[a]	1734.28 ± 438.79[a]	73.15 ± 0.97	31.27 ± 0.97
Cowslip creeper,	Raw		58.20 ± 9.55[a]	519.23 ± 114.21[a]		
flower	Blanching	3	41.57 ± 11.64[a]	516.14 ± 152.73[a]	68.42 ± 13.75[a]	31.58 ± 13.75[a]
	Boiling	5	38.99 ± 12.41[a]	491.22 ± 157.80[a]	68.54 ±13.61[a]	31.46 ± 13.61[a]
	Stir-frying	5	63.25 ± 15.03[a]	313.99 ± 79.71[a]	74.28 ± 15.33[a]	25.72 ± 15.33[a]
Sesbania, , yellow,	Raw		6.22 ± 1.21[ab]	51.99 ± 6.65[a]		
flower	Blanching	1	3.49 ± 1.03[b]	47.60 ± 12.12[a]	68.64 ± 10.12[a]	31.36 ± 10.21[a]
	Boiling	3	2.70 ± 0.50[b]	38.42 ± 5.96[a]	57.03 ± 5.60[a]	42.97 ± 5.60[a]
	Stir-frying	2	8.98 ± 2.48[a]	40.74 ± 10.88[a]	84.71 ± 2.05[b]	15.29 ± 2.05[b]

[1]Data reported in mean + SD of duplicate analysis, n=5

[2]Values within the same column with different superscripts are significant difference by one-way ANOVA with Sheffe's test and independent t-test at $p < 0.05$.

[3]Median and range values are reported in Appendix A

[4] Catechin content expressed in mg/100g of catechin equivalents

APPENDIX C

Table 13 Vitamin C content of raw and cooked vegetables per 100 g wet weight and one serving size

Food item	Cooking method	Time (min)	Vitamin C content (mg)		Recommended amount of food per meal (g) (2 rice-serving spoons)[3]
			per 100g wet weight[1]	Amount perone serving(% Thai RDI)[2]	
leaves and tender tips:					
Acacia pennata,	Raw		56.54 ±11.17	28 (47 %)	50
leaves and tender tips	Blanching	2	12.34 ± 2.85	7 (12 %)	60
Bittercucumber,	Raw		6.23 ± 2.05	3 (5 %)	45
leaves and tender tips	Blanching	1	1.13 ± 0.75	0.68 (1 %)	60
	Boiling	4	0.34 ± 0.35	0.20 (0.34 %)	60
	Stir-frying	3	1.66 ± 1.09	1 (2 %)	65
Cassia,	Raw		62.01 ± 10.14	25 (41 %)	40
leaves and tender tips	Boiling	4	16.90 ± 5.93	9 (15 %)	55
Indian pennywort,	Raw		22.83 ± 15.07	10 (16 %)	42
leaves	Blanching	1	11.42 ± 7.53	6 (10 %)	50
Leadtree,	Raw		10.77 ± 3.88	5 (8 %)	45
leaves and tender tips	Blanching	1	5.37 ± 1.94	3 (4 %)	50
Neem tree,	Raw		71.53 ± 1.07	27 (48 %)	40
leaves and tender tips	Blanching	2	46.48 ± 1.95	21 (35 %)	45

Table 13 Vitamin C content of raw and cooked vegetables per 100 g wet weight and one serving size

Food item	Cooking method	Time (min)	Vitamin C content (mg)		Recommended amount of food per meal (g) (2 rice-serving spoons)[3]
			per 100g wet weight[1]	Amount perone serving(% Thai RDI)[2]	
leaves and tender tips:					
Paco (Oke fern),	Raw		4.15 ± 1.93	2 (3 %)	45
leaves and tender tips	Blanching	1	2.49 ± 1.41	1 (2 %)	55
	Stir-frying	2	0.36 ± 0.41	0.22 (0.36 %)	60
Pagwanban,	Raw		78.46 ± 31.90	41 (68 %)	52
leaves and tender tips	Blanching	2	12.23 ± 5.59	7 (12 %)	60
	Boiling	4	10.26 ± 4.66	6 (10 %)	60
	Stir-frying	3	70.80 ± 19.80	50 (83 %)	70
Pagwanpa,	Raw		83.59 ± 27.93	38 (63 %)	45
leaves and tender tips	Blanching	1	43.88 ± 10.91	22 (37 %)	50
	Boiling	2	34.12 ± 9.25	17 (28 %)	50
	Stir-frying	1	67.88 ± 37.60	41 (68 %)	60
Paksien, pickled	Fermentation		41.27 ± 4.73	21 (34 %)	50

