PHYTOCHEMICALS TESTS ON PEANUTS (*Arachis hypogaea* L.) OF SABAH FOR SCREENING OF VARIOUS CHEMICAL COMPOUNDS

LIO BI INN

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF FOOD SCIENCE WITH HONOURS IN FOOD SCIENCE AND NUTRITION

FOOD SCIENCE AND NUTRITION PROGRAMME
SCHOOL OF FOOD SCIENCE AND NUTRITION
UNIVERSITY MALAYSIA SABAH
2006

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: PHYTOCHEMICALS TESTS ON PEANUTS (Arachis hypogaea L.) OF SABAH FOR SCREENING OF VARIOUS CHEMICAL COMPOUNDS

IJAZAH: SARJANA MUDA SAINS MAKANAN DAN PEMAKANAN

SESI PENGAJIAN: 2003/2004

Saya LIO BI INN

(HURUF BESAR)

mengaku membenarkan tesis (LPS/(Sarjana)/ Doktor Falsafah) ini di simpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. ** Sila tandakan (/)

[ ]	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
[ ]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukakan oleh organisasi/badan di mana penyelidikan dijalankan)
[/]	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: 35, JALAN PRI 6,
TMN PAYA RUMPUT INDAH,
76450 MELAKA.

DR MUHAMMAD IQBAL HASHMI
Nama Penyelia

Tarikh: 23/5/2006

Tarikh: 23/5/2006

CATATAN: * Potong yang tidak berkenaan.
* Jika tesis ini SULIT atau TERHAD, sila lampiran surat daripada pihak berkuasa/organsasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
* Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

DECLARATION

The materials in this thesis are original except for quotations, excerpts, summaries and references, which have been duly acknowledged.

(LIO BI INN)

HN2003-2385

13 April 2006

VERIFICATION

CERTIFIED BY

Signature

1. SUPERVISOR

(DR. MUHAMMAD IQBAL HASHMI)

2. EXAMINER – 1

(DR. CHYE FOOK YEE)

3. EXAMINER – 2

(MISS NOR QHAIRUL IZZREEN MOHD NOOR)

4. DEAN

(PROF. MADYA DR. MOHD. ISMAIL ABDULLAH)

ACKNOWLEDGEMENT

First and foremost, I would like to express my thankfulness and appreciation to my thesis supervisor, Dr. Muhammad Iqbal Hashmi. He had helped me a lots by discussing with me and sharing his opinions during the process of completing this research. With his guidance, supports and encouragements had helped me in completing this thesis.

With highest appreciation, I would like to send my regard and appreciation to Dean of School of Food Science and Nutrition, Prof. Madya Dr. Mohd. Ismail Abdullah and all the lecturers that had been helping me in completing my thesis.

Besides that, I would like to thank to the laboratory assistants in School of Food Science and Nutrition, University Malaysia Sabah by aiding me in the process of accomplish this thesis. I also would like to appreciate Mr. Mustaffa, IBTP Science Official in helping me to complete my laboratory works.

Nevertheless, I would like to appreciate my friends, Shakirah, Ting Soon, Tze Yin, Xue ni, Shy Chiou, Chee Ping, Kok Kheng, Alex, Dicken Wong and Mrs Susie who have helped me by giving me encouragements, advices and supports to me when carried out this thesis.

Finally, to my beloved family, thank you for their endless encouragements and supports to me.

ABSTRAK

UJIAN FITOKIMIA PADA KACANG TANAH (Arachis hypogaea L.) SABAH UNTUK PEMANTAUAN PELBAGAI KOMPONEN BAHAN KIMIA

Objektif utama kajian yang dijalankan adalah untuk mengenalpasti secara kualitatif pada kumpulan kelas organik yang hadir dalam kacang tanah di Sabah. Tiga variati kacang tanah iaitu Bandau Triplets, Kacang Raja dan MGNT VI telah diperolehi dari Jabatan Penyelidikan Agronomi Tuaran sebagai sampel analisis. Oleh kerana komponen utama dalam peanut adalah lemak, jadi penentuan kandungan lemak pada kacang tanah adalah salah satu objektif kajian. Kandungan lemak pada kacang tanah ditentukan secara kuantitatif dengan Kaedah Soxhlet manakala sterol dalam kacang tanah ditentukan secara kualitatif dengan menggunakan silica gel 60 HF254. Ujian fitokimia telah dijalankan dalam kajian ini untuk menguji kehadiran kumpulan alkaloids, flavonoids, saponin, tannin, antakuinon dan komponen fenolik dalam sampel kacang tanah. Kumpulan alkaloids, flavonoids, saponin dan kompone fenolik didapati hadir dalam ketiga-tiga sample kacang tanah manakala tannin dan antrakuinon adalah tidak hadir. Kandungan lemak yang paling tinggi didapati pada varieti Kacang Raja diikuti dengan varieti Bandau Triplets dan MGNT VI. Dalam TLC, komponen adalah di dalam Rf 0.11 dan diidentifikasikan secara kualitatif sebagai komponen sterol. Pembawa larutan yang diguna dalam TLC ialah hexane: diethyl ether: methanol dengan nisbah 25:25:50.

