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NITRATE CONCENTRATIONS AND POSTHARVEST QUALITY OF GREEN LEAFY VEGETABLES IN ORGANIC AND CONVENTIONAL CROPPING SYSTEMS, SELANGOR, MALAYSIA

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By

AZIMAH BINTI HAMIDON

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

Dedicated to my beloved parents, Azizah binti Moin and Hamidon bin Husain, my brother, Hamzah bin Hamidon for their endless love, support, understanding, sacrifces, motivation, advice and encourangement.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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The unregulated application of nitrogen fertilizers would result in luxury uptake of nitrate concentration in leafy vegetables, leading to the conversion of nitrate to nitrite and eventually to carcinogenic nitrosamines during storage. The greenness of green leafy vegetables is the result of fertilizer application to specifically nitrogen fertilizer. In Malaysia, the grade of nitrogen in most organic fertilizers is not ensured, thus leading to over application of nitrogen to the vegetable crop per growing season. The objective of the first experiment was to determine the leaf nitrate, nitrite and chlorophyll contents, nitrate reductase activity and colour of selected vegetables (*Amaranthus viridis*, *Brassica rapa*, *Lactuca sativa*) from three organic (ORG1, ORG2 and ORG3) and three conventional (CONV1, CONV2 and CONV3) farms in Selangor. The experiment was conducted in a completely randomized design with five replications. The organic *B. rapa* and *L. sativa* vegetables contained higher nitrate content, 1659 and 1119 mg/kg fresh weight (FW), respectively, compared to those of conventional vegetables. The nitrate reductase (NR) activity in organic *L. sativa* leaves was 56% higher than conventional. The leaf colour of the organic and conventional vegetables was deep, grayish or pale green. This could resulted in 31.1% and 23.6% higher chlorophyll content in conventional *A. viridis* and *L. sativa* leaves was than organic vegetables leaves. High nitrate content is associated with dark leaf colour, as well as high chlorophyll content. Thus, the consumers' perception regarding dark green leafy vegetables as being related to freshness and high quality is doubtful. The second experiment was carried out to determine the effects of cropping systems (organic and conventional) and storage duration (0, 3, 6 and 9 days) on postharvest quality characteristics, antioxidant activity, chlorophyll content and nitrate and nitrite contents of *A. viridis*, *B. rapa* and *L. sativa*. The leafy vegetables used were selected from the cropping systems that produced the highest nitrate contents as determined in the first experiment. The experiment was conducted using the randomized complete block design, arranged in a factorial experiment, with five replications. The *A. viridis*, *B. rapa* and *L. sativa* from the two cropping systems showed 4.5 to 8% weight loss and decreased firmness by 33 to 45.6% during storage. The organic and conventional vegetables showed the colour changes from dark grayish, deep and pale green to bright

grayish, grayish and yellowish dull green during storage. The colour changes were influenced by the degradation of chlorophyll content in *A. viridis*, *B. rapa* and *L. sativa* during storage. The NR activity of organic and conventional *B. rapa* and conventional *A. viridis* were 51 to 71% increased by storage day 3. The three organic and conventional leafy vegetables were 78 to 95.4% reduced in nitrate contents and 99% increased in nitrite content as storage days increased. It can be suggested that leafy vegetables, such as *A. viridis*, *B. rapa* and *L. sativa*, should not be kept under refrigerated (5 ºC) storage for more than 3 days. This is to prevent an increase in nitrite content, excessive water loss, firmness reduction, changes in total phenolic, ascorbic acid and antioxidant activity, and to maintain the postharvest quality of the vegetable. It is recommended that studies be carried out to determine the nitrate contents of other types of leafy vegetables grown under organic and conventional cropping systems. The soil nitrate contents where the vegetables are grown should also be determined. Future studies should be conducted to determine the optimum nitrogen requirement for leafy vegetables to prevent excess utilization of nitrogen fertilizers.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KEPEKATAN NITRAT DAN KUALITI LEPAS TUAI SAYURAN BERDAUN HIJAU DALAM SISTEM PENANAMAN ORGANIK DAN KONVENSIONAL, SELANGOR, MALAYSIA.