Table 13 Vitamin C content of raw and cooked vegetables per 100 g wet weight and one serving size

Food item	Cooking method	Time (min)	Vitamin C content (mg)		Recommended amount of food per meal (g) (2 rice-serving spoons)[3]
			per 100g wet weight[1]	Amount perone serving(% Thai RDI)[2]	
Sesbania,	Raw		72.48 ± 35.23	25 (42 %)	35
leaves and tender tips	Blanching	2	24.26 ± 11.18	9 (16 %)	40
	Boiling	5	16.93 ± 7.78	7 (11 %)	40
flower:					
Banana,	Raw		0.50 ± 0.29	0.23 (0.48 %)	55
flower	Boiling	5	0.22 ± 0.11	0.13 (0.22 %)	60
Cowslip creeper,	Raw		66.29 ± 16.09	36 (61 %)	55
flower	Blanching	3	43.95 ± 11.27	26 (44 %)	60
	Boiling	5	40.93 ± 10.78	25 (41 %)	60
	Stir-frying	5	64.42 ± 13.31	42 (70 %)	65
Sesbania, yellow,	Raw		14.87 ± 3.57	8 (14 %)	55
flower	Blanching	1	3.84 ± 1.87	3 (4 %)	65
	Boiling	3	2.64 ± 1.27	2 (3 %)	65
	Stir-frying	2	8.00 ± 3.14	6 (9 %)	70

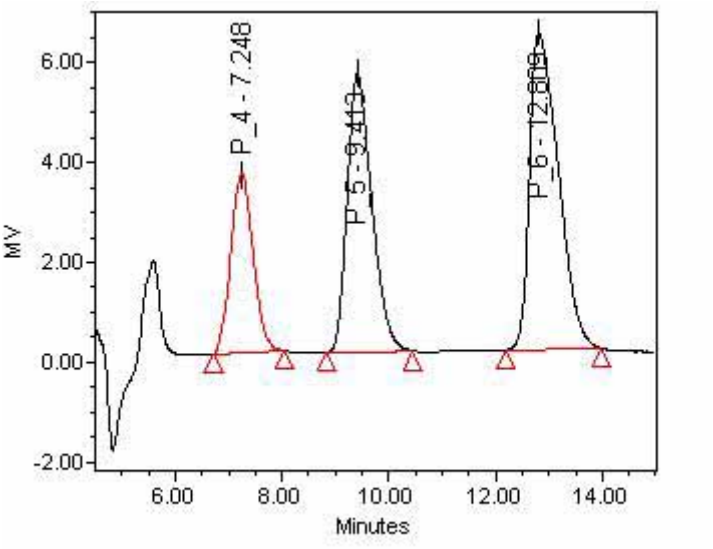
[1]Data reported in mean + SD of duplicate analysis, n=5

[2]Thai RDI for vitamin C = 60 mg/day

[3]Amount of food consumed per meal, recommended in the Thai FBDG, 2001, Ministry of Public Health, Thailand

APPENDIX D

Chromatogram of Inositol phosphates inositol tetraphosphates (IP4), inositol pentaphosphates (IP5), and inositol hexaphosphates (IP6)



APPENDIX E
Determination Method of Moisture
(Hot Air-Oven Method, AOAC, 1990)

Principle :

The individual well-homogeneous sample is dried in the hot air oven (usually at 100 ± 5 ^{0}C, 2-4 hr) until the constant weight is obtained. The weight lost represents the moisture content in the sample. The acid washed sand is mixed with the wet well-homogeneous sample prior to dry in order to increase area for rapid and complete evaporation of water from the wet sample.

