Mal J Nutr 9(1): 31-39, 2003

Determination of Vitamin C, β-carotene and Riboflavin Contents in Five Green Vegetables Organically and Conventionally Grown

Amin Ismail & Cheah Sook Fun

Department of Nutrition and Health Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

As consumer interest in organically grown vegetables is increasing in Malaysia, there is a need to answer whether the vegetables are more nutritious than those conventionally grown. This study investigates commercially available vegetables grown organically and conventionally, purchased from retailers to analyse β-carotene, vitamin C and riboflavin contents. Five types of green vegetables were selected, namely Chinese mustard (sawi) (*Brassica juncea*), Chinese kale (kai-lan) (*Brassica alboglabra*), lettuce (daun salad) (*Lactuca sativa*), spinach (bayam putih) (*Amaranthus viridis*) and swamp cabbage (kangkung) (*Ipomoea aquatica*). For vitamin analysis, a reverse-phase high performance liquid chromatography was used to identify and quantify β -carotene, vitamin C and riboflavin. The findings showed that not all of the organically grown vegetables were higher in vitamins than that conventionally grown. This study found that only swamp cabbage grown organically was highest in β -carotene, vitamin C and riboflavin contents among the entire samples studied. The various nutrients in organically grown vegetables need to be analysed for the generation of a database on nutritional value which is important for future research.

INTRODUCTION

A diet rich in vegetables (more than 5 servings per day) is recommended along with fruits and whole grains; an epidemiological study found that a diet of this composition has a negative association with the risk of chronic diseases. Antioxidant vitamins in vegetables are some of the important nutrients besides other vitamins, minerals, flavonoids and phytochemicals, which have been reported to contribute to health. Our local markets offer a variety of vegetables ranging from leafy to tubers for consumption. Malaysians mostly consume green vegetables such as Chinese mustard, Chinese kale, lettuce, spinach and swamp cabbage. According to the Nutrient Composition of Malaysian Foods, these green vegetables have been found to contain about 1825 – 4760 µg of β -carotene /100 g edible portion, 27.6 – 107 mg of vitamin C/100 g edible portion and 0.15 – 0.55 mg of riboflavin/100 g edible portion (Tee *et al*., 1997).

Beside the conventionally grown vegetables, currently, organically grown foods are gaining popularity among consumers, health educators, farmers and food retailers. Many consumers believe that organically grown vegetables are of better quality, healthier and more nutritious than conventionally grown ones. Organically grown vegetables are presently available in the Malaysian market, although the organic market is still new and is not as yet firmly established compared to the more developed countries. In these countries, consumer demand for organic foods has increased tremendously. This is due to a number of reasons which may vary from country to country such as safety, effect of environment, flavour, freshness, health benefits and nutritional value (Bourn & Prescott, 2002).

In general, organic foods are products produced by organic farming practices, grown without the use of chemical fertilizers, pesticides, fungicides and herbicides. They are usually fertilized solely with organic fertilizers such as animal waste, crop residues, green manures or off-farm organic wastes. Organically grown vegetables are believed to contain higher vitamins and minerals compared to conventionally grown vegetables. Smith (1993) reported that organic foods are more nutritious in terms of mineral content than conventional ones. Leclerc *et al*. (1991) found that carrots and celeriac roots grown organically were higher in ascorbic acid and β-carotene contents. In addition, they reported that the ascorbic acid content in potatoes grown organically was significantly higher than those grown under the conventional method. On the other hand, Svec, Thoroughgood & Hyp Chung (1976) reported no significant difference in the ascorbic acid content in potato, tomato and pepper grown using these two methods. Eggert and Kahrmann (1984) reported no differences in β-carotene content of carrots grown under organic and conventional fertilization.

Few studies have been conducted to investigate nutritional composition of organic vegetables purchased from retailers. Owing to the rapid growth in our country, research should be initiated to analyse the nutrient values of these vegetables for inclusion into the Malaysian Food Composition Database. In addition, the study should also be useful for consumers to know whether organic vegetables purchased from a supermarket are more nutritious than the conventional ones. Therefore, our first attempt is to analyse selected vitamins such as β-carotene, vitamin C and riboflavin, in five organically and conventionally grown commercially available green vegetables.

