EFFECT OF VARIOUS COOKING METHODS ON THE ANTIOXIDANT ACTIVITIES AND BIOAVAILABILITY OF MINERALS IN *Ceratopteris thalictroides* AND *Nephrolepis bisserrata*

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I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

The effects of various cooking methods on antioxidant activity and bioavailability of minerals in *Ceratopteris thalictroides* and *Nephrolepis biserrata* had been studied. The wild vegetables were blanched in 70 - 100 °C with 10 °C interval for 2, 5, 10, 15, and 20 minutes; steamed for 5, 10 and 15 minutes; and stir-fried for 1, 3, and 5 minutes. Antioxidant activities were assessed by measuring the DPPH free radical scavenging activity, TEAC equivalent antioxidant capacity, ferric reducing power, inhibition of beta-carotene bleaching, and total phenolic content. Bioavailability of minerals were determined by simulated gastrointestinal digestion. Effect of blanching did not decrease much on the antioxidant activities in *Nephrolepis biserrata*, but decreased in *Ceratopteris thalictroides* when the temperature and time of blanching were increased. The scavenging activity (36.23-73.79%) and ferric reducing power (6.08-15.15 μmol Fe²⁺ equivalent/g FW) in *Ceratopteris thalictroides* significantly increased after steaming from 5 min to 15 min and stir-fried till 3 min. Steaming for 10 and 15 min significantly (p<0.05) enhanced the scavenging activity and TEAC in *Nephrolepis biserrata*. On the other hand, blanching significantly (p<0.05) enhanced the bioavailability of calcium and iron in these two wild vegetables. The bioavailability of calcium (1.38-8.79%) and iron (5.97-12.17%) were significantly (p<0.05) increased after steamed from 5 min to 15 min in *Ceratopteris thalictroides* however bioavailability of calcium and iron were decrease from 3.17-0.37% and 23.00%-0.53% repectively in *Nephrolepis biserrata*. Calcium (8.51-8.89%) and iron (11.62-12.35%) bioavailability in *Ceratopteris thalictroides* significantly enhanced after stir-fried, but no significantly different after stir-fried for different duration. Stir-fried significantly (p<0.05) enhanced the bioavailability of calcium (3.11-4.82%), iron (19.31-23.64%), and zinc (12.24-20.75%) in *Nephrolepis biserrata* excluding magnesium (30.46-47.34%). Undoubtedly, the antioxidant activity and minerals bioavailability in selected wild vegetables depends on cooking methods despite on the type of vegetables.
**ABSTRAK**

Kesan beberapa kaedah memasak ke atas aktiviti-aktiviti antioksidan dan bio-kebolehdapatan mineral dalam *Ceratopteris thalictroides* dan *Nephrolepis biserrata*.