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Aplikasi baja nitrogen yang tidak terkawal akan menyebabkan pengambilan nitrat yang tinggi oleh daun serta pertukaran nitrat kepada nitrit dan akhirnya membentuk nitrosamina yang bersifat karsinogen semasa tempoh penyimpanan. Kehijauan sayur-sayuran berdaun hijau adalah hasil daripada aplikasi baja khususnya baja nitrogen. Di Malaysia, gred nitrogen yang digunakan dalam kebanyakan baja organik adalah tidak dijamin lalu menyebabkan aplikasi nitrogen yang berlebihan terhadap tanaman sayuran semusim. Objektif bagi eksperimen pertama adalah untuk menentukan kandungan nitrat, nitrit dan klorofil, aktiviti nitrat reduktase dan warna daun yang dipilih *Amaranthus viridis*, *Brassica rapa*, *Lactuca sativa*) daripada ladang organik dan konvensional di Selangor. Eksperimen dijalankan dalam rekabentuk rawakan lengkap (CRD) dengan lima replikasi. Daun organik *B. rapa* and *L. sativa* mengandungi kandungan nitrat yang lebih tinggi, iaitu 1659 dan 1119 mg/kg berat segar (BS), berbanding daun konvensional. Aktiviti nitrat reduktase (NR) dalam *L. sativa* organik adalah 56% lebih tinggi berbanding konvensional. Daun sayur-sayuran organik dan konvensional sama ada berwarna hijau pekat, hijau kelabu atau hijau pucat. Ini boleh memberi kesan 31.1% dan 23.6% lebih tinggi kandungan klorofil dalam konvensional *A. viridis* dan *L. sativa* daripada daun organik. Kandungan nitrat yang tinggi dikaitkan dengan daun yang berwarna gelap, dan juga kandungan klorofil yang tinggi. Oleh itu, persepsi pengguna mengenai sayur-sayuran berdaun hijau gelap ada kaitan dengan kesegaran dan berkualiti tinggi adalah diragukan. Eksperimen kedua telah dijalankan untuk menentukan kesan sistem penanaman (organik dan konvensional) dan tempoh penyimpanan (0, 3, 6 dan 9 hari) terhadap ciri-ciri kualiti lepas tuai dan kandungan nitrat dan nitrit dalam *A. viridis, B. rapa* dan *L. sativa*. Sayur-sayuran berdaun yang digunakan telah dipilih daripada sistem penanaman yang menghasilkan kandungan nitrat tertinggi seperti yang ditentukan dalam eksperimen pertama. Eksperimen ini dijalankan menggunakan reka bentuk blok lengkap rawak, disusun dalam uji kaji faktorial, dengan lima replikasi. Daun organik A. *viridis*, *B. rapa* dan *L. sativa* daripada kedua-dua sistem penanaman menunjukkan 4.5 hingga 8% peningkatan berat hasil dan 33 hingga 45.6% penurunan kekerasan semasa penyimpanan. Sayur-sayuran organik dan konvensional menunjukkan perubahan warna dari hijau kelabu gelap, hijau

pekat dan hijau pucat kepada hijau kelabu cerah, hijau kelabu dan hijau kusam kekuningan semasa penyimpanan. Perubahan warna dipengaruhi oleh kemerosotan kandungan klorofil dalam *A. viridis, B. rapa* dan *L. sativa* semasa penyimpanan. Terdapat peningkatan dalam aktiviti NR terhadap *B. rapa* organik dan konvensional, *A. viridis* konvensional dan *L. sativa* organik dalam 51 hingga 71% pada hari ke-3 penyimpanan. Kandungan nitrat dalam ketiga-tiga sayuran organik dan konvensional berkurang 78 hingga 95.4% dan kandungan nitrit didapati meningkat 99% selari dengan meningkatnya hari penyimpanan. Adalah disarankan bahawa sayur-sayuran berdaun seperti *A. viridis* , *B. rapa* dan *L. sativa* tidak boleh disimpan di dalam peti sejuk (5 ºC) simpanan lebih daripada 3 hari. Ini adalah untuk mengelakkan peningkatan kandungan nitrit, kehilangan air yang berlebihan, pengurangan kekerasan, perubahan dalam jumlah kandungan fenolik, asid askorbik dan aktiviti anti-oksida dan untuk mengekalkan kualiti sayuran lepas tuai. Adalah disyorkan bahawa kajian dijalankan untuk menentukan kandungan nitrat dalam jenis sayuran lain yang ditanam di bawah sistem penanaman organik dan konvensional. Kandungan nitrat tanah di mana sayur-sayuran ditanam juga perlu dikaji. Kajian masa depan perlu dijalankan untuk menentukan keperluan nitrogen optimum untuk sayur-sayuran berdaun bagi mengelakkan penggunaan baja nitrogen berlebihan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AA	Ascorbic acid
ADI	Acceptable daily intake
ANOVA	Analysis of variance
AVRDC	Asian Vegetable Research and Development Center
C*	Chromaticity
CIE	Commission Internationale de I'Eclairage / International Commission on Illumination
C/N ratio	Carbon nitrogen ratio
DMRT	Duncan's multiple range test
DPPH	2,2-Diphenyl-2-picryl- hydrazyl
EFSA	European Food Safety Authority
EC	European Comission
FAO	The Food and Agriculture Organization of the United Nations
FIA	Flow Injection Autoanalyzer
FW	Fresh weight
g	gram
h	Hour
h°	Hue angle
Hb	Haemoglobin
kg	kilogram
KNO_3	Potassium nitrate
L*	Lightness
metHb	methaemoglobin
mg	Miligram
mg/L	Milligram/liter
mg/kg	Milligram/kilogram