MATERIALS AND METHODS

Vegetables

Five types of green vegetables grown organically and conventionally were selected based on popular consumption among Malaysian. Conventionally grown vegetables (400 g) were purchased from a local wet market at Seri Kembangan, Selangor, while organically grown vegetables (400 - 500 g) were purchased from Abad Hijau Organik Enterprise at Seri Kembangan, Selangor. Convenience sampling was used to obtain the samples.

Preparation of samples

Upon arrival at the Department of Nutrition and Health Sciences laboratory, the fresh and healthy vegetables were immediately washed under tap water and excessive water dripped off. Edible portions (100 g) of the vegetables were cut into small pieces and homogenised using a blender (National; model MX-291N) for 2 min. The homogenised sample was transferred into an air-tight container and kept at -20°C before vitamin analysis. The above procedure was applied to both organically and conventionally grown vegetables. All procedures were carried out carefully without much exposure to light. All the chemicals and reagents used were of analytical grades or as otherwise stated.

Extraction of vitamins

Vitamin C

Vitamin C was extracted according to the modified method of Abdulnabi *et al*. (1997). The sample (10 g) was homogenised with an extracting solution containing meta-phosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask (wrapped with aluminum foil) and agitated at 100 rpm with the aid of an orbital shaker for 15 min at room temperature. The mixture was then filtered through a Whatman No. 4 filter paper to obtain a clear extract. The ratio of the sample to extraction solution was 1 to 1. All samples were extracted in triplicates.

β-Carotene

The *β*-carotene in the sample was extracted according to the method described by Tee *et al*. (1996) with slight modifications. The sample (10 g) was added with 40 ml of 99.8% ethanol and 10 ml of 100% (w/v) potassium hydroxide, and homogenised for 3 min using a blender. The mixture was saponified by means of a refluxing apparatus, and heated using a heating mantle for 30 min, and then cooled to room temperature. The mixture was frequently agitated to avoid any aggregation. For the extraction step, the mixture was transferred into a separation funnel and 50 ml of n-hexane was added. The funnel was inverted, vented and then shaken vigorously for a few seconds, and the layers were allowed to separate. The upper layer (hexane extract) was pipetted out, and the aqueous layer was re-extracted twice, each time with 50 ml of n-hexane. Then, the upper layer was pooled and washed with distilled water until free of alkali. Phenolphthalein solution (1%) was used to check for any alkali. The presence of alkali turns this indicator to pink. The extract was then filtered through anhydrous sodium sulphate to remove any water residue. The hexane residue was removed under reduced pressure at 45°C using a rotary evaporator (Laborata 4000, Heidolph Instruments GmbH & Co. KG, Germany). The resulting extract was diluted to 10 ml with n-hexane. All samples were carried out in triplicates.

Riboflavin

Riboflavin was extracted according to the method described in AOAC International (1990). One gram of sample was weighed and transferred into a 50 ml graduated polypropylene centrifuge tube. Then 17.5 ml of 0.1 N sulphuric acid was added to it. The mixture was shaken vigorously for 1 min, and then placed in boiling water for 30 min and shaken at 10 min intervals. The mixture was cooled in an ice bath before the addition of 2.5 ml of 2% a-amylase (Sigma Chemical Co., St. Louis, MO, USA). After a gentle mixing, the mixture was incubated at 55°C for 1 hr in a shaking water bath. The mixture was cooled and then diluted to 25 ml with deionised water. The resulting mixture was spun at 2500 rpm for 15 min at room temperature using a bench top centrifuge (Janetzki; model T32c). The supernatant was filtered through a 0.45 μm nylon filter disc before HPLC analysis. All samples were carried out in triplicates.

Determination of vitamins

The vitamins were determined by a reverse-phase HPLC technique. A Hewlett Packard HPLC Series 1100, USA equipped with degasser, quaternary pump, autosampler and diode array detector was used. A Ultrasphere octadecylsilyl (ODS) Hypersil C_{18}, 5 mm particle size, in a 250 mm length x 4.0 mm I.D stainless steel column (Hewlett Packard) was used to determine the vitamins. The separation conditions for the antioxidant vitamins and riboflavin are tabulated in Table 1.