ABSTRACT

PHYTOCHEMICALS TESTS ON PEANUTS (Arachis hypogaea L.) OF SABAH FOR SCREENING OF VARIOUS CHEMICAL COMPOUNDS

The main objective of this research is to identify qualitatively the different classes of organic compounds in local peanuts. Three varieties of peanuts; Bandau Triplets, Kacang Raja and MGNT VI which obtained from Tuaran Agriculture Research Centre are as the sample in this analysis. Since the main components of peanuts are lipids, therefore lipids determination on peanuts is interested in this research. Total lipids in peanuts are determined quantitatively by using Soxhlet method while sterols in peanuts are identified qualitatively by using thin layer chromatography silica gel 60 HF254. Phytochemical tests is carried out to test the present of alkaloids, flavonoids, saponin, tannins, anthraquinone and phenolic compounds in the peanuts sample. Alkaloids, flavonoids, saponins and phenolic compounds are present in three of the peanut samples while tannins and anthraquinone are absent in the sample. The highest total lipids yield is Kacang Raja follow by Bandau Triplets and MGNT VI. In the TLC sheets, the compound is in Rf 0.11 and qualitatively identified as sterol compounds. The developing solvent system in the TLC that manages to well separate the compounds is hexane: diethyl ether: methanol with the ratio 25:25:50.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS

ANFs	antinutritional factors
AOAC	*Association of Official Analytical Chemists*
AOCS	American Official Chemical Society
DMAPP	dimethylallyl pyrophosphate
$FeCl_3$	ferum chloride
FPP	farnesyl pyrophosphate
GGPP	geranyl geranyl pyrophosphate
H_2SO_4	*Sulphuric acid*
HCl	Hydrochloric acid
IPP	isopentenyl pyrophosphate
K_2O	Potassium oxide
LD	lethal doses
N_2	gas ammonia
Nd	Nitrogen deficiency
NH_4^-	ammonium
NH_4OH	ammonium hydroxide
NO_3^-	Nitrate
OSC	oxidosqualene cyclase
PAL	phenylalanine ammonia lyase
P_2O_5	Phosphorus peroxide
QAs	Quinolizidine alkaloids
TLC	Thin Layer Chromatography
UV	ultraviolet

SYMBOLS

%	Percentage
cm	Centimeter
g	Gram(s)
kg	Kilogram(s)
mL	Milliliter(s)
mM	miliMolar
mg	Milligram
°C	Degree Celsius

CHAPTER 1

INTRODUCTION

1.1. Phytochemicals at a glance

Phytochemicals are a major group that believes to contribute health effects to human nutrition. Phytochemicals includes lots of organic compounds and the novel compounds still continued to discover by researcher until this decade. When come to phytochemicals, the first thing come to our mind is the antioxidant properties that characterized them. There are too many phyotchemicals group that involved in this properties and therefore it is so familiar to people. Definitely, phytochemicals is not limited to the antioxidant properties only. They still have others health benefits properties such as reduce certain chronic disease such as cardiovascular disease and immune system boost up (Pennington, 2002).

The major groups of phytochemicals that may contribute to the total antioxidant capacity (TAC) of plant foods include polyphenols, carotenoids and the traditional antioxidant vitamins such as vitamin C and vitamin E. The vitamins are, however, not the only phytochemicals that can have a positive effect on the health of consumers. There are other phytochemicals present in plant foods that may have positive effects on the health of consumers and need further investigation. These phytochemicals may be present in small amounts but may be very important to the health of consumers (Lako *et al.*, 2006). Due to small amounts of phytochemicals

can bring up the wonder effect; therefore they are actually made up interest of not only researcher but to those people curious about it.

1.2. Interest in Phytochemicals

Societies nowadays are more health conscious. In terms of nutrition prospects, most of the plants compounds are in the dominant position if compare to animals compounds. Many natural compounds in plants had targeted by researcher in order to find out the benefits of those plants in human nutrition. Medicinal treatments are not any more become the hot topic in this century. The societies had realized that prevention is far better than treatment and natural compounds are more advantageous than synthetic products. Phytochemicals had raised the attention of researcher especially recent year because of the availability of discovered phytochemicals and lots of their health effect towards man.

Phytoestrogen shows health benefits effect on osteoporosis, cardiovascular, thyroid function, central nervous system and immune system (Boyle *et al.*, 2003). Plant phenols may also regenerate other antioxidants and act synergistically with chain-breaking antioxidants under other sets of conditions. Polyphenols such as the flavonoids and anthocyanidins have metal chelating properties and bind otherwise redox-active metal ions. Finally plant phenols protect plants against UV-irradiation and light-induced oxidative damage and may have similar effects in food and beverages (Pedrielli & Skibsted, 2002). Due to the health benefits that provided to human, phytochemicals contents in many food products such as nuts, legumes, cereals and vegetables had been continuously discovered and compared.