Kesan beberapa kaedah memasak ke atas aktiviti-aktiviti antioksidan dan bio-kebolehdapatan mineral dalam *Ceratopteris thalictroides* dan *Nephrolepis biserrata* telah dkaji. Sayur-sayuran liar yang terpilih telah dicelur pada 70 - 100 °C dengan setiap 10 °C selang untuk 2, 5, 10, 15, dan 20 minit; dikukus untuk 5, 10 dan 15 minit; dan digoreng untuk 1, 3, dan 5 minit. Aktiviti-aktiviti antioksidan telah diuji dengan mengukur kegiatan radikal bebas DPPH, keupayaan antioksid pertama persamaan TEAC, kuasa penurunan ferik, perencatan melunturkan beta karotena, dan jumlah kandungan fenolik. Bio-kebolehdapatan mineral telah ditentukan dengan menyerap penghadaman gastrousus. Aktiviti-aktiviti antioksid dalam *Nephrolepis biserrata* tidak menurun secara jelas selepas dicelur, tetapi aktiviti-aktiviti antioksid dalam *Ceratopteris thalictroides* menurun secara signifikan (p<0.05) apabila suhu dan masa penceluran meningkat. Kegiatan radikal (36.23-73.79%) dan kuasa penurunan ferik (6.08-15.15 μmol Fe²⁺ persamaan/g) dalam *Ceratopteris thalictroides* signifikan (p<0.05) selepas dicelur selama 5 min sehingga 15 min dan digoreng sehingga 3 min. Manakala, keupayaan antioksid persamaan TEAC dan kegiatan radikal meningkat secara signifikan (p<0.05) selepas dikukus selama 10 dan 15 min. Di samping itu, penceluran menyebabkan bio-kebolehdapatan kalsium dan besi meningkat secara signifikan (p<0.05) dalam kedua-dua sayur liar ini. Bio-kebolehdapatan kalsium (1.38-8.79%) dan besi (5.97-12.17%) meningkat secara signifikan (p<0.05) selepas dikukus selama 5 min sehingga 15 min dalam *Ceratopteris thalictroides*. Manakala, bio-kebolehdapatan kalsium dan besi meningkat dari 3.17-0.37% dan 23.00%-0.53% masing-masing dalam *Nephrolepis biserrata*. Bio-kebolehdapatan kalsium (8.51-8.89%) dan besi (11.62-12.35%) meningkat secara signifikan (p<0.05) selepas digoreng, tetapi tidak menunjukkan perbezaan yang signifikan selepas digoreng pada jangka masa yang berbeza. Menggoreng pula meningkatkan keterbiosediaan kalsium (3.11-4.82%), besi (19.31-23.64%), dan zink (12.24-20.75%) secara signifikan (p<0.05) dalam *Nephrolepis biserrata* ini kecuali magnesium (30.46-47.34%). Secara keseluruhann, aktiviti antioksid dan keterbiosediaan mineral pada sayur-sayuran liar bergantung kepada kaedah masakan dan juga jenis sayur.
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CHAPTER 1

INTRODUCTION

Wild vegetables referred to wild or non-domesticated plants normally collected from sources aside from farms and vegetable gardens or from the primary or secondary forests that are not privately managed for other crops (Noweg et al., 2003). According to FAO (1999), wild plants make an important contribution to the life of local communities especially in developing countries by providing foods and incomes. Wild vegetables have been the mainstay of human diet for centuries, providing millions of consumers with important micronutrients, such as vitamins and minerals to maintain health and strengthen immunity against infections (Flyman & Afolayan, 2006).

Although the mineral and vitamins content of wild vegetables are generally higher than cultivated vegetables (Ogle et al., 2001; Guerrero et al., 1998), the total amount of nutrients in a food does not reflect the amount that could be utilized for body functions. The proportion of a nutrient in food that can be absorbed from the gastrointestinal tract for normal body functions are termed as bioavailability (Watzke, 1998). Bioavailability of nutrient can be enhanced or inhibited by interactions with other dietary components in the gastrointestinal tract. Inhibitors such as phytate, tannins, oxalate, dietary fiber, and trypsin inhibitor; and enhancers such as ascorbic acid, and citric acid are commonly presence in vegetables (Harvey, 2001).

Vegetables are rich in nutritional antioxidant, such as vitamins C and E, and beta-carotene; and non-nutritional antioxidants, such as flavonoids, flavones, and other polyphenolic compounds (Lin & Chang, 2005). Dietary antioxidants are
important components because they help to protect human body against free radicals. Free radicals reactions are known to be the main contributor to degenerative diseases such as aging and are recognized as major factors causing cancer, cardiovascular disorders, and diabetes (Dini et al., 2008; Wu & Ng, 2008; Lim & Murtijaya, 2007). There are considerable amount of epidemiological evidence indicating an association between diets rich in fresh fruits and vegetables and a decreased risk of cardiovascular disease and certain cancers (Dauchet et al., 2006; He et al., 2006; Riboli & Norat, 2003; Chu et al., 2000; De & Dasgupta, 2007).