Identification and quantification of vitamins

Vitamin C

Two techniques were used to identify the peak of vitamin C on the chromatogram: comparing the retention time and spiking test with that of L-ascorbic acid (Sigma, Co. Chemical, St. Louis, USA). Ascorbic acid standard was prepared by dissolving 100 mg of L-ascorbic acid in a metaphosphoric acid (0.3 M) – acetic acid (1.4 M) solution at the final concentration of 1 mg/ml.

Table 1. HPLC conditions for separation and identification of vitamin C, β-carotene and riboflavin.

Parameters	Conditions		
	Vitamin C	*β*-Carotene	Riboflavin
Mobile phases	(1) 0.1 M potassium acetate, pH 4.9 (2) Acetonitrile-water (50:50)	Acetonitrile-methanol-ethyl acetate (88:10:2)	Methanol-water-acetic acid glacial (65:35:0.1)
Flow rate	1.5 ml/min	1.0 ml/min	1.0 ml/min
Detection	254 nm	250 nm	270 nm

β-Carotene

The peak of *β*-carotene was identified based on two techniques: comparing the retention time and spiking test with that of *trans*-*β*-carotene (Sigma, Co. Chemical, St. Louis, USA). Ten milligram of trans-*β*-carotene was weighed and dissolved in pure n-hexane to give a stock solution of 100 µg/ml. The solution was stored in a brown bottle and kept as stock in the fridge (4 – 5°C). The standard solution of 1µg/ml was prepared daily from the stock solution.

Riboflavin

For the identification of the riboflavin peak, comparison of the retention time and spiking test with that of riboflavin standard (Sigma, Co. Chemical, St. Louis, USA) was applied. The riboflavin was prepared by adding 20 mg riboflavin in deionised water with the addition of three drops of pure acetic acid glacial. It was warmed at 80°C in a water bath in order to dissolve the riboflavin. The final concentration of the standard was 100 µg/ml.

Statistical analysis

Data were expressed as mean value ± standard deviation. Independent t-test was applied to determine the significant difference at the level of $p<0.05$. A Statistical Package for Social Science (SPSS) for Windows version 10.01 was used to analyse the data.

RESULTS

Vitamin quantification was calculated from the curve generated by plotting the peak area of each authentic standard versus concentration.

Ascorbic acid

Organically (124.8 mg/100 g of fresh weight; CV = 15 %) and conventionally (114.7 mg/100 g of fresh weight; CV=16 %) grown Chinese mustard had the highest ascorbic acid content among the vegetables sampled (Figure 1). The lowest ascorbic acid content, as determined in conventionally grown lettuce, was 15.3 mg/100 g of fresh weight. There were significant differences ($p<0.05$) for Chinese kale, lettuce and swamp cabbage grown organically and conventionally. The results showed no significant difference ($p>0.05$) in ascorbic acid content of Chinese mustard and spinach grown conventionally and organically. Compared to the Nutritional Composition of Malaysian Foods and ASEAN Food Composition Tables, the results obtained on vitamin C content of conventionally grown vegetables were lower in Chinese kale, lettuce and swamp cabbage (Tee *et al*., 1997; Puwastien *et al*., 2000).

β-Carotene

The organic swamp cabbage had the highest content of *β*-carotene of 3503 µg/100 g fresh weight, while Chinese mustard (1994 µg/100 g fresh weight) and lettuce (2006 µg/100 g fresh weight) grown conventionally had the lowest *β*-carotene content among the vegetables (Figure 2). Organically grown swamp cabbage was highly significant ($p<0.01$) in *β*-carotene content compared to the conventionally grown ones. There was also significant difference ($p<0.05$) in *β*-carotene content of Chinese mustard grown using the two methods. Besides, lettuce grown conventionally and organically showed a similar *β*-carotene content. On the other hand, there were no significant differences ($p>0.05$) for Chinese kale, lettuce and spinach grown using the two different techniques. The *β*-carotene content in organically grown Chinese mustard and swamp cabbage was significantly higher ($p<0.05$) than that of the conventionally grown ones. Except for lettuce, the results obtained on *β*-carotene content were lower than that of Tee *et al*. (1997).