Although phytochemicals is well known for its health benefits, but not all phytochemicals are well applied in man. Some natural compounds in food may present in restricted amount or have difficulty in human digestion and absorption tract. Phytosterol is one of the phytochemicals which is not very popular in the past because of the characteristic stated above. Due to poor solubility and bioavailability of the free phytosterols, high doses (up to 25-50 g per day) of phytosterol required for efficacy (Moreau, Whitaker & Hicks, 2002). More over, practical applications of phytosterols in foods are limited due to their crystalline property. The solubility of phytosterols in edible oils is very low while their melting points are rather high which is in the range of 140° C - 150° C (Piironen, Toivo & Lampi, 2000; Vu *et al.*, 2004).

Every element are believed to have dual nature and phytosterol too, theoretically not abandoned. Therefore, phytosterol and other phytochemicals now are gaining the interest of researcher because the application of phytochemicals in man had discovered. The growing interest of phytochemicals and their availability in the functional foods also one of the reason they are interested. There are lots of phytochemicals that can be applied or ready available in foods such as flavonoids, catechins and limonoids. The phytochemicals compounds stated above can easily get from vegetables and fruits. Due to this, interest in phytochemical had brought up by researchers so that people can fully or wisely utilize the foods that available around us.

Synthetic food supplements are widely available in the market and people nowadays are more aware and tend to switch their diet practices to the natural products. Phytochemical is playing an important role because they have potential to replace the synthetic food supplement. Improving the bioavailability of natural food

supplements with nutritional and health benefits (nutraceuticals) are of a great interest to the food industry. Therefore, lots of phytochemicals products had try to incorporate into foods so that the phytochemicals products can be easily reached by people (Spernath *et al.*, 2003).

In Malaysia, research on phytochemicals is not a popular topic. However, people are awake at the benefits of phytochemicals. It is very obvious that the market trend preferences is more to natural products even most of the consumers do not understand the word of phytochemicals. Supplements and sundry goods which phytochemicals added are commonly available in every shopping market and pharmaceuticals shop outlets. For example, toothpaste with tea polyphenol compounds, beverage with flavonoids compounds and so on.

Given the importance of dietary habit and food components to health, the provision of phytochemical and antioxidant information of a range of foods is trying to ready so that they are available to support the future work in assessing the protective status of people from chronic degenerative disorders. The approach used is to be effective and culturally appropriate to the community. Food-based approaches are the essential for sustainable solutions to combat the alarming prevalence of chronic cancer, coronary heart diseases and diabetes (Lako *et al.*, 2006).

1.3. Relationship between peanuts and phytochemicals

Peanuts (*Arachis hypogaea L.*) in Sabah consist of three varieties which are MGNT, Bandau Triplets and Kacang Raja. Bandau Triplets is widely cultivated and mostly found in Tamu market, Sabah. MGNT and Kacang Raja are also available in the

market but in small quantities. Basically, peanut plantations in Sabah are in a small scale (Jabatan Pertanian Sabah, 2003). Peanuts are a favour ate foods in Malaysia, as a table snack, just to munch after roasted, added in local foods such as rojak and so on. Peanuts are considered as detrimental to one's health because they contain aflatoxin and are an allergen to most of the people. Indisputable, peanuts do contains some phytochemicals that benefits to health (Anderson & Deskins, 1995).

The phytochemicals function in the peanuts usually is act as antioxidant and applied for oil stability of the products. Naturally-occurring phytochemicals in peanuts such as tocopherols, carotenoids, and polyphenolics, may have a role in slowing or preventing lipid oxidation, due to their antioxidative nature. In model systems, the exogenous addition of phenolic compounds, such as catechin and rosemarinic acid, were shown to enhance oxidative stability during storage of peanut oil and were as effective as common synthetic antioxidants (Chu & Hsu, 1999; Talcott *et al.*, 2005). Flavonoids compound is present in peanuts. Flavonoids compounds in the peanuts are depends on the maturity of the peanuts. Because of the indeterminate fruiting characteristics of peanuts, seeds of varying maturity are on the plant at harvest time which means maturity is depending on the harvest time. From here, we know that harvest time is important for the phytochemical value in peanut (Daigle *et al.*, 1988).

1.4. Objectives

In qualitative analysis, numbers of advance equipment can either included or excluded. Phytochemicals test, saponification extracts and Thin Layer Chromatography identification was carried out in this research. Research carried out for phytochemicals tests on peanuts of Sabah for screening of various chemical compounds. Three types of peanuts; MGNT, Bandau Triplets and Kacang Raja were

obtained from Tuaran Agriculture Research Centre and as the samples in this research. Qualitative analysis is emphasized and simplified method was used to achieve the objectives.

- Estimate total lipids in peanuts using Soxhlet method.
- Qualitative identification of alkaloids, flavonoids, anthraquinone, saponins, tannins and polyphenol compounds in peanuts.
- Identification of sterols in peanuts using TLC.

CHAPTER 2

LITERATURE REVIEW

2.1. Discovery of phytochemical

In plant based, wood, rubber, alkaloids, isoprenoids, flower pigments-all of these secondary compounds are important to humans, and some are nearly essential. A number of groups have begun to create transgenic plants in which secondary metabolism is altered, and many of the approaches being taken were discussed at the meeting of the Phytochemical Society of North America (PSNA). Many could be better or more useful if altered in subtle or not-so-subtle ways. Such alterations could eventually result in more effective or less toxic drugs (Chasan, 1994).