Procedure :

1. Approximately weigh 20 g of acid washed sand into a porcelain dish containing a small glass stirring rod. Dry in the hot air oven before use at 95-105 ^{0}C for 30 minutes.
2. Remove the sand dish and cool in the desiccator at the room temperature.
3. Weigh sand dish (= a g) and then add proximate 5 g of the sample. Reweigh (= b g)
4. Add small amount of distilled water to disperse consistently the sample and evaporate the water as much as possible on the boiling water bath. The sample-sand mixture should be frequently mixed until dry.
5. Place the sample dish into the hot-air oven at 95-105 ^{0}C for 2 hr.
6. Remove the sample dish and cool in the desiccator until the weight at room temperature is obtained. Then weigh (= c g).
7. Return the sample dish to the hot-air oven and dry until a constant weight is obtained. Reweigh every 30 min.
8. The weight between each interval time should not differ more than 1-3 mg.

Calculation :

$$\% \text{ Moisture} = \frac{b-c}{b-a} \times 100 \text{ (w/w)}$$

APPENDIX F

Determination of Vitamin C

(Microfluorometric method, AOAC, 2000, 967.22)

Principle :

Ascorbic acid is oxidized to dehydroascorbic acid in the present of Norit (activated charcoal). The oxidized form is reacted with 1,2-phenylendiamin-dihydrochloride to produce a fluorophor. Fluorescent intensity is proportional to the concentration of vitamin C is prevented by forming a H_3BO_3 dehydroascorbic acid complex prior to addition of diamine solution.

Reagents :

1. Extraction solution (prepare freshly for each analysis: Dissolve 30 grams metaphosphoric (Merck # 1.00546.500) in 80 ml of Conc. Glacial acetic acid and stir until solution clear and then dilute to 100 ml with DI water.
2. Color reagent: dissolve 0.01 grams 1,2-phenylendiamine-dihydrochloride (Fluka # 78440) in 500 ml DI water.
3. 50 % sodium acetate solution: Dissolve 50 grams sodium acetate trihydrate (Merck # 1.06267.1000) in 100 ml of 50% sodium acetate.
4. Activated charcoal: Norit (Fluka # 05120).
5. Standard vitamin C: L+ ascorbic acid Merck # 1.00127 at concentration 1000 µg/ml
6. Blank solution (freshly prepare for each analysis): weigh 3 grams of Boric acid (Merck # 1.00165.100) and dissolve in 100 ml of vol. Flask with 50% sodium acetate.

Apparatus :

1. Spectrophotometer (Shimadzu ^{TM}; Model RF-540)
2. Vortex mixer
3. Altra-turrax

Procedure:

1. Weight 5-10 g of homogenous sample in to 250 ml beaker.
2. Add 30 ml extract solution into the sample and then mix well with Altra-turrax.
3. Pour the sample solution to 100 ml volumetric flask and dilute to 100 ml with extract solution.
4. Transfer the sample solution to 250 ml flask which contained 2 grams Norit (Activated charcoal) and mix well and the sample solution was filtrated by filter paper (Whatman # 42).
5. Pipette 5 ml supernatant of sample onto two flasks of 100 ml volumetric flask.
6. Add 5 ml of 50 % sodium acetate solution and dilute to 100 ml with DI water and the other flask add 5 ml of 3 % Boric acid (sample blank) and dilute to 100 ml with DI water and keep in dark for 15 min at room temperature.
7. Pipette 2 ml sample solution or sample blank solution (in duplicate analysis) into test tube.
8. Add 5 ml of color reagent and mix well.
9. Keep in dark at room temperature for 35 minutes.
10. Measure using Fluorescence spectrophotometer at excitation at wavelength 350 nm and emission at wavelength 430 nm.

Standard preparation:

1. Weigh accurately standard vitamin C 0.1 g into 50 ml of beaker and add small amount of extraction solution and mix well.
2. Carefully pour the standard vitamin C solution into 100 ml of volumetric flask and dilute to final volume with extraction solution.