Riboflavin

Only three types of B vitamins (riboflavin, thiamin and niacin) have been analysed and compiled in Nutrient Composition of Malaysian Foods Table (Tee *et al*., 1997). Riboflavin was selected in this study due to the established procedures in the laboratory. Furthermore, the present findings on this vitamin could be compared to a previous database generated by Tee *et al*. (1997).

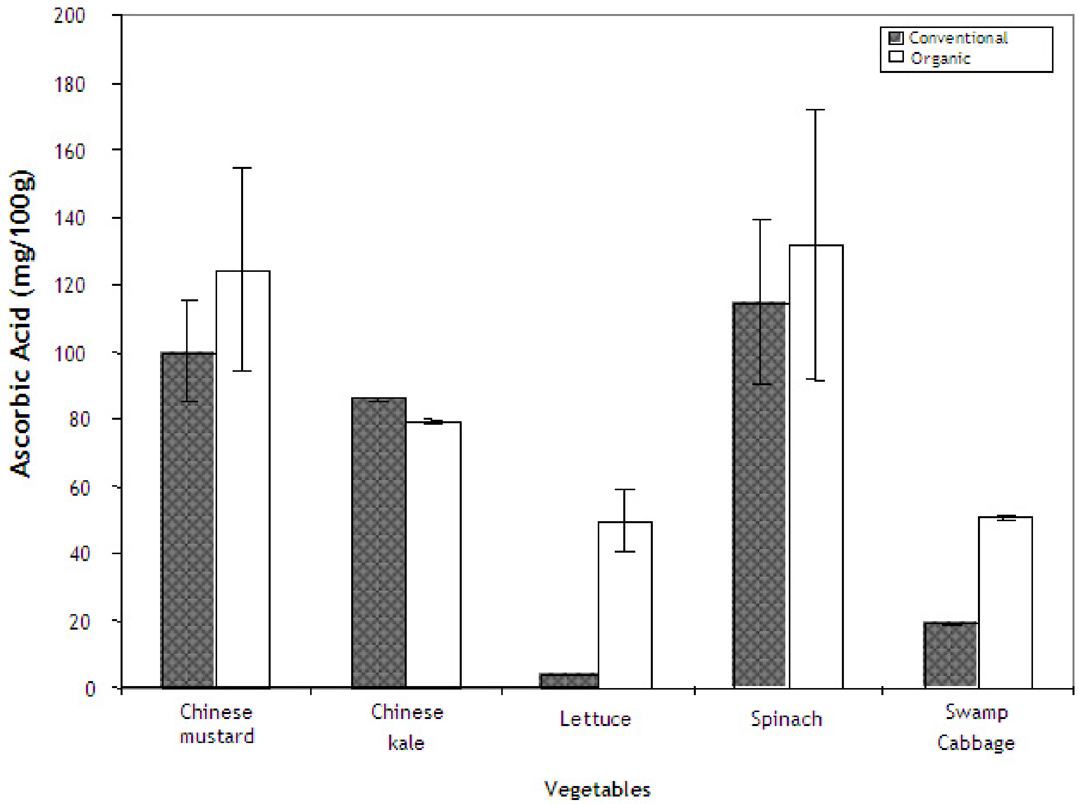

Figure 1. Ascorbic acid content in five types of green vegetables grown organically and conventionally. Asterisk (*) indicates a significant difference at the level $p<0.05$ between organically and conventionally grown vegetables.