mM	Millimolar
MOA	Ministry of Agriculture
N	Nitrogen
NR	nitrate reductase
NiR	nitrite reductase
nm	nanometer
ns	Non significant
NED	N-1-naphthyethyenediamine
NO^{3-}	Nitrate
NO^{2-}	Nitrite
oxyHb	haemoglobin
PE	Polyethylene
R^2	Coefficient determination
rpm	Rotation per minute
SAS	Statistical Analysis System
SOM	Malaysian Organic Scheme
TA	Titratable acidity
TPC	Total phenolic content
UPM	Universiti Putra Malaysia
WHO	World Health Organization
v/v	Volume per volume

CHAPTER 1

GENERAL INTRODUCTION

There was a 1% increase in the acreage of vegetable production in Malaysia from 2010 to 2012, from 52,793 to 53,322 ha, with a production of 878,975 tonnes in 2012 (MOA, 2013). The demand for vegetables is expected to increase from 1.6 million tonnes in 2010 to 2.4 million metric tons in 2020, with a growth of 4.5% per annum. Consistent with the trend in developing countries, the per capita consumption of vegetables is expected to grow by 2.6% per annum, from 55 to 70 kg per year (MOA, 2013*)*. Thus, the current demand for vegetables by consumers is high and would be even greater in the future. Consumption of green leafy vegetables is expected to be an upward trend due to preferences for a healthier lifestyle. The high demand for good quality leafy vegetables by the Malaysian consumer is in response to the increased in their income (Tey et al., 2009).

Leafy vegetables contain vitamins C, K, E, and B. Vegetables are also rich in iron, calcium, fibre, antioxidants and phytochemicals, which are key to good well being (Fahey, 2003; Podsedek, 2007; Jahangir et al., 2009) and significant in nutritional development (Olaniyi and Ajibola, 2008). Fruits, vegetables, grains and other plant food contain bioactive, non-nutrient plant compounds (Liu, 2004) and plant-derived chemicals (Onyeka and Nwambekwe, 2007) known as phytochemicals. Phytochemicals act as antioxidants to reduce and prevent the risk of major deteriorating diseases that are derived from different type and colour of fruits and vegetables. Cao et al. (1997) reported that all the compounds, such as phenolics, anthocyanins, and flavonoids, in the plant tissues are related to the plant antioxidant capacity. The phytochemicals in leafy vegetables are anthocyanins, steroid, tannin, alkaloid, flavonoids, carotenoids (Onyeka and Nwambekwe, 2007), indoles, lutein and phenolics (Abba et al., 2009; Farhan et al., 2012). Phenolics or polyphenols, including bioflavonoids, organic acids and phenolic acids, were reported to treat various chronic diseases, such as cancer, cardiovascular disease and diabetes (Scalbert et al., 2005) and as a protective agent against external stress and pathogenic attack.

Despite the health benefits of organic vegetables, their high nitrate and nitrite levels may pose a safety problem. After harvest, the nitrate in vegetables is converted to nitrite and carcinogenic nitrosamines, by the reaction with amines, amides and enzymes in the chloroplasts (Kross et al., 1992) during storage (Du et al., 2007; Bartsch et al., 1988). It is known that nitrosamines are carcinogenic, and eventually would reduce the quality of the produce. About 80-90% of the nitrogen from fertilizers or organic materials, absorbed in the form of nitrates by plants, are essential for protein synthesis (Chung et al., 2003; Santamaria et al., 1999). In 2008, nitrogen utilization of Brassica in Malaysia was 410 tonnes/ha, an increase of up to 12% from 361 tonnes/ha in 2003 (Sabri, 2009).