Working standard (conc. 10-100 µg/ml):

Concentration (µg/ml)	Vol. Pipette (ml)	Vol. Flask (ml)*
10	1	100
30	3	100
50	5	100
100	10	100

*Dilute to final volume (100 ml) with extraction solution

3. Weigh activated charcoal 2 grams into 250 ml Erlenmeyer flask.
4. Transfer each concentration of working standard into 250 ml Erlenmeyer flask which contained activated charcoal.
5. Filtrate the solution with filter paper (Whatman # 541 or 42).
6. Pipette 5 ml sample solution (in duplicate) into two flask of 100 ml volumetric flask and then add 5 ml of 3 % Boric acid and dilute to 100 ml with DI water.
7. Mix well and keep in dark room temp for 15 min.
8. Pipette 2 ml sample solution or standard blank solution (in duplicate analysis) into test tube.
9. Add 5 ml of color reagent and mix well.
10. Keep in dark at room temp for 35 minutes.
11. Measure using Fluorescence spectrophotometer at excitation at wavelength 350 nm and emission at wavelength 430 nm.

Calculation :

$$\text{Vitamin C (mg/100g)} = \frac{(\text{sample reading- blank reading}) \times \text{Conc.standard} \times \text{dilution} \times 100}{(\text{standard reading} - \text{standard blank reading}) \times \text{weight of sample} \times 1000}$$

APPENDIX G

Determination of Tannin and Catechin (Spectrophotometer Method)

Principle :

Phenolic compounds are extracted from food samples by dimethylformamide (DMF) in an acetate buffer. A ferric ammonium sulphate reagent is added and the resulting color is read spectrophotometrically at two wavelengths corresponding to the absorbance maxima of Fe-catechol and Fe-galloyl complexes. Food blanks and reagent blanks are subtracted (modified method from Brune *et al*., 1991).

Procedure :

1. Acetate buffer (0.1 M, pH 4.4):

   Solution A: add 11.5 ml acetate acid (CH_3COOH) (Merck # 1.0063.2500) to 1000 ml deionized water.

   Solution B: 16.4 g of sodium acetate (CH_3COONa) (Merck # 1.06267.1000) is dissolved and diluted to 1000 ml by deionized water.

Mixture solution A and B (Acetate buffer): Mix 305 ml of solution A and 195 ml of solution B, adjust the pH to 4.4 with sodium hydroxide (Merck # C591798) and diluted to 1000 ml with deionized water.

2. 50 % dimethyformamide acetate buffer (50% DMF-acetate solution): Carefully mix 500 ml dimethyformamide (Carlo Erba # 444923) with 500 ml 0.1 M, pH 4.4 acetate buffer.
3. 50 % Urea in acetate buffer: Dissolve 250 g Urea (H_2NCONH_2) (Univar # FOB271) in 500 ml 0.1 M. pH 4.4, acetate buffer.
4. 1 % Arabic gum: Dissolve 1 g Arabic gum (Sigma # G-9752) in 100 ml deionized water.

5. 5 % Ferric Amonium Sulfate (FAS): Dissolve 250 g (NH4 Fe(SO4)2.12H2O) (Merck # 1.03776.1000) in 100 ml 1M hydrochoric acid (HCl) (Merck # 1.00317.2500).
6. Food blank reagent: prepare by mixing, just before use;

   89 parts of 50 % urea in 0.1 M. acetate buffer solution
   10 part of 1 % Arabic gum
   1 part of 1 M HCl
7. FAS reagent (Iron reagent): Prepare by mixing, just before use;

   89 parts of 50 % urea in 0.1 M. acetate buffer solution
   10 part of 1 % Arabic gum
   1 part of 5 % ferric-ammonium sulphate

Apparatus:

1. Spectrophotometer (Unicam Beta)
2. Vortex mixer
3. Automatic pipette

Procedures:

1. Weigh 2.8 g of sample into a 125 ml Erlenmeyer flask.
2. Add 50 ml 50 % DMF-acetate solution.
3. Sample flask was covered with parafilm and shaking for 16 hours at room temperature.
4. Remove sample from shaker and filter through a paper filter (Whatman # No.42)
5. 2 ml of filtrate is vigorously shaken with 8 ml FAS-reagent in a 15 ml test tube. After 15 min the sample is read at 578 and 680 nm against in a regent blank consisting of 2 ml DMF-acetate and 8 ml of FAS-reagent.
6. The food blank is prepared by mixing 2 ml of the filtrate with 8 ml of food blank reagent in a 15 ml test tube. After 15 min the sample is read against a blank consisting of 2 ml DMF-acetate and 8 ml of food blank reagent at both wavelengths.
7. Values for food blank are subtracted from polyphenol extinction at each wavelength. The resulting net extinction values at 578 and 680 nm are used in the

calculations.