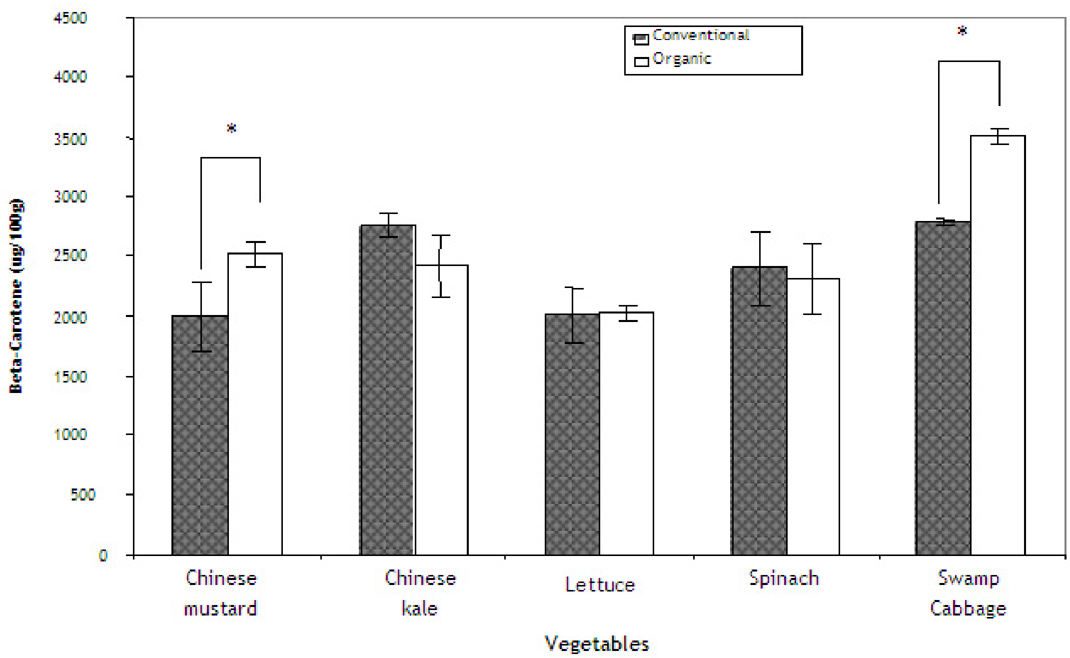

Figure 2. β-Carotene content in five types of green vegetables grown organically and conventionally. Asterisk (*) indicates a significant difference at the level $p<0.05$ between organically and conventionally grown vegetables.