Most vegetables undergo cooking processes rather than being eaten raw. The most common domestic cooking used include blanching, steaming, stir-frying, and microwaving. It is well known that cooking causes considerable changes in the nutrient content. However, several studies have shown that the effect of various cooking methods on the nutrient of vegetables depending on the type of nutrients, and vegetables (Hunter & Fletcher, 2002; Kala & Prakash, 2004). The minerals content in vegetables were not significantly affected by cooking treatment. However, there are a few exceptions, a loss of calcium was observed in cooking for some vegetables such as beans, and brinjal (Kala & Prakash, 2006; Kawashima & Soares, 2003). On the other hand, anti-nutrients in vegetables were significantly reduced by cooking where in some studies shown that blanching had the strongest effect on reduction compare to steaming, stir-frying, and microwaving (Mosha et al., 1995; Somsun et al., 2008; Yadav & Sehgal, 2003). The cooking effect on antioxidant activity of vegetables depends on the antioxidant components present in the vegetables. The antioxidants that are commonly loss are phenolic, ascorbic acid, and carotenoids (Zhang & Hamauzu, 2004; Chuah et al., 2008).

Wild vegetables constitute an important part of the diet in Sabah and most of the local communities eat at least one kind of wild vegetable in their daily meals. Wild vegetables are usually collected by the low-income groups for daily consumption and as a source of income (Andersen et al., 2003). Vegetable ferns are one of the wild vegetables that are always been a popular dish in Sabah. Vegetable fern such as Ceratopteris thalictroides (Jangkut kali/Sayur Kadok) and Nephrolepis biserrata (Pakis Sarawak) are commonly collected and marketed at town areas (Noweg et al., 2003). These two types of vegetable fern are selected in this study.
Food composition data, which are necessary tools for epidemiological and nutritional studies, are merely representative of foodstuffs consumed in their raw state. They cannot take into consideration the fact that nutrients content and also their bioavailability may change through cooking practices (Amin et al., 2006). From a nutritional point of view, the understanding of the consequences of cooking method on bioavailability of nutrients is one of the most important steps along the way to a correct interpretation and evaluation of study results regarding dietary habits and human health (Nicoli et al., 1999).

Many studies have analyzed the effect of cooking on antioxidant properties of a wide variety of cultivated vegetables such as spinach, broccoli, cabbage, peas, and carrot (Turkmen et al., 2005; Lin & Chang, 2005; Zhang & Hamauzu, 2004; Danesi & Bordoni, 2008). Yet, the effect of cooking treatments on the antioxidant activity of wild vegetables has seldom been reported. Hence, this study is aims to investigate the effect of various cooking methods on the antioxidant activity and bioavailability of minerals in selected wild vegetables.

The specific objectives of this study are:

1) To determine the effect of various cooking methods on the antioxidant activity in Ceratopteris thalictroides and Nephrolepis biserrata.

2) To determine the effect of various cooking methods on the bioavailability of minerals in Ceratopteris thalictroides and Nephrolepis biserrata.
CHAPTER 2

LITERATURE REVIEW

2.1 Edible Wild Plants
Tropical forests have long been recognized as housing a large percentage of the planet's biodiversity, and the plant species they contain are a major source of food and numerous other products. Not surprisingly, most of the wild-collected species are found in the tropics. The term "wild" when applied to plants or plant species refers to those that grow spontaneously in self-maintaining populations in natural or semi-natural ecosystems and can exist independently of direct human action (FAO, 1999).

Wild plants are essential for many rural households. At least one billion people are thought to use them. For instances, wild plants provide a greater share of the diet than domesticated cultivars in rural and have been identified as sources of emergency foods in Malaysia (Burlingame, 2000). Maundu (1996) found that wild vegetables are usually consumed as vegetables and salads and have become a frequent item on menus both at home and in restaurants. In remote rural settlements where plant cultivation is not practiced and market supplies are not organized, local inhabitants depend on edible wild plant for substituting in time of poor harvest of grown vegetables and enriching the diversity of food (Misra et al., 2008; Andersen et al., 2003).

Wild vegetables are important both for consumption and production, and in both cases, poor households rely more on these vegetables than more wealthy households. Further, they may serve as income sources and may be traded locally,
even internationally, and are important during periods of drought and or social unrest or war (Weinberger & Msuya, 2004). According to Vantommen et al. (2002), particularly in Southeast Asia, wild vegetables is potentially contribute to the intensification of shifting cultivation systems if appropriate cropping practices are developed using existing farming technique.