Nitrogen sources are either synthetically or organically produced. Synthetic nitrogenous fertilizers, such as urea, sodium nitrate, ammonium chloride and ammonium nitrate, are commonly used on conventional farms. Such fertilizers increased the yields and nitrate concentrations in leafy vegetables (Wang and Li, 2004). In organic vegetable farms, sources of nitrogen are from chicken dung, compost, Bokashi and commercial organic fertilizers. Studies have shown that organic vegetables have a tendency to have lower nitrate content than conventional vegetables (Worthington, 2001). However, in Malaysia, the quality of most organic fertilizers is not guaranteed in terms of C/N ratio and characterization of composted material (Kala et al., 2011). Thus, excessive and unregulated application of nitrogen fertilizers could result in luxury uptake of nitrate by the vegetables leading to an increase of nitrate accumulation in vegetables.

Most growers tend to use high amounts of nitrogen fertilizer to produce high biomass yield. There is also a preference by consumers for dark green leaves as they are considered fresh and healthier for consumption. Since nitrogen is a primary component of chlorophyll and other organic compounds (Chapman and Barreto, 1997), there is a positive correlation between nitrogen and chlorophyll (Tuncay, 2011). The higher nitrogen fertilizer rate would contribute to higher nitrate uptake and abundant pigment-protein complexes in the thylakoid of the chloroplast. Also, it can add to a darker green leafy vegetables as the excessive fertilizer contributes to the greenness of the colour. In Malaysia, information on nitrate and nitrite levels in leafy vegetables is currently lacking. Most of the available data are from temperate climates. Biochemical system in the tropical plant cell is different from those in the temperate because of nitrogen absorption coupling and photosynthetic electron transport in leaves, as it suggests light intensity, heat and water stress tolerance. These factors determine the nitrate content in plant.

Leafy green vegetables can build up high amounts of nitrates concentrations increased up to 6000 mg/ kg (De Martin and Restani, 2001). Even though the toxicity level of nitrates is small, but the accumulated compounds in the organisms threaten human health. Nitrite fixations in vegetables could rise to hoisted levels because of bacterial change of nitrate and nitrite, when stored at room temperature. Moreover, poor sanitation can increase the nitrite concentrations to a potentially harmful level (Chung et al., 2003). However, some components of vegetables (e.g. ascorbic acid and phenols) have been reported to restrict the adverse effects of nitrites (Walker, 1990). Differences in nitrate accumulation in plants could be due to several factors, such as harvesting period, amount and composition of fertilizers applied, amount and types of nutrients present in the soil, temperature and light intensity (Zhou et al., 2000).

The European Commission Regulation Union has established guidelines in February 1997 to monitor nitrate content in some vegetables, such as lettuce and spinach, by the growing season (EFSA, 2008). In China, the suggested maximum amount of 3100 mg kg^{-1} nitrate in vegetables has been established. Many countries do not have regulations in controlling nitrates in vegetables. The occurrence of nitrates and nitrites in vegetables has been studied in countries such as Korea (Chung et al., 2003), China (Zhou et al., 2000), Hong Kong (Chung et al., 2011), Australia (Hsu et al., 2009), France (Menard et al., 2008), Italy (Martin et al., 2003), Spain (Pardo-Marin et al., 2010), Sweden (Merino et al., 2006) and Turkey (Ayaz et al., 2007).

Presently, the studies on nitrate and nitrite contents of organic and conventionally produced vegetables in Malaysia is lacking. Notably, Berry et al. (1982) reported that nitrate and nitrite contents in Malaysian vegetables did not reflect the potential health hazards to consumers. Nevertheless, agricultural practices have changed significantly in the past 30 years. Similarly, nitrate and nitrite contents in vegetables could have changed. Thus, the objectives of the study were to (i) to determine nitrate, nitrite and chlorophyll contents, nitrate reductase activity and leaf colour of three types of organically and conventionally grown leafy vegetables (ii) to identify the effects of the cropping systems (organic and conventional) and storage duration (0, 3, 6 and 9 days of storage) on nitrate and nitrite concentrations and postharvest quality characteristics of the leafy vegetables. The three selected vegetables were *Amaranthus viridis*, *Brassica rapa* and *Lactuca sativa*, based on their consumption popularity. The quality of leafy vegetables can be measured by leaf colour, taste, firmness and nutrition benefit. Leaf colour can act as an index of physiological maturity, ripeness, or senescence and as an indicator of nitrate and nitrate content of leafy vegetables.

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