Phytochemical are natural products in plants. Although the structures vary immensely in size and complexity, most of them are derived from a limited number of core biosynthetic pathways. The biosynthetic origins of phenylpropanoid (including simple phenolics, flavonoids, anthocyanins and isoflavonoids), polyacetate, terpenoid, and alkaloid classes of metabolites are review in many researches. These compounds reported to be important in plant microbe, plant-animal and plant-plant interactions will be given as examples of each of these classes (Paiva, 2000).

Basically, phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases. Antioxidants are compounds that help delay and inhibit lipid oxidation and when

added to foods tend to minimize rancidity, retard the formation of toxic oxidation products, help maintain the nutritional quality and increase their shelf life (Fukumoto & Mazza, 2000).

2.1.1. Alkaloids

Alkaloid is an odorless, intensely bitter, nitrogen based compounds occur naturally in certain plants. Certain alkaloids, strychinine and cocaine are among the most powerful poisons and narcotics known to man (Anderson & Deskins, 1995). Alkaloids are loosely defined as compounds with a nitrogen-containing heterocyclic ring. The rings vary in size and oxidation state, ranging from aromatic to completely reduce. Thousands of alkaloids have been identified and are classified according to the structure of the ring system. Alkaloids are generally synthesized from amino acids (Paiva, 2000). Generally, the alkaloids compounds can extracted and separated easily if compare to others compounds that present in plant (Fasihuddin & Hasmah, 1993).

There is one phenomenon occur in alkaloids which is ergotism. Ergotism is the most notorious mycotoxicosis in human history, which is caused by consumption of grain, usually rye, contaminated with sclerotia of *Claviceps purpurea*. Ergot alkaloids represent a large family of mycotoxins that are derived from both amino acid and isoprenoid precursors, and include clavines, simple derivatives of lysergic acid, and structurally complex ergopeptines such as ergotamine. The core structural feature of ergot alkaloids is the ergoline nucleus, which is formed from 4-(g,g-dimethylallyl)tryptophan. Synthesis of 4-(g,g-dimethylallyl)tryptophan, the branch point step in ergot alkaloid biosynthesis, is catalyzed by the prenyltransferase, 4-

(g,g-dimethylallyl)tryptophan (DMAT) synthase, from dimethylallyl diphosphate and tryptophan (Desjardins & Hohn, 1997).

From figure 2.1, we can found the representative of alkaloids structure which includes pyridine, tropane, and isoquinoline alkaloid classes. Nicotinic acid (derived from aspartic acid and glyceraldehyde-3-phosphate) is a precursor of several pyridine alkaloids. Methylation of the nitrogen produces trigonelline, a compound found in many seeds including leguminous beans, wherein it has been shown to produce significant induction of nodulation-related genes in rhizobia. Nicotine, the infamous alkaloid component of tobacco, is produced by the fusion of nicotinic acid and another nitrogen-containing ring. Nicotine is insecticidal and inhibits feeding of herbivores. Tropinone, a precursor of the tropane alkaloids containing a bicyclic ring system, is derived from ornithine, which is cyclized and modified to form *N*-methyl-pyrrolinium cation, and malonyl CoA. Scopolamine, a powerful sedative, is formed from the ester of tropine (reduced tropinone) and an aromatic residue; cocaine is similarly formed. Lysine, its precursors and derivatives, can be converted to the six-membered rings, pyridine or piperidine rings, in several alkaloids (Paiva, 2000).

REFERENCES

Abidi, S. L. 2001. Chromatographic analysis of plant sterols in foods and vegetable oils. *Journal of Chromatography A.* **935**: 173-201.

Adesogan, E. K. 1973. Anthraquinones and anthraquinols from *Morinda Lucida*: the biogenetic significance of oruwal and oruwalol. *Tetrahedron.* **29**(24): 4099-4102.

Anderson, J. & Deskins, B. 1995. The Nutrition Bible. New York: William Morrow and Company.

AOAC. 2000. *Official Methods of Analysis.* (17th edition). Washington: Association of Official Analytical Chemists.

Balasundram, N., Sundram, K. & Samman, S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry.* **99**: 191-203.

Berkov, S., Zayed, R. & Doncheva, T. 2006. Alkaloid patterns in some varieties of *Datura stramonium*. *Fitoterapia.* **77**: 179-182.

Beveridge, T. H. J., Li, T. S. C. & Drover, J. C. G. 2002. Phytosterol content in American ginseng seed oil. *Journal of Agricultural and Food Chemistry.* **50**: 744-750.

Bisanda, E. T. N., Ogola, W. O. & Tesha, J. V. 2003. Characterisation of tannin resin blends for particle board applications. *Cement & Concrete Composites.* **25**: 593-598.