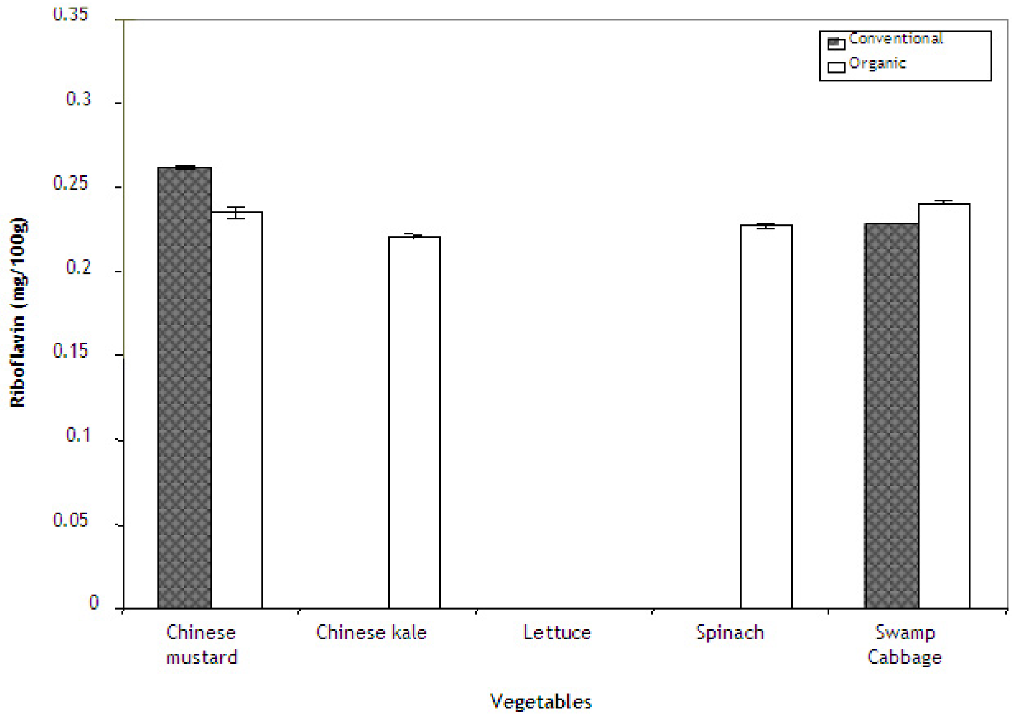

Figure 3. Riboflavin content in five types of green vegetables grown organically and conventionally. Asterisk (*) indicates a significant difference at the level p<0.05 between organically and conventionally grown vegetables.

The conventional Chinese mustard had the highest riboflavin content of 0.26 mg/100 g of fresh weight, while the lowest content of riboflavin was found in organically grown Chinese kale, that is 0.22 mg/100 g of fresh weight (Figure 3). In this study, riboflavin content in conventionally and organically grown lettuce was not detected. In addition, riboflavin content in conventionally grown Chinese kale and spinach was also not detected compared to the organically grown vegetables. The conventionally grown Chinese mustard was found to be significantly higher ($p<0.01$) in riboflavin content compared to that of organically grown ones. However, riboflavin was found to be significantly higher in organically grown swamp cabbage compared to conventionally grown ones. There were significant differences ($p<0.05$) in riboflavin and *β*-carotene contents between organically and conventionally grown swamp cabbage and Chinese mustard.

DISCUSSION

Three main factors can influence the nutritional composition of vegetables grown organically or conventionally, namely genetics, environment and post-harvest practices (Salunkhe & Desai, 1988). These factors are not controlled in vegetables purchased from retailers. In this study, although little or nothing is known about the origin of the vegetables analysed, any differences in vitamin values from both techniques of growing would be useful information to the consumers.

No significant difference ($p>0.05$) was found in vitamins C and *β*-carotene contents between Chinese mustard and spinach grown organically and conventionally. This finding was similar to a report published by Organic Retailers and Growers Association of Australia (ORGAA) which found no major differences in vitamin C and *β*-carotene contents of vegetables produced organically or conventionally (Anon, 2000). Schuphan (1974) found that spinach and lettuce grown organically were higher in ascorbic acid compared to those grown conventionally, using composted manure over organic fertilizers. Through high nitrogen fertilization, Hornick (1989) reported a lower content of ascorbic acid in kale grown organically compared to those grown conventionally. Based on a farming comparison study, Lairon *et al*. (1984) observed no difference in vitamin C content of kale and lettuce grown organically and conventionally.

As reported by Mercadante & Rodriguez-Amayua (1991), kale grown on farms using herbicides was found to contain lower *β*-carotene compared to organically grown kale. Schuphan (1974) found that inorganic fertilization slightly reduced the *β*-carotene content in spinach. Leclerc et al. (1990) reported no significant differences in *β*-carotene between lettuce grown with or without organic fertilization. Due to variation in the study designs by other researchers, it is extremely difficult to compare with our findings. In addition, post-harvest factors which may introduce variables such as maturity at harvest could well confound any apparent differences in vitamin content.

Furthermore, our study used the HPLC method to quantify the vitamins content compared to Tee *et al*. (1997). Probably some of the values obtained in the study were underestimated. For instance, the content of vitamin C in Chinese mustard grown conventionally was found to be higher compared to Tee *et al*. (1997). This could be due to an insufficient number of samples or/and experimental error during HPLC analysis. In the present study, we were not able to detect the presence of riboflavin in Chinese kale, lettuce and spinach grown conventionally, and lettuce grown organically. In food analysis, determination of riboflavin often uses HPLC equipped with a fluorescence detector. Riboflavin has a strong inherent fluorescence which allows it to be detected very exclusively and sensitively (Van Niekerk, 1988). This probably explains why our HPLC using UV detector could detect the riboflavin in some vegetables.