2.1.1 Multiple Roles of Edible Wild Plants
Throughout the world, and more especially in developing countries, wild plants make an important contribution to the life of local communities. They play a significant part in a wide range of agricultural systems as a source of wild foods and fuel wood, and they have an important socio-economic role through their use in medicines, dyes, fibers, and religious and cultural ceremonies (FAO, 1999; Kulip, 1996). The multiple roles of wild traditional vegetables as both food and medicinal sources have been well documented. These include: the listing of 28 medicinally important leafy vegetables by Ayodele (2005), the reported medicinal uses of 24 indigenous leafy vegetables in south western Nigeria by Adebooye and Opabode (2004), the documented antibacterial activities of various vegetables and fruits (Lee et al., 2003) and the cataloguing of wild vegetables with both therapeutic and dietary functions (Ogle et al., 2003).

In Malaysia, many wild plants are collected from their natural habitats for medicinal purposes. Some common edible wild plants used as medicinal plants are Eurycoma longifolia, Labisia pumila, Centella asiatica, Cinnamomum spp., Curcuma xanthorrhiza, Andrographis paniculata, Morinda citrifolia and Kaempferia galangal (Vantommen et al., 2002). Kulip (1996) found that wild plants are widely used for medicinal purpose in the local communities in Sabah. The traditional methods of healing using plants are still popular since many of the locals cannot afford to pay for the modern medicine. However, most of the plants are still proven could be curing many diseases and are of great uses in primary health care in the district.

2.1.2 Potential Role of Wild Vegetables to Improve Nutrition
Micronutrient deficiencies have become a serious global problem, especially in areas where the diet lacks variety. Iron, vitamin A and iodine deficiencies are the three micronutrient deficiencies of greatest public health significance in the developing world (Welch & Graham, 1999; Kennedy et al., 2003). It has been observed that
diets in many developing countries are not optimal (Johns, 2003) and that globalization and modernization in agriculture has resulted in simplification of diets and reliance on a few staple crops (Welch & Graham, 1999). Hence, a number of authors have advocated the use of food-based strategies to achieve optimal dietary requirements to combat micronutrient deficiencies (Ali & Tsou, 1997; Johns, 2003).

However, the limited supply of vegetables, especially during the off-season, higher market price and lower appreciation or awareness regarding their consumption are key factors that limit the vegetable consumption rate in the developing world. Encouraging the use of wild vegetables could play a role in increasing overall vegetable consumption. A review of species shown that edible wild plants could make a positive contribution to world food production because they are well adapted to adverse environmental conditions and are generally resistant to pests and pathogens. Integrating wild vegetables into diets has been promoted as the most practical and sustainable way to achieve this (Chadha & Oluoch, 2003), since such vegetables are efficient sources of several important micronutrients, both with respect to unit cost of production and per unit area of land (Ali & Tsou, 1997). Indeed, there is increasing consensus that wild foods could significantly contribute to alleviating hunger and malnutrition.

The fact that traditional rural communities are nutritionally successful, even during periods of drought, affirms the importance of recognizing and utilizing traditional wild food resources. Edible wild plants could be selected as a partial substitute for other cultivated commodities to alleviate nutrient deficiencies by extending production areas, increasing nutrient supplies, lowering the cost of production systems and complementing vegetable supplies during the off-season (Engle & Altoveros, 2000). In view of this, neglected crops, non-commercial foods, and wild foods are receiving renewed attention, with the recognition that they could become useful parents in breeding programs, convenient sources of income, and vehicles for improved nutrition and increased food supply (Burlingame, 2000).