Boyle, C., Moizer, K., Barlow, T., Jeffery, B. & Paul, S. 2003. Phytoestrogens and health. Johnson, I. & Williamson, G. (ed.). *Phytochemical functional foods.* England: Woodhead Publishing Limited and CRC Press.

Breinholder, P., Mosca, L. & Lindner, W. 2002. Concept of sequential analysis of free and conjugated phytosterols in different plant matrices. *Journal of Chromatography.* **777**: 67-82.

Brown, J. P. 1980. A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. *Mutation Research.* **75**: 243-277.

Chasan, R. 1994. Phytochemical forecasting. *The Plant Cell.* 3-9.

Chu, Y. H. & Hsu, H. F. 1999. Effect of antioxidants on peanut oil stability. *Food Chemistry.* **66**: 29–34.

Chung, I. M., Kim, J. J., Lim, J. D., Yu, C. Y., Kim, S. H. & Hahn, S. J. 2006. Comparison of resveratrol, SOD activity, phenolic compounds and free amino acids in *Rehmannia glutinosa* under temperature and water stress. *Environmental and Experimental Botany.* **56**: 44-53.

Cichewicz, R. H., Zhang, Y., Seeram, N. P. & Nair, M. G. 2004. Inhibition of human tumor cell proliferation by novel anthraquinones from daylilies. *Life Sciences.* **74**: 1791-1799.

Ciesiolka, D., Muzquiz, M., Burbano, C., Altares, P., Pedrosa, M. M., Wysocki, W., Folkman, W., Popenda, M. & Gulewicz, K. 2005. An effect of various nitrogen forms used as fertilizer on Lupinus albus L. yield protein, alkaloid and α-galactosides content. *Journal Agronomy & Crop Science.* **191**: 458-463.

Condo, A.M., Baker, D. C., Moreau, R. A. & Hicks, K. B. 2001. Improved method for the Synthesis of trans-Feruloyl-β-sitostanol. *Journal of Agricultural and Food Chemistry.* **49**: 4961-4964.

Daigle, D. J., Conkerton, E. J., Sanders, T. H. & Mixon, A. C. 1988. Peanut hull flavonoids: their relationship with peanut maturity. *Journal of Agricultural and Food Chemistry.* **35**: 1179-1181.

Desjardins, A. E. & Hohn, T. M. 1997. Mycotoxins in plant pathogenesis. *Molecular Plant-Microbe Interactions.* **10**(2): 147-152.

Dietrych-Szostak, D. & Oleszek, W. 1999. Effect of processing on the flavonoid content in buckwheat (*Fagopyrum esculentum* Moench) grain. *Journal of Agricultural and Food Chemistry.* **47**: 4384-4387.

Dikshith, T. S. S., Raizada, R. B. & Mulchandani, N. B. 1990. Toxicity of pure alkaloid of Tylophora astshamatica in male rat. *Indian Journal of Experimental Biology.* **28**(3):208-212

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouma, D., Stocker, P. & Vidal, N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry.* **97**: 654-660.

Dutta, P.C. & Appelqvist, L. A. 1996. Saturated sterols (stanols) in unhydrogenated and hydrogenated edible vegetable oils and in cereal lipids. *Journal of The Science of Food and Agriculture*. **71**: 383-391.

Ewald, C., Fjelkner-Modig, S., Johansson, K., Sjoholm, I. & Akesson, B. 1999. Effect of processing on major flavonoids in processed onions, green beans, and peas. *Food Chemistry*. **64**(2): 231-235.

Frankel, E. N., Huang, S-W., Kanner, J. & German, J. B. 1993. Interfacial phenomena in the evaluation of antioxidants. *Journal of Agriculture and Food Chemistry*. **42**: 1054-1058.

Fasihuddin, A. & Hasmah, R. 1993. Kimia hasilan semulajadi dan tumbuhan ubatan. Kuala Lumpur: Dewan Bahasa dan Pustaka.

Fedina, I., Georgieva, K., Velitchkova, M. & Grigorova, I. 2006. Effect of pretreatment of barley seedlings with different salts on the level of UV-B induced and UV-B absorbing compounds. *Environmental and Experimental Botany*. **56**: 225-230.

Fessenden, R. J. Fessenden, J. S. & Feist, P. 2001. *Organic Laboratory Techniques*. (3rd edition). Brooks/cole: Thompson Learning.

Fukumoto, L. R. & Mazza, G. 2000. Assessing antioxidant and prooxidant activities and phenolic compounds. *Journal of Agricultural and Food Chemistry*. **48**: 3597–3604.

Gang, Y. 2004. *Peanut production and utilization in the People's Republic of China*. Peanut in Local and Global Food Systems Series Report No. 4. University of Georgia.

Hakala, P., Lampi, A-M., Ollilainen, V., Werner, U., Murkovic, M., Wahala, K., Karkola, S. & Piironen, V. 2002. Steryl phenolic acid esters in cereals and their milling fractions. *Journal of Agricultural and Food Chemistry*. **50**: 5300-5307.

Ham, B., Butler, B. & Thionville, P. 2000. Evaluating the isolation and quantification of sterols in seed oils by Solid-Phase extraction and capillary Gas-Liquid chromatography. *LC GC*. **18**(11): 1177-1181.