8. Standard solutions containing tannic (TA) and catechin (C) are read with unknown samples, reagent blank and food blanks in each series.

Standard preparation:

1. Standard tannin: Dissolve 0.5 g of tannic acid (SIGMA CAT.No. T-0125) in 50 % DMF acetate and make up to 50 ml volume.
2. Standard catechin: Dissolve 0.5 g of catechin (SIGMA CAT.No. C-1788) in 50 % DMF acetate and make up to 50 ml volume.

Working standard (conc. 25-400 μg/ml) (stock standard conc. 1000 μg/ml)

Concentration (μg/ml)	Vol. pipette (ml)	Vol. Flask (ml)*	Pipette (ml)	FAS- regent (ml)	Final Vol. (ml)
25	0.25	10	2	8	10
50	0.5	10	2	8	10
100	1	10	2	8	10
200	2	10	2	8	10
400	4	10	2	8	10

*Dilute to final volume (10 ml) with 50 % DMF acetate

Note:

The food blank absorbance is subtracted from the food sample absorbance at 578 nm and 680 nm. The absorbance spectra of two kinds of Fe-pheolic complexes overlapped. The content of galloyl and catechol groups in the sample is, therefore, calculate using linear regression equations for standard curves, tannic acid (galloyl groups) and catechin (catechol proups) at two wavelengths. The resulting equation set is readily solved with a programmable calculator.

1. abbreviations
   A. Unknown samples;
      N578 = Net sample extinction at 578 nm
      N680 = Net sample extinction at 578 nm
      SW = Sample weight in grams
   B. Standard solutions
      St.ext TA 578 = Standard extinction tannic acid at 578 nm

St.ext TA 680 = Standard extinction tannic acid at 680 nm

St.ext TA 578 = Standard extinction catechin at 578 nm

St.ext TA 680 = Standard extinction catechin at 680 nm

St.cone.T = Standard concentration tannic acidμg/ml

St.cone.C = Standard concentration catechin μg/ml

Text.578 = True extinction at 578 nm (tannin extinction)

Text.680 = True extinction at 680 nm (catechin extinction)

2. Calculations

A. Extinction ration of standard:

$$K1 = \frac{\text{St.ext C578}}{\text{St.ext C 680}}$$

$$K2 = \frac{\text{St.ext TA 578}}{\text{St.ext TA 680}}$$

B. Calculation of content of tannin and catechin in unknown samples

Step I. Calculation of true extinctions:

$$\text{T ext.578} = \frac{N^{578} - K_1 N^{680}}{1-K_1K_2}$$

$$\text{T ext.680} = \frac{N^{680} - K_1 N^{578}}{1-K_1K_2}$$

StepII. Calculation of amount of catechin equivalents (mg/100g) and tannin equivalents (mg/100)

$$\text{Tannin equivalents (mg/100)} = \frac{\text{St. conc TA} \times 50 \times \text{T ext. 578}}{\text{SW} \times \text{ST. ext. TA 578}} \times 100$$

$$\text{Catechin equivalents (mg/100)} = \frac{\text{St. conc C} \times 50 \times \text{T ext. 680}}{\text{SW} \times \text{ST. ext. TA}} \times 100$$

APPENDIX H

Determination of Phytate in Food Samples

(Modified Method from Hotz's, 2001)

Principle

Base on Hotz's methods (2001), approximately 0.5 g of dried sample was extracted with 0.67 M HCl. The supernatant after centrifuged was removed and diluted with Millipore water and then percolated trough strong anion exchange columns (Sep-pak (anion-exchange column), commercial columns). Innositol phosphates were eluted and evaporated to dryness, and the residue was diluted with 1 ml of Millipore water before determined using ion-pair reverse-phase chromatography.