2.1.3 Ethonobotanical Study of Edible Wild Plants in Sabah
According to the surveys of Non-Timber Forest Products Traded in Sabah tamus, wild edible plants (32.1%) are the most products were collected and traded by local communities (Kodoh, 2005). Wild vegetables constitute an important part of the diet
in Sabah. Most of the locals eat at least one kind of wild vegetable in their daily meals and up to eleven different species of vegetables are consumed per week (Andersen et al., 2003). Noweg et al. (2003) indicated a large proportion (about 70.6%) of the Kadazandusun and Murut communities are involved in collection of wild plants for vegetables. Bamboo shoots appear to be the most frequently collected (45.96%), followed by low herbaceous plants (19.88%), wild banana (12.42%), and ferns (9.32%). Indigenous food plants that are most commonly found in 'Tamu' or local market in are Nephrolepis biserrata (Pakis), Solanum sp. (Tutan), Etlingera sp. (Tuhau), Stenochalena palustris (Lemiding), and Melastoma sp. (Komburiong) (Kulip, 1996).

a. Edible Wild Ferns in Sabah

More than 500 species of ferns are found in Malaysia. Fern are collected all year round and the fern stems, rhizomes, leaves, young fronds, and shoots are used for food. In Malaysia, the young shoots of fern Nephrolepis biserrata, Diplazium esculentum and Blechnum orientalis, and fronds of Ceratopteris thalictroides are commonly eaten as vegetables (Lee & Aban, 1985; Mannan et al., 2008; Noweg et al., 2003). Table 2.4 shown a list of edible ferns commonly found in Sabah.

Table 2.1: A List of Edible Ferns Commonly Found in Sabah.

<table>
<thead>
<tr>
<th>Local Name</th>
<th>Botanical Name</th>
<th>Family</th>
<th>Part(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemiding</td>
<td>Stenochlaena palustris</td>
<td>Blanchnaceae</td>
<td>Young shoot</td>
</tr>
<tr>
<td>Dungau</td>
<td>Blenchnum orientale</td>
<td>Blanchnaceae</td>
<td>Young shoot</td>
</tr>
<tr>
<td>Tree Fern</td>
<td>Cyathea moluccana</td>
<td>Cyatheaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>Golden Chicken Fern</td>
<td>Cibotium baromez</td>
<td>Dicksoniaceae</td>
<td>Rhizome</td>
</tr>
<tr>
<td>Pakis</td>
<td>Diplazium esculentum</td>
<td>Drypteridaceae</td>
<td>Young shoot</td>
</tr>
<tr>
<td>Paku / pakis</td>
<td>Nephrolepis biserrata</td>
<td>Oleandraceae</td>
<td>Young shoot</td>
</tr>
<tr>
<td>Sayur kodok/</td>
<td>Ceratopteris thalictroides</td>
<td>Parkeriaceae</td>
<td>Fronds</td>
</tr>
<tr>
<td>Jangkut Kali</td>
<td>Helminthostachys</td>
<td>Ophioglossaceae</td>
<td>Young shoot</td>
</tr>
<tr>
<td>Tongkat Langit</td>
<td>Pteridaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paku</td>
<td>Drynaria sp.</td>
<td>Polypodiaceae</td>
<td>Young leaves</td>
</tr>
<tr>
<td>Piai</td>
<td>Acrostichum aureum</td>
<td>Pteridaceae</td>
<td>Young shoot</td>
</tr>
<tr>
<td>Paku</td>
<td>Pteridium esculentum</td>
<td>Pteridaceae</td>
<td>Young shoot</td>
</tr>
<tr>
<td>Paku</td>
<td>Lygodium scandens</td>
<td>Schizaceae</td>
<td>Young shoot</td>
</tr>
</tbody>
</table>

Sources: Lee & Aban (1985), Mannan et al. (2008); Noweg et al. (2003).

*Ceratopteris thalictroides*, commonly known as water sprite, is an aquatic fern that is commonly found wild in rice fields and swampy locations. In Southeast Asia,
the young leaves of Ceratopteris thalictroides are either cooked or blanched and eaten as salad (Publishers & Grubben, 2004). On the other hand, Nephrolepis biserrata is very frequently found in lowland, particularly on the trunks of oil palms and rubber plantation. It is normally known as broad sword fern. Medicinally, in Malaysia, these two fern are used as a poultice against skin complaints where, Ceratopteris thalictroides is used as a drawing agent on carbuncles while Nephrolepis biserrata is often used to treat blisters or sores on the skin (Christensen, 1997).