Howard, L. R., Smith, R. T., Wagner, A. B., Villalon, B. & Burns, E. E. 1994. Provitamin A and ascorbic acid content of fresh pepper cultivars (Capsicum annuum) and processed jalapenos. *Journal of Food Science*. **59**(2): 362-365.

Husken, A., Baumert, A., Milkowski, C., Becker, H. C., Strack, D. & Mollers, C. 2005. Resveratrol glucoside (Piceid) synthesis in seeds of transgenic oilseed rape (*Brassica napus L.*). *Theory Application Genetic.* **111**: 1553-1562.

Jenson, P. N., Danielsen, B., Bertelsen, G., Skibsted, L. H. & Andersen, M. L. 2005. Storage stabilities of pork scratchings, peanuts, oatmeal and muesli: Comparison of ESR spectroscopy, headspace-GC and sensory evaluation for detection of oxidation in dry foods. *Food Chemistry.* **91**: 25-38.

Jimenez-Martinez, C., Hernandez-Sanchez, H., Alvarez-Manilla, G., Robledo-Quintos, N., Martinez-Herrera, J. & Davila-Ortiz, G. effect of aqueous and alkaline thermal treatments on chemical composition and ligosaccharide, alkaloid and tannin contents of Lupinus campestris seeds. *Journal of the Science of Food and Agriculture.* **81**(4): 421-428.

Karchesy, J. J. & Hemingway, R. W. 1986. Condensed tannins: (4β-8;2β-O-7) – Linked Procyanidins in *Arachis hypogea L. Journal of Agricultural and Food Chemistry.* **34**: 966-970

Khallouki, F., Spiegelhalder, B., Bartsch, H. & Owen, R. W. 2005. Secondary metabolites of the argan tree (Morocco) may have disease prevention properties. *African Journal of Biotechnology.* **4**(5): 381-388.

Khomova, T. V., Gusakova, S. D., Murdakhaev, Y. M. & Umarov, A. V. 1984. Dependence of the composition of the lipids of the seeds of Plectranthus glaucocalyn on the conditions of growth. *Chemistry of Plant Substances.* **1**: 30-35.

Lako, J., Trenerry, V. C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S. & Premier, R. 2006. Analytical, Nutritional and Clinical Methods: Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry.* **8**: 212-220.

Laporan Tanaman Kontan Di Sabah. 2003. Keluasan Tanaman Kontan Mengikut Daerah Sabah. Jabatan Pertanian Sabah. 2003.

Leeson, P. & Floter, E. 2002. Solidification behaviour of binary sitosteryl esters mixtures. *Food Research International.* **35**: 983-991.

Lou, H. X., Yamazaki, Y., Sasaki, T., Uchida, M. & Tanaka, H. 1999. A-type procyanidins from peanut skin. *Phytochemistry.* **51**: 297-308.

Maguire, L. S., O'Sullivan, S. M., Galvin, K., O'Connor, T. P. & O'Brien, N. M. 2004. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macademia nut. *International Journal of Food Sciences and Nutrition*. **55**(3): 171-178.

Marini-Bettolo, G. B., Nicoletti, M. & Patamia, M. 1981. Plant screening by chemical and chromatographic procedures under field condition. *Journal of Chromatography*. **213**: 113-127.

Miettinen, T. A. 2001. Phytosterols-what plant breeders should focus on. *Journal of the Science of Food and Agriculture*. **81**: 895-903.

Montiel-Herrera, M., Camacho-Hernandez, I. L., Rios-Morgan, A. & Delgado-Vargas, F. 2004. Partial physiochemical and nutritional characterization of the fruit of Vitex mollis (Verbenaceae). *Journal of Food Composition and Analysis*. **17**: 205-215.

Moreau, R. A. 2004. Plant Sterols in Functional Foods. Dutta, P. C (ed.). *Phytosterols as Functional Food Components & Nutraceuticals*. New York: Marcel Dekker. 319-349.

Moreau, R. A. 2005. Extraction and analysis of food lipids. Dutta, Moreau, R. A. *Methods of analysis of Food components and addictives*. USA: Taylor & Francis Group. 219-234

Moreau, R. A., Whitaker, B. D. & Hicks, K. B. 2002. Phytosterols, Phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Research*. **41**: 457-500.

Naczk, M. & Shahidi, F. 2004. Extraction and analysis of phenolics in food. *Journal of Chromatography A*. **1054**: 95-111.

Navarro, J. M., Flores, P., Garrido, C. & Martinez, V. 2006. Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chemistry*. **96**: 66-73.

Nunez Montoya, S. C., Comini, L. R., Sarmiento, M., Becerra, C., Albesa, I., Arguello, G. A. & Cabrera, J. L. 2005. Natural anthraquinones probed as Type I and Type II photosensitizers: singlet oxygen and superoxide anion production. *Journal of Phytochemistry and Photobiology B: Biology*. **78**: 77-83.

Paiva, N. L. 2000. An introduction to the Biosynthesis of chemicals used in plant-microbe communication. *Journal of Plant Growth Regulation.* **19**: 131-143.