Reagent

1. Tetrabutylammonium hydroxide solution (TBNA) (Flika # 86881)
2. Phosphoric acid 85% (Merck # 1.00573.1000)
3. Methanol (CH_3OH) (Baker analyzed # 9070-68)
4. Sulfuric acid (NH_2SO_4) (Merck # 1.00731.2500)
5. Inositol hexaphosphoric acid dodecasodium salt, from corn (P-8810, Sigma Chemical Co.)

Apparatus

1. Vortex mixer (Model: G-560E, Scienctific industries)
2. Centrifugation machine (Model: H-103 N series Kokusan enchinki Co.)
3. Sonicater (Model: 1510E-MT Bransonic ®)
4. Vacuum manifolds (Model: Vacuum manifold processing station, Agilent technologies)
5. Evaporator vacuum (Model: SpeedVac concentrator (SVC 100H), Savant instrument INC.)
6. HPLC system
7. Sep-pak, Vac 1cc. (100 mg) (Water® Accell™ Plus QMA cartridges Part No:WAT023620)

Prodecure

1. Approximately weigh 0.5 g of dried sample into test tube in duplicate.
2. Add 5 ml of 0.67 M HCl into each sample tube and homogenize by vortex mixer and sonicate in a bath for 30 minutes.
3. Centrifuge sample tubes at 3000 rpm for 10 minutes, then remove 2.5 ml of supernatant and dilute with 22.5 ml Millipore water.
4. Condition anion-exckange columns by adding 3 ml of 0.067 M HCl to sep-pak columns which is performed on vacuum manifolds and adjust flowrate to be 0.5-1 ml/min.
5. Add diluted samples (final volume about 25 ml) through the sep-pak column with flow rate 0.5-1 ml/min; discard eluted.
6. Add 2 ml of 0.067 M HCl; discard eluted.
7. Elute with 4 ml 2 M HCl ; collect this fraction in test tube.
8. Eluants are evaporated to dryness in evaporator vacuum at 40 °C.
9. The residue is dilute with 1 ml Millipore water and then sonicated for 10 minutes before injection into HPLC system

Preparation of the Moblie Phase for HPLC System

1. Add 1.1 ml phosphoric acid in 200 Millipore water.
2. Add 10 ml TBNOH in solution and then add 300 ml methanol (CH_3OH), let cool at room temperature (total volume is 500 ml).
3. Adjust pH to 4.0 with 9 N sulfuric acid (NH_2SO_4).
4. Filtrate the mobile phase by vacuum filtration through a 0.45 μm filter.

Preparation of the Standard Phytate

Stock Standard:

Weigh Dodecasodium phytate 0.09238 g and then dilute with 10 ml of Millipore water.

Standard Dilutions:

3.2 μmol/ml = 400 μl of stock standard + 600 μl Millipore water

1.6 μmol/ml = 200 μl of stock standard + 800 μl Millipore water

0.8 μmol/ml = 100 μl of stock standard + 900 μl Millipore water

0.4 μmol/ml = 50 μl of stock standard + 950 μl Millipore water

Apparatus and Chromatographic Condition for HPLC system

The HPLC system consists of pump equipped with a loop injector (515 HPLC pump water), ion-pair reverse-phase column (model: μ Bondapak C18 type: 125A°, 10 μm, size: 3.9 mm × 300 mm), and a reflective index detector (model: varian series RI3). The flow rate of the pump is 0.45 ml/min. The column is performed in a heater block at 45 °C.

Calculation:

The content of inositol phosphate (IP5 and IP6) is calculated in mg/100g of samples.

$$\text{Inositol phosphates (mg/100g)} = \frac{A \times B \times C \times D}{E \times F \times 10}$$

Where;

A = sample area

B = standard concentration (μmol/ml)

IP5 = $\frac{660.08}{580.1} \times$ % peak area std.IP5 × 3.2

IP6 = % peak area std.IP5 × 3.2

C = Molecular weight of inositol phosphates (IP5 or IP6)

IP5 = 580.1

IP6 = 660.08

D = dilution factor

E = standard area (IP5 or IP6)

F = weigh of sample (g)

BIOGRAPHY

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