CONCLUSION

It was found that not all of the organically grown vegetables were higher in vitamin contents compared to those grown conventionally. Vitamin C content was found to be significantly higher in Chinese kale, lettuce and swamp cabbage grown organically compared to the conventionally grown ones. Organically grown Chinese mustard and swamp cabbage were significantly higher in *β*-carotene and riboflavin content. In this study, factors such as environment and post-harvest practices that could influence vitamin content were not controlled. Thus it is extremely difficult to make a true comparison between the two techniques of growing. Nevertheless, this study is useful as a step towards further work on the generation of a vitamin content database for vegetables grown organically.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance rendered by the laboratory staff from the Department of Nutrition and Health Sciences throughout the research project and use of the laboratory facilities at Universiti Putra Malaysia.

REFERENCES

Abdulnabi AA, Emhemed AH, Hussein GD & Biacs PA (1997). Determination of antioxidant vitamins in tomatoes. *Food Chem* 60 : 207-212.

Anon (2000) Organic food is far more nutritious. *Newsletter of the National Association of Sustainable Agriculture Australia (NASAA)*. 10 February.

AOAC International (1990). Official methods of analysis. 15th ed. Arlington, VA, USA.

Bourn D & Prescott J (2002). A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Critical Rev in Food Sci and Nutr* 42 : 1-34.

Eggert FP & Kahrmann CL (1984). Response of three vegetable crops to organic and inorganic nutrient sources. In : Organic farming: current technology and its role in sustainable agriculture. Kral DM (ed). Madison, USA.

Hornick S (1989). Nitrogen overload may shrivel vitamin content. *Agric Res* 37 : 10-11.

Lairon D, Termine E, Gautier S, Trouilloud M, Lafont H & Hauton JC (1984). Effects of organic and mineral ferlizations on the contents of vegetables in minerals, vitamin C and nitrates. In: The importance of biological agriculture in a world of diminishing resources. H Vogtmann, E Boehncke & I Fricke (eds). Witzenhausen, Germany.

Leclerc J, Reuille MJ, Miller ML, Lefebvre JM, Joliet E, Autissier N, Martinez Y & Perret A (1990). Effect of climatic conditions and soil fertilization on nutrient composition of salad vegetables in Burgundy. *Sci Aliments* 10 : 633.

Leclerc J, Miller ML, Joliet E & Rocquelin G (1991). Vitamin and mineral contents of carrot and celeriac grown under mineral or organic fertilization. *Biol Agr and Horticulture* 7 : 339-348.

Mercadante AZ & Rodriguez-Amayua DB (1991). Carotenoid composition of a leafy vegetable in relation to some agricultural variables. *J Agric Food Chem* 39 : 1094.

Puwastein P, Burlingame B, Raroeengwichit M & Sungpuang P (2000). ASEAN Food Composition Tables. ASEAN Network of Food Data System (ASEANFOODS): Institute of Nutrition, Mahidol University Thailand.

Salunkhe DK & Desai BB (1988). Effects of agricultural practices, handling, processing, and storage on vegetables. In: Nutritional evaluation of food processing, 3rd ed. Karmas E & RS Harris (eds). New York: Van Nostrand Reinhold.

Schuphan W (1974). Nutritinal value of crops as influenced by organic and inorganic fertilizer treatments. *Plant Foods Hum Nutr* 23 : 333-358.

Smith BL (1993). Organic foods vs supermarket foods: elemental levels. *J. Appl Nutr* 45 : 35-39.

Svec LF, Thoroughgood CA & Hyp Chung SM (1976). Chemical evaluation of vegetables grown with conventional or organic soil amendments. *Communication Soil Science Plant Analysis* 7 : 213.

Tee ES, Kuladevan R, Young SI, Khor SC & Zakiyah HO (1996). Nutrient analysis of foods. Kuala Lumpur: Institute Medical for Research.

Tee ES, Mohd Ismail N, Mohd Nasir A & Khatijah I (1997). Nutrient composition of Malaysian foods. Kuala Lumpur: Institute Medical for Research.

Van Niekerk PJ (1988). Determination of vitamins. In : HPLC in food analysis. R Macrae (ed). London.