Palomar, M. K. (ed.). 1998. *Peanut in the Philippine Food System: A macro study.* Peanut in Local and Global Food Systems Series Report No. 1. Kasetsart University.

Peanuts/Groundnuts (online) American Peanut Council. www.peanutsusa.com. Printed 2002.

Pedrielli, P. & Skibsted, L. H. 2002. Antioxidant synergy and regeneration effect of quercetin, (-)epicatechin, and (+)-catechin on tocopherol in homogenous solutions of peroxidating methyl linoleate. *Journal of Agriculture and Food Chemistry.* **50**: 7138-7144.

Pennington, J. A. T. 2002. Study Review: Food composition databases for bioactive food components. *Journal of Food Composition and Analysis.* **15**: 419-434.

Philips, D. R., Rasbery, J. M., Bartel, B. & Matsuda, S. P. T. 2006. Biosynthetic diversity in plant triterpene cyclization. *Current Opinion in Plant Biology.* **9**: 1-10.

Piironen, V. & Lampi, A-M. 2004. Occurrence and Levels of Phytosterols in Foods. Dutta, P. C (ed.). *Phytosterols as Functional Food Components & Nutraceuticals.* New York: Marcel Dekker. 1-32.

Piironen, V., Lindsay, D.G., Miettinen, T.A., Toivo, J. & Lampi, A.-M. 2000. Plant Sterols: Biosynthesis, Biological Function and Their Importance to Human Nutrition. *Journal Of the Science of Food and Agriculture.* **80**: 939–966.

Piironen, V., Toivo, J. & Lampi, A-M. 2000. Natural sources of dietary plant sterols. *Journal of Food Composition and Analysis.* **13**: 619-624.

Pokorny, J. & Schmidt, S. 2003. The impact of food processing in phytochemicals: the case of antioxidants. Johnson, I. & Williamson, G. (ed.). *Phytochemical functional foods.* England: Woodhead Publishing Limited and CRC Press.

Poncet, C. & Remond, D. 2002. Rumen digestion and intestinal nutrient flows in sheep consuming pea seeds: the effect of extrusion or chestnut tannin addition. *Anim Res.* **51**: 201-206

Poutaraud, A. & Girardin, P. 2005. Influence of chemical characteristics of soil on mineral and alkaloid seed contents of *Colchicum autumnale*. *Environmental and Experimental Botany*. **54**: 101-108.

Price, K. R., Casuscelu, F., Colquhoun, I. J. & Rhodes, M. J. C. 1998. Composition and content of flavonol glycosides in broccoli florets (Brassica olearacea) and their fate during cooking. *Journal of Science and Food Agriculture*. **77**(4): 468-472.

Puupponen-Pimia, R., Hakkinen, S. T., Aarni, M., Suortti, T., Lampi, A-M., Eurola, M., Piironen, V., Nuutila, A. M. & Oksman-Caldentey, K-M. 2003. Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. *Journal of Science and Food Agriculture*. **83**: 1389-1402.

Racchi, M., Daglia, M., Lanni, C., Papetti, A., Govoni, S. & Gazzani, G. 2002. Antiradical activity of water soluble components in common diet vegetables. *Journal of Agriculture and Food Chemistry*. **50**(5): 1272-1277.

Ring, A. S., Waniska, R. D. & Rooney, L. W. 1988. Phenolic compounds in different sorghum tissues during maturation. *Biomass*. **17**: 39-49.

Rohmer, M., Oueisson, G. & Brandt, R. D. 1972. Hydrosoluble complexes of sterols, sterol esters and their precursors from *Zea mays L. European Journal Biochemistry*. **31**: 172-179.

Sanders, T. H., McMichael, R. W. & Hendrix, K. W. 2000. Occurrence of resveratrol in edible peanuts. *Journal of Agricultural and Food Chemistry*. **48**: 1243-1246.

Sensoy, I., Rosen, R. T., Ho, C-T. & Karwe, M. V. 2006. Effect of processing on buckwheat phenolics and antioxidant activity. *Food Chemistry*. **99**: 388-393.

Shaalan, E. A-S., Canyon, D., Mohamed Wagdy Faried Younes., Abdel-Wahab, H. & Mansour, A-H. 2005. A review of botanical phytochemicals with mosquitocidal potential. *Environment International*. **31**: 1149-1166.

Shahidi, F. & Naczk, M. 2004. Phenolics in Food and Nutraceuticals. Boca Raton: CRC Press.

Sharp, J. T., Gosney, I. & Rowley, A. G. 1989. Practical Organic Chemistry. London: Chapman & Hall.

Sheu, J-R. 1999. Pharmacological effect of Rutaecarpine, an alkaloid isolated from Evodia rutaecarpa. *Cardiovascular Drug Reviews.* **17**(3): 237-245.

Siddhuraju, P. & Becker, K. 2006. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chemistry.*

Sobolev, V. S. & Cole, R. J. 1999. Trans-resveratrol content in commercial peanut product. *Journal of Agricultural and Food Chemistry.* **47**: 1435-1439.