2.2 Nutritional Value of Wild Vegetables
Studies conducted by Ogle et al. (2001), and Gupta et al. (2005) in Asia have confirmed the importance of wild vegetables as sources of nutrients. Ogle et al., 2001 reported that wild vegetables contributed 21% of dietary folate in one Vietnamese district and 14% in another. In another study, it was reported that of the 70 wild vegetables eaten by the tribal people in south India, 36 had high vitamin A concentrations with only four having low contents (Rajyalakshmi et al., 2001). The wild leafy vegetables found in Nigeria are high in ascorbic acid (vitamin C), which is the ranged form 23 mg/100g to 232/100g (Achinewhu et al., 1995). Analysis of proximate composition of the wild leafy vegetables found in the forest and wetlands in India, revealed that some of the greens contained high amounts of crude protein (Shingade et al., 1995).

2.2.1 Comparisons of Nutritional Value in Wild Vegetables and Cultivated Vegetables
A large body of information exists on the nutrient composition of wild vegetables, generally indicating higher mineral and vitamin contents than in cultivated vegetables. Guerrero et al. (1998) compiled a comprehensive nutrient report of wild vegetables consumed by the first European farmers, and nearly all the species indicated good amounts of several micronutrients. In particular, Verbena officinalis was found to be an excellent source of calcium and magnesium, which were present at concentrations of 3 mg/g Ca and 1.6 mg/g Mg of fresh leaf mass respectively. In another study, in which 25 wild vegetables were analyzed for their mineral content in one district of Turkey, Turan et al. (2003) reported that the nitrogen, potassium, calcium, magnesium and protein contents of these vegetables were all higher than those of cultivated species, such as spinach, pepper, lettuce, and cabbage. However,
concentrations of iron, manganese, zinc, and copper were similar in both vegetable types.

Similarly, another study conducted in two Vietnamese districts, Ogle (2001) found that all but one of the 28 analyzed species had higher carotene concentrations than the locally cultivated species, with 14 of the wild species possessing higher calcium concentrations and 12 of the wild species possessing higher iron contents than the cultivated vegetables. The relatively high beta-carotene contents observed in 11 out of the 28 wild species ranged from 2.5 μg/g in Commelina communis to as high as 50 μg/g in Basella rubra, Limnocharis flava and Sauropus androgynus. A study in Sarawak, Malaysia found that six of the leafy indigenous vegetables have protein values of over 4%, which is higher than common leafy vegetables (Voon & Kueh, 1999).

Twenty wild vegetables in South Africa were analyzed for their nutrient contents. From this study, it was determined that twelve wild leafy vegetables, namely Amaranthus dubius, Amaranthus hybridus, Amaranthus spinosus, Ceratotheca triloba, Cleome monophylla, Cucumis metuliferus, Emex australis, Galinsoga parviflora, Justicia flava, Momordica balsamina, Physalis viscosa, and Wahlenbergia undulate provides mineral concentrations exceeding 1% of plant dry weight and are much higher than typical mineral concentrations in conventional edible leafy vegetables (Odhav et al., 2007). However, the nutritional quality of four wild vegetables analyzed in Ghana were found to be in the same range as conventional vegetables (Wallace et al., 1998), these findings implying the requirement for further studies, covering a wide range of species before wild vegetables can be recommended as substitutes for conventional ones.

2.2.2 Bioavailability of Nutrient and Antinutrient of Wild Vegetables
Little is known of the actual bioavailability of nutrients in wild vegetables, which is important from a consumptive perspective. de Pee et al. (1996) indicated increased vegetable consumption could improve micronutrient status, particularly with respect to iron and vitamin A, and evaluation of dietary are important approaches for combating the key micronutrient deficiencies. One potential solution is the selection of genotypes possessing good bioavailable sources of micronutrients, especially iron whose bioavailability in vegetables is known to be poor (Ruel & Levin, 2000). In one
REFERENCES