Spernath, A., Yaghmur, A., Aserin, A., Hoffman, R. E. & Garti, N. 2003. Self-diffusion Nuclear Magnetic Resonance, microstructure transition, and solubilization capacity of phytosterols and cholesterol in Winsor IV food-grade microemulsions. *Journal of Agricultural and Food Chemistry.* **51**: 2359-2364.

Sukharomana, S., Dobkuntod, B., Rhoades, R. & Nazarea, V. 2003. *Peanut in the Thai Food System: A macro perspective.* Peanut in Local and Global Food Systems Series Report No. 8. University of Georgia.

Talawar, S., Rhoades, R. E. & Nazarea, V. 2003. World Geography of Groundnut: Distribution, Production, use and trade (online) http://www.lanra.uga.edu/peanut/publications/wgg.cfm. Printed 2003.

Talcott, S. T., Duncan, C. E., Pozo-Insfran, D. D. & Gorbet, D. W. 2005a. Polyphenolic and antioxidant changes during storage of normal, mid, and high oleic acid peanuts. *Food Chemistry.* **89**: 77-84.

Talcott, S. T., Passeretti, S., Duncan, C. E. & Gorbet, D. W. 2005b. Polyphenolic content and sensory properties of normal and high oleic acid peanuts. *Food Chemistry.* **90**: 379-388.

Teissedre, P. L., Frankel, E. N., Waterhouse, A. L., Peleg, H. & German, J. B. 1996. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *Journal of the Science of Food and Agricultural.* **70**: 55-63.

Thanh, T. T., Vergnes, M-F., Kaloustian, J., El-Moselhy, T. F., Amiot-Carlin, M-J. & Portugal, H. 2006. Effect of storage and heating on phytosterol concentrations in vegetable oils determined by GC-MS. *Journal of the Science of Food and Agriculture.* **86**: 220-225.

Toivo, J., Piironen, V., Kalo, P. & Varo, P. 1998. GC determination of major sterols in edible oils and fats using solid-phase extraction in sample preparation. *Chromatographia*. **48**: 745-750.

Tsuda, T., Ohshima, K., Kawakishi, S. & Osawa, T. 1994. Antioxidative pigments isolated from the seeds of *Phaseolus vulgaris* L. *Journal of Agricultural and Food Chemistry*. **42**: 248-251.

Verstraeten, S. V., Hammerstone, J. F., Keen, C. L., Fraga, C. G. & Oteiza, P. I. 2005. Antioxidant and membrane effects of Procyanidin Dimers and Trimers isolated from peanut and cocoa. *Journal of Agricultural and Food Chemistry*. **53**: 5041-5048.

Vu, P-L., Shin, J-A., Lim, C-H. & Lee, K-T. 2004. Lipase-catalyzed production of phytosteryl esters and their crystallization behaviour in corn oil. *Food Research International*. **37**: 175-180.

Wu, J. Y., Wong, K., Ho, K. P. & Zhou, L. G. 2005. Enhancement of saponin production in *Panax ginseng* cell culture by osmotic stress and nutrient feeding. *Enzyme and Microbial Technology*. **36**: 133-138.

Yang, J., Li, H., Chen, Y-Y., Wang, X-J., Shi, G-Y., Hu, Q-S., Kang, X-L., Lu, Y., Tang, X-M., Guo, Q-S. & Yi, J. 2004. Anthraquinones sensitive tumor cells to arsenic cytotoxicity in vitro and in vivo via reactive oxygen species-mediated dual regulation of apoptosis. *Free Radical Biology and Medicine*. **37**(12): 2027-2041.

Yen, G-C., Duh, P-D. & Tsai, C-L. 1993. Relationship between antioxidant activity and maturity of peanut hulls. *Journal of Agricultural and Food Chemistry*. **41**: 67-70.

Yoshida, H. Hirakawa, Y., Tomiyama, Y., Nagamizu, T. & Mizushina, Y. 2005. Fatty acid distributions of triacylglycerols and phospholipids in peanut seeds (*Arachis hypogaea* L.) following microwave treatment. *Journal of Food Composition and Analysis*. **18**: 3-14.

Young, J. C., Chen, Z-J. & Marquardt, R. R. 1983. Reduction in alkaloid content of Ergot Sclerotia by chemical and physical treatment. *Journal of Agricultural and Food Chemistry*. **31**: 413-415.

Yu, J., Mohamed, Ahmedna. & Goktepe, I. 2005. Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolic. *Journal of Agricultural and Food Chemistry*. **90**: 199-206.

Zangenberg, M., Hansen, H. B., Jorgensen, J. R. & Hellgren, L. I. 2004. Cultivar and Year-to Year Variation of Phytosterol Content in Rye (*Secale cereale* L.). *Journal of Agricultural and Food Chemistry*. **52**(9): 2593-2597.

Zwickenpflug, W., Meger, M. & Richter, E. 1996. Occurrence of the Tobacco Alkaloid Myosmine in nuts and nut products of *Arachis hypogaea* and *Corylus avellana*. *Journal of Agricultural and Food Chemistry*. **46**: 2703-2706.